CHANGES IN THE AIR–BLOOD BARRIER OF THE LUNGS DURING INHALATION OF HELIUM–OXYGEN MIXTURES

E. V. Rozova, T. N. Kovalenko, and M. M. Seredenko

UDC 612.223.1:546.291]:612.261

KEY WORDS: helium–oxygen mixtures; air–blood barrier of the lungs; mean arithmetic thickness; mean harmonic thickness.

Previous investigations into the use of helium–oxygen mixtures have not provided an unequivocal answer to the question of the possible mechanisms of their effect on man and animals. It is thus necessary to examine the more likely pathways of specific action of helium on living structures. The site of action of an inert gas is considered to be the cell membrane, and it must probably be manifested as a change in the membrane mechanisms of O_2 transport into the cell [8]. It has been suggested that helium may have an inhibitory action [8] and also that it may facilitate the passage of O_2 through the cell membrane [2, 5]. The probable cause of this effect may be a change in the properties of the plasma membrane under the influence of helium, leading to changes either in its permeability [7] or metabolic reactions connected with the membrane [11]. Deviations are also possible in the physical characteristics of various subcellular structures which have a high degree of affinity for an inert gas [10].

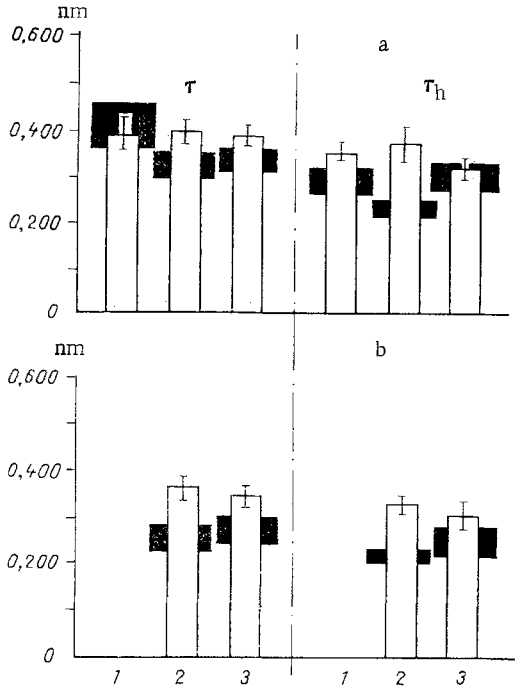

Fig. 1. Changes in arithmetic mean (τ) and harmonic mean (τ_h) thickness of ABB of the lungs (in nm) produced in rats by inhalation of helium–oxygen mixtures. Black background indicates level of parameters during inhalation of corresponding nitrogen–oxygen mixtures. 1) 11% O_2, 2) 21% O_2, 3) 40% O_2. a) Adult animals, b) rats aged 2 weeks.

A. A. Bogomolets Institute of Physiology, Academy of Sciences of the Ukrainian SSR, Kiev.
(Presented by Academician of the Academy of Medical Sciences of the USSR D. F. Chebotarev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 4, pp. 107-110, April, 1983. Original article submitted August 16, 1982.

