Development and involution of the notochord in the human spine

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Summary
The availability of a collection of fetal and embryonic spines made possible a review of the involution of the notochord. Results of this histological, histochemical and immunohistochemical study are in accord with the dominant view in the literature that the notochord does not contribute materially to the adult nucleus pulposus. It is also consonant with the expectation that, normally, all chordal cells have disappeared during early childhood, but is not sufficiently detailed to assess the possibility of occasional survivors.

Introduction
In the years between 1870 and 1910 the arguments about the development of the nucleus pulposus were well established, and have been re-considered at intervals since then. Gegenbauer[1], Hertwig[2], Williams[3] and Keyes and Compere[4] supported the view that it is formed by notochordal tissue, which persists in the disc throughout embryonic life. Against this was the opinion of Virchow[5], Heiberg[6], and Weiss[7] that the origin was from peri-notochordal embryonic cartilage. From his studies Peacock[8] concluded that in the pre-natal period there is a modification of the notochordal tissue within the nucleus pulposus, and that after this there was a progressive substitution of chordal material by fibrocartilage from the surrounding tissues, which was completed between the ages of one and three years. Confirmatory experimental evidence is provided by Ruggeri[9], who showed this to be the case in chick embryos. The observation that keratan sulphate is absent from the human notochord[10], but is present in adult human nuclei pulposii, implies it must be synthesized entirely by chondrocytes. This is consistent with the reduction in the number of chordal cells in the early postnatal period, and virtual disappearance by 10 years[11-13]. In this study the development and involution of the human notochord has been investigated in embryonic, fetal and infant spines with histological, histochemical and immunohistochemical methods.

Materials and methods
Intervertebral discs were obtained at autopsy from 38 human vertebral columns, ranging from fetuses of 8 weeks to children of 10 years. Fetal gestational age was estimated from morphological features[14]. The age ranges of up to 8 weeks gestation (n=2), 9-13 weeks gestation (n=5), 14 weeks gestation to 12 months postnatal (n=2), and 1-10 years (n=6) were chosen because the spinal morphology within them shows some common features.

Prenatal specimens consisted of the entire spine; those from infants were limited to three dorsal or lumbar vertebrae with two IV discs. The specimens had been preserved in formalin for many months before this study. Ethylene di-amine tetra-acetic acid was used for decalcification; the end point was determined by X-ray, and was reached after several weeks. Embedding was in paraffin. Sections were cut in the sagittal or coronal plane and stained with either solochrome, haematoxylin-eosin or Alcian blue. Sections from each age group were stained with the anti-epithelial monoclonal antibodies CAM 5.2 and HMFG-2, which have been used previously to demonstrate human notochordal cells[15]. CAM 5.2 recognizes low molecular weight cytokeratins[16], whereas HMFG-2 recognizes an epitope expressed on a variety of epithelial cell membranes[17]. Sections were stained by the avidin-biotin peroxidase complex technique[18]; those for CAM 5.2 were pre-treated with trypsin[19]. All sections were counterstained with haematoxylin. Positive controls were of vermiform appendix and kidney; negative controls were made by substituting Tris-buffered saline for the primary antibody.

Observations
Features common to all the spines of the same age group will be described first; the unusual features found in some specimens will then be presented.

Group 1
In 8 week embryos the notochord was ovoid in the long axis of the spine, and extended into the cartilaginous model of the vertebral bodies, where it was reduced to a band of acellular material which stained less than the cartilage matrix and had a mucoid or vesicular aspect (Figure 1). The chordal tissue, demarcated from the surrounding cartilage by a layer of acellullar matrix (commonly referred to as the notochord sheath), was very cellular, with scanty intercellular substance. About a half of the cells had a dispersed nuclear chromatin; nucleoli were not seen.

