

THE BEHAVIOUR OF GRAFTS OF PRIMITIVE STREAK BENEATH THE PRIMITIVE STREAK OF THE CHICK

By M. ABERCROMBIE¹ AND C. H. WADDINGTON²

From the Strangeways Research Laboratory and the Sub-Department of Experimental Zoology, Cambridge

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(With Fourteen Text-figures)

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I. INTRODUCTION

THE experiments summarized in this paper serve two purposes: first, they complete the previous paper (Abercrombie, 1937), which dealt with the behaviour of all regions of the epiblast other than the primitive streak when placed under the primitive streak of the host blastoderm; and secondly, they extend the work of Waddington & Schmidt (1933), which studied the mutual action of two individuation fields brought into fairly close contact. The method employed was exactly the same as that used in the previous paper (Abercrombie, 1937), except for the difference in the place of origin of the graft. In the present experiments, however, the endoderm was usually left on the graft. The work is based on fifty specimens, representing various positions in the donor (classified into three types, and referred

¹ Hastings Scholar, The Queen's College, Oxford.

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to as anterior, middle or posterior grafts), various positions in the host (also classified into three types, and referred to as grafts in the anterior, in the middle, or in the posterior), various sizes of graft (though not by any means an exhaustive series), and three different orientations of the anterior-posterior axis of the graft, that is, like the host (referred to as aa.), opposite to the host (ap.) and at right angles across beneath the host primitive streak (crosswise). By no means all the possible combinations of the various positions and orientations were tried (sixteen different variations were actually used). It is not at present worth while doing such experiments in exhaustive fashion, since until some method of distinguishing graft and host tissues by other than morphological criteria is developed, no detailed account of the interaction of host and graft can be given. For a discussion of how certainly in such experiments graft and host can be distinguished, see Waddington (1932); to this it may be added that graft-derived somites and notochord seem to remain distinct from the corresponding host tissues to almost the same extent as graft-derived neural tissue.

II. DIFFERENTIATION OF THE GRAFT

(a) *Self-differentiation*

The differentiation which the graft undergoes is the outcome of the tendency of the graft to self-differentiate, and the influences which the host brings to bear on it. Anterior and middle grafts, but not posterior grafts, are sufficiently determined to self-differentiate, in some specimens, more or less perfectly; and all kinds of graft are sufficiently labile to be influenced by the host so that, in other specimens, they self-differentiate poorly or not at all. The conditions which determine whether a graft will submit more or less completely to the influences of the host, so far as they are known, will be discussed in detail below. On the whole, it can be said that the size of the graft, either in relation to the donor or to the host, makes no observable difference; nor does the age of donor or host (sufficiently varied experiments were not carried out to confirm the finding already established by Waddington & Schmidt (1933), that grafts from old blastoderms self-differentiate better than grafts from young ones); nor does the orientation of the graft, dv. or dd., aa. or ap. On the other hand grafts orientated crosswise, self-differentiate less well than those placed aa. or ap.; posterior grafts do not self-differentiate at all; and grafts in the posterior of the host are very greatly influenced, their further development being suppressed if they stay under the primitive streak of the host, while grafts elsewhere may be comparatively slightly affected.

Grafts in the posterior of the host axis are inhibited from further differentiation by the action of the host; but some cases occur of lack of differentiation apparently in spite of the influence of the host: thus in specimens QQ and QAD,¹ middle grafts, placed in the head of the host, have failed to differentiate, incidentally partially suppressing the development of the host head; and in several specimens,

¹ The various specimens are referred to either by a set of capital letters beginning with Q, or by a number prefixed by "35-".

especially where the graft was placed crosswise, graft-derived mesenchyme is found amongst the axial organs of the host.

A few of the grafts have differentiated into a good deal more than their presumptive fate. This may possibly show a tendency for the graft to complete itself, as found by Waddington & Schmidt (1933), but in these specimens it is probably more easily explained as due to the action of the host.

The host affects the graft in a number of different ways, which may, however, be divided into those where the influence of the host acts directly on the graft (since it can occur whether or not an induction by the graft has taken place), and is probably produced by the host axis, in short, the actions of the host individuation field; and those effects on the graft produced by the interaction of the graft and the reacting tissues of the host when the graft performs an induction.

(b) Influence of the host individuation field

The effects of the host individuation field can be classified, perhaps rather arbitrarily, into the following types: First, suppression of some of the tissues which the graft would normally form. Secondly, reversal of the antero-posterior orientation of the graft when it is opposite to that of the host. Thirdly, determination of the tissue differentiation of the graft to correspond with the region of the host axis in or near which the graft lies. Fourthly, the suppression of further differentiation in the posterior end of the host primitive streak. Fifthly, the arrangement of graft tissues, in certain ways, with regard to the host axis. Sixthly, a greater or lesser degree of fusion of the graft and the host tissues, in a few cases producing complete incorporation. None, one, or several of these effects may occur in a single graft.

Very few anterior grafts self-differentiate anything like perfectly. Head mesenchyme is the tissue most regularly formed, and neural tissue is the most often missing. Notochord and somites are intermediate in frequency of occurrence. These tissues fail to differentiate, presumably because of conditions in the host; but the only regularly occurring effect of the host individuation field of this type which can be discerned is one where the graft has been hindered or prevented from forming neural tissue in the posterior part of the host (not under the host axis): of seven anterior grafts which have self-differentiated to some extent in the posterior part of the host, four have formed no neural tissue and in three it is very weak or doubtfully present; while of ten which have self-differentiated in the anterior part of the host, two have formed none, two have it doubtfully or weak, and six have formed the normal amount of neural tissue.

