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The Influence of Axial Structures on Chick Somite Formation

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The influence of the axial structures on somite formation was investigated by culturing, on a nutritive agar substrate, segmental plates from chick embryos having 8 to 20 pairs of somites. In the first set of experiments, segmental plate was explanted together with adjacent notochord and approximately the lateral halves of the neural tube and node region. These explants formed 18 to 20 somites within 30 hr. In a second series of experiments, the notochord and neural tube were included as before, but further regression movements in the explants were prevented by removing the node region. These explants formed only 11.9 ± 1.1 somites. Finally, explants of segmental plate that included no neural tube, notochord, or node region were made. These explants had formed 10.7 ± 1.1 somites 14 to 17 hr later. When such explants were cultured for periods longer than 17 hr, there was a marked tendency for the more posterior somites to disperse and for all of the somites to develop a peculiar "hollow" morphology. It was concluded from these results that during the period of development when chick embryos possess 8 to 20 pairs of somites, the segmental plate mesoderm (1) represents about 12 prospective somites, (2) may segment into its full complement of somites without further contact with the axial structures, but (3) requires continued intimate contact with the axial structures for normal somite morphologic differentiation and stability.

INTRODUCTION

The striking periodicity and symmetry of somite formation in the chick embryo has attracted the attention of many investigators. Unfortunately, one finds little consensus as to the factors that might influence somite mesoderm determination and segmentation. On different occasions it has been suggested that paraxial mesoderm forms somites under the influence of Hensen's node (Fraser, 1954), putative "somite centers" (Spratt, 1955), regression movements (Bellairs, 1963), neural tissue (Fraser, 1960; Lipton and Jacobson, 1974) and the notochord (Nicolet, 1970, 1971a).

Nicolet (1970, 1971a) has convincingly demonstrated that explants of chorda bulb and the posterior portion of the notochord, from early chick embryos, have the ability to promote the organization of somites in primitive streak mesoblast. Yet, it has been consistently shown that somites can form in the complete absence of notochord (Grabowski, 1957; Fraser, 1960; Bellairs, 1963; Butros, 1967; Lipton and Jacobson, 1974). Some investigators have interpreted their data to indicate that the presence of the neural tube is essential for somite formation in the chick (Fraser, 1960; Menkes *et al.*, 1961). However, other investigators have clearly demonstrated that somite mesoderm can segment when isolated from the neural tube (Bellairs, 1963; Nicolet, 1971b; Sandor and Amels, 1970; Lanot, 1971; Christ, Jacob, and Jacob, 1972; Brustis and Gipoulous, 1973). Yet, when a section of neural plate and epidermis is rotated 180° in head process stages, the rotated neural plate overlies lateral plate mesoderm and induces somites in it (Lipton and Jacobson, 1974). This experiment suggests that neural plate is sufficient to

induce somites, but does not exclude notochord as also being a potential inductor of somites.

It seems probable that both neural plate and notochord participate in somite determination and that segmental plate is sufficiently induced before it actually segments to form somites with no further induction. This view is consistent with the patterns of determination found in other organs such as the lens, nose, and ear (Jacobson, 1966), and with the available data from all sources on somite determination.

The interpretation of experiments concerning the establishment of any organ requires that one know whether the organ is determined at the time of experimental intervention. Accordingly, we have decided to investigate in detail the ability of chick paraxial mesoderm to form somites in the absence of various tissues with which it normally comes into contact. Embryos at Hamburger and Hamilton (1951) stages 9 through 13 (8–20 pairs of somites) have been chosen for study because the long discrete segmental plates present at these times seem particularly suitable for experimentation. We demonstrate that the entire segmental plate, when maintained *in vitro*, is able to segment into somites in the absence of the adjacent lateral plate mesoderm or the neural tube and notochord. It is further noted that under these conditions, normal morphologic differentiation of formed somites does not proceed in the absence of the notochord and possibly the ventral neural tube.

MATERIALS AND METHODS

Fertile White Leghorn chicken eggs were obtained from Spafas, Inc., of Norwich, Conn. The eggs were stored at 9°C until needed and then incubated at 38°C in a humidified atmosphere for approximately 48 hr. Each embryo was removed from the yolk and transferred into a 35-mm plastic petri dish containing a nutritive substrate. This substrate was a mixture of one part 6% agar and three parts egg supernatant. The egg supernatant was made by centrifuging the thoroughly mixed contents of several unincubated eggs at 15,900 *g* for 30 min. After spreading out the blastoderm on the substrate, with the ectoderm up, the excess supernatant was removed. Microsurgical procedures were performed with tungsten wire needles electrolytically sharpened in a saturated solution of sodium nitrite. Following experimental manipulation, donor embryos and tissue explants were cultured at 38.5°C in an atmosphere of 95% O_2, 5% CO_2 which was bubbled through water in the bottom of the culture chamber. After approximately 14 hr of culture, a small amount of fresh egg supernatant was added to each dish. The cultures were photographed at various intervals, fixed in Romeis' (1948) fixative and prepared for histological examination in a routine manner. Most experiments were on embryos with between 8 and 20 pairs of somites (stages 9+ to 13+; Hamburger and Hamilton, 1951).

