

Development of the notochord in normal and malformed human embryos and fetuses

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ABSTRACT In view of its possible involvement in early embryogenesis and teratogenesis, the developmental characteristics of the human notochord were studied by light and electron microscopy and immunohistochemistry on 20 human conceptuses (5th-22nd week). At the earliest embryonic stages examined, the notochord is closely related to both the pharyngeal endoderm and the neuroectoderm of the posterior (tail) end of the neural tube. In both regions the interspace is bridged by slender cytoplasmic processes, lined with basal lamina and filled with amorphous extracellular material containing collagen types III and IV and laminin. The notochordal cells express cytokeratin brightly and vimentin weakly. As embryonic age progresses, the notochord gradually separates from the epithelia, becomes the axis of developing spinal column and undergoes progressive cell degeneration and rearrangement within the vertebral bodies. This is associated with extensive production of extracellular material and the first appearance of fibronectin. Intracellularly, the expression of vimentin gradually increases, while that of cytokeratin slightly weakens. Changes in the notochord parallel other developmental events in axial organs. In anencephalic fetuses the course of the notochord is irregular and partly interrupted with segments outside the basichondrocranium in specimens with craniorachischisis.

KEY WORDS: *notochord, human embryos, skull base, axial structures*

Introduction

Although a transitory embryonic organ, the notochord plays an important role in early embryogenesis (Willis, 1962; Carlson, 1973; Strudel, 1976). It probably acts as a primary organizer for adjacent embryonic organs (Jurand, 1974) inducing formation of the neural tube and the vertebral column (van Straaten and Drukker, 1987; Smith and Schoenwolf, 1989). In addition, the notochord is the first embryonic cellular structure that actively produces fibrillar extracellular matrix (Frederickson and Law, 1971; Carlson and Upson, 1974; Strudel, 1976), which is essential for the process of chondrogenesis in the surrounding sclerotomal mesenchyme (Strudel, 1967; Zilliken, 1967; Carlson, 1973; Strudel, 1976). Later in development it degenerates and participates in the formation of the nucleus pulposus of the intervertebral disc (Shaffer, 1930; Peacock, 1951, 1952; Wolfe *et al.*, 1965). Notochordal vestiges in man have a distinct pathology (Willis, 1962) and chorda-mesoderm disturbances are considered to be the primary cause of anencephaly (Marin-Padilla, 1965a,b, 1966a,b, 1979). This explains the great interest which the notochord has elicited among biologically and medically oriented scientists for several decades.

Knowledge about the notochord has mostly been based on experimental work done on different classes of animals and only a

few light and electron microscopic studies (Kunimoto, 1918; Peacock, 1951, 1952; Trout *et al.*, 1982a,b; Shinohara and Tanaka, 1988) were performed on human samples.

The main interest in studying human notochord has been oriented towards the origin of the nucleus pulposus, and there is still insufficient knowledge concerning the notochord in early human embryogenesis, its relationship with neighboring structures and its possible involvement in the genesis of developmental anomalies of the body axis.

