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Interactions between somite cells: the formation and maintenance of segment boundaries in the chick embryo

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Summary

We have investigated the interactions between the cells of the rostral and caudal halves of the chick somite by carrying out grafting experiments. The rostral half-sclerotome was identified by its ability to support axon outgrowth and neural crest cell migration, and the caudal half by the binding of peanut agglutinin and the absence of motor axons and neural crest cells. Using the chick–quail chimaera technique we also studied the fate of each half-somite.

It was found that when half-somites are placed adjacent to one another, their interactions obey a precise rule: sclerotome cells from *like* halves mix with each other, while those from *unlike* halves do not; when cells from unlike halves are adjacent to one another, a border is formed.

Grafting quail half-somites into chicks showed that the fates of the rostral and caudal sclerotome halves are similar: both give rise to bone and cartilage of the vertebral column, as well as to intervertebral connective tissue. We suggest that the rostrocaudal subdivision serves to maintain the segmental arrangement when the mesenchymal sclerotome dissociates, so that the nervous system, vasculature and possibly vertebrae are patterned correctly.

Key words: pattern formation, segmentation, chick embryo, somites, sclerotome, segment borders, vertebral column.

Introduction

In higher vertebrate embryos, the somites first form as pseudostratified epithelial spheres. Some 6–8 h later, each somite disperses into three components: the *dermatome* (presumptive dermis), the *myotome* (presumptive skeletal muscle) and the *sclerotome* (presumptive vertebral column and ribs). At this time, the dermatome and myotome remain as an epithelial cap (known as *dermomyotome*) over the dorsolateral surface of the sclerotome. The sclerotome itself has by now lost its epithelial structure and appears as a loose mesenchyme. If the mesenchymal cells of the sclerotome are free to move, what are the mechanisms that prevent the cells from adjacent sclerotomes from mixing and destroying the segmental pattern? In this paper we have addressed this question experimentally in the chick embryo.

Each sclerotome is divided into a rostral and a caudal half. Motor nerves and neural crest cells emerging from the developing spinal cord region are only able to traverse the rostral half of each sclerotome (Keynes & Stern, 1984, 1985; Rickmann, Fawcett & Keynes, 1985), and it is this restriction that leads to the segmented pattern of the peripheral nervous system. There must be, therefore, some intrinsic difference between the cells in the two halves of the sclerotome (Keynes & Stern, 1984, 1985). The question remains as to the precise fate of each half. In spite of the well-known descriptions of resegmentation during the development of the vertebral column (for reviews see Baur, 1969; Verbout, 1976; Dalgleish, 1985), it is still uncertain whether both sclerotome halves can form bone or whether the rostral half, in fact, develops only into intervertebral connective tissue. We have approached this

issue using the quail–chick chimaera technique (Le Douarin, 1973).

In the experiments reported here, we have also investigated the properties of the interactions between the two sclerotome halves by constructing 'compound somites' with microsurgical techniques. The two halves of the sclerotome can be distinguished from one another by the ability of rostral half-sclerotome to support axonal outgrowth and neural crest cell migration, and by the binding of peanut agglutinin (PNA) to the caudal half-sclerotome (Stern, Sisodiya & Keynes, 1986). We have used these differences to assay for rostral and caudal cells in the compound segments.

Materials and methods

Embryo techniques

Fertile hens' eggs (Light Sussex or Rhode Island Red) and quails' eggs (obtained from Houghton Poultry Research Station, Huntingdon, Cambs) were incubated at 38°C for about 48 h to Hamburger & Hamilton (1951) stages 11–13.

The embryos were operated *in ovo* by the following procedure. About 300 μl thin egg albumen were removed from the blunt end of the egg with a hypodermic syringe. With the egg on its side, a square window (approx. 15×15 mm) was cut in the eggshell with a scalpel blade, and the piece of shell and underlying shell membrane were removed. The yolk was then floated up to the level of the window by adding calcium- and magnesium-free Tyrode's solution (CMF). In order to improve the contrast between the embryo and the yolk, about 30–50 μl of a 1:5 dilution of Indian ink (either Pelikan Fount India or Rowney Kandahar) in CMF were injected into the sub-blastodermic space. The edges of the window were then surrounded by a wall of high-vacuum silicone grease (BDH), which supported a standing drop of fluid over the embryo. The standing drop, when combined with tangential optical fibre illumination, improved the optical quality further (Hara, 1970). The drop was made with a 0·1 % solution of trypsin (1:250, DIFCO) in CMF, which greatly facilitated the operations and had no deleterious effect on the embryo.

The vitelline membrane was cut at an appropriate position for the operation. Operations were performed using fine tungsten needles sharpened in molten sodium nitrite and fine iridectomy microknives (Weck, 15° angle). The following operations were performed (by double-rostral or double-caudal segments we mean segments of rostral–rostral–caudal or rostral–caudal–caudal composition, respectively). (1) Double-rostral segments (Fig. 1) were made either by (A) removing the caudal half of one somite (24 embryos) or (B) removing a whole somite in a host embryo and grafting a rostral half-somite from a donor embryo in its place (8 embryos). (2) Double-caudal segments (Fig. 2) were made either by (A) removing the

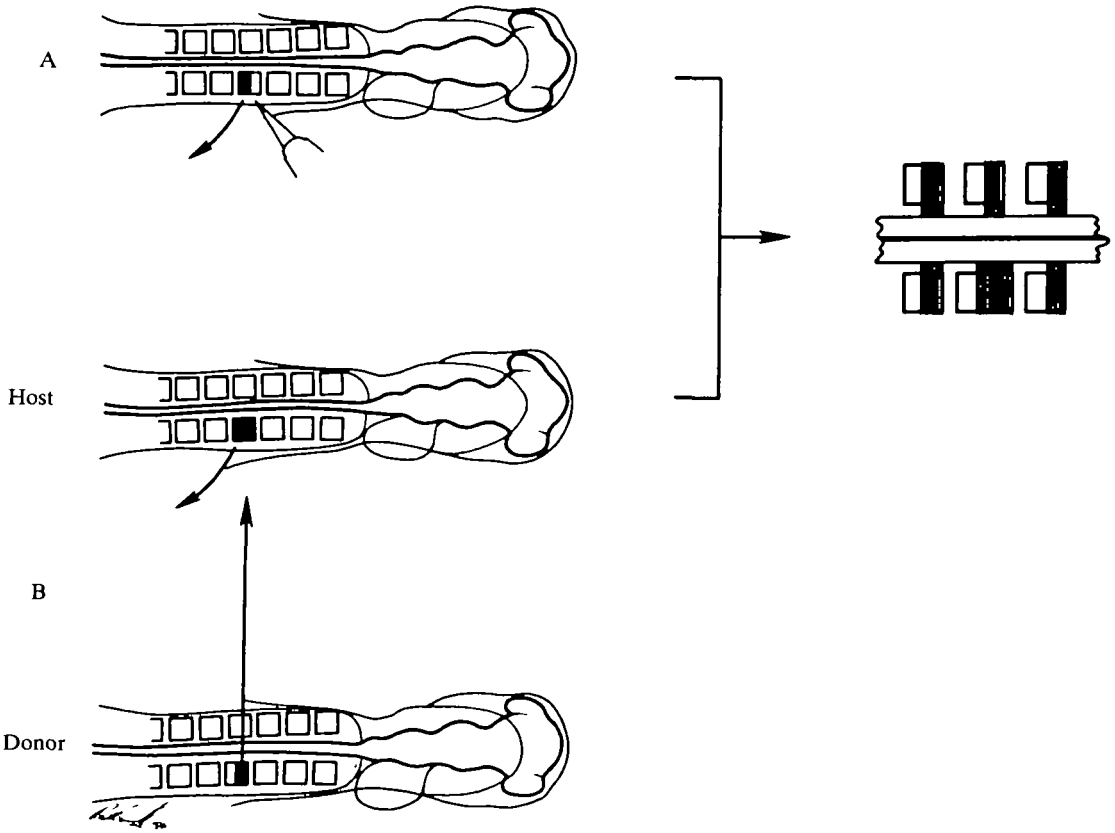


Fig. 1. Diagrams showing the two alternative procedures used to construct double-rostral somites. (A) The caudal half of one newly formed somite is removed in one embryo. (B) A whole newly formed somite is removed from a host embryo, and a rostral half-somite from a donor embryo at the same stage of development is grafted in its place.

