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Role of cartilage canals in osteogenesis and growth of the vertebral centra

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INTRODUCTION

Studies of the prenatal growth of vertebrae have been made by several workers and there appear to be two different views of the mechanism of development. Bick & Copel (1950), Walmsley (1953) and Schmorl & Junghans (1971) were of the opinion that the vertebral body grows precisely as does the diaphysis of a long bone with the earliest formation of bone occurring in a periosteal position. However, Tondury (1953), Larsen & Nordentoft (1962) and Ogden (1979) considered early ossification in the centra analogous to the development of a secondary ossification centre of a long bone.

A further difference of opinion is observed regarding the time of appearance of the primary centre of ossification in the vertebral body. Radiological studies by Bagnall, Harris & Jones (1977) and an alizarin red method study by Noback & Robertson (1951) indicate that the primary centre appears in the lower thoracic and upper lumbar vertebrae around the tenth week of intrauterine life; histological examinations by Bick & Copel (1950) and Walmsley (1953) describe it as appearing around week fifteen.

Although the critical role held by bone vascularisation in bone development was recognized by Brookes (1971) and Crock (1960), not many authors relate the development of vertebrae to its blood supply. Cartilage canals in developing vertebrae were observed by Smith (1931), Coventry, Ghormley & Kernohan (1945), Ferguson (1950), Harris & McNab (1954), Theiler (1965), Brookes (1971), Ogden (1979), Ratcliffe (1981) and Whalen, Wesley, Mazur & Stauffer (1985) but they were interpreted mainly in relation to their nutritional role; Whalen *et al.* (1985) state that the cartilage canals have no role in the formation of ossification centres in the human vertebrae.

The role of cartilage canals in the osteogenesis of secondary centres of long bones has been described by Wilsman & Van Sickle (1970), Lutfi (1970) and Gray & Gardner (1969). This study seeks to examine the role of cartilage canals in relation to osteogenesis in the centra and its growth in early fetal life.

MATERIAL AND METHODS

The lower thoracic and upper lumbar vertebrae of eight fetuses ranging from 30 mm C.R. length to 185 mm C.R. length were examined. The bones were fixed in 10% formalin, decalcified with formic acid and embedded in paraffin. The tissue was

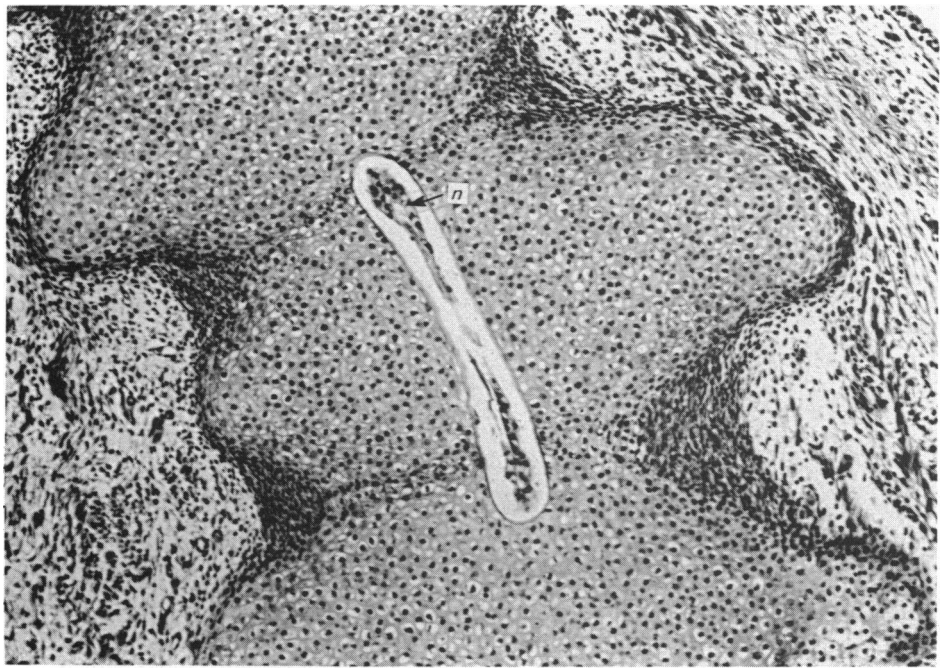

Fig. 1. 30 mm C.R. length, coronal section (thoracic). Centrum is cartilaginous with notochord present; no blood vessels in centrum. *n*, notochord. H and E. ×31·25.

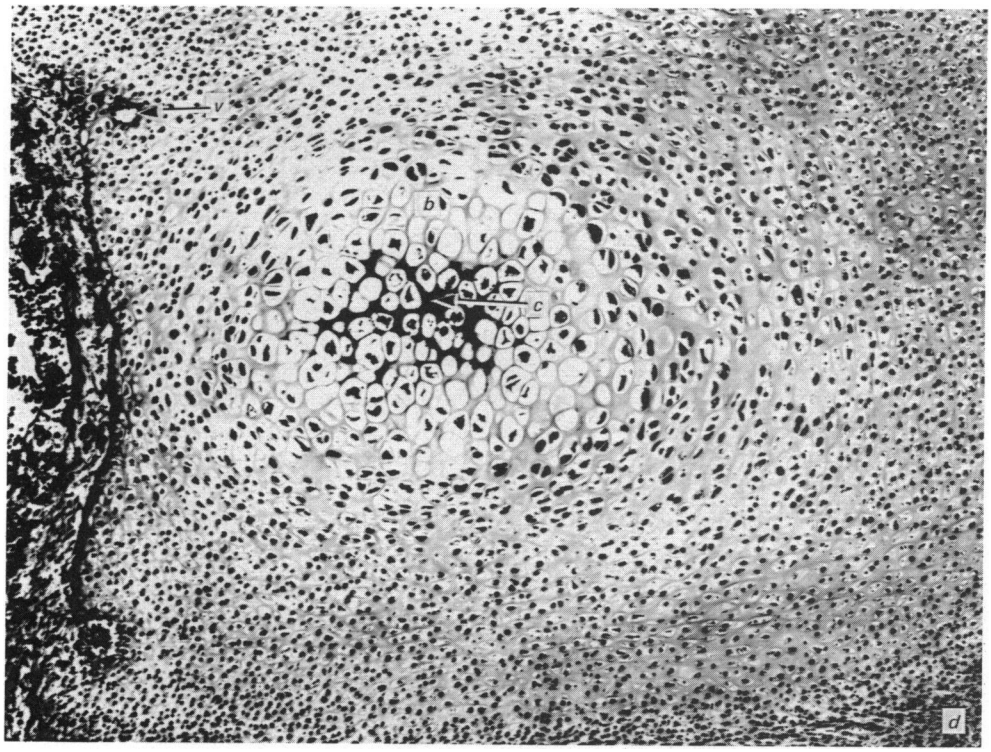

Fig. 2. 55 mm C.R. length, sagittal section (thoracic). Centrum shows calcification in the centre with hypertrophic zone around the central area, blood vessels on dorsal aspect and vascular indentation near intervertebral discs. *c*, calcification; *b*, blood vessel; *v*, vascular indentation; *d*, disc. H. and E. ×60.

