Early postnatal growth of skeletal muscle blood vessels of the rat

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Abstract. The development of blood vessels during the first three postnatal weeks was studied in the ventral stripe of the spinotrapezius muscle of the rat by use of India ink-gelatine injections, and electron microscopy. The number of terminal arterioles and collecting venules remained unchanged postnatally in the observed area. A remarkable proximodistal gradient of vascular development was apparent: while the basic structure of the hilar vessels remained unchanged in the time studied, the intramuscular arteries and veins matured gradually. More peripherally, gradual maturation of terminal and precapillary arterioles was observed. The capillary endothelium and the pericytes showed immature features, and remained unchanged during the time studied. An intense rebuilding activity was found in the endothelial cells of the growing venules, expressed by various forms of gaps, covered by an intact basal lamina and pericytes. Numerous mast cells and macrophages were found along all vessels. Intramuscular lymphatics were not present prior to the first postnatal week.

Key words: Postnatal growth – Skeletal muscle – Blood vessels – Rat (Wistar)

Introduction

In the skeletal muscle, an intensive inner reconstruction takes place early postnatally correlated with the gradual morphological and functional maturation of the muscular tissue. A slight increase in the number of muscle fibers and growth is characteristic of this process (Schippel et al. 1975; Ontell and Dunn 1978). The postnatal maturation of muscle fibers is accompanied by architectural rearrangement of the microcirculatory bed, and by a change in the capillary density as well as the capillary to fiber ratio (Welt et al. 1974; Scheller et al. 1977; Sillau and Banchero 1977; Ripoll et al. 1979; Aquin and Banchero

1981; Sarelius et al. 1981; Bogusch 1984). These studies paid attention mainly to the capillaries.

In order to obtain basic morphological data concerning postnatal growth and maturation of the whole vascular network of the skeletal muscle, we decided to carry out a combined injection and electron-microscopical investigation, facilitated by our previous experiences with the study of the structure of microvessels in various tissues of growing and adult experimental animals (Rhodin 1967 and 1968; Stingl 1971a; Rhodin and Fujita 1989). As a model, we used the ventral stripe of the spinotrapezius muscle of the rat. This muscle is very useful for such a study, because of its very simple vascular architecture and the fact that it has one stable point of orientation during the entire postnatal life - the hilar region. Therefore, it was possible to study consecutively the same vascular area, e.g., the hilar vessels and their nearest intramuscular branches, including the microcirculatory bed. The results obtained by the study of the growth of hilar vessels, intramuscular arteries and veins, arterioles, capillaries, and venules, are presented in this paper.

Materials and methods

Wistar rats of both sexes were used, reared under optimal laboratory conditions, and without any previous experimental interference. In the first experiment, we injected the vascular system of the animals under ether anesthesia via the left ventricle with a solution of India ink in 10% gelatine. Twenty-five animals were used, divided into 5 groups (5 animals in each group) at ages of 1 day, 1, 2, 3 weeks, and adults (weighing 350–450 g). Injected animals were fixed in 10% formalin for 10 days. The muscles were then dissected, mounted in glycerine and the vascular architecture was recorded (Fig. 1).

For the electron-microscopical investigation 44 rats were used, in age groups of 1 day (12 animals), 1 week (10), 2 weeks (10), and 3 weeks (12). In these animals, also anesthetized by ether, the muscles were fixed in situ by immersion in Dalton's fixative (Dalton 1955) and, after dehydration in a series of graded alcohols, embedded in a thin layer of Epon. By this procedure all parts of the vascular bed were well visible in the blocks and it was possible to cut out small regions containing previously selected vessels. The
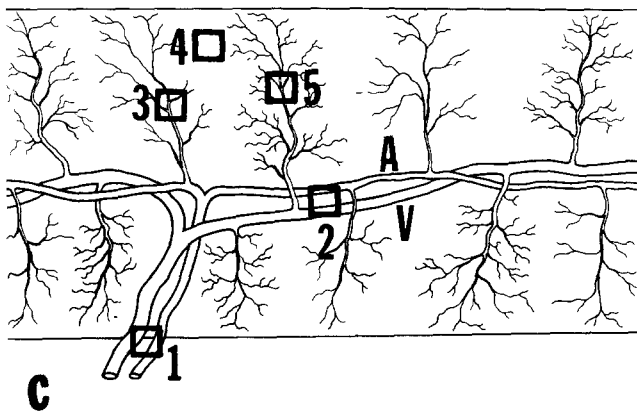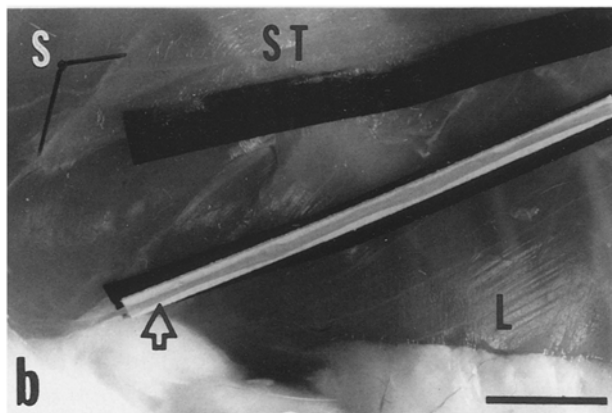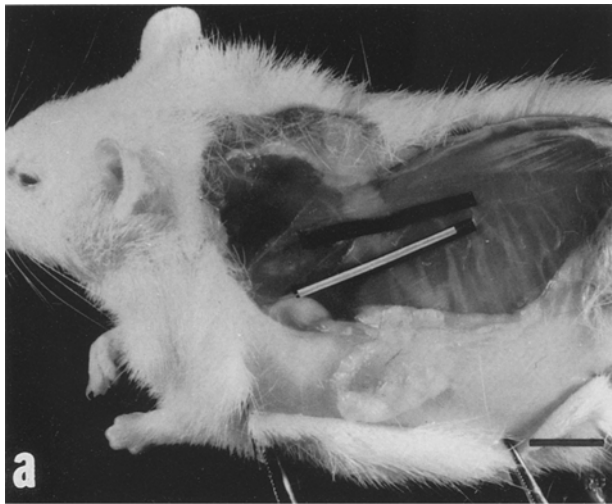
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Fig. 1a–c. Ventral stripe of spinotrapezius muscle of the rat. a Dissected left stripe. *Bar:* 1 cm. b Same muscle in detail. *L* Latissimus dorsi muscle; *S* spina scapulae; *ST* spinotrapezius muscle. *Arrow* Hilar region. *Bar:* 0.5 cm. c Scheme of general arrangement of blood vessels in hilar region of studied muscle. *Squares*Areas where samples were taken: *1* hilar vessels; *2* central vessels, *3* terminal and precapillary arterioles; *4* capillaries (not depicted); *5* postcapillary and collecting venules. *A,V* Central artery and vein

Fig. 2a–e. Architecture of injected intramuscular blood vessels. a One-day-old rat. b One-week-old rat. c Two-week-old rat. d Three-week-old rat. e Adult stage. *A, V* Central artery and vein. *Empty arrows* Stems of terminal arterioles; *full arrows* stems of collecting venules. *Arrowheads* Chain-like loops of venules. *Bars:* 0.25 mm. Magnification of all figures × 60
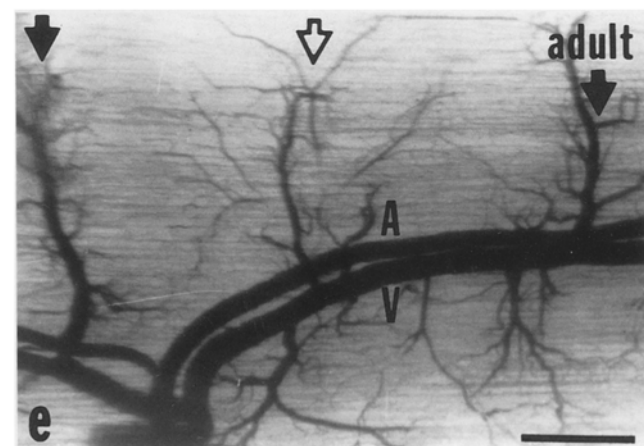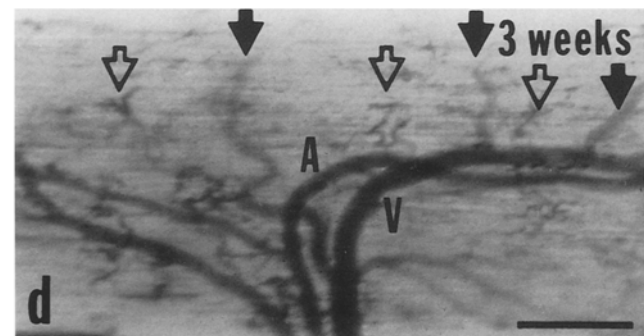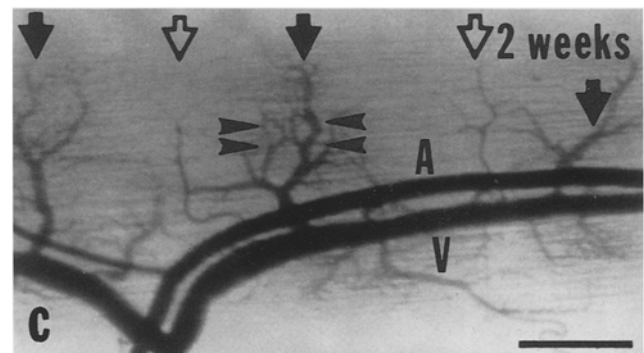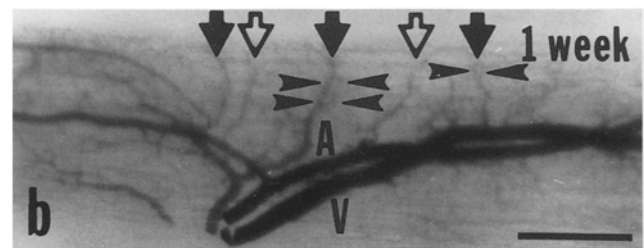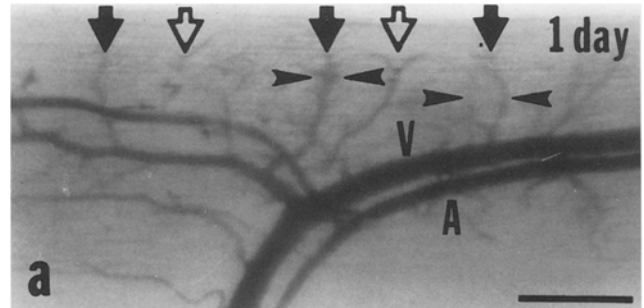
semithin sections (1 μm) were stained with toluidine blue and py-ronin. Thin sections were contrasted with uranyl acetate and lead citrate, and observed in a Philips 300 electron microscope (this part of the study was conducted in the Department of Anatomy, University of Hamburg, Hamburg, Germany).

Results

Architectural development of the intramuscular vessels

In the muscle studied (Fig. 2) both the hilar artery and vein (with inner diameters of about 100 μm and 120 μm in adult animals) branched off after entrance into the muscle to form one anterior and one posterior branch, running parallel to the longitudinal axis of the muscle. From these central vessels (artery 60 μm, and vein 80 μm in diameter) branched off the stems of terminal arterioles and collecting venules, by alternately changing their position, and having a perpendicular or oblique orientation to the muscle fibers. The stems of terminal arterioles (50 μm) branched into precapillary arterioles (20–10 μm), continuing into the capillary network. The capillaries formed a three-dimensional loose plexiform network on the surfaces of the muscle fibers, that continued into the postcapillary venules, emptying into the stems of the collecting venules (80 μm).

In all groups studied, only the hilar and central vessels had the same arrangement. The architecture of the terminal vessels developed gradually from immature shapes in one-day-old rats (Fig. 2a), to their final features in adult animals (Fig. 2e). The average length of the stems of terminal arterioles increased from about 250 μm in one-day-old rats to 600 μm in the adults. The average distances of the stems of neighboring terminal arterioles and collecting venules increased continuously from about 100 μm in one-day-old rats to 450 μm in the adults.

