III. *Experiments on the Development of Chick and Duck Embryos, cultivated* in vitro.

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[Plates 22—28.]

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Introduction.

The experimental study of the developmental mechanics of the birds, and of the higher vertebrates in general, has hitherto been particularly difficult, since it has been impossible to keep the embryos alive in a situation in which the necessary manipulations can be successfully carried out. In the circumstances our knowledge of the subject is practically confined to such aspects as can be investigated by the method of isolation; that is to say, chiefly to the aspect of self-differentiation. This has been studied by Hoadley (1926, *a*), Murray and Selby (1930), Hunt (1929, *a*, *b*, 1931), Willier and Rawles (1931), and Umanski (1931), in the early stages of the fowl embryo, by means of transplantation to the chorio-allantoic membrane, and by several investigators, in the later stages, both by the chorio-allantoic method and recently by the method of cultivation *in vitro*. It is the purpose of this paper to show that the latter method can be applied to the study of the developmental mechanics of the young fowl blastoderm, and to give some of the results which have been obtained by its application. A rather wide field of inquiry has already been covered by the use of this method. The object which has been kept in view during this preliminary study has been, not so much the detailed and complete investigation of one particular problem, but rather the general mapping out of developmental mechanics of the avian embryo during the stages between the laying of the egg and the appearance of the head process. It was hoped that in this way the limitations of the technique would become clearer, and that data would be accumulated which would enable one to decide, among the detailed problems which present themselves for investigation, which are the most likely to yield fruitful results.

At the beginning of the investigation, it was thought possible that the most interesting difference between the developmental mechanics of birds and of the Amphibia, would be closely connected with the differences in the process of gastrulation and chordagenesis in the two groups. While in the Amphibia both endoderm and mesoderm are formed simultaneously, and at the same place, namely the blastopore, in the chick the work of Wetzel (1925, 1929) and Gräper (1929) has shown that there are at least two processes involved; first the formation of the endoderm, probably by invagination of the posterior margin of the blastodisc and then, considerably later, the formation of mesoderm and notochord by invagination through the primitive streak. The data which have been obtained on these two invaginations are here separated, the invagination of the endoderm being dealt with in Part II, while the somewhat fuller material concerning the invagination of the mesoderm is to be found in Part III. Part I deals with the technique employed and the general characteristics of chicken and duck embryos cultivated *in vitro*. The individual sections, into which the three parts are divided, usually open with a short description of the experiment to be dealt with, any particular technical difficulty encountered is mentioned, and a summary of the main results of the experiment is given: this introduction is followed by a description

of typical operated specimens, and a section in which the results are interpreted and discussed.*

PART I.

1*a*. *Cultivation of entire Blastoderms* in vitro.

Entire chick blastoderms were cultivated *in vitro* by McWHORTER and WHIPPLE (1912), who used the hanging-drop technique. They obtained no differentiation of embryos explanted before the formation of the head process: their best results were obtained with 10–12 somite embryos, one of which lived for 31 hours. The method was also used by SABIN (1919) to watch the formation of the blood vessels; the average life of her cultures was about 5 hours.

Mention should perhaps be made of the experiments of BRACHET (1912, 1913) and MAXIMOV (1925), who used the *in vitro* technique for the cultivation of young blastocysts of the rabbit. It is hoped that in the near future, a similar method may enable a study of their developmental mechanics to be undertaken.

The technique used in this study is the watch-glass technique used by FELL and ROBISON (1929), for the cultivation of embryonic organs *in vitro*. A similar procedure was used some years earlier by D. H. and T. S. P. STRANGEWAYS for the cultivation of entire blastoderms, but only a few experiments were made and the results were never published. The technique is as follows. (Precautions must be taken throughout to preserve sterility.)

Cultivation is carried out in a watch-glass, preferably of Jena glass, which is placed in a Petri dish kept moist by cotton wool soaked in 20–30 c.c. of boiled water, fig. 1.

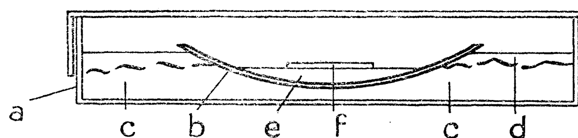


FIG. 1.—Culture vessel. *a*, Petri dish. *b*, watch-glass. *c*, cotton-wool. *d*, boiled water, *e*, plasma-extract clot. *f*, embryo.

If it is desired to observe the culture by reflected light, the outside of the watch-glass can be painted black, but it is usually more convenient to leave the glass unpainted, and to cut a hole in the cotton wool on the floor of the Petri dish, so that transmitted

* This work was begun in June and July, 1929, when a few preliminary experiments were made, chiefly directed towards testing various modifications of the technique. The application of the technique to a study of the developmental mechanics of the chick was begun in October, 1929. The cultures were numbered consequtively from that time, beginning at 1, to the present, when rather more than 650 cultures have been made.

During this period I received a Senior Research Award from the Department of Scientific and Industrial Research, for which I should like to make grateful acknowledgment. A preliminary communication of the results to be described in this paper was made in "Nature" (1930).

light may also be used. The culture medium, which is placed in the watch-glass, consists of equal parts of blood plasma from an adult fowl and "embryo extract." The latter is made, in the usual way, by mincing up an 8 or 9-day embryo, mixing the macerated tissue with saline solution (Pannett-Compton's) and centrifuging, the clear supernatant liquid being the extract used. Usually about half a cubic centimetre, or less, of each constituent is placed in the watch glass.

If a series of cultures is being made, the sterilised Petri dishes are placed in a row on the bench, the water is pipetted on to the cotton wool, a certain number of drops of embryo extract are placed in each watch glass and then, after the pipette has been washed out with sterile distilled water, the same number of drops of plasma are added from the same pipette. The mixture in each watch glass is stirred and spread into an even pool of liquid immediately the plasma is added: in a minute or two it coagulates into a fairly firm clot.

The same method, using fowl plasma and chick embryo extract, has been used for the cultivation of duck embryos.

The blastoderm is brought on to the clot in the following way. The egg is broken by tapping with the handle of a scalpel, the cap of the shell is lifted off, and the contents of the egg poured into a sterile Petri dish in such a way that the blastoderm lies at the uppermost pole of the yolk. The vitelline membrane is then cut at two diametrically opposite points, care being taken first that the membrane does not rapidly split along a radius passing through the blastoderm (a most frequent mishap, but avoidable if care is exercised), and secondly that the current of yolk passing out of the membrane does not drag away the half-digested yolk from beneath the blastoderm. Four cuts are then made, freeing the blastoderm from the main part of the vitelline membrane, and the blastoderm with a piece of vitelline membrane above and a certain amount of yolk underneath, is lifted out on the end of a flat spatula (a nickelled section lifter is very suitable for this purpose). Meanwhile a Petri dish containing a watch glass has been made ready by putting about one or one and a half cubic centimetres of saline solution on the surface of the clot. The blastoderm is transferred to this watch glass from the spatula, so that it lies with the vitelline membrane against the clot and the yolk uppermost. The yolk can then be scraped away with knives and pipetted off with a wide-mouthed pipette. Clean saline is added and the culture, still in the Petri dish, is transferred to the stage of a dissecting microscope for operation. It is advisable to perform under a cover all the manipulations after the blastoderm is removed from the egg.

1*b*. *Histological methods.*

Most of the explants were fixed in Bouin's fluid, but a few of the earlier ones in Zenker's. Fixation was carried out with the explant still attached to the clot on which it had been growing, but it was freed from the clot before being passed through the alcohols to Cedarwood Oil or hæmotoxylin. In some cases the entire specimen was

stained with dilute hæmatoxylin before being cleared and mounted, and some of these whole mounts were later dissolved out again and sectioned. Other specimens were sectioned before being stained. All sections described were cut at 10 μ, unless otherwise stated ; they were stained in Delafield's hæmatoxylin and differentiated in acid alcohol.

Some attempts have been made to use Lehmann's technique for preserving the colour of pieces of tissue stained with Nile Blue Sulphate, but the avian tissue has not proved very suitable for this.

2. *The Normal* in vitro *Development of the Chick and Duck.*

Under favourable conditions a chick embryo usually remains alive *in vitro* for 2 to 3 days. During this time the embryo pursues its normal course of differentiation, with some divergencies which are noted below. At the end of this period, the culture probably needs transferring to a new medium. Such transference is a rather difficult matter, since the blastoderm is thin and large in area, and adheres closely to the surface of the clot. These difficulties could, however, probably be overcome, but no determined effort to work out a satisfactory technique of transference has been made, since for the purposes of the present experiments, 1 or 2 days' cultivation is sufficient to give the required results.

There seem, however, to be other factors which limit the possible life of the embryo *in vitro*. First, there is an extremely critical period during the time when the circulation begins to come into action. No difficulty is experienced in obtaining embryos in which the heart is beating, and blood formation in the vascular area invariably occurs, but there frequently seems to be a maladjustment between the two, so that embryos are often obtained in which the heart is beating, probably not very strongly, whilst the blood instead of circulating in the blood vessels, is concentrated in large masses in the posterior part of the area vasculosa.

Secondly, in those embryos in which the circulation functions properly, it is nearly always found that after 12–24 hours the heart slows down and stops, and the embryo dies. It is possible that in such cases a change of medium would suffice to prolong life for a time, but the facts rather suggest that there is something lacking in the conditions of cultivation for embryos of this age, since if an embryo with a functional circulation is removed from the egg and explanted *in vitro* it rarely survives for more than 24 hours.

Fig. 26, Plate 23, shows an embryo which had 9 somites when transplanted and which was cultivated for 43 hours : this specimen shows almost the latest stage of development to which embryos attained in these experiments. Plate 22 shows a series of photographs of a living chick blastoderm, taken during *in vitro* cultivation. They exhibit very well the essential normality of the process of differentiation *in vitro*, but there are a few points which require notice.

There is a general tendency for ectoderm to form cysts *in vitro*, and these are usually found in the outer parts of the *area vasculosa* of young embryos. As will be described

later, such formation of cysts also affects the ectoderm of the *area pellucida* when it is cultivated after removal of the endoderm.

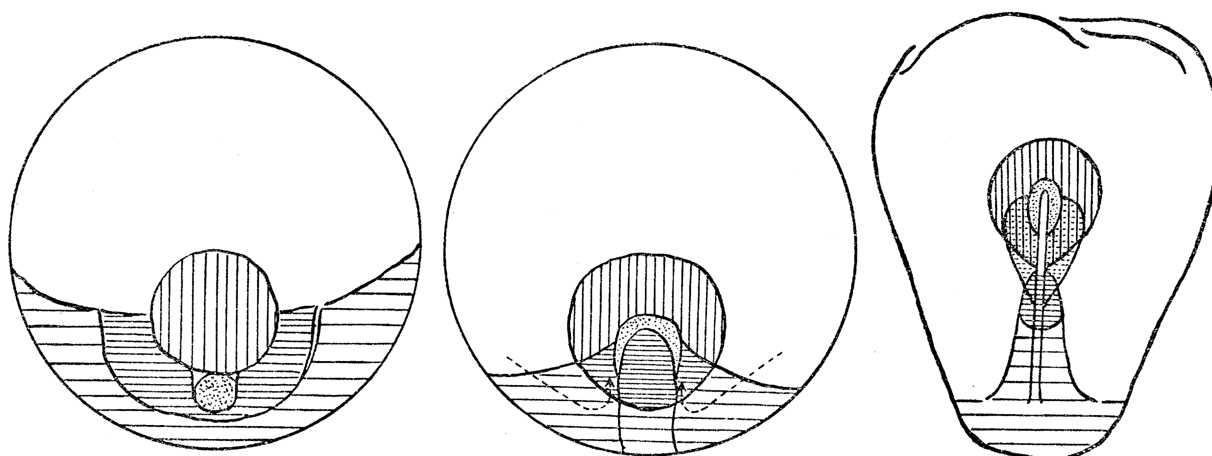
The rates both of growth and of differentiation are slower *in vitro* than *in vivo*. No exact study of the alterations of rate has been attempted but certain remarks can be made from a consideration of material which has accumulated. According to LILLIE's (1919) chart, 1·04 hours are required, on the average, for the formation of one somite under normal conditions, between the stages of 2 and 27 somites. The same figure, for 34 specimens grown *in vitro*, is 1·65 with a variation between 2·44 and 1·15. Both figures apply to incubation at approximately 37·5° C. For accurate results more carefully controlled experiments would be necessary. Nevertheless, the ratio of about 1 : 1·5 between the *in vivo* and *in vitro* differentiation rates, can probably be taken as approximately correct. There is no evidence that this slowing of the rate of development affects the young stages more than the old or *vice versa* ; for example, a blastoderm transplanted after 2 hours' incubation had 20 somites at the end of 72 hours, a stage which would be reached by a normal embryo in about 43 hours, giving a ratio of about 1 : 1·7.

The rate of growth (increase in length) is very variable *in vitro*, but is probably always considerably slower than *in vivo*. The stage of differentiation attained by an embryo is very largely independent of the absolute size, and, if a blastoderm is explanted at an early age and thus is affected by the slowing of the growth rate for the greater part of its life, embryos may be formed which, compared with the normal, are very much too small for their stages of differentiation. Thus an embryo cultivated for 72 hours after a preliminary incubation for 2 hours, has some 18-20 somites, although its length is only about 2 mm., whereas the normal length for such a stage should be about 5·3 mm. (from LILLIE's table). It cannot be decided, with the material at hand, whether the slowing of the growth rate is greater if the blastoderm is transplanted at an early stage, or whether the marked effects seen in such embryos is entirely due to the fact that the slower rate of growth has been in action for a greater proportion of the total life. It can be seen that the embryo shown in Plate 22 has grown remarkably little during the 32 hours covered by the series of photographs. The normal *in vitro* development of the duck appears to be similar to that of the chick, in that only rather unimportant modifications are caused by the abnormal environment.

3. *Location of the Presumptive Organ-forming Materials in the early Blastoderm of the Chick.*

The work of WETZEL (1925, 1929) and GRÄPER (1929) enables one to fix, at least in a provisional manner, the location of the organ-forming materials in the early stages of the blastoderm. WETZEL has provided four figures (fig. 124-127, p. 313 of 1929 paper) with reference to this point and GRÄPER has given five diagrams (Plate II, V.1-V.5). GRÄPER's diagrams concern the presumptive medullary plate, presumptive chorda,

etc., but remain only diagrammatic ; WETZEL's figures, though more exact, concern only the material which will ultimately form the primitive streak. It would perhaps be useful here to attempt to combine the two and to give sketches, as exact as possible, of the distribution of the presumptive medullary plate, etc., at various stages (figs. 2-4). We can neglect the earliest stage of GRÄPER, V.1, when the endoderm has not been invaginated, since this occurs as a rule before the egg is laid. In GRÄPER'S stage, V.2, corresponding more or less with WETZEL's fig. 124, it is probable that the medullary material should be more posterior than GRÄPER has drawn it (cf. WETZEL's figs. 91 and 92) ; (see fig. 2 ; on this figure the boundary of the axial mesoderm corresponds to line 4 of WETZEL's fig. 124).



FIGS. 2–4.—Location of the presumptive organ-forming materials in the blastoderm of the chick. Fig. 2.—Circular blastoderm before the appearance of the primitive streak. Fig. 3.—Circular blastoderm with short primitive streak. Fig. 4.—Fully-grown primitive streak.

Dotted—notochord material, vertical lines—neural material, horizontal lines—axial mesoderm, spaced horizontal lines—nonaxial mesoderm, dotted horizontal lines—invaginated mesoderm.

GRÄPER's fig. V.3 probably corresponds fairly closely to the conditions found in the young primitive streak stage before any mesoderm is formed, that is, to WETZEL's fig. 125, which is a considerably younger stage than GRÄPER's figure would indicate. The required alteration has been made in fig. 3, which otherwise corresponds with GRÄPER's drawing.

During the formation of the stage shown in fig. 3 from that shown in fig. 2, two main movements have been going on : a pushing forward of the primitive streak material along the mid-line, and a wheeling movement which brings new material from the sides into the mid-line (see dotted arrows in fig. 3). GRÄPER appears to think that this pushing forward affects even the posterior part of the blastodisc, but the work of WETZEL makes it probable that the posterior edge remains comparatively unmoved, the main movements taking place rather anterior to it.

Soon after the stage of fig. 3, mesoderm begins to be formed by the primitive streak, and material can be shown to be moving in from the sides to the primitive streak.

Perhaps the main dynamical difference between this stage and the last is that the transverse movement is now not accompanied by so much forward movement. This results in the condition shown in fig. 4, which is the stage most usually dealt with in the experiments to be described in this paper. This diagram is very similar to GRÄPER'S fig. V.4; in particular, the notochord material is drawn as extending some distance posteriorly to the primitive pit. Some independent evidence for this posterior extension of the notochord material was obtained in the experiments described in this paper (p. 189). Fig. 4 applies to a stage midway between those of WETZEL'S figs. 126 and 127. In the further development towards the conditions of WETZEL'S fig. 127, the main movement is the continued flowing of presumptive mesoderm into the primitive streak in the middle region, *cf.* WETZEL'S fig. 94.

In the later development, after the stage of the head process shown in WETZEL'S fig. 127, the main movement is a backward one along the primitive streak, by which the presumptive neural material and axial mesoderm, which were previously concentrated at the anterior end of the streak, are carried to their final position. This posterior drag is complicated by a wheeling movement which, however, does not concern us in this paper.

Certain experiments have been made during the course of this study which directly concern the localisation of the presumptive areas. They may be divided into three groups: (*a*) experiments in which the blastoderm has been cut into two parts in various ways, (*b*) defect experiments in which pieces of the primitive streak have been removed, and (*c*) experiments in which pieces of the primitive streak have been allowed to differentiate in isolation, usually as grafts in the extra-embryonic portions of other blastoderms. Of these, the last two groups of experiments were made incidentally to a study of the potentialities for induction possessed by various parts of the primitive streak; they are described later, in connection with the grafting experiments. Most of the experiments in group (*a*) were made in the early stages of the investigation, when the technique was still being elaborated; they have not been carried very far since the method does not appear to be so useful as the vital staining technique of WETZEL. As far as they go, they support the conclusions already drawn by WETZEL and GRÄPER, and since they are not without importance as confirmatory evidence, a few typical cases are described here. All the specimens were operated on at about the stage represented in fig. 6, except 180, which was rather more developed.

The Differentiation of portions of Blastoderms.

1. *Anterior Portions.*—If, shortly before the appearance of the head process, the blastoderm is cut through the primitive pit or just anterior to it, no embryonic structures usually appear on the anterior portions, except occasionally a little neural tissue.

If, however, the cut is made just posterior to the primitive pit, a head appears and usually a thin "tail." This "tail" consists of neural plate, somites and a notochord,

and stretches back from the head, jutting out sharply from the more or less straight cut edge of the blastoderm segment; this result shows that there is a very much more powerful tendency for backward growth in the line of the primitive streak than in the rest of the *area pellucida*. The further posteriorly the cut is made, the less marked is the tendency to form a tail.

*Description of specimens.**

325. A 22¼-hour blastoderm with no head process was sectioned just behind the primitive pit, and the culture incubated for 19½ hours. The tail is shown in the figure, fig. 27, Plate 23.

324A. The blastoderm was sectioned through the primitive pit after 22 hours' cultivation. A little neural tissue developed on the anterior portion, fig. 28, Plate 23. The posterior portion is described later.

189. In this case the blastoderm, which was similar in age to the last, was not cut in half, but the second anterior quarter of the primitive streak (*i.e.*, not the quarter including the primitive pit, but the one just posterior to this) was removed by cuts which extended rather far transversely across the *area pellucida*. The hole thus made became enlarged during cultivation, probably because there was an accumulation of liquid; the anterior part of the blastoderm formed a "tail" projecting into this space. This "tail" includes rather more of the primitive streak than that of 325, fig. 29, Plate 23.

Fig. 30, Plate 23, shows a typical section through a "tail," with somites, notochord and neural plate.

408A. This is the anterior portion of a 20-hour blastoderm, cut just posteriorly to the primitive pit, and incubated for 22 hours. The cut in this case was, intentionally, not exactly transverse to the primitive streak but inclined at an angle of about 45° to it, so that the explanted piece reached further posteriorly on the right side than the left. In these circumstances, as may be seen from the figure, no "tail" is formed, but the right neural fold continues further along the edge of the cut than the left fold and normally arranged somites are present on the right side, fig. 5.

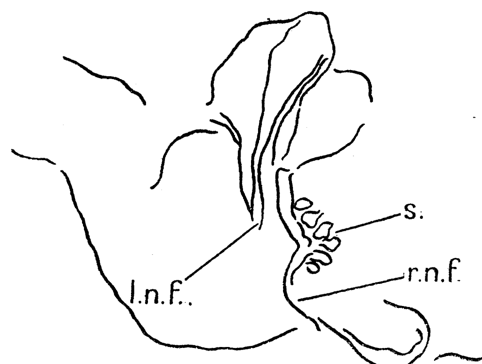


FIG. 5.—408A (20–22) Anterior half of blastoderm cut obliquely just posteriorly to primitive pit. Camera lucida sketch of whole specimen: *l.n.f.*, end of left neural fold; *r.n.f.*, right neural fold; *s.*, somites. (× 50).

2. *Posterior Portions.*—If the blastoderm is cut through the primitive pit, the posterior half develops all the axial structures except part of the head; the experiments have

* In the descriptions of the specimens, the age of the embryo at operation, in hours of incubation at 37° C., is always given. It has been found, however, that these figures give rather little information as to the stage of differentiation which has been attained, and where more accurate data are required, it has been necessary to fall back on an arbitrary classification based on the length of the primitive streak. (See note to Protocols, p. 197.) When the head process had appeared at the time of operation, this is always stated, and when no remark is made, it can be taken that that structure was absent. The head process appears considerably later in many of these eggs than appears to be usual. This is probably due to the fact that much of the work was done in the winter, since I have found that in warm weather the embryos are considerably more developed than in cold.

not yet been made accurately enough to determine from exactly which level the head is lacking.

If the cut is made a short distance posterior to the pit, the floor of the medullary plate and the chorda are absent and the two neural folds are therefore separate, and in the entire specimen they are seen to be spread out in the form of a V.

If the cut is still further posterior, *e.g.*, as far back as the middle of the primitive streak, there is usually little or no trace of embryonic structure in the posterior half.

In this connection, the most interesting question is: how far posterior to the pit can the cut be made so as to include the presumptive floor of the neural plate and the chorda? This has not been determined exactly, but it appears that the cut *can* be made slightly posterior to the pit.

Description of specimens.

324P. This is the posterior portion of a 22-hour blastoderm, sectioned through the primitive pit and then cultivated for 20 hours. The floor of the neural groove is lacking in the anterior end, and so, probably is the notochord; both are present at the posterior end of the specimen. The heart rudiments have not yet appeared, fig. 31, Plate 23.

135P. The blastoderm was sectioned through the primitive pit after 20 hours' incubation; it had a fully-developed primitive streak but no head process. The posterior end of the primitive streak was slightly injured during the operation. The specimen was fixed after 22 hours' cultivation, fig. 32, Plate 23. In the living specimen, the two heart rudiments, which can be seen in the figure, were beating, but not synchronously with one another. It will be seen that the anterior part of the embryo appears to project forward in front of the cut edge of the blastoderm. This forward projection is not nearly so marked as the backward projection in those anterior portions which have formed "tails." It is probably due, partly to the tendency of the cut edge of the blastoderm to curl up except in the region where the development of the axial structures gives greater rigidity to the tissue, but also partly to a real tendency towards regeneration.

172. The blastoderm, after 20 hours' incubation, was cut through the primitive pit, and the posterior portion cultivated for 44 hours. In sections, the most anterior part of the neural tube is seen to consist of the roof only, the floor being absent. Slightly posterior, the tube becomes completed, and shortly behind this the otic pits are seen. Still further posterior the notochord appears and also, on the right side, a heart rudiment; the embryo was injured in the left side and the left heart rudiment has not developed, fig. 33, Plate 23.

The anterior part of the embryo is slightly raised above the surface of the clot; the space thus left beneath the embryo represents the foregut, which is open anteriorly, posteriorly and ventrally. At the sides of the space, the endoderm is pushed in to form two pouches, which represent the lateral evaginations of the foregut.

The posterior part of the embryo is practically normal.

304P. This is the posterior part of a 22-hour blastoderm, cut slightly posterior to the primitive pit and cultivated for 26 hours. In the whole mount, the embryo was seen to be in the form of a V, of which the left limb was considerably longer than the right and contained 5–6 somites. Fig. 34, Plate 23, shows a section passing through this left limb, but not through the right. The side wall of the neural plate is joined to both the ectoderm and the endoderm. The neural plate disappears before the two limbs of the V join.

180. The specimen consists of the posterior portion of a 19½-hour blastoderm cut very slightly posterior to the primitive pit. An early head process was present at the time of operation. The head process and

primitive pit were cultivated as a graft at the posterior end of the blastoderm and yielded neural tissue and notochord. The embryo is split along the neural plate for the greater part of its length, forming a shape like a capital Y (fig. 6). It is interesting to note that each limb of the Y contains, in addition to the side wall of the neural plate, a small piece of notochord material. It is probable, therefore, that the presumptive notochord material extends slightly posterior to the primitive pit. (The possibility of regeneration of notochord in this specimen is considered later.) In the most posterior part, the floor of the neural groove is present.

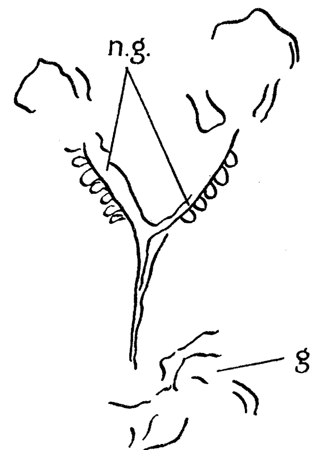


FIG. 6.—180 (19½–32) Posterior half of blastoderm cut very slightly posteriorly to primitive pit. Camera lucida sketch of entire specimen. *n.g.*, neural groove. *g*, graft mass (× 50).

Interpretation of Results.—The development of the anterior portions is very easy to interpret on the basis of WETZEL and GRÄPER'S work. In particular, the formation of "tails" by halves cut just behind the primitive pit shows very clearly how the materials of the axial organs are at first concentrated in the anterior part of the primitive streak and are brought to their final position by a powerful backward movement. One can scarcely appeal to regeneration as a complete explanation of the phenomena, since one would expect, under those conditions, an embryo which was more harmonious in its proportions, probably smaller throughout, and without such marked growth of the posterior margin. It is, however, possible that regenerative processes have contributed to the result in a minor degree. 408A is interesting, since in this case, owing to the obliqueness of the cut, the backward movement could proceed along the cut edge on one side, so that on that side the embryonic organs are present in a region originally outside the primitive streak; that is, in a region which would not normally contain them. It must be supposed that the transverse movements of the earlier stage have played a part here as well as the longitudinal backward movement.

The general results of the cultivation of the posterior halves are also quite consistent with the scheme of development given above; if the cut is made through the primitive pit, the floor of the neural groove and the notochord are present; if more than very slightly behind the pit, these two regions are absent; if the cut is posterior to the middle of the primitive streak, no embryonic structures develop. The phenomena found in the most anterior region of the posterior segment when the cut has been made through the primitive pit will be dealt with in a later paper.

In 180 a notochord is present although there is no floor to the anterior part of the neural groove. This probably indicates that the presumptive notochord material is longitudinally extended.

The removal of the area lying anterior to the primitive pit hinders the formation of a foregut. As a result, the two heart rudiments usually develop separately, one on each side (*cf.* 172 and 135P). The best examples of this have been found in experiments

on the regeneration of the head, and the conditions found in the separated hearts will be discussed in a further communication dealing with these experiments.

The experiments described in this section contradict the results of HUNT (1929, *a*, *b*, 1931) and WILLIER and RAWLES (1931), who, using the chorio-allantoic technique, found that the posterior portion of the primitive streak is not capable of self-differentiation in the absence of HENSEN'S node (*i.e.*, of the area lying immediately round the primitive pit). They have argued from these results that HENSEN'S node is similar in its developmental importance to the dorsal lip of the blastopore in Amphibia, the capacity of which for self-differentiation is well known, and further, that, like the dorsal lip, the node exerts an organising influence on its surroundings. Naturally, the fact that an embryonal area has a capacity for self-differentiation does not prove that it is an organiser. But there are further grounds for such an assertion if it can be shown that no other part of the embryo has a similar capacity. As regards the organising function of the node, therefore, the important part of HUNT and WILLIER'S work is the negative conclusion that the posterior part of the streak is not capable of self-differentiation: their results on this point are shown to be inconclusive by the experiments described above and by others to be described in the section dealing with grafts. One might suppose that the failure of development in their experiments was due to some mechanical cause, but without an intimate knowledge of the technique, it is difficult to make any definite suggestions. Possibly the grafted tissue becomes rolled up in such a way as to interfere with the movements of cell masses: such an explanation is perhaps suggested by the fact that WILLIER and RAWLES did not obtain differentiation of the posterior part of the trunk even in grafts of the entire blastoderm. Conceivably, the fact that their grafts were allowed to differentiate to a much later stage than that obtained in my experiments may have been of importance. UMANSKI (1931) also failed to get differentiation of the posterior half of the primitive streak on the chorio-allantois, but from his diagrams of the operation it appears likely that the fragments which he transplanted did not actually contain any presumptive neural material, so that his failure to obtain this tissue is not surprising.

It will be shown later, by positive experiments, that the node possesses an organising capacity, which, however, is not confined to it, but is shared at any rate by the anterior part of the primitive streak.

PART II.—THE FORMATIVE INFLUENCE OF THE ENDODERM.

The experiments described in this part of the paper were designed to test whether the formation of the endoderm plays an organising rôle in the development of the embryo. It has as yet been impossible to test this directly in the usual way by transplantation experiments, since the endoderm is formed at a very early stage when the blastoderm shows no distinctive marks by which it can be orientated; moreover, even if the orientation could be carried out, the cultivation of such young embryos would probably be very difficult.

The investigation has therefore had to proceed by more roundabout methods. In the first series of experiments, the endoderm was removed and the epiblast allowed to differentiate alone. In this case little evidence was found that the endoderm plays an important rôle, since the epiblast developed almost normally, but, again, technical difficulties prevented the experiments being carried out at a very early stage. In the second series the spatial relations of the two layers relative to one another were altered. In this case a definite influence of the endoderm was found.

1. *Cultivation of the Isolated Endoderm and Epiblast.*

In embryos of 16–24 hours' incubation, when the primitive streak stretches half or nearly two-thirds of the way across the *area pellucida*, it is easy to separate the endoderm from the epiblast. The blastoderm is laid on its upper or epiblast side, and, by manipulation with knives the endoderm is gradually pulled away. It is best to begin the removal of the endoderm at the edge of the *area pellucida*, and to free it first all round the circumference, afterwards working inwards towards the primitive streak, from which the coherent sheet of endoderm is finally pulled away at the very end of the operation. Usually, a considerable amount, probably the greater part, of any mesoderm which has been formed comes away with the endoderm, leaving the upper sheet of tissue, which is usually referred to as the ectoderm. Since, however, the work of WETZEL and GRÄPER has shown that some of this tissue is destined to be invaginated through the primitive streak and thus formed into mesoderm, it is not desirable to refer to the whole tissue-layer as the ectoderm, and the term "epiblast" is used in this work.

The two layers, the endoderm and the epiblast, have been cultivated in isolation from each other.

Section 1a.—*Cultivation of the Isolated Endoderm.*

The endoderm is usually slightly torn by the operation of removal. It shows a strong tendency to curl up into a roll or a ball: in the process of flattening and spreading the curled up tissue on the surface of the clot, further injury is often inflicted. The tissue can, however, be held flat and the saline solution in which the operations have been made removed; the endoderm then lies spread out on the surface of the clot. Any tears which have been made in it very soon heal and cell division and cell wandering from the edges of the tissue take place actively. No anatomical differentiation took place in explants kept alive for two or three days.

Section 1b.—*Cultivation of the Isolated Epiblast.*

Isolated and untorn epiblasts can be easily prepared. Like the isolated endoderm, they show a tendency to curl up, but they can be flattened and spread on the surface of the clot, by the use of knives and a judicious sucking away of the saline solution in

which they have been prepared. Their successful cultivation appears to depend to a rather large extent on the success with which this flattening is done. The most common result of cultivation is to find that the tissue has formed a number of bubbles or blisters filled with liquid. It is probable that the bubbles arise from the small empty spaces left between the epiblast and the surface of the clot as a result of incomplete flattening of the former. This phenomenon, which is usually seen in the *area opaca* of normal blastoderms cultivated *in vitro*, is probably a manifestation of the general tendency towards cyst-formation which is characteristic of ectoderm *in vitro*. It is difficult to obtain cultures in which the blister formation is entirely absent; there is a series, at one end of which are cultures in which the formation of bubbles has prevented or obscured anatomical differentiation, while at the other end are cultures in which very few bubbles are present and morphogenesis is fairly normal. In between the extremes lie the cases in which the anatomical differentiation can be seen, more or less distorted by the bubbles. In my experiments, "bubbly" and distorted explants have been considerably more common than those of the undistorted type.

The epiblasts prepared in the above-mentioned way are always associated with a marginal ring of yolk-sac endoderm underlying the *area opaca*. Moreover, it is not possible merely from the method of separation, to be absolutely certain that the whole of the endoderm has been cleared away from the *area pellucida*. In the specimens sectioned after cultivation, however, it is found that either there is no endodermal material, except for that derived from the marginal ring of yolk-sac endoderm, or that there are in addition a few small epithelial tubes present which are probably derived from pieces of endoderm left attached to the epiblast. The presence of these tubules appears to show that if any significant quantity of endoderm is left unremoved beneath the *area pellucida* in the separation of the layers, its presence can be detected in the cultivated specimen. Hence in cases where there is no manifest sign of the presence of endoderm, it can be assumed that all, or all but an insignificant amount, has been removed.

Description of specimens.

293. The culture consisted of the epiblast of a 25-hour chick; at explanting the head process had not yet been formed. After 23½ hours the embryo had three pairs of somites. The specimen was fixed after 42 hours, when there were seven or eight pairs of somites.

The contracted neural folds of the head are shown in fig. 35, Plate 24, with the subcephalic pocket below. Twenty-two sections further posteriorly, fig. 36, Plate 24, the neural plate appears more normal and there is a space which may represent the heart. Still further posteriorly the neural plate is small and overlies a large notochord which is embedded in thick masses of mesoderm. The mesoderm is full of spaces, some of which have an epithelial lining which may be derived from small pieces of endoderm left adherent to the epiblast. Some of these epithelial areas, however, are found to be connected with folds in the ectoderm. At the most posterior end, about 75 sections from the last figure, the whole floor becomes covered by a thick layer of yolk-sac endoderm, fig. 37, Plate 24.

319. The culture consisted of the epiblast from a 21-hour chick blastoderm and was cultivated for 26 hours. The blastoderm was under-developed when explanted and had only a young primitive streak, which extended not quite half way to the centre of the still circular *area pellucida*. During cultivation

a bubble of liquid formed under the *area pellucida* ; in sections the floor of the bubble is seen to be covered with yolk sac endoderm which has grown inwards from the sides, while on the upper surface the ectoderm has formed a neural plate and a certain amount of mesoderm, fig. 38, Plate 24. The notochord appears to be absent. No trace of head-formation is distinguishable ; possibly tensions, due to the formation of the bubble, prevented the development of a head fold.

227. The epiblast was $20\frac{1}{4}$ hours old at explantation and was cultivated for $22\frac{1}{2}$ hours. A fairly straight and comparatively well-formed embryo could be seen in the cleared specimen. In sections, the posterior part of the neural groove is remarkably normal in appearance. In the middle part there is a bend and some disturbance of the normal symmetry, which is however more or less regained further anteriorly. Towards the anterior region, part of the ventral edge of the mesoderm begins to form a thin membrane lining the cavity between the mesoderm and the plasma ; and a sheet of yolk-sac endoderm has grown along the surface of the plasma and provided a cellular floor for this space, fig. 39, Plate 24. As the sections are followed towards the anterior end, the cavity lined by the membrane is seen to assume a shape rather like that of the foregut, except that it is always open below into the space above the plasma ; it does, however, enclose a space which pushes out laterally into the head mesoderm of the embryo, fig. 40, Plate 24. This is most marked on the left side, where the membrane is also thicker. It is possible that part of the membrane in this region may be derived from endoderm which was left after the operation, but from the histological appearance this appears to be unlikely, and certainly cannot apply to more than a small part of the membrane. Meanwhile the neural folds have widened out, but are not properly folded ; this lack of closure of the folds is probably the commonest malformation of the head in cultivated embryos, both with and without endoderm.

402. The blastoderm was operated on after 24 hours' incubation and was cultivated for 19 hours. The only part requiring notice is the head, where the neural plate is divided into two. In about the middle of the head region there is a space lying below the neural plate, in approximately the position which ought to be occupied by the foregut ; this space is lined by a thin membrane apparently formed from the contiguous mesoderm, fig. 41, Plate 24. Posteriorly, the membrane constituting the floor of the space becomes confluent with a piece of yolk-sac endoderm, which has grown in from the side ; in places this endoderm takes part in the formation of the floor, without, however, showing any sign of giving rise to a structure resembling a foregut. The lumen, with its membranous lining, eventually disappears quite suddenly.

Interpretation of results.—The main conclusion to be drawn from these experiments is that a considerable amount of differentiation can take place in blastoderms from which the endoderm has been removed in middle or late primitive streak stages. Neural plate, somites and notochord are found in the explant after cultivation, somewhat disturbed in their morphological relations by the presence of " bubbles " or blisters, but easily recognisable. No really satisfactory heart has been formed in the cultures, but in a culture of two apposed epiblasts (No. 479, p. 205) a beating heart was developed ; this shows that a heart can develop in the absence of endoderm.

The notochord has formerly been referred to as an endodermal derivative ; yet it develops in these cultures. Now, at the primitive pit, from which the notochord originates, the ectoderm and endoderm, and also the mesoderm, are fused together and cannot be distinguished from one another. It is therefore impossible to be certain that all the endoderm has been removed from this region ; but it is certain that at least the lowest layer of tissue has always been removed, so that at any rate the notochord cannot originate in the lowest layer. Recent work, however, shows that the notochord

material is originally in the epiblast and later becomes invaginated at the primitive pit region, so that the development of notochord in explanted epiblasts is easily explicable.

It would be very interesting to determine to what extent the endoderm can be regenerated, and in particular whether it can be regenerated perfectly enough to be capable of forming a foregut. The yolk-sac endoderm from the edge of the disc frequently grows centripetally and covers the entire area below the *area pellucida,* but in no case has it shown any signs of being able to take part in the formation of a foregut. On the other hand, it is known from other experiments (see Part II, section 2) that the neural plate is capable of forming a foregut from non-presumptive endoderm. It may therefore, not be impossible that a foregut might be formed from yolk-sac endoderm in favourable cases. But it is unlikely that a regenerated endoderm could ever be fully functional in the sense of exerting a directive influence on the growth of the neural plate similar to that which, as will be shown in the next section, is exerted by the normal endoderm.

In connection with the formation of a false or regenerated foregut, the mesodermal membranes seen in 227 and 402 are interesting. In both cases, the formation of a membrane may be due merely to the fact that the cells were probably in contact with the surface of a liquid filling the cavity below them. But, even if the formation of the membrane is not to be taken to be a regenerative phenomenon, the shape of the cavity, at least in 227, suggests that the mesoderm as a whole has left a space into which a foregut could be fitted.

The experiments described here have been concerned with middle and late primitive streak stages; very young blastoderms have not been used to any great extent, since they are usually very badly distorted by the formation of blisters. This distortion might perhaps make it impossible to prove the negative result, that epiblasts of less than a certain age could not differentiate. Development has, however, been obtained from a duck epiblast in which the primitive streak at explanting only stretched about a quarter of the distance across the *area pellucida.* In another case, the formation of a layer of yolk-sac endoderm beneath the *area pellucida* seems to have prevented the occurrence of " bubbling " and quite a good embryo was obtained from an epiblast explanted at a very early stage (4 hours' incubation), but there is a slight doubt whether the operation was properly performed, since the sections show a certain amount of tissue which looks remarkably like endoderm, left attached to the epiblast.

Epiblasts have, however, been used which were young enough to show that the period in which self-differentiation is possible overlaps with the period in which the endoderm can be shown to have a directional influence on the growth of the primitive streak and medullary plate (see next section). It should be noticed that the medullary plates which develop from the isolated epiblasts, though frequently somewhat distorted by blisters, nevertheless lie in the general direction of the primitive streaks from which they developed, and never show curvatures comparable to those described in the next section.

Several of the cultures suggest that the primitive streak has not continued its normal growth in length after the removal of the endoderm; the neural plates formed after cultivation were very much shorter than normal. This is most noticeable in cultures of very young epiblasts, such as the duck culture mentioned above.

It will be noticed that the differentiation of the isolated epiblast is frequently slower than that characteristic of the unoperated blastoderm *in vitro*. Thus 293 had only developed seven pairs of somites after 42 hours. In other cases this slowing is not so noticeable.

2. *The mutual influence of the Epiblast and Endoderm.*

A series of cultures have been made, in which the epiblast and endoderm from a single blastoderm were separated and then brought together again in such a way that their original longitudinal axis were approximately at right angles. It was hoped that by this derangement of the normal relations, some mutual influence of the layers on one another, or of one layer on the other, would become apparent. A positive result was obtained: it was found that if the operation is performed on a blastoderm in which the primitive streak is not yet full grown, the direction of growth of that structure is influenced by the position of the endoderm. Further it was found that, although the head of the resulting embryo might lie above endoderm which would not normally be associated with it, yet, in nearly all cases, a foregut was formed with its normal relation to the (misplaced) head, and no such structure was found in the position now occupied by the presumptive gut endoderm. It is then necessary to consider, at least, two formative influences, which are to some extent opposed: in one, the earlier to be active, the endoderm influences the formation of an ectodermal structure, namely, the primitive streak; while in the second and later, the ectoderm influences the endoderm in the formation of the gut.

It was pointed out in the previous section that when the epiblast is cultivated with no endoderm present, the axial structures develop in the direction of the primitive streak, except in so far as they are distorted by the occurrence of bubbling. In the experiments described in this section, since these structures constantly show a deflection towards the anterior end of the endoderm, it would appear that this deflection is a significant result and indicates a real influence exerted by the endoderm.

The operations were, as usual, carried out in saline solution on the culture dish. It is particularly important in these experiments to leave the endoderm attached to the primitive streak until it has been freed everywhere else, since in itself it has no marks by which its longitudinal axis can be distinguished. At the end of the operation, it must be pulled away from the primitive streak, working from the anterior end backwards, and once it is finally free a careful watch must be kept on it while the epiblast is being reversed and put into place. When the operation is complete and the saline solution has been removed from the clot, a stroke is made on the bottom of the Petri

dish with a diamond or grease pencil and the culture is carefully orientated under the microscope, by moving the watch glass so that the primitive streak points straight along the mark on the glass. Once orientated, the culture vessel should be handled gently, but the watch glass sits fairly firmly on the hole in the cotton wool and will only move in response to a rather severe jerk.

In such cultures, besides the mutual influence of the layers, there is, of course, another factor to be considered, namely, the satisfactoriness of the healing of the wound separating the layers: probably the degree to which the layers can influence one another depends on their being in organic connection. As far as can be seen in the living specimens, the healing usually appears to be completed quite soon, within four or five hours of the operation. Viewed by transmitted light, a newly-operated culture as a rule shows somewhere a ragged edge of endoderm, darkened by its burden of yolk, dimly visible through the epiblast, while around the edges of the epiblast may be lighter spots where there is no underlying endoderm. In four or five hours such lighter spots are usually filled up and the dark masses of yolk, formerly on the edges of the endoderm, have disappeared. This healing is nearly always complete enough to prevent the formation of bubbles or blisters in the *area pellucida*; they are, of course, found in the *area opaca* as usual. In a few cases the anatomical differentiation of the neural tube is to some extent disarranged although there has been no formation of bubbles. On the whole, however, the embryos obtained are strikingly normal in this respect. But although the healing of the wound may be satisfactory as judged by the morphological differentiation, this does not perhaps necessarily prove that it is satisfactory in the sense that the two layers can exert their influences freely upon one another. As regards the direction of growth of the primitive streak, the only criterion by which we can decide whether the endodermal influence has been exerted is the actual occurrence of a bent primitive streak: if a straight primitive streak or neural tube develops, the possibility must always be borne in mind that this may be due, not to the ineffectiveness of the endodermal influence itself, but to unfavourable conditions of healing which have prevented its successful action.

It is not possible to be certain *a priori* that all the endoderm has been removed from a blastoderm; small pieces of tissue may have been left attached to the epiblast. It might be argued that this uncertainty is sufficient to cast doubt on the validity of the method. But if the turning round of the endoderm has an effect on the direction of the primitive streak in any particular case, then in that case at least, any such endodermal fragments left attached to the epiblast have clearly not had an important influence. On the other hand, if there is no apparent influence of the endoderm, it is possible that this may be due to an inadequate separation of the two layers, but although this is possible, reasons have been put forward in the last section for supposing that in fact the removal of the endoderm is usually fairly complete.

