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The contribution of the primitive streak to the somites in the avian embryo

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SUMMARY

Chick embryos were removed from the egg at stages 6–11 and explanted in culture. The greater part of the postnodal primitive streak of each embryo was replaced with a similar region taken from a corresponding quail embryo. The reciprocal experiment was also carried out, chick primitive streak being grafted in place of quail. After further incubation, the grafted primitive streak cells were found to contribute to lateral plate mesoderm, somites and intermediate cell mass. In an additional series of experiments, the postnodal primitive streak was extirpated and the embryo allowed to heal without a graft being inserted; after further incubation, many more somites formed in these embryos. It is concluded therefore that the contribution of cells from the primitive streak shown in the first experiment may not be essential for somite formation. It is suggested moreover that two major morphogenetic movements are taking place simultaneously in the mesoderm during this period: one is the mediolateral migration of cells after ingression through the streak, whilst the other is an anteroposterior movement associated with regression.

INTRODUCTION

In the chick embryo the presumptive somite region lies at the full-length primitive streak stage in a crescent-shaped area on either side of Hensen's node and immediately behind it (Pasteels, 1937; Spratt, 1955; Rosenquist, 1966; Nicolet, 1970). This region apparently becomes stretched out along the axis during the regression stage of gastrulation. The evidence is based on experiments in which different areas of the embryo have been labelled by vital dyes or by tritiated thymidine. The relationship between the presumptive somite areas and the primitive streak is however far from clear. According to Gallera (1975) some at least of the future somite cells are still in the ectoderm at stage 4 of Hamburger & Hamilton (1951). Apparently, however, they undergo a 'massive invagination' into the primitive streak at stage 5 (Nicolet, 1967).

The problem of what happens to these cells after they enter the primitive streak has however received little attention. There appear to be two possibilities. Either

Key words: avian embryo, somite, primitive streak, chick/quail grafts.

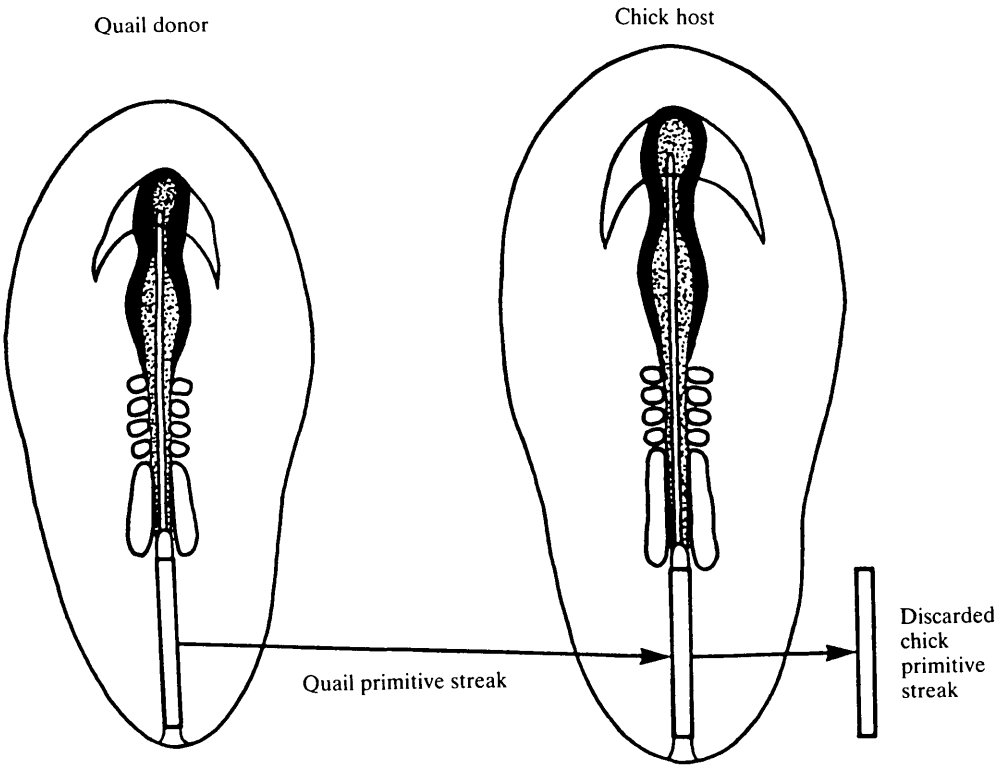


Fig. 1. Diagram to show the grafting experiment. Most of the primitive streak which lay caudal to Hensen's node and to the segmental plate in a chick host embryo was replaced by a corresponding region from a quail donor embryo.

they remain for some time in the primitive streak and leave it gradually in an anteroposterior sequence, or they immediately migrate laterally. Thus, when the presumptive somite cells become drawn caudally during regression, are they still in the primitive streak, or have they already become mesoderm lying in bands on either side of the primitive streak? The question is of significance not only for an understanding of the development of the somites themselves but also because of the light it throws on the morphogenetic movements involved in regression.

MATERIALS AND METHODS

Chick and quail eggs were incubated until the embryos were at stages 6–12 of Hamburger & Hamilton (1951). Embryos were then removed from the yolk and placed dorsal side uppermost in a Falcon plastic Petri dish, directly on the plastic. The stage of the embryo and the number of its somites were recorded, the operation was performed and the embryo was re-incubated for about 2–3 h at 37 °C. If the operated region had then healed, the embryo was transferred to a vitelline membrane and cultured according to the technique of New (1955) for a period of between one and two days. The operations were of two types.

