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INDUCTION BY THE ENDODERM IN BIRDS.

by

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With 17 figures in the text.

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HOADLEY (1927) and GRAPER (1929) have suggested that in the birds the formation of the endoderm probably plays an organising rôle similar to that of the invagination which takes place at the blastopore in the Amphibia. The purpose of this paper is to determine in how far this suggestion is true. It will be shown that the endoderm can indeed exert an organising influence on the epiblast, but that this influence is not exactly paralleled by the induction performed by the roof of the primitive gut in the Amphibia, which corresponds more nearly to the induction by the mesoderm arising from the primitive streak in the birds. The function of the endoderm in the birds appears to be one which is as yet unknown in other groups.

In a recent publication (WADDINGTON 1932) I described some experiments in which the endoderm was separated from the epiblast, rotated through 90° and then replaced, so that the anterior-posterior axes of the two layers were at right angles to one another. The experiments demonstrated an effect of the anterior-posterior axis of the endoderm on the direction of elongation of the primitive streak, which became curved so that its anterior end pointed towards the original anterior region of the endoderm. This suggests that the tissue movements in the epiblast, which build up the primitive streak, go on in a direction which is determined by the endoderm. In the experiments just mentioned, no decision could be reached as to whether the endoderm merely guided the movements once they had started, or whether it was also able to initiate them. In the experiments to be described in this paper, the endoderm was turned so that its anterior-posterior axis was diametrically opposed to that of the epiblast. It was thought that, if the endoderm did in fact induce a new set of tissue movements, there would be a greater likelihood of these leading to the formation of a new primitive

[1] This work was done while I was in receipt of a part-time grant from the Medical Research Council, for which I should like to express my thanks.

streak if they were diametrically opposed to the original set of movements, since if the two sets were only at right angles to one another, it is easy to imagine that they might merge into one another and give rise to a single primitive streak in an intermediate, resultant position.

In the experiments with the endoderm turned through 180° in this way, a sufficient number of positive results have been obtained to show that the endoderm can induce a new set of movements, which give rise to a new primitive streak, which itself can of course proceed to develop into an embryo. This appears to be a case of induction which is in some ways more radical than anything which is known in the Amphibia, since here the process of mesoderm formation is determined as well as the fate of the ectoderm. But, as will be pointed out in the discussion, the induction performed by the endoderm is probably an induction of form-building movements, rather than of a specific organ or tissue.

Experimental procedure.

The blastoderms (chick or duck) were removed from the egg and cultivated *in vitro* by the method previously described (WADDINGTON 1932). Essentially, the technique consists in keeping the blastoderm on the surface of a clot composed of equal parts of blood plasma from an adult fowl and of a saline extract of a chick embryo of 8 or 9 days' incubation. The clot is made in a watch glass, which is placed in a Petri dish containing a ring of cottonwool soaked in boiled distilled water, which serves to keep the atmosphere moist.

The method of operation is essentially the same as that described in my former paper, except of course that the epiblast is rotated through 180° instead of 90° before being replaced over the endoderm. The blastoderm is laid on its ectodermal surface with the posterior part nearest the operator and the anterior part furthest away. The endoderm is then loosened from the epiblast and eventually peeled off from it by being turned through 180° about a horizontal axis running across the field of view from left to right. At the end of this manoeuvre, the endoderm is lying with its lower face to the clot and with its anterior end nearest the operator. The epiblast is then turned 180° about its anterior-posterior axis, leaving it with its lower face on the surface of the clot, and its posterior end nearest the operator. The epiblast is then placed on top of the endoderm, and the liquid in which the operation has been performed is pipetted off, care being taken that the two layers of tissue do not move relatively to one another during this process. The direction of the anterior-posterior axis of the epiblast is then marked with an arrow drawn in grease pencil on the lower surface of the PETRI dish. It is often advantageous to make a series of radial cuts around the edge of the epiblast, since this makes it easier to flatten this tissue, which is really shaped like part of the surface of a sphere.

The operation has in many cases been performed on blastoderms which have only a very short primitive streak. In such cultures it is sometimes difficult to mark the direction of the anterior-posterior axis very accurately, since the primitive streak may be difficult to see after the two layers have been brought together again. But this slight inaccuracy does not seem to be very important, since it is common for the embryos developed in these cultures to diverge by as much as 30^0 from the marked directions, probably owing to some sort of inhibition of the form building movements. In any case, there is no question of confusing the epiblastal with the endodermal directions.

Before the appearance of the primitive streak it is difficult to be sure, in the living specimen, of the position or direction of the anterior-posterior axis. In many cases, I have not dared to make a definite identification. But sometimes in the chick the endoderm can be seen to form a small circular sheet of coherent tissue in one part of the blastoderm at a stage when the rest of the endoderm is loose and, as it were, fluffy. This coherent patch, which is the Entodermhof of WETZEL (1929) lies excentrically in the *area pellucida*, being displaced slightly towards the anterior. It thus provides a mark which can be used for orientation. The position of the axis as determined in this way has always proved at least approximately correct, and so has its direction except in a few exceptional cases which are interpreted as inductions. (See specimens 796 and 797.) Shortly before the appearance of the primitive streak, the coherent part of the endoderm has spread backwards, and when the primitive streak appears the endoderm beneath it is already a definite and easily manipulated layer of tissue. In the duck, the endoderm seems to become coherent at a relatively earlier stage, and orientation by the *Entodermhof* is rarely possible.

The cultures of very young blastoderms, which must be cultivated for two days before they develop a embryo of satisfactory size, are unfortunately badly affected by the liquefaction of the clot. In two days cultivation, there is always considerable liquefaction, whatever the age of the blastoderm at explanting. But here, owing to the small size of the blastoderms (the growth rate *in vitro* is very much smaller than in the egg) the liquefaction has a greater effect than when the culture is started with an older embryo. It may even be the case that the younger blastoderms have a greater liquefying effect than older ones, but this is uncertain. It would be possible to change the blastoderms on to a new clot after one day's cultivation, but for these experiments in particular, where it is essential to keep the endoderm as intact as possible, this would not be advisable, since in changing the embryo it would be difficult avoid tearing this tissue, which adheres closely to the medium. In one set of experiments including 742 and 745, the plasma formed a soft clot which liquefied particularly extensively. The whole clot, with

the attached blastoderm, was therefore washed and slid out of its watch-glass on to the surface of another clot in another watch-glass; but this treatment was not very effective, since although the embryos apparently remained alive, which they probably would not have done if they had been left, they did not proceed with their development.

