Analysis of Normal Somite Development

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We describe how the first 6 somite pairs form, using the third somites as examples. This history is based upon time-lapse movies of carbon-marked embryos and histological studies by light and electron microscopy of embryos fixed *in situ* with glutaraldehyde and osmium tetroxide. At head-process stage a continuous sheet of mesoblast occupies the regions of the future third somites. Mesoblast cells attach either to hypoblast or to overlying neural plate which is already a simple pseudostratified columnar epithelium. Prospective somite cells are those attached to the neuroepithelium, and they extend laterally exactly as far as the neural plate does. By head-fold stage, regression of the node down the midline is shearing the sheet of mesoblast into right and left halves. Somite cells hang from the bottom of the neural plate. As the neural plate condenses toward the midline, attached somite cells are compacted. When the somite segments, somite cells are tightly apposed to one another, and, in addition to junctions binding their basal ends, new junctions appear between their apical ends. This leads to reorganization into the typical somite rosette configuration. Spaces filled with extracellular materials form around the whole somite.

INTRODUCTION

The early chick blastoderm is a totipotent embryonic field (Spratt and Haas, 1960), from which the patterned structures of the embryo gradually emerge. The subdivision of the embryonic field of the blastoderm into organ fields with specific restricted spatial arrangements and, finally, the appearance of patterned morphological structures raise many questions.

One of the most intriguing problems in embryology is to identify those mechanisms that are responsible for coordinating the cells of the embryo in forming patterned structures. An interesting example of early pattern formation is the development of the basic metamerism of higher animals. This pattern becomes apparent with the formation and subsequent development of the somites. The somites appear early in development, form many important organs, and impose their segmental pattern on other parts of the embryo, for example, on the spinal ganglia.

Somite development has been the object

of numerous investigations. However, only a few of these are descriptive studies; among them are Duval (1889), Platt (1889), and Patterson (1907). Recently, Hay (1968) presented a brief survey of somite development on the fine structural level as part of a study on epithelial-mesenchymal interactions in the developing chick. Most reports deal with the mechanisms of somite formation and have suggested that segmentation of the somites is dependent upon the actions of other structures. Among controlling elements implicated in chick somite morphogenesis are a somite-forming center (Spratt 1954, 1957), the neural plate (Fraser, 1960; Butros, 1967), the notochord (Nicolet, 1970a) and regression movements (Bellairs, 1963).

One of the problems in analyzing the results of these earlier studies is that all are experimental investigations based on a system whose normal development has not yet been clearly described. It seems only reasonable that, before one can ask the right questions, one must define the system. Therefore, it is the object of this report to analyze normal somite morphogenesis on both the light and electron

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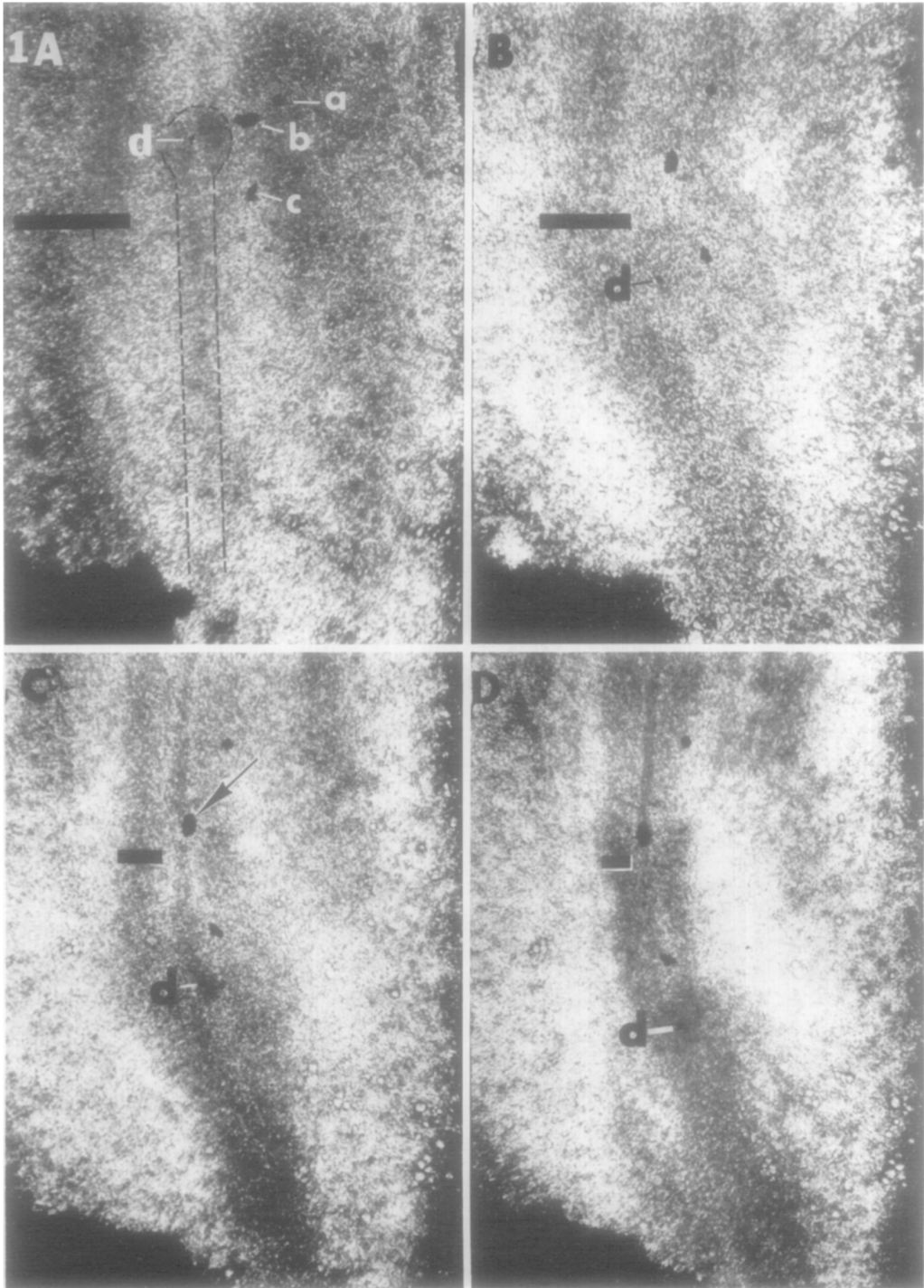
FIG. 1. Frames from a time-lapse movie of a carbon-marked embryo at head-process stage. Four carbon marks (*a*, *b*, *c*, and *d*) have been placed on the epiblast surface. Marks *a*, *b*, and *c* have been placed on the presumptive neural plate lateral to the streak, and either anterior (*a*), at the level of (*b*) or posterior (*c*) to Hensen's node. The smallest carbon particle, *d*, is placed over the posterior portion of the node. × 75.

(A) Embryo at the start of the film. Dashes outline the area of the primitive streak. The horizontal bar on the *left* indicates the width of the area giving rise to the somite mesoderm. This area is identifiable by its greater density due to the multilayering of cells in the mesoblast (see Fig. 4).

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microscopic levels. We find that there are two distinct phases in somite formation. The first 6–8 somites are formed somewhat differently from later ones. This report primarily focuses on the development of somites 1 through 6.

The descriptive analysis presented in this study provides insight into the mechanism of somite morphogenesis. A cooperative interaction among the developing neural plate, notochord, and somite tissues is suggested by this study. An experimental investigation of these interactions and a hypothesis of somite development are presented in a following paper.

MATERIALS AND METHODS

Fertilized eggs of White Leghorn chickens were incubated at 38°C and staged by the criteria of Hamburger and Hamilton (1951).

Time-lapse cinematography. Embryos were explanted into 35-mm plastic petri plates containing a saline-agar substrate (DeHaan, 1967). The plates were sealed with silicone grease and placed into a specially constructed microscope stage incubator. A temperature of 38°C was maintained by a circulating water bath in conjunction with forced hot (40°C) air, the latter essential for preventing moisture condensation on the top of the culture plate. The embryos were photographed with a Sage time-lapse unit through a microscope utilizing a Zeiss 1.5–6× objec-

tive lens and transmitted and oblique lighting.

Histology. Eggs were opened into a dry finger bowl and the yolk positioned with the embryo facing upward. A glass ring 15 mm in diameter by 5 mm in height was placed on the yolk, making a well around the embryo. The fixative (2% glutaraldehyde buffered in 0.05 M sodium cacodylate with 2.6% sucrose) was placed into the well and also injected into the yolk underlying the embryo. After 15 minutes, the glutaraldehyde was removed and replaced with Millonig's osmium tetroxide (Millonig, 1962). During osmification, the embryos were excised from the yolk and then resuspended in fresh osmium for a total period of 45 min. The tissues were rinsed in 1% NaCl, dehydrated in a graded series of alcohol and propylene oxide and then embedded in Epon 812.

