J. Anat. (1997) **191**, pp. 259–267, with 11 figures Printed in Great Britain

The architecture of internal blood vessels in human fetal vertebral bodies

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(*Accepted 22 April 1997*)

ABSTRACT

The internal vascular system of vertebral bodies was investigated in 17–24 wk human fetuses by acrylic dye injection and by corrosion casting/scanning electron microscopy. The regions of intervertebral spaces did not contain blood vessels. The radial metaphyseal vessels were at the stage of centripetal ingrowth into the vertebral body cartilage and their terminal, blindly ending segments had a form of cuff-like capillary plexuses. The anterolateral equatorial arteries communicating with the vessels of the ossification centre were only rarely found. The centre was usually supplied by 2 posterior (nutrient) arteries which branched into an arcade-like array of arterioles equipped with occasional sphincters and giving origin to a dense network of peripherally located capillaries. Numerous blind capillary buds formed the advancing border of the ossification centre. The veins usually accompanied the arteries. In the ossification centre the venous compartment consisted of sinuses drained by larger posterior veins. In the 17 wk fetus, an axial avascular area was observed in the place of notochord localisation, indicating the formation of a ring-shaped ossification centre around the notochord remnants at earlier stages of fetal development.

Key words: Vasculature; notochord; corrosion casting; spine.

INTRODUCTION

The vascularisation of the human spine has been the subject of anatomical and microanatomical studies for almost a century. Attention has mostly been focused on the external segmental blood supply and drainage of the vertebral bodies (Lexer et al. 1904; Hanson, 1926; Böhmig, 1930; Batson, 1940; Willis, 1949; Harris & Jones, 1956). Only a few papers have described the intraosseous arteries of the vertebral bodies in detail (Wagoner & Prendergrass, 1932; Ferguson, 1950; Wiley & Trueta, 1959; Guida et al. 1969). Among more recent studies, a comprehensive description of both extra- and intraosseous vessels of the vertebral bodies was presented by Crock et al. (1973), Crock (1976) and Ratcliffe (1980, 1981, 1982). The latter author proposed the most convincing classification of the intraosseous arteries supplying the vertebral body, dividing them into (1) the equatorial arteries, including the nutrient arteries originating from the postcentral longitudinal anastomosis and 2 anterolateral arteries arising from the segmental artery, (2) the metaphyseal, radially disposed arteries arising from the periosteal vascular network and (3) short, centripetal peripheral arteries originating from the same source.

Almost all authors studying the vascularisation of the vertebral bodies employed x-ray angiographic methods which have not permitted visualisation of the capillary networks due to the high density of the contrast media. We decided to study the human fetal vertebral bodies using acrylic dye injection for light microscopy and corrosion casting for scanning electron microscopy. The latter method offers complete visualisation of the vascular system, significantly higher resolution and quasi-3-dimensional images which are especially useful in the investigation of the microvascular architecture (Lametschwandtner et al.

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1990). It was occasionally used for investigating the vertebral vasculature in animals including the rat (Konerding & Blank, 1987) and rabbit (Oki et al. 1994), but never in human material.

MATERIALS AND METHODS

Twenty-five fetuses (15 male, 10 female) with crown–rump length ranging from 160 to 240 mm, i.e. 17–24 wk of conception age according to Pineau's tables (Pineau, 1965), were used for the study. The fetuses were obtained after spontaneous abortions from the Institute of Gynaecology and Obstetrics, Jagiellonian University School of Medicine, Craców. All abortions were due to maternal disorders and on inspection the fetuses showed no malformations. The study was in accordance with the institutional requirements for the use of human material.

About 2 h after the abortion, the thorax of each fetus was opened to expose the heart and large vessels. The heart apex was cut off and a cannula inserted via the left ventricle into the ascending aorta and secured by a ligature. The vascular system of the fetus was then perfused manually with a sequence of solutions, efflux occurring via the umbilical veins and incised posterior tibial vessels.

Dye-injected specimens

The perfusion began with heparinised, prewarmed (37 °C) saline and was continued until the effluent saline was devoid of any visible traces of blood. The fetuses (11 male, 7 female) were then perfused with green aqueous acrylic emulsion (Liquitex, Binney & Smith, USA). When the emulsion began to flow out of the umbilical and tibial vessels, they were ligated and the perfusion was continued until a green tint appeared in the oral mucosa and conjunctivae. After termination of the perfusion and ligation of the aorta, the fetuses were fixed for 2 wk in 10% buffered formalin.

The lower thoracic and lumbar spine was dissected out, decalcified for 7 d in 5% trichloroacetic acid changed every day and dehydrated in graded series of ethanol. The removed fragments of the vertebral columns were cut into 2–5 mm thick sections in the coronal, sagittal and transverse planes. The sections were cleared first in absolute ethanol, next in ethanol/methyl salicylate solutions (3:1, 1:1, 1:3) and finally in pure methyl salicylate.

Cleared sections were examined in the Technival 2 (Zeiss Jena, Germany) stereo microscope.