The specimens were fixed in BOUIN's fluid and stained in dilute DELAFIELD's haematoxylin. Only a few have been sectioned, since it is the direction of the embryonic axis, not the details of its structure, which is interesting. Those which have been sectioned were cut at 15 μ and stained in DELAFIELD's haematoxylin.

Description of specimens.

The results of the experiments are very variable. The embryos obtained fall into the following eight groups:

1. Embryo nearly normal, having the direction of the anterior-posterior axis of the epiblast, or nearly that direction.

2. Embryo similar, but very short, or not stretching across the entire *area pellucida*.

3. Main part of the embryo inclined at a large angle (up to 90°) to the original epiblastal direction.

4. The embryo developes in the epiblastal direction, but at some stage a transitory primitive streak appears in the opposite, endodermal direction, and later vanishes.

5. Semicircular embryos, with the anterior part in the epiblastal direction and the posterior part in the endodermal.

6. Two embryos develop, in opposed directions, with their heads together.

7. An embryo developes in the endodermal direction, and the original primitive streak disappears.

The protocol numbers of the embryos in these various group, and their developmental stage at operation are given in Table 1. The developmental stage is indicated by an arbitrary classification of the primitive streaks into short (S), medium (M) and long (L). Short primitive streaks are in a round *area pellucida* and stretch halfway across it or less, medium streaks are in a pear-shaped *area* and stretch about halfway across it or a little more, long streaks are in a very elongated pear-shaped *area* and stretch two-thirds of the way across it (i. e. it is the stage just before the appearance of the head-process, "definitive primitive streak"). In addition, there are the intermediate stages SM and LM, and the stage "very short" (VS) for blastoderms younger than the S stage.

As well as the embryos recorded in the table, at least an equal number of operations have been made which yielded such distorted specimens that they have been discarded. Quite a large number of cultures have also been lost by infection.

Table 1.
Length of primitive Streak.

Group.	None	VS	S	SM	M	Total
1	773 799 780 32—121	32—122	778 32—44	32—64 32—101	727	10
2		32—120	782 32—98	724 725 729 728 777	726 32—9	10
3	32—123		32—82		32—163	3
4			709 748			2
5	32—91	32—103		798		3
6	32—95		716 742			3
7	796 797	745	32— 68 32— 99 32—100	32—65 737		8

The first three of these groups are clearly not very sharply divided from one another. They represent the failures, in which no induction has taken place. One or two illustrative examples of each group are described. The other groups are more important, and all the embryos included in them are mentioned below.

Group I. The embryos falling in this group are those in which the rotation of the endoderm has had least effect. Certainly in some specimens the embryonic axis deviated slightly from the marked direction, and this deviation may probably be attributed to a tendency of the endoderm to set in train tissue movements opposing the elongation of the primitive streak, but this effect is not very striking or conclusive. The more markedly deviating embryos are classed in group 3.

There is also a tendency for the anterior parts of some of the embryos in this group (and of those in the next) to be further developed than the more posterior parts. Nos. 778 and 780 are described as examples. (And see 725 in group 2.)

778. Chick, 14 hours, S pr.s.[1] 24 hours after the operation, the embryo was in the head-fold stage, with a head-process nearly as long as the primitive streak. The axis nearly coincided the epiblastal direction. After 47 hours cultivation, the head possessed well formed optic vesicles and the primary divisions of the brain, but in the trunk region the neural plate was flat and unfolded and no somites were present, although the notochord is thick and well developed. The foregut and heart are present but are not well formed (Fig. 1).

780. Chick, $14^1/_2$ hours, no pr.s. The orientation was not quite certain, but the embryo which developed probably lay in the epiblastal direction. After 46 hours

[1] After the protocol number of the specimen to be described is given its species (chick or duck), the age of the blastoderm at operation in hours of incubation at 37,5° C, and the length of the primitive streak.

cultivation the embryo has six pairs of somites, but the optic vesicles are characteristic of a much later stage. The neural tube is not properly closed in the mid-brain region, but this is a common abnormality to which no importance can be attached. The foregut is fairly normal (Fig. 2).

Fig. 1. No. 778. 47 hours cultivation (× 35). Note: All figures are orientated so that the original anterior-posterior axis of the epiblast was parallel to the long edge of the page, the original posterior being at the bottom of the page, and the original anterior at the top.

It is difficult to suggest a reason for this inhibition of the posterior part of the embryo. It may be that the most anterior region, particularly the pre-chordal region which always lies anterior to HENSEN's node, does not in normal development undergo so large a movement as the posterior parts, and is therefore less affected by the antagonistic action of the endoderm on this movement.

It is interesting to compare the inhibitory effect of the reversed endoderm on the development of the posterior end with the results obtained by HUXLEY (1927) with temperature gradients. In general, an anatagonistic temperature gradient, i. e. one in which the apical end of the axial gradient was cooled, led to a reduction in size, and sometimes to an inhibition of development, of the apical end relatively to the basal end. In the present experiments, it is possible that the same thing is true; probably the posterior region of the blastoderm is the apical end of the axial gradient system of which the endoderm is a part. But the interpretation of the facts is made very insecure by the circumstance that by the time the primitive streak is developed, the apical region has shifted to the region round HENSEN's node, and the posterior end of the streak is the basal end of the gradient.

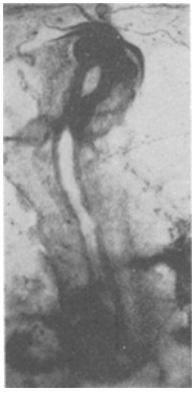


Fig. 2. No. 780. 46 hours cultivation. Note large optic vesicles (× 30).