rostral half of one somite (48 embryos) or (B) removing a whole somite in a host embryo and grafting a caudal half-somite from a donor embryo (12 embryos). (3) Triple-rostral and quadruple-rostral or triple-caudal and quadruple-caudal segments were made by removing a whole somite and grafting two or three rostral or caudal half-somites in its place (22 embryos). (4) Double-rostral or double-caudal segments were made as described in 1(B) and 2(B) above but using a quail donor embryo to construct chimaeric chick–quail compound somites (27 chimaeras). (5) To study the fate of rostral half-segments, a rostral half-somite was removed from a host chick embryo and a rostral half-somite from the same position in an identically staged donor quail embryo was grafted in its place. An equivalent procedure was followed to investigate the fate of caudal half-segments (20 chimaeras).

After the operation, the trypsin was removed and the surface of the embryo washed gently several times with CMF. 1–2 ml thin albumen were then removed with a hypodermic from the hole in the blunt end of the egg, which lowered the yolk. In most experiments 3–4 drops of a solution of penicillin-G (10^5 units ml^{-1}), streptomycin sulphate (10 mg ml^{-1}) and amphotericin-B (25 μg ml^{-1}) in 0·9 % sodium chloride (Sigma A9909) were added to the egg. The silicone grease was then wiped off the shell, and the window and side hole covered with PVC tape. The eggs were then reincubated at 38°C for the desired time. Embryos destined for zinc iodide/osmium tetroxide staining were incubated for about 2 days after surgery, by the end of which they had reached stages 18–22. Operated embryos destined for HNK-1 or PNA staining were incubated overnight, by which time they had produced about 10–12 somites.

The host embryos for grafting operations were prepared as described above, but the donor embryos were explanted and pinned out on a Sylgard dish containing 0·1 % trypsin in CMF at room temperature. The pieces of tissue to be grafted were transferred from donor to host using a siliconized Pasteur pipette that had been pulled to a fine tip.

Rostral/caudal sclerotome assay

The ability of rostral half-sclerotome to support motor axon outgrowth and neural crest migration and the binding of PNA to caudal half-sclerotome were used as biological assays for the sclerotomal cell subpopulations. To visualize the motor nerves, we used a modification of the zinc iodide/osmium tetroxide method. The embryos were split along the midline, eviscerated and stained directly in a mixture of 6 ml zinc iodide: 1·75 ml (2 %) osmium tetroxide (prepared as described previously, Keynes & Stern, 1984) for 90 min at 55°C. They were then bleached for a few seconds with a saturated aqueous solution of potassium periodate to remove background stain from the surface of the embryo. Embryos were then dehydrated in alcohol, cleared with xylene or Cedarwood oil and mounted in Canada Balsam or Permount, between two glass coverslips. This staining method could only be used up to stage 22 or

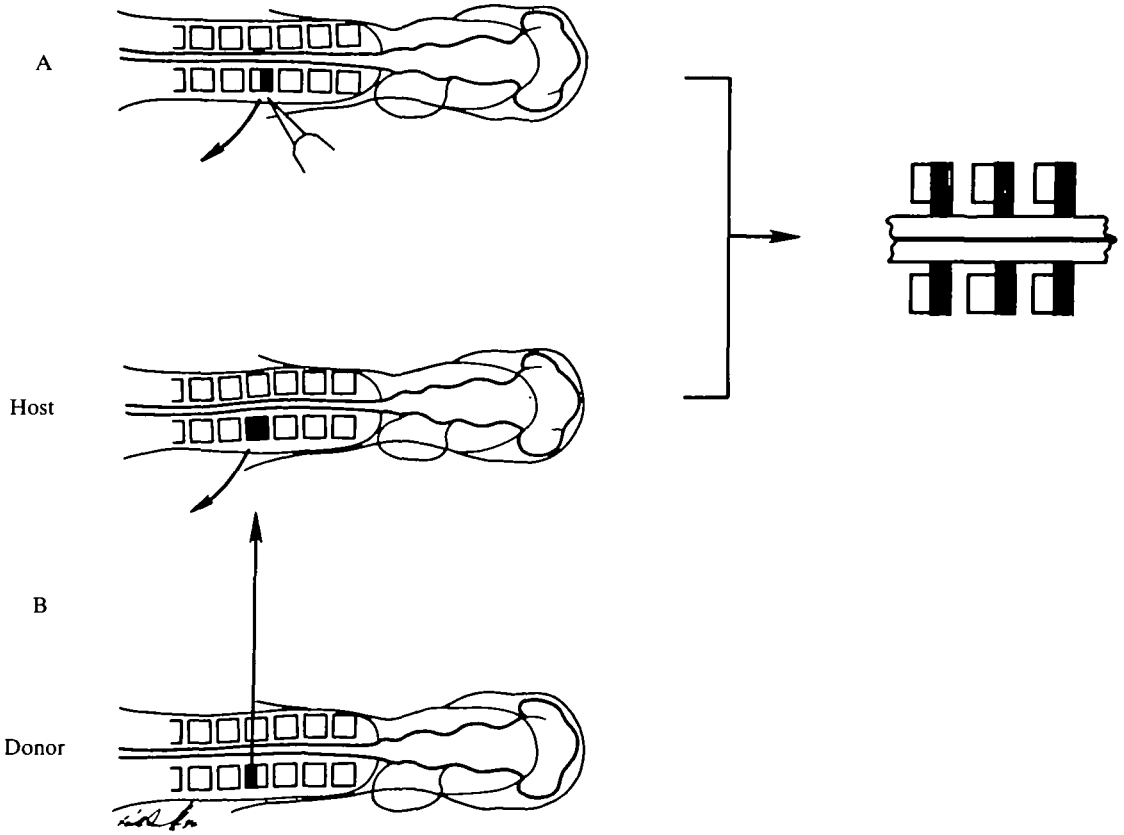


Fig. 2. Diagrams showing the two alternative procedures used to construct double-caudal somites. (A) The rostral half of one newly formed somite is removed in one embryo. (B) A whole newly formed somite is removed from a host embryo, and a caudal half-somite from a donor embryo at the same stage of development is grafted in its place.

so, after which the flank of the embryo became too thick for whole-mount examination.

Neural crest cells were visualized with the monoclonal antibody HNK-1 (Becton Dickinson). The staining procedure used has been described previously (Stern *et al.* 1986). Cryostat sections of gelatine-embedded embryos that had been fixed for 20 min in 0·25 % glutaraldehyde in PBS were used.

Peanut lectin (PNA) binds preferentially to the caudal half-sclerotome and was used as an assay for these cells. Horseradish-peroxidase-labelled PNA (Sigma) was applied to cryostat sections of embryos fixed in ethanol or glutaraldehyde, and visualized using 3-3′ diaminobenzidine (DAB, Sigma or Aldrich) as the substrate, as described previously (Stern *et al.* 1986).

Processing of chimaeric embryos

Chimaeric (chick–quail) embryos, operated as described above, were incubated to the desired stage: 24–36 h for compound somite experiments, or for up to 7 days (stages 20–36) for fate mapping experiments. They were then fixed in Zenker's fixative for 3–12 h, washed for several hours in running tap water, dehydrated, cleared and embedded in paraffin wax for sectioning at 8–12 μm on a rotary microtome. The sections were mounted on slides and stained either by Feulgen's method (see Stern & Ireland, 1981 for details) or with Harris's haematoxylin after acid hydrolysis (Hutson & Donahoe, 1984) to demonstrate the position of quail cells, which can be distinguished by their prominent nucleoli (Le Douarin, 1973).

