Scanning Electron Microscopy

Volume 1985
Number 1 *1985*

Article 27

1-15-1985

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SCANNING ELECTRON MICROSCOPY/1985/I (Pages 257-267)
SEM Inc., AMF O'Hare (Chicago), IL 60666 USA

0586-5581/85$1.00+.05

CELL MOVEMENT AND CONTRACTION IN SOMITE DEVELOPMENT

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(Paper received January 19 1984, Completed manuscript received January 15 1985)

Abstract

During somite formation the segmental plate mesoderm, lying on either side of the axial organs, reorganizes into roughly spherical pairs of epithelial structures. This segmentation process includes changes in cell shape and position, cell-cell and cell-substratum adhesive properties and accumulation of extracellular matrix material which proceed down the anterior-posterior axis. Later in somite development the sclerotome region "disperses", migrating around the spinal cord where it produces the cartilage model of the vertebral column. Experimental manipulation of segmentation and sclerotome dispersal with drugs affecting microfilaments, microtubules and calcium-dependent contraction suggest that cells migrate into position, elongate, and undergo apical contraction as part of the segmentation process. This process of calcium-dependent, possibly calmodulin-mediated, contraction can be both stimulated precociously and inhibited, showing similarities with contractile morphogenetic events in epithelial organ systems such as eye and thyroid. Similar experiments with drugs affecting contractile microfilaments demonstrate that active cell movement, along with extracellular matrix production, is involved in sclerotome dispersal.

KEY WORDS: organogenesis, segmentation, segmental plate mesoderm, somite formation, sclerotome dispersal, calcium-dependent contraction, cell shape changes, microfilaments, microtubules, extracellular matrix

Introduction

Segmentation, in vertebrate embryos, is the process of forming a series of paired, transitory "vesicular" structures called somites from a primary mesodermal tissue (segmental plate) found on either side of the early axial structures (notochord, neural plate/tube). Somite formation and development has been described as a transition in tissue organization from mesenchymal to epithelial and back to mesenchymal form. A collection of loosely associated (mesenchymal) cells in the segment plate reorganizes to closely apposed cells that form the epithelial somite. Later in somite development there is a return to mesenchymal organization as the ventromedial wall of the somite vesicle breaks down and the sclerotome portion of the somite "disperses". The sclerotome is the cartilage-forming region of the somite. Somitic cartilage eventually surrounds the spinal cord and establishes the framework for the vertebral column. The dorsal portion of the somite forms the dermamyotome, which eventually gives rise to the dermis of the skin and to the skeletal muscles. These reorganizations of tissue structure involve changes in cellular adhesivity, specialized cell junctions, extracellular matrix (ECM) material, and cytoskeletal organization. My own studies of somitogenesis have involved establishing the existence of a calcium-dependent contraction event in segmentation and examination of the contribution of cell movement to somite formation and to sclerotome dispersal. In this paper I will describe my own work in relation to other studies of segmental plate organization, segmentation, and sclerotome dispersal and try to integrate the various observations on morphogenetic forces underlying somitogenesis using examples from my own scanning electron microscopy (SEM) studies. All of the micrographs I will show are of stage 14-15 chick embryos (staging as in Hamburger and Hamilton, 1951), which occurs at approximately 2.5 days of incubation. There are 22 pairs of somites at stage 14, and the segmental plate mesoderm is in the trunk region (between the prospective limb bud areas).

Segmental Plate Organization

The early events in formation of the segmental plate mesoderm include shearing of the primary mesoderm from anterior to posterior (cranial to caudal) by the regression of Hensen's node (avian) and formation of the notochord, association of the tissue with neural epithelium, and condensation toward the midline (in anterior regions - Lipton and Jacobson, 1974a). Accompanying changes in cell shape and orientation have been described in detail by Bellairs (1979). Somite formation proceeds from anterior to posterior and the segmental plate mesoderm lies on either side of the axial structures extending from the last somite caudally to Hensen's node (avian) or to the anterior end of the primitive streak (mammalian). (Fig. 1). The dorsal and ventral surfaces of the segmental plate are covered by ectoderm and endoderm, respectively (Figs . 2-4).

Figure 5 shows the dorsal side of normal segmental plate with the ectoderm dissected away after fixation. Cells appear loosely associated and contact each other with numerous cell processes. On the ventral side (Fig. 6), with the endoderm removed, cells lie in a planar arrangement. In more anterior portions of the segmental plate, more ECM is seen on both dorsal and ventral surfaces (Fig. 7a,b,c,) and more ECM accumulates with time. In sagittal fracture, (Fig. 8) segmental plate appears as a loosely organized mesenchymal tissue. The cells are stellate and slightly flattened along the dorsal-ventral axis. The tissue appears the same in cross-fracture. The segmental mesoderm is attached, initially, to the neural tube (Fig. 9) and the overlying ectoderm (Bellairs & Portch, 1977) by fine cell processes. The ventro-medial portion of the segmental plate is connected with the notochord ECM by matrix fibers (Fig. 10) composed of collagen and glycosaminoglycan (Lash & Vasan, 1978).

