

Somite formation in the early chick embryo following grafts of Hensen's node

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SUMMARY

Quail grafts of Hensen's node were examined for their potential to induce somites in chick blastoderms. The origin of the structures induced depended on the distance of the graft from the host's midline. Nodes placed at the margin of the *area pellucida* resulted in structures differentiated from the cells of the graft, whereas medially the graft organized host cells to form rows of somites. The results are discussed in terms of competence of graft and host mesenchyme and a positional signal from the node.

INTRODUCTION

To understand somite formation we need to know both how the presumptive somitic mesoderm is specified and how this material becomes segmented. Bellairs & Portch (1977) in reviewing somite formation have suggested that these are two distinct processes. They argue that there is no strong reason to believe that the somites are specified by Hensen's node, as suggested by Nicolet (1970, 1971*a, b*). They also point out that regression movements, which have been stressed by many workers, are not essential. Lipton & Jacobson (1974) have supported the idea that regression of the node is a mechanical requirement for somite segmentation, but argue that it is the neuro-epithelium that can induce the mesoderm to form somites. However, as Bellairs & Portch (1977) point out, other interpretations are possible. Mechanisms for segmentation are considered by Cooke (1977), Lipton & Jacobson (1974) and Bellairs & Portch (1977). In this paper we are only concerned with the specification of the somitic region and not its segmentation, and we have investigated the development of somites following grafts of Hensen's node.

Many researchers have studied the formation of structures resulting from

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grafts of Hensen's node and more caudal parts of the primitive streak at different stages of development – Waddington (1934), Grabowski (1957), Mulherkar (1958) and Vakaet (1965) – but have been primarily concerned with the induction of neural tissue (reviewed Gallera, 1971). The most extensive investigations in recent years were not only concerned with the structures resulting from different regions of the primitive streak at different stages, but also whether the host exerts an influence on the graft. When a graft was placed in the area pellucida and another in the area opaca, no difference was found in the response of the two sites up till stage 5 of the host. Rather little attention has been paid to somites appearing in these induced or self-differentiated secondary axes.

Our approach has been to investigate the origin of somites in secondary axes produced by grafting Hensen's node to see if somites were always of donor origin or could be induced to arise from host tissue. To do this we used the node from quail embryos, in which the cells carry a nucleolar marker recognizable in sections stained with Feulgen (Le Douarin, 1969). We thus grafted the node at different distances from the host axis and examined the resulting structures both in whole mounts and in sections.

Most of the donors and hosts were from stage 4, since at this stage the maximum elongation of the primitive streak has been reached and the presumptive fate of the cells within the node is to become notochord. Hensen's node now ceases to be an invagination centre and regression begins, the notochord being laid down. However, invagination through the primitive streak is still continued at stage 5, giving rise to mesoblast for the head formation, the precardiac tissues, prospective somites, prospective lateral plate tissue and extra-embryonic mesoblast (Nicolet, 1971*b*).

MATERIALS AND METHODS

White Leghorn eggs were incubated at 38 ± 1 °C for 18–24 h. The embryos were then washed in Pannet and Compton BSS with the addition of 0.9% glucose and explanted, using the culture technique of New (1955). The stages of Hamburger & Hamilton (1951) were employed to determine the age of the blastoderms. The quail eggs were obtained from our own breeding stock and incubated for 15–20 h to give comparable stages to the chicks.

The culture method of New simulates the situation *in ovo* with one important difference. The embryo lies with its ventral side facing outwards. Operations were carried out with tungsten needles which were electrolysed to sharp points and then bent with watchmaker's forceps to the shape required.

