The development of the notochord in the chick embryo, studied by scanning and transmission electron microscopy

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SUMMARY

The notochord of the chick embryo between stages 5 and 23 inclusive has been studied by scanning electron microscopy, supplemented by transmission electron microscopy. Three main phases of development are described, and these have been designated: bilaminar; rod-like, unvacuolated; rod-like and vacuolated. The change in shape of the organ from bilaminar to rod-like is accompanied by changes in the shape, orientation and position of the cells, an increase in the complexity of the cell contacts, and the laying down of a basal lamina. The change from the unvacuolated to the vacuolated phase is accompanied by increasing complexity within the cytoplasm. Most of the vacuoles are intracellular and appear empty though some contain a granular material. The notochordal sheath appears to be secreted by the notochordal cells and fine fibrillar material has been seen in the intercellular spaces. By stage 23, most of the notochordal cells have become so highly vacuolated that the cytoplasm has become closely packed around the nucleus.

INTRODUCTION

The notochord is one of the few truly midline structures in the embryo, and plays an important role in establishing bilateral symmetry in the axis. During the period of its own formation, it induces the neural plate in the overlying ectoderm, and for some time remains in electrical communication with the neural tube (Sheridan, 1968). Later, it becomes involved in cartilage induction (Kosher & Lash, 1975), and it provides a focal point for the first deposition of the cartilage of the vertebral centra; in this way, it ensures that the vertebrae become laid down in the appropriate position in relation to the neural tube.

The present paper is, to the best of our knowledge, the first to report on the structure of the notochord as seen by scanning electron microscopy (SEM). We have supplemented our investigations by parallel studies using transmission electron microscopy (TEM) and have been able to confirm and extend observations of Jurand (1962) and Ruggeri (1970, 1972). We have also carried out a histochemical analysis of the notochord and its sheath, but since many other histochemical studies on the chick notochord have been reported in the literature.

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(e.g. Johnston & Comar, 1957; Leeson, Threadgold & Sinclair, 1961; O'Connell & Low, 1970; Kvist & Finnegan, 1970a, b; Carlson & Low, 1971; Frederickson & Low, 1971; Carlson, 1973a, b), we will consider our own histochemical results only briefly.

MATERIALS AND METHODS

Eighty-nine chick embryos have been used, ranging in age from about 18 h to about 3 days of incubation. They were staged according to the Normal Table of Hamburger & Hamilton (1951).

(A) Scanning electron microscopy (SEM). Specimens were fixed for periods of 4–24 h in 3 % glutaraldehyde made up in 0.15 M cacodylate buffer, or in half strength Karnovsky's fixative (Karnovsky, 1965). The pH of the fixative was 7.2. Some specimens were also treated with cetyl pyridinium chloride (CPC), which was added as a 1 % solution to the fixative. After washing in cacodylate buffer, the specimens were immersed in 1 % osmium tetroxide for 30 min and then washed again in cacodylate buffer. They were dehydrated in graded ethanols and dried in a Polaron critical point drying apparatus from liquid CO₂. They were mounted on stubs with UHU glue (Fishmar Ltd., Waterford, Eire), coated with carbon and gold, or with carbon alone, and examined in a Cambridge Stereoscan S 4–10 or Cambridge Stereoscan IIA electron microscope.

(B) Transmission electron microscopy (TEM). The embryos were usually fixed whole in half-strength Karnovsky's fixative (Karnovsky, 1965) for 1 h at about 15–20 °C and at a pH of 7.2. A further 15 specimens were also treated with CPC, which was added as a 1 % solution to the fixative (as above). Specimens were washed three times in either cacodylate or phosphate buffer, and then treated with a 1 % osmium tetroxide fixative for 1 h at the same temperature. They were subsequently washed three times in maleate buffer (pH 6.0) and stained in block with a 1 % uranyl acetate solution in maleate buffer of pH 5.2. After dehydration in ethanols the specimens were trimmed and embedded in Spurr resin. Sections were cut on a Porter Blum mark II ultramicrotome, and stained with aqueous lead citrate. They were examined with a Siemens Elmiskop 1 electron microscope.