Results

All operations were carried out on the two or three most recently segmented (i.e. most caudal, still epithelial) somites of stage 11–13 embryos, to avoid complications brought about by the presence of neural crest cells in the rostral halves of older sclerotomes (see Rickmann *et al.* 1985; Stern *et al.* 1986). Both procedures used to construct double-rostral or double-caudal segments ((A) and (B) in Materials and methods) gave similar results. We found that if a somite, or a portion of a somite, was removed from a host embryo, the gap produced always closed within 2–3 h of the operation. Using carmine marks, we concluded that this was due to a shift of the more caudal somites in the rostral direction.

Double-rostral and double-caudal compound segments

Of the 32 embryos with double-rostral segments, 26 were stained with zinc/osmium to visualize spinal nerve roots, 3 with monoclonal antibody HNK-1 to localize neural crest cells and 3 with PNA to localize caudal sclerotome cells. In 23 of the 26 embryos stained for motor nerves, the spinal root was wider than normal (Fig. 3). Two of the three embryos stained with HNK-1 had a wider area of sclerotome containing neural crest cells (Fig. 4). Each of the three embryos stained with PNA showed normal caudal halves in the operated region.

Of the 60 embryos operated to construct double-caudal segments, 53 were stained with zinc/osmium for nerves (Fig. 6), 3 with HNK-1 for neural crest cells and 4 with PNA for caudal half-sclerotome (Fig. 7). In 18 of the 53 embryos stained for motor nerves, the caudal half-sclerotome in the operated region was wider than normal. The remaining embryos were of normal appearance.

When examining whole-mounted or sectioned embryos that had developed after these operations, we noted that in the majority of cases (58/92 = 63 %) no boundary could be seen between adjacent *like* half-sclerotomes. For example, after excision of a caudal half-somite, no border could be seen separating the resulting rostral half-sclerotome from the adjacent rostral half-sclerotome, which had developed from the neighbouring, unoperated somite. Of the remaining embryos, 9 (10 % of the total) had a clear border. In most of the remaining 25 (27 %) embryos, there appeared to be a partial border, perhaps indicating that there had been some contamination by somite cells from the incorrect half, which the operation had failed to excise.

Multiple-rostral or multiple-caudal compound segments

22 embryos were grafted: 1 quadruple-caudal, 2 quadruple-rostral, 10 triple-caudal and 9 triple-rostral compound somites were constructed. The rostral/

Figs 3–5. Compound rostral segments.

Fig. 3. An embryo that had been operated to construct a double-rostral segment, stained with zinc iodide/osmium tetroxide 2 days later to visualize the spinal nerves. A wide spinal nerve can be seen at the site of the operation. Rostral is to the right. The arrows mark the position of the intersegmental borders. Bright field optics. Bar, 50 μm.

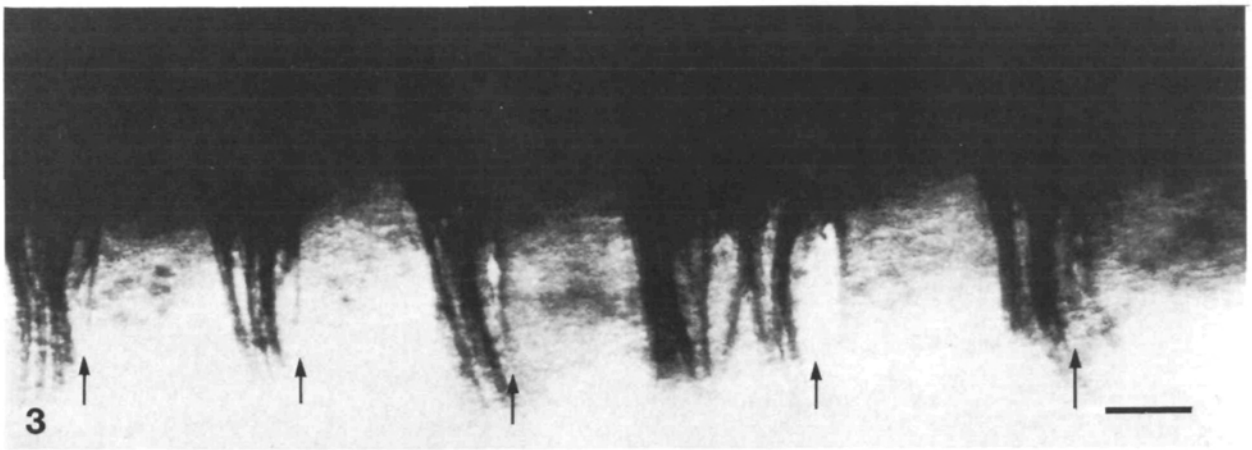
Fig. 4. An embryo that had been operated to construct a double-rostral segment, stained by indirect immunoperoxidase histochemistry with HNK-1 antibody 1½ days later to visualize the neural crest cells. A wide region of HNK-1 binding can be seen at the site of the operation. The arrows mark the position of the intersegmental borders. *C*, caudal-half-sclerotome; *R*, rostral-half-sclerotome. Rostral is to the right. Bright field. Bar, 50 μm.

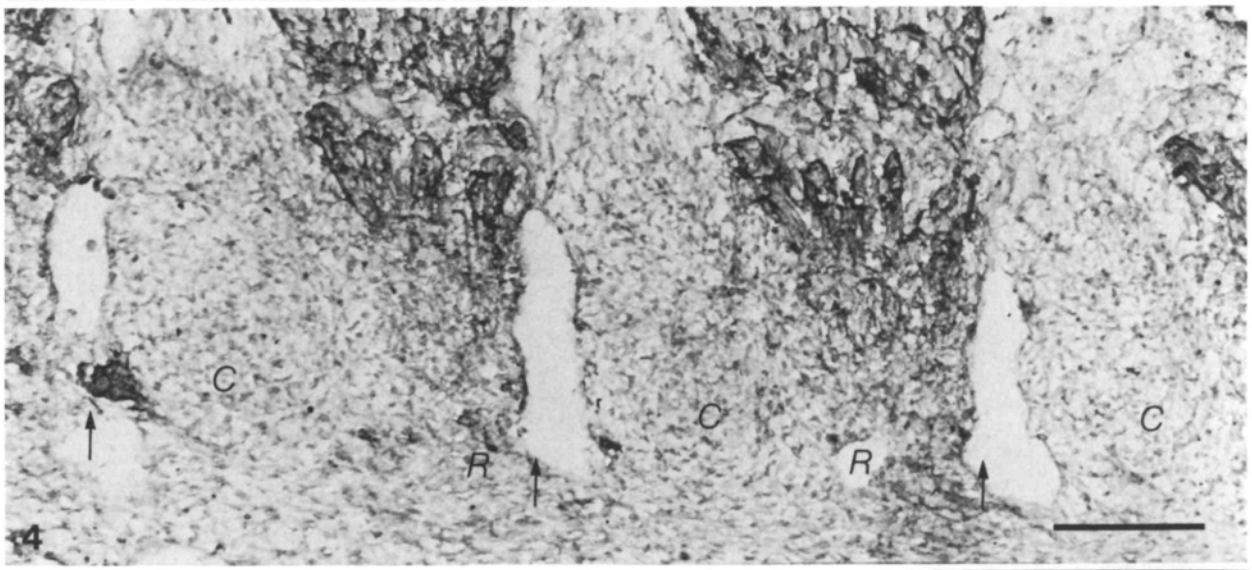
Fig. 5. An embryo that had been operated to construct a triple-rostral segment, stained with zinc iodide/osmium tetroxide 2 days later to visualize the spinal nerves. A very wide region with spinal nerves can be seen at the site of the operation. The arrows mark the position of the intersegmental borders. Rostral is to the right. Bright field. Bar, 50 μm.