The stems of the terminal arterioles had the same slim, tree-like, and poorly branched forms in all groups studied. From the first postnatal day the collecting venules were clearly wider and more branched. Their stems formed loops of various shapes and sizes in numerous places in continuation with the regions of postcapillary

venules. These meshes were well visible from one day after birth to the second week, when they gradually disappeared (Figs. 2a-d and 3). The number of stems of terminal arterioles and collecting venules did not increase postnatally in the areas observed.

The capillary network in one-day-old rat muscles consisted of numerous small meshes, very irregular in size and shape. During the following three postnatal weeks, the architecture of the capillary network gradually developed into a characteristic system of long meshes, parallel with the course of muscle fibers, and having numerous short transverse connections. At the end of the third week, the architectural arrangement of capillaries was similar to that in adult muscles. No evidence of sprouting activity was found in injected specimens in any of the studied vascular areas.

Electron-microscopical findings

Hilar vessels. The hilar vessels showed very mature shapes from the beginning of postnatal life. The wall of the artery consisted of a well developed endothelial layer, a continuous internal elastic lamina, two layers of smooth muscle cells, and a loose adventitial coat consisting of an incomplete external elastic lamina, unmyelinated nerve fibers, and several layers of fibroblasts (Fig. 4a). The basic structure of the arterial wall remained unchanged after the third week (Fig. 4b), and only a continued maturation of all wall compartments was apparent.

The wall of the hilar vein consisted of endothelium, a very thin and incomplete internal elastic lamina, one layer of very flat smooth muscle cells, poor innervation, and a layer of free fibroblasts. No basic differences in the structure of the venous walls in one-week-old and three-week-old rats were observed (Fig. 4c,d).

In close proximity to the hilar vessels, well developed lymphatic capillaries were constantly present since the first postnatal day, with a layer of endothelium much thinner than that of the blood vessels.

Central vessels. In one-day-old rats there was only a small difference in the structure of the walls of the central artery and vein (Fig. 4e,f). In both vessels one layer of immature smooth muscle cells was present, covered with a very thin and finely granulated basal lamina. Both vessels had
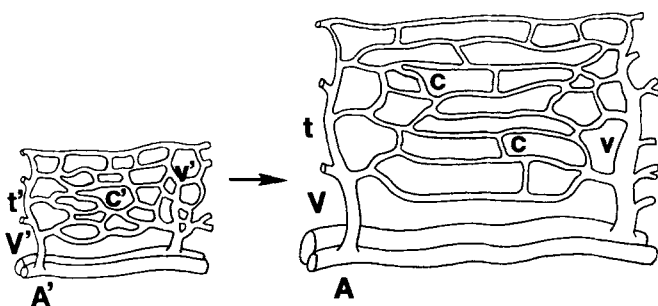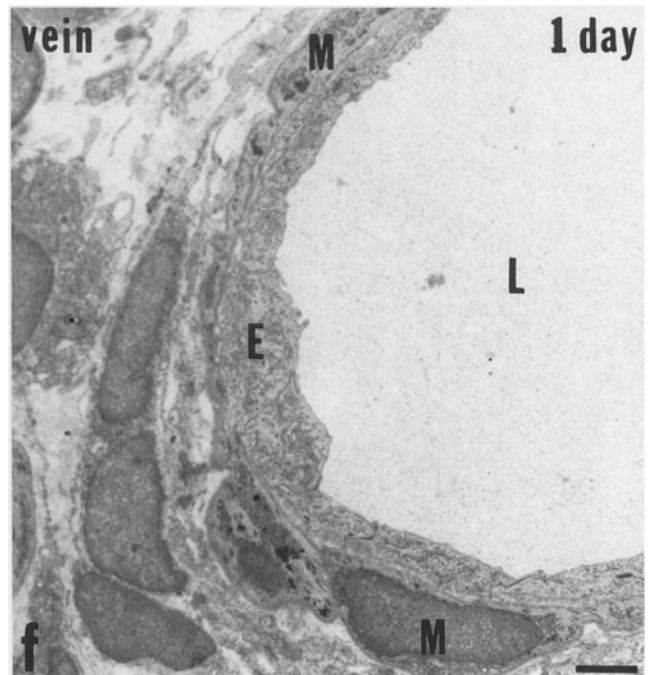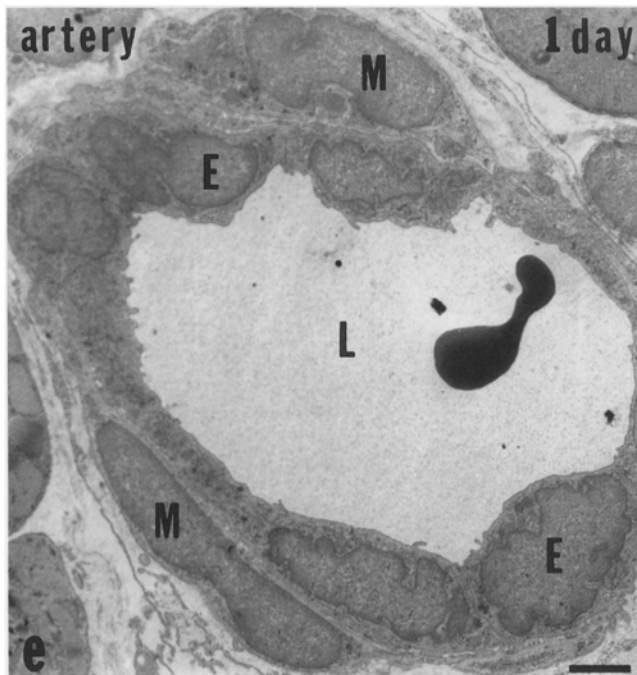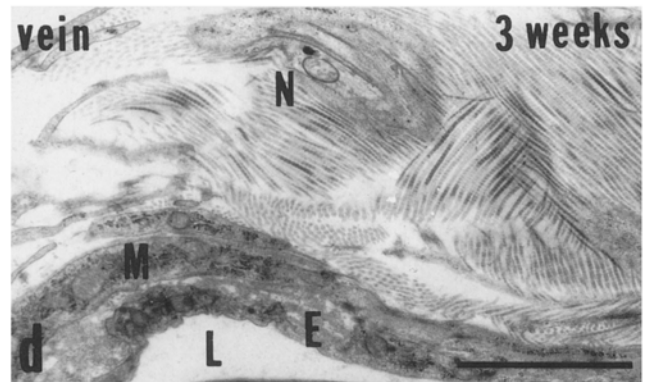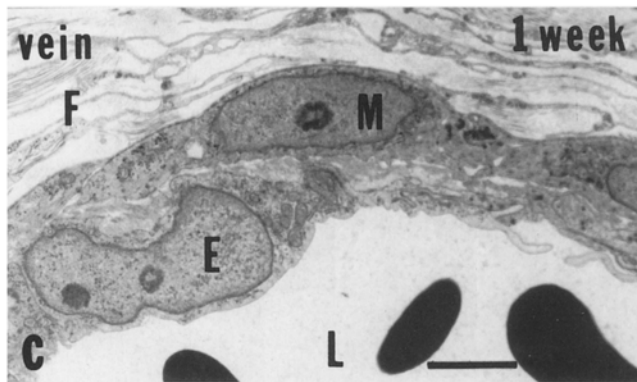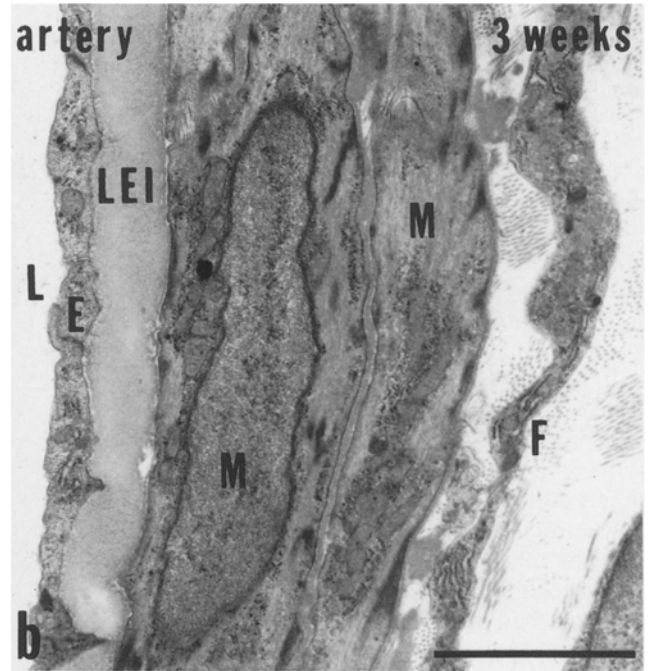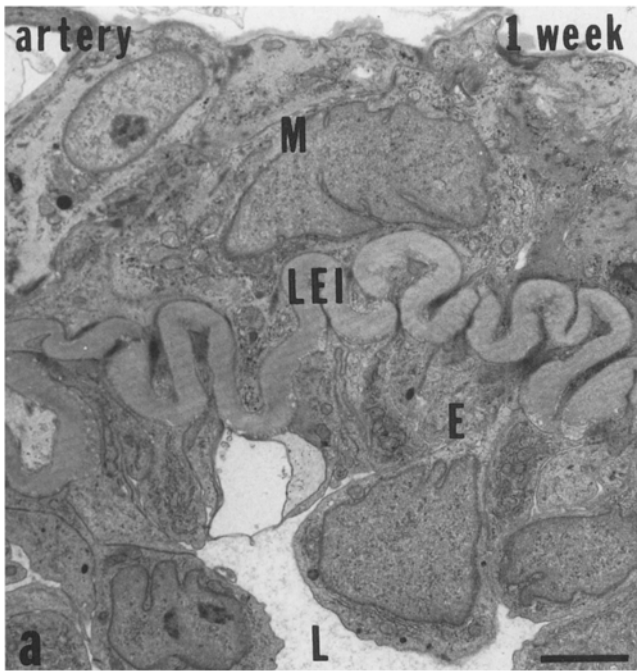

Fig. 3. Schematic representation of postnatal changes of intramuscular microvasculature. In one-day-old rats (*left*), capillaries (*c'*) forming dense meshwork of short interconnected channels between terminal arterioles (*t'*) and postcapillary venules and collecting venules (*v'*). In third postnatal week (*right*) meshwork changed to elongated intramuscular capillaries with widely spaced, short interconnecting channels. *A'* and *A* Central artery; *V'* and *V* central veins