Experiment 1. The greater part of the primitive streak (excluding Hensen's node) was removed from a chick and replaced by a similar piece of primitive streak from a quail (see Fig. 1). Attempts were made to use a chick and a quail of the same stage of development although the quail was smaller than the chick. This meant that the graft consisted of almost the

entire postnodal length of the quail primitive streak, but it often replaced only about the anterior three quarters of the chick postnodal primitive streak; the posterior quarter of the host primitive streak thus remained *in situ*. Reciprocal exchanges were also made, chick primitive streak being grafted in place of quail primitive streak.

Care was taken to make the lateral cuts for both host and donor as close as possible to the primitive streak. The following dimensions were recorded: length of graft (l), width of graft (w), distance of anterior border of graft from posterior border of last somite, (see Fig. 1). 84 embryos in this group of experiments were fixed, 14 being subsequently discarded because the donor cells could not be found.

Experiment 2. The postnodal region of the primitive streak was extirpated but no graft was inserted. Instead, the two cut edges were allowed to heal together. 60 embryos in this group were fixed.

In 12 further embryos, the entire primitive streak (including Hensen's node) was removed and the cut edges allowed to heal together.

After the operated embryo had been further incubated, the number of somites was recorded. Those embryos which had healed well were fixed in Zenker's or Carnoy's fluid. Serial sections, both transverse and sagittal, were prepared and stained by Feulgen's and light green (for experiment 1), or by haematoxylin and light green (for experiment 2).

Controls. For both series of experiments control embryos were grown under the same conditions and at the same time, but without operation. 38 control embryos developed normally. In 5 additional controls the primitive streak was extirpated and replaced *in situ*.

RESULTS

(1) *Grafting experiments*

General. The size of the graft was not standardized but depended on the condition of each pair, host and graft. In general however, grafts used with the younger embryos (i.e. stages 6, 7 and 8) were longer than those used with older embryos (i.e. stages 9, 10, 11). Thus the mean length for the younger stages was $730\,\mu m \pm 217$, compared with that for the older ones of $666\,\mu m \pm 207$. This difference reflected the progressive shortening of the primitive streak as regression occurs. The width of the individual grafts also varied, ranging from $125\,\mu m$ to $500\,\mu m$.

The distance from the anterior border of the graft to the posterior border of the last somite also varied considerably even in embryos of identical stages, e.g. in two embryos at stage 7, the distance was $1375\,\mu m$ and $950\,\mu m$ respectively, owing to differences in length of their segmental plates. Of the 70 specimens successfully healed and analysed, 14 were fixed after 18 h, 46 after 24–28 h and the remainder after 42 h incubation.

Healing. This had usually taken place within 3 h of grafting. With good healing it was usually impossible to recognize the graft in the living embryo the next day. The stage of development reached, as judged by the number of somites which had developed, was usually comparable to that of the control embryos. Thus, specimens explanted at stage 9 (7 prs of somites) had reached about the equivalent of stage 11 after 18 h, stage 12 after 24 h and stage 15 after 42 h. After sectioning, the quail and chick cells were distinguishable from one another in the Feulgen-stained preparations (technique of Le Douarin, 1969). In some embryos, patches

of pyknosis were visible at the junction of the graft and host and were interpreted as evidence of trauma due to the operation, since similar patches were visible in some of the replacement control embryos. If the region of pyknosis was extensive the embryo was discarded.

Location of the graft cells. In healthy, healed specimens, the graft cells were found in the following locations: somites and/or segmental plate, intermediate mesoderm and the proximal part of the lateral plate. The most anteriorly situated graft cells were usually found either in the somatic lateral plate (Fig. 2) or in the somites (Figs 3, 4, 5). Further posteriorly, the graft cells were usually present in large numbers throughout the somites (Fig. 3) though in some embryos they remained restricted to the dorsal side, or more rarely, were found only on the ventral side. Graft cells could be found however in any part of a somite including the central lumen (Fig. 6), but some host cells were usually still visible in the caudal somites or the segmental plate. In most embryos the graft cells were found in similar positions in the right and left sides of the host, but occasionally the two sides differed, e.g. in one embryo quail cells could be seen mainly at the dorsal side of the left somite and the ventral side of the right somite, though further caudally quail cells are found throughout the left and the right somites.

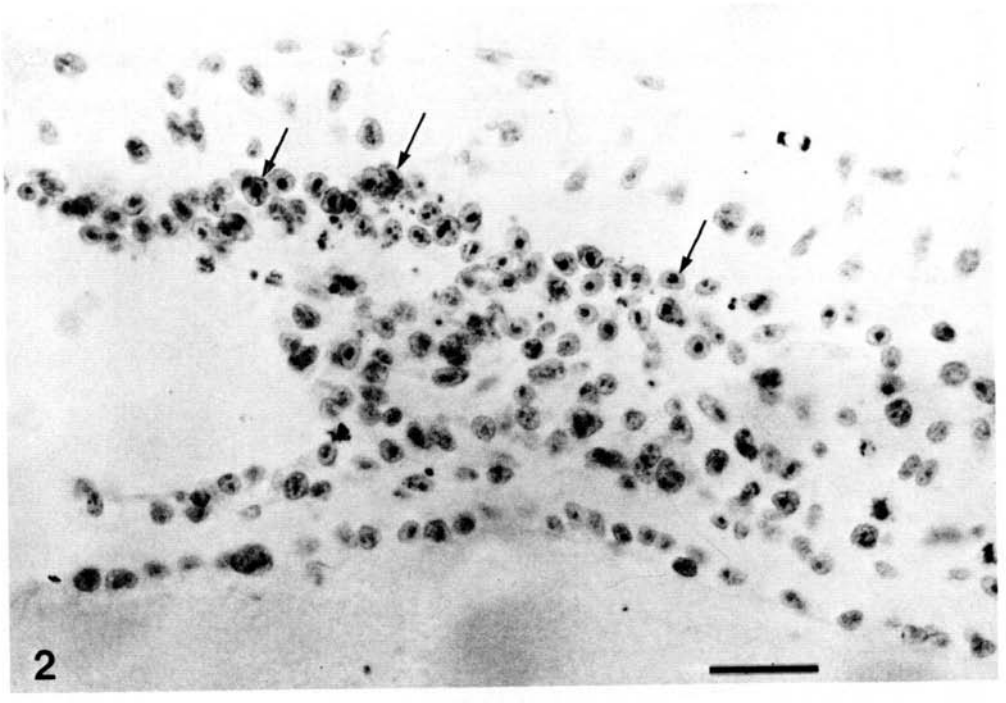
In six embryos an additional though incompletely separated row of somites was visible at the time of fixation on either one or both sides. In some of these embryos the distal part of each double somite was composed largely of quail cells, whilst the proximal row was of chick cells (Figs 2, 4, 5), but in other embryos both proximal and distal somites each consisted of a mixed population of chick and quail cells.