Thick and thin sections were cut on a Porter-Blum MT-2 microtome. The thick (1 μm) sections were stained with 1% methylene blue made up in a 1% sodium borate solution. The thin sections were doubly stained with uranyl acetate and lead citrate (Venable and Coggeshall, 1965) and photographed with a Siemens 1A electron microscope.

RESULTS

Time-lapse movies of control and carbon-marked embryos developing *in vitro* illustrate a sequence of morphogenetic

(B) After 4 hr, the node has regressed 0.28 mm as indicated by the posterior translocation of carbon mark d. Since mark d is more widely separated from particle b and has bypassed particle c, shear lines must exist in the epiblast between the node and the lateral marks. While the regression of the node is occurring, the embryo or area pellucida is elongating as indicated by the increased distances between marks a, b, and c. In addition, the neural plate and presumptive somite mesoderm are condensing toward the midline as indicated by the medial movements of the carbon particles a, b, and c and by the narrowing of the dense region of the somite mesoderm (horizontal bar).

(C) After 6.5 hr, the node has regressed a total of 0.36 mm. The condensation of the neural plate is indicated by the change in position of the large carbon particle b. Originally this particle was lateral to the node; now it lies directly over the forming notochord (arrow). Also, condensation is evident from the continued narrowing of the presumptive somite mesoderm toward the midline of the blastoderm.

(D) At the initiation of somite segmentation, 8.5 hr after the start of the movie, the node has regressed a total of 0.46 mm. This frame also shows the further condensation of both the overlying neural plate and the somite mesoderm. Originally marks b and c were lateral to the midline; now both overlie the notochord in the midline of the embryo. The prospective somite mesoderm has condensed to about one-third of its earlier width.

movements which corroborates many of the data presented in earlier marking studies (reviewed by Nicolet, 1971). During the stages analyzed in this report (Stages 4–9), a complex series of movements collectively regarded as regression occur within the embryo. The most striking aspect of regression is the posterior translocation of Hensen's node. As illustrated in carbon-marked embryos followed by time-lapse cinematography, the tissues of Hensen's node (chorda bulb plus overlying epiblast and underlying hypoblast) actually cut a pathway down the length of the embryo during regression, creating shear lines between the node and the adjacent tissues (Fig. 1). Shear lines are best seen in movies, where they appear as sharp boundaries between cell domains moving in different directions. As the node regresses it gives rise to the notochord, which is laid down in its wake.

Time-lapse films also record several other important related events that occur at the same time as the node is regressing. In addition to a general anteroposterior elongation of the embryo or area pellucida, the neural plate, as it elongates, shows a simultaneous condensation toward the midline anterior to the regressing node (Fig. 1). This decrease in width of the neural plate is an important process in the transformation of the plate into the neural tube.

The position changes of the axial mesoderm are easily identifiable in time-lapse films because of the greater density of the mesoderm compared to the other germ layers. This increased density of the mesoblast is due to the multilayering of mesoblast cells in contrast to the monolayer organization of the overlying epiblast and underlying hypoblast. The area of the presumptive somite mesoderm elongates and narrows to form the segmental plate anterior to the regressing node. This activity of the mesoderm occurs simultaneously and in synchrony with the condensation of the overlying neural plate (Fig. 1). The seg-

mentation of somites 1 through 3 occurs prior to the final condensation of the segmental plate. When first blocked out these somites exhibit a chevron-shaped configuration (Fig. 2A). However, with a continued medial condensation of the mesoderm they assume a typical blocklike configuration (Fig. 2B). This transformation in the shape of the first 3 somites has been beautifully illustrated by Duvall in 1889 (see his Plate IV, Fig. 70–77). During formation of the somites, as viewed in time-lapse films from a dorsal perspective, cleavage of the segmental plate is always initiated at the lateral side of the condensed mesoderm.

In order to follow the morphogenesis of the somites on the cellular level, thick sections were cut through the region of the presumptive third somite at each stage examined between the definitive primitive streak (Stage 4) and formation of the eighth somite (Stage 9). By comparing these sections, one can follow the changes which occur within a specific region during the course of development (Figs. 3–9).

Primitive Streak

Sections through the presumptive third somite region at this stage (Fig. 3) show cells apparently fixed in the course of migrating from their origin at the primitive streak. These mesoblast cells appear to migrate laterad by adhering to the overlying epiblast or its basement lamina, and by directly adhering to the cells of the hypoblast (Fig. 10). It is important to note that even at this early stage, the epiblast lateral to the primitive streak is organized as a simple pseudostratified columnar epithelium which is morphologically identical to neural plate. These sections, supplemented by data from the time-lapse studies, clearly show that these columnar epiblast cells constitute the forming neural plate. The border between the streak cells of the epiblast and the presumptive neural plate is easily identifiable in thick sections because of the differences in cell morphology
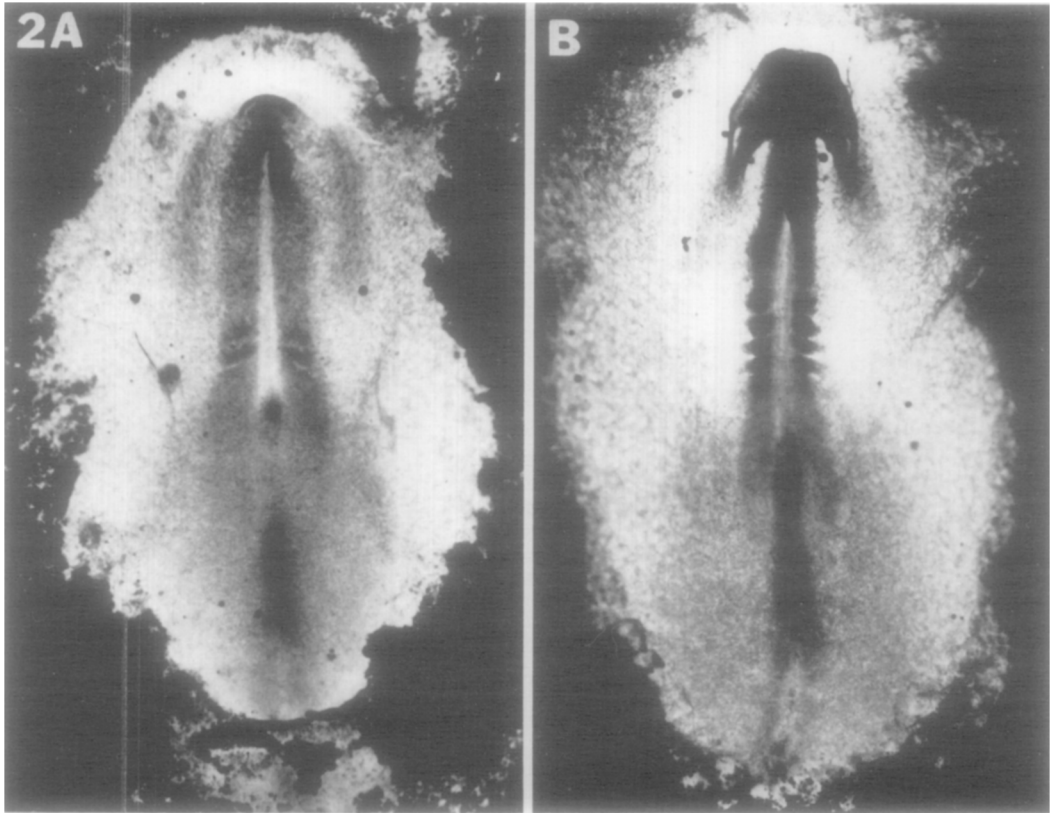
FIG. 2. Low power micrographs showing the changes in the morphology of somites 1–3. × 24. (A) Stage 7 embryo (24 hr). When first formed, these somites have an elongate rectangular morphology organized in a chevron-shaped configuration. (B) The neural plate overlying somites 1–3 has condensed prior to the closure of the neural tube (see Fig. 1). A synchronous condensation of the underlying mesoderm results in a narrowing of somites 1–3.

(Fig. 3). The region of transition between presumptive neural plate and primitive streak is also identifiable on the fine structural level since the basal surface of the epiblast is covered with a basal lamina, except in the region of the streak where cells are involuting and giving rise to the mesoderm (Fig. 11). The lateral border between the presumptive neural plate and the epidermis is imperceptible at this stage since the epiblast exhibits a gradual gradation between the columnar cells of the plate and the cells of the epidermis.