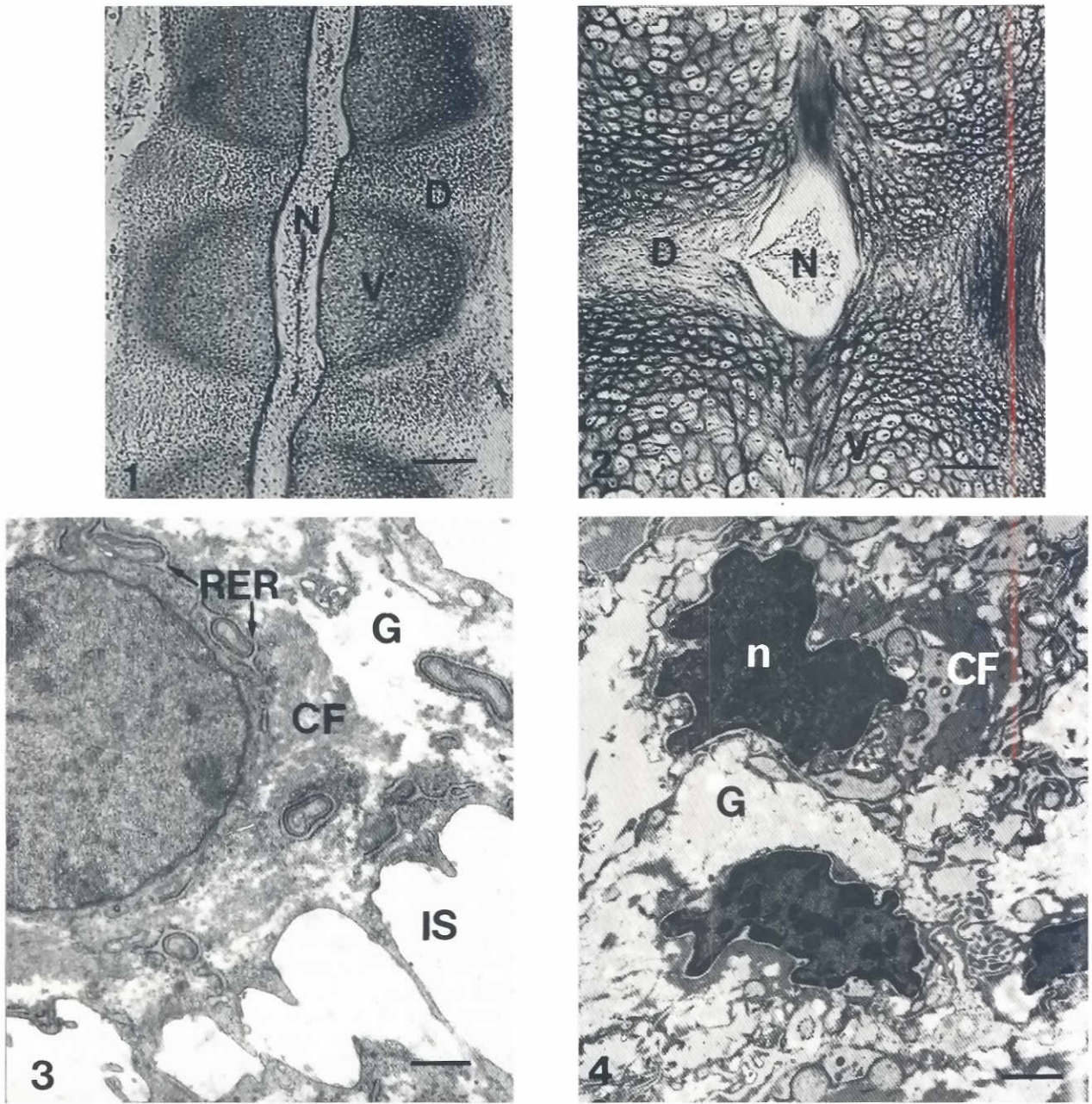
Here we give a concise survey of our studies performed on human embryos and fetuses ranging in age from the 5th to the 22nd week of pregnancy. The normal course of development was investigated at the thoracic level of embryos and fetuses. Furthermore,

Abbreviations used in this paper: A, dens axis; ACV, area cerebrovasculosa; BC, basichondrocranium; bl, basal lamina; C, vertebral column; CF, cytofilaments; CNS, central nervous system; CR, coccygeal rest; D, intervertebral disc; E, pharyngeal endoderm; ECM, extracellular matrix; G, glycogen; H, hypophysis; IS, intracellular spaces; M, mesenchyme; N, notochord; n, nucleus; O, ossification center; R, Rathke's pouch; RER, rough endoplasmic reticulum; S, spinal cord; V, vertebral bodies.