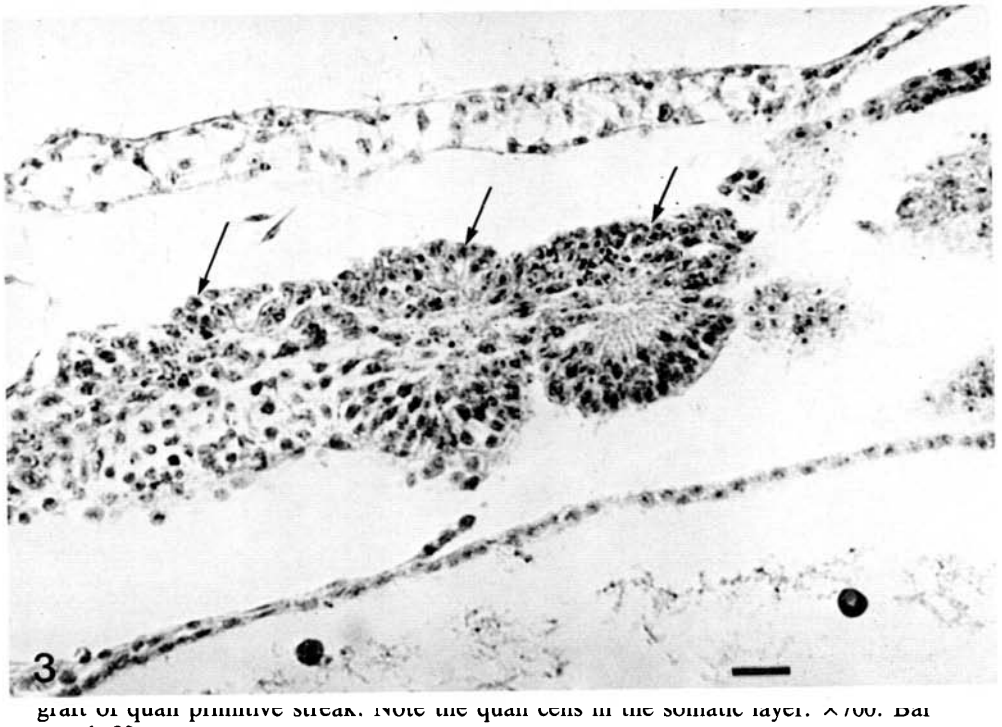
In the lateral plate, graft cells were restricted to the proximal region and were usually in the somatic layer (Fig. 2). Occasionally they were also found in the splanchnic layer. Graft cells were often present in the intermediate cell mass.

Graft cells were never found in the neural tube or notochord, which were composed exclusively of host cells which must therefore have moved caudally. Host cells were also found in the tail bud surrounded by graft cells (Fig. 9). Similarly, graft-derived cells were not found in the ectoderm or the endoderm throughout the region of the graft, although they were sometimes seen in the most medially situated endoderm of the tail bud (Fig. 10).

In embryos with incomplete healing, the pattern of graft cell distribution was modified. For example, in two specimens where the graft failed to heal at one side, small somites on the unhealed side were entirely host-derived, whilst in four others no somites were present in the regions lateral to the unhealed wound.

No correlation could be found between the distribution of graft-derived cells and the length of graft, or width of graft. The stage of the host or donor at the time of operation did not appear to affect the mediolateral distribution of the graft-derived cells. Graft cells were found in the lateral plate when the operation had been performed in embryos at all stages between 7 and 11 (see Table 1). Furthermore, similar results were obtained when a chick graft was inserted into quail host (Fig. 8). The younger the host embryo at the time of operation however the more anteriorly the labelled cells were discovered.





graft of quail primitive streak. Note the quail cells in the somatic layer. ×700. Bar equals 20 μm.

Fig. 3. Longitudinal section through somites and segmental plate of a chick host which had received a quail primitive streak. Note the quail cells in the dorsal side of the mesoderm. ×370. Bar equals 20 μm.

(2) *Extirpation of primitive streak*

Healing. Again, healing usually took place within about 3 h though it was often possible to recognize the region of the operation at a later stage.