A variety of experiments have suggested that there is a pre-patterning of the segmental plate mesoderm prior to the actual segmentation event. When separated from the neural tube and notochord surgically, 10 to 12 somites (in chick and quail) form at one time in the segmental plate, after a lag, instead of in sequence. (Packard and Jacobson, 1976; Packard, 1980). Meier (1979) demonstrated a morphological basis for the prepatterning by showing 10 to 12 areas of circular organization on the surfaces of the segmental plate. These areas, termed somitomeres, have now been shown to correlate with the location of the somites (Packard and Meier, 1983). Somitomeres are difficult to see and require stereo SEM imaging. The forces that produce this pre-patterning remain under investigation. The involvement of Hensen's node, the axial structures, ectoderm and endoderm have all been considered (Bellairs and Portch, 1977; Meier and Jacobson, 1982; Packard and Meier, 1983; Christ et al., 1972, 1974; Sandor and Fazakas-Todea, 1980).

FIGURE 1. Control stage 14 chick embryo in ring culture (see Chernoff and Lash, 1981). Explanted with surrounding extraembryonic membranes grown ventral side up over L-15 culture medium. Shown from heart level to posterior end. OA= omphalomesenteric artery. Arrows mark last fully formed somite (som), notochord (noto, dark structure), and neural tube (NT, bright structure). Light micrograph. Bar=100μm

FIGURE 2. This figure and Fig. 5 are a set showing cross-fracture through the nascent somite pair (nasc. som.) from a stage 14-15 embryo. Both halves are shown to give an impression of the three dimensional arrangement of cells in the forming somite. end=endoderm, ect=ectoderm, NT=neural tube, no=notochord. Bar=100μm

FIGURE 3. This half of the fracture pair retains more of the ECM that surrounds the somites and forms the perinotochordal sheath. ECM=extracellular matrix. Bar=100μm

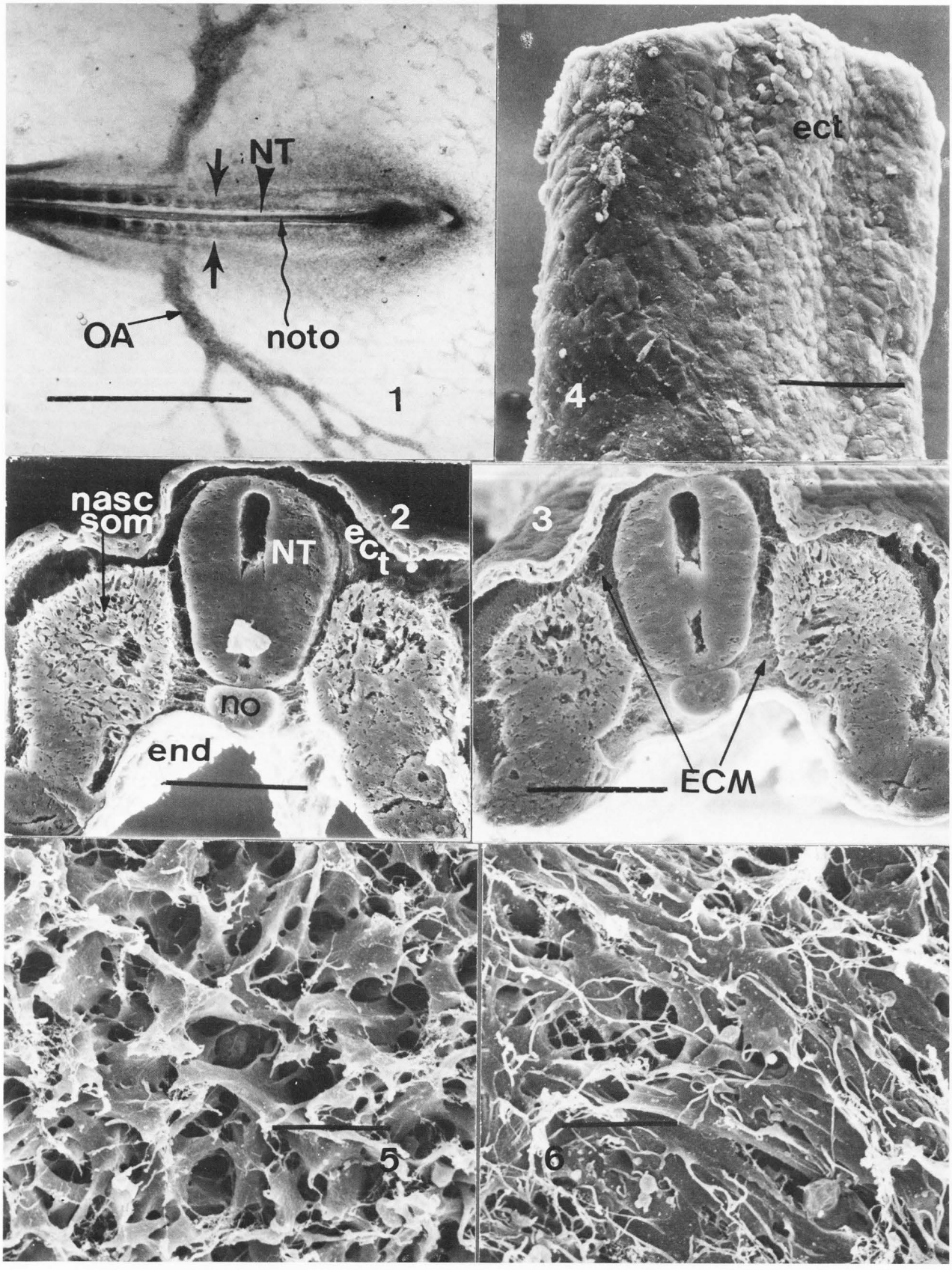
FIGURE 4. Dorsal surface of cultured trunk segment like that used in figures 2 and 3 (see Chernoff and Hilfer, 1982) shows close packed polygonal cells of the covering ectoderm(ect). Bar=100μm