RESULTS

Role in Somite Formation of Tissues Lateral to Segmental Plate

The most direct method of testing the ability of the segmental plate to form somites is to remove it from the embryo and place it in culture. In preliminary experiments, isolated segmental plates failed to form somites because the tissues dispersed. To provide mechanical support and prevent dispersion, we wished to include the lateral plate mesoderm and associated ectoderm and endoderm in explants of segmental plate. In order to be certain that these tissues were not themselves essential for segmentation of the segmental plate, a rather simple experiment was performed. All tissues lateral to the right segmental plate from the level of the last formed somite pair to a point just posterior to the node were removed (Fig. 1). In every

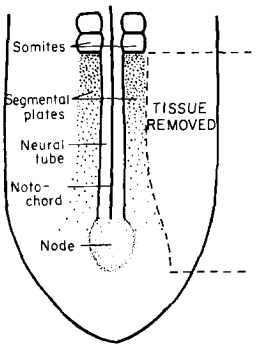

FIG. 1. Operation scheme to remove the tissues lateral to the segmental plate. The tissues include lateral plate mesoderm, epidermis, and endoderm.

case (seven cases), an identical number of somites formed on each side of the neural tube (Fig. 2). Somites did not appear in the explanted lateral tissues.

Role in Somite Formation of Axial Structures

This series of experiments was designed to demonstrate the ability of explants to form somites under our experimental conditions. Explants were taken from donor embryos having from 8 to 20 pairs of somites by making two cuts extending through all three germ layers of the embryo (Fig. 3). The first cut was made perpendicular to the embryonic axis, immediately posterior to the last formed somite. The second cut was parallel to the embryonic axis and was made along the left border of the notochord, nearly bisecting the neural tube and node region. Both cuts were extended out through the area opaca to free the explant from the donor embryo. The explants were pulled to the side of the culture dish prior to incubation. The explants thus formed contained the right segmental plate, the right lateral plate, the notochord, approximately one-half of the neural tube, node and primitive streak regions, and the associated ectoderm and endoderm. As seen in Fig. 4, regression movements and somite formation continued in a routine manner. A cross section of a typical somite formed in these explants is shown in Fig. 5. The morphology and arrangement of the tissues appears normal. From a count of the number of somites in such explants after various times in culture (Fig. 6A), it is apparent that somites continued to be formed for at least 31 hr after explantation.

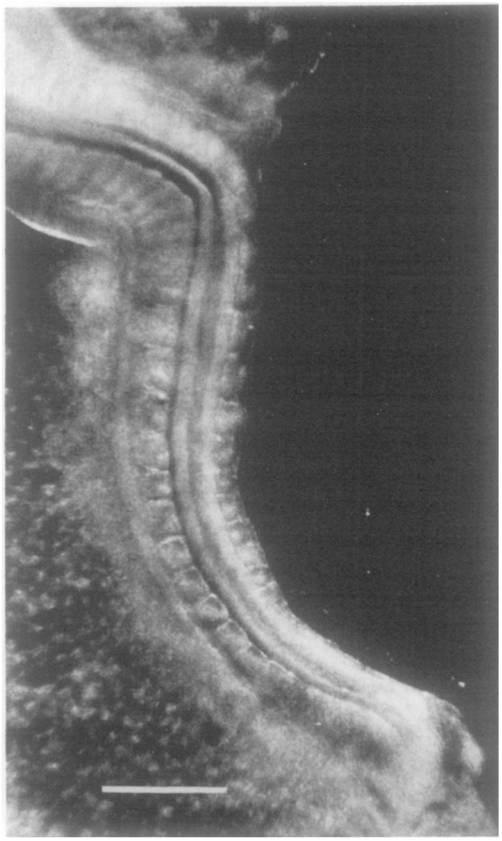

FIG. 2. Results of operation illustrated in Fig. 1. Equal numbers of somites form on each side of the neural tube. Bar = 0.5 mm.

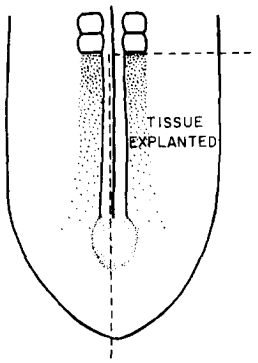

FIG. 3. Operation scheme to explant segmental plate with notochord and lateral halves of neural tube and node region.

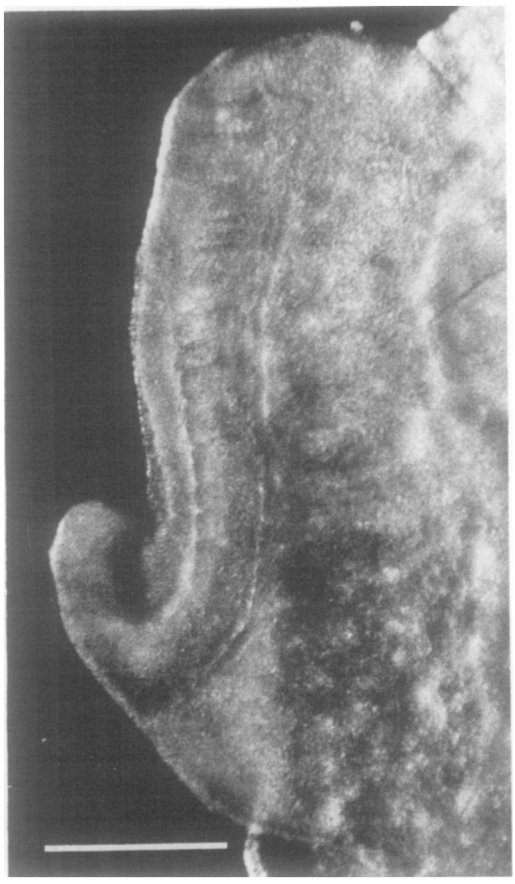

FIG. 4. Results in explant produced as shown in Fig. 3. Somites have formed and regression movements continued normally. Bar = 0.5 mm.