Number of new somites. Most specimens were fixed at 18–24 h after the operation, and the number of new somites was then counted (Fig. 12). Unoperated control embryos usually formed between 10 and 16 pairs. Fifty-five experimental embryos from which the postnodal primitive streak had been removed, were fixed at this time. Nine of these were judged to be retarded since they had each formed less than 10 pairs of new somites. Twenty-one of this group of experimental

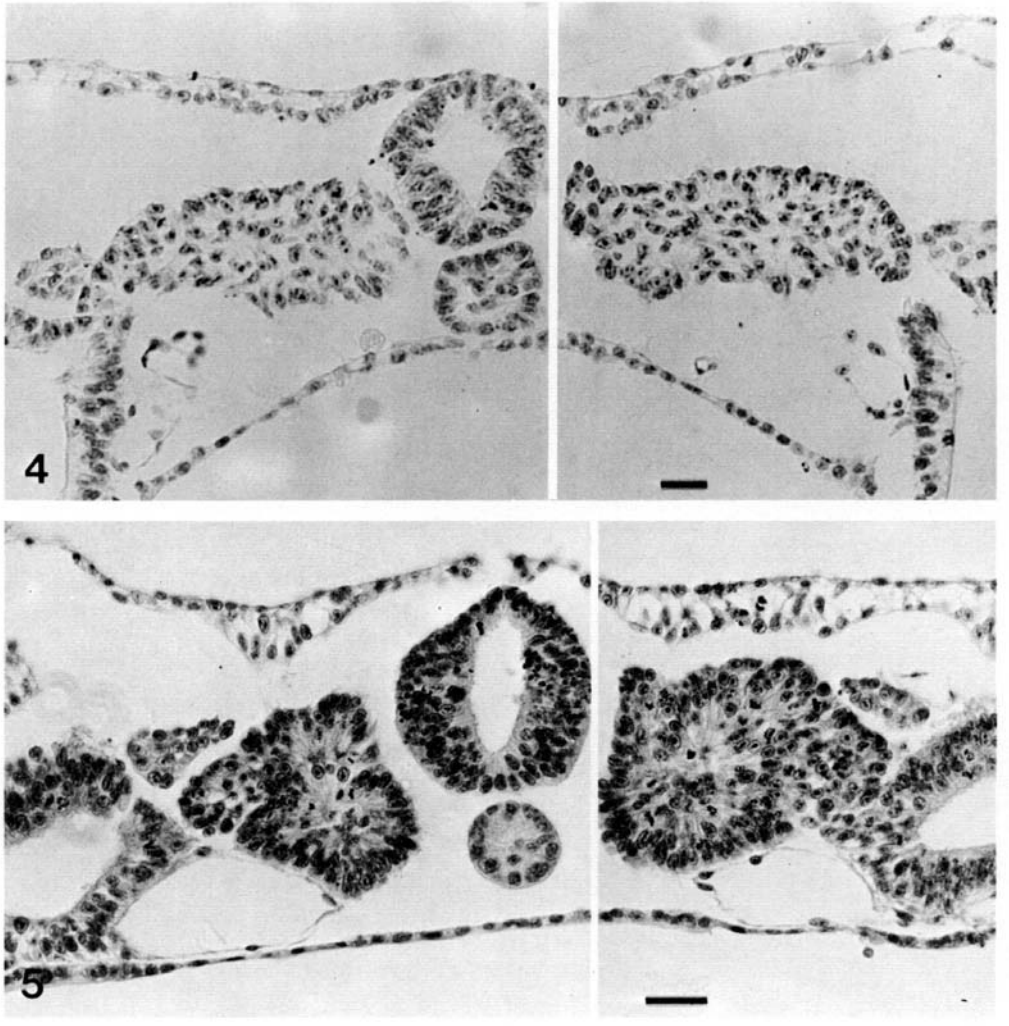
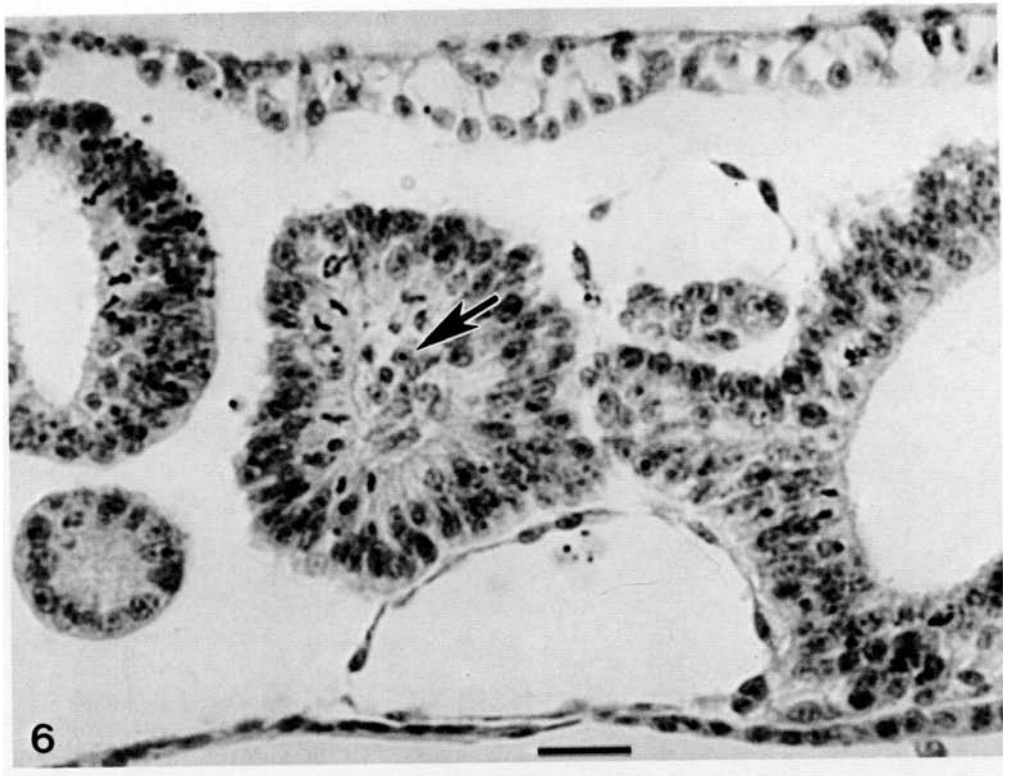


Fig. 4. Transverse section through a chick host which had received a quail primitive streak. Note the quail cells are distributed throughout the segmental plate. ×300. Bar equals 20 μm.