(C) Light microscopy and histochemistry. Thick sections were removed from the blocks which had been prepared for transmission electron microscopy, and were examined by phase-contrast microscopy. Other specimens, having been fixed in half-strength Karnovsky's fixative (with or without CPC), were dehydrated with graded ethanols and embedded in paraffin wax. Serial sections were prepared and stained with PAS-alcian blue techniques (see Pearse, 1968). Companion specimens were digested with either testicular hyaluronidase for 3 h (see Lillie, 1965) or neuraminidase for 18 h (see Lillie, 1965) prior to staining. Controls were simultaneously incubated in phosphate buffer. Sections of full term human umbilical cord were stained simultaneously as further controls, both with and without enzyme treatment.
RESULTS

The anterior end of the notochord, which begins to form at about stage 5, is known as the head process and lies anterior to Hensen's node. The main part of the notochord, however, is laid down as Hensen's node migrates posteriorly. During the succeeding stages of development, morphological changes take place in the organ, and since each of these begins at the anterior end of the embryo and gradually passes posteriorly, the anterior regions are always developmentally in advance of the more posterior ones. For this reason we will relate our findings to the morphological phases of the notochord itself, rather than to the chronological stage of the embryo. Between Hamburger and Hamilton stages 5 and 23, the notochord gradually passes through three morphological phases which we will designate bilaminar; rod-like, unvacuolated; rod-like and vacuolated. In addition, it begins to secrete an extra-cellular sheath around itself toward the end of the rod-like, unvacuolated phase.

1. The bilaminar phase

Fig. 1 shows a stage-7 embryo which was transversely fractured about midway along the notochord. At this stage, the notochord seems to consist of two layers of cells, the more dorsal of which lies immediately beneath the neural plate, and the more ventral of which is situated directly above the endoderm. The cells of the dorsal layer have a well-defined dorso-ventral orientation, whereas those of the ventral layer are orientated medio-laterally. Short filopodia extend out from the dorsal cells toward the overlying neural plate, and when similar regions are examined by TEM the filopodia are found to touch the basal lamina of the neural plate (Fig. 3). Similar processes from the ventral cells contact the underlying endoderm. Laterally, the edges of the notochord are not always well defined and it is sometimes difficult to discover the boundary between the notochord and mesenchyme, whether by SEM or by TEM. This is probably exaggerated by the absence of the basal lamina around the notochord at this stage.

Within the notochord itself, the cells are fairly loosely packed and there are extensive intercellular spaces between them. Numerous points of contact are present between the notochord cells, both within the dorsal and ventral layers, and between the cells of the two layers (Fig. 2). Most of these contacts appear to be of a simple kind which consist of paired thickenings along the borders of apposed cells, though desmosomes with their characteristic accompaniment of bands of microfilaments have also been seen. At this stage the cytoplasm is still of the type characteristic of young embryos, in that it is rich in ribosomes and glycogen, but poor in endoplasmic reticulum. Lipid drops are frequently seen in the cells and these are probably remnants of intracellular yolk drops. The mitochondria tend to be round in TS and their cristae are easily damaged.
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Microfilaments and microtubules are present but scarce. The nuclei usually possess one or two nucleoli and tend to be elongated along the length of the cell.

2. The rod-like, unvacuolated phase

With further development the notochord becomes separated from the adjacent mesenchyme and acquires a clear outline. Although it is now rod-like, it is not uniform along its length and this variation appears to be related to the segmental pattern of the somites. The notochord is widest at the intersomitic position and narrowest at the point half-way antero-posteriorly along each somite. This alternation of wide and narrow regions has been illustrated elsewhere by us (Bancroft & Bellairs, 1975). The depth of the notochord does not appear to be affected, but since the width varies quite substantially, the shape of the organ varies between being nearly circular to roughly oval in T.S.

Fig. 4 is an SEM micrograph of the fractured surface of the notochord in the anterior region of a stage-8 embryo. It is oval in shape and the lateral edges are now distinct from the adjacent head mesenchyme. This clear boundary is also visible in sections examined by TEM (Fig. 5) and it is present even when no basal lamina can be seen around the notochord. At its posterior end this notochord still resembles the one illustrated in Fig. 1.

The notochord during this phase still consists of two layers of cells, the dorsal ones being orientated dorsoventrally. Filopodia can still be seen extending from the dorsal cells toward the neural tube and from the ventral cells to the endoderm. When sections are examined in the TEM, the filopodia can be seen to be pressed up against the basal lamina of the neural tube as in the earlier stages. In the notochord illustrated in Fig. 4, the outlines of the cells are somewhat obscured because CPC was included in the glutaraldehyde fixative, but it is nevertheless apparent that large intercellular spaces are still present. These intercellular spaces are also visible in Figs. 5 and 6 and it can be seen that the cells are loosely packed. Many of the cells possess filopodia and these appear to

Figures 1–5

Fig. 1. SEM micrograph of a transversely fractured notochord. (Bilaminar phase from a stage-7 embryo.) d, dorsal cells; e, endoderm; np, neural plate; v, ventral cells. × 1600.