Next, the experiment was altered somewhat so that somites derived from the segmental plate present at the time of explantation could be distinguished from somites derived from the segmental plate formed after explantation. If the node and primitive streak are present in the explant, regression movements continue and additional segmental plate is formed. If regression movements could be prevented in the explants, no further formation of segmental plate would occur. Any somites found in such an explant must, then, be derived from segmental plate present at the time of explantation. Exclusion of the node itself was not sufficient to halt regression. Increasing amounts of the node region were excluded from the explants until the point shown in Fig. 7 was reached. Explants created in this fashion showed little or no signs of regression and formed somites to the posterior limit of the segmental plate (Fig. 8). Again, the histological appearance of the somites and other tissues in the explants was normal (Fig. 9). Counts of the number of somites in these explants at various times after explantation (Fig. 6B) indicate that the number of somites reaches maximum value within 18 hr after explantation and remains at that level through the remainder of the observation period. From these data one could conclude that the segmental plate represents about 10–13 prospective somites. However, since these data represent results of experiments utilizing donor embryos from a range of developmental stages, the possibility that there were differences in the number of somites formed in each explant due to the age of the donor embryo was investigated. The data were reexpressed as maximum number of somites formed in the explant versus number of somite pairs possessed by the donor at the time of explantation (Fig. 10). The donors ranged from 8 to 20 pairs of somites. No correlation is seen to exist between the stage of development of the donor embryo and the number of somites formed by the segmental plate present at the time of explantation. In Fig. 6B, if the points from 17 to 34 hr are considered to represent the maximum number of somites formed, the

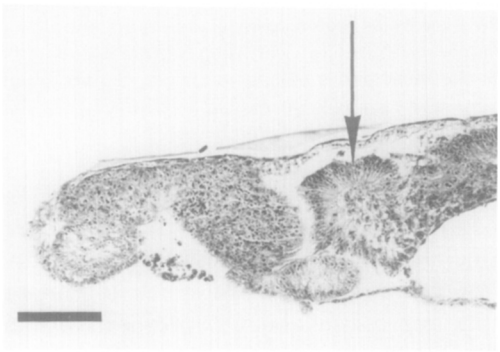

FIG. 5. Cross section through a somite (arrow) in an explant, such as is shown in Fig. 4. Neural tissue is to the left of the somite, notochord to lower left, lateral plate to right. Bar = 0.1 mm.

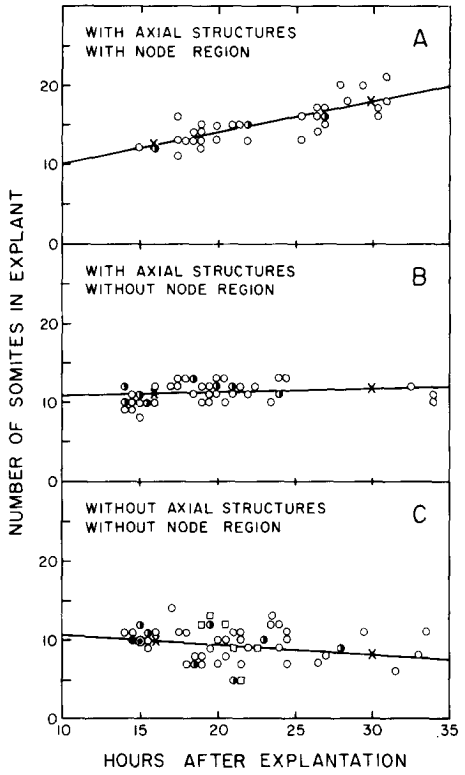

FIG. 6. The numbers of somites that form from segmental plate explanted with or without various axial tissues are shown at different times after explantation. Open circles represent one case, half-filled circles two cases, filled circles three cases. The line is positioned with the aid of a regression analysis. (A) Segmental plates explanted with axial structures (notochord and neural tube) and with the node region present continue to form somites through 30 hr after explantation. (B) In segmental plates explanted with neural tube and notochord, but without node region, regression movements are halted and the somite number is maximum by 18 hr. (C) Segmental plates isolated without axial structures or node region form a maximum number of somites within 15 to 17 hr, then begin to lose somites as the most posterior ones disperse. Square points represent results with explants cultured in a dish separate from the donor embryo.

mean number of somites formed by these explants is then 11.9 ± 1.1. We, therefore, conclude that between the stages of 8 and 20 pairs of somites, the chick segmental plate represents about 12 prospective somites.

With respect to the conclusion presented above, it is interesting to note that the length of the segmental plate can be shown to increase considerably as the embryo gets older (Fig. 11). The reason that segmental plates of different lengths yield equal numbers of somites is not clear, al-

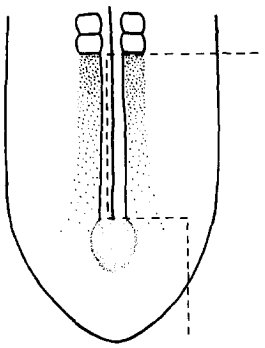

FIG. 7. Operation scheme to explant segmental plates with axial structures, but excluding node region that contributes to regression movements.

