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Fenestrated capillaries in 'venous patch' adventitia

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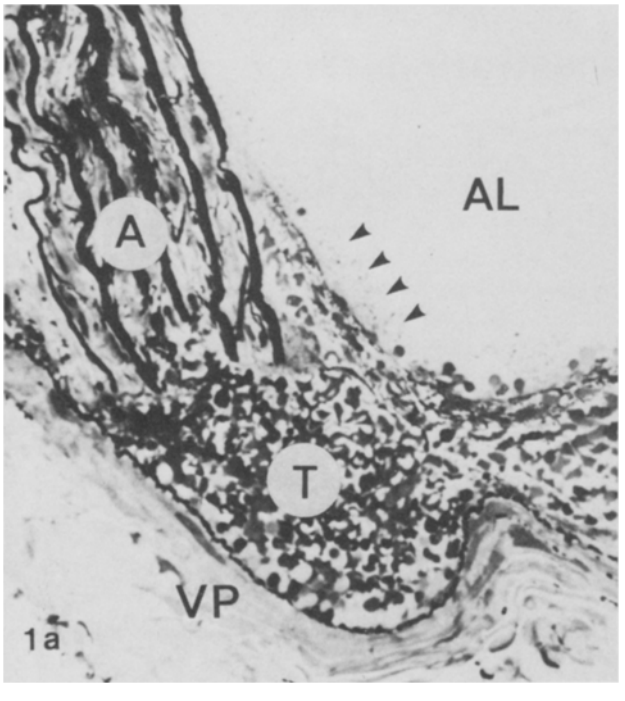
Summary. Fenestrated capillaries in venous patch adventitia are seen during the 2nd week after microsurgery. The fenestrae contain diaphragms. These capillaries are possibly transitory structures which appear during injury cicatrization. The importance of these capillaries during transcappillary exchange in injuried tissue is discussed.

When grafted to animal arteries less than 1 mm in external diameter, the venous patch graft provides the opportunity to study, among other problems, the morphological changes in patch vasa vasorum capillaries. Tight junctions open and pinocytotic vesicles increase in number, whereas the basal lamina remains unchanged[2]. 6 days after surgery, the most external part of the venous patch wall presents new capillaries formed by the proliferation of intact capillaries of arterial vasa vasorum, situated close to the suture[2]. Endothelial cells exhibit tight junctions characteristic of mature endothelium[3]. The capillaries ramify throughout the adventitia, usually stopping at the external border of the media. During the 1st 3 weeks after surgery, capillaries of the venous patch vasa vasorum greatly increase in number and these are lined by a continuous endothelium with an uninterrupted basal lamina. 2 weeks after surgery, the venous patch and the host artery intima presented a muscular hyperplasia[2] and patch revascularization occurred at the same time. In order to correlate hyperplasia and revascularization of the patch, we employed the venous patch technique and, since revascularization is evident 2 weeks after surgery, we studied animals at the period and we found (unexpected) fenestrated capillaries in the venous patch wall. The purpose of the present study was to analyze such types of capillaries.

Material and methods. Experiments were performed on 16 male Wistar rats weighing around 250 g. All animals had free access to standard rat chow and water. Nembutal (30 mg/kg) was injected i.p. With the help of an operating microscope, a 10–12 mm long segment of the saphenous vein was extirpated and longitudinally incised on a rectangular strip ('venous patch') that was immediately placed in a saline solution. The right common carotid artery was then dissected and clamped as caudally and cranially as possible with microvascular clip, and a 5–8 mm longitudinal ar-



