

# CONVERSION OF PRESUMPTIVE ECTODERM TO MESODERM IN THE CHICK

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(With One Plate)

## INTRODUCTION

THE greater part of descriptive embryology is based on the concept of the three germ-layers—ectoderm, mesoderm and endoderm. As we acquire more information about the physiology of development, it is natural to ask whether the same concept is as fundamental for a consideration of individual development as it is for comparative morphology. The investigations on the Amphibia have already provided a negative answer for that group. Mangold (1925), in a review on the subject, pointed out that presumptive ectoderm could be caused to undergo differentiation into endodermal organs or mesodermal organs. More recently, Lopashov (1935) and Töndury (1936) have shown that mesoderm can become ectoderm. Only the heavy yolk-laden endoderm cannot be changed into anything else, a disability which is probably not an expression of any fundamental determination but rather an adventitious circumstance depending on the presence of large masses of inactive yolk.

Mangold describes two ways in which ectoderm can become converted into mesoderm; either the presumptive ectoderm can be grafted into the blastopore or it can be inserted directly into the blastocoel cavity. In the first case, it takes part in the gastrulation movements of the host, but the fact that mesodermal organs are also formed by grafts made in the second way show that these movements are not essential for subsequent mesodermal differentiation. In the chick, the analogue of placing a graft into the blastocoel cavity would appear to be placing it under the primitive streak, beneath the invaginating mesoderm and the endoderm. This experiment has been performed by Abercrombie (1937), who found that under such circumstances the graft did not usually develop as mesoderm, but became induced to form neural tissue in a rather peculiar way which he has fully discussed. His work, and that of Waddington and Abercrombie, in which grafts of primitive streak material were placed under the primitive streak, made it probable that an essential preliminary to the development of mesodermal tissues is the breaking down of the epithelial structure found in the epiblast. It therefore appeared that it might be possible to obtain more success in converting presumptive

<sup>1</sup> This work was done while I held a Senior Studentship of the Royal Commissioners of the Exhibition of 1851, for which I should like to make grateful acknowledgement.

ectoderm into mesoderm if the ectoderm could be caused in some way to lose its structure. In order to do this, pieces of presumptive epidermis from primitive streak blastoderms were placed in small holes made by removing a suitable amount of tissue from the primitive streaks of host blastoderms. The sizes of the grafts varied, but they were mostly rather small, of the order of a sixth or less of the primitive streak in length and not much wider than it. The operations were performed on chick embryos cultivated *in vitro* in the usual way.

#### DESCRIPTION OF THE RESULTS

Different specimens varied very much in the extent to which the grafts healed into the primitive streak. In some cases, the graft shifted in position before any healing took place, and then appeared, after the period of cultivation, as a small separate mass of tissue, usually lying below the host embryo immediately in contact with the plasma clot; in some such specimens the hole in the primitive streak became enlarged into a long split, running longitudinally along the greater part of the host's axis, while in others the wound was repaired and the host appeared entirely normal. Entirely normal hosts also occur in which no trace of an ejected graft can be found. It appears almost certain that in these the graft has become perfectly healed into the primitive streak and has taken part in the formation of the host embryo, developing like its surroundings into mesodermal tissue of the somites. This conclusion does not, however, rest solely on the fact that no ejected graft-mass can be found. There is a complete set of transitional stages between embryos in which the graft has only very imperfectly healed into the streak, through those in which the graft-mass is partly brought into conformity with the host's anatomy (cf. Pl. I, figs. 3, 4, 5), and embryos in which the only sign of the graft is a slight enlargement of some host tissues.

The series of partially healed grafts not only suggest the possibility that complete healing may sometimes occur, but they also provide examples in which the conversion of ectoderm to mesoderm can be placed beyond doubt. Thus in Pl. I, fig. 6 the graft is found as a large mass of tissue sunk below the main axis of the host, and this mass consists clearly of somitic mesoderm, arranged in the characteristic way, but considerably larger in volume than a normal somite. Similarly, in Pl. I, fig. 2, the graft-mass can be identified with certainty and there is no doubt of the mesodermal nature of the greater part of it. In No. 105 (Pl. I, fig. 1) the graft seems to have slipped below the streak at a fairly early stage and behaves much like the grafts placed beneath the streak which were described by Abercrombie (1937); that is to say, a large induced neural tube has been formed, though here again the presence of somitic mesoderm is undoubted.

#### DISCUSSION

These results place beyond doubt the possibility of causing presumptive ectoderm to differentiate into mesodermal structures and tissues. Some of the grafts certainly contained endoderm as well as ectoderm, but it would be unreasonable to suppose that the large masses of mesoderm which are found were

formed exclusively from the thin layer of endoderm, which, moreover, is known from previous work to take little part in the masses formed from such grafts in other parts of the area pellucida, and often apparently to disappear entirely. Some of the grafts which contained endoderm may also have included a few mesoderm cells, but in most cases the grafts were taken from the edge of the area pellucida or from the anterior part of it, and both these are regions to which the mesoderm has not penetrated at the stage in question, so that one is justified in assuming that the grafts were free of mesoderm.