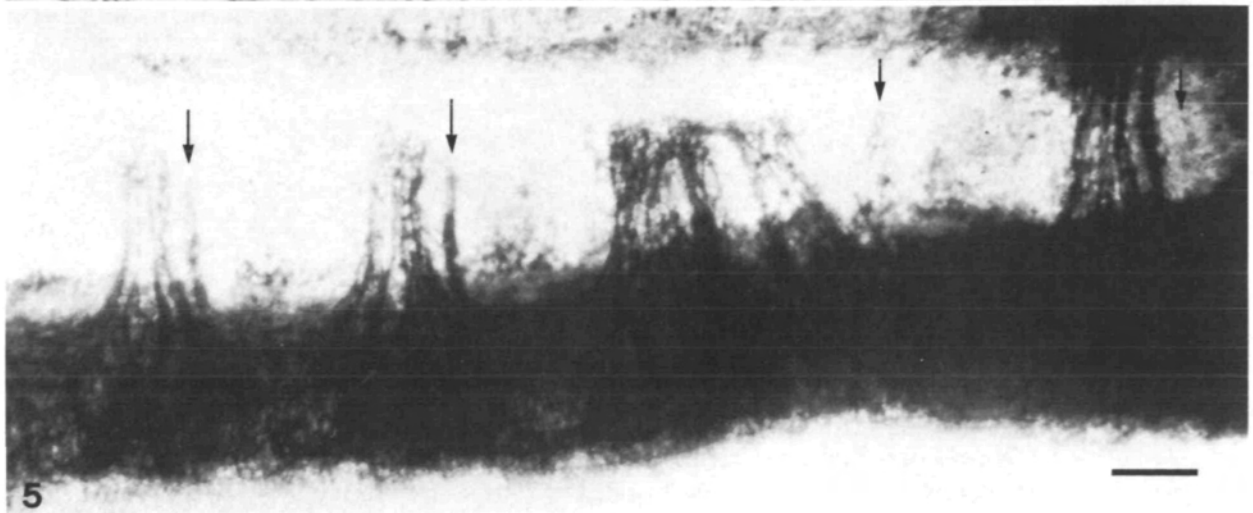
caudal sclerotome composition of these compound segments was examined; as before, multiple-rostral compound sclerotomes had a wider region occupied by a spinal nerve and neural crest cells (Fig. 5), while multiple-caudal compound sclerotomes had a wider region of PNA binding. Also as before, the border between adjacent *like*-sclerotome halves was absent or reduced (in all but two cases). In many cases, however, a boundary was seen between adjacent half-*myo*tomes.

Chimaeric chick–quail compound somites

These experiments were designed to establish whether there is cell mixing between adjacent *like* half-sclerotomes. The chimaeric embryos were stained either by Feulgen's technique, or with







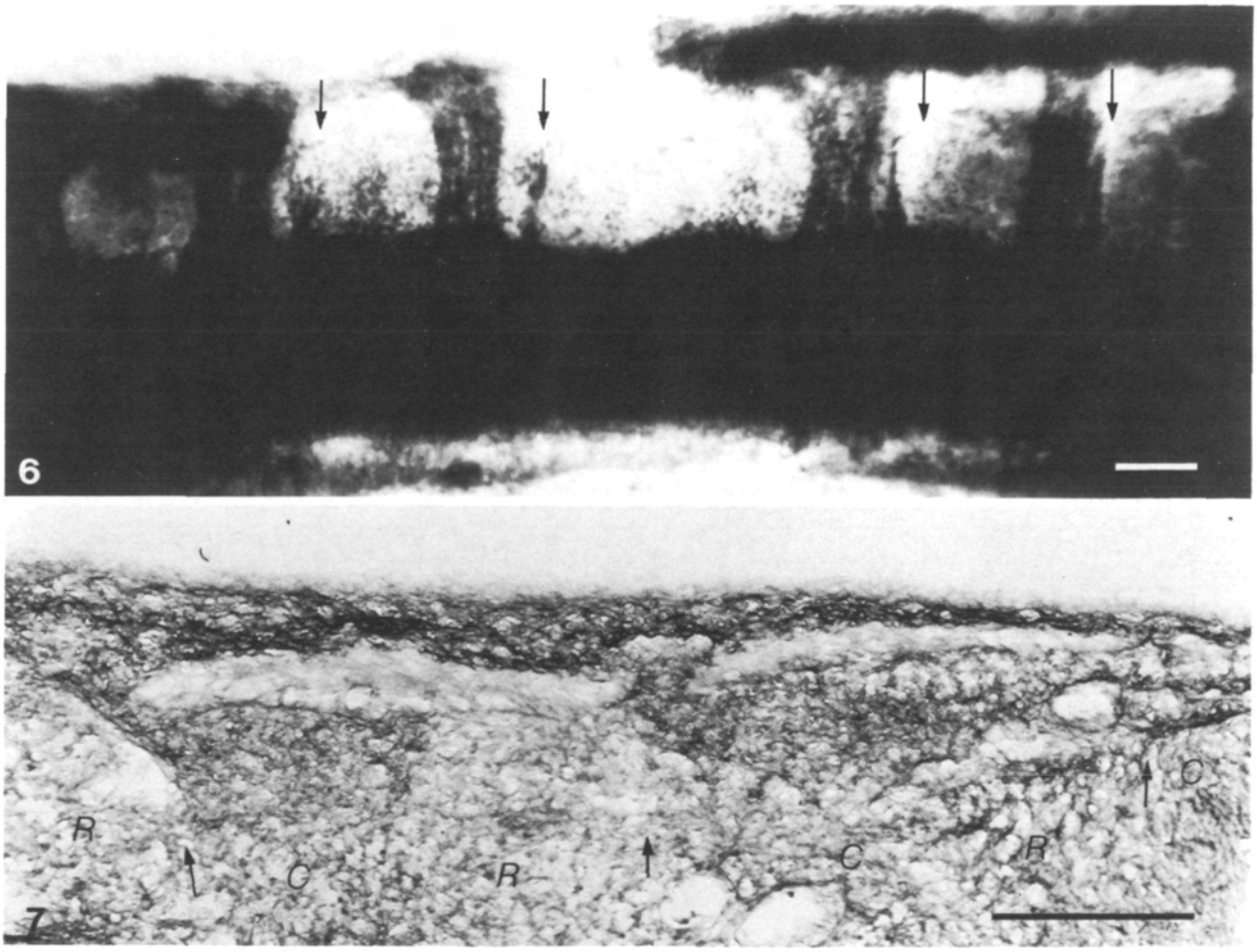
Harris's haematoxylin following the method of Hutson & Donahoe (1984), to localize the grafted cells. If, when adjacent, *like*-sclerotome halves can mix but *unlike* halves give rise to a boundary, we would expect a border to be present between the grafted half-somite and the host *unlike*-half sclerotome, but not between the former and the adjacent *like* half. We would also expect the cells of the donor and host *like*-half sclerotomes to mix.

20 such grafting experiments were carried out successfully and the results obtained confirmed the prediction. A boundary could be seen on one side of the grafted tissue but not on the other. Where graft cells confronted *like*-host cells, the cells mingled and no border was present (Figs 8–12). Mingling of cells was seen in all cases and no border between *like*-halves was seen in 16 of the 20 successful chimaeras. That the chimaeric half-segment was truly mixed could be established for certain only in the case of compound *caudal* half-sclerotomes, because rostral half-sclerotomes are invaded by neural crest cells, which in chimaeras will be of host chick origin.

