MECHANISMS OF REPARATIVE REGENERATION OF THE VENOUS ENDOTHELIUM

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KEY WORDS: venous endothelium; reparative regeneration; migration; proliferation.

Reparative regeneration of the arterial endothelium has been and is being studied on a sufficiently wide scale. According to research in this field, the endothelial defect is restored by the formation of a monolayer [3, 7, 8, 10]. According to the observations of Shevchenko [6] and Gololobov [1], reparative regeneration of the venous endothelium differs significantly from that of arterial, and does not end with monolayer formation; instead, the newly formed endothelium consists of surplus intramural and endovasal proliferation. In these investigations the regeneration process was modeled by injection of 2.5% silver nitrate solution into a blood vessel. We consider that it is the specific character of the injurious agent and modification of the subjacent layers of the vessel wall after its injection that distorted the process of reparative regeneration of the venous endothelium. The absence of any clear boundaries of the lesion did not permit the time course of the reparative process to be studied sufficiently accurately, and the use of only film preparations prevented assessment of interaction between blood cells and the wound surface.

The aim of this investigation was to study the characteristics of cellular responses of the venous endothelium during reparative regeneration under conditions when the structure of the underlying substrate was preserved and when the area of the defect was accurately measured.

EXPERIMENTAL METHOD

Experiments were carried out on 34 Kyoto-Wistar (normotensive) albino rats aged 25-50 weeks, receiving a standard diet. The animals were anesthetized by intramuscular injection of 1% pentobarbital solution in a dose of 40 mg/kg body weight. After a midline laparotomy the posterior vena cava was mobilized. A copper rod with a base 3 mm in diameter, previously cooled to −190°C, was applied to the vessel wall for 30 sec [7, 8]. Material for investigation was taken immediately after de-endothelization, after 4 and 24 h and also 2, 3, 4, and 5 days after the operation. Preparations for scanning electron microscopy were obtained by the use of retrograde perfusion through the common iliac artery, under the average arterial pressure for each animal, initially with medium 199 with the addition of heparin (10 U/ml) for 60 sec, and later with 2.5% glutaraldehyde solution in medium 199 (pH 7.5) for 5 min, followed by immersion postfixation for not less than 24 h [4]. The cell boundaries were observed after impregnation with silver [3]. Subsequent treatment of the specimens included dehydration in acetone, critical point drying in liquid carbon dioxide, ionic spraying with gold, and examination in "Tesla B-350" and "Hitachi S-405a" scanning electron microscopes. Preparation of the material for scanning autoradiography included injection of ^{3}H-thymidine solution (1 mCi/g body weight, volume of solution not more than 0.5 ml) intramuscularly 1 h before sacrifice of the rats, perfusion fixation, dehydration, drying, and application of type M photographic emulsion by immersion. After exposure for 3 weeks the autoradiographs were developed [2], dried in air, sprayed with gold, and examined in the scanning electron microscope (SEM). Material for transmission electron microscopy was prepared by the standard method [5].

EXPERIEMENTAL RESULTS

The luminal surface of the intact venous epithelium appeared flattened on the scans. It was smooth and had virtually no microvilli. The nucleus containing zone of the endothelial cells projected a little into the lumen of the vessel. Connective-tissue fibers ran

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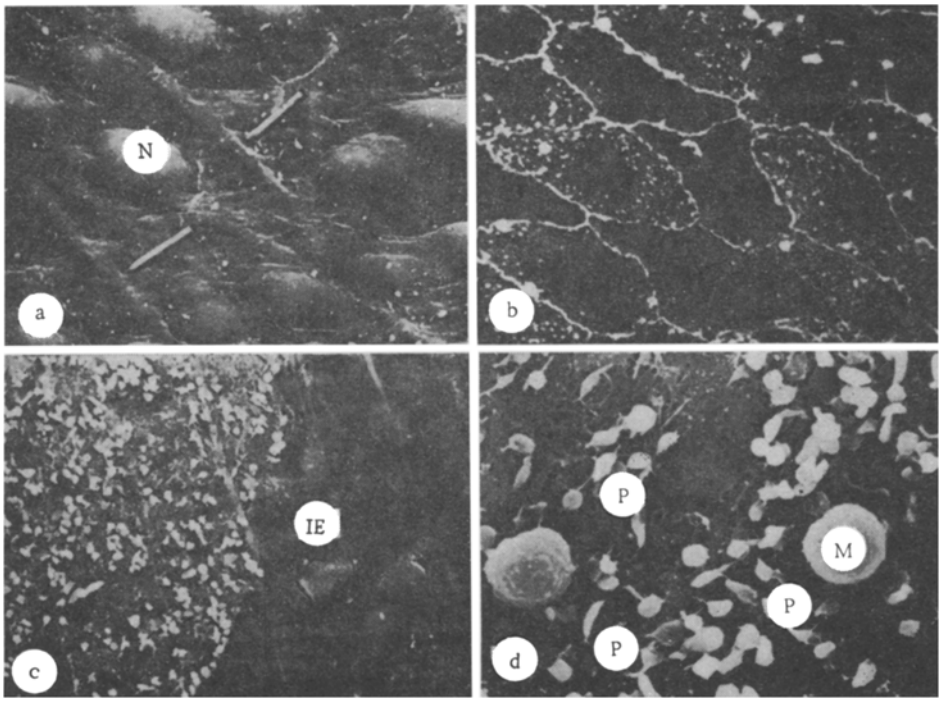

