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THE VASCULARIZATION OF THE VERTEBRAL COLUMN OF RATS

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Abstract

The intrinsic and extrinsic blood supplies of the vertebral column of rats have been examined by corrosion cast with Mercox CL2B(R). The close functional and topographic relationship of vessels to supplied structures were exposed by fractionated maceration of the soft tissues. The segmental arteries diverge into an anterior, median and posterior group with various anastomoses between their branches. Vertebral bodies and intervertebral discs are preferably supplied by branches of the anterior and median section. The vessels reaching the vertebral body from the dorsal side diverge in a treelike branching mode into the direction of the vertebral endplate. The venous drainage takes place by the segment-overlapping internal and external vertebral venous plexuses. Bloodflow regulating sphincters can be identified within all parts of the arterial and venous system. This fact seems to be of great importance regarding pathophysiological processes of degeneration and likewise regeneration even of the intervertebral discs.

KEY WORDS: Vertebral column, vertebral body, intervertebral disc, vascular corrosion casts, blood supply, intravertebral vessels, microvasculature, vertebral venous plexus, sphincter, rat.

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Introduction

Knowledge about vascularization of the spine is indispensable for the comprehension of the complex physiological and pathophysiological processes of growth, degeneration and regeneration. The significance of vertebral vascularization has been pointed out, e.g., for the development of vertebral bodies (Mineiro, 1965), bone growth (Foley and Kirkaldy-Willis, 1979) and fracture healing (Appuzzo et al., 1978).

The studies of Crock et al. (1973) and Ratcliffe (1980, 1981) demonstrate conclusively that nutritive factors are also responsible for the ethiopathogenesis of damages to the intervertebral disc, in addition to mechanical reasons. Even for the development of structural scolioses, vascular factors are considered as possible causes (De Salis et al., 1980).

Examinations such as angiography, microangiography and autoradiography in combination with precision preparatory and histological techniques have enabled essential understanding but allow only a two-dimensional view with limited resolution. Batson (1957) as well as Crock and Yoshizawa (1976,1977) already referred to the necessity for corrosion cast preparations.

This work aims at making visible the topographic correlations of vertebral vascularization to various elements of hard and soft tissues even in the capillary region. At the same time it should be verified whether further essential results on the vertebral vascularization can be obtained by scanning electron microscopy of vascular corrosion casts.

Materials and Methods

In the procedure 68 rats of the Wistar and RCS breed, aged between 6 and 32 months, between 200-430 g, were used. Half an hour before the perfusion, 1000 IU Heparin (Liquemin(R)) per 100 g body weight were applied intraperitoneally. The rats were thoracotomized under deep pentobarbital anaesthesia (Nembutal(R), 8 mg per 100 g body weight intraperitoneally).

Following recommendations of Lametschwandtner et al. (1984) and Hodde et al. (1980) we have chosen the following procedure: After opening the right auricle, a button-cannula was introduced

into the left ventricle, then Ringer solution of +36°C-39°C was perfused in order to exsanguinate the animal. 360 ml at a pressure of 80-90 mm Hg were required. Fixation was carried out with 200 ml of a modified solution of Karnovsky (1965) (2.5% glutaraldehyde, 770 mosmol, ph 7.40) over a period of 5 minutes at a pressure of 90-110 mmHg. As casting medium we utilized 30-40 ml Mercox CL-2B(R) (Japan Vilene Comp., Tokyo) with 2 % catalyzing substance. For the injection into the arterial system the cannula was either advanced into the ascending aorta and fixed in that position or bound into the thoracic aorta after decapitation. All vessels leading cranial and into the extremities were fastened or closed with clips. When injecting into the venous system, the cannula was advanced through the right superior vena cava via right atrium into the inferior vena cava till caudal of the joining hepatic veins in order to avoid bursting of the liver and the upper abdomen. The carcasses hardened in water at 40°C for about 6 h. In a previous preparation, the cutis, head and extremities were taken off and the carcasses eviscerated.