The putative node, measuring 200 by 200 μm , was excised from a quail or chick blastoderm, transferred to the host by means of a fine pipette or ladle-shaped micro-spatula, and positioned near the graft site of the chick blastoderm. Care was taken to ensure that the orientation of the donor node, with respect

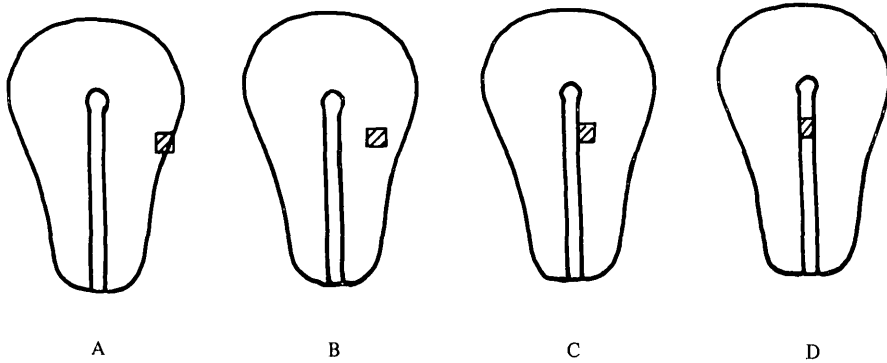


Fig. 1. The area pellucida of a chick blastoderm at stage 4. The crosshatched square indicating the site of the graft. (A) 500–600 μm away from the primitive streak. (B) 200–300 μm away from the primitive streak. (C) Immediately adjacent to and touching the primitive streak. (D) Replacing tissue of the primitive streak.

to the host, remained harmonious with the dorso-ventral and cranial-caudal axis of the host. Two methods of performing the operation were employed and both gave the same results.

(1) A square was cut marginally smaller than the graft through all three cell layers of the blastoderm and the donor tissue inserted.

(2) An incision was made in the host's endoblast which was eased off the loosely adherent mesoderm and the graft inserted into this pocket. The rapidly regenerating endoblast ensured perfect healing of graft into host.

Only one graft was made per host, and when not in the midline this was always on the host's left-hand side.

In our experience, the length of the primitive streak at stage 4 is very variable and can be between 1.4 and 2.2 mm. To take into account the different lengths of the primitive streak, the graft was placed in the same relative position with respect to the anterior-posterior axis. Grafts were placed in four different lateral positions (Fig. 1).

(A) 500–600 μm away from the primitive streak; (B) 200–300 μm away from the primitive streak; (C) immediately adjacent to the primitive streak; (D) replacing tissue in the primitive streak itself. All measurements were made with an ocular graticule. Explants were left to heal for 3–4 h at room temperature after grafting, before incubation continued. This was found necessary to prevent the blastoderm from tearing at the site of the graft due to expansion of the tissue.

The embryos were examined 24 h later, drawn, photographed then fixed in $\frac{1}{4}$ strength Karnovsky (1965) fixative for 45 min and embedded in araldite. Sections, 2 μm thick, were cut on a Cambridge Huxley Ultramicrotome through graft and host at 20 μm intervals in groups of five. Alternative groups were stained with toluidine blue and Feulgen.

RESULTS

When Hensen's node was grafted to the area pellucida lateral to the host primitive streak it normally produced additional axial structures. The structures formed included notochord, neural tissue and somites. By using the quail cell marker we were able to distinguish between tissues formed from the graft (self differentiated) and those formed from the host (induced).

If there was a notochord it was always composed of quail cells exclusively. Little mention will be made of the origin of the neural tissues which were found to consist of chick cells, or quail cells, or both in the secondary axes. We confined our interest to the origin of the somites.

(A) *Nodes grafted 500–600 μm away from the primitive streak*

This was in most cases at the margin of the area pellucida. The host-graft area formed small clusters of two to six somites, or very rarely a secondary axis with several pairs of somites in two columns with a notochord between them (Table 1). The clusters were either a group of somites which were not accompanied by a notochord, or the somites were arranged like beads on a string alongside the notochord. Normally the secondary axis was straight and converged on the host axis. This is reminiscent of the observations of Cooke (1972) on the amphibian embryo. When the graft was from a quail donor, all somites were found to consist of quail cells as did the entire notochord when present (Figs. 2A and 3).

The somites of the secondary axis appeared smaller than those of the host.