Group 2
In older fetuses (9-13 weeks gestation) the chordal tissue, demarcated by the acellular sheath (stained by Alcian blue), was present only within the intervertebral disc, and had a rounded or oval shape, now with the long axis perpendicular to that of the spine. In the centre of the cartilaginous model of the vertebral body the chondrocytes were hypertrophic and their intervening matrix was calcified. Occasionally a band, an acellular remnant of the notochord,

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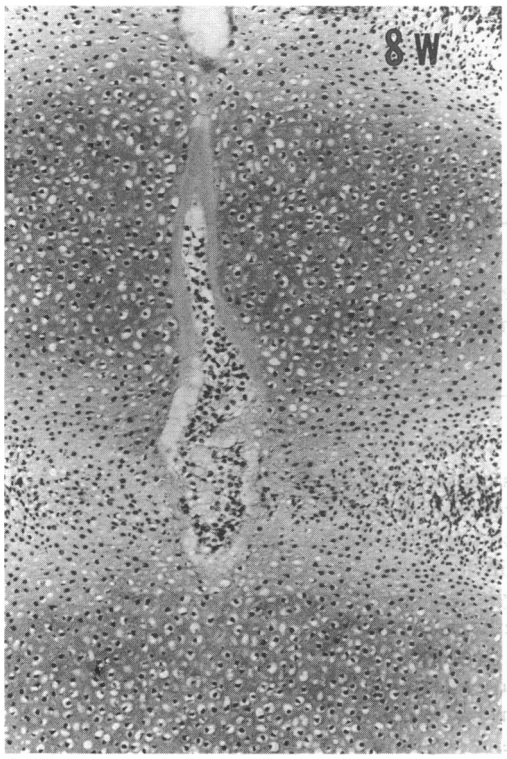

Figure 1. Eight week embryo. The notochord is ovoid in the long axis of the spine, and extends into the cartilaginous vertebral body. The sheath is tenuous in the region of the disc, but well developed where it is in contact with the body. H & E×100.

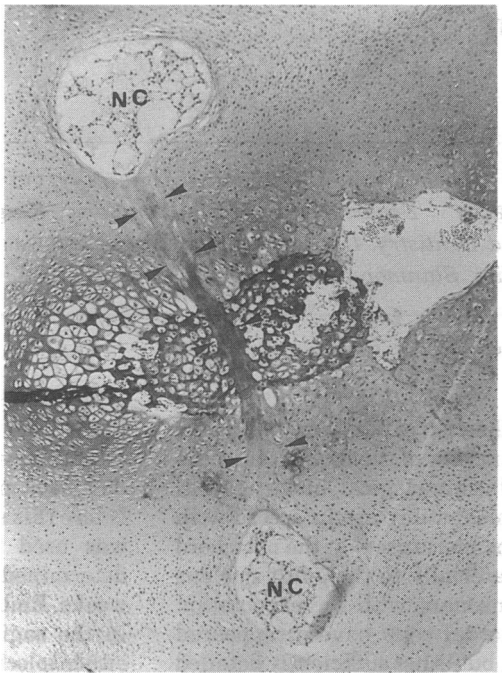

Figure 2. Ten week embryo. Delicately ensheathed pools of notochordal tissue, lying in the discs on either side of a vertebral body which is starting to ossify, are connected by an acellular band closely resembling the sheath. H & E×100.

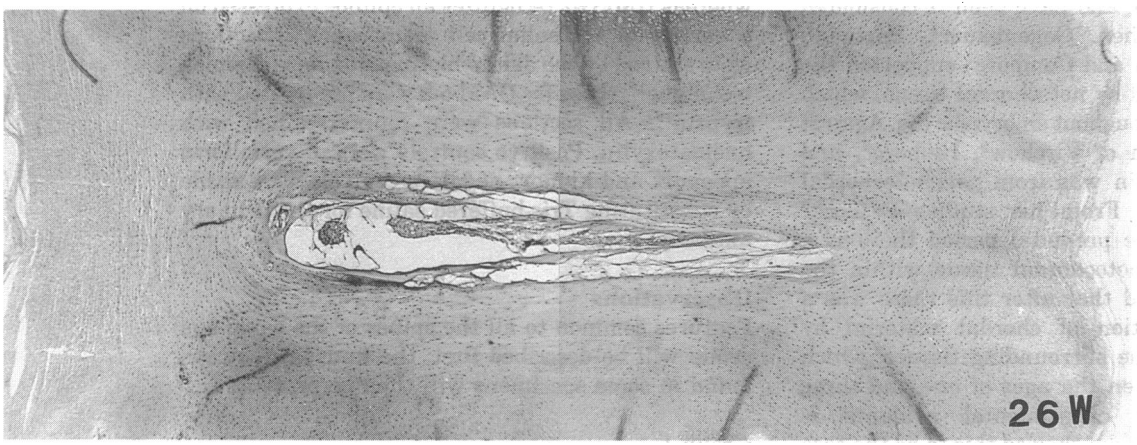

Figure 3. 26 week embryo. A transverse, ensheathed, mucinous pool containing chordal cells, in the disc. This is greatly expanded as compared with Figures 1 and 2, although there is little to choose in cellularity. Beyond this age the chordal tissue becomes progressively less distinct. H & E×22.5.