Fig. 5. Transverse section through a chick host which had received a quail primitive streak. Note the quail cells in the dorsal part of the somite. ×400. Bar equals 20 μm.



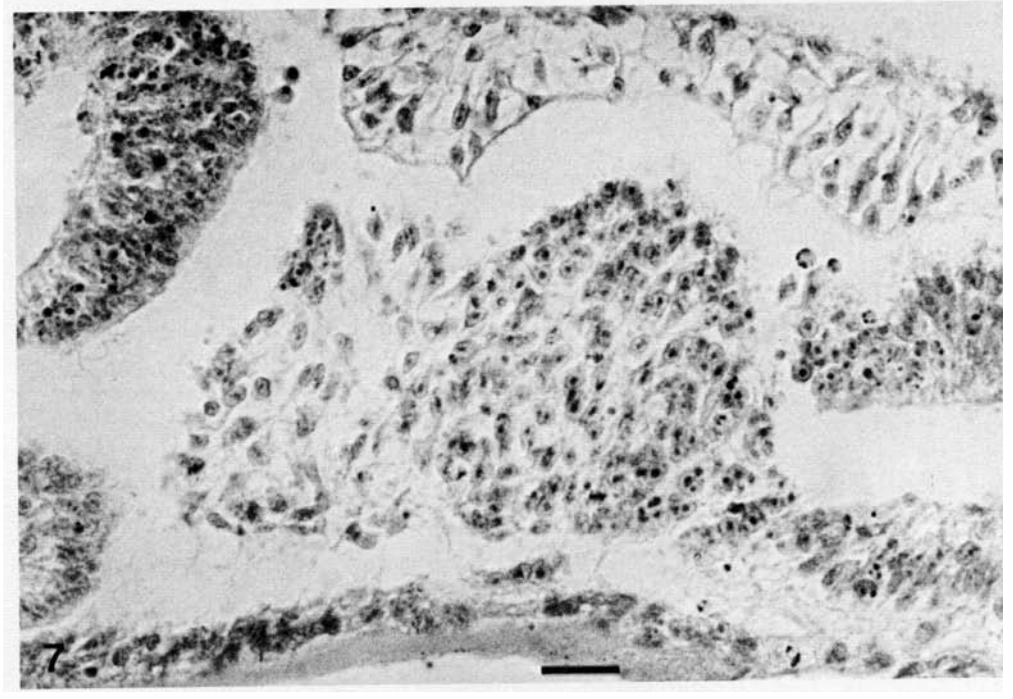
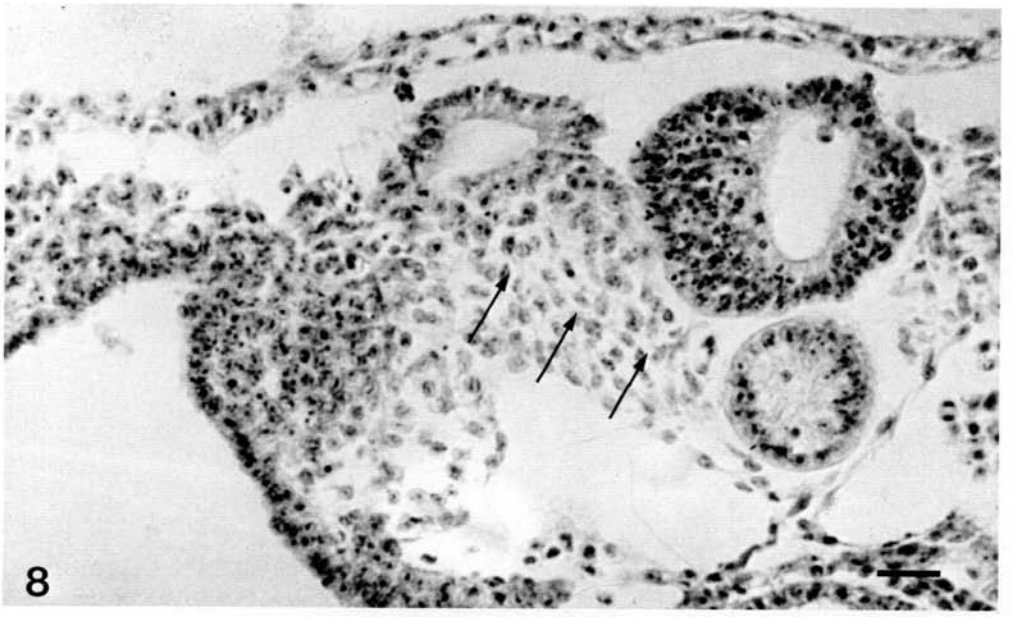


Fig. 6. Transverse section across a chick host which had received a quail primitive streak. Quail cells lie in lateral plate and somite. Note quail cells in lumen of somite (arrow). ×600. Bar equals 20 μm.

Fig. 7. Transverse section across a chick host which had received a quail primitive streak. Note the double somite, the medial portion being composed mainly of chick, the lateral part being mainly quail. ×500. Bar equals 20 μm.



