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Differentiation

Differentiation of the Metameric Pattern in the Embryonic Axis of the Mouse

II. Somitomeric Organization of the Presomitic Mesoderm

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The formation of the embryonic axis is brought about by the continuous recruitment of cells from the primitive streak, and at later stages from the tail bud. Presumptive somitic cells are first incorporated into presomitic mesoderm before they emerge as metamerically arranged somites. When the presomitic mesoderm was examined in stereo with the scanning electron microscope (SEM), mesenchymal cells were found to be already organized into segmental units. These segmental units are called somitomeres because of their striking similarity to structures in the embryonic axis of the chick embryo described by Meier [16]. Cells within the somitomere are arranged in concentric whorls about a core center, bisected by a medio-lateral seam which subdivides the cell population into anterior and posterior halves. The concentric configuration of the cells is most easily observed along the medial face of the presomitic mesoderm when it is generally wedge-shaped. Even tough the units are tandemly contiguous, somitomeric interfaces are distinguished by abrupt change in cellular orientation. Despite a nearly two-fold fluctuation in the overall size of the presomitic mesoderm during embryonic development, a relatively constant number of somitomeres (six) is found in tandem sequence. Somitomeric maturation culminating in somite formation involves compaction of the cell population, more orderly alignment of cells, reduction in extracellular space, and changes in the shape of the somitomere concomitant with neurulation. Though the more mature somitomere is about 70% the size of the most recently formed somitomere at the caudal end of the presomitic mesoderm, the average size of each somitomere is adjusted proportionally to the overall length of the presomitic mesoderm. In vitro culture of the presomitic mesoderm shows a direct developmental lineage between the somitomere and the somite, suggesting that somite formation is a morphologic manifestation of a somitomeric pattern laid down at an earlier stage in development. The somitomeric pattern in the paraxial mesoderm is the earliest recognizable morphologic evidence of metamerism in the embryonic axis. This pattern is later emulated by other tissues that are topographically associated with the paraxial mesoderm.

Introduction

During the development of the mouse embryo, the metameric pattern of the mesoderm is first visualized at the light microscopic level with the formation of somites. Somites are generated in cranio-caudal succession by the segmentation of the paraxial mesoderm located under either side of the neural plate. There is always a portion of the paraxial mesoderm situated caudal to the most recently formed somite that shows no overt somitic segmentation. This tissue is called the presomitic mesoderm in the mouse embryo [12, 27, 31], and is homologous to the paraxial mesoderm of the amphibian embryo and to the segmental plate of embryos of birds and snapping turtles [14, 19–22].

Various studies on the developmental potential of this overtly unsegmented portion of the mesoderm have shown that its cells are patterned. For amphibian and chick embryos, the removal or addition of presumptive somitic tissue results in the production of the usual numbers of somites, although somite size is adjusted proportional to the amount of tissue present [6, 18, 30]. When the normal orientation of presomitic mesoderm is reversed in the embryo, the original cranio-caudal sequence of somite segmentation is still maintained [18, 23]. Generation of somites occurs when the segmental plates of embryos of birds and snapping turtles are explanted in vitro, and when the segmental plate is deprived of associated axial structures such as neural plate, notochord, and surface epithelia [1, 3, 19–22]. Furthermore, it has been demonstrated that regardless of the size of the segmental plate obtained from embryos of different developmental stages, a consistent number of somites is always formed after culture in vitro [19–22]. It has been suggested that a pattern for prospective somite formation has been established once the presumptive somitic mesoderm has been organized into the segmental plate [14, 22]. It is thought that sequential appearance of segmented units is the result of a chronological sequence of prospective somite specification [6, 8, 10, 23] that is manifested cellularly by a graded series of morphologic events, including increased adhesion among cells and differential matrix production [2, 4, 5].

The morphologic manifestation of such a pattern of prospective somites in the presomitic mesoderm has been demonstrated with the use of scanning electron microscopy

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(SEM) and stereo image analysis [16]. The mesoblast in the segmental plate of chick embryos is parceled into tandemly aligned circular domains called somitomeres. The somitomere can be discerned by the prominent concentric whorling of its component mesenchymal cells. The cells have their long axes directed towards the core center which contains a small primitive coelomic space. Ten to eleven such segmental units are found in the segmental plate of stage 8–9 chick embryos. Even though the morphology of somitomeres in different parts of the segmental plate differs, together they demonstrate a sequence of somitomeric morphogenesis that culminates in somite formation.

In the present study, we use stereo SEM to examine the somitomeric organization of presumptive somitic cells in the presomitic mesoderm of mouse embryos. We provide experimental evidence for the direct developmental relationship between somitomeres and somites in a mammalian embryo using in vitro cultures. Our results suggest that the specification of a somitic pattern does not occur in the presomitic mesoderm, but occurs elsewhere at an earlier stage. Presumptive somitic cells originating from the primitive streak or the tail bud become spatially arranged into somitomeres during axis formation.

Methods

CF-1 random bred mice were used in this study. Embryos were isolated as before [17]. Embryos aged 8.0–11.0 days post coitum (PC) were washed in phosphate buffered saline and fixed with half-strength Karnovsky's fixative [13] in 0.1 M cacodylate buffer (pH 7.3) for 30–45 min at room temperature. Fixed embryos were dissected with electrolytically sharpened needles in 0.1 M cacodylate buffer under a dissecting stereo microscope using transmitted light. The caudal portion of the embryo, containing the presomitic mesoderm, was isolated and split into lateral halves by a cut along the midline. The neural and surface ectoderm was then peeled away, exposing the paraxial mesoderm. After several rinses with buffer, dissected specimens were transferred to 1% osmium tetroxide solution for 60–70 min and processed for SEM as previously described [17]. Altogether, about 140 specimens and over 740 photographs have been analyzed in this study.

For light microscopy, specimens were fixed as described in half-strength Karnovsky's fixative and then in 1% osmium tetroxide solution. After post-fixation in osmium, specimens were dehydrated in alcohols and embedded in Epon 812 plastic [15]. Sections 2 μm thick were collected and stained with a solution of 1% methylene blue in 1% borax.

The presomitic mesoderm of 8.5–9.5 day embryos was cultured in vitro in order to demonstrate the developmental potential of the somitomeres. Embryos were dissected from the decidua and the extraembryonic membranes. Three cuts were made as shown in Fig. 23a, so that a strip of the presomitic mesoderm, plus one or two somites and half of the neural plate was obtained. Primitive streak or tail bud was not included in the explant and sometimes the hind-gut and lateral mesoderm were also removed. From each embryo, one of the halves was immediately fixed and processed for SEM study and the other half was cultured for 12–15 h in either agar coated (1% agar in Dulbecco's modified Eagle's medium, DMEM, GIBCO) or poly-L-lysine coated (0.2% aqueous solution, Sigma) Falcon plastic culture dishes. The culture medium was either whole rat or mouse serum, or serum diluted 1 : 1 with DMEM [29]. Cultures were maintained at 37.5° C in 95% O_2 and 5% CO_2. At the end of the culture period the specimen was fixed in Karnovsky's fixative and processed for SEM study.

Results

Between 8.0 and 8.5 days PC, extensive morphogenesis of the neural plate leads to the formation of the cephalic neural folds with primary subdivisions of brain parts. The heart tube and the foregut portal are also well developed at this stage (Fig. 1). The first pair of somites is formed at about 8.3 days PC, and by 8.5 days PC five to six pairs of somites are present in the embryo. When the embryo is bisected longitudinally and the neural ectoderm is removed, the whole expanse of the paraxial mesoderm can be examined by SEM (Fig. 2). Despite the advanced morphogenesis of the head mesenchyme, the seven cranial segments underneath the cephalic neural folds can still be identified (Fig. 2). The eighth and subsequent segments differentiate into somites, which are separated by prominent intersomitic fissures. Caudal to the most recently segmented somite, the paraxial mesoderm forms a longitudinal band of presomitic mesoderm that extends to the very caudal end of the embryonic axis (Fig. 2). When it is viewed from the dorsal side, the presomitic mesoderm and the primitive streak form a horseshoe-shaped structure flanking the sides of the neural plate (removed in Fig. 3). Medially, the two bands of presomitic mesoderm are separated by the neural plate and notochord (Fig. 4), except at the primitive streak area where cells designated for the presomitic mesoderm intermingle with the invaginating epiblast cells (Fig. 5). In transverse sections, the presomitic mesoderm is triangular in shape, with its greatest concavity in the side facing the neural plate. The dorsal apex of the presomitic mesoderm fits exactly into the crest of the neural fold and marks the lateral boundary of the neural plate. Ventrally, the presomitic mesoderm is associated with the endoderm and at a later stage, with the dorsal aorta.