traversed the body (Figure 2). The volume of chordal tissue was increased relative to younger specimens (Figure 3), although cellularity per unit volume probably diminished. The amorphous, vesicular or mucoid intercellular substance did not stain with Alcian blue in contrast with the surrounding cartilage matrix. This failure to stain with Alcian blue may be an artefact. The inference that formalin fixation may be responsible is probably unfounded as it is generally considered acceptable for all mucins except hyaluronic acid in the free state. EDTA is known to preserve mucins, more so than some of the other decalcifying agents which could have been chosen. Additionally, mucins are better preserved in paraffin than in frozen sections. The cells had a densely packed chromatin, no nucleoli and a faintly stained cytoplasm with an irregular outline due to the many processes extending from them. Additionally, they were uniformly distributed, and constituted no more than 1/5 to 1/10 of the volume of the nucleus pulposus.

Group 3

In the next age group (from 14 weeks gestation to 12 months postnatal) the calcified cores of the vertebral bodies were ossifying. Some discs presented features identical to those already described, with a further increase in volume of chordal mucins (Figure 3); in others notochordal cells were clustered and most of the nucleus pulposus was filled by the vesicular substance. Although segregation of chordal substance from the surrounding structures was still evident, inside the nucleus there was now detectable Alcian blue positivity, indicating the presence of acid mucin.

Group 4

In the last age group (1-10 years) demarcation of the notochordal material from the vertebral end plates and annulus fibrosus was absent. A few cells were observed inside the amorphous material of the nucleus, but they failed to react with CAM 5.2 and HMFG-2 monoclonal antibodies. Most of the disc nuclear substance was stained by Alcian blue; the regions of vesicular substance were few.

The unusual features that were observed concerned, firstly, four embryos aged 13, 16, 20 and 21 weeks in whom residual notochordal cells (with the same characteristics as the 8 week embryos (Figure 1) were found in the cartilage model of the sacral vertebrae. This may be a reflection of sampling; our resources did not extend to the sectioning of all the available tissue, a procedure which might reveal that residual cells were more common than the present observations suggest. Secondly, two centres of ossification were present in some vertebral bodies of three embryos in the third group. It is recognized[20] that the centrum is sometimes ossified from two lateral centres and our observations are consistent with this.

Discussion

The volume of the notochord was observed to increase steadily with age, in proportion to the growing vertebral bodies and IV discs. At 8 weeks the notochordal tissue was very cellular and no intercellular matrix was observed, while at older ages, up to about one year, most of its volume was occupied by the vesicular, mucoid material which stained differently from the peri-notochordal matrix: this supports the view that the initial mucoid material is the product of chordal cells, which are actively secreting until term or the first months of post-natal life.

Since, with growth, the total number of notochordal cells remains the same[21] and mitoses are not observed after the 3.5 mm stage, corresponding to 4-5 weeks of gestation[11], only the production of mucoid material by the cells already present is responsible for the increased volume. Thus, at these early stages, at least, chordal cells have an active role in the development of the nucleus pulposus.

Using the anti-epithelial monoclonal antibodies, we were not able to demonstrate notochordal cells after 4 years of age. Nevertheless they have been reported both in this age group[8,22-24] and in adults[25,26]. However, their recognition by light microscopy must be uncertain when the nucleus has already been invaded by fibrocartilage. Estimates have been made of their densities in human discs of fetal and early post-natal life[12] by counting their number in measured areas and converting to cells/mm^2. The values declined exponentially from 2000/mm^2 at 6 weeks to 100/mm^2 at one year, indicating a more or less static total number of cells which is diluted by growth and development.

We believe that a far more detailed study would be required to assess the question of residual chordal tissue in vertebrae. Our observations are similar to those of Taylor[27], but the significance for the development of chordoma is not further clarified thereby.