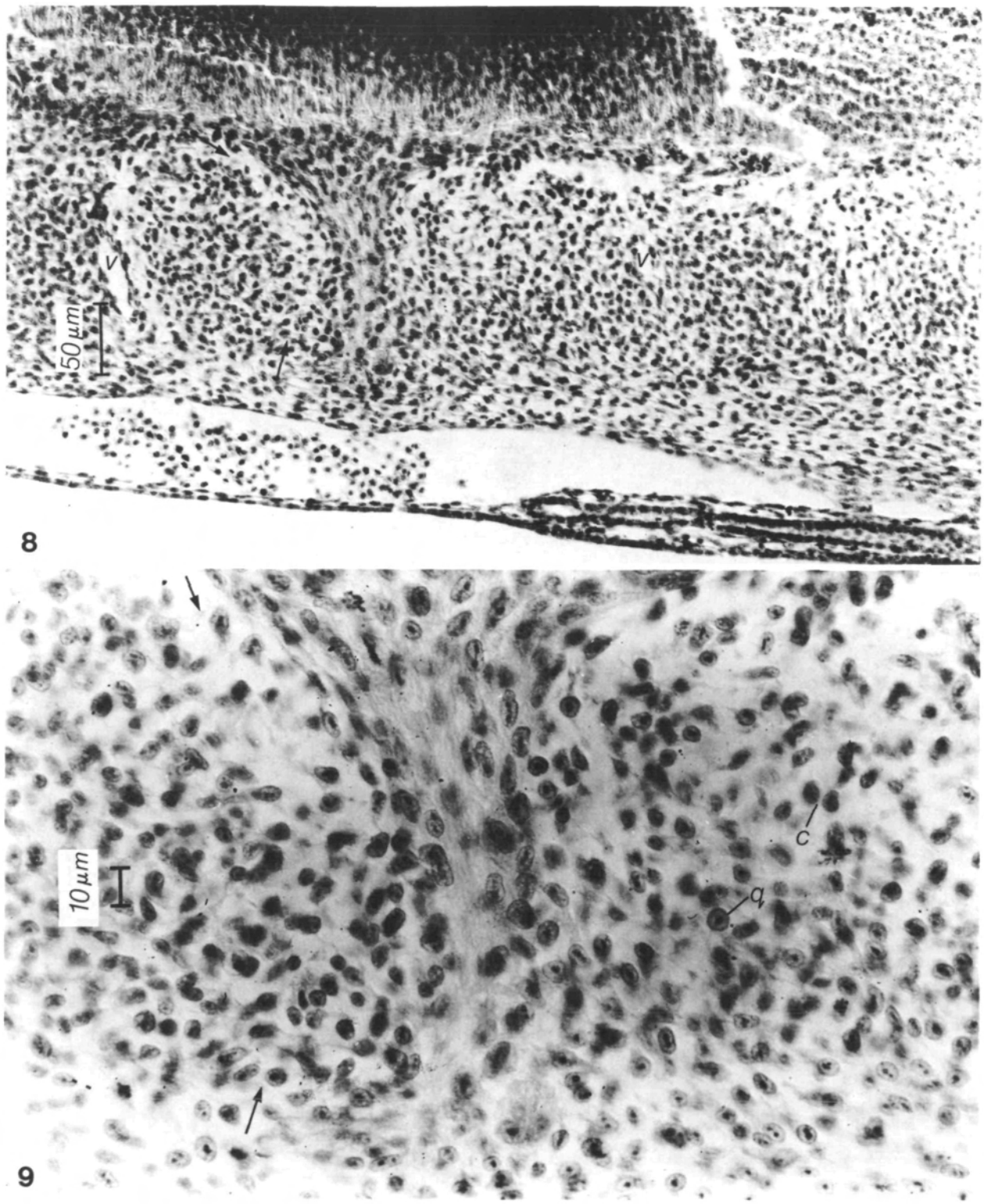
To make certain that quail half-sclerotomes behave in an equivalent manner to their chick counterparts with respect to outgrowing motor nerves and migrating neural crest cells, seven double-rostral chimaeric embryos made as described above were stained with zinc/osmium (six) or HNK-1 (one). The results confirmed that quail rostral half-sclerotome was capable of supporting the growth of chick host motor nerves and the migration of chick neural crest cells.

Experiments to determine the fate of individual half-somites

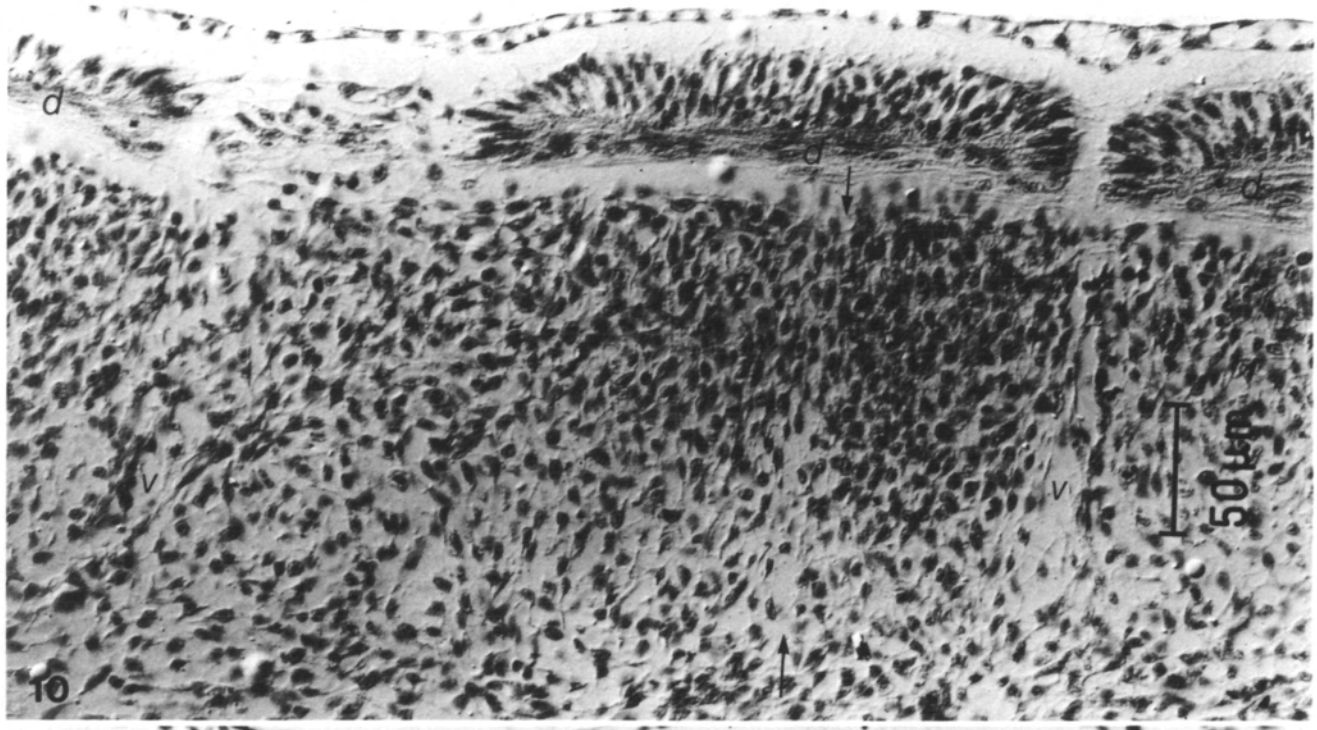
To investigate whether both halves of the sclerotome have equivalent developmental fates, one half of a



Figs 6, 7. Compound caudal segments.
Fig. 6. An embryo that had been operated to construct a double-caudal segment, stained with zinc iodide/osmium tetroxide 2 days later to visualize the spinal nerves. A wide region devoid of nerves can be seen at the site of the operation. The arrows mark the position of the intersegmental borders. Rostral is to the right. Bright field. Bar, 50 μm.
Fig. 7. Embryo that had been operated to construct a double-caudal segment, stained with PNA 1 day later to visualize the caudal half-sclerotome. A wide region of PNA-positive cells can be seen at the site of the operation. Bright field. A normal somite (to the left) and the double-caudal somite (to the right) can be seen. The arrows mark the position of the intersegmental boundaries. *C*, caudal-half-sclerotome; *R*, rostral-half-sclerotome. Rostral is to the right. Bar, 50 μm.



