LITERATURE CITED

1. V. K. Vasilevskii, L. D. Zherebtsov, and S. A. Bremzen, Vest. Akad. Med. Nauk SSSR, No. 12, 54 (1978).
2. V. K. Vasilevskii, V. I. Semkin, L. D. Zherebtsov, and I. N. Mikhailov, Vopr. Antropol., 62, 76 (1979).
3. I. M. Gurevich, Color and Its Measurement [in Russian], Moscow–Leningrad (1950).
4. D. B. Judd and G. Wyszecki, Color in Business, Science and Industry, New York (1975).
5. L. D. Zherebtsov, V. K. Vasilevskii, and S. A. Bremzen, Vopr. Antropol., 56, 146 (1977).
6. L. D. Zherebtsov, V. K. Vasilevskii, and S. A. Bremzen, Arkh. Patol., No. 12, 52 (1977).
7. L. D. Zherebtsov, V. K. Vasilevskii, and S. A. Bremzen, Dokl. Akad. Nauk SSSR, 239, No. 4, 996 (1978).
8. W. R. Buckley and F. Grum, Arch. Dermatol., 83, 249 (1961).
9. E. A. Edwards and S. G. Duntley, Am. J. Anat., 65, 1 (1939).
10. K. Jimbow, O. Oikawa, S. Sugiyama, and T. Takeuchi, J. Invest. Dermatol., 73, 278 (1979).
11. R. P. van Oort, I. I. T. Rosch, and P. C. F. Borosboom, J. Soc. Cosmet. Chem., 32, 1 (1981).
12. R. Scheuplein, J. Soc. Cosmet. Chem., 15, 111 (1964).
13. R. T. Tregear, Physical Functions of Skin, London (1966).
14. S. Wan, R. R. Anderson, and J. A. Parrish, Photochem. Photobiol., 34, 493 (1981).
15. H. P. Wasserman, Ethnic Pigmentation, Amsterdam (1974).

CHANGES IN VENULAR ENDOTHELIUM OF RAT MESENTERY IN RESPONSE TO HYDROGEN PEROXIDE APPLICATION

V. V. Banin, I. D. Senatova, and K. T. Zaitsev

UDC 616.149.2/.3-018.74-02:615.31:546.215

KEY WORDS: microvessels; permeability; hydroperoxides.

Three oxygen radicals (FOR), intermediate products of oxygen reduction, play a very important role in cell injuries. During inflammation, production of short-living FOR, superoxide anions, and hydroxyl radicals, as well as of the more stable compound, hydrogen peroxide, is associated with polymorphonuclear leukocyte activation [4, 13]. The use of enzyme–substrate systems, such as hypoxanthine–xanthine oxidase, as the source of free radicals leads to a sharp increase in permeability of the walls of microvessels for plasma proteins [2, 8]. Electron-microscopic studies of lung capillaries [7, 10, 12] have demonstrated injuries of the endothelium which can be linked with the action of FOR. It is not clear, however, precisely what structural transformations of the endothelial lining of the microvessels increase its permeability.

The aim of this investigation was to reveal injuries to the endothelium of mesenteric venules in response to surface application of hydrogen peroxide (H_2O_2) solutions.

EXPERIMENTAL METHOD

Experiments were carried out on 23 albino rats weighing 180-250 g. During intravital luminescence microscopy of the mesentery, 0.5 ml of a 10% solution of human albumin, labeled with fluorescein isothiocyanate (FITC-albumin), was injected into the femoral vein of the animals, anesthetized with pentobarbital (4 mg/100 g body weight). With illumination provided by an Hg-200 mercury vapor lamp (BG-12, BG-38, and K-530 filters) the dynamics of protein transport through the walls of the venules was recorded photographically in the control series of experiments and after application of H_2O_2 for 10 min. The luminence of FITC-albumin near the vessel walls was estimated by densitometry of negatives, in conventional units relative to nonfluorescent areas of the mesentery. Areas of mesentery of the small intestine

Laboratory of Electron Microscopy and the Microcirculation, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 10, pp. 498-502, October, 1988. Original article submitted October 16, 1987.

