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The Blood Supply of the Growth Plate and the Epiphysis: A Comparative Scanning Electron Microscopy and Histological Experimental Study in Growing Sheep

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Abstract. The blood supply of the growth plate has been described in the late 50s and early 60s, and there was controversial discussion about the existence of transphyseal vessels. The vascular supply of growth plate and epiphysis of the proximal tibia was reinvestigated using a modern technique, the Mercox®-perfusion method, in six sheep aged 6–24 weeks. A comparison was made among pure perfusion specimens, the corrosion casts, and histological sections. The metaphyseal, epiphyseal, and perichondral blood supply systems were confirmed. However, there was evidence of regular transphyseal anastomoses between the metaphyseal and epiphyseal system. Based on the histological arrangement of the blood vessels, the arterial blood flow would appear to be from the metaphysis to the epiphysis. The existence of transphyseal arterial vessels originating metaphyseally and seen both in cast preparations and histological sections was added to the present description of the blood supply of the growth plate. Age-related differences in the vascularization of the growth plate were not found in this study.

Key words: Growth plate — Corrosion casts — Vascular anatomy — Transphyseal vessels

The growth plate and the epiphyseal region of long bones are supplied by three different vascular systems: the epiphyseal, metaphyseal, and perichondral arterial blood supply [1, 2]. Eighty percent of the metaphyseal vessels derive from the nutrient artery which divides into several major branches and multiple smaller arteries and capillaries in a tree-like fashion [3]. The epiphyseal arterial network is nourished by the epiphyseal arteries which enter the epiphysis from different sides [1]. The perichondral vessels surround the growth plate by a circular vascular system sending anastomoses to both the metaphyseal and the epiphyseal arteries [2, 4].

It is common knowledge that interconnecting vessels between the metaphyseal and the epiphyseal vascular systems do not exist within the bone; the growth plate is regarded as an avascular structure [2, 3, 5]. However, recent studies on the blood supply of the growth plate using most modern injection techniques have revealed vessels that do cross the growth plate in rabbit cast preparations [6]. In the past, the existence of transphyseal vessels had been discussed by several authors who accepted their presence during a short postpartal period in different species such as foals, chicken, and even humans [7–9]. The direction of the blood flow was from the epiphyseal to the metaphyseal side [8, 9]. In rats, a metaphyseal arterial perfusion system and an epiphyseal drainage system had been described by other authors using the same injection technique [10]. These studies suggested a species-specific blood supply which could not be generalized.

These uncertainties and discrepancies in the understanding of the blood supply in the region of the growth plate justify further studies using the most modern and accurate injection methods.

Materials and Methods

The perfusion studies were performed in 6 Merino sheep aged 6–24 weeks. There were 3 male and 3 female animals. The injection studies were made at three different ages, at 6, 12, and 24 weeks, having two animals at each time point. For perfusion, both hindlegs were used with a preference for the knee joint region. The study was done on both proximal tibiae.

The perfusion studies used Mercox® (Vilene, Tokyo), which is a well-recognized method [10–15] for manufacturing the cast preparations. It was started under general anesthesia (50 mg/kg body weight) using intramuscular administration of Ketavet® (Parke, Davis & Co, Berlin) and Rompun® (4 mg/kg b.w.) (Bayer AG, Leverkusen). The first step of perfusion implied the intravenous administration of heparine for 15 minutes. For dilatation of the vessels, a sodium-nitroprusside solution was given. After rinsing the vascular system with a physiological sodium-chloride solution, the large intraabdominal vessels were exposed through a median laparatomy. The aorta was opened infrarenally, and the lower half of the vascular system was carefully rinsed with 4 liter heparinized Ringer solution. By opening of the v. cava, the clearance of the vascular system from blood could be followed, and the rinsing procedure was stopped when there was clear reflux. Two other cannulae were placed in both iliac arteries for