Corrosion casts

Following a thorough perfusion with prewarmed (37 °C) saline carried out as described above, perfusion fixation was performed with 300–600 ml of 0.66% paraformaldehyde/0.08% glutaraldehyde in 0.2 M cacodylate buffer, pH 7.3 (Paine & Low, 1975) containing 0.2% lidocaine, at 37 °C. Finally, 60 ml of casting medium consisting of 8 ml Mercox CL-2B (Vilene, Tokyo) and 2 ml methyl methacrylate (Fluka) containing 0.2 g initiator per 10 ml of the final casting medium was injected. Following the injection, the fetuses (4 male, 3 female) were kept overnight in water at 55 °C in order to temper, accelerate and complete resin polymerisation (Miodoński et al. 1981).

After polymerisation of the resin, the lower thoracic and lumbar sergments of the spine were dissected out, the surrounding soft tissues were removed and the vertebrae were decalcified in several passages of 5% trichloroacetic acid for 7 d. Following rinsing in distilled water, the specimens were macerated in 10–15% potassium hydroxide for several days, the solution being changed every day after careful washing in hot distilled water.

The resulting vascular casts were carefully and thoroughly cleaned in 5% trichloroacetic acid, followed by washing in distilled water for the next few days. Some of the casts were cut to separate the vascular systems of individual vertebrae. The casts were then freeze-dried, mounted onto specimen stubs using colloidal silver and 'conductive bridges' (Lametschwandtner et al. 1980), coated with gold and examined in a JEOL JSM 35-CF scanning electron microscope at 20–25 kV.

RESULTS

Three concentric zones could be distinguished in the vascular architecture of the fetal vertebral bodies: (1) the peripheral zone of perichondrial vessels, (2) the intermediate zone of radial vessels and (3) the central zone of the ossification centre vessels.

Dye-injected specimens

In dye-injected specimens examined by light microscopy (Figs 1, 2), the venous system was practically not visualised, because the dye did not pass freely the capillary network. The perichondrial vessels formed a

relatively narrow layer from which fine arterioles were sometimes seen emerging from the inner circumference, especially from the anterolateral surface of the vertebral body, and forming a shallow, centripetally directed arcade-like array.

The radial arteries originated from the segmental arteries and their branches or from the perichondrial plexus. They were regularly distributed and in the sagittal view their blindly ending branches were directed towards the vertebral end-plates. The terminal segments of these vessels showed thickened, irregular contours. Occasionally, some branches seemed to communicate with the vessels of the ossification centre (Fig. 2).

The ossification centre was mostly supplied by 2 arteries of similar size (Fig. 1), originating from the spinal branches of basivertebral artery (postcentral anastomosis), entering the vertebral body from the posterior aspect and extensively branching into a lenticular network with a fan-shaped, centrifugal arrangement of the vessels.

The intervertebral spaces and end-plate regions were completely devoid of blood vessels (Fig. 2).

Corrosion casts

In corrosion casts examined by scanning electron microscopy, the perichondrial capillary network displayed in some areas a conspicuous, square grid-like pattern (Figs 3, 5). Three types of vascular structures were observed to protrude centripetally from the plane of the perichondrial network. (1) Paired vessels surrounded by elongated capillary plexuses in a cuff-like manner ended blindly above or below the ossification centre vessels, but were never seen to communicate with them (Figs 3–5). (2) Very rare arterioles showing only occasional side branches entered the ossification centre from its anterolateral aspects (Figs 3, 10). (3) Elaborate tortuous capillary plexuses were distributed quite regularly around the anterolateral perimeter of the vertebral body, forming low 'vascular folds' (Fig. 5).

The main arterial supply and venous drainage of the ossification centre were located at its posterior pole (Fig. 6). The arteries after entering the centre extensively branched into arterioles which formed multiple anastomosing arcades in its peripheral area, following the circular course of the advancing border of the centre (Fig. 7). In a few arterioles, sphincter-like constrictions could be observed just after the site of branching (Fig. 8).

The extreme periphery of the centre was occupied mainly by a dense capillary network. The capillaries terminated at the bone/cartilage interface, forming blind buds or, occasionally, irregular loops (Fig. 9). The peripheral capillary network was drained by a system of sinusoidal vessels converging in a tree-like manner into 1 or 2 main venous stems leaving the centre at its posterior pole (Fig. 6). They finally drained into the posterior inner vertebral plexus.

In the youngest, 17 wk fetus, an axial avascular area, ~ 0.7 mm in diameter, was present in the middle of the ossification centre (Fig. 10). Capillaries were seen to invade this area centripetally, as manifested by buds and occasional loops protruding from the surrounding vascular network (Fig. 11).

DISCUSSION

The general architecture of the blood vessels present in the vertebral bodies of fetuses from the 2nd trimester shows the pattern observed by other authors. Although some observations of the fetal vertebrae were mentioned in the early papers of Hanson (1926), Böhmig (1930) and Willis (1949), our results can be compared primarily with those reported by Ferguson (1950), Guida et al. (1969) and Ratcliffe (1981) who specifically devoted their studies to the developmental aspect. However, they investigated fetuses from the 3rd trimester and at term. It seems therefore that differences observed in the present study stem from the earlier stage of fetal development that was studied.