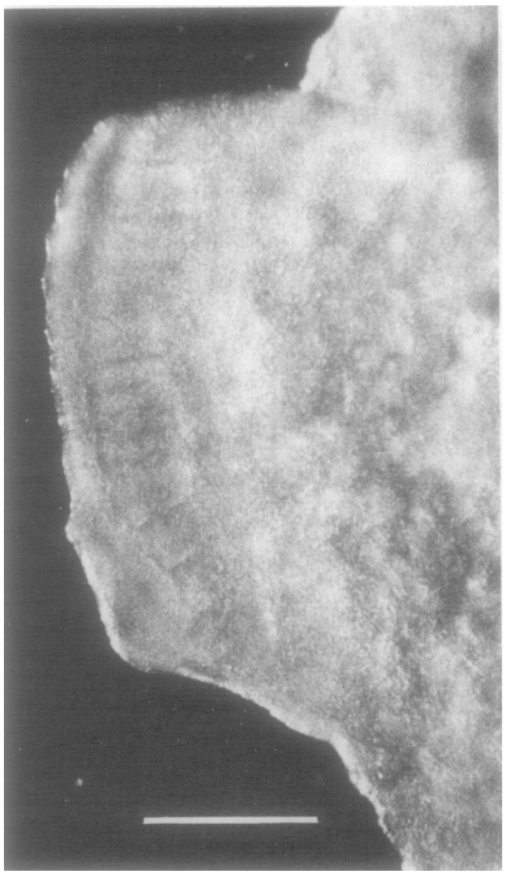

FIG. 8. Results in explant produced as shown in Fig. 7. No additional segmental plate has formed from regression movements since node region is lacking. Somites have formed to the posterior border. Bar = 0.5 mm.

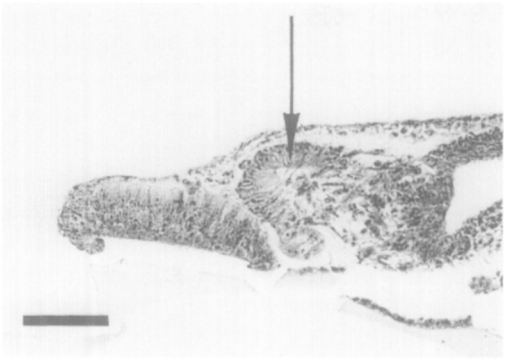

FIG. 9. Cross section through a somite (arrow) formed in an explant, such as shown in Fig. 8., cultured 14 hr. Bar = 0.2 mm.

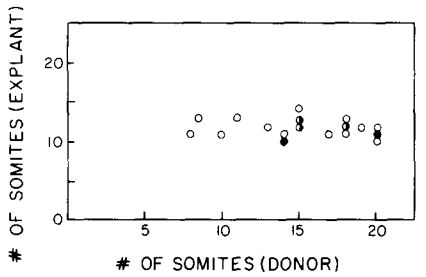

FIG. 10. The maximum number of somites formed in explants of segmental plate with axial structures but lacking node region is shown for donors of different ages. Donor age is given by the number of somites the donor had when the explant was made.

though we have noticed that somites formed in segmental plates of older embryos are initially longer in the anteroposterior axis.

It was now possible to test the role of the axial structures in somite formation. If segmental plate explants were made without including the axial structures or the node region (Fig. 12), one would expect fewer than 12 somites to form if some essential role in somite formation is played by the adjacent neural tube and notochord. In fact, such explants formed somites to the posterior limit (Fig. 13). These somites were spherical in appearance and often lacked cells in the center (Fig. 14). The numbers of somites formed in these explants at various times after explantation are shown in Fig. 6C. Explants observed between 14 and 17 hr after explantation contained 10-12 (10.7 ± 1.1) somites. These results are not significantly different from those obtained in the same time period with explants which contained the axial structures. However, at times later than 17 hr after explantation, a marked tendency for a reduction in somite number was noted. When this occurred, it always involved the dissociation of the most posterior somites.

These results suggest that the continued presence of the axial structures is not es-

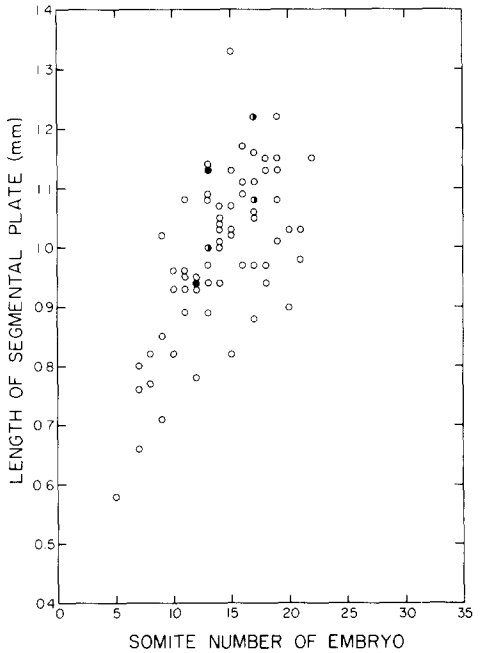

FIG. 11. Data illustrating that the segmental plate is longer in older embryos (in embryos with more somites).

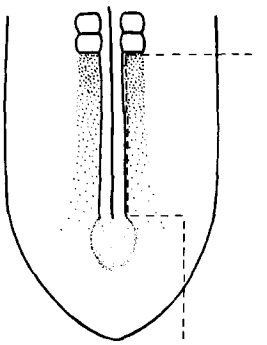

FIG. 12. Operation scheme to explant segmental plates without axial structures or node region.

