THE EFFECTS OF ANTERO-POSTERIOR REVERSAL OF LENGTHS OF THE PRIMITIVE STREAK IN THE CHICK

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In chick blastoderms at primitive streak stage, lengths of the primitive streak were cut out and replaced with their antero-posterior orientation reversed. In some experiments the region immediately in front of the primitive streak (presumptive prechordal head) was also included in the excised-piece. Control operations involving excision and replacement without reversal were also performed. The embryos were subsequently grown *in vitro* by Waddington's technique. After reversal of a variety of different parts of the streak at various developmental stages, many cases of regulative development were obtained. In these, the original orientation of the blastoderm was maintained, and while there were abnormalities of various kinds in the embryos, they were no different from the abnormalities found in the controls. Very occasionally the regulated axis was partially doubled after a reversal, though not after a control operation. A few specimens which had undergone reversal of long pieces of the primitive streak and had completely healed showed a failure of regulation in that there was some tendency for the reversed-piece to develop according to its own orientation. But at best this reversed differentiation was very distorted and incomplete. Evidently the orientation of the primitive streak does not at any stage control the orientation of the embryo; and the primitive streak, when it is fully developed and contains most of the presumptive axial material, is highly labile in its powers of differentiation. In spite of its well-known 'organizer' activity, the primitive streak is subject to control by the surrounding blastoderm.

I. Introduction

The primitive streak has been shown to play an important part in early chick development (Waddington 1932 and later). An investigation of its internal organization by an interchange of its parts seemed therefore likely to be fruitful. Technically the simplest operation of this kind consists in the reversal of part of the length of the primitive streak, so that the original anterior border of the part becomes posterior; and it is with the results of such operations that the present paper is concerned. In view of the known functions of the

primitive streak, the outcome of these experiments was expected to be a severe disturbance of the embryo. The results were, however, unexpected, in that many specimens developed substantially normally after the operation.

II. Method and terminology

The *in vitro* technique of Waddington (1932) was used. At the time of operation the blastoderms were in the primitive streak stage. They were removed from the yolk, and a rectangular piece of the primitive streak, consisting of its full visible width and a varying extent of its length, was isolated by four cuts which passed through all three germ layers. The lateral cuts were made sufficiently far apart (usually about 0·2 mm.) to pass through the epiblast adjacent to the region of cell immigration of the streak. The isolated piece was sometimes (control experiments) simply replaced; more usually it was reversed, so that its posterior border lay anteriorly, and then replaced. The original antero-posterior axis of the blastoderm was marked on the culture vessel and measurements of the blastoderm and of the reversed piece noted. Culture *in vitro* lasted about 24 hr. with occasional interim inspections. After fixation in Bouin, the specimens were stained whole, cleared and sketched with the aid of a camera lucida; then embedded, sectioned serially, stained and re-examined. In all, 195 specimens (thirty-four of them controls) were obtained, but seventy (nine of them controls) were not sectioned because of gross failure of development or of healing.

The piece of the primitive streak which was isolated will in general be referred to as the *excised-piece*; when it was also reversed as the *reversed-piece*; and the remainder of the blastoderm as the *residual blastoderm*. The term *primitive streak* is used so as to include the primitive node. *Primitive* (Hensen's) *node* is used in the sense of Wetzel (1929) to refer to the mass of tissue (first appearing at *LM* stage, see below) in front of *and at either side of* the primitive pit. *Trunk* refers to the region of the embryonic axis where there are somites; *head* to the anterior region where the axial mesoderm is in the form of mesenchyme.

In designating the stage of the blastoderm at the time of operation, Waddington's terminology is used (Waddington 1932). In this system *S* (short) stands for a blastoderm with circular area pellucida, and an ungrooved primitive streak of about one-third to half the length of the area pellucida; *M* (medium) for a blastoderm with ovoid area pellucida, and a primitive streak still ungrooved of about two-thirds of the area pellucida length; and *L* (long) for a blastoderm with strongly ovoid or pear-shaped area pellucida, and a primitive streak with groove and node and of about three-quarters of the area pellucida length (at this stage the primitive streak reaches its maximal development). Intermediate stages, *SM* and *LM*, are also distinguished. The *L* stage is succeeded by the *head-process* stage, during which the head-process forms and the node moves backwards.

The following types of excised-piece were used, each one at a variety of developmental stages. They are illustrated in figure 1, together with maps of presumptive areas in *M* and *L* stages.

Type IA. The anterior end of the primitive streak; in length one-tenth to one-quarter of the total length of the primitive streak (i.e. excised-piece 0·13 to 0·40 mm. long). Thirteen reversed and three control specimens.

Type IB. As IA, but one-third to three-quarters of the primitive streak in length (i.e. excised-piece 0·28 to 1·2 mm. long). Sixty-one reversed and sixteen control specimens.

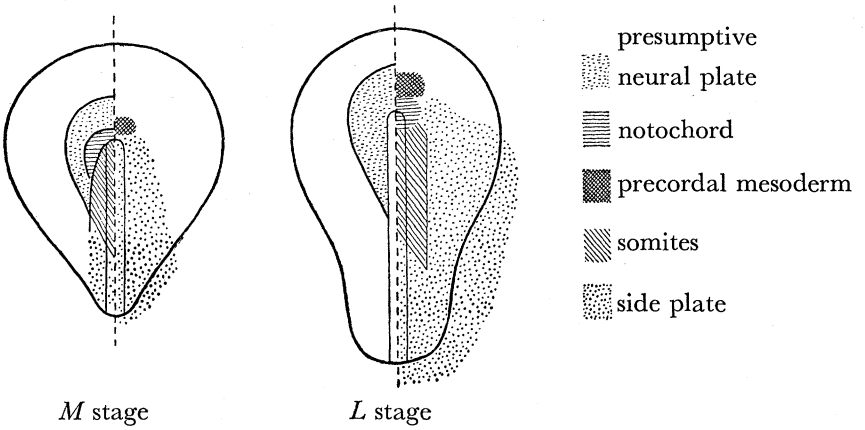

FIGURE 1*a*. Maps of presumptive areas according to Pasteels (1937). On the left of each figure, areas of the epiblast; on the right, areas which have migrated into the mesodermal layer. The *S* stage differs from the *M* stage only in that the epiblast areas at the sides of the primitive streak are laterally displaced.

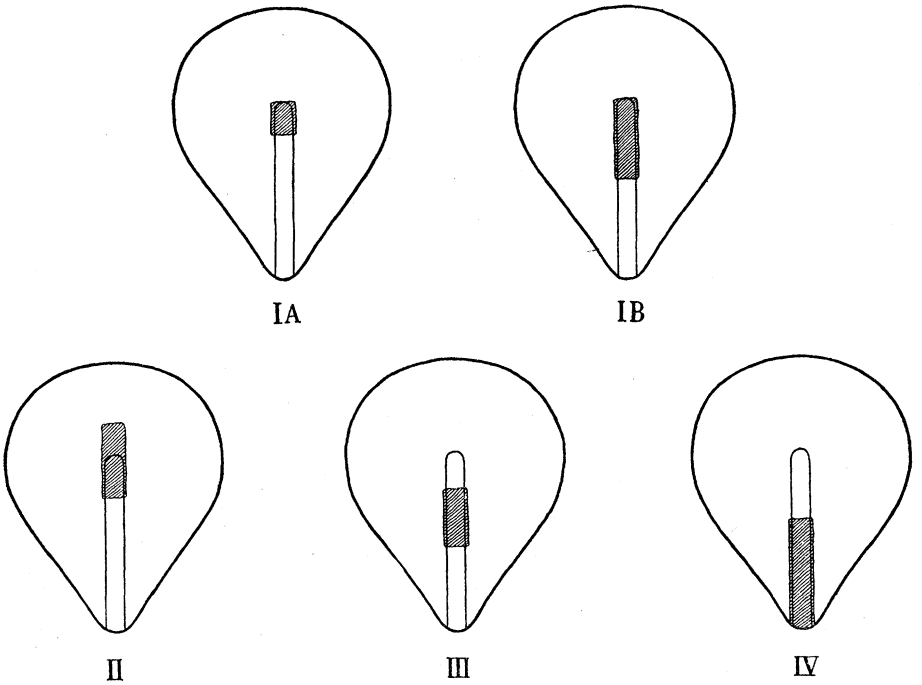

FIGURE 1*b*. Scheme of types of operation. The excised-piece is indicated by the shaded area. The blastoderm has been arbitrarily represented as *M* stage.

Type II. As IA or IB, but in addition including a region of the area pellucida extending 0·11 to 0·40 mm. forward from the anterior edge of the primitive streak (the length of this additional piece is equivalent to between one-tenth and one-third of the primitive streak length). Thirty-seven reversed and eight control specimens.

Type III. A middle region of the primitive streak; in length usually about one-third of the primitive streak length, with its anterior limit one-tenth to one-third of the primitive streak length behind the anterior edge of the primitive streak. Thirty-seven reversed and six control specimens.

Type IV. The posterior end of the primitive streak; in length one-half to three-quarters of the total primitive streak length. Thirteen reversed and one control specimens.

In describing any specimen, the following information is given: type of operation; stage at operation; measurements at operation, namely, primitive-streak length, the percentage this length is of the overall area pellucida length, length of head-process (when present), length of excised-piece, with (in type II operations) the length of epiblast in front of the primitive streak included in the reversed-piece, and (in type III operations) the distance between the anterior incision of the excised-piece and the anterior limit of the primitive streak.

The operations lead to a number of different kinds of results, and a preliminary analysis of specimens is given in table 1.

TABLE 1. PRELIMINARY ANALYSIS OF RESULTS OF OPERATION. FREQUENCY OF DIFFERENT KINDS OF SPECIMEN ACCORDING TO TYPE OF OPERATION

1 operation type	2 not healed	3 healed, no development	4 healed distorted	5 healed normal	6 total
		after reversal operation			
IA	2	0	6	5	13
IB	26	12	10	13	61
II	24	1	4	8	37
III	7	9	6	15	37
IV	3	1	4	5	13
total	62 (39%)	23 (14%)	30 (19%)	46 (29%)	161
		after control operation			
IA	2	0	0	1	3
IB	5	2	2	7	16
II	5	0	1	2	8
III	2	0	1	3	6
IV	1	0	0	0	1
total	15 (44%)	2 (6%)	4 (12%)	13 (38%)	34

III. SURVEY OF RESULTS

A substantial proportion of operated blastoderms has almost completely failed to heal (column 2). The proportion (39%) amongst those which had undergone reversal does not differ appreciably from the proportion (44%) amongst the controls. Reversal certainly does not decrease the chances of healing in these experiments. The results of type IB and type II operations show significantly poorer healing than the rest (at the 5% level of probability by chi-square test). After type IB operations the failures of healing were predominantly among those specimens where a very long piece of the primitive streak had been reversed. After type II operations, the failures were mainly among operations done at the *L* stage, when there seems to be difficulty in obtaining healing of the epithelial part of the blastoderm in front of the primitive streak (involved in this type of operation only) because its cut edges tend to roll up.

Considering now only those specimens whose healing was adequate, a proportion (23% of all those healed after reversals) failed altogether to develop an embryonic axis (column 3). These embryos belong almost exclusively to operation types IB and III, which had been operated at a stage younger than *L*, and in which large pieces involving most of the anterior half of the primitive streak were reversed. The effect of such operations is not peculiar to

reversals, since two controls also failed to develop. If operation is performed at the *L* stage or later, suppression of development is very rare, whatever the type of operation.

The remaining specimens, those which have both healed and developed axial tissues, fall into two classes. A number (column 4) has gross distortions, while a larger group (column 5) has developed fairly normally. After reversal operation the embryos in the latter group are oriented not in the direction of the reversed-piece but in that of the residual blastoderm; and these are the embryos to which I shall refer as *regulated*. The final proportion of all embryos regulated after reversal (29%) is not very much less than, and certainly not statistically significantly different from, the proportion of normal embryos resulting from control operations without reversal (38%).

In the following sections of the paper, some of these types are considered in more detail. First (§ IV) the specimens healed and regulated after reversal (column 5 of table 1). Then the healed but distorted specimens (column 4 of table 1) of which there are three major types, namely: (§ V) those which have a doubling, more or less complete, of the axis (four specimens); (§ VI) those with a tendency towards axial differentiation in the orientation of the reversed-piece (eleven specimens); and (§ VII) those with the midline of the neural plate and the notochord undifferentiated, probably owing to a failure of the reversed-piece but not of the residual blastoderm to develop (seven specimens). The specimens which, though healed, failed altogether to develop an embryonic axis (column 3 of table 1) were not sectioned and are not considered further. Of the embryos which had grossly defective healing (column 2 of table 1), are described (§ VIII) only a group of twenty-four specimens which have a characteristically split axis with the excised-piece more or less completely isolated. The different kinds of development characteristic of each type of operation are then summarized (§ IX).