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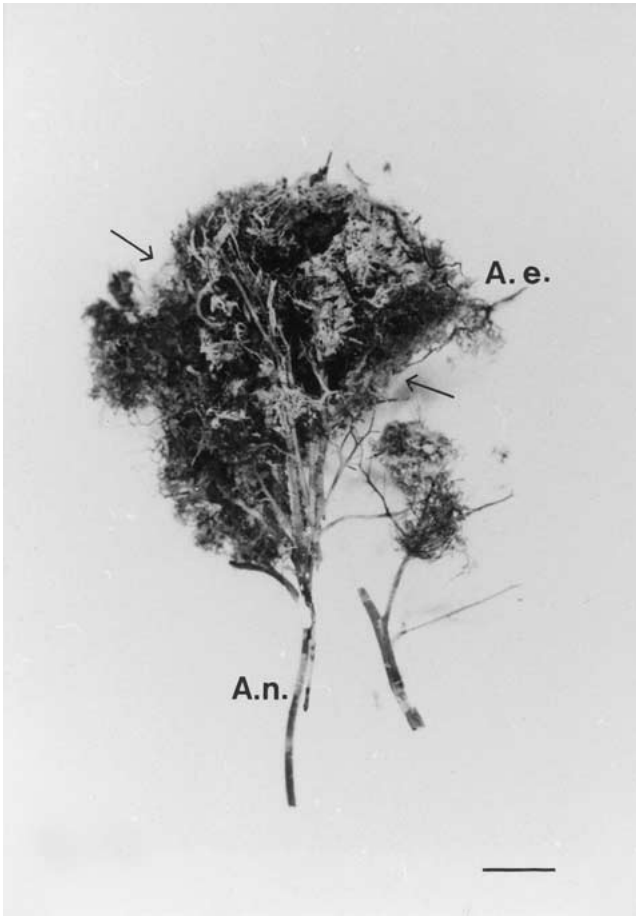


Fig. 1. Mercox®-cast preparation of the proximal tibia of a 6 week old **male** sheep under light microscopy (×10). A.n. = arteria nutricia, A.e. = arteria epiphysaria. The plane of the growth plate lies between the arrows. Bar: 3 mm.

fixation of the vessel walls with 2 liter 0.5% glutaraldehyde solution on either side at a temperature of 37°. After 20 minutes of fixation the perfusion with Mercox®, a soluable methylmethacrylate derivative, was initiated. It is crucial to inject the solution under as high pressure as possible. Damage to the vessel walls must be avoided. Both legs were perfused separately with 150–250 ml of Mercox®-solution. The perfused specimen was left at room temperature for 24 hours to allow full polymerization of the solution. Thereafter, the specimen was watered in 60° warm fluent water for three hours.

The left tibia was used for corrosion cast preparation and the right tibia was further processed for conventional light microscopy. Cast preparation followed the classic method described elsewhere [13, 14, 16–19]. Soft tissues were carefully removed from the specimens which were then treated intermittently with 30% KOH solution and 5% trichloracedicacid solution for maceration. Complete maceration frequently took 3–4 months. The specimens were frozen at −20° and later freeze-dried for up to 7 days. They were then prepared for scanning electron microscopy. Two photographs of the same area were taken at an angle of 6° allowing three-dimensional viewing under the stereomicroscope.

For histological evaluation, sections using a modified sawing and grinding technique were made [20]. Only the proximal tibia was processed and fixed in 1% glutaraldehyde for 7 days. A 5-mm section was then cut in the frontal plane and frozen in liquid nitrogen. The specimen was freeze-dried and embedded in methylmethacrylate for 10 days. After polymerization, the sections were made using a precision microsawing system to a thickness of 100 μm. Later they were ground to a thickness of 20–30 μm by the precision microgrinding system. Finally, the sections were stained with toluidine blue.

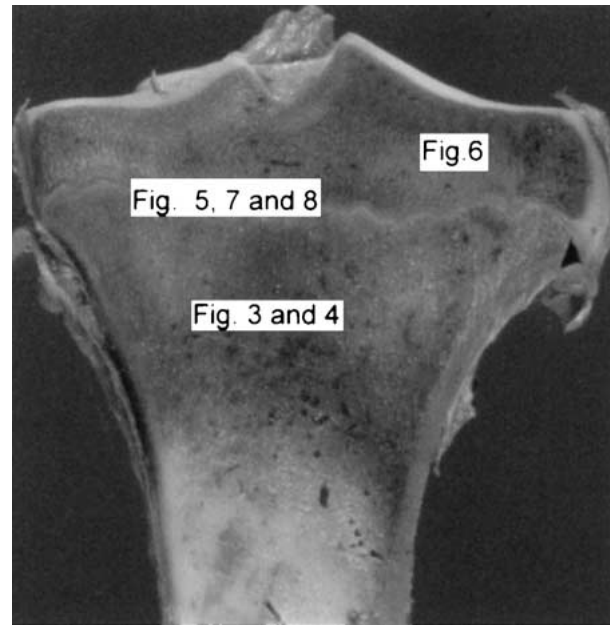


Fig. 2. Section of a perfused proximal tibia in a 12-week-old male sheep ready to be processed for histological analysis (×2). This specimen illustrates the location of Figures 3–8.