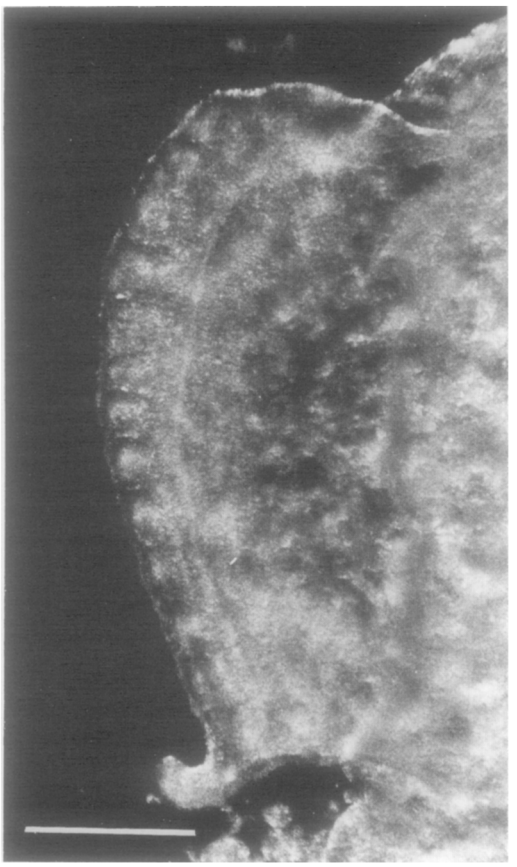

FIG. 13. Results in explant produced as shown in Fig. 12. Somites formed the length of the segmental plate. Bar = 0.5 mm.

sential for somite formation from the segmental plate. To confirm this conclusion, an additional control experiment was done. The explants studied in the above experiments were cultured in the same dish as their donor embryos. If the axial structures exert a somite-promoting influence on the segmental plate through some diffusible substance(s), it may have been possible for the donor embryos to "condition" the fluid culture medium in such a way as to promote somite formation in explants that did not include axial structures. To investigate this possibility, the following experiment was performed. In six cases, explants without axial structures or the node region were removed from the donor's culture dish immediately after surgery. These explants were washed in a second culture dish filled with fresh egg supernatant and finally introduced into a third dish for culture in the routine manner. The number of somites observed in each of these explants is presented as square points in Fig. 6C. These results are indistinguishable from those obtained with explants grown in the same dish with the donor embryo. We conclude from these results, together with the results presented above, that (1) between the stages of 8 and 20 pairs of somites, the segmental plate may segment entirely into somites in the absence of further contact with the axial structures, and (2) at these same stages, continued presence of the axial structures stabilizes formed somites.

The Role of Axial Structures in Somite Morphogenesis

It was briefly mentioned above that some differences in somite morphology were noted in explants that did not contain the axial structures. These differences were found to be greatly exaggerated after longer periods in culture. In cross sections through explants containing axial structures and cultured for approximately 24 hr (Fig. 15), the histologic appearance of the somites is very similar to somites found in control embryos having clearly developed dermatome, myotome, and sclerotome regions. In cross sections through explants

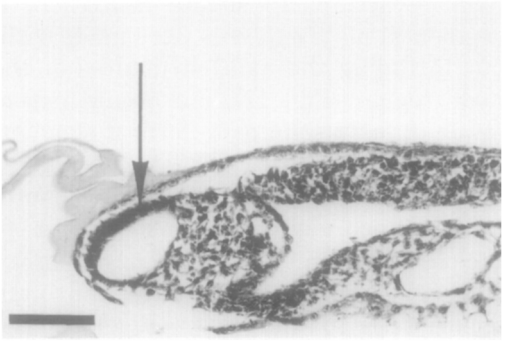

FIG. 14. Cross section through a somite (arrow) in an explant such as shown in Fig. 13. In these explants lacking axial structures, the somite has an abnormal "hollow" morphology. This explant was cultured 14 hr. Bar = 0.1 mm.

without axial structures and cultured for approximately 24 hr (Fig. 16), the morphology of the somites is distinctly abnormal, having the appearance of a flattened, hollow sphere. A sagittal section through some of the somites of a similar explant is shown in Fig. 17.

In an effort to confirm these observations and to gain some insight into how close an association between somite and axial structures is required to maintain a normal somite morphology, several explants were made from which only a short length of the axial structures was excluded. An example of such an explant is shown in Fig. 18. The same explant is shown again in Fig. 19 after 18 hr in culture. Note that only two somites lack adjacent axial structures. A horizontal section of this same explant is shown in Fig. 20. It is obvious that only the two somites not immediately in contact with the axial structures have the abnormal "hollow" morphology.

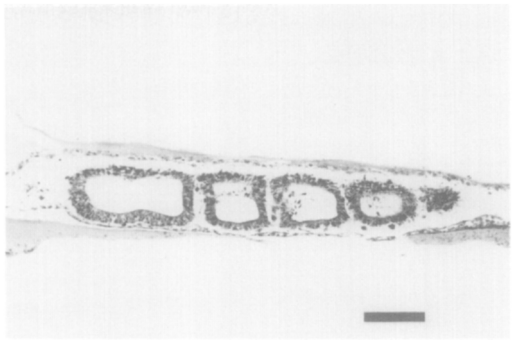

FIG. 17. Sagittal section through somites in an explant of segmental plate without axial structures cultured 24 hr to illustrate that all of the somites formed in such explants have abnormal morphology. Bar ≈ 0.1 mm.

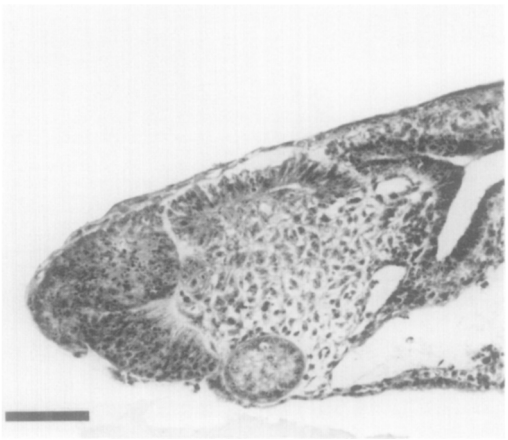

FIG. 15. Differentiated somite in cross section of an explant of segmental plate with adjacent axial structures cultured 24 hr. Dermatome, myotome, and sclerotome are evident. Bar = 0.1 mm.