IV. Regulated specimens

Of the healed and normal specimens included in column 5 of table 1, those which had undergone a control operation have differentiated as expected. Interest centres on those which had undergone a reversal operation. In spite of the reversal, they have developed a more or less normal embryonic axis, with the orientation of the residual blastoderm. They have therefore undergone a regulation during development of the disarrangement produced by the operation. They have been arbitrarily divided into those which have achieved complete regulation, and those which show only partial regulation.

By *complete regulation* is meant development, from the disarranged tissues, of an embryonic axis as normal as that developed from an unoperated blastoderm *in vitro*. Careful inspection is necessary to establish whether complete regulation has involved the tissues of both reversed-piece and residual embryo. Waddington (1932) has shown that if the primitive streak is simply excised from a blastoderm, the cut edges may heal together, a new primitive streak be constituted, and normal development proceed. Waddington & Cohen (1936) have found analogous behaviour in the pre-nodal region. If the reversed-piece fails to heal essentially the same regeneration may take place *dorsal to* the reversed-piece, which is therefore left isolated and inconspicuous ventral to a normal embryo. This has happened in three specimens (which have been included in column 4 of table 1). It is, however, hardly possible for such an isolated reversed-piece to disappear without trace, and in the

majority of cases of complete regulation the healing of the reversed-piece to the residual blastoderm was actually observed during development; so there can in fact be little doubt that the cases of complete regulation involve the incorporation of the reversed-piece into the resulting embryo. Since the embryos have not been carried beyond a 10- to 15-somite stage, normality refers only to structures developed at this stage. For this reason, the attainment of complete regulation is often not of the same significance after operations of types III and IV as after the other types, since such reversals may have involved tissues still little differentiated at the time of fixation. It may be added that *rate* of development of completely regulated embryos operated at *S* or *M* stages seemed often to be abnormally slow.

By *partial regulation* is meant the development of an embryonic axis in the orientation of the residual blastoderm, but showing minor traces of the operation. Such traces are however of a kind met with among the unreversed controls, and are not indicative of any tendency of the reversed-piece to develop according to its own orientation. The imperfections of development concerned are of the following kinds: (1) extra abnormally situated tissue, usually loose mesenchyme amongst the somites, occasionally epithelial tissue amongst the primitive streak mesoderm; (2) strongly marked bilateral asymmetry, usually involving the disproportionate size of one row of somites or duplication of part of the length of a row, with corresponding asymmetry of the neural plate; (3) small and poorly differentiated axial organs, usually in the somitic region; (4) an unusually massive notochord near the front end of the embryo, due perhaps to an obstruction of the backward movement of the node; (5) a small hole dorso-ventrally through the axis, due to failure of healing, usually in the posterior part of the embryo, and commonly associated with absence of notochord behind the defect.

It is unfortunately impossible to list all regulated specimens, but the following is a record of the more important operations which were found to be compatible with regulation. The figures are lengths in mm., and the following contractions are used in all subsequent pages where measurements are involved:

p.s. = primitive streak. *a.p.l.* = area pellucida length.
h.-p. = head-process. *r.-p.* = reversed-piece.

After type IA operation, complete regulation at *L* stage (*p.s.* 1·8, *r.-p.* 0·15) and at head-process stage (*p.s.* 2·2, *h.-p.* 0·3, *r.-p.* 0·15). *After type IB operation*, complete regulation at *S* stage (*p.s.* 1·2, *r.-p.* 0·8) and at *M* stage (*p.s.* 1·1, *r.-p.* 0·55); partial regulation at *LM* stage (*p.s.* 1·7, *r.-p.* 1·1) and at *L* stage (*p.s.* 1·8, *r.-p.* 0·65). *After type II operation*, complete regulation at *S* stage (*p.s.* 0·8, *r.-p.* 0·35 of streak with 0·25 of anterior epiblast), at *M* stage (*p.s.* 1·4, *r.-p.* 0·30 with 0·25), at *LM* stage (*p.s.* 1·8, *r.-p.* 0·25 with 0·25) and at *L* stage (*p.s.* 1·6, *r.-p.* 0·3 with 0·1). *After type III operation*, complete regulation at *L* stage (*p.s.* 1·7, *r.-p.* 0·55 excluding 0·25 of the anterior end of the streak) and at head-process stage (*p.s.* 2·0, *h.-p.* 0·1, *r.-p.* 0·5 excluding 0·65); partial regulation at *M* stage (*p.s.* 1·0, *r.-p.* 0·7 excluding 0·25), *L* stage (*p.s.* 2·0, *r.-p.* 0·7 excluding 0·45) and head-process stage (*p.s.* 2·3, *h.-p.* 0·55, *r.-p.* 0·75 excluding 0·25). *After type IV operation*, complete regulation at *S* stage (*p.s.* 1·1, *r.-p.* 0·8) and at *SM* stage (*p.s.* 1·5, *r.-p.* 1·0); partial regulation at *M* stage (*p.s.* 1·5, *r.-p.* 1·2) and *LM* stage (*p.s.* 2·3, *r.-p.* 1·1).

The following are illustrative descriptions of a few of these specimens. The determination of the presumptive areas involved in the reversed-piece is based on Pasteels (1937).

Specimen no. 1 (type IA operation at *h.-p.* stage; *p.s.* 2·2 mm. 70% of *a.p.l.*, *h.-p.* 0·3 mm.; *r.-p.* 0·15 mm., i.e. primitive node). The operation reversed the presumptive notochord of at least the entire trunk, while it was undergoing backward extension. At fixation, though the anterior end of the head is distorted by failure of the neural tube to roll up normally, a common defect of cultured embryos, the entire trunk including the remains of the primitive streak is quite normal (figure 2, plate 21). There were fifteen pairs of somites.

Specimen no. 2 (type IB operation at *L* stage; *p.s.* 1·8 mm., 75% of *a.p.l.*; *r.-p.* 0·55 mm.). The operation must have placed the invaginated presumptive notochord (primitive node) in the position of part of the invaginated presumptive somite region and vice versa; and would similarly have exchanged presumptive lateral with median neural plate. The regulation of this specimen has been partial. The notochord probably does not extend as far forward into the head as it ought to; the trunk region has rather poorly differentiated somites (figure 3, plate 21); and the notochord stops short of the hind end of the trunk, a small hole appearing in the middle of the axis in this region, marking a place where healing was observed to fail during development. Nevertheless, a recognizable embryonic axis in normal orientation has developed.

Specimen no. 3 (type IB operation at *S* stage; *p.s.* 1·2 mm., 50% of *a.p.l.*; *r.-p.* 0·8 mm.). This reversal of two-thirds of the primitive-streak length would not have affected the main presumptive areas; the entire reversed-piece was presumptive side-plate, superficial or invaginated. It might, however, have produced a conflict of tissue movements, and if the early primitive streak has an inductive influence on the later, this operation should show it. The site of the operation was observed to heal satisfactorily and regulation was substantially complete at fixation (when there were four pairs of somites); though the primitive streak region at the posterior end of the embryo is slightly distorted, and there is some asymmetry of the axis (figure 4, plate 21).

Specimen no. 4 (type II operation at *LM* stage; *p.s.* 1·7 mm., 70% of *a.p.l.*; *r.-p.* 0·28 mm. of *p.s.* plus 0·23 mm. in front). The partly invaginated presumptive notochord would probably have been exchanged for invaginated presumptive head mesoderm by this operation, and vice versa. Healing was observed, and at fixation regulation was complete in the head region (figure 5, plate 21) and trunk; but behind the node there was some distortion, with an irregular mass of cells, which it seems possible represents the epithelial part of the reversed-piece, originally in front of the node and containing the presumptive head mesoderm, which has been carried right back.

Specimen no. 5 (type II operation at *S* stage; *p.s.* 0·8 mm., 45% of *a.p.l.*; *r.-p.* 0·34 mm. of *p.s.* plus 0·23 mm. in front). The operation probably displaced presumptive notochord, head mesoderm and side-plate, of which only the latter would be invaginated at this stage. Healing was observed, the graft remaining at the front end of the primitive streak which elongated backwards. At fixation regulation had been complete (figure 6, plate 21).

Specimen no. 6 (type III operation at *L* stage; *p.s.* 2 mm., 71% of *a.p.l.*; *r.-p.* 0·40 mm., its anterior edge 0·23 mm. behind the front end of the *p.s.*). The operation should have reversed a section of the (invaginated) presumptive somite region. Failure to regulate might have been manifested by a confusion of the trunk region, due to the reversed-piece elongating

according to its presumptive fate, in opposition to the tissue movements of the residual blastoderm. Healing was observed, but it was not complete, and a small hole persists in the primitive-streak region of the fixed embryo. The trunk region is rather poorly developed, and rather distorted by asymmetry of the somites, notochord and neural plate (figure 7, plate 21). The orientation of the reversed-piece has, however, conformed to that of the residual embryo, and the specimen is therefore a case of partial regulation.

Specimen no. 7 (type IV operation at *SM* stage; *p.s.* 1·5 mm., 60% of *a.p.l.*; *r.-p.* 1 mm.). Reversal of the posterior two-thirds of the primitive streak at this stage would not exchange major presumptive areas, since the whole reversed-piece is presumptive side-plate, but might at least show itself in a derangement of inductive influence of early on later primitive streak, or in a conflict of tissue movements. Actually the entire embryo at fixation is perfectly normal.

Generalizing about the powers of regulation revealed by all the specimens, it appears that complete regulation has on occasion followed each type of operation, up to the maximum size of reversed-piece compatible with healing, with one exception. If the blastoderm at the time of operation was in the *LM* stage or older, then reversals involving the front end of the primitive streak do not lead to complete regulation unless the reversed-piece is very small; about 10% of the primitive-streak length was the longest reversal actually found to be compatible with complete regulation, though the series of experiments was not adequate to determine an upper limit with any accuracy. Partial regulation is not limited in this way.

Discussion of the actual changes of developmental processes involved in regulation meets a serious limitation, in that the precise actual fates of the reversed-piece and residual blastoderm are usually unknown. We can often be confident that they have not developed according to their presumptive fate, but we do not usually know how they have in fact developed. This is particularly so as regards the development of the anterior end of the primitive streak. Here the tissue movements are complex and the presumptive areas crowded, and it is always uncertain whether the changes from normal presumptive fate which must have occurred were in the reversed-piece only or also in the residual blastoderm. Another difficulty occurs with young blastoderms in that the reversed-piece was often seen to persist in the axis, owing to delayed healing, instead of forming side-plate as would be expected from its position immediately after the operation; and when the time for the formation of the axis arrived, the reversed-piece occupied the position of, and may have developed into, axial material. The exact fate of the reversed-pieces of young blastoderms may vary therefore with rate of healing. Finally, it is not certain in a completely regulated embryo that an anterior reversed-piece has even played the part of normal primitive streak, since after an operation the axis does not necessarily develop in the precise position of the primitive streak, especially of the young streak (Abercrombie & Waddington 1937; Woodside 1936). The bilateral asymmetry of some partially regulated specimens suggests that the reversed-piece is to one side of the middle of the axis. In three specimens (included in column 4 of table 1) an extra fragment of embryonic axis occurring beside the main axis probably indicates more extreme displacement of the latter from the position of the original primitive streak, leaving the reversed-piece to develop autonomously to one side. It is conceivable that a presumptively axial reversed-piece might be absorbed without

trace into the lateral body wall by such an occurrence, and its fate quite misinterpreted. Such displacement of the final axis is probably, however, unusual, judging by observation of developing specimens, and by the many imperfect embryos where the reversed-piece can be located with considerable probability. But the possibility of its occurrence makes interpretation unsure in some cases.

In view of these causes of uncertainty, detailed discussion of the readjustments presumed to have occurred during regulation cannot be undertaken. The experiments at least prove that reversed orientation of large regions of the young primitive streak (which contains no axial tissues) does not disturb the functioning of the later primitive streak (which forms the axis). They prove that there is great lability in the development of the presumptive axial tissues contained in the primitive streak at the *L* stage, since the process of development of these tissues must usually be very seriously altered, involving changes from presumptive fate, when a regulated embryo results from the operations. We can be confident from the operations of type IB that presumptive notochord in the *L* stage can be converted into other tissues (probably somites and/or side-plate); and in the same experiments some other tissue (presumptive somite probably) must form notochord. The backward movement of the node occurs in spite of the reversal of the node, even in the head-process stage when the movement has already started (e.g. in specimen no. 1 above; it is interesting that Spratt (1947) has recently suggested that this movement is a relatively passive one). After type II operation on *S*, *M* and *LM* stages, complete regulation probably means that the epithelial tissue from in front of the node is converted to primitive-streak material (similar conversion has been described by Waddington & Taylor 1937). Occasionally, in partially regulated specimens, such conversion fails, and the epithelium persists in the primitive-streak region of the embryo, or in one case is induced to a patch of extra neural plate beneath the axis. After type II operations also, from the *M* stage onwards according to the maps of Pasteels (1937), the prechordal head region is constructed out of non-presumptive tissues in regulated specimens. Many other transformations must of course occur during regulation, but their detailed specification requires some technique for differentially marking regions of the blastoderm.