The second type of effect consists in the reversal of the antero-posterior orientation of the graft when it is opposite to that of the host. This orientation can frequently be recognized in the fixed specimen by the relative positions of head mesenchyme and somites. Occasionally the size and position of the graft neural tissue has to be used as a criterion. In twenty-three specimens a recognizable antero-posterior differentiation has been established in grafts which were originally put in ap. In four which were from the posterior and one which was from the middle of the donor, the original orientation has been reversed to aa.; in some of

these specimens the graft is not in the host axis, but has formed an induction out to the side. Of the eighteen anterior grafts, all those (ten) specimens where the graft is outside (lateral to, behind, or in front of) the host axis have maintained their ap. orientation. Two of those immediately alongside the host have been reversed, and one has remained ap. Four of those which have stayed in the axis have been reversed, while one has remained ap., and this was under the anterior end of the host head. The reversal of anterior grafts therefore only takes place alongside or in the host axis, while the posterior grafts are reversed irrespective of their distance (within the limits of the experiments) from the axis. All grafts are reversed when they lie in the host axis in the trunk and the posterior half of the head regions. There is no correlation between size of graft, or age of host or donor, and the occurrence of reversal of orientation.

The behaviour of crosswise grafts may be mentioned here. They have usually failed to maintain their orientation. Three of them have performed inductions at right angles to the host axis, and have themselves self-differentiated in the position they were placed. The others (seven specimens) have either been incorporated at their ends into the host, while the part beneath the axis has been incorporated into the host axial organs, and hence acquired a new orientation (e.g. QAQ); or they have been drawn out into a strand of tissue down the host axis, and have also acquired a new orientation, that of the host (e.g. QAV).

The dorso-ventral orientation was noted in all grafts, but it is difficult to discover it in the fixed specimens. It can in fact only be recognized in the graft neural tissue, when this has been formed. When it is present it is found that the neural tissue has always maintained the original orientation of the graft; this is the case also with grafts from other parts of the epiblast (Abercrombie, 1937). At the age of these specimens, the mesoderm has no visible orientation, but its relations to the induced neural plates in the complementary inductions (see below) strongly suggest that it is reversed in these, if it was originally dv.

The third type of effect of the host occurs in the case of aa. grafts, and of ap. grafts which have been reversed. In these the host may influence the graft so that the differentiation of its mesoderm and notochord corresponds with the differentiation of the host axis at the same level. This may involve suppression of part of the normal prospective differentiation of the graft, as is found sometimes in anterior grafts in the region of the host head, which may form no somites (QE, QL, QAA, QW, Fig. 1); in QAA this effect has apparently been produced on a graft which has maintained its original ap. orientation. It may involve the acquirement of new or extended regions by the graft (in comparison with its prospective fate), as in QAE, where a middle graft, placed in the head and much elongated, has formed a full-length axis, with head (Figs. 2, 3); or as in QT and QAI, where small anterior grafts, which have been considerably elongated, have formed full-length somite regions (Figs. 5, 6). It is possible that this may be partly due to a tendency of the graft to complete itself, but such a tendency is not in these experiments found under other circumstances (e.g. in grafts which have remained ap.); it was, however, observed by Waddington & Schmidt (1933).

The prospective fate of the graft may be completely altered in the case of posterior grafts, which may be caused to form head mesenchyme (35-045), head and trunk mesoderm (QY, QAH), or trunk mesoderm alone (QAW, QAO, QAJ) (Figs. 7, 8, 9); such grafts acquire at the same time the power to evocate neural tissue in the host ectoderm, if they no longer lie in the host axis. In some of these (QY and perhaps QAH) and also in the middle graft QAE the graft has formed notochord although it did not contain presumptive notochord region. In QAE there is a particularly interesting arrangement of tissues, since the same strip of graft tissue is anteriorly somitic mesoderm (not, however, divided into separate somites), and posteriorly a notochord. This seems to show that the differentiation of the notochord depends upon a "situation stimulus" within the undifferentiated presumptive chorda-mesoderm, and not upon the different origin and much greater elongation of the notochord tissue compared with the somitic tissue; with a slight difference in the "situation stimulus" a particular part of the undifferentiated presumptive chorda-mesoderm forms somitic mesoderm. Complete alteration of the prospective fate occurs also in grafts placed crosswise, which, after being elongated down the axis of the host, may have their redistributed material regionally determined by the host into head and trunk mesoderm; this has happened in QAP (anterior graft) and QAV (middle graft) (Fig. 10). The regional determination by the host individuation field affects both grafts which have remained in the host axis, and those which have not. It is notable that when a graft has been in this way regionally determined, it never forms neural tissue unless presumptive neural tissue was present originally; thus none of the posterior grafts which have been converted to an anterior differentiation have formed any neural tissue. Nor do crosswise grafts which have been regionally affected produce neural tissue.

Quite often, when a graft extends into both head and trunk regions of the host, and is regionally determined by the host, the border between head and trunk is at exactly the same level in the host and graft axes; and this may apply also to other levels, such as the anterior end of the notochord and the position of the foregut. But more often there is only a rough general correspondence between the levels of the two axes: thus in QAE the graft head starts farther posteriorly than that of the host, and the host is for a short space somitic where the graft has head mesenchyme. The correspondence between graft and host intersomitic grooves, which has been noticed before in chicks (Waddington & Schmidt, 1933) and in Amphibia (Spemann, 1931), only occurs in about half the specimens where graft and host somitic regions are contiguous.