Results

Under light microscopy, the principles of the vascular anatomy of the proximal tibia become visible (Fig. 1). The nutrient artery runs from the diaphysis in metaphyseal direction and divides dichotomously in several main branches. These branches split into a thick network of arterioles and capillaries which are directed to the metaphyseal end of the growth plate. The growth plate area itself can be hardly identified as a band of lesser vascularity. At the epiphyseal side a very thick vascular network is obvious which is nourished by separate epiphyseal arteries.

By scanning electron microscopy a differentiated visualization of all vessels is possible. In order to better relate the following scanning electron microscopical photomicrographs to their anatomic region, Figure 2 indicates the locations they are taken from. The method allows the discrimination between arteries and veins with the arteries being covered by multiple oval dots which represent the negative impressions of the nuclei of the epitheloid cells [13]. Veins appear wider, and the greater tension of the arterial walls is missing (Fig. 3). The discrimination of arteries and veins allows the assessment of the direction of the blood flow. The nutrient arteries can be clearly identified and the direction of all arterial vessels be deduced from the dichotomous division pattern. So, the direction of the blood flow is according to this division pattern.

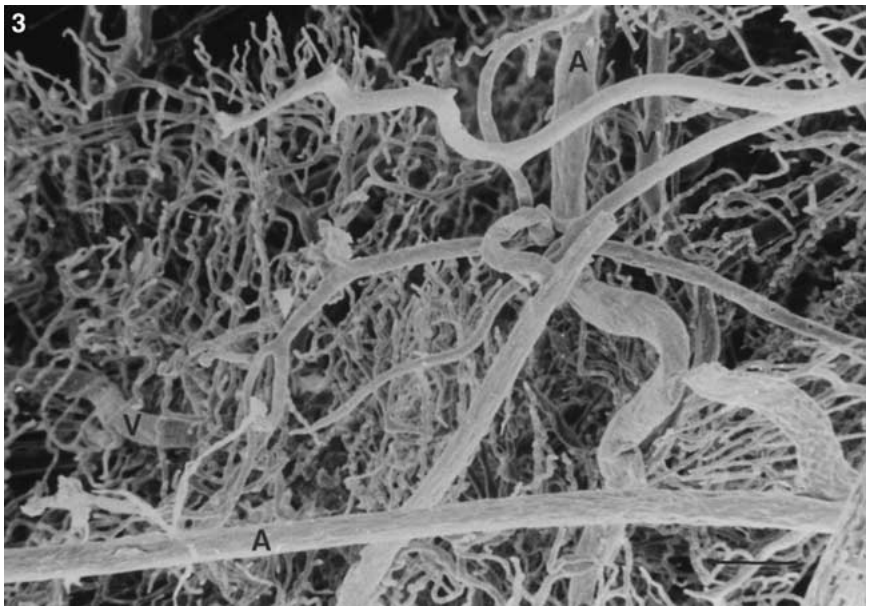


Fig. 3. Discrimination of arterial and venous vessels after Mercox® perfusion by scanning electron microscopy in a 12-week-old male sheep. The arteries (A) show multiple oval dots on the surface. The veins (V) appear wider with a lower tension of the vessel wall. Bar: 200 μm.

