ULTRASTRUCTURAL CHARACTERISTICS OF THE CAPILLARY WALL IN DISTURBANCES OF ITS BARRIER FUNCTION

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Electron-microscopic investigations have shown that disturbance of vascular permeability in the lungs, kidneys, liver, and spleen in radiation pathology may be based on certain changes in the ultrastructure of the blood capillaries.

While many electron-microscopic investigations have been made of the blood capillaries under both normal and pathological conditions [1-12,14,16,17], and the ultrastructural mechanisms of disturbance of capillary permeability have thereby been explained, the almost total absence in the literature of data concerning the fine changes in the capillaries in radiation sickness is remarkable [13], despite the fact that this condition is accompanied by severe disturbances of vascular permeability.

The object of the present investigation was to study the capillaries in the parenchymatous organs of animals after whole-body x-ray irradiation.

EXPERIMENTAL METHOD

Experiments were carried out on male albino mice weighing 20-25 g. Control and irradiated animals (5 animals in each group) received an intravenous injection of preliminarily purified and heated ink (the labeled vessels method) in a volume of 0.2 ml per animal. The animals were decapitated 1 h later and pieces of organs (lungs, kidneys, liver, and spleen) were taken for electron-microscopic investigation. The animals were irradiated on the RUM-3 apparatus (180 kV, 15 mA, filters 0.5 mm Cu and 1 mm Al, skin-focus distance 40 cm, total dose of x-ray irradiation 600 R).

The pieces of organs were fixed in 1% buffered (pH 7.4) OsO_4 solution in the cold for 2 h. Dehydration was carried out with cold ethanol and the tissues embedded in a mixture of prepolymerized methacrylate (methyl–butyl 1 : 5). Ultrathin sections were cut on an LKB ultratome, using glass knives, and these were mounted on copper grids with a formvar bedding. Sections were stained with saturated uranyl acetate solution (solvent 30% ethanol) in the course of cutting the ultrathin sections, the bath being filled with this solution, and they were counterstained with a saturated aqueous solution of lead citrate for 3 min. The sections were studied in a JEM-7 electron microscope.

EXPERIMENTAL RESULTS

Investigation of the capillaries in the organs of the control animals yielded results basically in agreement with those obtained by other workers [3-6,8,9,15]. The capillaries of the studied organs differed significantly in structure. In the lungs, for instance, the endothelium of the capillaries is continuous and has no pores, and the basement membrane is well-marked and consists of homogeneous and microfibrillary components. The endothelium of the glomerular capillaries (in the region of the glomerular filter) has numerous pores (or windows), and it is extremely thin, whereas the basement membrane is well-developed and has the typical structure. In the specific blood capillaries of the liver and spleen (the sinusoids and sinuses) the endothelium is interrupted, and the basement membrane in most cases is absent, although in some parts it appears and has the typical structure. In the lungs and renal glomeruli, a few ink particles were found only in the lumen of the blood vessel. In the liver and spleen, particles were found in the

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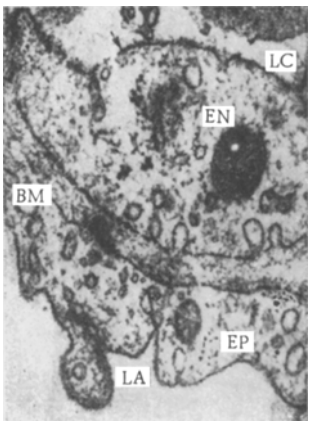

Fig. 1

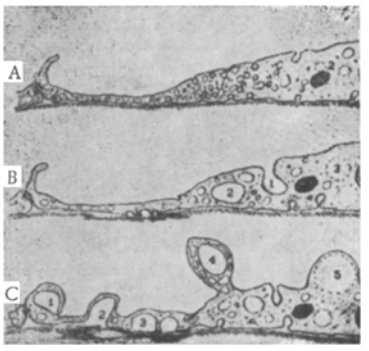

Fig. 2

Fig. 1. Commencing local disintegration of an endothelial cell (marked by an arrow). Irradiated animal. EN – endothelial cell; LC – lumen of capillary; BM – basement membrane; EP – alveolar epithelium; LA – lumen of alveolus. 50,000 ×.