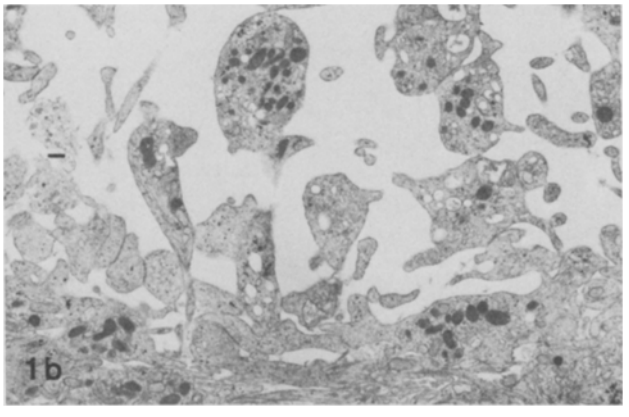


Figure 1. *A* Confluence area between artery (A) and venous patch (VP). Arrows point out the platelets. T, red thrombus; AL, Arterial lumen. Rat in the 1st day after surgery. × 1380. *B* Platelet aggregation in the suture line. 1st h after surgery. × 5700.

teriotomy was performed. The autologous venous patch was grafted onto the edges of the carotid incision with interrupted sutures of 10-0 Nylon monofilament. The animals were sacrificed at different times during the 2nd week after surgery. The techniques used in the perfusion, fixation and tissue selection for electron microscopy have been described in detail in a previous report[4]. Ultrathin serial sections were examined in a Phillips EM 301 electron microscope equipped with a liquid nitrogen anticontamination device.

Results and discussion. Ultrathin section showed 2 types of capillaries of venous patch vasa vasorum: continuous and fenestrated. Fenestrated capillaries, which are seldom detected during the 2nd postoperative week, were lined with an endothelium interrupted by pores of 24–44 nm in diameter and bridged by a thin membrane or diaphragm (figs 2,A and B and 3) from 6 to 9 nm. All fenestrations occurred in areas where the endothelial cell layer was relatively thin. Fenestrated capillaries were found in every rat studied. They were more numerous at the beginning of the 2nd postoperative week; that is, in the suture area, they are more numerous than continuous ones (3/1). On the 14th day, all sections showed almost exclusively continuous capillaries. The fenestrated capillaries of venous patch vasa vasorum exhibited the same features which have been reported in regenerating capillaries[5-7]: thin endothelial wall and increased pinocytosis. The fenestrations are not necessarily seen in all transections of capillaries because the sieve area in which they occur is usually rather small. Fenestrations were generally observed on only 1 side of the capillaries which were surrounded by a continuous basal lamina (fig. 2,A and B). The thicker part of the endothelial cell cytoplasm contained long oval nuclei, prominent perinuclear Golgi, scattered mitochondria, polyribosomes and rough endoplasmic reticulum. There were more pinocytotic vesicles in the luminal than in the abluminal part.

Our observations of fenestrated capillaries in the venous patch wall are very similar to those reported by McKinney et al.[7] in skeletal muscle capillary regeneration, but they differ greatly in that we observed a pericapillar basal lamina in regenerating vessels. These conflicting results could reflect the different methodological procedures of tissue preparation for electron microscopy, i.e. fixation by perfusion or immersion.

Regeneration of endothelial cells requires the presence of basal lamina[8,9]. This structure forms the matrix upon which endothelial cells migrate and proliferate to reform vascular channels[8,9]. The sequential production of extracellular matrix (basal lamina, collagen and/or glycosaminoglycans) by developing vessels has been shown to affect their final differentiation[10]. The mechanism for vascular regeneration have been identified and platelets seem to have an important role in endothelial regeneration[11]. In our experimental model, the injury of vasa vasorum capillaries is associated with local platelet aggregation; in the suture line, collagen fibers of host artery and venous graft touch the blood flow producing a platelet aggregation (fig. 1A and B); so, the platelets may be able to induce the endothelial migration and proliferation from the intact endothelial cells of arterial vasa vasorum close to the suture. The transit of macromolecules seems to be much faster through fenestrae than through interendothelial junctions or via vesicular