The thoracic and lumbar vertebral bodies were taken out en bloc together with proximal paravertebral musculature and immersed into 15 % potassium hydroxide for maceration for several weeks. After the removal of the perivertebral tissue, decalcification was carried out by electrolysis in a modified solution of Richman et al. (1946) of 410 ml aqua bidest., 50 ml formic acid 85 % and 40 ml HCl 25 %, till the result of Shipley's test on calcium contents (Romeis, 1968) was negative. After that the specimens were further macerated gradually in potassium hydroxide. Alternatively, the decalcification by electrolysis was carried out before maceration. The advantage of the shorter period of maceration of the carcasses was smaller risk of vessel rupture because the para- and perivertebral and thin subperiostal vessels were still surrounded by a protecting coat of soft tissues.

The single steps of maceration to the finished corrosion cast specimens were carried out under a stereo loupe for documentary evidence. After separation and dustfree drying in an incubator at +30°C the specimens were mounted with Leit-C-carbon cement(R) and then sputtered with gold in argon-atmosphere. The scanning electron microscopic examinations were carried out with a Stereoscan 180 scanning electron microscope (Cambridge) at an acceleration voltage of 10 kV.

In order to compare the experiments we made sections of India ink-injected vertebral columns. For this we injected a mixture of 10 ml India ink (Merck, Darmstadt) and 30 ml 30 % gelatin according to the above described procedure. The vertebral bodies which were not decalcified were embedded in methylmethacrylate, cut at different levels, ground between rotating glass discs down to 40-60 µm and mounted on specimen holders for light microscopy.

Results

In the thoracic and lumbar region hard tissues as well as the spinal cord with its meninges and the paravertebral structures up to the cutis are supplied by branches of the segmental arteries.

These intercostal v. lumbar arteries which have their source in the aorta on the anterolateral planes of the vertebral body divide in front of, respectively, at the same level with the foramina intervertebralia into three groups of vessels:

1. An anterior group of arteries which have their source on the anterolateral planes of the vertebral bodies and which supply the paravertebral tissues (fig. 1). Part of these vessels are connected with the intravertebral vessels by tiny nutrient foramina.

2. A medial group the branches of which enter the spinal canal by the intervertebral foramina (fig. 2). They supply the vertebral body, intervertebral disc, pedicles, vertebral arch, and spinal cord.

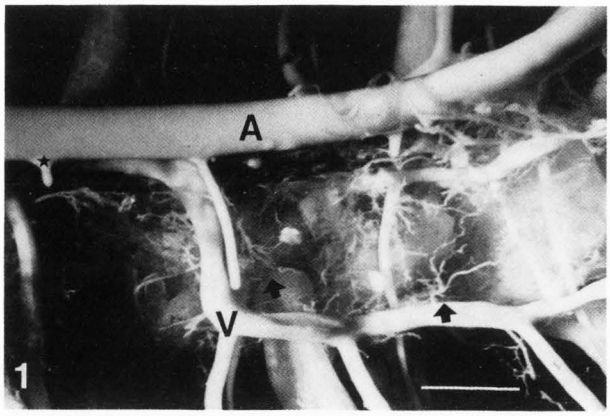
3. A posterior group the arteries of which lead dorsally, outside the spinal canal and supply the dorsal elements, the paravertebral musculature and superficial tissues.

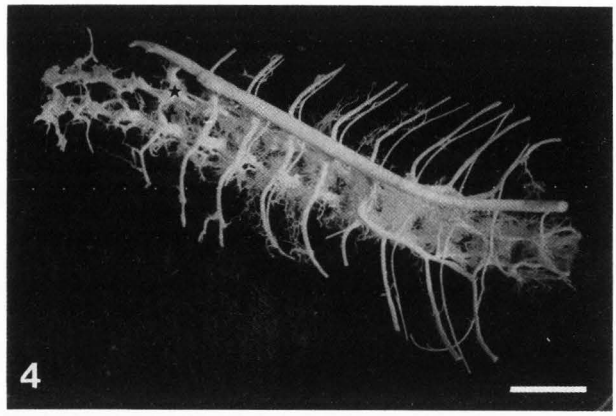
Between the arteries of the above groups distinct connections can be viewed in certain parts. Thus, tight vascular and functional connections, even segment-overlapping, arise between separate parts of the vertebral column. Nevertheless, the completely macerated specimens show that the vessels which supply musculature and cutis can be associated with distinct segments (fig. 3). This also applies partially to the venous part, too, in spite of the fact that drainage takes place by segment-overlapping longitudinal venous systems like the external and internal vertebral venous plexus (figs. 3 and 4).