FIGURE 5. Control segmental plate mesoderm, dorsal surface (ectoderm dissected off following fixation). Fine cell processes and some ECM fibers are visible. Bar=10μm

FIGURE 6. Control segmental plate, ventral side (endoderm off). Cells lie in a more planar arrangement on this surface. Bar=10μm

Segmentation

The segmental plate mesoderm lying on either side of the notochord forms somites by a process of segmentation that proceeds from the anterior to the posterior end of the embryo. A number of morphogenetic forces are involved in the transition from segmental plate mesoderm to epithelial somite. Progressive changes in cellular adhesivity appear to occur along the embryonic axis as somites form (Bellairs and Portch, 1977; Bellairs, 1979). Cell-cell and cell-substratum adhesivity increase from segmental plate to somite stages (Bellairs et al., 1978, 1980). Changes in cell junctions accompany this process (Trelstad et al., 1967; Lipton and Jacobson, 1974a; Solursh et al., 1979; Bellairs, 1979). The role of extracellular matrix material (ECM) in somitogenesis has been an active area of investigation. One ECM component, fibronectin, seems to stimulate segmentation in vitro (Cheney et al., 1980) presumably by affecting cell adhesion. Perinotochordal sheath fibers may 'stabilize' newly formed somites (Lipton and Jacobson, 1974a,b). It has been suggested that collagen fibers of the segmental plate and surrounding tissues anchor nascent somite cells and aid in elongation (Bellairs, 1979) (Figures


NT
OA
noto
1
ect
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6

2,3,10,11). However, these cell-matrix interrelationships alone cannot account for the major changes in cell organization during somitogenesis.

Changes in cell shape during segmentation have been described in a variety of contexts (Williams, 1910; Lipton and Jacobson, 1974a; Bellairs, 1979; Meier, 1979). Of principal interest here is the elongation of cells and apical constriction during formation of the epithelial somite (i.e., a contractile event). The existence of a contractile event in segmentation is suggested by two lines of experimental evidence. The changes in cellular morphology during segmentation are consistent with contraction. The detailed studies of Bellairs (1979) describe the changing appearance of the surface of the segmenting mesoderm. Segmentation seems to begin on the dorsal side of the segmental plate with elongation of cells in that region (Platt, 1889; Lipton and Jacobson, 1974a; Bellairs, 1979). The cells of the somite epithelium are elongated and tend to be narrower toward the lumen (Figs. 12,13). The position of the nucleus in a cell of the somite epithelium varies with the stage of the cell cycle (Fig. 13). Nuclei move toward the apical end of the cell (center of the somite) in preparation for cell division (Langman and Nelson, 1968; Bellairs, 1979). The elongated somite cells contain many microtubules whereas few are reported in segmental plate cells (Bellairs, 1979). Microtubules in segmental plate cells are probably just not as highly oriented as those in somites. The epithelial somite appears to be under tension through attachment to neural tissue, ectoderm, endoderm and the dorsal aorta (Bellairs, 1979; Lipton and Jacobson, 1974a). The presence of microtubules and tension forces are thought to contribute to somite cell elongation. This elongation and narrowing of the cell apices is reminiscent of morphological changes that occur in epithelial systems in which contraction has been found to play a role (Wessells et al., 1971; Burnside, 1973; Schroeder, 1973). A second line of thought has grown out of studies on early stages of somitogenesis and observations on segmentation in the absence of the axial structures. As stated in the previous section, Meier (1979) described somitomeres, circular areas of orientation visible on the dorsal and ventral mesoblast surfaces. These appear during condensation of the paraxial mesoblast as the segmental plate forms. They represent a stage in the process that results in segmentation. In Meier's stereo SEM study, somitomeres seem to indicate the boundaries on the segmental plate of 10 or 12 prospective somites. This is consistent with the observation (Lipton and Jacobson, 1974b; Packard and Jacobson, 1976; Packard, 1978) that 10-12 somites will form simultaneously from segmental plate separated from the axial structures. This simultaneous formation has been cited as evidence for a contraction event in somitogenesis (Meier, 1979). It should be noted that the response is simultaneous and not instantaneous segmentation; segmentation took 14-17 hr in the posterior (Lipton and Jacobson, 1974b) and approximately 10 hr in the anterior region (Packard and Jacobson, 1976). The situation may not be one of contraction upon release of tension or physical restraint through anchoring to adjacent tissues but, instead, may reflect release from axial control of sequential segmentation.