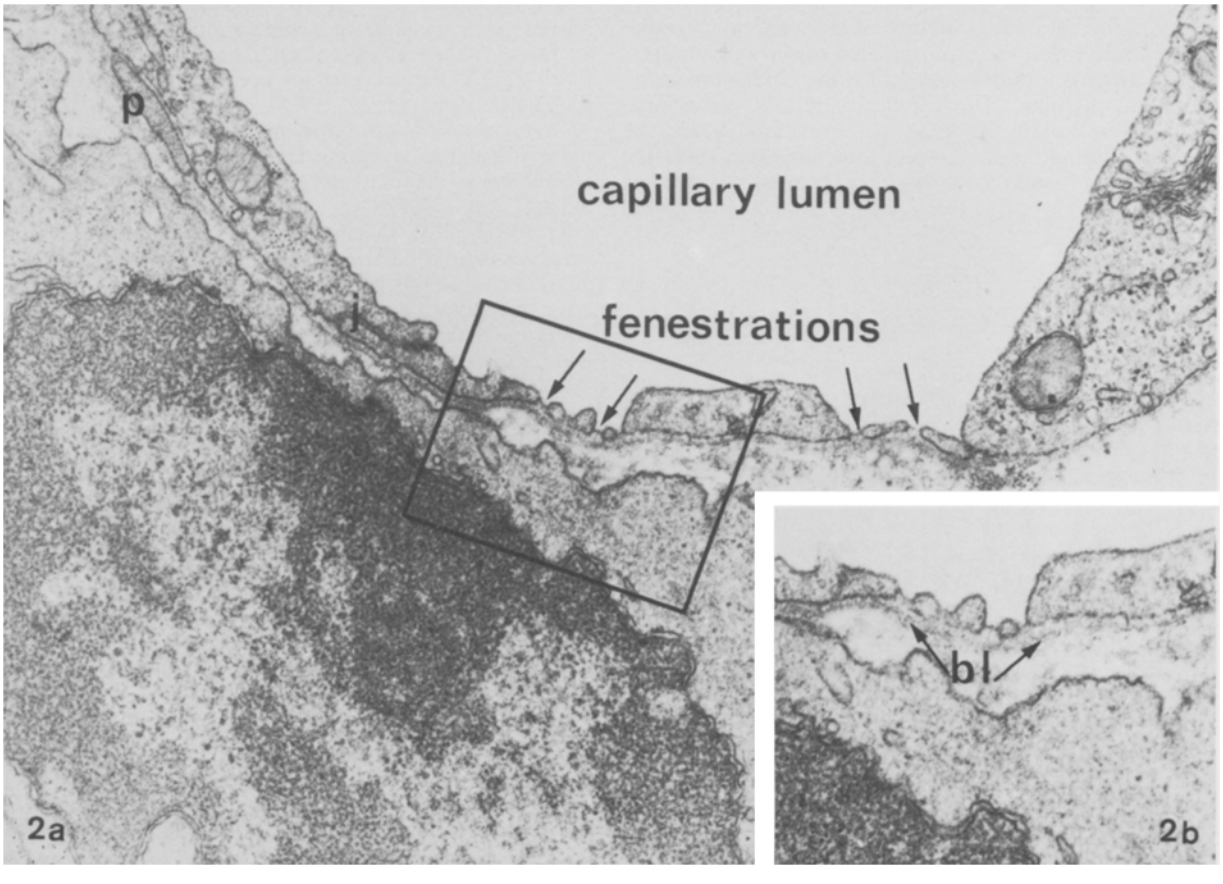


Figure 2. *A* Capillary of vasa vasorum patch graft, 12 days after surgery. The endothelium is thin and contains fenestrae covered by a diaphragm; p, pericyte; J, endothelial cell junction. ×33,900. *B* A higher magnification of the rectangled area in figure 2A. bl, basal lamina. ×49,500.

transport[12,13]. Large molecules were found to pass more rapidly through fenestrated than through continuous capillaries indicating that fenestrations represent the preferential pathway for rapid transcapillary exchange[12-15]. The regenerating vessels are 3–4 times more permeable than normal skeletal muscle vessels to macromolecules[16]. The diaphragms may permit selective molecular sieving during transcapillary exchange[7]. The presence of fenestrated capillaries in injured tissues may have an important role in facilitating the marked transcapillary exchange occurring in areas proximal to injury[7]. Although the significance of fenestrated capillaries in the venous patch adventitia has not been determined according to McKinney et al.[7], they probably exist transitorily during the repair period ad integrum of the suture edges between the rat common carotid artery and the venous patch, since they have not been detected after the 2nd postoperative week (manuscript in preparation). More attention should be directed to investigation of transcapillary exchange in wound capillaries and their surrounding environment.