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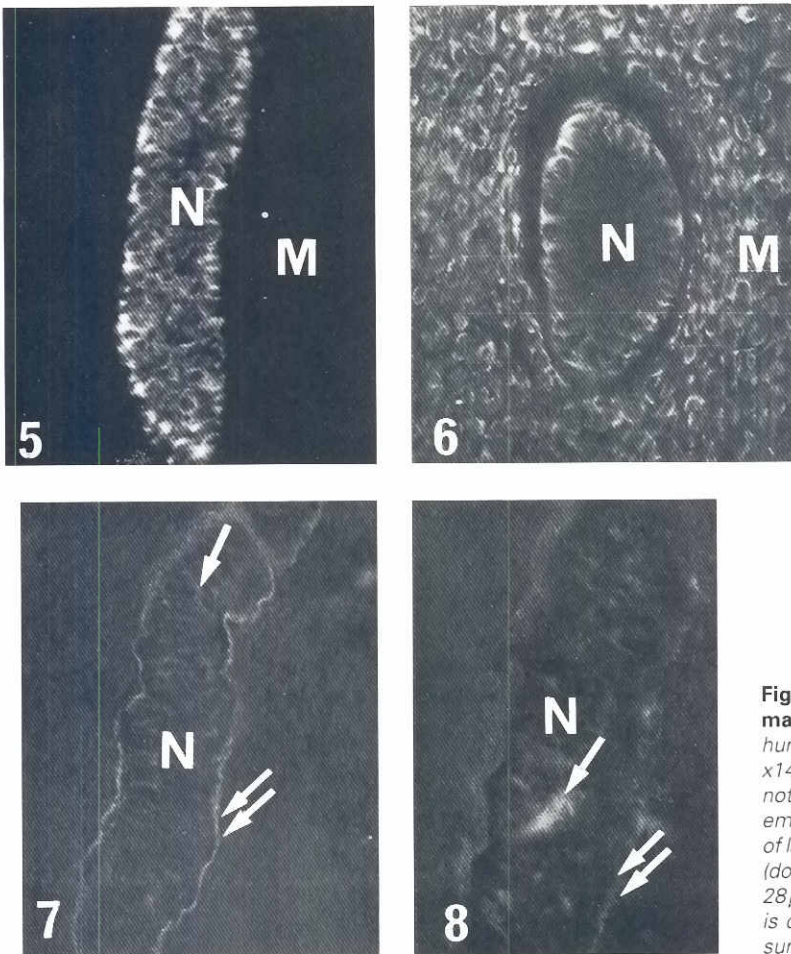
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Figs. 1-4. Morphological features of the human notochord. (1) Mid-sagittal section through the vertebral column of a 5-week-old human embryo. The notochord (N) extends as a solid rod of cells through the future vertebral bodies (V) and intervertebral discs (D). Sirius red x90 (scale bar 110 μ m). (2) Mid-sagittal section through the vertebral column of a 12-week-old human fetus. The notochord (N) forms the fetal nucleus pulposus of the intervertebral disc (D), while it degenerates inside the vertebral bodies (V). Sirius red x90 (scale bar 110 μ m). (3) Viable notochordal cell of an 8-week-old human embryo. Its cytoplasm contains mitochondria enclosed by rough endoplasmic reticulum (RER), cytofilaments (CF) and glycogen (G). Cytoplasmic extensions of the neighboring cells enclose numerous intercellular spaces (IS) - x12000 (scale bar 0.83 μ m). (4) Notochord of a 22-week-old fetus. Degenerating notochordal cells with picnotic nuclei (n), discharged glycogen (G) and homogenized masses of cytofilaments (CF) form the fetal nucleus pulposus. x9600 (scale bar 1 μ m).

we have given special attention to both ends of the notochord (head and tail regions) because of their possible relationship with the development of the skull and the fore-gut and the regression of the tail, respectively. Moreover, these are regions in which the major

anomalies of the body axis as well as tumorous vestiges of the notochord (chordomas) most often occur. Finally, we studied the anomalous course of the notochord within the skull base of anencephalic fetuses.



Figs. 5-8. Immunohistochemical changes in the developing human notochord. (5) Intensive cyokeratin expression in a 5-week-old human notochord (N). The surrounding mesenchyme (M) is negative. $\times 140$ (scale bar $70 \mu m$). (6) Vimentin expression in the peripheral notochordal cells (N) and in surrounding mesenchyme (M) (5-week-old embryo). $\times 200$ (scale bar $50 \mu m$). (7) Immunohistochemical localization of laminin in an 8-week-old embryo on the surface of the notochord (N) (double arrows) and in the intercellular spaces (arrows). $\times 350$ (scale bar $28 \mu m$). (8) Human notochord in a 6-7-week-old embryo (N). Fibronectin is distributed in the intercellular spaces (arrows) and weakly on the surface of the notochord (double arrows). $\times 400$ (scale bar $25 \mu m$).

Normal development

Light and electron microscopy

In 5-6-week-old embryos the notochord is a solid rod of cells which extends through the entire developing vertebral column (Fig. 1). Most of the cells have euchromatic oval nuclei, scattered cysternae of the granular endoplasmic reticulum, some of which intimately envelope mitochondria. The Golgi body is prominent, and a large part of the cytoplasm contains glycogen and intermediate filaments. Some cells are closely packed and connected by desmosomes, while other cells form cytoplasmic extensions surrounding extracellular spaces of different sizes. The same ultrastructural features are retained during subsequent weeks in all viable cells (Fig. 3). The basal lamina covers the surface of the cells and fine granular and fibrillar material is seen in both intercellular spaces and perinotochordal space (Saraga Babic *et al.*, 1984; Galic *et al.*, 1986).

