J. Anat. (1980) **130**, 1, pp. 75–81
With 12 *figures*
Printed in Great Britain

A morphological and histological study of the postnatal development of intervertebral discs in the lumbar spine of the rabbit

N. A. SCOTT, P. F. HARRIS AND K. M. BAGNALL
Department of Anatomy, University of Manchester

(*Accepted* 2 *February* 1979)

INTRODUCTION

The rabbit intervertebral disc has been used by a number of workers as a model for studying the metabolism (Davídson & Small, 1963; Souter & Taylor, 1970), nutrition (Brodin, 1955) and pathology of discs (Smith & Walmsley, 1951; Bobechiko & Hirsch, 1965; Haimovici, 1970), in the hope of elucidating the mechanisms underlying human intervertebral disc prolapse.

The use of the rabbit intervertebral disc as an experimental model for investigating disc pathology prompted the present study to define in greater detail certain morphological and histological features of the developing lumbar intervertebral disc. Of particular interest were the influence of the curvature of the lumbar spine on disc thickness, the orientation of the lamellae of the annulus fibrosus in relation to curvature, and the pattern of secondary ossification in the region of the vertebral body, with special reference to the cartilage end plates.

MATERIALS AND METHODS

By careful dissection the whole of the lumbar spine was removed from ten male and female rabbits, of either New Zealand White or Dutch breeds, aged between birth and twenty two months. Anteroposterior and lateral radiographs were taken of each spine using Kodak PE4006 film with an exposure of 180 mA at 45 kV and anode-film distance of 100 cm. After radiography each lumbar disc, together with parts of the adjacent vertebral bodies, was removed from the spine and fixed in Bouin's solution. The specimens were decalcified, dehydrated and embedded in wax.

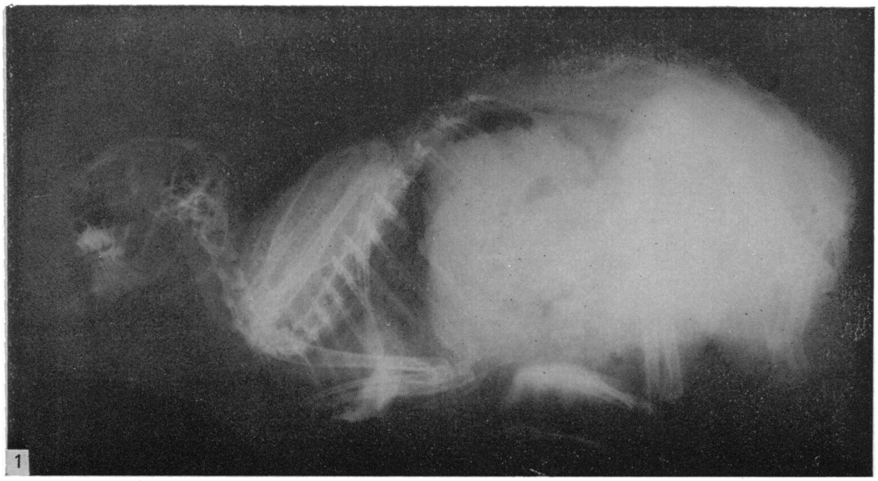
Histological sections were prepared from each disc and stained with haematoxylin and eosin, Van Gieson's stain or 0·1 % toluidine blue.