In my own work, the existence of a calcium-dependent process in somite formation was investigated in the embryonic chicken using drugs and conditions that inhibit shape changes in other systems (Hilfer et al., 1981; Brady and Hilfer, 1982). Calcium-dependence of segmentation was investigated by culturing embryonic trunk explants with and without Ca^{++} in the medium and by treatment with calcium antagonists and agonists. Calcium activation of non-muscle contractile systems is thought to reside with calcium dependent regulatory proteins, such as calmodulin (Cheung, 1980; Klee et al., 1980). The participation of calmodulin in segmentation was investigated by using calmodulin antagonists in culture (Brady and Hilfer, 1982). The effects of the calmodulin antagonists were compared with those of cytochalasin D (CD), which affects contractile microfilaments by another mechanism (Brown and Spudich, 1979; Lin, et al., 1980; MacLean-Fletcher and Pollard, 1980). A calcium agonist, the Ca^{++} ionophore A23187 was used to stimulate contraction of somitic tissue to observe precocious somite

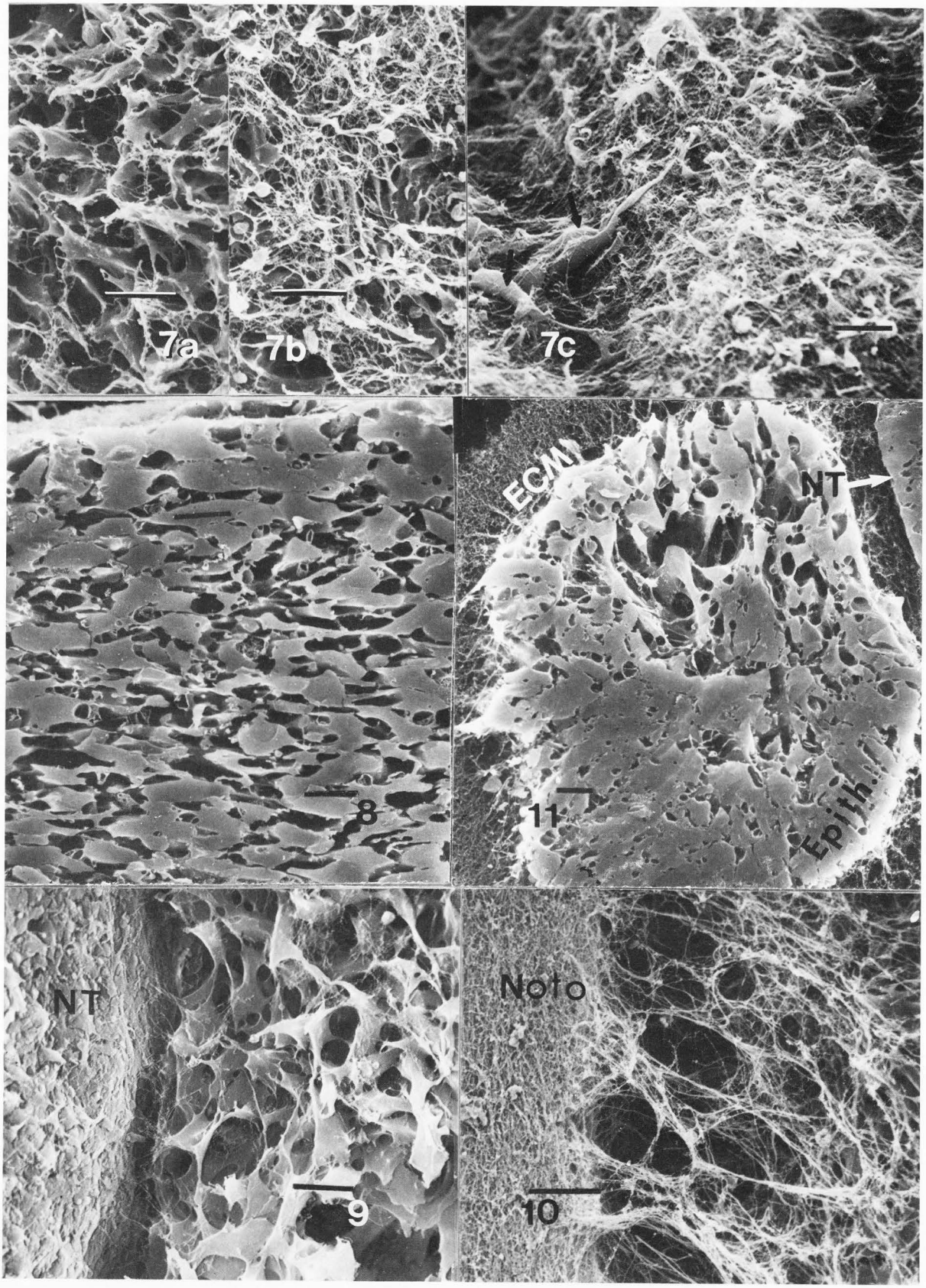
FIGURE 7. This series of three micrographs shows accumulation of ECM with time. Sequence is posterior to anterior. (a) shows dorsal surface of segmental plate as in figure 5. (b) shows more anterior region of segmental plate in same stage 15 embryo with covering of ECM fibers. (c) shows dorsal surface of 2 fully-formed somites at the level of the omphalomesenteric artery. The somites are densely covered with ECM. Arrows show neural crest cells migrating across the somites. Bar=10μm