Head-Process

By head-process stage the population of mesodermal cells has increased, resulting in a multilayering of the cells of the meso-blast (Fig. 4). In Fig. 12, the mesoblast is seen to consist of a single sheet of cells that is continuous from the left side of the embryo, through the streak, to the right side. The border between the neural plate and the presumptive epidermis can now be readily identified as the point of transition between the columnar organization of the plate and the more flattened and vacuolated morphology of the epidermal cells (Fig. 12A).

Head-Fold

By the time the head fold forms (Stage 6), two distinct populations of cells, based on their behavior and relationships, can be identified in the mesoblast in the region of the presumptive third somite. One group,
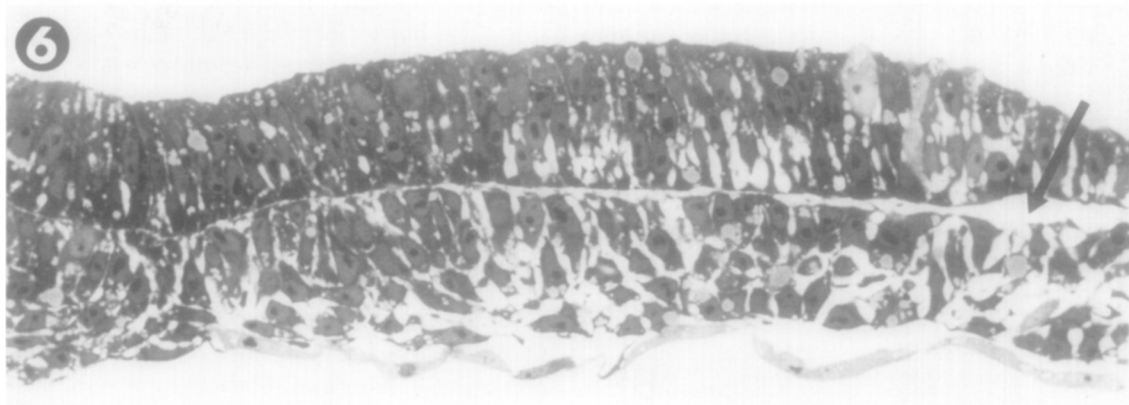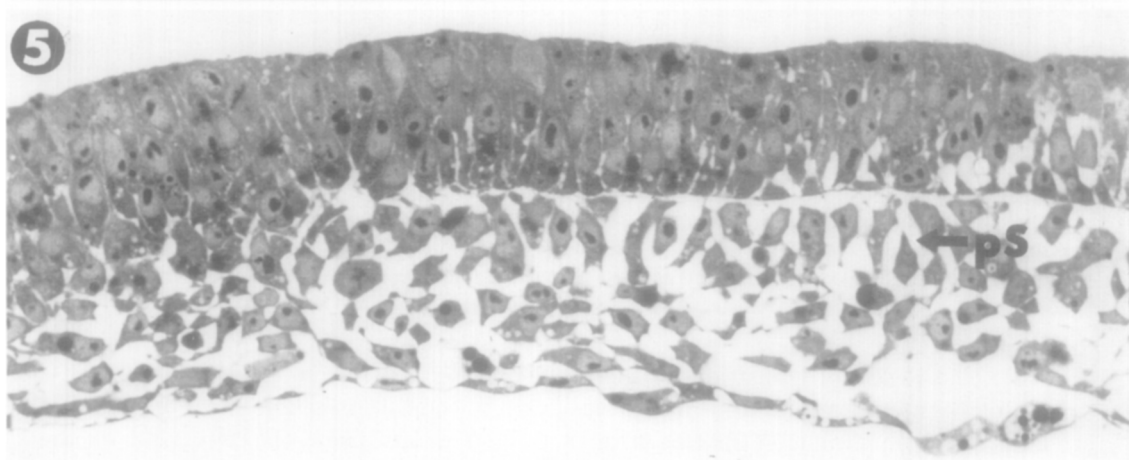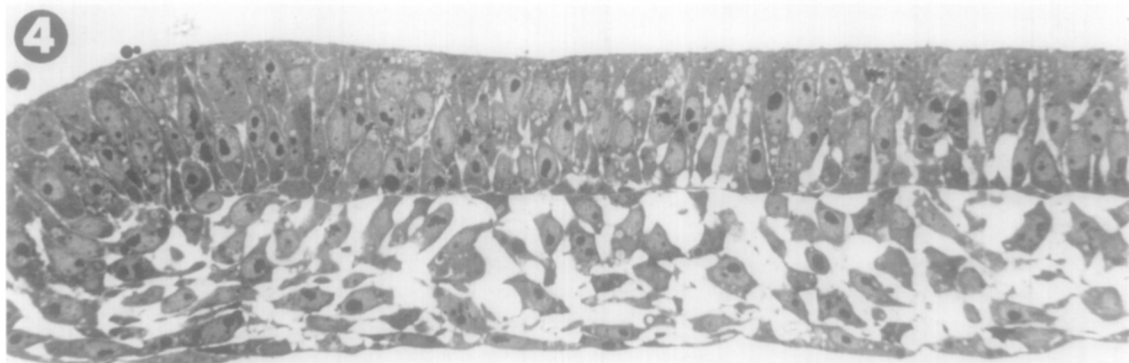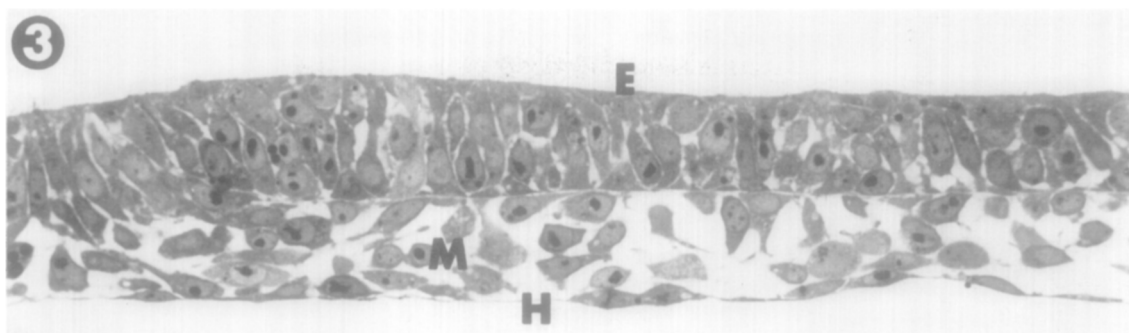
the presumptive somite cells, forms a loosely arranged columnar epithelium whose basal surface is attached to the basal lamina of the overlying epiblast (Fig. 5). These cells are also bound to each other via thin cellular processes at their basal surfaces (Fig. 13).

The other population of mesoblast cells possesses a more flattened mesenchymal configuration and is most likely contributing to the lateral plate mesoderm (Figure 5).

Of particular importance at this stage is the relationship between the epiblast and the mesoblast. Cells of the mesoblast are found to adhere to the epiblast only in the region of the neural plate, not in the region of the presumptive epidermis (Fig. 12B). Those cells which adhere to the basal lamina under the neural plate compose that population of cells identified above as the presumptive somite mesoderm. Therefore, there is a one-to-one correlation of the area of the presumptive somite with the area of the overlying neural plate.

At this stage of development, Hensen's node has regressed to the level of the presumptive third somite and is evident in the region of the streak as a condensed mass of mesoderm (Fig. 12B). Further regression of the node results in a shearing of the once continuous sheet of mesoderm into right and left halves (Fig. 14) separated by the notochord.

Somites

Somites 1–3. While the first somite is forming, the cells of the neural plate in the region of the presumptive third somite have elongated, causing the neural plate to condense toward the midline. The underlying and adhering presumptive somite cells, once loosely arranged with large extracellular spaces between them, have become more tightly packed and condensed, mimicking the activity of the overlying neural plate (Figs. 6, 7, and 15). During this condensation, which results in the formation of the segmental plate, the extracellular space between these mesodermal cells is virtually obliterated as the cells become tightly apposed to one another (Fig. 16). Earlier, these cells were bound to each other only at their basal surfaces; however,

FIG. 3–9. Transverse sections, all through the level of the third somite, at different stages between stage 4 (primitive streak) and stage 9 (8-somite). Sections are at the same magnification and are all arranged with the midline of the embryo on the left. × 500.

FIG. 3. Primitive-streak stage. Cells of the mesoblast (*M*) fixed during their lateral migration from the streak are bound to the overlying epiblast (*E*) and underlying hypoblast (*H*). The epiblast shown in this section consists of presumptive neural plate that is continuous with the primitive streak at the midline (left edge of photograph). The neural plate cells are organized as a simple pseudostratified epithelium. The basal surface of the neural plate is flat and a basal lamina is present (see Figs. 10, 11, and 13). The region of the primitive streak has an extremely uneven and irregular basal surface from which cells appear to emerge and enter the mesoblast layer.