 0007-4888/88/0010-1504$12.50

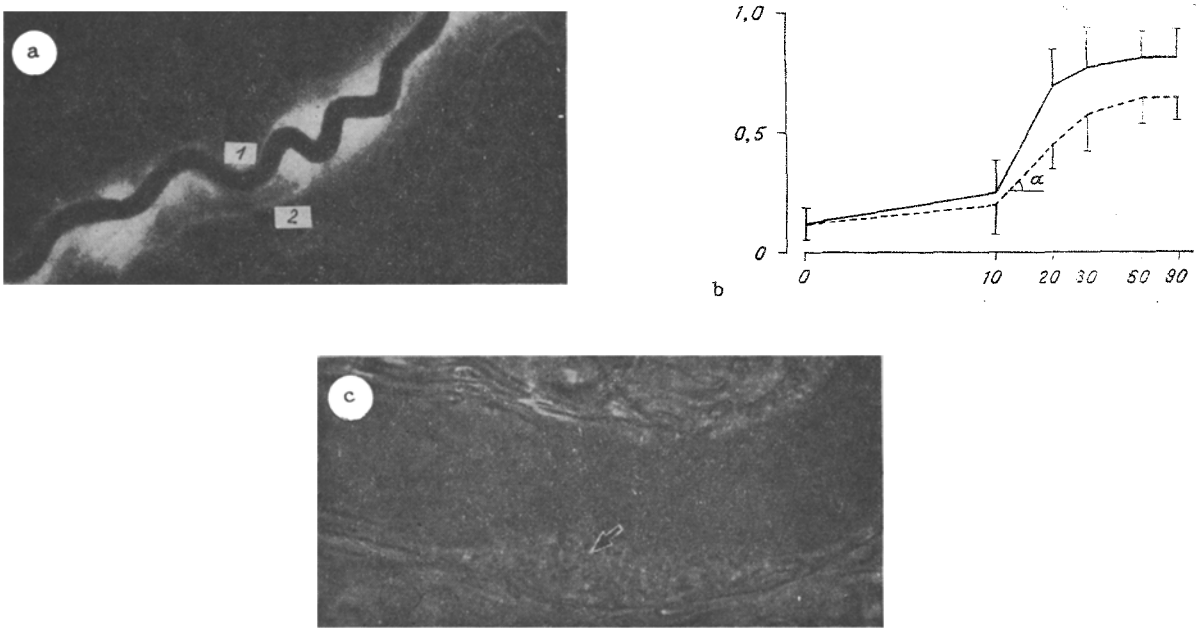

Fig. 1. Effect of application of H_2O_2 on permeability of venular walls for protein and on thrombus formation. a) Fluorescence of FITC-albumin near wall of venule with increased permeability 30 min after H_2O application (1 — arterioles; 2 — venule, 120 ×); b) dynamics of protein accumulation near outer surface of venules in control (1) (tan α = 1.01 ± 0.17) and after application (2) of hydrogen peroxide (tan α = 1.75 ± 0.30); c) platelet aggregation (indicated by arrow) in lumen of a large venule (350 ×).

for electron-microscopic study were fixed in situ with 2.5% glutaraldehyde under control conditions and after irrigation with a 10^{-6}-10^{-4} M solution of H_2O_2 (five rats in each group). Subsequent processing of the material for transmission electron microscopy was carried out by the usual methods.

EXPERIMENTAL RESULTS

Application of H_2O_2 solution (10^{-5} M) to the surface of the mesentery was accompanied by rapid and massive outflow of FITC-albumin from the lumen of the microvessels into the surrounding tissue. Regions of high permeability covered a large part of the surface area of the microvessels. As a rule these were postcapillary venules or larger collecting venules up to 50 μ in diameter (Fig. 1a). Protein transport was distinctly diffuse-focal in character: regions of the vessel wall with high permeability alternated with regions of moderate outflow of albumin. Increased permeability of the walls of the venules was manifested not only by enlargement of the exchante surface available for protein transport, but also by the more intensive transfer of albumin through the vessel walls. The results of densitometry showed that the fluorescent marker accumulated in the paravasal connective tissue more rapidly after application of H_2O_2 than under control conditions. The maximal value of the tangent of the angle of slope of the curve reflecting the dynamics of albumin transfer under the influence of H_2O_2 was more than 1.5 times greater than the control values (Fig. 1b).

