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# Notochord action on spinal development

## A histologic and morphometric investigation

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Forty-seven human embryos and fetuses of a gestational age from 5 to 13 weeks were studied histologically and histomorphometrically in an attempt to elucidate the possible action of the notochord on spinal development, in particular as it relates to segmentation. Segmentation occurred first in the tissue surrounding the notochord and not in the notochord itself; notochordal development at its most cranial part lagged behind that of its surrounding tissue. We also found that in later stages the decrease of notochordal cells at the level of the body was due to degeneration and not to migration of cells toward the intervertebral space. This observation puts into doubt the theory of squeezing or migration of notochordal cells from their vertebral position into the intervertebral disc space.

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Isolated malformations of the anterior part of the spinal column in humans have been attributed to malformation or functional insufficiency of the notochord (Shapiro & Eyre 1981). In fact these authors stated that congenital scoliosis results from a malformation of the notochord, an interpretation based on studies of notochordal development in lower vertebrates.

We have studied human embryos in an attempt to elucidate the possible action of the notochord on spinal development, in particular as it relates to segmentation.

## Material and methods

Forty-seven human embryos and fetuses with a crown-rump length from 7 to 85 mm and a gestational age according to Patten (1968) between 5 and 13 weeks formed the basis of this study. Whereas in embryos of a crown-rump length of less than 20 mm the whole specimen was sectioned, in larger specimens the vertebral column was dissected and embedded in 10% neutral formalin, radiographed and decalcified in 12% EDTA. After embedding in paraffin, sagittal and frontal serial 6 μm sections were made and stained with hematoxylin-phloxine-saffron and Mann Dominici.

To determine the stage of development of each embryo, the mean segment height was measured (S.H.: distance between the middle of the adjacent dark zones or intervertebral disc). The shapes of the notochord as outlined by the marginal notochord sheath at the vertebral body level changed during development and permitted a classification into five stages. In Stage 1, the shape was cylindrical (age less than 5½ weeks, S.H. between 220 and 290 μm, 4 embryos); in Stage 2, it was convex (age between 5½ and 6½ weeks, S.H. between 250 and 350 μm, 11 embryos); in Stage 3 it was intermediate (age between 6½ and 8 weeks, S.H. between 320 and 490 μm, 10 embryos); in Stage 4 it was concave (age between 8 and 9 weeks, S.H. between 400 and 560 μm, 9 embryos); and in Stage 5 only a mucus streak remained (age between 9 and 13 weeks, S.H. between 700 and 1500 μm, 13 embryos).

The sclerotomic tissue was subdivided into three areas. The number of nuclei was counted using a Zeiss integrated eye-piece ruler allowing visualization of an area of 8.8 μm length and 56.8 μm in width for a total of 500 $\mu m^2$.

## Results

### A. Segmental height and development of the spine

When plotting values for segmental height and the age derived from measurements of crown-rump length, a close but not exact relationship was noted. Up to 13 weeks the S.H. values were more reliable for the determination of age than the crown-rump length.

### B. Evidence of segmentation

Observations of the sclerotomic tissues surrounding the notochord were facilitated by defining three distinct areas. Area A was closest to the notochord and had a thickness of a few cell layers. The adjoining area B comprised the wide middle portion of the anterior part of the spinal column. Area C constituted the peripheral portions of the anterior part of the spinal column eventually forming the anulus fibrosus and the ligaments.

*Stage 1* (Figure 1 and 2). Embryos did not show any evidence of segmentation of the notochord nor of area A. The size, shape, spatial arrange-