(B) *Nodes grafted 200–300 μm away from the primitive streak*

In these experiments there were many more supernumerary somites. The gross appearance was a well organized secondary axis with one or two and, in one case, three columns of somites (Table 1). More important, when the graft was of quail origin some of the somites were entirely of chick cells. The secondary axis always converged towards the caudal end of the host axis. The grafted node regressed to the same level as the host's node and eventually they merged. The arrangement and composition of the somites is as follows:

(i) One extra column of somites: quail grafts always gave all quail somites which could be on either side of the notochord (Fig. 2A).

(ii) Two extra columns of somites: these were in pairs, one on each side of the newly formed notochord. In quail grafts the column furthest away from the host axis was always of quail cells, as were the most cranial somites on the host side. But, where the medial column converged on the host axis caudally, the somites were always composed of chick cells (Fig. 2B). There were never any quail somites more caudal to a chick somite in the same column, and as far as we could tell none of the somites were of mixed origin.

(iii) Three extra columns of somites developed in only one case and the graft

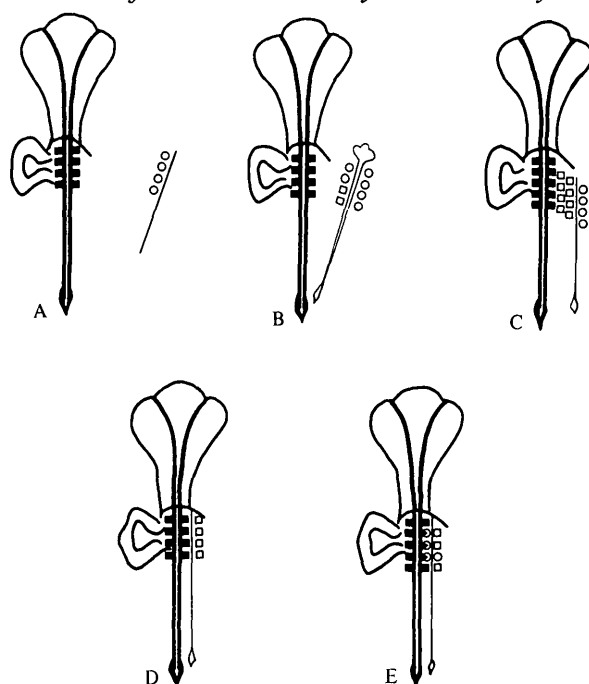


Fig. 2. Diagram of the embryo 24 h after the operation showing a variety of secondary axes obtained. (A) Grafts performed 500–600 μm away from the primitive streak gave small numbers of somites, with or without a notochord which was straight or curved, composed of quail cells. (B, C) Grafts placed 200–300 μm away from the primitive streak gave in many cases two extra columns of somites and in one case even three extra columns of somites and a notochord. The latter always consisted of quail cells and so did the column of somites which was furthest away from the host. Somites in-between the host and the notochord of the secondary axis were mainly of chick and a few of quail cells. (D) Grafts adjacent to and touching the primitive streak resulted in one extra column of somites entirely composed of chick cells divided from the host only by the width of a notochord which consisted of quail cells. (E) Replacing tissue of the primitive streak gave one extra column of somites and a quail notochord. The medial column was found to have some somites of mixed origin while the left lateral somites at the level of the graft were of quail cells the somites anterior and posterior to it were of chick cells. The diagram shows the embryo from the ventral side. ■, Host somites; □, extra somites of chick cells; ○, somites of quail cells; ●, somites of mixed origin.

was from a quail donor. The original host axis had two columns of somites, and the induced secondary axis had two columns, each five somites long. In between these two axes lay a fifth column of five somites (Fig. 2C). The two axes were parallel throughout their length. All five columns were approximately an equal distance apart from one another and in register, but with the graft induced somites displaced a half somite width to the rear. The most lateral column was entirely of quail cells, as was the adjacent notochord. All the other somite columns were made up of chick cells. The neural tube was entirely of chick origin and had extended to overlies both notochords and the intermediate structures.