In 8.0–9.0 day old embryos, mesenchymal cells in the presomitic mesoderm are organized into somitomeres. Altogether six such segmental units can be identified in the presomitic mesoderm (Fig. 3). They are arranged in a tandem series between the emerging somite and the primitive streak. The very young embryos, at early-somite-stages, have a smaller presomitic mesoderm and only five distinct somitomeres are sometimes found. The mesodermal cells immediately lateral to the primitive streak usually show no definite patterns of organization, but those cells lying adjacent to the cranial end of the streak are found to be patterned into somitomeres (Fig. 6). In cross-section, the newly formed somitomere is generally wedge-shaped with a concave medial surface. Viewed dorsally in stereo, cells in the somitomere have extensive processes and projections which form concentric ridges swirling about the center of the unit. Within each somitomere, the cell population is subdivided into cranial and caudal halves by a line of cell orientation that bisects the unit perpendicular to the embryonic axis (Figs. 6 and 7). Fractures through the somitomere reveal that the cells are generally aligned with their long axes pointing towards the core center. The cells make extensive contact with neighboring cells by extending filopodia and lamellipodia. Large intercellular spaces, originally filled with extracellular matrix, are present within the somitomere. The boundary between somitomeres is often accentuated by an abrupt bifurcation in the general

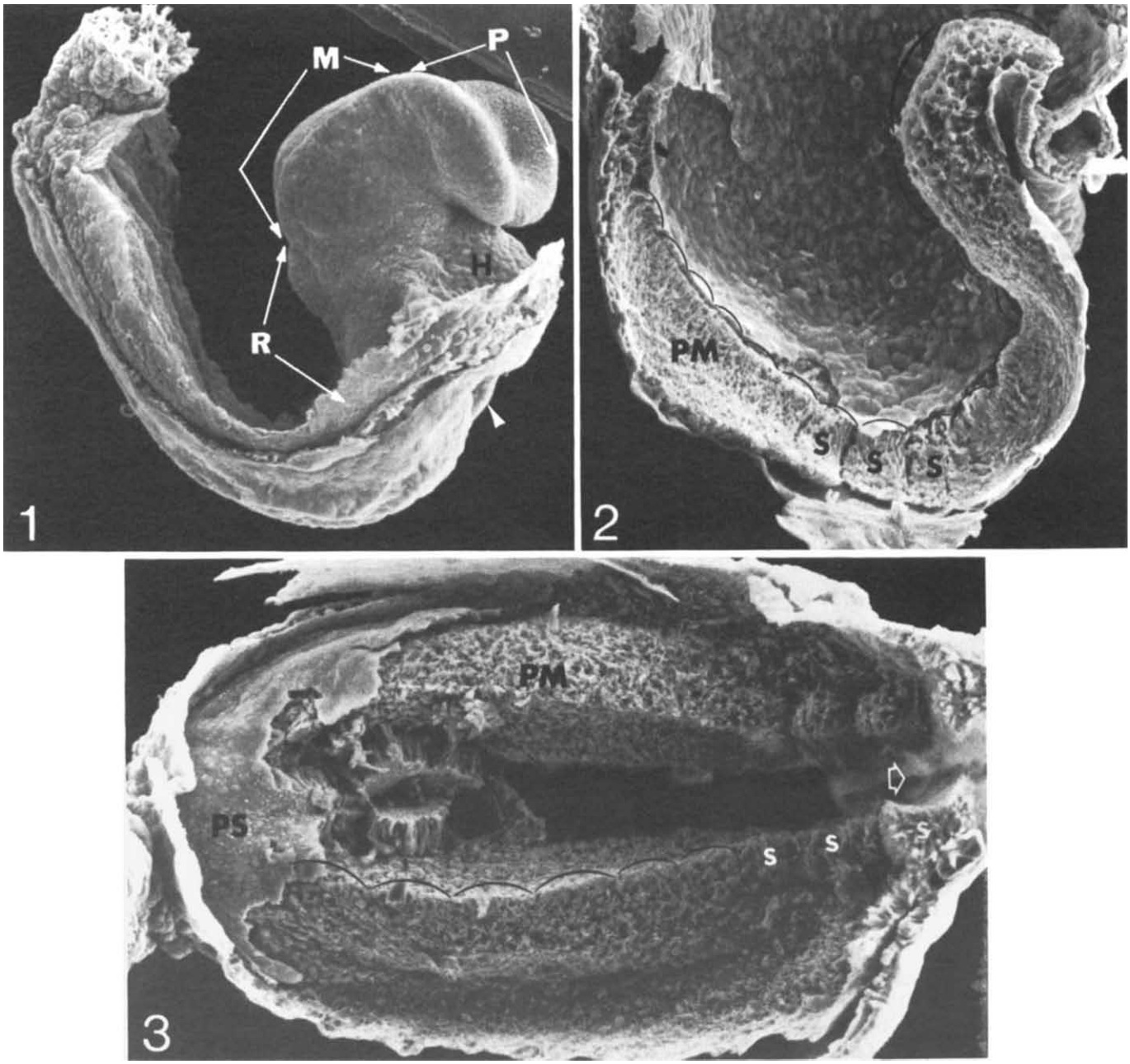


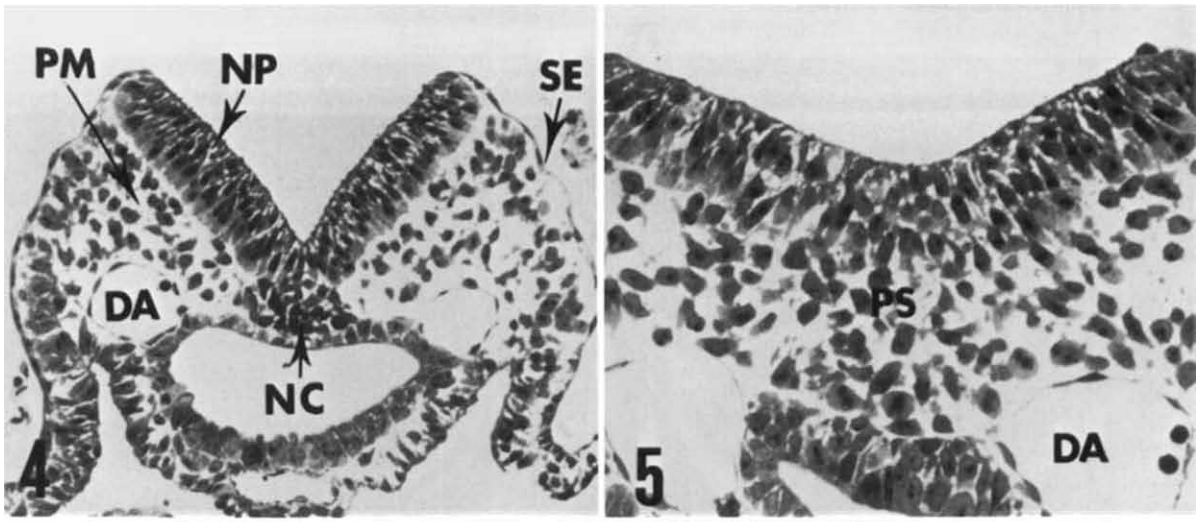
Fig. 1. SEM of an embryo with six somites at 8.5 days PC, showing well developed cephalic folds, heart *(H)* and foregut portal *(white arrowhead)*. The prosencephalon (*P*) has flexed ventrally so that the optic evaginations are facing posteriorly. The broad cranial folds correspond to the mesencephalon (*M*). The rhombencephalon (*R*) is subdivided into metencephalon and myelencephalon. A distinctive boundary between neuroepithelium and surface ectoderm is present at the crest of the neural fold. × 95

Fig. 2. SEM of the left half of an embryo with three somites, from which the neuroepithelium is removed to expose the paraxial mesoderm. Seven segments are found in the cranial region (*curved lines*). The eighth to tenth segments have undergone morphogenesis into somites (*S*). The presomitic mesoderm (*PM*) is found by stereo SEM to be segmented into six somitomeres. The torn caudal portion of the presomitic mesoderm (*arrowhead*) is contiguous with the primitive streak. × 130

Fig. 3. SEM of a dorsal view of the presomitic mesoderm (*PM*) after the overlying ectoderm, including neural tissue, is removed. Six somitomeres are found in the presomitic mesoderm between the primitive streak (*PS*) and the somites (*S*). *Open arrow* points cranially. × 148

orientation of the cells and by a trough or groove in the extracellular space (Figs. 6–8).

Somitomeres in different regions of the presomitic mesoderm are slightly different in morphology, probably reflecting the progressive series of morphogenetic events that culminate in the segmentation of somites. The more mature somitomeres found towards the cranial end of the presomitic mesoderm have more cells and the cells are closely packed. Cells near the crest of the wedged somitomere (the lateral edge) tend to align dorso-ventrally, but those near the midline still retain their