FIG. 4. Head-process stage. The number of mesodermal cells has greatly increased. As a result of multilayering in the mesoblast, the region of the presumptive somite exhibits an increased density when viewed with transmitted light (see Fig. 1).

FIG. 5. Head-fold stage. Two populations of cells are evident in the mesoblast based on their behavior. One group, the presumptive somite mesoderm (*pS*) forms a loosely arranged columnar epithelium whose basal surface is bound to the basement lamina of the overlying neural plate. Although there are large spaces between the presumptive somite cells, they are bound to each other by thin cytoplasmic processes at their basal surfaces (see Fig. 13). The other group of cells, with the more flattened mesenchymal configuration, probably contributes to the lateral plate mesoderm.

FIG. 6. 1-Somite stage. The neural plate has narrowed toward the midline in advance of neural tube closure. The underlying and adhering presumptive somite cells have become more tightly apposed during the condensation. On the right side of the micrograph the lack of adhesion between the mesoblast cells and the presumptive epidermis is evident (arrow). Note the abrupt transition from neural plate to epidermis.
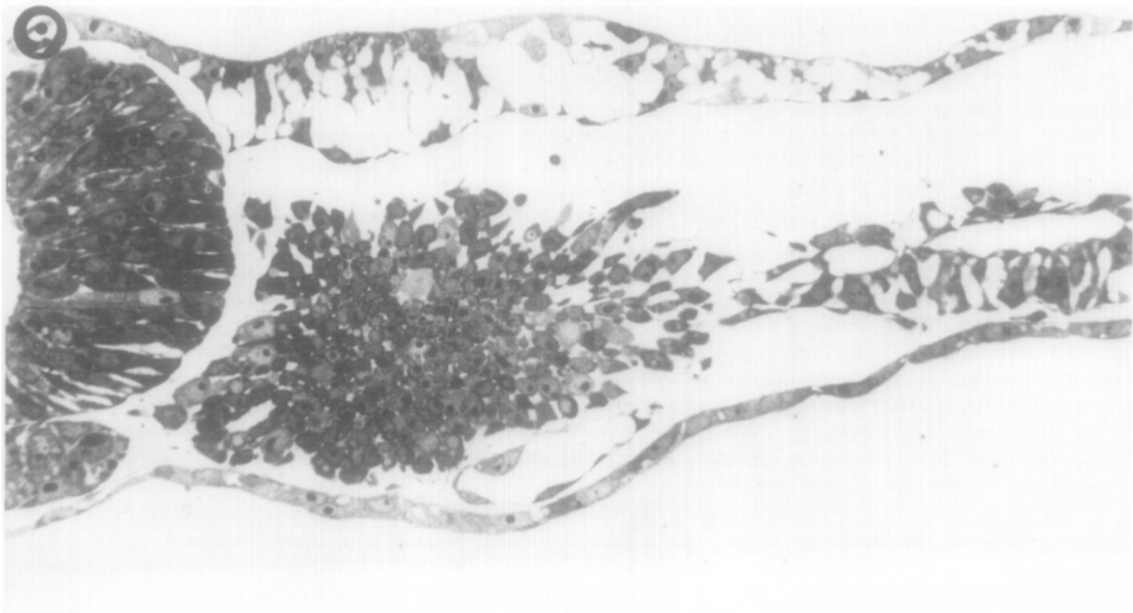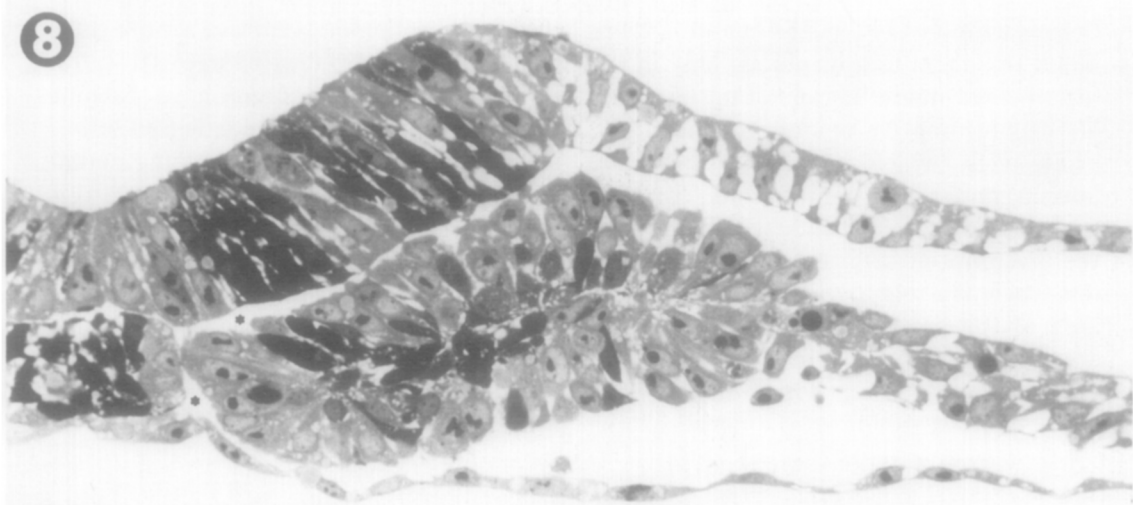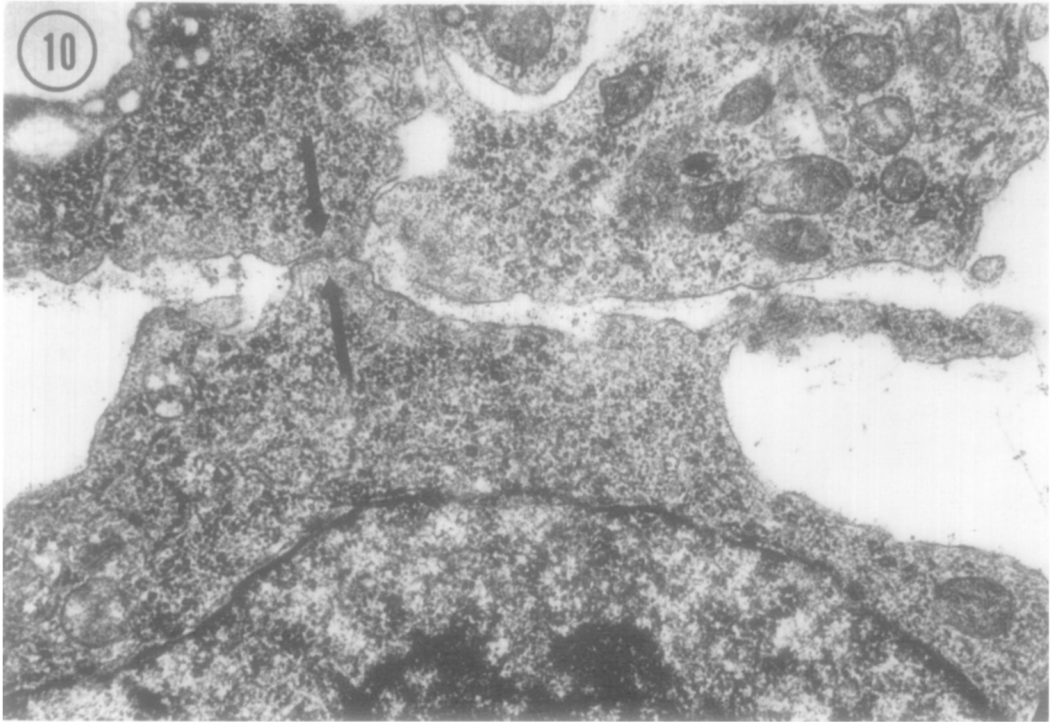
FIG. 10. Electron micrograph showing area of contact between a migrating mesoblast cell and the overlying presumptive neural plate in a primitive-streak embryo. Although the mesodermal cells adhere to the basement lamina of the epiblast, occasional discontinuities of the basement lamina allow direct cell–cell contact (arrows). When contacts occur, focal tight junctions are usually present (Trelstad *et al.*, 1967; Hay, 1968). × 38,600.

new complex junctions now are evident at their apical surfaces (Fig. 17). These new junctions, primarily fasciae adhaerentes, result in the presumptive somite mesoderm assuming a rosette configuration (Fig. 8).