The foregut appears always to be normally formed, unless the neural tube overlying it is disarranged; in such cases either the distorted neural tube has been unable to exert

its normal influence or some cause of distortion has arisen which has affected equally both the ectodermal and endodermal structures of that region.

Protocols of the experiments are as follows, disregarding cultures lost by infection (Tables I and II).

The length of the primitive streak at the time of operation is classified into—S, short; M, medium; and L, long; with the intermediate classes SM and LM. Short primitive streaks are those in a circular *area pellucida,* stretching about a third or a quarter of the length of this area; medium streaks are in definitely pear-shaped areas, and are about half as long as the area; long primitive streaks are in very markedly pear-shaped areas and are about two-thirds as long as the area. The classification is quite arbitrary; a further idea as to the significance of the terms will be obtained from the figures. The word "bent" applied to the direction of the neural tube means that the tube was curved away from the anterior end of the ectoderm towards the anterior end of the endoderm. The word "straight" means that the neural tube developed in the direction of the longitudinal axis of the ectoderm.

Description of specimens.

Chick.

233. After operating the endodermal axis pointed,* at right angles, to the left of the ectodermal axis, Twenty-three and a half hours after the operation the neural folds were closing in the head region and the general curvature of the embryo towards the left was clearly visible. In the specimen, fixed after 44 hours' cultivation, this curvature is exaggerated by the presence of a hole in the blastoderm, which surrounded an area of liquefaction of the medium. The specimen is shown here as an example of a bent chick embryo with a well-formed foregut, fig. 7.

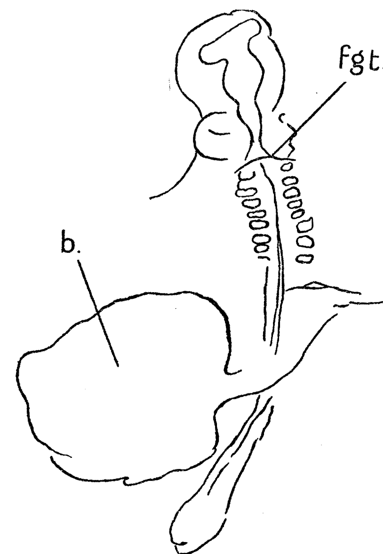


FIG. 7.†—233 (21–44) Ectoderm and endoderm turned. Camera lucida (× 50). *b.*, hole in blastoderm. *Fgt.*, foregut.

290. Fig. 42, Plate 25. The angle between the ectodermal and endodermal axis was rather more than a right angle, the endodermal one pointing to the left of the ectodermal. The blastoderm was $17\frac{1}{2}$ hours old at the time of operation and had a medium sized primitive streak. After cultivation for 29 hours, the neural folds had appeared, lying exactly in the ectodermal direction. The figure shows the stained specimen, fixed after 43 hours' cultivation. The head is considerably shortened and the closing of neural folds has not progressed very far posteriorly. It is impossible to say how far these abnormalities are due to incomplete healing of the two layers or how far they are merely due to the usual risks of culture *in vitro*; such deformities could easily be paralleled among specimens of unoperated blastoderms cultivated *in vitro*, although the majority of such specimens would be more nearly normal. The main axis of the embryo shows no sign of curvature. The posterior margin of the foregut can be distinguished, with a rather bulbous heart lying anterior to it.

* When the word "point" is applied to the axial structures of an embryo, whether to the anterior or to the posterior part, the direction of pointing is always taken to be from the posterior towards the anterior of the part in question. Thus, in fig. 15, the anterior part of the neural groove points to the right, the posterior part to the left. The length of the primitive streak at operation in the described specimens will be found in the third columns of Tables 1 and 2.

† In figures 7–16 the original ectodermal axis is arranged parallel to the long side of the page.

TABLE I.—*Chick Embryos.*

Ref. No.	Age at explantation in hours.	Length of primitive streak and remarks on operation.	Hours cultivated.	Direction of neural tube.
335	20	SM, endoderm much crumpled	44	Slightly bent.
226	20½	M	47	Endoderm not well healed, no true neural folds, straight.
233	21	M	44	Bent.
290	17½	M	43	Straight.
323	22	M	45½	Straight.
517	18	M	45	After 17 hours slightly bent, later straight.
563	20	M	21	Very irregular, straight.
564	20	M	21	Rather bent, but not well healed.
565	20	M	21	Bent.
569	21	M	24½	Sinuous neural tube, main part bent.
567	21	LM	20½	Slightly sinuous neural tube, main direction straight.

TABLE II.—*Duck Embryos.*

Ref. No.	Age at explantation in hours.	Length of primitive streak and remarks on operation.	Hours cultivated.	Direction of neural tube.
415	23	S	41¼	Bent.
435	23½	S	24	Bent, later infected.
436	24	S	29¼	Bent, not well formed.
473	23½	S	30	Bent.
474	23¾	SM	30	Bent.
437	25	SM, endoderm not concentric with *area pellucida*, but with anterior edge near line of primitive streak	28½	Straight.
465	27½	SM	44	Slightly bent.
543	28½	SM	21½	Bent.
478	24	M	25½	Bent.
536	26¼	M	19¾	After 7½ hours primitive streak bent, later not curved but inclined about 20 to ectoderm axis, neural folds badly formed.
537	26½	M	25½	Bent, later straighter.
540	27	M, endoderm not concentric with *area pellucida*, but one side margin of endoderm near anterior end of primitive streak	6½	Early neural folds straight, later infected.
533	27½	LM	21½	Bad healing.
560	28	LM	29	Slightly bent.
561	28	LM	29	Bent.
548	37	L	28	Straight.
549	37	L	28	Badly healed, straight.
550	37	L	28	Straight.
551	37	L	28	Straight.
552	37	L	28	Bent, but this may be due to a hole in the blastoderm.
573	40	L	24	Straight.

517. The endodermal axis pointed, at right angles, to the right of the ectodermal. The embryo, operated after 18 hours' incubation, had at that stage a medium or short-medium streak and after $19\frac{1}{2}$ hours the specimen still showed only a late primitive streak and a head process ; the axis pointed slightly away from the original line of the ectodermal axis, towards the right. When the specimen was finally fixed, after 45 hours, necrosis was beginning. The embryonic axis had now straightened itself out and the resulting embryo lies in the ectodermal direction. The embryo as a whole is short and the head is not very well formed. The foregut can clearly be seen, fig. 8.

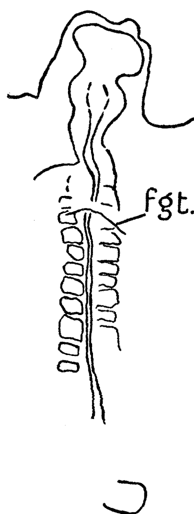


FIG. 8.—517 (18–45) Ectoderm and endoderm turned. *Fgt.*, edge of foregut (× 50).

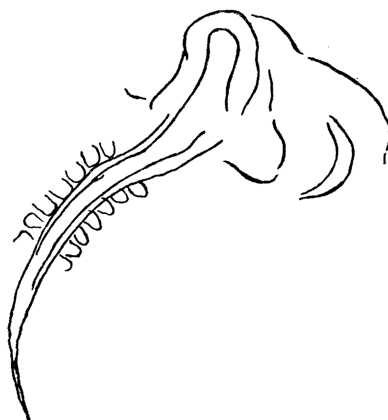


FIG. 9.—565 (20–21) Ectoderm and endoderm turned (× 50).

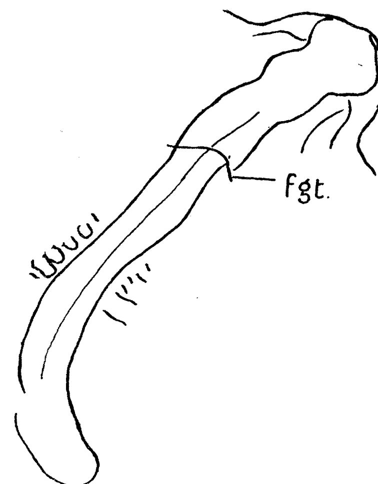


FIG. 10.—473 ($23\frac{1}{2}$–30) Ectoderm and endoderm turned. Duck. *Fgt.* foregut (× 50).

565. The endodermal axis was at right angles to the ectodermal, pointing to the right of it. The embryo has developed with a considerable curvature of the main part of the axis towards the right, but the head bends back away from the prolongation of the curve. The neural tube in the head region is not closed although a closed tube has been formed posteriorly, fig. 9.

323. The endodermal axis was inclined at 60° to the left of the ectodermal. The embryo developed exactly in the ectodermal direction. The section, fig. 43, Plate 25, shows the foregut, which is in its normal relation to the other embryonic structures. This foregut was presumably formed by non-presumptive endoderm.

Duck.

473. The endoderm pointed, at right angles, to the right of the ectodermal axis. The primitive streak at the time of explantation only occupied about one-fifth of the diameter of the blastoderm. The embryo resulting is very much bent, the anterior end being directed to the right, while the posterior part of the axis points to the left, fig. 10.

437. The endoderm pointed, at right angles, to the right of the ectoderm ; it was so arranged that the anterior end of the endoderm lay just along the right side of the primitive streak, fig. 11*a*. After 20 hours neural folds had appeared in the ectodermal direction. The specimen fixed after $28\frac{1}{2}$ hours is straight and lay in the ectodermal direction, fig. 11*b*. The head and foregut are well formed. It is suggested (see later) that the failure of bending in this embryo and in 540 may be due to the excentric position of the endoderm.

543. The endoderm pointed at about 45° to the left of the ectoderm, fig. 12*a*. After 5 hours the primitive streak had grown considerably in length and the anterior end was definitely bent to the left, fig. 12*b*.

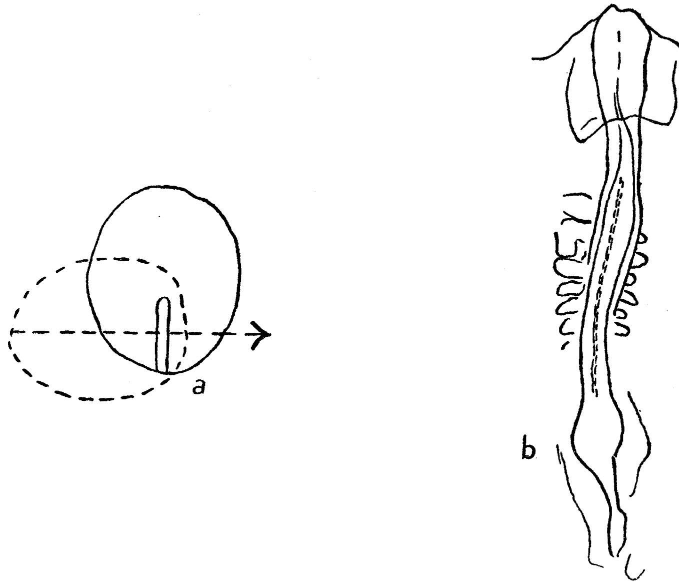


Fig. 11.—437 (25–28½) Ectoderm and endoderm turned. Duck. *a*, diagram of operation, endoderm dotted. *b*, embryo obtained ($\times$ 50).

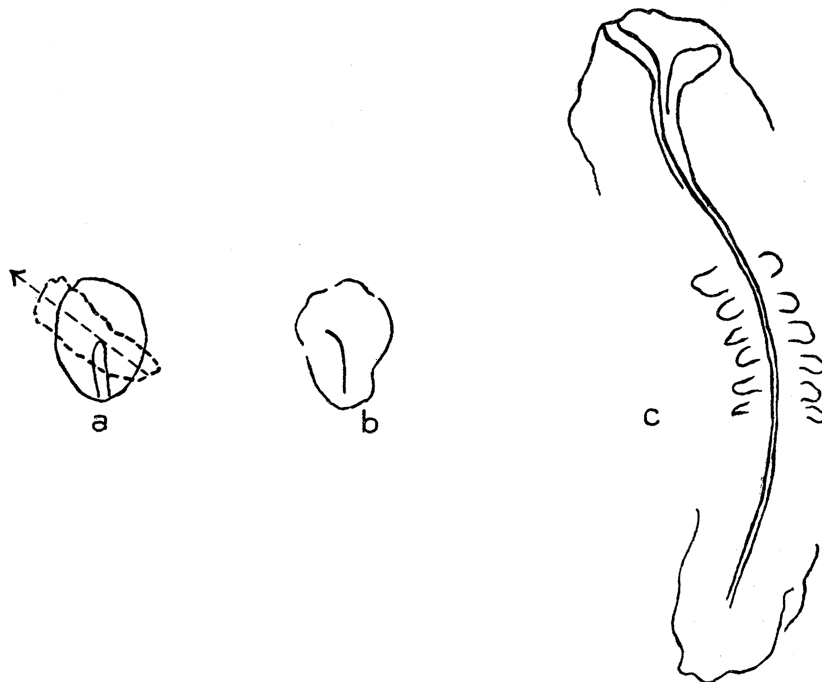


Fig. 12.—543 (28½–21½) Ectoderm and endoderm turned. Duck. *a*, diagram of operation, endoderm dotted. *b*, blastoderm after 5 hours *in vitro* ($\times$ 40). *c*, embryo after 17½ hours *in vitro* ($\times$ 40).

After 17⅔ hours' cultivation, the neural folds were formed, also bent to the left, but the most anterior part of the folds bent back again slightly to the right, fig. 12*c*. During the course of the next 3⅔ hours

the neural tube began to straighten. coming to lie more and more nearly along a straight line pointing to the left of the original ectodermal axis. This straightening took place by a backward shifting of the point where the rightward bending of the head began. Unfortunately the specimen has been lost and the full extent of this straightening cannot be shown. The head and foregut presented a very normal appearance.

537. The endoderm pointed, at right angles, to the right of the ectodermal axis, fig. 13*a*. After $7\frac{1}{3}$ hours there apparently was a slight bend of the primitive streak to the left, but the structures were not very easy to distinguish. After $19\frac{1}{2}$ hours the course of the embryonic axis was sinuous, the main middle part of the axis pointing towards the anterior of the endoderm, the two extremities away from it, fig. 13*b*. Later the anterior part of the embryo began to get straighter, by a retreat posteriorly of the point where the leftward bending of the head began; meanwhile the leftward bending of the posterior portion became more pronounced. The formation of the head and foregut is very abnormal, fig. 13*c*.

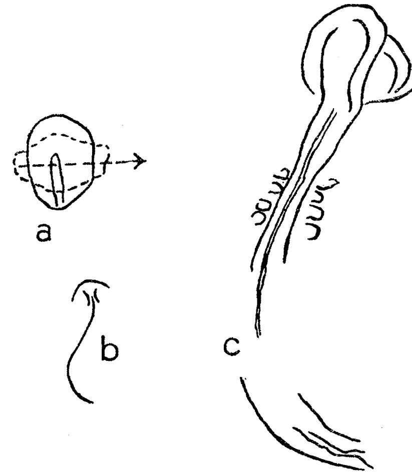


FIG. 13.—537 ($26–25\frac{1}{2}$) Ectoderm and endoderm turned. Duck. *a*, diagram of operation, endoderm dotted. *b*. head fold and axis of embryo after $19\frac{1}{2}$ hours, *in vitro* ($\times$ 40). *c*, embryo after $25\frac{1}{2}$ hours ($\times$ 50).

540. The endoderm pointed, at right angles, to the right of the ectodermal axis, fig. 14. It was so arranged that an edge of the endoderm lay against the primitive pit, so that anterior to the primitive streak was an area without endoderm. After $6\frac{1}{2}$ hours this area had been filled up by spreading of the endoderm, and neural folds had appeared, exactly in the ectodermal direction. The specimen later became infected and was discarded.

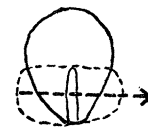


FIG. 14.—540 ($27–6\frac{1}{2}$) Ectoderm and endoderm turned. Duck. Diagram of operation, endoderm dotted.

561. The preparation was a particularly good one, with the endoderm pointing, at right angles, to the right of the ectodermal axis, and the primitive pit lying almost exactly over the middle of the endoderm, fig. 15*a*. After 20 hours the neural folds had appeared and were very markedly bent, fig. 15*b*, and the bend remains, though rather lessened, in the specimen fixed after $29\frac{1}{3}$ hours, fig. 15*c*.

FIG. 15.—561 (28–29) Ectoderm and endoderm turned. Duck. *a*, diagram of operation, endoderm dotted. *b*, after 20 hours ($\times$ 40). *c*, after 29 hours ($\times$ 40).

550. The age of this embryo at operation is uncertain, but the primitive streak occupied about $\frac{2}{4}$ of the diameter of the *area pellucida*, or perhaps rather more. The endoderm pointed, at right angles, to the left of the ectodermal axis. The resulting embryo is, to all intents and purposes, quite straight, the slight curvature which can be detected not being large enough to be significant. The foregut is well formed, fig. 16.

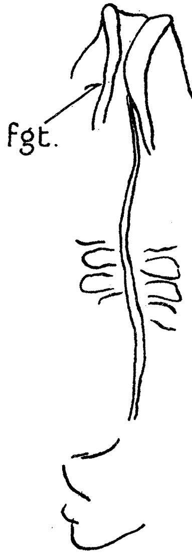


FIG. 16.—550 (37–28) Ectoderm and endoderm turned. Duck. *Fgt.*, edge of foregut (× 50).

Interpretation of Results.—The general result of these experiments is clear: if the endoderm and ectoderm are placed so that their longitudinal axes make an angle with one another, the anterior part of the primitive streak, and of the resulting neural folds, is bent towards the anterior end of the endoderm, if the experiment is performed at a young enough stage. Moreover, although abnormalities of the head and foregut are not uncommon, a normal foregut can be formed in cases where it is clear that the head is lying above endoderm which would, in the ordinary course of events, not form a foregut.

Besides the factors of age and of the degree of healing of the wound which have been mentioned previously, there may be a third factor which influences the amount of curvature. From some of the experiments it is suggested that the influence of the endoderm is most marked if the anterior end of the primitive streak lies immediately over the middle point of the endoderm. Such was the case in specimens 543 and 561. In specimens 540 and 437 two types of excentricity of the endoderm are well exemplified, in which the anterior end of the primitive streak did not lie over the middle of the endoderm, and in both cases the resulting embryos are straight and in the ectodermal direction.

Similar excentricity certainly existed to various, though slighter, degrees in other specimens, since it is difficult to adjust the two layers once the ectoderm has been placed on the top of the endoderm, as the upper layer is then the greater in area and prevents easy manipulation of the lower. The two cases of excentricity mentioned were accidents, as efforts were always made to arrange the endoderm concentrically with the *area pellucida*.

In those specimens in which bending of the axis occurred, the bending of the posterior part so as to point away from the anterior end of the endoderm is almost as constant a feature as the bending of the anterior part of the axis so as to point towards it. This might perhaps be due to some mechanical properties of the embryo, such as elasticity, and thus be merely a secondary phenomenon associated with the primary bending of the anterior part. It is possible, however, that it is due to an attractive influence of the anterior end of the endoderm causing the late backward growth of the primitive streak to be deflected towards this part of the endoderm, just as it had directed the forward growth of the anterior part at an earlier stage. If this hypothesis is adopted, it would

be true to say that the anterior end of the endoderm causes a growing primitive streak to bend towards it. An alternative view would be that the anterior part of the endoderm attracts the anterior end of the primitive streak, whilst the posterior part is indifferent to it, and that, as suggested above, mechanical factors are involved. The first hypothesis, however, that both ends of the primitive streak are attracted by the anterior part of the endoderm seems to fit the facts best. The question should be investigated by a series of experiments in which arrangements such as that in specimen 540 are employed, where the main attraction of the endoderm is concentrated on the posterior part of the primitive streak instead of the anterior part.

In several specimens which show a marked bend (*e.g.*, 565, 543, 537), the most anterior end of the neural tube bends backward again, away from the anterior part of the endoderm, towards which the embryonic axis as a whole has been directed. This slight bending of the head seems to be an expression of a regulative tendency towards the straightening out of the bent axis. Thus, on several occasions it has been observed that although this anterior secondary flexion may be quite pronounced at first, it is then always confined to the very furthest anterior end of the neural tube, while later it tends to progress backwards and to become less noticeable. This process causes the whole embryonic axis to move back again nearer to the original ectodermal line. The reason for this behaviour is obscure: it may be connected with the control which the ectoderm eventually obtains over the endoderm, as is shown by the formation of the foregut.

It is clearly very important that these experiments should be repeated and carried further by the use of vitally stained marks. The technique for this is known (see WETZEL, 1925) and I hope to perform the experiments in the near future. At present, although it is clear that the endoderm has an important influence on the differentiation of the ectoderm, it is impossible to decide whether this influence affects only the form-building movements, or whether it affects also the actual qualitative developmental fate of the tissues. That is, it cannot be stated definitely whether, in embryos with a bent neural tube, the neural material is really derived from presumptive medullary material, or whether non-presumptive material has been caused to take part in formation of the tube. Since the whole bent neural plate is continuous and since at the time of operation part of the primitive streak had already been normally formed, it is likely that the former alternative is the correct one, but even so, it would perhaps be possible for the endoderm to induce the formation of a neural plate from non-presumptive neural material, if the operation could be performed at an early enough stage.

The process of formation of the endoderm is usually completed by the time the egg is laid, and is certainly completed by the time these experiments were carried out. Since the completed endoderm has an effect on the form-building movements in the epiblast, it is perhaps a likely assumption that the process by which the endoderm is formed actually initiates these form-building movements.

Part III.—The Formative Influence of the Mesoderm.

The experiments described in the following Part were concerned with the organising influence of the primitive streak. In the first series the inductive capacity of the entire structure was investigated, in the second series that of isolated pieces.

Section 1a.—*Cultivation of two Apposed Epiblasts.*

In this series of experiments two blastoderms were taken into a quantity of saline solution on the surface of the clot, cleared of endoderm, and then arranged one on top of the other so that their ventral or mesoderm faces were together; that is, the lower lay with its ectodermal face against the plasma, while the upper lay above with the ectodermal surface uppermost. They were so arranged that the primitive streaks were not in contact along their whole length. The operation is attended with considerable difficulty, since as soon as the endoderm is removed from the blastoderm, the remainder of the blastoderm tends to curl up into a ball; and if this is allowed to occur it is very difficult to arrange two blastoderms on top of each other so that the preparation is flat and the two pieces of tissue are evenly in contact with one another. Attempts were made to hold one blastoderm firmly stretched on the surface of the clot by means of glass pins, the clot for this purpose being deepened by a layer of tough agar jelly below the normal plasma and extract medium; but this method was not found to be very useful. It is a considerable help to leave the first operated blastoderm still attached to the vitelline membrane while the endoderm is being removed from the second. It is also important that as much yolk and yolk sac endoderm as possible be removed, as, if these are left, they may interfere with the desired contact between the two epiblasts; in many cases the yolk sac endoderm grows to such an extent that it entirely separates the two epiblasts, which then have no effect on each other.

On cultivation, both epiblasts of such preparations usually give rise to neural folds. The course of these folds is frequently somewhat irregular, as was seen to be the case in single isolated epiblasts. Unfortunately, the preparations are usually very opaque, due to the presence of a certain amount of yolk, and it is almost impossible to examine the whole specimens satisfactorily; one can only note the general direction in which the neural tubes run and then have recourse to sections. In specimens which have been incubated for more than about one day, the development of blood and of masses of mesoderm makes the interpretation of the whole specimens particularly obscure, and frequently the sections are also so complicated that their interpretation is difficult.

Sections of the cultures show that opposite a normal set of neural folds there is frequently a set of neural folds induced in the other epiblast. These induced folds are most often seen opposite the neural folds of the lower epiblast, and only occasionally and rather doubtfully opposite those of the upper. The reason for this difference appears to be that the lower epiblast rapidly dies and degenerates in these cultures; the cause of the degeneration is not known, but it may be the relative absence of oxygen, or the accumulation of toxic substances, both of which factors would affect the lower rather than the upper epiblast.

Description of specimens.

495. Both blastoderms were 21 hours old when explanted. The surface view of the entire preparation, after 20 hours' cultivation, showed the presence of two medullary plates, making an angle of about 40°. The sections reveal that one of these plates is double, consisting of the normal plate of the lower epiblast and an induced plate in the upper.

The section plane is approximately transverse to both embryos. In going through the series of sections, the double embryo appears first, the inducer below being in the form of a tube, above which is a large tubular mass of induced neural tissue derived from the upper ectoderm ; these two masses of tissue appear within three sections of each other, fig. 44, Plate 25. Ten sections later a notochord can be seen as a plate stretching between the two neural grooves, which are still some distance apart. A few sections later there is a fold in the lower epiblast and the inducing and induced fold come to lie nearer each other. The normal neural plate of the upper epiblast does not become apparent until some way further down the series ; by this time the mesoderm between the induced and inducing neural plates has become arranged symmetrically into a series of somites, fig. 45, Plate 25. The mesoderm beneath the normal upper neural plate never forms somites and no notochord ever appears here ; this neural plate remains very undeveloped and short, and it is not possible to be absolutely certain which is its anterior and which is its posterior end, but appearances suggest that its polarity is opposed to that of the induced-inducing pair, so that it is the posterior end which appears in fig. 44. It disappears suddenly, leaving only a thin layer of ectoderm. The middle region of the normal plate is shown in fig. 46, Plate 25.

About 15 sections further posterior than fig. 46 the inducing set of folds becomes contorted and the mesoderm is no longer arranged into somites. The induced plate continues some distance further, accompanied by folded and thickened ectoderm of the lower blastoderm, which may be neural in nature ; it becomes wider and thinner and eventually disappears.

500. The two blastoderms were aged $18\frac{1}{4}$ hours at operation and were noted as being considerably cut about, during operation, in an attempt to flatten them. In the cleared specimen, fixed after 22 hours' cultivation, two sets of neural folds could be seen on the upper surface, both terminating in heads. The two sets were approximately parallel and were polarised in the same direction, but the structures in the posterior parts were obscure. The section plane was approximately transverse to both sets of folds.

The first structure to appear is the highly contorted head of the normal upper set of folds. The transverse section of the neural tissue is in the shape of a W. Later the two lower angles separate off from the upper part and form a tube with a U-shaped fold above. This tube is already beginning to disappear by the level of fig. 47, Plate 25, when the induced head can be seen as a tube overlying a deep V-shaped piece of the inducing lower folds. The induced tube soon opens out on to the surface and we get to the condition seen in fig. 48, Plate 25. The normal upper folds continue to be small, and are asymmetrical. Further posteriorly these normal upper folds have increased in size again and form a large V. Still later the ventral part of the V pinches off again and rounds up to a tube and disappears. In the most posterior part of the embryos all three neural plates are flat and thin.

479. The blastoderms were 17 hours old at the time of operation and were cultivated for $49\frac{1}{3}$ hours. The living specimen was extremely obscure, but a heart could be seen beating. In the cleared and stained specimen it was possible to see that there were two neural tubes in the upper surface, lying with their heads together and making an angle of about 120°, fig. 17. It was not possible to be certain whether the two neural tubes were continuous. The section plane is approximately transverse to one neural tube. Details of the arrangement are exceedingly complicated and only a general description is necessary. The sections first show the posterior part of the normal upper folds, accompanied by a notochord and somites ; below is a thin layer, closely adhering to the somites, which is presumably the lower ectoderm.

After some time this lower layer disappears, and, still later, some more tissue appears in contact with

the lower surface of the upper neural tube. Again, this material is presumably ectoderm of the lower blastoderm; and soon it is seen to form a well-shaped neural groove, fig. 49, Plate 26, which, however, only extends for some 20 sections, before becoming converted again into a piece of fairly typical ectoderm;

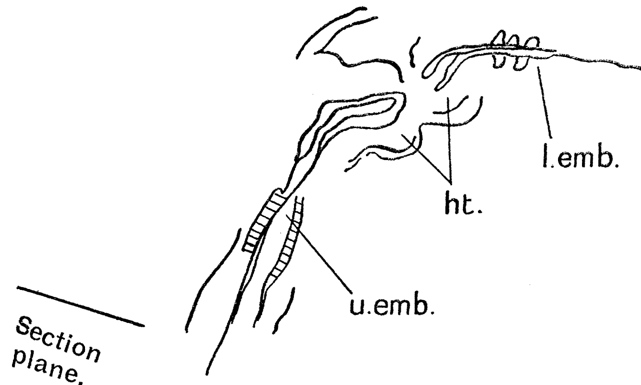


FIG. 17.—479 (17–49½) Two epiblasts. Camera lucida sketch of entire specimen (somewhat diagrammatic). *u.emb.*, embryo of upper epiblast. *l.emb.*, lower embryo and induced embryo. *ht.*, heart.

at intervals this piece of ectoderm becomes thickened and appears as if it were a young neural plate. The poor development of this plate is perhaps due to the extensive degeneration which has gone on in this region. Rather further along the series of sections, the right heart rudiment appears first as a mesodermal sac lined by the endocardium. Its place of origin is obscure. At about this level the normal upper neural tube becomes broken by the disappearance of part of the floor, and a little later it almost entirely vanishes. The upper induced neural tube, which soon appears, is, however, probably just continuous with the anterior end of the normal upper tube. The anterior part of the normal lower tube is extensively degenerated but quite recognisable. The middle part is well seen in such a section as that shown in fig. 50, Plate 26, and it continues to be strongly marked till it vanishes posteriorly. The upper induced set of folds, which are well developed anteriorly, become posteriorly very thin and flat and the meagreness of their appearance is increased by the fact that they are obliquely cut, fig. 51, Plate 26.

This specimen, then, shows parts of four sets of neural folds, namely, the normal upper folds which appear first and induce a somewhat spasmodic appearance of an induced set of folds in the lower ectoderm and the normal lower folds which have induced a fourth set in the upper ectoderm.

At about the same level as the induced upper embryo, a sac of mesoderm appears on the *left*; no beating structure was observed in the living specimen on this side, but this mesodermal sac is very probably the left heart rudiment.

350. The blastoderms were 18⅔ hours old at operation. The culture was arranged so that the posterior parts of the primitive streaks crossed one another, while the anterior part diverged at an angle of about 60°. In the sections at least three neural plates can be distinguished, but the normal upper plate and the plate induced in the upper epiblast are very near together, partly because of the position of the primitive streaks, and partly because the specimen sank, during cultivation, into a cut in the plasma clot. The specimen, therefore, when taken by itself is not very convincing as a proof of induction. It is described here because of the arrangement of the somites between the two upper neural plates.

The section, fig. 18, shows three neural plates, with two somites. The lower epiblast is very necrotic, and has largely fallen to pieces. However, a small piece of neural plate derived from the lower epiblast can be seen. It lies at the left side of the induced plate, and extends through 35 sections. In the region of the section, this piece of neural plate is not continuous with the ectoderm of the lower epiblast, but is separated from it by a space filled with necrotic cells.

There is a somite lying between the inducing and the induced plates, and it is impossible to be certain with which plate it is associated. But, a few sections away, fig. 19, four somites appear. There are three neural plates with which these somites might be associated, namely, the normal lower (inducing) plate, the normal upper and the induced upper plates; actually the inducing plate is no longer visible, having at

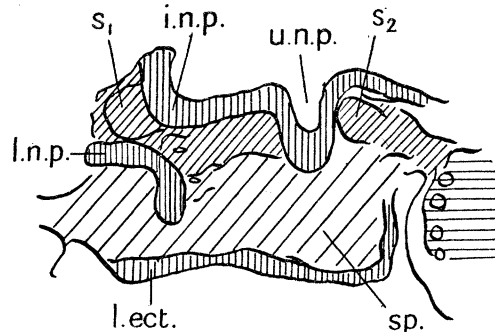


FIG. 18.—350 (18–26) Two epiblasts. Diagrammatic section. *l.n.p.*, neural plate of lower epiblast. *i.n.p.*, induced neural plate in upper epiblast. *u.n.p.*, normal neural plate of upper epiblast. s_1, s_2, somites. *sp.*, space filled with necrotic cells. *l.ect.*, lower ectoderm.

this point shared in the general disintegration of the lower epiblast, but there can be little doubt that it originally existed in a position similar to that shown in fig. 18. Of the somites, one (s_2) is associated with the normal upper plate, another (s_3) probably with the same plate perhaps partly with the induced plate, another (s_1) with the induced plate or the inducing plate, and finally one (s_4) is almost certainly associated

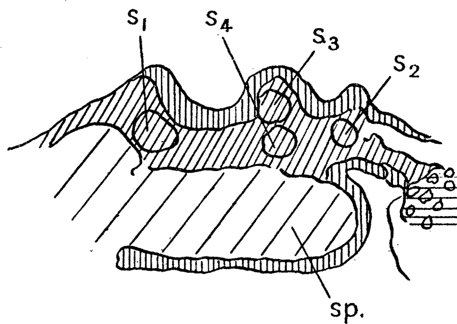


FIG. 19.—Same specimen. s_1, s_2, s_3, s_4, somites (see text).

with the induced plate. If this somite is really associated with the induced plate it becomes very probable that invagination of mesoderm has taken place in connection with the formation of the plate. That is to say, the process of induction of a medullary plate does not merely involve the conversion of presumptive epidermis into neural tissue, but the setting in train of the whole series of processes which normally take place in the development of a medullary plate.

349. The blastoderms were 18 hours old at operation. They were arranged so that the primitive streak of the upper epiblast, which was the less developed, pointed at right angles to that of the lower epiblast. After 28 hours' cultivation, only one embryo could be seen on the upper surface. This embryo pointed in the direction of the axis of the primitive streak of the *lower* epiblast; sections show that for most of its length it overlies the normal lower neural plate, and it is thus presumably induced by it. No embryo developed in the direction of the normal upper primitive streak.

The head end of the normal lower plate appears in 30 sections before the induced upper plate is seen. Somewhat posteriorly to this point, both plates are well developed and there is a single notochord between them, the mesoderm being arranged symmetrically in a common set of somites, fig. 52, Plate 26. Still further posteriorly, the lower normal plate flattens out and can be seen only as a thickened piece of ectoderm, while the notochord persists and somites are found symmetrically near the induced upper plate. The induced plate can be traced for some distance further. Unfortunately, the specimen was broken in the embedding and the posterior part of the induced plate is not connected to the anterior part. It is clear, however, that the posterior end of the induced plate continues somewhat beyond that of the inducing lower plate. Its most posterior part overlies a thick layer of mesoderm.

SUMMARY OF SUCCESSFUL CULTURES WITH TWO EPIBLASTS.

No.	Condition of operation.	Result.
349	Lower blastoderm more developed than upper, primitive streaks at right angles	Only one induced set of folds in upper ectoderm, running in direction of lower primitive streak and overlying lower neural plate.
351	Directions unknown	Only one set of neural folds on upper ectoderm, overlying for most of their length a neural plate in the lower ectoderm.
350	Primitive streaks diverged at about 60°	Two neural plates on upper ectoderm near together, one overlying the neural plate of lower ectoderm.
500	Primitive streaks parallel, pointing in the same direction	Two neural plates in the upper ectoderm, quite separate, one overlying the lower neural plate.
496	Directions unknown	The upper neural plate well developed, the lower not certainly identifiable. Formation of a foregut-like structure from lower ectoderm. Possibly induction of neural tissue in lower ectoderm by upper neural plate.
479	Primitive streaks nearly parallel, pointing in opposite directions	Two neural plates in upper ectoderm, just confluent in head region. Induction from lower to upper and also in places from upper to lower. Beating heart.
495	Directions unknown	Two neural plates in upper ectoderm, entirely separate, one overlying the neural plate of lower ectoderm.
329	Primitive streaks crossing, diverging at an angle of about 45°	Two well-formed heads in the upper ectoderm, the neural plates joining further posteriorly. Lower ectoderm much degenerated and no neural plate visible in it.

Interpretation of Results.—The experiments described in this section provide definite evidence that a developing primitive streak can induce the formation of a medullary plate in an epiblast with which it is in contact. Such cases as 495, and 500, where the two medullary plates in the upper ectoderm are entirely separate, leave no doubt on this point. Even cases like 329, where the medullary plates become united for some part of their length, are very much more convincing proof of induction in this material than they would be in Amphibian material. In the latter, the form-building movements are proceeding along the length of the medullary plate and branching of the plate may be accidental, while in the chick the movements at the relevant stage, that is, the invagination of mesoderm, are proceeding perpendicularly to the length of the plate, as WETZEL and GRÄPER have shown.

The possibility arises that the induced medullary plate may be formed from presumptive neural material which has been prevented from moving to its normal place; that is, the induction may have affected the form-building movements only, and not the qualitative differentiation of the cells. This hypothesis is, I think, untenable. In specimen 500 the induced plate occupies a position which would be well out at the edge of the *area pellucida,* and unless the normal movements were not only hindered but actually reversed, the presumptive neural tissue could not have reached this situation. In case 479, the induced folds of the upper ectoderm stretch out anterior to the head of the normal embryo and probably pass into ectoderm which originally overlay the yolk sac endoderm; it is very difficult to see how presumptive neural tissue could have reached this anterior position. Moreover, it is known from other experiments, in which the primitive streak, after injury, forms a complete medullary plate, that non-presumptive neural ectoderm can, in fact, be caused to form nervous tissue at this stage (see later).

It will be remembered that, according to WETZEL and GRÄPER, there are two main form-building movements going on in the middle and later primitive streak stages, that is, subsequent to the stage at which these experiments were made. First, there is a movement of material from the sides towards the primitive streak, where the material becomes invaginated to form mesoderm. Secondly, at a later stage, there is a movement along the primitive streak, from the anterior towards the posterior, by which the material of the notochord and somites is drawn away from its original concentration in the anterior part of the primitive streak, and comes to lie in its final position. There is no particular reason for supposing that these movements would be present in the inducing epiblast but not in the induced, therefore it is to be expected that the induced neural plate would be co-extensive with the inducer. In other words, conditions here are not parallel to those found in the Urodeles: in that group, the neural plate stretches away in front of the region of invagination and an implanted dorsal lip of the blastopore may therefore induce a plate not all of which overlies graft tissues. In the chick, since the neural plate is co-extensive, or nearly so, with the region of invagination, it is to be expected that the whole of the induced plate will overlie implanted material. This is, in fact, usually the case. In some cases, however, the induced plate extends rather further posterior than the inducing plate; this is probably due to the development of the posterior part of the inducing plate being obscured or prevented by the unfavourable conditions under which it is growing.

The question of the possible orientation of induced plates is of interest; the orientation is, of course, determined by that of the inducing plate, but it is interesting to inquire whether a medullary plate can be induced at any angle to the normal neural plate of the same epiblast. It appears to be true that they *can* be induced in any orientation. Thus in 500 they are nearly parallel, in 459 they make an angle of about 40° (or perhaps 130°), in 479 they are at about 150°. The most interesting case is 349. Here the primitive streaks are at right angles, but only one medullary plate is visible in the

upper epiblast, and that is clearly the induced plate. Now, in other similar experiments in which heteroplastic cultures with duck and chick material have been used, which will be described in the future, a case has been observed in which the induced plate is at right angles to the original plate in the upper epiblast; but in that case the two primitive streaks did not actually cross one another, since the lower lay under the anterior part of the upper *area pellucida*. It is possible, therefore, for two plates to co-exist at right angles if they do not cross, and 329 demonstrates that two plates may cross if they are not at right angles. Perhaps, though, it is impossible to obtain two plates which both cross and are at right angles to one another. On the other hand, in 349, the lower epiblast was considerably more developed at the time of explanting than the upper, and this factor may have played some part in the suppression of the upper normal medullary plate. Further work on the subject is needed.

Section 1b.—*Cultivation of Epiblast and entire Blastoderm.*

Attempts have been made to test the capacity for induction of the upper surface of the primitive streak by growing an epiblast, free of endoderm, on the upper surface of a normal blastoderm, but considerable technical difficulties were encountered. Ten cultures have been grown. In nine of these, there was an entire failure of the two components to attain organic contact with one another. The upper epiblast remained entirely free on the ectodermal surface of the lower blastoderm. In the remaining case, the two components grew together in one place, near the head of the lower embryo, and in this place the ectoderm of the upper epiblast is thickened. It is probable that induction has taken place in this specimen, but the normal embryo of the upper epiblast cannot be found in the sections so that there is room for doubt on this point.

Attempts are being made to overcome the technical difficulties of experiments of this type.

2. *Grafting Experiments.*

The grafting of fragments is probably more difficult in the chick embryo than in Urodeles. If a hole is made in the blastoderm and a fragment of tissue placed in it, the graft very rarely unites with the host. Usually the edges of the wound in the host and the edges of the fragment curl up and thicken, without union of the two pieces. The technique which has been adopted is to push the grafted fragment in between the endoderm and the epiblast of the host. This operation can be performed through a hole either in the epiblast or in the endoderm. In the first case, the thickened edges of the wound may appear very like the beginnings of a neural plate in sections, and the method is therefore not advisable. Most of the grafts to be described have been performed through the endoderm. A hole is made near the edge of the *area pellucida* and the graft inserted and pushed so that it lies between the uninjured endoderm and the uninjured epiblast. The wound in the endoderm heals fairly easily.

No adequate technique of vitally staining the grafts has as yet been found. The cells contain few fat globules, and although the tissues can be stained with Nile blue

sulphate (or neutral red) so that the colour is recognisable for some time in the entire specimen, the dyes frequently appear to be fatal and they do not stain intensely enough to be visible in sections, which is necessary if they are to be of much use. Attempts to use particulate suspensions, such as carmine, have not as yet been successful. Moreover, there are not sufficient differences between duck and chick tissues to enable one to be recognised as a graft in the other. In sections, therefore, the evidence as to which piece of tissue is derived from the graft and which from the host is obtainable only from the morphological relationships. In practice, the graft usually appears fairly distinct from the host since it tends not to heal with the host tissue, but to form a separate mass of its own. If the graft differentiates into mesoderm, one usually finds a large mass of which the major part probably has come from the graft; and if the mass is in a position normally fairly free from mesoderm, such as the head region, one can be fairly certain that this is the case. If the graft gives rise to neural tissue, this is usually quite separate from any neural tissue which may be induced in the ectoderm. But difficulties in interpretation do sometimes arise. Thus cases are found where there is a large mass of neural tissue which is continuous both with the host's neural tube and with the host's ectoderm, but which is not in conformity with the former. In such cases some of this tissue is presumably derived from the graft, but it is impossible to say how much.

When a mass of mesoderm is found lying beneath a neural plate apparently induced in the ectoderm of the host, the possibility arises that the neural plate is really derived from the graft tissue and is only secondarily joined to the host's ectoderm. This seems improbable, especially in grafts made through the endoderm, for the following reasons. (*a*) The host's ectoderm above such grafts was not injured in the operation and the general difficulty of persuading two such pieces of embryonic chick tissue to heal together makes it extremely unlikely that such neat healing has happened. (*b*) Very many cases are found in which the graft has given rise to neural tissue, and this is not united to the surface ectoderm. (*c*) Several cases occur in which the union of graft neural tissue to the ectoderm has apparently taken place, but in these the combined graft and induced neural plate is badly shaped and shows signs of its composite origin. (*d*) Finally, most of the grafts were made with pieces placed dorso-ventrally (in HARRISON's sense), so that the neural tissue, if any, would lie below the mesoderm, not above. Cases are known in which there is, at the bottom of the graft mass, a piece of neural tissue with mesoderm above it, while above the mesoderm is a neural plate continuous with the host ectoderm, and in such cases it is difficult to suppose that the apparently induced plate is really derived from the graft.

Description of specimens.

572. The graft consisted of the anterior third of the primitive streak, from a chick embryo of $21\frac{2}{3}$ hours; it was planted without endoderm, dorso-ventrally, between the layers at the right anterior region of another embryo of the same age. After 19 hours, a structure occupying the position of the graft could be seen at the margin of the *area opaca*.

Sections show, at the most anterior end of the graft structure (anterior as regards the host embryo, but perhaps posterior as regards the induced plate), a neural plate joined to the host ectoderm and overlying somite-tissue derived from the graft, amongst which is a small piece of material which is probably neural tissue, fig. 53, Plate 26. The neural tissue, if such it really is, very soon disappears, and for a considerable time the sections show only somitic material underlying the induced neural plate. After some time the mesoderm underlying the induced plate becomes very thin, fig. 54, Plate 26, and later the endoderm is torn away from the right edge of the induced plate. Unfortunately, the endoderm is lacking from beneath the whole induced plate by the time the host neural folds enter, fig. 55, Plate 26. Since the endoderm is lacking in this region, it is possible that there was graft neural tissue below the induced plate, and that this has also been lost with the endoderm. Perhaps this is even probable, since the anterior third of a primitive streak would be expected to give more neural tissue than the very small piece found in the anterior of the graft mass.