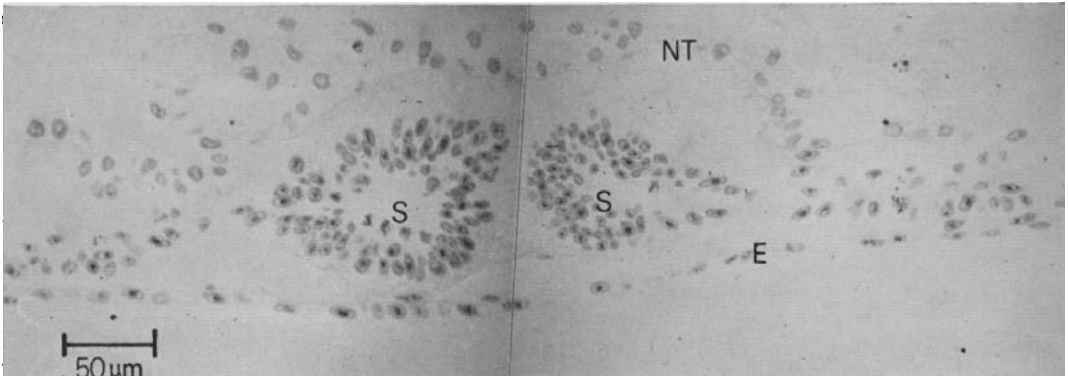


Fig. 3. Section through two somites formed in a cluster 500 μm away from the host axis. All cells in the somites are of quail origin, a notochord is absent, the endoderm consists of quail cells while the ectoderm has formed a neural tube all of chick cells. S, somite; E, endoderm; NT, neural tube.

(C) Nodes grafted adjacent to and touching the primitive streak

A second notochord developed together with a single additional column of somites (Table 1). The secondary notochord ran more or less parallel to the host's notochord at a distance of just over the width of one somite (Figs. 2D and 4). The somites between the two notochords were chick. The column of somites lateral to the secondary notochord contained all chick somites, but in one case the most cranial somite in a column of six was of quail cells. In one other case in which the graft was from a quail donor we found a complete secondary axis with two columns of somites. However, only the first two pairs of somites of the graft were normal, the more caudal somites of the secondary axis were fused across the graft notochord to form a single column of very large somites all of chick cells and all in register with the host's somites.

(D) Nodes grafted caudal to the host's node, replacing tissue of the primitive streak

This graft again produced an additional lateral column of somites (Table 1). The somites were usually of chick origin except for two cases. One had one quail somite most cranial in the lateral row. The other was more unusual. The somites in the centre column were of mixed origin (Figs. 2E and 5). The number of quail cells rapidly diminished from a majority in the most cranial somite to zero by the fourth or fifth somite. This was the only case in all the experiments in which we observed clearly mixed somites. In the lateral column of the secondary axis the most cranial two somites were of chick origin followed by one somite of quail cells: the remaining somites were chick. This was the only case in which quail somites were found caudal to chick somites in the same column.