In dye-injected specimens, the localisation and the mode of branching of the radial arteries permitted their identification as the metaphyseal arteries, according to the classification of Ratcliffe (1980), while the occasional shallow arcades protruding from the perichondrial network correspond—as far as their size and arrangement is concerned—to the peripheral vessels. The equatorial vessels entering the vertebral body from an anterolateral direction and communicating with the vasculature of the ossification centre were found only rarely.

The interpretation of the images provided by scanning electron microscopy of vascular casts was sometimes difficult because of extreme fragility of the casts especially in the weakly supported region between the perichondrial network and the ossification centre vessels. As a result, the vessels of the ossification centre and the perichondral network were well preserved, while the vascular interconnections between them, if any, were often broken and displaced.

The observed capillary cuffs surrounding pairs of larger vessels are typical vascular arrangements of the developing cartilage canals (Skawina et al. 1994*b*).

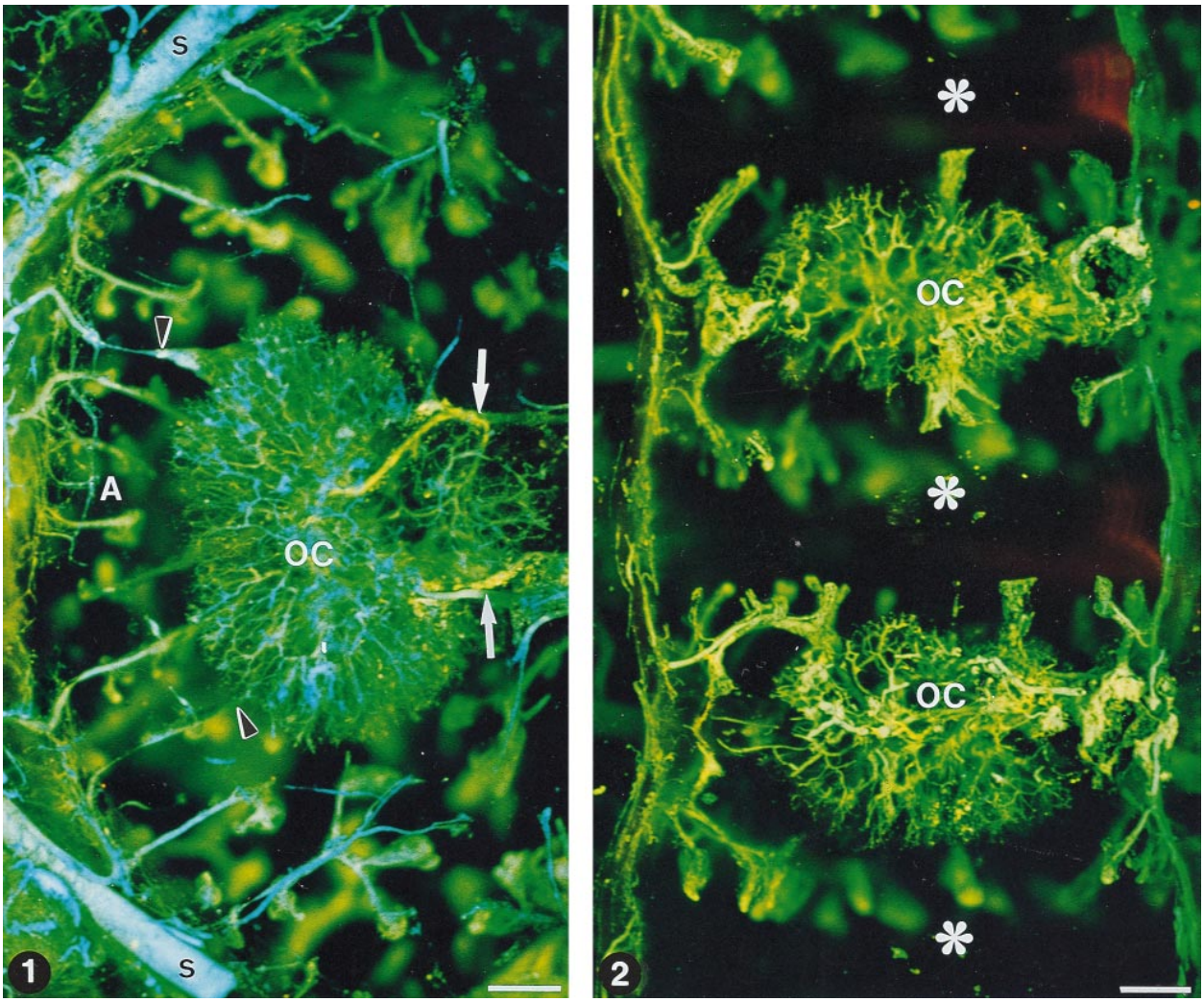


Fig. 1. Dye-injected vasculature of a vertebral body in 21-wk fetus (transverse section). Blindly ending radial arteries with extended irregular terminal segments are directed towards the ossification centre (OC), supplied by 2 posterior arteries (arrows). Two sites of communication between the radial arteries and the ossification centre are indicated by arrowheads. S, segmental artery; A, subperichondrial arteriolar arcade. Bar, 750 μm.