RESULTS

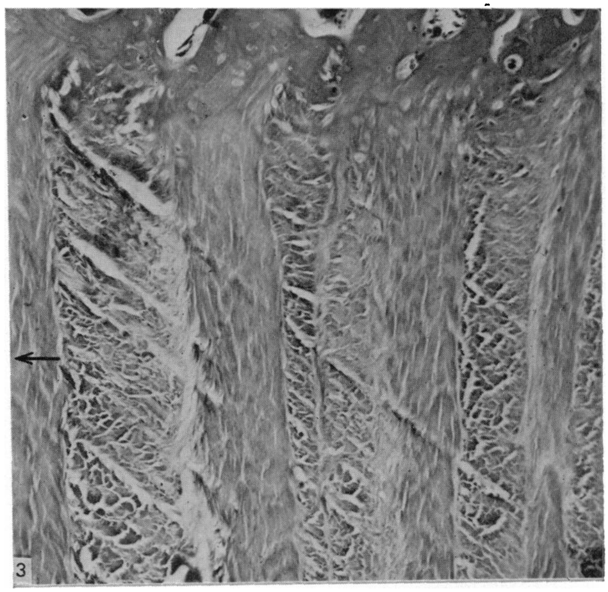
Gross anatomy

A lateral radiograph of the intact lumbar spine in an adult rabbit showed that during life it has the form of a primary curve with the concavity facing ventrally (Fig. 1). On sagittally bisecting the isolated lumbar spine in the median plane the *ventral* aspect of each lumbar disc was seen to be thicker than the posterior aspect (Fig. 2).

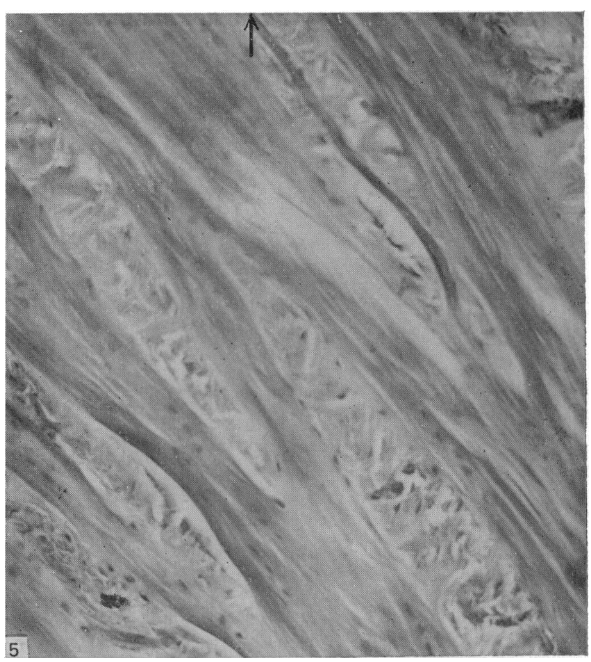
0021-8782/80/2828-7100 $02.00


1


2


3


4


5

Histology

The annulus fibrosus

On staining the intervertebral discs with haematoxylin and eosin the peripheral zone of the annulus fibrosus was seen to be strongly eosinophilic whilst the deeper part, adjacent to the nucleus pulposus, was basophilic. The exposed ends of adjacent lamellae in the annulus fibrosus differed in their appearance according to the orientation of the fibres (Fig. 3). The arrangement of the lamellae also differed depending on whether they were located in the anterior or posterior parts of the annulus. Lamellae in the anterior region traverse the intervertebral disc in an essentially vertical direction (Fig. 3). However, those in the posterior region of the annulus take a U-shaped course with a distinctly posterior inclination (Fig. 4). It was noted that the U-shaped configuration is evident at a very early stage, even in the newborn rabbit.

Transverse sections of the lumbar intervertebral discs showed that as some of the lamellae pass posteriorly they fuse together (Fig. 5).

The nucleus pulposus

Over the age range examined, the nucleus pulposus consisted mainly of a basophilic gel with no evidence of any fibrous replacement. The most striking change observed in the nucleus pulposus during this time was in its cellularity.

At birth a significant proportion of the nucleus pulposus was occupied by large groups of cells. Each cell appeared to be considerably distended by a cytoplasmic vacuole, the remainder of the cytoplasm, together with the nucleus, being compressed around its periphery (Fig. 6). The nuclear chromatin had a dispersed pattern and nucleoli were prominent.