398. The graft consisted of the primitive pit region of a $22\frac{3}{4}$-hour chick blastoderm, which was planted with endoderm, dorso-ventrally, and probably anterio-anteriorly, between the layers on the right of its own blastoderm. The wound from which the primitive pit was removed did not heal, but on the contrary the hole enlarged, so that the whole embryo is split longitudinally along the centre of the neural folds. The graft mass is hidden away below the mesoderm of the host and does not come sufficiently into contact with the host ectoderm for an opportunity for induction to arise.

The most anterior part of the graft mass consists mainly of notochord and perhaps a little somitic mesoderm. This is the only part of the graft which comes near the host ectoderm, which is in fact slightly thickened, but it is very doubtful if any significance can be attached to this thickening. More posteriorly, fig. 56, Plate 26, the notochord is accompanied by somites and the neural tube appears. The notochord is absent in the most posterior part of the graft, which consists of somitic mesoderm and neural tissue.

596B. The graft consisted of the anterior half of the primitive streak of the donor to 596A (see later, p. 213). It was planted dorso-ventrally, without endoderm, transversely in the left anterior region of a host of the same age. After 19 hours' cultivation the host embryo had formed normally and a structure derived from the graft could be seen to the left, running transversely. Sections show that the left end of this structure consists of a neural plate derived from the host ectoderm and underlain probably by mesoderm only (the endoderm is lacking, having adhered to the plasma when the specimen was removed from the clot). A little to the right, fig. 57, Plate 26, a small neural tube appears presumably derived from the graft; this tube soon opens out and becomes an inverted U shape. The combination of graft plate and induced plate can be traced for some considerable length.

Finally, new masses of neural tissue appear and attach themselves to both the induced and the graft plates, which thus become very irregularly shaped. Shortly after this both disappear, the induced plate rather the later, and an area of ordinary *area pellucida* follows before the host embryo is reached.

575. The graft consisted of the middle third of the primitive streak of a *duck* blastoderm with a long primitive streak (? 40 hours' incubation). It was planted together with endoderm, dorso-ventrally between the layers in the anterior part of another duck embryo of the same age. After 23 hours the specimen was fixed and when cleared and stained showed a structure derived from, or induced by, the graft, lying anterior to the head of the host embryo. Sections through the graft show first a bag of host ectoderm filled with graft mesoderm. The bag soon becomes a mere bulge of the surface of the host ectoderm, and a neural plate appears on the bulge. In one place a small piece of neural tissue can be seen at the base of the thick layer of mesoderm, fig. 58, Plate 26. This piece of neural tissue soon disappears and the induced neural plate continues for a considerable distance, overlying mesoderm only, fig. 59, Plate 26. It finally ends in a knob of thickened ectoderm and is succeeded by a considerable interval of *area pellucida* before the head of the host embryo is reached.

611A. The graft consisted of the middle third of a primitive streak of a 19 hours chick blastoderm, planted between the layers without endoderm, dorso-ventrally in the anterior region of a blastoderm of similar age, which, however, was rather further developed and had an early head process. The culture was grown for 28 hours. In sections the anterior part of the graft mass is seen to consist of a mass of mesoderm nearly enclosed in a bag of host ectoderm which forms a thick and obvious neural plate, fig. 60, Plate 26. The bag gradually sinks down to the general level of the host ectoderm, but the neural plate becomes smaller. Thirty sections later, fig. 61, Plate 26, a piece of neural tissue makes its appearance in the graft mass; by this time the induced plate has nearly disappeared, being represented only by a groove, made of comparatively thin ectoderm. In later sections the graft mass, including a piece of neural tissue, lies free from the surface ectoderm, which is slightly thickened, but cannot be said to form a clear neural plate. The neural tissue derived from the graft is, for some distance, connected with the host endoderm. The final sections of the graft show it again to consist only of mesoderm, which forms a thickened part of the host's side-plate.

555. The graft consisted of the middle third of the primitive streak of the donor to 554 (see later, p. 214), planted without endoderm, probably but not certainly dorso-ventrally, between the layers in the anterior part of another blastoderm of the same age. In this case the graft has given rise to a considerable mass of nervous tissue. Going through the sections from the posterior toward the anterior end, the graft mass is first seen as a separate mass of nervous tissue in the heart region of the host, fig. 62, Plate 27. This nervous tissue soon joins up with the host neural tube, which becomes contorted, and also pushes out towards the right where it underlies the surface ectoderm, without apparently performing any induction on it, fig. 63, Plate 27, and finally, by the disappearance of the edge of the host ectoderm lying above it, comes to lie free on the surface of the host ectoderm. This specimen illustrates the tendency of graft neural tissue and host neural tissue to fuse together and is also an informative example of the manner in which the graft neural tissue may appear on the surface of the host; not by direct fusion with the host ectoderm, but by first becoming joined to host neural plate.

518. The graft was the middle third of a chick primitive streak of about 15 hours, planted without endoderm, dorso-ventrally between the layers in the right side of a blastoderm of 18½ hours. It was stained with Nile blue sulphate and was originally planted near the edge of the *area pellucida*. When the specimen was fixed, after about 24 hours, it could be seen that the green tissue had moved inwards to the right row of somites. In sections the stain is, of course, not visible (Lehmann's technique was not adopted in this case), but a mass of rather necrotic tissue can be seen, of mesodermal nature, supplementing the normal somitic material on the right side. This mesoderm is presumably the graft material. It lies near the right of the neural plate of the host, which is rather larger on that side than on the other. The graft mesoderm runs as a longitudinal strand and at most places along its length shows a tendency to be arranged in a manner suggesting somites, fig. 64, Plate 27. No nervous tissue can be distinguished among the mesoderm.

428. The graft consisted of the middle third of a chick primitive streak, planted on the right, with endoderm, between the layers of its own blastoderm, at the age of 20¼ hours. The hole from which the grafted piece was taken repaired perfectly in the early stages of cultivation. The embryo fixed after 29½ hours had seven pairs of somites and was entirely normal, except that the middle three or four somites on the right were doubled, fig. 65, Plate 27. The extra row of somites are presumably derived from the graft.

596A. The graft consisted of the posterior half of a primitive streak of 20½ hours, planted between the endoderm and epiblast of a blastoderm of the same age, in the left anterior region. The graft was cleared of endoderm and planted dorso-ventrally. The specimen was fixed after 27 hours. The host was normal except that the neural folds diverged in the region of the head. Anterior to the head was a knob of tissue derived from the graft. In sections, fig. 66, Plate 27, this knob is seen to consist of a mass of mesoderm underlying a covering of host ectoderm, which is thickened and grooved and thus gives some indication

of the presence of an early neural plate or a primitive streak, either of which would represent an induction. No neural tissue derived from the graft can be seen.

554. The graft consisted of the posterior third of a 20-hour chick primitive streak, planted dorso-ventrally, without endoderm, between the layers in the anterior part of another blastoderm of the same age. The specimen was fixed after 31 hours. The posterior part of the host embryo is quite normal, but the head is badly formed; the neural folds are not closed, and on the right side the ectoderm has the appearance of neural tissue. It also overlies a much larger quantity of mesoderm than is found on the left side. Further, anteriorly the neural folds of the head of the host become very flat and the neuralisation of the ectoderm on the right side spreads still further, fig. 67, Plate 27. The mesoderm underlying the neuralised ectoderm presumably represents the graft. No nervous tissue can be detected in it.

Formation of Nervous Tissue by Grafted Pieces of Primitive Streak.

Region from which graft comes.	Formed nervous tissue.	Formed no nervous tissue.
Anterior quarter of primitive streak, including primitive pit	398, 459, 481, 468, 462, 426D, 443, 572, 400	442, 280 ?
Anterior third of primitive streak, including primitive pit	173, 510, 591, 595C ...	—
Anterior half of primitive streak, including primitive pit	596B, 597B, 613B ...	—
Middle third of primitive streak	555, 582, 593B, 594B, 611A, 575	570, 518, 428.
Posterior two-thirds of primitive streak	—	444.
Posterior half of primitive streak	463...	596A, 613A.
Posterior third of primitive streak	—	300 ? 554, 610A.

Interpretation of Results.

(A) *Differentiation of Grafted Pieces of Primitive Streak.*—As can be seen from the description of individual specimens, grafted pieces of the primitive streak frequently differentiate in such a way that it is possible to determine, with a fair degree of certainty, which structures originate from the graft and which from the host. It is therefore possible to discuss, in a preliminary manner, the correlation between the position from which the grafted piece was derived, and the type of tissue which it yields after differentiation. The conclusions drawn must, however, at present be accepted with caution, although it is hoped that in the future they will be tested by vital staining techniques. They appear to stand in good agreement with the results to be deduced from the work of WETZEL and GRÄPER.

Probably all the grafts form a certain amount of mesoderm; but it is not always possible with this tissue to make out with certainty the boundary between the graft mass and the host tissues. As regards the neural tissue, the table shows that anterior pieces of the primitive streak frequently form this material, the middle piece sometimes do and sometimes do not, while the posterior pieces hardly ever do so. The records would be entirely self-consistent, were it not for the specimens 280 and 442. Of these 280 is exceedingly difficult to interpret and it is by no means certain that no nervous

tissue is present. In 442 the grafted piece was extensively injured during the operation and the failure to form neural tissue may be due to this fact.

Thus, from the grafts recorded in the table, it might be possible to argue that the anterior end of the primitive streak always formed neural tissue if conditions were favourable, while the posterior end never did. The varying results obtained with middle thirds would then depend solely on whether they extended far enough anteriorly to include any of the presumptive medullary material. There is, however, another factor to be considered. These grafts were prepared primarily for the purpose of testing the inducing capacities of the grafted tissue, and in several cases, when the graft structures could not be seen after a day's cultivation, the culture was discarded and not sectioned. In such cases it is very probable that the graft had formed mesoderm only, since neural tissue is usually easily seen in inspection of the culture. While it is almost certain that an examination of these cultures would not have altered the statistical fact that the anterior end of the primitive streak forms neural tissue more often than the posterior end, it might very well have made impossible the absolute statement that the anterior end *always* forms such tissue. The possibility of the anterior end not developing neural tissue is suggested by specimen 442 ; here there is no doubt as to the fact that no nervous tissue is present, but there is the unusual circumstance that the graft was considerably damaged during the operation. We must admit then, that while grafted pieces of the anterior end of the primitive streak usually self-differentiate, giving some neural tissue, this self-differentiating capacity may on occasion remain in abeyance. When this is so, the graft may yield mesoderm which is fairly harmonious with the body of the host (*e.g.*, 442). We do not yet know, however, whether the host can force a grafted piece to develop more or less harmoniously with itself, when the self-differentiating capacity of the graft has not previously been checked by some such influence as the damage which was done to 442. The enormous range of conditions found in grafts of the middle parts of the primitive streak does, however, strongly suggest some influence of the host on the graft. Some of the grafts show a considerable quantity of neural material, and appear very like grafts of the anterior primitive streak. But in 518 and 428, the graft was near the host body and not only does not show any neural tissue but also shows a distinct tendency for the graft-mesoderm to be arranged in somites, while in 444 the graft has apparently been so harmoniously incorporated in the host as to be no longer recognisable. In such cases it is clear that the host has influenced the final morphological differentiation of the graft, and it is perhaps probable that it has also influenced the histological differentiation, into mesoderm as opposed to neural tissue. On the other hand, such grafts as 575 and 570 are far removed from the host body, but also show very little or no neural tissue. The morphological influence of the host seems to be quite commonly effective with posterior pieces of the primitive streak, which often disappear entirely, and are presumably incorporated into the host's anatomy.

At the early stage at which most of these specimens were fixed, it is not always

possible to identify the notochord with certainty. However, all the cases in which the graft has developed a notochord, or where there is even a probability that a notochord is present, are found to be grafts of the anterior part of the primitive streak, including the primitive pit. Grafts of the middle and posterior regions have never shown anything suggesting such a structure.

(B) *Induction by Grafts of fragments of the Primitive Streak.*—The evidence at present available shows that the anterior and middle thirds of the primitive streak can, during their development as grafts, induce the formation of a neural plate by non-presumptive host ectoderm; but whether this power can be manifested by the posterior third is more doubtful. The great majority of grafts which have succeeded in inducing neural plates are those which have themselves differentiated into nervous tissue (among other things); a clear case in which the grafted and induced neural tissue was nearly equal in extent is 596B, and there are several others not described here. Such cases are commonest in grafts of the anterior part of the streak, which usually contain a considerable quantity of presumptive neural tissue. Other specimens, which are typically derived from the middle part of the streak, show grafts which have only developed a very small mass of neural tissue, considerably smaller than the induced neural plate. A good case is 575. Perhaps 572 is also of this type, although derived from an anterior third, but since part of the graft is broken away, there may have been more neural tissue than appears. Finally, there are grafts, typically derived from the posterior part of the streak, which develop no nervous tissue. The question as to whether these grafts can induce neural plates needs further discussion.

Since there is little reason to expect an inducing influence in the chick to extend further longitudinally than the region in which it originates, the evidence of such grafts as 575 and 611A, where the induced medullary plate lies above mesodermal tissue, strongly suggests that induction by mesoderm is possible in the entire absence of nervous tissue. Of the grafts which develop no nervous tissue, some, like 428, become more or less incorporated into the host and are probably not to be expected to induce, while the others are mostly derived from the posterior third of the streak. As WETZEL has shown, the posterior third of the streak probably takes little part in the formation of the axial structures of the embryo, but becomes part of the side plate mesoderm, and in that case, perhaps, it is reasonable to suppose that grafts derived from this region are unlikely to induce. In point of fact, grafts of this kind, such as 610A from a posterior third, form merely masses of mesodermal or indifferent tissue above which the ectoderm is slightly thickened, but shows no other sign of forming a neural plate. In order to obtain grafts containing no neural tissue, which yet succeed in inducing, the only chance appears to be to graft a piece of streak which does not reach sufficiently anteriorly to include medullary material, but which is sufficiently anterior to include axial mesoderm. The boundaries of the presumptive tissue-areas are not known exactly enough for this to be done at will, but we have, perhaps, such cases in 570, 596A, the first of these being derived from a middle third and the last from a posterior half. In these cases,

the graft tissues consist of a large mass of mesoderm. Above this the host ectoderm is thickened to a greater extent than in cases like 610A, and has also formed a groove, so that the general appearance is as though a primitive streak or very early neural plate had been induced. There are also cases like **554**, in which a posterior third has apparently not developed any neural tissue, but, lying near the host neural plate, has caused the latter to spread beyond its normal limits.

Thus, there is as yet no absolutely satisfactory case in which a graft, which has itself not given any neural tissue, has induced a good medullary plate. But the tendency towards inductions by such grafts, and the fact that induced plates frequently lie above the mesodermal parts of grafts which also include neural material, suggests strongly that the axial mesoderm is capable of induction in the absence of graft neural material.

Attempts have been begun to demonstrate the inducing capacity of mesoderm by making grafts of notochord from older embryos into younger blastoderms. Three grafts have been made from a 50-hour embryo into a 20-hour blastoderm, but, although the notochord lived and probably developed normally, no induction occurred. The number of experiments is, of course, insufficient to prove a negative, and even if further work gives the same result, that negative will apply in the first instance only to a later stage than that of the grafts of primitive streak.

If it is granted that the induction obtained with pieces of primitive streak is, at least in part, due to the mesoderm which is developed, important differences between this induction and induction by the roof of the primitive gut in Amphibia immediately demand notice. In the Amphibian case, all the material which is invaginated through the blastopore appears to be capable of functioning as an organiser, and in fact probably does so in normal development. In the present case, on the other hand, only the last invaginated mesoderm, that is, the most axial parts, can possibly act as an organisation centre in normal development. The invagination goes on approximately perpendicularly to the length of the future neural tube. For the same reason, the induced neural plates in these experiments do not usually extend much beyond the pieces of tissue by which they have been induced. (They do, indeed, extend beyond the pieces of neural tissue derived from the grafts, but this, it is urged, is to be taken as evidence that the induction is partly caused by the mesoderm, beyond which they do *not* extend.)

3. *Repair and Regeneration in the Primitive Streak.*

If pieces of the primitive streak are removed and cultivated separately, as grafts, they are found to differentiate to neural tissue, somitic mesoderm, etc., as described in the last section. It is now necessary to discuss the fate of the injured primitive streak.

There are in practice two possibilities ; either the hole made by the removal of tissue heals and closes, or it enlarges. In the former case, a considerable amount of regeneration takes place, in the latter probably very much less. But even in the latter case, the walls

of the neural tube are usually complete from the tail to the head, and they are frequently accompanied by somites. Since any cross-section of the anterior part of the primitive streak contains some material destined to form the wall of the medullary groove, and, considering the size of the pieces eliminated, probably some somitic mesoderm as well, a certain amount of regeneration must have occurred in these cases also.

Description of specimens.

418. The third quarter (counting from the primitive pit backwards) was removed from a 24-hour blastoderm in which the head process had not yet appeared. In the specimen fixed after 17½ hours the wound is quite healed and the embryo is fairly normal, except perhaps that the sinus rhomboidalis and the region posterior to this, is shorter than normal *in vitro*, fig. 68, Plate 28.

176. The middle third of the primitive streak was removed from a 21½-hour blastoderm. The embryo fixed after 43 hours is nearly normal, fig. 69, Plate 28.

153. The middle third of the primitive streak was removed from a 22½-hour blastoderm. The embryo fixed after 22½ hours is nearly normal, fig. 70, Plate 28.

145. The primitive pit region was removed from a 21½-hour blastoderm. In the specimen fixed after 20½ hours, the hole is healed over, but the somites are disarranged in the anterior part of the embryo. The notochord is visible, stretching unbroken through the disordered region and reaching well into the posterior part of the specimen, fig. 71, Plate 28.

398. The primitive pit was removed from a 22¾ hour chick and grafted into its own blastoderm. The fate of the graft is described on page 212; it formed neural tissue, notochord and somites. The embryo is split along the middle of the neural groove, the floor of which is lacking. The neural tissue is, however, continuous from the anterior to the posterior end of the embryo; there is always a greater quantity of it on the right than on the left side, fig. 72, Plate 28. No notochord is present. The conditions pertaining to regeneration in the head (pre-primitive pit region) will be discussed in a separate paper.

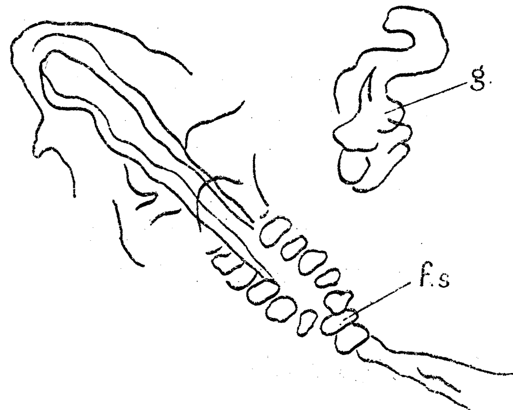


FIG. 20.—173 (20–25½) Autoplastic graft of primitive pit region. Camera lucida sketch of entire specimen. *g.*, graft. *fs.*, fused somites.

173. The primitive pit region was removed from a 20-hour embryo and grafted into the side of the same blastoderm. The graft yielded neural tube, notochord and somite material. In spite of this, the host is quite normal from the head region down to the level of the 6th pair of somites, fig. 20. At this place the notochord swells up into a round lump and is lacking in the more posterior parts. Behind the point of disappearance of the notochord, the somites have fused in the mid-line beneath the neural plate, fig. 73,

Plate 28. An exactly similar phenomenon is found in 403, where the fusion takes place after the 4th pair of somites.

407. The entire primitive streak, including the primitive pit and knot, was removed from a 20-hour blastoderm. The culture was incubated for 28 hours. In the fixed specimen a nearly normal head can be seen (the neural tissue in the left side has been torn during mounting), fig. 74, Plate 28. The embryo has 7–8 somites; there is a deep fold in the blastoderm between the head and the body of the embryo, so that they appear to be separated from one another. The embryo seemd to have been formed by movement of material from the sides towards the position of the primitive streak, this movement being most rapid opposite the anterior part of the removed primitive streak. It is uncertain whether a notochord is present or not.

423. The entire primitive streak, with a fairly wide area on each side of it, was removed from a $17\frac{1}{2}$-hour blastoderm. The culture was incubated for 29 hours, by which time some 5–6 somites were present and the specimen resembled 407 in appearance. Sections show a fairly normal head. The heart region is shown in fig. 75, Plate 28; it will be seen that a notochord is present and a normal neural tube. The two heart rudiments are still separate, and the somite region is quite normal, fig. 76, Plate 28. The neural plate continues some distance further posterior than the somite region, becoming flat and perhaps rather larger than normal. At the most posterior part it is split into two and soon after dies out altogether.

Interpretation of Results.—There are two sets of tissue-movements to be considered in connection with these instances of regeneration; the transverse movement of the earlier stage and the backward longitudinal movement which supervenes later. The closing of the hole made by the elimination is probably due either to general growth of the cut edges or to the transverse movements, since when the primitive pit is removed it is difficult to see how the longitudinal movements could accomplish the closure unless they are temporarily reversed in direction. Since the pieces of tissue removed were usually longer in the longitudinal direction than in the transverse, it is probable that, if the closure is due to general growth from the cut edges, it would be the lateral edges which were responsible for the greater part of the healing; that is, in this case also, the healing would in effect be due to a transverse movement.

One must suppose that the later longitudinal movements go on whether the wound has been closed or not, since in either case the somites are normally arranged and must therefore have been pulled backwards into place. If the primitive pit has been removed, and the wound has not closed, there will be no floor to the neural groove and the embryo will be split longitudinally along the axis for part of its length. This means that the longitudinal movements must also be divided. Cases in which these movements must have gone on, although the embryo is split along the axis, also occur in the cultivation of posterior half blastoderms.* In the specimens in which the primitive pit has been successfully regenerated, it is probable that the wound has been closed over by transverse movements before the longitudinal movements begin. There is some evidence that once the head process has begun to form, and the transverse movements have ceased in the primitive pit region, regeneration of this part of the blastoderm is no longer possible.

* *Cf.* 304P, p. 188.

It will be noticed that the notochord is usually lacking if the floor of the neural groove is lacking and is usually present if that structure is present. There are, however, one or two exceptions. Thus, in 173, the notochord is only present in the anterior part of the embryo, although the floor of the neural groove is present throughout. Perhaps this is due to an insufficiency of notochord material having been regenerated. In 180 (p. 188), there was a notochord present although there was no floor of the neural groove. It is possible that this notochord was regenerated; but even if it was, the fact that there could be a notochord in the absence of the floor of the neural groove probably indicates that the presumptive notochord stretches some little distance further longitudinally than the presumptive floor of the neural tube.

It is interesting to note that when the notochord is lacking from part of the embryo, the somites have, in that part, become fused in the mid-line (173, and another undescribed specimen). A similar phenomenon is known in Amphibia.

4. *Discussion of the Period during which the Mesoderm is formed.*

The phenomena of regeneration make it clear that no discussion of the developmental factors at work in the blastoderm at this stage can be complete if it is confined to a consideration of the primitive streak. The epiblast must be considered as a whole; the endoderm can probably be neglected, although it is possible that that layer may be at least a contributory factor to some of the processes, in particular to the movements, going on in the upper layer.

In the epiblast, then, the following factors would appear to be concerned:—

(1) A superficial movement of material from the sides into the primitive streak, and, at a deeper level, a corresponding movement of mesoderm from the streak towards the sides.

(2) A movement of material backwards along the primitive streak. This becomes of greater importance in the later stages of development.

(3) A capacity for induction possessed by the primitive streak.

(4) A probable, but hypothetical, increasing labile determination of the presumptive tissues and a hypothetical potentiality of these tissues, when determined, for homoiogenetic induction. This labile determination, if it is present, probably depends on the movements mentioned in 1 and 2, but these are more far-reaching, since they can cause the differentiation of neural material when most of the presumptive medullary material has been removed.

As regards the capacity for induction, it is known to be manifested by pieces of primitive streak which develop neural tissue as well as mesoderm (the endoderm being neglected). It is also probable, but not certain, that induction can be shown by pieces which develop only mesoderm. Whether this is so or not, it is still quite possible that in those pieces which do yield neural tissue, this tissue plays some part, by way of

homoiogenetic induction, in the total result. We cannot, then, say with certainty that the organisation process is the process of formation of the axial mesoderm, since this would depend on the proof that pieces yielding only mesoderm can induce. But at the same time, it is very unlikely that all the induction obtained from the primitive streak is homoiogenetic induction, and some, probably the greater part, can be presumed to be due to the process of axial mesoderm formation.

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. As causative agents for the differentiation which is obtained, one is thrown back upon the movements of material. It is, perhaps, easy to imagine that the transverse movements might bring the cut edges together, that a new primitive streak would be formed, and that the transverse movements would then be forced to turn downwards, thus leading to the formation of mesoderm. This mesoderm could then be regarded as controlling the differentiation of neural material, while the movement along the length of the streak would also be enabled to take place. It is, however, rather more difficult to give a plausible scheme which applies to those cases where the floor of the medullary plate is lacking, but the walls are continuous from front to back. It cannot be the case here that the transverse movements close the cut which is later opened again by the failure of the longitudinal movements, to which WETZEL supposes the floor of the medullary plate to be due. One must suppose that, in so far as the movements of material are the causative agents in the regeneration, it is the transverse movements which are effective.

It is probable that the presumptive medullary material has some power of self-differentiation, although this has not yet been tested in any way. If this is so, when a piece of the primitive streak induces non-presumptive ectoderm to form neural tissue, it is doing more in the way of determination than it does when it is in its normal place in a blastoderm. Similarly, when the primitive streak is eliminated and regeneration takes place, it is almost certain that the agents to which this regeneration is due, the movements of material or whatever else they may be, are accomplishing more in the way of organisation than normally falls to their share.

Considerations such as these lead one to regard the normal development of the blastoderm as being the resultant of a set of influences. Speaking in the terms of a material model, one might compare the course of differentiation to a ball impelled by a set of different forces along a plane surface; the course which it actually takes is the resultant of those forces. The procedure of experimental embryology is, usually, to eliminate one or more of these forces. Thus a presumptive organiser is planted into the region where the influence of the material movements is absent or at least different from normal; or the presumptive organising region is removed and the material movements left without its aid. In such circumstances differentiation follows a course which is the resultant of those influences which remain. The organiser induces in spite of the absence of the transverse movements, or the movements are responsible for the

building up of a new embryonic region without the aid of a normal organisation centre. These influences, which have been unaltered by the experiment, are then exhibiting greater potentialities than they are called upon to exert in the normal course of events. There are no grounds for supposing that the unaided primitive streak, in normal development, induces the medullary plate from non-presumptive ectoderm, since under normal conditions it is aided by the material movements, which have been initiated at an earlier stage. Even if the transverse movements of material are originally initiated by the primitive streak, this does not affect the argument given above which applies to the organising capacity of the primitive streak of later stages, from which the inducing grafts were made. Similarly, it is clear that the transverse material movements themselves do not in normal development give rise to the axial region of the embryo, since they are in fact added by the primitive streak, which has been built up by the longitudinal movements of a still earlier stage.

General Discussion.

As was stated in the Introduction, the previous experimental work on the development of the chick has been largely confined to a study of self-differentiation. Olivo (1928) has described the self-differentiation of heart muscle explanted into tissue culture from young blastoderms. The most important previous work in this field is probably that of Hoadley (1926*a*, *b*, 1927), who has published a series of papers on the self-differentiation of various parts of the blastoderm, transplanted at various ages to the chorio-allantoic membrane of older chicks. His results make it clear that the various regions of the blastoderm are, even in very early stages of development, no longer indifferent but possess a capacity for self-differentiation, and this capacity appears to increase with the age of the embryo from which the explant is taken. Hoadley has discussed his data in terms of a process of " differential dichotomy " or " embryonic segregation," by which the capacity of the individual cells for self-differentiation becomes itself differentiated. According to Hoadley, an early embryonic cell should be capable of self-differentiating only into a rather generalised, lowly organised cell-type. As the process of differential dichotomy goes on, however, the descendants of such a cell acquire more specialised potentialities which have been formed from the parental generalised potentiality by a process of segregation of parts. Thus, if a piece of tissue was isolated from an early blastoderm (the isolation is taken to stop the process of segregation), it differentiated only into generalised epidermis and gut: its weak capacity for self-differentiation could carry it only a short distance along a generalised path. If a similar isolation was made at a later stage, an eye was obtained: the stronger capacity of the daughter cells had carried them a longer distance along a more specialised path.

It will be noted that the idea of segregation contains two parts: first, that the process gives rise to parts with new potentialities, as Hoadley concluded, and secondly, that it involves a restriction of potentiality from a general to a particular. Lillie

(1929), who has discussed the concept from a general standpoint, defines it as the " process of origin of parts of the embryo possessing irreversible prospective value as tested by self-differentiation," and characterises it as being a process of segregation. The difficulty here arises that self-differentiation techniques do not in fact test irreversible prospective value. In Triton the ectoderm can be shown to possess the capacity to self-differentiation to epidermis, and probably to neural plate, at a stage before it is restricted to this fate. True irreversible prospective value can only be tested by interchange experiments. But if segregation is limited to being the process of origin of parts possessing irreversible prospective value as tested by interchange experiments, it becomes identical with determination, and it loses the first element mentioned above, namely, that it is responsible for the origin of parts with new potentialities.

Such new potentialities do in fact arise. Consider a single-stage process of differentiation of a piece of tissue; for example, the differentiation of neural plate from blastula ectoderm in Triton. At first the blastula ectoderm is quite indifferent as regards this process, later it is definitely determined either to become neural plate or not; only in the middle period does the differentiation to neural plate exist as an undetermined potentiality. In this middle period it is *competent* to form neural plate, and this competence is a new potentiality which has arisen.

The idea of competence covers two concepts already in use, *reactionsfähig* and *labil determiniert*. Reactionsfähig means that the tissue to which the adjective is applied is competent to differentiate to a certain other tissue but requires a definite stimulus, while labil determiniert means that it is competent so to differentiate without a definite stimulus. It is, however, difficult to determine what is a definite stimulus, as can be seen from the different results obtained by HOLTFRETER (1929, 1931), BAUTZMANN (1929), KUSCHE (1929), and others, in the case of self-differentiation of the ectoderm of the early gastrula of Triton. Since, as this work shows, it appears to be necessary to specify the conditions under which a " self-differentiation " takes place, the idea of self-differentiation must be discarded as an exact concept for theoretical discussion. We retain then the three concepts, non-competent, competent to differentiate to the tissue in question under certain conditions, and determined. This terminology has the advantage that it makes, even by implication, no assumptions as to the specificity of any given set of conditions, such as the roof of the primitive gut, or a salt solution, for the ectoderm of a Triton gastrula, or an implanted ear-vesicle for the limb-mesoderm at a later stage.

Very little is known as to the origin of competence. It is naturally limited by the restriction of potentialities involved in the last process of determination which the tissue has undergone. But when one distinguishes, as we have done, between the origin of potentiality and its restriction by determination, there remains no particular reason for regarding the former process as being a segregation. Certainly it does not seem to involve a dichotomy, since, undoubtedly, quite a large number of competencies can exist side by side, and it is very probable that several such competencies can arise at the same time.

We have as yet discussed competence only as regards a single-stage process of differentiation. HOADLEY's experiments tested competence in our sense, but with reference to a series of processes which was, in different cases, halted at different stages. HOADLEY argues as if the stage attained in this series was a direct measurement of the potentiality with which the processes were started in the first place, so that he could say that in one case the tissue was isolated when it had a definite potentiality to form primitive epidermis, and in another case when it was capable of differentiating to brain. But it is probable that the tissue when isolated was, in fact, non-competent with regard to the later processes of differentiation which it finally performed. In such a series of processes it is probable that the later members are dependent on the harmonious (spatial or otherwise) realisation of the earlier. It is thus possible that HOADLEY's older explants differentiated further than the young, not only because they were competent for further stages of differentiation but also partly because they were more competent for the earlier stages and thus performed them more harmoniously. MANGOLD (1928) has pointed out that exceptional cases of very complete differentiation do occur in isolated pieces of tissue.

It is also unfortunate that HOADLEY isolated the ectoderm and endoderm together, so that it is impossible to say where the competencies are localised; while the added possibilities for the occurrence of induction make the interpretation of a series of processes of differentiation still more difficult.

HOADLEY's work does not in any way deal with "the origin of parts possessing irreversible prospective value": he made no attempt to reverse (*i.e.*, alter) the prospective value, so that the definitive test was lacking. Similarly, WETZEL (1929), and HUNT (1929*a*, *b*, 1931), and WILLIER and RAWLES (1931) and UMANSKI (1931) have made speculations as to the position of the organisation centre in the chick embryo, based on self-differentiation experiments. But the characteristic feature of an organiser-region is not that it can self-differentiate but that it can alter the prospective value of other tissue. Experiments designed with this fact in view have been described in this paper, and it has been shown that alteration of the prospective value can occur.

In the first place there is an influence of the endoderm on the growth of the primitive streak. This is probably effected by an influence exerted on the material movements taking place in the epiblast, and it is suggested that, probably, the endoderm not only affects the movements but also originates them.

In the second place, we have the induction of medullary plate by the primitive streak, probably by the mesoderm which is formed therefrom.

Lastly, the axial epiblastic structures can cause the formation of a foregut from endoderm which would normally not form such a structure.

The relations between these three organising processes present interesting problems for future investigation.

Summary.

I.

(1) Entire chicken and duck blastoderm, removed from the egg during the first two days of incubation, have been cultivated *in vitro* by the watch-glass method. A short description of the technique is given.

(2) The embryos develop nearly normally as regards their anatomy, but the rates both of differentiation and of growth are considerably slower than *in vivo*, the rate of growth being the more affected by the unusual conditions, so that embryos are obtained which are much too small for the stage of differentiation which they have reached.

(3) The work of Wetzel and Gräper on the localisation of the presumptive organ-forming regions is summarised, and some experiments bearing on the same question are described. Blastoderms with a well-formed primitive streak, but no head process, were cut into portions transversely at various places along the primitive streak. The results of cultivating the portions were :—

(*a*) Anterior portions :—

Cut through primitive pit : either no embryonic organs, or a little disordered neural tissue.

Cut just posterior to primitive pit : a head with a "tail," consisting of somites and notochord, which juts out posteriorly behind the cut edge.

Cut further posteriorly : the extent to which the tail juts out behind the cut edge becomes less as the cut is made further posterior.

(*b*) Posterior portions :—

Cut through primitive pit : everything present except the head. Heart rudiments separated.

Cut slightly posterior to primitive pit : no notochord, or floor to neural groove, so that the embryo is split longitudinally and forms a V.

Cut further posteriorly : no embryonic structures.

In one case a notochord is present in the absence of the floor of the neural groove ; this is taken to indicate that the notochord rudiment extends very slightly posterior to the primitive pit.

II.

(1) The isolated endoderm has not been cultivated with any success. The isolated epiblast, cultivated from young or medium primitive streak stages, yields neural groove, notochord, somites, etc. The *area pellucida* is affected by the cyst formation which is always seen in the *area opaca*, and the axial organs may be disturbed by this. These organs, if undisturbed, lie in the direction of the primitive streak but are perhaps shorter than those of normal embryos.

The yolk-sac endoderm may cover the entire floor of the *area pellucida*, but has not

in the present experiments ever formed a foregut. A structure resembling a foregut may be formed by head mesoderm.

(2) The endoderm and epiblast were separated and then brought together again so that their original longitudinal axes made an angle with each other. If the experiment is performed in a young primitive streak stage, the primitive streak, as it lengthens, is deflected towards the anterior end of the endoderm. In later stages the endodermal influence is not effective. The bent embryos later straighten themselves by a bend in the opposite direction, which affects the anterior part first.

The period in which bending of the axis can be caused in this way overlaps the period in which self-differentiation of the epiblast can be obtained.

The foregut is nearly always regularly formed, frequently from non-presumptive endoderm.

III.

(1) Two epiblasts, cleaned of endoderm, were cultivated together, placed so that their mesoderm faces were in contact, and it was found that both develop neural grooves, but the lower soon dies and degenerates. A normal neural groove may induce an extra neural groove in the other epiblast; usually there is induction performed by the lower primitive streak on the upper epiblast.

Invagination of mesoderm probably goes on in the induced groove, but no satisfactory case of an induced notochord has been found in this material.

If the induced and normal grooves in one epiblast do not actually cross, they can lie at any angle to each other: if the primitive streaks crossed, the neural grooves developed from them can lie at a considerable angle, but the evidence suggests that if the primitive streaks lay at right angles to one another, one of the neural grooves is suppressed.

(1*a*) If an isolated epiblast is cultivated on the upper surface of an entire blastoderm, the two components of the preparation do not usually make organic contact with one another, and the experiments have therefore been indecisive up to the present, but there is some indication that induction may be possible in such cultures.

(2*a*) Fragments of the primitive streak, cleared of endoderm, were grafted between the endoderm and epiblast of other blastoderms of similar age, the epiblast not being cut by the operation.

Vital staining which can be recognised in sections is not yet possible, but the anatomical relations are frequently sufficient to make it clear which structures are derived from the graft, and which from the host.

Anterior pieces of the primitive streak, usually or always give rise to neural tissue, notochord and (somitic) mesoderm: middle pieces give rise to mesoderm, with or without neural tissue; posterior pieces probably never give rise to neural tissue.

There is considerable evidence of the host influencing the anatomical differentiation of the graft in some cases.

(2*b*) Grafts of the middle and anterior thirds of the primitive streak have induced neural grooves in the host ectoderm: it is doubtful if the posterior third can do this.

No satisfactory case of induction is known in which the graft has not given rise to some neural tissue, but the induced groove frequently lies above the mesodermal, non-neural parts of the graft, and it is suggested that probably the axial mesoderm is capable of induction without the presence of neural tissue.

(3) Any part of the primitive streak, or even the whole of it, can be regenerated.

The appearance of the embryo depends chiefly on whether the notochord and neural groove floor is regenerated or not; if they are absent the embryo is split down the axis.

Even in the split embryos, the somites are more or less normally arranged, that is, they have been shifted into place by the longitudinal movements in the ordinary way. It is suggested, then, that the regeneration probably depends on the transverse movements of the earlier stage.

(4) It is suggested that development is to be looked upon as the resultant of several forces, the metaphor being applied as strictly as possible. The procedure of experimental embryology is, usually, to eliminate one or more of the forces and then to test the remainder. In these circumstances it is probable that the remaining forces are collectively more causally effective than they are in normal circumstances.

DESCRIPTION OF FIGURES.

Plate 22.

Figs. 21–25. Five stages of the development of a chick blastoderm *in vitro*. Taken from a cinematograph film made by Dr. R. G. Canti. The blastoderm was 18 hours old at explantation.

Fig. 21. After 4 hours.

Fig. 22.—After 14 hours.

Fig. 23.—After 18 hours.

Fig. 24.—After 24 hours.

Fig. 25.—After 32 hours.

Magnification the same for all figures.

Plate 23.

Fig. 26.—Embryo explanted at 9 somite stage, cultivated for 43 hours *in vitro*. (× 10.)

Fig. 27.*—325 (22¼–19½). Anterior portion of blastoderm sectioned just posterior to primitive pit. *t.*, tail. (× 23.)

Fig. 28.—324A (22–26). Anterior portion of blastoderm sectioned through primitive pit. *n.t.*, neural tissue. (× 32.)

Fig. 29.—189 (22¼–33). Blastoderm from which the second quarter of the primitive streak was eliminated. *t.*, tail. *ht.*, heart. (× 27.)

* In the legends to the figures, the protocol number of the specimen is given first (*e.g.*, in this case 325), then the age of the blastoderm at operation in hours of incubation (22¼), and then the number of hours for which the explanted embryo was cultivated before fixation (19½).

FIG. 30.—177A (20¼–24). Section through "tail," from embryo sectioned slightly posterior to primitive pit. *n.p.*, neural plate. *s.*, somites. *nch.*, notochord. (× 175.)

FIG. 31.—324 (22–20). Posterior portion of blastoderm sectioned through primitive pit. (× 23.)

FIG. 32.—135P (20½–17½). Posterior portion of blastoderm sectioned through primitive pit. *l.ht.*, left heart rudiment. *r.ht.*, right heart rudiment. (× 32.)

FIG. 33.—172 (20–44). Section through posterior portion of blastoderm cut through primitive pit. *n.t.*, neural tube. *l.ht.*, left heart rudiment. *fg.*, foregut. (× 80.)

FIG. 34.—304P (22–26). Section through the left side of posterior portion of blastoderm cut just posterior to the primitive pit. *n.g.*, side wall of neural groove. *s.*, somite. (× 175.)

PLATE 24.

FIG. 35.—293 (25–42). Section through the head region of embryo cultivated after removal of the endoderm. *n.f.h.*, neural folds of head. *scph.s.*, sub-cephalic space. (× 110.)

FIG. 36.—Same specimen. Section through central region. *n.p.*, neural plate. *nch.*, notochord. *ht.*, space representing heart ? (× 110.)

FIG. 37.—Same specimen. Section through posterior region. *n.p.*, neural plate. *nch.*, notochord. *end.*, yolksac endoderm. (× 110.)

FIG. 38.—319 (21–26). Section through embryo cultivated after removal of endoderm. *n.p.*, neural plate. *bl.*, blister. (× 60.)

FIG. 39.—227 (20¼–22½). Section through anterior part of embryo cultivated after removal of the endoderm. *n.p.*, neural plate. *mem.*, mesodermal membrane. *end.*, yolksac endoderm. (× 175.)

FIG. 40.—227. Same specimen. Section still further anteriorly. *sp.*, space resembling foregut.

FIG. 41.—402 (24–19). Section through anterior region of embryo cultivated after removal of endoderm. *np.*, neural plate (divided). *sp.*, space enclosed by membrane. (× 175.)

PLATE 25.

FIG. 42.—290 (17¼–43). Ectoderm and endoderm turned. The original ectodermal axis was parallel to the longer side of the page. Straight embryo. *fg.*, edge of foregut. *ht.*, heart. (× 23.)

FIG. 43.—323 (22–45). Ectoderm and endoderm turned, straight embryo. Section through heart region. *n.t.*, neural tube. *fg.*, foregut. *ht.*, heart.

FIG. 44.—495 (21–20). Two epiblasts cultivated together. Section through anterior region. *l.n.g.*, neural tube of lower epiblast (inducer). *i.n.g.*, induced neural tube in upper epiblast. (× 260.)

FIG. 45.—Same specimen. *u.n.g.*, neural plate of upper epiblast. *l.n.g.*, neural plate of lower epiblast. *i.n.g.*, induced neural plate in upper epiblast. *s.*, somites. *nch.*, notochord. (× 90.)

FIG. 46.—Same specimen. Letters as last figure. (× 175.)

FIG. 47.—500 (18¼–22). Two epiblasts. Section through anterior region. *u.n.g.*, neural plate of upper epiblast. *l.n.g.*, neural plate of lower epiblast. *i.n.g.*, induced neural plate in upper epiblast. (× 60.)

FIG. 48.—Same specimen, section further posteriorly. Letters as in last figure. (× 175.)

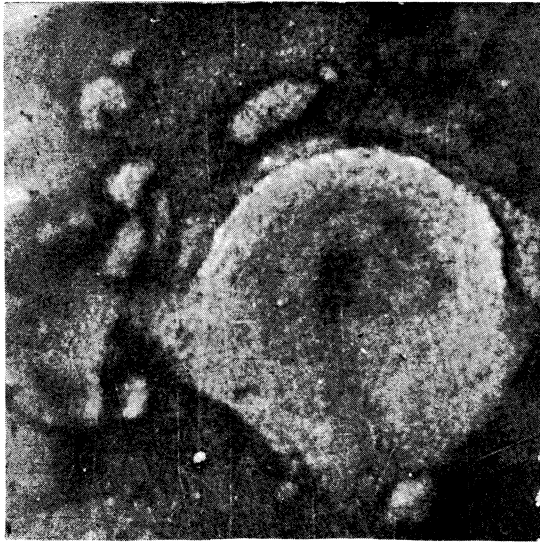
PLATE 26.

FIG. 49.—479 (17–49). Two epiblasts. Section. *u.n.g.*, neural groove of upper epiblast. *i.l.n.g.*, neural groove induced in lower epiblast. (× 110.)

FIG. 50.—Same specimen. *l.n.g.*, neural groove of lower epiblast. *i.u.n.g.*, neural groove induced in upper epiblast. *ht.*, ? heart rudiment. *nch.*, notochord. (× 75.)

FIG. 51.—Same specimen. Letters as last figure. (× 75.)

FIG. 52.—349 (18–28¼). Two epiblasts, section through central region. Letters as in last figure. (× 60.)