Fig. 1. Endothelium of rat vena cava under normal conditions (a, b) and after local de-endothelization (c, d): a) structures of venous endothelium stand out in relief: nucleus-containing zones (N) of endothelial cells, subendothelial connective-tissue fibers (arrow). SEM. 700×; b) shape and orientation of endothelial cells of vena cava. SEM. Treated with $AgNO_3$. 650×; c) boundary between intact endothelium (IE) and zone of injury after de-endothelization. SEM. 650×; d) Adherent platelets (P) and monocytes (M) on de-endothelized surface. SEM. 1000 ×.

in different directions and "shone" through the endothelial layer (Fig. 1a). Impregnation of the cell boundaries with silver nitrate showed that the venous endothelium consisted of irregularly shaped cells, each of which was in contact with five or six neighbors. The orientation of the cells along the axis of the vessel was quite clear (Fig. 1b).

Immediately after local injury to the endothelium the platelets adhered to the wound surface and formed a monolayer. Some of them formed pseudopodia on contact with the substrate. Three states of the platelets here could be distinguished: the first were completely spread out, the second were round in shape and had pseudopodia, whereas the third were spherical, and only touched the de-endothelized surface. Some platelets were adherent (Fig. 1c). The boundary of the defect was distinct in preparations obtained 4 h after de-endothelization (Fig. 1d).

During the first day after injury marked restructuring of the endothelial monolayer located near the defect was observed. Wide polygonal cells, mingled with more elongated, appeared. Often the endotheliocytes assumed a complex configuration because of the presence of cytoplasmic processes. Toward the end of the first day after the operation the reactively changed endothelium near the edge of the defect was characterized by a lower numerical density, and the majority of endotheliocytes were oriented along the axis of the vessel; those located proximally and distally to the zone of de-endothelization were longer, moreover, than cells located laterally to the defect. The endotheliocytes of the first row of the migrating sheet of cells were polarized and tapering. The edges of the cells facing the defect were considerably widened and constituted and advancing "lamella" [4] (Fig. 2a). As it migrated over the wound surface, the endothelium removed with it the adherent platelets, as shown by their absence in transverse sections beneath the migrating monolayer. As the reparative processes unfolded, not only migration of endotheliocytes could be observed. Disturbance of the integrity of the endothelium is a powerful stimulus for proliferation [9, 11], as was manifested by the increased synthetic activity of the cells located alongside the de-endothelized region. The histoautoradiographic investigation, carried out on the second day of regeneration, revealed a mosaic pattern of ^3H-thymidine incorporation into endotheliocyte nuclei of the reactively changed zone. Numerous villi and vesicles were formed on the surface

