EFFECT OF MECHANICAL STIMULATION ON VASCULARIZATION OF THE WALL OF A LARGE ARTERY

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The vasa vasorum of large arteries usually lie in the outer membrane of the vessel [4]. In atherosclerosis intensive vascularization of the arterial wall is observed [5, 9]. Vascularization of an atherosclerotic plaque ultimately leads to hemorrhages into the plaque followed by its destruction and the formation of an occlusive thrombus in the lumen of the affected artery [10]. The mechanisms of induction of angiogenesis in the arterial wall in atherosclerosis have not been adequately studied. It is considered that a definite role in this process is played by macrophages and platelets [3, 5]. The influence of mechanical factors on vascularization of the arterial wall, however, remains virtually unstudied [1, 2], although the attention of research workers has recently been concentrated increasingly on the study of mechanical stimulators of the processes of angiogenesis [7, 8].

The aim of this investigation was to study the effect of continuous longitudinal stretching on the state of the vasa vasorum of a large artery.

METHODS

Experiments were carried out on five adult mongrel dogs kept on a standard diet. Under pentobarbital anesthesia (40 mg/kg) the bones of both of the animals' legs were fractured by transverse corticotomy. The bone fragments were fixed by application of Ilizarov's apparatus [1, 2]. Gradual stretching of the bony fragments of one limb began 5 days after the operation, at a rate of 1 mm at a time daily, under roentgenographic control. On the 30th day of distraction the arteries in the legs of the anesthetized dogs were fixed intravitally by perfusion with 2% solutions of paraformaldehyde and glutaraldehyde (pH 7.4). Postfixation was carried out in 1% osmium tetroxide solution. Fragments of arteries from the zone of distraction were prepared by the standard method and embedded in a mixture of Epon and Araldite. Semithin and ultrathin sections were cut on an LKB-3 Ultrotome. The semithin sections were stained with methylene blue, the ultrathin sections with uranyl acetate and lead citrate. Two-dimensional preparations of the outer membrane of the artery were obtained by separating it from the media, and they were stained with hematoxylin and mounted in balsam. Investigations at the light-optical level were carried out on the MBI-15 microscope. Ultrathin sections were studied with the ÉVM-100 AK electron microscope. Arteries of the contralateral limbs (application of the apparatus but without distraction) and limb arteries of adult intact dogs were studied as the control.

RESULTS

The vasa vasorum of the control arteries were located in the outer membrane. They were few in number (Fig. 1a). The bulk density of the microvessels averaged $4.7 \pm 1.3\%$ ($p < 0.05$). In two-dimensional preparations of the

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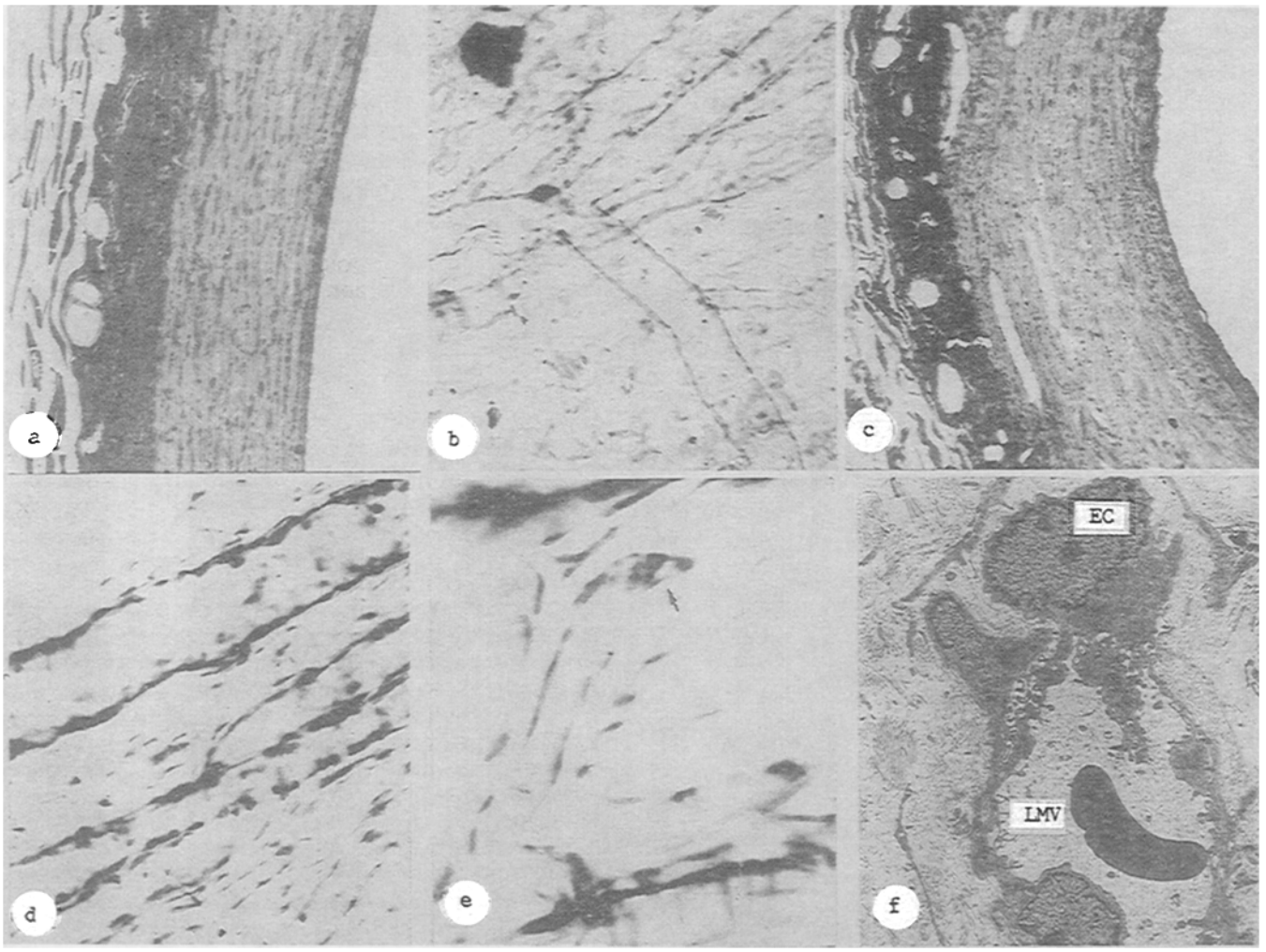