FIGURE 8. Sagittal fracture of normal segmental plate mesoderm in culture 4 hours. Most cells are slightly flattened along the dorso-ventral axis (dorsal surface is at top of figure) and stellate in profile. Bar=10μm

FIGURE 9. Fine cell processes connect the segmental plate (stage 14, ectoderm off) with the neural tube (NT). Bar=10μm

FIGURE 10. On the ventral surface (stage 15, endoderm off) ECM surrounds the notochord (Noto) and ECM fibers connect the notochord and the segmental plate mesoderm. Bar=10μm

FIGURE 11. Higher magnification view of cross-fractured nascent somite shown in figures 2 and 3. Cells are accumulating into an epithelial arrangement (Epith) at the somite periphery and elongating. Cells seem to be accumulating at the somite core. The tissue is surrounded by ECM. NT=neural tube. Bar=10μm


7a
7b
7c
8
ECM
NT
11
Epith.
NT
9
Noto
10

formation. The role of microtubules in somite cell shape changes was explored with nocodazole, an inhibitor of microtubule polymerization. Scanning electron microscopy of frozen, fractured (Humphreys, et al., 1974) somites and segmental plate tissue was used to analyze the contributions of cell movement, elongation, and apical constriction to somitogenesis. The results are consistent with the involvement of a calcium-dependent process in somite formation (Chernoff and Hilfer, 1982).

The responses of segmental plate and somites to the drugs used in this study emphasize that the segmentation process has a number of components. Ca^{++} antagonists (verapamil, papaverine) (Chernoff and Hilfer, 1982), calmodulin antagonists (trifluoperazine (TFP)), cytochalasin (CD), and nocodazole all reversibly arrest somite formation at the stage of drug treatment (Chernoff and Hilfer, 1982; Figs. 14-16). The importance of cell movement in segmentation is illustrated by the prevention of accumulation of segmental plate cells into the somite periphery. (Compare drug-treated segmental plates; Fig. 14-16; and drug-treated nascent somites; Figs. 17,18, with normal tissue at the same time of incubation (Figs. 11 ,12).

TFP and CD prevented the apical constriction necessary to form the epithelial somite (compare Figs. 19, 20 with Fig. 12). The effects of nocodazole show that segmental plate cell morphology and somite epithelial cell elongation are heavily dependent upon cytoplasmic microtubules (Figs. 18,21). Microtubules have been implicated in directionality of cell movement in other systems (e.g., Gail and Boone, 1971). The Ca^{++} ionophore A23187 has a very rapid, specific effect on somitogenesis. The nascent somite pair of a trunk segment in culture rapidly separates from the segmental plate, directly implicating apical contraction (Chernoff and Hilfer, 1982). Segmental plate tissue merely condenses in the presence of Ca^{++} ionophore.

Sclerotome Dispersal

Later in somite development the sclerotome "disperses". Some cell junctions are lost and ECM is elaborated (Fisher and Solursh, 1977; Solursh et al., 1979). The sclerotome then expands into the space around the notochord, a process that has generally been considered to mark the onset of active sclerotome migration (Trelstad et al, 1967; Hay, 1968; Ebendal, 1977). Both ECM production and active cell movement are important in sclerotome migration. ECM can play different roles in a tissue at different stages in development. The ECM may stabilize the sclerotome in early development (Lipton and Jacobson, 1974a,b). It may later serve as a substrate during migration into the perinotochordal area (Ebendal, 1977). Later still, this matrix is a stimulator of somite chondrogenesis (Lash and Vasan, 1978). Solursh, et al., (1979) showed that treatment

FIGURE 12. Cross-fracture of epithelial (fully formed) somite. Typical elongated epithelial cells (epith) with narrowed apices surround core cells (core). Bar=10μm