Fig. 2. SEM micrograph of a transversely fractured notochord. (Bilaminar phase from a stage-7 embryo.) d, dorsal cell; v, ventral cell. × 3350.

Fig. 3. TEM micrograph of a section through the border between notochord (n) and neural plate (np). (Bilaminar phase from a stage-7 embryo.) Note the filopodia (arrowed). × 15000.

Fig. 4. SEM micrograph of a transversely fractured notochord. (Rod-like, unvacuolated phase from a stage-8 embryo.) This specimen was treated with CPC. d, dorsal cell; v, ventral cell. × 1800.

Fig. 5. TEM micrograph through the lateral border of a transversely sectioned notochord. (Rod-like, unvacuolated phase from a stage-9 embryo.) There is no basal lamina. Note the filopodia extending into the intercellular spaces. × 5000.
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be extending into the intercellular spaces. Tight junctions and desmosomes are still present and are especially conspicuous near the margin of the notochord (Fig. 6).

About stage 8, the extracellular materials begin to appear around the notochord at its anterior end, though there is some variation from one embryo to another. These extracellular materials can be seen by SEM (Fig. 7) and are also visible in specimens examined by TEM as wisps of extracellular materials (Fig. 6) which appear at about the same time as the first signs of the basal lamina. The amount that is present varies, however, from one specimen to another as well as from one level of the embryo to another. During these early stages of sheath formation, the cytoplasm appears to be similar to that in the previous stage.

By about stage 9, further changes have already taken place at the anterior end of the notochord. This can be seen in Fig. 7, which shows marked differences from the less mature structures seen in the earlier figures. The cells are apparently mainly flattened in an antero-posterior direction and show the beginnings of radial arrangement. Most of the cells show a tendency towards a triangular shape in which their apices lie centrally and their bases at the outer edge of the notochord. Intercellular spaces are considerably less extensive than in the earlier stages, and cellular contacts are correspondingly more difficult to distinguish by SEM. Jurand (1962) noticed an increase in the numbers of cell contacts at about this stage.

In Fig. 7, small amounts of extracellular material can be seen surrounding the outer edges of the notochord, in one place running between the notochord and the endoderm. The regular smooth appearance of the notochordal margins might suggest that a well-formed basal lamina is now present, though TEM sections show that it is still in a rudimentary state as compared with the later

**Figures 6-10**

Fig. 6. TEM micrograph through the lateral border of a transversely sectioned notochord. (Rod-like, unvacuolated phase from a stage-9 embryo.) *ecm*, early signs of extracellular materials beginning to appear around the notochord; white arrows, desmosomes and tight junctions. × 20000.

Fig. 7. SEM micrograph through a transversely fractured notochord. (Rod-like, unvacuolated phase from a stage-9 embryo.) This specimen was treated with CPC. *ecm*, wisps of extra-cellular materials around the notochord. Note that the cells are becoming radially arranged. × 1600.

Fig. 8. SEM micrograph of a longitudinally fractured notochord. (Rod-like, unvacuolated phase from a stage-10 embryo.) Note the extracellular fibrils on both dorsal and ventral surfaces. × 7000.

Fig. 9. SEM micrograph of a notochord seen both from the dorsal aspect and in a transversely fractured plane. (Rod-like, unvacuolated phase taken from region anterior to the auditory pit of a stage-11 embryo.) *scl*, sclerotome cells. × 750.

Fig. 10. SEM micrograph of a transversely fractured notochord. (Rod-like, unvacuolated phase from a stage-12 embryo.) This specimen was treated with CPC. Note that many fine fibrils pass from the notochord to the sclerotome cells (*scl*) and from the notochord to the neural tube. × 1900.
stages (described below) and is still present only as a patchy layer. In the SEM micrographs, the notochord no longer appears to be making cellular contacts with the basal lamina of the neural tube, and there is no longer any close contact with the endoderm of the type seen in the earlier stages. The cytoplasm is beginning to take on a characteristic secretory appearance and so appears to be more differentiated than in the earlier stages. Ribosomes are still plentiful, but there appears to have been an increase in the amount of granular endoplasmic reticulum and of the Golgi apparatus. The endoplasmic reticulum is, however, still present as scattered profiles and not as stacks, whilst mitochondria and lipid drops resemble the structures seen earlier. Microtubules have increased in number and tend to run longitudinally along the elongated cells and in some places microfibrils lie in bands beneath the cell membrane and, extend into the filopodia. There is little change in the structure of the nuclei, which still tend to be round or oval in TS and to possess one or two nucleoli.