In the embryos of this and the next group, it is difficult to suppose that the foregut is formed from normal presumptive foregut endoderm. Yet all, or nearly all, the embryos have a foregut which is conformable with the head. The formation of these foreguts must be determined by the overlying epiblast and therefore does not fall within the scope of this paper as set out in the title. An example of the induction of a foregut by a grafted piece of primitive streak has been described in a former communication (WADDINGTON 1933), and the phenomenon is more fully discussed elsewhere (WADDINGTON and SCHMIDT, 1933). One good example is figured here.

32—101. Chick, $8^1/_2$ hours, SM pr.s. The culture was fixed after $27^1/_2$ hours. The embryo lies almost exactly in the epiblastal direction, and a normal foregut can clearly be seen (Fig. 3).

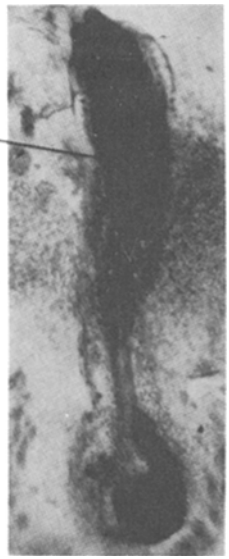


Fig. 3. No. 32 – 101. $27^1/_2$ hours cultivation. *fgt.* posterior edge of foregut (× 30).

Group 2. The embryos in this group really fall roughly into two types; firstly, embryos which do not stretch right across the *area pellucida*, and secondly, very short embryos. But embryos of both types, although lying in the epiblastal direction, show, more clearly than those in group 1, the effect of the reversed endoderm in hindering the forward growth of the primitive streak. No 32—120 is an example of the first type, 725 of the second.

32—120. Duck, $18^3/_4$ hours, VS pr.s. The operation was noted as being particularly neatly performed. The embryo developed as nearly as can be expected in the epiblastal direction. As fixed after $46^1/_4$ hours, it has 8 pairs of somites and a well formed foregut. It is slightly, but not very much, shorter than normal, but the *area pellucida* is very large, so that the embryo only extends halfway across it. (Fig. 4.)

725. Chick, $14^3/_4$ hours, SM pr.s. After 20 hours incubation there was an embryo with the neural folds just closing in the head region. It lay in the epiblastal direction. The culture was fixed after 44 hours. There are only 6 pairs of somites present but the brain has large optic vesicles. The heart and foregut are present. The whole embryo, including the brain, is very short, and as it were telescoped. (Fig. 5.)

Group 3. This group consists of only three embryos, in which the slight deviation from the epiblastal axial direction, characteristic of groups 1 and 2, is present in an exaggerated degree. In other respects, 32—82 and 32—123 would be classed in group 1, and 32—163 in group 2.

Fig. 4. No. 32 – 120. $46^1/_4$ hours cultivation (× 15).

32—123. Duck, 19 hours, no pr.s. The anterior end could be determined with fair accuracy. After 26 hours cultivation, a fairly long primitive streak was present, markedly curved to the right of the epiblastal axis, the point of inflection being in the posterior half of the streak. By the next day, after 46 hours cultivation, a wellformed embryo was present, also curved to the right, the point of inflection being by this time well in the posterior part of the embryonic axis. (The point of inflection in 32—82, which is about the same stage of development, is also at the posterior end.) The head and foregut are very well shaped. (Fig. 6.)

32—163. Chick, 18 hours, SM pr.s. The blastoderm was more transparent than most, so that a photograph could be taken which shows the appearance of an operated embryo. (Fig. 7a.) The endoderm can be seen as a darker area lying under the middle of the primitive streak. All round the endoderm is a lighter area between it and the edge of the area

opaca; this area was originally occupied by loose, fluffy endoderm, which was scraped off and lost during the operation. One can also see the radial cuts round the edge of the blastoderm, which help in the flattening of the epiblast.

On the day after the operation, a small embryo had appeared, markedly inclined to the epiblastal axis, and not occupying the whole of the *area pellucida*, in which, moreover, it was not symmetrically placed. (Fig. 7*b*.) By the evening of the same day, after 31 hours cultivation, 6 pairs of somites were present. (Fig. 7*c*.) The embryonic axis had moved back into a more symmetrical position, but the head remained sharply deflected to the right. The somites on the left side of the embryo lie some distance away from the neural plate. The reason for this is not clear. A similar appearance on both sides of the embryo is sometimes seen in

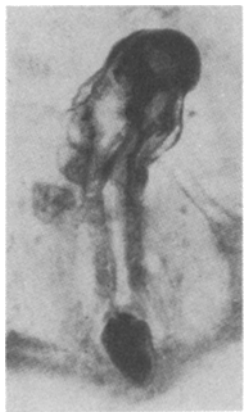


Fig. 5. No. 725. 44 hours cultivation (× 30).

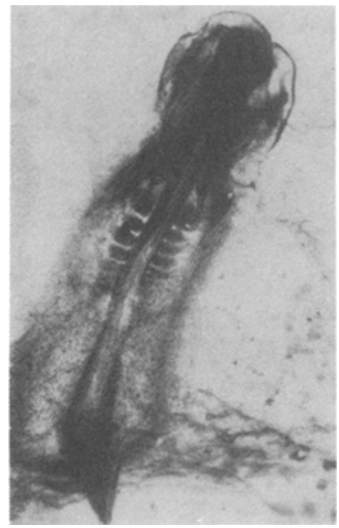


Fig. 6. No. 32—123. 46 hours cultivation (× 35).

specimens which are in the early stages of necrosis, but this specimen seems to be quite healthy. Possibly it is connected with the regulative tendency of the embryonic axis to move back into a more symmetrical position. The foregut is conformable to the head.

Group 4. In three specimens, a transitory primitive streak, induced by the endoderm, was seen in the living specimen, but had been obliterated by the development of the normal primitive streak before the specimen was fixed.

32—91. Duck, 21½ hours, no pr. s. The anterior posterior axis could be determined with fair accuracy from the position of the *Entodermhof*. On the day after the operation, there seemed to be two primitive streaks, which coalesced at their anterior ends. One lay in the marked epiblastal direction, the other in the diametrically opposite direction. The culture was later badly affected by the liquefaction of the medium and had to be discarded.