Figs 8, 9. Quail–chick chimaeric double-rostral somites.
Fig. 8 is a low-power view of a double-rostral segment. A spinal nerve can be seen traversing the chimaeric double-rostral sclerotome. Neural tube towards the top of the picture, rostral to the right. *v*, intersegmental blood vessels. The arrows show the limit of the double-rostral sclerotome, between them and the blood vessel to their right, and of the single-caudal sclerotome, between the arrows and the blood vessel to their left. Bright field. Rostral is to the right.
Fig. 9 is a higher power view of the chimaeric sclerotome in Fig. 8. Quail cells (*q*) can be seen intermingled with chick host cells (*c*) throughout the double-rostral sclerotome.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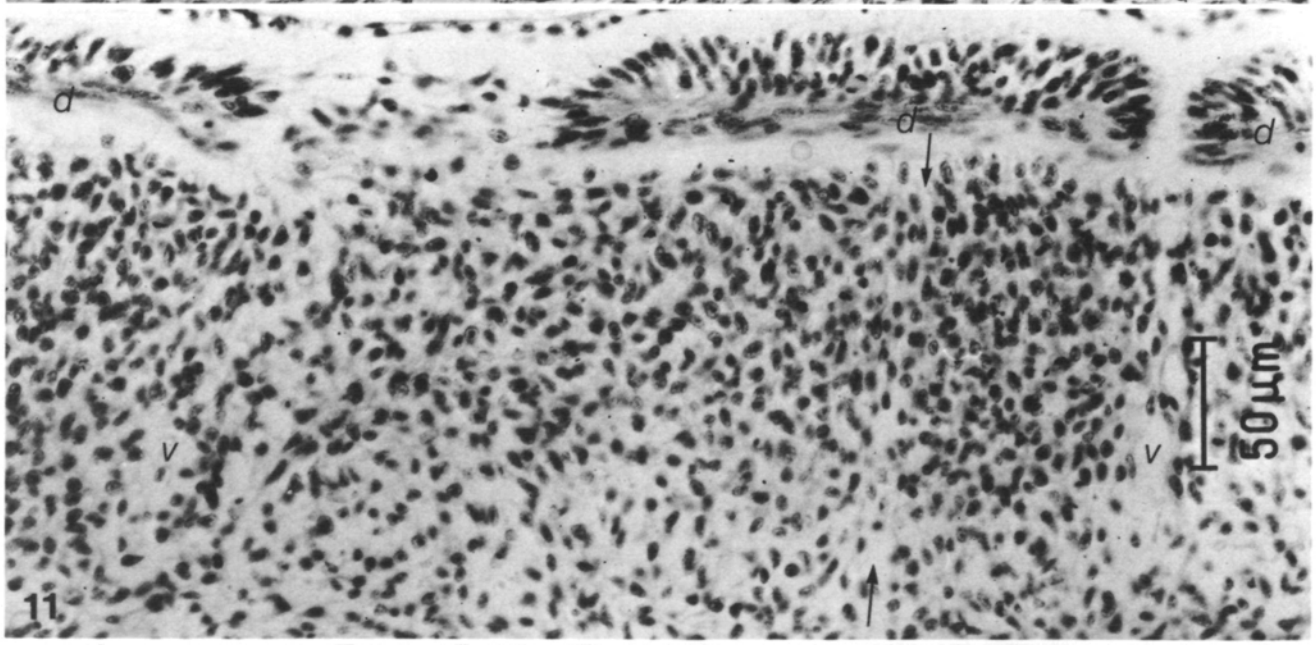
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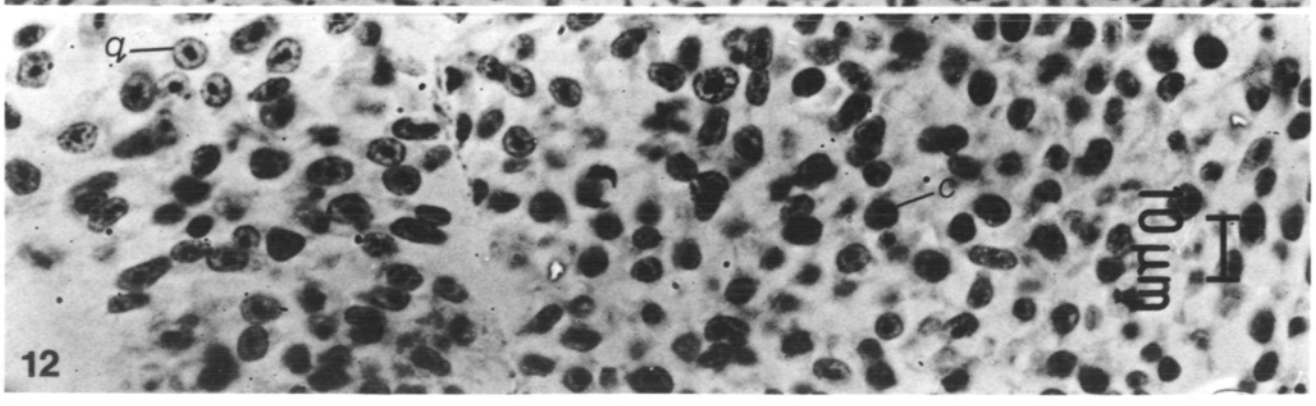
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newly segmented somite was replaced, in a host chick embryo, with an equivalent quail half-somite. In all cases (20 successful experiments: 15 rostral and 5 caudal grafts), donor and host embryos were at the same stage of development; the half-somite to be grafted was taken from the same position in the donor as that into which it was to be grafted. The resulting chimaeric embryos were cultured *in ovo* up to stages 20–36, fixed in Zenker's fixative and sectioned along the transverse, sagittal or coronal plane.

Both the rostral and the caudal halves of the somite were found to contribute to the same tissues. After grafting either half of a quail somite, quail cells were found in one or two vertebrae, their periostia and intervertebral discs, one or two ribs and their periostia, axial and intercostal muscles and the dermis of the trunk.

Discussion

Our results can be summarized as follows.

(1) When grafted into another region of a host embryo, a newly formed half-somite develops half-sclerotome properties characteristic of its origin as rostral or caudal.

(2) Multiple-rostral or multiple-caudal 'compound' somites can be constructed by any method that brings two or more *like* half-somites into adjacent positions.

(3) Multiple-rostral compound somites develop a sclerotome through which passes an abnormally wide spinal nerve.

(4) Multiple-caudal compound somites give rise to a sclerotome whose caudal portion is unusually wide, devoid of motor nerves and neural crest derivatives, and whose cells are coated with PNA receptor.

(5) Neighbouring *like*-half sclerotome cells mix with each other, while *unlike*-half sclerotome cells do not. The latter are separated by a boundary.

(6) Both somite halves have similar developmental fates. Both contribute to the vertebrae and ribs, periostium, intervertebral discs, axial and intercostal muscles and the dermis of the trunk.

Newly formed somites are a mosaic of presumptive rostral and caudal cells

Our results confirm that the newly formed epithelial somite is a 'mosaic' of committed rostral and caudal cells: whether or not the half-sclerotome that develops can support axonal growth and neural crest cell migration depends upon its origin and not upon the surrounding tissues. It follows that by the time of segmentation, the epithelial somite contains cells already committed to rostral and caudal fates. These fates will be expressed when the sclerotome forms, some 4–6 somites (6–8 h) later.