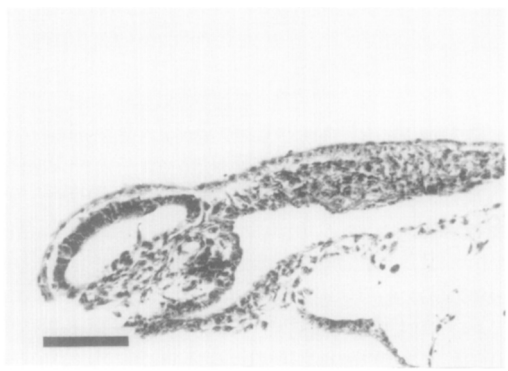

FIG. 16. Cross section of a somite in an explant of segmental plate without axial structures cultured 24 hr. "Hollow" morphology is typical of all such somites. Bar ≈ 0.1 mm.

Is this apparent effect upon somite differentiation associated with the presence or absence of both the neural tube and the notochord, or is only one of these tissues responsible for the effect? The morphology of somites in eight donor embryos from which explants containing axial structures had been taken was examined histologically. It will be recalled that when explants with axial structures were made, only one-half to two-thirds of the neural tube was included with the notochord in the explant. The remainder of the neural tube was retained in the donor embryo (Fig. 21). If somite differentiation was found to proceed normally in these donors, one could conclude that the presence of the neural tube alone is sufficient. In fact, as shown in Fig. 22, even in the presence of ample neural tissue, somites acquired the abnormal morphology.

As a final check on the possibility that some variations in culture conditions could be responsible for the observed variations in somite differentiation, the following ex-

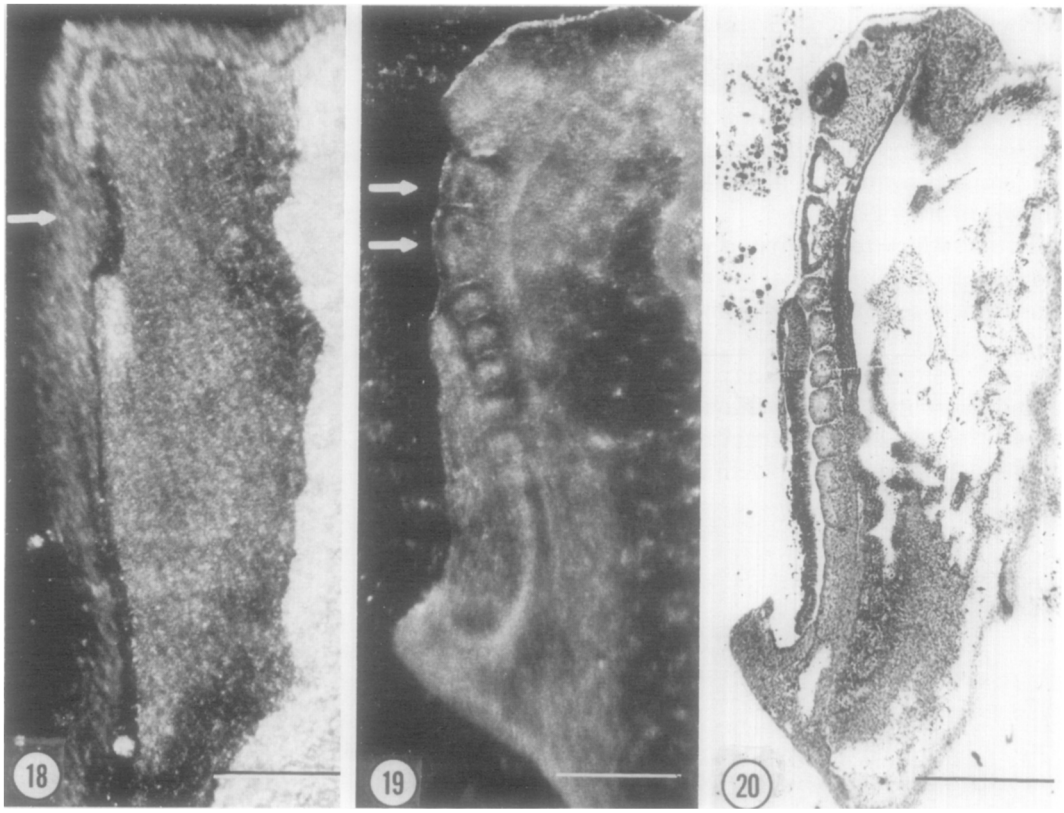

FIG. 18. An explant of segmental plate with axial structures, except for a short length (arrow) in which the notochord and neural tube were excised. Bar = 0.5 mm.

FIG. 19. The same explant shown in Fig. 18 after 18 hr in culture. Somites have formed in the segmental plate in the region lacking axial structures (arrows). Bar = 0.5 mm.