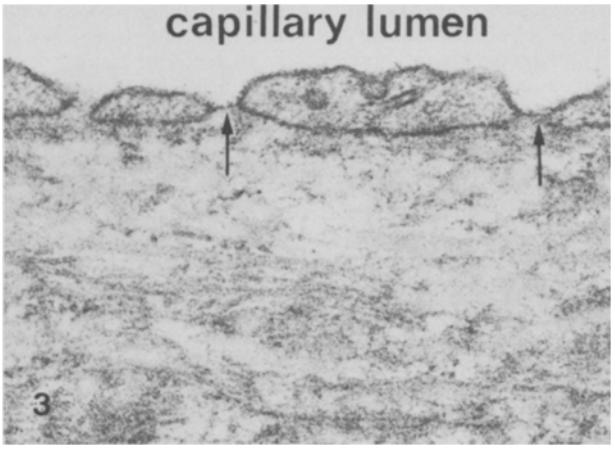


Figure 3. Capillary endothelium of vasa vasorum patch graft 14 days after surgery. The endothelial wall is thin and contains 3 fenestrae. 2 diaphragms are present (arrows). The trilayered cell membrane is visible. ×67,500.

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The morphology of the Schwann cells and the unmyelinated fibers of a nerve supplying an immobilized muscle

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Summary. Hind limbs of cats were immobilized in the resting position for varying periods and the nerve supplying the medial head of the gastrocnemius muscle was studied while it was undergoing immobilization atrophy. Degenerative changes in the unmyelinated fibers and the Schwann cells, followed by an abundant increase in collagen, were noticed after prolonged immobilization. Electron microscopic evidence that Schwann cells produce the collagen is discussed.

The effects of immobilization of a limb on the morphology, physiology and biochemistry of the skeletal muscle have been thoroughly investigated and widely reported, whereas similar studies on the nerve supplying the immobilized muscle are not numerous. After tenectomy, in albino rats, the mean nerve fiber diameter and the number of myelinated fibers present in the nerve supplying the inactive muscle were found to be significantly reduced[1]. Further, it was reported that, in young animals, immobilization of a limb retards the myelination of the nerve supplying the atrophied muscle[2]. In a similar study, Eisen et al.[3] observed a significant reduction in the diameter of the larger myelinated nerve fibers in the nerve to the immobilized muscle. But further studies at the electron microscopic level are lacking and the present experiment reveals changes, as observed under the electron microsope, in the morphology of the Schwann cells and of the unmyelinated fibers of a peripheral nerve supplying an immoblized muscle.

Materials and methods. Adult cats, *Felis domestica,* weighing 2–3.8 kg were used for the study. 8 cats, irrespective of sex, were weighed and anesthetized using I/P sedium pentothal (30 mg/kg b.wt). The right or left hind limb was cleaned and immobilized in a plaster cast, keeping the knee and ankle joints in the resting position. The tissues of the contralateral hind limbs and also of these of another group of animals, not immobilized but subjected to the same dose of anesthesia and kept in identical surroundings, served as controls. The casts were removed under anesthesia from 2 cats at a time, at the end of 4, 6, 8, and 10 weeks of immobilization. The gastrocnemius muscle and the nerve to its medial head were exposed. Approximately 1 cm length of the nerve, just proximal to its entry into the muscle, was cut, stretched on a filter paper and fixed initially in Karnovsky's fixative[4]. The specimen was further washed with buffer (phosphate buffer, pH 7.4) and cut into smaller pieces and then fixed in buffered 6% glutaraldehyde for 2 h