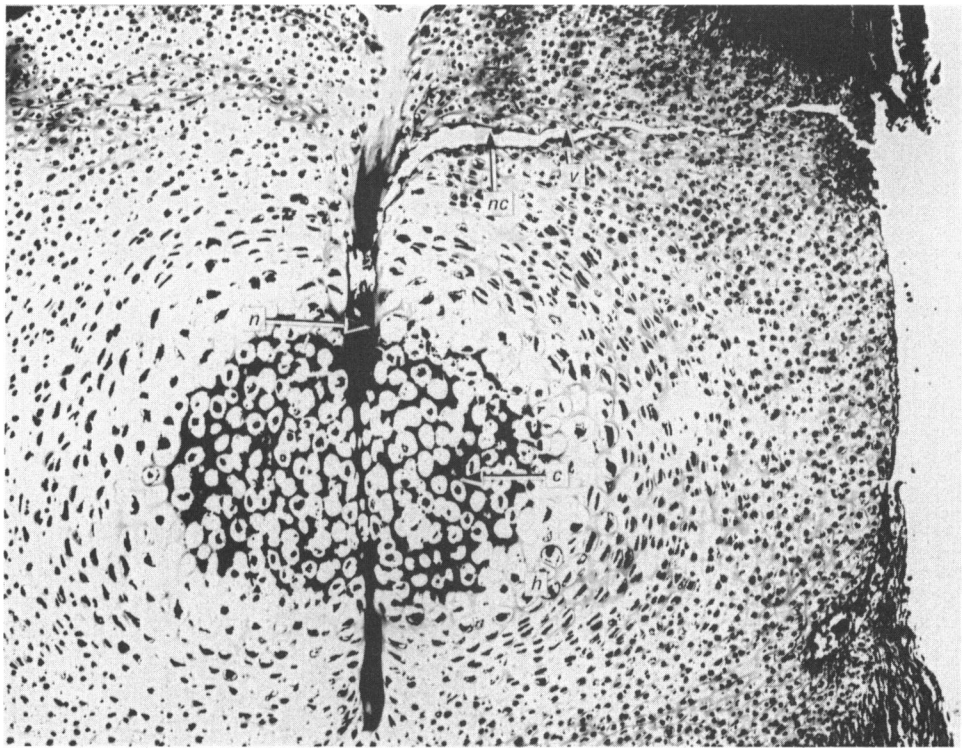

Fig. 3. 55 mm C.R. length, coronal section (thoracic). Central calcification, zone of hypertrophy, notochordal remnant and vascular canal with red cells and nucleated cells reaching central area. *c*, calcification; *h*, hypertrophy; *n*, notochord; *v*, vascular canal; *nc*, nucleated cells. H and E. ×60.

serially sectioned at 5 μm thickness, in the transverse, coronal and sagittal planes. Every twentieth section (except at 55 mm C.R. length, where every tenth section was taken) was stained with haematoxylin and eosin. From this series some sections were selected and stained with periodic acid–Schiff reagent, Masson's trichrome and toluidine blue.

RESULTS

30 *mm C.R. length* (*Fig.* 1)

The lower thoracic vertebrae were sectioned in the coronal plane, the upper lumbar vertebrae were sectioned in the transverse plane. The centra and vertebral arches at this stage were both cartilaginous. Intense metachromasia of the cartilage was observed. Remnants of notochordal tissue were found in the centra. No vascularisation of the vertebral bodies or disc was seen.

55 *mm C.R. length* (*Figs.* 2, 3)

The last two thoracic and the first lumbar vertebrae were examined. The lumbar vertebra was sectioned in the transverse plane, one of the thoracic vertebrae was sectioned coronally and the other in the sagittal plane. Every tenth section of this stage was examined. The centra showed a central region of calcified cartilage surrounded by a zone of hypertrophic cartilage (Fig. 2). The hypertrophy reached the dorsal surface of the centrum where it was in close opposition to blood vessels; anteriorly it fell short of the ventral surface of the centra. A vascular channel was observed within the lower

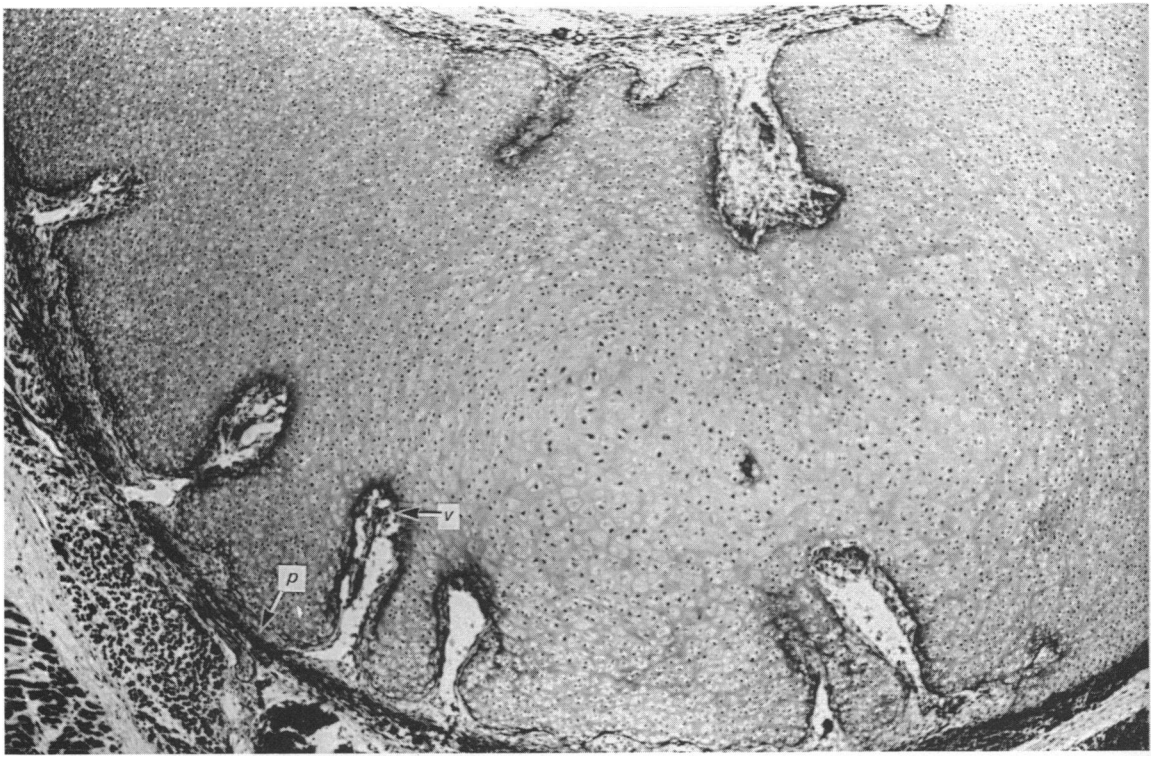

Fig. 4. 85 mm C.R. length, transverse section (lumbar). Note vascular canals from perichondrial region entering the centrum. *v*, vascular canal; *p*, perichondrium. H and E. ×60.

thoracic centrum (Fig. 3); lying within this channel were red cells and other nucleated cells (probably of bone marrow origin) which reached the central calcified area. In sagittal section two vascular channels lying adjacent to the intervertebral discs were observed, on the dorsal aspect of the centrum (Fig. 2).