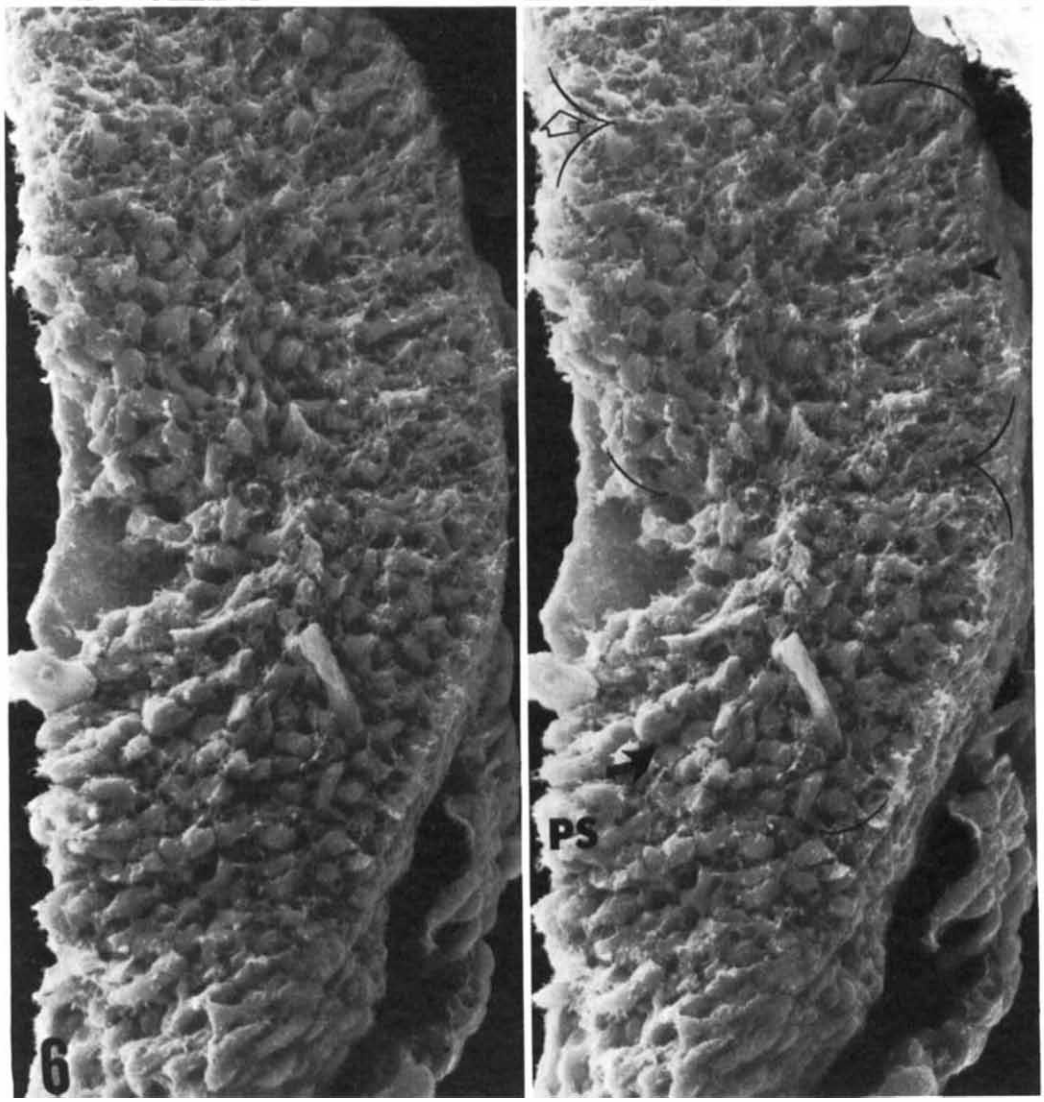


Fig. 4. Light micrograph of a plastic transverse section through the presomitic mesoderm (*PM*) of an 8.5-day embryo, showing its relationship with neural plate (*NP*), notochord (*NC*), dorsal aorta (*DA*) and surface ectoderm (*SE*). × 350

Fig. 5. Light micrograph of a plastic transverse section through the primitive streak (*PS*) of an 8.5-day embryo, showing the invagination of cells from the epiblast and the organization of these cells into presomitic mesoderm. *DA*, dorsal aorta. × 550

Fig. 6. A stereo SEM (tilt angle 10°) of the presomitic mesoderm adjacent to the primitive streak (*PS*) in an 8.5-day embryo as viewed from the midline. The mesenchyme cells on leaving the streak are displaced laterally (direction of *arrow*) to from the presomitic mesoderm. More anteriorly, the mesenchyme cells become organized into a definitive somitomere (*curved lines*) with a prominent middle seam (*arrowhead*) bisecting the somitomere. *Open arrow* points cranially. × 520

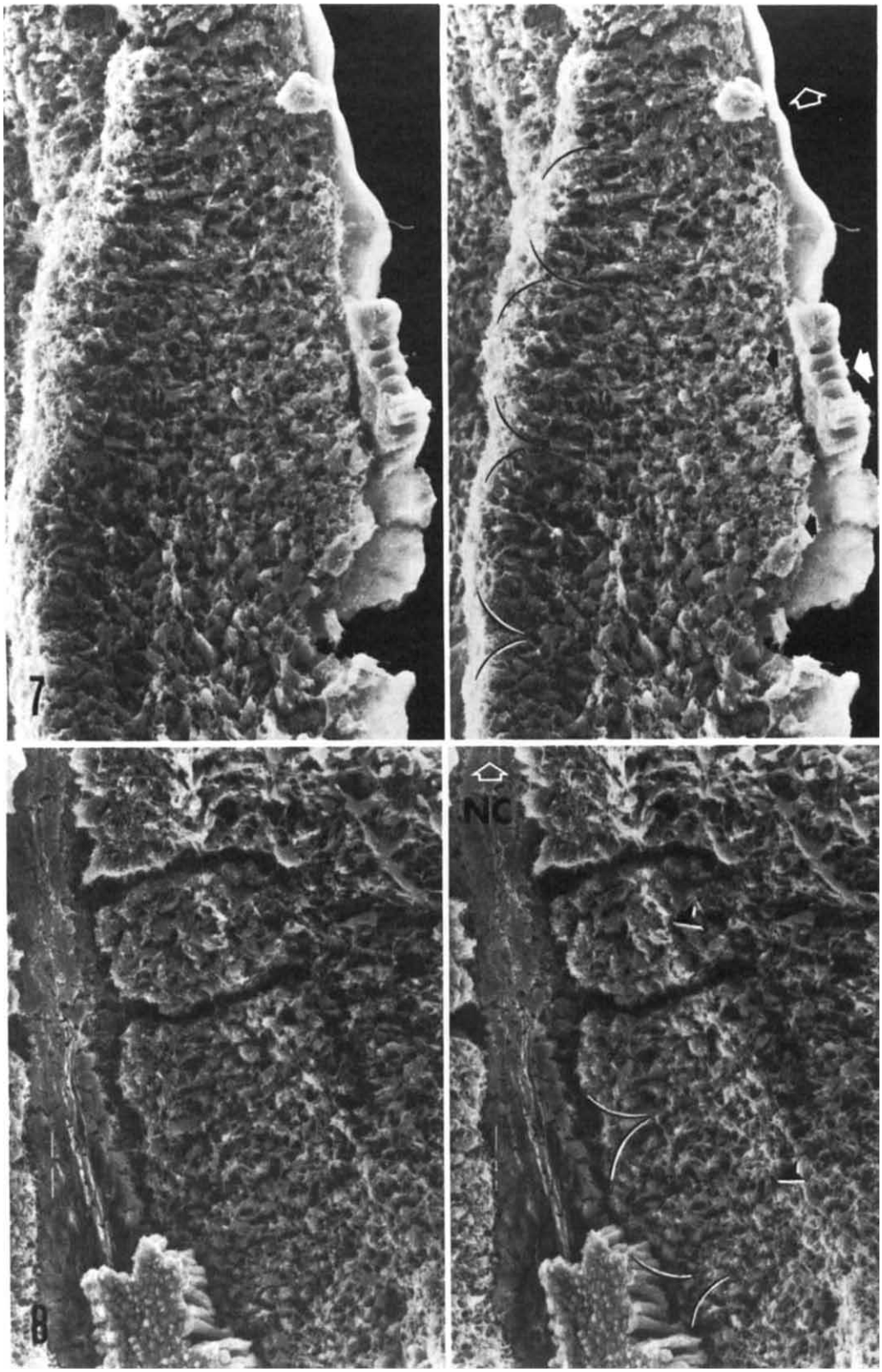
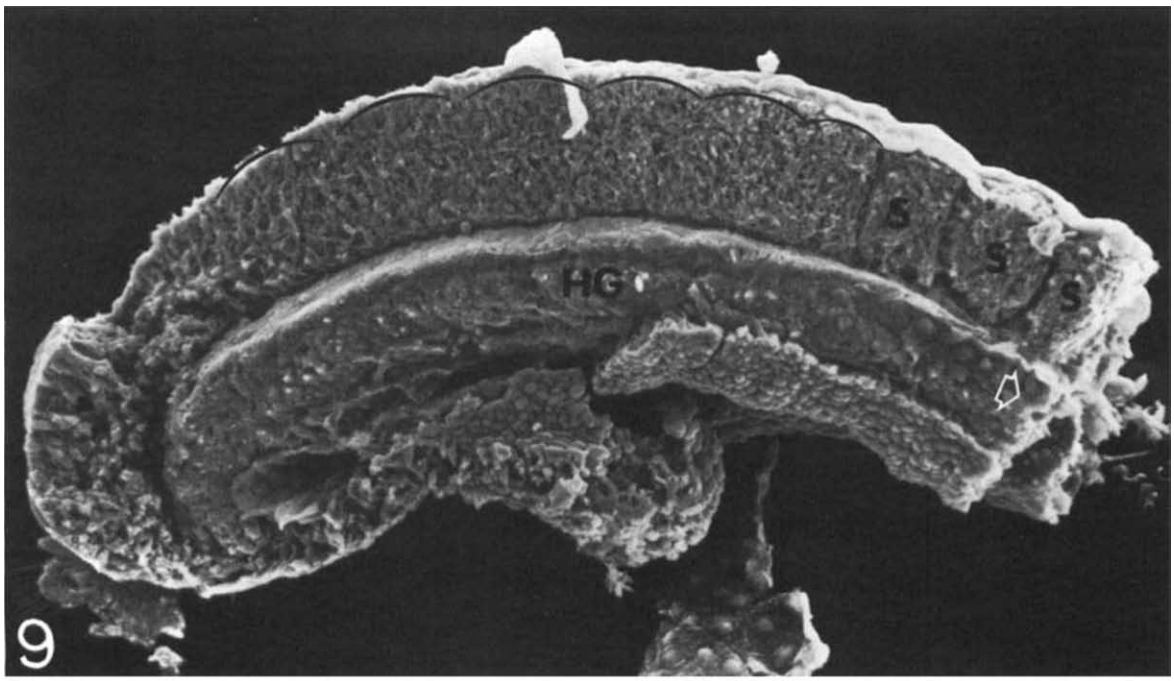
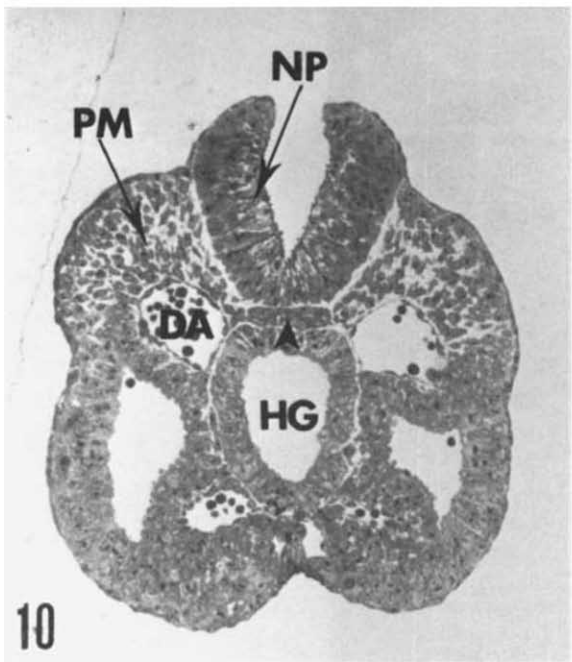


