ELECTRON-MICROSCOPIC CHARACTERISTICS OF "STRESS LUNG"

E. V. Rozova, M. M. Seredenko,
and F. Z. Meerson

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KEY WORDS: air-blood barrier of the lungs; immobilization stress; arithmetic mean thickness; harmonic mean thickness.

An exceptional adrenergic effect, such as regularly arises during prolonged exposure to stress, entails activation of lipid peroxidation (LPO) and of phospholipases and lipases, leading to an increase in concentrations of free fatty acids and lysophosphatides and, ultimately, to damage to cell membranes and disturbances of function of the heart, liver, endocrine glands, and other organs [5]. The question of the comparative stress sensitivity of different target organs still remains largely unexplained. In particular, we do not yet know which ultrastructural changes arise under the influence of long-term exposure to stress in lung tissue.

The aim of this investigation was to study the effect of prolonged immobolization stress on the ultrastructure of lung tissue and, in particular, on the state of the air-blood barrier (ABB), through which oxygen passes from the alevolar air into the blood.

EXPERIMENTAL METHOD

Experiments were carried out on six male rats weighing 180-200 g. Material was taken for electron-microscopic investigation of the lung tissue after induction of stress by the usual method by immobilization for 6 h [8]. The criterion of development of a stress reaction was the presence of ulceration on the gastric mucosa [5]. After decapitation of the rats pieces of tissue were excised from identical areas of the lower lobes of both lungs, fixed in glutaraldehyde and OsO_4, and then embedded in Epon. Ultrathin sections 40-60 nm thick were stained with uranyl acetate and lead citrate and examined in the JEM-100 CX electron microscope. A random set of samples was treated by Weibel's method [2]. Morphometric measurements of the arithmetic mean (τ) and harmonic mean (τ_h) thickness of ABB of the lungs were made on photomicrographs [14]. To monitor the development of arterial hypoxemia, the value of pO_2 of the arterial blood was measured on a type OP-210 biological microanalyzer (Hungary). Rats not subjected to any of these procedures served as the control.

EXPERIMENTAL RESULTS

Immobilization stress caused marked ultrastructural disturbance in the lungs, which were most marked in the region of ABB.

Analysis of the electron micrographs revealed considerable outflow of the liquid part of the blood and of erythrocytes into the lumen of the alveoli in the lung tissues of animals exposed to stress, i.e., the development of an adematous-hemmorrhaic syndrome (Fig. 1a). When these changes are evaluated, the pathological disturbances arising in the lungs during stress, and manifested as focal hemorrhages, inflammatory infiltration, local edema, and disturbances in the mitochondrial apparatus of the cells, developing both in the first few minutes of exposure to stress and over a period of several days in the case of chronic stress, must be called [3, 7]. Edematous-hemorrhagic changes of this kind also are observed in the lungs in shock [1, 15].

A. A. Bogomolets Institute of Physiology, Academy of Sciences of the Ukrainian SSR, Kiev. Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 12, pp. 735-738, December, 1989. Original article submitted April 27, 1988.