32—103. Duck, 21 hours, VS pr. s. After 22 hours cultivation, the blastoderm possessed two primitive streaks, as described in 32—91. But in this case, the culture was more transparent, so that the primitive streaks were certainly recognisable as such, and the two posterior ends could be clearly seen. The induced streak

was shorter than the normal. After 47 hours cultivation, an embryo of 6 pairs of somites was present, lying in the epiblastal direction, and the induced streak had disappeared.

798. Chick, 17 hours, S pr.s. After 24 hours, the culture presented a somewhat obscure appearance which was interpreted as showing a primitive streak in the endodermal direction. After 44 hours, only a embryo in the epiblastal direction was present.

Thus of the three cases in this group, only 32—103 is at all satisfactory. The obliteration of an induced primitive streak by the normal one, or *vice versa* as in group 7, does not seem surprising when one remembers the assimilation of grafted pieces of primitive streak into the host's body which has been mentioned before (WADDINGTON, 1932/a, this is further discussed in WADDINGTON and SCHMIDT, 1933).

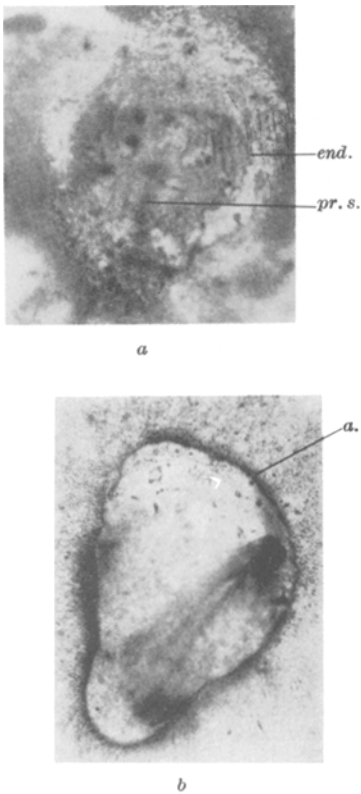


Fig. 7*a—c*. No. 32—163. 7*a* blastoderm shortly after operation, *pr. s.* primitive streak, *end.* edge of endoderm (× 35). 7*b* after 20 hours cultivation, *a. p.* margin of *area pellucida* (× 30). 7*c* after 31 hours cultivation (× 30).

Group 5. This group consists of two semicircular embryos. That is, in these embryos the anterior part corresponds to the epiblastal axis, the posterior part to the endodermal axis. The other possible type of semicircularity, in which the induced part of the axis is the anterior part, has not been found, but there is probably no reason why it should not occur The two embryos are rather different from each other, since in 709 the embryo is quite large and occupies nearly the whole *area pellucida* except for part of the originally posterior region, whereas in 748 the embryo is very minute, and only the anterior portion of the original primitive streak has taken part in its formation, the posterior part of the streak

surviving as a groove which occupies the originally posterior part of the *area pellucida*.

709. Chick, $14^1/_2$ hours, S pr.s. On the day after the operation, the structures were obscure and could not be identified with certainty. On the following day, after 46 hours incubation, a well developed embryo was present, with the anterior end pointing in the epiblastal direction, but the posterior end curled round so as to point in the opposite, endodermal direction. (Fig. 8.) The anterior part of the embryo and the somite region is well formed, but the posterior end is misshapen, with a large knob of neural tissue, and what appeared, in the whole mount, to be another irregular set of somites. This suggested that the semicircular embryo is really made up of two embryos united head to tail, the posterior one being incomplete, but stretching as far forwards as its somite region.

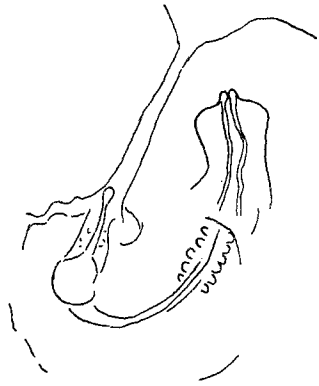


Fig. 8. No. 709. 46 hours cultivation. Camera lucida drawing (× 25).

Most unfortunately, the specimen was injured when it was taken out of the whole mount for embedding. But the sections are sufficiently complete for one to see

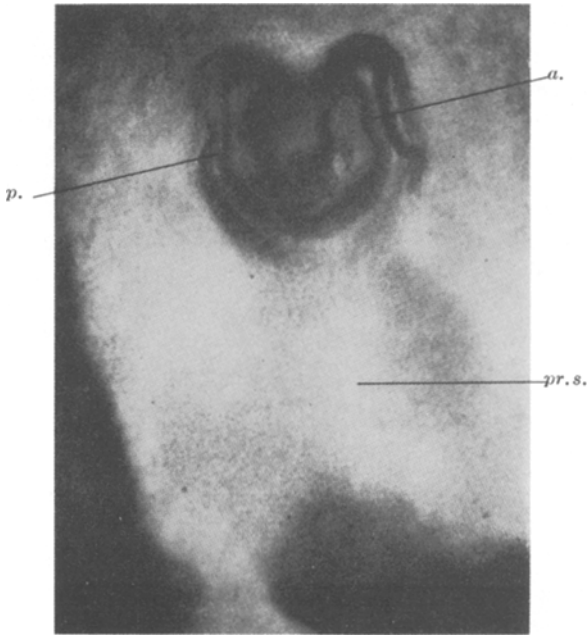


Fig. 9. No. 748. About 48 hours cultivation, *a.* anterior end of embryo, *p.* posterior end of embryo, *pr. s.* remains of primitive streak (× 70).

that the neural plate forms a good brain in the anterior part of the embryo, then opens out on the surface and becomes a shallow and quite small groove in the middle region where the somites are, and then, in the most posterior part where the presence

of extra somites was suspected, becomes larger again and more deeply folded, and eventually forms another nearly closed tube. It thus supports, to some extent, the suggestion made above that the specimen really consists of two embryos fused together. But the suspected somites were found not to be somites at all, but mere lumps of loose mesoderm.