Fig. 7. A stereo SEM (tilt angle 10°) of the middle portion of the presomitic mesoderm of the left side of an 8.5-day embryo as viewed from the midline. Cells in the dorsal region of the somitomere (*brackets*) are aligned dorso-ventrally, as contrasted to the more circular arrangement near the midline. *Arrowheads* point to the middle seams in the somitomeres. The patch of columnar epithelium (*solid white arrowhead*) on the midline is part of the notochordal plate above the archenteron and is continuous with the flat notochord shown in Fig. 8. *Open arrow* points cranially. × 400

Fig. 8. A stereo SEM (tilt angle 10°) of the newly segmentd somite and the cranial portion of the presomitic mesoderm. Cells in the somite and somitomere are subdivided by a prominent seam (*arrowhead*) into cranial and caudal portions. Somitomeres are marked by curved lines. The notochord (*NC*) is made up of flattened cells with overlapping edges. Longitudinal rifts and ridges of the surface are characteristic of the notochord at this stage. *Open arrow* points cranially. × 300





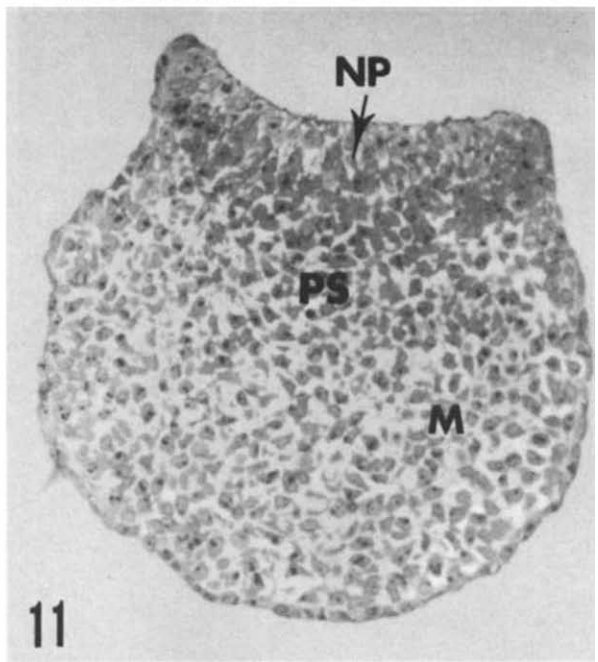


Fig. 9. SEM of an overview of the presomitic mesoderm of a 27-somite (9.5 day) embryo. Six somitomeres are arranged in tandem (*curved lines*) caudal to the newly formed somite (*S*). Both the neural tube and the notochord are removed. Segmental pattern seen in the presomitic mesoderm is reflected by the undulating dorsal surface of the hind-gut (*HG*) (see also Fig. 14). (Open arrow) points cranially. × 190

Fig 10. Light micrograph of a plastic transverse section through the middle portion of the presomitic mesoderm (PM) of a 9.5-day embryo, showing its topographical relationship with the neural plate (*NP*), notochord (*black arrowhead*), hind gut (*HG*), dorsal aorta (*DA*) and surface ectoderm. × 320

Fig. 11. Light micrograph of a plastic transverse section through the open neural plate area (*NP*) of a 9.5-day embryo, showing the continued invagination of superficial cells to a mesodermal population (*M*), which is reminiscent of the primitive streak (*PS*) area shown in Fig. 5. × 500

primary concentric organization (Fig. 7). In conjunction with the closure of the neural plate, the medial faces of somitomeres converge toward the midline. Further compaction of the somitomeric cell population results in the diminution of the intercellular space (Fig. 8). Somite segmentation follows the formation of an intersomitic cleft and the grooved partitioning of the somitomere from the more lateral mesoderm. The newly 'segmented' somite still retains a midline seam that divides the somitic cell population in two (Fig. 8). Examination of the ventral side of the neural plate and the lateral ectoderm overlying the mesoderm reveals undulations in these sheets that correlate precisely with the somitomeric pattern of the

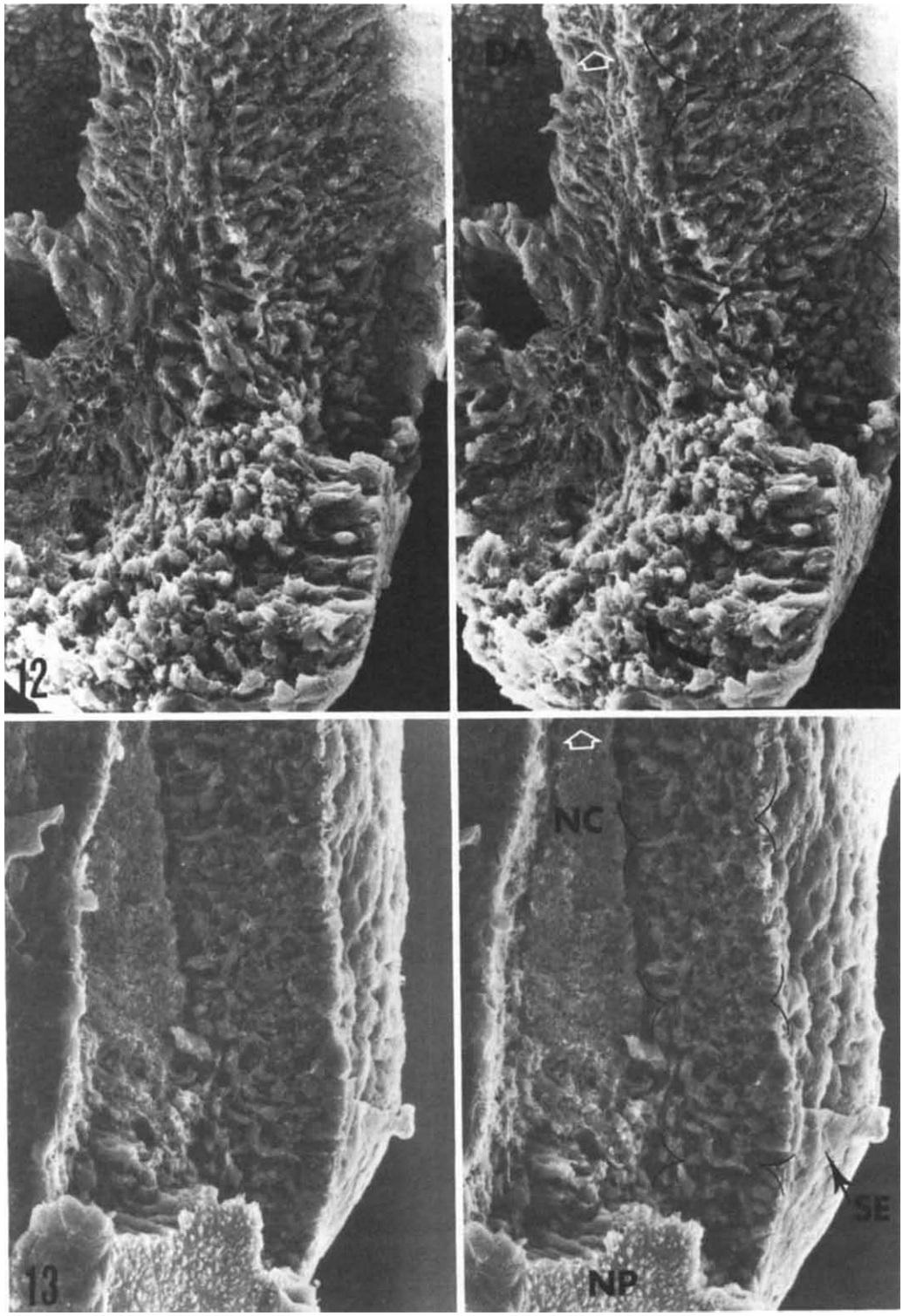


Fig. 12. A stereo SEM (tilt angle 10°) of the caudal portion of the presomitic mesoderm bordering the posterior neuropore, showing the invagination of cells (*black arrow*) and the organization of mesenchyme into the presomitic mesoderm. One somitomere, with a prominent center, can be seen and a new one is forming immediately caudal to it (*star*). *White arrow* points cranially. *DA*, dorsal aorta. × 360

Fig. 13. A Stereo SEM (tilt angle 10°) of the presomitic mesoderm, showing the difference in surface morphology of cells facing the neural plate (*NP*) and the surface ectoderm (*SE*). *NC*, notochord. *White open arrow* points cranially. Somitomeres are marked by *curved lines*. × 305

presomitic mesoderm they cover. No mesodermal cells were found attached to the undersurfaces of these epithelia as mesenchymal cells at this stage seem to adhere more to each other and to the endodermal layer.