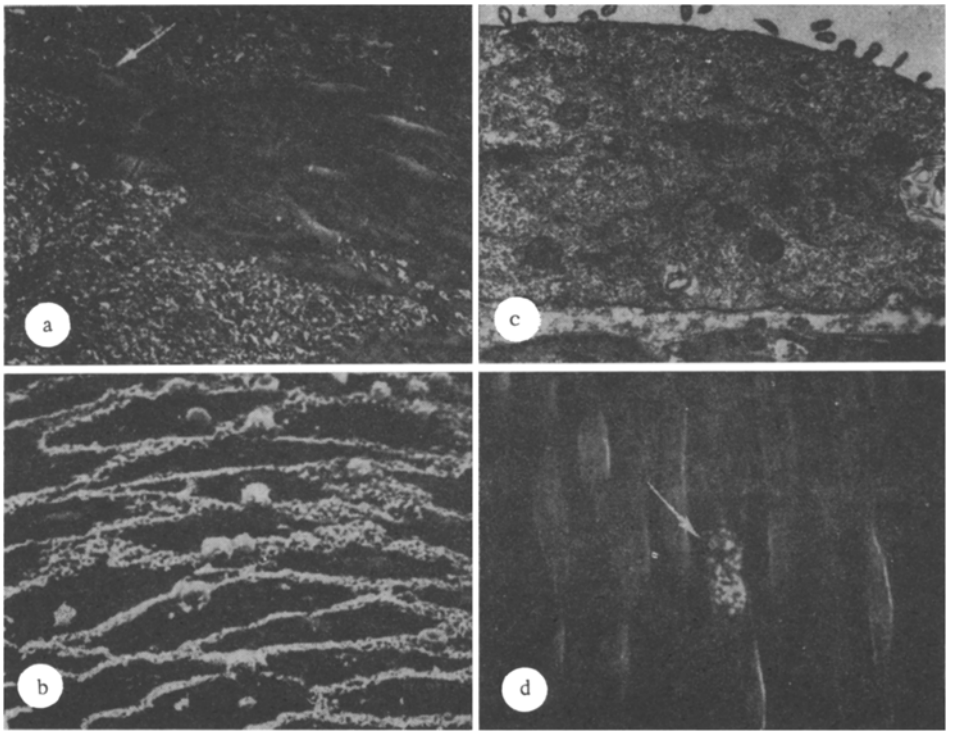

Fig. 2. Migration of endothelial cells during reparative regeneration (a), newly formed endothelium (b, c, d). a) Leader cells (arrow) SEM. 650×; b) shape and orientation of newly formed endotheliocytes. SEM. Treatment with $AgNO_3$. 700×; c) Transverse section through young, undifferentiated endothelial cell 3 days after cold injury. Transmission electron microscopy. 14,000×; d) Incorporation ^3H-thymidine into nuclei of regenerating endotheliocytes (arrow). SEM. 650×.

of the mitotically dividing endothelial cells. Numerous microvilli, formed by cytolemma, could be seen in transverse sections through these cells, and chromosomes were visible.

As it spread over the wound surface in a monolayer, the endothelium gradually covered it. The newly formed endothelial lining consisted of long spindle-shaped cells oriented along the axis of the vessel, each having five or six neighbors. The numerical density of the cells in this monolayer exceeded that in the control by 2.1 times ($p < 0.05$; Fig. 2b). Spindle shaped cells were round in shape in transverse sections. Single microvilli were seen on the luminal surface of these cells. The cytoplasm of the young relatively undifferentiated cells, contained many organelles. In the basal segments there were many microtubules, oriented mainly along the cell. The number of micropinocytotic vesicles was small (Fig. 2c). By the time the defect was completely covered (3rd day) only solitary DNA-synthesizing cells were found (Fig. 2d). The sharp decline in the number of endotheliocytes containing radioactive label at the moment of restoration of the monolayer was evidently associated with the onset of contact inhibition of proliferation, blocking DNA synthesis [12].

Under these experimental conditions, ruling out any disturbance of the structure of the subendothelium, we did not observe any surplus proliferation of endothelial cells. Nor was it found during regeneration of the aortic endothelium [3, 8, 10]. It must be pointed out, however, that the rate of re-endothelization in the vena cava is rather higher than in the aorta. The repair process in the vein is complete on the 3rd after injury. The corresponding endothelial defect in the abdominal aorta is not covered until the 4th-5th day after de-endothelization [3].

The investigations thus showed that there is no difference in principle in the mechanisms of reparative regeneration of the venous and arterial endothelium. Venous endothelium also restores its integrity with a connected monolayer through migration and proliferation with the formation of endotheliocytes elongated parallel to the blood flow.

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MORPHOLOGICAL MANIFESTATIONS OF COMPENSATORY AND ADAPTIVE PROCESSES IN THE LIVER DURING AGING

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Aging is characterized by reduced reliability of the maintenance of homeostasis, the material basis of which is regeneration. Meanwhile, a combination of compensatory and adaptive mechanisms aimed at preserving viability under conditions of a changed external environment operates in the body [9]. An important feature distinguishing changes in the liver during aging is reduction of the number of hepatocytes due to their death from external and internal causes, with the result that the organ atrophies. The aim of this investigation was to study compensatory and adaptive processes and mechanisms of regeneration developing in this situation.

EXPERIMENTAL METHOD

Experiments were carried out on 15 male Wistar rats belonging to three age groups: 8, 24, and 30 months (five animals in each group). Pieces of liver for light microscopy were fixed in acetic-alcohol-formalin by Brodskii's method; paraffin sections 7 μ thick were stained with hematoxylin and eosin, with magnification of 90, 1.6, and 10 times, in fields of vision with an area of 0.78 mm^2, taken as the unit of measurement; the total number of hepatocytes, the number of binuclear hepatocytes and the number of sinusoidal cells were counted; the mean area of the mononuclear hepatocytes and of their nuclei was determined on the Leitz ASM instrument, using a KONFI program. Cytochrome oxidase was determined by Burstone's method. For electron microscopy (EM) material was fixed in 3% glutaraldehyde in phosphate buffer, pH 7.4, postfixed in 1% osmic acid solution, dehydrated, and embedded in Epon 812. Sections cut on the LKB-III Ultrotome were stained by Reynolds' method and studied in the JEM-100B electron microscope.

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