FIG. 20. Horizontal or frontal section of the explant shown in Fig. 19. The two somites in the area lacking axial structures have the "hollow" morphology. Bar = 0.5 mm.

periment was performed. An embryo was placed on the agar medium and appropriate cuts made to establish an explant with axial structures but without the node region (Fig. 7). In this case, the cuts were not extended out through the area opaca. Thus, the explant remained closely associated with the donor embryo. This embryo-explant combination was cultured for 19 hr, fixed, and examined histologically. As shown in Fig. 23, somites found in the explant maintained a close association with both neural tissue and notochord and proceeded to differentiate in a routine manner. On the other hand, somites found in the donor embryo, despite a close association with neural tissue, developed the abnormal morphology. It therefore seems that forming somites must be in immediate association with the notochord and possibly the ventral neural tube in order to develop histologically discernible dermatome, myotome, and sclerotome regions.

DISCUSSION

If the axial structures induce somite formation in paraxial mesoderm, one would expect that the longer the mesoderm is exposed to the inductive influences, the more it will be inclined to form somites (Jacobson, 1966). Since the axial structures and the segmental plates of the chick form in a cranial to caudal sequence, mesoderm at the anterior end of the segmental plate has been in contact with the axial structures for a greater length of time than

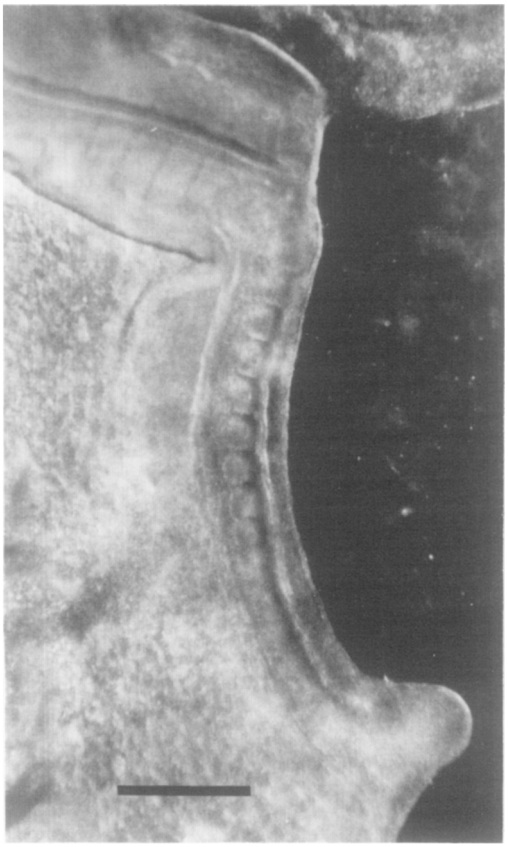

FIG. 21. Donor embryos from which the right lateral halves of the neural tube and node region, the notochord, and the segmental plate were removed, as shown in Fig. 3. The remaining left segmental plate, associated with the remaining half neural tube, has formed somites. Bar = 0.5 mm.

mesoderm at the posterior end. One could assume the induction of segmental plate to be complete anteriorly. For this reason, a demonstration that a portion of the unsegmented somite mesoderm is able to form at least some somites in the absence of the axial structures is not sufficient to exclude a possible role for the axial structures in somite formation. Segmentation of the anterior portion of the isolated segmental plate might proceed because induction by axial structures is already sufficient there, but more posterior segmental plate could require further induction by neural plate and notochord before it is sufficiently induced to proceed with somite formation on its own.

A putative continuing essential role for the axial structures in the segmentation of somite mesoderm could be ruled out by demonstrating that the segmental plate present at a given stage of development can segment along its entire length when isolated from the axial structures. The present study has provided such a demonstration. We have shown that the segmental plate of 8 to 20 somite chick embryos represents 11.9 ± 1.1 prospective somites. In our experiments, when segmental plate explants not accompanied by the axial structures were cultured, they had formed 10.7 ± 1.1 somites between 14 and 17 hr after explanation, a number not significantly different from the number found in segmental plates with axial structures. Thus, it is clear, that during the developmental period studied, the segmental plate does not require further contact with the axial structures in order to form its full complement of somites. In addition, we have shown that segmentation of the entire segmental plate occurs in the absence of the tissues normally found lateral to it.

We have also shown that the number of somites present continues to increase with time in explants including axial structures and node region (Fig. 6A), but, after an initial period of somite formation, little or no further increase in somite number is

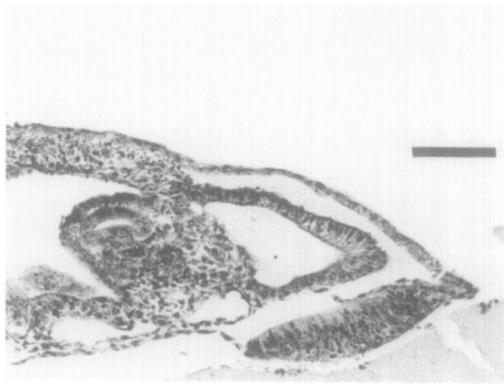

FIG. 22. Cross section through a somite in the former segmental plate region of the donor embryo shown in Fig. 21. This somite is "hollow" despite its close association with neural tissue. This region of the donor lacks notochord. Bar = 0.1 mm.