With further condensation, the neural plate rolls up into the definitive neural tube. During closure of the tube the underlying somite cells, formerly bound to the basal lamina of the epiblast, are no longer adherent to the undersurface of the neural plate (Fig. 9). As a result of this change in adhesion, a large extracellular space develops between the somite and neural plate.

Sagittal sections illustrate the cleavage of the segmental plate into the somite blocks. As viewed in sagittal section (Fig. 18), segmentation of the plate is initiated at the dorsal surface. The first indication of the presumptive cleavage is the presence of

FIG. 7. 2-, 3-Somite stage. The cells of the third somite whose anterior and posterior boundaries are just forming have become tightly packed, obliterating most of the extracellular space between the cells. The cells of the somite, which were formerly only bound to each other at their basal surfaces, have formed new junctions at their apical ends (see Figs. 16 and 17). The cells of the forming notochord (*NC*) are present in the midline.

FIG. 8. 4-Somite stage. The neural plate has lifted toward the midline during closure of the neural tube. The cells of the underlying somite have become organized into a rosette configuration. At this stage of development, separations (*) between the formerly adhering mesoderm and adjacent neural plate and notochord become evident (see also Fig. 14).

FIG. 9. 8-Somite stage. The neural tube has completed closure. The tightly packed cells of the third somite have not yet organized into the sclerotome, dermatome, and myotome subdivisions. Large spaces, filled with extracellular material (Hay, 1973), surround the somite. Fibrils, presumably collagen, are found binding the somite to the neural plate and notochord (see Fig. 19).
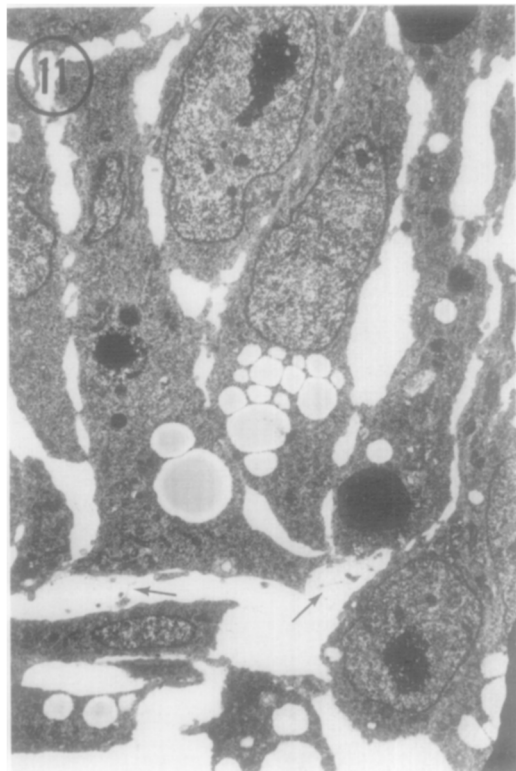
FIG. 11. Junction between the presumptive neural plate and the primitive-streak cells. The basal surface of the neural plate is flat and shows development of basal lamina material (arrows). In the region of the streak to the right, no basal lamina is evident and the basal surface is quite irregular. One streak cell has been fixed, apparently while in the process of migrating into the mesoblast. × 4200.

a large gap between the overlying neural plate and the segmental plate mesoderm. Cleavage initiation at the dorsal surface of the segmental plate has also been reported by both Platt (1889) and Patterson (1907).

Somites 4-8. With further development, the cells of the third somite become tightly packed, revealing very little extracellular space (Fig. 9). A loose matrix of fibrils, most likely of a collagenous nature (Cohen and Hay, 1971; Hay, 1973), is found encapsulating the somite block. In addition, larger bundles of fibrils are found binding the somite to both the differentiated notochord and the neural tube (Fig. 19). The subsequent development and fate of the third and other occipital somites has recently been reviewed by Hazelton (1970).

Two technical aspects of this study deserve special comment. The first concerns the use of carbon particles in fate-mapping experiments. In the course of this study, carbon-marked embryos were regularly photographed using time-lapse cinematography. Analysis of the films showed that occasionally carbon particles became dislodged and moved independently of the cells of the embryo. Carbon marking experiments are therefore unreliable unless verified by time-lapse cinematography. Observing only the beginning and final posi-

FIG. 12. Two low power micrographs of cross sections through the area pellucida of head-process and head-fold stage embryos. × 72.

(A) Head-process stage. At this stage of development the mesoblast consists of a single sheet of cells extending from one side of the embryo to the other, through the midline. The epiblast can be divided into three portions; streak cells (S), presumptive neural plate (NP), and presumptive epidermis (E). The epidermal cells can be distinguished from neural plate by both their lower profile and the greater amount of extracellular space between adjacent cells.

(B) Head-fold stage. In the center of the embryo the densely packed cells of the regressing node split the previously single sheet of mesoderm into right and left halves. The presumptive somite cells are bound to the overlying neural plate. No mesodermal cells adhere beneath the presumptive epidermis, which is lateral to the neural plate.

FIG. 13. An electron micrograph showing the association between somite mesoderm and neural plate in a head-fold embryo. The basal surface of the presumptive somite epithelium is bound to the basement lamina of the overlying epiblast. In addition, thin cytoplasmic processes (arrows) are found extending between the basal portions of adjacent mesoderm cells. × 21,100.