Fig. 4a–f. Development of hilar and central vessels. **a** Constricted hilar artery of one-week-old rat (cross section). *E* Endothelium; *L* lumen; *LEI* internal elastic lamina; *M* smooth muscle cells. *Bar*: 2 μm; × 5700. **b** Hilar artery of three-week-old rat (cross section). *E* Endothelium; *F* fibroblasts; *L* lumen; *LEI* internal elastic lamina; *M* smooth muscle cells. *Bar*: 2 μm; × 13 200. **c** Hilar vein from same specimen as in Fig. 3a. *E* Endothelium; *F* fibroblasts; *L* lumen; *M* smooth muscle cells. *Bar*: 2 μm; × 5000. **d** Hilar vein of three-week-old rat (cross section). *E* Endothelium; *L* lumen; *M* smooth muscle cells. *Bar*: 2 μm; × 11 500. **e** Central artery of one-day-old rat (cross section). *E* Endothelium; *L* lumen; *M* smooth muscle cells. *Bar*: 2 μm; × 4100. **f** Central vein of one-day-old rat (cross section; same specimen **e**). *E* Endothelium; *L* lumen; *M* smooth muscle cells. *Bar*: 2 μm; × 4100
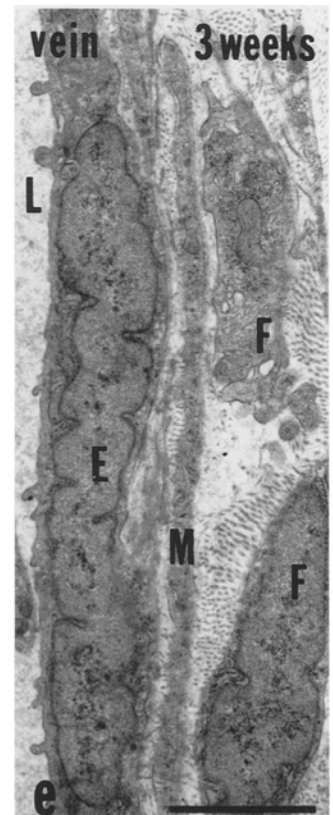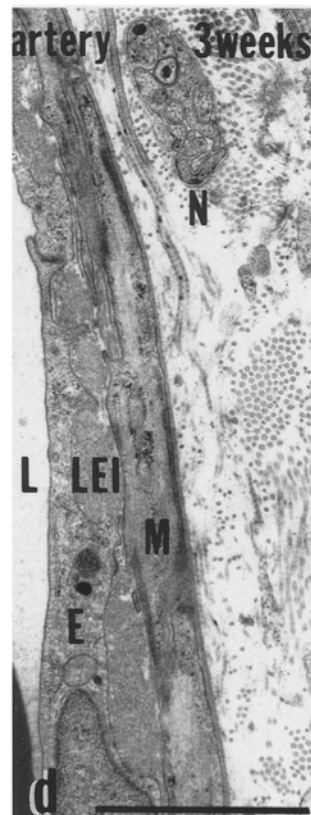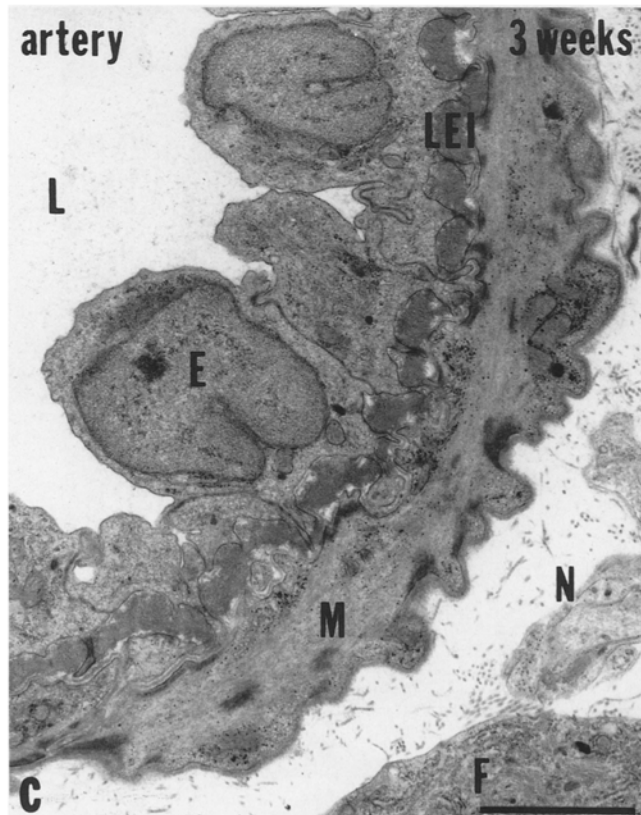
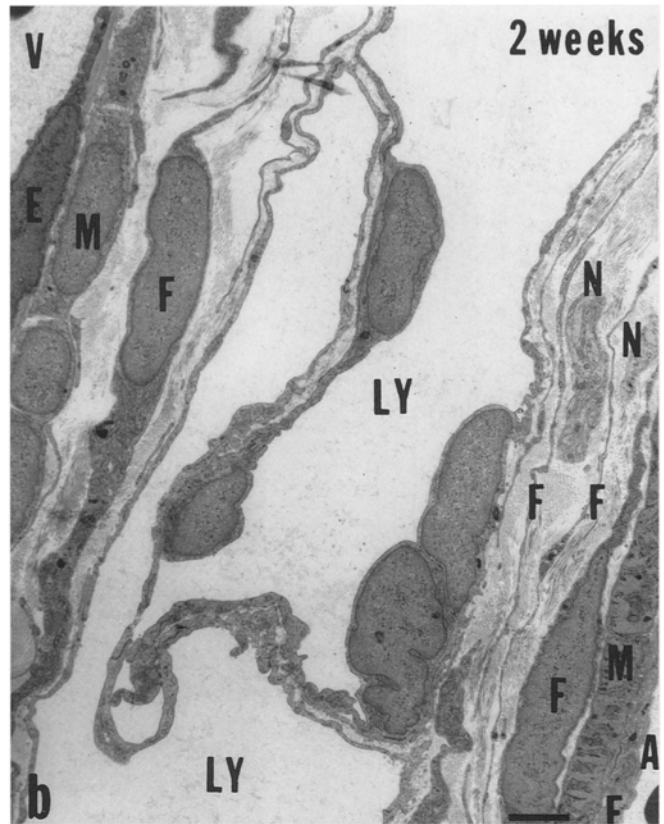artery — 1 week (a); artery — 3 weeks (b); vein — 1 week (c); vein — 3 weeks (d); artery — 1 day (e); vein — 1 day (f)
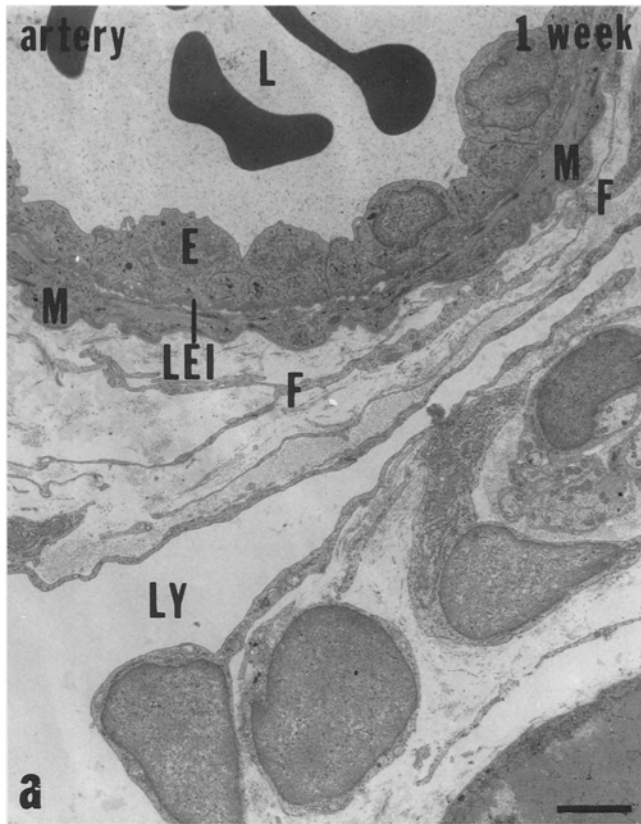
Fig. 5a–e. Development of central vessels. **a** Central artery from one-week-old rat (cross section). *E* Endothelium; *F* fibroblasts; *L* lumen; *LEI* internal elastic lamina; *LY* lymphatic capillary; *M* smooth muscle cells. *Bar*: 2 µm; × 4800. **b** Central vessels in two-week-old rat (longitudinal section). *A* Central artery; *E* endothelium; *F* fibroblasts; *LY* lymphatic capillaries; *M* smooth muscle cells; *N* nerve fiber; *V* central vein. *Bar*: 2 µm; × 4000. **c** Slightly constricted central artery; three-week-old rat (cross section). *E* En-

dothelium; *F* fibroblast; *L* lumen; *LEI* internal elastic lamina; *M* smooth muscle cell; *N* nerve fibers. *Bar*: 2 µm; × 10 200. **d** Dilated central artery; three-week-old rat. *E* Endothelium; *L* lumen; *LEI* internal elastic lamina; *M* smooth muscle cells; *N* nerve fiber. *Bar*: 2 µm; × 14 100. **e** Central vein; three-week-old rat (longitudinal section). *E* Endothelium; *F* fibroblasts; *L* lumen; *M* smooth muscle cells. *Bar*: 2 µm; × 9900

a 1 day

b 1 day

c 1 week

d 1 week
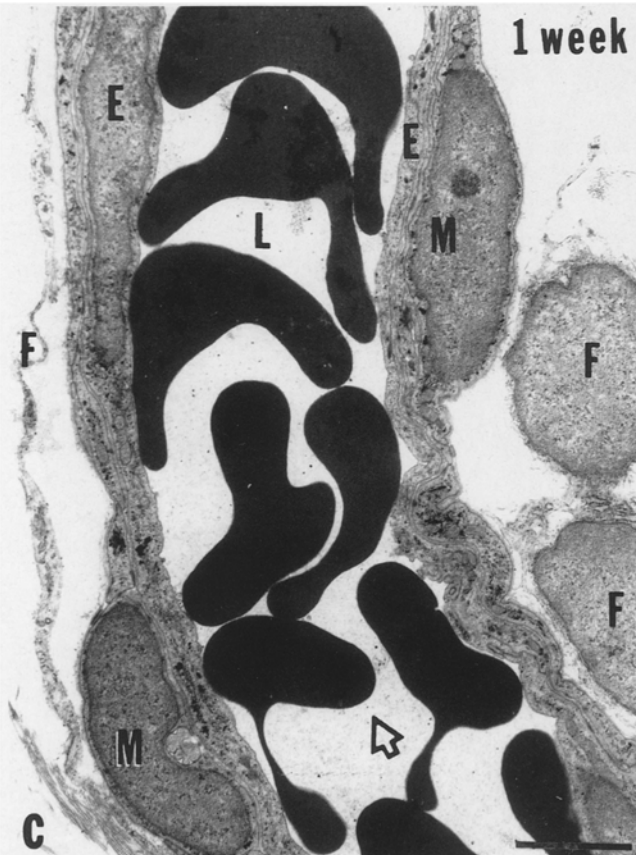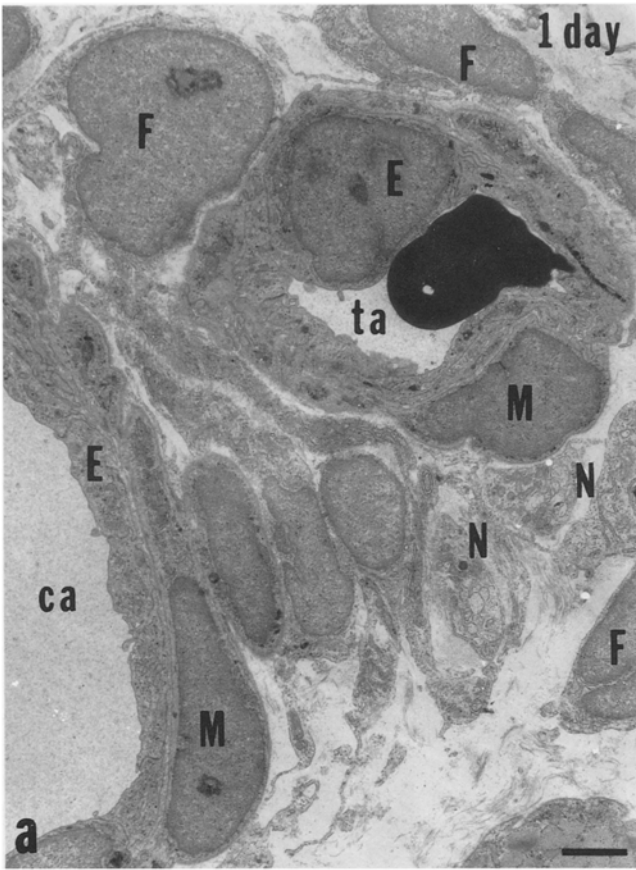
poor innervation and an incomplete coat of fibroblasts. Lymphatics were not observed in the proximity of the central vessels at this age group.