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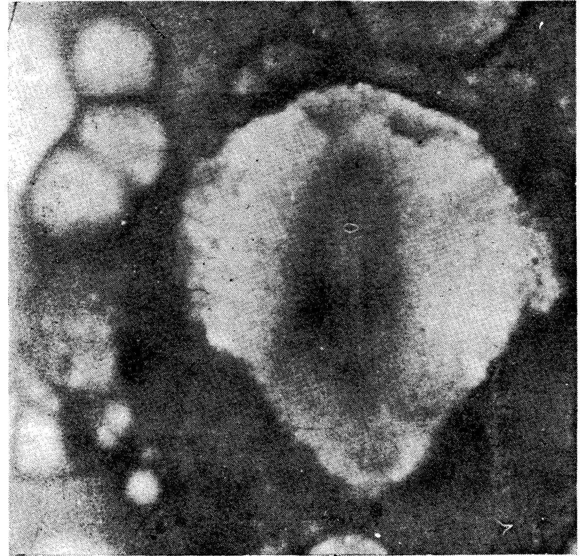

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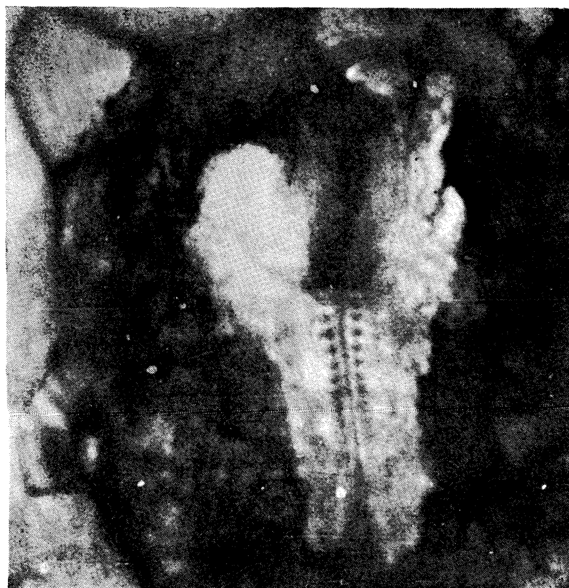

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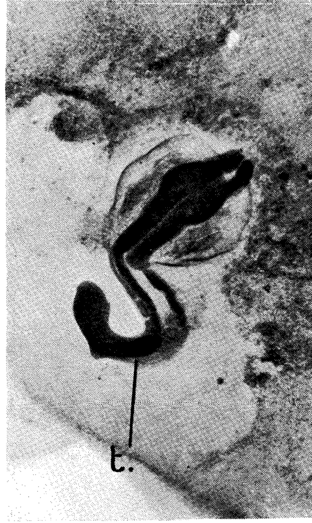

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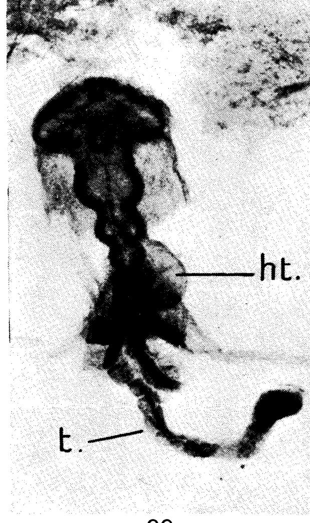

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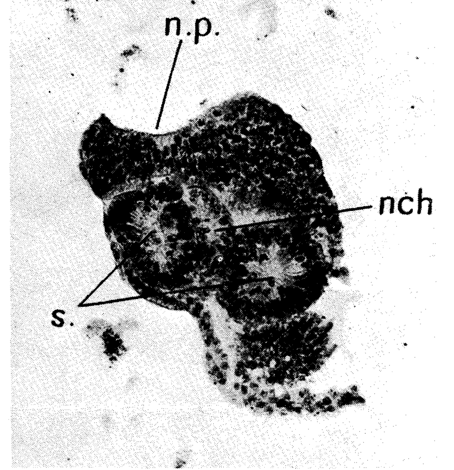

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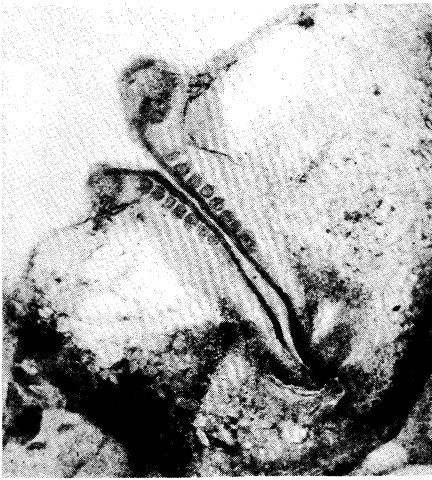
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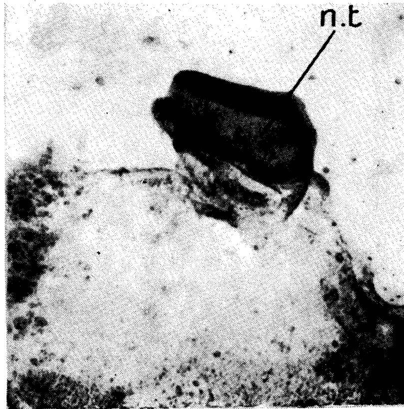

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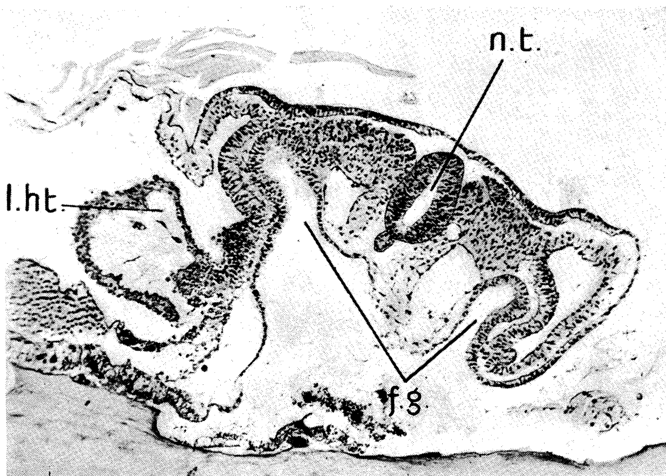

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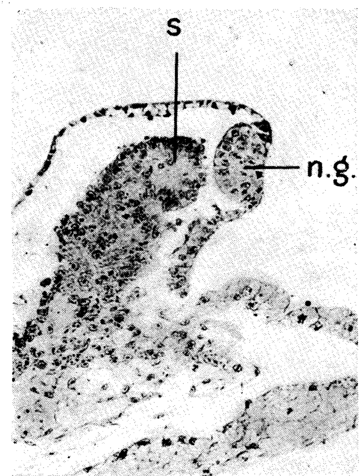

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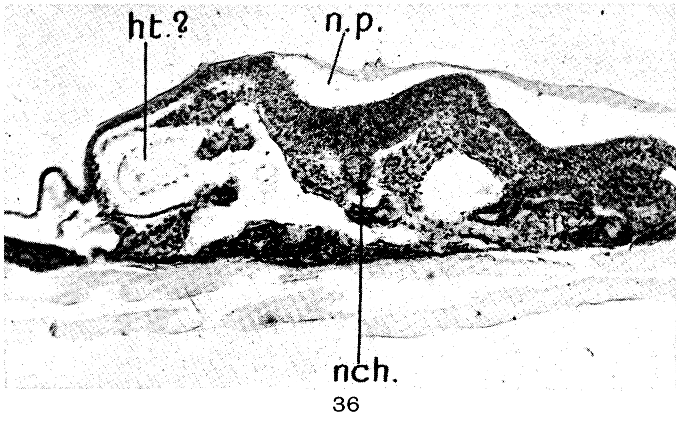

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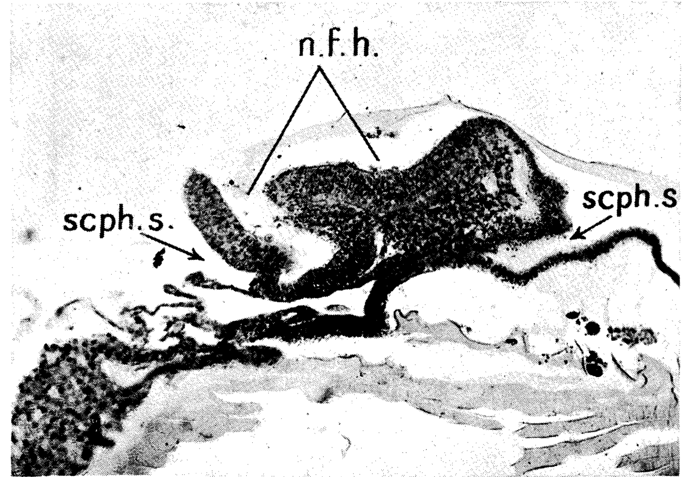

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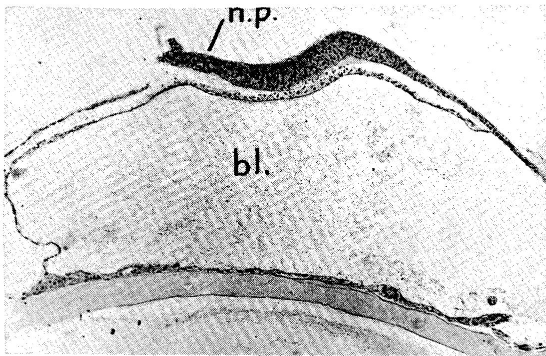

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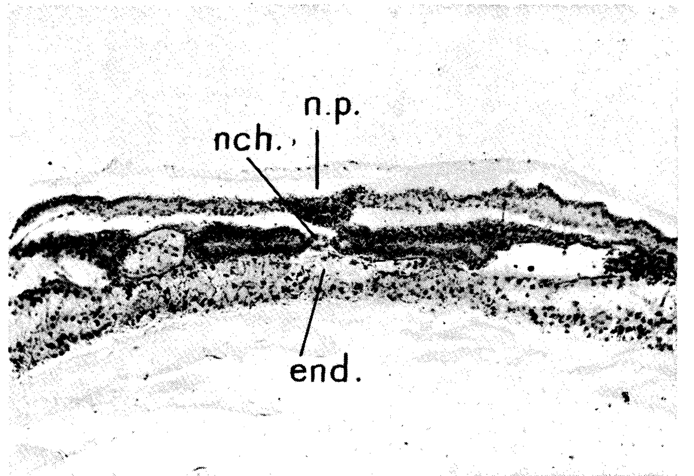

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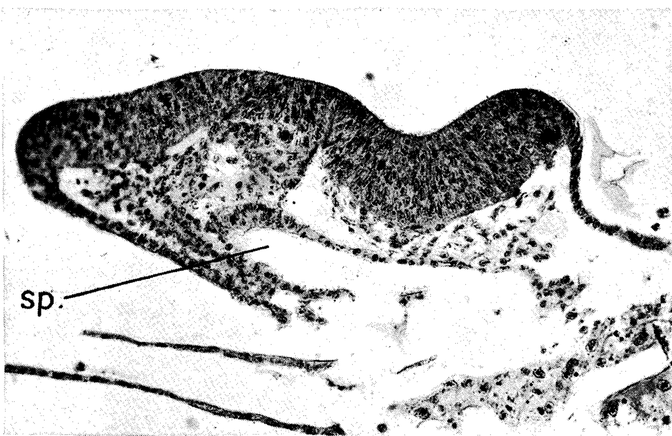

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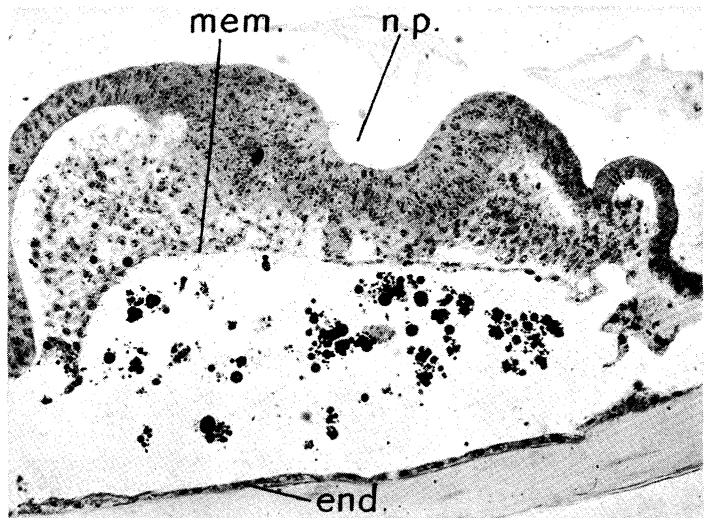

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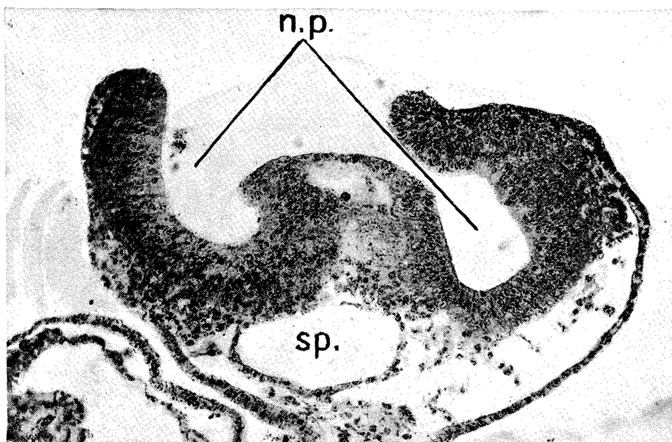

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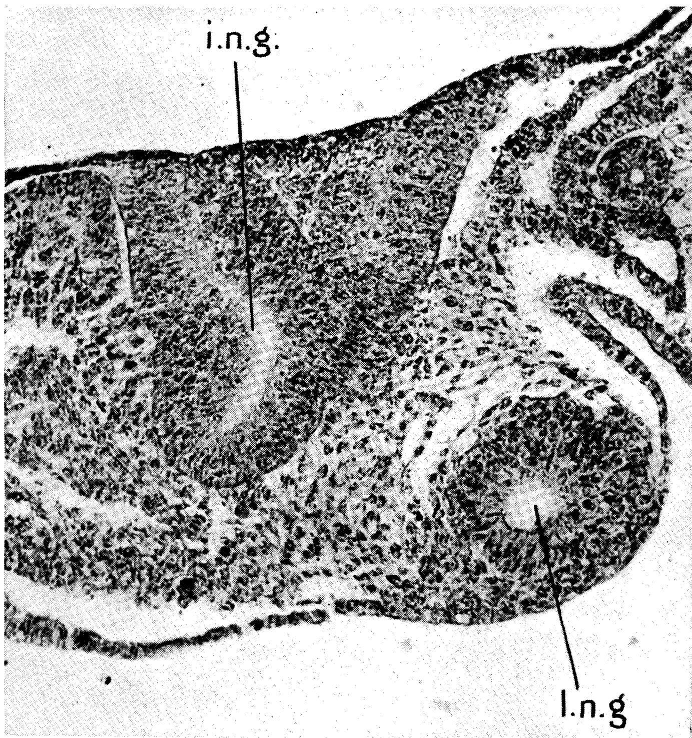

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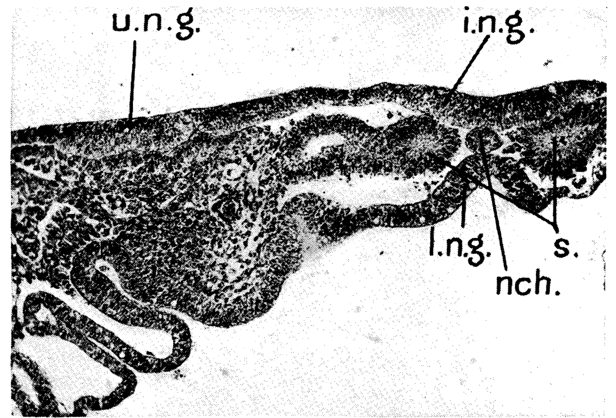

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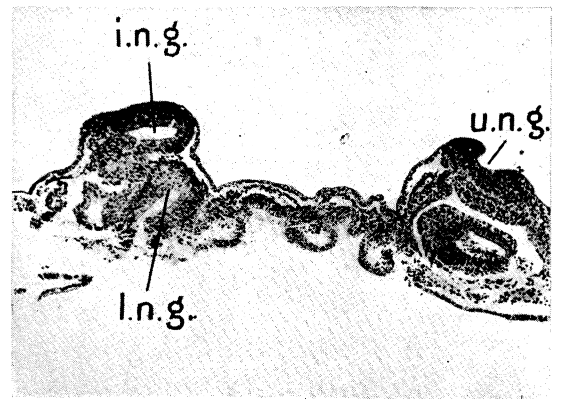

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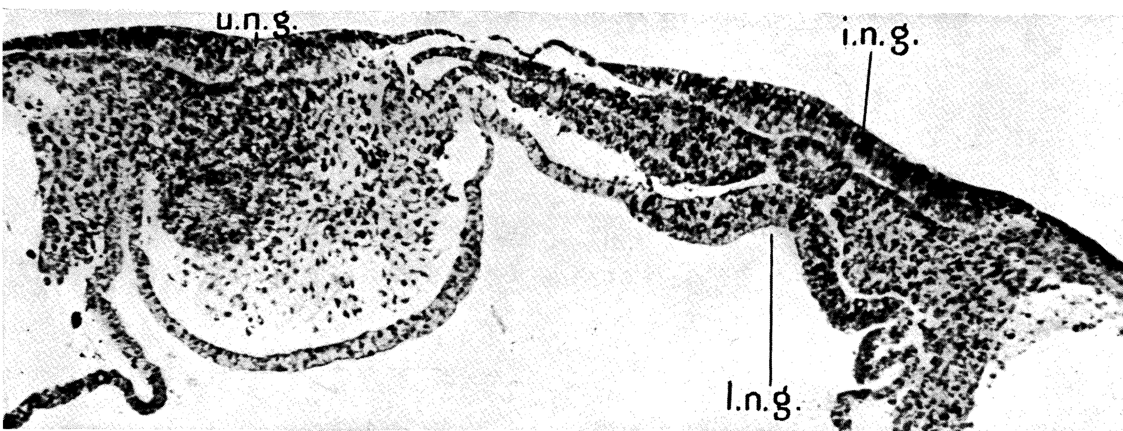

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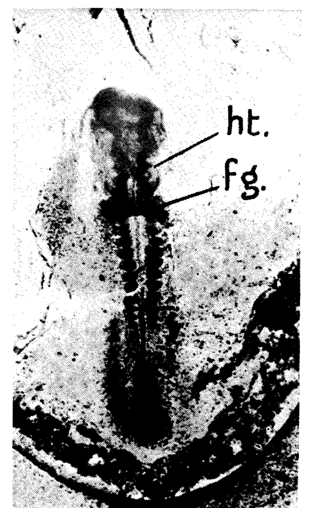

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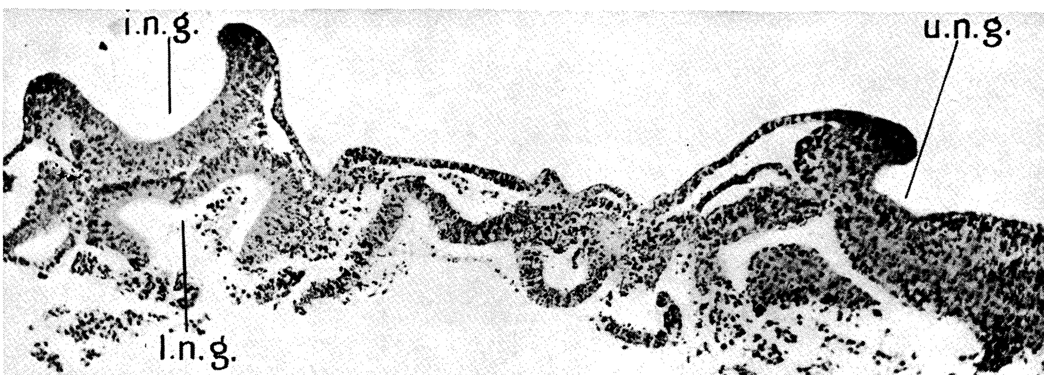

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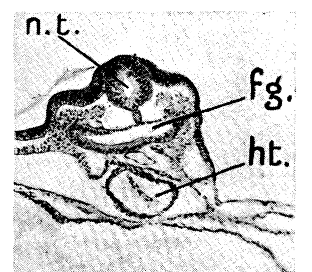

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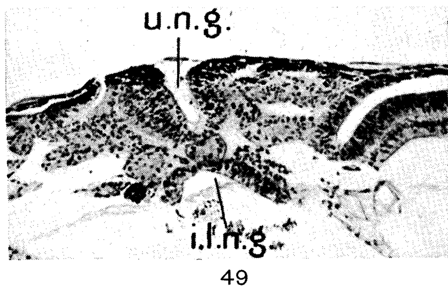

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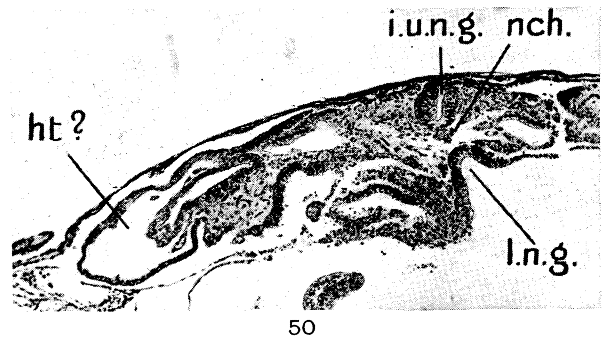

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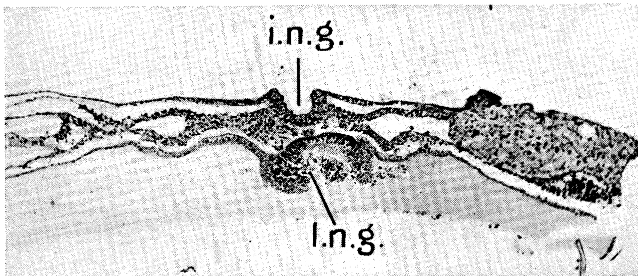

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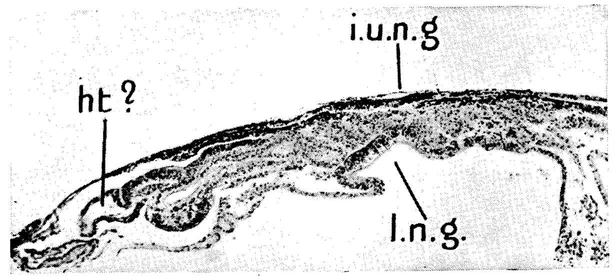

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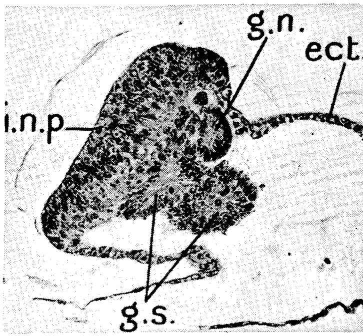

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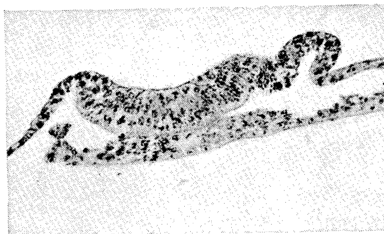
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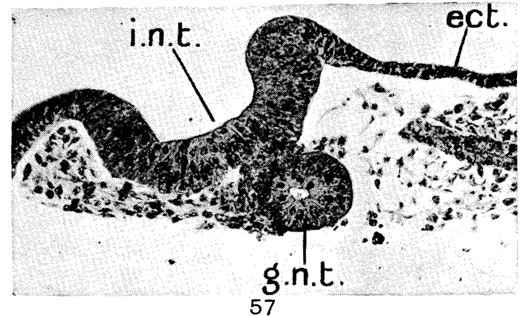

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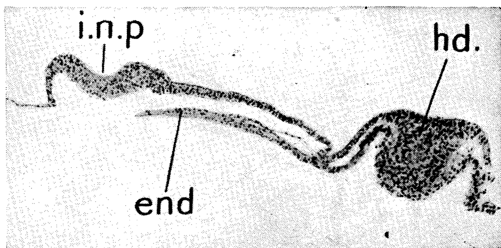

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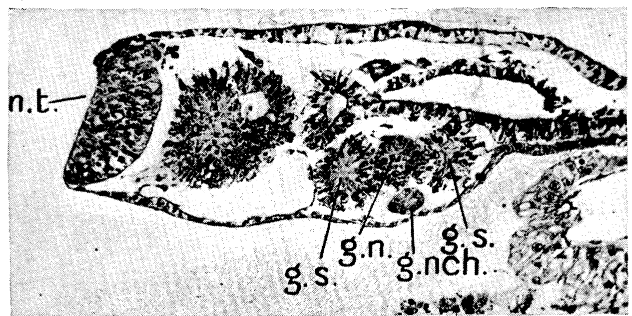

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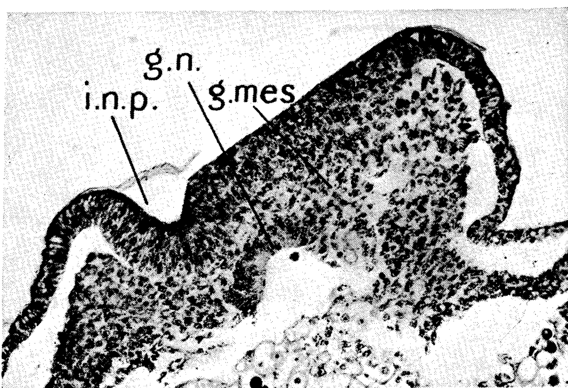

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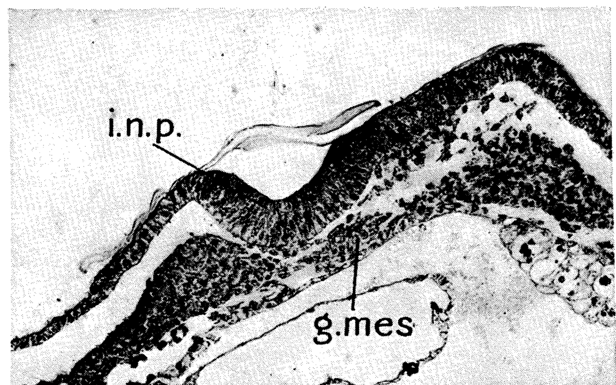

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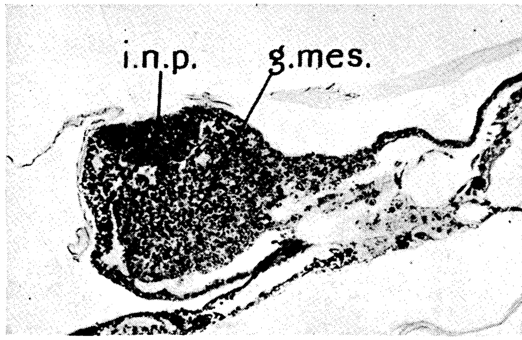

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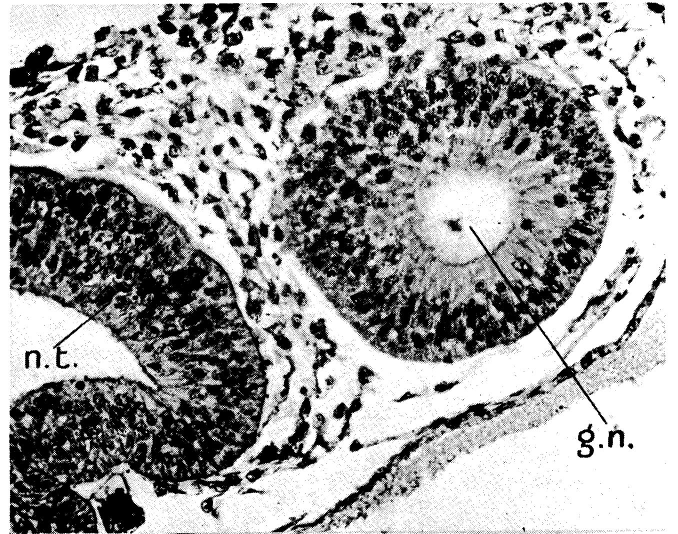

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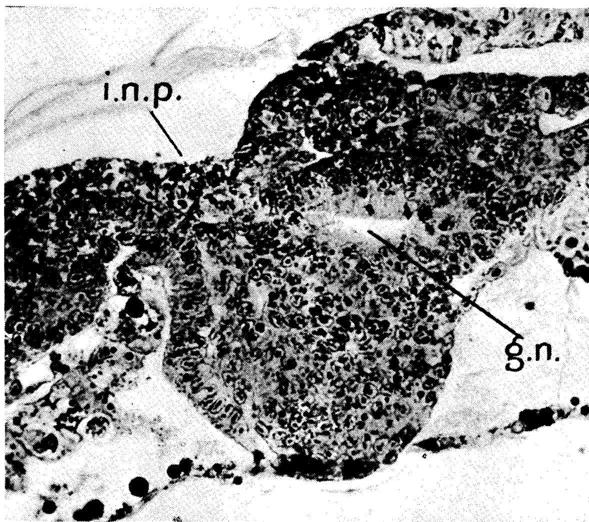

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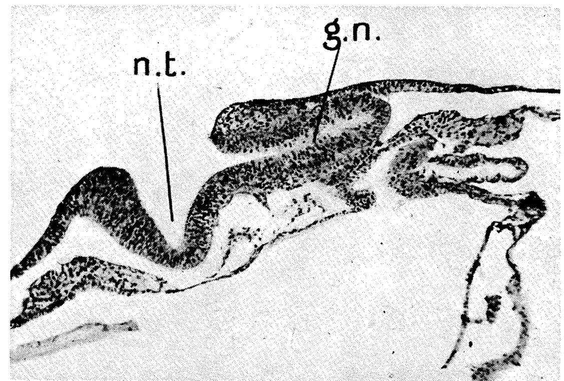

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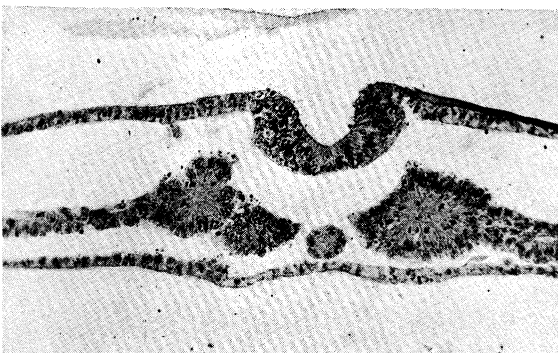

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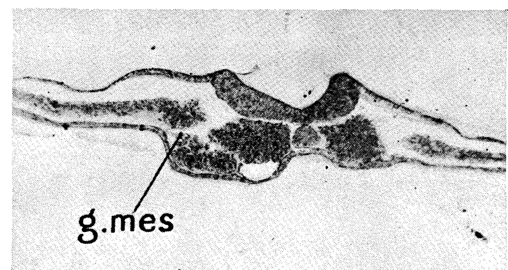

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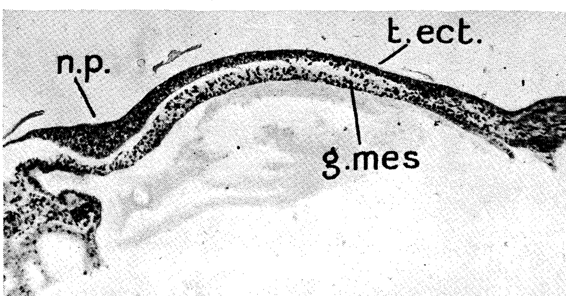

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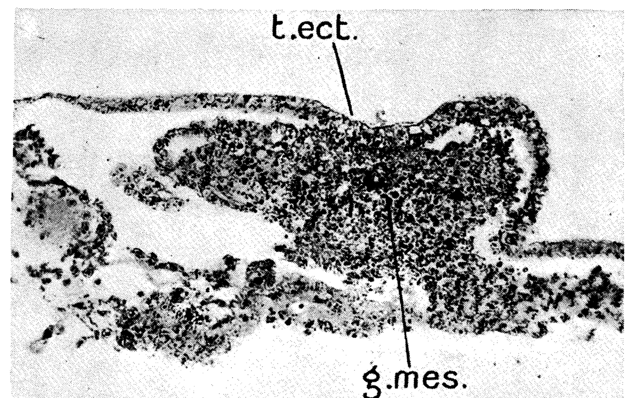

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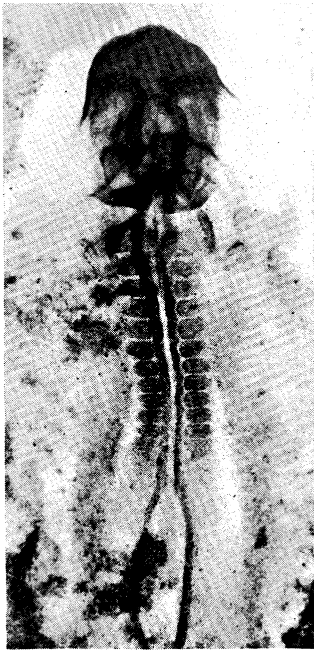
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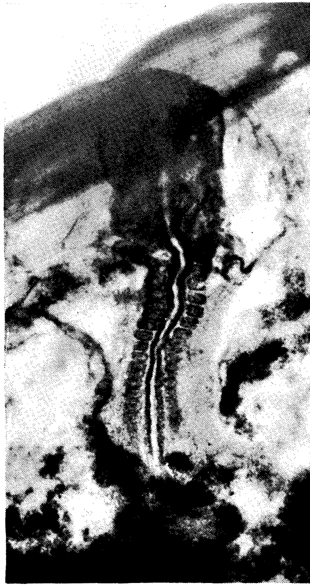
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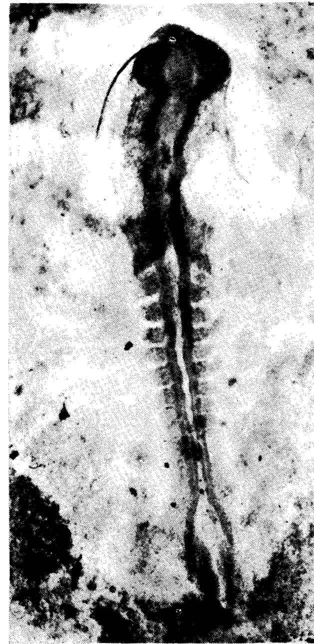
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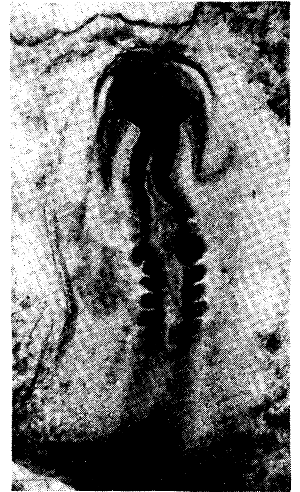
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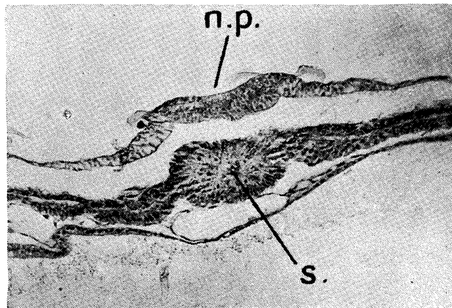

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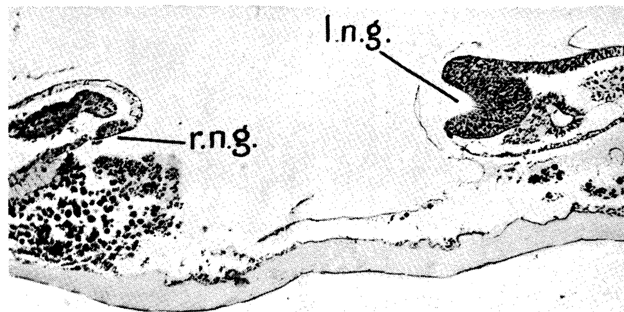

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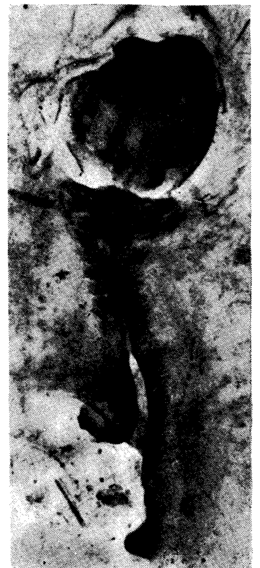
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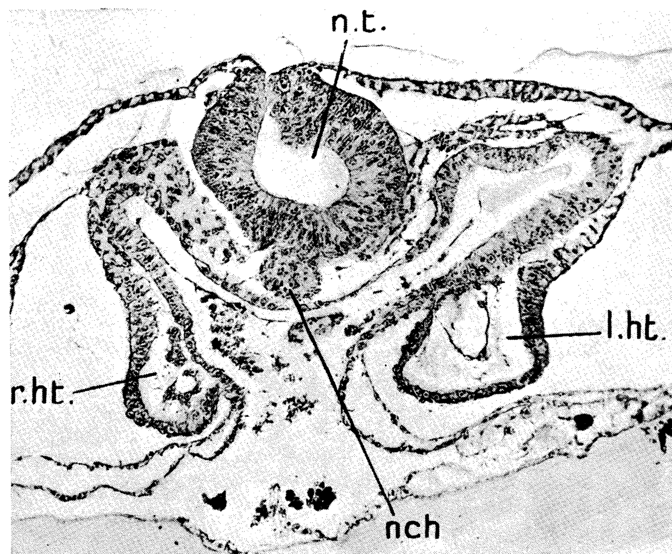

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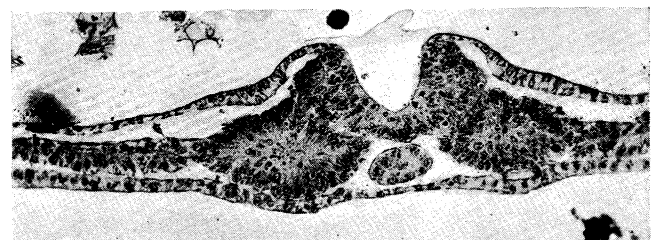
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FIG. 53.—572 (21½–19). Homoplastic* graft of anterior third of primitive streak. Section through anterior region. *ect.*, host ectoderm. *i.n.p.*, induced neural plate. *g.s.*, somites derived from graft. *g.n.*, neural material derived from graft.

FIG. 54.—Same specimen, section further posteriorly. Induced neural plate underlain by a thin layer of graft mesoderm.

FIG. 55.—Same specimen, section still further posteriorly. *i.n.*, induced neural plate. *hd.*, head of host embryo. *end.*, torn endoderm. (× 60.)

FIG. 56.—398 (22¾–22). Autoplastic graft of primitive pit region. Section through central region of graft. See also Plate 28, fig. 72. *n.t.*, sidewall of host neural tube. *g.s.*, somites derived from graft. *g.n.*, graft neural tissue. *g.nch.*, graft notochord. (× 175.)

FIG. 57.—596B (20½–25). Homoplastic graft of anterior half of primitive streak. Section. *g.n.t.*, graft neural tube. *i.n.p.*, induced neural plate. *ect.*, host ectoderm. (× 175.)

FIG. 58.—575 (40 ?–23). Duck, homoplastic graft of middle third of primitive streak. Section. *i.n.p.* induced neural plate. *g.mes.*, mesoderm from graft. *g.n.*, neural tissue from graft. (× 175.)

FIG. 59.—Same specimen. Induced neural plate underlain by graft mesoderm. (× 175.)

PLATE 27.

FIG. 60.—611A (19–28). Homoplastic graft of middle third of primitive streak. *g.mes.*, graft mesoderm. *i.n.p.*, induced neural plate. (× 175.)

FIG. 61.—Same specimen. *g.n.*, graft neural tissue. *i.n.p.*, induced neural plate. (× 250.)

FIG. 62.—555 (20–31). Homoplastic graft of middle third of primitive streak. *n.t.*, host neural tube. *g.n.*, graft neural tissue. (× 250.)

FIG. 63.—Same specimen. *n.t.*, host neural tube. *g.n.*, graft neural tissue. (× 90.)

FIG. 64.—518 (18½–c. 24). Homoplastic graft of middle third of primitive streak of blastoderm aged 15 hours. Graft stained with Nile Blue Sulphate. *g.mes.*, graft mesoderm. (× 90.)

FIG. 65.—428 (20¼–29¼). Autoplastic graft of middle third of primitive streak. Note doubled somite on left side. (× 90.)

FIG. 66.—596A (20½–27). Homoplastic graft of posterior half of primitive streak. *g.mes.*, graft mesoderm. *t.ect.*, thickened host ectoderm. (× 175.)

FIG. 67.—554 (20–31). Homoplastic graft of posterior third of primitive streak. *n.p.*, host neural plate. *t.ect.*, thickened ectoderm. *g.mes.*, graft mesoderm. (× 60.)

PLATE 28.

FIG. 68.—418 (24–17½). Third quarter of primitive streak removed. (× 27.)

FIG. 69.—176 (21½–43). Middle third of primitive streak removed. (× 30.)

FIG. 70.—153 (22½–22½). Middle third of primitive streak removed. (× 27.)

FIG. 71.—145 (21½–20). Primitive pit region removed. (× 30.)

FIG. 72.—398 (22¾–22). Autoplastic graft of primitive pit region. Section anterior to graft region. See also Plate 26, fig. 56. *r.n.g.*, right side of neural groove. *l.n.g.*, left side of neural groove. (× 80.)

FIG. 73.—173 (20–25). Autoplastic graft of primitive pit region. Section posterior to graft. See also fig. 20. *n.p.*, neural plate. *s.*, fused somites. (× 80.)

FIG. 74.—407 (20–28). Entire primitive streak removed. (× 30.)

FIG. 75.—423 (17½–29). Entire primitive streak removed. Section through heart region. *n.t.*, neural tube. *nch.*, notochord. *l.ht.*, left heart rudiment. *r.ht.*, right heart rudiment. (× 175.)

FIG. 76.—Same specimen. Section further posteriorly. (× 175.)

* Homoplastic: a graft into an animal other than the donor, but belonging to the same species.

Autoplastic: a graft into the donor, in these experiments always into a place different to the place of removal, *i.e.*, always heterotope.