By the age of six weeks cells formed a much smaller proportion of the nucleus pulposus (Fig. 7). They had either an annular arrangement or were in small groups, and intracellulular vacuoles were much less conspicuous. After the age of six weeks, cells were seen only infrequently in the matrix of the nucleus pulposus. By 11 months cells could not be clearly defined at all.

The position of the nucleus pulposus in relation to the remainder of the disc was noted both in histological and in gross specimens of lumbar intervertebral discs. Its general position tended to be more posterior than central.

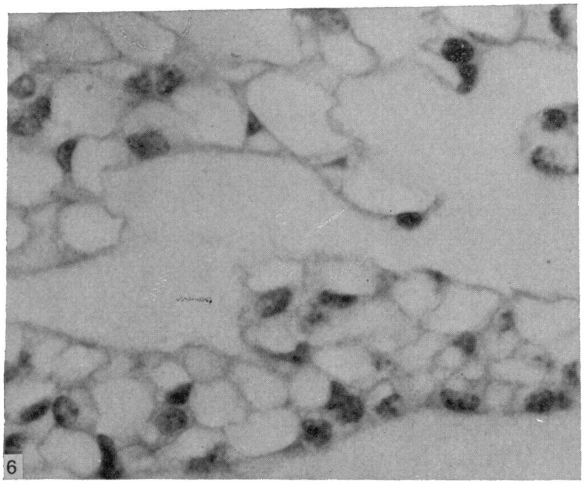
Fig. 1. Lateral radiograph of intact vertebral column in the living rabbit. Note the primary curvature throughout the lumbar region.

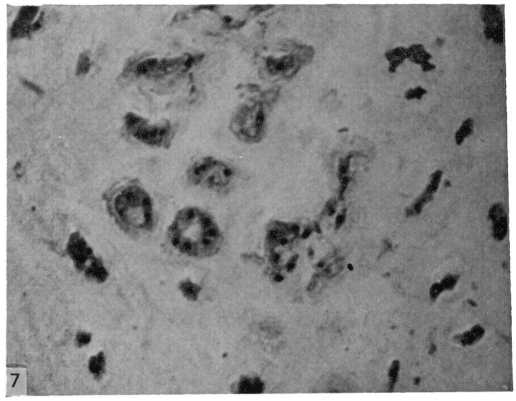
Fig. 2. Sagittal section of rabbit lumbar intervertebral disc showing thick ventral aspect and thinner posterior aspect. (Anterior is indicated by arrow).

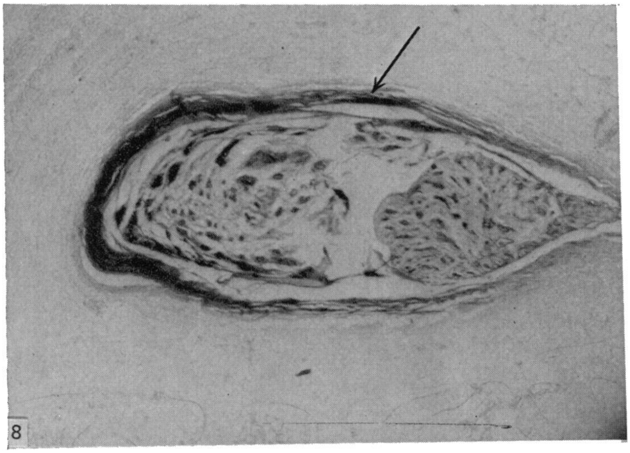
Fig. 3. Sagittal section of the anterior lamellae of the annulus fibrosus. This shows differing orientation of fibres within adjacent lamellae and their essentially vertical direction. (Anterior is indicated by arrow). ×88.