748. Chick, age unknown, S. pr. s. On the day following the operation, the structure could not be interpreted nor indeed could very much be seen in the living specimen on the following day when the culture was fixed. But in the stained specimen, which was orientated by means of blisters etc. on the *area opaca*, the embryonic structures can easily be seen (Fig. 9). The original primitive streak is present, somewhat longer than at operation but otherwise undeveloped. It consists of a wide groove. At its anterior end lies a minute embryo. The anterior end of the embryo lies to the right, running in the direction of the epiblastal axis and is in the stage of the closure of the neural folds. There is a well developed head-fold. The posterior part is to the right and runs in the direction of the endodermal axis. The remains of the primitive streak cut the embryonic axis in the middle of the curved portion which joins the anterior and posterior parts.

The only explantaion which can be offered for these remarkable embryos is that they are the result of the coalescence of two streaming movements in the epiblast, one, already determined at the time of operation and proceeding from the posterior to the anterior of the epiblast, and another induced by the endoderm and proceeding in the opposite direction. These movements must be imagined to have met each other not quite head on, and thus to have given rise to a vortex motion. The situation is therefore imagined to be essentially very similar to that in the embryos of group 6, where again there were two opposed streaming movements which were more or less equal in intensity. But in that group the movements seem to have met each other more squarely, so that no vortex was set up.

Group 6. This group consists of specimens in which two primitive streaks have developed, one in the epiblastal direction and one in the endodermal. As has been said above, these streaks are probably dependent on two sets of tissue movements, neither of which was powerful enough to obliterate the other, and which met each other so squarely that no vortex could be formed.

32—95. Duck, $23^1/_2$ hours, no pr. s. The anterior-posterior axis could be determined with some certainty. After 25 hours incubation, there was a short primitive streak in the epiblastal direction. A few hours later, rapid development had occurred, and it was easy to make out two primitive streaks running in opposite directions, and with their anterior ends meeting in the centre of the *area pellucida* (Fig. 10). In this central area of fusion, the thickened edges of each primitive streak separated from one another and joined up with the edges of the other primitive streak, thus making a fairly large oval thickening which enclosed a more transparent, probably thinner area. This may possibly indicate a tendency to form a *Duplicitas cruciata*. The normal primitive streak was slightly larger than the induced one.

Early on the following morning, after 44 hours cultivation, the clot on which the specimen was growing had liquefied and the specimen was much folded and in an advanced stage of necrosis.

716. Chick, $14^3/_4$ hours, S pr. s. During the operation, the right anterior part of the area opaca was lost, but the endoderm was neatly removed over the entire

area pellucida and replaced before its edges had time to curl inwards. The diagram (Fig. 11*a*) shows that even so the endoderm is not so large in area as the area pellucida; this is partly explained by the loss of some fluffy endoderm from the marginal parts, but it must also be remembered that it is impossible to apply a tension to the endoderm once the epiblast has been placed on top of it, and that it is therefore never stretched out to its full extent in these experiments.

After a day's cultivation, the structure could not be made out, partly, perhaps, because this was an early experiment of this type, and I was not experienced in interpreting the cultures. On the second day, after 50 hours cultivation, two embryos were present, lying with their anterior ends together in the middle of the area pellucida. They were already moribund and slightly disintegrated when fixed (Fig. 11*b*).

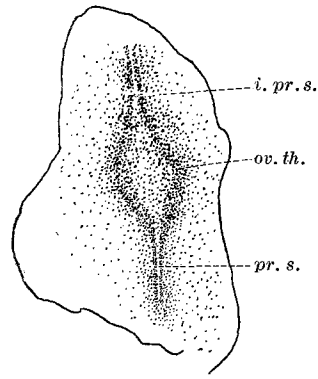
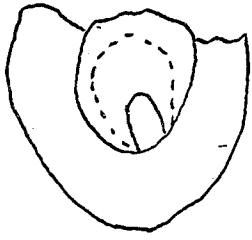


Fig. 10. No. 32—95. 28 hours cultivation. *pr. s.* normal primitive streak, *i. pr. s.* induced primitive streak, *ov. th.* oval thickening (× 25).

One of the embryos is developed from the original primitive streak, although it diverges quite considerably from the marked direction. The other embryo lies in the exactly opposite direction, and must have been induced by the endoderm. Each embryo has a neural plate, notochord and somites. On each side of the fused anterior ends is a heart; that on the left of the normal embryo was beating strongly when the culture was fixed, that on the right either not at all or only very weakly. The structure of the fused anterior ends cannot be determined with certainty from the entier specimen, but it appears that the most anterior ends of the two heads are missing and that the two neural plates are continuous at about the level of the ears. The specimen has not been sectioned, since it is more



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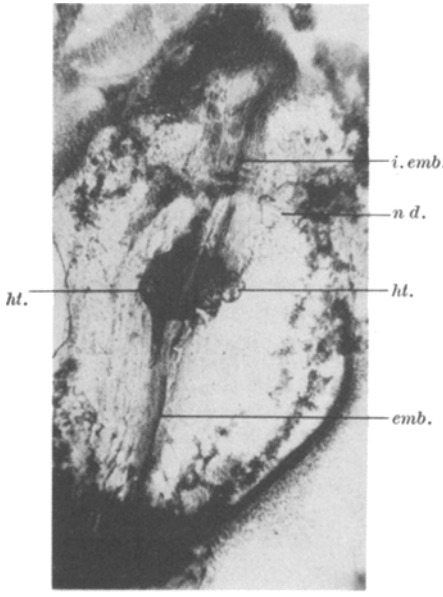


b

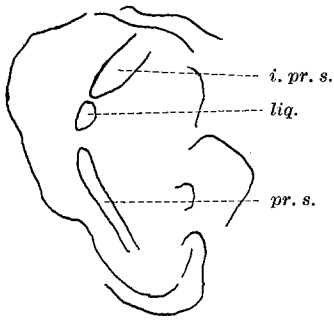
Fig. 11*a—b*. No. 716. 11*a* diagram of blastoderm after operation, outline of endoderm dotted. 11*b* after 50 hours cultivation, *emb.* normal embryonic axis, *i. emb.* induced embryonic axis, *end.* edge of hole in endoderm, *ht. ht.* heart rudiments (× 22).

important to preserve a record of the directions of the two embryos than of the details of the structure. Actually it is doubtful if it would be possible, even from

sections, to state definitely if the anterior parts of the heads were present, since at this stage, the optic vesicles, which would be the only criterion, would in any case not be well developed.