85 mm C.R. length (Figs. 4–7)

The lower thoracic and first lumbar vertebrae were sectioned in the transverse, sagittal and coronal planes. As in the previous stage, one vertebra, the lowest thoracic, was sectioned transversely, the other in the coronal plane and the lumbar vertebra in the sagittal plane. Transverse sections showed several vascular canals invading the centrum from its periphery (Fig. 4). Each of the invading vascular canals had a membrane continuous with the perichondrium; within the canal was found an arteriole and a venule surrounded by loose connective tissue cells. The canals were observed to branch within the centrum. Examination of the coronal sections revealed that the site of entry of these vascular canals was located above and below the intervertebral disc (Fig. 5). In addition to the eight to ten vascular canals on the anterior and four on the posterior surface, two larger vascular canals were observed to enter the mid-dorsal surface of the centrum. At their central termination within the centrum, both at the disc margin and in the centre of the vertebral body, each of the terminal branches further subdivided into a capillary plexus (glomerulus).

The influx of blood vessels (cartilage canals) within the centrum was associated with erosion of the calcified cartilaginous matrix, vascular and connective tissue invasion

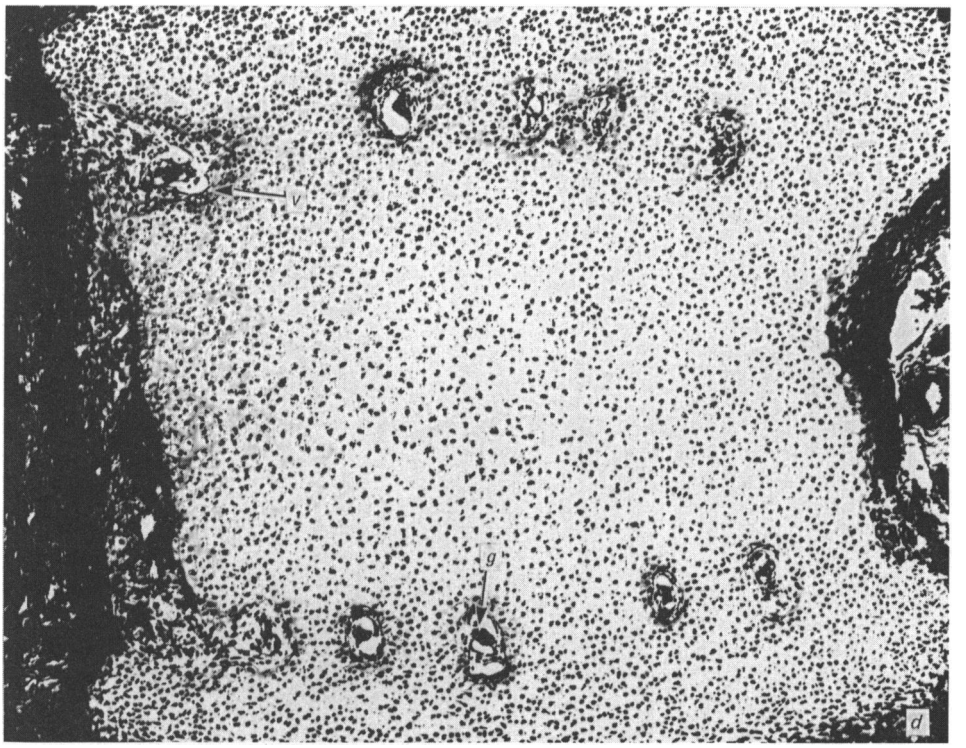

Fig. 5. 85 mm C.R. length, coronal section (thoracic). Termination of the vertical branches of vascular canals near intervertebral disc, glomerular tuft adjacent to the disc. *g*, glomerular tuft; *v*, vascular canal; *d*, disc. H and E. ×60.

of this region, and formation of spicules of bone in relation to the calcified cartilage in the central region of the centrum. No periosteal bone was seen. The zone of hypertrophy reached the posterior surface (Fig. 6). Some chondrocytes immediately adjacent to the margin of the cartilage canal appeared to be enclosed partially in the cartilaginous matrix and partially in the connective tissue (Fig. 7).

95 *mm C.R. length* (*Fig.* 8)

The lower three thoracic and upper three lumbar vertebrae were examined. Sections were made as before, two lumbar vertebrae being cut transversely, the first lumbar and last thoracic sectioned coronally, the tenth and eleventh thoracic vertebrae in the sagittal plane.

Vascularisation was well marked. Blood vessels on the perichondrial surface of each vertebra, accompanied by a collar of perichondrial connective tissue, entered the centrum at several sites. Two large foramina at the mid-dorsal surface gave entry to two large vessels; ten to twelve smaller vessels were observed on the anterior, lateral and posterior surfaces of the centrum adjacent to the intervertebral discs. The vessels and their surrounding connective tissue were surrounded by an eosinophilic and PAS-positive membrane which was continuous with the perichondrium. The cartilage canals in the ventrolateral regions branched within the centrum, giving a medial (centrally directed) and one or more vertical branches towards the disc. Glomeruli were observed at the termination of these branches. The central axial area of the centrum contained the largest number of cartilage canals.

Three zones could be recognised in the centrum at this stage, a peripheral zone of

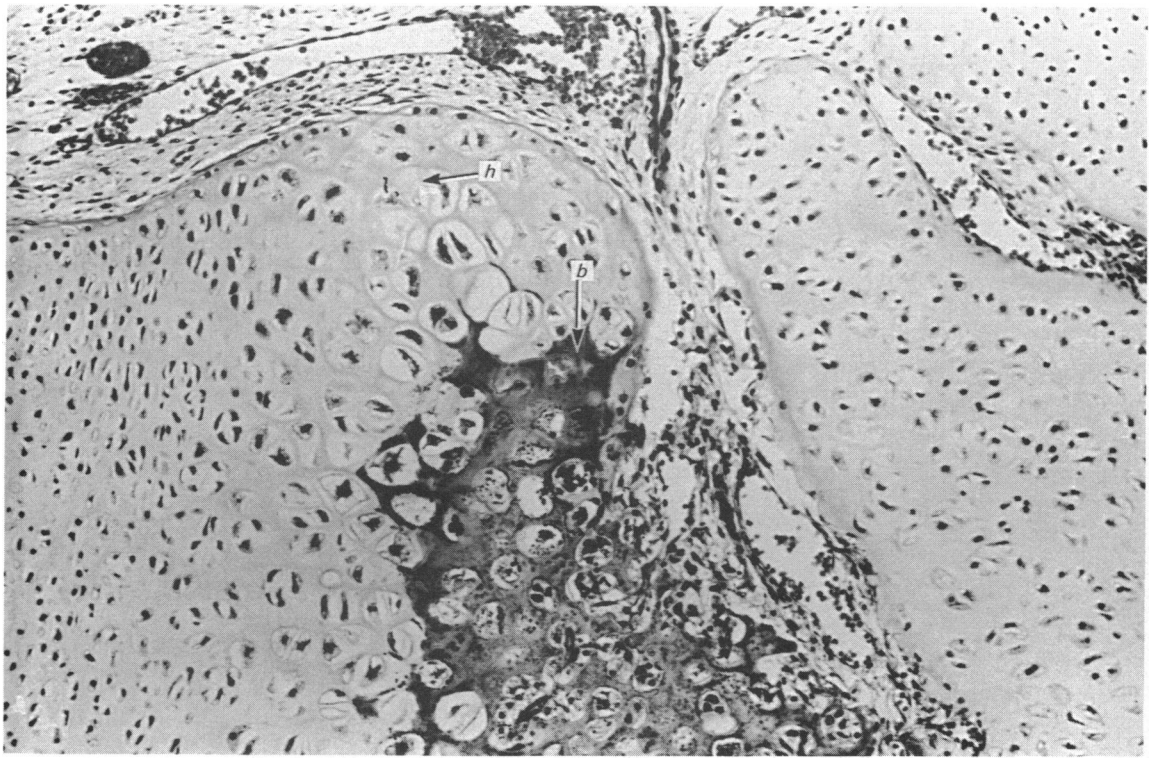

Fig. 6.