In one- and two-week-old rats (Fig. 5a,b), an increase in the size and further maturation of the inner structure of smooth muscle cells were evident in the arterial wall. In the venous wall, the same cells remained small and flat, and their inner structure lacked some of the characteristics of fully developed arterial smooth muscle cells. A thin, incomplete, and poorly visible internal elastic lamina was present in the artery. The coat of fibroblasts on the arterial wall was complete, while on the venous wall, it consisted of only isolated single cells.

Since the first postnatal week, well developed lymphatic capillaries were present close to the central blood vessels, bordered by thin flat cells. The thickness and inner structure of these endothelial cells were more similar to those of the neighboring fibroblasts than to those of blood vessel endothelium (Fig. 5a,b).

At the end of the third week (Fig. 5c,d) the artery had a fully developed internal elastic lamina, one layer of typical smooth muscle cells, rich innervation, and a complete coat of 1–2 layers of fibroblasts. The vein (Fig. 5e) had a much thinner wall because of lack of the internal elastic lamina, and a very thin layer of smooth muscle cells, distended in the form of a flat sheath around the endothelium. There was an incomplete coat of fibroblasts and poor innervation on the outer surface of the central vein.

Terminal and precapillary arterioles

In one-day-old rats, the terminal arterioles branched off from the central artery, having an inner diameter of about 3 μm to 6 μm (Fig. 6a). The layer of smooth muscle cells continued from the wall of the artery to the stems of the terminal arterioles. The smooth muscle cells in the arteriolar wall were small, and usually two to three cells encircled the endothelium. Delicate nerve fibers ran along the stems of terminal arterioles.

The layer of smooth muscle cells continued from the stems of terminal arterioles to the precapillary arterioles in the form of short cuffs, encircling the beginning of the smallest arterioles. More peripherally, their walls were formed by endothelium only, and by continuous branch-

ing they became true capillaries. In the branching areas of the precapillary arterioles, single cells were located close to the surface of the endothelium (Fig. 6b). Their inner structure was very similar to that of fibroblasts, but their round shapes were rather typical for immature smooth muscle cells. The surface of these cells was partially covered by a very thin and discontinuous basal lamina.

In the following three weeks, the inner diameter of terminal arterioles remained about 5–8 μm. The smooth muscle cells on the stems of terminal arterioles became bigger and had a typical spiral orientation around the endothelium (Fig. 6c). During the first week, mesenchymal cells or fibroblasts continued to be located near the endothelium of the precapillary arterioles and presumably became transformed into immature smooth muscle cells (Fig. 6d). From the second week on, this process was no longer apparent and all new smooth muscle cells were separated from the surrounding space by a basal lamina (Fig. 7a,c). In the third week very fine nerve fibers reached the segments of newly matured precapillary arterioles (Fig. 7b,d). No signs of sprouting or mitotic activity of endothelium were found in the arteriolar area. Numerous mesenchymal cells or fibroblasts, mast cells, and macrophages were regularly present in the interstitium surrounding the arteries and arterioles in all groups studied.

Capillaries

The inner diameters of the investigated capillaries in all age groups ranged from 2 μm to 4 μm (Fig. 8b,d,f) and remained unchanged during the time studied. Usually two to four endothelial cells participated in the building of the capillary wall. Various types of contacts between neighboring endothelial cells were found, independently of age. Some cells had simple flat or overlapping contacts (Fig. 8b,d,f), but especially in longitudinal sections frequently more or less complicated contacts were apparent (Fig. 8a,c,e).

The inner structure of endothelial cells remained unchanged during the first three postnatal weeks. It was characterized mainly by fine granulated cytoplasm containing few mitochondria, minute cisterns of rough endoplasmic reticulum, and numerous free ribosomes. A rich pinocytotic activity on both luminal and abluminal surfaces of endothelial cells was apparent since the first postnatal day. The basal lamina, covering the abluminal surface of the endothelium, remained very thin and finely granulated during the three postnatal weeks studied. Numerous single collagen fibrils were attached to it. No sprouting activity or mitoses of endothelial cells were found in the investigated capillary area.

Pericytes were usually closely attached to the endothelial cells (Fig. 8b,c,d) and covered by their own thin basal lamina. Their contacts with the endothelium were not always complete, and irregular free spaces between both kinds of cells were often apparent (Fig. 8a). In other cases some extensions of pericytes were far away from the endothelial surface and not completely covered with basal lamina (Fig. 8f).

Fig. 6a–d. Development of arterioles. a Cross section through stem of terminal arteriole (ta) near its origin from central artery (ca); one-day-old rat. E Endothelium; F fibroblasts; M smooth muscle cells; N nerve fibers. Bar: 2 μm; × 4100. b Longitudinal section through peripheral branching of precapillary arteriole (pa) in one-day-old rat. E Endothelium; F fibroblasts; ta stem of terminal arteriole; x_1, x_2 two cells, close to endothelium near branching place. Arrows Blood flow direction. Bar: 2 μm; × 5000. c Stem of terminal arteriole (one-day-old rat, longitudinal section). E Endothelium; F fibroblasts; L lumen; M smooth muscle cells. Arrows Blood flow direction. Bar: 2 μm; × 7700. d Branching of precapillary arteriole (one-week-old rat, longitudinal section). E Endothelium; F fibroblast; M smooth muscle cells. 1 Cell with fibroblastic characteristics near endothelium, 2 nuclear part of this cell, continuing peripherally as a typical fibroblastic process (3). Arrowhead Process of smooth muscle cell. Arrows Blood flow direction. Bar: 2 μm; × 5200
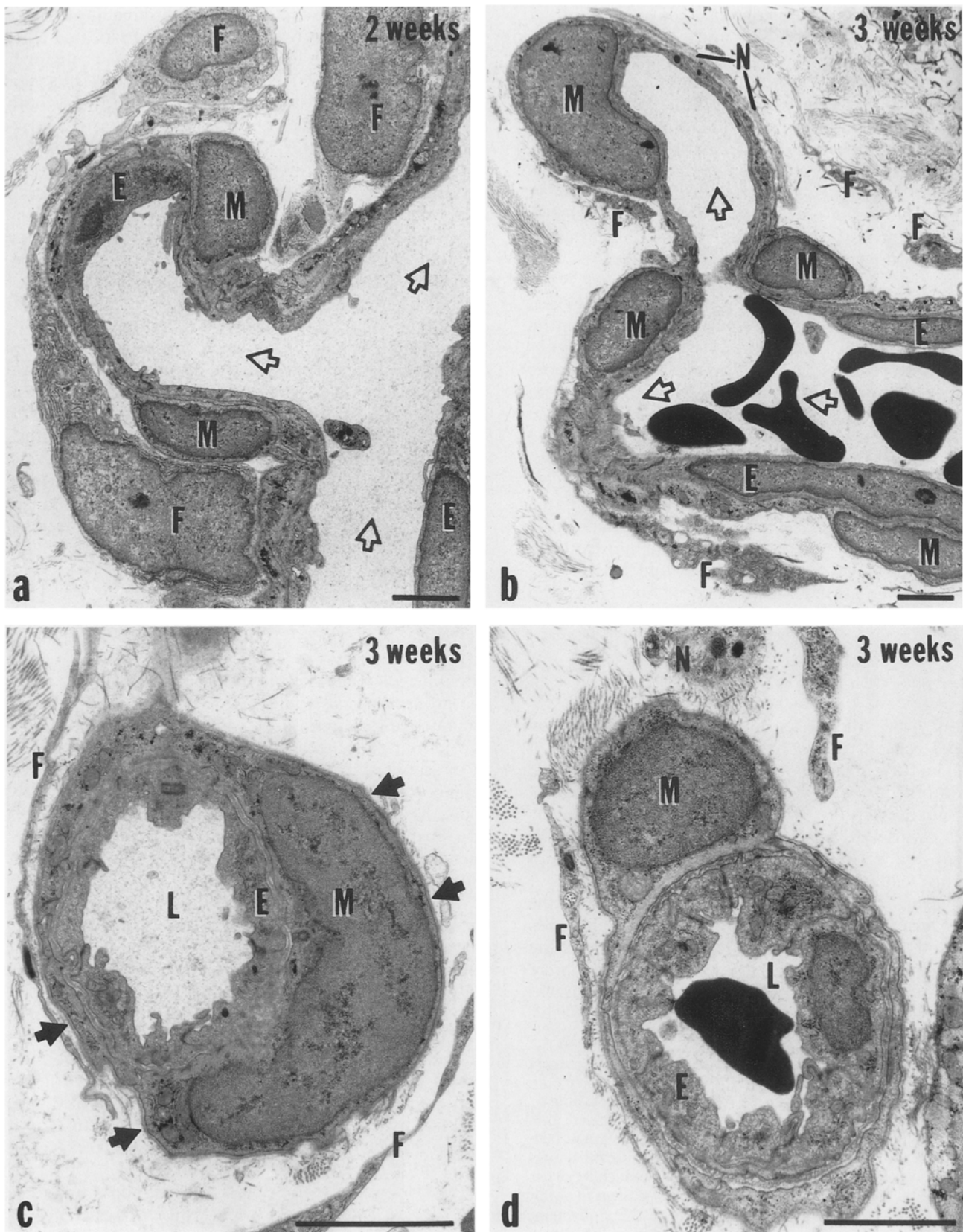
Fig. 7a–d. Structure of terminal and precapillary arterioles. **a** Precapillary arteriole branching from stem of terminal arteriole (two-week-old rat, longitudinal section). *E* Endothelium; *F* fibroblasts; *M* smooth muscle cells. *Arrows* Blood flow direction. *Bar*: 2 μm; × 5900. **b** Precapillary arteriole branching from stem of terminal arteriole (three-week-old rat, longitudinal section). *E* Endothelium; *F* fibroblasts; *M* smooth muscle cells; *N* nerve fiber. *Arrows* Blood flow direction. *Bar*: 2 μm; × 5000. **c** Precapillary arteriole of three-week-old rat in cross section. *E* Endothelium; *F* fibroblasts; *L* lumen; *M* smooth muscle cell. *Arrows* Basal lamina. *Bar*: 2 μm; × 14 000. **d** Constricted precapillary arteriole of three-week-old rat (cross section). *E* Endothelium; *F* fibroblasts; *L* lumen; *M* smooth muscle cells; *N* nerve fibers. *Bar*: 2 μm; × 12 000
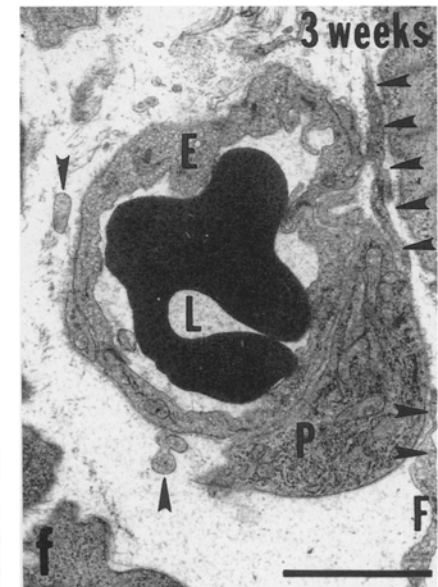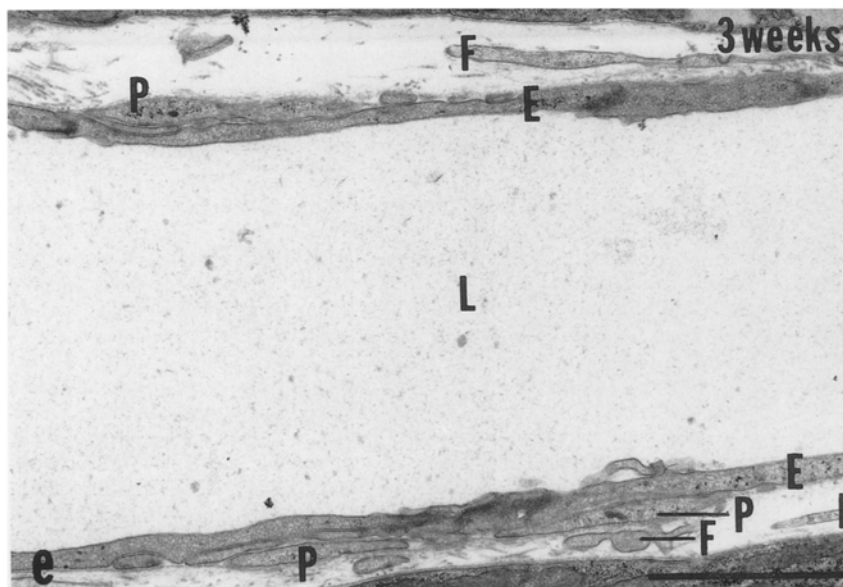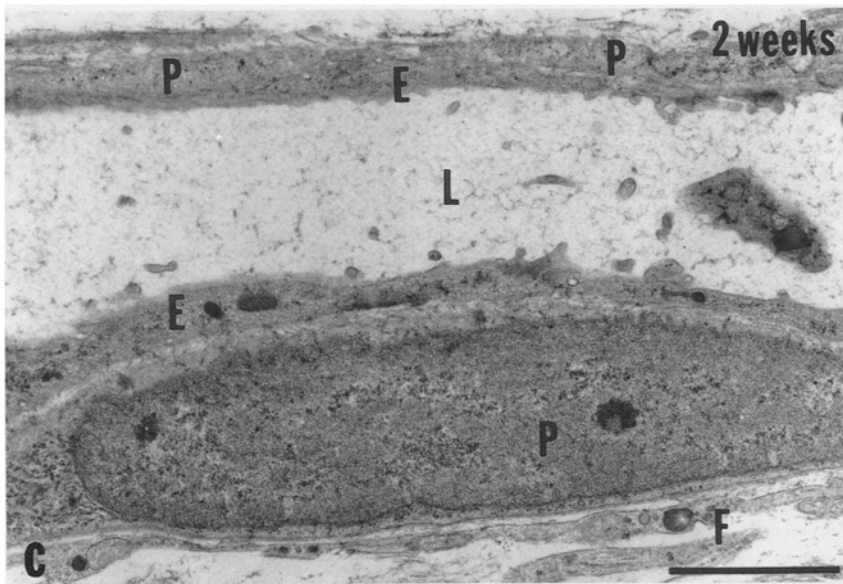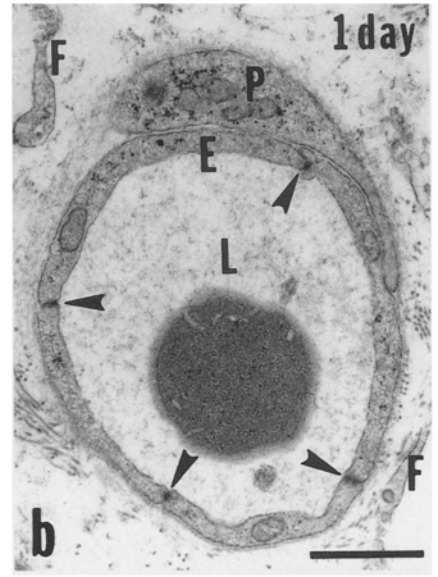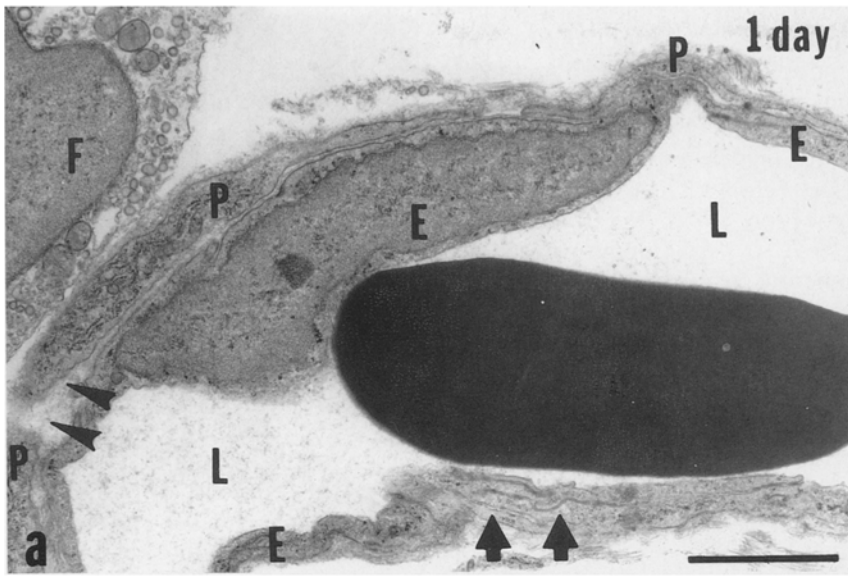
The inner structure of pericytes also remained unchanged during the period studied. Very typical for their cytoplasm were numerous cisterns of rough endoplasmic reticulum and free ribosomes. This structural arrangement was so similar to that of the neighboring fibroblasts, that in some cases it was not possible to distinguish with certainty to which type of cell the processes belong, especially when the basal lamina on the pericytic processes was incomplete or not well apparent (Fig. 8e,f).