FIG. 14. Transverse section through region of the third somite of a 4-somite embryo. The closely packed cells of the notochord (NC), containing a high concentration of lipid droplets, are found tightly bound to the overlying neural plate and underlying hypoblast. Extensive extracellular space is found between the condensed somite mesoderm and the adjacent neural plate and notochord. × 5800.
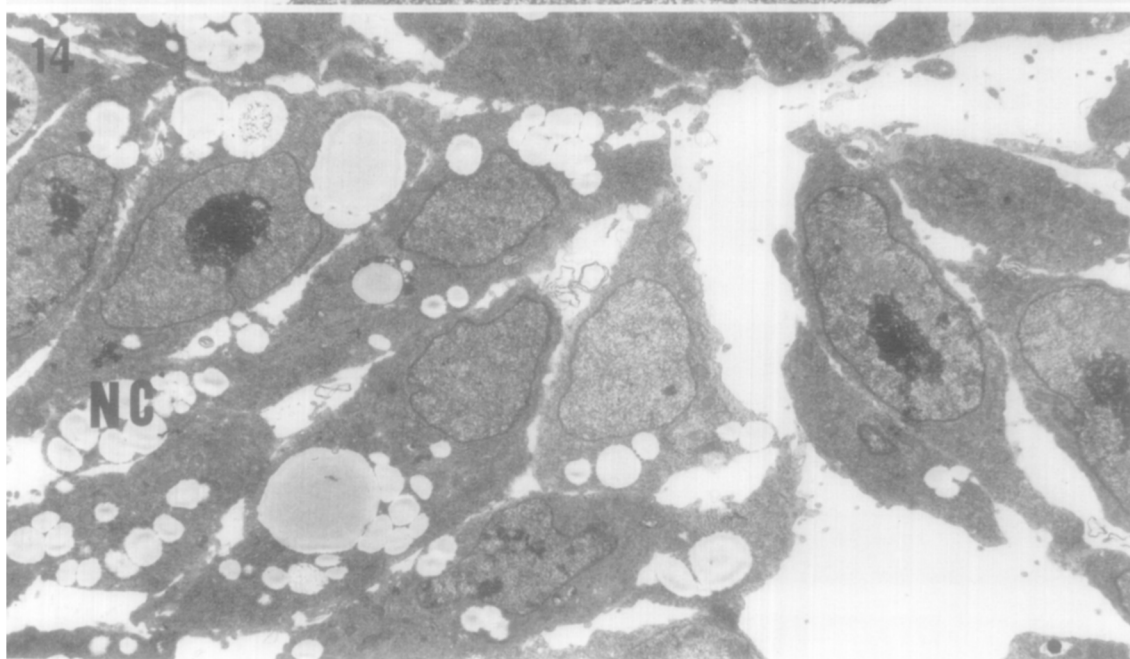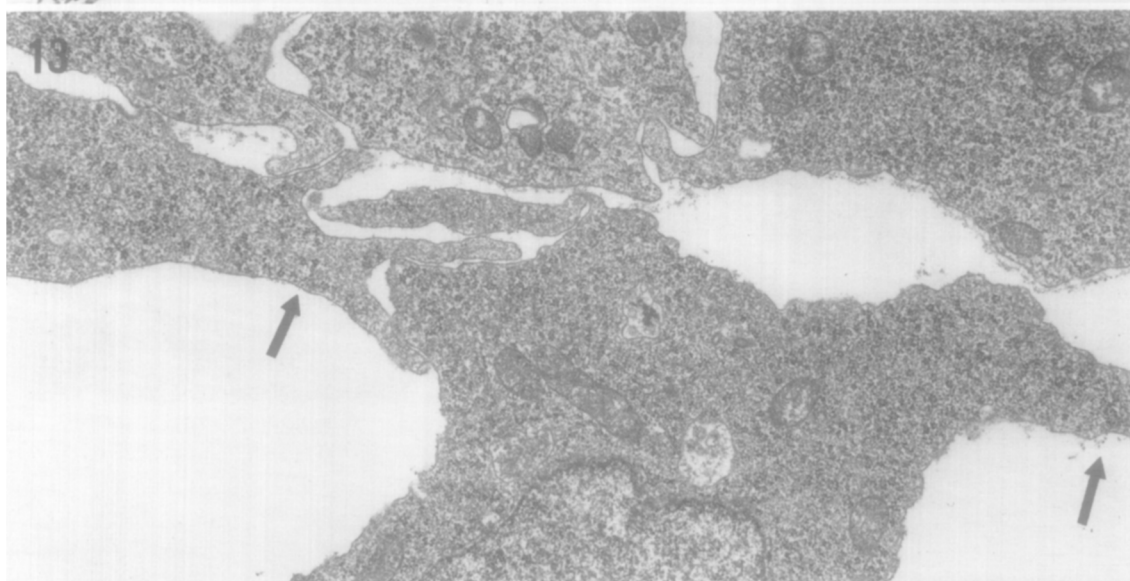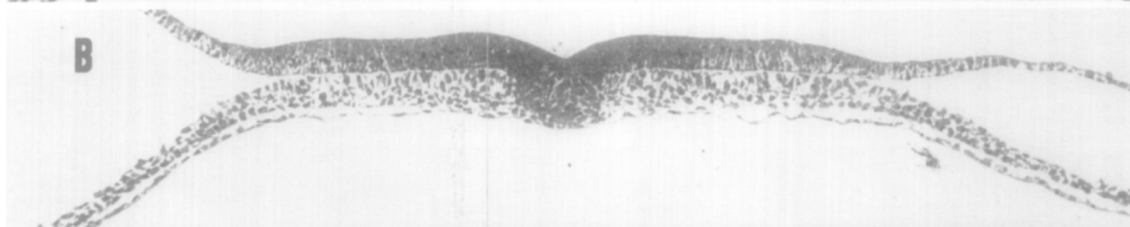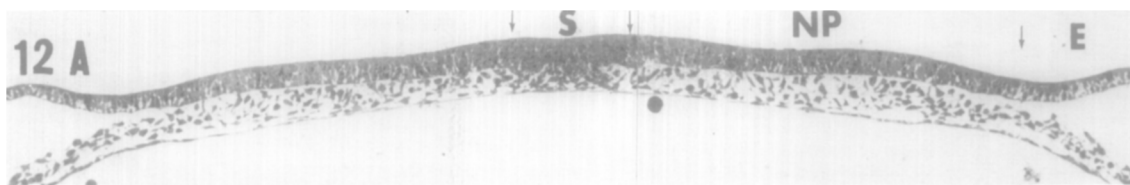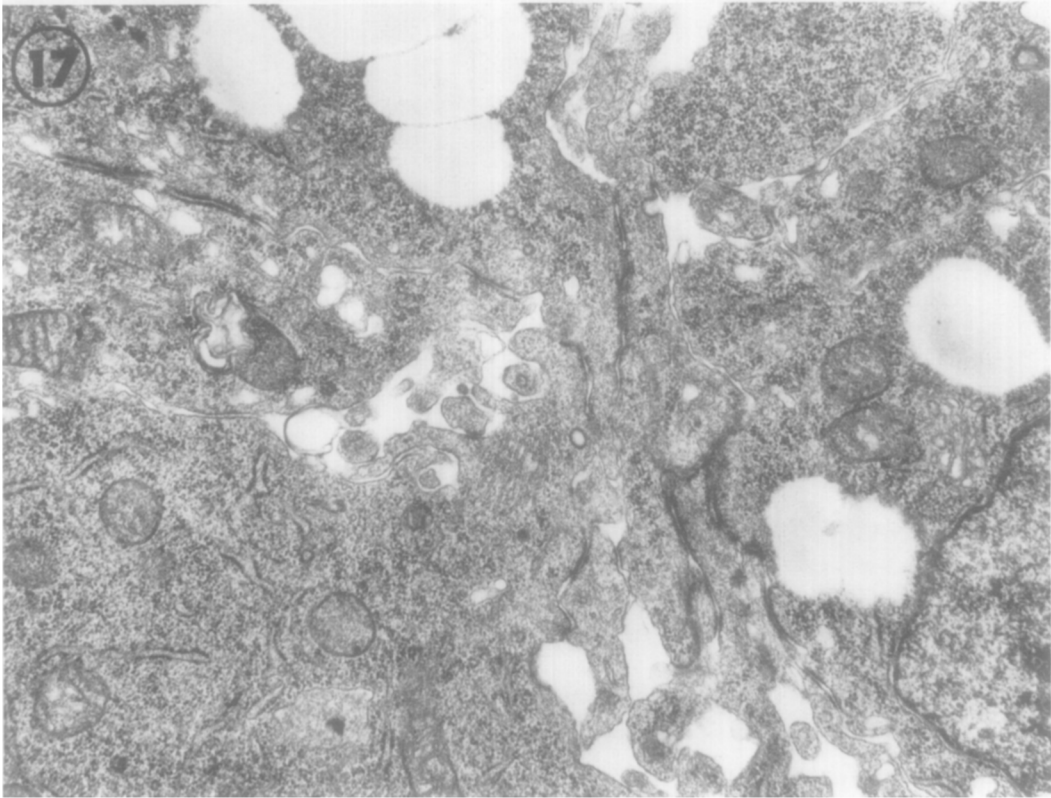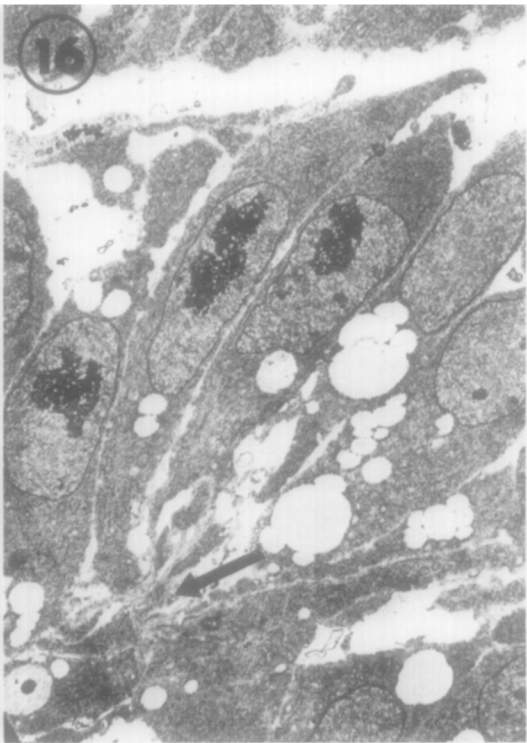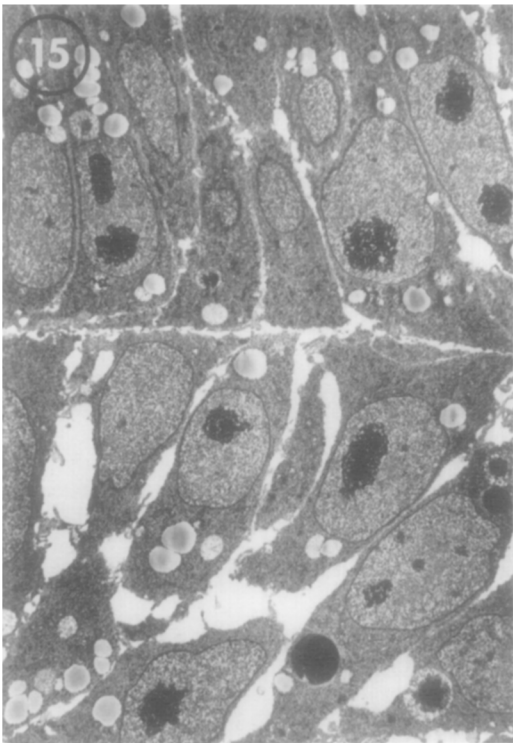
tions of carbon particles would not reveal aberrant movements and could give a ralse interpretation of cell displacements. Therefore, fate maps based only upon the observations of the final positions of these carbon particles may not accurately reflect the normal morphogenetic movements of the embryo. It is advisable that, when possible, time-lapse filming should be used in conjunction with marking experiments to ensure accuracy in subsequent mapping analysis. In addition, time-lapse movies provide detailed timing information.