Fig. 2. Microvesiculation, vacuolation, and formation of larger vesicles in the capillary endothelium of irradiated animals. A) Microvesiculation; 1) micropynocytotic vesicles; 2) microvesicles formed as a result of a local collection of fluid in the cytoplasm; B) vacuolation; 1) vacuoles formed by a mechanism of micropynocytosis; 2) vacuoles resulting from union of separate microvesicles with each other or by growth of some of them; 3) vacuoles formed by a local collection of fluid in the cytoplasm; C) larger vesicle formation; 1) formation of endothelial vesicles with participation of marginal or superficial folds (by a mechanism of macropynocytosis); 2) formation of vesicles by separation of endothelium from basement membrane; 3) formation of vesicles as a result of disturbance of detachment of micropynocytotic vesicles on basal surface of endothelium; 4) endothelial vesicles formed as a result of microvesiculation and vacuolation; 5) formation of endothelial vesicles as a result of a local collection of fluid in the cytoplasm.

reticulo-endothelial cells inside the compact phagocytic vacuoles with a clearly defined membrane. Single particles could be seen in the Desse's spaces, but none were found in the liver cells.

Investigation of the lungs of the irradiated animals revealed severe edema of the pericapillary tissues and also various changes in the capillaries. In some parts of the endothelium a diffuse edema was found, with local destruction of the endothelial cells. According to these observations, local destruction began with the cytoplasmic membrane and spread into the depth of the cytoplasm (Fig. 1). No connection was found between this process and other changes in the endothelium. It is interesting to note that local destruction was found only in the peripheral parts of the endothelial cells and it always began from the surface of the cells facing the lumen of the capillary. These facts suggest, on the one hand, that the damaging agent acts from the side of the blood stream, and on the other hand, that the peripheral parts of the endothelial cells are least resistant to its action. As well as the changes just described, an increase in the degree of microvesiculation vacuolation and vesicle formation was also found in the endothelium. The interconnection between these vesiculatory processes and the great variety of ultrastructural mechanisms of vesiculation, especially the formation of large vesicles, which was revealed is shown schematically in Fig. 2. Vesicle formation in radiation sickness is a heterogeneous process embodying different ultrastructural mechanisms which evidently play different roles in the pathology of the endothelium and in the disturbance of its barrier function. This problem clearly requires special investigation. Other widespread forms of changes in the capillaries under pathological conditions were a moderate dilatation of the intercellular spaces in the endothelium and its separation from the basement membrane, as the corresponding observations showed. As a result of detachment of the endothelium, characteristic endothelial vesicles were formed (Fig. 2). Changes in the basement membranes in the capillaries of the lungs of the irradiated

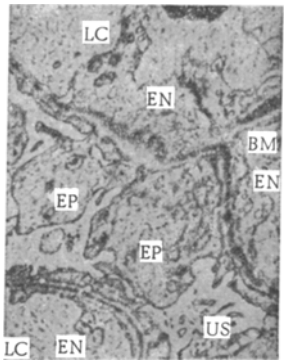

Fig. 3. Wavelike changes in density of basement membrane in glomerular capillaries. Irradiated animal. BM – basement membrane; LC – lumen of capillary; EN – endothelium; EP – glomerular epithelium; US – urinary space. 20,000 ×.

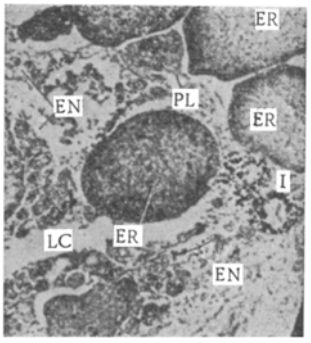

Fig. 4. Destruction of cells of endothelium (EN) lining splenic sinuses and dissemination of ink particles (I) throughout cytoplasm. Irradiated animal. LS – lumen of sinus; ER – erythrocyte; PL – platelet, 6000 ×.

animals took the form of their homogenization, with the appearance of granules and partial or complete disintegration. No connection was found between these various types of change in the basement membranes. Different mechanisms may perhaps lie at the basis of these changes.