Similar although less marked changes in permeability of the venular walls also were found when less concentrated solutions (10^{-6} M) of hydrogen peroxide were used. Conversely, when more concentrated solutions (10^{-4} M) were applied, intensive transmural outflow of albumin ceased in some venules after 20-30 min. It was noted that in these areas the working lumen of the vessels was sharply constricted due to massive juxtamural deposits of platelets (Fig. 1c). With the course of time, widespread stasis if blood developed in the adjacent fragments of the venules and capillaries.

Ultrastructural investigation of the walls of the venules after application of H_2O_2 to the surface of the mesentery for 2 min revealed substantial structural transformations of the endothelial lining. Peripheral zones of endothelial cells, often reduced in thickness, sometimes appeared to be interrupted in sections; the extent of the "breaches" or "ports" formed here varied, and in some cases amounted to several microns. These "ports" were often the site of penetration of blood cells into the surrounding interstitial tissues (Fig. 2a). In some

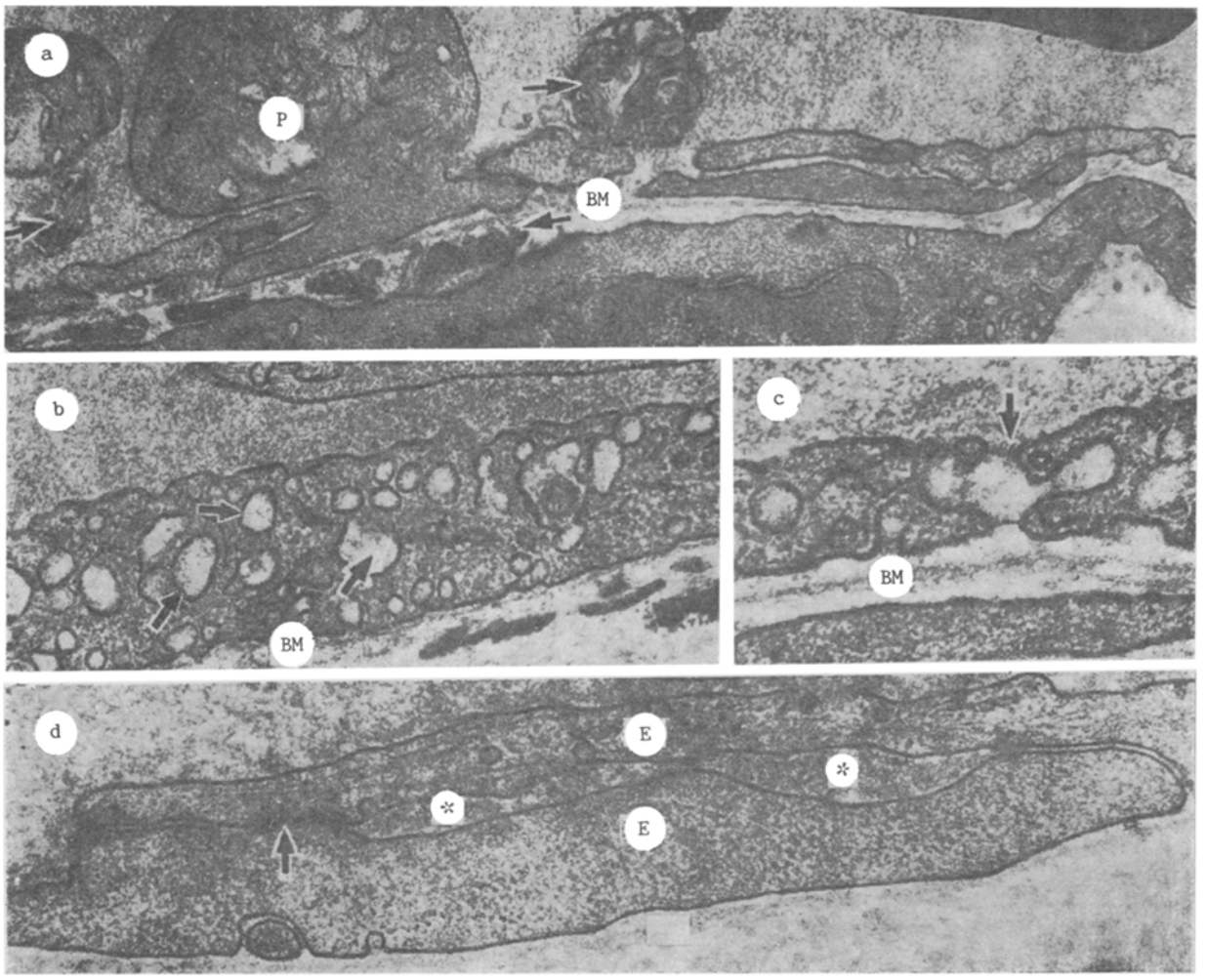

Fig. 2. Changes in endothelial lining of venules after application of 10^{-5} M H_2O_2 solutions. a) Platelet passing through "port" in venular endothelium; myelin-like structures (arrows) on surface of an endotheliocyte and platelet and near pericyte, 35,000 ×; b) concentration of plasmalemmal vesicles (arrows) in cytoplasm of endotheliocyte, with deformation of their walls, 40,000 ×; c) transendothelial channel (arrow), 100,000 ×; d) widening of interendothelial space (asterisks), preservation of structure of tight junction (arrow), 40,000 ×. Here and in Fig. 3: E) endotheliocyte; LV) lumen of venule; BM) basement membrane; P) platelet.

