traditional structural proteins of the retroviruses were revealed in corresponding quantitative portions (Fig. 3a). For instance, p27 of M-PMV, p30 of RD-114, and p30 of R-MuLV, the principal internal porteins of these retroviruses, stained most brightly. This same gel, after staining with silver, is illustrated in Fig. 3b. The intensity of staining of p27 and p10 of M-PMV, of p12 of RD-114, and of p15E, p15, and gp70 of R-MuLV was increased. However, the intensity of staining of p10 of M-PMV, p30 of RD-114, and p12 and p10 of R-MuLV was sharply reduced. Other disparities also were observed between the results of original staining and of staining with silver, further evidence of the selectivity of binding of silver ions with proteins.

The combined use of two stains — Serva blue and Amido black 10B — did not lead to increased sensitivity of staining. Moreover, the brightness of the protein bands was reduced, indicating competition between the dyes for the binding sites on the protein.

The data given above are evidence of the higher sensitivity (relative to the generally accepted level) of the method of protein staining with dyes of the Coomassie G-250 type, which for most proteins is not more than 70-80 ng. On the whole, however, silver staining is 18-30 times more effective for many proteins, but silver staining takes place selectively. Some proteins stain weakly with silver, whereas others may not be revealed by it at all. All these facts indicate that in order to obtain reliable results in the study of unknown protein or of complex protein mixtures, combined staining of the gel is essential.

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RAPID METHOD OF VISUALIZING ENDOTHELIUM IN UNFIXED TISSUE

V. G. Sharov, Yu. P. Vedernikov, A. A. Anikin, and A. M. Vikhert*

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After it had been shown that the endothelium influences the response of vessels to certain drugs [3, 4] research began into its role in the regulation of vascular tone. However, interpretation of the physiological parameters of the vessel wall is largely determined by the integrity of the endothelial lining, and this raises the question of a simple and rapid method of detecting endothelial cells. Several methods are used nowadays to verify integrity of the endothelium. The physiological method consists essentially of recording responses of vessels to substances releasing endothelial relaxation factors (acetylcholine, calcium ionophore A 23187, etc.). The method is simple but does not allow the state of the endothelium to be observed directly. Certain species differences also may exist in the physiological response of the vessels [5]. It is often necessary to resort to the use of film ("Häutchen") preparations impregnated with silver [2, 7]. The two-dimensional film prepara-

*Corresponding member of the Academy of Medical Sciences of the USSR.

Department of Human Cardiovascular Pathology, All-Union Cardiologic Science Center, Academy of Medical Sciences of the USSR, Moscow.

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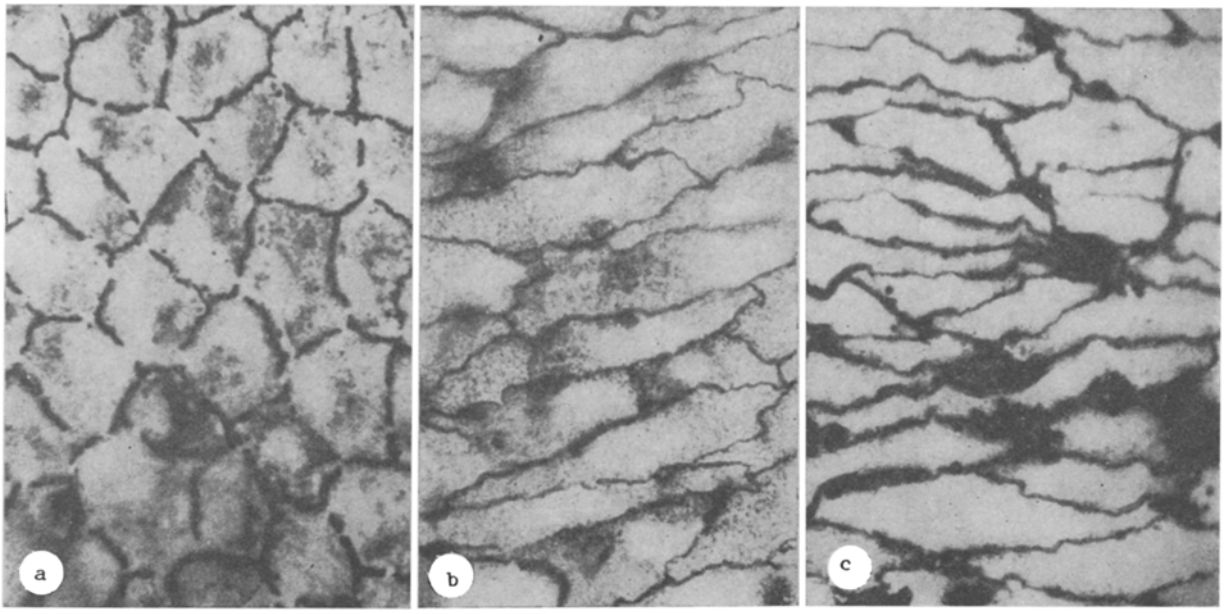