It is interesting to note that, while all the cells of the newly formed somite appear to be committed to rostral or caudal half-sclerotome, not all the cells of the somite will become sclerotome cells. Gallera (1966) showed that the differentiation of the epithelial somite into dermomyotome and sclerotome arises as a result of inductive interactions with the adjacent epiblast and endoderm. This finding indicates that a somite cell is specified as dermomyotome or sclerotome after a somite forms but before it subdivides, 6–8 h later. Since by the time of segmentation rostral and caudal cells are already specified as rostral or caudal, the rostral/caudal decision must precede the commitment to become sclerotome or dermomyotome. For a presumptive dermomyotome cell, therefore, the latter decision must override the former. Alternatively, the somite could be even more of a mosaic than hitherto supposed, comprising, at the time of its formation, fully committed dermomyotome and rostral and caudal sclerotome cells. If so, Gallera's findings would have to be explained by cell sorting under the influence of the surrounding epiblast and endoderm.

Compound somites: differences between rostral and caudal half-sclerotome

There was a difference in the proportion of embryos in which a larger sclerotome was visible when double-rostral or double-caudal sclerotomes were constructed. While 28/32 (88%) embryos with double-rostral sclerotomes had a wider rostral region, only 18/53 (34%) of double-caudal sclerotomes displayed a wider caudal sclerotome region. This difference is difficult to explain. When double-caudal sclerotomes were constructed using a quail donor

Figs 10–12. Quail–chick chimaeric double-caudal somites.

Fig. 10 is a low-power view of a double-caudal segment. Dermomyotome (*d*) towards the top of the photograph, rostral to the right. *v*, intersegmental blood vessels. The arrows mark the boundary between the double-caudal sclerotome, between them and the vessel on their left, and the single-rostral sclerotome, between them and the vessel to their right. In this case, the dermomyotome is entirely chick-derived and spans the normal distance, rather than the entire compound segment. Nomarski optics.

Fig. 11. The same section viewed by bright-field optics, to show the quail cells intermingled with chick host cells throughout the chimaeric double-caudal sclerotome. Symbols as in Fig. 10.

Fig. 12. A higher power view, showing the quail cells (*q*) intermingled with chick host cells (*c*) in the compound sclerotome. Symbols as in Fig. 10. Bright field. Rostral is to the right in these three figures.

half-somite, the quail cells were seen to be interspersed with chick host cells in the double-caudal sclerotome. This argues against the possibility that the extra caudal cells are eliminated. Moreover, when triple-caudal or quadruple-caudal sclerotomes were constructed, all of the operated embryos did have a wider region devoid of nerves. These considerations suggest that the difference must reside in the way the additional cells are packed. It is worth remembering that, at a later stage, the caudal half-sclerotome develops a higher cell density than the rostral half (see for example, Keynes & Stern, 1984). It is possible, therefore, that the caudal half-sclerotome is more effective than its rostral counterpart at absorbing greater cell numbers at a higher density.

Segmental borders and their maintenance

Our results suggest that the interactions between adjacent sclerotome cells obey a precise rule: 'Cells from *like* halves mix with each other, while those from *unlike* halves do not; when cells from unlike halves are adjacent to one another, a border is produced'.

On the grounds that 'cells originating from the posterior half of one somite together with cells from the anterior half of the next somite form one vertebra', Meinhardt (1982) inferred that each somite must consist of at least a rostral and a caudal portion. His conclusion was based on the classical descriptions of vertebral column development, about which there is some controversy (see below). Nevertheless, our results confirm the idea of a twofold subdivision of the somite. Meinhardt argued further that 'a juxtaposition of A[nterior, = rostral] and P[osterior, = caudal] cells cannot be the signal to form a segment border (or a cleft), since a second A–P confrontation is present in the centre of each segment, without a border being induced'. In order to explain why borders do not form in the middle of each segment, he suggested that segments are in fact subdivided threefold, with a third region, 'S', the segment border forming at the P/S transition. However, in the sclerotome an *intra*segmental boundary does exist, and was first described by von Ebner in 1888. This 'von Ebner's fissure' has been regarded by some as an artefact of fixation (Baur, 1969; Verbout, 1976), but it can be seen in living, unfixed embryos (see Keynes & Stern, 1985), and it is clear in sagittal (Fig. 13) or coronal sections and in scanning electron micrographs after removal of the dermomyotome (Fig. 14). While our results do not exclude the existence of a third region such as that postulated by Meinhardt, they do not argue in favour of it. It seems unnecessary to postulate any more than a twofold subdivision to explain our results or the pattern seen in the normal embryo. We can conclude that juxtaposition of rostral and caudal cells in the normal embryo always produces a border, whether between adjacent sclerotomes or within a sclerotome.

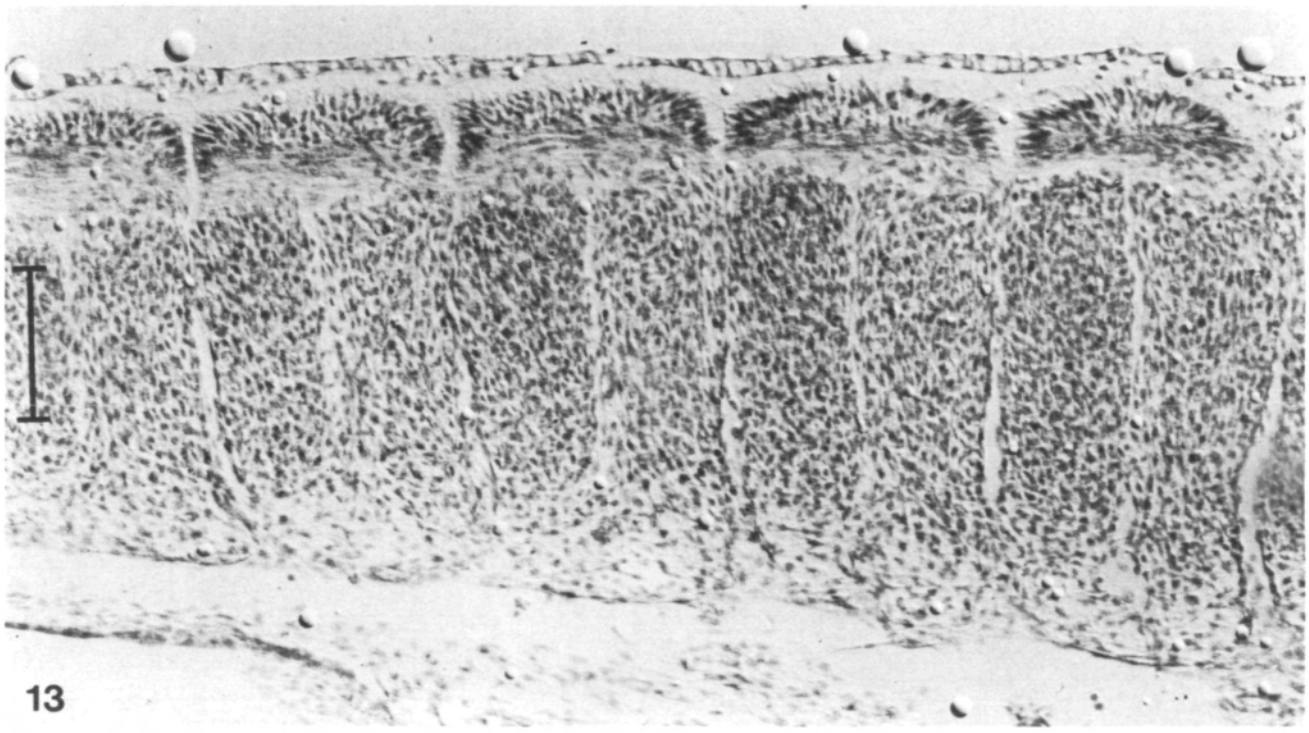

Fig. 13. Sagittal 8 μm paraffin section through a stage-16 embryo, stained with haematoxylin. Rostral is to the right. The intrasegmental or 'von Ebner's' fissure appears similar to the intersegmental border. The difference can be detected only by reference to the extent of the dermomyotomes, towards the top of the photograph. Bar, 50 μm.