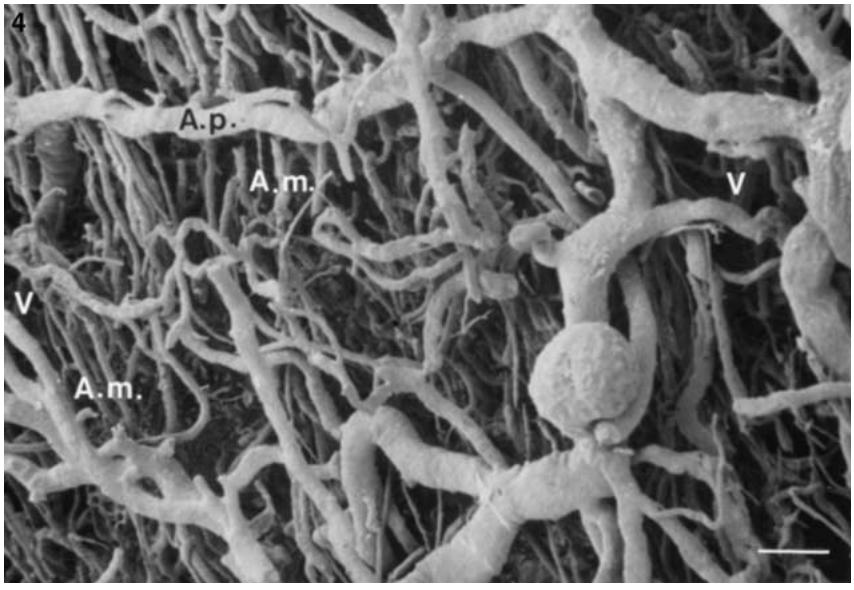


Fig. 4. Scanning electron microscopical illustration of the metaphyseal vascular systems (×100) in a 24-week-old female sheep. A longitudinal arterial and venous system with dichotomous branching pattern can be identified as well as a circular periosteal vascular system. A.p. = periosteal artery; A.m. = metaphyseal artery; V = metaphyseal vein. Bar; 100 μm.

Metaphyseally, two different vascular systems exist (Fig. 4). There are multiple bundles of arteries that run from the diaphysis to the metaphysis and are accompanied by venous vessels which derive from the nutrient artery or from a corresponding vein. This longitudinal vascular system is surrounded by a circular net of small arteries which represent the periosteal vascularization. There are multiple anastomoses between both systems which can be best observed three-dimensionally by looking at the corresponding photographs under the stereo-microscope.

At the metaphyseal side of the growth plate most arterioles end. The growth plate itself appears as an undulating band of lesser vascular density, however, there are vessels that cross the growth plate (Fig. 5a). These vessels enter or build up a capillary network at the epiphyseal side of the growth plate. A higher magnification clarifies the anatomic situation at the metaphyseal end of the growth plate. The metaphyseal vessels form multiple loops when they turn, these loops appear to lie within one horizontal plane which is located beneath the growth plate in the zone of already mineralized bone. From this plane other arterial vessels arise, some of which represent the transphyseal arteries (Fig. 5b). At their end, capillary sprouts are visible which again seem to lie within one plane. These capillaries mark the beginning of the network at the epiphyseal side of the growth plate. Other arterial vessels

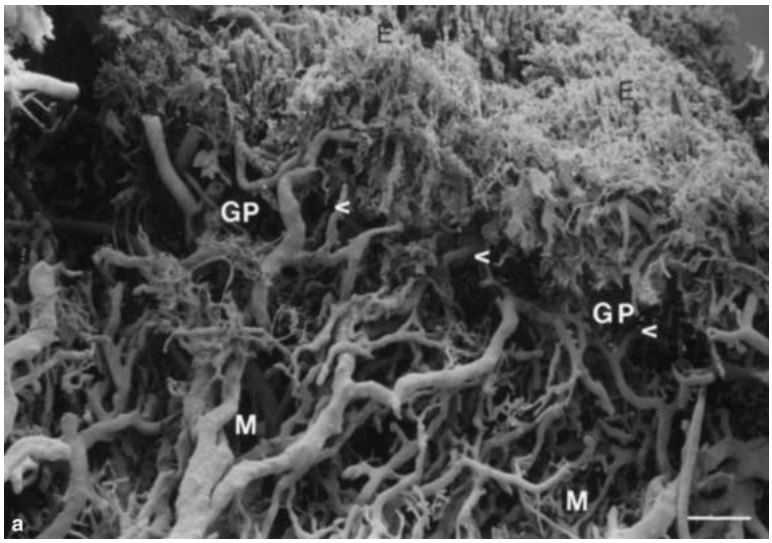


Fig. 5a. Vascular supply of the proximal tibial growth plate region (x20) of a 12-week-old female sheep. Most metaphyseal vessels end there. Some vessels cross the plate and take part in the epiphyseal blood supply (arrow). E = epiphysis, GP = growth plate, M = metaphysis. Bar: 500 μm. **(b)** Scanning electron micrograph at the level of the growth plate (×125) of a 12-week-old female sheep. There are metaphyseal vessels that build up vascular loops (L) that all are lying in the same plane. This region corresponds to the calcification zone. Some branches (arrow) cross the growth plate and divide at the epiphyseal side (+). Bar: 100 μm.

ascend to the last row of the hypertrophic chondrocytes of the growth plate (Fig. 7a).