V. Doubling of axis

In four specimens, all of them the result of reversal operations, there has been a tendency to form, not a single regulated axis, but a more or less completely doubled axis, with the orientation of the residual blastoderm. These specimens, taken in order of decreasingly distinct doubleness, are as follows:

Specimen no. 8 (type IB operation at *S* stage; *p.s.* 1·3 mm., 60% of *a.p.l.*; *r.-p.* 0·75 mm.) has anteriorly two small but complete divergent, embryonic axes (figure 8, plate 21) which fuse posteriorly in the trunk region. In the anterior part of the somitic region there is a central somite-like mass (reversed-piece) in common between the two axes. Farther back the two neural plates join, though still appearing double in section; then the central somitic mesoderm disappears, the two notochords fuse, and the neural plate takes on the normal single form. The overall length of the embryo is unusually short.

Specimen no. 9 (type IB operation at *L* stage; *p.s.* 2·3 mm., 80% of *a.p.l.*; *r.-p.* 1·1 mm.) has two separate very distorted heads (figure 9, plate 21). These lead back to a common

trunk with a wide neural plate, having a single central somite series bordered on each side by a notochord, and the usual outer somite series lateral to each notochord (figure 10, plate 21). This doubled axis continues back as far as the large hole which developed in the posterior part of the blastoderm.

Specimen no. 10 (type II operation at *L* stage; *p.s.* 1·8 mm., 80% of *a.p.l.*; *r.-p.* 0·55 mm. of *p.s.* with 0·3 mm. in front) is similarly arranged except that the head is externally single, with, in section, a normally shaped neural plate but two symmetrically placed poorly developed notochords (figure 11, plate 22). The trunk has a common central somite series, two notochords, and a more irregular neural plate than has specimen no. 9. The doubling persists back to the node.

Specimen no. 11 (type IA operation at *S* stage; *p.s.* 1·3 mm., 50% of *a.p.l.*; *r.-p.* 0·3 mm.) is also externally single throughout its length. It has two notochords, which are always very close together, and fuse (rather imperfectly) for a space in front of the first somite (figure 12, plate 22), farther back separating again. There is no central somite series between the notochords; but where the notochords are separate there is a little mesenchyme between them. The doubling of the notochord continues back to the hole which developed in the posterior part of the blastoderm.

It is not possible in these specimens to distinguish tissues derived from the reversed-piece and those from the residual embryo, except in no. 11, which entirely failed to heal. The reversed-piece in this specimen lies quite undifferentiated in the middle of the large hole, contributing nothing to the doubled axis.

Two rather different explanations of these specimens are possible. It may be that one axis (or part-axis as the case may be) has formed from the reversed-piece and the other from the residual blastoderm which has regenerated. Specimens rather similar to nos. 10 and 11 were obtained by Abercrombie & Waddington (1937) from the contiguous differentiation of host and grafted primitive streaks, which supports this explanation. On the other hand, it may be that the residual blastoderm has formed axes (or part-axes) on either side of the original mid-line of the primitive streak, with or without a contribution from the reversed-piece to the median part of the doubled axis. The position of the reversed-piece in no. 11 supports this explanation. So does the subsequent discovery (Abercrombie & Bellairs, unpublished) that similar doubled axes are consistently produced by this second mechanism, when a piece of the posterior end of the primitive streak is substituted for the primitive node in *L* stage blastoderms. Further discussion of doubling will be deferred in view of this later work.

VI. Tendency to reversed development

In this section are considered all those specimens which healed almost perfectly after a reversal operation, and may be said to show some sign of development according to the orientation of the reversed-piece. No tendency to such reversed development is found after any control operation. To begin with there are three specimens whose final orientation, in the region of the reversed-piece, is uncertain. They had undergone type III operations; and the posterior region of the trunk is of uniformly poor differentiation, being devoid of somitic mesoderm. This may conceivably be due to the fact that presumptive side-plate

had replaced presumptive somite, while the presumptive somite had been transferred too far posteriorly to differentiate at the time of fixation.

Eight further specimens show a tendency to reversed development. In all of these a large piece of the primitive streak was reversed.

In the sections of specimen no. 12 (type III operation at *h.-p.* stage; *p.s.* 2·0 mm., 71% of *a.p.l.*, *h.-p.* 0·25 mm.; *r.-p.* 1·2 mm., 0·25 mm. back from anterior end of primitive streak) at approximately the anterior end of the reversed-piece the notochord becomes unusually massive, while the lateral mesoderm is very poorly differentiated (figure 13, plate 22). Farther back the notochord narrows to a rod only one cell in thickness accompanied by much mesoderm, some of which is roughly in the form of somites (figure 14, plate 22). Farther back still, now towards the original anterior end of the reversed-piece, the notochord becomes large again; it is associated with a mass of mesoderm, which is to some extent necrotic (figure 15, plate 22). An axis has therefore partly differentiated. But the unusual state of the notochord may well reflect the influence of the reversed-piece, which may have inhibited differentiation in its original posterior (presumptive side-plate) region. Orientation may therefore be regarded as to some extent a compromise.

Specimen no. 13 (type IB operation at *L* stage; *p.s.* 2·3 mm., 73% of *a.p.l.*; *r.-p.* 1·0 mm.) has, behind a normal head, a trunk which in its anterior part consists of a very thin and wide neural plate overlying a little mesoderm (figure 16, plate 22). Farther posteriorly, in the primitive-streak region, the mesoderm becomes more and more bulky; and thick neural tissue (continuous with the thin sheet of neural tissue in front) lies at either side of the region of cell immigration (figure 17, plate 22). This massive and atypical primitive-streak region stops suddenly at the posterior end, and is succeeded by a tiny primitive streak for a short distance. What was originally the anterior end of the reversed-piece has therefore apparently remained a mass of mesenchyme. It has induced the epidermis above it to neural tissue; but it has formed no suggestion of notochord or somites. Axial differentiation of the residual blastoderm or of the original posterior end of the reversed-piece has almost failed to occur.

In two other specimens (posterior two-thirds of the primitive streak reversed at *LM-L* stages) a similar primitive-streak region with wide neuroid tissue at each side occurs. As is to be expected, however, the anterior part of the trunk is comparatively well developed also, since the original anterior end of the primitive streak was left *in situ*.

In three specimens, no. 14 (type IB operation at *LM* stage; *p.s.* 1·8 mm., 70% of *a.p.l.*; *r.-p.* 1·1 mm.); no. 15 (type IB operation at *L* stage; *p.s.* 2·2 mm., 79% of *a.p.l.*; *r.-p.* 1·3 mm.); and no. 16 (type IB operation at *M* stage; anterior three-quarters of *p.s.* reversed), the reversed differentiation has gone farther than in the preceding specimens, though the influence of the orientation of the residual blastoderm is also clearly apparent. The prechordal head, which is in the usual position, is very distorted in two of the specimens though normal in no. 14 (figure 18, plate 22). Behind it is an aberrant region containing no notochord. In no. 14 this region consists of poor somites, with extra mesenchyme, under a thin neural plate. In no. 15 there is a flat neural plate with undifferentiated mesenchyme and epithelial tissue beneath it (figure 19, plate 22). In no. 16, in which the mesoderm is throughout very massive, there are somites, varying from one to four in a transverse row, beneath a flat neural plate. Farther towards the posterior end of the

residual embryo in these three specimens the self-differentiation of the reversed piece begins to be evident. In no. 16 there appears in the posterior trunk region a short stretch of notochord, to one side of the mass of somites which fills the axis (figure 21, plate 23) and which is continuous with the mass of somites farther forward. This notochord is not at the original anterior of the reversed-piece, for behind it (in the sense of the residual blastoderm) the mesoderm of the reversed-piece continues. It is here mesenchymal, resembling head mesoderm, and it has induced a small well-formed neural plate (figure 22, plate 23). There is no primitive streak at the most posterior end of the area pellucida. In the posterior trunk region of no. 14 and no. 15 there is a region of wide somitic mesoderm, and, towards the original anterior end of the reversed-piece, a brief laterally placed notochord (figure 20, plate 23). In both these specimens there is a small split at either side of this end of the reversed piece. Its walls, formed by the residual embryo, show some neural tissue with axial mesoderm, followed by a structure typical of primitive streak.

In these three specimens, the prechordal head has maintained the original orientation of the blastoderm. But no fully functional node has appeared in the position corresponding to this orientation, that is, in the original posterior end of the reversed-piece; though some somites have formed here in two specimens. It is quite possible that tissue movements in the residual embryo have proceeded in their normal direction, since in two specimens neural tissue belonging to the residual embryo can be identified with some likelihood in the posterior part of the area pellucida, where it is in normal development carried by the backward movement. On the other hand, the tissue movements in the reversed-piece must have been very aberrant; but a short length of notochord associated with somitic mesoderm, suggesting a partial functioning of a node, has in each case formed near the original anterior end of the reversed-piece.

The last specimen in this series is no. 17 (type IB operation at *L* stage; *p.s.* 2·1 mm., 82% of *a.p.l.*; *r.-p.* 0·7 mm.). Behind a fairly normal anterior region of the head, the reversed-piece appears fused into the axis. But a little farther back the neural plate of the residual blastoderm divides into two which pass at either side of the reversed-piece, to which they are healed (figure 23, plate 23). Behind the reversed-piece, in the posterior of the trunk region, the neural plates join again in the mid-line and lead to a primitive-streak region. There is very little axial mesoderm under the neural plates alongside the reversed-piece, but behind the reversed-piece good somites develop. The reversed-piece consists of somitic mesoderm in the head-region of the residual blastoderm (figure 24, plate 23). Farther towards the original anterior end of the reversed-piece the somites give place to a large mass of notochord tissue, which is not covered by a neural plate (figure 23, plate 23). It seems clear that residual embryo and reversed-piece in this specimen have each largely self-differentiated, a unique situation amongst healed specimens.

This series of specimens includes every instance where a reversed-piece can possibly be described as tending to differentiate according to its own orientation. An important condition for any such differentiation seems to be that the reversed-piece should be large; and it is probable that delay in healing, which was observed in most of these specimens, is also important in that it prevents successful regulation. The frequency and extent of reversed development is impressively small. There is no question in these experiments of a simple reversal of the polarity of development of the whole embryo. The reversed-piece

never imposes its orientation on development as a whole, in the same way that the residual blastoderm so frequently and successfully does, as described in § IV. In the specimens described in the present section both orientations are found to be represented simultaneously, usually, however, in an extremely distorted form. The mutual distortion is minimal in specimen no. 17, where the reversed-piece has remained structurally fairly independent of the residual embryo, in spite of eventual healing. Self-differentiation of the reversed-piece also occurs where the structural independence is greater still, owing to failure of healing (§ VIII).

VII. Specimens with mid-line undifferentiated

Very occasionally, in specimens well healed after small anterior reversals, there has been a more or less complete failure to differentiate on the part of the tissues normally derived from the primitive node, i.e. notochord and neural tube floor.

Four typical specimens, all the result of reversal operations, were obtained: one operated (type IB) at *M*, two (type IA) at *LM* and one (type IA) at head-process stage. In these, the mid-line of the neural plate, along a varying length of the axis, consists of a strip of thin non-neural epithelium. The notochord in the same region is missing, the somites are usually poorly developed and there may be extra mesenchyme in the axis (e.g. figure 25, plate 23, specimen no. 18; type IA operation at *LM* stage; *p.s.* 1·5 mm., 65% of *a.p.l.*; *r.-p.* 0·3 mm.). As in the split specimens described later, the notochord and central strip of the neural plate are present in the posterior part of the embryo. In specimen no. 18 there is an irregular and feeble notochord in front of the defective region, and a normal prechordal head; while in the specimen operated at head-process stage there is of course a well-developed notochord, derived from the head-process, and prechordal head in front of the defective region. Occasionally (two reversal and two control specimens) the same sort of defective neural plate occurs when notochord is present (e.g. figure 26, plate 23; specimen no. 19; type III operation at *h.-p.* stage; *p.s.* 2·0 mm., 80% of *a.p.l.*, *h.-p.* 0·25 mm.; *r.-p.* 0·25 mm., 0·75 mm. behind anterior end of *p.s.*). This is due presumably to some dissociation of the processes of the backward movement.

In these specimens it is of course impossible to identify the tissues of the reversed-piece with certainty. But it seems probable that the residual blastoderm has developed according to its presumptive fate; while the reversed-piece has failed to differentiate and in so doing has been responsible for the defect in the embryo at the time of fixation. Such a failure is reminiscent of the undifferentiated tissue sometimes found in an otherwise normal axis, referred to in the description of partial regulation. It may seem surprising that the reversed-piece can be responsible for the undifferentiated mid-line in spite of the fact that in the *M* stage and perhaps in the *LM* stage blastoderms it can hardly have contained the presumptive notochord and neural plate subsequently missing. But slow healing, which was observed in these specimens, may have resulted in the reversed-piece occupying the position of the primitive node at the time of the backward movement. From such a position it perhaps underwent the tissue movement of node material but not the tissue differentiation. Slow healing may, as with the tendency to reversed development previously discussed, be part of the explanation of the failure to regulate to a single normal axis (cf. the poor differentiation of many unhealed excised-pieces described in § VIII). It is probable

that the residual embryo has undergone normal backward movement of its axial tissue, despite the absence of a functioning primitive node. Wolff (1936) has obtained rather similar embryos by X-irradiation of the node *in vivo*.