The intrasegmental border is in some ways similar to the intersegmental border: it is rich in extracellular matrix and contains only a few cells, aligned at right angles to the rostrocaudal axis of the embryo. The confrontation between rostral and caudal cells presumably leads to the secretion of matrix, which acts to keep the two populations separate. There are, however, a number of differences between the intersegmental and intrasegmental borders. First, the intersegmental border is rich in fibronectin and laminin, whereas these are not concentrated in von Ebner's fissure (Rickmann *et al.* 1985). Second, the intersegmental border contains a blood vessel, while von Ebner's fissure does not. Third, while von Ebner's fissure only encompasses the sclerotome, the intersegmental border includes a boundary between adjacent dermomyotomes as well (Fig. 13). Collectively, these may be the reasons why, in a whole-mounted or living embryo, the two boundaries appear to be somewhat different.

We can account for these differences: the newly formed epithelial somite is surrounded by a basal lamina rich in fibronectin and laminin. After the somite disperses into dermomyotome and sclerotome, some of this basal lamina material probably persists between adjacent somites. The differential distribution of these molecules could then play a role in determining the pattern of angiogenesis. Finally, the dermomyotome spans both sclerotome halves presumably because it persists as an epithelial cap during the period of sclerotome dispersal.

Why is the sclerotome subdivided? Immediately after formation, each somite is an epithelial sphere, which then disperses into an epithelial dermomyotome and a mesenchymal sclerotome. In the absence of some restraining mechanism, the loosely packed cells of the sclerotome would tend to mix with each

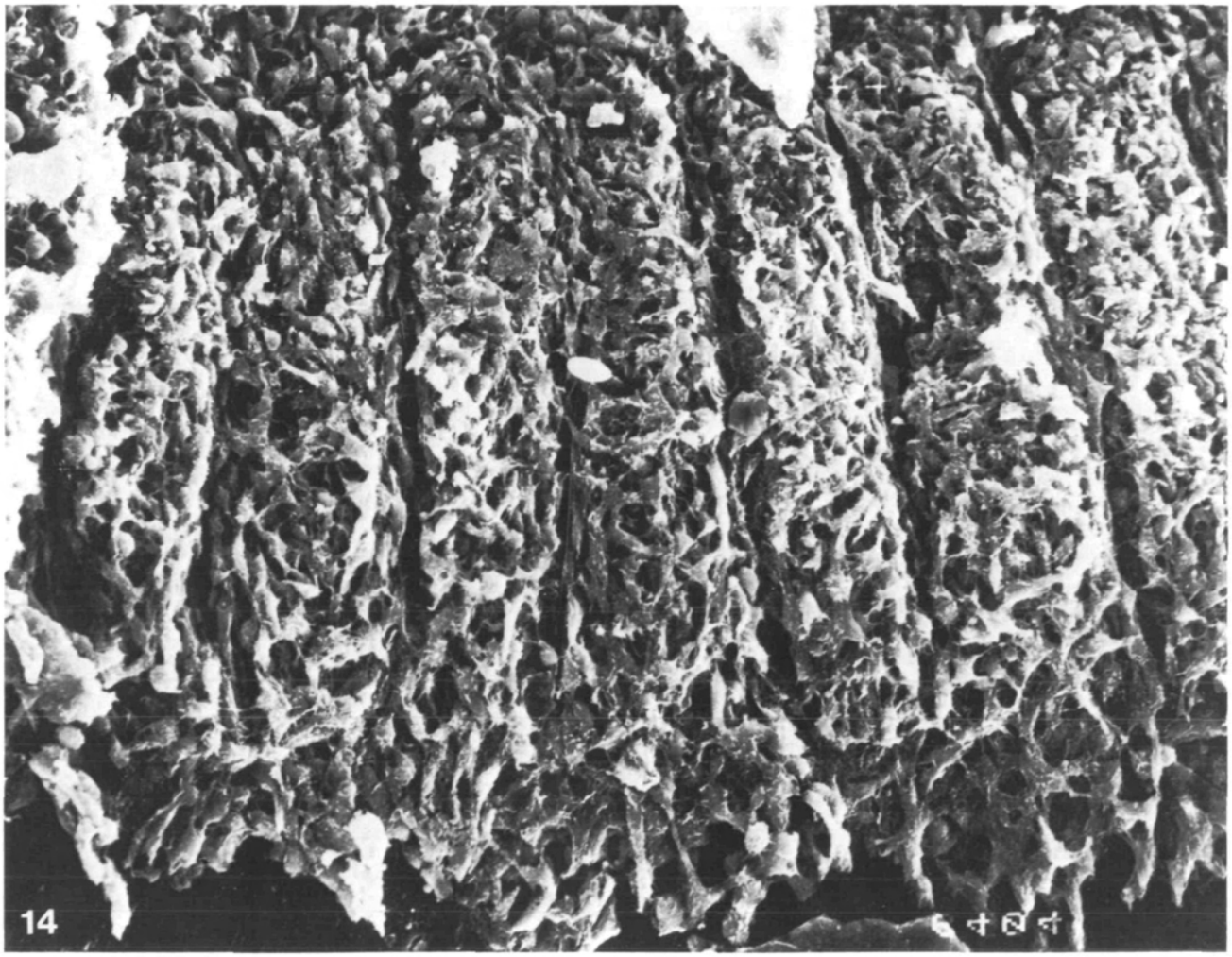

Fig. 14. Scanning electron micrograph of an embryo from which the epiblast and dermomyotomes were removed. Each 'block' is a half-sclerotome (the picture therefore shows 3½ whole segments). The half-sclerotomes are viewed from a dorsolateral direction. There is little, if any, apparent difference between the intersegmental and intrasegmental borders. Rostral is to the right, the neural tube towards the bottom of the photograph. Photograph taken and supplied by Dr Stephen Meier and reproduced with the kind permission of Dr A. Jacobson. ×250.

other, destroying the segmental pattern. The immiscibility of rostral and caudal sclerotome cells probably serves to maintain their segmental arrangement.

Why is it important to preserve the segmental arrangement? One function of the subdivision of the sclerotome is presumably to impose segmental organization on the peripheral nervous system and vasculature. Another function, often discussed in the literature concerning the development of the vertebral column, could be to generate a punctate arrangement of vertebrae. It has been widely accepted that each vertebra develops from a combination of the rostral half of one sclerotome and the caudal half of the next rostral sclerotome, on each side of the embryo. Thus, vertebra formation comprises a resegmentation of the sclerotomal mesenchyme across the intersegmental border, and von Ebner's fissure becomes the intervertebral boundary. This process was termed 'Neugliederung' (resegmentation) by Remak (1855), who introduced the concept in order to explain why each axial segmental muscle spans two adjacent vertebrae, an arrangement that is necessary to produce flexion-extension movements of the vertebral column. However, the original descriptions of resegmentation are open to criticism (Baur, 1969; Verbout, 1976; Dalgleish, 1985). Moreover, it is difficult to reconcile our finding that the fates of the two halves of the sclerotome are equivalent with the idea of resegmentation. The issue of resegmentation should therefore be reexamined.

Formation of the intersegmental border

If the phenomenon of resegmentation does exist, it is interesting to speculate on the mechanisms that determine which half-sclerotomes fuse with one another to make a vertebra. One consideration of potential importance is that the border appearing at the cranial end of the segmental plate, separating the newly forming somite from the next, actually defines both the caudal edge of the newly forming somite and the rostral edge of the next somite to form. Thus, the caudal half of any one somite and the rostral half of the next caudal somite are of the same developmental age. Perhaps the formation of the intersegmental border starts some sort of clock, so that the two sclerotome halves adjacent to that border later mature together, for example to adhere to one another (Stern & Keynes, 1986).

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