It will be seen that the endoderm is missing from a large part of the specimen; this is probably a late development due to the liquefaction of the clot on the second day of cultivation.

742. Chick, 14 hours, S pr. s. The endoderm was replaced rather to the side of the primitive streak. After 27 hours cultivation, two primitive streaks were clearly visible. The longer lay nearly in the epiblastal direction, but diverged to some extent to the left of it; the shorter lay nearly in the endodermal direction. The streaks were not exactly in opposite directions but make an angle of about 140° with one another. The induced streak, although the shorter of the two, was broader



a

Fig. 12*a*–*b*. No. 742. 12*a* after 33 hours cultivation, *pr. s.* normal primitive streak, *i. pr. s.* induced primitive streak, *liq.* area of liquifaction, Camera lucida drawing (× 25). 12*b* 48 hours cultivation (× 30).

and more robust in appearance than the original streak, which was narrow and difficult to see, particularly in its anterior end, where it approached the anterior part of the induced one.

Six hours later the two streaks were still visible, and had joined together with an oval thickening similar to that described in 32—95. (Fig. 12*a*.) But an area of liquefaction had appeared in the central thin part inside this thickening, and a hole had been made in the blastoderm here. The whole clot was washed with saline and transferred to the surface of a new clot, to try to obtain further differentiation of the primitive streaks, but next morning, after a total of 48 hours in vitro, no further development had taken place, and the hole in the blastoderm had become slightly larger. (Fig. 12*b*.) The specimen was therefore fixed.

Group 7. This group consists of specimens in which only the induced primitive streak has developed, the normal primitive streak being suppressed.

796. Chick, age unknown, no pr. s. The orientation was not very certain, but the embryo which developed lay in the direction which had been marked as the endodermal.

797. Chick, $14^3/_4$ hours, no pr. s. The embryo could be orientated with considerable certainty in this case, by means of the position of the *Entodermhof*. After

24 hours cultivation, a primitive streak was present in the endodermal direction; either no streak ever developed in the epiblastal direction, or its had already disappeared. After 44 hours, the induced streak had developed into a small embryonic axis, which sections show to consist of neural plate underlain by a thick layer of mesoderm to which the endoderm is closely adherent. (Fig. 13.)

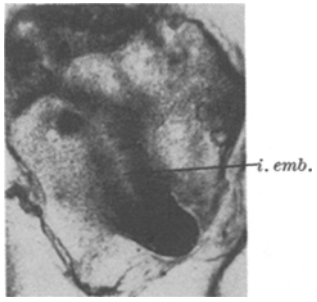


Fig. 13. No. 797. 44 hours cultivation, *i. emb.* induced embryonic axis (× 30).

745. Chick, 15 hours, VS pr. s. After $26^1/_2$ hours there was a clear primitive streak in the endodermal direction, and

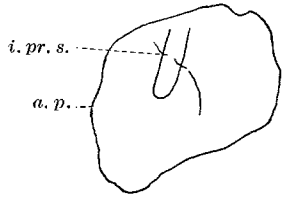


Fig. 14. No. 745. $26^1/_2$ hours cultivation, *i. pr. s.* induced primitive streak, *a. p.* edge of area pellucida (× 18).

the original streak was no longer visible (Fig. 14). The culture suffered the same arrest in development as 742, described above; like it, the whole culture was transferred to a new watch-glass, but with even less success, since by next morning degeneration was so extensive that the culture had to be discarded.

32—68. Duck, $21^1/_2$ hours, S pr. s. The epiblast and the endoderm were both considerably damaged during the operation. By the following day, no further development seemed to have occurred; the original streak was still visible. On the second day, however, this streak had disappeared, and an embryo was present

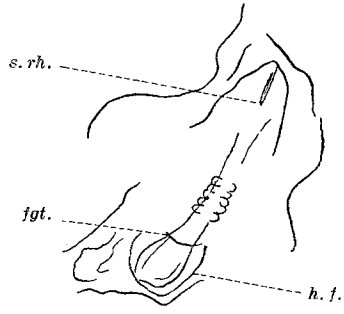


Fig. 15. No. 32—68. about 48 hours cultivation, *h. f.* head fold, *fgt.* posterior margin of foregut, *s. rh.* sinus rhomboidalis (× 40).

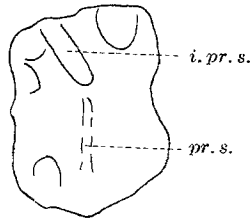


Fig. 16. No. 32—100. $27^1/_2$ hours cultivation, *pr. s.* remains of normal primitive streak, *i. pr. s.* induced primitive streak (× 18).

in the endodermal direction. (Fig. 15.) In the head region there are neural folds which are just coming together to form a closed tube; posteriorly to this the folds are open, with the notochord lying below, and at the most posterior end the remains of the induced streak is present. This was morphologically one of the most normal of the induced embryos, but the specimen became somewhat folded during fixation, probably owing to the liquid consistency of the clot.

32—99. Duck, 28 hours, S pr. s. The incubation of this, and of the next, blastoderm took place in two periods, which perhaps explains its early stage of deve-

lopment. A ring of yolk-sac endoderm was left attached to the central endoderm and turned with it during the operation. After $20^1/_2$ hours there was a primitive streak in the endodermal direction and the original streak had disappeared. The blastoderm was later lost owing to liquefaction.

32—100. Duck, 28 hours, S pr.s. After one day's cultivation, a primitive streak could be clearly seen in the endodermal direction, and traces of the original streak were still visible. (Fig. 16.) On the following day, after 43 hours in vitro, the clot had liquefied and the culture degenerated in the same way as 32—99, but in this case the head of the embryo was still recognisable. It was clearly the head of an induced embryo, since it lay in what had been the most posterior part of the epiblast, and with an anterior-posterior axis corresponding to the endodermal axis. The normal streak must have disappeared, since there was no room left for it.