Fig. 2. Midsagittal view of the dye-injected vasculature of 2 adjacent vertebral bodies in 23-wk fetus, showing blindly ending radial arteries and their branches dircted towards the end-plates. The intervertebral regions (asterisks) are devoid of blood vessels. OC, ossification centre. Bar, 1000 μm.

Their appearance corresponds with the widened and irregular segments of the metaphyseal vessels seen in the dye-injected specimens. Cartilage canals directed towards the end-plates were demonstrated in dye-injected human fetal and rabbit vertebral bodies by Whalen et al. (1985) who suggested that they provided an important source of nutrition to the avascular intervertebral disc region during fetal development. Since the interconnections between the metaphyseal arteries reported by Ratcliffe (1981) could not be seen in our material, it seems that in the 2nd trimester these arteries are at the stage of ingrowth into the vertebral body cartilage. They become fully incorporated into the ossification centre about the 36th wk of fetal life (Ratcliffe, 1981).

Occasional arterioles entering the ossification centre from the periphery probably represent the antero-lateral equatorial vessels, sometimes seen also in dye-injected specimens. However, they did not occur consistently at the developmental stage investigated in this study, although they had been described in 7–10 mo old fetuses by Ferguson (1950), Guida et al. (1956) and Ratcliffe (1981).

The fold-like capillary plexuses with their regular distribution around the inner perimeter of the vertebral body may be precursors of the later peripheral vessel system. Ratcliffe (1981) did not find peripheral arteries in his material but it seems that the development of peripheral vessels may just begin in the 2nd trimester, albeit only in some local sectors.

The vasculature of the ossification centre clearly shows that the posterior vessels are mainly, if not