There is a regional correspondence with the host, induced by the host or the result of the placing of the graft, in almost all aa. or reversed ap. grafts. In QM, however, an aa. graft placed immediately in front of the host primitive streak has split the host head, so that the graft trunk region lies between the two halves of the host head (Figs. 11, 12). Ap. grafts do not of course correspond regionally with the host.

Anterior grafts are capable of differentiating into head and trunk anywhere round the host axis—head behind the host axis, or beside its posterior part, trunk in front of it, or beside its anterior part, are all found. The differentiation of an

anterior graft into a head is never regionally altered, wherever the graft may lie. A posterior graft is always regionally determined by the host.

A fourth type of effect of the host is one closely like the regional determination described above; it is found in all grafts which remain under the posterior end of the host primitive streak. Whatever the place of origin of the graft, it undergoes no further differentiation in this position, but remains a mass of primitive streak mesoderm, usually of exactly the same histology as the host primitive streak above it, and with a complete absence of any epithelium. Nine specimens show this effect. It is not produced on grafts lying to one side of the posterior end of the host axis.

The fifth type of effect is where the host has influenced in various ways the arrangement of the differentiated graft tissues when these lie in the host axis. There is no tendency for the graft neural tissue to be centred beneath the host axis. This is in contrast with the behaviour of ectoderm grafts, where centring of the graft neural plate is usual (Abercrombie, 1937); the difference is probably due to the fact that ectoderm grafts elongate, under the influence of the host, exactly in the centre of the host axis and here become neural tissue, while grafts of primitive streak are only seldom influenced in this way. There is sometimes, however, a tendency for the graft notochord to be displaced towards the centre of the host neural plate: in QAE the graft notochord is found on the side of the graft tissues nearest the midline of the host, with the graft somites lateral to it (Fig. 3); and in QAR the graft and host notochords are arranged symmetrically under the host neural plate, equidistant from its midline, with a somite series between them (Fig. 13). The somites and notochord derived from the graft are usually arranged in the same horizontal plane as the host somites and notochord, when they lie in or adjacent to the host axis; only seldom do they occur below the host axial organs, in the position in which the graft was originally placed.

The last type of effect of the host individuation field is the fusion which occurs between graft and host tissues, culminating in complete incorporation of the graft into the host. When graft somites occur adjacent to host side-plate mesoderm, they are generally attached at their outer edges to the side-plate, in the normal way; graft head mesenchyme is similarly continuous with host side-plate when this is possible (Figs. 2, 10, 11). Graft-head mesenchyme, when it lies adjacent to host-head mesenchyme, is nearly always fused to it, so that no line of demarcation can be found between host and graft tissues. They form together a single mass of mesenchyme, and because the neural plate stretches right across over this mass (often quite symmetrically) and the foregut right across beneath it, perfect incorporation often seems to have occurred. But in the region of the head where the host notochord is present, the asymmetry of the mesenchyme with respect to the original midline is usually clear (e.g. QAI, Fig. 4). In such a specimen, despite the fusion of graft and host-head mesenchyme, the graft notochord is always distinct from the host's, and the graft somites form a separate series in the trunk of the host (e.g. QY, QAI, Fig. 5). In general the graft somitic mesoderm maintains its autonomy to a remarkable extent. Thus in QAH there is almost perfect incorporation of the graft in the posterior part of the host head, forming a wide mass

of head mesoderm, with central notochord, normal neural plate above, and symmetrical foregut diverticula below; yet in the trunk region of the host the graft somites and notochord are distinct.

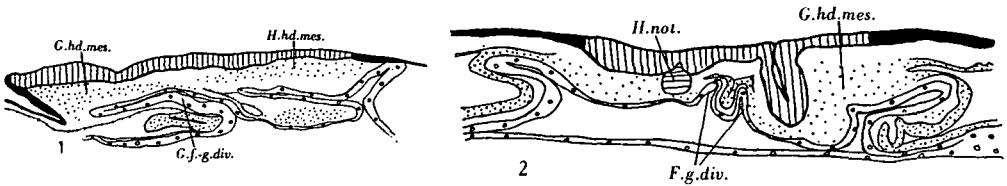
Fusion of at least part of a series of graft somites with those of the host does, however, occur (e.g. QAM, QAI, Figs. 3, 6); and graft-derived mesoderm may be added to the host mesoderm and the whole mass then divided up into somites, forming several rows of symmetrically placed and evenly spaced somites: this has probably happened in QAJ, which was a posterior graft, and has been regionally determined by the host (Fig. 9). Fusion may also occur in the case of the notochords (e.g. QAP, QJ, Fig. 6) and of the neural tissue (QAI, QJ, Figs. 5, 6). This fusion of part of the graft with the corresponding host tissue, resulting usually in some part of the host being unusually bulky and out of proportion with the rest, is an elementary form of incorporation. How far grafts which have remained distinct have also contributed to host organs without disturbing their normal proportions, it is of course impossible to say, but, if the chick resembles the Amphibia in this, it must be to a considerable extent. Several grafts have obviously in part become perfectly incorporated into the host, since they have decreased considerably in size without any abnormal disturbance of the host anatomy. This has happened to grafts of various kinds: anterior in anterior (QE), anterior in middle (35-034), anterior in posterior (QAZ), middle in middle (QAM), posterior in middle (QAX). The complete disappearance of a graft in the head and trunk regions of the host, leaving only slight traces of unincorporated tissue and only slight irregularities in the host, occurred in five specimens, of which three were from the posterior of the donor primitive streak, one from the middle, and one was a whole primitive streak placed crosswise. This indicates that complete incorporation takes place more easily in the case of posterior grafts.