In the entire investigated capillary area, numerous free mesenchymal cells or fibroblasts, macrophages, and mast cells with discharged granules were present in close proximity to the capillaries in all age groups studied.

Venules

The capillaries merged at their venous ends, giving rise to short stems of postcapillary venules (Fig. 9a). The walls of the postcapillary venules consisted of endothelium and an incomplete layer of pericytes, whose processes were in some places not completely covered by basal lamina. The postcapillary venules, usually about 5 μm to 8 μm in diameter, emptied into wider stems of collecting venules. No basic differences were found in the structure of the postcapillary and collecting venules, but the layer of pericytes was complete in the walls of collecting venules. The inner structure of the endothelium and pericytes of the venules displayed the same features as that of capillaries, and remained unchanged during the whole studied period.

Our attention was further concentrated on those venular segments that formed irregular loops in younger animals and later were transformed to the definitive postcapillary and collecting venules. The average inner diameter of these venules was about 6–8 μm, depending on whether their position was closer to capillaries or to the stems of collecting venules. The results of our analysis seem to indicate that there was an extremely high activity of venular wall remodeling in these areas.

The most prominent structural changes were found in the endothelial layer, where various forms of discontinuity were frequent. In some places, only small simple gaps of various sizes were apparent, completely covered on the outside by an intact basal lamina and pericytes (Fig. 9b). In other cases, larger adjacent spaces were observed,

filled with plasma and very often also with single erythrocytes and numerous platelets. These spaces were always covered on their outer surface either by the basal lamina, pericytes alone, or by larger adjacent processes of endothelial cells (Fig. 9c, d). In cross sections it was evident that these spaces were sometimes very large and encircled great parts of the outer surface of the original vessel (Fig. 10). The endothelial cells sent numerous polymorphous projections of pseudopodial character to the outer periphery, stretching the intact basal lamina, which often was not covered by pericytes. The new periendothelial spaces were in some places subdivided into several smaller compartments by extensions of basal lamina of neighboring endothelial cells (Fig. 10a). Other new spaces were narrow, and their endothelial walls had smooth surfaces (Fig. 10b). Numerous mast cells and fibroblasts were regularly present in close proximity to the venules.

The remodelling activity of the venular endothelium was also quite apparent in oblique sections (Fig. 11). Particularly striking were the short pseudopodial projections, forming very complicated multilocular gaps on the entire outer surface as well as between the endothelial cells. These processes apparently not only pushed the basal lamina to the periphery, but also made the neighboring endothelial cells become separated. No mitotic activity or capillary sprouting were observed in any of the investigated parts of postcapillary or collecting venules.

Discussion

The results obtained can be compared with only few morphological data, based on similar studies of postnatal vascular development in other organs. By comparison of the same injected intramuscular vascular areas in all age groups studied, it was evident that during the postnatal period no new terminal arterioles or collecting venules arise and, therefore, only growth of previously formed blood vessels takes place in the investigated muscle. Similar results were also obtained in the growing rat brain and intestine (Bär 1983; Unthank and Bohlen 1987).

Our muscle model makes it possible to detect and evaluate growth changes in all segments of skeletal vas-

Fig. 8a–f. Ultrastructure of postnatally growing capillaries. a One-day-old rat, longitudinal section. E Endothelium; F fibroblast; L lumen; P pericytes. Arrowheads Free space between endothelium and pericytes. Arrows Pericyte/endothelial contacts. Bar: 2 μm; × 10 000. b One-day-old rat; cross section. E Endothelium; F fibroblasts; L lumen; P pericyte. Arrowheads Simple types of endothelial contacts. Bar: 2 μm; × 7400. c Two-week-old rat; longitudinal section. E Endothelium; F fibroblasts; L capillary lumen; P pericytes. Bar: 2 μm; × 11 250. d Two-week-old rat; cross section. E Endothelium; L lumen; P pericytes. Bar: 2 μm; × 9000. e Three-week-old rat; longitudinal section. E Endothelium; F fibroblasts; P pericytes. Bar: 2 μm; × 13 000. f Three-week-old rat; cross section. E Endothelium; L lumen. Arrowheads Similarity of peripheral processes of pericytes (P) and fibroblasts (F). Bar: 2 μm; × 9700