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References

1 Gegenbaur C. *Elements of comparative anatomy.* London: Macmillan and Co, 1878
2 Hertwig O. *Lehrbuch der Entwicklungsgescichte des Menschen und Wierbeltiere.* Jena: Gustav Fischer, 1890
3 Williams LW. The later development of the notochord in mammals. *Am J Anat* 1908;**8**:251-84
4 Keyes DC, Compere EL. The normal and pathological physiology of the nucleus pulposus of the intervertebral disk. *J Bone Joint Surg* 1932;**14**:879-935
5 Virchow R. *Untersuchungen uber die Entwicklungen des Schadelgrundes im gesunden und wrankhaften Zustande und uber den Einfluss derselben auf Schadelform.* Berlin: Gesichtsbildung und Gehirnban, Reimer, 1857
6 Heiberg J. Uber die Zwischenwerbeigelenke und Knockenkerne der Werbeisaule bei den Neugeboren und ihre Verhaltniss zur Chorda. *Mitt Embryol Inst Univ Wien* 1880;**1**:119-29
7 Weiss A. Die Entwicklung der Wirbensanle der weissen Ratte, besonders der vardensten Halswirbel. *Z Wiss Zool* 1901;**69**:492-533
8 Weiss A. (1952) Observations on the post-natal structure of the intervertebral disc in man. *J Anat* 1952;**86**:162-79
9 Ruggeri A. Ultrastructural, histochemical and autoradiographic studies on the developing chick notochord. *Z Anat EntwGesch* 1972;**138**:20-33
10 Salisbury, JR, Watt FM. Lack of keratan sulphate in the human notochord. *J Anat* 1988;**157**:175-9
11 Peacock A. Observations on the pre-natal development of the intervertebral disc in man. *J Anat* 1951;**85**:260-74
12 Salisbury, JR. Personal communication, 1987
13 Walmsley R. The development and growth of the intervertebral disc. *Edinburgh Med J* 1953;**60**:341-63
14 Streeter GL. Weight, sitting height, head size, foot length, and menstrual age of the human embryo. *Contrib Embryol* 1921;**11**:143-70
15 Salisbury JR, Isaacson PG. Demonstration of cytokeratins and epithelial membrane antigen in chordomas and human foetal notochord. *Am J Surg Pathol* 1985;**9**:791-7
16 Makin CH, Bobrow LG, Bodmer WF. Monoclonal antibody to cytokeratin for use in routine histopathology. *J Clin Pathol* 1984;**37**:975-83.
17 Burchell J, Durbin H, Taylor-Papdimitriou J. Complexity of expression of antigenuc determinants recognised by monoclonal antibodies HMFG-1 and HMFG-2 in normal and malignant human mammary epithelial cells. *J Immunol* 1983;**131**:508-13
18 Hsu S-M, Raine L, Fanger H. The use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques. *J Histochem Cytochem* 1981;**29**:577-80
19 Poston RN, Sidhu YS, Makin C, Mason DV. Specific enzyme treatment is required for individual monoclonal antibodies in immunohistochemistry with formalin fixed sections. *Proc Protides of the Biological Fluids* 1984; **32**:557-60
20 Williams RL, Warwick R, eds. *Gray's anatomy,* 36th edn. Edinburgh: Churchill Livingstone, 1980:282
21 Loewe L. Zur Kentnisse der Saengetierchorda. *Arch mikr Anat* 1879;**16**:597-612
22 Amprino R, Bairati A. Studi sulle trasformazioni delle cartaligini dell'uomo nell'accrescimento e nella senescenza. Parte III. *Z Zellforsch* 1934;**21**:440-82
23 Prader A. Die Frühembryonale Entwicklung der menslichen Zwischenwirbelscheibe. *Acta Anat* 1947;**3**:68-83
24 Schaeffer J. Die Ruckenseite der Saugtiere nach der Geburt nebst Bemerkungen uber den Ban und die Vermocherung der Wirbel. *S B Akad Wiss Wien (Abt III)* 1910;**119**:409-65
25 Fick R. *Handbuch der Anatomie und Mechanik der Gelenke.* Part I. Jena: Gustav Fischer, 1904
26 Koelliker A. *Entwicklungsgeschicte des Menchen und hoeren Tiere.* Leipzig: Wilhelm Engelmann, 1879
27 Taylor JR. Persistence of the notochordal canal in vertebrae. *J Anat* 1972;**111**:211-17

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