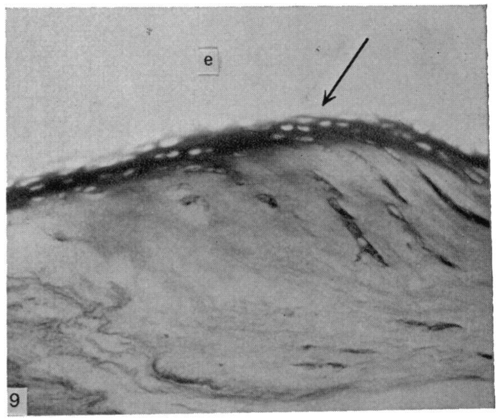
Fig. 4. Sagittal section of the posterior lamellae of the annulus fibrosus showing the U-shaped course of these lamellae. (Anterior is indicated by arrow). ×30.

Fig. 5. Transverse section of lumbar intervertebral disc. The lamellae fuse as they pass posteriorly. (Anterior aspect is indicated by arrow). ×88.

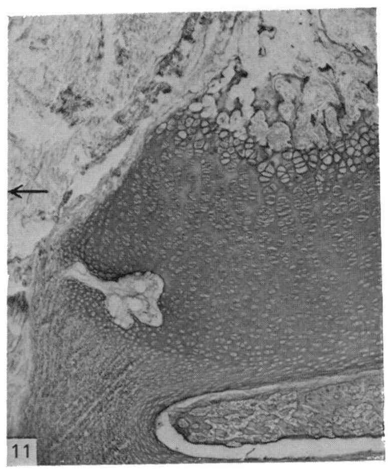

6

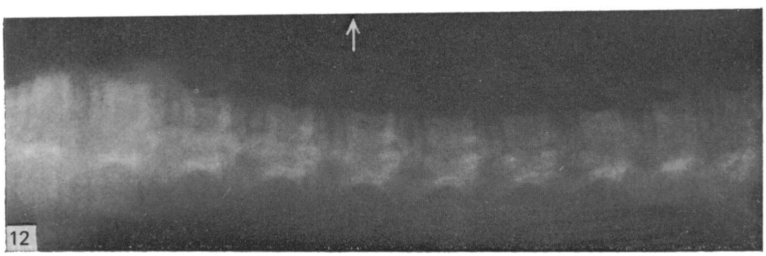

7


8


e
9


10


11


12

Cartilage end plates

Using toluidine blue an age-related change was noted in the metachromatic material lying above and below the nucleus pulposus. At three and six months of age a metachromatic zone was seen around the nucleus pulposus. Within this zone, in the sagittal plane, collagen fibres encircled the nucleus (Fig. 8). By sixteen months these metachromatic zones appeared to have been transformed into bands of hyaline cartilage adjacent to the respective surfaces of the vertebral bodies (Fig. 9).

Radiography

From a series of radiographs of the isolated vertebral columns, between birth and 16 months of age, the sequence of epiphyseal formation was studied.

Radiographs of vertebral columns at birth showed adjacent vertebral centra to be separated from each other by a single radio-lucent space with no evidence of secondary ossification centres (Fig. 10). However, even at this stage, in one specimen the histological sections of the vertebral column showed evidence of secondary ossification (Fig. 11) beginning in what had appeared to be a completely radio-lucent space.

By 12 days of age the radiographic appearance of the spine was very different. A pair of epiphyses had appeared between adjacent vertebral centra. These epiphyses were evident as radio-opaque plates limiting the superior and inferior surfaces of the intervertebral discs (Fig. 12).

DISCUSSION

The division of the annulus fibrosus of the lumbar intervertebral disc in the rabbit into a peripheral fibrous zone and an inner fibrocartilaginous region was described by Souter & Taylor (1970). A similar arrangement in the human intervertebral disc was described by Peacock (1952). In the annulus fibrosus, the orientation of the fibres in adjacent lamellae in opposite directions is termed biaxial orientation. This was recently described in detail by Inoue (1973) in the discs of human and other species.