Fig. 9a–d. Postnatal development of venules. a One-day-old rat; longitudinal section through stem of postcapillary venule (PV), formed by confluence of two capillaries (C_1, C_2), and emptying into collecting venule (CV). F Fibroblast; P pericyte. Empty arrows Blood flow direction; full arrows free peripheral endothelial surface of postcapillary venule. Arrowheads Processes of pericytes or fibroblasts not covered by basal lamina. Bar: 2 μm; × 13 000. b One-day-old rat; cross section of postcapillary venule with endothelial gap (arrow). E_1, E_2 Neighboring endothelial cells; L lumen; MC mast cell; P pericyte. Bar: 2 μm; × 11 300. c Two-week-old rat; wall of collecting venule in longitudinal section with developing accessory space (x), containing plasma and erythrocyte. E Endothelium; F fibroblast; L lumen; P pericyte. Arrowheads Intact endothelial basal lamina. Bar: 2 μm; × 9500. d Two-week-old rat; cross section of postcapillary venule wall with wide adjacent space (X). E Endothelium; F fibroblast; L lumen; P pericyte. Bar: 2 μm; × 11 600
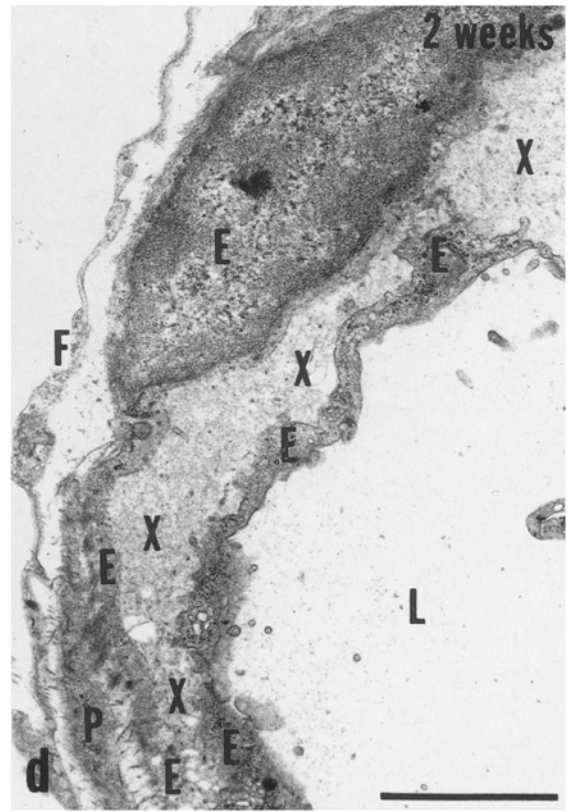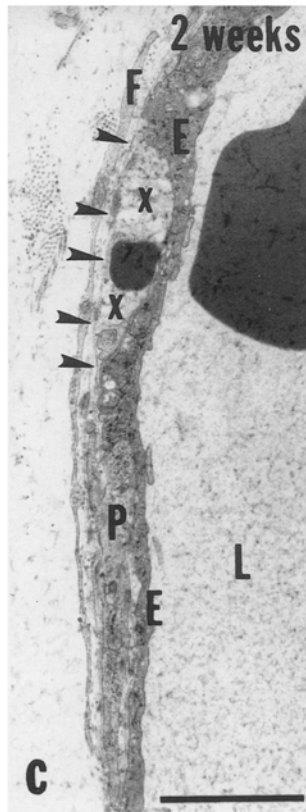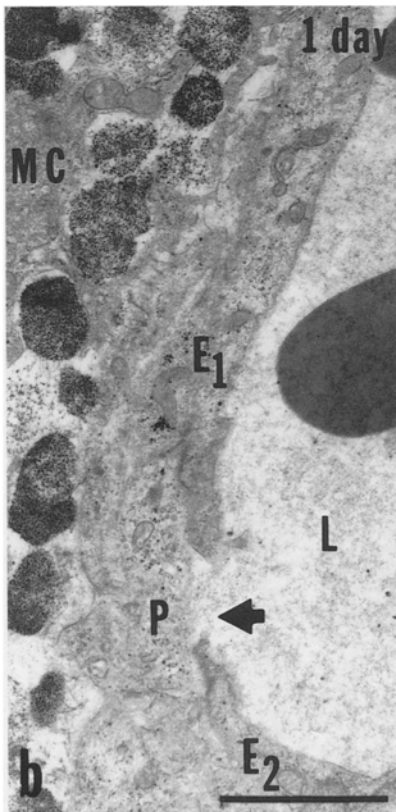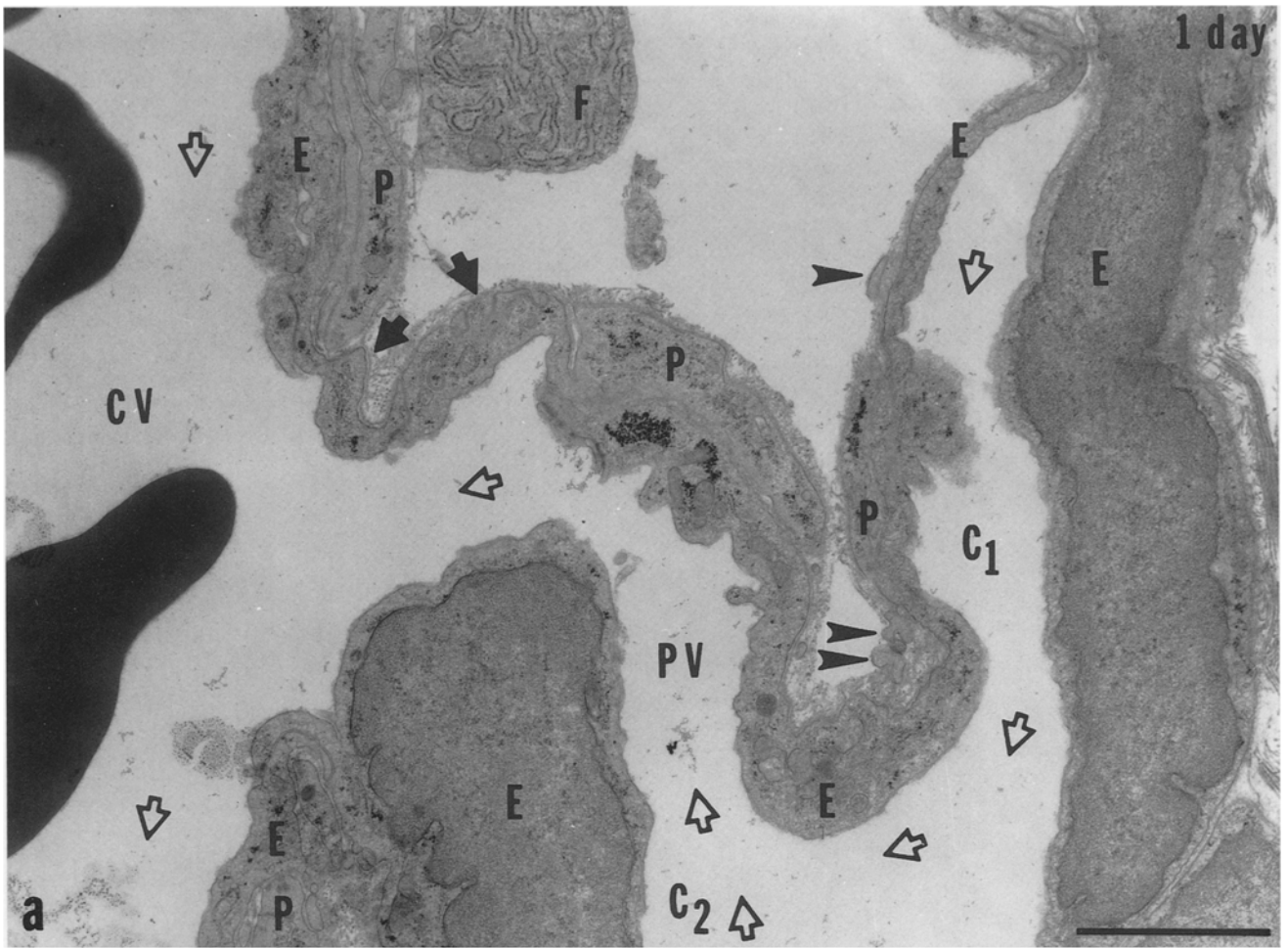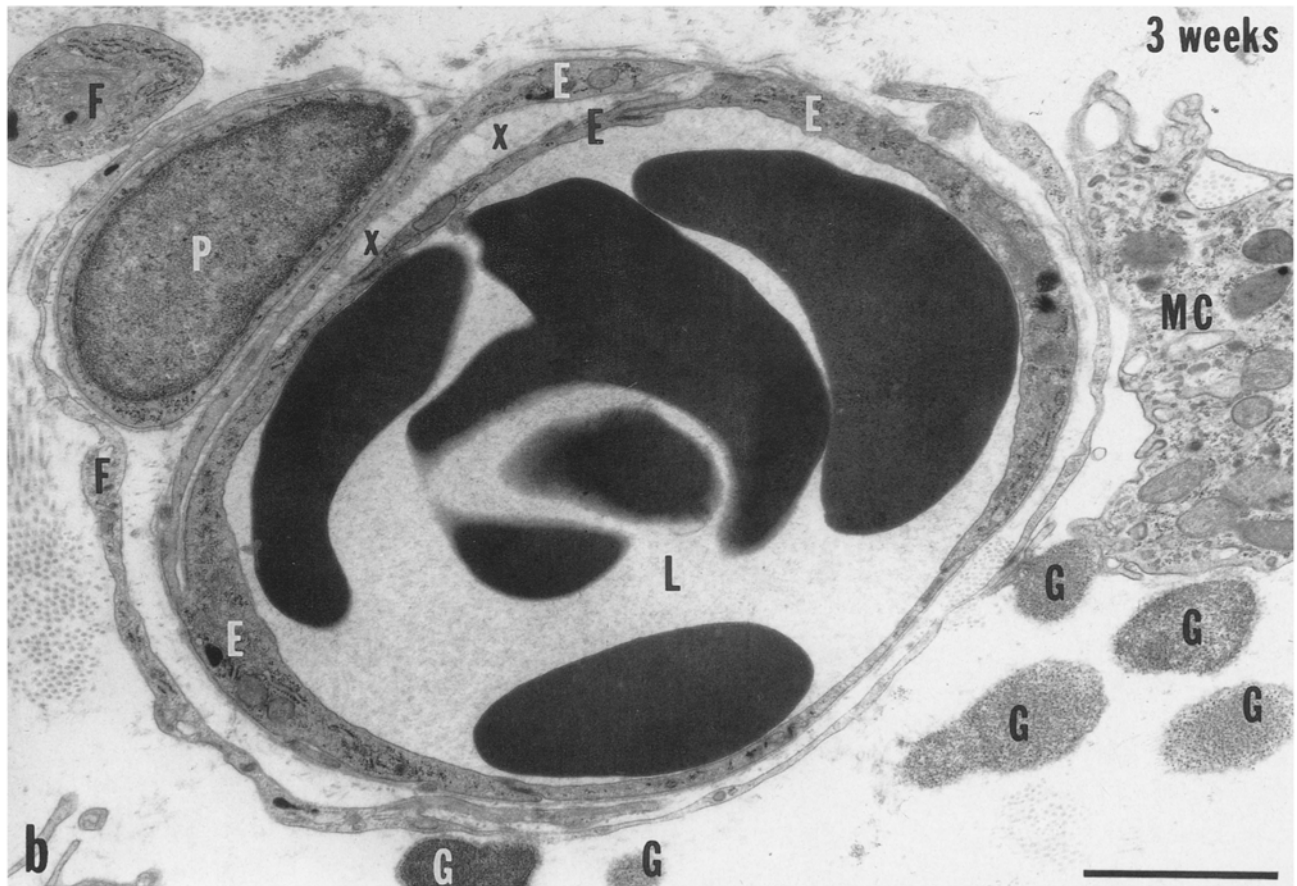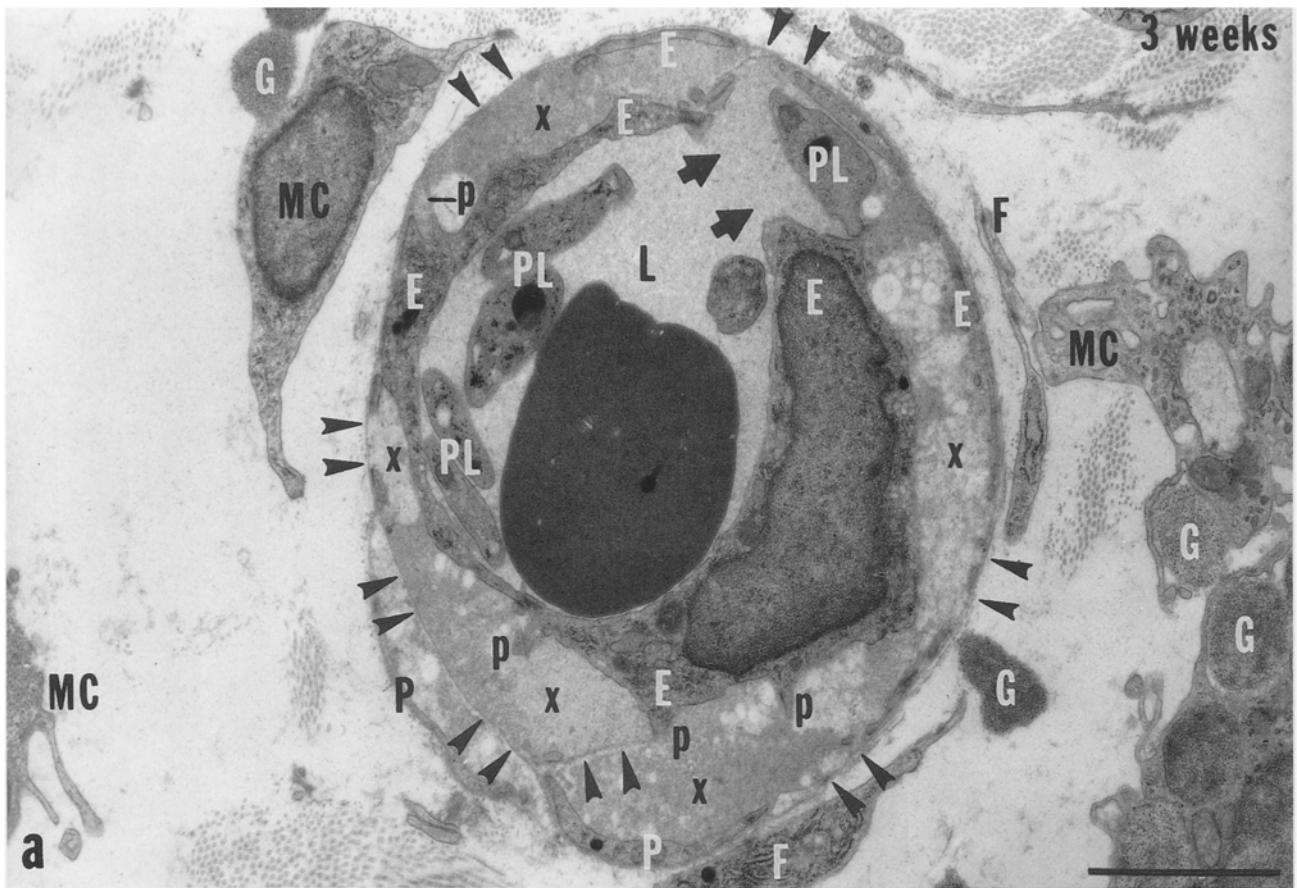
Fig. 10a,b. Growing venules in cross sections. **a** Three-week-old rat; venule with wide endothelial gap (*arrows*), and large extraendothelial space (*x*). *E* Endothelium; *F* fibroblast; *G* granules of mast cells; *L* lumen; *M* macrophage; *MC* mast cell; *PL* platelets; *p* projections of endothelial cells. *Arrowheads* Intact basal lamina of endothelial cells. *Bar*: 2 μm, × 12 500. **b** Three-week-old rat; another venule with narrow adjacent space (*x*). *E* Endothelium; *F* fibroblasts; *G* free granules of mast cells; *L* lumen; *M* macrophage; *P* pericyte. *Bar*: 2 μm; × 12 800
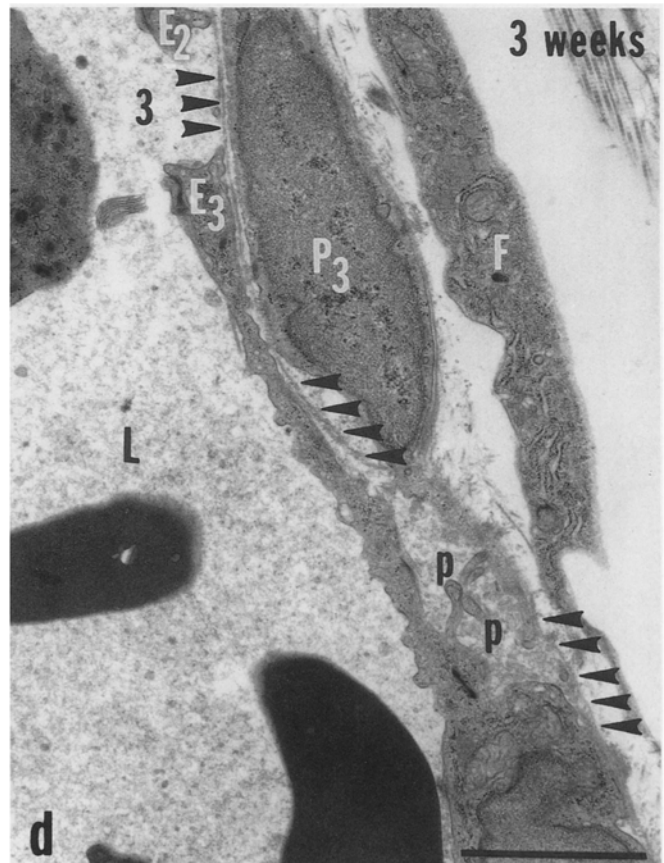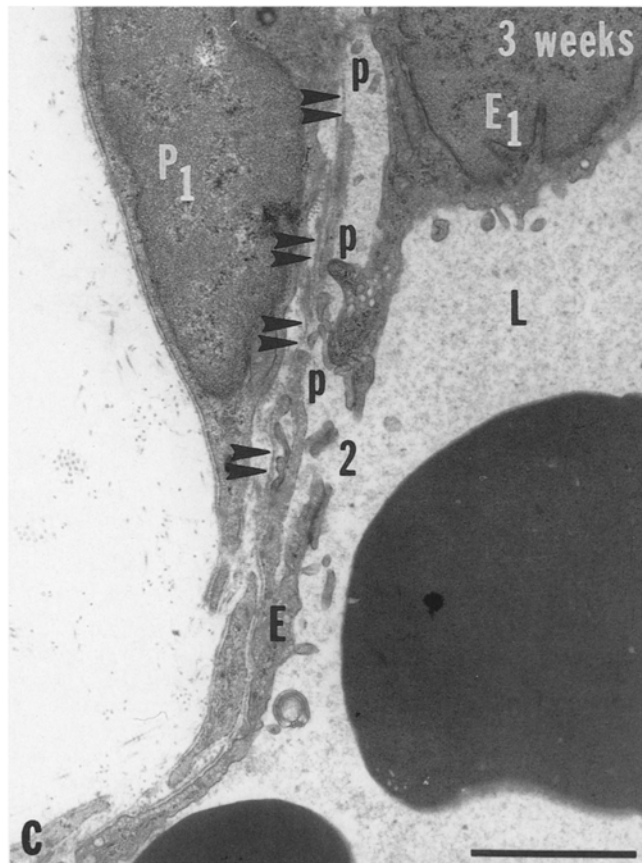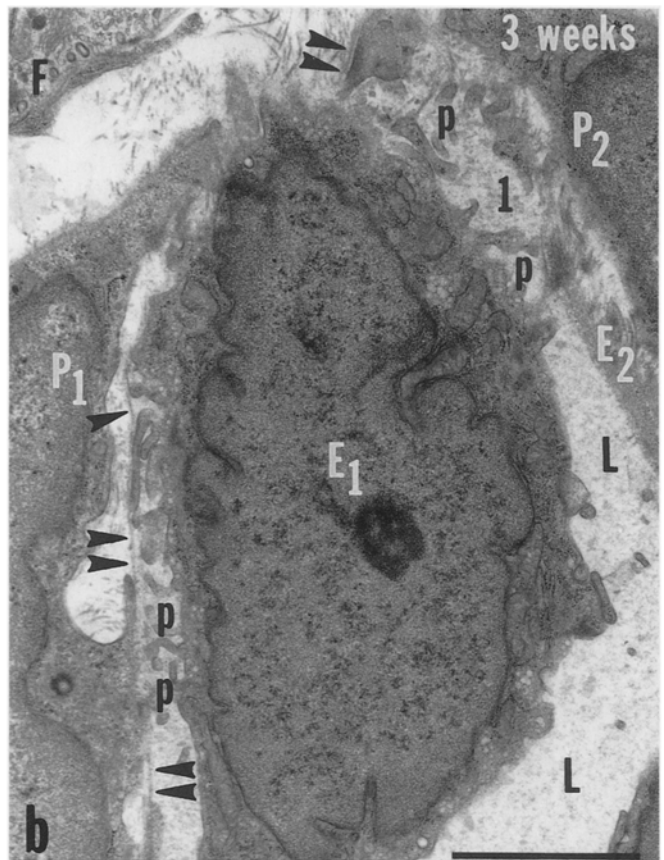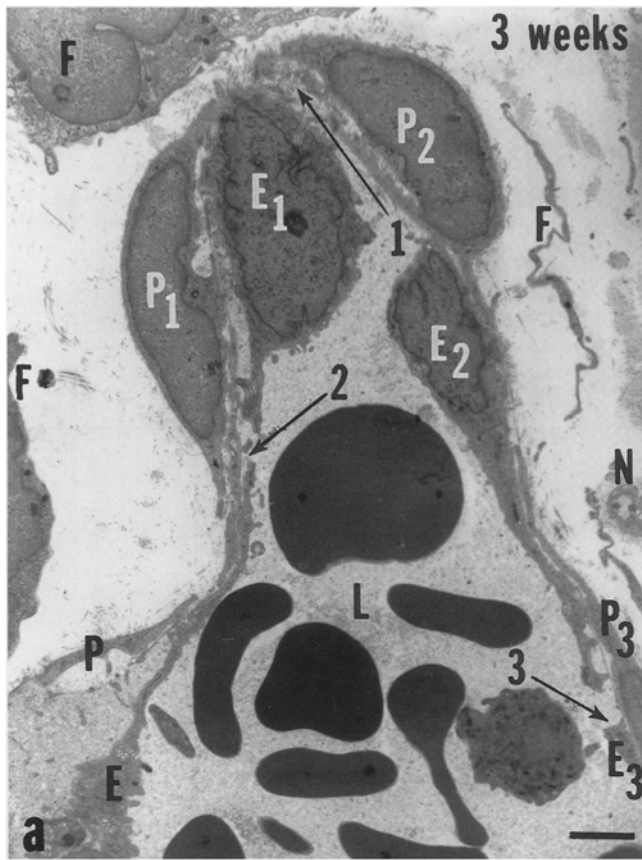
Fig. 11a–d. Growing venule in oblique section; three-week-old rat. **a** Two pericytes (P_1; P_2) covering region of newly arising gap (1) between neighboring endothelial cells (E_1, E_2). Two other gaps (2, 3) between endothelial cells E_1 and E, and E_2, E_3, respectively, covered by pericytes P_1 and P_3. *F* Fibroblasts; *L* lumen; *N* nerve fiber. *Bar*: 2 μm; × 4200. **b** Detail from Fig. 5a, showing gap 1 area. *L* Lumen of venule; *p* projections of endothelial cells. *Arrowheads* Intact endothelial basal lamina. Other symbols as in **a**. *Bar*: 2 μm; × 12 500. **c** Detail of gap 2 area from **a**. Symbols as in **a** and **b**. *Bar*: 2 μm; × 11 200. **d** Detail of gap 3 from **a** and adjacent distal part of venular wall. Symbols as in **a–c**. *Bar*: 2 μm; × 12 300