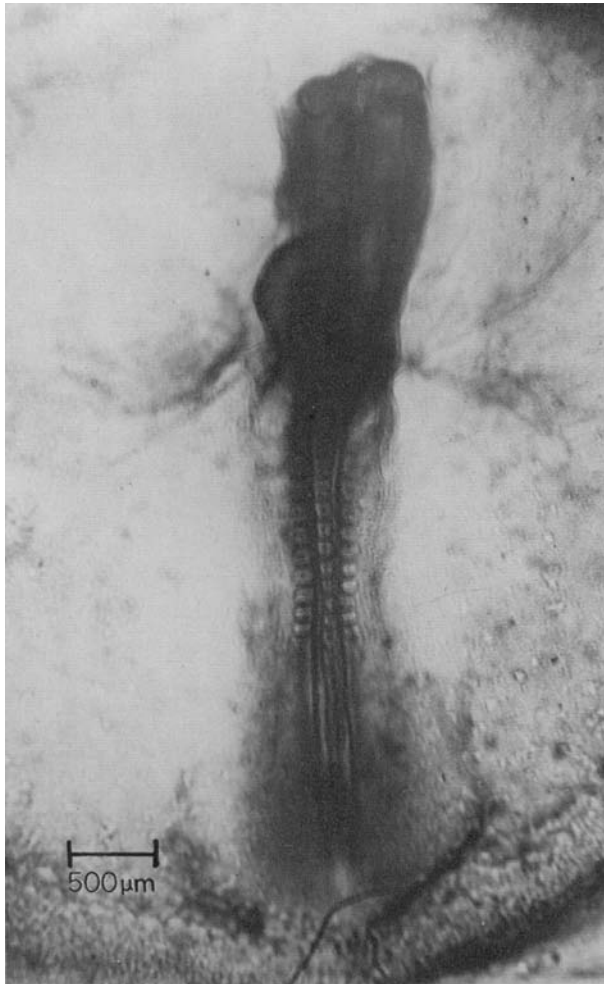


Fig. 4. Photograph of an embryo in culture 24 h after the operation of a type C experiment. Note the three columns of somites.

Table 1. *Somite formation following grafts of Hensen's node to different positions*

Position of graft relative to primitive streak	No. of operations	Stages of host/donor	No. of extra somites	Arrangement of additional somites		
				Dis-organized	Single column	Double column
(A) 500–600 μm	14	4/4–5	2–12	3	9	2
(B) 200–300 μm	17	4/4–5	3–10	5	6	6
(C) Adjacent	5	4	3–12	1	3	1
(D) Replacing	6	4/4–5	3–7	–	5	1

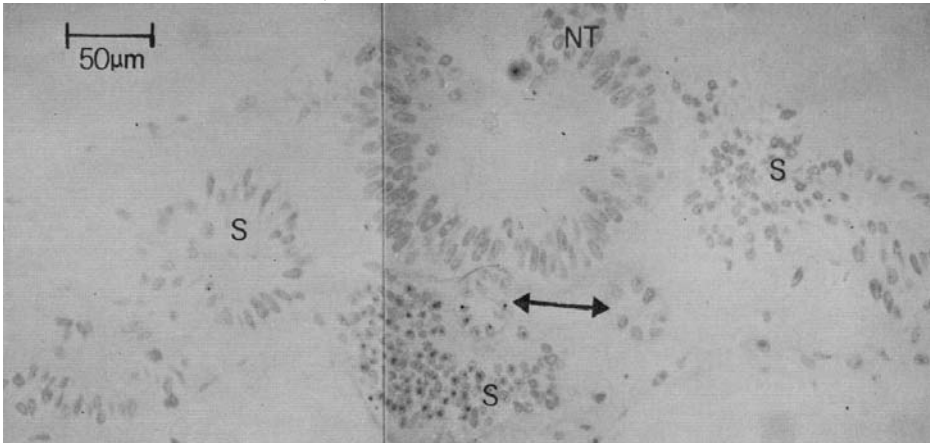


Fig. 5. Transverse section of a type D experiment. There are again two notochords (arrows) one of chick cells and the other of quail cells. The medial somite consists of many quail cells and some chick cells. S, somite; NT, neural tube; \longleftrightarrow , notochord.