VIII. Split specimens

In the table in § III there are listed seventy-seven specimens with a failure of healing so gross as seriously to affect their development. About half of this group was discarded before sectioning because of an accompanying failure of differentiation. Of the rest, which have undergone considerable if distorted differentiation, twenty-four specimens have defective healing of a particular kind. Residual blastoderm and excised-piece have developed largely independently, owing to their spatial separation. This style of development occurs after control operations as well as after reversals. The excised-piece in these specimens contained all or part of the primitive node, within which lies the presumptive material of notochord and neural tube floor. At the time of fixation the residual blastoderm has a longitudinal mid-line split through the whole dorso-ventral thickness of the embryo. In the region of the split the tissues of the mid-line, i.e. the notochord and probably the central strip of the neural plate, whose presumptive areas were in the reversed-piece, are missing altogether. The fate of the central strip of endoderm is unknown. The excised-piece very rarely develops according to its presumptive fate, particularly as regards its tissue movements.

Embryos of this kind result almost entirely from type II operations performed on *L* or head-process stage blastoderms. Fifteen were obtained after reversals of this type. Four control specimens of type II operations developed in the same way. The presence of an epithelial part in the excised piece, which tends to roll up, seems to be responsible for the failure of healing. Split embryos rarely result from other types of operation involving the node, but three were obtained after type IB and two after type III operations at *L* stage.

The residual blastoderm. Embryos of basically similar structure have been described before by several workers, using a variety of techniques, all of which prevent the backward movement of the node material (e.g. Waddington 1932, 1935; and pre-eminently Wetzel 1936).

When the split specimen results from an operation performed at the *L* stage, with excised-piece involving the node but no part of the primitive streak behind the node, the usual structure is as follows (ten specimens). The edges of the split bear the lateral part of the neural plate (what should be their inner free edges being joined to the endoderm) and contain axial mesoderm. The split closes posteriorly where the strands of neural plate at each side become joined to each other by a thin epithelium, connecting their inner edges; and a little farther posteriorly this may give place to an apparently normal neural plate. The posterior end of the notochord is also present behind the split (one exception); and less of the notochord is usually missing than of the presumptive neural tube floor, the notochord being present under the thin epithelium, and often farther forward, a little up one side of the split. The amount of notochord and presumptive neural tube floor present behind the split varies considerably and independently after apparently identical operations. The posterior notochord, typical of such *in vitro* experiments, is not found in split specimens produced *in vivo* (Wetzel 1936). The primitive node and primitive-streak region of these specimens is otherwise normal.

In six specimens the reversed-piece included, besides the node itself, a considerable section (one-third or more) of the primitive streak behind the node. In five of them the entire notochord is eliminated from the residual embryo and the neural plate is split right back to the primitive-streak region. In these specimens the somites may be affected as well; they are missing entirely in two of them. In the sixth specimen the reversed-piece has fused to one side of the split, and has probably contributed the notochord found there (see p. 332). The lateral strands of neural plate are present in these specimens. In two further specimens type III operations were performed in which the reversed-piece, 0·3 to 0·4 mm. long, reaching up to 0·1 mm. from the anterior end of the primitive streak, included some of the node. Split embryos resulted, but involved only the posterior part of the trunk, where notochord and neural plate centre were missing. Somites were very feeble throughout.

Where a split is produced after a type II operation at an early head-process stage (reversal of node and of head-process) the morphology of the embryo usually differs slightly from that produced by an operation at *L* stage; *all* the notochord tends to be eliminated, and the full length of the chordal neural plate floor above the notochord (three out of four specimens). In one further specimen the anterior part of the head-process was not included in the excised piece, and the anterior end of the axis is normal. In another specimen the posterior part of the node was also excluded from the excised piece, and correspondingly the posterior end of the notochord and neural plate floor developed in the embryo.

The development of the residual blastoderm in the specimens so far considered in this section seems to show that the mid-line of the axis can be eliminated by suppression of development of the corresponding presumptive areas at the *L* stage or head-process stage, without affecting the differentiation of what is left of the axis. Partial elimination of these presumptive areas (e.g. removal of the anterior or posterior part of the presumptive notochord) results in corresponding partial elimination of the axial material. There is clearly a strong tendency towards a 'mosaic' development according to presumptive fate.

The excised-piece in these split embryos does not show a 'mosaic' style of development. It is always separated from the residual embryo to some extent by the failure of healing. Commonly it is attached to the residual embryo anteriorly; indeed, it is usually well healed in there. Behind and at the sides of the excised-piece lies the hole in the axis. But the excised-piece is often loosely connected with one or both sides of the hole by a thin sheet of epithelium, and it is occasionally fused closely to one side. Rarely it lies entirely free.

The presumptive fate of the excised-pieces in these specimens was neural tissue and notochord in all specimens; together with prechordal head mesoderm in the *L* stage type II operations, and somites when part of the primitive-streak material behind the node was included.

In four of the fixed specimens the excised-piece consisted of mesenchyme and indifferent epithelium only; only one of these specimens had undergone a type II operation. In twelve others, almost all the results of type II operations both at *L* and head-process stages, the excised-piece has also formed neural tissue. These specimens vary from those where only a little very poorly differentiated neural tissue occurs amongst much indifferent epithelium, to those where the neural tissue is extremely abundant and indifferent epithe-

lium practically absent. In four of the strongly neuralized specimens the excised piece, which is almost completely fused into the head of the residual embryo, has developed a distinctly head-like structure, devoid of notochord. A fore-gut occurs under one (figure 27, plate 23; specimen no. 20; type II operation at *h.-p.* stage; *p.s.* 1·8 mm., 80% of *a.p.l.*, *h.-p.* 0·1 mm.; *r.-p.* 0·1 mm. of *p.s.* plus 0·25 mm. in front). These four were at the head-process stage when operated. In none of the sixteen specimens mentioned so far has the excised-piece undergone any elongation, which is its main presumptive tissue movement.

In eight specimens the excised-piece has developed axial tissues besides neural tissue and mesenchyme. In two of these the excised-piece has formed somites but no notochord. In the other six it has formed notochord with or without somites. Two of them form short axes (figure 28, plate 23; specimen no. 21; type II operation at *L* stage; *p.s.* 2·6 mm., 78% of *a.p.l.*; *r.-p.* 0·3 mm. of *p.s.* plus 0·25 mm. in front) with their original (i.e. reversed) polarity, lying almost freely in the hole of the residual embryo, and one of these is considerably elongated. Another one, also lying free, is of uncertain orientation. Three of them are fused to one side of the split residual embryo. In these there is a notochord all the way along one side of the split, terminating posteriorly in a primitive node behind the split and anteriorly in the mass of partly free tissue of the excised-piece. The most reasonable interpretation is that the node, included in the original excised-piece, has functioned almost normally by retreating down one side of the split in the axis. These excised-pieces are probably elongated, but their extent cannot be ascertained because of confusion with residual embryo tissue. One of these specimens was a control, while in the other two the original polarity of the reversed-piece has been converted to that of the residual blastoderm.

The excised-piece in these experiments therefore frequently develops only neural tissue and mesenchyme, in spite of the fact that it contains node tissue. This may often be a non-specific effect of its isolation from the residual blastoderm. Even when well healed however into the head region of the latter, the node tissue still does not develop according to its presumptive fate, but forms head-like structures instead; which suggests a direct influence of the residual blastoderm. Sometimes, especially when laterally fused to the chordal level of the residual embryo, it may form its presumptive axial tissues; in some cases probably rather more than its presumptive tissues, since somites appear from what is supposed to be presumptive notochord.

IX. Operation type and differentiation

A preliminary analysis of the different kinds of differentiation appears in § III. It now remains to supplement this analysis in the light of the data subsequently presented, and summarize it according to type of operation.

Operation type IA. Healing usually occurred. There were no cases of failure of development (as judged by the whole mounts) of the entire embryonic axis. The commonest defect was failure of differentiation of the mid-line tissues (probably of the excised-piece). Regulation is common after reversals of this type.

Operation type IB. Many failed to heal. A very few embryos with the characteristic split axis described in § VIII occurred; but the majority of specimens in which healing

failed showed little trace of differentiation and were not sectioned. Many of those healed after operation at young stages had their axial development suppressed. The majority of all those specimens obtained with doubled axis, and of those with a tendency to reversed development, occurred after this type of operation. Regulation was, however, fairly common after reversals of this type, amongst the embryos which succeeded in healing; it was usually perfect in the blastoderms young at operation (*S* to *M*), imperfect in the older.

Operation type II. Although excised-pieces were usually small, failure to heal was much the commonest result, producing the characteristic kind of embryo with split axis. Suppression of all axial development practically never occurred. There were several instances of regulation after reversal of this type.

Operation type III. Failure of healing was relatively uncommon. In several specimens young at operation, mostly involving large reversed-pieces, axial development was suppressed. Most of the cases of defective development belong to the group with uncertain orientation of reversed-piece (discussed at the beginning of § VI). Regulation after reversal of this type was the commonest outcome, but in view of the relatively undifferentiated state at fixation of the region in question, it is of less significance than is regulation after the preceding operation types.

Operation type IV. Differentiation was much as after type III operations. Two cases of reversed development, after reversal of very long pieces, occurred.

X. General discussion

These experiments emphasize particularly clearly a point which Waddington (1935) has made: that while experimental elimination of a region of the chick blastoderm at primitive-streak stage often leads the remainder to undergo a surprisingly 'mosaic'-like development, reminiscent of a fully determined embryo; yet other experiments show that this remainder is really still highly labile. Mere defects do not necessarily disrupt the normal relations of tissues sufficiently to demonstrate their lability. Reversal of part of the fully formed primitive streak may on the one hand lead, through failure of healing, to an embryo with a large defect, a split embryo. In such a case, the nature and extent of its missing tissues seem to be directly related to the presumptive areas included in the excised-piece. This is especially obvious with regard to notochord. On the other hand, a similar operation may be followed by complete healing, and regulation to form a single axis. This may involve considerable departures from the presumptive fate of the axial material. Presumptive notochord, for instance, must have been caused to develop into tissues other than notochord in many of the regulated specimens.

There is, of course, no contradiction between the 'mosaic'-like development of the split embryos and the labile state of the axial tissues demonstrated in the regulated embryos. Both seem to be due to an autonomy of the blastoderm other than the streak, together with control of the streak by the rest of the blastoderm when there is physical continuity between them. In the mid-line position a given part of the primitive streak evidently tends to develop according to its antero-posterior position in the blastoderm. This generalization seems to hold within wide limits regardless of the presumptive fate of the piece of primitive streak concerned (in the regulated embryos); and regardless of the presence or absence of other levels of the primitive streak (in the split embryos). The blastoderm around the primitive streak

has its antero-posterior organization from early primitive-streak stages onwards, as shown by the reversals at these stages. A polarity, presumably the precursor of the antero-posterior organization which controls the primitive streak, has been detected experimentally in the unincubated egg (see Wolff 1948).

This subjection of the primitive streak to control by its surroundings contrasts with the well-known autonomy of the primitive streak on transplantation. Waddington (1932 and subsequently) showed that when suitably grafted it formed a secondary axis, inducing host ectoderm to neural tissue. Such a secondary axis may be longitudinally differentiated into head, trunk and posterior primitive-streak mesoderm. This antero-posterior regional organization depends wholly on the original antero-posterior orientation of the grafted primitive streak, provided the graft has developed well away from the host axis (Waddington & Schmidt 1933; Abercrombie & Waddington 1937). Even the early primitive streak, in spite of being presumptive side-plate mesoderm, can induce a secondary axis, maintaining its own polarity (e.g. Waddington 1934, p. 212); incidentally a state of affairs without obvious parallel in amphibian development.

The primitive streak therefore has an inherent polarity, capable of controlling the polarity of a secondary embryo, and consequently, it might have been presumed, of the primary embryo. This inherent polarity shows itself occasionally in the present work, though always imperfectly, in the specimens which tend to a reversed development. Its manifestation in these may be only the result of delayed healing, which prevents the full exertion of the residual blastoderm's influence. It also shows itself in a few of the isolated reversed-pieces of split embryos. These reversed-pieces developed in their own orientation, when they had any orientation at all, if they remained unhealed to the residual blastoderm. But if well fused to it, even though only at one side, their polarity became that of the residual embryo. Of course the outcome of the development of an isolated or grafted primitive streak may be a structure with *no* polarity. This is exemplified by most of the excised-pieces of the split embryos described in the present paper. But though the inherent polarity is easily destroyed especially by isolation from the surrounding blastoderm, it undoubtedly exists.