As the embryonic axis elongates between 9–10 days, the site of fusion of the neural tube covers the site of somite segmentation. Consequently, the presomitic mesoderm is partly associated with a closed neural tube and partly with the

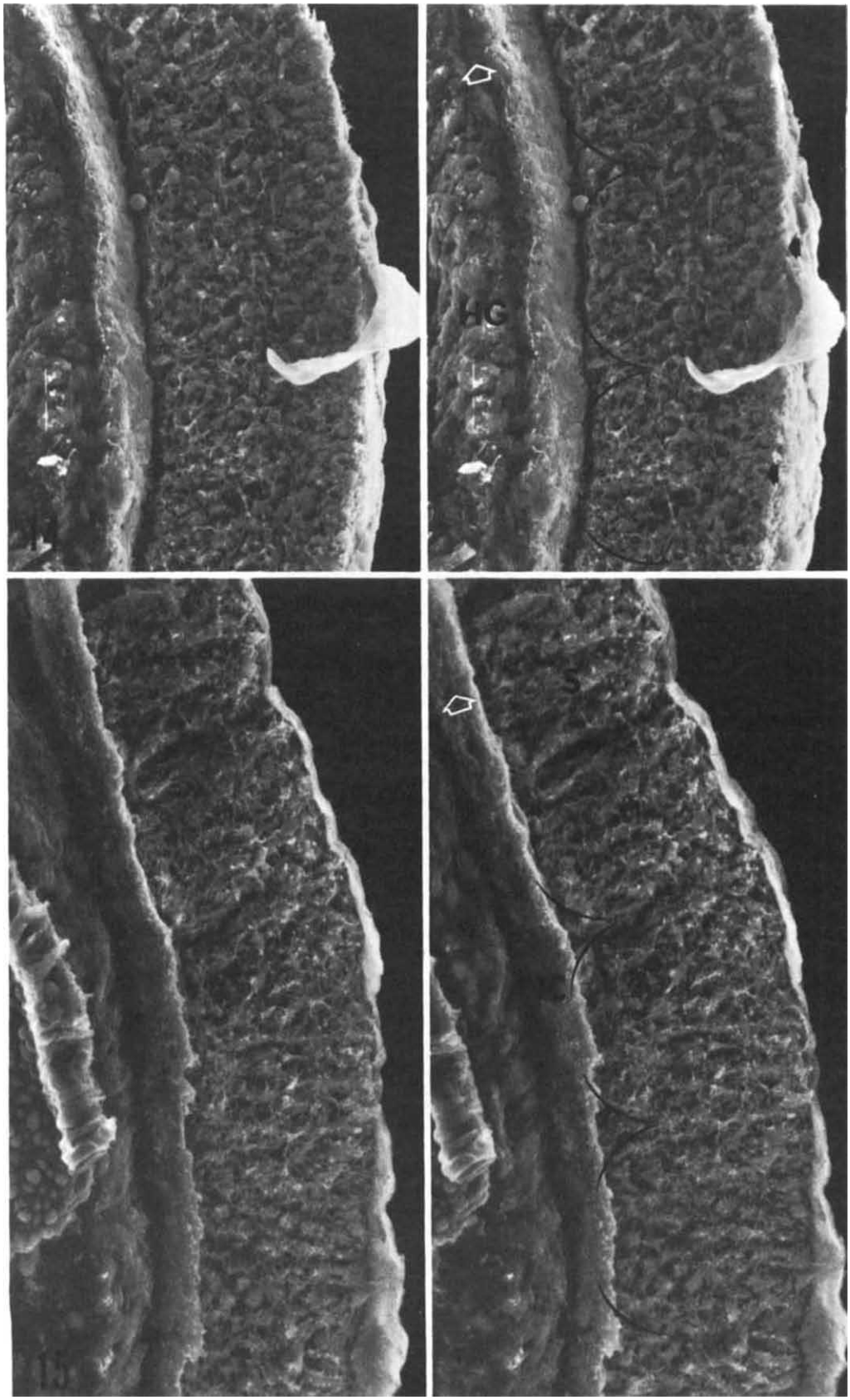


Fig. 14. A stereo SEM (tilt angle 10°) of the middle portion of the presomitic mesoderm (shown in Fig. 9), showing the maturing somitomeres (*curved lines; arrowheads* point to seams). The segmental pattern can also be seen on the dorsal surface of the hind gut (*HG*), which is normally covered by the notochord (removed). *White arrow* points cranially. × 350

Fig. 15. A stereo SEM (tilt angle 10°) showing the formation of somites (*S*) at the cranial end of the presomitic mesoderm. Considerable condensation of the somitomere (*curved lines*) occurs prior to their separation into somites. *NC*, notochord. *White arrow* points cranially. × 430

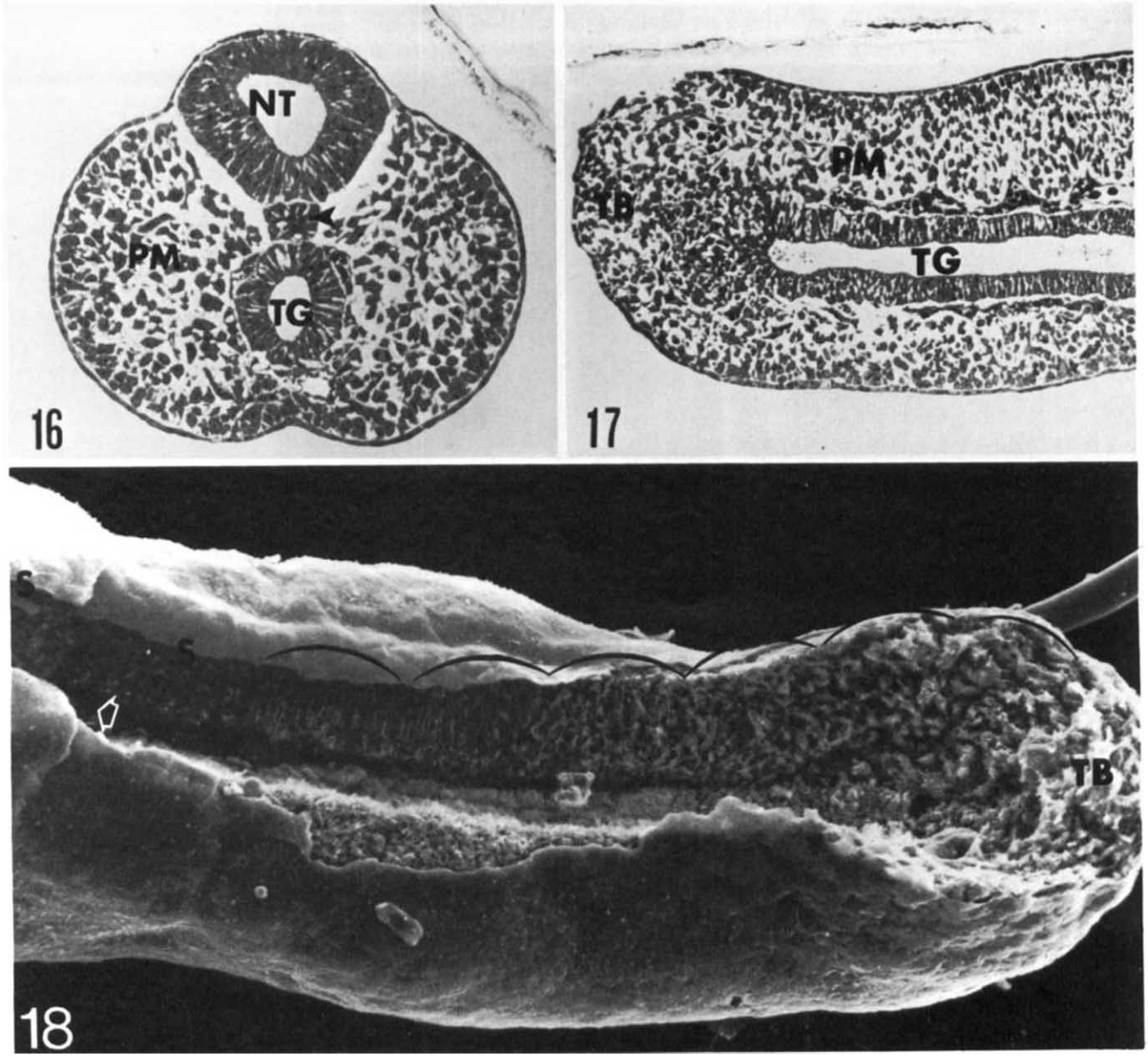


Fig. 16. Light micrograph of a plastic transverse section through the tail of a 10.5-day embryo, showing the relationship of the presomitic mesoderm (*PM*) with the neural tube (*NT*), notochord (*arrowhead*), and the tail gut (*TG*). × 350

Fig. 17. Light micrograph of a plastic longitudinal section through the tail of a 10.5-day embryo showing the contiguity of tissues in the presomitic mesoderm (*PM*) and in the tail bud mesenchyme (*TB*), *TG*, tail gut. × 320