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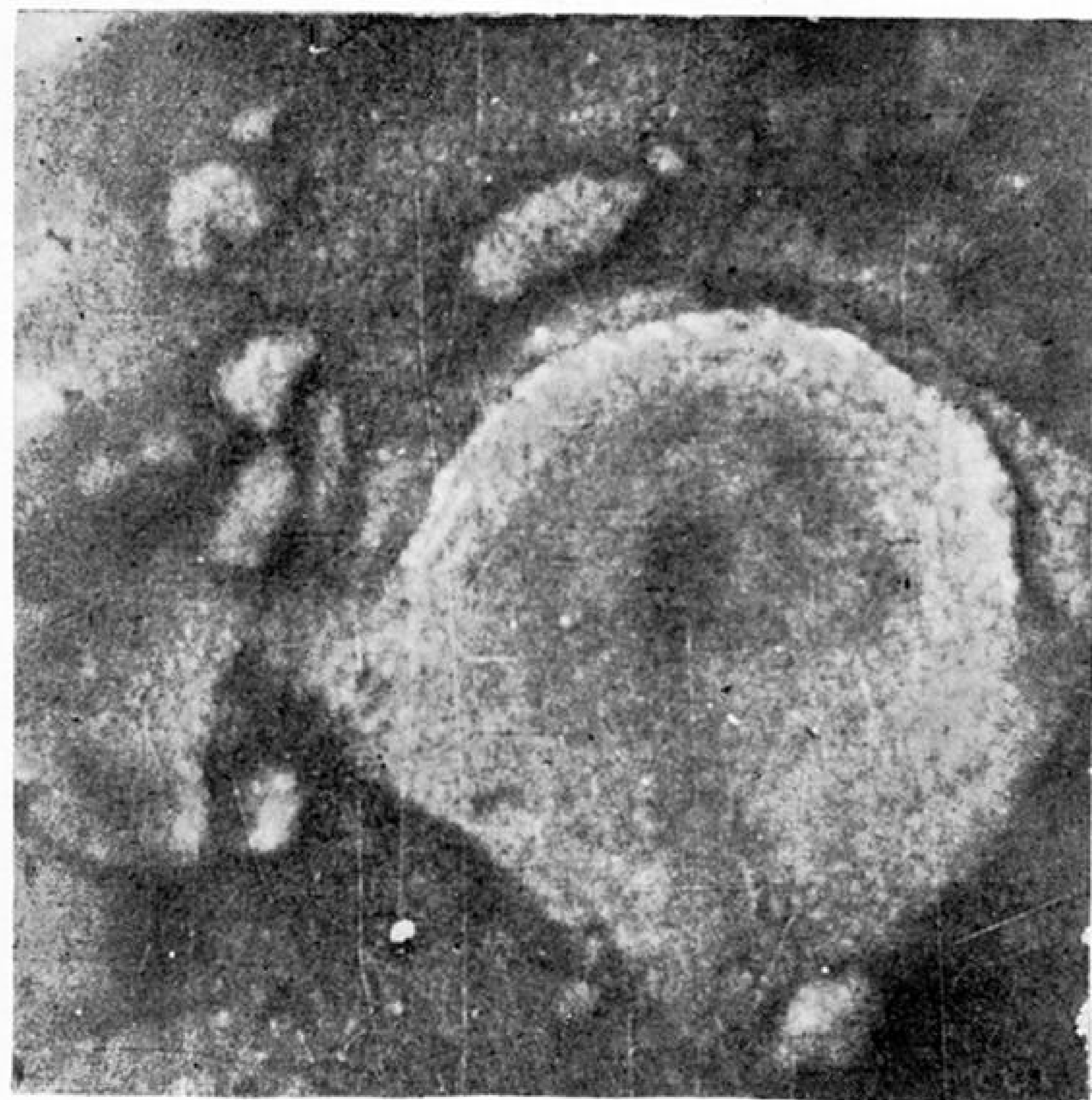
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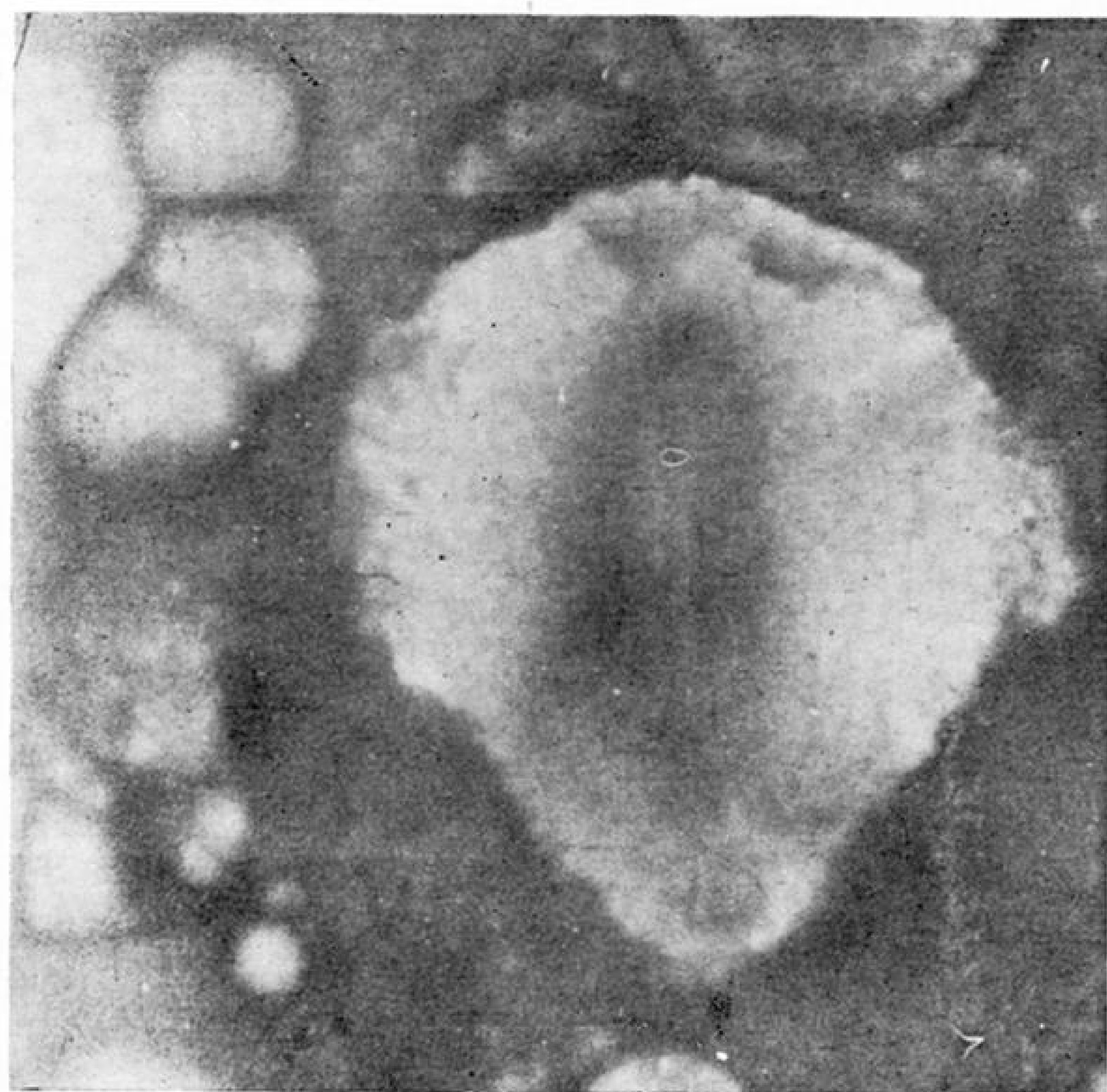
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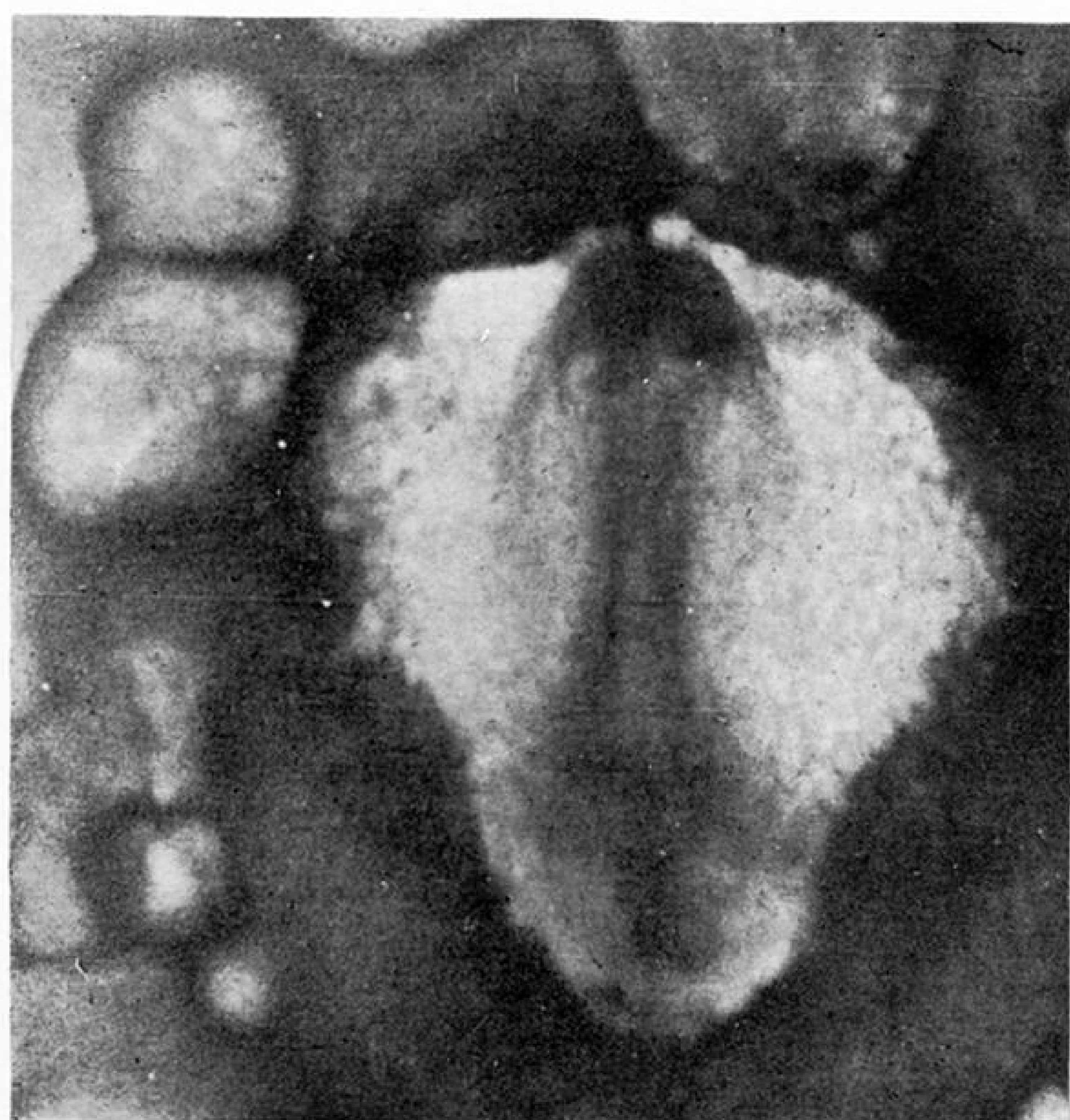
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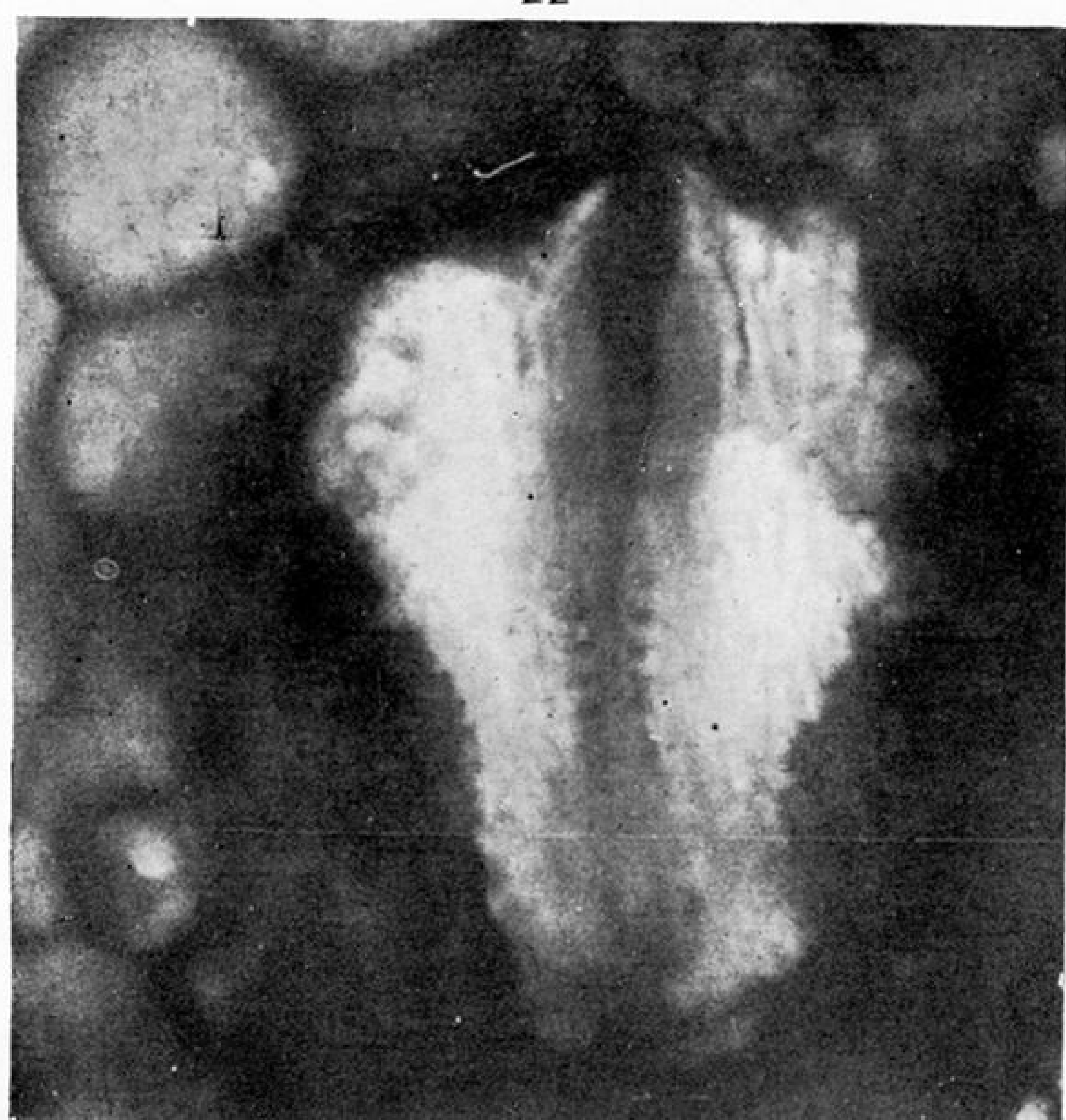
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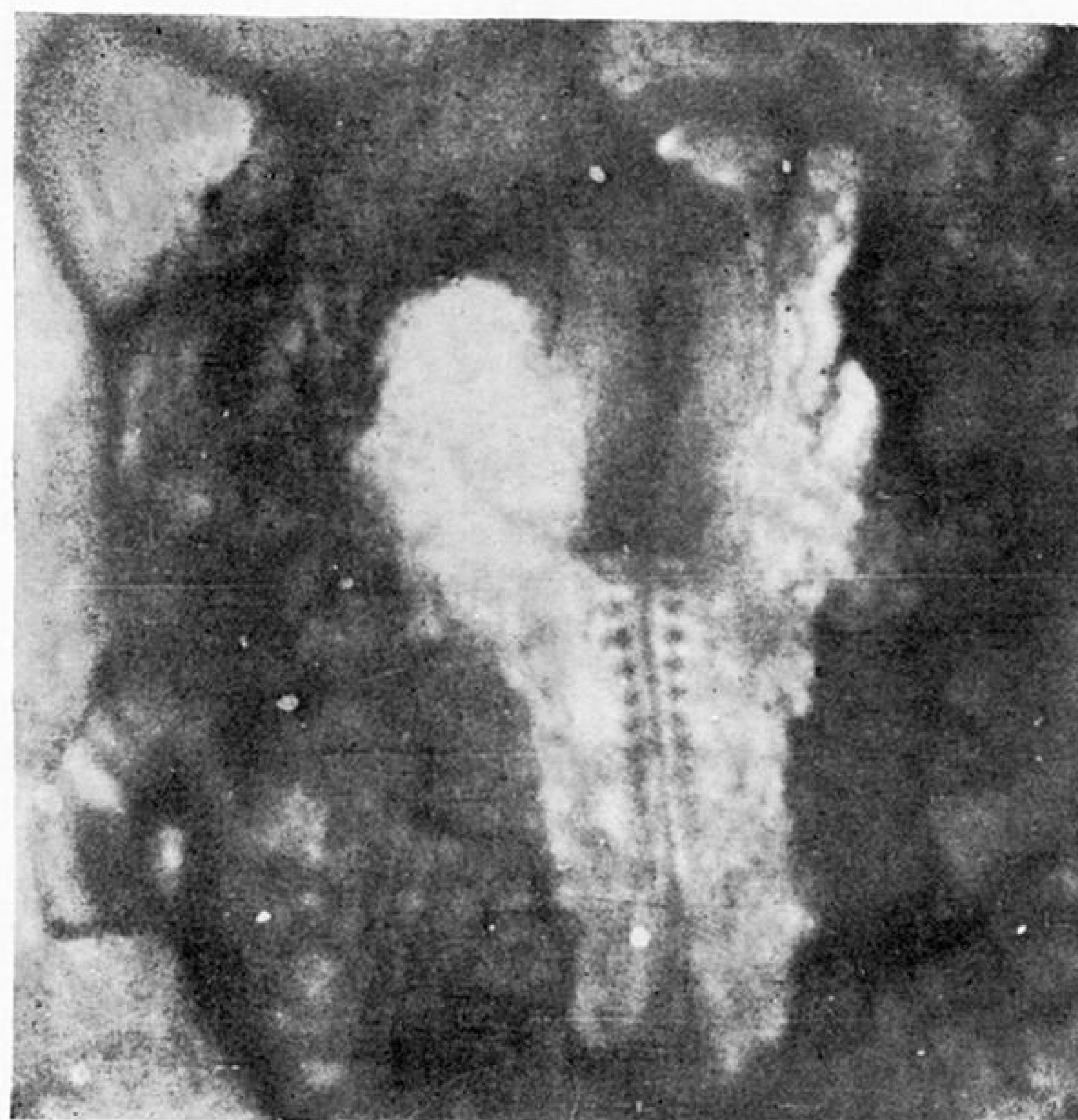
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Plate 22.

Figs. 21–25. Five stages of the development of a chick blastoderm *in vitro*. Taken from a cinematograph film made by Dr. R. G. Canti. The blastoderm was 18 hours old at explantation.

Fig. 21. After 4 hours.

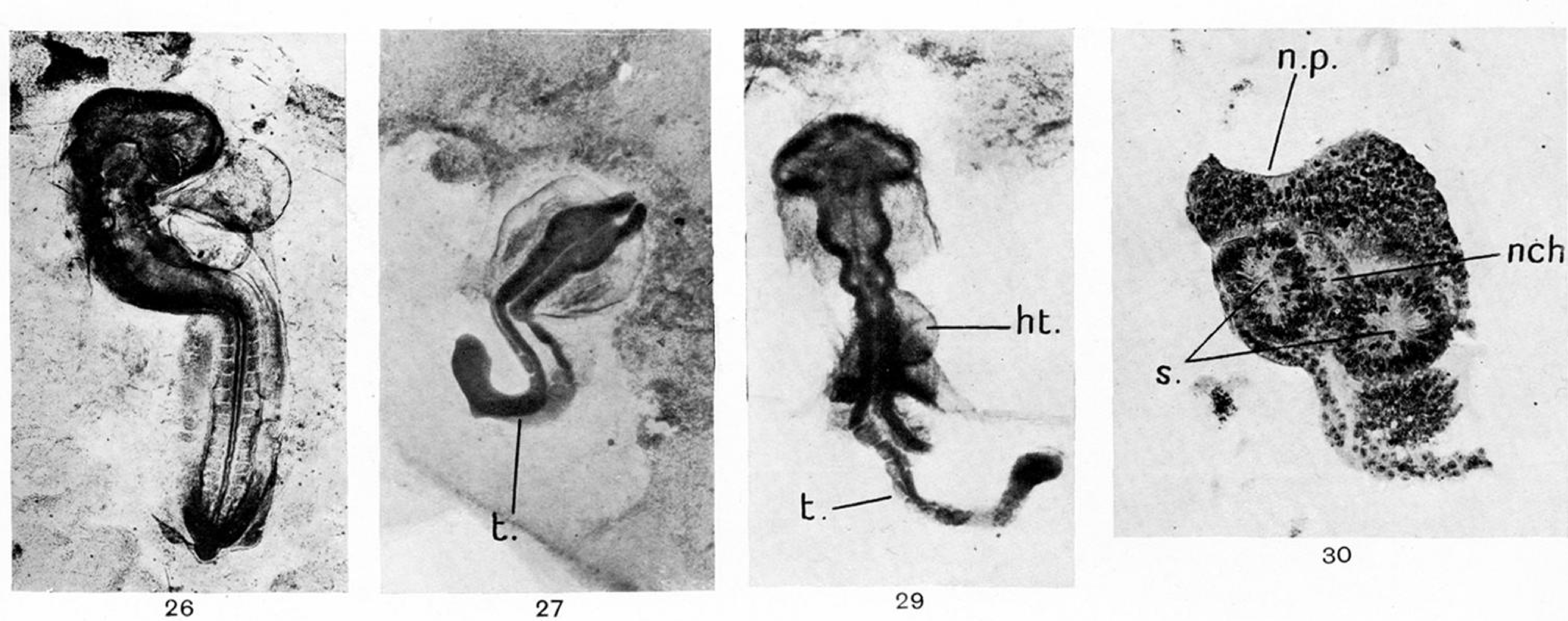
Fig. 22.—After 14 hours.

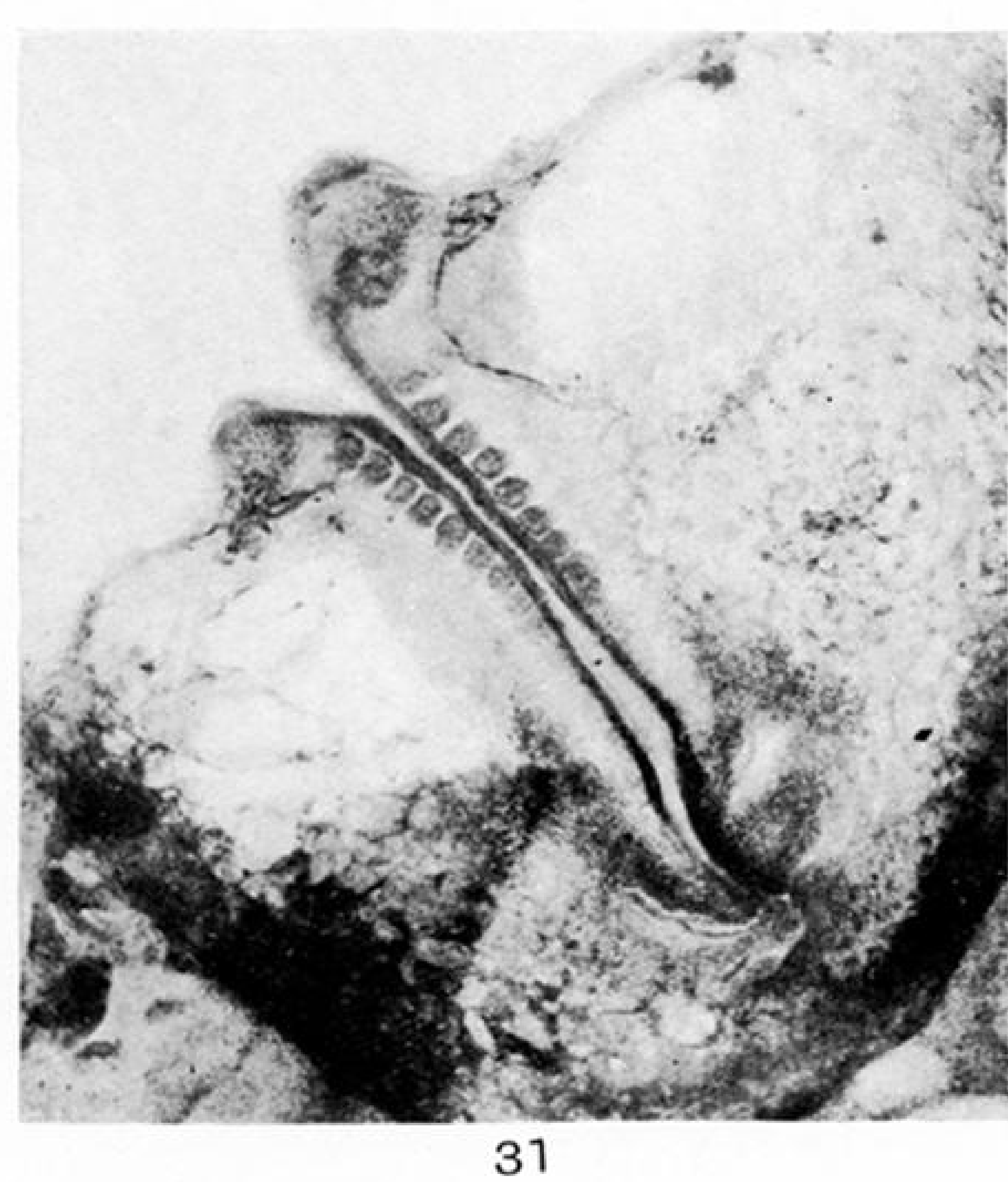
Fig. 23.—After 18 hours.

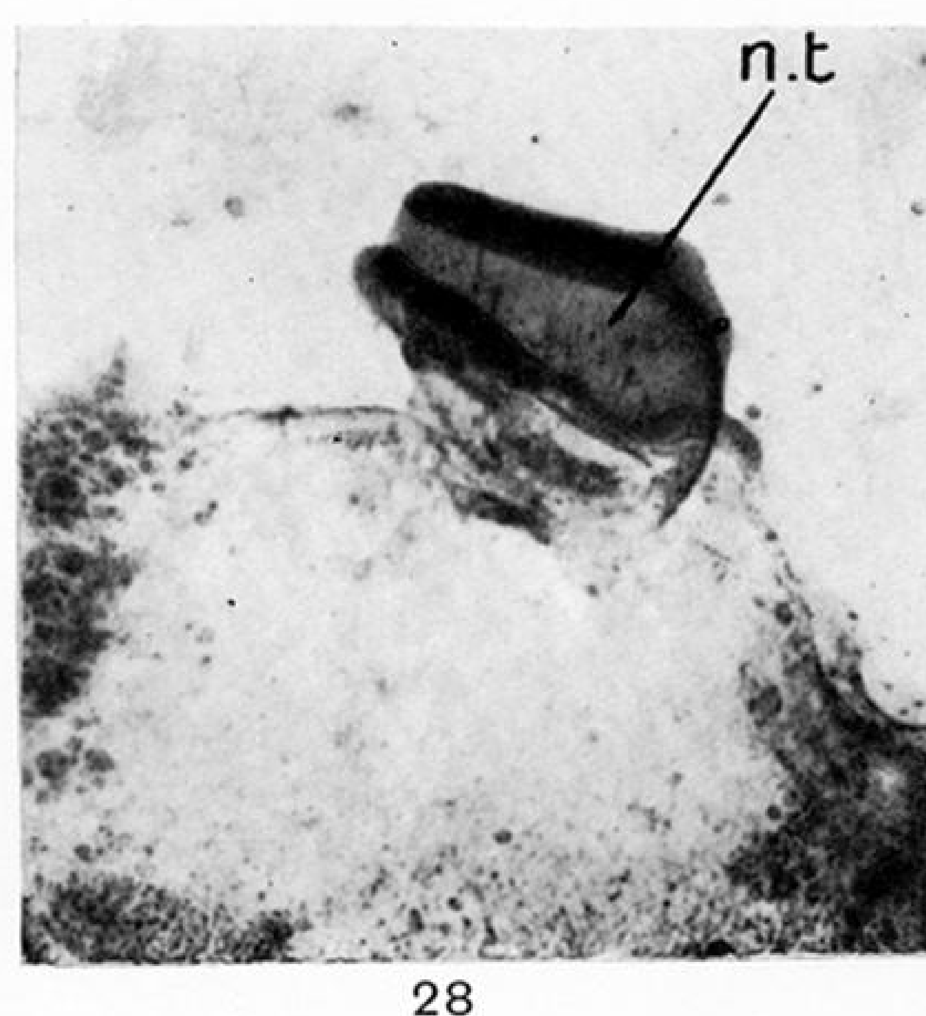
Fig. 24.—After 24 hours.

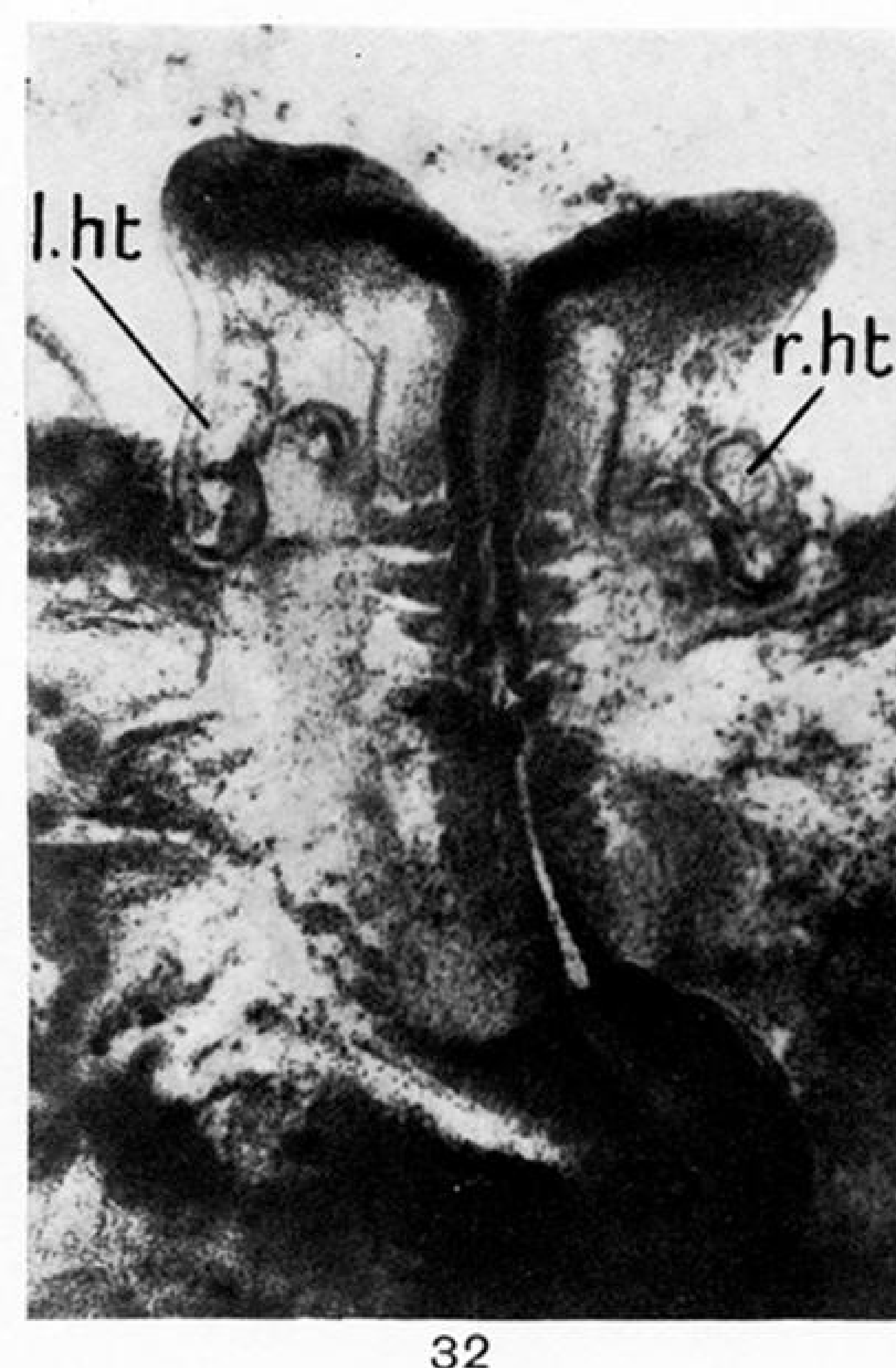
Fig. 25.—After 32 hours.

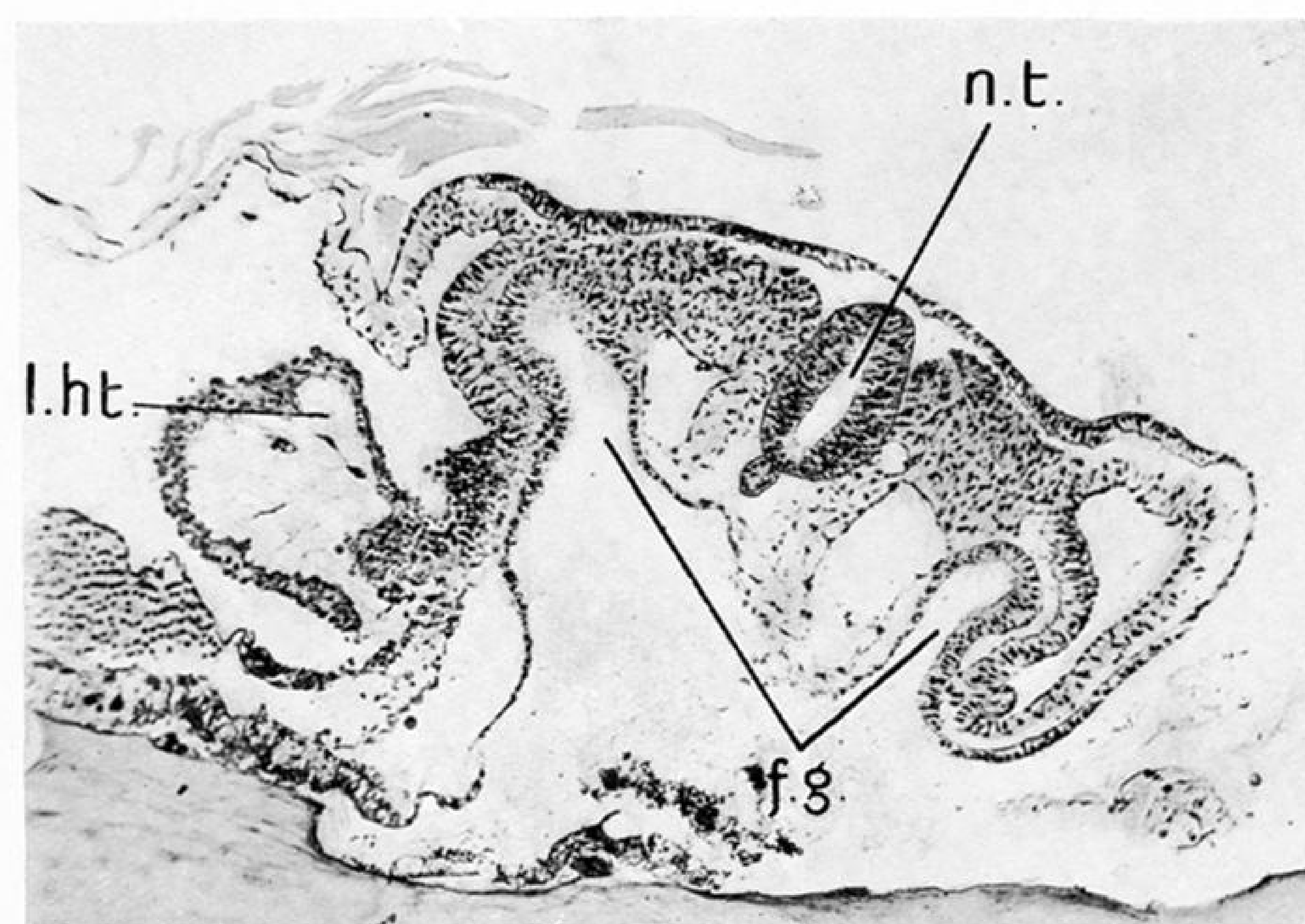
Magnification the same for all figures.











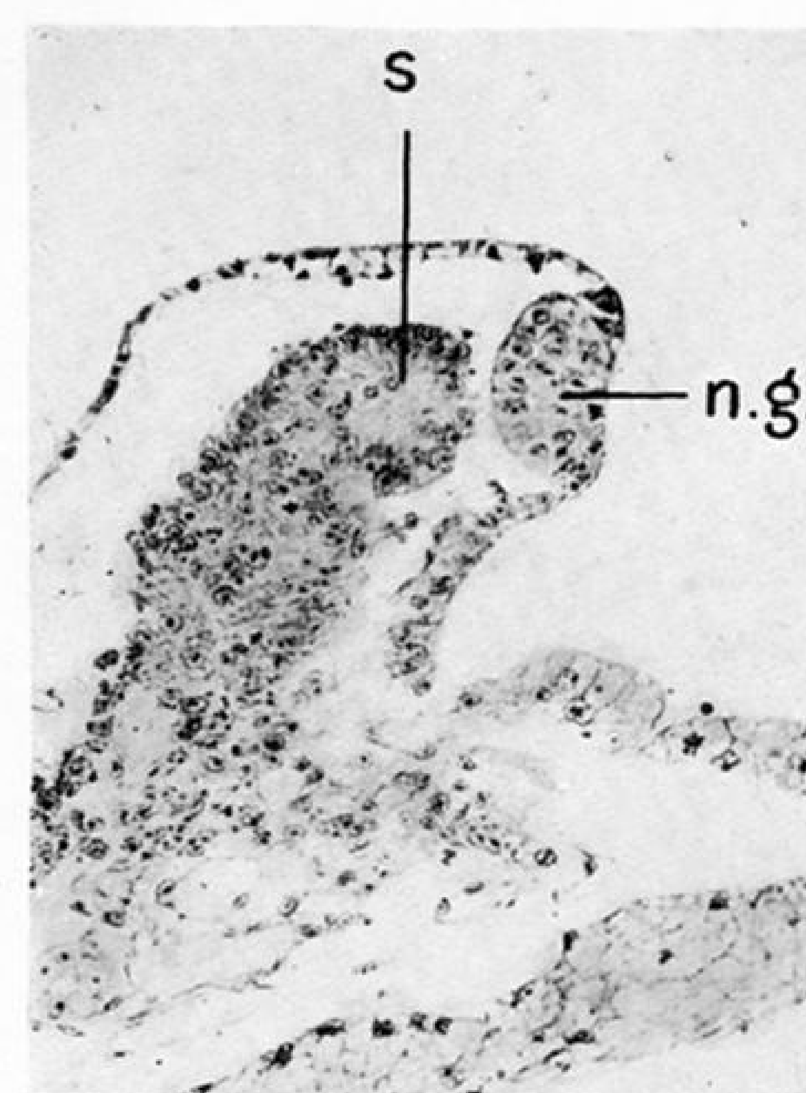


PLATE 23.

FIG. 26.—Embryo explanted at 9 somite stage, cultivated for 43 hours *in vitro*. (× 10.)

FIG. 27.*—325 ($22\frac{1}{4}$–$19\frac{1}{2}$). Anterior portion of blastoderm sectioned just posterior to primitive pit. *t.*, tail. (× 23.)

FIG. 28.—324A (22–26). Anterior portion of blastoderm sectioned through primitive pit. *n.t.*, neural tissue. (× 32.)

FIG. 29.—189 ($22\frac{1}{4}$–33). Blastoderm from which the second quarter of the primitive streak was eliminated. *t.*, tail. *ht.*, heart. (× 27.)

* In the legends to the figures, the protocol number of the specimen is given first (*e.g.*, in this case 325), then the age of the blastoderm at operation in hours of incubation ($22\frac{1}{4}$), and then the number of hours for which the explanted embryo was cultivated before fixation ($19\frac{1}{2}$).

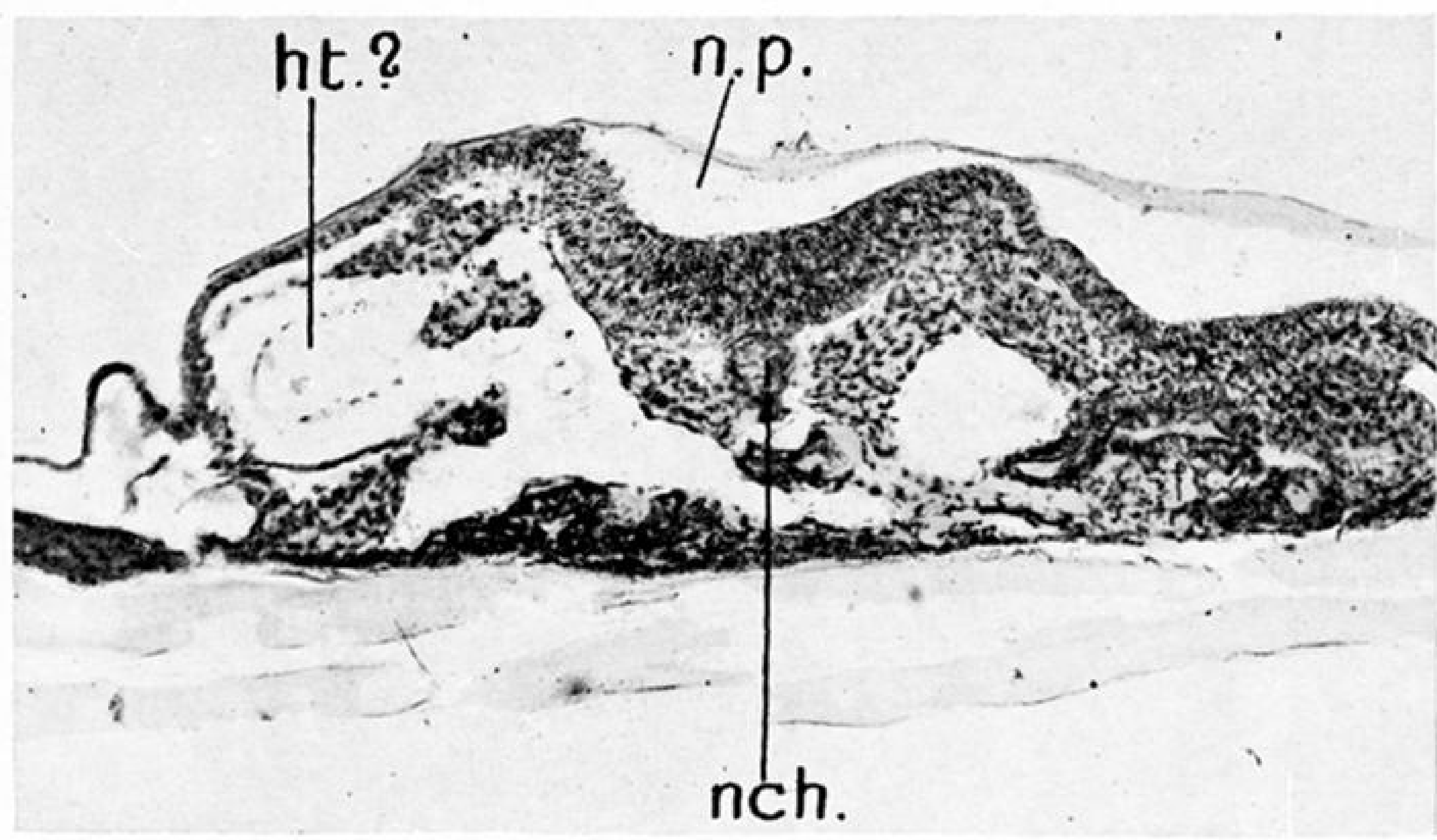
FIG. 30.—177A ($20\frac{1}{4}$–24). Section through "tail," from embryo sectioned slightly posterior to primitive pit. *n.p.*, neural plate. *s.*, somites. *nch.*, notochord. (× 175.)

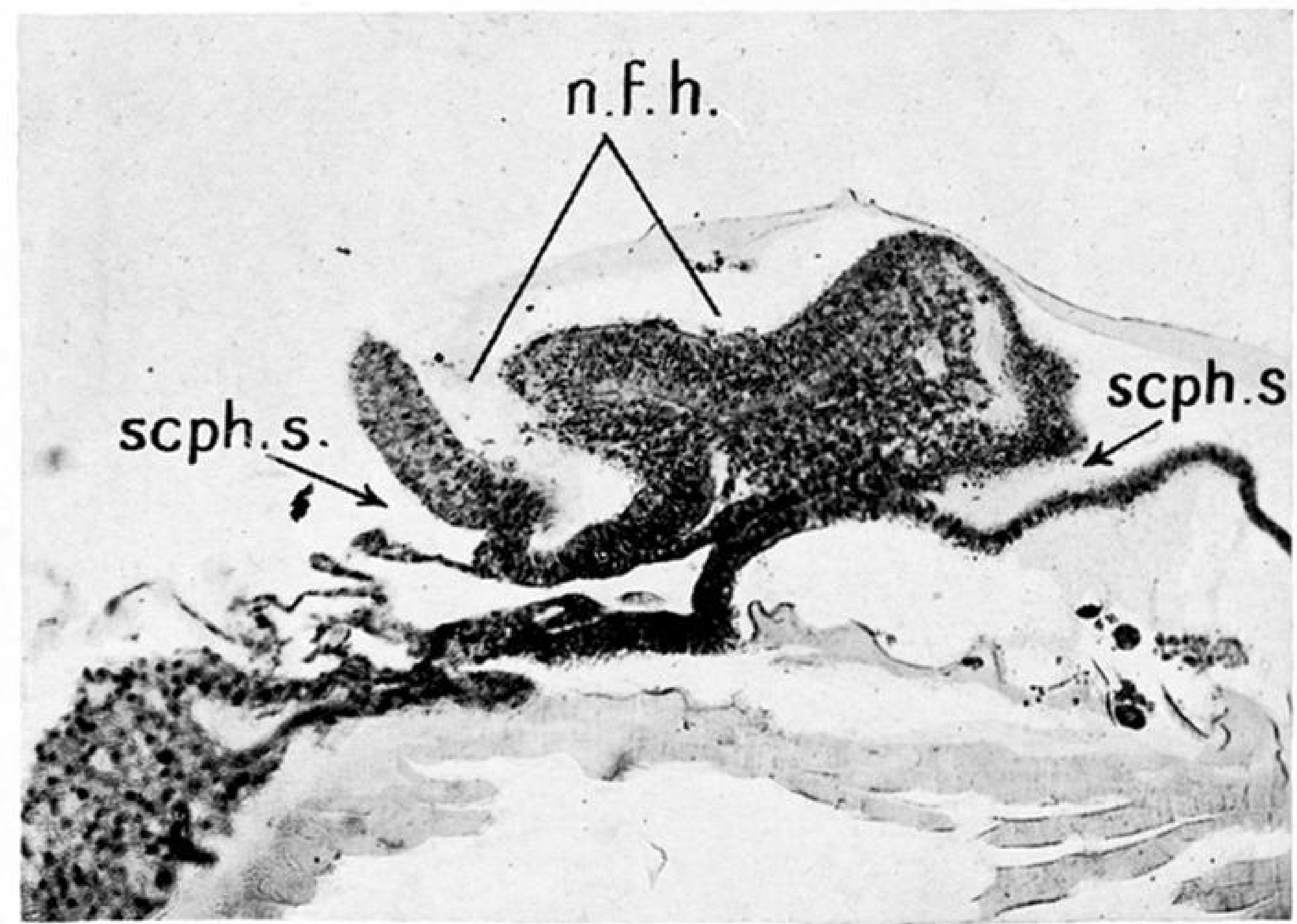
FIG. 31.—324 (22–20). Posterior portion of blastoderm sectioned through primitive pit. (× 23.)