In the endothelium of the glomerular capillaries of the irradiated animals changes were found similar to those described above, but more severe. Examination of the basement membranes revealed fluctuating changes in their density (Fig. 3) and also the appearance of hernialike invaginations on the side of the glomerular epithelium. Areas of complete rupture of both glomerular capillaries and of capillaries in the lungs, occasionally found, probably indicate that the pathological changes run in the same direction in the capillaries of both these organs.

In the sinuses of the spleen and sinusoids of the liver, the early changes took the form of destruction of endothelial and reticulo-endothelial cells against a background of increased vacuolation (Fig. 4).

No escape of ink particles from the pulmonary and glomerular capillaries was found in the irradiated animals. Particles likewise were not found in the microvesicles, vacuoles, and endothelial vesicles. However, collections of particles were occasionally found in the lungs outside the venules, probably as the result of dilatation of the intercellular spaces in the endothelium of the venules and destruction of the basement membrane. The liver and spleen particles were found in the reticulo-endothelial cells, in which they lay inside the vacuoles or as clusters resembling the vacuoles in shape, but sometimes they were diffusely distributed throughout the cytoplasm (Fig. 4). Intravenously injected particles also were found in the liver cells, in compact vacuoles of phagocytic type. It is interesting to note that hardly any areas were found in the sections where reticulo-endothelial cells contained no ink particles, whereas cells were frequently seen whose cytoplasm was mainly filled with phagocytic vacuoles. The phagocytic activity of the cells in these organs of the control animals was much less marked. The increase in vesiculatory processes (microvesiculation, vacuolation, and larger vesicle formation) in the capillaries of the lungs and the renal glomeruli, and also the increase in phagocytic activity of the reticulo-endothelial cells in the sinuses and sinusoids suggests that an increase in activity of its cell surface is the typical reaction of the endothelium of all types of blood capillaries to general irradiation. The significance of this reaction for the state of the capillary permeability is not clear.

The results thus demonstrate that 3 h after whole-body irradiation of animals definite changes take place in the ultrastructure of all types of blood capillaries capable of substantially modifying their permeability.

LITERATURE CITED

1. A. I. Klembovskii, Arkh. Pat., No. 7, 24 (1964).
2. A. M. Chernukh and A. K. Krantchev (A. K. Kranchev), in: 4th European Conference on Microcirculation, Proceedings, Basel (1967), p. 390.
3. V. A. Shakhlamov, Arkh. Anat., Gistol. i Émbriol., No. 1, 24 (1967).
4. H. S. Bennet, J. H. Luft, and J. C. Hampton, Am. J. Physiol., 196, 381 (1959).
5. R. du Boistesselin, Rhumatologie, 15, 243 (1963).
6. H. David, Dtsch. Gesundh.-Wes., 21, 1319 (1966).
7. H. Friederici and C. L. Pirani, Lab. Invest., 13, 250 (1964).
8. K. Hama, Acta Path. Jap., 13, 207 (1963).
9. J. H. Luft, in: B. W. Zweifach (editor), The Inflammatory Process, New York (1965), p. 121.

10. G. Majno, International Symposium on Injury, Inflammation, and Immunity, Proceedings, Baltimore (1964), p. 58.
11. P. R. Oudea., Lab. Invest., 12, 386 (1963).
12. G. E. Palade, J. Appl. Physiol., 24, 1424 (1953).
13. T. L. Phillips, Radiology, 87, 49 (1966).
14. D. E. Philpott, Changes in Fine Structure of the Frog Lung Induced by Substances Altering Transcapillary Exchange, Doct. dissertation, Boston (1963).
15. A. Policard and C. A. Beau, Submicroscopic Structures of Cells and Tissues under Normal and Pathological Conditions [Russian translation], Leningrad (1962).
16. H. Ruska, in: Internat. Symp. on Injury, Inflammation,and Immunity. Proc., Baltimore (1964), p. 35.
17. R. E. Yodaiken, in: Electron Microscopy, Vol. B, Prague (1964), p. 419.