3. The development of the notochordal sheath

Once the notochord becomes rod-like, its overall appearance undergoes little further change for a considerable period, at least when viewed by SEM. However, alterations occur especially in the notochordal sheath, and later in the cells themselves which become vacuolated. Both these developments are visible by SEM and TEM.

Extracellular fibrils were seen in small numbers in the space around the notochord, about half-way along its length at stage 9 (Fig. 7). These increase with time and are more conspicuous at stage 10. Fig. 8 is an SEM micrograph of the notochord fractured in LS, and the extracellular material can be seen lying dorsal and ventral to the notochord. Extracellular fibrils also pass between the notochord and the sclerotoma at this stage. By stage 11, extracellular fibrils form a dense mat around the entire notochord (Fig. 9). In this micrograph, which is taken at the region anterior to the auditory pit, it can also be seen that the mesenchyme cells have a close relationship with the notochord, making many contacts on its outer surface.

The extracellular coat also makes contacts with the ventral surface of the neural tube (Fig. 10). It appears that the basal lamina of the notochord and that of the

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**Figures 11–13**

Fig. 11. SEM micrograph of the dorsal side of the notochord, covered in notochordal sheath (*not s*), and the ventral side of the neural tube, covered with neural sheath (*ns*). Note the connexions between the notochordal and neural sheaths (rod-like, unvacuolated phase from a stage-11 embryo). x 4000.

Fig. 12. TEM micrograph of a section through a similar region to the one shown in Fig. 11. Note the fibrils passing between the notochordal and neural sheaths. (Rod-like, vacuolated phase from a stage-15 embryo.) x 25000.

Fig. 13. TEM micrograph of a section through the ventral border of a notochord. (Rod-like, vacuolated phase from a stage-23 embryo). *bl*, basal lamina; *mf*, microfilaments; *not s*, notochordal sheath; *sel*, sclerotome cell. x 25000.
neural tube are now connected to one another by many fine fibrillar processes, and these can also be seen in Fig. 12 which is a section through a similar region in an older embryo. Similarly, extracellular fibrils pass from the notochord to the endoderm at this stage.

With further development, sclerotome cells begin to migrate between the notochord and the endoderm, although the close contacts between the notochordal sheath and the neural sheath remain (Fig. 10).

The extracellular coat is shown at higher magnification in Fig. 11 which was taken at stage 11. Its appearance by SEM changes very little, at least until about stage 20. It consists of a mass of fine fibrils which appear to be without overall orientation, though in some areas there is a tendency toward an antero-posterior orientation, especially on the ventral surfaces. Sections examined by TEM show that the notochordal sheath increases in thickness during this period. It is most dense near the balsa lamina of the notochord which has now become well defined (cf. Figs. 12 and 13). Short cellular processes can sometimes be seen passing through the basal lamina from the notochord into the surrounding space. This type of process may perhaps permit direct cell to cell contact between sclerotome and notochordal cells.

The notochordal sheath is similar in appearance to the neural sheath, by both SEM and TEM, although the notochordal sheath appears slightly earlier in development. Its structure in TEM has already been described in detail by others (e.g. Frederickson & Low, 1971; Lauscher & Carlson, 1975). Like Frederickson and Low, we have found fibrils measuring about 10 nm near the basal lamina, and rather thicker fibrils further distally. Beaded structures can be seen on many of the fibrils. Our histochemical studies on the notochordal sheath are also in general agreement with those of previous workers (see Discussion).

4. The rod-like, vacuolated phase

Soon after the notochordal sheath starts being laid down, striking changes are seen taking place in the cells of the notochord. At about stage 14, rounded intracellular vacuoles begin to be visible and these soon increase in number.

Figures 14-17

Fig. 14. SEM micrograph of a section through the notochord and base of the neural tube. (Rod-like, vacuolated phase from a freeze-fractured stage-16 embryo.) Note the vacuoles (arrowed) at the cut surface of the notochord; nt, cut surface of the neural tube; not. s, notochordal sheath. × 670.