The conversion of presumptive ectoderm into mesoderm in these grafts placed into the streak, coupled with the rarity of such a conversion in the grafts placed under the streak (Abercrombie, 1937), is strong evidence for Abercrombie's suggestion that a preliminary stage in the conversion is the breaking down of the epithelial structure of the ectoderm. In this way we can probably explain the discrepancy of our results for the chick with those described for the *Amphibia*. In the latter group, presumptive ectoderm becomes converted into mesoderm without going through the gastrulation movements (Mangold, 1925), while in the chick the gastrulation movements appear at first sight to be essential. But the cell arrangements in the various tissues involved are very different in the two groups. In the *Amphibia* the mesoderm, including the somites, is formed from the sheet of tissue invaginated through the blastopore, part of which forms the roof of the primitive gut, and the degree to which the cells of this mesoderm sheet are arranged in an epithelium does not differ very much from the conditions found in the blastula ectoderm. In the chick, on the other hand, the cells of the epiblast before invagination are arranged in a rather definite epithelium which, probably because the blastopore is no longer open but is represented by the fused sides of the primitive streak, becomes completely broken down during the invagination, so that the immediate precursor of the somites is a comparatively disorderly mass of cells. Thus what we might call the "gastrulation movements" involve very different things in the two cases; in the *Amphibia* merely a movement of a sheet of cells as a whole, in the chick a phase in which the sheet of tissue breaks down and the units of movement are the individual cells. It seems therefore quite understandable that the chick ectoderm, unlike the amphibian, can only be converted into mesoderm if it takes part in the gastrulation movements of the primitive streak, because this is the only way known at present in which the epithelial structure is broken down. There is, however, no reason to suppose that the mere fact of passing from the upper to the middle layer plays any greater part in the chick than in the newt.

The importance of the experimental changing of germ-layer quality for embryological theory has been discussed by Mangold. The germ-layers are entities defined on a morphological basis and experiments such as those described for the *Amphibia* and the present ones on the chick do not in any way lessen the usefulness of the germ-layer concept for morphological analysis. What is demonstrated is that the division into germ-layers does not correspond with any specialization of physiological functions concerned with development. Mangold expresses it by saying that there is no period in which the parts of the embryo are determined

simply as belonging to a definite germ-layer, without at the same time being determined as a definite organ of that layer. Thus from the moment when a part of the blastula tissues becomes determined to be ectoderm, that part is also determined either as neural plate or as epidermis. The same conclusion is, as we have seen, true of the ectoderm and mesoderm of the chick. It does not follow immediately of the endoderm as opposed to the ectoderm and mesoderm together. There may well be a stage in the development of the chick in which the tissues are simply determined either as endoderm or as ectoderm or mesoderm. All the evidence which is available suggests that pieces of epiblast, which are still indifferent as to whether they develop into ectoderm or mesoderm, are already incapable of becoming endoderm. If this is indeed the case, we see that the more profound morphological distinction between endoderm and epiblast, which is characteristic of the chick as opposed to the newt, does involve a physiological specialization as well.

#### SUMMARY

1. Fragments of epiblast, or of epiblast together with endoderm, were placed in holes cut in the primitive streaks of young chick embryos, and the embryos then cultivated *in vitro* for about 24 hours. The grafts were sometimes extruded, but in many cases they healed more or less well. In those cases in which partial healing occurred the grafted presumptive ectoderm has usually developed into mesodermal structures, such as somites, though in a few cases it has been induced to form neural tissue. In some cases healing was so complete that no trace of the graft can be found, and in these embryos also the greater part of the presumptive ectoderm must have formed mesoderm.

2. The conversion of presumptive ectoderm into mesoderm has only been accomplished by the method of the above experiments, which involves the performance by the graft of the normal gastrulation movements. In the *Amphibia* the performance of such movements is unnecessary, and it is suggested that in the chick the importance of the movements is not that they are a part of the gastrulation process but merely that they involve the breakdown of the epithelial structure of the grafted ectoderm.

3. The relevance of these results to the germ-layer theory is discussed, and it is pointed out that, although it is clear that there is no fundamental physiological distinction between the ectoderm and mesoderm before invagination, it is still possible that such a distinction may exist between the endoderm and the ectoderm-mesoderm system.

#### REFERENCES

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Fig. 1.

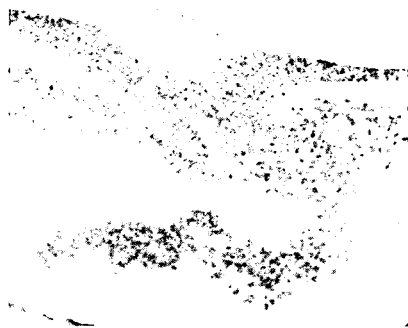


Fig. 2.



Fig. 3.



Fig. 4.



Fig. 5.



Fig. 6.

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EXPLANATION OF PLATE I

Fig. 1. No. 105. The graft, from an embryo with a medium primitive streak into a young head-fold stage, has sunk below the host axis and been induced to form a neural tube and somites. Cultivation  $26\frac{1}{2}$  hours.

Fig. 2. No. 35-051. The graft consisted of ectoderm and endoderm from the anterior of a 14-hour blastoderm with long primitive streak, and was grafted into an embryo of the same age. It has formed an irregular-shaped mass of mesoderm and has possibly taken part in the formation of the neural plate, which is flat in this region. Cultivation 22 hours.

Fig. 3. No. 36-67. The graft and host were both in the long primitive streak stage (18 hours). After 24 hours' cultivation, the graft forms a thick mass of mesoderm below the host's somites in the left side, and has also formed some neural tissue which is united with the host's neural plate, forming an abnormally wide and deep groove.

Fig. 4. No. 113. The graft, from a long primitive streak stage into one of the same age, has for a short distance formed an extra row of somites on the left side, and above it the host neural fold is larger than on the right side; it is impossible to state whether graft tissue has contributed to this enlargement of the fold or whether it is a secondary effect of the increased quantity of mesoderm. Cultivation 25 hours.

Fig. 5. No. 119. The graft, from a short primitive streak stage into a medium stage, forms a second row of somites on the right side partly incorporated into the host somites. Cultivation 25 hours.

Fig. 6. No. 36-64. The graft, which was from a long-medium stage and did not include endoderm, has formed an enormous single somite lying nearly symmetrically under the host axis. In the anterior it has also formed a short piece of neural tube. Cultivation 24 hours.