FIGURE 13. A higher magnification view of somite epithelium shows the varying levels of nuclei within the single cell layer (arrowheads). Location of the nucleus is cell-cycle-dependent. Bar=10μm

FIGURE 14. Sagittal fracture of segmental plate treated with TFP for 4 hrs. Cells have rounded up and tissue is more condensed than in control (figure 8). During time of drug treatment control explants have formed 1-2 new somite pairs. Bar=10μm

FIGURE 15. Dorsal surface of segmental plate mesoderm (ectoderm off) treated with CD for 5 hours. Cells have rounded up. ECM fibers cover the surface (compare with normal tissue, figures 5 and 7). Bar=1μm

FIGURE 16. Cross-fracture of segmental plate treated with nocodazole. Cells have completely lost their stellate shape and tissue is highly condensed. NT=neural tube. Bar=10μm

FIGURE 17. TFP-treated nascent somite viewed in cross-section. Compare with figure 11. Cells are rounded up and distinction between accumulating epithelial cells and core cells is largely lost. Bar=10μm

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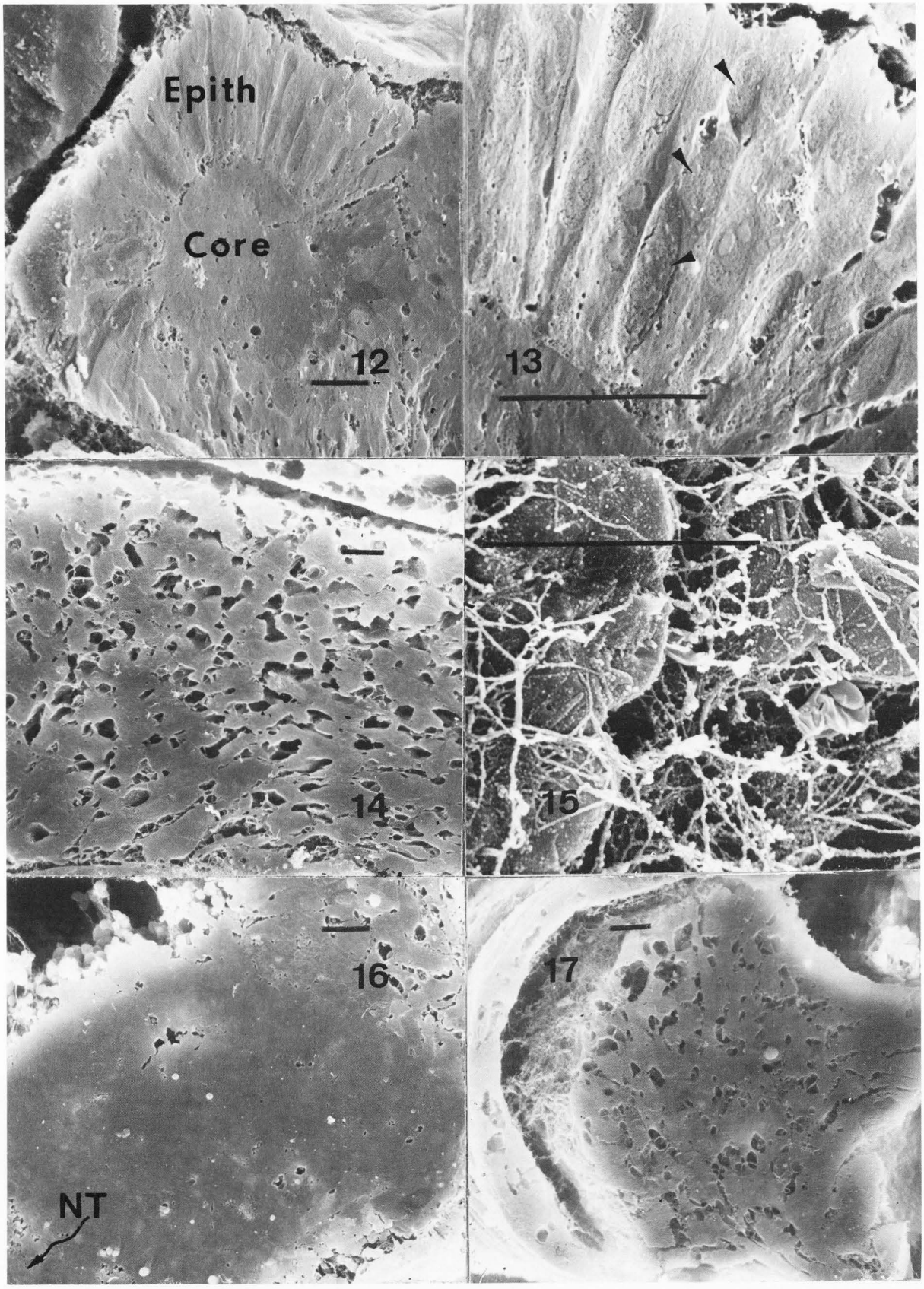
of sclerotome-forming somites with glycosaminoglycan lyases, (enzymes which digest important ECM components) caused the collapse of sclerotome and halted dispersal. They suggested that sclerotome cells are passively pushed apart by the accumulating ECM. However, sclerotome cells assume a filopodial, lamellipodial morphology as they disperse and move toward the notochord (Hay, 1968; Ebendal, 1977). This morphology is consistent with that of actively moving cells in other tissues of the embryonic chick (Trelstad et al., 1967; Ebendal, 1976; Chernoff and Overton, 1977; Bard et al., 1975; Nelson and Revel, 1975; Ho and Shimada, 1978). Treatment of somites that have formed distinct dermatome and sclerotome with CD (Figs. 22,23) shows sclerotome dispersal to be arrested. If active cell movement was not involved in sclerotome dispersal, then the sclerotome cells should have been passively pushed apart by ECM formation in the presence of CD, since CD did not affect ECM production (Chernoff and Lash, 1981). Active cell movement is also consistent with the increase in cell-substratum adhesivity during somite development (Bellairs and Portch, 1977; Bellairs, et al., 1978). It is likely that cell-substrate adhesivity continues to increase as ECM accumulates. Bellairs et al. (1980) have shown that sclerotome tissue does spread more in vitro than do nascent somites.