Fig. 18 SEM overview of the newly formed somite (*S*) and the presomitic mesoderm of a 35-somite (10.5-day) embryo. The neural tube, the notochord, and the dorsal portion of the tail bud are removed. Five definitive somitomeres and one that is forming in the tail bud are found. *White arrow* points cranially. × 340

posterior neuropore (Fig. 9). The dorso-lateral face of the mesoderm is now covered by the surface ectoderm (presumptive epidermis). Ventrally, the presomitic mesoderm is associated with the wall of the hindgut and the dorsal aorta (Fig. 10). Sections taken through the posterior border of the open neural plate show that the superficial pseudostratified epithelium contributes cells to a deeper mesodermal layer. Cell relocation here is very similar to that observed for the primitive streak (Fig. 11). Immediately cranial to this region of cell invagination, the mesenchymal population becomes contiguous with the presomitic mesoderm (Fig. 12). Once the cells are incorporated into the presomitic mesoderm, they become organized into somitomeres (Fig. 12). Somitomeres here are more cuboidal in shape than those of younger embryos, but the cells are similarly arranged in characteristic concentric whorls when viewed in stereo (Figs. 12 and 13). Mesenchymal cells send out numerous processes to neighboring cells and to the adjacent neural epithelium. However, those cells facing the epidermal ectoderm have a very different appearance (Fig. 13). Intercellular space is drastically reduced as cells are packed more closely to each other. Cells contact each other with very broad and overlapping appendages and a few

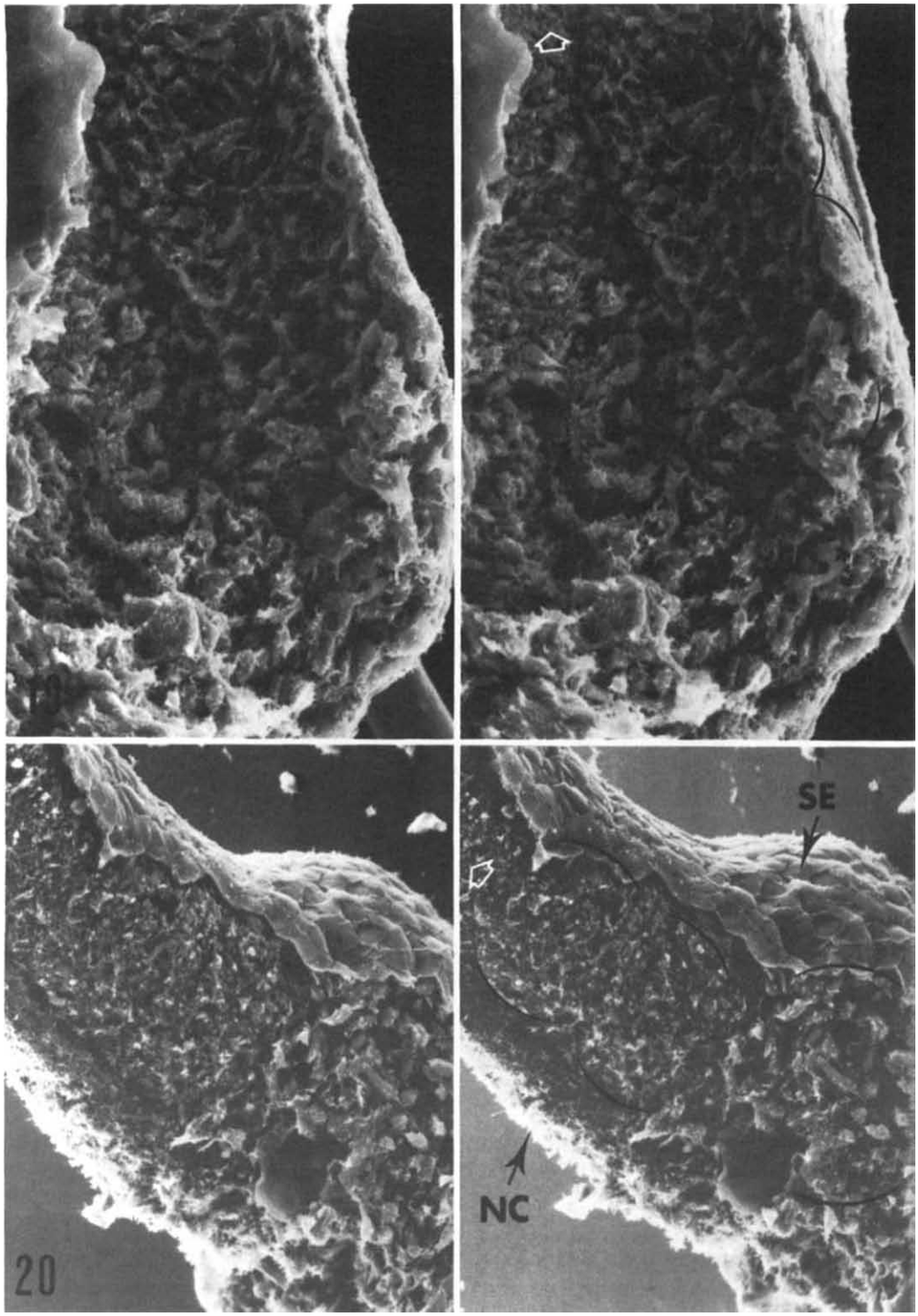


Fig. 19. A stereo SEM (tilt angle 10°) showing the organization of mesenchyme cells coming from the tail bud into the presomitic mesoderm. Somitomeres are marked by curved lines. *White arrow* points cranially. × 440

Fig. 20. A stereo SEM (tilt angle 10°) of the undersurface of the medullary cord and the tail bud, after removal of the presomitic mesoderm (*white arrowhead* points cranially). The medullary cord bears a circular arrangement of cells at the site of somitomere formation and the imprint of already formed somitomeres as well (*curved lines*). *NC*, notochord, *SE*, surface epithelium. *White arrow* points cranially. × 470

filopodia, therefore giving a smooth contour to this face of the somitomere. Generally, six somitomeres are found in a tandem series in the presomitic mesoderm of the 18–30 somite embryos at 9–10 days (Fig. 12). In some cases, however, up to seven somitomeres can be identified. The most cranial one is usually condensed and resembles more a somite than a somitomere, except that the intersomitic fissures separating it from its adjacent somite and somitomere are not completely

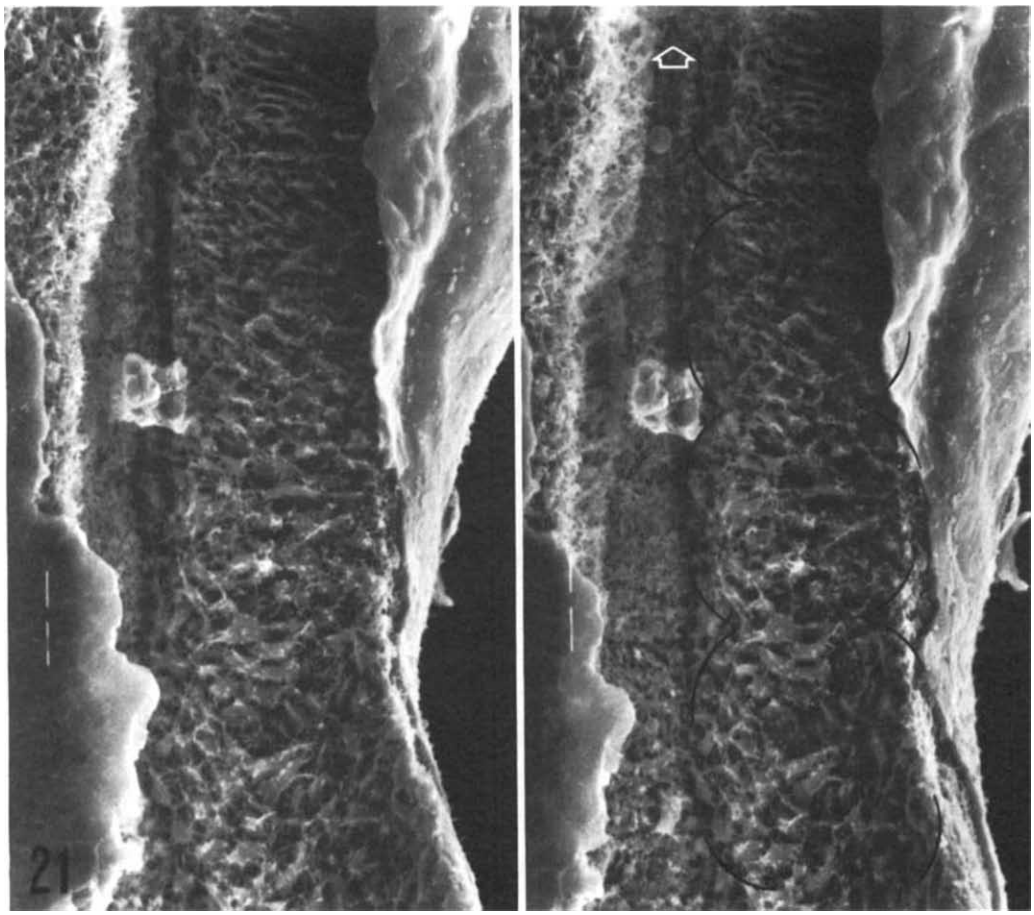


Fig. 21. A stereo SEM (tilt angle 10°) of the presomitic mesoderm shown in Fig. 18, showing the change in packing density and orientation of cells during the maturation of the somitomere. *White arrow* points cranially. Somitomeres are marked by *curved lines*. × 430

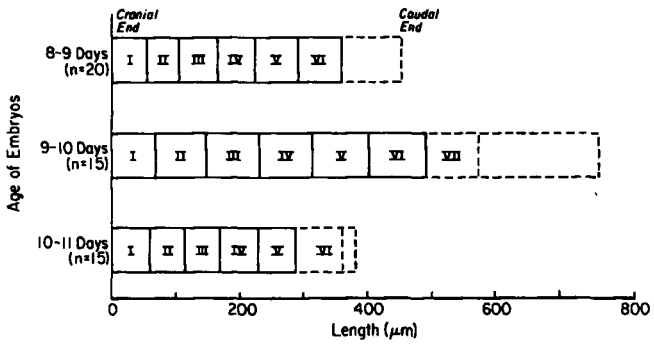


Fig. 22. The number and size of somitomeres in the presomitic mesoderm of 8–11 day mouse embryos. A relatively constant number of six somitomeres is found in presomitic mesoderm of different sizes. The size of the somitomere is adjusted according to the overall size of the presomitic mesoderm during different stages of development. More mature somitomeres at the cranial end are generally more condensed and smaller in size. The *dash-line box* represents the region of mesenchymal cells associated with either the primitive streak or the tail bud

formed. Increases in the abundance of cells, increased packing density of cells, medial convergence of the somitomere, and gradual alignment of mesenchymal cells in a dorso-ventral direction are features of maturing somitomeres (Figs. 14 and 15), and are similar to those observed in the younger embryos at 8–9 days. The intersomitic fissure is initiated medially and forms progressively in a medio-lateral direction. Concomitantly, the cells in the somitomere become organized into a somitic epithelium (Fig. 15).