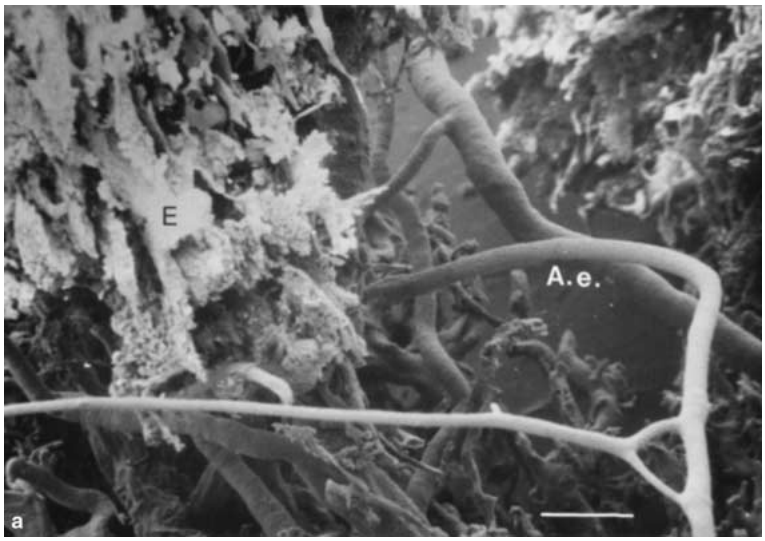
The arterial blood supply of the epiphysis originates from separate epiphyseal vessels which enter the epiphysis from several sides and build up a thick arterial and capillary network (Fig. 6a). One part of the arteries participates in the blood supply of the epiphyseal side of the growth plates and forms anastomoses with the transphyseal vessels. The other part nourishes the epiphyses itself and ends in sinusoid-like formations which cover the epiphyseal surface like corals in the sea (Fig. 6b). This marks the area of the subchondral bone just underneath the joint line. The venous drainage of the epiphysis corresponds to the arterial supply. The blood of the sinusoids drains into venoles which build up small veins that form major venous vessels in order to transport the blood to the periphery of the bone (Fig. 6c).

The interpretation of cast preparations is complicated by the lack of anatomical landmarks since they have all been lost during the maceration procedure. Therefore we attempted to obtain good histological sections which still contained Mercox®, with the anatomical structures preserved. The best results were achieved with a freeze-drying method. No other methods were applicable as the intravascular dye would have been washed out by the chemicals needed. So artefacts, in particular shrinking artefacts, due to the method used were unavoidable. However, the identification of the growth plate was undoubtfully possible. The metaphyseal vessels which run straight to the calcification zone of the proximal metaphysis could be clearly identified as dark lines. They formed loops, as previously described. Some smaller vessels branched off from these loops into the direction of the mineralization and lower hypertrophic zone without further division (Fig. 7a). The number of vessels diminished dramatically in the proximal metaphysis. At the epiphyseal side of the growth plate a significant number of vessels could be identified as those that have crossed the growth plate and have divided in the resting zone to form capillary arcades there (Fig. 7b). These findings are consistent with the detection of vascular structures crossing the growth plate, identified by conventional histology in a previous experiment unrelated to this study (Fig. 8).

The vascular supply of the proximal tibial growth plate in sheep appears to be more complex than described before. Figure 9 summarizes the findings of both the cast preparations and the histological sections.

Discussion

The basic knowledge of the blood supply of the growth plate region comes from studies that have used the techniques of Berlin blue, bariumsulphate, or Latex injection [2, 3, 21]. The Spalteholz technique for tissue preparation was also frequently used so the vascular anatomy could be investigated within fully preserved tissue. For three-dimensional analysis serial-cut speci-