Fig. 15. TEM micrograph of a section through a notochordal cell. (Rod-like phase from a stage-17 embryo.) Note the Golgi profiles containing granular material, and the microtubules. × 18000.

Fig. 16. TEM micrograph of a section through a notochord cell. (Rod-like, vacuolated phase from a stage-17 embryo.) Note that the endoplasmic reticulum contains granular material; v, vacuole. × 25000.

Fig. 17. TEM micrograph of a section through a notochord cell. (Rod-like, vacuolated phase from a stage-16 embryo.) The fine fibrous material is probably intercellular. × 23000.
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They can be seen especially clearly in freeze-fractured specimens examined by SEM because the fracture plane obtained with this method tends to pass through the cytoplasm and thus exposes the vacuoles as holes (Fig. 14). The holes appear to be distributed mainly at random at the broken surface, though they seem to be less numerous near the periphery. The appearance of these vacuoles corresponds with dramatic changes in the nature of the cytoplasm. The cells are still rich in ribosomes, but the amount of both granular endoplasmic reticulum and Golgi material has increased, and each of these profiles is packed with a fine granular material (Fig. 15). Some of the intracellular vacuoles appear to be empty (Fig. 16) but others contain a mass of granular material. Vacuoles containing fine fibrous material similar to the notochordal sheath as seen in Fig. 17 are probably intercellular, though the angle of section often makes it difficult to decide. At the same time the morphology of the mitochondria has changed to that which is generally considered to be that characteristic of secretory tissue (Fig. 16). Many microtubules (Fig. 15) are present and bundles of microfilaments come to lie beneath the cell membrane, especially around the periphery (Fig. 13). Lipid drops appear to be scarcer. The nuclei show little change, though mitotic figures are still visible, and midbodies and telophase bridges have been seen which are comparable to those already described for the epiblast (Bellairs & Bancroft, 1975).

At this stage the cells have become more closely packed, have retained their tendency to be wedge-shaped with their apices toward the centre of the notochord. When these cells were examined in stereo by SEM they were found to be flattened in an antero-posterior direction. When sections were examined by TEM, two different types of cells were sometimes visible, one being more electron opaque than the other. These two categories of cells have previously been distinguished by Jurand (1962) but their significance is not known. Both by SEM and TEM it can be seen that most of the intercellular spaces have

**Figures 18-22**

Fig. 18. SEM micrograph of a transversely fractured notochord. (Rod-like, vacuolated phase from a stage-23 embryo.) not. s, notochordal sheath; nt, neural tube; scl, sclerotome cells. × 330.

Fig. 19. SEM micrograph of an enlarged region of the vacuolated notochord seen in Fig. 18. Note that the cytoplasm is restricted to a narrow band around each vacuole. × 2010.

Fig. 20. TEM micrograph of a section through a notochord. (Rod-like, vacuolated phase from a stage-23 embryo). c, cytoplasm; n, nucleus; not. s, notochordal sheath; scl, sclerotome cells; v, vacuole. × 2500.

Fig. 21. TEM micrograph of a section through the cytoplasm from the notochord shown in Fig. 20. The edge of the notochord and the accompanying basal lamina is on the left. Note the granular endoplasmic reticulum and its continuity with an intracellular vacuole. × 25000.

Fig. 22. TEM micrograph of a section through the junction of three cells from the specimen shown in Fig. 21. d, desmosomes. × 15000.
become much reduced and in many places persist only as small gaps between close neighbours. Some of the intercellular spaces appear to have become larger, however, and these frequently contain fibrillar material. Although it seems possible that some of these spaces may be intercommunicating, we found no evidence of any central lumen. True desmosomes were seen at cell junctions, especially near the periphery of the organ, and structures which appeared to be either tight or gap junctions were also visible.

Between about stages 17 and 23 the number of vacuoles increases so much that almost every notochord cell seems to consist of a very large vacuole surrounded by a thin layer of cytoplasm (Figs. 18, 19). Fig. 20 is a TEM micrograph of a section across the notochord at this stage and it can be seen that the cytoplasm tends to be packed closely around the nuclei. Stacks of granular endoplasmic reticulum are still present and may be seen at higher magnifications, and occasionally they are in direct continuity with intracellular vacuoles (Fig. 21). Desmosomes are frequent (Fig. 22) and bands of microfibrils are conspicuous. Small intercellular spaces still persist, but the large spaces are intracellular and contain fibrillar material. Pinocytotic-like invaginations of the type described by Jurand (1962) and the membrane-bound bodies of Carlson (1973) have also been seen.