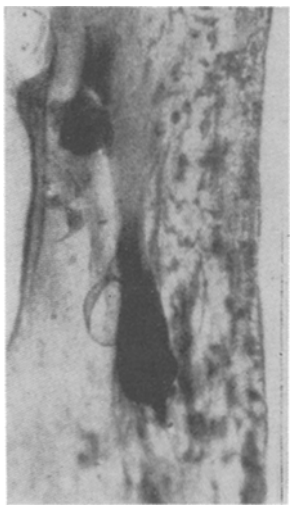


Fig. 17. No. 32—65. $44^1/_2$ hours cultivation (× 30).

32—65. Duck, $20^1/_2$ hours, SM pr.s. By the end of the day following the operation, the specimen was still very obscure, and no definite primitive streaks could be identified with certainty; there seemed to be indications of two streaks running in opposite directions. On the second day, after $44^1/_2$ hours, the specimen was somewhat degenerated, but there was clearly only one embryo present, and this lay in the endodermal direction. (Fig. 17.) Sections show that the neural tube is closed in the head region; in the middle part of the embryo it is in the form of a thin flat groove, but becomes better developed again in the posterior part.

737. Chick, 15 hours, SM pr.s. After 24 hours cultivation a primitive streak in the endodermal direction could be seen. The specimen was later lost by infection.

The embryos of groups 5,6 and 7 provide conclusive evidence of the induction of a new primitive streak by the endoderm. In group 5, the identification of the induced streaks in 32—91 and 798 was doubtful, and in group 7 there was some uncertainty as to the orientation of 796. This leaves one certain case in group 5, three in group 6 and seven in group 7, making eleven in all, which seems a large enough number to put the phenomenon beyond all reasonable doubt.

The occurrence of a transitory primitive streak in the embryos of group 5, and the disappearance of the normal streaks in those of group 7, is not difficult to interpret on the theory that the primitive streak is dependant on a system of tissue movements. It is only necessary to assume that one set of movements has been powerful enough to annul or reverse the other set. The fact that the reversal of the endoderm does not always suffice to induce a new primitive streak even in blastoderms in which the streak has not yet appeared, shows that the first stage of the determination of these movements takes place at a very early stage in normal development; probably it is contemporaneous with the formation of the endoderm. At least, one must imagine that in all the

embryos which were employed in this investigation the epiblast was no longer indifferent as regards these movements.

Perhaps a word should be said on the question as to whether the agent responsible for these inductions might be the mesoderm, and not the endoderm at all. This question was really answered in the earlier investigation (WADDINGTON 1932) in which it was shown that the bending of the embryonic axis by rotation of the endoderm through 90° could only be obtained if the operation was performed in an early stage when there in very little mesoderm present. In the same way, it will be noticed here that all the embryos in groups 5, 6 and 7 were very young at operation, SM or less. The M blastoderms all gave embryos falling in groups 1 and 2. Comparatively few of these older stages have been used, since it seemed certain that no positive results would be obtained from them. In my earlier paper referred to above, I stated that any mesoderm which was present usually came away with the endoderm when that is removed. This has been found to be much less usually the case than I thought at that time. In older blastoderms, it is true, some mesoderm, but probably not by any means all, is removed with the endoderm; but in the younger material which is in question here, there is in the first place very little mesoderm already formed from the primitive streak, and what there is usually remains attached to the streak after the endoderm is stripped away, and can be seen as a darker area with a ragged edge. Even in the older blastoderms, it is mainly the peripheral mesoderm which is removed; the more central mesoderm nearly always remains attached to the streak. Finally, any mesoderm which may be already invaginated at the stages when these operations were carried out, would almost certainly not be axial mesoderm, but would be destined to migrate outwards to the side of the *area pellucida*, and the supposition that this mesoderm may be responsible for the induction is therefore not *a priori* very plausible.

It should be noted that the induction which is known to be performed by the mesoderm derived from the primitive streak in later stages is not believed to involve any of the tissue movements leading to the forward growth of the primitive streak. It would not therefore be possible to account, by a hypothesis of mesodermal induction, for the formation of curved or shortened or semicircular embryos, without making subsidiary hypotheses as to the capabilities of the mesoderm as an inductive agent.

Discussion.

Throughout the presentation of the results, it has been implicitly assumed that the thing which is induced by the endoderm is a set of tissue movements. This hypothesis has the advantage that it gives a single explanation for the bending of the embryonic axis previously reported, and for the inductions which are the subject of this paper.

Moreover, it seems to be the only hypothesis which is capable of explaining the details of some of the embryos which have been obtained. In particular, it is difficult to imagine an explanation for the semicircular embryos 709 and 748 other than that given; namely, that the formation of a primitive streak is dependent on movements of some kind.

If it is granted that the endoderm induces the tissue movements of the epiblast necessary for the formation of a primitive streak, several questions as to the nature of this induction arise. In the first place, is the induction performed by a localised part of the endoderm, or must one assume that it is dependant on some sort of a "gradient system", or "field", of the endoderm as a whole? This question cannot be confidently answered until experiments have been made with the rotation of parts of the endoderm, but the technical difficulty of such experiments would probably be very great, and it may therefore be worth while to draw attention to one fact which may be suggestive in this connection. GRÄPER has shown that in normal development the primitive streak is built up by a polonaise-like streaming of material along the sides of the *area pellucida* to the posterior, where the two streams unite and proceed forwards again up the centre. If the induced movements are essentially similar, as one would expect them to be, and if all the effects of the rotated endoderm are due to a local induction of movements, one would expect that whenever the endoderm had any effect at all, there would be a short primitive streak formed over the posterior part of the endoderm. Now in the case of the bending of the normal primitive streak, such short induced streaks have not been found at all, and they are rare in cases of shortening of the original streak (but see 742). It looks, therefore, as though the normal primitive streak can be affected even when the endoderm does not induce an entire new set of tissue movements; and this suggests that it is affected by the endoderm lying immediately below it.