areas of endotheliocytes concentrations of plasmalemmal vesicles were observed. Together with small (50-90 nm), round vesicles, larger vesicles and vacuoles, whose walls appeared somewhat deformed (Fig. 2b), also were found. Sometimes the vesicles formed clusters or chains, giving rise to transendothelial through channels, linking the lumen of the vessel with the subendothelial space (Fig. 2c). Vacuoles or large cavities could also be seen in the cytoplasm of pericytes, mainly in the perinuclear zone of the cells. After application of H_2O_2 the gaps in the junctions between endotheliocytes were widened and were filled with floccular material, which was denser than in the lumen of the vessels (Fig. 2d). However, the structure of the tight junctions appeared unchanged. In the subcortical zones of the cytoplasm of the endotheliocytes and pericytes bundles of microfilaments, oriented mainly along the cell axis, were quite frequently seen.

The damaging action of H_2O_2, in different concentrations, on the venular endothelial cells was manifested by the formation of myelin-like figures, resembling lamellar bodies, arranged concentrically in the cytoplasm or in the form of extrusions on the cell surface (Fig. 2a). Myelin-like bodies also were found near blood cells and pericytes.

When relatively high concentrations of H_2O_2 (10^{-5} and 10^{-4} M) were used, the endotheliocyte membranes were not clearly distinguishable, for they differed only a little from the cytoplasm in density. On some parts of the cells the plasmalemma could not be identified at all and the cytoplasmic matrix was altered (Fig. 3a, b). These pictures were interpreted as mani-