0007-4888/83/9504-0540$07.50
© 1983 Plenum Publishing Corporation

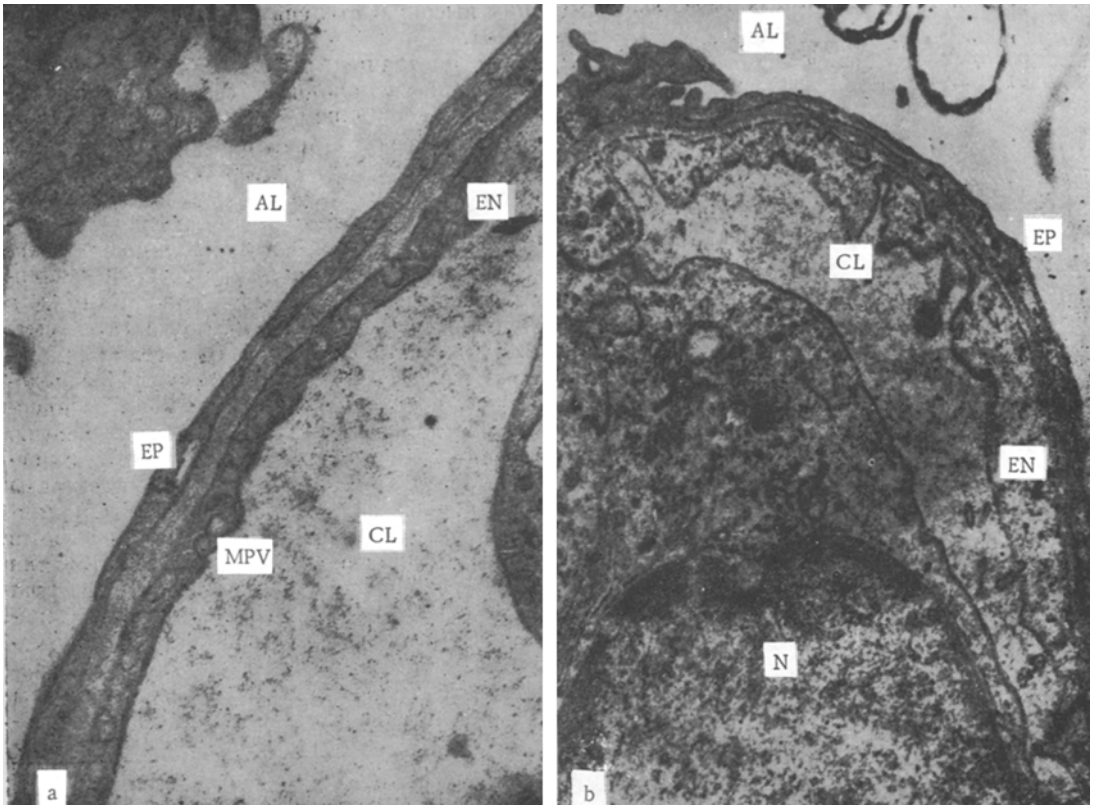

Fig. 2. Ultrastructure of ABB of adult rat lung. a) During inhalation of ordinary atmospheric air, b) of helium–oxygen mixture with 21% O_2. AL) Alveolar lumen, CL) capillary lumen, EP) alveolar epithelium, EN) capillary endothelium, MPV) micropinocytotic vesicles, N) nucleus of endothelial cell. 2800 ×.

The object of this investigation was to study the effect of helium–oxygen mixtures with different O_2 content on the ultrastructure and morphometric characteristics of the air–blood barrier (ABB) of the lungs.

EXPERIMENTAL METHOD

Experiments were carried out on laboratory albino rats of two age groups: 2 weeks and 10-14 months. The adult animals inhaled helium–oxygen mixtures containing O_2 in concentrations of 11, 21, and 40% for 30 min, whereas the young rats inhaled mixtures containing 21 and 40% O_2 in helium. Each series consisted of 10-12 animals. The rats were decapitated and pieces of tissue were taken from identical regions of the lower lobes of both lungs, fixed in glutaraldehyde and OsO_4, and then embedded in Epon. Ultrathin sections cut to a thickness of 40-60 nm were stained in uranyl acetate and lead citrate and examined in an electron microscope (Tesla BS-613 or IEM-7A). A random sample of specimens was obtained by Weibel's method [1]. Morphometric measurements of the arithmetic mean and harmonic mean thickness of the ABB of the lungs were made on electron micrographs [12]. Results obtained by similar methods on animals inhaling nitrogen–oxygen mixtures served as the control [3, 4].

EXPERIMENTAL RESULTS

The study of the effect of a normoxic helium–oxygen mixture on the thickness of ABB of the lungs in adult rats showed that its arithmetic mean thickness was increased a little ($P > 0.1$) compared with that found in animals breathing air (Fig. 1a). Meanwhile the harmonic mean thickness was considerably increased ($P < 0.01$), indicating that under these conditions thickened regions of the ABB predominated. It can be postulated that helium has a direct action on the ABB, which is manifested as a change in permeability of the cell membranes constituting the barrier under its influence. According to some workers [6, 9], this effect may be due to destruction of protein and lipid regions of the membrane and separation of its layers. Under these conditions changes also are observed in the ultrastructure of