Between about stages 16 and 23, a dramatic increase in the size of the notochord takes place. At about stage 16, the notochord tends to be about 30 \( \mu \text{m} \) in depth, though of variable width, and there are about 13 or 14 cells visible in transverse view. By stage 23 the notochord is about 115 \( \mu \text{m} \) deep by about 160 \( \mu \text{m} \) wide. The cells are difficult to count with precision, but there are usually about 60 present in any transverse section.

DISCUSSION

There are three major points for discussion.

1. Changes in the shape of the notochord

When the notochord begins to develop, it has a bilaminar structure and is not clearly delineated from the mesoderm at either side. Subsequently, it becomes rod-like, with most of the cells wedge-shaped in TS, and the organ now becomes marked off from the lateral mesoderm. Later it becomes vacuolated, and consequently larger in diameter. Several factors may be correlated with the change from the bilaminar to the rod shape, though it is difficult to decide which play the most fundamental role. The delineation from the adjacent mesoderm begins to take place just before the laying down of the basal lamina and seems to be associated with the rearrangement of the cells. In our SEM studies we saw that the more ventral cells in the two layered stages were elongated in a medio-lateral direction, whereas by about stage 9 they had reoriented themselves so that they extended in an antero-posterior direction and in TS all the cells were
beginning to show signs of a radial arrangement. The changes in cell shape are accompanied by an increase in the specialized contacts between the cells, especially around the periphery of the organ. In the early stages these are either terminal bars (tight junctions), as reported by Jurand (1962), or rather simple desmosomes. Later, more elaborate desmosomes become apparent. At the same time as the cells come into closer contact, the intercellular spaces become reduced, as Jurand (1962) has already reported. This author has suggested that increased cell-to-cell contact is the morphogenetic factor responsible for causing the notochord to become rod-shaped, and we are in agreement that it plays an important role.

We have found that the changes in the cell shape are accompanied by certain intracellular changes which may be of importance in the alteration of the shape. These include an increase in the numbers of microtubules and many of these tend to be orientated along the length of the cells, and therefore radially in relation to the whole organ. It has been suggested that microtubules by aligning themselves within a cell help to establish its direction of elongation. Burnside (1971), who demonstrated an orderly arrangement of the microtubules in the elongating neural cells of the newt, *Taricha torosa*, concluded that these helped to promote cell elongation during neurulation. It may be noted, however, that the numbers of microtubules are considerably less in the chick notochord than in the newt neural cells, so that their relative importance in shape changes may also be less.

A more fundamental role may be played by the bands of microfibrils which appear beneath the cell membrane at the periphery of the notochord. These have previously been described by Ruggeri (1972). They become denser and more numerous with further development (cf. Figs. 12 and 13) and it seems possible that by contracting, they enable the notochord to form a firm boundary at a time when it begins to increase in size due to vacuolation. It seems possible that the production of a basal lamina and then of the acellular sheath may also enable a firm edge to be retained. It seems likely that this rearrangement of the cells not only assists in the delineation of the notochord, but may also help it to elongate. We found both from our SEM and TEM studies that at stage 6 the anterior tip of the notochord was about 50 µm wide, at about stage 8 about 30 µm wide, and by stage 9 only about 20 µm wide. This reduction was coupled with a decrease of about 30% in the numbers of cells seen in each TS. These findings imply that the cells have reorientated themselves so that the major axis now lies along the antero-posterior axis of the embryo. It seems likely that the inward migration of some of the peripheral notochordal cells into the central area could also contribute to this reduction in cell numbers seen in TS, as well as the reduction in width which is about 60% between stages 6 and 9. A further reduction in width also occurs between stages 10 and 13 (Jurand, 1962).

In addition to the rearrangement of the cells, it has been suggested that mitotic division plays a role in increasing the length of the notochord (Jurand,
1962). Although we have made no special study of the mitotic figures or their distribution, we have frequently noted cells in division and have also seen telophase bridges similar to those present in the epiblast (Bellairs & Bancroft, 1975) and each of these marks the site of a former mitotic division.