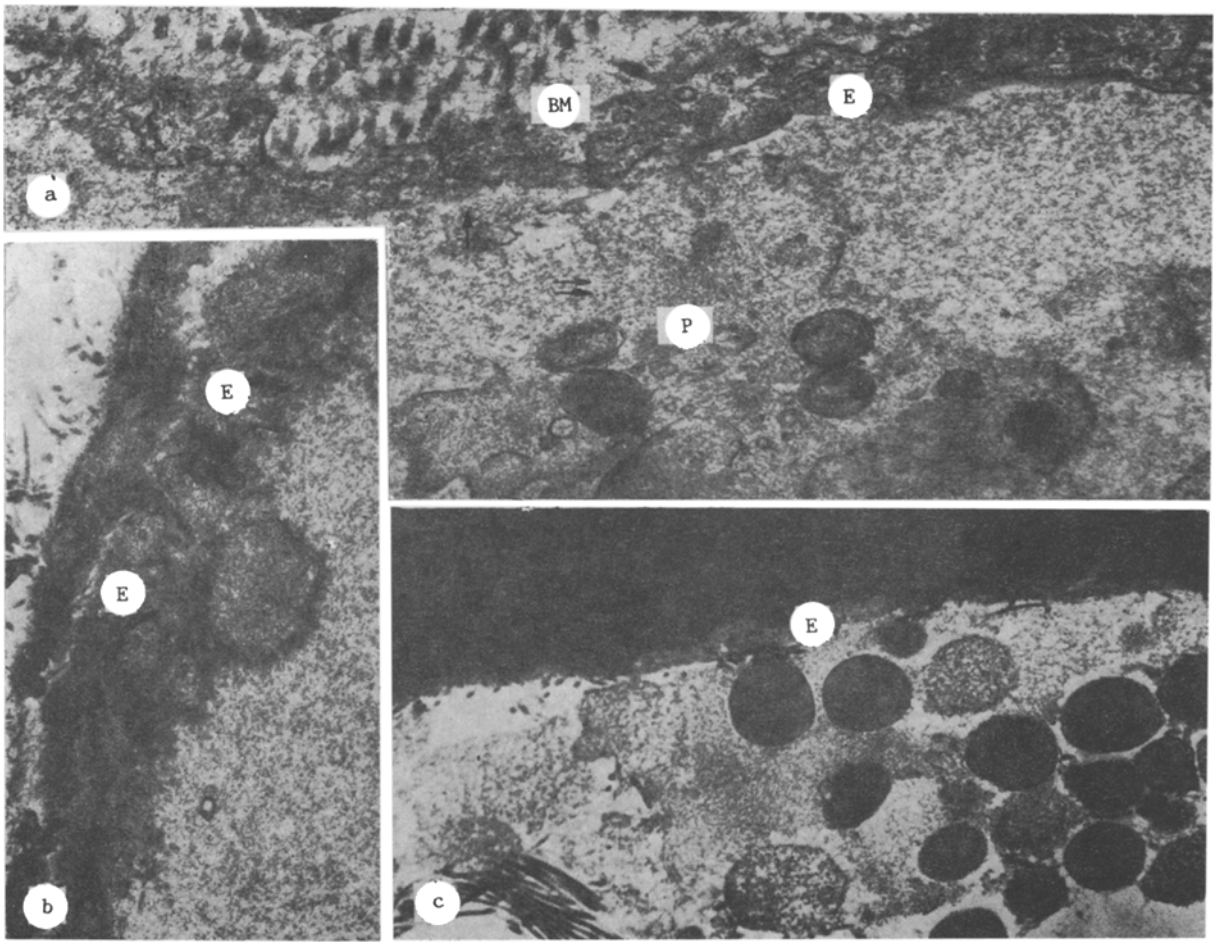

Fig. 3. Destructive changes in endothelium, platelets, and mast cells. a) Regions of lysis of endotheliocyte (single arrow) and platelet (double arrows) membranes, 10^{-4} M H_2O_2, 38,000 ×; b) fragmentation and necrotic changes in an endothelial cell, 10^{-4} M H_2O_2, 175,000 ×; c) lysis of some granules of a mast cell near wall of venule (stasis of erythrocytes in its lumen), 10^{-6} M H_2O_2, 175,000 ×.

festations of destruction. On the luminal surface of the injured endotheliocyte membranes polymorphonuclear neutrophils with an undulating surface could be detected, evidence of their activation and adhesion to the endothelium. Relatively preserved or destroyed platelets (Fig. 3a) frequently formed juxtamural aggregations. Granules of mast cells (tissue basophils), swollen and partially destroyed, were often found in the immediate vicinity of the vessel walls (Fig. 3c). The basement membranes appeared loose in texture, fragmented, and in some places could not be identified (Fig. 3).