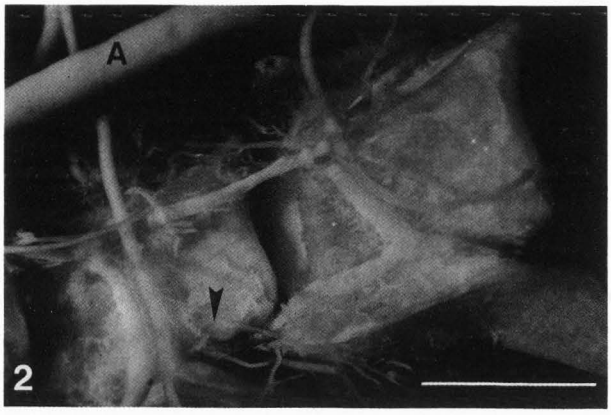
The intravertebral vessels which mostly enter the vertebral body by one or two nutrient foramina dorsally, divide in tree-like branching mode in the direction of the vertebral endplate (fig. 5). Only very few distorted vessels reach into the area of the vertebral endplate cartilage. Most vessels branch off into the medullary cavities so that here a greater density of vessels is to be found than in the compacta (fig.6).

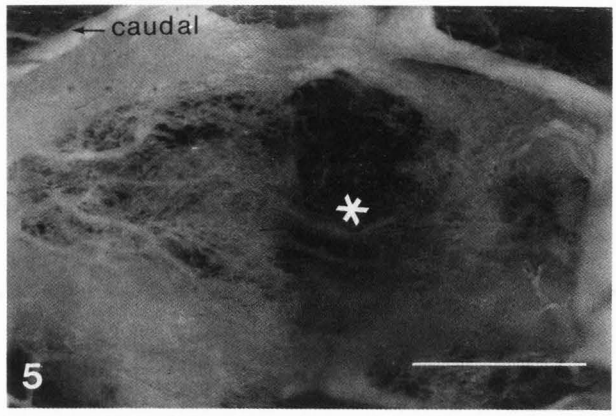
Fig. 1: View from anterolateral to the middle thoracic region after maceration of the soft tissues. On the anterolateral vertebral body there are several branches of the anterior vertebral body artery group (➧). A = descending thoracic aorta, V = hemiazygos vein, * = filling artifact on segmental artery. Bar = 5 mm

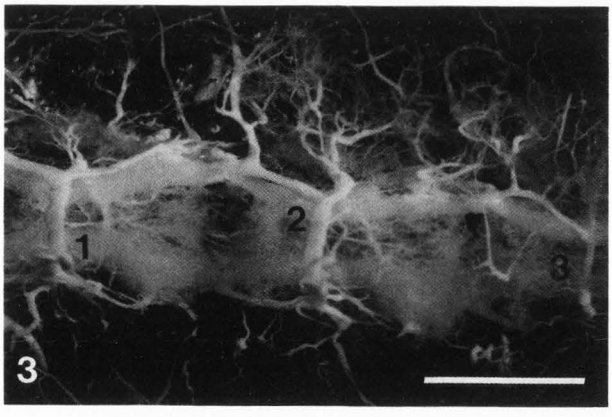
Fig. 2: View from anterolateral to partly macerated vertebral bodies in the thoracolumbar region. A = aorta, ➧ entrance of the medial branches of segmental arteries into the intervertebral foramen. Bar = 5 mm











