ULTRASTRUCTURAL CHANGES IN THE WALL OF THE RAT POSTERIOR VENA CAVA DURING ACUTE OBSTRUCTION TO THE VENOUS OUTFLOW

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The study of the early stages of pathological processes is important for the understanding of the mechanisms of their development and it can be undertaken mainly under experimental conditions. For instance, during analysis of the principles of formation of structural changes in blood vessels characteristic of arteriosclerosis or phlebosclerosis, examination of the earliest changes both in single cells of the vessel wall and in intercellular relations is extremely important. For arteries, the concept of the endothelial–muscular unit [3] has already been postulated for arteries, and the role of such cooperative structures in atherogenesis has been indicated. Meanwhile, in the case of veins the importance of endothelial–muscular relations under normal and pathological conditions has simply been suggested [1]. The possibility of migration of extravascular cells into the wall of veins with establishment of new intercellular connections has received even less study.

In this connection an electron-microscopic study of the abdominal part of the posterior vena cava of rats in the early stages after experimental production of coarctation of that vessel was undertaken [2]. Special attention was paid to the state of the endothelium, the smooth muscle cells (SMC), the presence of platelet aggregates on the inner surface of the vessel, and the dynamics of migration of blood cells into the thickness of the venous wall.

EXPERIMENTAL METHOD

Experiments were carried out on 22 noninbred male rats weighing 260-300 g. Under ether anesthesia the posterior vena cava was constricted in 18 animals by the method described previously [2], and four rats whose circulation was undisturbed were used as the source of control material. Pieces of the wall of the vena cava were taken from all animals under deep ether anesthesia, and in the case of animals undergoing the operation, every 2 h during the first 24 h after coarctation. Material was taken the first time 1.5 h after the operation. Fixation, processing, and examination of the material were carried out by the scheme tested previously [2].

EXPERIMENTAL RESULTS

Marked injury to the endothelial lining of the vein was found 1.5 h after acute disturbance of the venous outflow (the hemodynamic characteristics were described previously [2]). For a distance of 3-5 mm from the constricting ligature the endothelium was absent and the inner elastic membrane (IEM) was exposed for a considerable distance (Fig. 1b). For a great distance from the site of coarctation (10-20 mm) accumulation of finely granular material (evidently plasma proteins) took place between the IEM and the basement membrane of the endotheliocytes; the endotheliocytes under these circumstances became irregular in shape and their cytoplasm showed marked osmiophilia (Fig. 1a).

On the exposed surface of IEM amorphous masses of low electron density, not containing platelets or other blood cells, were deposited in places. Elsewhere such masses (most probably coagulated plasma proteins) surrounded shrunken (stellate) endotheliocytes, which had become detached from the IEM. In connection

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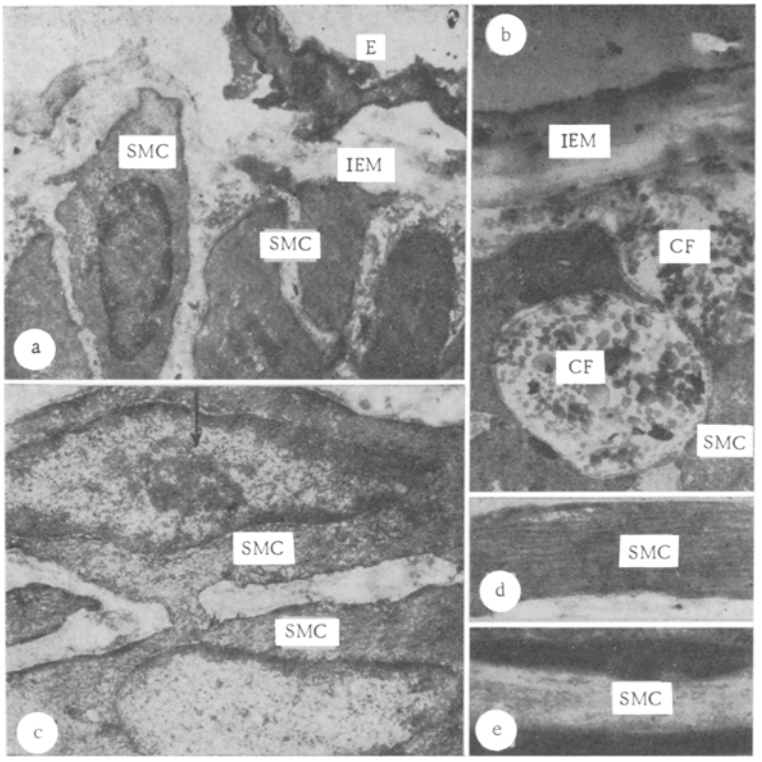