It was in those areas where fragments of disintegrated plateletes or lysed granules of mast cells were found that the destructive changes in the endothelium were most marked (Fig. 3a, c).

Exposure of the microvessels to FOR and, in particular, to superoxide radicals leads to increased permeability of their walls for macromolecules [2, 8]. As the results of the present investigation showed, hydroperoxides, another active oxygen compound — have a similar property. Intensification of transport of labeled protein through the walls of mesenteric venules, observed after H_2O_2 application, is evidence of the formation of large pores or "leaks" in the walls of the vessels, allowing free convective transport of macromolecules. The results of ultrastructural analysis show that these "leaks" may arise in various ways. One way is connected with the formation of fairly large "ports," bounded by an unchanged cell membrane. Transformation of the endothelium of this type is typical of "histamine damage" [5]. Other evidence that endogenous histamine may be involved in the response to hydrogen peroxide application is given by the intensive vesiculation in endothelial cells and the formation of transendothelial channels and large vacuoles [1]. In this case the main source of endogenous histamine is evidently the mast cells, degranulation of which near venules followed by lysis

of the granules are observed when different concentrations of H_2O_2 are applied. Increased protein transport through the venular walls may also be connected with transformation of interendothelial junctions. Some of them evidently become permeable for protein, although the tight junctions preserve their structure.

Local injuries to membranes of endothelial cells and their cytoplasm, disturbing the selectivity of the endothelial barrier, are another probable method of massive outflow of protein into the tissues. Membrane injuries characteristic of lipid peroxidation processes [6, 9] are found more often when H_2O_2 is used in high concentrations. Besides the direct toxic action of the peroxide, effects connected with the production of other FOR, such as superoxide anions, may also play a role in the genesis of these injuries. Activated forms of leukocytes, in direct contact with endothelial cells, can be regarded as their source [11]. Biologically active substances set free during adhesion and destruction of platelets must be yet another factor disturbing the integrity of the endothelium. In the presence of hydrogen peroxide, platelet activation by various agents is substantially intensified [3].

It can be tentatively suggested that the qualitative features of endothelial injuries arising under the influence of active forms of oxygen differ depending on the concentration of the agent in the medium. In particular, with relatively low concentrations, the possibility of a mechanism mediated by endogenous histamine cannot be ruled out.

LITERATURE CITED

1. I. D. Senatova, Arkh. Patol., No. 7, 60 (1974).
2. R. F. Del Maestro, J. Björk, and K. E. Arfors, Microvasc. Res., 22, 239 (1981).
3. D. Del Principe, A. Menichelli, W. de Matteis, et al., FEBS Lett., 185, 142 (1985).
4. K. Donaldson, J. Slight, D. Hannant, and R. E. Bolton, Inflammation, 9, 139 (1985).
5. J. Fox, F. Galey, and H. Wayland, Microvasc. Res., 19, 108 (1980).
6. I. Fridovich, Science, 201, 875 (1978).
7. K. J. Johnson, J. C. Fantone, J. Kaplan, and P. A. Ward, J. Clin. Invest., 67, 983 (1981).
8. K. Ley and K. E. Arfors, Microvasc. Res., 24, 25 (1982).
9. J. Luotola, Med. Biol., 62, 115 (1984).
10. W. J. Martin, Am. Rev. Resp. Dis., 130, 209 (1984).
11. T. Sacks, C. F. Moldow, P. R. Craddock, et al., J. Clin. Invest., 61, 1167 (1978).
12. P. A. Ward, K. J. Johnson, and G. O. Till, Acta Physiol. Scand., Suppl. 548, 79 (1986).
13. S. J. Weiss, J. Young, A. E. LoBuglio, and A. Slivka, J. Clin. Invest., 68, 714 (1981).