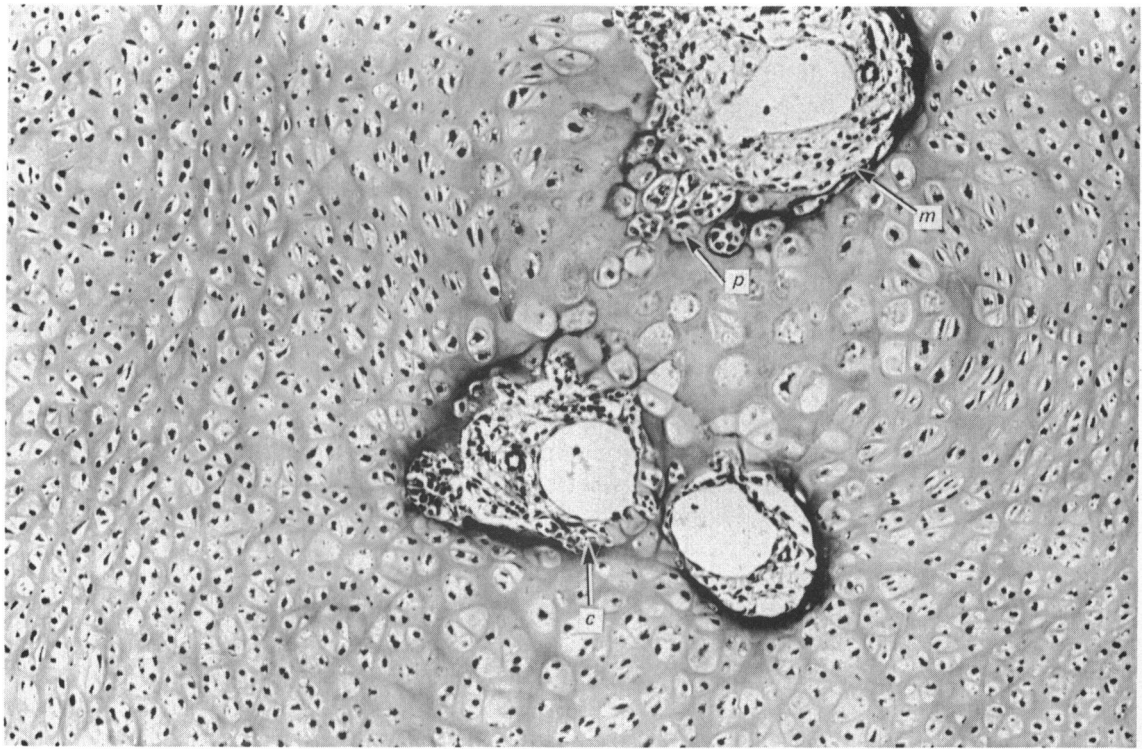

Fig. 7.

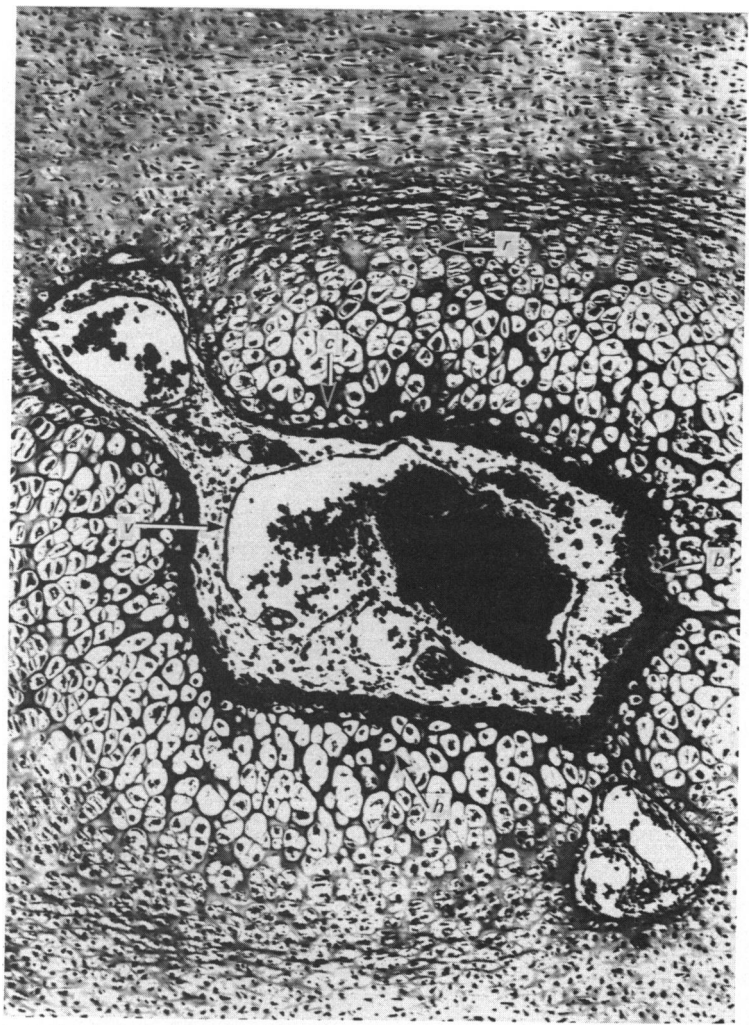

Fig. 8. 95 mm C.R. length, coronal section (thoracic). Bone spicules on walls of cartilage canal, connective tissue cells and blood vessels within canal. Resting cartilage, hypertrophy, calcification around central area. *b*, bone spicules; *v*, blood vessels; *r*, resting cartilage; *h*, hypertrophy; *c*, calcification. Masson's trichrome. ×60.

resting cartilage, a middle hypertrophic zone and a central area of ossification where the calcified cartilage was replaced by bone. The zone of calcifiation extended to the mid-dorsal region of the centrum, and very early evidence of periosteal bone was observed in the first lumbar vertebra. Multiple foci of ossification were observed in the cartilage canals present in the hypertrophic zone of the cartilaginous zone of the

Fig. 6. 85 mm C.R. length, transverse section (lumbar). Central area showing spicule of bone. Hypertrophic zone reaching dorsal surface. *b*, spicule of bone; *h*, hypertrophy. H and E. ×31·25.

Fig. 7. 85 mm C.R. length, coronal section (lumbar). Cellular proliferation within cartilage canals. Chondrocytes partly within connective tissue matrix in canal and partly outside in cartilaginous matrix. *p*, proliferating cells; *c*, cartilage canal; *m*, membrane around cartilage canal. Masson's trichrome. ×31·25.