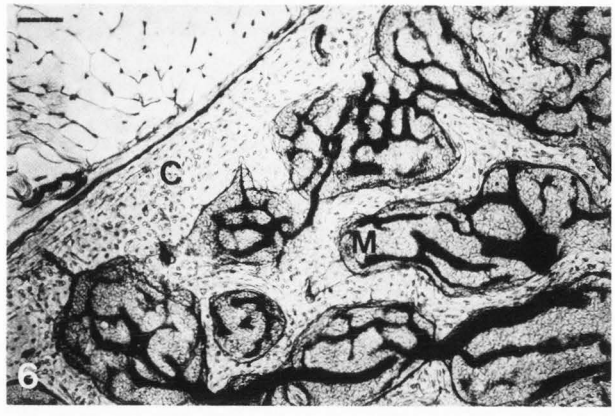
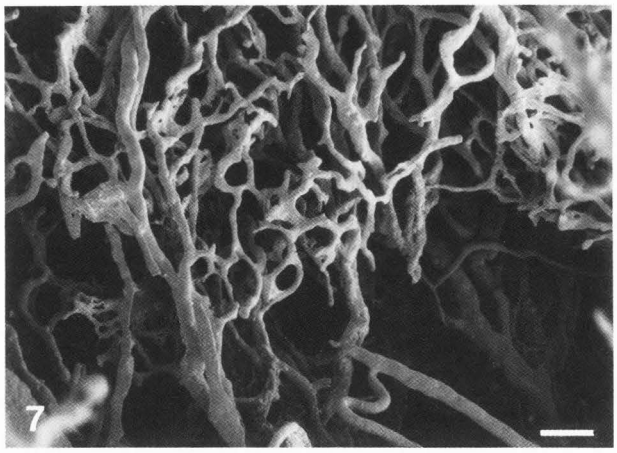


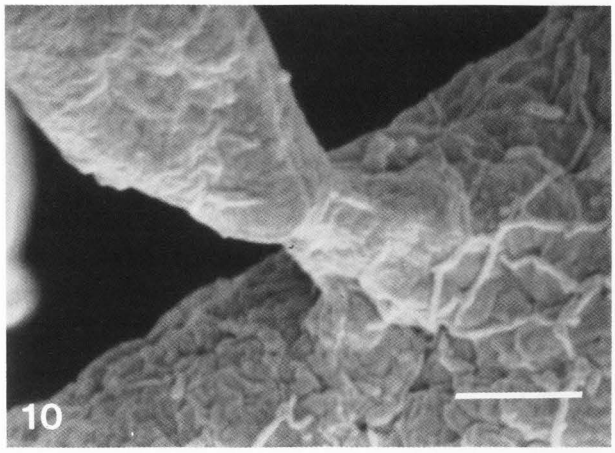
Fig. 3: View from ventral to the fully macerated corrosion cast of the lumbar vertebral column. The aorta was dissected. 1,2,3 = origin of the 1., 2., 3. lumbar arteries. Several muscular and cutaneous rami (upper part of the photo) can be traced back to the segmental vessels. Bar = 5 mm

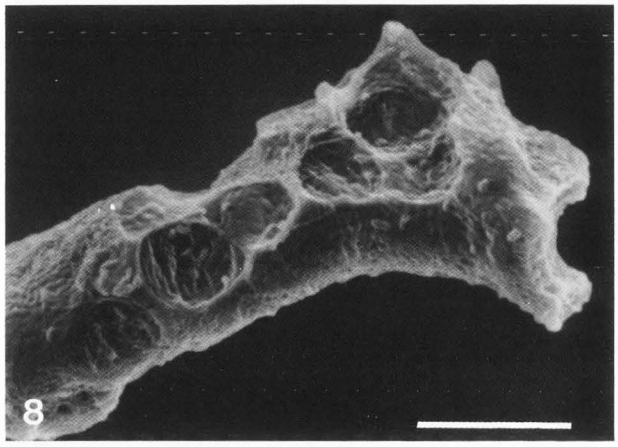
Fig. 4: Vascular corrosion cast of the thoracic vertebral column. In the foreground are the aorta and segmental vessels, in the background are the large longitudinal inner vertebral venous plexuses (*). Bar = 10 mm