Fig. 1. Microcirculatory bed of a large artery: a) control artery (semithin section, objective 40, ocular 7); b) control artery ("en face" preparation, objective 60, ocular 7); c) artery from zone of distraction (number of microvessels in outer membrane sharply increased, vasa vasorum are invading the tunica media of the artery, semithin section, objective 40, ocular 7); d) "en face" preparation of artery from zone of distraction (microvessels oriented to correspond to direction of stretching force, objective 60, ocular 7); e) vascular bud (arrow) (blood cells can be seen in the terminal part, "en face" preparation, objective 60, ocular 7); f) migration of endothelial cells (EC) from lumen of microvessel (LMV) (transmission electron microscopy, magnification 15,000 ×).

tunica adventitia irregularly oriented microvessels could be seen forming anastomoses with one another and a widely spaced network (Fig. 1b).

In the zone of distraction the bulk density of the microvessels in the outer membrane of the artery was increased by more than 1.5 times, to an average of 7.6 ± 0.4% ($p < 0.05$) (Fig. 1c). Vascularization of the tunica media of the artery also could be observed. Microvessels were growing inward from the t. adventitia virtually to the middle of t. media.

Examination of two-dimensional preparations of t. adventitia showed that the density of the network of the vasa vasorum was increased in the zone of distraction, and the vessels there were oriented as a rule longitudinally, in the direction of the acting stretching force (Fig. 1d). In some cases thinner outgrowths, with pointed ends and ending blindly, branched from the microvessels; these were vascular buds, and accumulations of blood cells were visible in their terminal part (Fig. 1e).

Ultrastructural investigation of these terminals verified that they were microvessels, in whose lumen erythrocytes and blood plasma could be seen (Fig. 1f). No pericytes were present in their wall. We also observed endothelial cells migrating from a capillary toward the connective-tissue matrix. The migrating endotheliocytes were irregular in shape, with a large eccentric nucleus. The contours of their basal cytolemma were uneven, and the luminal cytolemma formed numerous microvilli, while concentrations of microvesicles were visible close to the luminal cytolemma.

Thus, in the zone of distraction, vascular buds were formed in the t. adventitia of the artery, their number and the total area of the vasa vasorum increased, and these vessels were found not only in the outer membrane, but also in the t. media of the artery.

Most of the known mechanical factors inducing angiogenesis are linked with a local increase in the blood flow, hemodynamic stress, and damage to the endothelial cells [7]. In the zone of distraction we did not observe death of the endotheliocytes in the vasa vasorum. Probably under the experimental conditions used, significant damage to the endothelium of the microvessels did not take place, for the stretching force was not applied directly to the arterial wall, but was transmitted to it through surrounding tissues. Under these circumstances, possibly only the junctions between individual endothelial cells were damaged, and this itself may have induced their proliferation [6]. Disturbance of intercellular contact interactions also can induce activation of various proteolytic enzymes in the endotheliocyte membrane [7], and this in turn creates conditions for cell migration. The possibility likewise cannot be ruled out that smooth muscle cells in t. media of an artery, when exposed to stretching, secrete angiogenic factors, which induce growth of microvessels toward t. media.

Thus continuous longitudinal stretching of a large artery induces processes of angiogenesis in the microvessels of its outer membrane, with invasion of t. media of the artery by its vasa vasorum.

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