From the 7th to the 12th week of development, the notochordal tissue gradually disappears within the future vertebral bodies which undergo chondrification and ossification. The remaining notochordal tissue forms fetal nucleus pulposus (Fig. 2). It contains the above-described viable cells (Fig. 3), but also an increased number of progressively degenerating cells which are characterized by irregularly shaped pre-picnotic nuclei and an increased amount of glycogen and cytoplasmic filaments. Cell processes enclose numerous

intercellular spaces of different sizes, filled with extracellular matrix components formed on fibrillar and flocculent material (Saraga Babic *et al.*, 1984; Galic *et al.*, 1986).

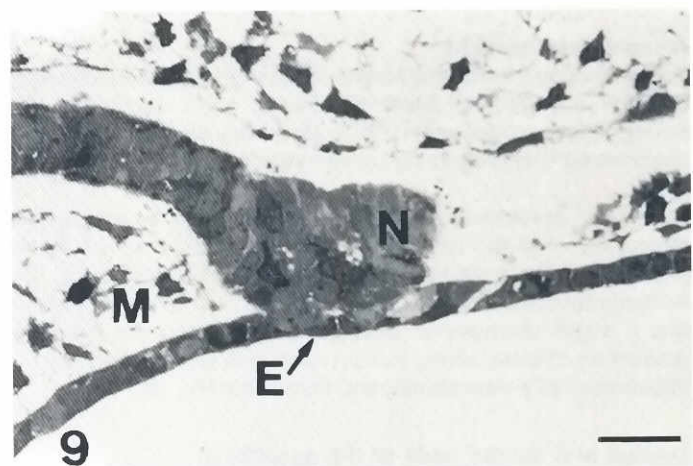
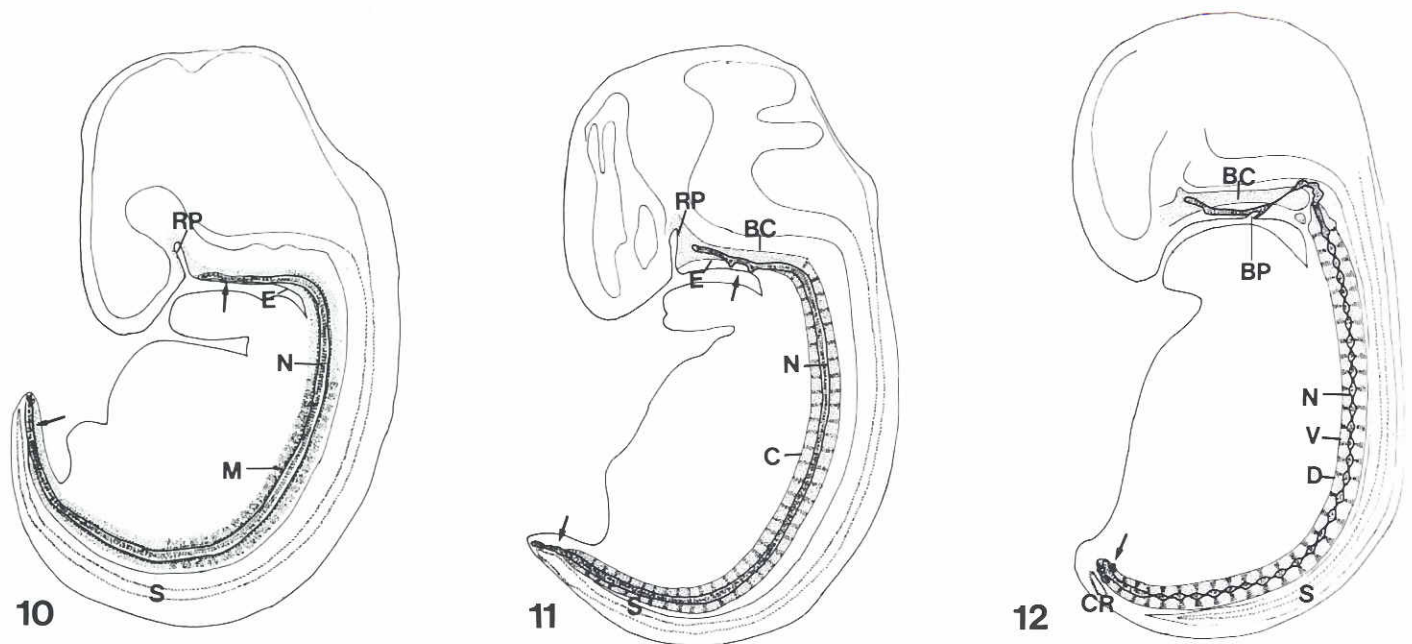


Fig. 9. Semi-thin sagittal section through the head of a 5-week-old human embryo. The site of attachment of the notochord (N) to the endoderm of pharynx (E). Mesenchymal cells (M) $\times 160$ (scale bar $40 \mu m$).