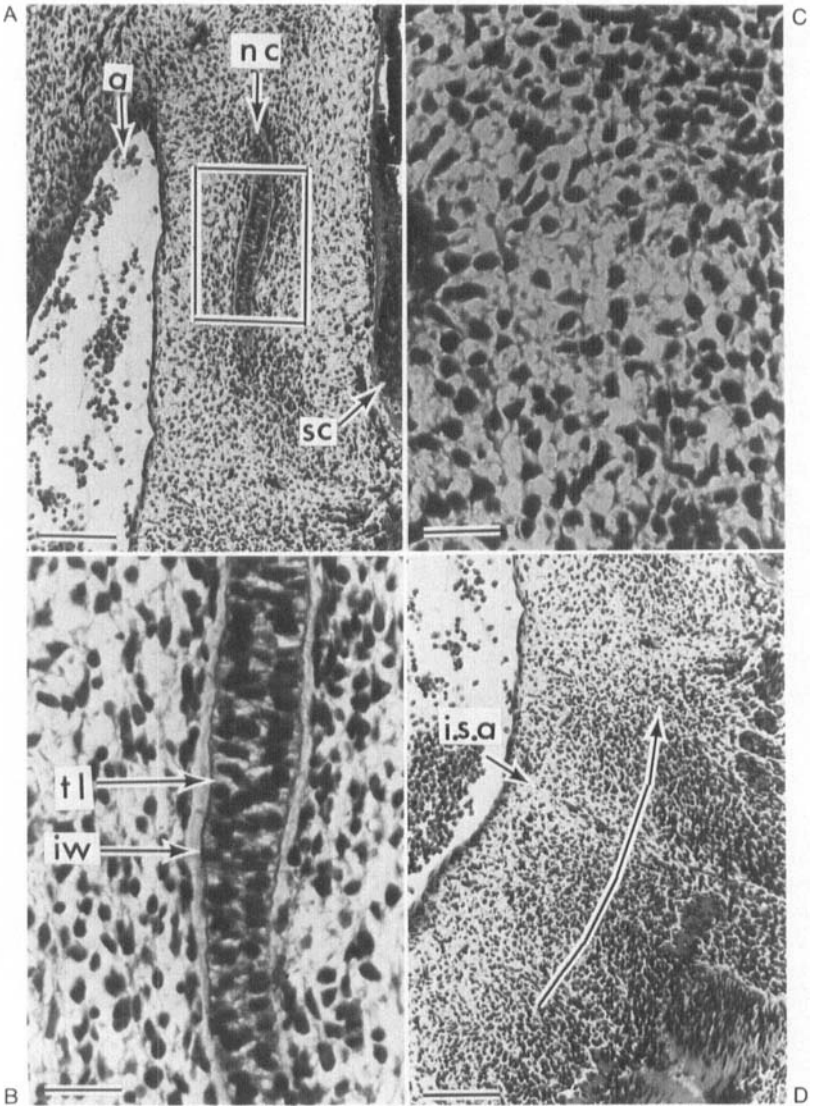


Figure 1. Photomicrographs of an embryo in early Stage 1, S.H. 223 μm, estimated age 5 weeks, sagittal section, HPS.

A Mid-sagittal section. a. aorta; n.c. notochord; s.c. spinal cord; scale 100 μm.

B Neither notochord nor sclerotomic cells around it (area A) show segmental appearance. i.w., inner wall of the notochordal sheath; t.l., transparent layer; scale 25 μm.

C Slightly lateral sagittal section (area B). Sclerotomic cells without segmental appearance except for a slight difference in density. Scale 25 μm.

D Latero-sagittal section (area C). Sclerotomic tissue showing segmental appearance. Segments separated by intersegmental arteries (i.s.a.). Arrow indicating the site where nuclei were counted. Scale 100 μm.