In the present study the gel-like nature of the nucleus pulposus persisted even up to twenty two months of age. This is similar to the observations of Souter & Taylor (1970), who noted that the rabbit nucleus pulposus remains unchanged until the age

---

Fig. 6. Vacuolated cell groups present in the nucleus pulposus at birth. Within the nuclei the chromatin has a dispersed pattern, and the nucleoli are prominent. × 200.

Fig. 7. The less conspicuous groups and rings of cells present in the nucleus pulposus at 6 weeks of age. × 180.

Fig. 8. Sagittal section of lumbar intervertebral disc at three months stained with toluidine blue. Collagen fibres (arrow) traverse the metachromatic zone around the nucleus pulposus. (Anterior to the right.) × 30.

Fig. 9. Sagittal section of vertebral disc boundary at sixteen months stained with toluidine blue. A band of hyaline cartilage (arrow) is now present adjacent to the vertebral epiphysis (*e*) whose location is marked since it does not stain with toluidine blue. × 88.

Fig. 10. Lateral radiograph of rabbit lumbar spine at birth. A single radio-lucent space separates adjacent vertebral centra. (Anterior aspect is indicated by arrow).

Fig. 11. Sagittal section of rabbit lumbar intervertebral disc at birth showing the beginning of secondary ossification. (Anterior is indicated by arrow). × 65.

Fig. 12. Lateral radiograph of the rabbit lumbar spine at twelve days of age. Epiphyses are now evident as radio-opaque plates bounding the intervertebral disc. (Anterior aspect is indicated by arrow).

of twenty four months. On the other hand, in the human nucleus pulposus, Coventry, Ghormley & Kernohan (1945) described the transformation of the nucleus pulposus by the fifth decade from a moist structure into one whose consistency resembled that of the annulus fibrosus.

The embryological development of the rabbit intervertebral disc involves expansion of the notochord in the intervertebral regions. Part of this expansion is thought to be due to vacuolisation of notochordal cells (Leeson & Leeson, 1958). In the present study this is supported by the vacuolated appearance of the cells of the nucleus pulposus noted in the rabbit disc at birth.

Walmsley (1953) studied the *human* intervertebral disc and described the lamellae in the posterior part of the annulus fibrosus as having a U-shaped configuration. The gap between two adjacent vertebral bodies was also stated to be wider *anteriorly* than posteriorly. It is of considerable interest that these features were also noted in the present study of developing intervertebral discs in rabbits.

The explanation of Walmsley (1953) for this arrangement is that *at birth* the anterior and posterior lamellae are of the same length and pass in a gentle curve between adjacent vertebrae. He suggests that it is the formation of the secondary lumbar curvature in man (which accompanies adoption of an upright posture) that widens the anterior part of the disc and compresses the posterior part. Compression in the latter site is also responsible for causing the posterior lamellae to deviate and adopt a U-shaped course. However, in the present study *all* the lumbar discs examined showed a difference in thickness between anterior and posterior parts and also a deviation in the course of the posterior lamellae. These features are seen in the lumbar region of the rabbit's vertebral column even though this animal is a quadruped whose lumbar spine has only a primary curvature. It therefore seems unlikely that such an arrangement is produced by forces acting on the intervertebral disc as a consequence of the development of a secondary lumbar curvature associated with an upright posture.

On the vertebral aspect of the cartilage plates bounding the human intervertebral discs the cells are arranged in columns as in growth plates, whilst on their other face they are continuous with the intervertebral disc (Taylor, 1973). Thus the two functions of longitudinal vertebral growth and de-limitation of intervertebral disc boundaries are present in the same cartilage plates. In the human this plate remains as a single entity since secondary ossification here produces a peripheral ring of bone – the ring apophysis (Bick & Copel, 1951).