Figs. 10-12. Relationship of the notochord to the adjacent axial structures during human development. (10) Drawing of a mid-sagittal section through a 5-week-old human embryo demonstrates the relationship of the notochord (N) and adjacent axial organs: mesenchyme of the vertebral column and skull base (M) and neuroectoderm of the spinal cord (S). Areas of close association between the notochord and pharyngeal endoderm (E) in the head region and between the notochord and the neuroectoderm of the spinal cord in the tail region are indicated by arrows. Rathke's pouch invagination (RP). (11) Drawing of a mid-sagittal section through a 5-6-week-old embryo. The initial separation of the notochord (N) from the pharyngeal endoderm (E) and its partial incorporation into the developing basichondrocranium (BC) is indicated by an arrow. The separation (arrow) of the caudal end of the notochord from the spinal cord (S) is accompanied by shortening of the tail. Vertebral column (C), Rathke's pouch (RP). (12) Drawing of a mid-sagittal section through a 12-week-old human fetus. The notochord (N) degenerates inside the vertebral bodies (V) and widens in the intervertebral discs (D). In the head region, the partial degeneration of the notochord inside the basi-chondrocranium (BC) and development of bursa pharyngea is demonstrated. In the regressed tail region (sacrocoxygeal vertebrae) the arrow indicates branching of the notochord. Coccygeal rest (CR) of the regressing spinal cord (S).

In 22-week-old fetuses, dying cells are predominant. Vacuolated remnants of their cytoplasm, condensed masses of glycogen and filaments from neighboring cells merge into heterogeneous extracellular material forming the nucleus pulposus (Fig. 4) (Svajger *et al.*, 1984).

Immunohistochemistry

In earlier developmental stages, when treated with antibodies to different intermediate filament proteins, the notochordal cells strongly express cytokeratin (Fig. 5) but weakly vimentin (Fig. 6). The extracellular matrix contains collagen types III, IV and laminin (Fig. 7).

In later developmental stages, characterized by progressive degeneration of the notochordal cells, a change occurs in the expression of both the intracellular and extracellular proteins. The notochordal cells show a distinct increase in vimentin expression and a slight decrease in cytokeratin expression. An extensive production of extracellular matrix material is accompanied by the appearance of a new component, fibronectin (Fig. 8).

Cranial and caudal ends of the notochord

Head region

In early 5-week-old embryos the notochord extends in a wavy course throughout the condensed mesenchyme of the future skull

base, establishing several contacts with the pharyngeal endoderm (Figs. 9, 10).