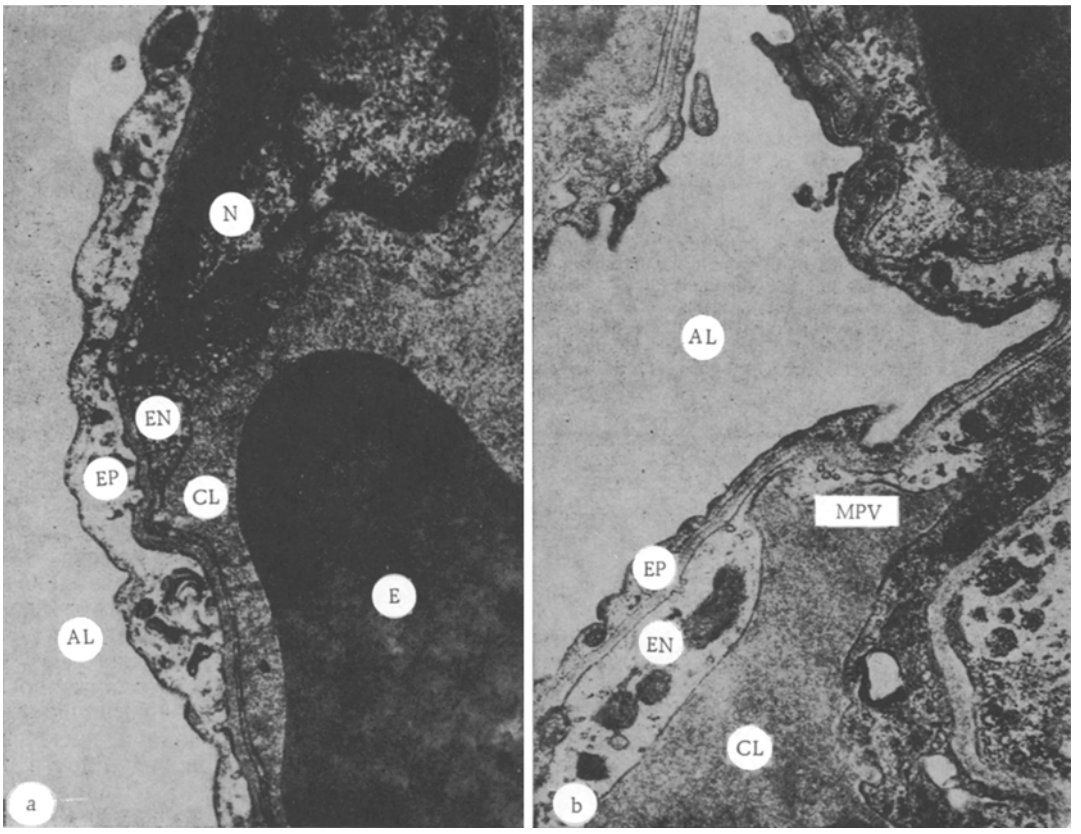

Fig. 3. Changes in ultrastructure of ABB of lungs in adult rats after inhalation of helium–oxygen mixtures. a) Edema of alveolar epithelial cell after inhalation of mixture of 40% O_2 in helium; b) edema of capillary endothelium after inhalation of mixture of 11% O_2 in helium. 22,000 ×. E) Erythrocyte; remainder of legend as to Fig. 2.

ABB of the lungs, with the formation of micropinocytotic vesicles in cytoplasmic processes of the capillary endothelial cells and type I pneumocytes. Sometimes vesicles about 100-150 nm in diameter with pale contents were present. Invaginations of the plasma membrane, which could reach almost to the basement membrane, to form indented, uneven cell outlines, were observed on the luminal surface of the endothelial cells (Fig. 2b). Thickening of the ABB was due chiefly to the capillary endothelial layer.

Raising (to 40%) or lowering to (to 11%) the O_2 concentration in the helium–oxygen mixture was not accompanied by any significant changes in either the arithmetic or the harmonic mean thickness of ABB of the lungs in adult animals. It is this which distinguishes helium–oxygen from nitrogen–oxygen mixtures, for keeping animals in nitrogen–oxygen hypoxic and hyperoxic environments affected the arithmetic mean and, in particular, the harmonic mean thickness of the barrier, which was considerably increased (Fig. 1a).