At 10–11 days of development, and after complete closure of the posterior neuropore, the site of cell recruitment is shifted from the primitive streak to the tail bud at the posterior border of the neural tube. A bulbous core of proliferating mesenchyme cells is found in the tail bud. No clear tissue boundary exists between this core of mesenchyme and the well-defined surrounding tissues of the presomitic mesoderm, medullary cord, notochord, and lateral mesoderm (Fig. 17). Immediately cranial to the zone of cell proliferation, and lateral to the developing medullary cord and notochord, the mesenchyme is organized into presomitic mesoderm (Figs. 16 and 18) and definitive somitomeres can be identified (Fig. 19). Examination of the complementary surface of the tail bud, which has been torn free from this region, reveals similar sized circular arrangements of adherent tail bud mesenchyme at the sites of somitomere formation. Furthermore, the wall of the neural tube (the medullary cord of the tail) bears the imprint of the established somitomeric pattern of the presomitic mesoderm (Fig. 20). The initial concentric circular arrangement of

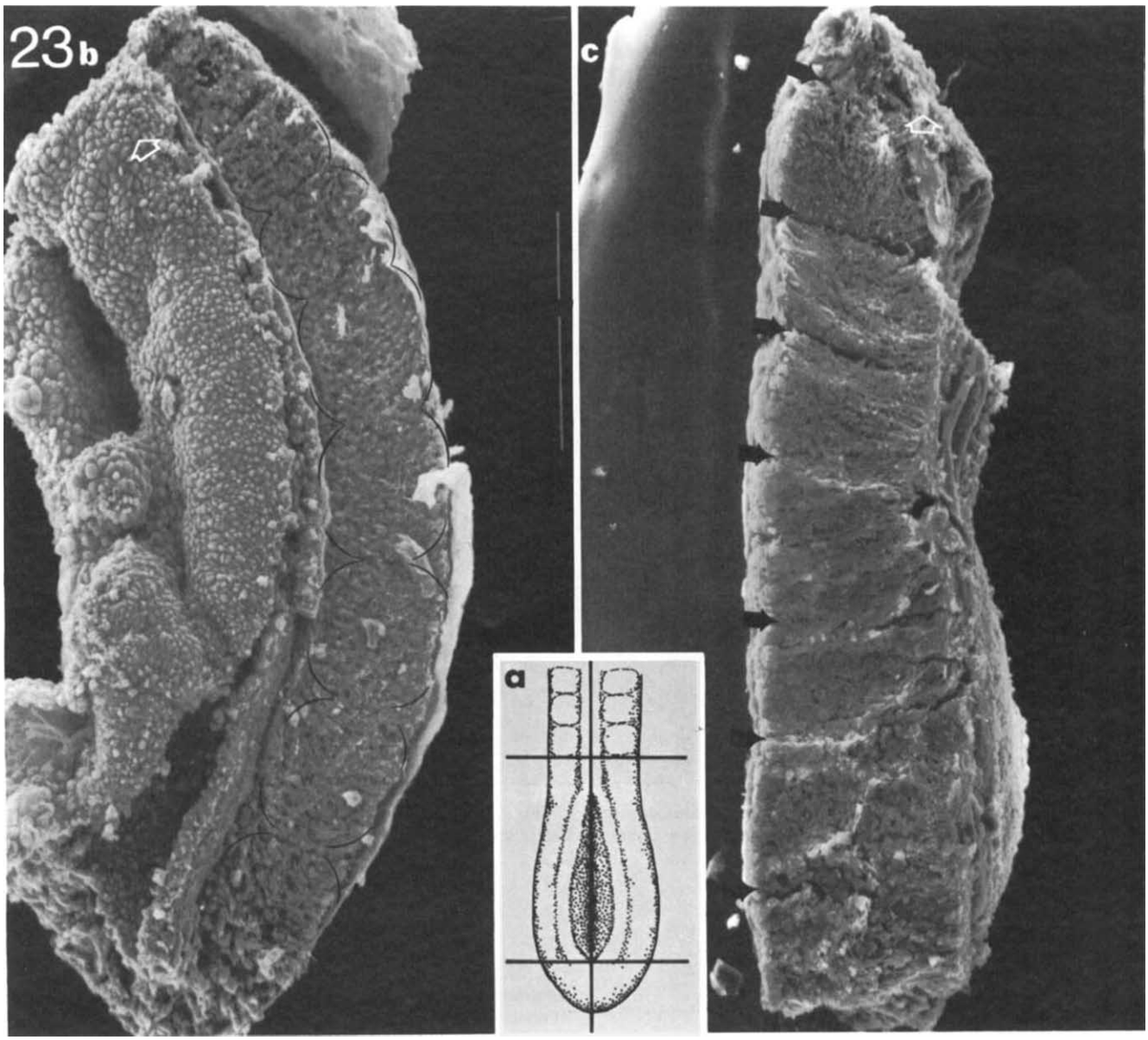


Fig. 23. **a** A diagram showing the three cuts (two transverse and one sagittal) that are made to explant the presomitic mesoderm from 8.5–9.5-day embryos. **b** SEM of a piece of presomitic mesoderm fixed before culture, showing the presence of one somite and six whole somitomeres. × 240. **c** SEM of the other half of presomitic mesoderm from the same embryo after culture 12 h in vitro. Seven somites are found (intersegmental fissures marked with *arrows*). × 300

cells within the somitomere is modified as component cells become more explicitly aligned dorso-ventrally (Fig. 21). Nevertheless, the overall cell pattern still allows unequivocal identification of the core center and intersomitomeric boundaries (Fig. 21). Maturation of somitomeres in these late stage embryos mostly involves progressive condensation of the segmental unit. Normally, five somitomeres can be identified in this much-shortened presomitic mesoderm (Fig. 18).

The size of the presomitic mesoderm changes during embryonic development. When size is expressed as the cranio-caudal length of the tissue between the newly segmented somite and the most recently formed somitomere, a two-fold fluctuation in length can be measured between 8–9 days to 9–10 days, and between 9–10 days and 10–11 days (Fig. 22). However, the number of somitomeres found in each of these stages does not vary from the average number of six by more or less than one. Five somitomeres are sometimes found in the shorter presomitic mesoderm of very young and very old embryos. Measurements of individual somitomeres show that their sizes are regulated with regard to the overall length of the presomitic mesoderm. However, the absolute size of somitomeres as well as the presomitic mesoderm may be greater than the measured value because of the inevitable shrinkage of tissues during specimen preparation for SEM. Assuming that the degree of shrinkage is generally comparable for all the tissues, more mature somitomeres in the presomitic mesoderm are about 70% the size of the most recently formed somitomeres adjacent to the primitive streak or the tail bud.

The normal cranio-caudal sequence of somite segmentation is observed in vitro when the development of explanted presomitic mesoderm is monitored at regular intervals over a

10–12 h period. Normal somite morphogenesis and maintenance of proper tissue topography is dependent on the presence of axial and associated epithelial structures. Culture of presomitic mesoderm explants on agar-coated dishes often results in curling of the specimen and some tissue necrosis, but somites can still be identified and counted. The best preservation of tissue topography is achieved by attaching the explants to a poly-L-lysine coated surface. In the absence of further addition of cells by the primitive streak and the tail bud, somite formation ends abruptly when the entire presomitic mesoderm has been converted into 'somites' (Fig. 23). The number of somites that can be generated from presomitic mesoderm of embryos at different stages should be related to the amount of tissue initially included in the explant. Examination of about 50 specimens from 8.5–9.5 day-old embryos indicates that there is a one-to-one relationship (Chi square value = 1.64, d.f. = 50, $p < 0.001$) between the number of somitomeres that can be identified initially in the presomitic mesoderm and the number of somites that is eventually formed when the complementary lateral half is cultured in vitro (Fig. 23).

Discussion

Well before the emergence of the somitic pattern, metamerism of the embryonic body can be visualized as a result of the formation of somitomeres in the paraxial mesoderm. We have previously described the presence of cranial segments in the mouse embryo [17]. The first seven somitomeres undergo morphogenesis into head mesenchyme, but the fundamental segmentation can still be recognized at an advanced stage of cephalic fold formation. The eighth and all the succeeding somitomeres differentiate into somites. A tandem series of somitomeres can be identified in the presomitic mesoderm at all stages of development using stereo SEM. The mesenchymal cells in the somitomere are arranged in concentric whorls about a core center. Such a cellular pattern is most obvious at the medial face of the somitomere where cell processes form ridges and grooves in a swirling configuration. The long axes of the cells are aligned radially towards a central space that may be the future myocoelic cavity of the somite. Furthermore, the cell population in the somitomere is subdivided into anterior and posterior halves. Such subdivision is still apparent in the newly segmented somites and has been suggested to be the early indication of the partitioning of the sclerotomal cell population in the differentiating somite [26].