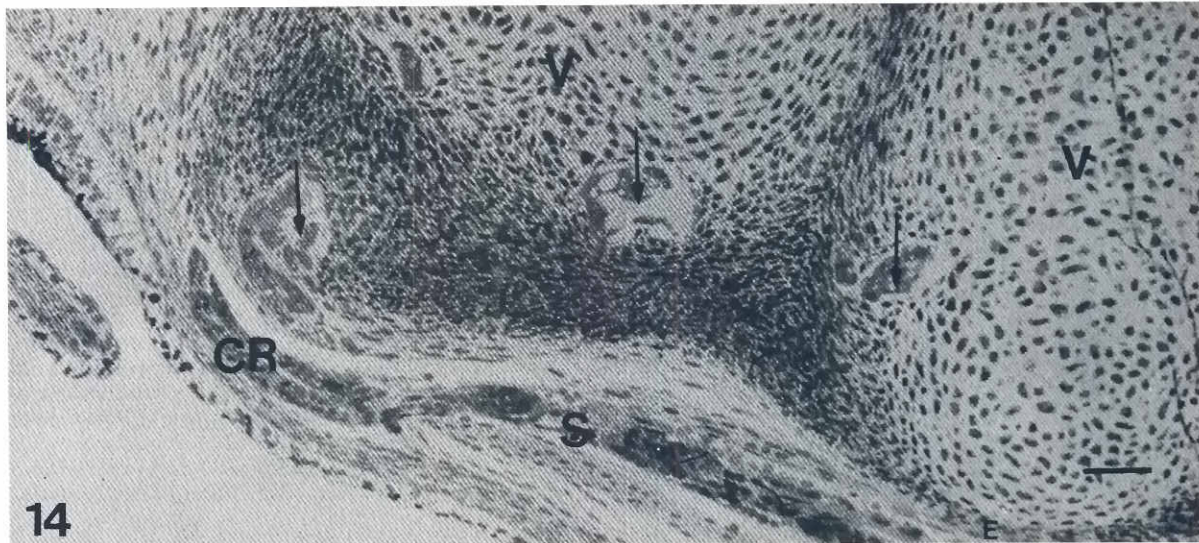
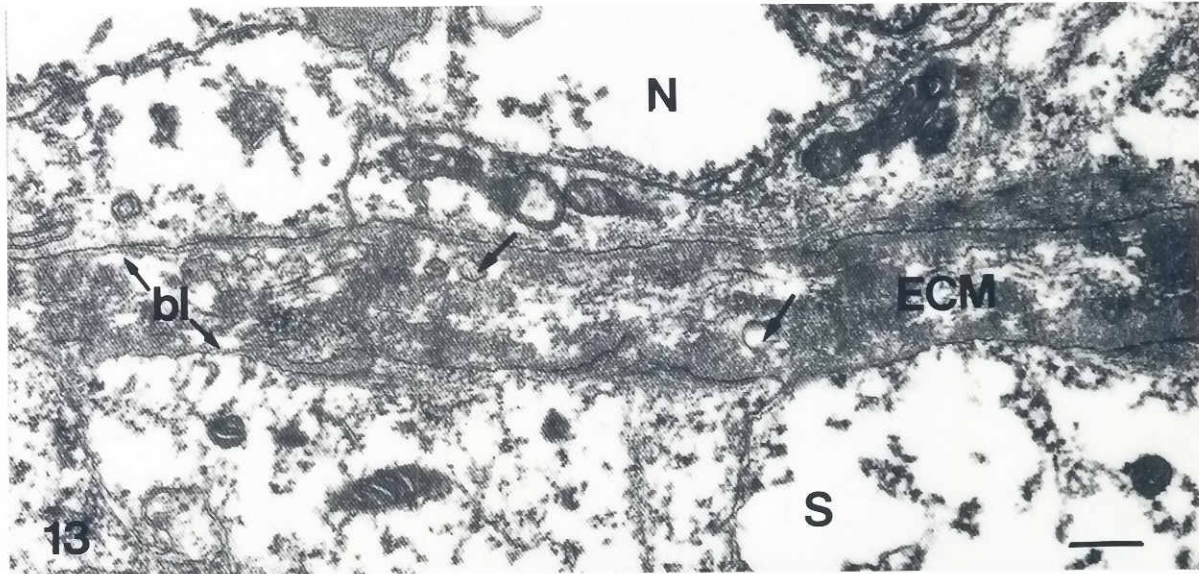
In areas of close contact between the two epithelia, poorly developed basal laminae and cell processes from both cell types are observed. On the other hand, basal laminae are prominent in areas where the notochord and the endoderm are separated by a space filled with extracellular matrix and mesenchymal cells.

In later developmental stages, the notochord branches and gradually separates from the pharyngeal epithelium by the interposition of connective tissue (Fig. 11), except in the area of the bursa pharyngea where it remains associated with the pharyngeal epithelium (Fig. 12) (Saraga Babic, 1990).

Between the 7th and the 12th weeks of development, the notochord changes its relationship with the developing skull base, into which it is partly incorporated (Fig. 12). The progressive degeneration of notochordal tissue, first observed inside the chondrifying and ossifying skull base, and later on also around the skull base, is finished by the end of the 16th fetal week.

Tail region

In the tail region the caudal end of the notochord undergoes a process very similar to the one observed at its cranial end. Close association between the notochord and the ventral wall of the spinal cord is observed in the most caudal part of the tail in the 5th developmental week (Fig. 10). In the long area of their close contact,



Figs. 13-14. Notochord in the tail and head region. (13) Close association between the notochord (N) and the spinal cord (S) in the tail of a 5-week-old embryo. Cross sections through small cell projections (arrows), basal laminae (bl) lining both apposed epithelia and extracellular matrix components (ECM) are visible within their interspace. $\times 10000$ (scale bar $1 \mu m$). (14) Sacrococcygeal region of a 10-week-old fetus. Branching of the notochord (arrows) within the last two coccygeal vertebrae (V) and regressive changes within the spinal cord (S). Coccygeal rest (CR). Semi-thin section, Toulidine blue $\times 500$. (scale bar $20 \mu m$).