The overriding control of the polarity of the primitive streak imposed by the surrounding blastoderm fits well, however, with some previous experimental results in the chick. For instance, it accords with Waddington's discovery (1932) that when the entire primitive streak is excised a new one regenerates and develops into an embryo with the original polarity. The control by the host of the polarity of a primitive-streak graft placed between endoderm and epiblast, provided the graft develops close to the host axis (Waddington & Schmidt 1933; Abercrombie & Waddington 1937) is probably due to the same forces that control the reversed primitive streak in the present experiments. Abercrombie & Waddington (1937) found that even when the grafted streak was placed and remained in reverse orientation immediately ventral to the host streak, it produced no sign of any reciprocal influence on the host polarity. This also accords with the apparently subservient role of the primitive streak in determination of polarity in the central part of the blastoderm.

At first sight the important role assigned to the surroundings of the primitive streak may not seem to accord with earlier interpretations of chick development in terms of Spemann's concept of the organization centre. If the primitive streak is taken to be the whole organization centre of the chick blastoderm, then on the usual interpretation of that term its

subservience to its surroundings in the way I have demonstrated would certainly be surprising. Waddington has at times seemed to suggest that the anterior end of the primitive streak is the organization centre, and this view has gained some currency. For instance, 'If the entire primitive streak is removed, both the normal organisation centre of the blastoderm and a great amount of the, perhaps labilely determined, presumptive axial organs, have been eliminated' (Waddington 1932, p. 221); 'The structure which arises in response to the organising stimulus' (from the endoderm) 'is the primitive streak, and from the streak a new organising stimulus is exerted on the rest of the embryo. The proof that the streak is an organisation centre was also given with the help of the *in vitro* technique' (Waddington 1939, p. 4). Professor Waddington has, however, kindly informed me that, taken in their context, such remarks were not intended to imply that the whole organization centre is located in the primitive streak. He has always considered that it may well extend farther laterally. Discussion of its lateral extent has not, however, been possible, because there are no experimental data yet for the chick on the lateral extent of regions capable of induction, that is to say, data such as define the size of the amphibian organization centre.

The presumptive fate of the tissues immediately surrounding and presumably controlling the reversed-piece in these experiments is not really precisely known. At young stages these tissues include presumptive chorda-mesoderm in the epiblast layer. At the full-length (*L*) streak stage they may well include a certain amount of invaginated presumptive axial material; though according to present information a smaller amount than is included in any reversed-piece involving the anterior end of the streak. If presumptive axial material is present in the residual embryo of operations at *L* stage, then these experiments are comparable with those in Amphibia in which a central strip of the presumptive chorda-mesoderm is reversed. In *Discoglossus* (Waddington 1941) the result of such experiments is quite unlike the result in the chick; the reversed-piece tends strongly to maintain its autonomy. Professor Waddington and Dr Yao, however, kindly allow me to refer to work in the press which shows that in *Triton* regulation is the commonest result of such an operation, in conformity with my results on the chick.

Whatever its exact presumptive fate, the area surrounding the primitive streak has a considerable variety of developmental potencies as tested by chorio-allantoic grafting (see Rudnick 1944). Hitherto there has been little to link the organization of potencies in the area pellucida revealed by the chorio-allantoic technique with the results of Waddington's transplantation technique, which by concentrating on the primitive streak has seemed to give the area pellucida a relatively passive role. The present work, by assigning a controlling influence to the surroundings of the primitive streak, may perhaps begin to bridge the gap.

Finally, the area pellucida organization is not to be regarded as rigid. At times it certainly shows 'mosaic'-like development as in the residual embryo of split specimens, and probably of those embryos with their mid-line tissues undifferentiated. Its lability has, however, been most clearly shown by Waddington's classical experiments (1932, 1933) when he rotated the endoderm and thereby altered or even reversed the polarity of the embryo. Those experiments throw a suggestive light also on the development of the organization.

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Description of plates 21 to 23

All are transverse sections. A fuller description of the operation will be found where each specimen is referred to in the text. The linear magnification of all photographs is × 175 unless otherwise stated. Abbreviations: *f.-g.* fore-gut; *n*, notochord; *n.t.* neural tissue; *r.-p.* reversed-piece; *s.* somitic mesoderm.

Plate 21

Figure 2. Specimen 1. Regulated trunk region. Type IA operation at *h.-p.* stage.

Figure 3. Specimen 2. Regulated trunk region. Type IB operation at *L* stage.

Figure 4. Specimen 3. Regulated, though rather asymmetrical, trunk region. Type IB operation at *S* stage.

Figure 5. Specimen 4. Regulated head region. Type II operation at *LM* stage.

Figure 6. Specimen 5. Regulated posterior head region. Type II operation at *S* stage.

Figure 7. Specimen 6. Partially regulated, asymmetrical trunk region. Type III operation at *L* stage.

Figure 8. Specimen 8. Doubling of axis. Twin axes in head region, both very small. Type IB operation at *S* stage. (Magn. × 110.)

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Figure 10. Specimen 9 again. Partly doubled trunk region with central somite.

Plate 22

Figure 11. Specimen 10. Doubling of axis. Two notochords in otherwise normal head. Type II operation at *L* stage.

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Figure 13. Specimen 12. Tendency to reversed development. Massive notochord anteriorly. Type III operation at *h.-p.* stage.

Figure 14. Specimen 12 again. Farther posteriorly than figure 13. Very reduced notochord (below leader line), extra mesoderm in axis.

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Figure 18. Specimen 14. Tendency to reversed development. Normal anterior region of head. Type IB operation at *LM* stage.

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PLATE 23

FIGURE 20. Specimen 15 again. Region containing notochord at posterior end of trunk.

FIGURE 21. Specimen 16. Tendency to reversed development. Region containing notochord and somitic mass at posterior end of trunk. Type IB operation at *M* stage.

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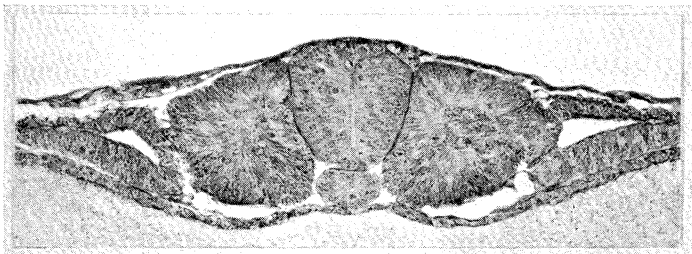

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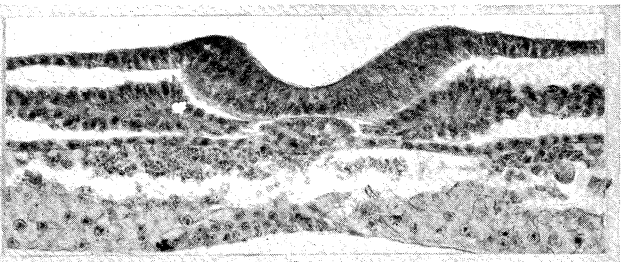

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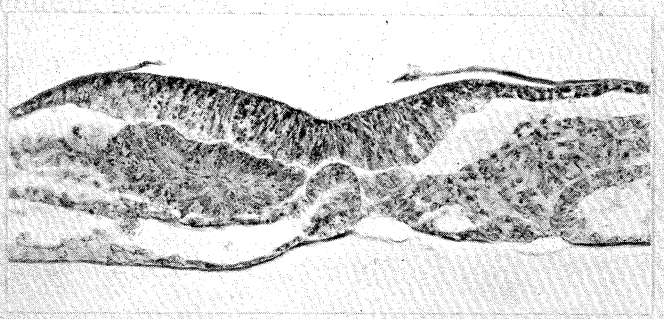

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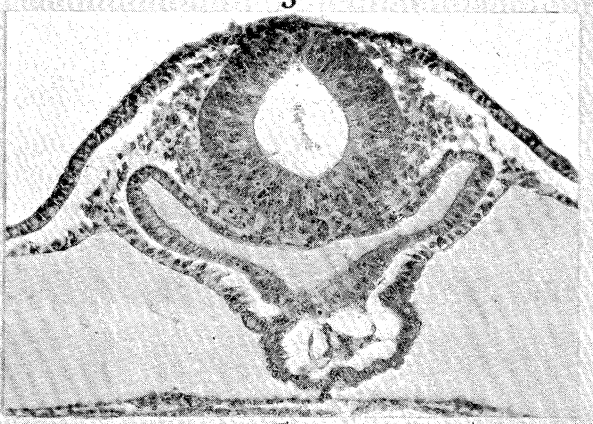

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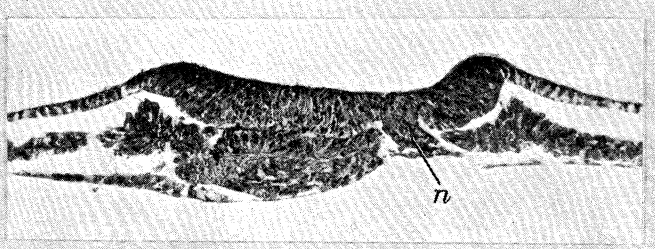

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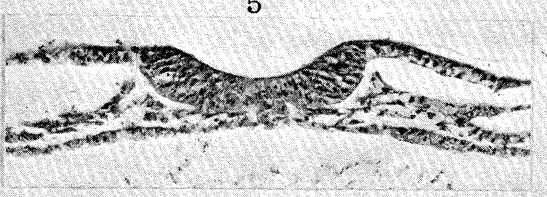

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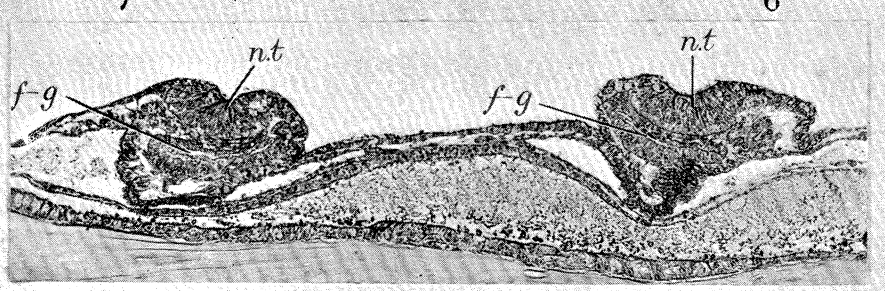

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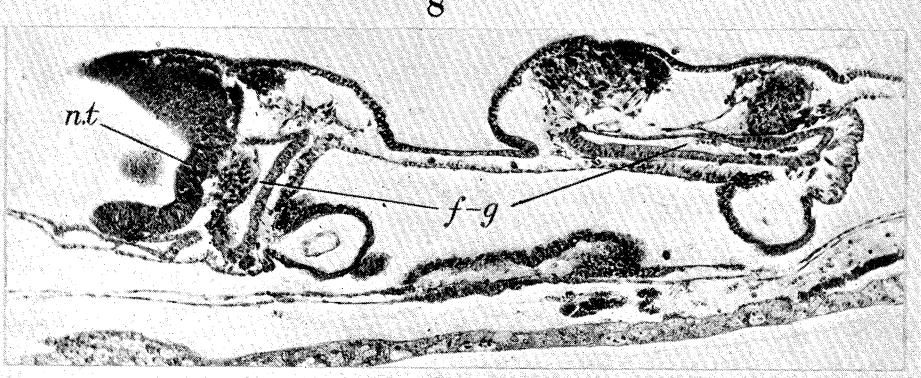

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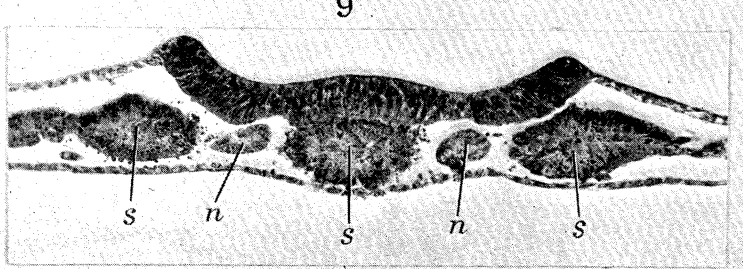

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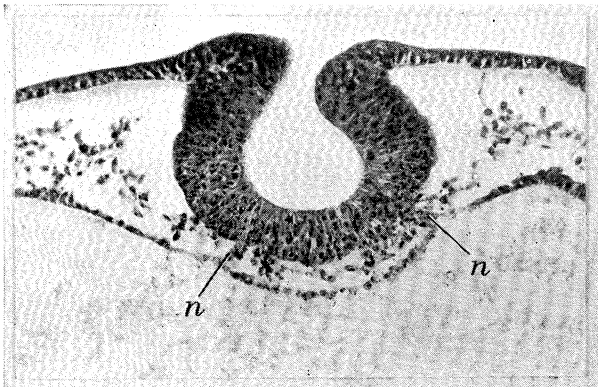