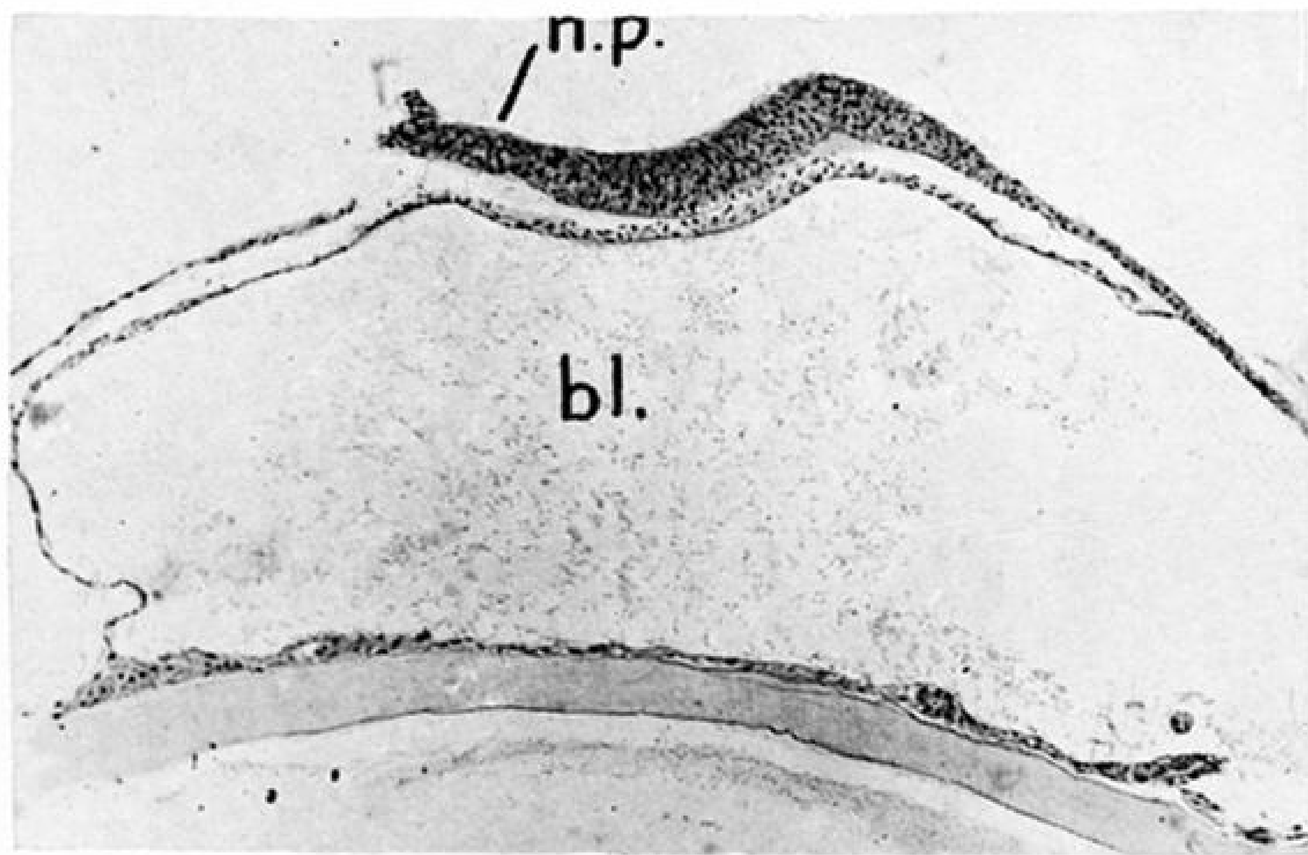
FIG. 32.—135P ($20\frac{1}{2}$–$17\frac{1}{2}$). Posterior portion of blastoderm sectioned through primitive pit. *l.ht.*, left heart rudiment. *r.ht.*, right heart rudiment. (× 32.)

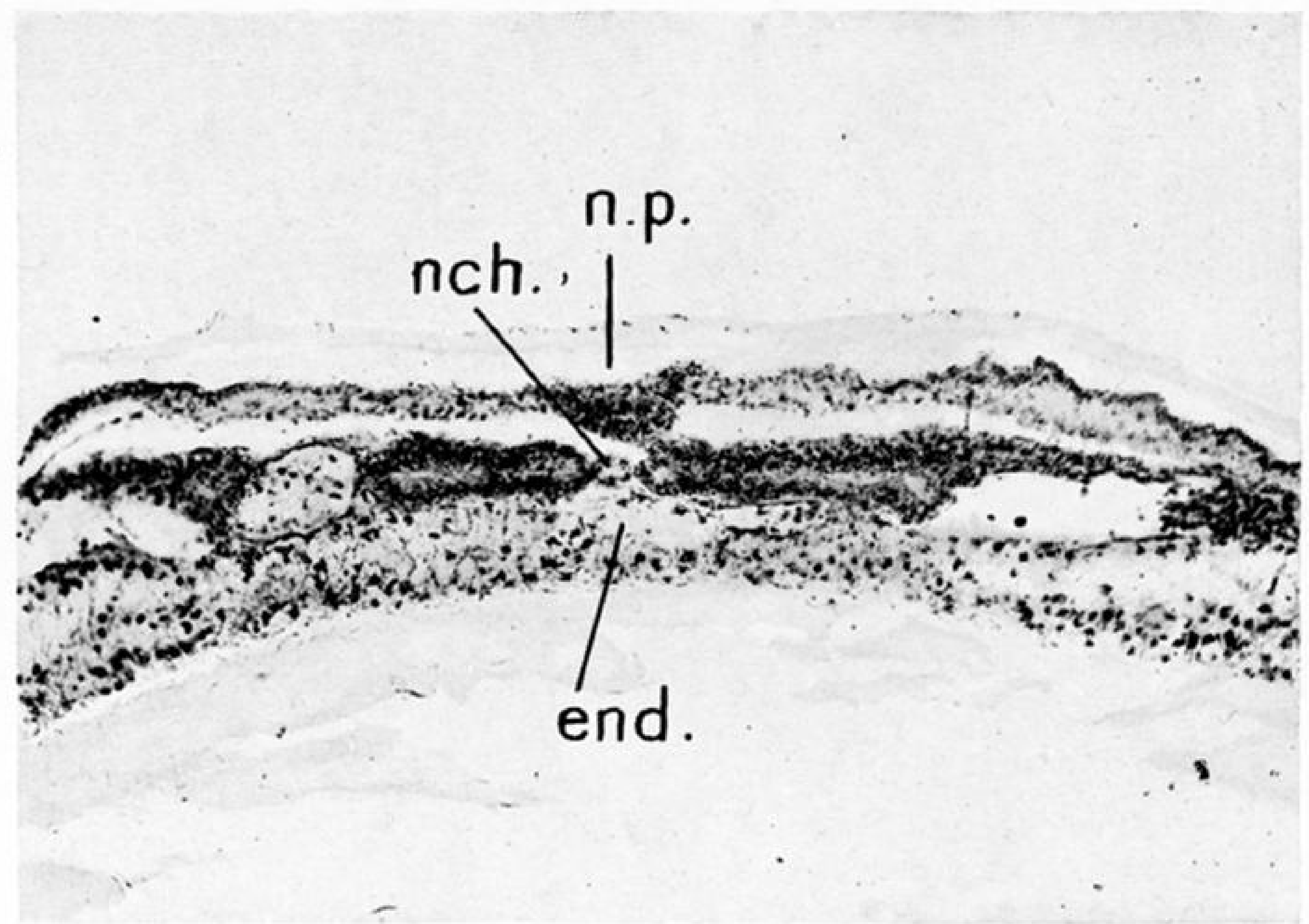
FIG. 33.—172 (20–44). Section through posterior portion of blastoderm cut through primitive pit. *n.t.*, neural tube. *l.ht.*, left heart rudiment. *fg.*, foregut. (× 80.)

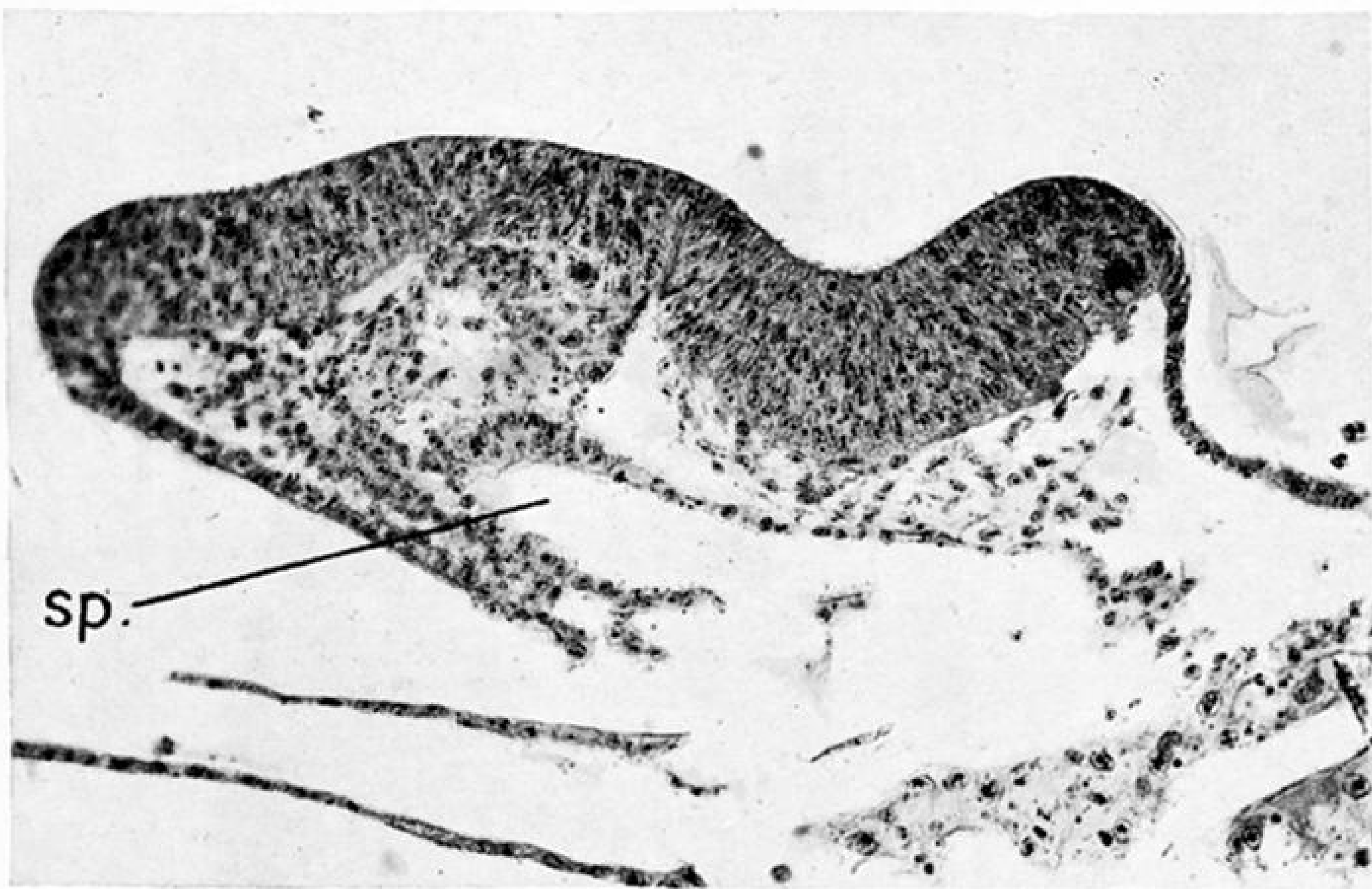
FIG. 34.—304P (22–26). Section through the left side of posterior portion of blastoderm cut just posterior to the primitive pit. *n.g.*, side wall of neural groove. *s.*, somite. (× 175.)

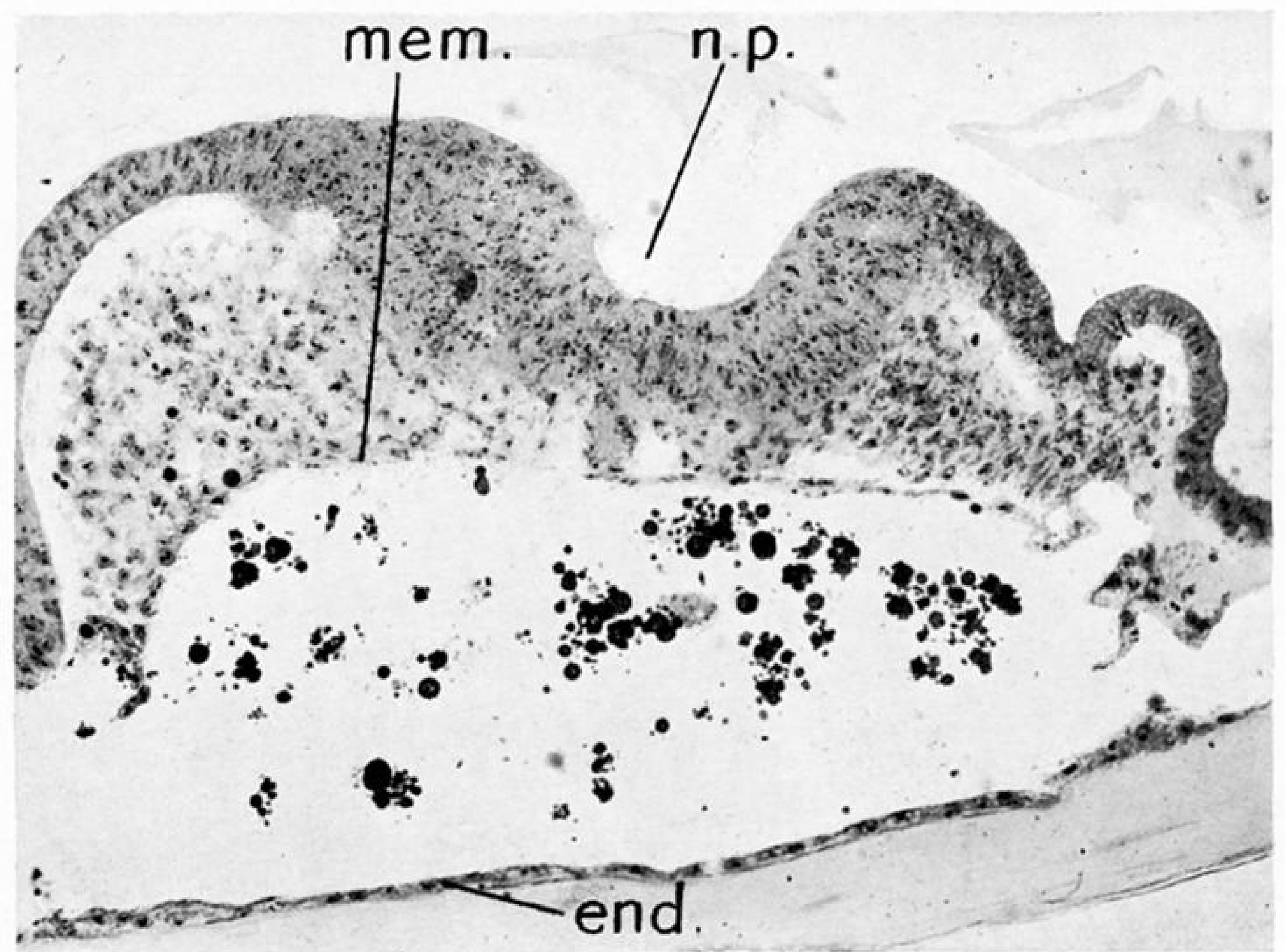












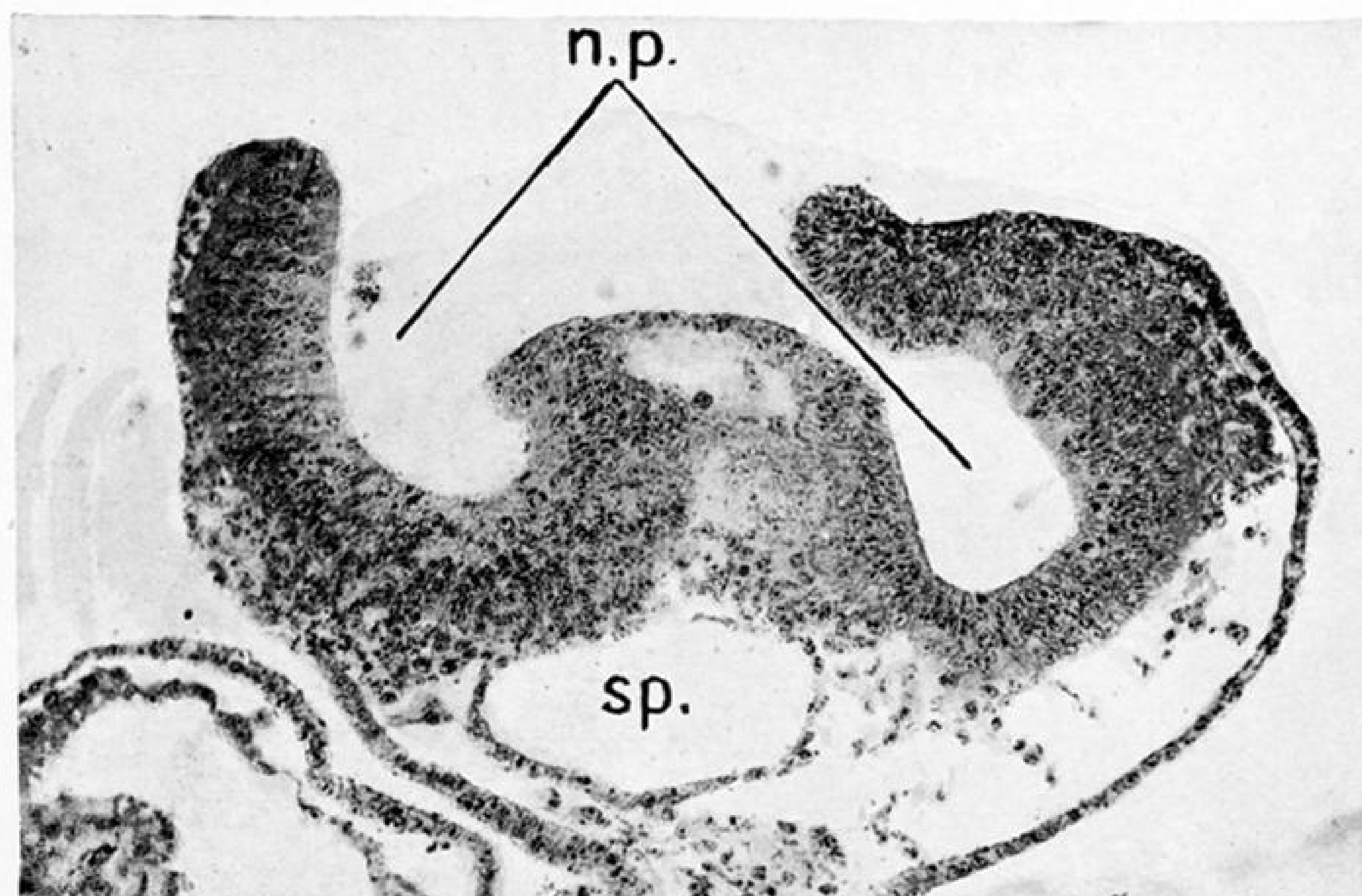


PLATE 24.

FIG. 35.—293 (25–42). Section through the head region of embryo cultivated after removal of the endoderm. *n.f.h.*, neural folds of head. *scph.s.*, sub-cephalic space. (× 110.)

FIG. 36.—Same specimen. Section through central region. *n.p.*, neural plate. *nch.*, notochord. *ht.*, space representing heart ? (× 110.)

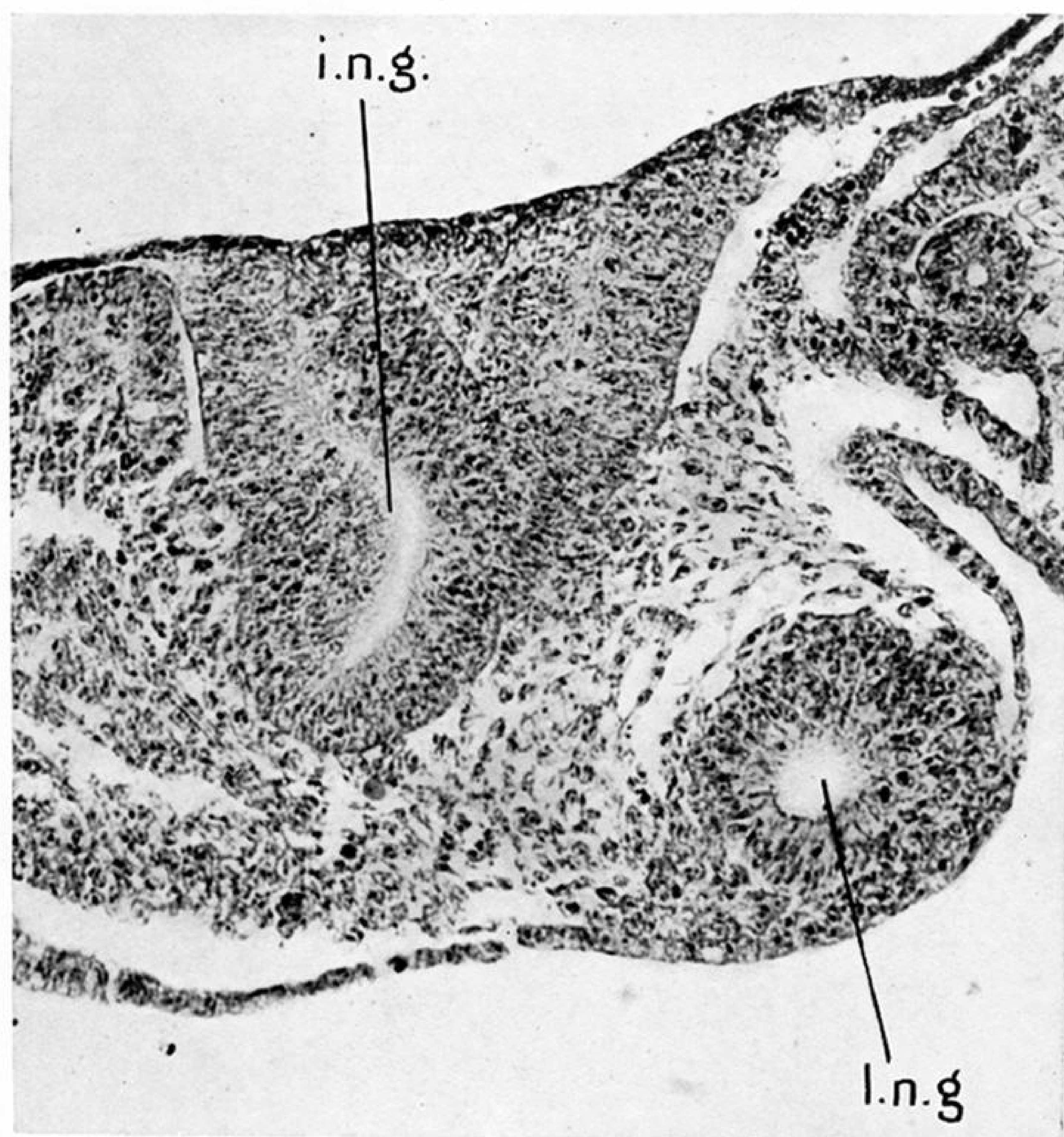
FIG. 37.—Same specimen. Section through posterior region. *n.p.*, neural plate. *nch.*, notochord. *end.*, yolksac endoderm. (× 110.)

FIG. 38.—319 (21–26). Section through embryo cultivated after removal of endoderm. *n.p.*, neural plate. *bl.*, blister. (× 60.)

FIG. 39.—227 ($20\frac{1}{4}$–$22\frac{1}{2}$). Section through anterior part of embryo cultivated after removal of the endoderm. *n.p.*, neural plate. *mem.*, mesodermal membrane. *end.*, yolksac endoderm. (× 175.)

FIG. 40.—227. Same specimen. Section still further anteriorly. *sp.*, space resembling foregut.

FIG. 41.—402 (24–19). Section through anterior region of embryo cultivated after removal of endoderm. *np.*, neural plate (divided). *sp.*, space enclosed by membrane. (× 175.)



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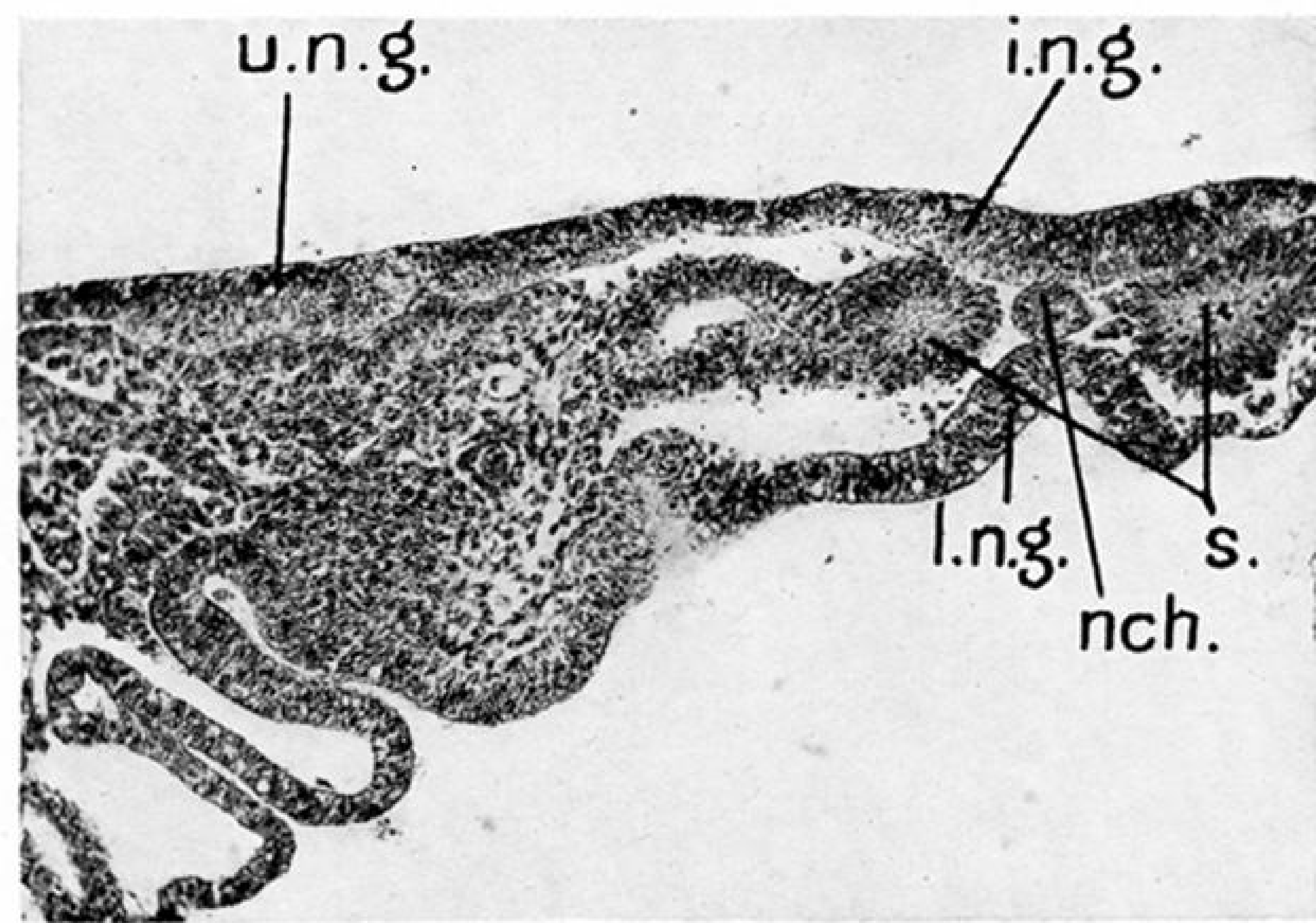

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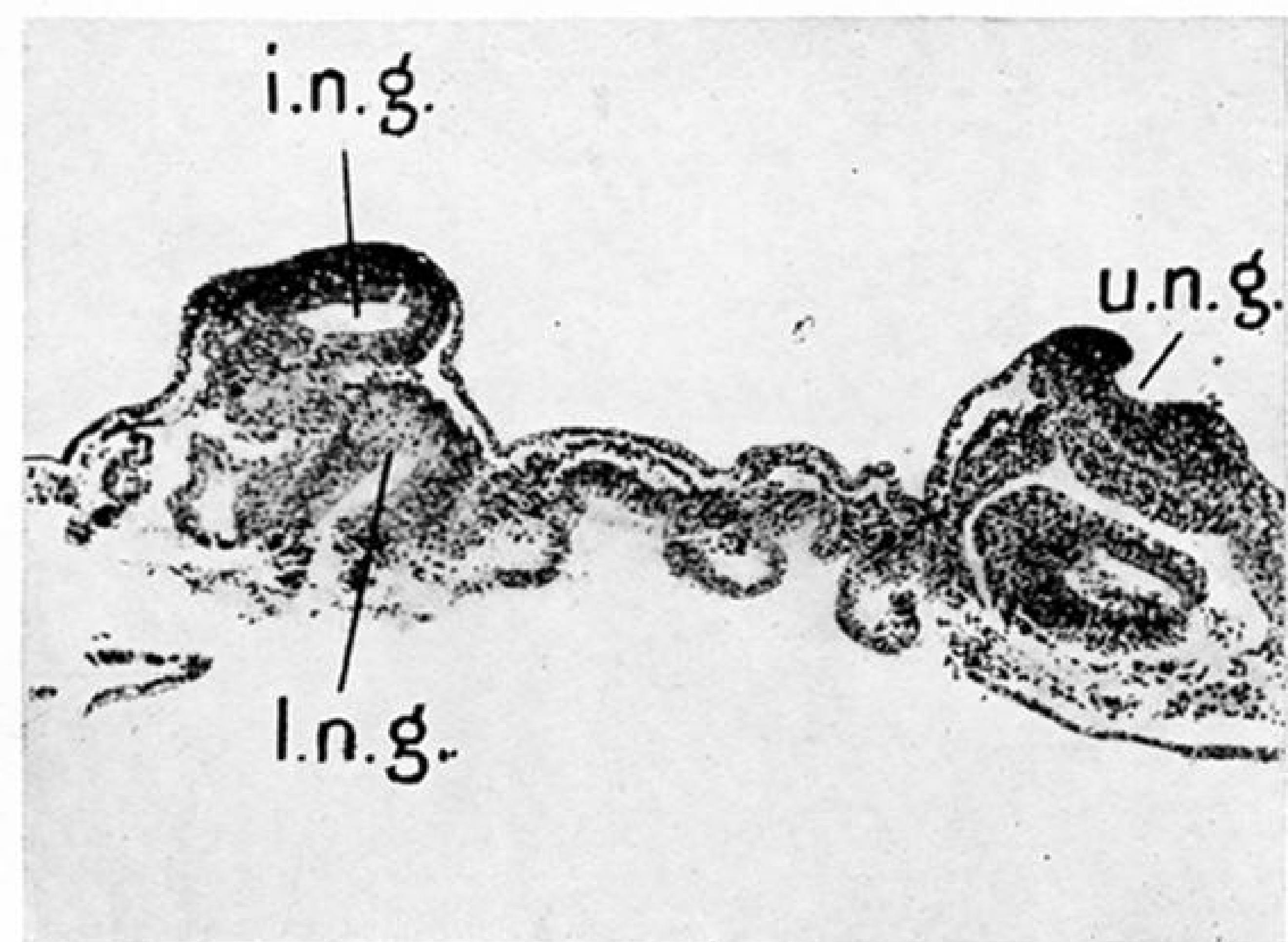

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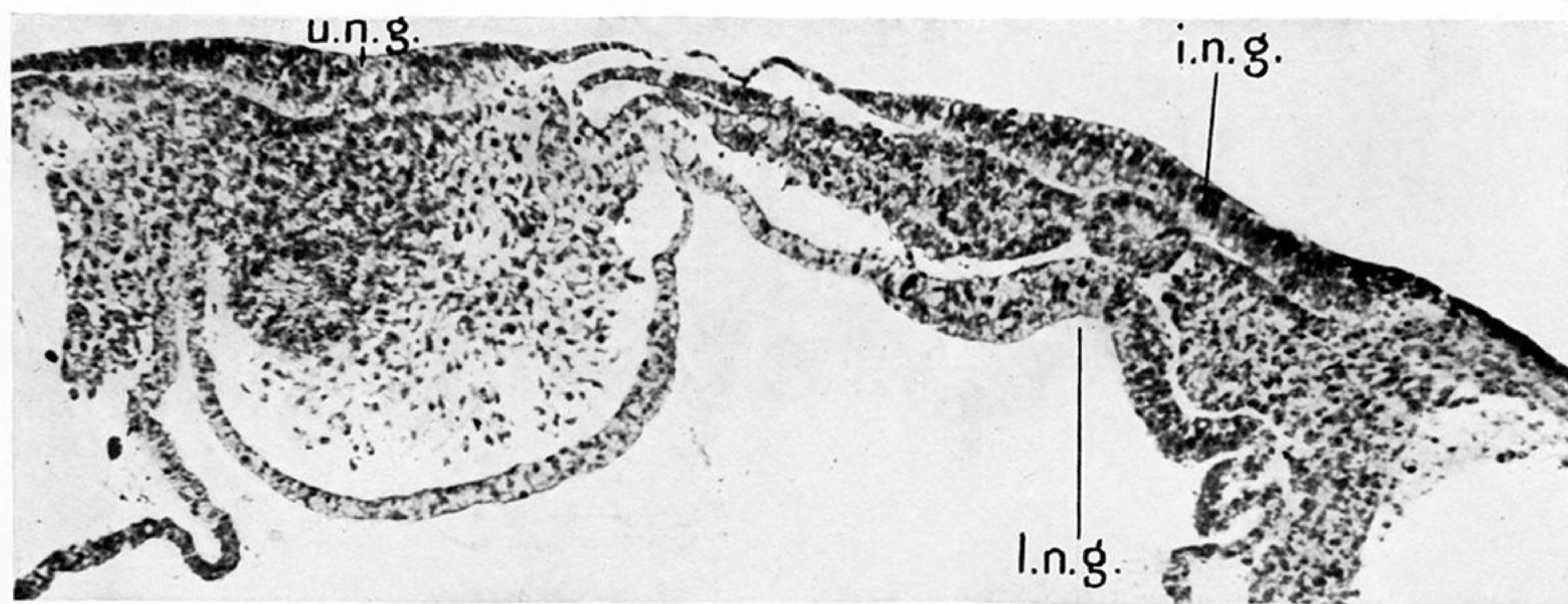

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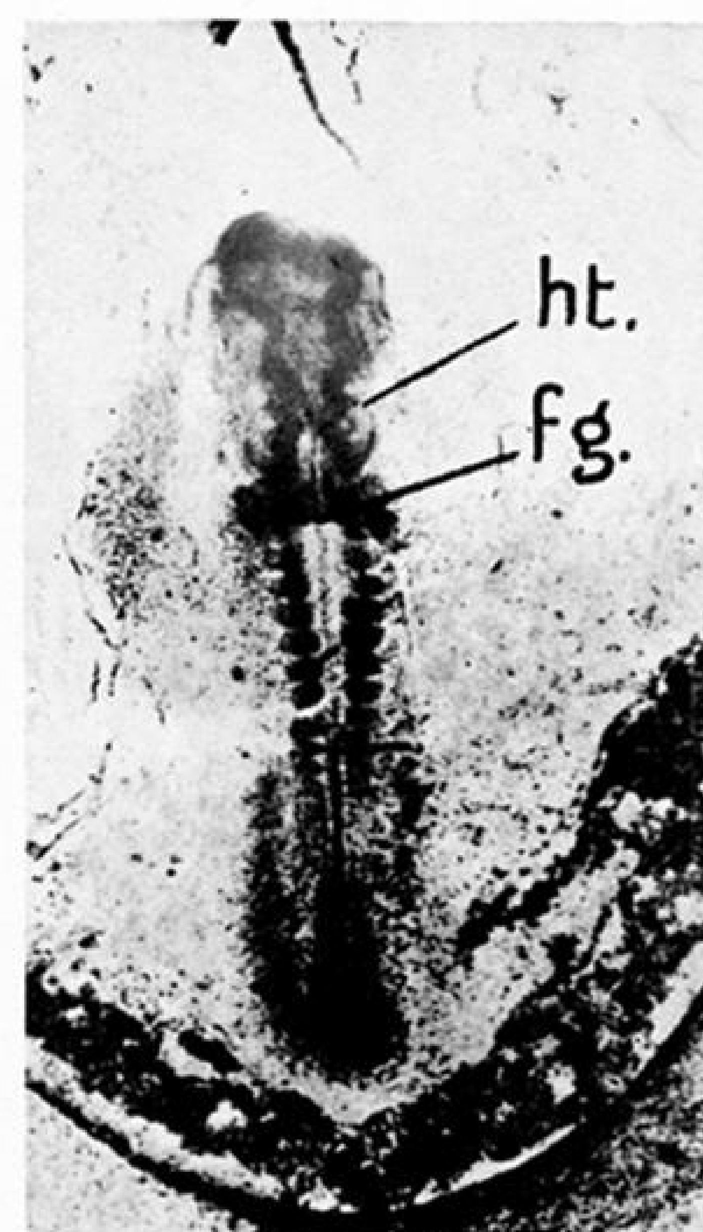

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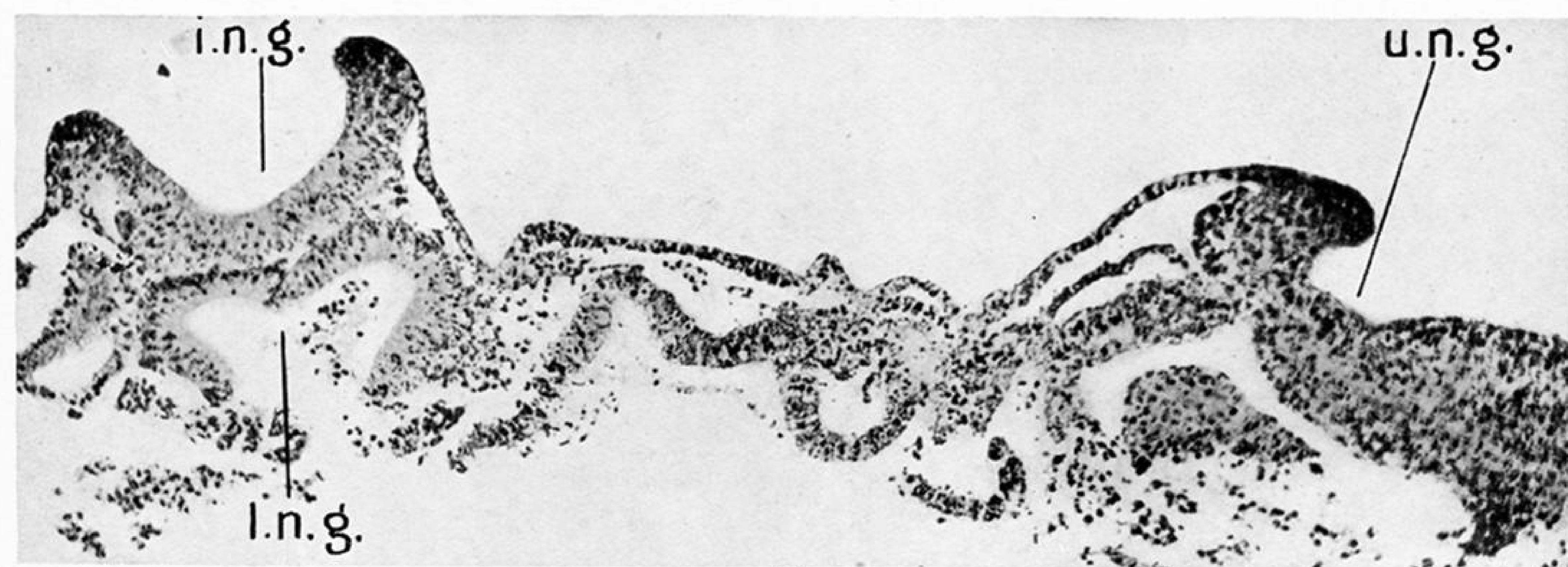

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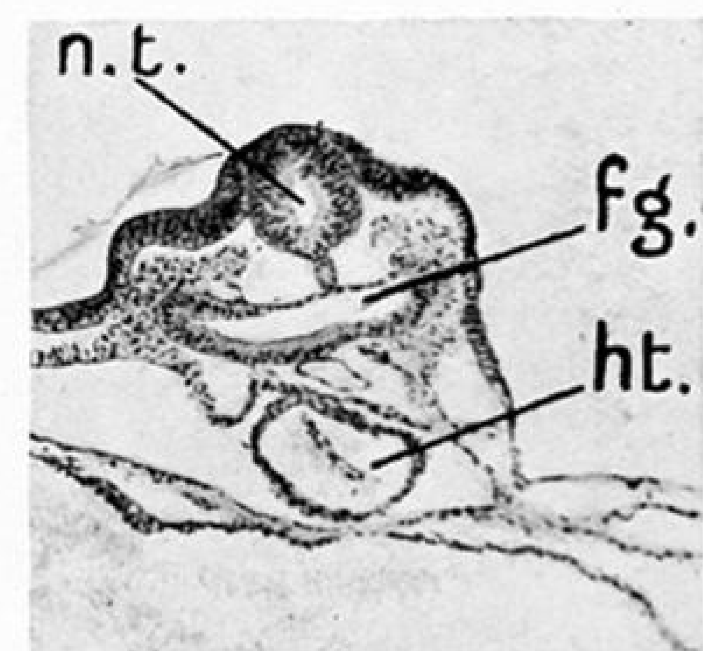

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PLATE 25.

FIG. 42.—290 (17¼–43). Ectoderm and endoderm turned. The original ectodermal axis was parallel to the longer side of the page. Straight embryo. *fg.*, edge of foregut. *ht.*, heart. (× 23.)

FIG. 43.—323 (22–45). Ectoderm and endoderm turned, straight embryo. Section through heart region. *n.t.*, neural tube. *fg.*, foregut. *ht.*, heart.

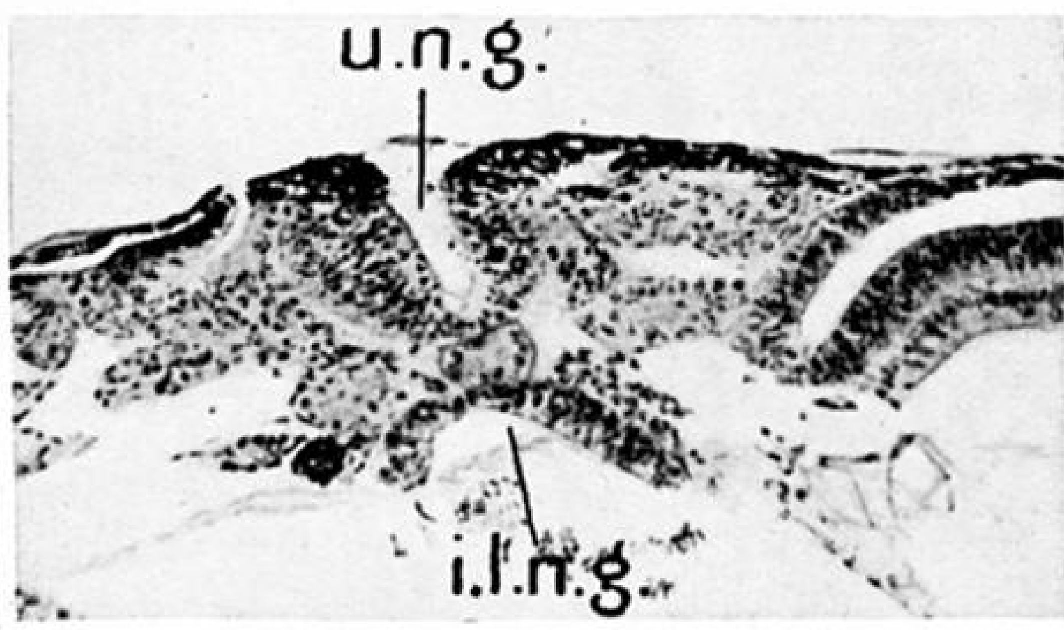
FIG. 44.—495 (21–20). Two epiblasts cultivated together. Section through anterior region. *l.n.g.*, neural tube of lower epiblast (inducer). *i.n.g.*, induced neural tube in upper epiblast. (× 260.)