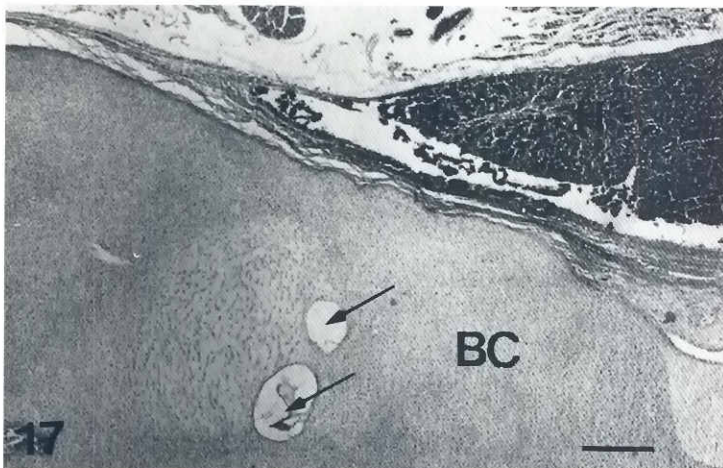
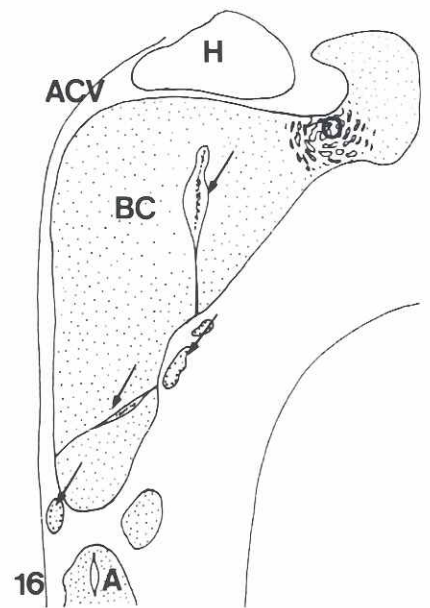
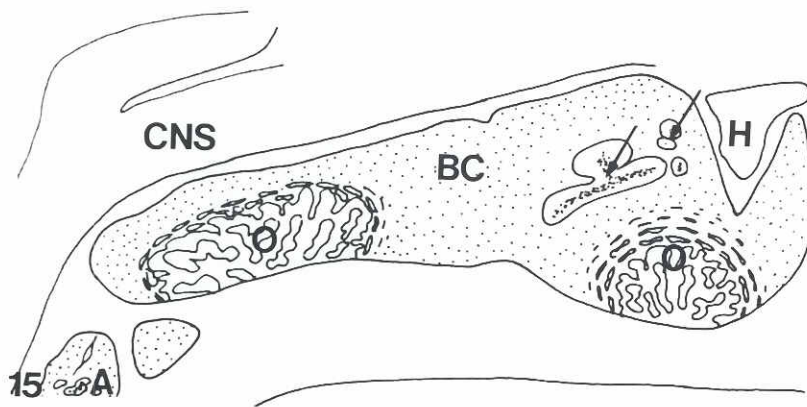
small cell projections and amorphous extracellular material are seen within their interspace. Well-developed basal laminae cover both apposed epithelial cells (Fig. 13).

Between the 6th and the 12th developmental week, a progressive separation of the notochord from the spinal cord by interposition of mesenchymal cells of the future spinal column occurs in a cranio-caudal direction (Figs. 11, 12). During the same period, regression of the tail is accompanied by the chondrification of the coccygeal vertebrae, extensive branching of the notochord and degeneration and connective tissue transformation of the caudal part of the spinal cord, except for its most caudal end which remains

as the epithelial coccygeal rest (Fig. 14) (Saraga Babic *et al.*, 1989). In the fetal period the complete notochordal tissue inside the vertebral bodies degenerates parallel to the ossification of the vertebrae (Stefanovic *et al.*, 1989).