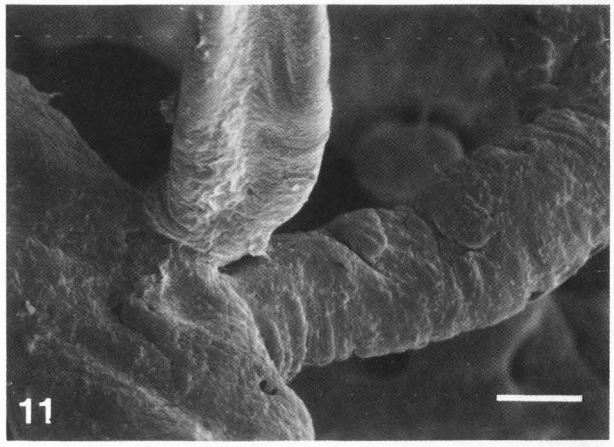
Fig. 5: Intraosseus vessel convolute of two neighbouring vertebral bodies. The vessels almost invade the former subchondral area. The vessel-free area shows the place where the intervertebral disc was located (*). In the background are parts of the internal vertebral venous plexus. Bar = 3 mm

Fig. 6: Horizontal cut through the compacta (C) and medullary parts (M) of a vertebral body after ink injection. Note the closeness of the vessels in the medullary cavities. Bar = 200 µm









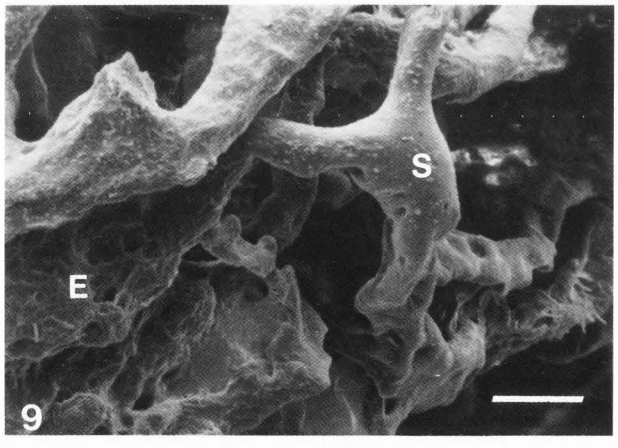


Fig. 7: Intravertebral vessels with several anastomoses on the vertebral endplate. The vessel-free area shows the place taken by the intervertebral disc. Bar = 100 µm

Fig. 8: Imprints with crista-like surface structure on a subchondral vessel. Bar = 10 µm

Fig. 9: Sinuses in medullary cavities of the center of the vertebral body (S). On the foreground presumptive extravasations (E) with different surface structure. Bar = 20 µm

Fig. 10: Sphincter on an intravertebral arteriole. Bar = 5 µm

Fig. 11: Sphincterlike constriction on an intravertebral vein. Bar = 15 µm

The scanning electron microscope shows that the intravertebral vessels form an extensive network with numerous ring-anastomoses. Especially in the area of vertebral endplates recurrent tracks of vessels in loop-line are typical (fig. 7). In this region we have frequently seen blind ending vessels with impressions of cells sized 6-10 µm (fig. 8). It should be discussed whether these imprints with crista-like surface structure were caused by perivascular cells such as osteoblasts or by intravascular leukocytes.

In the medullary cavities vessels preferably lead into sinuses (fig. 9). Sometimes extravasations can only be defined by their surface structure. In intravertebral arterioles (fig. 10) as well as in corresponding veins (fig. 11) sphincter-like constrictions of the vessel diameters can be demonstrated in the proximal segment - as

in all other sections of vessels examined in the vertebral column. In proportion to the phase of contraction of the smooth perivascular muscle cells they are more or less distinctly visible.

Discussion

Methods of examination such as radiography and microangiography which have often been employed for studies of vertebral vascularization (Crock et al., 1973; Domisse, 1975; Crock and Yoshizawa, 1977; Ratcliffe, 1980, 1981, 1982) only indirectly permit results beyond a two-dimensional view, since specimens are too thick and have to be analysed by several levels. The reason for this trial is to study further corrosion cast techniques. Irino et al. (1975) and Draenert and Draenert (1980a, b) have already demonstrated the plasticity of presentation of vessels in long bones with methyl methacrylates. Since there is only little experience in application of corrosion cast techniques for presentation of intraosseous vessels, it was thought wise to utilize an experimental animal which was easy to handle as well as to procure. The relatively high conformity of the extravertebral vessels compared with humans (see Bowsher, 1954; Halpern, 1953) recommended the rat.