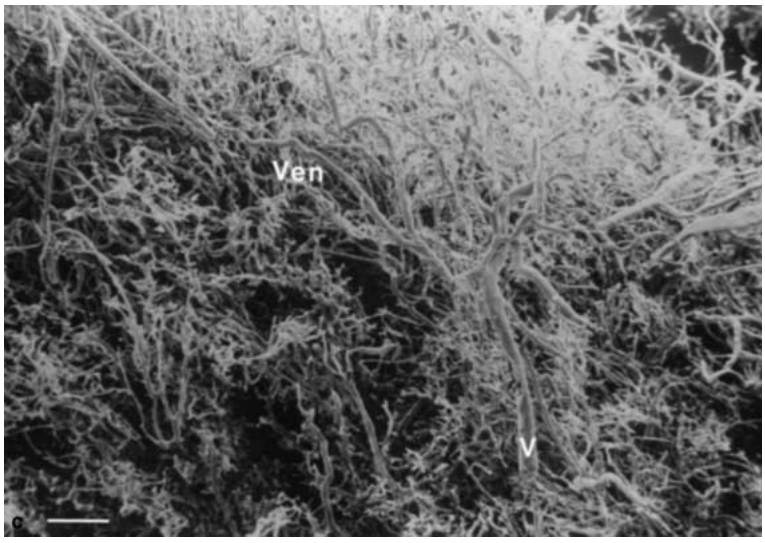
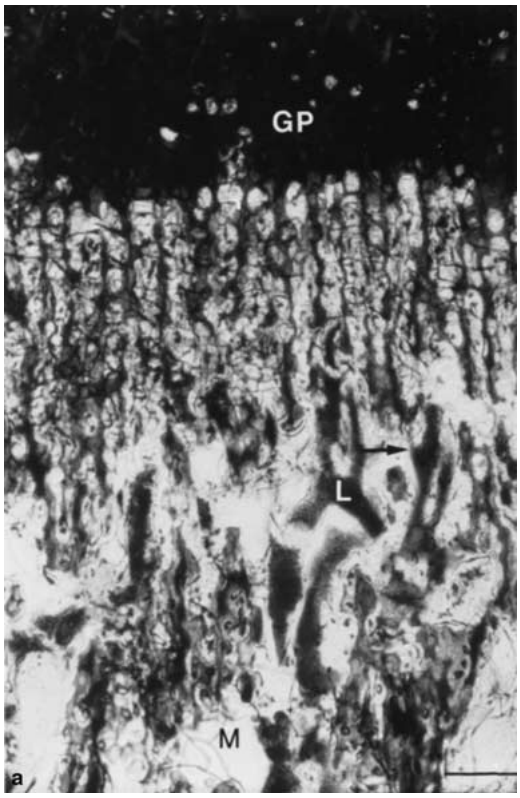


Fig. 6a. Scanning electron photomicrograph of the proximal tibia of a 24-week-old female sheep. Vessels that enter the epiphysis (A.e.) arise from a circular vascular net. They mainly participate in the epiphyseal blood supply (x80). E = epiphysis. Bar: 200 μm. **(b)**. Scanning electron microscopy of the vascular surface of the epiphysis (x20) in a 24-week-old female sheep. Many sinusoid-like structures covering the whole epiphyseal surface can be seen. Bar: 500 μm. **(c)**. Venous drainage from the epiphyseal region under the scanning electron microscope (x20) in a 6-week-old male sheep. Small venoles (ven) collect the blood from sinusoids of different regions and drain it into bigger veins (V) to the periphery. Bar: 500 μm.

mens were necessary. The Mercox® -perfusion technique allows visualization of the vascular anatomy three dimensionally by contemplating two identical photographs taken in a 6° angle under the stereomicroscope facilitating the much greater understanding of the cast preparation. Another great advantage of the Mercox® method is the possible discrimination of arteries and veins by their characteristic appearance under the scanning electron microscope [13], thus, the direction of the blood flow can be determined additionally. The main disadvantage of the Mercox®-perfusion method, however, is the loss of any anatomical and histological landmark. In this study we attempted to eliminate this problem by comparing the cast preparations with non-decalcified, perfused histological sections.

This perfusion study based on cast preparations and histological sections confirmed the previous work of many authors which demonstrated three different main arterial systems in the blood supply of the growth plate region [1, 2, 4, 12, 22]. The arterioles of the metaphyseal system end either in the form of capillary loops at the level of the zone of calcification or in capillary sprouts in the same area [1–4, 6, 10, 23, 24]. These findings could be reproduced in this study in sheep. The vascular loops appear to be located beneath the growth plate, and only the capillary sprouts reach the last row of hypertrophic chondrocytes of the growth plate. These findings resemble reports on the metaphyseal microvascular pattern of growing rats [23]. Age-related changes in the shape of these metaphyseal capillaries have been ob-