Of the grafts which have formed primitive streak mesenchyme under the primitive streak of the host, most have remained recognizably distinct masses of tissue, although closely associated with the host tissues. Two (35-046 and 35-070) have disappeared entirely, and may have been perfectly incorporated into the posterior mesoderm of the host.

One type of incorporation, or perhaps resorption of tissues, seems to occur regularly; no trace of the endoderm, when it was present in a graft, can generally be found.

(c) Influence of induction

Inductions occur in about half the specimens, owing to the tendency for the graft to cease lying under the host axis, and hence to come into contact with the host ectoderm. In some cases when an induction takes place, the interaction of the graft and the reacting tissues of the host suppresses the formation of graft neural tissue, and perhaps affects the occurrence of other graft tissues. This occurs in those cases where the secondary axis is of entirely normal cross-section, the neural plate of this axis being induced from the host ectoderm, while the mesoderm and notochord, at any rate in part, are provided by the graft. In such an axis graft neural tissue would clearly be superfluous, and it is in fact suppressed (Fig. 14). Thus



Epidermis black, *neural tissue* vertical lines, *notochord* horizontal lines, *endoderm* circles, *somites* radiating lines, *other mesoderm* dots (*mesenchyme* dots not enclosed by lines).

Fig. 1. Specimen QL. Graft of the anterior third of primitive streak of medium streak stage donor; placed ap. under and in front of anterior end of streak of medium streak stage host. Grown 28 hours. Section through posterior part of head. The original ap. orientation has been reversed, and the grafts form a complementary induction of a head, at the level of the host head, free anteriorly, but in the section laterally fused with the host head. One of the foregut diverticula (*G.f.-g.div.*) of the host further anteriorly forms a closed foregut beneath the graft side of the neural plate. *G.hd.mes.*, *H.hd.mes.* graft and host-head mesenchyme.

Fig. 2. Specimen QAE. Graft of the middle third of medium streak stage donor; placed ap. under and in front of anterior end of medium streak stage host. Grown 20 hours. Section through the head. The original ap. orientation has been reversed, and the graft has formed head mesenchyme (*G.hd.mes.*) beside the host head. One of the foregut diverticula is associated with the graft, the other with the host; and between host and graft-head mesenchyme is a short stretch of mesothelium, below which is a double endoderm diverticulum (*F.-g.div.*), probably an attempt at two further foregut diverticula. The graft has induced a neural tube, which is laterally continuous with the host neural plate. *H.not.* host notochord.

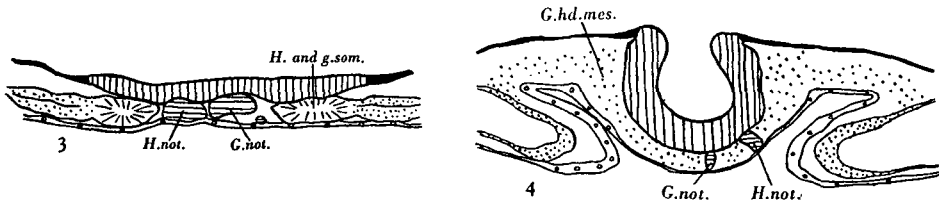


Fig. 3. The same specimen, in the trunk region. The graft has been considerably elongated down the host axis, forming a series of somites and a notochord, alongside the host axis. The neural plate, composed of host and induced neural plates fused laterally, is clearly of double origin. The graft notochord (*G.not.*) is displaced towards the host notochord (*H.not.*), and vice versa; there is no mesoderm between them. *H. and g.som.* host and graft somite series fused.

Fig. 4. Specimen QAI. Graft of the primitive node of head-process stage donor; placed ap. under anterior end of long-medium streak stage host. Grown 20½ hours. Section through the head. The host and graft-head mesenchyme is perfectly fused together, and the neural tube (partly induced by the graft) is almost normal in shape. The asymmetry of the specimen is, however, shown by the notochords, the host notochord (*H.not.*) lying in what is probably the true midline of the host, and the graft notochord (*G.not.*) displaced so as to be alongside it. The foregut diverticula are wider apart than usual owing to the extra head mesenchyme. *G.hd.mes.* graft-head mesenchyme.

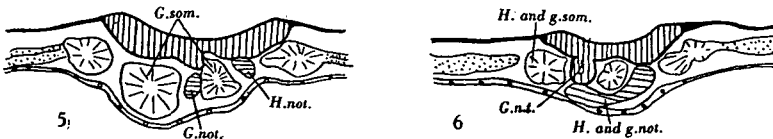


Fig. 5. The same specimen, in trunk region. The neural plate is slightly asymmetrical (the bump on it is due to the fusion with it of graft-derived neural tissue, and it is also widened by induction by the graft). Graft somites (*G.som.*) are quite distinct, although the head mesenchyme was incorporated. *G.not.*, *H.not.*, graft and host notochord.

Fig. 6. The same, more posteriorly. The lateral row of graft somites has fused with the host row of somites (*H. and g.som.*). The graft neural tube (*G.n.t.*) is partially free from the host neural plate (which is symmetrical), and the host and graft notochords (*H. and g.not.*) are fusing ventrally to the central graft somite (they eventually fuse completely).