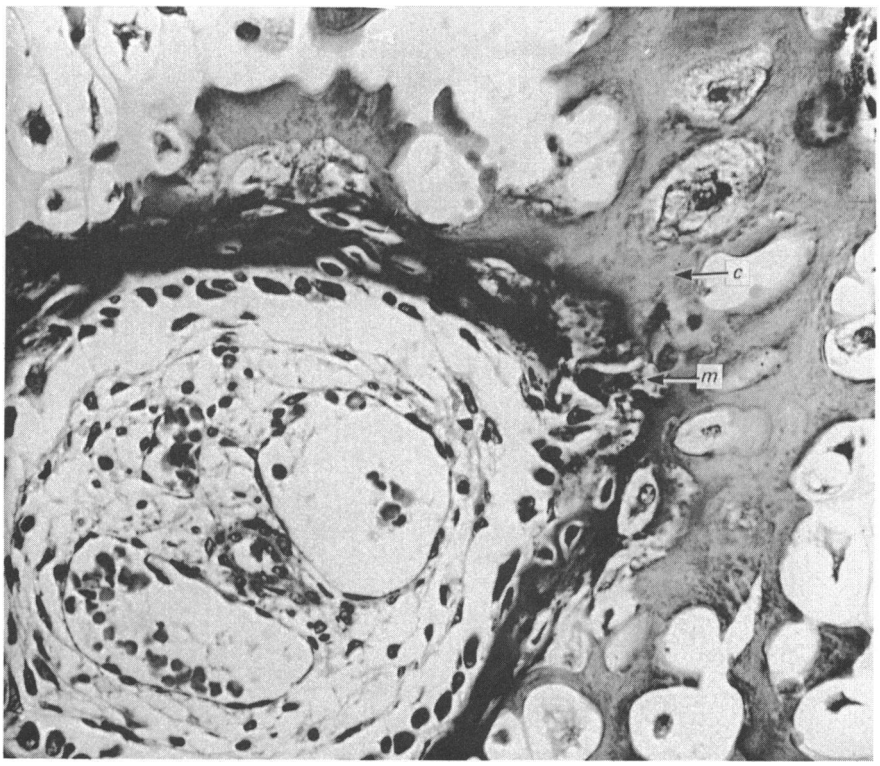

Fig. 9.

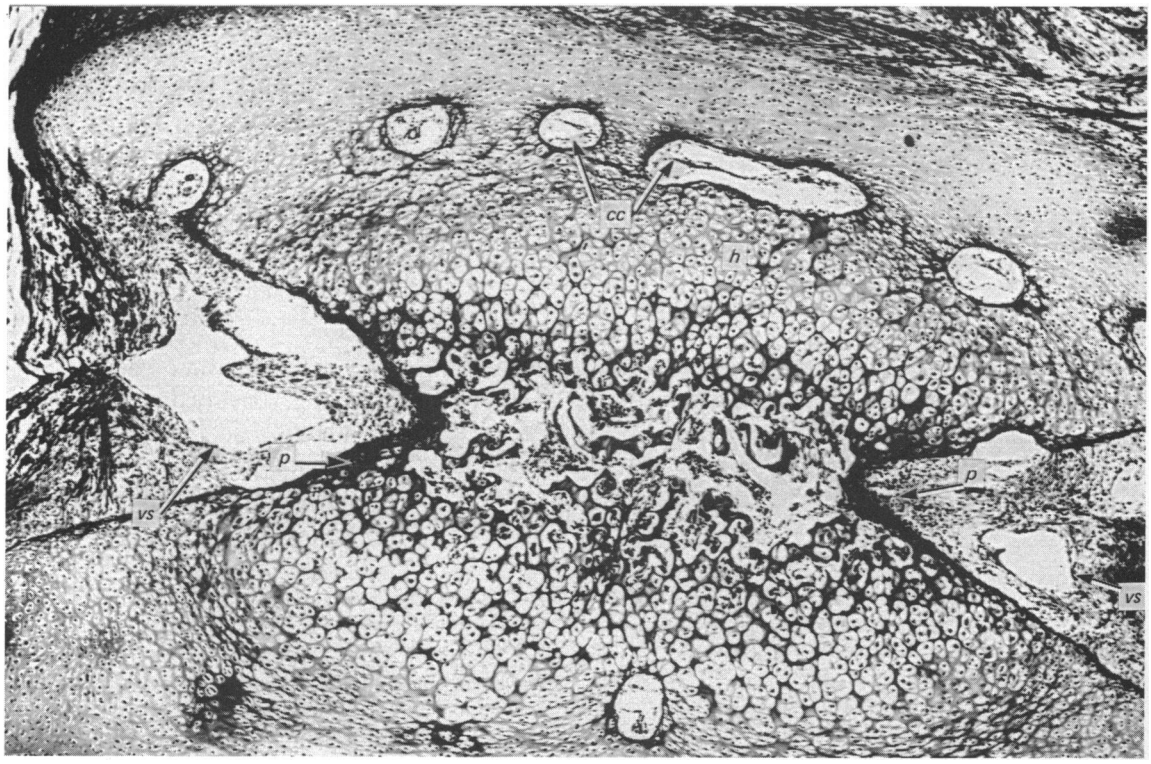

Fig. 10.

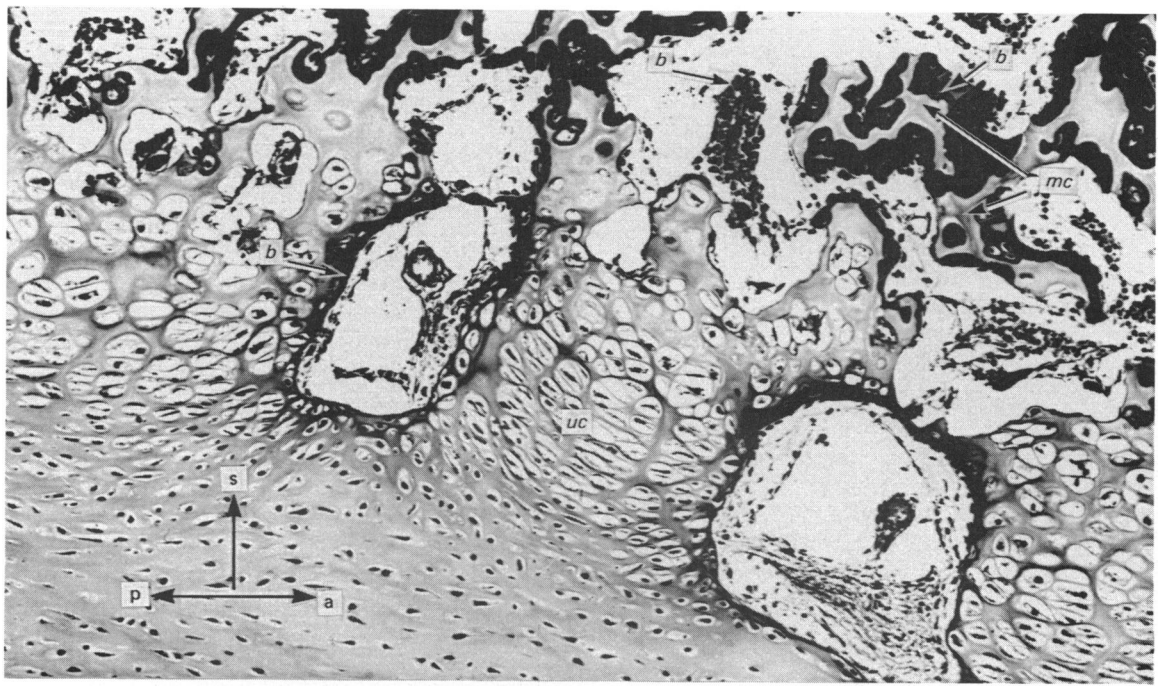

Fig. 11. 125 mm C.R. length, sagittal section (lumbar). Bone spicules in relation to cartilage canal in hypertrophic zone. Bone formation on mineralised and unmineralised cartilage. *b*, bone spicules; *mc*, mineralised cartilage; *uc*, unmineralised cartilage; *s*, superior; *a*, anterior; *p*, posterior. Masson's trichrome. × 31·25.

centrum (Fig. 8). Spicules of bone were found on the walls of the cartilage canal, a feature peculiar to ossification in the vertebral centrum; that is bone tissue being formed on the walls of the canal. In addition evidence of cartilage cells and hypertrophy of cartilage cells was also found within the walls of the cartilage canal.