culature. First, a remarkable proximodistal gradient in the maturation of blood vessels is apparent. The hilar vessels are well developed in muscles of newborn rats and only slight further progress in the maturation of their inner structure is recorded in the following three weeks. Secondly, the central artery and vein, which are structurally very similar in one-day-old rats, undergo marked developmental changes during the following three postnatal weeks. Most noticeable in the progressing maturation is the increasing thickness of the internal elastic lamina in the wall of the central artery, and the final difference in the structure of arterial and venous smooth muscle cells. This is in agreement with previous findings (Rhodin 1980; Miller et al. 1985; Bizuneh et al. 1991; Nehls and Drenckhahn 1991).

In the area of precapillary arterioles, an assumed continuing transformation of mesenchymal cells and/or fibroblasts to new smooth muscle cells is observed. We recognize that other technical approaches are necessary to prove the correctness of this assumption. This would entail immunofluorescence and antibody reactions to cell organelles such as filamentous F-actin in fibroblasts, intermediate filaments (desmins) in pericytes and alpha-actin in smooth muscle cells. Our findings indicate that the most peripheral parts of the arterioles undergo not only qualitative but also quantitative developmental changes during the period of postnatal growth of skeletal muscle. The process of fibroblast movement toward the endothelium and the acquisition of a basal lamina is important, because only then are these cells stabilized in their positions, and subsequently transformed into smooth muscle cells. Once these changes are finished, nerve fibers grow into newly developed arteriolar segments. The presence of nerve fibers on the arteriolar surface is another expression of structural and functional maturation. From this point of view, our results are in full agreement with the conclusions of Gray (1973), who observed that the reactivity of skeletal muscle blood vessels in growing rats was comparable to that of the adults in the third postnatal week.