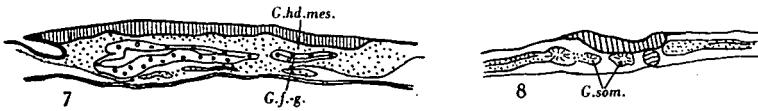


Fig. 7. Specimen QAH. Graft of posterior third of medium streak stage donor; placed ap. under and in front of anterior end of medium streak stage host. Grown 29 hours. Section through head. Very wide neural plate, one side induced by the graft, the other host. Separate closed foreguts, each derived from one of the host foregut diverticula, which are very widely spaced owing to extra graft-derived head mesenchyme (*G.hd.mes.*). *G.f.-g.* closed foregut associated with graft.

Fig. 8. Specimen QAW. Graft of posterior third of medium streak stage donor; placed ap. under anterior end of long-medium streak stage host. Grown 23 hours. Section through trunk region. Graft-derived somites (*G.som.*) cause asymmetry of neural plate.



Fig. 9. Specimen QAJ. Graft of a minute piece from posterior end of head-process stage donor; placed ap. under anterior end of medium streak stage host. Grown 23½ hours. Section through trunk. Graft has merely produced extra somites, on both sides of midline, symmetrically arranged. Probably the somites are formed from mixed graft and host tissues. *I.som.* graft-derived somite almost at intersomitic groove, out of phase with the other somites.

Fig. 10. Specimen QAV. Graft of second quarter from the anterior of medium streak stage donor; placed crosswise under anterior end of long-medium streak stage host. Grown 20 hours. Section through head. Graft has been elongated backwards and converted into a head and a trunk alongside the host axis, and hence completely re-orientated. The foregut diverticula (*F.g.div.*) are symmetrically disposed beneath the wide double neural plate and head mesenchyme mass. *G.hd.mes.* graft head mesenchyme, *H.n.pl.* host neural plate, *H.not.* host notochord, *I.n.pl.* induced neural plate.

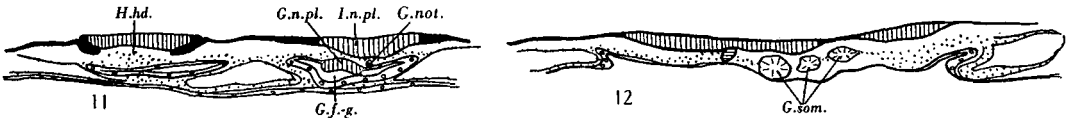


Fig. 11. Specimen QM. Graft of anterior third of long streak stage donor; placed aa. under and in front of anterior end of medium streak stage host. Grown 27 hours. Section through anterior end of specimen, with graft head beside host half-head (*H.hd.*) (the host head is split, but the other half does not extend so far anteriorly as this). The graft, which elsewhere forms a complementary induction, has here a patch of neural tissue (*G.n.pl.*), which is not however arranged with relation to the other graft tissues. The closed foregut (*G.f.-g.*) is induced from non-presumptive endoderm. *G.not.* graft notochord, *I.n.pl.* induced neural plate.

Fig. 12. The same, further posterior. The graft-induced neural plate is here fused with one side of the host-head neural plate. The graft forms three series of somites (*G.som.*) between the two masses of host-head mesenchyme. The foregut diverticula are very widely spaced, and only one closes off to form the host closed foregut.

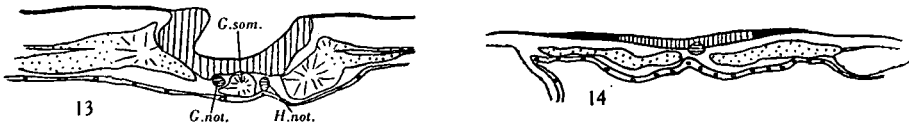


Fig. 13. Specimen QAR. Graft of anterior quarter of long-medium streak stage donor; placed ap. under anterior end of long-medium streak stage host. Grown 22½ hours. Section through trunk. The graft and host notochords (*H.not.* and *G.not.*) are symmetrically placed beneath the neural plate, with a (graft) somite (*G.som.*) between them (cf. QAI, Fig. 5).

Fig. 14. Specimen QAC. Graft of whole streak of short streak stage donor; placed ap. under and in front of anterior end of medium streak stage host. Grown 22 hours. Section in front of host head, in trunk region of the ap. graft. The graft has formed a perfect complementary induction. More posteriorly it has split the host head.

in QG, the graft (the anterior two-thirds of the donor primitive streak) has formed a full-length secondary axis, ap., beside and in front of the host axis. Its neural tissue has been induced from the host, while the graft has formed head mesenchyme with notochord, then a somitic region also with notochord, and finally a posterior mesenchyme region which is probably an induced primitive streak. Such an induction may be referred to as a complementary induction (Mangold, 1932).

(d) Discussion of the differentiation of the graft

The final differentiation of each graft is the product of the interaction between the individuation field of the graft and that of the host. The individuation fields consist of those influences which tend to build the tissues into a single normal axis. The graft individuation field tends to maintain the autonomy of the graft, and to cause it to self-differentiate. The host individuation field does the same for the host; but it also acts on the graft, producing the various effects which have been described, and all of which can be considered as varying degrees and kinds of conformity between host and graft tissues, culminating in complete incorporation. It is not supposed that each different type of effect of the host on the graft represents a specific and distinct type of influence proceeding from the host; some may as easily represent different results of the same influence. It is characteristic of these specimens that it is the host individuation field which forces the graft to conform, and except for rare exceptions, not vice versa.