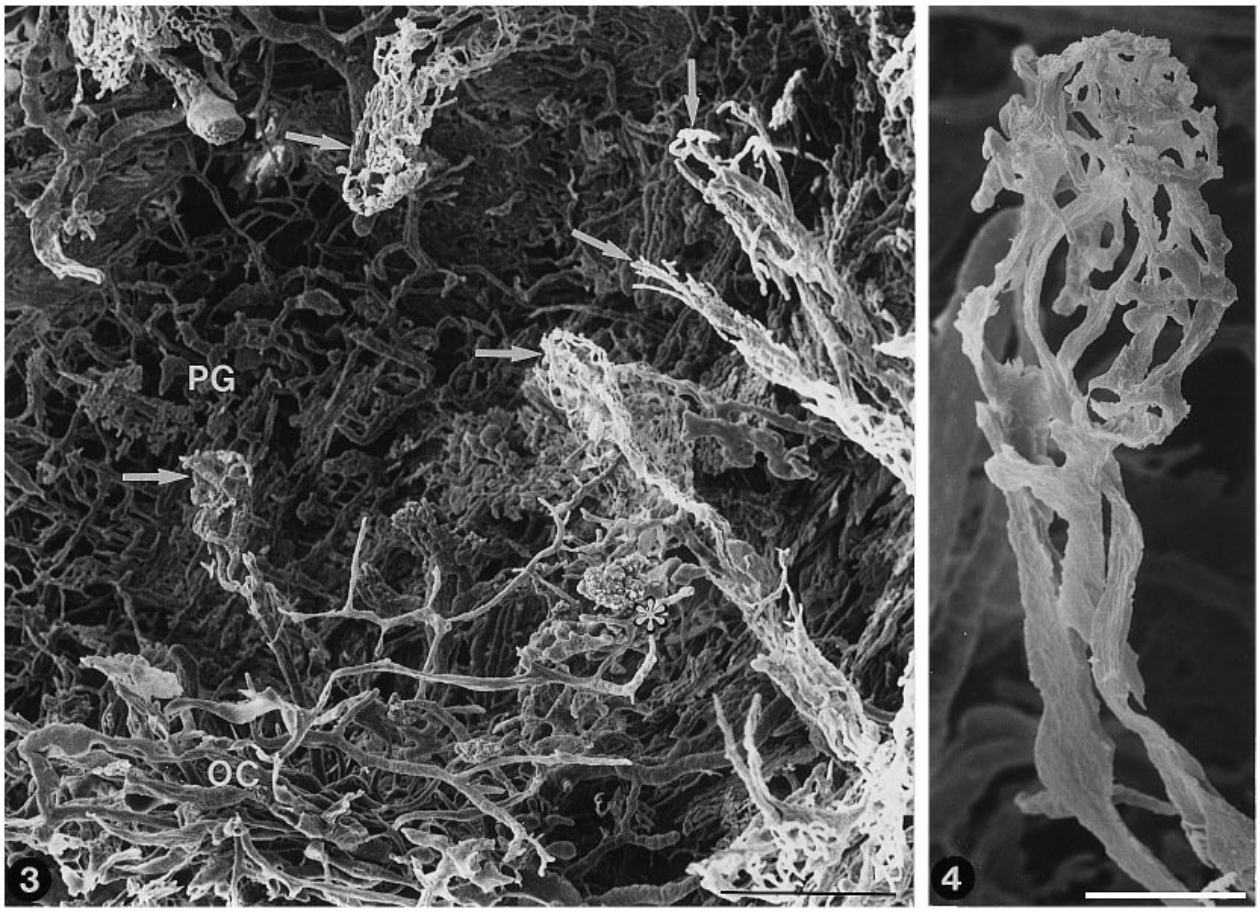


Fig. 3. Anterior inner perichondrial area of the vertebral body viewed from the inside. Radial vessels protruding from the perichondrial capillary grid (PG) have a form of elongated cuff-like capillary plexuses (arrows). Asterisk indicates arteriole communicating with blood vessels of the ossification centre (OC). Corrosion cast, SEM. Bar, 500 μm.

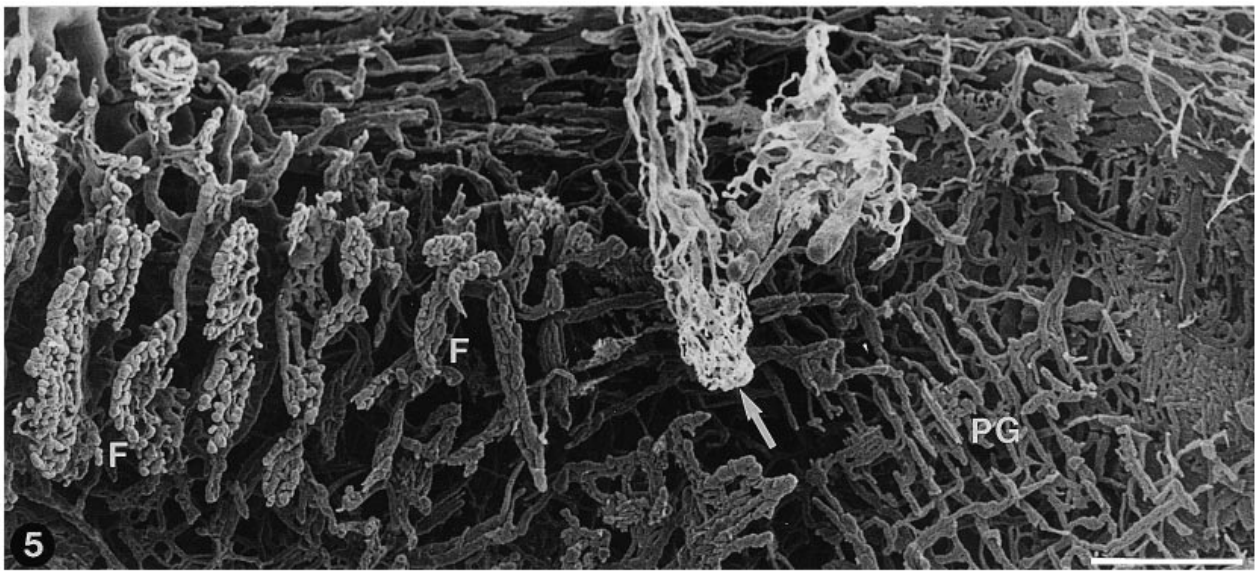
Fig. 4. A short radial vascular structure under higher magnification, showing 2 parallel vessels terminating in a capillary plexus. Corrosion cast, SEM. Bar, 100 μm.

exclusively, responsible for the supply and drainage of the centre in the 2nd trimester. The number of the posterior (nutrient) arteries has been a matter of controversy, ranging from 1 (Ferguson, 1950) to 4 (Guida et al. 1969). In our material, most of the vertebral bodies had 2 nutrient arteries, while the others were supplied by just 1. These results are in agreement with the observations of Ratcliffe (1981).

The presence of arterial sphincters has not yet been reported in the intraosseous vasculature of human vertebral bodies, but such sphincters, both arterial and venous, were described in the rat (Konerding & Blank, 1987). Interestingly, in our material the circular sphincter-like constrictions were found exclusively in the blood vessels of the ossification centre. Sphincters may control blood supply to the specific areas of the centre, locally influencing the ossification process.

The appearance of the capillary vessels at the advancing border of the ossification centre is similar to that of the 'vascular besom' described by us (Skawina et al. 1994*a*) in corrosion casts of the femoral vessels directed towards the metaphyseal plate and it seems to be a typical arrangement of capillaries at the bone/cartilage interface in the ossification areas. The presence of sinusoidal vessels, mainly belonging to the early venous compartment, previously reported by Ferguson (1950), is also consistent with our observations of the femoral metaphyseal region. Such sinusoids developing in the ossification centre will later remain in the bone marrow.