The somitomere undergoes morphogenesis that leads to somite formation. There is an increase in cell density and an increase in the order of cellular arrangement. Condensation of the somitomere is associated with the obliteration of intercellular space and reorganization of cells into an epithelium. In the chick embryo, the maturing somitomeric cells make cell junctions with neighboring cells along the apical surface facing towards the primitive myocoelic space, and deposit patchy basement membrane basally [16]. In mouse, similar surveys of cell junctions have not been analyzed specifically at the somitomeric level. However, it has been reported that mesenchymal cells in the presomitic mesoderm of the mouse embryo make only simple, focal contacts with wide lacunae between cell membranes. More specialized contacts, such as gap junctions, are rarely found [11].

Besides direct visualization, the in vitro study of the somite-forming capacity of explanted presomitic mesoderm provides the most compelling evidence for the direct developmental relationship between somitomeres and somites. The number of somitomeres identified in the presomitic mesoderm is nearly always the same as the number of somites that are formed from explants obtained from the same embryo. That the number of segmental units is preserved in an in vitro condition and that the sequence of appearance of somites remains the same, suggested that the metameric pattern is stable once somitomeres are formed. This evidence supports the contention that a prepattern of prospective somites is already present in the presomitic mesoderm or segmental plate of mouse and chick embryos [14, 22, 27].

Despite a two-fold variation in the size of the presomitic mesoderm at different stages of development, a consistent number of about six somitomeres is always found. Studies on the somite-forming capacity of the segmental plate coming from different stages of snapping turtle [20], quail [21], and chick embryos [14, 22] show that a relatively constant number of somites is always formed from the explanted segmental plate. Particularly, the number of prospective somites that is present in the chick segmental plate (9–12) corresponds remarkably well with the number of somitomeres identified in the segmental plate by stereo SEM [16]. In this study, we have measured the size of the somitomeres in the presomitic mesoderm of embryos at different ages and found that the size of somitomeres is regulated according to the overall size of the presomitic mesoderm. This size adjustment would tend to keep a constant number of somitomeres in the presomitic mesoderm or the segmental plate at any one time in development.

The presence of somitomeric organization in the paraxial mesoderm of the cranial region [17] and of the remaining part of the embryonic body indicates that embryonic axis formation is likely to occur by addition of successive segments. In a future paper we will present morphologic evidence for the establishment of a somitomeric pattern in the mesoderm of very young embryos of primitive streak stages [28]. A somitomere is generated during early development by the arrangement of mesodermal cells as they are relocated by invagination through the primitive streak. The early association of the presomitic mesoderm with the primitive streak suggests that somitomeres formed at these stages are of streak origin. However, at later stages the streak disappears and the posterior site of cell invagination is replaced by a caudally located core of proliferating mesenchyme. Such a transition of the source of cells for the embryonic axis has been described extensively in the chick embryo [9, 24, 25]. It has also been shown experimentally that the tail bud mesenchyme does contribute cells to the formation of somites in the tail region. The transition from a streak origin to a non-streak origin of somitic cells probably occurs simultaneously with closure of the posterior neuropore, when the embryo has about 26–29 somites. It seems, therefore, in the mouse embryo, about forty segments of the body (seven cranial somitomeres, 26 somites, and six somitomeres in the already formed presomitic mesoderm) are generated through the activity of the primitive streak. Since the metameric pattern is already established once the cells leave the primitive streak or the tail bud, the time and site of specification of the somitic pattern must have occurred earlier and at a different site than heretofore realized [12, 27].

Normal regulation or perturbation of the somitic pattern is most likely to take place as the cells are spatially laid down as somitomeres. Heat shock treatment of the amphibian embryo, and ultraviolet irradiation of the chick embryo produce abnormalities in the segmentation of somites always at a

predictably regular, but developmentally later, stage than the time of treatment [3, 7, 8, 10, 23]. It has been suggested that these experimental treatments disrupt the normal formation of the pattern by deranging cellular organization at the time when the cells are recruited into the somitic pattern. We suggest that if such a perturbation would affect the formation of the somitomere, it would result in an abnormality in the somites derived from those affected somitomeres. Somitomeric formation is, therefore, a morphological manifestation of the so called 'prior wave of somite determination' described for amphibian embryos [6, 8].

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References

1. Bellairs R (1963) The development of somites in the chick embryo. J Embryol Exp Morphol 11: 697
2. Bellairs R (1979) The mechanism of somite segmentation in the chick embryo. J Embryol Exp Morphol 51: 227
3. Bellairs R, Veini M (1980) An experimental analysis of somite segmentation in the chick embryo. J Embryol Exp Morphol 55: 93
4. Bellairs R, Sanders EJ, Portch PA (1980) Behavioral properties of chick somitic mesoderm and lateral plate explanted in vitro. J Embryol Exp Morphol 56: 41
5. Cheney CM, Seitz AW, Lash JW (1981) Fibronectin, cell adhesion and somite formation. J Cell Biol 87: 94a
6. Cooke J (1977) The control of somite number during amphibian development. Models and experiments. In: Ede DA, Hinchcliffer JR, Balls M (eds) Vertebrate limb and somite morphogenesis. Cambridge University Press, Cambridge, p 433
7. Cooke J (1978) Somite abnormalities caused by short heat shocks to preneurula stages of *Xenopus laevis*. J Embryol Exp Morphol 45: 283
8. Cooke J, Elsdale TR (1980) Somitogenesis in amphibian embryos. III. Effects of ambient temperature and of developmental stage upon pattern abnormalities that follow short temperature shocks. J Embryol Exp Morphol 58: 107
9. Criley B (1969) Analysis of the embryonic sources and mechanisms of development of posterior levels of chick neural tubes. J Morphol 128: 465
10. Elsdale T, Pearson M (1979) Somitogenesis in amphibia. II. Origins in early embryogenesis of two factors involved in somite specification. J Embryol Exp Morphol 53: 245
11. Flint OP, Ede DA (1978) Cell interactions in the developing somites; in vivo comparisons between *amputated (am/am)* and normal mouse embryos. J Cell Sci 31: 275
12. Flint OP, Ede DA, Wilby OK, Proctor J (1978) Control of somite number in normal and *amputated* mutant mouse embryos: an experimental and a theoretical analysis. J Embryol Exp Morphol 45: 189
13. Karnovsky MJ (1965) A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J Cell Biol 27: 137a
14. Lipton B, Jacobson AG (1974) Experimental analysis of the mechanisms of somite morphogenesis. Dev Biol 38: 91
15. Luft JH (1961) Improvements in epoxy resin embedding methods. J Biophys Biochem Cytol 9: 409
16. Meier S (1979) Development of the chick embryo mesoblast. Formation of the embryonic axis and establishment of the metameric pattern. Dev Biol 73: 25
17. Meier S, Tam PPL (1982) Metameric pattern development in the embryonic axis of the mouse. I. Differentiation of the cranial segments. Differentiation 121: 95
18. Menkes B, Sandor S (1977) Somitogenesis: regulation potencies, sequence determination and primordial interaction. In: Ede DA, Hinchcliffer JR, Balls M (eds) Vertebrate limb and somite morphogenesis. Cambridge University Press, Cambridge, p 405
19. Packard DS Jr (1978) Chick somite determination: The role of factors in young somites and the segmental plate. J Exp Zool 203: 295
20. Packard DS Jr (1980a) Somite formation in cultured embryos of the snapping turtle, *Chelydra serpentina*. J Embryol Exp Morphol 59: 113
21. Packard DS Jr (1980b) Somitogenesis in cultured embryos of the Japanese quail, *Coturnix coturnix japonica*. Am J Anat 158: 83
22. Packard DS Jr, Jacobson AG (1976) The influence of axial structures on chick somite formation. Dev Biol 53: 36
23. Pearson M, Elsdale J (1979) Somitogenesis in amphibian embryos. I. Experimental evidence for an interaction between two temporal factors in the specification of somite pattern. J Embryol Exp Morphol 51: 27
24. Schoenwolf GC (1977) Tail (end) bud contributions to the posterior region of the chick embryo. J Exp Zool 201: 227
25. Schoenwolf GC, Delango J (1980) Ultrastructure of secondary neurulation in the chick embryo. Am J Anat 158: 43
26. Solursh M, Fisher M, Meier S, Singley CT (1979) The role of extracellular matrix in the formation of the sclerotome. J Embryol Exp Morphol 54: 75
27. Tam PPL (1981) The control of somitogenesis in the mouse embryos. J Embryol Exp Morphol (Suppl) 65: 103
28. Tam PPL, Meier S (1982) The origin and establishment of a somitic pattern in the mesoderm of the gastrulating mouse embryo. Am J Anat (in press)
29. Tam PPL, Snow MHL (1980) The in vitro culture of primitive-streak stage mouse embryos. J Embryol Exp Morphol 59: 131
30. Waddington CH, Deuchar EM (1953) Studies on the mechanism of meristic segmentation. I. The dimensions of somites. J Embryol Exp Morphol 1: 349
31. Woo Youn B, Keller RE, Malacinski GM (1980) An atlas of notochord and somite morphogenesis in several anuran and urodelean amphibians. J Embryol Exp Morphol 59: 223

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