Summary

Since the somites form in a temporal sequence along the embryonic axis, different events take place at a given time at different


Epith
Core
12
13
14
15
16
17
NT

levels of the embryo. Moreover, within each somite the events do not occur in a strictly coordinated fashion; some somite epithelial cells may be undergoing apical constriction while others are still migrating into position. In this regard the somite system is unlike the epithelial systems in which cells are organized as sheets at the start of the morphogenetic event. Invagination or branching in epithelial systems is a coordinated process of cell elongation and apical constriction. It does appear that somite segmentation shares underlying mechanisms with epithelial systems such as thyroid eye, and neural tube, including Ca^{++}-dependent (possibly calmodulin-mediated) apical constriction and microtubules dependent cell elongation. The process of sclerotome dispersal involves active cell migration through ECM-filled spaces and the elaboration of ECM within the sclerotome itself.

Acknowledgements

The studies on cell movement and contraction in somite formation were performed in the laboratory of Dr. S. Robert Hilfer, Department of Biology, Temple University, Philadelphia, PA 19122. The study of sclerotome dispersal was performed in the laboratory of Dr. James W. Lash, Department of Anatomy, University of Pennsylvania School of Medicine, Philadelphia, PA. 19104, and completed in the laboratory of Dr. Hilfer.

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FIGURE 18. Cross-fractured nascent somite treated with nocodazole. Elongation of peripheral cells is lost (Epith). NT=neural tube. Bar=10μm

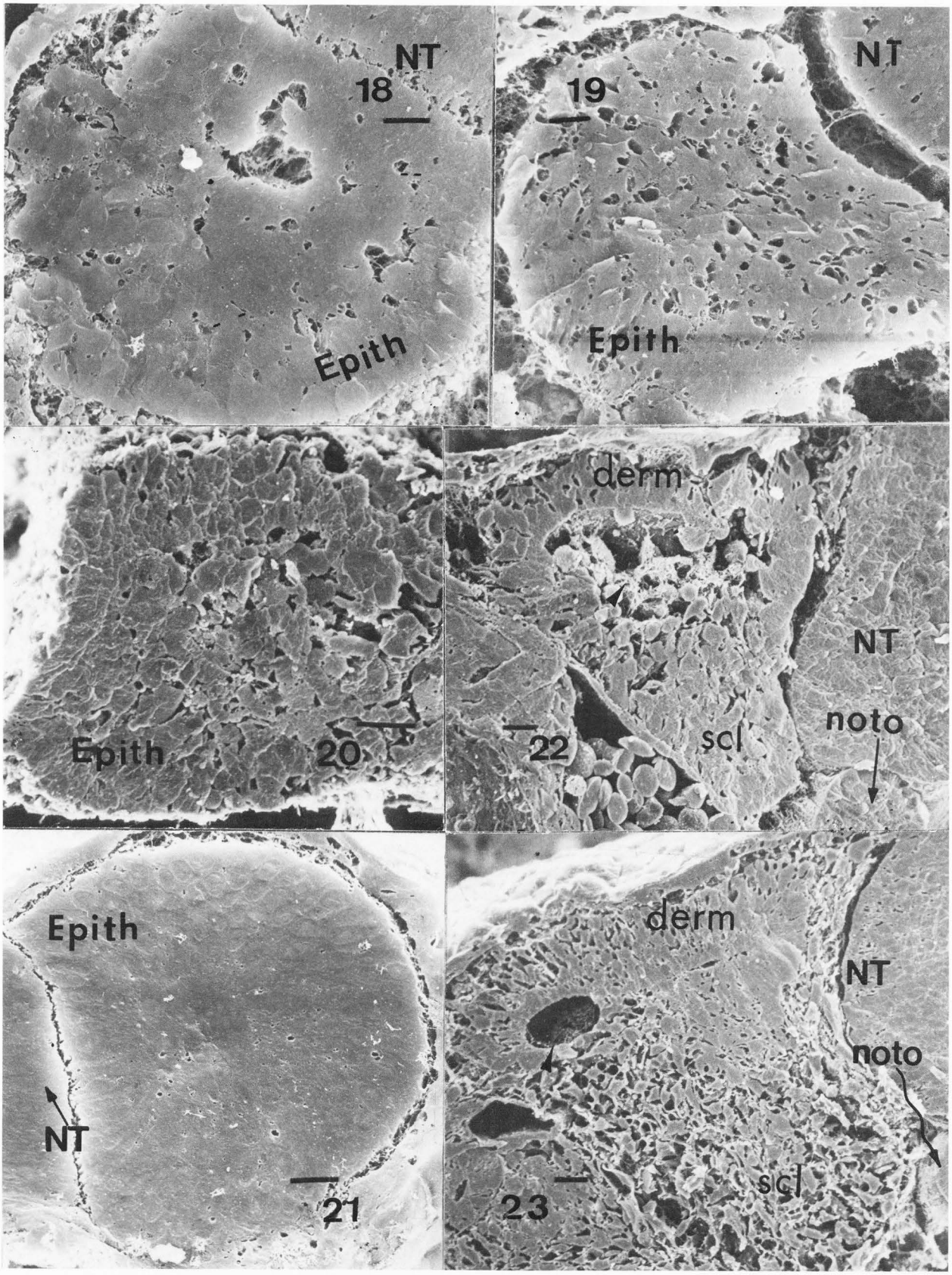
FIGURE 19. TFP-treated somite that has fractured slightly obliquely. Cells rounded. Some cells appear elongated, but apical constriction is lost. (compare with figure 12) NT=neural tube, epith=epithelium. Bar=10μm