The internal venous system of the fetal vertebral bodies generally followed the course of the arteries, as also seen in adults (Crock et al. 1973; Crock, 1976), although some specific venous arrangements such as the subchondral network and the horizontal collecting vein of the endplate region described by Crock et al. (1973) have not yet been developed. Only Ferguson (1950) mentioned the presence of blood vessels in the region of fetal end-plates and even of the annulus



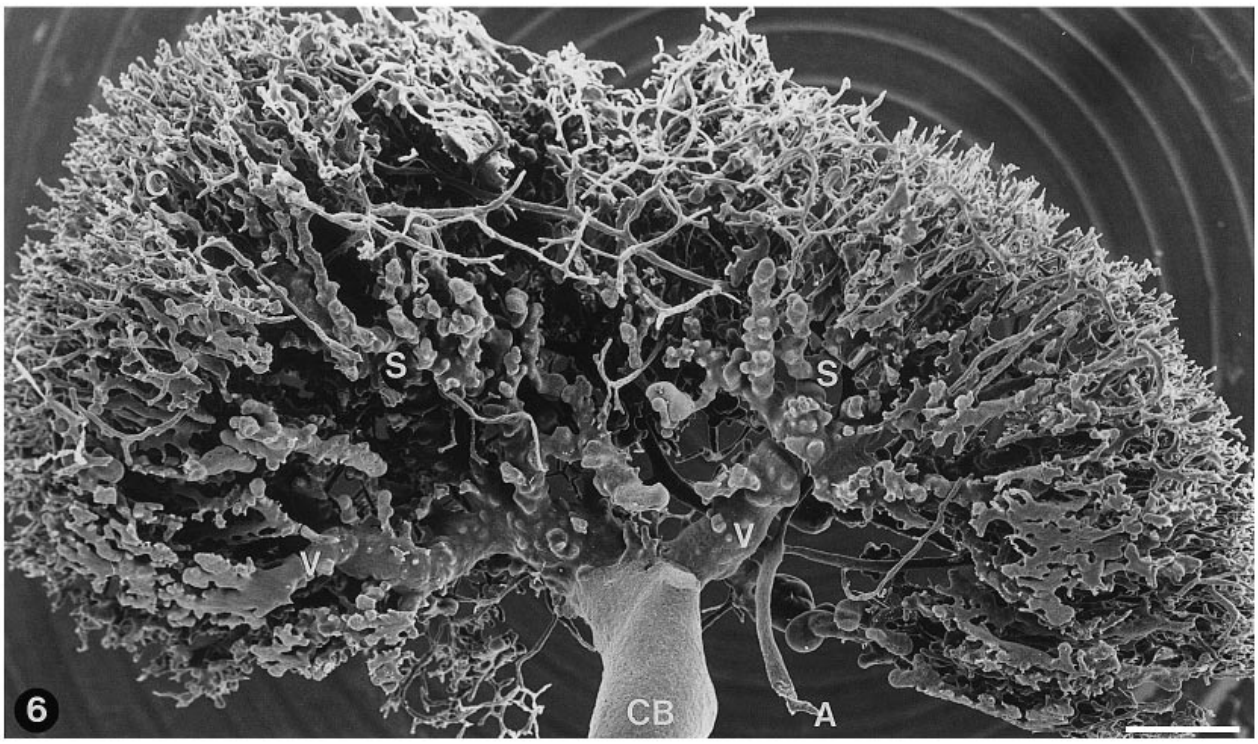


Fig. 5. Perichondrial capillary grid (PG), a radial vessel (arrow) and fold-like capillary plexuses (F) on the inner anterior surface of the vertebral body. Corrosion cast, SEM. Bar, 500 μm.

Fig. 6. Vasculature of the ossification centre with centrifugally advacing capillary 'besom' (C) and a tree-like system of venous sinuses (S) draining into veins (V). A, posterior (nutrient) artery; CB, conductive bridge. 24-wk fetus, corrosion cast, SEM. Bar, 500 μm.