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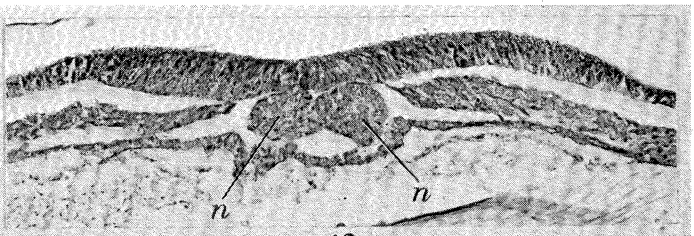

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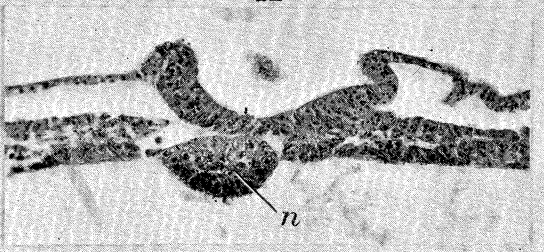

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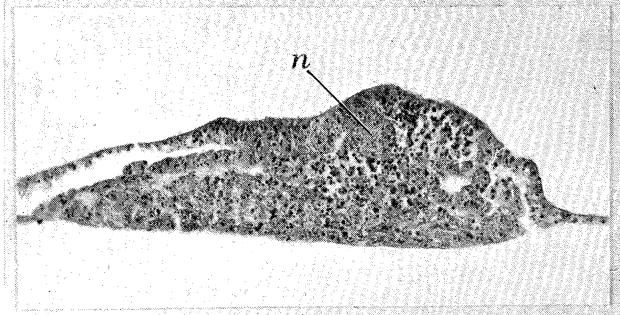

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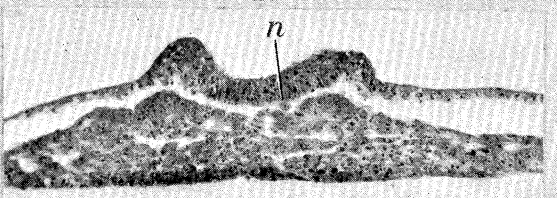

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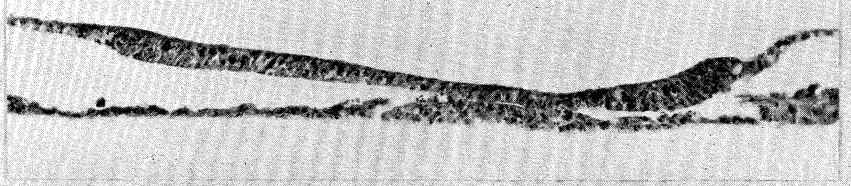

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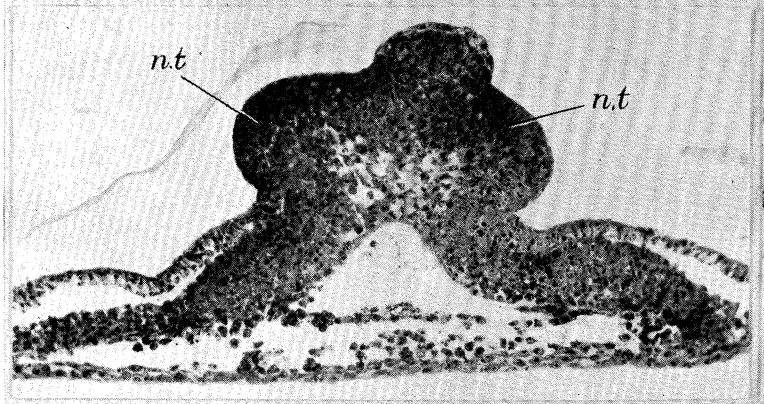

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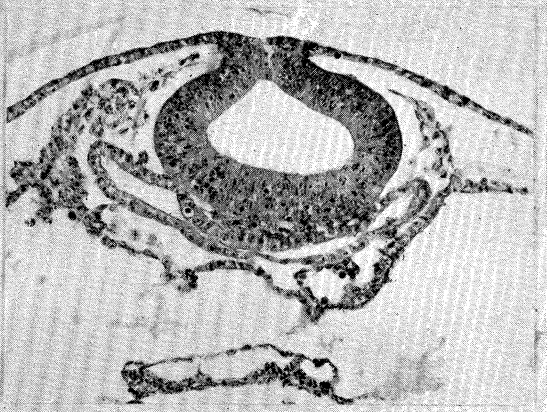

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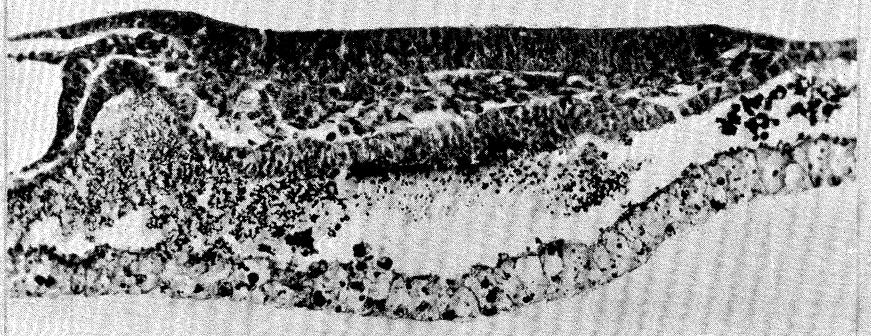

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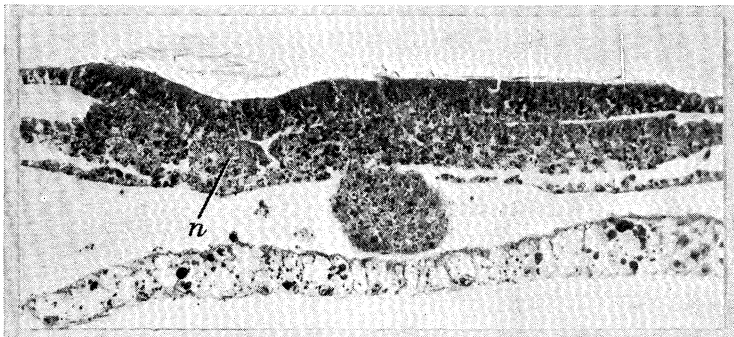

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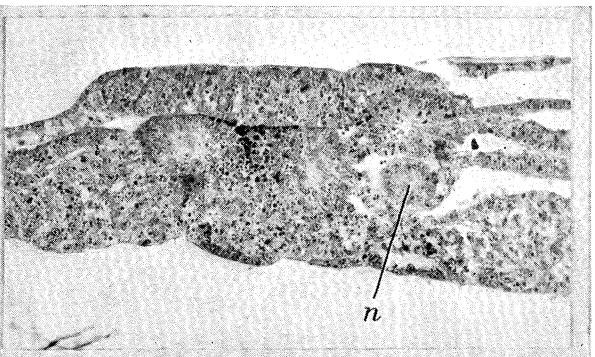

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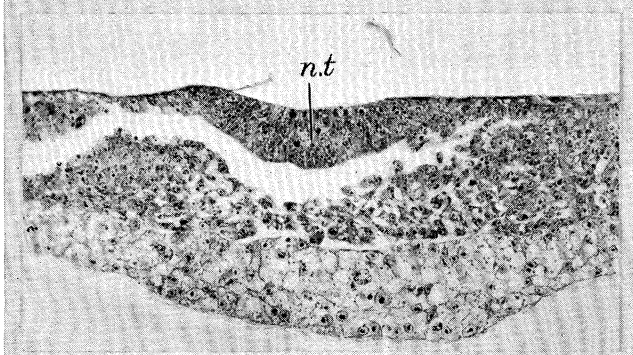

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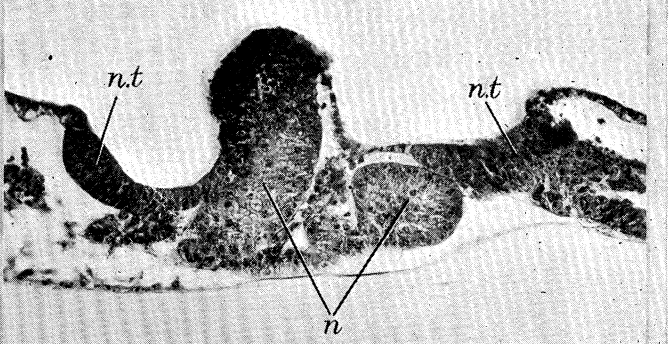

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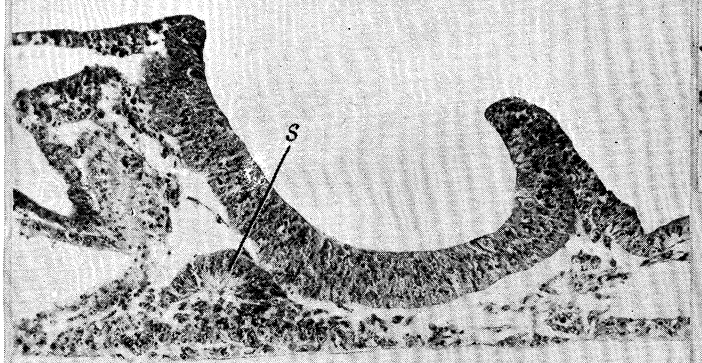

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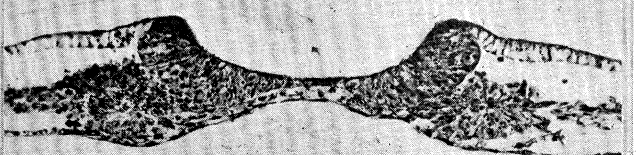

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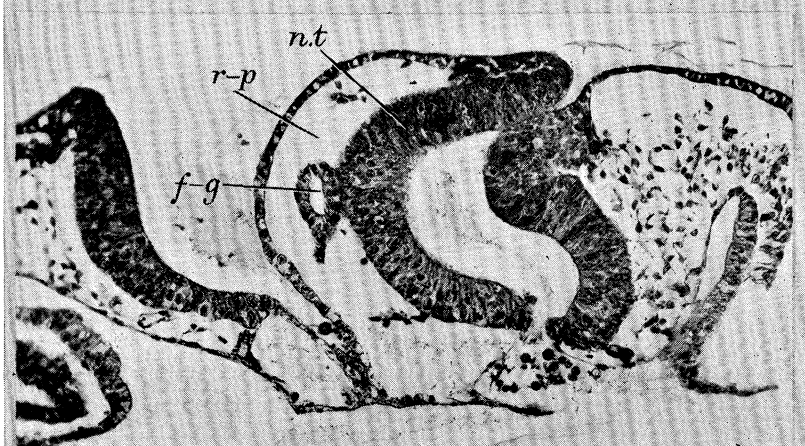

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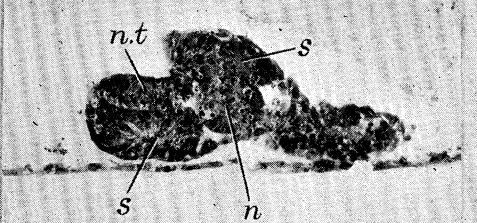

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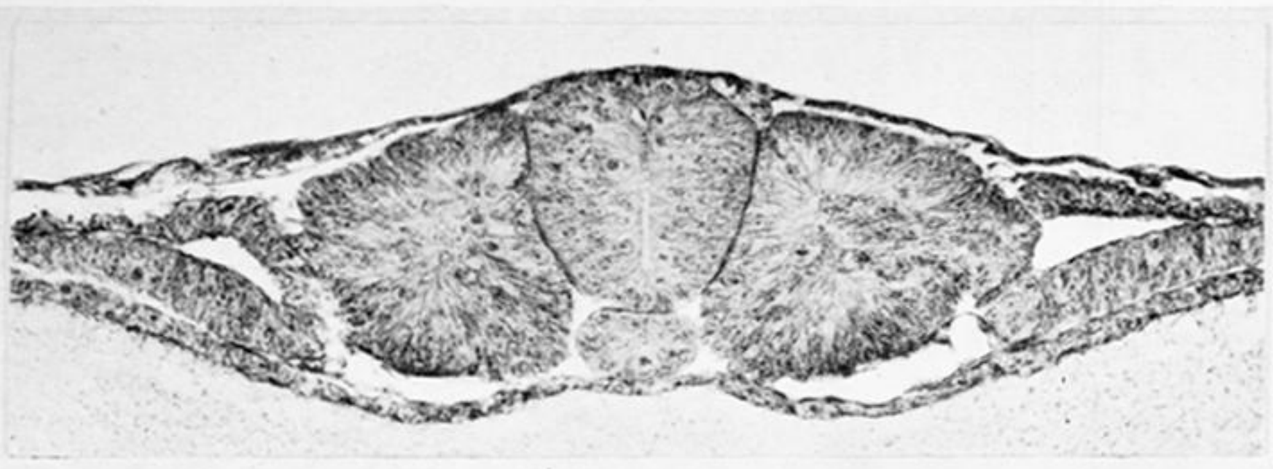