FIG. 45.—Same specimen. *u.n.g.*, neural plate of upper epiblast. *l.n.g.*, neural plate of lower epiblast. *i.n.g.*, induced neural plate in upper epiblast. *s.*, somites. *nch.*, notochord. (× 90.)

FIG. 46.—Same specimen. Letters as last figure. (× 175.)

FIG. 47.—500 (18¼–22). Two epiblasts. Section through anterior region. *u.n.g.*, neural plate of upper epiblast. *l.n.g.*, neural plate of lower epiblast. *i.n.g.*, induced neural plate in upper epiblast. (× 60.)

FIG. 48.—Same specimen, section further posteriorly. Letters as in last figure. (× 175.)



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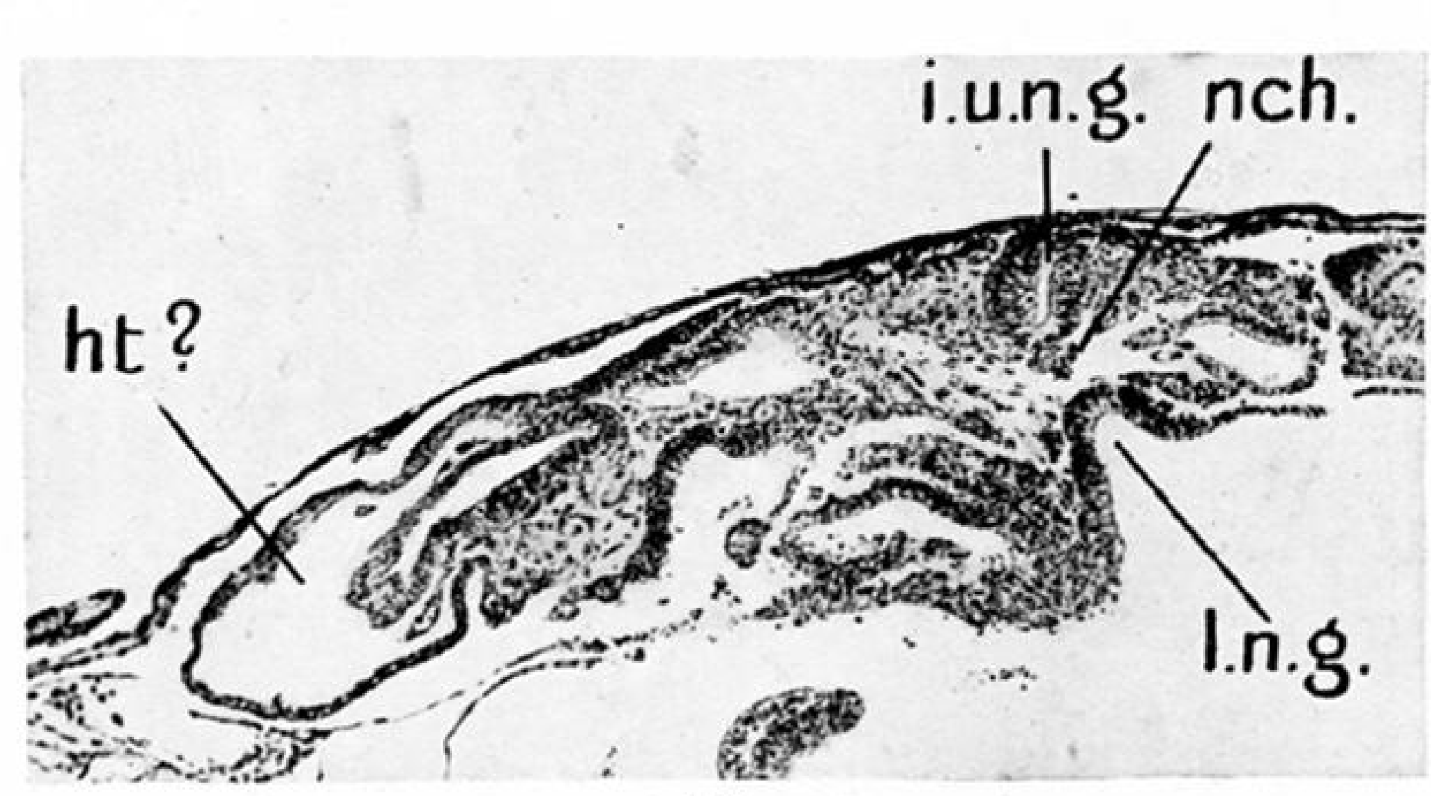

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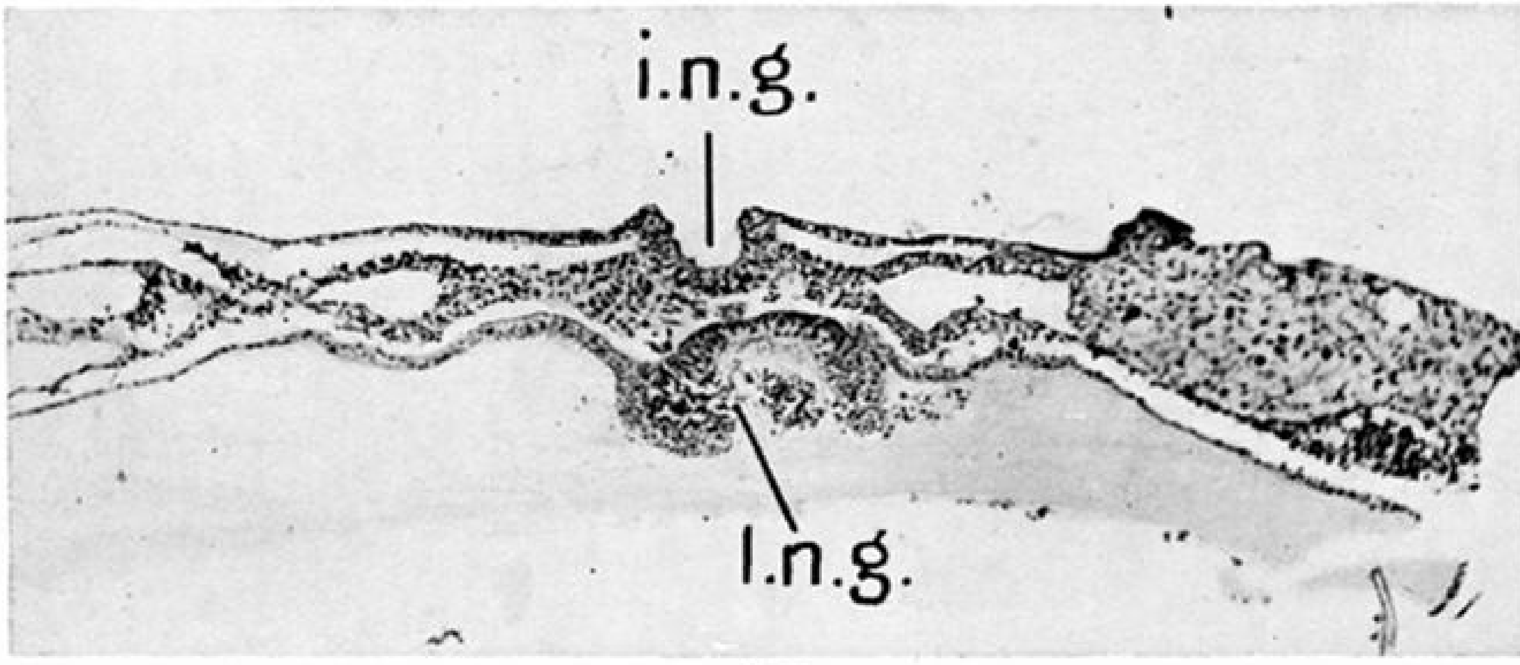

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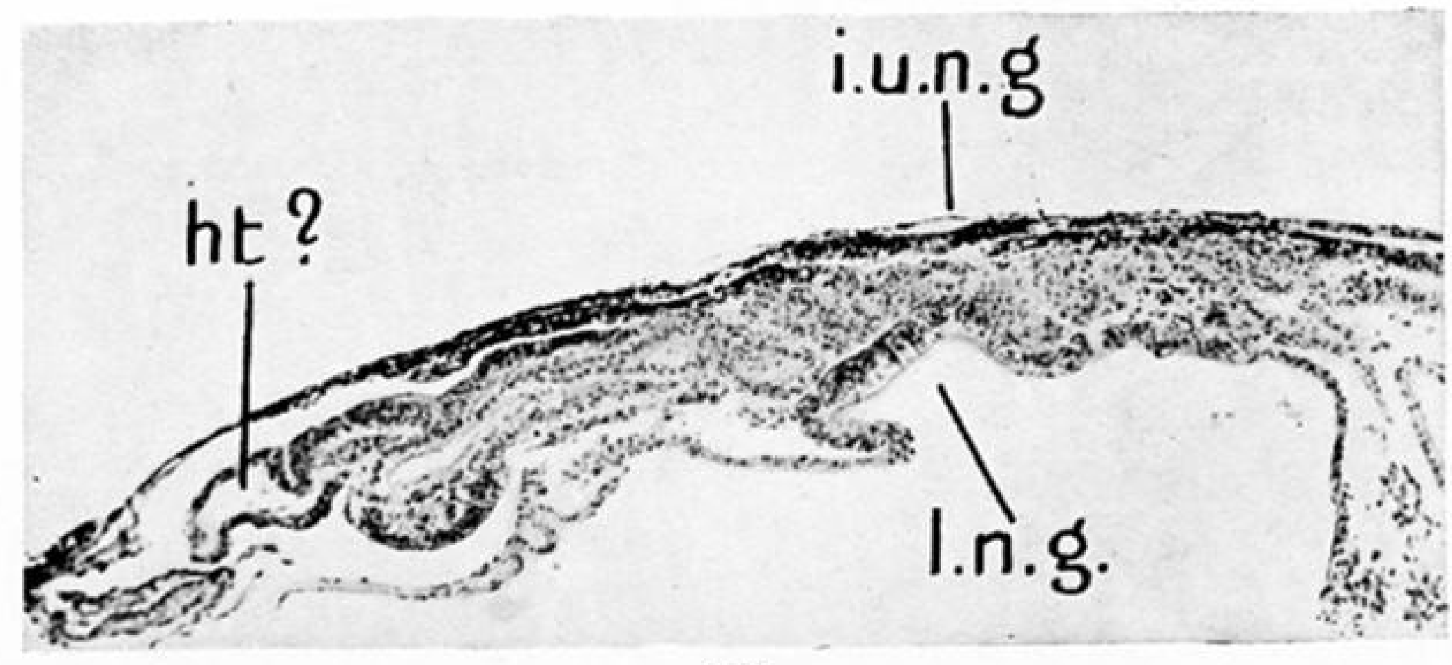

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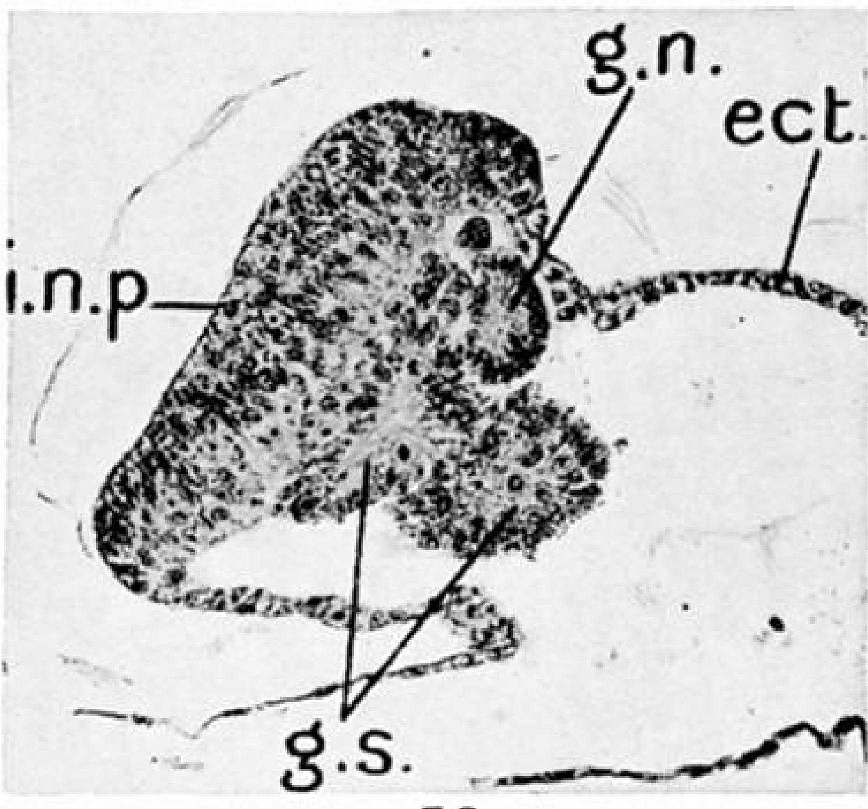

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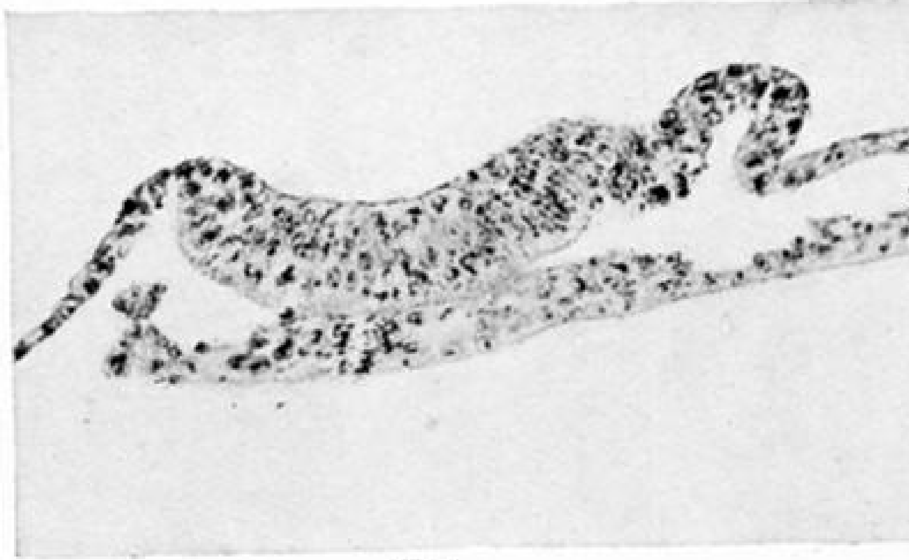
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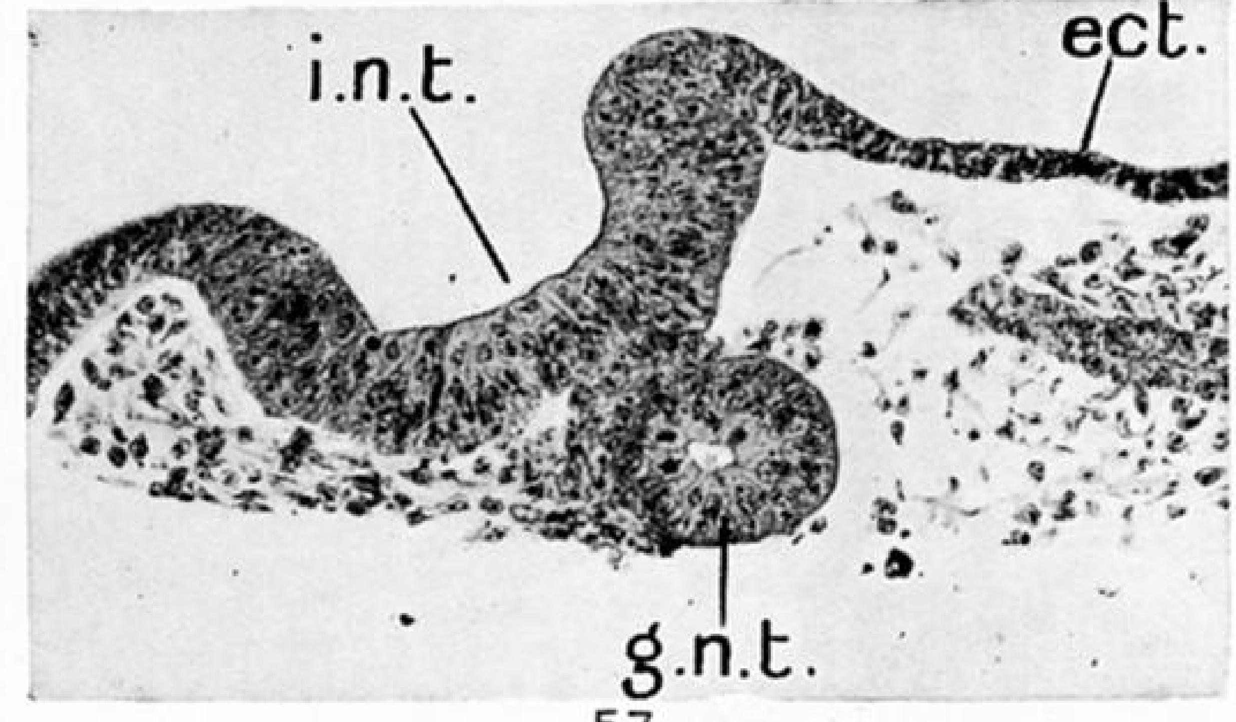

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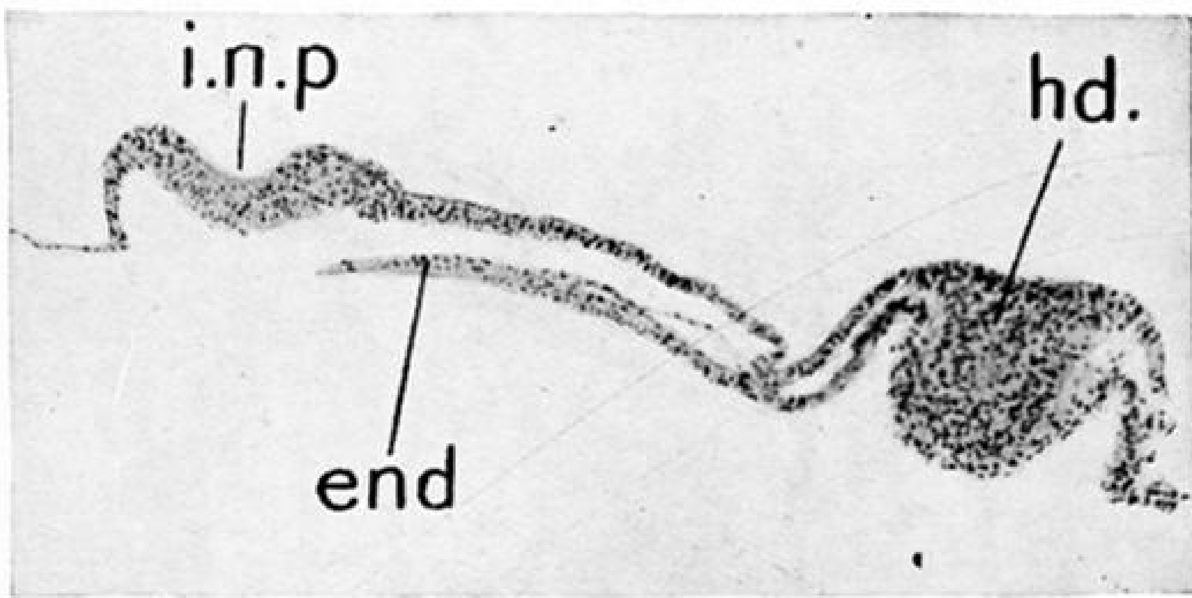

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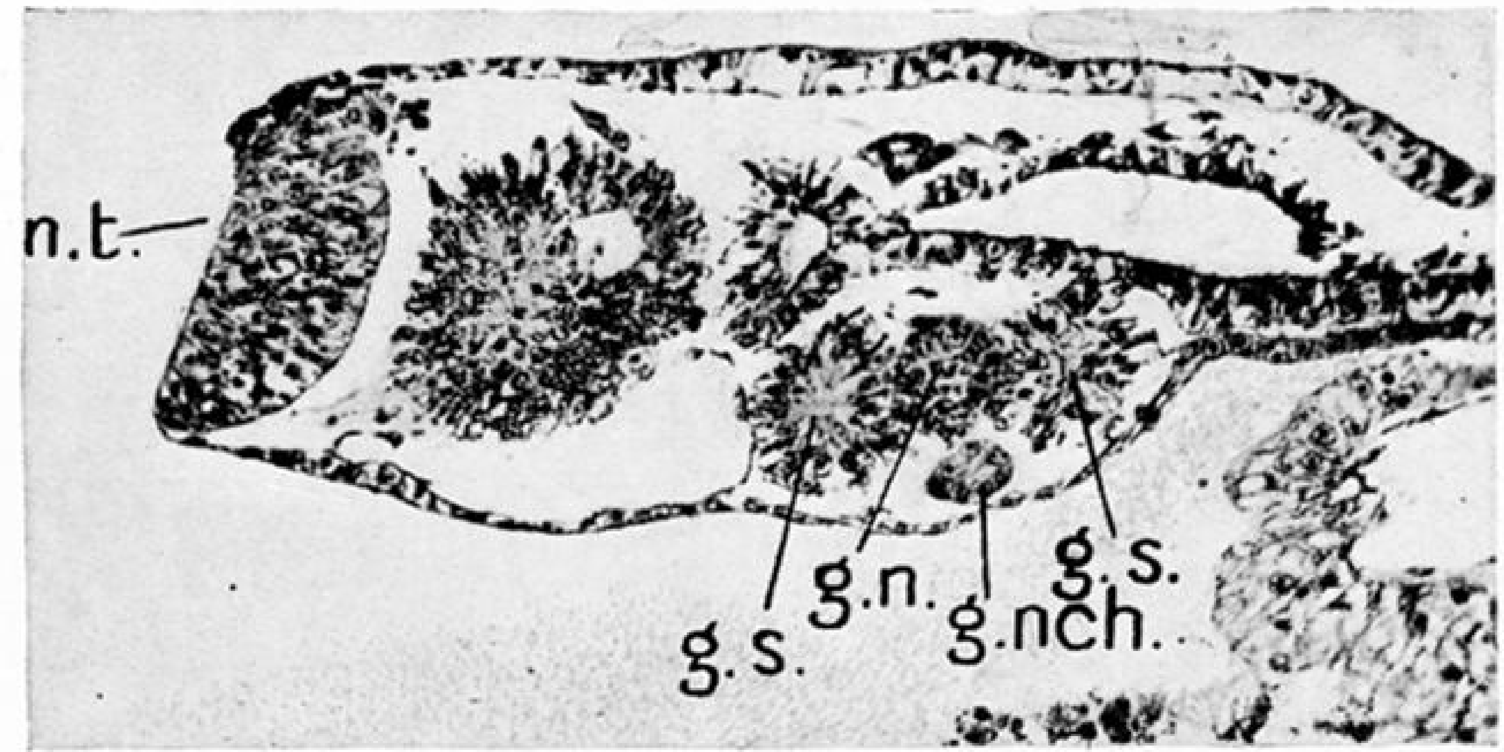

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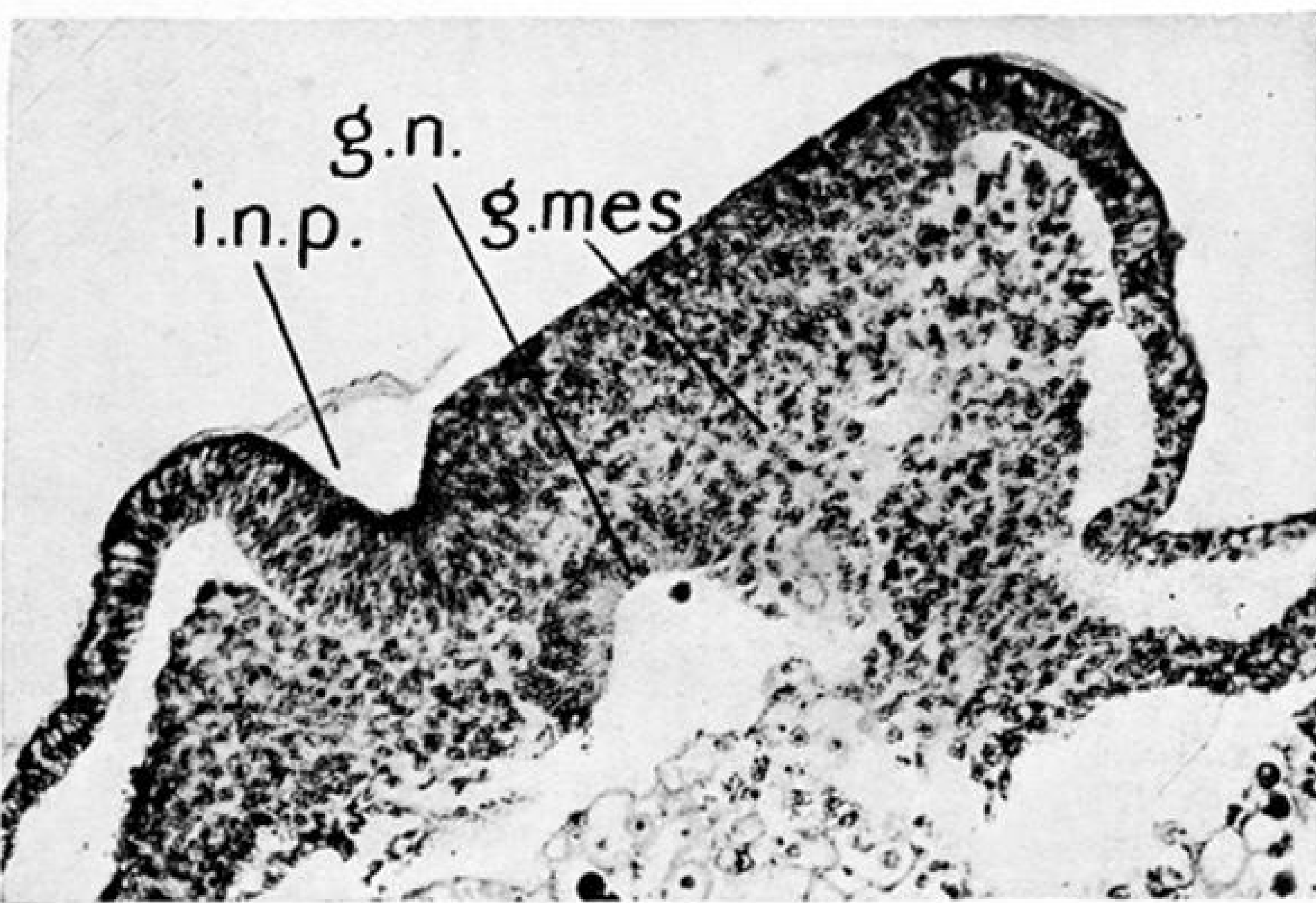

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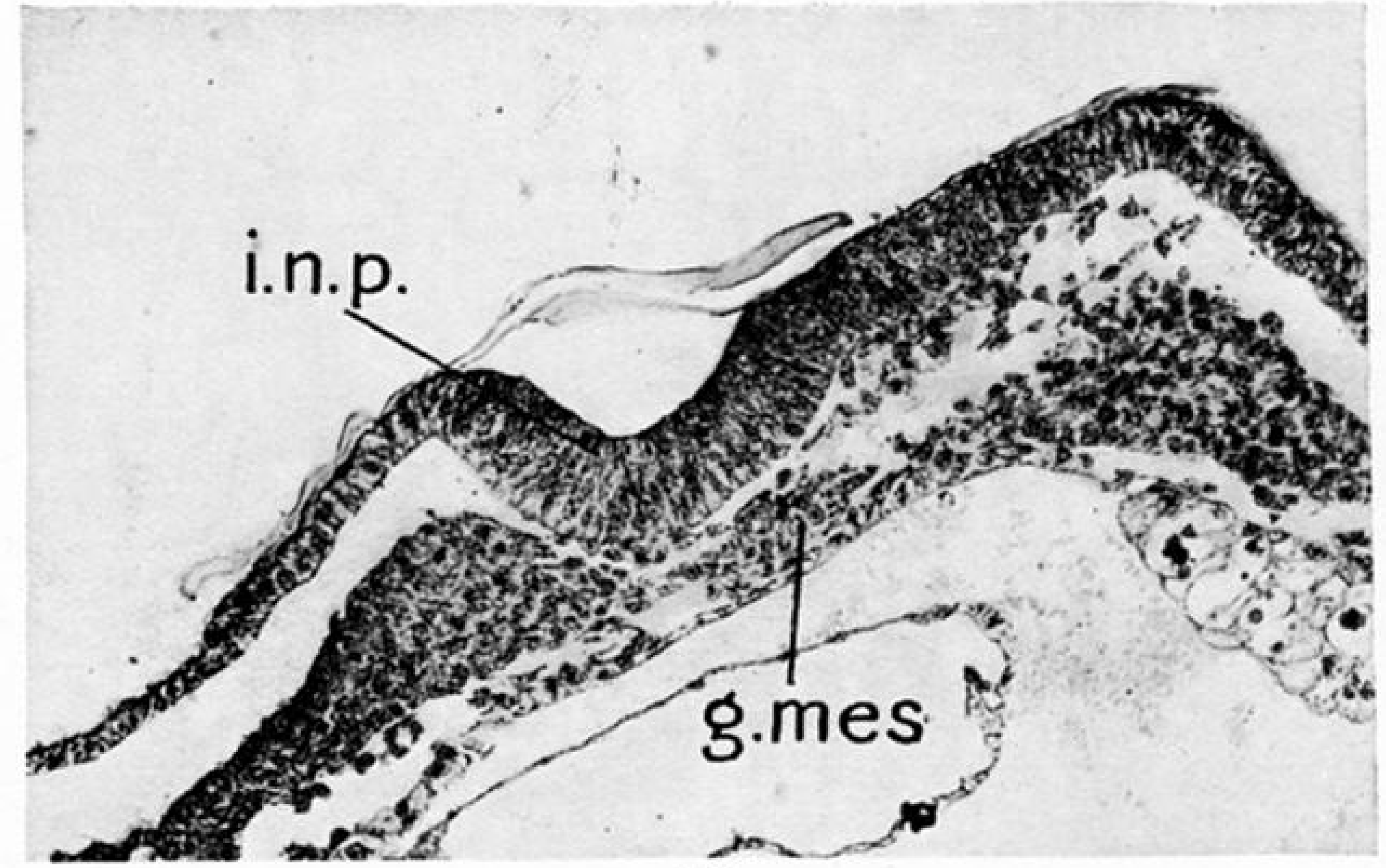

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PLATE 26.

FIG. 49.—479 (17–49). Two epiblasts. Section. *u.n.g.*, neural groove of upper epiblast. *i.l.n.g.*, neural groove induced in lower epiblast. (× 110.)

FIG. 50.—Same specimen. *l.n.g.*, neural groove of lower epiblast. *i.u.n.g.*, neural groove induced in upper epiblast. *ht.*, ? heart rudiment. *nch.*, notochord. (× 75.)

FIG. 51.—Same specimen. Letters as last figure. (× 75.)

FIG. 52.—349 (18–28¼). Two epiblasts, section through central region. Letters as in last figure. (× 60.)

FIG. 53.—572 (21½–19). Homoplastic* graft of anterior third of primitive streak. Section through anterior region. *ect.*, host ectoderm. *i.n.p.*, induced neural plate. *g.s.*, somites derived from graft. *g.n.*, neural material derived from graft.

FIG. 54.—Same specimen, section further posteriorly. Induced neural plate underlain by a thin layer of graft mesoderm.

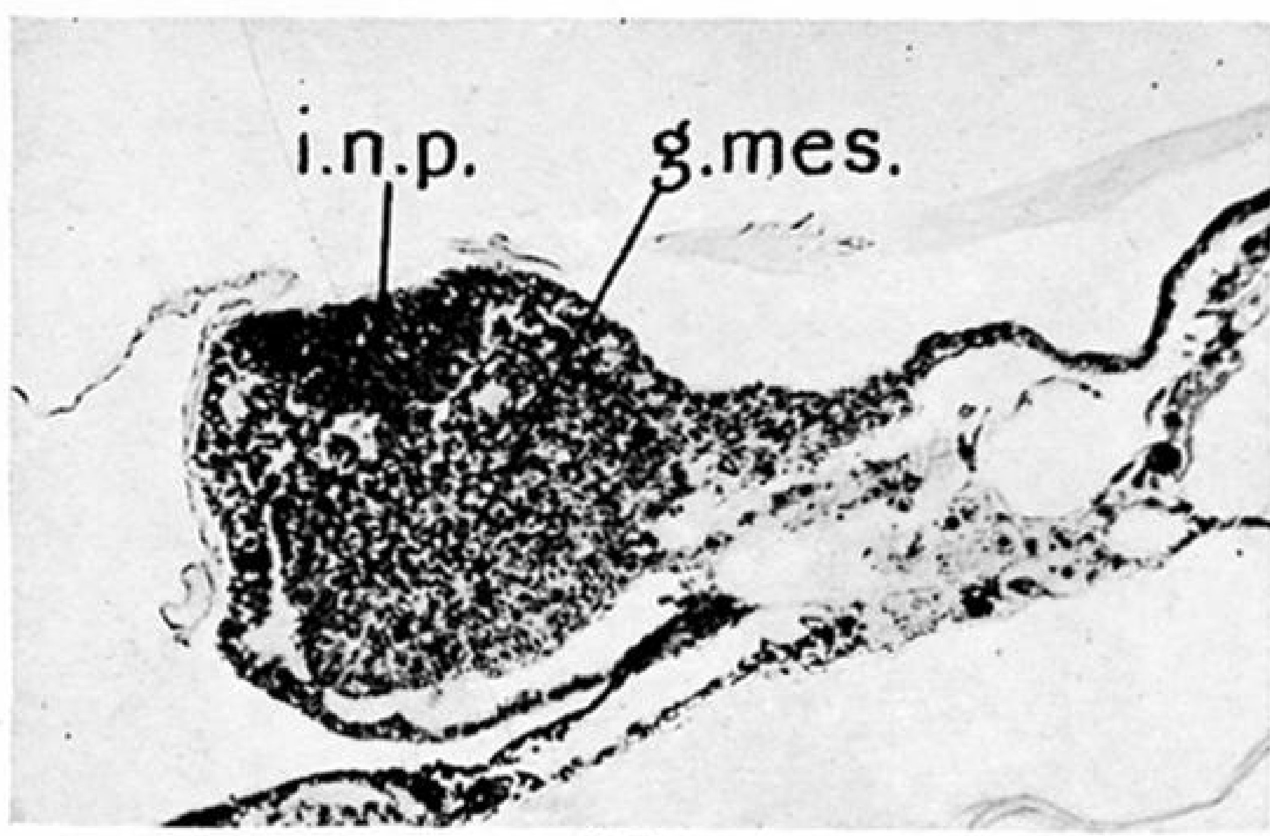
FIG. 55.—Same specimen, section still further posteriorly. *i.n.*, induced neural plate. *hd.*, head of host embryo. *end.*, torn endoderm. (× 60.)

FIG. 56.—398 (22¾–22). Autoplastic graft of primitive pit region. Section through central region of graft. See also Plate 28, fig. 72. *n.t.*, sidewall of host neural tube. *g.s.*, somites derived from graft. *g.n.*, graft neural tissue. *g.nch.*, graft notochord. (× 175.)

FIG. 57.—596B (20½–25). Homoplastic graft of anterior half of primitive streak. Section. *g.n.t.*, graft neural tube. *i.n.p.*, induced neural plate. *ect.*, host ectoderm. (× 175.)

FIG. 58.—575 (40 ?–23). Duck, homoplastic graft of middle third of primitive streak. Section. *i.n.p.* induced neural plate. *g.mes.*, mesoderm from graft. *g.n.*, neural tissue from graft. (× 175.)

FIG. 59.—Same specimen. Induced neural plate underlain by graft mesoderm. (× 175.)



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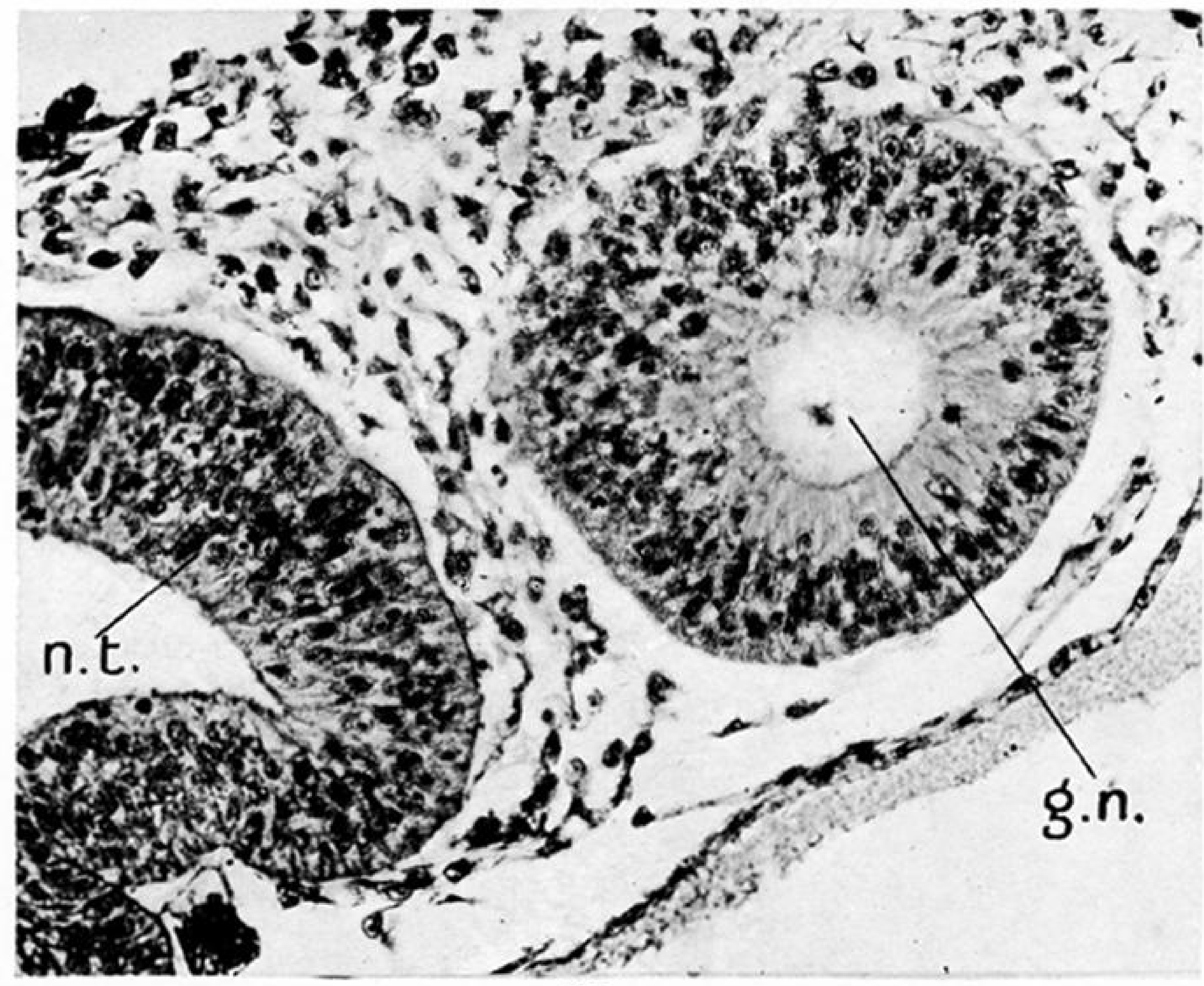

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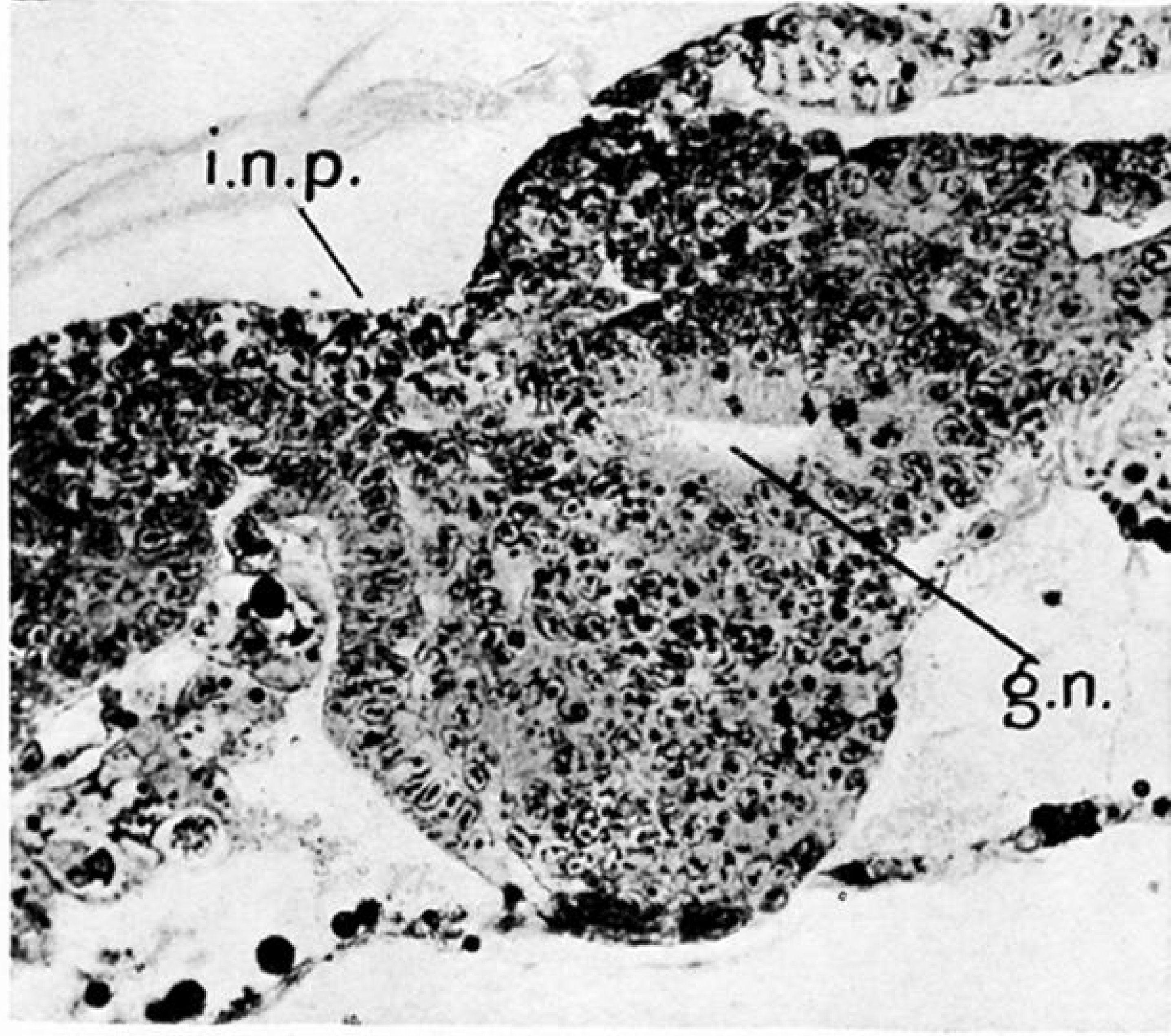