The vacuolation of the notochord does not begin until after the sheath has begun to be laid down. It is generally considered that during its vacuolar stage, the notochord becomes turgid and provides a rigidity for the embryonic axis in the stages before the development of the vertebral column (Romanoff, 1960). Like other investigators, we have found that some of the vacuoles are intracellular (Ruggeri, 1972; Jurand, 1962) whilst others are intercellular spaces (Carlson, 1973a). The intracellular vacuoles sometimes possess faint amorphous contents, but often appear to be empty (Fig. 16), whilst the intercellular spaces tend to contain fibrillar material. It seems likely that the contents of both types of vacuole are highly hydrated, but the way that this water enters the cells is not understood. Jurand (1962) has suggested that water enters the notochordal cells by pinocytosis, since he has found pinocytotic-like invaginations and vesicles, and we have confirmed his observations. Recently, however, Jurand (1974) has described similar invaginations in the notochord of 11-day-old mouse embryos, but these do not appear to result in vacuolization. It now seems possible, therefore, that pinocytosis in the notochord of the chick may not be totally or even partially related to vacuolization. We have seen that microtubules are often present in the notochord, and in TS tend to be orientated from the more central regions to the periphery, though it is difficult to know whether they play a part in reorganizing the general shape of the cells. It seems possible, however, they may transport fluid into the most central part of the organ at a later stage.

2. The notochordal sheath

It was noted by Ruggeri & Scandrioglio (1968) that the perichordal fibrillar coat began to appear at exactly the same time as the notochordal cytoplasm acquired the appearance characteristic of secretory cells. Ruggeri (1972) found that this event had begun by stage 10, and we have been able to show that it has started even at stage 9. We have confirmed the observations of earlier workers (Duncan, 1957; Leeson et al. 1961; Jurand, 1962; Low, 1970) that the extracellular materials continue to increase around the notochord during the following stages. Ruggeri (1972) concluded that some of these extracellular materials diffuse away from the notochord and become distributed among the sclerotome cells.

According to Low and his collaborators (Low 1968, 1970; O'Connell & Low, 1970; Carlson & Low, 1971; Frederickson & Low, 1971; Carlson, 1973a), the smaller type of fibril which has a diameter of about 10 nm and is digestible with hyaluronidase, may be a precursor of the larger type, which is about 20 nm in diameter and is digestible with collagenase.

It has been known for many years that the notochord stimulates chondro-
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genesis in somite mesoderm, both in vivo and in vitro (Lash, 1963, 1968a, b, c) and this occurs at the time when it is secreting both collagen and proteoglycans (e.g. Kvist & Finnegan, 1970a, b; Bazin & Strudel, 1972; Linsenmayer, Trelstad & Gross, 1973; Lauscher & Carlson, 1975). In an elegant and highly controlled series of experiments Kosher, Lash & Minor (1973) and Kosher & Lash (1975) have implicated the proteoglycan components in somite chondrogenesis; these are chondroitin-4-sulphates (40 %), chondroitin-6-sulphates (40 %), and heparan sulphates (20 %). Our own histochemical results support their conclusions. There is as yet no evidence as to whether the collagen fibrils play a major role in chondrogenesis. Nor is it known whether the cellular contacts between the Sclerotome cells and the notochordal sheath are important in the control of the process. The immunological role of proteoglycans in promoting cell recognition is now well established (see, for example, Roth, McGuire & Roseman, 1971). It seems possible, therefore, that as the proteoglycan component of the notochordal sheath comes into contact with the sclerotome cells, it directs their movement toward the notochord, thus promoting a chemotactic response.

3. The notochord as an epithelium

It is often stated in the literature that the notochord is an epithelium (e.g. Hay, 1968; Hay & Meier, 1974; Carlson, 1973a). This idea appears to derive from the era prior to TEM studies when it was thought that a single central lumen was present (Kuhlenbeck, 1930). The present TEM studies confirm the findings of Jurand (1962) that no single central lumen is present, but only a number of irregular intercellular spaces. Furthermore, unlike cells in a true epithelium, the cells bordering the spaces do not appear to be bounded by an apical series of tight junctions. Although we know that certain epithelial features are present, such as the fact that the cells lie close together in an orderly arrangement, and that a basal lamina comes to surround the organ, the cells are not arranged in a sheet as a characteristic epithelium might be and indeed some of the cells lie medially in the organ (see Figs. 7 and 8). For these reasons, we feel that to regard the notochord as an epithelium is a misleading concept.

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REFERENCES


Development of notochord in chick embryo


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