Fig. 1. Ultrastructure of changed endothelial barrier and SMC of posterior vena cava in early stages after obstruction of venous outflow. a) Pycnosis and desquamation of endotheliocyte (11,000×); b) "denudation" of IEM, swelling of intermuscular collagen fibers (18,000×); c) fungiform contact between two SMC, hypertrophy of nucleolus (arrow) (17,000×); d) degeneration of SMC with appearance of ribbon-like filamentous structures (20,000×); e) degeneration of SMC with clearing of central zone and increased density of peripheral zone of cytoplasm (26,000×). E) Endotheliocyte; CF) collagen fibers.

with damage to the endothelial barrier, the liquid part of the blood penetrated into the substance of the venous wall. This was demonstrated by the appearance of granular or floccular material between the SMC, and by widening of the spaces between the individual myocytes and collagen fibers. Transverse sections through the latter showed that the individual fibers were stellate and differed in thickness (pathological collagen fibers [7]) (see Fig 1b). The SMC appeared relatively intact, with a well-defined basement membrane (see Fig. 1b). The nuclei of the SMC were characterized by a diffuse distribution of the chromatin and by the presence of large, loosely packed nucleoli (Fig. 1c). Besides the contacts that were preserved between the processes of SMC, new contacts also appeared, organized in the manner of fungiform projections (see Fig. 1c).

Marked vesiculation of the cytoplasm was observed 6 and 8 h after constriction of the vein in the residual endotheliocytes: the vesicles were small and mainly intracytoplasmic (closed). At the same time there was a marked increase in the number of ribosomes and polysomes. In some areas on the surface of the endotheliocytes with disturbance of the integrity of the apical part of the cytoplasm, the formation of layers of coarse fibrin threads and platelets could be seen. The platelets also adhered in small groups (of two or three) to the undamaged endothelium and were surrounded by finely granular masses. At this period round, amorphous inclusions (evidently lipoproteins) appeared in the SMC close to the nucleus, and the basement membrane of these cells remained clearly visible. Individual polymorphs, located in the subendothelial part of the intima and in the media of the venous wall, could be seen.

Single SMC with modified ultrastructure were found 10 h after constriction of the vein. For instance, in individual SMC in the absence of appreciable changes in the contractile filaments, cigar-shaped long mitochondria were observed. In others, a sharp increase in the density of the cytoplasm was found, with the ap-