There are thus some grounds for supposing that the induction is performed by the endoderm by virtue of a quality which is spread throughout that tissue; that is to say, by virtue of some gradient system. Even if this supposition should prove not to be well founded, it will be necessary to assign some such structure to the localised inducing part of the endoderm, since this controls not only the occurrence but also the direction of the movements which it induces.

In this connection it should be noticed that the endoderm affects not only the primitive streak, but the whole *area pellucida*. Thus an induced streak is accompanied by an accumulation of blood islands opposite its posterior part, and in some cases, notably 32—163, it looks as though an extra piece of area pellucida had been induced, although the primitive streak required to fill it has not been developed. But it is impossible to be certain whether these are direct effects of the endoderm or are secondarily attained through the agency of the induced streak.

The idea that the formation of the primitive streak is directly dependent on certain tissue movements may seem at first sight to be similar in some respects to GOERTTLER's (1927) theory of dynamic determination. But I do not here contemplate as wide a field of application for this idea as GOERTTLER has postulated for it in the Amphibia. All that is suggested is that the primitive streak is dynamically determined. Now the primitive streak is to be regarded as a mere temporary morphological entity rather than as a definite organ; it is analogous, in this respect at least, with the Amphibian blastopore. Like the latter, it is the place of origin of the mesoderm, and it is perhaps tempting to regard it as a histological unit defined by the type of histology characteristic of the mesoderm. In this way it might be possible to argue that the determination of the mesoderm in birds is at first a dynamic determination; that is, a determination to take part in the movements which give rise to the streak. An analogous assertion regarding the Amphibian mesoderm might seem to have some plausibility, since it is certainly true that the presumptive mesoderm has a strong tendency to perform invagination movements, as MANGOLD (1923, 1925) showed. But the same author was at the same time able to produce some evidence that the determination as of the mesoderm and of the tendency for movements were two different things, since mesoderm can be obtained from tissue which had never undergone the appropriate movements. In the chick there are as yet no data on this point, but clearly it is necessary to keep an open mind on the subject. The argument given at the beginning of this paragraph prejudged the question by the assumption that the determination of the mesoderm is synonymous with the determination of the primitive streak: in reality it is quite possible that the former occurs at a much later stage, while the mesoderm is actually being proliferated from the streak, for example.

Concerning a possible "dynamic determination" of the neural plate, nothing is known in birds at this stage. This question, and that of the possible significance of the tissue movements in the epiblast for the inductive capacity of the primitive streak, will be discussed rather more fully in a later communication.

In the Amphibia, nothing is known about the origin of the movements which lead up to the formation of the blastopore. MANGOLD (1923, 1925) has shown that they are already determined in the blastula, and it is difficult to see how they can be due to any ordinary process of induction. So far as can be seen, they probably arise in the egg as an inherent "field", perhaps defined in the first place by the segregation of the presumptive material of the organisation centre in the grey crescent. It is possible that a further comparative study of the independent origin of these movements in Amphibia and their dependent origin in birds will throw new light on the nature of induction.

HOADLEY, when he isolated fragments of the blastoderm on the chorioallantoic membrane (1926a, b), included the underlying endoderm in his grafts. The fact that he obtained differentiation of fragments of blastoderms, transplanted before the appearance of HENSEN's node, and of fragments in which the node was not present, show, as he claims, that the node is not the primary organisation centre of the embryo. But the proof which is brought forward in the present paper, that the endoderm exerts an organising influence, which HOADLEY himself foresaw in 1927, makes it impossible to accept his isolations as giving any information about the degree of determination of the fragments at the time of isolation. The differentiation which they performed after isolation was presumably due to the inclusion in the isolated fragment of part of an organisation centre, namely the endoderm.

Summary.

1. The endoderm was separated from the epiblast in chick and duck embryos of young primitive streak stages, and then the two layers of tissue were replaced so that their longitudinal axes were diametrically opposed: i. e. the anterior part of the endoderm lay under the posterior region of the epiblast, and the posterior part of the endoderm under the anterior region of the epiblast. The combination was then cultivated *in vitro* by the watch-glass technique.

2. The displaced endoderm had in most cases an effect on the development of the epiblast. It either (1) hindered the normal growth in length of the primitive streak, or (2) caused the development of a new primitive streak. In some cases the secondary (induced) streak disappeared on further cultivation, in some cases both streaks persisted and gave rise to a double monster, and in still other cases the secondary streak persisted and the primary disappeared. In two cases primary and secondary united to form a single semicircular embryo.

3. It is argued that the endoderm does not induce the differentiation of a definite tissue, but that it induces the form-building movements which lead to the development of the primitive streak.

Zusammenfassung.

1. Bei Hühner- und Entenembryonen im frühen Primitivstreifenstadium wurden Entoderm und Epiblast voneinander gelöst und darauf wieder in umgekehrter Richtung vereinigt: d. h. der vordere Teil des Entoderms kam unter das Hinterende des Epiblasts, und der hintere Teil des Entoderms unter das Vorderende des Epiblasts zu liegen. Diese Kombination wurde dann *in vitro* mit der Uhrschälchenmethode gezüchtet.

2. In den meisten Fällen übt das verlagerte Entoderm einen Einfluß auf das darüberliegende Epiblast aus. Es konnte entweder 1. das normale Wachstum des Primitivstreifens verhindern, oder 2. die Bildung eines

neuen Primitivstreifens veranlassen. Dieser neue induzierte Primitivstreifen lag dann zu dem primären in entgegengesetzter Richtung. In einigen Fällen verschwand dieser sekundäre Streifen bei weiterer Züchtung, in anderen kamen beide Streifen zur Weiterentwicklung und führten so zu einer Doppelbildung, in wieder anderen Fällen verschwand der primäre Streifen, während der sekundäre sich weiterentwickelte. Bei zwei Experimenten vereinigten sich Primäre und Sekundäre zu einem halbkreisförmigen Embryo.

3. Es wird daraus gefolgert, daß das Entoderm nicht die Differenzierung wohl definierten Gewebes induziert, sondern die Formbildungsbewegungen, die zur Primitivstreifenbildung führen.

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