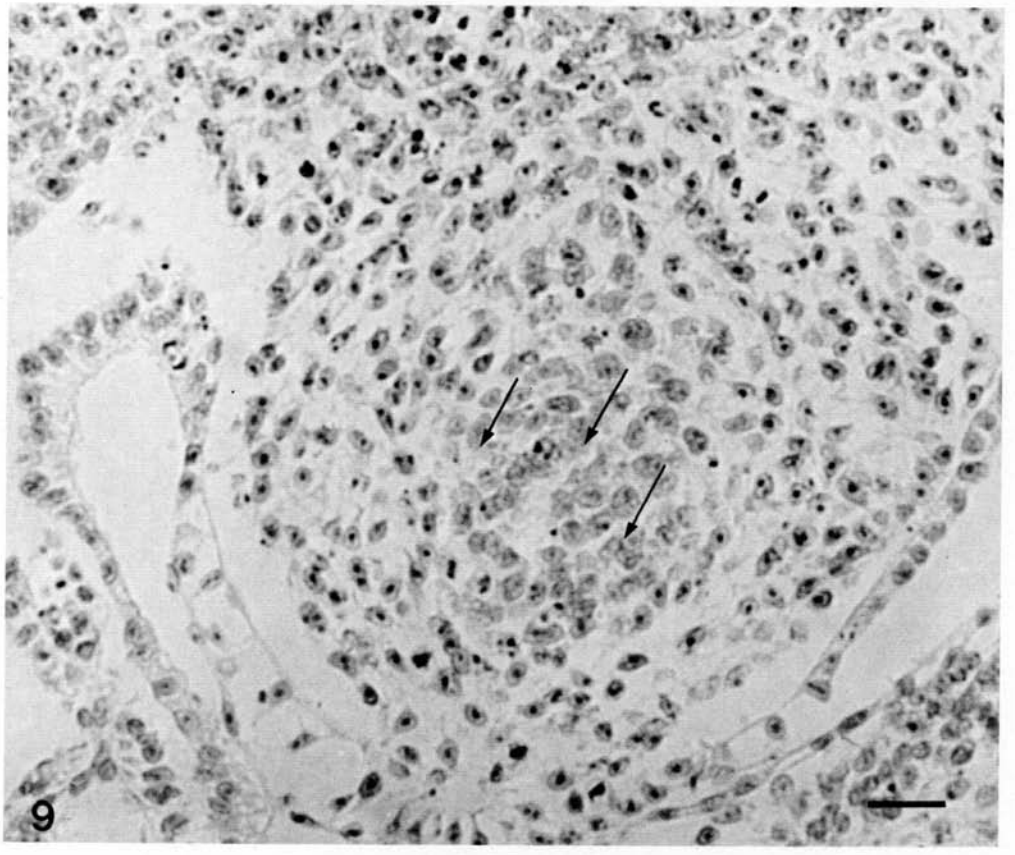
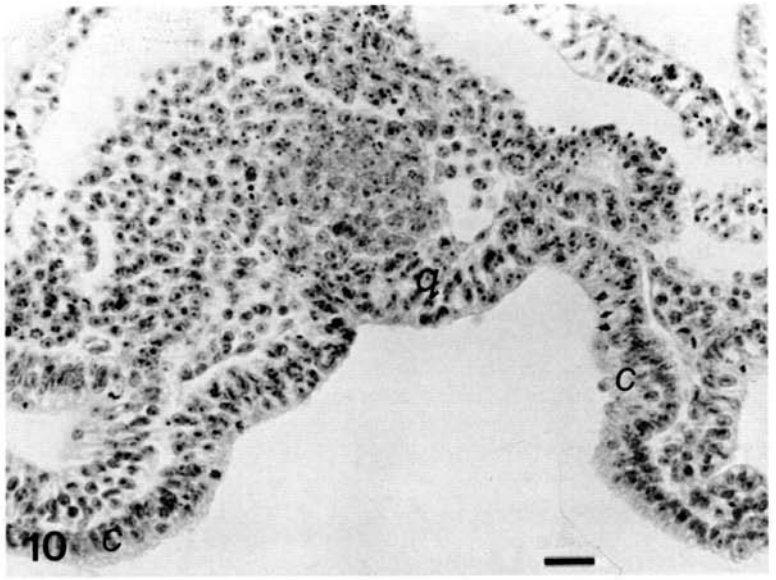


Fig. 8. Transverse section across quail host which had received a chick primitive streak. Note the chick cells in the somite. ×400. Bar equals 20 μm.

Fig. 9. Transverse section across the tail bud of a chick host which had received a quail primitive streak. Note that host cells (arrowed) are surrounded by quail cells. ×500. Bar equals 20 μm.