115 *mm C.R. length* (*Fig.* 9)

The lower thoracic and upper lumbar vertebrae were examined. The appearances observed at this stage were very similar to those of the 95 mm C.R. length embryo. Figure 9 illustrates a multinucleated cell in relation to an area of calcified cartilage that is being eroded.

125 *mm C.R. length* (*Figs.* 10–12)

The lower two thoracic and upper lumbar vertebrae were sectioned in the transverse, coronal and sagittal planes. The central ossifying area had expanded

Fig. 9. 115 mm C.R. length, coronal section (thoracic). Multinucleated cell at mouth of cartilage canal invading calcified cartilage. *m*, multinucleated cell; *c*, calcified cartilage. Masson's trichrome. × 78·25.

Fig. 10. 125 mm C.R. length, sagittal section (lumbar). Resting cartilage, hypertrophic zone, cartilage canals with bone spicules on walls, central area of calcified cartilage, bone marrow. Periosteal bone on anterior and posterior surface. Venous sinuses. *cc*, cartilage canals; *h*, hypertrophic zone; *p*, periosteal bone; *vs*, venous sinuses. Masson's trichrome. × 12·5.

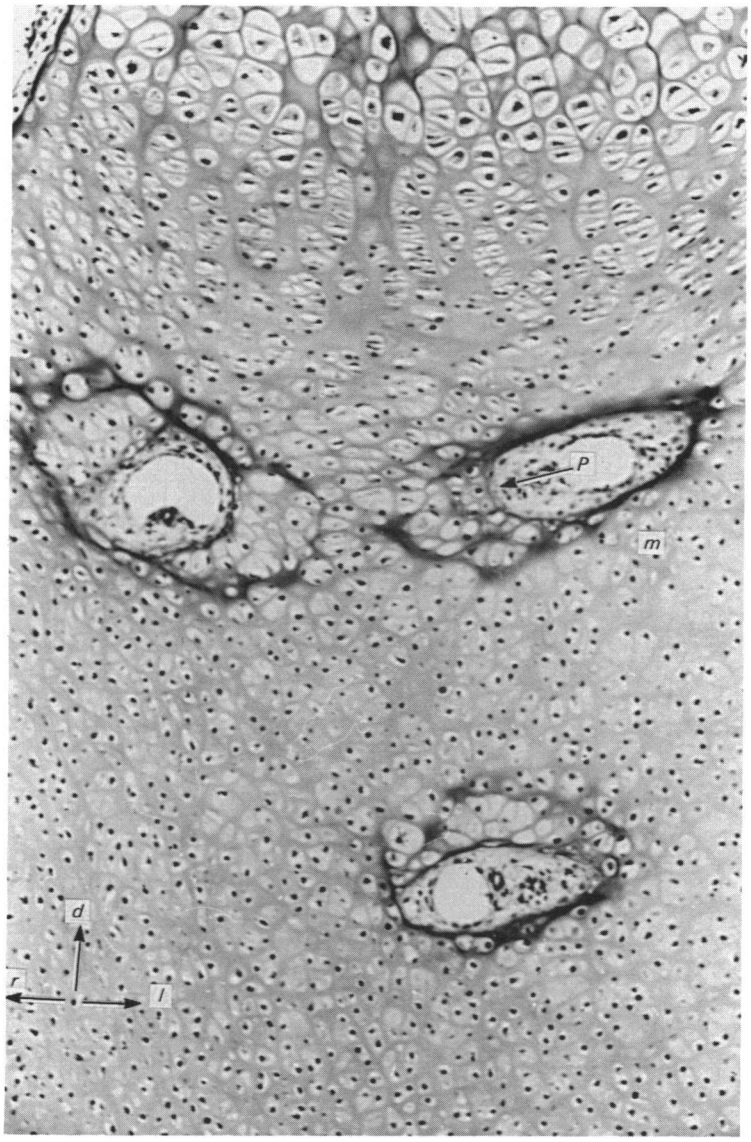

Fig. 12. 125 mm C.R. length, transverse section (lumbar). Cellular activity within cartilage canal contributing to anterolateral growth. *p*, cellular proliferation within cartilage canal; *m*, membrane; *d*, dorsal; *r*, right; *l*, left. Masson's trichrome. ×31·25.

centrifugally. The hypertrophic zone, referred to at the 95 mm C.R. stage, was now composed of three to five rows of hypertrophic cartilaginous cells. On the inner aspect of the hypertrophied area were remnants of eroded calcified cartilage and spicules of bone. The most central region contained red cells and marrow cells, in addition to the irregular mass of bone and calcified cartilage. There were multiple foci of ossification in relation to the cartilage canals in the hypertrophic zone (Fig. 10) and bone formation was observed on the internal and external aspects of the hyphertrophic zone (Fig. 11). Some of the cartilage canals within the hypertrophic zone had lost their eosinophilic (PAS-positive) membrane, and at these sites increased cartilage cell proliferation and hypertrophy were observed. Some periosteal bone was found on the

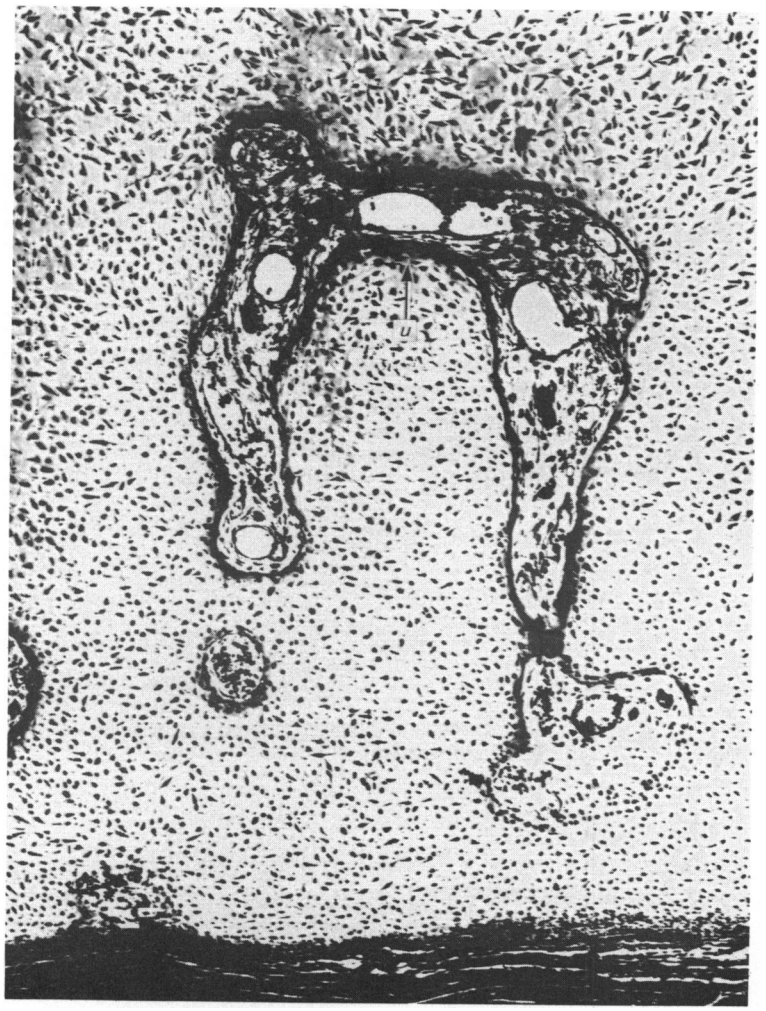

Fig. 13. 147 mm C.R. length, transverse section (lumbar). U-bend in cartilage canal. *u*, centrifugal bend of cartilage canal. H and E. ×60.

anterior and posterior surfaces of the centrum; this appeared as irregular spicules confined to the mid-region of the body. A superior and an inferior convexo-concave growth cartilage could be defined in the sagittal view. The cartilage canals seen in transverse sections in the peripheral resting cartilage exhibited cellular proliferation and chondrocytes within the canals (Fig. 12).