Under hyperoxic conditions created by means of a helium–oxygen mixture many free ribosomes and micropinocytotic vesicles could be clearly identified in the cytoplasm of the endothelial cells. In the capillary lumen large vesicles with pale contents, surrounded by closely interconnected membranes, were sometimes seen. Myelin figures could be observed inside the vesicles. Type I pneumocytes sometimes had a translucent cytoplasm. Increased liberation of osmiophilic contents — reserve surfactant — into the alveolar lumen, where it formed networks of membranous structures, was observed in the type II pneumocytes (Fig. 3a). In hypoxia, separation of the basement membranes into layers could be observed. In some places destruction and loss of integrity of the cytoplasmic processes of the endothelial cells were observed. In type II pneumocytes the quantity of osmiophilic material in the lamellar bodies was reduced. These bodies had the appearance of vacuoles with electron-translucent contents (Fig. 3b). Similar results were obtained in rats aged 2 weeks inhaling helium–oxygen mixtures (Fig. 1b). Under normoxic conditions both the arithmetic mean and the harmonic mean thickness of ABB was significantly ($P < 0.01$ and $P < 0.001$, respectively) increased compared

with values characteristic of air breathing. Practically no differences were found in the values of the thicknesses studied under these conditions in adult animals and in young rats. In the course of the investigations no change in these parameters could be found in young animals on changing to inhalation of a hyperoxic helium–oxygen mixture ($P > 0.5$).

These investigations thus suggest that helium has a direct effect on ABB of the lungs which is practically independent of the O_2 concentration in the respiratory gas mixture and of the animals' age. It can be postulated on the basis of the electron-microscopic findings that among the various structures of ABB the capillary endothelium is that which is most sensitive to the action of helium–oxygen mixtures.

LITERATURE CITED

1. E. R. Weibel, Morphometry of the Human Lungs [Russian translation], Moscow (1970).
2. P. A. Gul'tyaev, V. V. Boriskin, V. P. Zaval'nyuk, et al., in: Proceedings of the 10th Congress of the I. P. Pavlov All-Union Physiological Society [in Russian], Vol. 2, Moscow–Leningrad (1964), p. 235.
3. T. N. Kovalenko, Fiziol. Zh. (Ukr.), No. 6, 762 (1981).
4. T. N. Kovalenko, V. P. Pozharov, and M. M. Seredenko, Byull. Éksp. Biol. Med., No. 1, 51 (1980).
5. G. V. Troshikhin and Zh. A. Donina, Kosmich. Biol., No. 3, 54 (1979).
6. S. Daniels, W. D. M. Paton, and E. B. Smith, Br. J. Pharmacol., 65, 229 (1979).
7. G. A. Harrison and J. D. Solomon, Aviat. Space Environ. Med., 46, 21 (1975).
8. D. A. Maio and J. R. Nevill, Aerospace Med., 38, 1049 (1967).
9. K. W. Miller, W. D. M. Paton, and R. A. Smith, Mol. Pharmacol., 9, 248 (1973).
10. H. R. Schreiner, R. C. Gregorie, and J. A. Lawrie, Sciences, 136, 652 (1962).
11. F. South and S. Cook, J. Gen. Physiol., 37, 335 (1954).
12. E. R. Weibel and B. W. Knight, J. Cell Biol., 21, 367 (1964).

EFFECT OF ADMINISTRATION OF LIPOSOMES DIFFERING IN CHOLESTEROL AND PHOSPHATIDYLCHOLINE CONTENT ON ULTRASTRUCTURE OF MYELIN-LIKE PARTICLES IN THE MOUSE LIVER

S. M. Chebanov, V. P. Torchilin, V. R. Berdichevskii, A. S. Loginov, and V. N. Smirnov

UDC 616.36-008.939.22-02:615.31:547.922

KEY WORDS: liposomes; myelin-like particles; cholesterol; liver.

The idea of using liposomes as carriers of drugs and physiologically active compounds *in vivo* in order to correct the cell metabolism of target organs is bound up, as has been discovered, with the solution of more difficult problems than was hitherto considered. The main obstacle and the way to achieving the presumed final effect of liposomes, even if these particles preserve their stability during circulation, is the ingestive function of the liver, leading to rapid removal of lipid vesicles from the blood flow [2, 6], and this is accompanied by accumulation of myelin-like structure in the cells and extracellular spaces of the liver [4, 8, 13]. However, all the electron-microscopic evidence that these myelin-like structures are identical with liposomes is based on their external similarity, and considerable misgivings have been expressed [12].

In recent years, with improvements in methods of culture of the sinusoidal and parenchymatous cells of the liver, evidence has been obtained to suggest that its ingestive function includes not only the primary uptake of large liposomes by Kupffer cells, but also the trans-

Laboratory of Electron Microscopy, Research Institute of Gastroenterology, and Laboratory of Engineering Enzymology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 4, pp. 110-113, April, 1983. Original article submitted July 22, 1982.

© 1983 Plenum Publishing Corporation