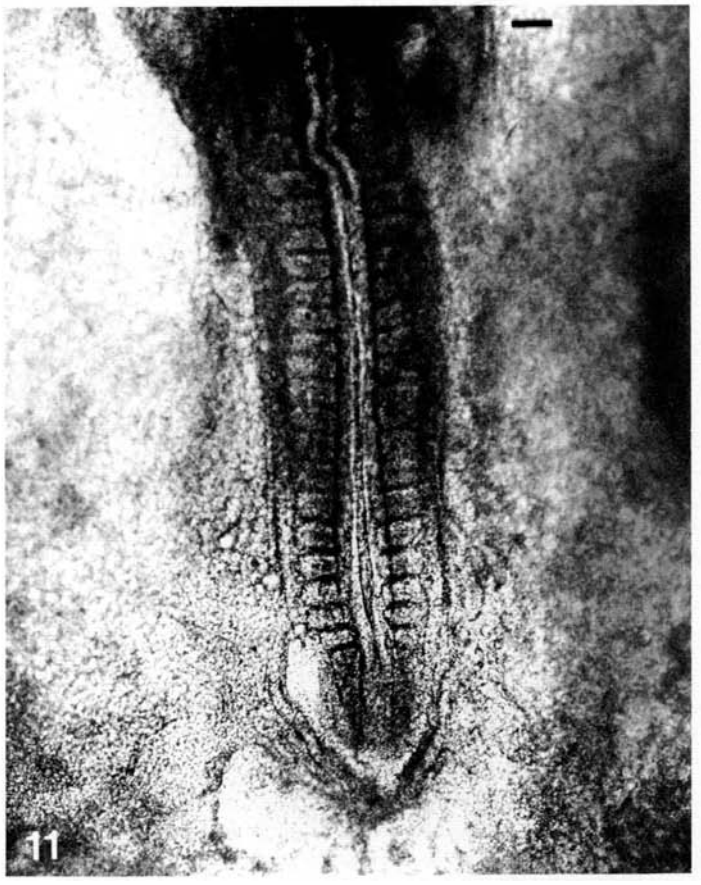


Fig. 10. Transverse section across the tail bud of a chick host which had received a quail graft. Note that the medial endoderm is of quail origin. *c*, chick; *q*, quail. ×320. Bar equals 20 μm.

Fig. 11. Chick embryo from which the postnodal primitive streak was removed at stage 8. After further incubation the embryo had developed an additional 19 pairs of somites. ×26. Bar equals 20 μm.

Table 1. *Destination of graft-derived cells*

Stage	Som. No.	S.M. & L.P.	S.M. alone	L.P. alone	N.
6–7+	0–2	3	1	–	4
8	3–4	10	–	–	10
8+	5–6	19	7	2	28
9	7–8	17	4	1	22
11	9–13	5	1	–	6
					70

The stage of the host and the number of somites possessed by the host at the time of operation are shown in columns 1 and 2. Destination of graft cells is shown in columns 3, 4 and 5, each figure indicating the number of embryos concerned.

Som. No. = number of somites at time of operation
S.M. = somitic mesoderm (i.e. somites and/or segmental plate)
L.P. = lateral plate
N = total number of embryos

embryos had however formed 14 or more pairs of new somites during the same period. In nine further embryos (not shown in Fig. 12) in which the node had been removed at the same time as the primitive streak, a similar range of new somites was formed.

Five further specimens from which the postnodal primitive streak had been removed were fixed at 42 h and were found to have formed correspondingly more somites (Fig. 11). In seven of the fifty-five embryos, a small fragment of baby's

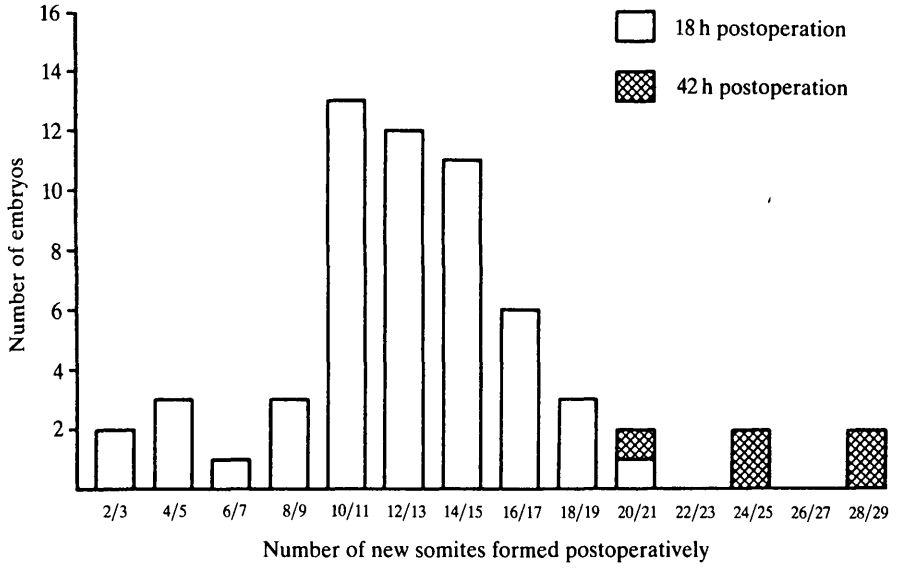


Fig. 12. Diagram to show the number of new somites which formed in embryos from which the postnodal primitive streak had been removed but not replaced by a graft. Twenty-one experimental embryos had formed 14 or more pairs of somites after 18 h of further development. Five other embryos which were fixed after 42 h had formed even more somites.

hair was inserted at the lateral edge of the area pellucida at the level of the anterior end of the cut, and in each case this hair was subsequently found nearer the posterior end of the area pellucida. In six other specimens, carbon particles were jabbed into the anterior border of the wound; some particles were subsequently found at the posterior end of the embryo, including the tail bud, though the main body of carbon remained further anteriorly. The new somites which formed in the streakless embryo, were usually comparable in size to those of the controls, though occasionally very small somites were seen especially in the operated region. Little can be deduced from that observation however, since in two of the control embryos similar miniaturized somites were seen at the posterior end.

Sections cut through the trunk region of the experimental embryos showed that the neural tube and notochord often became smaller and smaller, until at the extreme posterior end they disappeared, a thick band of segmental plate-like mesoderm, being sandwiched between thin sheets of ectoderm and endoderm. In other embryos however the neural tube and notochord ran continuously down into the tail bud.