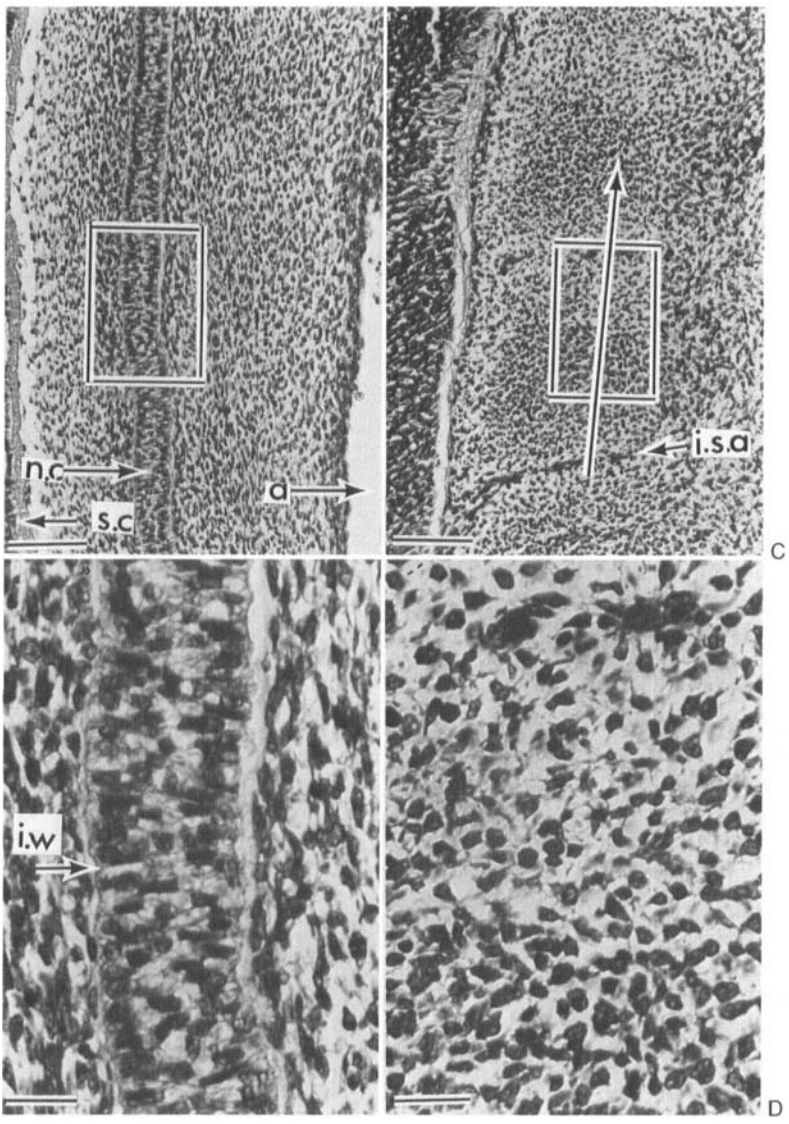


Figure 2. Photomicrographs of an embryo in late Stage 1, S.H. 272 μm, 5½ weeks, sagittal section, HPS.
A Mid-sagittal section. n.c. notochord; s.c. spinal cord; a. aorta. Scale 100 μm.
B No segmental appearance of notochord or sclerotomic cells around it (area A). i.w., inner wall of the notochordal sheath. Scale 25 μm.
C Latero-sagittal section. Segmentation evident in area B. s.c. spinal cord; i.s.a. intersegmental artery. Arrow pointing out site where nuclei were counted. Scale 100 μm.
D Sclerotomic cells at area C showing evidence of segmentation not only in respect to cell density but also to shape and size of nuclei. Scale 25 μm.

ment and number of cells and their nuclear chromatin content were uniform.

In area B all nuclei were identical in early Stage 1 but a slight difference in cell density could be detected. The dense segments were situated between two segmental arteries either exactly at the mid-part or slightly caudal to it. Segmentation became more obvious during late Stage 1 and was due to the difference in size and shape of nuclei and less to variation in cell density.

At area C sclerotomic fissures and intersegmental arteries were present. Already in early Stage 1 (Figure 1) the alternation of cell density was evident. This segmental appearance became more marked in late Stage 1 (Figure 2). Clear and dark zones were separated by sclerotomic fissures.

Histomorphometry revealed a small difference in number of cells in dark and clear zones but not enough to cause an appearance of segmentation. The latter was mostly due to differences in size, shape and chromatin density of nuclei. A decrease in size and an increased variety in nuclei gave the impression of a clear segment where sclerotomic cells would later differentiate into cartilaginous cells.

The peak of cell density was observed at the

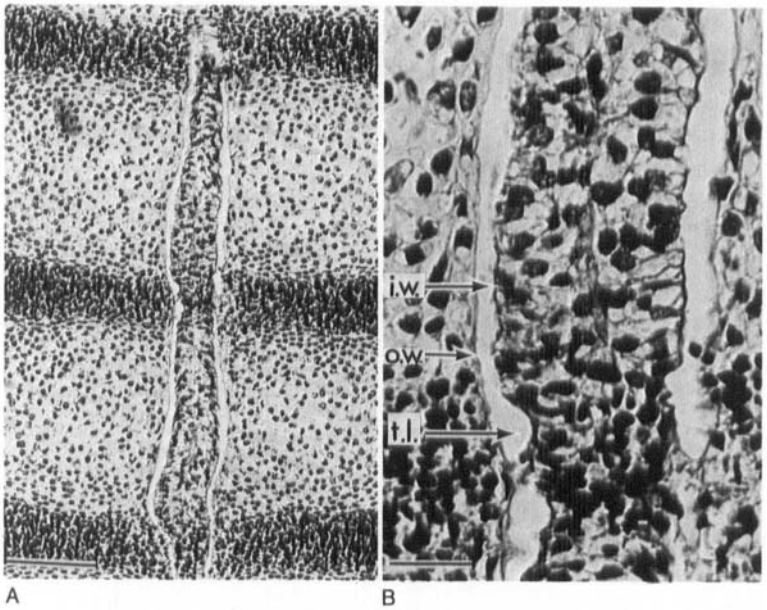


Fig. 3. Photomicrographs of embryos in late stage 2. Frontal section S.H. 272 μm, age 6 weeks. Mann-Dominici. Notochord shows segmented appearance. Chromatin density and number and size of cytoplasmic vacuoles vary alternately between disc space and vertebral body level. Note outer wall (o.w.) of the notochordal sheath. i.w. inner wall of the notochordal sheath; t.l. transparent layer. Scale A 100 μm, B 25 μm.

mid-part of its adjoining caudal part of each segment in stage 1 and thus caudal to the sclerotomic fissure.