147 *mm C.R. length* (*Fig.* 13)

The lower two thoracic and upper lumbar vertebrae were serially sectioned as before in the transverse, coronal and sagittal planes. Some of the cartilage canals in the anterolateral region of the centrum were observed to make a U-turn and travel centrifugally towards the periphery of the centrum (Fig. 13). Branches of cartilage canals passing towards the disc and to the central ossifying area were also observed. Spicules of bone were observed on the internal and external aspects of the hypertrophic cartilage. The narrow spaces were larger in the central area; remnants of calcified cartilage and bone trabeculae were also present.

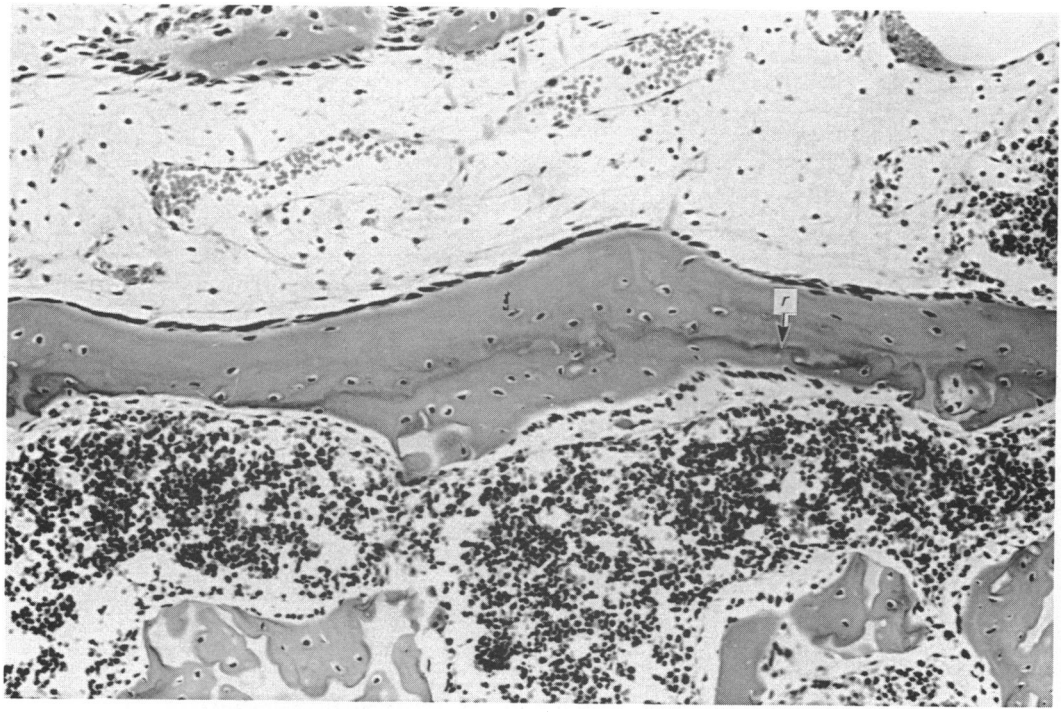

Fig. 14. 185 mm C.R. length, transverse section (lumbar). Cement (reversal) line on periosteum of dorsal surface. *r*, reversal line. H and E. ×31·25.

185 *mm C.R. length* (*Fig.* 14)

The lower two thoracic and upper lumbar vertebrae were serially sectioned as before in the transverse, coronal and sagittal planes. In addition to a total increase in size at this stage, there was evidence of periosteal bone resorption indicated by the reversal (cement) line on the dorsal surface of the centra (Fig. 14), while it was not found on the anterolateral surface. Large periosteal venous sinuses were observed anteriorly and posteriorly in the central region of the vertebral body. The central area had larger trabeculae of bone in the transverse and longitudinal planes. These trabeculae were continuous with the periosteum on the dorsal surface. Multiple foci of osteogenesis were observed in relation to the cartilage canals in the hypertrophic zone of cartilage.

DISCUSSION

The results of this study indicate that the earliest changes in the developing centra occur in the 55 mm C.R. length fetus and consist of hypertrophy and calcification of cartilage in the vicinity of blood vessels (Figs. 2, 3). This finding supports the conclusion made by Wilsman & Van Sickle (1970) and Kugler, Tomlinson, Wagstaff & Ward (1979) who studied the process of secondary ossification in the long bones of animals, and observed that the centres of ossification formed in a well vascularised area and not in an ischaemic area. The earliest spicules of bone observed at the 85 mm C.R. length stage were found within the centrum in relation to the central branches of the cartilage canals (Fig. 6) and not on the dorsal surface of the centrum as described by Walmsley (1953). Osteogenesis in the centra, therefore, appears analogous to secondary ossification in the epiphysis of a long bone as observed by Wilsman & Van

Sickle (1970) in dogs, Kugler *et al.* (1979) in rats and Gray & Gardner (1969) in human fetal material. Ogden (1979) was also of the opinion that cartilaginous centra ossify in a manner similar to a secondary centre in a long bone; however, he did not have any histological evidence nor did he specify the precise role of cartilage canals in osteogenesis. Although Walmsley (1953) and Bick & Copel (1950) examined histological sections of human material in their studies, they appear to have missed some earlier stages of development in their series, hence their conclusion that a vertebral body grows precisely as does the diaphysis of a long bone differs from our own observations.

The earliest bone formation in this study, recognised by its staining characteristics with the stains used, was found within the centrum and not in a periosteal position on the dorsal surface. The earliest trace of periosteal bone was first observed on the dorsal surface of the centrum at 95 mm C.R. length, at which stage large spicules of bone were found on the walls of the cartilage canals within the centrum (Fig. 8). The time at which the earliest bone formation was observed was at 85 mm C.R. length, and not at 55 mm C.R. length, as observed by Bagnall *et al.* (1977) and Noback & Robertson (1951). The method used by Bagnall *et al.*, namely radiological examination, would have detected the earliest change, that is calcification at 55 mm C.R. length, which was observed histologically by Walmsley (1953) and in this study. Noback & Robertson used the alizarin red method to detect the earliest vascularisation that occurs at 55 mm C.R. length. The alizarin red method by itself, however, would not be sufficient, because vascularisation of the cartilaginous centra prior to ossification has been observed by Streeter (1949), Walmsley (1953) and in the present investigation. As Noback & Robertson (1951) correctly remarked, the most suitable method of study is a serial examination of the developing bone, as described in this paper. Although calcification and vascularisation occur at the 55 mm C.R. length, the actual bone is laid down at about the 85 mm C.R. length.