In contrast, secondary ossification in the rabbit vertebral body produces a continuous plate of bone which lies between the epiphyseal growth cartilage and the intervertebral disc. However, the existence of a further plate of hyaline cartilage in the rabbit lying between the intervertebral disc and the vertebral epiphysis has been disputed. A cartilage plate bounding the rabbit intervertebral disc is illustrated by Souter & Taylor (1970), but Haimovici (1970) states that the main difference between the human and rabbit intervertebral disc is in the absence of cartilage end plates in the rabbit. In the present study the distribution of metachromatic material at three months suggests that analogies of these plates may exist at this stage, but they are obscured by fibrous tissue passing through them. With increasing age the fibrous envelope above and below the nucleus pulposus diminishes, and by sixteen months of age bands of cartilage are apparent, on the intervertebral aspect of the vertebral bodies.

Since the presence of a definitive cartilage plate bounding the intervertebral disc is an age-related phenomenon in the rabbit, controversy over the existence of these plates may have resulted from comparing the findings in rabbits of differing ages.

SUMMARY

Some basic features in the development of the structure of the annulus fibrosus and nucleus pulposus in the rabbit, as described by previous workers, have been confirmed in the present study.

However, the greater thickness of the anterior part of the disc, as compared with the posterior region, and the distinctive arrangement of lamellae in the posterior part of the disc, cannot be attributed, as conventionally claimed from studies of the human spine, to a secondary curvature in the lumbar spine associated with an upright posture: for these features are present in the lumbar spine of the quadrupedal rabbit with its primary curvature.

Secondary ossification produces a plate-like epiphysis separating the growth cartilage from the intervertebral disc. A distinct cartilaginous plate, limiting the nucleus pulposus in the rabbit intervertebral disc, only becomes apparent when collagen fibres cease to traverse the area above and below the nucleus pulposus.

REFERENCES

BICK, E. M. & COPEL, J. W. (1951). The ring apophysis of the human vertebrae. *Journal of Bone and Joint Surgery* **33** A, 783–787.

BOBECHIKO, W. P. & HIRSCH, C. (1965). Auto-immune response to nucleus pulposus in the rabbit. *Journal of Bone and Joint Surgery* **47** B, 574–580.

BRODIN, H. (1955). Paths of nutrition in articular cartilage and intervertebral discs. *Acta orthopaedica scandinavica* **24**, 177–183.

COVENTRY, M. B., GHORMLEY, R. K. & KERNOHAN, J. W. (1945). Part II. Changes in intervertebral discs concomitant with age. *Journal of Bone and Joint Surgery* **27**, 233–247.

DAVIDSON, E. A. & SMALL, W. (1963). Metabolism (*in vivo*) of connective tissue mucopolysaccharides. I. Chondroitin sulfate (C) and keratosulfate of nucleus pulposus. *Biochimica et biophysica acta* **69**, 445–452.

HAIMOVICI, E. H. (1970). Experimental disc lesion in rabbits. The effect of repeated ACTH. *Acta orthopaedica scandinavica* **41**, 505–521.

INOUE, H. (1973). Three dimensional observations of collagen framework of intervertebral discs in rats, dogs and humans. *Archivum histologicum japonicum* **36**, 39–56.

LEESON, T. S. & LEESON, C. R. (1958). Observations on the histochemistry and fine structure of the notochord in rabbit embryos. *Journal of Anatomy* **92**, 278–285.

PEACOCK, A. (1952). Observations on the postnatal structure of the intervertebral disc in man. *Journal of Anatomy* **86**, 162–176.

SMITH, J. W. & WALMSLEY, R. (1951). Experimental incision of the intervertebral disc. *Journal of Bone and Joint Surgery* **33** B, 612–625.

SOUTER, W. A. & TAYLOR, T. K. F. (1970). Sulphated acid mucopolysaccharide metabolism in the rabbit intervertebral disc. *Journal of Bone and Joint Surgery* **52** B, 371–383.

TAYLOR, J. R. (1973). Growth and development of the human intervertebral disc. Ph.D. Thesis, Edinburgh University.

WALMSLEY, R. (1953). The development and growth of the intervertebral disc. *Edinburgh Medical Journal* **60**, 341–364.