Anencephalic fetuses

Anencephaly is the most severe disturbance of development of both brain and skull. The brain tissue is reduced to atypical remnants combined with vascularized connective tissue (area cerebrovasculosa). The skull vault is practically absent. The anomaly



Figs. 15-17. Notochord in anencephalic fetuses. (15) Schematic reconstruction of cephalic structures in a 16-week-old anencephalic fetus with cranioschisis. Slightly curved basichondrocranium (BC) contains residual branches of the notochord (arrow) which end close to the hypophysis (H). The central nervous system (CNS) structures are partly developed. Dense axis (A), ossification centres (O). (16) Schematic reconstruction of cephalic structures in a 16-week-old anencephalic fetus with craniorachischisis. Considerably curved basichondrocranium (BC) shows delayed ossification (O). Variable remnants of the notochord (arrows) are seen inside and around the basichondrocranium. The central nervous system is reduced to area cerebrovasculosa (ACV), hypophysis (H) is abnormally positioned. Dense axis (A). (17) Part of the skull base in a 22-week-old anencephalic fetus with craniorachischisis. Curved basichondrocranium (BC) contains irregular notochordal branches (arrows) which end close to the abnormally developed hypophysis (H). x20 (scale bar 500 μ m).

can occur as an isolated entity (cranioschisis) or it can be combined with the analogous dysraphic anomaly of the spinal cord and the vertebral column (craniorachischisis) (Willis, 1962). To our knowledge we performed the first histological analysis of the complete skull basis in anencephalic fetuses. We gave special emphasis to the expected anomalous features of the notochord in this region.

Cranioschisis

When compared with normal human fetuses, the skull base of malformed fetuses is slightly curved and its angle towards the vertebral axis is enlarged. The remnants of the notochord are restricted to the interior of the basichondrocranium, where their persistence outlasts that of normal fetuses (Fig. 15). Between variably deformed vertebral bodies, the nucleus pulposus is sometimes displaced (Stefanovic *et al.*, 1989).

Craniorachischisis

The skull base is considerably curved and erected to a vertical position. Variable remnants of the notochord reside both inside and

around the basichondrocranium, which is characterized by delayed ossification (Saraga Babic and Svajger, 1987; Figs. 16, 17). Besides changes in the width and shape of the vertebral bodies, their fusion and hemivertebrae are sometimes found. Many irregular notochordal branches and abnormal ossification centres characterize these vertebrae (Stefanovic *et al.*, 1989).

Concluding remarks

During early human embryonic development, the notochord shows close associations with the pharyngeal endoderm in the head region, and with the neuroectoderm of the spinal cord in the tail region. At other levels the notochord forms the core of the future spinal column. The cranial and the caudal ends of the notochord establish intimate relationships with adjacent epithelia via thin cell processes. In both head and tail regions the apposed epithelia are lined with basal laminae and their interspaces are filled with small amounts of amorphous extracellular material. This type of contact has previously been demonstrated between the notochord and

endoderm in mouse and chick embryos (Jurand, 1974; Bancroft and Bellairs, 1976) and between the notochord and ectoderm in amphibians (Grunz and Staubach, 1979; Tacke and Grunz, 1988). Although transitory in nature, these early embryonic tissue contacts could be indicative of developmentally relevant interactions between the notochord and neighboring epithelia, independently of their nature and embryonic origin.

In later developmental stages, changes in the course and degeneration of the intracranial notochord are related to formation of other structures in the head area such as bursa pharyngea and the skull base (Saraga Babic, 1990). Various regional cell interactions between the notochord and the neighboring structures in the head area could therefore be important for the normal formation of cephalic structures.

In the tail region, the separation of the notochord from the spinal cord coincides with degeneration and shape changes of the notochord, the spinal cord and the most caudal somites (future coccygeal vertebrae). These spatially and temporally coincidental changes of the relationship between the notochord and the spinal cord should have a developmental significance in the regression of the human tail (Saraga Babic *et al.*, 1989).

After having accomplished its role in early embryogenesis, the notochord becomes the axis of the developing vertebral column. The transition of the epithelial features of the notochordal cells into mesenchymal ones during development of the vertebral column is characterized by changes in the general appearance of a notochord as a tissue, the rate of viable and dying cells (Galic *et al.*, 1986), and changes in the production and expression of intracellular and extracellular proteins. Both the intracellular and extracellular products of the notochordal cells increase in amount and complexity at later developmental stages. All these morphological and biosynthetic changes terminate with the programmed death of the notochord, which is its final destiny.

Finally, our analysis of anencephalic fetuses confirms the correlation between anomalies of the notochord and the abnormal formation of axial structures. There is an evident relationship between affection of the notochord and the central nervous system with its enclosing skeletal structures.

In conclusion, our morphological and immunohistochemical study of the human notochord supports the concept of its different interactions with adjacent organ structures of ectodermal, mesodermal and endodermal origin. Therefore, although a transitory embryonic structure, the notochord probably plays an important although not yet sufficiently clarified role in normal and abnormal morphogenesis of axial structures of the body. To determine the exact nature of these interactions, more specific methods should be applied.

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