The structure of the cartilage canal was found to vary according to its function and the length of time it had been established. The earliest canal observed at 55 mm C.R. length was an unbranched narrow canal which contained red blood cells and other nucleated cells, possibly of marrow origin (Fig. 3) which reached the central calcified zone. When the total size of the centra had increased, at 85 mm C.R. length, several cartilage canals, wider in diameter and containing well defined vessels and loose connective tissue cells within the canal, were seen. These canals branched within the centra (Figs. 4, 5); those branches directed towards the intervertebral disc had a glomerular plexus at their termination (Fig. 5). The central branches which reached the calcification zone showed, in addition to the glomeruli, evidence of connective tissue cell proliferation and differentiation (Figs. 6, 7). By 95 mm C.R. length the spicules of bone found on the walls of the cartilage canal gave evidence of the osteogenic activity of cells within these canals. The presence of multinucleate cells at sites where cartilage canals invade calcified cartilage (Fig. 9) lends support to the phagocytic (chondroclastic) activity of connective tissue cells within these canals.

There appears to be a relationship between the cellular activity within the cartilage canal and the surrounding hypertrophic zone of cartilage in the developing centra. Kugler *et al.* (1979), who worked on secondary ossification in rabbit long bones, considered it probable that the hypertrophic cartilage is responsible for the proliferation and differentiation of the connective tissue cells within the cartilage canals. The cartilage canals located in the hypertrophic zone of the convexo-concave growth plates of the 125 mm C.R. length fetus in this study (Figs. 10, 11) illustrate multiple foci of cartilage and bone formation within them. Such foci of cell

differentiation are significant, because the enhanced growth of the centra in the anterolateral regions could be a direct result of the presence of such cartilage canals (Fig. 12). There were more cartilage canals in the anterolateral segment of the centra than in the posterior segment. Ratcliffe (1981), having studied the arterial anatomy of the human lumbar vertebrae by a microradiographic method, observed that growth on the anterolateral surfaces of the vertebral body is greater than on the posterior surface, and that vertebral diameter increases through growth on the anterolateral surfaces. Knuttson (1961) made observations on the postnatal growth of vertebrae and he too observed growth in diameter to be predominantly on the anterolateral surface. Further evidence of the central role cartilage canals play in vertebral growth is seen in their changing pattern that occurs during growth. The U-turn (centrifugal) in the cartilage canal observed in the 147 mm C.R. length fetus (Fig. 13) illustrates the direction of growth in the centra. This observation is in agreement with that of Ratcliffe (1981), who observed that intraosseous arteries in developing vertebral bodies followed the advancing zone of ossification in a centrifugal manner.

The contents of the cartilage canals, in addition to providing cells which initiate osteogenesis in the vertebral centra, appear to contribute to the horizontal and anterolateral growth in the centra. Figure 7 (85 mm C.R. length) and Figure 12 (125 mm C.R. length) indicate that the proliferation and differentiation of cells within the cartilage canals to form chondrocytes permit such growth. Such a differentiation of cells within the cartilage canals was also observed by Kugler *et al.* (1979) in the rat epiphysis.

This study therefore lends histological evidence that supports the observations made by Ratcliffe (1981) and Knuttson (1961), who observed anterolateral growth in vertebral bodies to be greater than the anteroposterior growth.

The presence of reversal (cement) lines on the dorsal aspect of the 187 mm C.R. length fetus illustrates a difference in the growth pattern on the posterior surface.

The cartilage canals in the developing vertebrae appear to permit a very rapid process of bone formation to occur and thus contribute to the rapid increase in size. Crisman & Low (1974), who examined the development of chick vertebrae, observed multiple foci of ossification on both mineralised and unmineralised cartilaginous matrix. Lutfi (1970) also made similar observations. The appearance of multiple foci of bone formation in an uncalcified zone of cartilage, observed in this study in the 125 mm C.R. length fetus (Figs. 10, 11), in which bone tissue was observed on the walls of the cartilage canal, is different from normal endochondral ossification; in fact it could be considered analogous to intramembranous ossification. The walls of the cartilage canals were found to be continuous with the perichondrium in the 85 mm C.R. length fetus (Fig. 4). Similar bone formation within the cartilage canal was demonstrated in the epiphyses of long bones by Gray & Gardner (1969).

The findings of this study, taken as a whole, indicate that the cartilage canals in human fetal vertebrae perform similar functions to those observed in the secondary centres of ossification in long bones, as studied by Lutfi (1970), Wilsman & Van Sickle (1970, 1971), Gray & Gardner (1969) and Kugler *et al.* (1979). Although Tondury (1953), Larsen & Nordentoft (1962), Theiler (1965) and Ogden (1979) considered vertebral body ossification to be analogous to secondary ossification, they did not define the precise role of cartilage canals in such osteogenesis. Whalen *et al.* (1985), however, state that cartilage canals in human vertebral bodies play no role in the formation of ossification centres. Such an opinion could well be due to the fact that they studied fetal vertebrae from 16 weeks of gestation (which could exceed 100 mm C.R. length) to full term, and missed the early phases of development.

Why cartilage canals invade the centra around 55 mm C.R. length, as observed in this study, and also by Brookes (1970) and Theiler (1965), remains unanswered. Whether hypertrophy of cartilage cells in the vicinity of the dorsal vessels leads to an environmental change in cartilage, resulting in an altered enzyme activity as shown by Reddi & Kuettner (1981), and speculated on by Stockwell (1971), is possible. The hypertrophy of the cartilage certainly starts the sequence of events leading to calcification, resorption and osteogenesis, as in endochondral ossification and osteogenesis of uncalcified cartilage. The latter feature appears to accelerate the growth and expansion of the centra.

According to Brookes (1970) it was Prochaska in 1810 who first proposed that cartilage canals convey osteoprogenitor cells to secondary centres of ossification. Although several workers have supported this concept, including Haines (1933), Lutfi (1970), Wilsman & Van Sickle (1970), it was Kugler *et al.* (1979) who gave cytological evidence of this process, and defined the function of the connective tissue cells in the cartilage canals. The present study gives histological evidence of the role of cartilage canals in the initiation of vertebral body ossification. In addition, this study demonstrates that the bone tissue in the centra is not all formed by a process of endochondral ossification. The presence of bony trabeculae in cartilage canals varies with the length of time they have been established. The centrifugal turn in cartilage canals supports the concept that the centra grow in an anterolateral direction. Juxtadiscal terminations of the cartilage canal were observed and will be followed in later stages of development in a subsequent study.

SUMMARY

Bone formation in the vertebral centra commences within the centrum and is in this respect analogous to the secondary ossification which occurs in the epiphysis of a long bone. Bone tissue first appears at about the 85 mm C.R. stage and not in the 55 mm C.R. length embryo; at the latter stage blood vessels and calcification alone were observed.

The connective tissue cells within the cartilage canal appear to assist osteogenesis by providing osteogenic cells which lay down bone in the walls of the cartilage canal, and provide cells which remove calcified cartilage found at the periphery of the canal; they assist growth by producing an appreciable number of chondrocytes that permit lateral expansion of the centra. Osteogenesis appears to occur in multiple foci within the growth plate of the older embryos and could account for the rapid rate of growth of vertebrae. Bone formation occurs in both mineralised and unmineralised matrix (as seen on the walls of the cartilage canals). The blood vessels within the growing vertebra tend to follow the zone of cartilage hypertrophy.

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