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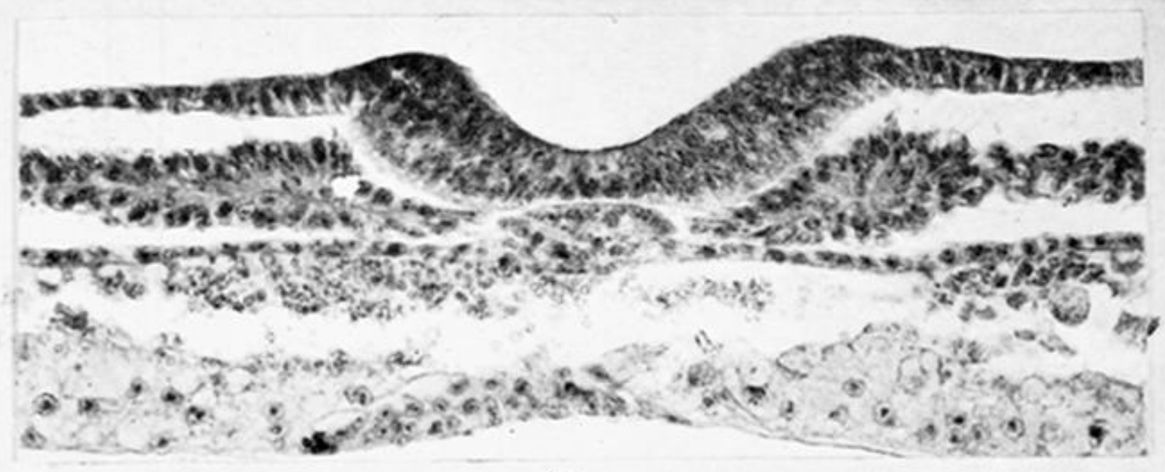

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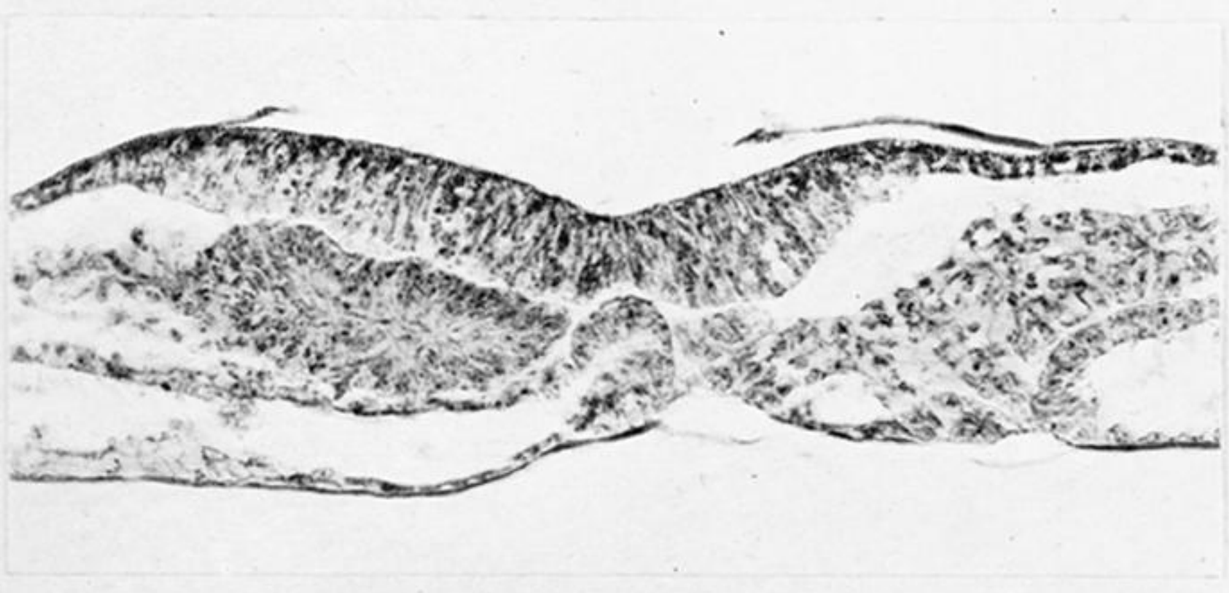

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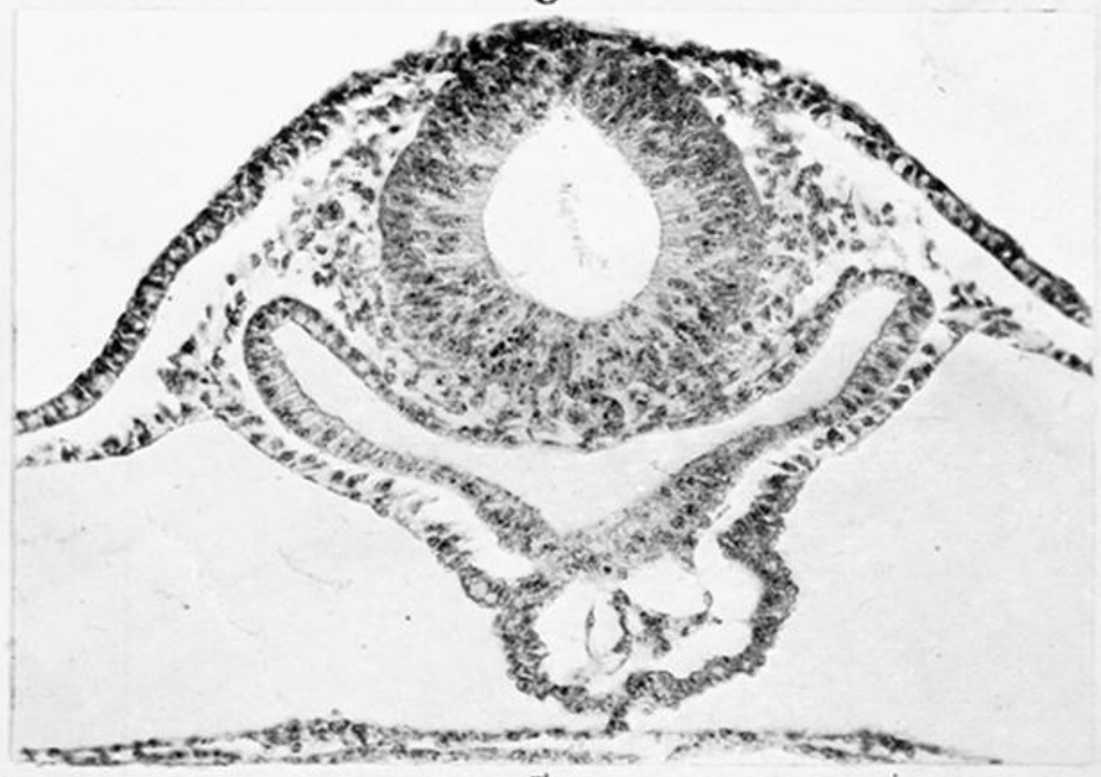

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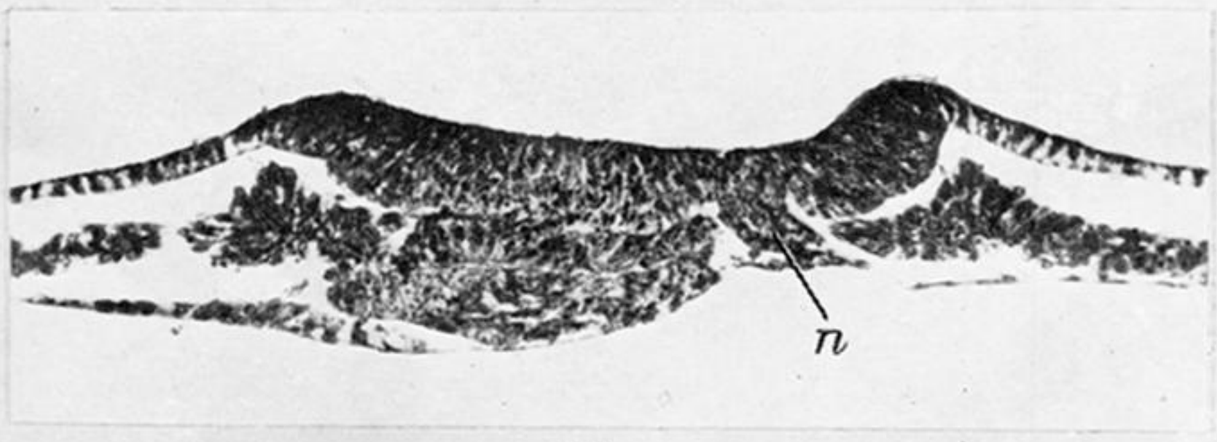

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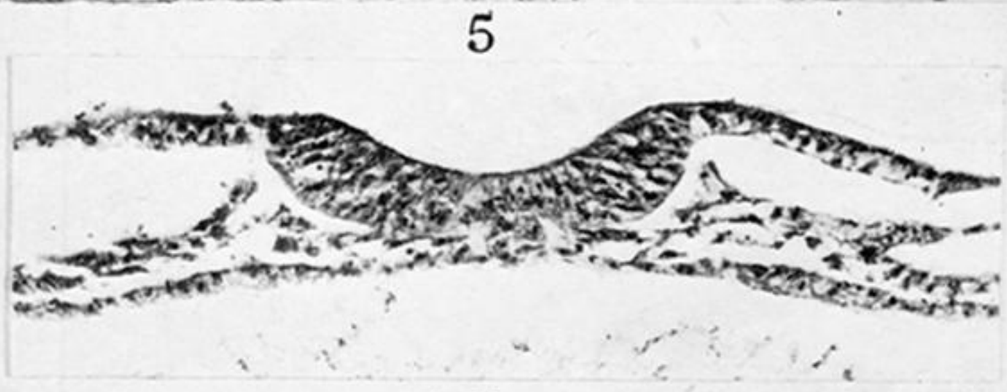

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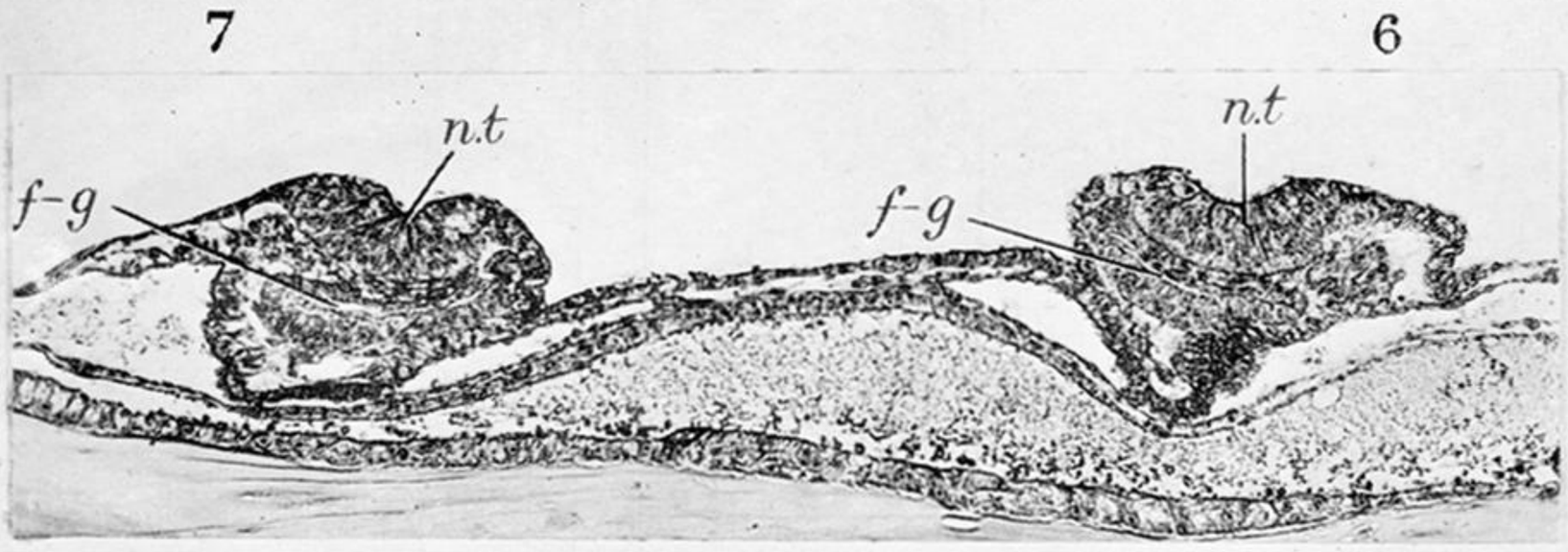