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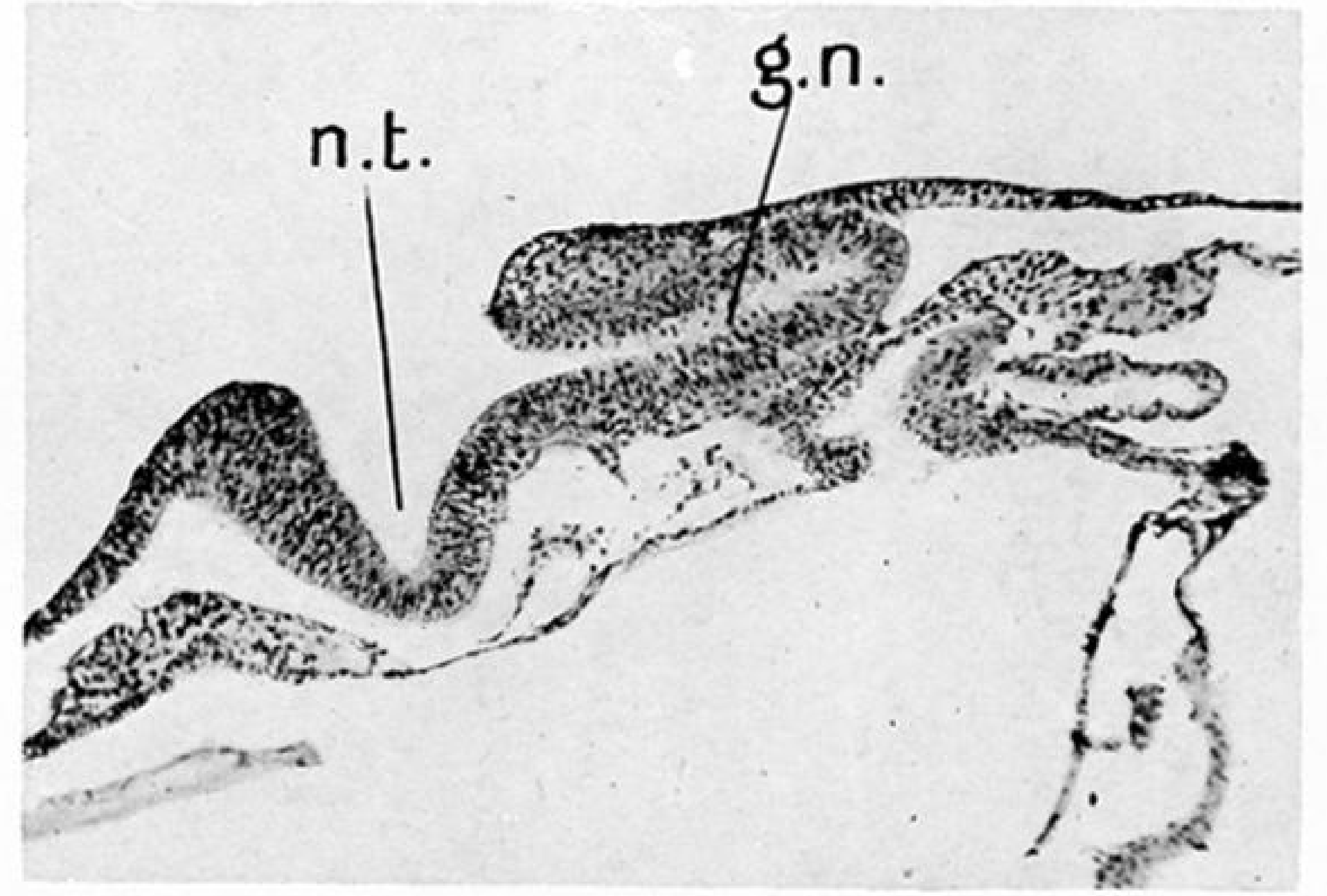

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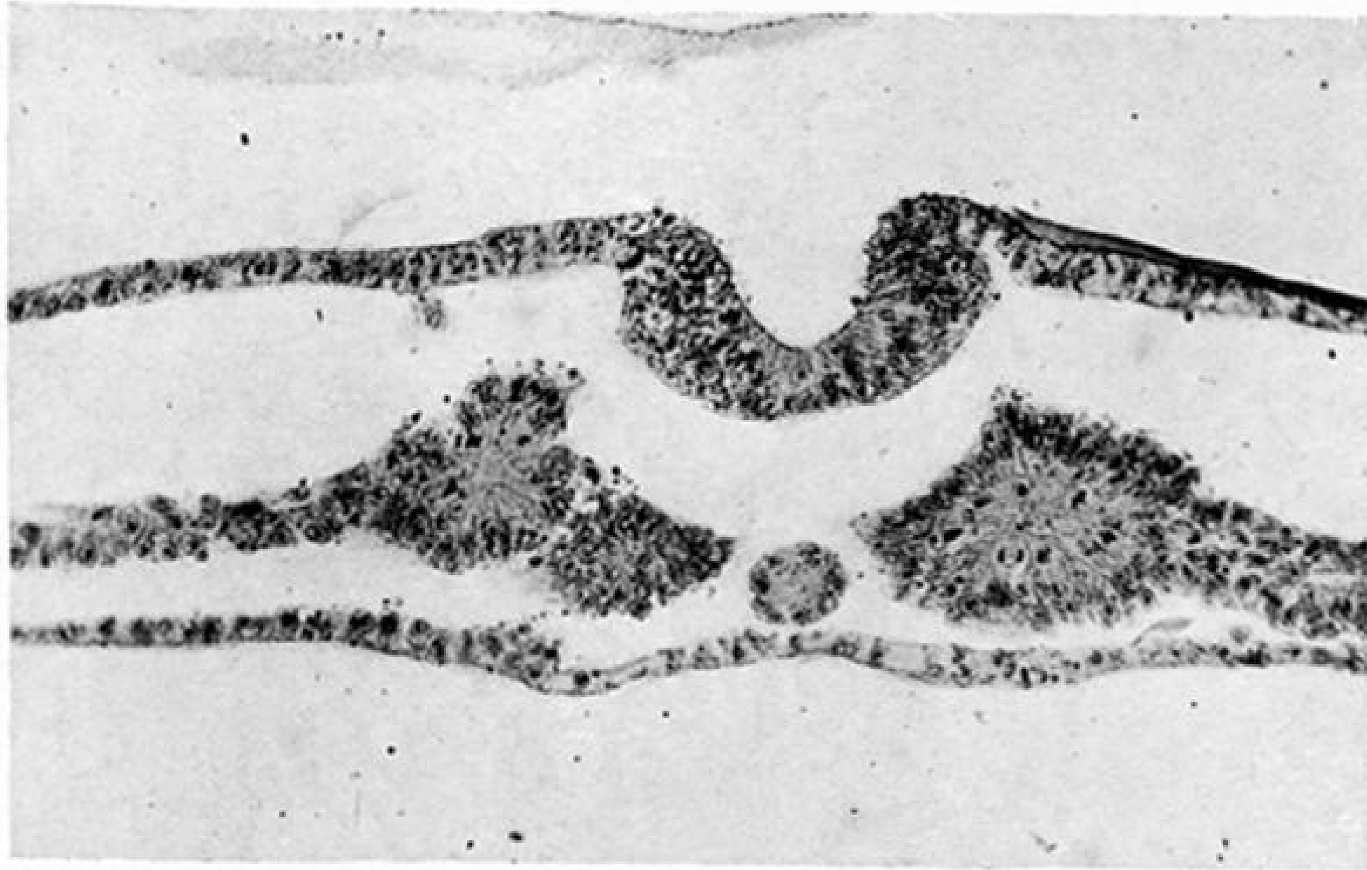

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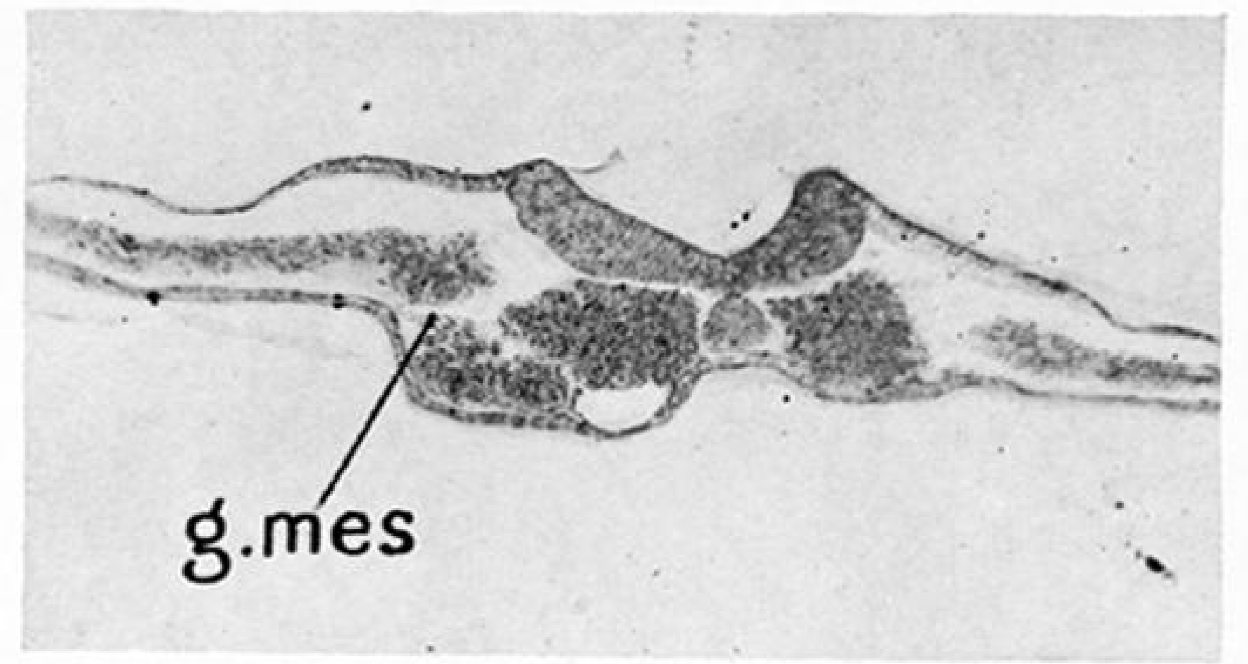

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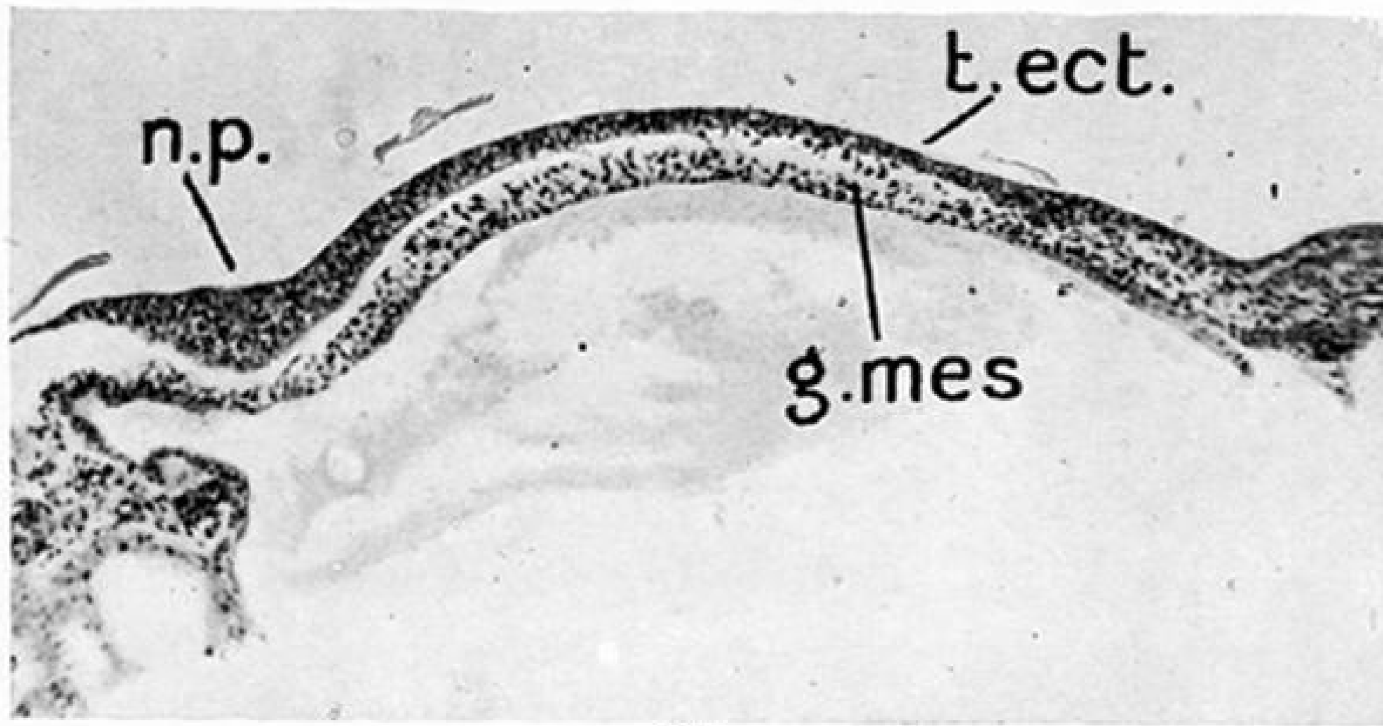

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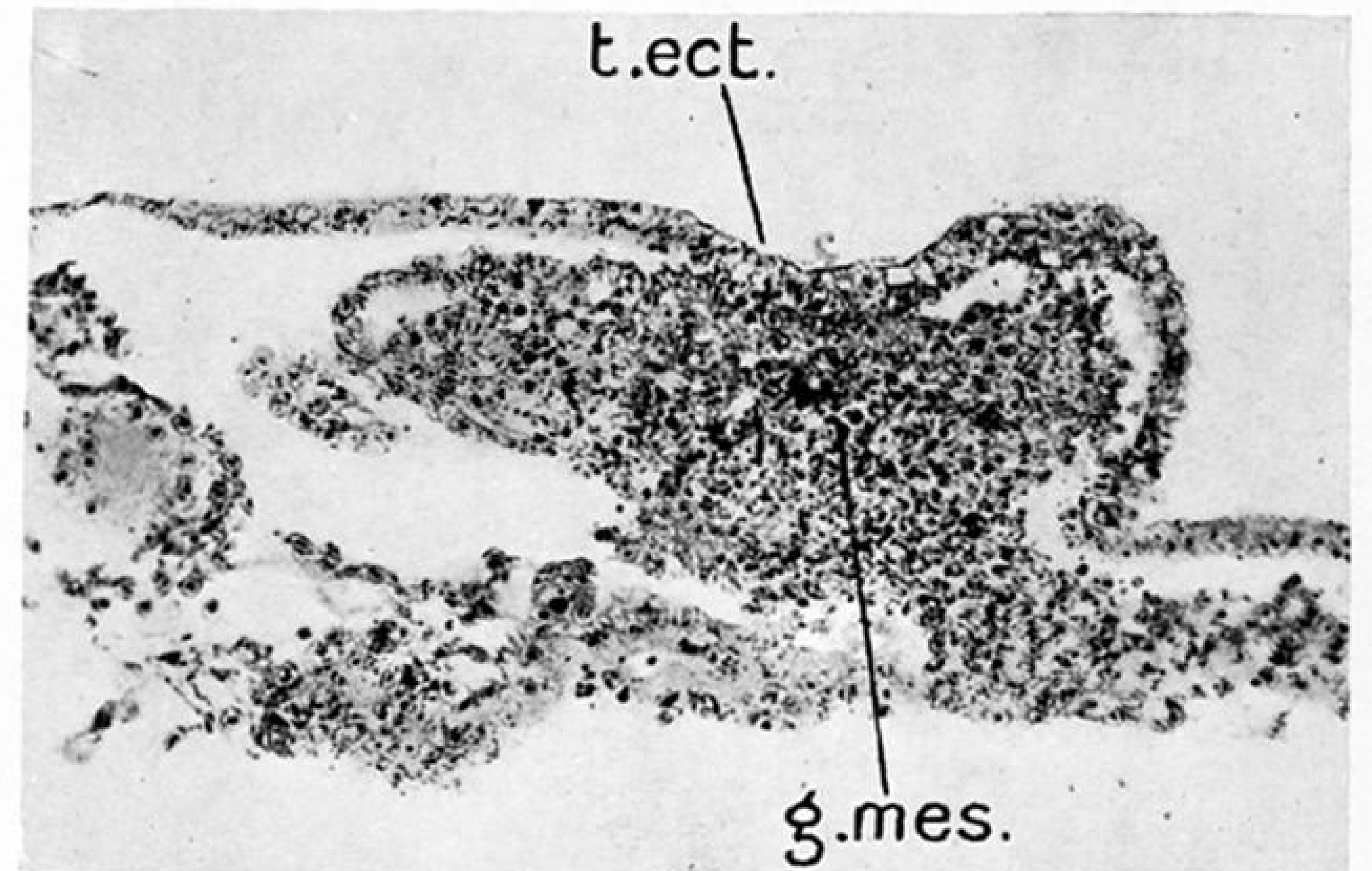

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PLATE 27.

FIG. 60.—611A (19–28). Homoplastic graft of middle third of primitive streak. *g.mes.*, graft mesoderm. *i.n.p.*, induced neural plate. (× 175.)

FIG. 61.—Same specimen. *g.n.*, graft neural tissue. *i.n.p.*, induced neural plate. (× 250.)

FIG. 62.—555 (20–31). Homoplastic graft of middle third of primitive streak. *n.t.*, host neural tube. *g.n.*, graft neural tissue. (× 250.)

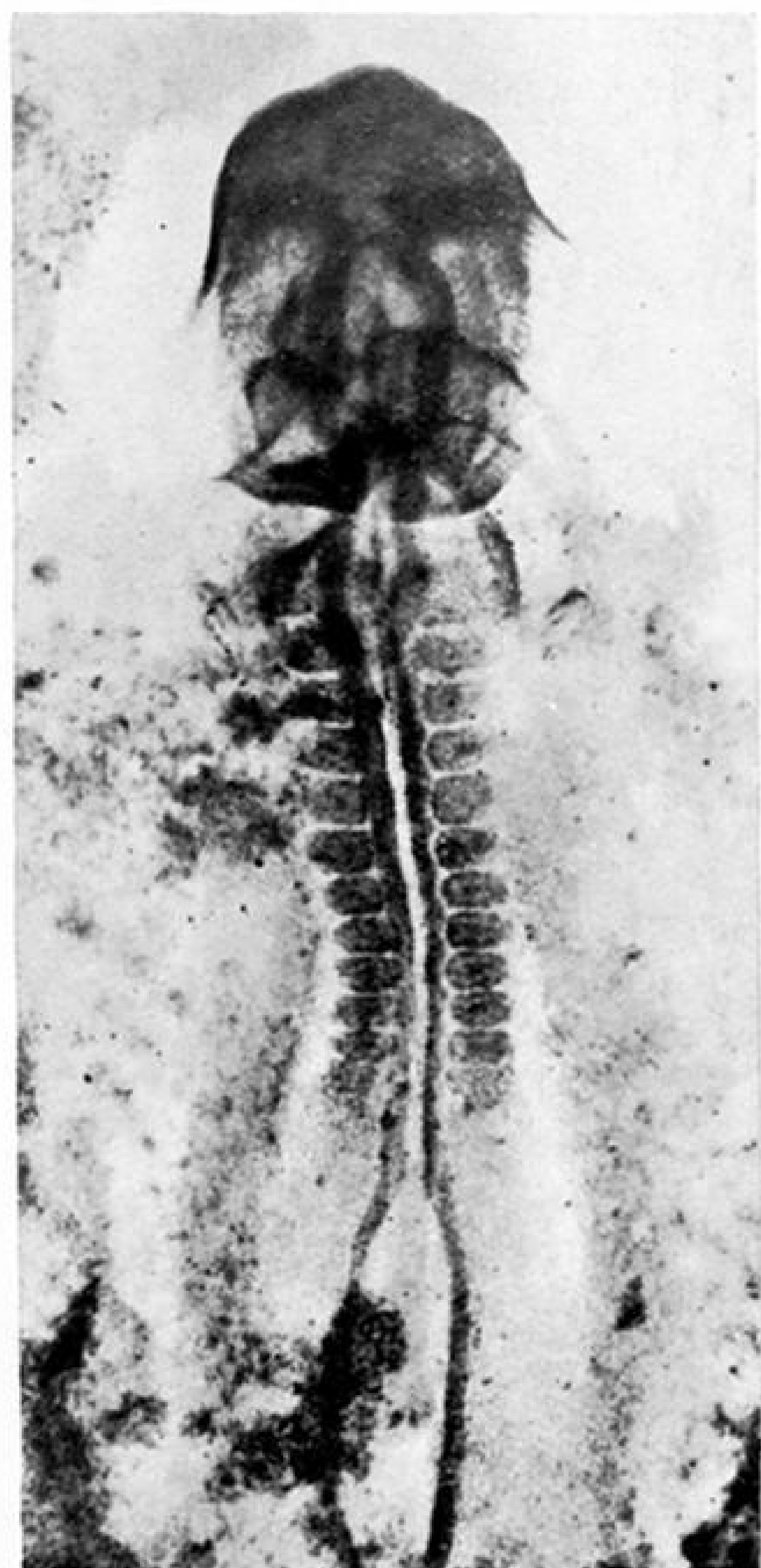
FIG. 63.—Same specimen. *n.t.*, host neural tube. *g.n.*, graft neural tissue. (× 90.)

FIG. 64.—518 (18½–c. 24). Homoplastic graft of middle third of primitive streak of blastoderm aged 15 hours. Graft stained with Nile Blue Sulphate. *g.mes.*, graft mesoderm. (× 90.)

FIG. 65.—428 (20¼–29¼). Autoplastic graft of middle third of primitive streak. Note doubled somite on left side. (× 90.)

FIG. 66.—596A (20½–27). Homoplastic graft of posterior half of primitive streak. *g.mes.*, graft mesoderm. *t.ect.*, thickened host ectoderm. (× 175.)

FIG. 67.—554 (20–31). Homoplastic graft of posterior third of primitive streak. *n.p.*, host neural plate. *t.ect.*, thickened ectoderm. *g.mes.*, graft mesoderm. (× 60.)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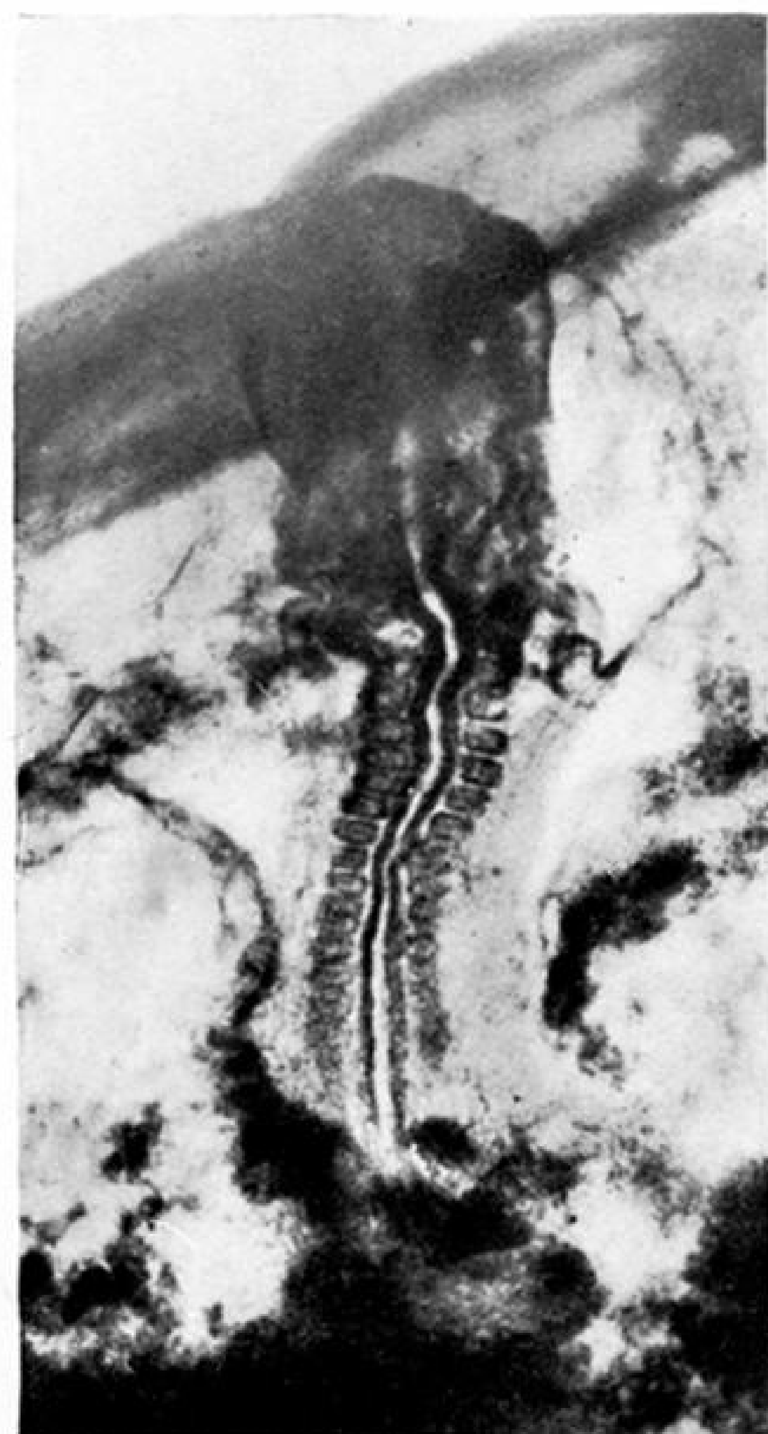
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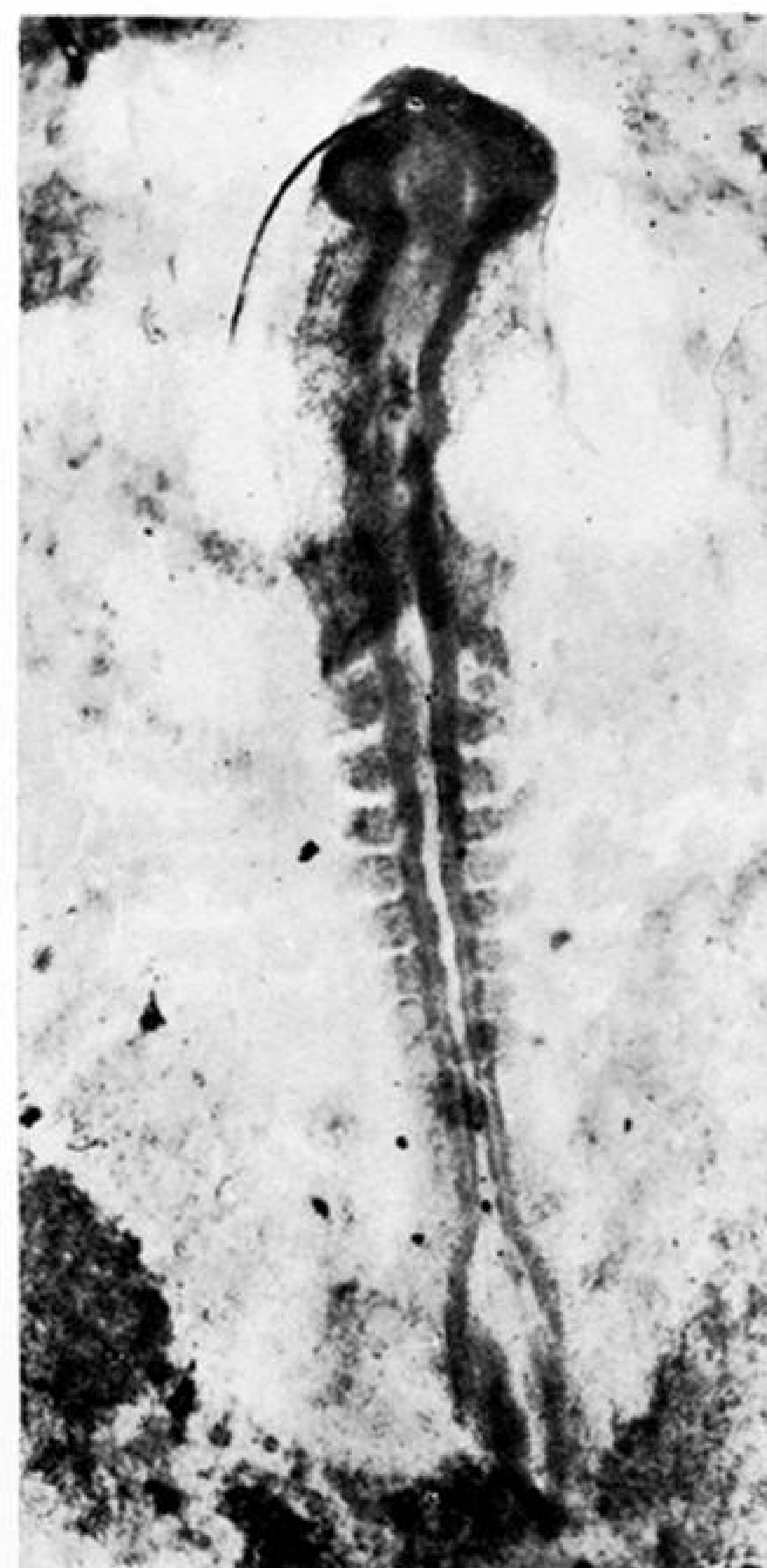
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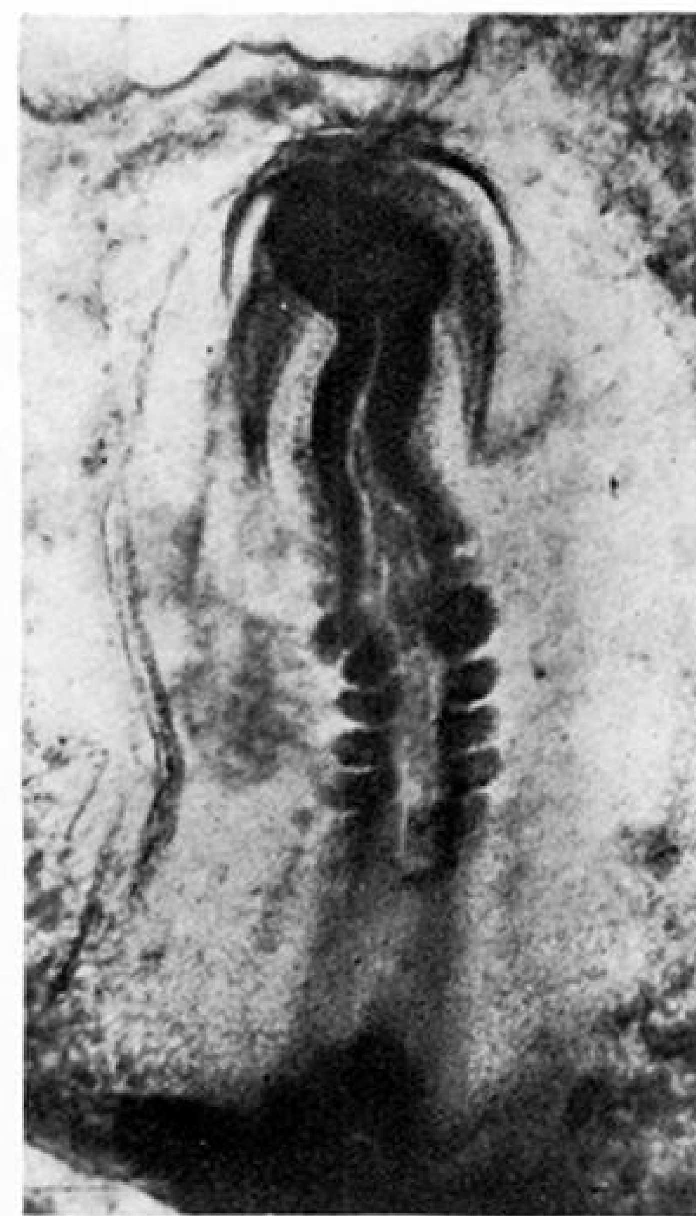
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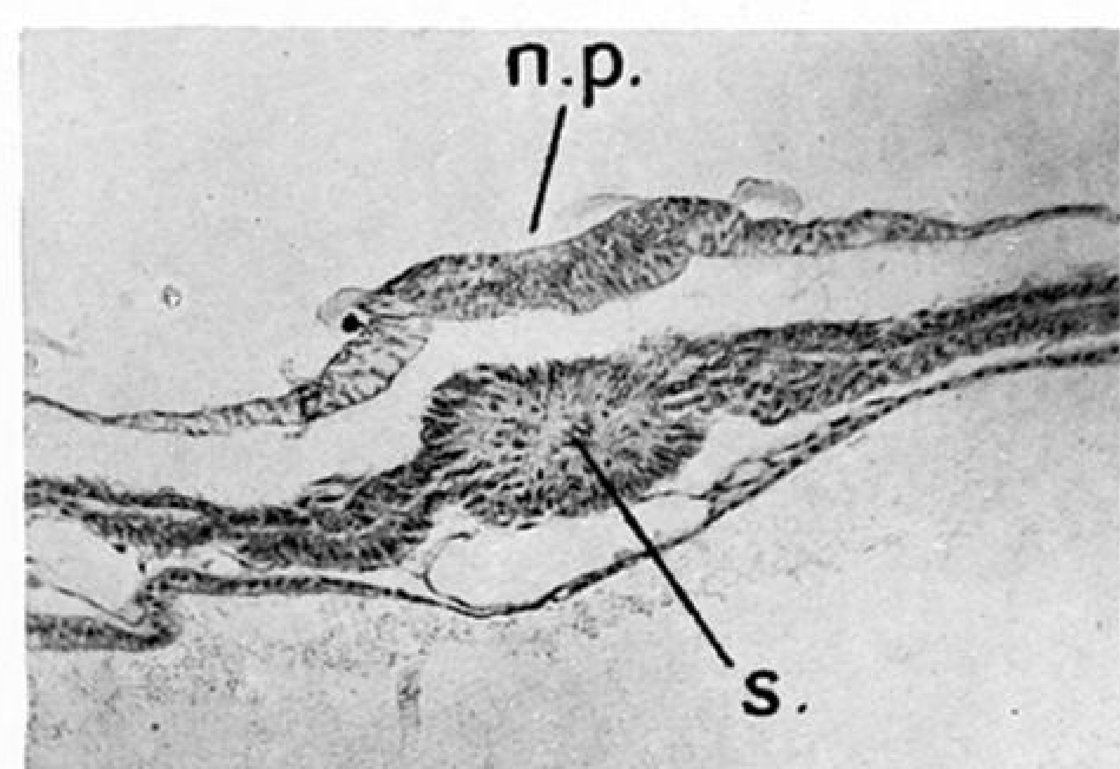

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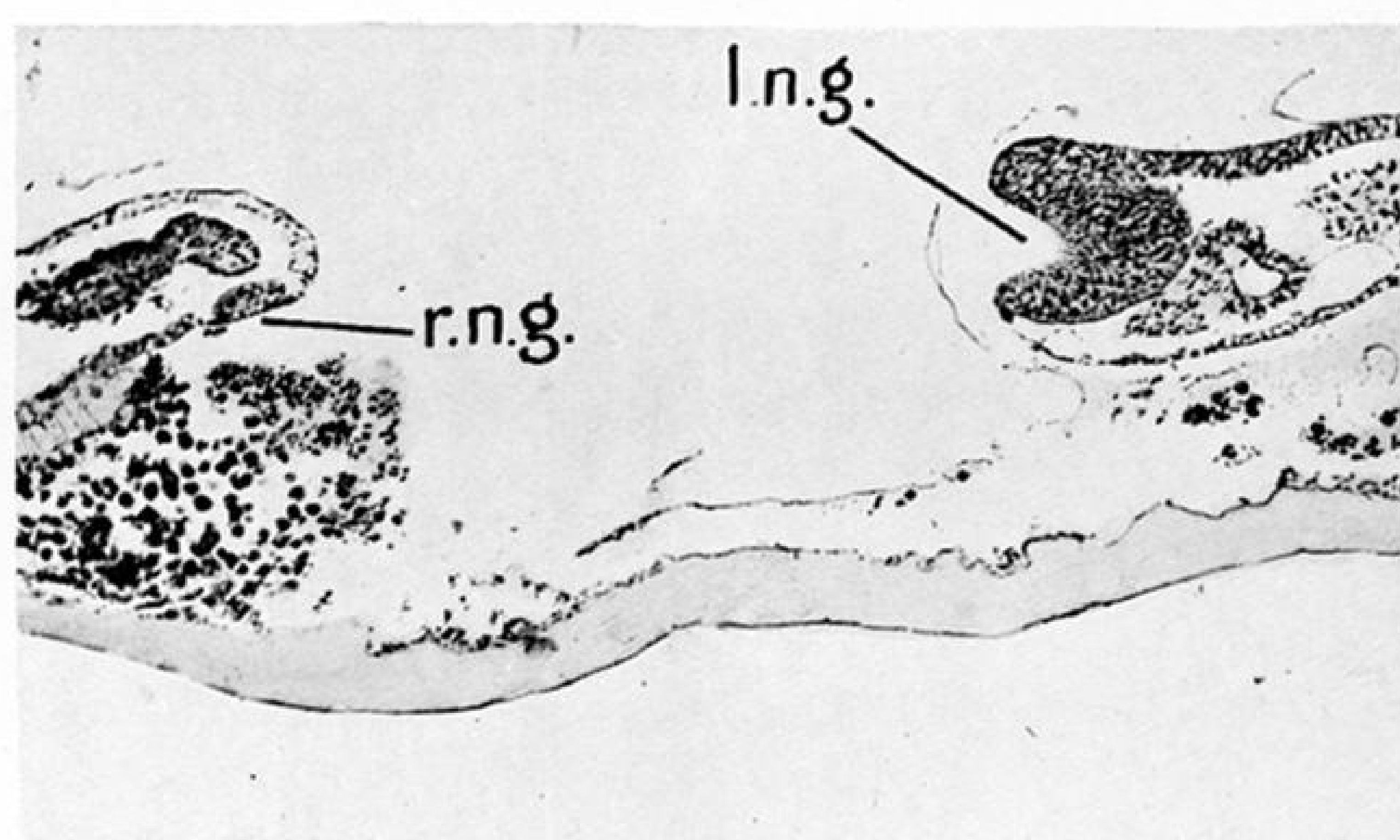

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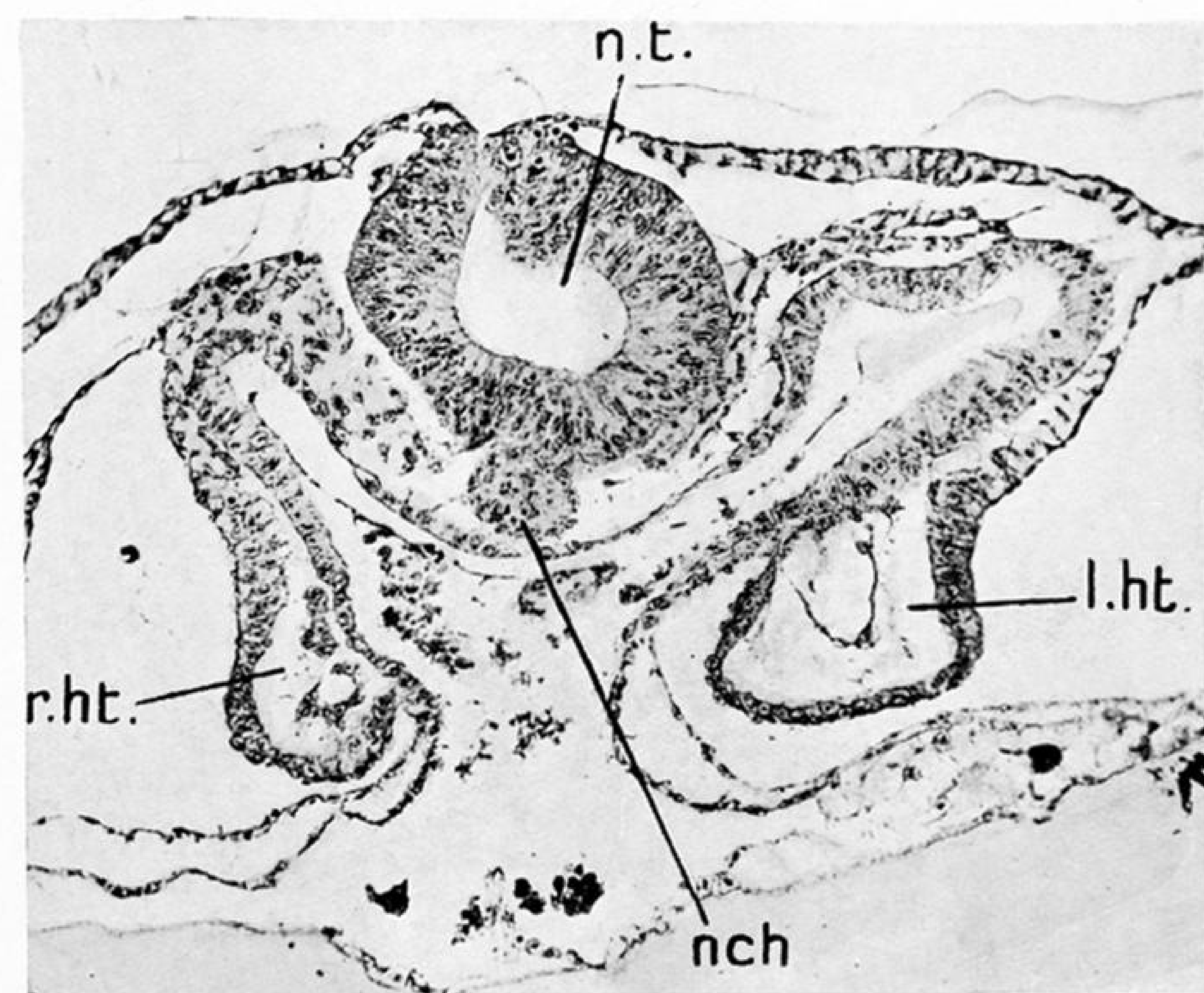

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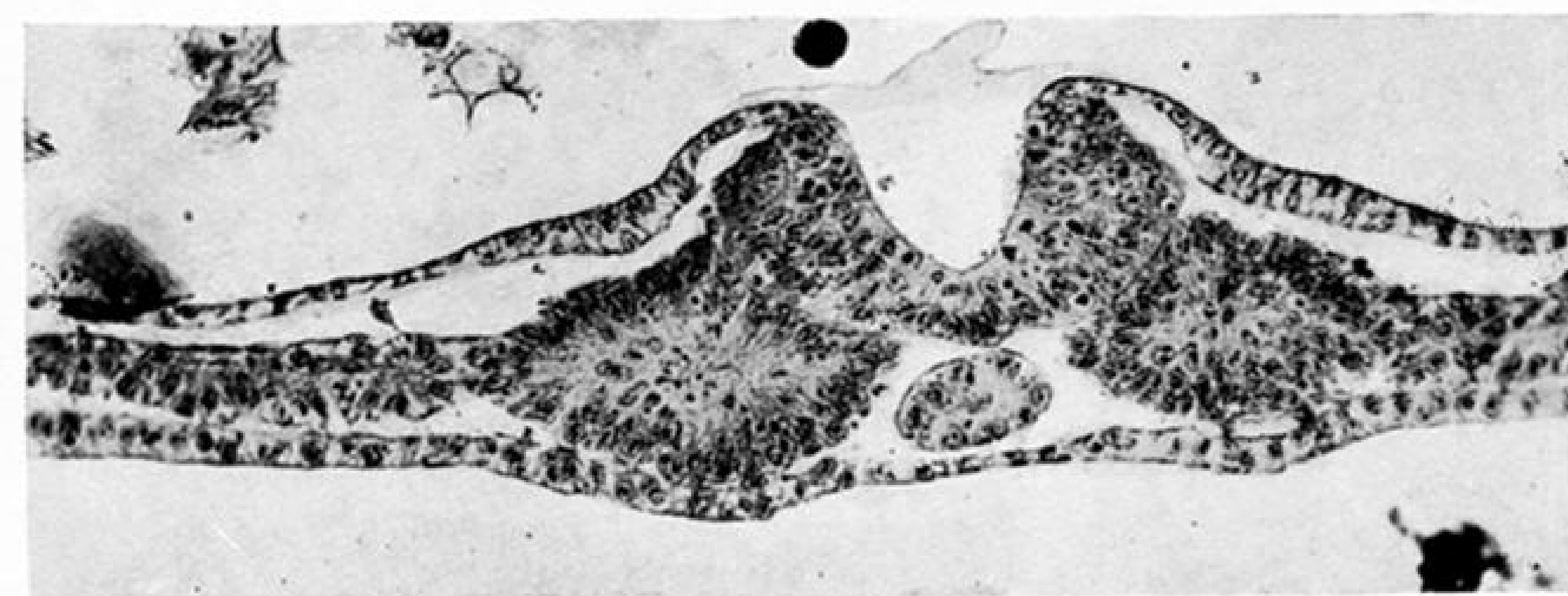
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PLATE 28.

FIG. 68.—418 (24–17½). Third quarter of primitive streak removed. (× 27.)

FIG. 69.—176 (21½–43). Middle third of primitive streak removed. (× 30.)

FIG. 70.—153 (22½–22½). Middle third of primitive streak removed. (× 27.)

FIG. 71.—145 (21½–20). Primitive pit region removed. (× 30.)

FIG. 72.—398 (22¾–22). Autoplastic graft of primitive pit region. Section anterior to graft region. See also Plate 26, fig. 56. *r.n.g.*, right side of neural groove. *l.n.g.*, left side of neural groove. (× 80.)

FIG. 73.—173 (20–25). Autoplastic graft of primitive pit region. Section posterior to graft. See also fig. 20. *n.p.*, neural plate. *s.*, fused somites. (× 80.)

FIG. 74.—407 (20–28). Entire primitive streak removed. (× 30.)

FIG. 75.—423 (17½–29). Entire primitive streak removed. Section through heart region. *n.t.*, neural tube. *nch.*, notochord. *l.ht.*, left heart rudiment. *r.ht.*, right heart rudiment. (× 175.)

FIG. 76.—Same specimen. Section further posteriorly. (× 175.)

* Homoplastic : a graft into an animal other than the donor, but belonging to the same species.

Autoplastic : a graft into the donor, in these experiments always into a place different to the place of removal, *i.e.*, always heterotope.