The elaborate fixation techniques described in the methods section were utilized in order to avoid the artifacts that are often produced by standard histological procedures. Normally, blastoderms are excised from the yolk and the overlying vitelline membrane is removed. The embryos are then immersed in or rinsed with saline in order to remove excess yolk prior to fixation. As a result of these manipulations and the subsequent handling during paraffin embedding, the tenuous associations among the germ layers usually become grossly distorted. Figure 20 illustrates two common examples of artifacts produced by shrinkage and deformation of the germ layers that occur during these procedures.

In using the techniques described in this study, glutaraldehyde fixation alone, although greatly reducing the extent of distortion, does not completely eliminate artifacts. Apparently the postosmification serves to thoroughly harden the tissues and preserve their structural relationships.
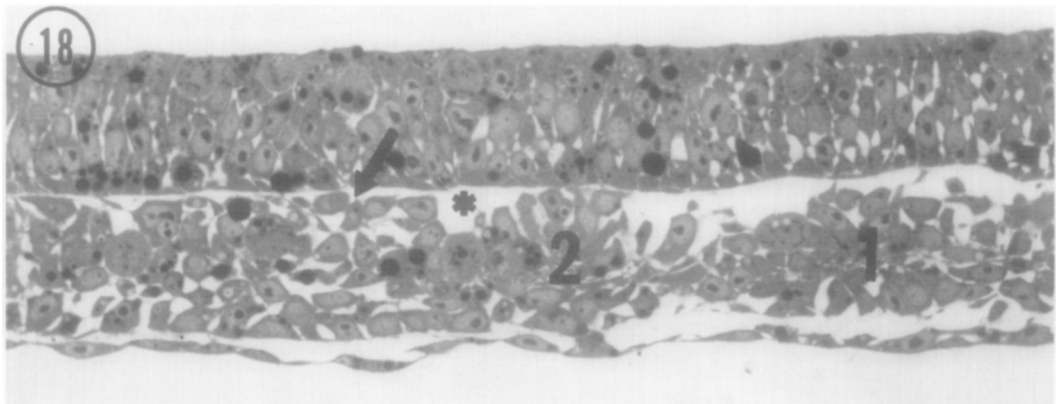

FIG. 18. Sagittal section through the somite mesoderm of a 2-somite embryo. Somites one and two are numbered. The intersomitic furrow between the second and third somite is initiated by a "release" of the mesoderm from the undersurface of the neural plate (*). Note the extracellular space between the formed somites and the overlying neural plate and compare it to the close binding between the unsegmented mesoderm and the epiblast above (arrow). × 500.

FIG. 15. Cross section through a region of the third somite in a 1-somite embryo. The organization of the presumptive somite epithelium is equivalent to that shown in Fig. 6. The cells of the presumptive somite have undergone a mediolateral condensation by becoming more tightly apposed to one another. Compare the amount of extracellular space between these cells and the presumptive somite cells in Fig. 5. × 4250.

FIG. 16. The somite cells have become organized into a rosette configuration and have established complex junctions (arrow) at their apical ends (see Fig. 17). The somite cells are no longer bound to the basement lamina of the overlying neural plate, and a large extracellular space is now evident between the neural plate and somite. × 4550.

FIG. 17. A higher magnification of the complex junctions found in the center of the somite rosette, similar to one shown in Fig. 16. × 29,550.

DISCUSSION

During the course of this investigation it became apparent that there are at least two distinct phases in the development of the axial structures of the chick embryo. The distinction between the phases is based on the mechanisms of notochord formation. In the first phase, notochord
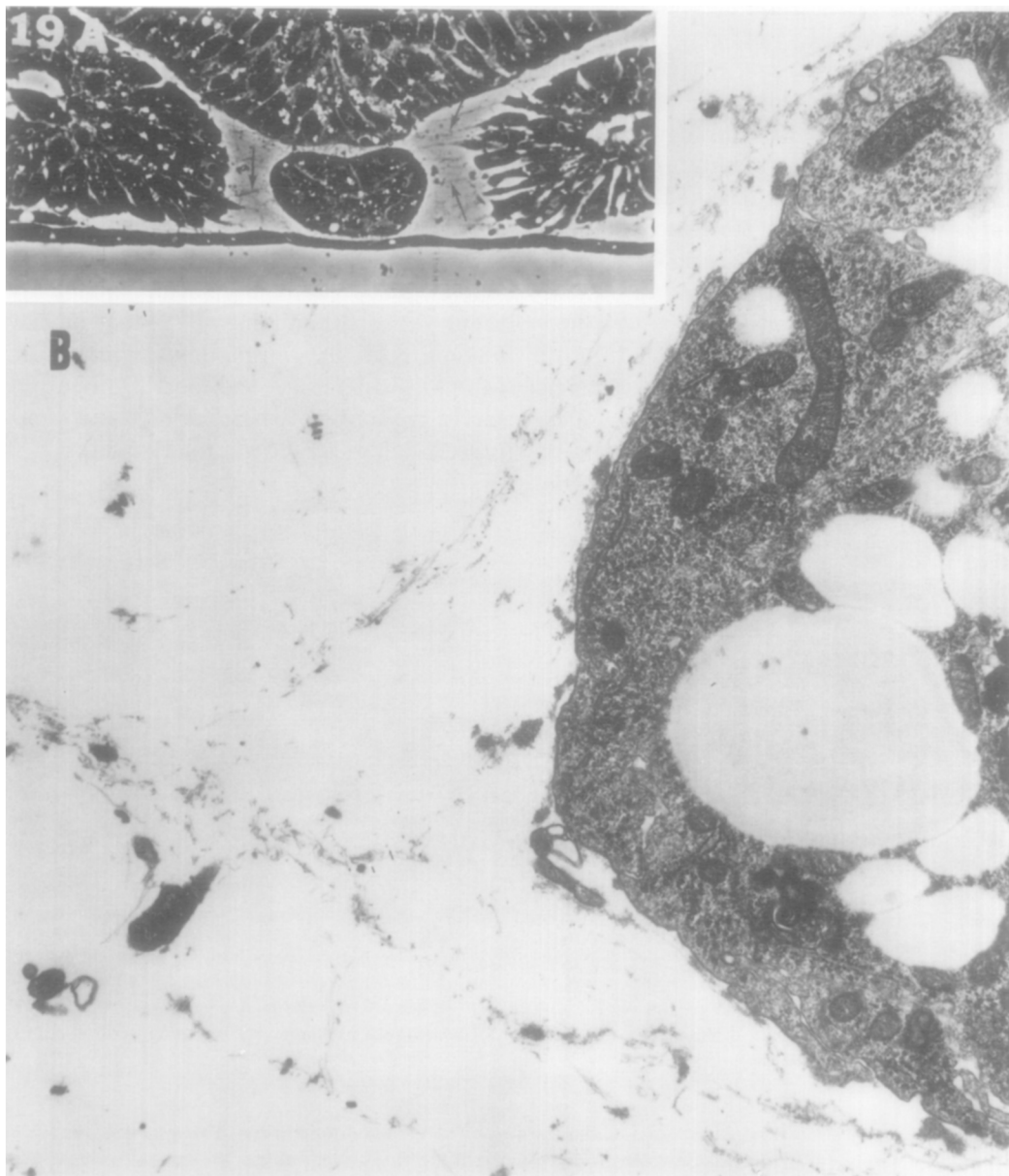