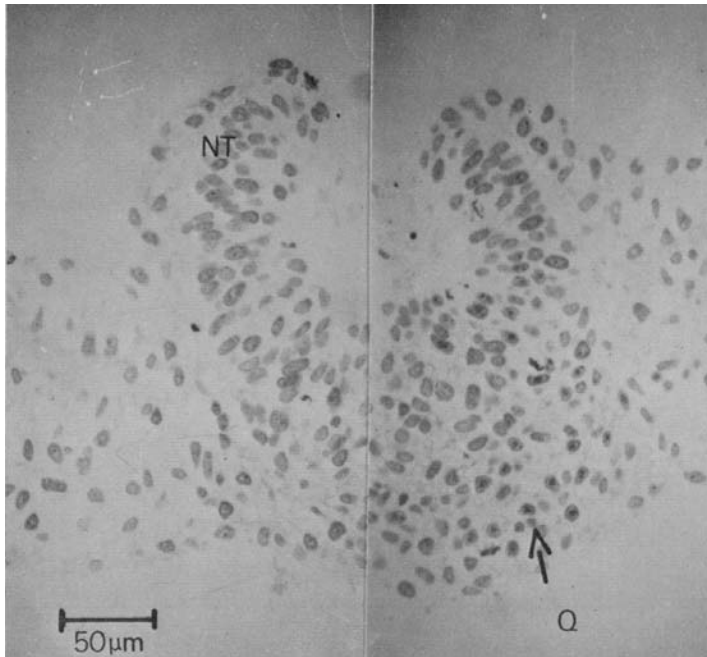


Fig. 6. Transverse section through the node area of a 12-somite chick embryo with a type C operation. The quail cells are mixing freely with chick cells before forming the notochord.

All grafts

The grafted node caused the formation of a secondary axis. When the graft came from a quail donor the notochord of the secondary axis was always entirely of quail cells (Fig. 6).

We never obtained a secondary axis which contained somites without the presence of a notochord. We did, however, observe in two of the cases that a notochord was absent. This was seen only where the somites had not formed in a column but were randomly arranged in clusters. It is of interest to note that neural tissue was present in both these cases.

DISCUSSION

The main finding is that the position of the graft determines the pattern of the induced somites and whether the induced somites are of host or graft origin. When the graft is more than about 500 μm from the host axis the somites formed appear small and are entirely derived from graft tissue. When the graft is between 200 and 300 μm away the additional somites formed may be of both host and graft origin. Those of host origin are always between the two notochords. Individual somites are not of mixed origin. Posterior somites in a row may be host even though anterior ones are from the graft. When the node is grafted adjacent to the primitive streak or replacing Hensen's node, additional rows of somites form but these are almost always of host origin.

These results clearly show that the grafted node can provide some sort of signal such that host tissue forms additional somites.

It seems probable that chick somitogenesis is basically a two-step process (Bellairs & Portch, 1977), in which tissue is first programmed to form presumptive somite mesoderm which later undergoes segmentation. We feel that our results say little about the segmentation phase except to provide the tantalising clue that it seems to be very difficult to produce a somite of mixed (chick/quail) origin. This observation accords well with the suggestion of Bellairs & Portch (1977) that segmentation essentially involves a wave of changing pattern of cell adhesion. If either the absolute timing or the relative adhesion of chick and quail cells were different then one might expect them not to collaborate easily in forming a single somite.

The ability of the node to organize mesoderm to form somites is relevant to the phase of programming of presumptive somite mesoderm. We have shown that both host mesoderm within 300 μm of the midline, and graft cells surrounding the node are – in certain circumstances – able to form somites. This observation argues that, in classical terms, all of these cells are competent. It also suggests that the mesoderm cells more than 300 μm away from the midline are not competent. The problem then is why additional somites form from host tissue under the influence of the node, and also, why graft tissue, while clearly competent, does not give rise to somites when close to the host node.

It may be possible to explain the results by a mechanism falling within the general framework of positional information (Wolpert, 1969, 1971). We have illustrated this below by using the example of a simple diffusion model similar to that used to describe the actions of the zone of polarizing activity (ZPA) in