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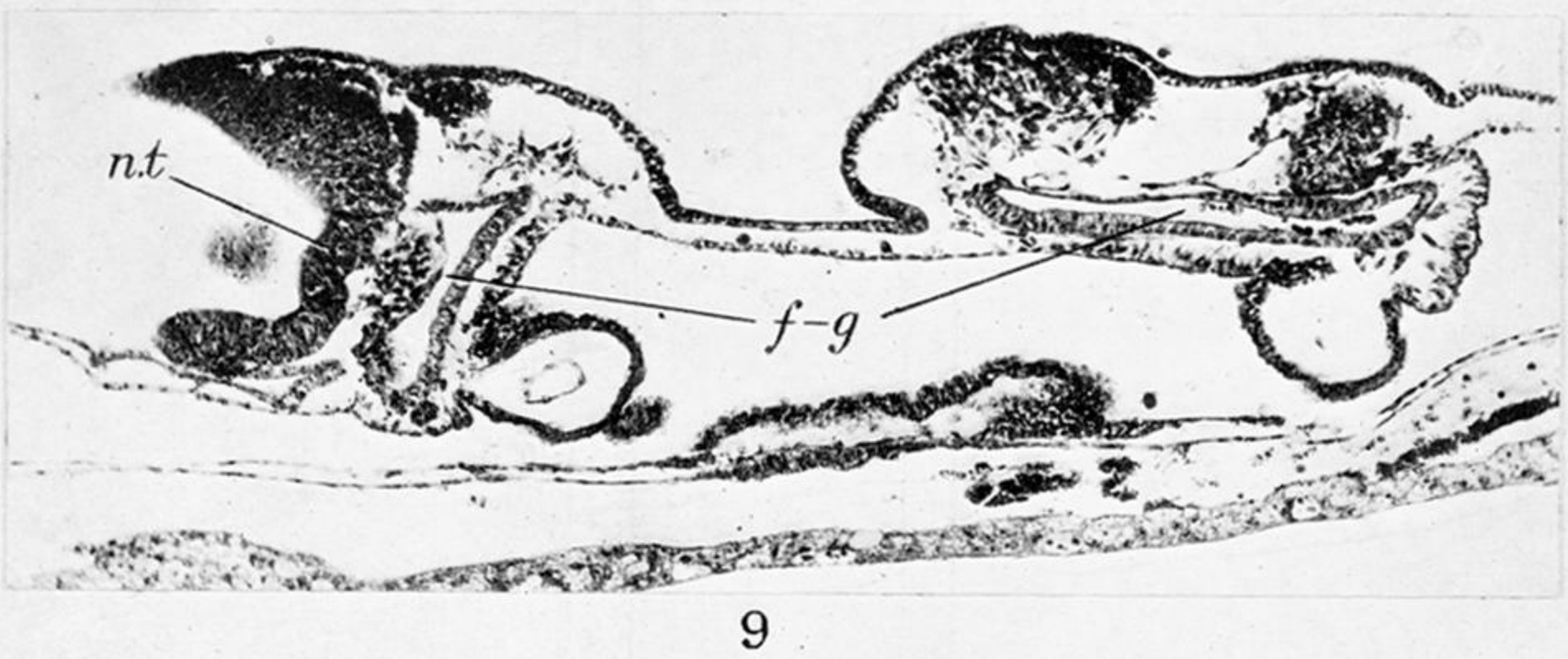

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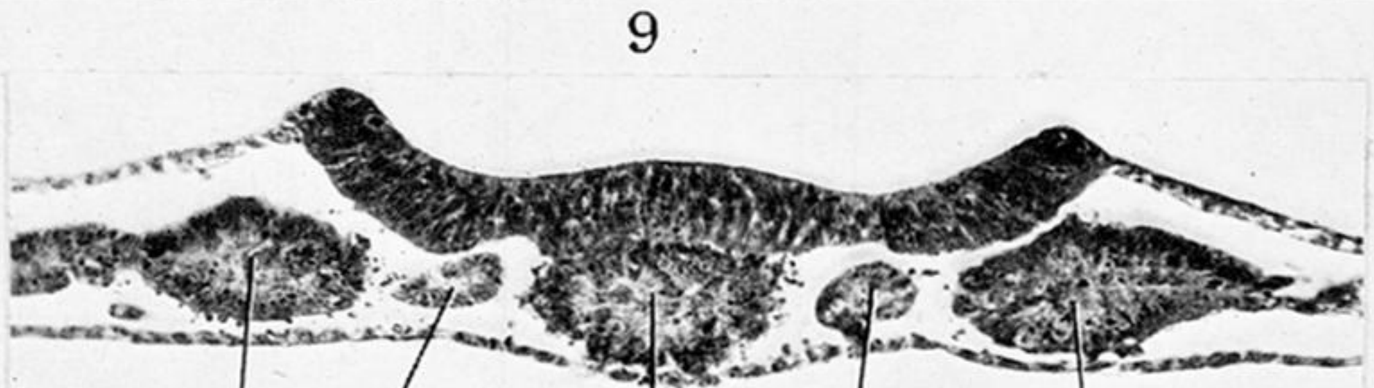

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PLATE 21

FIGURE 2. Specimen 1. Regulated trunk region. Type IA operation at *h.-p.* stage.

FIGURE 3. Specimen 2. Regulated trunk region. Type IB operation at *L* stage.

FIGURE 4. Specimen 3. Regulated, though rather asymmetrical, trunk region. Type IB operation at *S* stage.

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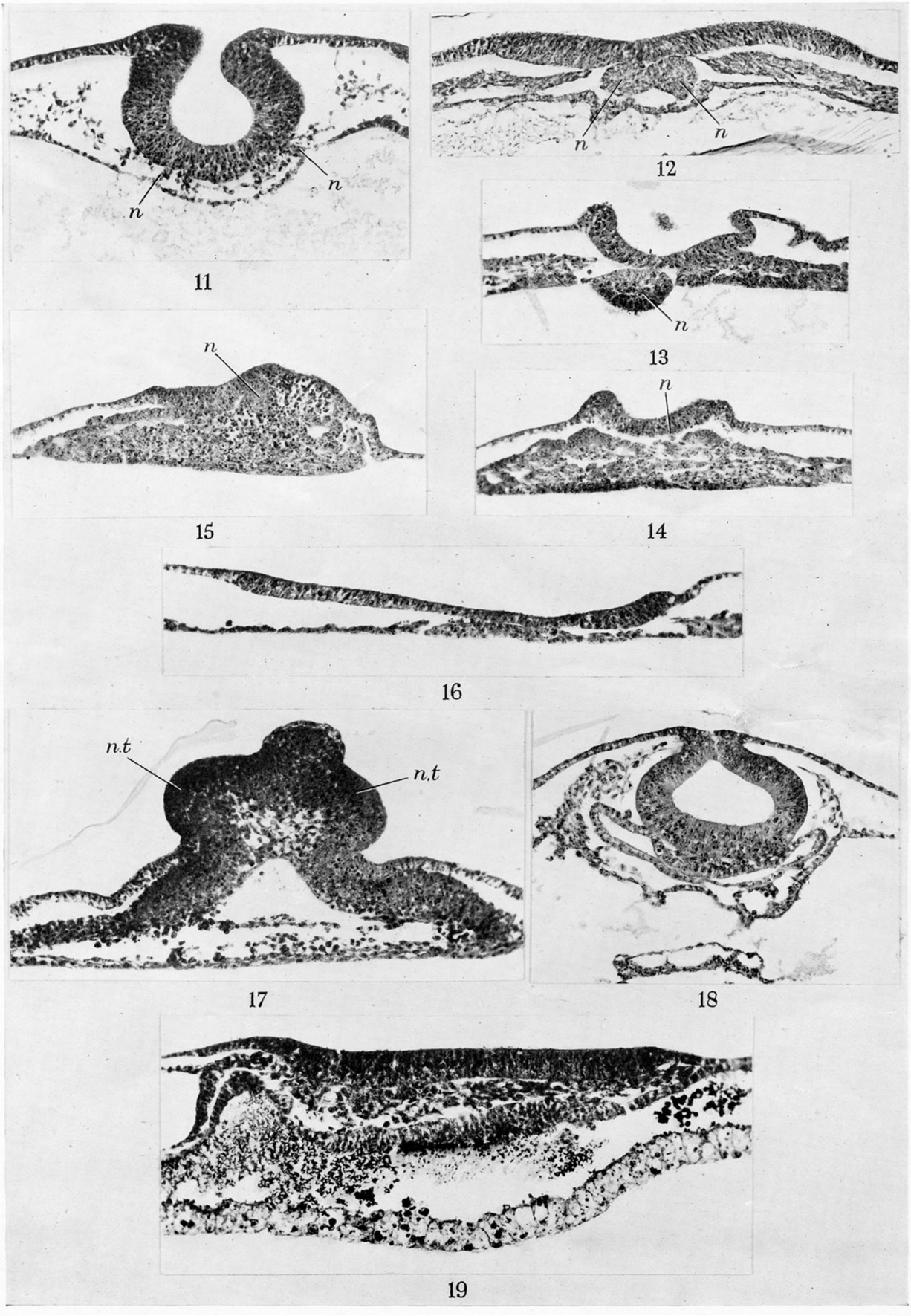

PLATE 22

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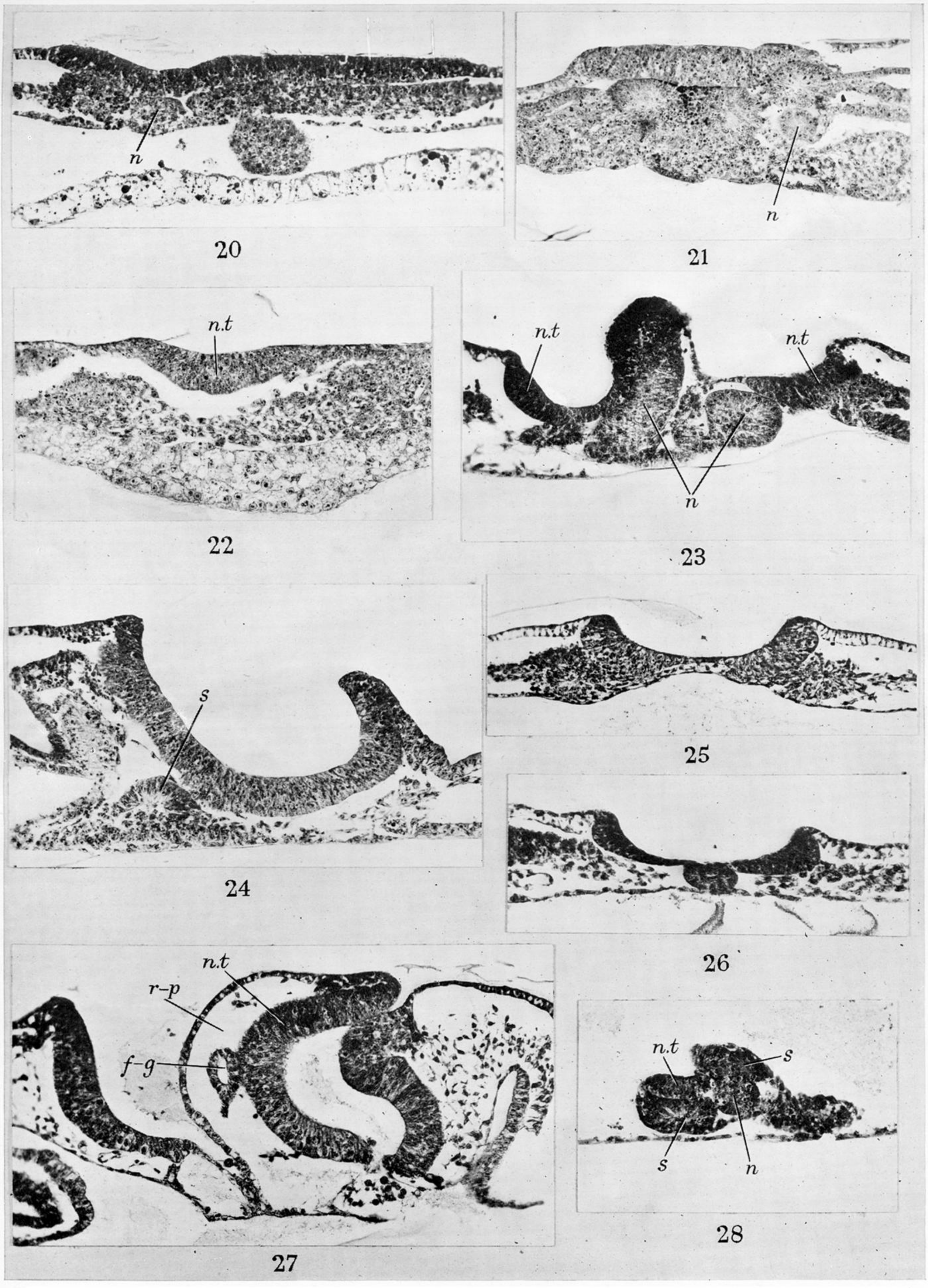

PLATE 23

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