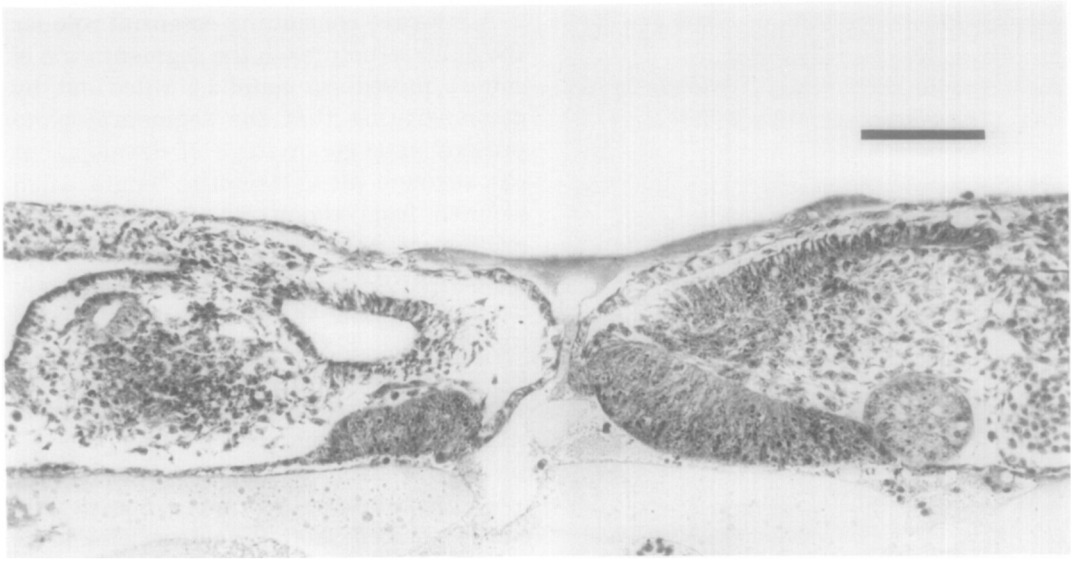

FIG. 23. After a cut was made, as shown in Fig. 7, the "explant" (right) and the "donor" embryo were cultured in close association for 19 hr, then sectioned through the somites that formed in the segmental plate. Donor part (left), lacking notochord, has abnormal somites. Bar = 0.1 mm.

seen with time in explants that include axial structures but not the node region (Fig. 6B). In this latter case, the segmental plate formed 11.9 ± 1.1 somites. Similar numbers were obtained in explants lacking axial structures and node region (Fig. 6C). It therefore seems probable that the most effective induction of somites occurs around the chorda bulb and primitive streak where future notochord cells are located in the bulb and streak midline and future neural plate cells are lateral over the prospective somite cells. This idea is also supported by experimental results of Shoger (1960), Butros (1962), Bellairs (1963), Nicolet (1970, 1971a), and Lipton and Jacobson (1974). Anteriorly, where segmental plate is already formed by the regression movements, the segmental plate is by then sufficiently induced to form all the somites that normally come from it, even in the absence of the axial tissues. The formed notochord and neural plate and tube of these anterior areas probably still retain, however, the capability of inducing somites.

An interesting finding of this study is that the number of prospective somites in the segmental plate is remarkably constant despite the pronounced lengthening of the segmental plate with increasing embryo age. This observation may be related to the work of Cooke (1975) who manipulated the lengths of *Xenopus* embryos and found no effect on total somite number or relative somite positions.

Lipton and Jacobson (1974) observed that when somites formed in early chick explants that included neural plate but not the notochord, most of the somites dispersed within 24 to 30 hr after explantation. We have observed that in explants not containing the axial structures there is a definite tendency for the more posterior somites to dissociate when cultured for periods longer than 17 hr. These observations suggest that the association of forming somites with the notochord and possibly the neural tube tends to stabilize the somites and prevent their dissociation. Lipton and Jacobson (1974) suggest that the large extracellular fibrils that bind somites to the notochord may serve to stabilize the formed somites and prevent them from migrating away laterally.

Our experiments strongly indicate that

segmental plate is already determined to form somites, but they do not eliminate the possible continuing inductive role of overlying ectoderm and underlying endoderm. Sandor and Amels (1971) have reported that removal of chick ectoderm or endoderm from the last intersomitic cleft posterior to about the length of four to five prospective somites does not inhibit segmentation. But these experiments tested only the anterior segmental plate that one would expect to be highly induced. Whether overlying ectoderm and underlying endoderm have a role in determination of posterior segmental plate is still an open question.

Lanot (1971) has suggested that as one somite forms, it causes the mesoderm immediately posterior to it to initiate segmentation. The formation of somites in segmental plates isolated from formed somites, as in our experiments, does not directly test this contention. However, experiments of Lipton and Jacobson (1974), showing that segmentation of several somites can occur simultaneously tend to contradict this hypothesis.

We have demonstrated that developing somites, when not in contact with the axial structures, fail to form histologically discernible dermatome, myotome, and scleratome regions. Rather, these somites appear as flattened, epithelioid spheres. Somites with a similar abnormal morphology have been noticed by other investigators (Sandor and Amels, 1970; Sandor, 1971; Christ, Jacob, and Jacob, 1972). In each case, the abnormal somites were developing without contact with the axial structures. We have further shown that this abnormal somite morphology is due to a lack of direct contact with the notochord and possibly the ventral neural tube. It is interesting to speculate that this unusual somite morphology may be related to the tendency for these somites to dissociate. The notochord and ventral neural tube may play a critical role in scleratome formation. The ability of these structures to stimulate the formation of scleratomal cartilage in somitic mesoderm *in vitro* is well known (Flower and Grobstein, 1967; Kosher and Lash, 1975). In the absence of these structures one might then expect the mesenchymal prospective scleratomal cells to disperse, leaving only the epithelial portions of the somite. Apparently the epithelial portions may also migrate away with time if not anchored to axial structures. This would help explain both the abnormal morphology of such somites and their tendency to disperse. It must be noted, however, that Sandor and Amels (1970) observed cartilage and myoblast development in chick embryos *in ovo*, despite the extirpation of the axial structures prior to somite formation.

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