DISCUSSION

There are four main points for discussion:

(1) *Fate of the cells in the primitive streak*

At the stages used in the present experiments, the anterior part of the embryo has already formed neural tube and notochord, and some somites and segmental plate are present. The caudal end is however still undergoing gastrulation. The fate of the primitive streak at this stage has hitherto received little attention although there is some implication in the work of Rosenquist (1966) that it gives rise to lateral plate, but the evidence is not clear. The results of our chick/quail grafting showed that the primitive streak cells contribute to a variety of tissues, lateral plate, somites, and intermediate cell mass, but no correlation could be found, within the limits of our experiments, to suggest that the destination of the primitive streak cells was related to the size or position of the graft or to the age of the host. We do not know what guides the cells to their destinations but it seems likely that the basal lamina of the ectoderm continues to play an important role, just as it does in the earlier primitive streak stages (Sanders, 1983). If this is so, it may explain why the most anteriorly situated graft cells tend to be located dorsally, irrespective of whether they are situated in a somite or in the lateral plate.

The cells which enter the lateral plate extend distally along it for only about 0·2 mm. It is possible that this lateral limit corresponds with the future junction between the embryonic and extraembryonic coeloms, the cells of the distal lateral plate having passed through the primitive streak at an earlier stage.

(2) *Interpretation of primitive streakless embryos*

When the primitive streak was extirpated some segmental plate had already formed and this remained in the embryo. There is evidence both from scanning electron microscopy (Meier, 1981) and from experiments (Packard & Meier, 1983) that each segmental plate contains the rudiments of 10–12 somites. The cells in the segmental plate are already determined for somite formation (see discussion by Bellairs, 1985). In deciding therefore whether any new somites have formed at the level of the missing primitive streak we must subtract at least 12 from the total number of new somites formed after the operation. In Table 1 it can be seen that twenty-nine operated embryos had formed at least 14 new pairs of somites (i.e. at least 2 of these pairs were probably formed from the postsegmental plate region). More importantly, sixteen embryos had formed 16 or more pairs (i.e. at least 4 postsegmental plate). Moreover in the five embryos left to develop for a longer length of time, even more additional somites had formed in the streakless region. This indicates that the contribution of cells from the primitive streak to the somites, which we showed in experiment 1, may not be essential for their formation.

(3) *The origin of the somitic mesoderm*

The results of our grafting experiments however, provide strong evidence that the new segmental plate which forms between stages 8 and 12, and the somites which form from that segmental plate, are derived at least in part from the primitive streak. These findings are therefore in harmony with the idea of Tam (1981) that in mammalian embryos the primitive streak gives rise to the somites, though they provide no evidence about his idea that commitment occurs during the process of leaving the streak. Indeed it seems more likely that the cells are not fully committed until they have taken up their positions within the segmental plate. There is however evidence that these may not be the only cells which contribute to the segmental plate. Firstly, we have found host cells mingling with the graft cells in the segmental plate and somites. Secondly, there is experimental evidence that a thick band of mesoderm which corresponds morphologically to segmental plate can develop in the absence of the primitive streak (Bellairs & Veini, 1984). Finally, in some of the embryos from which the primitive streak had been extirpated many new somites developed, and the most posterior of these were sometimes miniaturized.

At present we do not have firm evidence as to the origin of these additional host cells which may often be seen in the somites nor can we claim with confidence that they are present in all somites. One possibility is that they are derived from cells which left the primitive streak at more anterior levels. Indeed they may even have formed part of the original presumptive somite area. Recently it was suggested that somites are normally formed both from programmed cells in the presumptive somite mesoderm which become drawn posteriorly during regression, and from a population of as yet unprogrammed cells which migrate from the primitive streak

to supplement them (Bellairs & Veini, 1984; Bellairs, 1985). The present results support these ideas. Further experiments are now in progress to investigate whether presumptive somite mesoderm cells do indeed become drawn so far caudally. Whatever their origin however, these particular cells which were found in embryos of experiment 1, appear to have moved caudally, during the period of regression. (The alternative possibility that these additional cells have moved anteriorly from the remnant of host primitive streak at the posterior end of the area pellucida, seems less likely since such a movement would be contrary to the direction of all the regression taking place at this time.)

In those 6 embryos which possessed additional rows of somites, the dual origin is especially clear. The significance of these additional rows is however probably related less to the origin of the component cells than to the mechanical conditions encountered during the early stages of the experiment, since multiple rows of somites can be obtained in unoperated embryos explanted under conditions which interfere with stretching of the area pellucida (Stern & Bellairs, 1984).

(4) *The morphogenetic movements*

During the period of our investigation, two major morphogenetic movements are taking place. Ingression is occurring through the primitive streak, which results in a mediolateral migration of the mesoderm cells. And regression is taking place in an anteroposterior direction. There is ample evidence from experiments in which Hensen's node has been labelled, that it regresses to the caudal end of the primitive streak, laying down notochord as it goes (Spratt, 1955; Pasteels, 1937; Nicolet, 1971; Vakaet, 1970; Rosenquist, 1966). Our present experiments in which we replaced the postnodal region of the primitive streak provide even more evidence, since the notochord was always derived from the host. Regression should not however be regarded merely as a property of Hensen's node. In the present experiments we have shown that it involves neural tissue, ectoderm and endoderm. The same experiments also show that mesoderm which contributes to the somites becomes drawn backwards during the process. It appears therefore that two different morphogenetic movements are occurring in the mesoderm, probably simultaneously. They are the mediolateral migration of ingressing cells and the anteroposterior movement associated with regression. It seems possible that the two streams interact with one another, the cells which leave the primitive streak tending to pass more dorsally.

Once the cells have entered the segmental plate, they become committed to form not only somites, but also to form somite derivatives, such as limb bud mesenchyme. Indeed, Wachtler, Christ & Jacob (1982) have shown that the type of tissue which cells will subsequently form is related to the position which they occupy in the segmental plate.

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