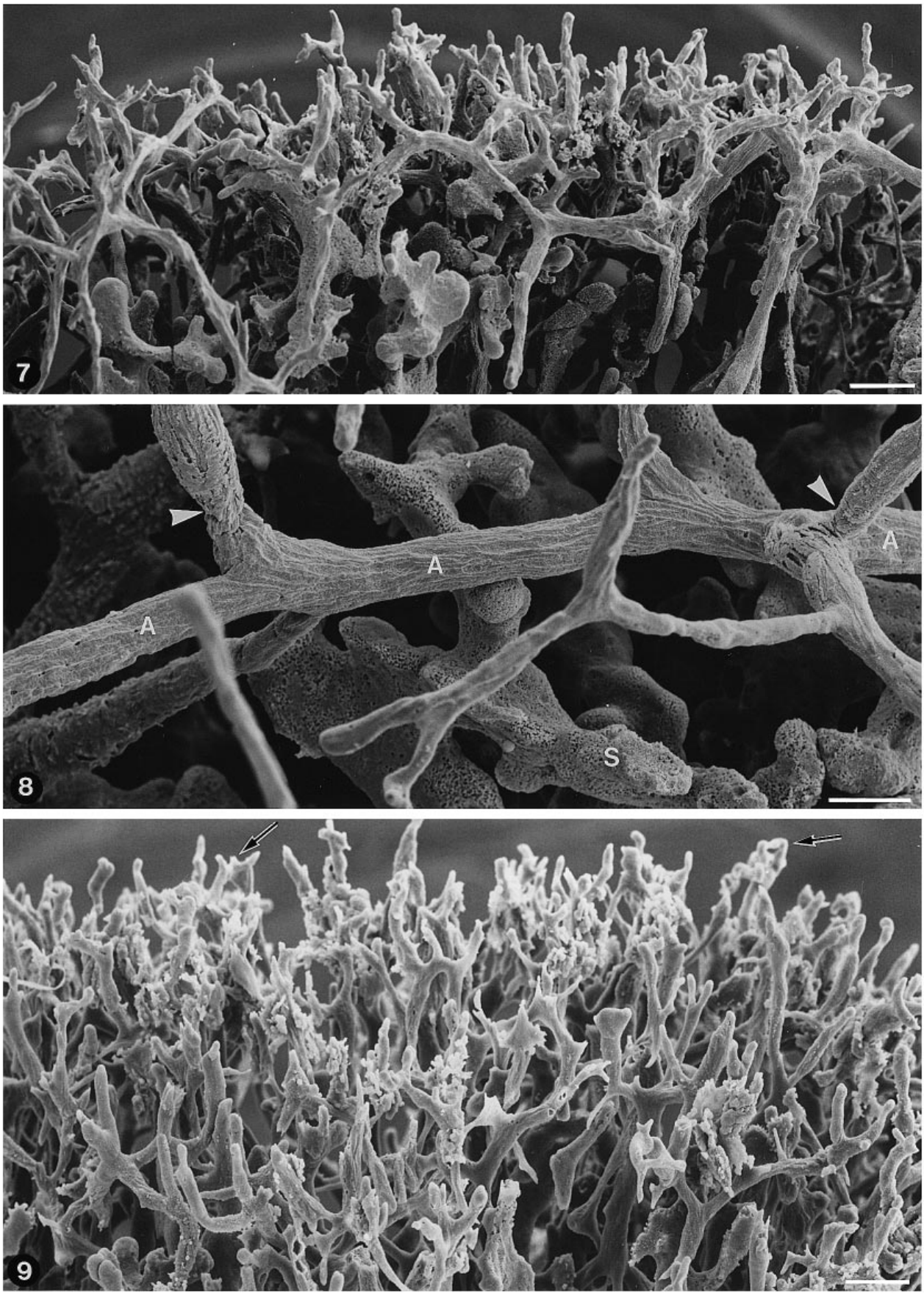
fibrosus. Our observations, however, agree with those of Guida et al. (1969) and Ratcliffe (1981) who also found the end-plate area avascular.

A striking observation was the axial avascular area in the centre of the vertebral bodies of the youngest fetus examined, corresponding to the location of the notochord and making the vasculature of the ossification centre ring-shaped. According to our knowledge,

Fig. 7. Arteriolar arcades near the advancing border of the ossification centre. Corrosion cast, SEM. Bar, 100 μm.

Fig. 8. Branched arteriole (A) from the ossification centre with 2 sphincter-like constrictions near the sites of branching (arrowheads). S, sinusoids. Corrosion cast, SEM. Bar, 50 μm.

Fig. 9. Capillary buds and occasional loops (arrows) at the bone/cartilage interface of the ossification centre. Corrosion cast, SEM. Bar, 100 μm.



Figs 7–9. For legend see opposite.