Tight topographic relations of elements participating in construction of the vertebral column imply tight connections of vessels with the same source which supply the osseous, nervous and ligamentous structures. The segmental arrangement of the vertebral column is still clearly distinct in the osseous elements; in other structures as in the autochthonous musculature, the former segmental arrangement is only partially retained. So the arterial supply also has to be segment-overlapping, too. MacNab and Dall (1971) assigned great importance to the vascularization of vertebral bodies after surgical interventions to these segment-overlapping anastomoses.

If one compares human and rat purely anatomically, many parallels are found in relation to the distribution of the larger vessels. Thus, the course of the abdominal aorta and the branching mode of the segmental arteries and veins in rats do not reveal major differences in humans and rats (see Bowsher, 1954; Domisse, 1975). However, comparisons of the supplied areas of separate groups of vessels or else supply of separate structures clearly prove differences. Thus, we found that the vertebral body obtains its supply for the most part from dorsal by rami canalis spinalis originating from the medial group of arteries of the vertebral body whereas Ratcliffe (1980) established 10-20 arteries which enter the human vertebral body anterolaterally and supply great parts of it.

The tree-like branching mode of intravertebral vessels with invasion of the vertebral endplate by central vessels is parallel to the results of Guida et al. (1969). The great number of anastomoses among the intravertebral arteries, sinuses and veins make it unlikely that separate areas of the center of a rat's vertebral body get supply from one artery exclusively.

Even the presence of presumably blind ending vessels as shown in fig. 8 does not prove that certain areas get an exclusive supply since we are not able to exclude possible artifacts with certainty. The surface structure of the imprints in the casting medium might also be caused by large, rounded up leucocytes obliterating the lumen. Although Grunt et al. (1986 a,b) have demonstrated conclusively the existence of blind ending vessels in tumors it is still questionable whether such blind "cul de sacs" exist in normal tissue, too. This problem requires further comparing electron microscopic studies.

This concerns the differentiation of evasation versus real existing structure, too: Previous studies on the features of bone marrow endothelium suggest various surface structures (Irie et al., 1986). Thus evasations and casted sinuses can sometimes be discerned only by their imprints.

Our studies confirm the works of Irino et al. (1975) and Draenert and Draenert (1980a), who have given evidence of arterial sphincters in the vascular system of the bone marrow. The existence of sphincters in all parts of the vascular system of the vertebral body might well have consequences for the regional supply of separate parts. It is to be expected that by the segmental nociceptive connection irritations in paravertebral tissues like cutis and subcutis or musculature with the consequence of regulative changes in blood supply will lead as well to intravertebral changes of circulation. This is made possible by the fact that all structures which phylogenetically make up the vertebral column get their innervation by rami dorsales of the spinal nerves.

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Discussion with Reviewers

E.D.F. Motti: Do you think "cul de sac" vessels exist in normal bone tissue?

Authors: Till now we could not prove the existence of such vessels in bone tissue of adult animals by corrosion casting or transmission electron microscopy, but based on studies of angiogenesis we think that they occur in developing vessel buds. Ohtani et al. (1982) have shown sinusoidal blind-ends "feeling that some blind-ends are real features", whereas Draenert and Draenert (1980 a,b) have documented such vessels without commenting on them as possible artifacts or real existing structures.

E.D.F. Motti: Do you think corrosion casting is an adequate method of investigation to document the existence of "cul de sac" vessels? Do you think a preparation where the A-V path has not been documented may give information on blind-ending vessels?

Authors: The studies of Grunt et al. (1986 a,b) have shown convincingly that it is even possible to discern different forms of the development of capillary sprouts in corrosion casts. Since these works show high conformity with transmission electron microscopic studies (Hammersen et al.,1983), it should be possible to prove the existence of "cul de sac" vessels by means of corrosion casting. Of course a complete filling of the vascular system without artifacts is indispensable.

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