The fine structure of skeletal muscle capillaries does not change markedly during the first three weeks of postnatal growth. Also, the inner capillary diameter remains unchanged at that time. Compared to the mature capillaries in muscles of adult rats (Stingl 1971a; Tilton et al. 1979; Sims 1991) the growing capillaries differ above all by showing an abundant endoplasmic reticulum in the pericytes, and a very thin and immature basal lamina associated with the pericytes and endothelium.

Mitotic activity of capillary endothelium was not detected in any age group studied. This is in agreement with the generally accepted opinion that endothelial mitoses are extremely rare, and therefore only exceptionally detectable. On the other hand, we assume that the number of endothelial cells does not increase substantially during the postnatal period, and that the capillaries grow mainly by elongation of preexisting endothelial cells.

Capillary sprouts are not found in our material either in the injected specimens or those analyzed by electron microscopy. Neither did we find them in other growing rat skeletal muscles (Stingl 1971b). On the other hand, a decreasing number of sprouts was described in the triceps brachii muscle of the postnatally growing rats during the first three weeks (Welt et al. 1974; Scheller et al. 1977). This discrepancy can be explained as the result of different rates of postnatal development of the postural back muscles and those of the limbs. Nevertheless, a thorough morphological comparison of the dynamics of the early postnatal development of both groups of muscles is necessary to clarify this point.

Processes of the capillary and venular pericytes are very often markedly extended peripherally into the surrounding interstitium, lacking a basal lamina. This spatial arrangement and the immature inner structure are reminiscent of the process of an assumed transformation
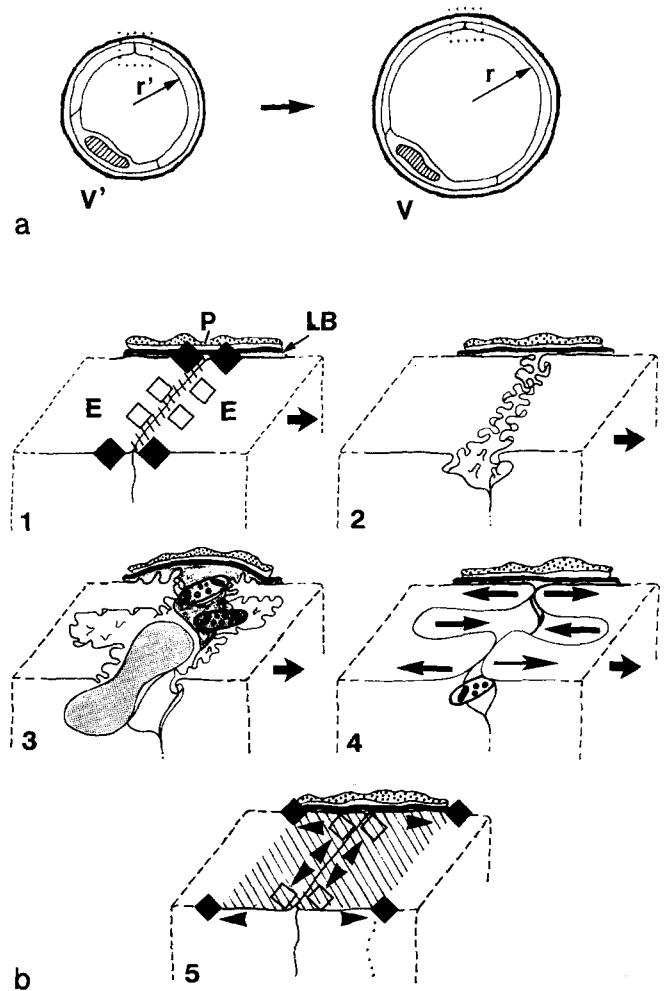

Fig. 12a,b. Schematic illustration of endothelial activity in postnatally growing venules. a Growth of developing venule (V) to definitive size (V). r, r' Radiuses of venules. Developmental changes of endothelial contact in dotted areas depicted in b. b Basic stages of endothelial rebuilding by venular growth. 1 Initial stage with complete contact of neighboring endothelial cells (E). LB Basal lamina; P pericyte. Full and empty squares Parts of endothelial cells to be shifted to new positions. 2 Start of endothelial rebuilding by creation of numerous pseudopodial processes in arising gap. 3 Enlarged gap, filled with plasma, platelets, and an erythrocyte. 4 Smooth surface of gap space, and shifts of newly created endothelial surfaces. 5 Final stage with new location of originally closely adjacent endothelial surfaces, and enlarged endothelial cells (oblique hatching)

of fibroblasts to smooth muscle cells, described during the growth of arterioles. Therefore, we assume that also in the capillary and venular regions the only sources of new pericytes are the free perivascular fibroblasts or mesenchymal cells gradually approaching the endothelial surface during postnatal vascular development.

The picture of dynamic endothelial rebuilding in growing venules substantially differs from the structure of these vessels in muscles of adult rats (Stingl 1978). We consider it important that this remodelling process takes place in these meshes, identified in injected specimens as vascular structures (Fig. 3) which gradually are transformed into postcapillary venules and parts of the stems of collecting venules. The same meshes were described earlier by use of injecting techniques in other postnatally growing muscles of the rat, and in human fetal muscles (Stingl 1971b). It is possible to consider these segments of venules as remnants of prenatally developed capillary networks that postnatally are rebuilt into the final parts of the vascular bed. Regarding the apparent proximodistal gradient of maturation of blood vessels in the growing muscle, the venules are the very last region in which this dramatic rebuilding process takes place even postnatally, while the main structural components of the walls of other parts of the vascular bed are basically developed prenatally. The most important factor which ensures this vascular rebuilding is the activity of endothelial cells which enlarge their size by numerous pseudopodial processes and by moving newly created surfaces to the periphery (Fig. 12). This endothelial activity is quite similar to that described by Hunter and Arsenault (1990) in the growing metaphyseal capillaries. Another important factor in this rebuilding is the continuous integrity of the basal lamina, below which the described changes take part. In this way the venular growth differs markedly from the normal postnatal capillary sprouting, and the tumor-related angiogenesis (Rhodin and Fujita 1989; Paku and Paweletz 1991).

An important role in the venular growth is probably also played by the pericytes, very often covering the places of endothelial gaps. Their participation in this process is likely very active, because their shape resembles that of the umbrella cells observed in the capillary sprouting of rat mesentery (Rhodin and Fujita 1989). Numerous erythrocytes and platelets are often found in the developed gaps. Except for the possible mechanical influence of enlarging gap spaces, one must also consider the biochemical importance of growth factors which the platelets may release directly into the gaps (Ross et al. 1986). The venules probably keep their ability for morphological rebuilding also in the adult, as indicated by the formation of gaps in response to various experimental influences (Sims et al. 1990), or by the initiation of postnatal angiogenesis, mainly by capillary sprouting (Myrhage and Hudlicka 1978).

Our study was performed in a simple vascular model, but one located in a complex environment, e.g., the growing muscle. Although only the main characteristic developmental features of intramuscular vessels were presented, we suggest that the collected morphological data can serve as a base for further investigations of the postnatal development of the vascular bed under normal, pathological, and experimental conditions.

References

Aquin L, Banchero N (1981) The cytoarchitecture and capillary supply in the muscle of growing dogs. J Anat 132:341–356
Bär T (1983) Patterns of vascularization in the developing cerebral cortex. In: Nugent J, O'Connor M (eds) Development of the vascular system (Ciba Foundation Symposium 100), Pittman, London, pp 20–32
Bizuneh M, Bohlen HG, Connors BA, Miller BG, Evan AP (1991) Vascular smooth muscle structure and juvenile growth in rat intestinal venules. Microvasc Res 42:77–90
Bogusch G (1984) Development of the vascular supply in rat skeletal muscles. Acta Anat (Basel) 120:228–233
Dalton AJ (1955) A chrome-osmium fixation for electron microscopy. Anat Rec 121:281
Gray SD (1973) Developmental changes in vascular reactivity in neonatal skeletal muscles. Bibl Anat 121:376–382
Hunter WL, Arsenault AL (1990) Endothelial cell division in metaphyseal capillaries during endochondral bone formation in rats. Anat Rec 227:351–358
Miller BG, Overhage JM, Bohlen HG, Evan AP (1985) Hypertrophy of arteriolar smooth muscle cells in the rat small intestine during maturation. Microvasc Res 29:56–69
Myrhage R, Hudlicka O (1978) Capillary growth in chronically stimulated adult skeletal muscle as studied by intravital microscopy and histological methods in rabbits and rats. Microvasc Res 16:73–90
Nehls V, Drenckhahn D (1991) Heterogeneity of microvascular pericytes for smooth muscle type alpha-actin. J Cell Biol 113:147–154
Ontell M, Dunn RF (1978) Neonatal muscle growth: a quantitative study. Am J Anat 152:539–556
Paku S, Paweletz N (1991) First steps of tumor-related angiogenesis Lab Invest 65:334–346
Rhodin JAG (1967) The ultrastructure of mammalian arterioles and precapillary sphincters. J Ultrastruct Mol Struct Res 18:181–223
Rhodin JAG (1968) The ultrastructure of mammalian venous capillaries, venules and small collecting veins. J Ultrastruct Mol Struct Res 25:452–500
Rhodin JAG (1980) Architecture of the vessel wall. In: Bohr DF, Somlyo AP, Sparks HV Jr (eds) Handbook of Physiology, Sect 2: The cardiovascular system, vol II. American Physiological Society, Bethesda, Maryland, pp 1–31
Rhodin JAG, Fujita H (1989) Capillary growth in the mesentery of normal young rats. Intravital video and electron microscope analyses. J Submicrosc Cytol Pathol 21:1–34
Ripoll E, Sillau AH, Banchero N (1979) Changes in the capillarity of skeletal muscle in the growing rat. Pflugers Arch 380:153–158
Ross R, Raines EW, Bowen-Pope DF (1986) The biology of platelet-derived growth factor. Cell 46:155–169
Sarelius IH, Damon DN, Duling BR (1981) Microvascular adaptations during maturation of striated muscle. Am J Physiol 241: H317–H324
Scheller W, Welt K, Schippel G (1977) Licht- und elektronenmikroskopische Untersuchungen zur postnatalen Entwicklung von Kapillaren im *M. triceps brachii* der weissen Ratte bis zum 20. Monat. Verh Anat Ges 71:701–705

Schippel K, Schippel G, Welt K, Scheller W (1975) Untersuchungen zur postnatalen Differenzierung von Skelettmuskelfasern. Beitr Orthop Traumatol 22:535–537

Sillau AH, Banchero N (1977) Effect of maturation on capillary density, fiber size and composition in rat skeletal muscle Proc Soc Exp Biol Med 154:461–466

Sims DE (1991) Recent advances in pericyte biology. Implications for health and disease. Can J Cardiol 7:431–443

Sims DE, Miller FN, Donald A, Perricone MA (1990) Ultrastructure of pericytes in early stages of histamine-induced inflammation. J Morphol 206:333–342

Stingl J (1971a) Beitrag zum Studium der Ultrastruktur des terminalen Gefässbettes der Skelettmuskulatur. Acta Anat 80:355–372

Stingl J (1971b) Architectonic development of the vascular bed of rat skeletal muscles in the early postnatal period. Folia Morphol (Praha) 19:208–213

Stingl J (1978) Ultrastructure of microcirculation of the skeletal muscle. Plzen Lék Sbor [Suppl 39]: 5–41

Tilton RG, Kilo C, Williamson JR (1979) Pericyte-endothelial relationships in cardiac and skeletal muscle capillaries. Microvascul Res 18:325–335

Unthank JL, Bohlen HG (1987) Quantification of intestinal microvascular growth during maturation: techniques and observations. Circ Res 61:616–624

Welt K, Schippel K, Schippel G, Scheller W (1974) Zur Ultrastruktur von Kapillaren im Skelettmuskel der weissen Ratte vom 19. Fetaltag bis zum 2. Tag post partum. Z Mikrosk Anat Forsch 88:465–478