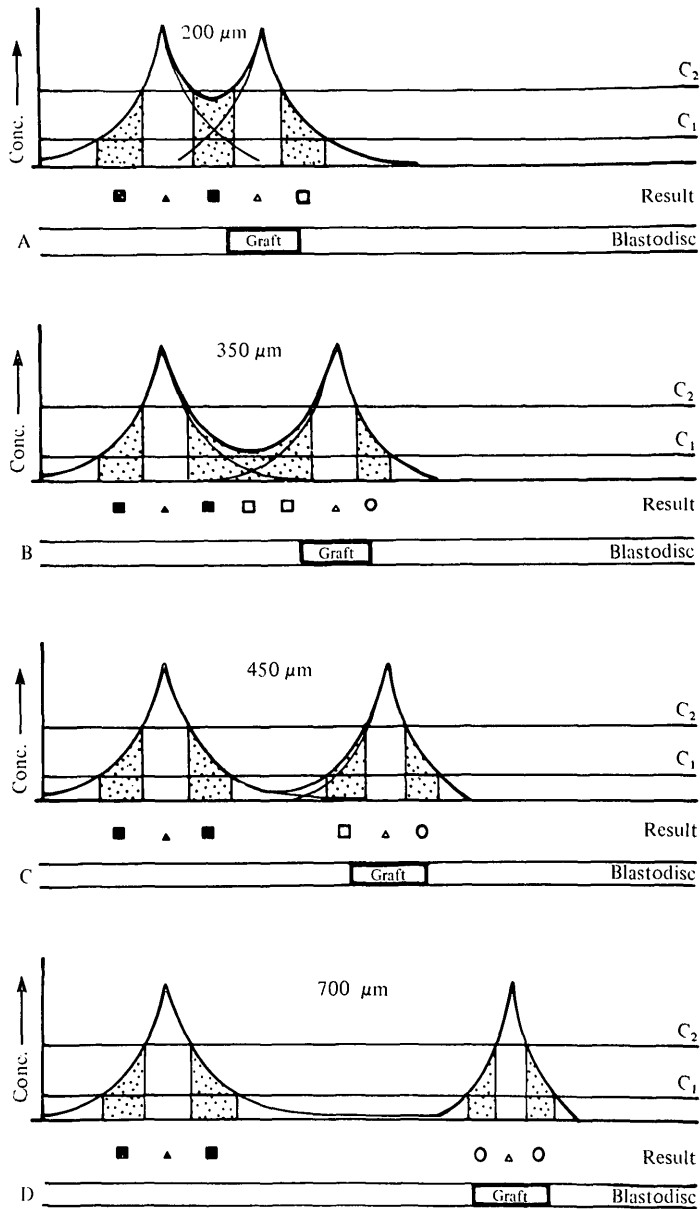


Fig. 7. Concentration profiles of a morphogen across the medial-lateral axis. The source is fixed at constant profiles and the morphogen diffuses freely through the mesoderm where it is degraded. The flux through the mesoderm is non-uniform being fast medially and slower laterally. Beneath the concentration profiles are the results of the experiment, and the original position of the graft in the blastodisc: (A) graft node 200 μm from host node; (B) graft node 350 μm from host node; (C) graft node 450 μm from host node; (D) graft node 700 μm from host node. ■, Host somites; □, extra somites of chick cells; ○, somites of quail cells; ▲, chick notochord; △, quail notochord; □, quail node graft; stippled, concentration range specifying presomitic mesoderm.

chick limb-bud (Wolpert, 1969; Tickle, Summerbell & Wolpert, 1975; Summerbell & Tickle, 1977). We assume that the node is the source of a morphogen which is able to diffuse out into the surrounding tissues where it is destroyed by the cells. This produces the concentration profile, as shown in Fig. 7. We also need to assume that the blastodisc is non-uniform so that diffusion is more rapid near to the midline than it is towards the periphery. This has the effect of giving the concentration profile a steeper gradient in the periphery. Cells at the appropriate concentrations in the field are specified as being presomitic mesoderm and will eventually give rise to the somites. The model is illustrated for four different graft positions in Fig. 7. With the graft distant from the host axis, the concentration profile around the graft node is very steep so that presomitic mesoderm is specified within the graft. The somites are formed from quail cells. That part of the concentration profile within the host mesenchyme is too low to specify somites (we do not know if chick mesoderm within this region is competent to form somites). When the graft is near to the midline the concentration profile is sufficiently shallow for the presomitic mesoderm to be specified outside the graft. The somites are formed from chick cells. When the graft is in the intermediate position the presomitic mesoderm is specified outside the graft towards the midline, and within the graft towards the periphery. The medial column is of chick cells and the lateral column of quail cells. The model explains only how presomitic mesoderm is specified across the medio-lateral axis. It does not explain segmentation along either the medio-lateral or the anterior-posterior axis.

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