The reversal of the ap. orientation of a graft clearly means that to a considerable extent the various presumptive tissues it contains cannot be self-differentiating. Presumptive somite material will perhaps be forming head mesenchyme, and vice versa, though presumptive somite material may also be forming somites (in the reverse orientation). Nevertheless, the graft is self-differentiating in so far as it is forming these tissues at all, and is not incorporated; and regarded as a whole the graft seems uninfluenced by the reversal of its ap. as to the tissues it forms. Thus a reversed ap. graft may form head mesenchyme in the somite region of the host if it contained presumptive head mesenchyme (35-034); and reversed ap. grafts form neural tissue if they originally contained presumptive neural tissue (QAR, QAI, Fig. 6), but not if they did not contain it (as in posterior grafts). But the graft is influenced as to whereabouts in itself the various tissues will appear; in 35-034 the graft mesenchyme is at the new anterior end of the graft, and in QAR the neural tissue is also at the anterior (in QAI it occurs right through the graft). The graft therefore, in these specimens, appears to be conditioned by its individuation field to form a certain set of tissues (those of its prospective fate), but the order in which they appear is dependent on an orientation, which is reversible by the host individuation field. It is, however, difficult to obtain confirmation of this supposition, because of the frequent occurrence of an influence of the host which alters the determination by the graft individuation field of what tissues the graft will form.

The influence of the host tending to reverse the ap. orientation of the graft is exerted only in the anterior of the host axis, or in the region lateral to the anterior end. In confirmation of the findings of Waddington & Schmidt (1933), the influence

seems to be most intense in the host axis, and to fall away with increasing distance from it.

The determination of the regional differentiation of the graft by the host involves further subordination of the graft individuation field. Although in most cases the reversal of an ap. orientation has been accompanied by regional determination, the reversal of the orientation can occur alone (35-034). But the regional differentiation is usually not affected unless the ap. orientation conforms to that of the host. As in the case of the reversal of the ap. orientation, neural tissue is produced in a graft which has been regionally determined only if it was part of the prospective fate of the graft; posterior grafts never produce it. These grafts are then conditioned by the graft individuation field to form part of an autonomous axis, containing or not containing neural tissue, but which part they form is decided by the host.

The grafts from the posterior maintain their autonomy also under the influence of the graft individuation field. It is interesting that this autonomy is, by the influence of the host, expressed in the form of a separate axis, although the grafts consisted of presumptive side-plate mesoderm, and were, at least in the experiments of Waddington & Schmidt (1933), placed in a region (beside the host axis) where the host mesoderm itself actually takes the form of side-plate.

The influence which suppresses the differentiation of the graft when it lies under the posterior of the host primitive streak differs from the influence altering the regional differentiation in that it is not exerted laterally to the axis, and at the same time it affects all grafts, without exception. It appears that differentiation in the posterior part of the primitive streak is impossible, and this influence is exerted not only on the host tissues temporarily there and in process of moving out to the sides (where they proceed to differentiate), but on tissues lying stationary below the axis. It is not therefore a function of the extensive invagination and movement of the host tissues there. No trace of epithelium is found in these grafts, although many of them must have originally contained some. Their behaviour is therefore comparable with that of ectoderm grafts in a similar situation (Abercrombie, 1937), which also undergo no differentiation, and tend to lose their epithelial character. Grafts of primitive streak which have for a short time been under this part of the host primitive streak, and have then ceased to lie there, are not affected in their subsequent differentiation, except possibly in the suppression of neural tissue, referred to as the first type of effect of the host.

Incorporation is the type of differentiation where the graft individuation field almost ceases to play a part. The tendency of the graft to remain autonomous was a factor in all the previously discussed types of differentiation, however much the autonomous graft was otherwise influenced by the host. But when incorporation occurs this tendency is overcome.

(e) Elongation of the graft

An elongation of the graft takes place normally in all fairly small grafts which differentiate to form an axis of head mesenchyme and somites. It occurs in anterior grafts whether they were originally aa. (e.g. QT), or were ap. and have been reversed

(e.g. QAI), whether they have stayed in the host axis (e.g. QAR) or whether they have not (e.g. QAE). It also occurs in posterior grafts which have been converted to an anterior differentiation (e.g. QY, QAH). It is unfortunately impossible to say whether the apparent absence of elongation in grafts which have maintained their original ap. orientation is of any significance. Elongation does not appear to take place in grafts which have differentiated only to head mesenchyme (with or without neural tissue QE, QL); nor in grafts consisting of all or almost all of the donor primitive streak (QG, QAC) (this does not apply when such grafts were placed crosswise). Crosswise grafts are not noticeably elongated in the long axis of the graft, but their material tends to be drawn down the axis of the host (QF, QJ, QAV, QAP). The elongation of the grafts is probably mainly due to an autonomous movement in the graft, more or less assisted by the movement down the host axis; in the case of the crosswise grafts the elongation down the host axis is probably mainly the result of the host movement, which is known to be able to influence grafts of epiblast (Abercrombie, 1937). No sign of a conflict between the host and ap. grafts in the host axis were observed; the graft has in these cases always been reversed in orientation. It is quite possible that the direction of tissue movements induced in the graft by the host is the first step in the reversal of the ap. orientation of these grafts.