FIGURE 20. Cross-fracture of epithelial somite from CD-treated explant. The epithelial cells (epith) are rounded up and apical narrowing is lost. (compare figure 12). Bar= 10μm

FIGURE 21. Epithelial somite treated with nocodazole. Epithelial cells have rounded compared with controls, but apical constriction can still be seen in some regions of the periphery. NT=neural tube. Bar=10μm

FIGURE 22. Cross section of CD-treated stage 15 embryo, somite number 20, in culture 8 hours. The forming dermamyotome (derm) and sclerotome (scl) are indicated. ECM is visible in the somitocoel (arrowhead). NT=neural tube, noto=notochord. Bar=10μm

FIGURE 23. Cross-fracture of control, somite number 20 from explant of stage 15 embryo in culture for 8 hours. Only small somitocoel remains (arrowhead). Dermamyotome (derm) formation is more advanced than in the drug-treated somite (see figure 22) and sclerotome dispersal has started. NT=neural tube, noto=notochord. Bar=10μm


NT
18
Epith
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19
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Epith
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Discussion with Reviewers

S.R. Hilfer: It has been suggested that oriented collagen fibers direct the migration of sclerotome cells toward the notochord and spinal cord. Do you find evidence for such a phenomenon?

Author: The extracellular matrix fibers (collagen with hyaluronic acid and chondroitin sulfate proteoglycan) direct sclerotome migration in the sense that the cells use the fibers as their physical support for migration. The perinotochordal matrix is a dense meshwork and except for a general extension of the fibrous mass between the notochord and somite, there was little indication of radial orientation in my own samples or the micrographs from other studies. In fact, sclerotome cell processes are seen on fine fibers oriented in any direction as the cells move in the general direction of the notochord. It should be noted that movement toward the notochord is only the first stage in sclerotome migration. As the process continues the sclerotome surrounds the spinal cord, and the matrix around the developing spinal cord (neural tube) is a dense mat of randomly-oriented fibers.

S. R. Hilfer: What is the current opinion of how spinal cord and notochord induce somite formation?

Author: The role of neural tube and notochord in somitogenesis is still a matter of active investigation. A number of studies have suggested that the shearing of the primary mesoderm by the regression of Hensen's node (notochord formation) is a key step in triggering the start of somite formation (Lipton and Jacobson, 1974a, b; Bellairs, 1979). Transplanted nodes or primitive streaks can induce supernumerary somites (summarized in discussion section of Packard and Meier, 1983). The implication is that nodal regression is associated with somitomere formation. Nothing is yet known about the nature of the interaction.

Contact with notochord or neural tube may be important in other aspects of somite formation. Continued contact of newly formed anterior somites with the axial structures is required for stability of the somites. The more posterior somites form is a "more advanced" stage than the anterior somites with regard to morphology, stability when separated from the embryonic axis, and chondrogenesis in vitro. This could result from the relatively longer exposure of posterior somitomeres to the axial structures or their extracellular matrix. It should be pointed out that the overlying ectoderm and endoderm and their matrix material may also interact with forming somites and could be involved in the same processes.