Fig. 1. Structure of endothelium: a) endothelium of left ventricle of a mini-pig; b) endothelium of abdominal aorta of a rat; c) endothelium of human circumflex coronary artery after keeping for 24 h in medium 199 with Hanks' salts. 900×.

tions of endothelium removed from the vessel wall, obtained in this way, are sometimes stained to reveal nuclei and studied in the light microscope in transmitted light. The method is time-consuming (takes several hours), it requires highly trained personnel, it is not free from artefacts of fixation and mechanical damage during separation of the endothelium from the vessel wall, it is more suitable for use with the endothelium covering a surface without any marked relief, and it cannot be used to monitor the surface of the epithelium itself. The examination of silver-impregnated endothelium, not removed from the intima, in bright transmitted light [1] is possible only with thin-walled vessels and it is made much more difficult by the presence of numerous superfluous details in the field of vision. The method of detecting endothelium by scanning electron microscopy is technically even more difficult, time-consuming, and requires expensive equipment; it is therefore unsuitable for systematic rapid analysis.

The facts described above explain the urgent need for development of a rapid and simple method of visualizing endothelium, which not only will greatly simplify the study of morphological and functional characteristics of the vessel wall, but also will greatly speed up the study of the role of the endothelium in the pathogenesis of atherosclerosis and thrombosis.

In this paper a simple method of rapid visualization of unfixed endothelium, not removed from the vessel wall, is suggested. It is based on the method of intravital staining of endotheliocytes with the fluorescent dye thioflavine, and impregnation of the endothelium with silver, well known previously. Intravital staining of endothelium with thioflavine is widely used to reveal zones of ischemia in experimental myocardial infarction [6], and impregnation of endotheliocytes with silver is used to visualize these cells in conjunction with fixation of the material and the use of film preparations. The method is based on a combination of the two methods mentioned above — the silver-impregnated endothelium is examined in reflected fluorescent light of thioflavine-stained endotheliocytes and intima or other subjacent tissue.

EXPERIMENTAL METHOD

Segments of the blood vessel or pieces of another tissue (subendocardium, valves, chordae tendineae, etc.). The specimens are rinsed in normal Krebs–Henseleit solution and immersed in 5% glucose solution in distilled water, for 3-5 min before staining.

This is followed by impregnation with silver. Pieces of tissue are immersed for 30-60 sec in a freshly prepared 0.25-0.3% solution of silver nitrate, then rinsed to remove excess of silver in 5% glucose solution for 30-60 sec.

In the method now described the tissue was stained in a 10^{-4}-10^{-3} M solution of thioflavine-T (from Sigma, USA) in physiological saline for 15-20 sec. The solution must be freshly prepared and filtered. The final stage is washing in 5% glucose solution for 30-60 sec to remove excess of the dye.

The tissue to be studied, e.g., a segment of blood vessel, is first cut longitudinally, turned inside out, and placed on a thin Porolon backing, which is glued to a slide with the endothelium uppermost, covered with a coverslip glued around its perimeter, and pressed with a weight of 15-20 g. By using the Porolon backing the surface of the endothelium covering a tissue with complex relief can be made sufficiently smooth. Such a preparation can be investigated at once or kept for several days in a refrigerator at 4°C. The Lyuman I-3 microscope was used to examine the preparations. Luminescence was excited by blue-violet light and examined in the yellow-green region of the spectrum.

EXPERIMENTAL RESULTS

During exposure of the preparation to light the silver is reduced and after about 1 min the tissue acquires maximal contrast. Granules in the cytoplasm of the endothelial cells give bright yellow-green luminescence and are surrounded by clearly defined dark boundaries, marked by reduced silver (Fig. 1a, b). By contrast with the stained granules, the unstained cell nuclei are visualized as pale homogeneous ovals. Various deposits, possibly microthrombi [3], are often seen on the cell surface. The endothelium of human coronary vessels can be well visualized by this method after keeping for 24 h in nutrient solution (medium 199 with Hanks' salts) or in Krebs–Henseleit solution (Fig. 1c).

The suggested method thus takes only a few minutes, requires no special training, is free from artefacts of fixation or mechanical damage during preparation of the specimen, can be used regardless of the complexity of relief or the thickness of the tissue covered with endothelium, and can be used to visualize the surface of the endothelium in reflected light.

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