*Stage 2* (Figure 3). There was evidence of segmentation of the notochord due to a higher incidence of chromatin dense nuclei at the disc level. At area A segmentation was evident. Chondrification reached into area A in the clear zone, whereas mesenchymal cells occupying area A during the preceding stage would be limited to the dark zone. Segmentation became even more conspicuous during the later phase of this stage, when enlargement of the cartilaginous vertebral body progressed rapidly and the future disc zone was marked by a relative decrease in height. Simultaneously, fibrous tissue appeared in area C of the future disc zone.

*Stage 3 and 4.* The notochord showed a decrease in number and chromatin content of cells at the body level as compared to the disc space during Stage 3. During Stage 4, notochordal cells at the body level became sparse and showed signs of degeneration, whereas cells at the disc level proliferated, leading to a widening of the notochord. In area A, small hypertrophic cells appeared at the disc level. Although the degree of hypertrophy varied from one specimen to another, these cells resembled those of the vertebral body. Cartilage tissue was well formed in area B of the body, whereas the more peripheral area C was the site of smaller hypertrophied cells. At the intervertebral space fibrous tissue occupied area C and proliferating mesenchymal tissue was seen in area B.

*Stage 5.* At the body level only a mucous streak remained, whereas at the disc level notochordal cells continued to proliferate. The body was the site of hypertrophy of chondrocytes in areas A, B and C. In the disc zone the small hypertrophic cells in area A as well as mesenchymal cells at the boundary between body and disc started to decrease.

## C. Cranial notochordal development

As seen in two specimens, the development of the notochord in the cranial portion of the spine was delayed not only when compared to its thoracolumbar portion but also to the surrounding tissues. However, the sclerotomic tissue cranially was developing into cartilaginous tissue (Stage 2), indicating a lack of synchronization of development of the notochord and sclerotomic tissue cranially.

## Discussion

The importance of the notochord in the development of the spine has been well studied in lower vertebrates (Bancroft & Bellairs 1976). In tissue cultures, Cooper (1965) observed that the matrix secreted by the notochord induced a chondrification of sclerotomic tissue. This significant notochordal function of induction has been supported by extirpation methods (Watterson et al. 1954).

These results lend support to the universally accepted concept that the notochord in its secretory phase induces both condensation and chondrification of sclerotomic tissue of the anterior spinal column, and it has been surmised that these inductive properties are also operational during human spinal development.

In lower vertebrates the size of the notochord at the secretory stage is as large as the area of the anterior spinal column (Ruggeri 1972). In humans, as shown in this study, the notochord-anterior spinal column ratio is much smaller. Here the segmentation of the more peripheral tissue precedes secretory changes of the notochord and thus its segmentation. The gradient of segmentation is directed lateromedially in each stage. Application of the anterior spinal column-notochordal ratio of lower vertebrates to the human situation would permit only the inclusion of area A, which would neglect a larger part of the future vertebral bodies and invertebral discs where we observed the earliest evidence of segmentation. This segmentation, already obvious in late Stage 1, is caused by an alternation between clear and dark zones. This is believed to be due to a variation in the cell density (Bardeen & Lewis 1901, Williams 1908, Peacock 1951). Our histomorphometric results contradict this interpretation. During late Stage 1 at the clear zone of area C precartilage cells are present, having smaller nuclei without clearly identifiable cell membranes, which accounts more for the clear aspect than the reduced number of cells. Our results are in accordance with those of Keyes & Compere (1932) who stated that the early demarcation of the vertebrae is the result of differential growth and not of condensation. Chondrification in late Stage 1 evidently starts before any secretory change of the notochord occurs.

Based on studies in pigs, Williams (1908) believed that the expansion of the vertebral body exerts a pressure on the notochord, squeezing its cells toward the disc. Keyes & Compere (1932), Peacock (1951), Parke (1975) and Tsou (1978) transposed this idea to human notochordal development and thought that the migrated notochordal tissue contributed to the formation of the nucleus pulposus. Our observations made during Stages 3 and 4 indicate that the decrease of notochordal cellular contents at the vertebral body level is caused by degeneration and not by migration. There is no evidence of compression of the notochord from outside. On the contrary the thickness of the transparent layer between the inner and outer wall of the notochordal sheath is increasing. The notochord also maintains its size at the disc level during Stages 2 and 3. Rapid proliferation of notochordal cells only starts in Stage 4.

The delayed development of the upper cervical notochord seems to constitute another proof that the notochord does not play an essential role in vertebral segmentation. This was observed in two of our specimens. Here a development of the notochord was delayed in comparison to the development of the notochord at the thoracolumbar level, although the development of the tissues surrounding the notochord was identical over the entire extent of the spine.

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