III. INDUCTION BY THE GRAFT

An induction takes place wherever host non-neural ectoderm covers graft-derived head or trunk mesoderm. The induction can occur, as it usually does, because of the placing of the graft, in the epidermis lateral to the host axis, though it is often laterally fused with the host neural plate. Inductions can be obtained immediately lateral to the host primitive streak (e.g. QV, QAY) which shows that the host ectoderm is competent at this level, long before neural tissue is present. Grafts placed crosswise sometimes induced neural plates at the sides at right angles to the host axis, either on both sides of the host axis (QU; the host axis is, however, almost non-existent posterior to the graft), or only on one side (QBA). Inductions occur immediately in front of the host head, often with splitting of the head, so that the induction lies partially between two half-heads (e.g. QAC). And ap. inductions also occur in line with and immediately behind the host primitive streak region. In these the primitive streak may be common to both the host and the induced axes, so that a double-ended embryo results (e.g. QBC); or the posterior end of the graft may be over (QAG) or under (35-047) the posterior end of the host axis.

The induction is almost without exception controlled in its development, as regards its regional differentiation and antero-posterior orientation, by the graft; this is so for the antero-posterior orientation even where the induction is ap. and is for a space laterally fused to the host (QAC). The induction of course frequently conforms with the host, because the graft has itself differentiated under the influence of the host. But in two cases (QG, QB) it is possible that where the induced neural plate of an ap. graft has fused laterally with the host neural plate, the induced neural

plate has had its orientation reversed so as to conform with the host—the induced neural plate narrows down towards the posterior.

The induced neural plate tends, however, to be incorporated laterally into the host neural plate, independently of the degree of incorporation of the graft mesoderm into the host mesoderm, when the induction lies close to the host axis. Various degrees of incorporation can be traced in this. The induced neural plate can be quite free from, though near the host neural plate (QM): the two plates can be laterally fused in one part, but free in the rest (35-034); they can be laterally fused throughout, but only by their edges, so that the combined neural plate is clearly double (QAE, Fig. 3); they can be fused so that a single, but markedly asymmetrical plate is present (QAW, Fig. 8); and finally the symmetry of the single plate can show intermediate degrees of perfection (QAI, Fig. 5) culminating in a perfectly normal single neural plate (QAH). In all these, the graft mesoderm is, in the trunk region, distinct from that of the host, though of course adjacent to it. An interesting case is QAV where the neural plate anteriorly is of normal form, but has a deep crevice in the middle of it; it appears that one half is induced, and the other half is the host neural plate, with the notochord below it.

In the more usual form of induction the tissues of the graft develop and are arranged without apparent relation to the induced neural plate, which is as it were a fortuitous reaction of the host tissues, without influence on the graft; sixteen of the inductions are of this type. But thirteen inductions are of the complementary type, as described above. A complementary induction seems to involve the suppression of the graft neural tissue, and the arrangement of the graft notochord and mesoderm in relation to the induced neural plate (and perhaps to some extent vice versa). If any other tissue is contributed by the host to the secondary axis, then it must be arranged in relation to the other tissues of the axis, and the corresponding tissue in the graft must be suppressed or rearranged. A complementary induction of a full-length axis is found in five specimens. In QM and QBC there is a long complementary axis, but in it a short patch of graft neural tissue occurs; this neural tissue is not, however, arranged centrally below the graft notochord, and therefore in its normal position in the graft tissues, but it is displaced to one side; the graft mesoderm and notochord, in the region of the graft neural tissue continue to be arranged in relation to the induced neural plate (Fig. 11). A good complementary induction of part of an axis occurs in QL, where the graft has formed head mesenchyme only, inducing a foregut and headfold as well as a neural plate; and in QC, where, in the anterior half of the graft, the graft somites and notochord are arranged around the graft neural plate, in the form of an axis, close against the endoderm of the host, but in the posterior half of the graft the graft neural plate disappears, an induced neural plate appears, and the notochord and somites move up from their ventral position to become normally arranged immediately below the induced neural plate.

In six of the complementary inductions the posterior part of the secondary axis is apparently a primitive streak, which must of course have been induced by the graft (e.g. QBC). This suggests that most complementary inductions are the

result of the induction of a primitive streak in the host ectoderm by the graft, the graft fusing (perhaps sometimes not quite completely) with this primitive streak as it is being formed; and that this primitive streak then differentiates like a normal axis. If this is so it makes it much easier to see how the differentiation of the graft and induction is controlled by a single individuation field. In this case it would not be accurate to refer to the mesoderm and notochord of such an axis as graft-derived; there must be an admixture of host tissues. The ordinary inductions take place on the other hand by evocation of the competent host ectoderm direct by the inducing tissues of the graft. Some complementary inductions also apparently occur this way (e.g. QC), with suppression of the graft neural plate and rearrangement of the graft mesoderm and notochord.

The individuation of these complementary inductions takes place in exactly the same way as in the normal inductions; that is, they depend mainly on the graft, but the induced neural plate may be fused laterally with the host neural plate.

A closed foregut and a headfold are both rather rarely induced. The closed foregut of the graft may be formed by one of the foregut diverticula of the host axis, when the graft lies adjacent to the host (QL, QAH, Fig. 7); in these cases the host foregut is formed from the other diverticulum. Or it may be induced from endoderm which is non-presumptive foregut (QS, QM, Fig. 11). In only one specimen is it formed in a graft that has maintained an ap. orientation (QS); and no vestige of a foregut or of a head fold is ever induced by the grafts which have differentiated ap. behind the host axis. A headfold is only induced in two specimens, both with aa. grafts in the anterior (QL, QM).