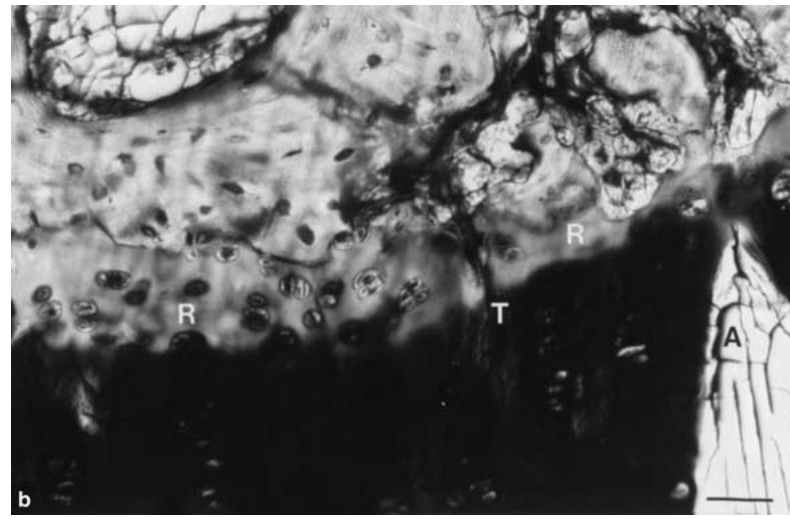


Fig. 7a. Light micoscopy of a 30-μm undecalcified histological section of the proximal tibia after Mercox® perfusion and freeze-drying procedure (x100) in a 12-week-old male sheep. There are vessels that run straight to the growth plate (GP), others form loops (L), and some demonstrate dichotomous division (arrow). M = metaphysis. Bar: 100 μm. (**b**) Light microscopy of the growth plate region in higher magnification (x200) of a 12-week-old male sheep. The vessels that cross the growth plate (transphyseal vessels, T) divide at the epiphyseal side of the plate. R = reserve cell zone, A = shrinking artefacts during freeze-drying procedure. Bar: 50 μm.

served in the rat [25]. Our specimens also pictured the epiphyseal and periosteal vessels in both the cast preparations and the histological sections. Furthermore, both methods revealed vessels that crossed the growth plate and divided at the epiphyseal side.

The present investigation, like other studies [15] could not reveal major differences in the organization of the arterial and venous vascular systems. We found that the veins run parallel to the arteries at the proximal metaphysis of the tibia. We also demonstrated that the arterial blood of the epiphysis was collected in superficial sinusoids and drained through venoles into bigger epiphyseal veins. The so-called metaphyseal arterial perfusion system and the epiphyseal venous drainage system described by Draenert [10, 24] in rats is not present in sheep. There is a separate venous drainage in the metaphysis and the epiphysis in sheep. Furthermore, in contrast to the findings in the rat [10, 24], a specific peripheral venous drainage of the metaphyseal region was not detected in sheep.

The present study describes transphyseal connections between the metaphyseal and epiphyseal arteries in addition to the already known extraosseous anastomoses [2]. The transphyseal communication of both arterial systems has been frequently discussed in the past. Early in this century Lexer et al. [26] demonstrated vessels by angiography which interconnected the epiphysis and metaphysis. These findings could not be confirmed, and the growth plate was regarded as an avascular barrier [27]. The current literature suggests a species-specific vascular supply of the growth plate. Transphyseal vessels have been found in many species [2, 5, 6, 9, 25, 28] most numerously found in rabbits [6, 28], and seem to be much less frequent in sheep. Most authors have seen them for a limited period in infancy only [8, 9, 29]. Other studies stated that in older animals only the larger vessels persisted [8]. Also, the development of a peripheral periosteal vascular system seemed to decrease the number of transphyseal vessels [30]. Those authors who described transphyseal vessels stated that their numbers were higher at the periphery [6–8, 30].

Epiphyseal and metaphyseal vessels are known to have distinct functions. Interference with the epiphyseal blood supply to the growth plate leads to severe disorders within the columnar structure and in the function of the proliferating chondrocytes [21, 31], disturbance of the metaphyseal blood supply results in an increase of thickness of the proliferative and hypertrophic cell zone with abnormal endochondral ossification [21].

Some information in the literature is incompatible with the generally accepted structure and function of the vascular supply system of the growth plate. Fluorescing substances, e.g., spread from the metaphysis to the epiphysis [28]. In contrast to Trueta and Amato [21], other authors have reported subsidence of all longitudinal growth after interruption of the epiphyseal blood supply in dogs [32]. These reports suggest a closer vas-