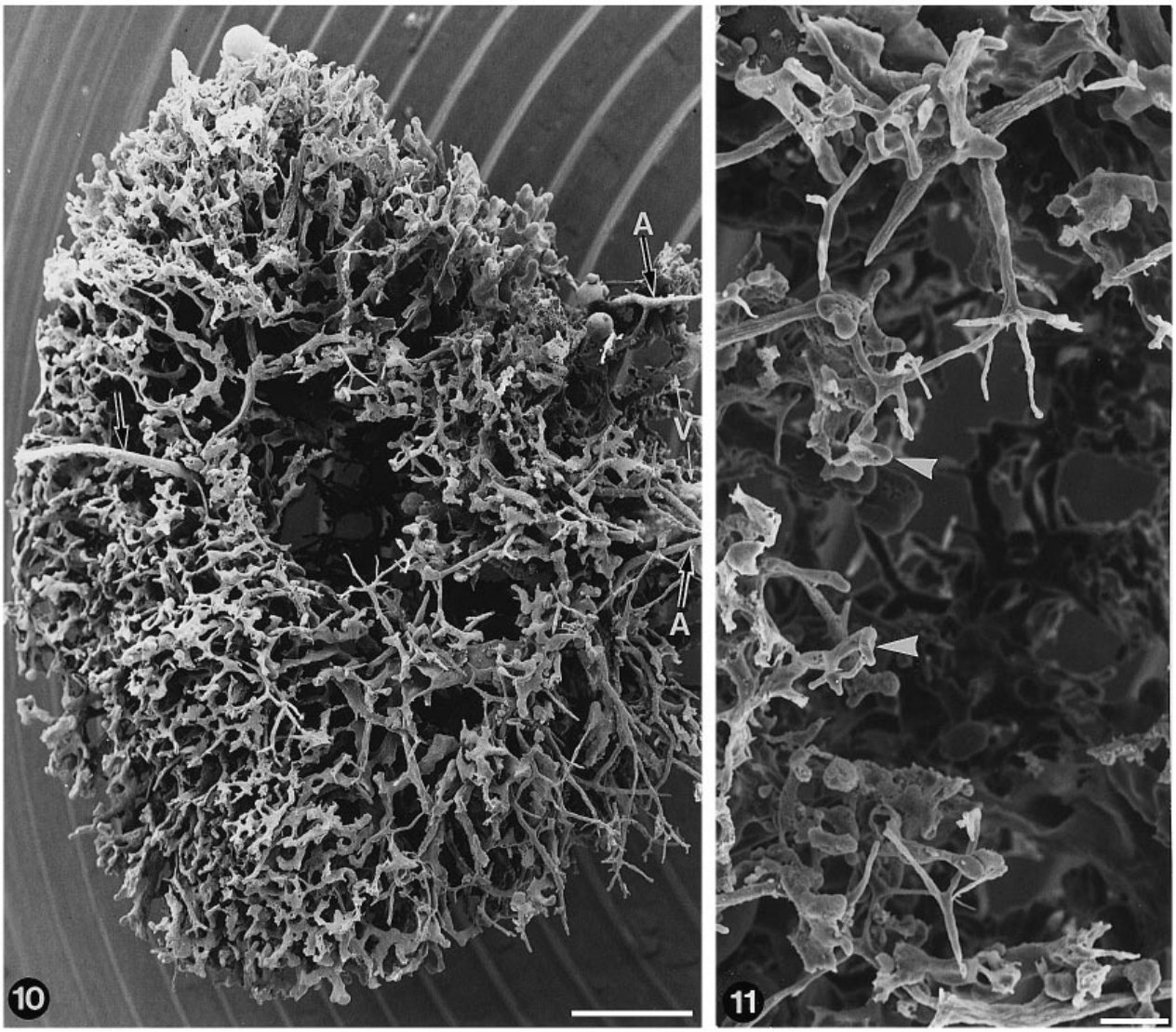


Fig. 10. Vasculature of the ossification centre from vertebral body of a 17-wk fetus. Note the axial avascular area. A, nutrient artery; V, efferent vein; arrow, radial artery entering the ossification centre. Corrosion cast, SEM. Bar, 500 μm.

Fig. 11. Capillary buds and loops (arrowheads) growing into the avascular area shown in Figure 10. Corrosion cast, SEM. Bar, 100 μm.

this is the first description of such vascular array in the fetal vertebral body. Böhmig (1930) reported the occurrence of an axial artery running along the previous localisation of the notochord, but later nobody could confirm his observation. The replacement of mesenchyme by cartilage in the centre of the vertebral body proceeds from the 6th week on by growth and coalescence of 2 chondrification centres flanking the notochord and ultimately surrounding its remnants (Tondury,1958). The notochord disappears completely on the turn of the 1st trimester, while the earliest ossification centres in the vertebral bodies have been observed as early as in the 8-wk fetuses (Bareggi et al. 1994). It seems therefore that the ossification centre is first formed around the remnants of the notochord and later—at the beginning of the 2nd trimester—the vessels advance in both directions: centrifugally, increasing the size of the centre, and centripetally, invading the formerly avascular central region. The observed arterial arcades located medially to the advancing border of the ossification centre might be the manifestation of the initial ring-shaped arrangement of the centre. It can be speculated that the notochord cells may contain some angiogenesis-inhibiting factors acting locally on the developing vessels of the ossification centre. Delayed vascular penetration and, subsequently, ossification of that region can probably lead to such rarely occurring congenital malformations of the vertebral bodies as the persistent notochord canal or even cleft vertebral body (Hensinger & MacEwan, 1975).

Taken together, the observations on 2nd trimester fetuses (this study), 3rd trimester fetuses and infants (Ratcliffe, 1981, 1982) as well as adults (Crock, 1976;

Ratcliffe, 1980) suggest a biphasic development of the internal (intrachondral and intraosseous) vascular architecture of the human vertebral body. In the fetal and early infantile period, the vessels grow centripetally from the periphery and centrifugally from the ossification centre, until they meet and form an integrated, extensively anastomosing system. Afterwards, during late childhood and adolescence, the radial vessels are gradually withdrawn and the anastomoses disappear, dividing the vertebral body into separate compartments supplied and drained by individual arteriovenous pairs.

ACKNOWLEDGEMENTS

This study was supported by a statutory grant no. BNS/501/KL/111/L from the Jagiellonian University School of Medicine. The authors wish to thank Dr M. Nowogrodzka-Zagórska and J. Urbaniak for their skilled technical assistance.

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