FIG. 19. Micrographs showing the associations between the notochord and adjacent somites at stage 12. (A) Light micrograph of a stained thick section photographed in phase contrast. Numerous large filaments (arrows) are found binding the somites to the adjacent neural plate and notochord. × 470. (B) An electron micrograph showing a portion of a notochord from an area similar to that illustrated in (A). Bundles of filaments, presumably collagen, are found extending between the developing sheath of the notochord and the adjacent somite. × 15,900.
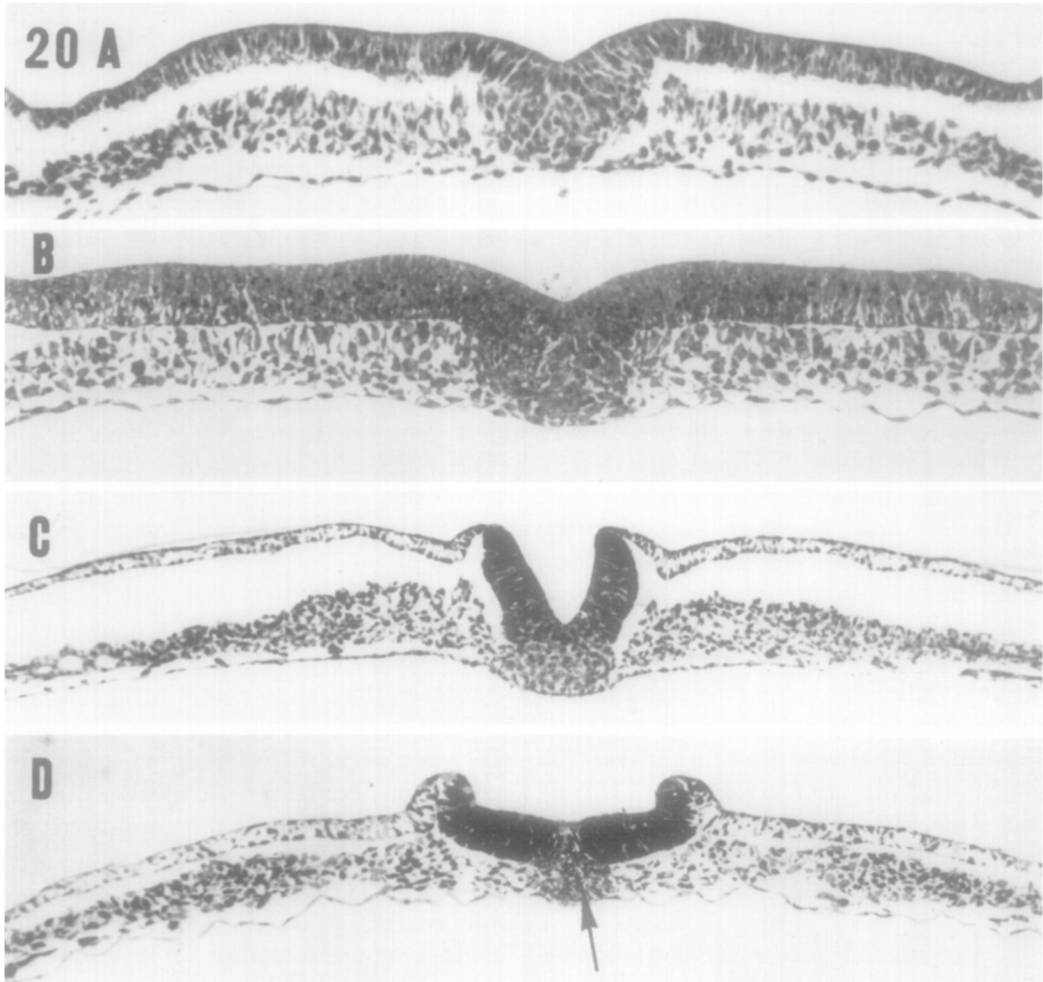
FIG. 20. Sections showing the effects of different fixation techniques on structure of early chick embryos. Sections A and C are from paraffin-embedded blastoderms that were excised from the yolk and vitelline membrane prior to fixation in glutaraldehyde (Section A) or Bouin's (Section C). Sections B and D are thick plastic sections of embryos fixed *in situ* with both glutaraldehyde and OsO_4 prior to their removal from the yolk. × 135.

(A) A 5 μm paraffin section through the level of the third somite in a head-process embryo. During preparation, the tenuous association between the epiblast, mesoblast, and hypoblast have been broken. As a result of subsequent shrinkage, abnormal separations exist between these germ layers.

(B) A 1-μm section through a similar region of a plastic-embedded embryo that was fixed *in situ*. The normal association of adhering germ layers is retained by this procedure.

(C) Transverse section through the rhomboidal sinus of a 12-somite blastoderm that was fixed in Bouin's using standard procedures. The distorted v-shaped appearance of the neural plate is typical of most embryos fixed in this manner. Also note the abnormal amount of extracellular space between the epidermis and underlying mesoderm.

(D) Plastic section of a similar region of an embryo fixed *in situ*. The neural plate has retained its normal flat morphology. In addition one can identify the presence of a cellular condensation (arrow) which gives rise to the notochord. Because of the obvious distortion in the paraffin section (Fig. 20C), this mass of cells is not distinguishable as a separate entity from the neural plate.

formation results from a "laying down" of mesodermal cells anterior to the regressing node. Evidence has accumulated that the cells of the notochord, at least in these early stages, are all of chorda bulb origin (Nicolet, 1970b, 1971). As illustrated in carbon-marked embryos, the cells of Hensen's node, including the chorda bulb, are actually translocated posteriorly by shearing a pathway down the center of the embryo during regression. The shearing is most obvious in time-lapse cinematography, where one can observe abrupt boundaries between the cells of the node and the cells of the neural plate as they move side by side in different directions. These boundaries are not evident in either thick or thin sections. However, such shearing between epithelial cells has recently been found in the development of amphibian neural plate (Jacobson and Gordon, 1973) and in monolayers of cultured epithelia (Steinberg, 1973). In summary, all the cells giving rise to the notochord in the first phase of development are contained within the chorda bulb prior to regression. During regression, as the node shears down the center of the embryo, bulb cells are left behind and condense into the definitive notochord.

In the second phase of development, after Stage 9, the notochord apparently forms by a different mechanism as suggested by time-lapse studies and correlated examination of thick sections. In time-lapse films, the shearing between node cells and the neural plate is either absent or greatly reduced. Thick sections suggest that the notochord cells are derived directly from the cells of the overlying epiblast and that a definitive chorda bulb, evident in the first phase, is nonexistent. We have in preparation a study further elucidating the mechanisms of notochord and neural development during these phases.

Since there are two distinct mechanisms of notochord formation, one must keep in mind that this descriptive study and the following experimental analysis are restricted to somites formed in the first phase of development, somites 1 through 6. Some of the observations in these reports may not apply to the somites that form in the second phase of development.

During somite formation the cells of the mesoblast, which arise from the primitive streak, migrate laterad by adhering to the overlying epiblast basement lamina and the underlying hypoblast cells. The fine structural aspects of mesoblast migration have been reported earlier by Trelstad et al. (1966, 1967) and Hay (1968). In the early stages of laterad migration, these mesoblast cells constitute the primary mesenchyme. A portion of these cells, the presumptive somite mesoderm, becomes organized as a loosely arranged columnar epithelium bound to the basement lamina of the overlying epiblast. The presumptive somite epithelium is found attached only to those regions of the epiblast possessing the pseudostratified columnar epithelial morphology characteristic of neural plate. In areas lateral to the neural plate, in regions of presumptive epidermis, no mesodermal cells are bound to the overlying epiblast. These observations indicate that there is a strict correlation between the original lateral extent of the neural plate and that of the presumptive somite mesoderm.

The observations in this report show that a very close relationship exists between the developing neural plate and the underlying somite mesoderm. This structural relationship correlates well with earlier studies that have implicated the neural plate as playing an active role in somite morphogenesis (Spratt, 1957; Fraser, 1960; Butros, 1967). Additional evidence of this relationship is indicated in the morphogenesis of somites 1-3. These somites block out under a wide area of the neural plate that will form the posterior portion of the hind brain. Initially the lines of cleav-

age between these somites have the same oblique angle as the demarcation between prospective hind brain and spinal cord in Spratt's (1952) fate map of the neural plate (his Fig. 4). When first formed, these somites appear to be two to three times as wide as subsequent somites. However, as the overlying neural plate cells condense toward the midline during closure of the neural tube, the underlying and attached somite cells also condense toward the midline. By the time the neural tube has closed, the once rectangular somites have condensed to a cuboidal configuration typical of the later somites which form beneath the narrower spinal cord portion of the neural tube.

In the literature there is some disagreement about the distance between Hensen's node and the posterior limit of the neural plate (Waddington, 1935; Spratt, 1952; Nicolet, 1971). We find that the neural plate at Stage 5 extends about 0.8 mm posterior to Hensen's node. However, by Stage 9 the distance between the node and the posterior limit of the plate is reduced to about 0.3–0.4 mm. These observations suggest that the regression of the node proceeds more rapidly than any posterior formation or extension of the neural plate that may occur, with the result that the distance between these points decreases during development.

Just prior to closure of the neural tube, the underlying condensed segmental plate mesoderm loses contact with the overlying epiblast. The lack of stainable material between the neural tissue and somite mesoderm in thick and thin sections suggests that the separation of these tissues might result from a change in their adhesive properties. However, autoradiographic evidence by Johnston and Comar (1957), Manasek (1970), and Hay (1973) shows that, prior to closure of the neural tube, the neural plate and notochord actively synthesize acid mucopolysaccharides, which become localized between these structures

and the adjacent somite mesoderm. Therefore, it is quite reasonable to assume that local accumulation of these mucopolysaccharides initiates the separation of the neural and mesodermal tissues.

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