IV. DIFFERENTIATION OF THE HOST

(a) *Shifting of the axis*

In spite of the fact that the graft was always placed under the host primitive streak (though sometimes partially anterior or posterior to it), at fixation it is, in twenty-five out of the fifty specimens, at one side of the host axis. This is in some cases clearly due to a shifting of the graft, under the influence of the host tissue movements; this must be the explanation when the graft, with one end under the host axis, projects sideways at an angle to the host axis, which has remained straight. But in the majority of cases it was probably the host primitive streak which shifted to one side of the graft. For it is known that grafts of ectoderm are very rarely shifted sideways by the host tissue movements (Abercrombie, 1937); and it would certainly be no easier to shift a graft of primitive streak. In QP and QAI the host axis was observed to shift during development, but usually it was not possible to make direct observation. In 35-033, where the graft was placed under the posterior of the primitive streak, the host axis has bent away from the graft almost at right angles. The explanation of this shifting is at present obscure.

In some specimens the host head is turned to one side at an angle to the rest of the host axis, with the graft beside it. This is presumably due to deflexion of the head process mesoderm.

(b) Alteration of regional differentiation

A partial suppression of the host head seems to have taken place in three specimens. In AQ and AAD the graft, from the middle of the donor primitive streak, has remained an undifferentiated and mainly epithelial, but not unduly necrotic, mass under the front end of the host head. Above the graft is a very little head mesenchyme and a very poor and rather necrotic neural plate, which peters out anteriorly with no further structural alteration. Immediately behind the graft, head mesoderm at once appears in the normal amount, a proper neural plate appears above, and a foregut below. In QAA, the anterior end of the host overlies the posterior end of an ap. graft in front of it. Here also the head mesenchyme is reduced, there is no foregut, and the neural plate is wide and thin; while behind the graft the head becomes more normal.

(c) Splitting of the head

Three cases occurred where the host head has been split by the graft. In QM the graft was aa., and was placed immediately in front of the anterior end of the host primitive streak. Not only is the head split, but, by the posterior extension of the graft, the split extends right back into the beginning of the somite region. The left side of the split is much smaller than the right, which forms a normal head (Figs. 11, 12). In QS the host head is split on an ap. graft placed in front of the host primitive streak. Considerable distortion and compression of the resulting half-heads has occurred, so that they actually lie partly in the level of the host somites. Each half-head has a closed foregut formed from one foregut diverticulum, and its own normally formed mass of head mesenchyme. The head of QAC has been split by a graft similar to that of QS, but the half-heads are not compressed. Each half-head has a deep foregut diverticulum (which does not close off). The specimen is peculiar in that the split is apparent far behind the position of the graft, the host-head mesenchyme always forming two distinct masses, and the host-neural plate being split by a narrow non-neural strip far down the trunk. In both these embryos, which resemble the specimen described in Abercrombie (1937), the split has presumably been caused by a splitting of the forward growing head process mesoderm.

(d) The foregut diverticula

In a large number of specimens the graft-derived head mesenchyme lies immediately adjacent to and fused with the host-head mesenchyme. When this happens, the combined head mesenchyme has a considerable lateral extension; at its edges it passes over into the mesothelium of the side-plate mesoderm. In all these cases the foregut diverticula, at their posterior origin, are found at the outside edge of the total expanse of head mesenchyme; there are never any cases, when graft and host-head mesenchyme are continuous, even if the graft is ap. (QG), in which even a rudimentary foregut diverticulum is present, at the side on which the graft is, in its normal position in relation to the host-derived head mesenchyme (Figs. 4, 10). Eleven specimens show this. This fact strongly suggests that the

foregut diverticula are induced where head mesenchyme joins the lateral plate mesothelium. Confirmation of this comes from other specimens. In QAE the graft posteriorly forms head mesenchyme continuous with the host's, and the foregut diverticula are on the outside edge of this head mesenchyme; but anteriorly the graft diverges rather from the host axis, and between the head mesenchyme of graft and host there appears a very short stretch of mesothelium; and below this mesothelium a rather feeble double foregut diverticulum is here and only here developed (Fig. 2). In QAD and QQ the head mesenchyme of the host is almost entirely suppressed at the most anterior end of the head, and the graft here lies between the head mesenchyme and the endoderm; in this region there are no foregut diverticula. But in the posterior part of the head, at the place where the graft dies away, and the head mesenchyme becomes well developed, and in contact with the side-plate and with the endoderm, foregut diverticula develop.

The conditions which determine the closing off of the foregut diverticula to form a closed foregut cannot yet be ascertained. But in almost all the cases where the host head is split, or the graft forms a head alongside the host head, so that the foregut diverticula are widely separated, one foregut diverticulum becomes associated with each head, and usually closes off (five specimens) (Fig. 7). Nor is the extent of the closed foregut dependent on the neural plate, since in QE it occurs, in association with head mesenchyme, well lateral to the neural plate, and in QAR, also with head mesenchyme, anterior to where the neural plate has ceased.

V. SUMMARY

1. Grafts consisting of pieces of primitive streak from blastoderms in the primitive streak stage were placed under the primitive streak of blastoderms also in this stage.
2. Various effects of the host on the graft are described, particularly the reversal of the antero-posterior orientation of the graft, the alteration of the regional character of the graft so as to conform with the host tissues at the same level, the suppression of differentiation in the posterior end of the primitive streak, and the incorporation of the graft tissues into the host.
3. A considerable number of inductions occurred, since the host axis often apparently shifts to one side of the graft. The inductions are of two kinds, the normal evocation by graft mesoderm, resulting usually in the formation of superfluous neural tissue; and the complementary induction of a normal secondary axis, which it is supposed is most often due to the preliminary induction of a primitive streak in the host.
4. Various effects of the graft on the host occur. In particular the disturbance of the head mesenchyme suggests that foregut diverticula are produced where head mesenchyme joins lateral plate mesothelium.

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