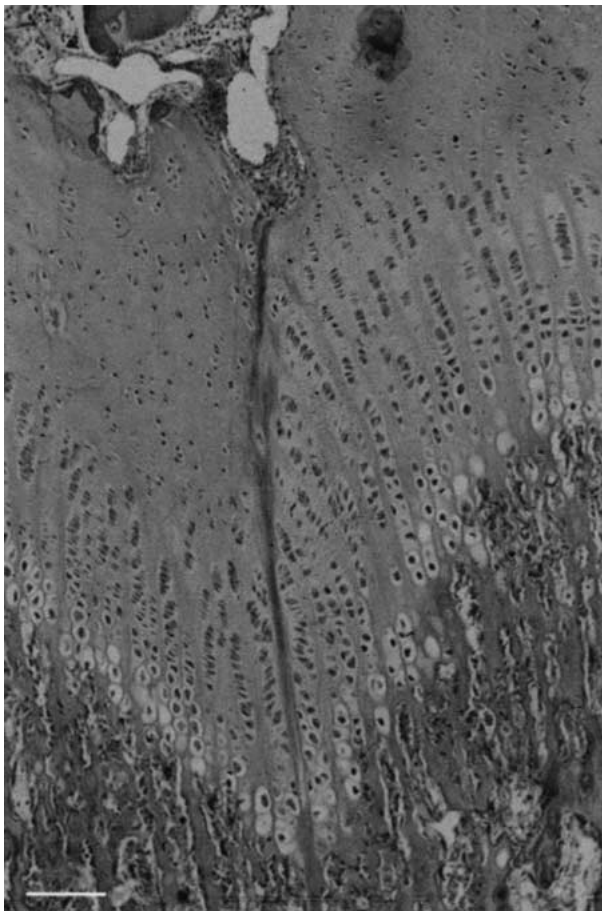


Fig. 8. Illustration of a transphyseal vessel in a conventional histological section in a 6-week-old sheep, hematoxylin-eosin stain, ×40. It divides into several branches at the epiphyseal end of the growth plate. Bar: 250 μm.

cular relation between the metaphyseal and epiphyseal side of the growth plate.

Currently, the physiological role of the transphyseal arteries is unclear and will remain speculative. Brodin [28] found cartilage canals that run parallel to the cartilaginous cell columns in the rabbit. Ogden [34] has described such "arterial–sinusoidal" communication between epiphysis and metaphysis in humans up to the age of 15–18 months of age and, like others, linked their existence to the formation of the secondary center of ossification [9, 33]. Other authors reported on cartilage canals which appeared during certain age and growth periods in order to modulate the endochondral ossification [25]. In our study, the transphyseal vessels have been found up to the age of 24 weeks, which makes it unlikely that they are only involved in the formation of the ossific nucleus. Furthermore, the spread of metaphyseal infections into the epiphysis was attributed to the existence of transphyseal vessels [7, 34].

The Mercox® method reliably distinguishes arteries from veins [13]. We can therefore say that the arterial blood flow is from the metaphysis to the epiphysis and not the opposite way [8].

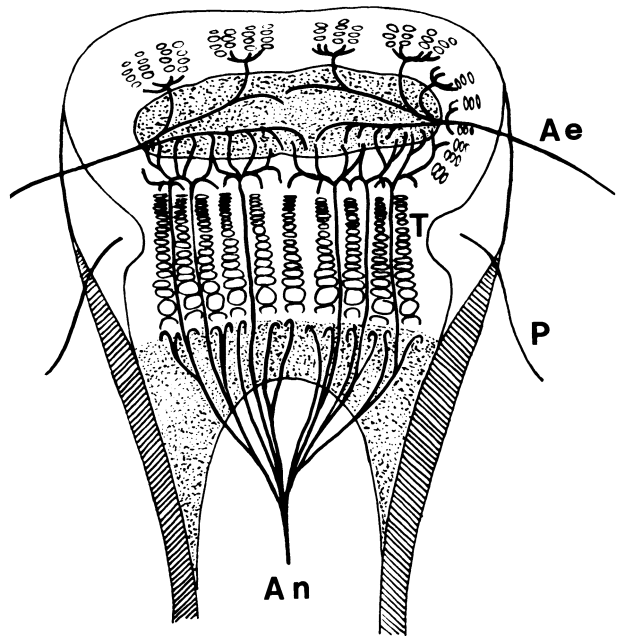


Fig. 9. Schematic illustration of the arterial vascular supply of the growth plate. A.n. = A. nutricia, A.e. = A. epiphysaria, T = transphyseal vessels, P = perichondral vessels. The blood supply of the reserve cell zone is partly accomplished by the transphyseal vessels.

The physiological role of the transphyseal vessels can only be determined when the effect of their selective obstruction on growth plate function can be discovered. Otherwise, their existence and significance will remain speculative.

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