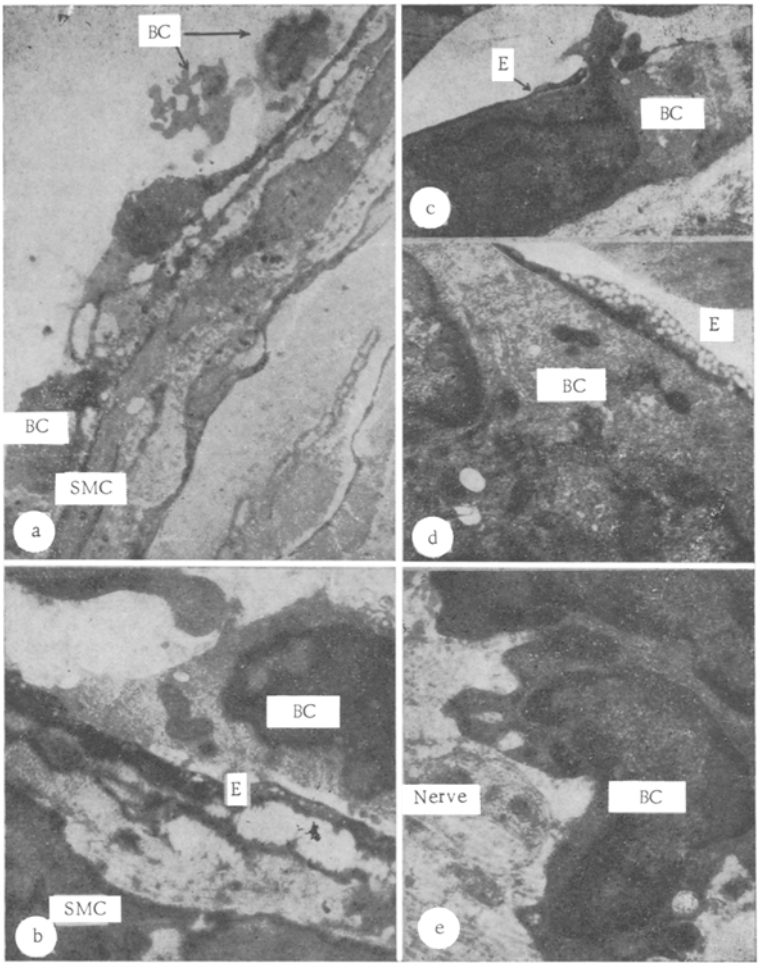

Fig. 2. Stages of migration of blood cells into thickness of venous wall after obstruction of venous outflow: a) general view of blood cells at different stages of penetration into vessel wall (4400×); b) formation of close contact between blood cell and endotheliocyte (16,500×); c, d, e) stages of penetration of monocyte-like cells to venous wall (11,000, 20,000, and 18,700× respectively). BC) Blood cell; remainder of legend as in Fig. 1.

pearance of short, dense structures of "ribbon" type [6] (Fig. 1d). In a third group of SMC a sharp but homogeneous increase in the density of the part of the cell beneath the plasmalemma was observed, whereas the central part was less dense and had lost its structure (Fig. 1e). At this same period, but especially 14-16 h after constriction of the vein, marked migration of blood cells resembling monocytes into the venous wall was observed. In the lumen of the vein these cells had a round cytoplasm and nucleus, but when they adhered to the endothelium (Fig. 2a, b) they became elongated in shape, with an oval or goblet-shaped nucleus (Fig. 2b-e). The cytoplasm of these cells was dense and finely granular, and contained few organelles. During penetration into the wall of the vein these cells spread out on the apical surface of the endotheliocytes, with which they formed close contacts (Fig. 2b). These cells then passed into the subendothelial space [through the greatly thinned cytoplasm of the endotheliocyte (Fig. 2c) or in the zone of interendothelial junctions]. The IEM was evidently an obstacle to their migration into the depth of the wall, and the monocyte-like cells at first spread out on the IEM (Fig. 2c), but later they succeeded in penetrating into the depth of the wall of the vein. Under these circumstances these cells had microvilli filled with cytoplasm. These migrating cells were not found close to SMC surrounded by a basement membrane, but they could be in close proximity to the basement membrane of efferent axons (Fig. 2e). Some of the monocyte-like cells were converted into macrophages in the substance of the wall of the vein; phagosomes in such cases were formed evidently through phagocytosis of parts of the degenerating cells of the vessel wall. However, most such cells, judging from the

previous study of the ultrastructure of the venous wall on the 2nd or 3rd day after obstruction to the venous outflow, and from light-optical data, move out into the perivenous connective tissue.

This investigation thus showed that the endothelial lining of the venous wall is a readily vulnerable component of this vessel. The most marked injuries to the endothelial barrier coincided in topography with the region of formation of an intimal fibromuscular thickening during coarctation of the vein [2]. The changes in SMC which were discovered and described in that paper, and which should more correctly be regarded as a manifestation of irreversible dystrophic changes, also were found in this same region. Since these changes in SMC appeared a few hours later than de-endothelization, they can be connected with disturbance of the integrity of the endothelial barrier and, evidently, with the plasmorrhagia accompanying this process. Sometimes directly, sometimes indirectly through injury to SMC, de-endothelization in the veins presents as the basic factor determining migration of SMC into the intima and the formation of the intimal venous plaque. Such a situation, as we know, occurs in arteries also during arteriosclerosis [4]. The question of the possible importance of factors whose source is the aggregates of platelets, for the formation of intimal thickenings, must be considered. Such factors in tissue culture stimulate growth of SMC [5]. In the model now used, no strict topographic connection could be discovered between the formation of platelet aggregates and the intimal fibromuscular thickenings. This question requires further study. In our view the most important result is the discovery of intensive migration of blood cells into the venous wall. It is not just a question that single clone-forming cells may be present among them. A more important aspect is that, at a certain stage of acute disturbance to the venous outflow, marked changes took place in the composition and structure of the cell system, with the formation of new, not previously existing intercellular connections.

The study of the ultrastructure of the early phase of the process thus showed that disturbance of the venous outflow does not simply give rise to diapedesis of leukocytes, as can be seen in the light microscope, but causes complex systemic reactions, which must be taken into account during analysis of the mechanisms of structural changes in the vessel walls in pathology.

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