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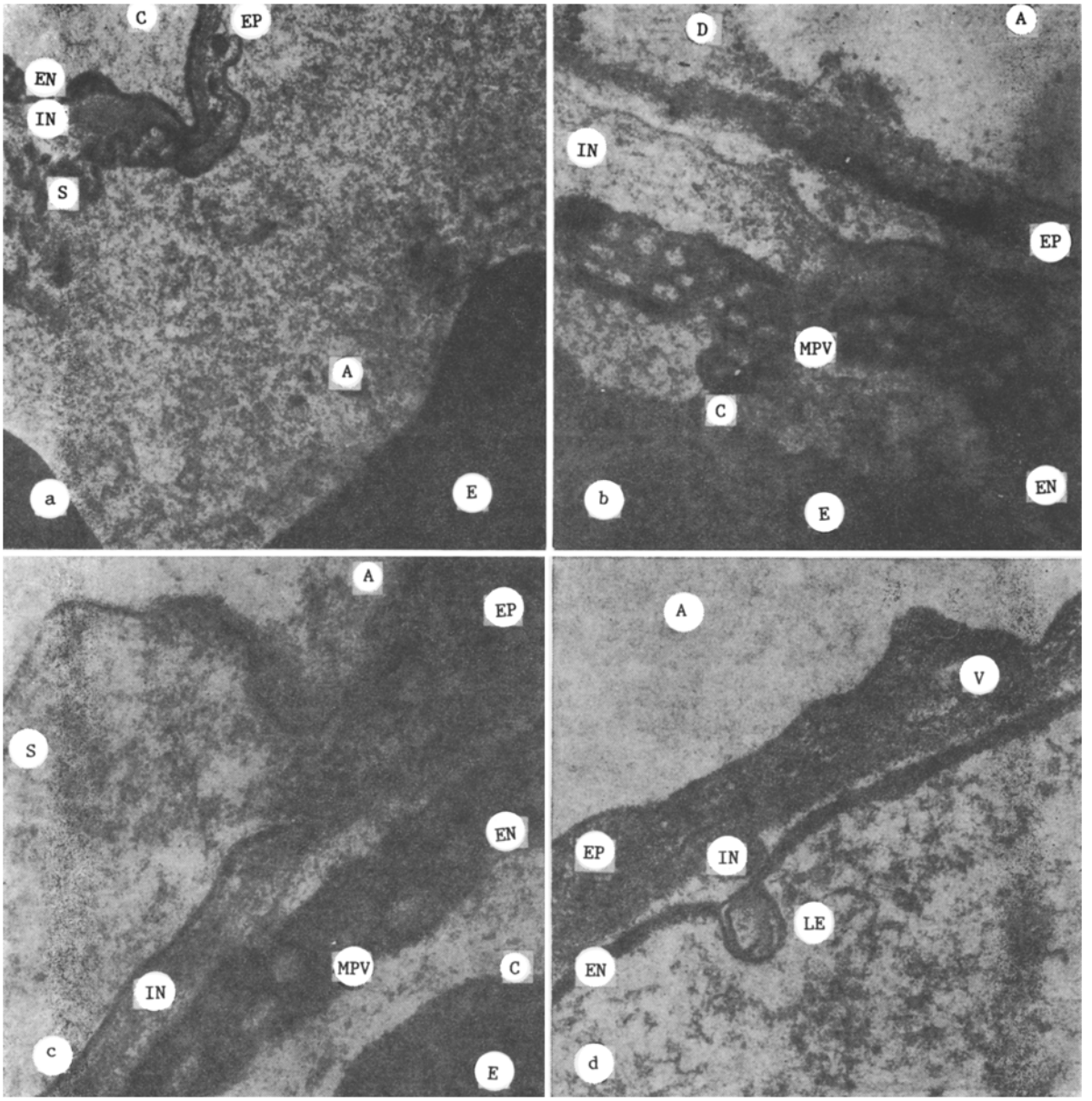

Fig. 1. Ultrastructural changes in ABB of lungs due to immobilization stress. A) Alveolus, C) capillary, E) erythrocyte, EP) epithelial layer, IN) interstitial layer, EN) endothelial layer, MPV) micropinocytotic vesicles, V) vacuolar formations, LE) local subendothelial edema, D) destructive changes, S) sail-like outgrowths, CL) clefts in intercellular junctions, L) lysosomes. Magnification: a, e-h) 22,500; b) 103,000; c, d) 64,000.

The most characteristic features of the alveolar epithelium were areas of total blood flow of type I pneumocytes with sail-like projections (Fig. 1b), evidence of increased saturation of the lung tissue with water. Areas of total destruction of the alveolar epithelium also were found relatively often (Fig. 1c). Areas of thickening, indicating increased accumulation of liquid, also were observed in the interstitial layer (Fig. 1d).

The endothelium of the pulmonary capillaries also showed considerable changes, and areas of local subendothelial edema in the form of vesicles, shedding the endothelial layer, attaining a diameter of 2.7 μ, and filled with electron-optically-translucent contents with arc floccules, resembling plasma proteins, were frequently found.

Another marked response to stress-induced damage was the intensification of pinocytosis detected on the electron micrographs, and particularly conspicuous in the endothelial layer of the pulmonary capillaries, but also observable in the alveolar epithelium; this last

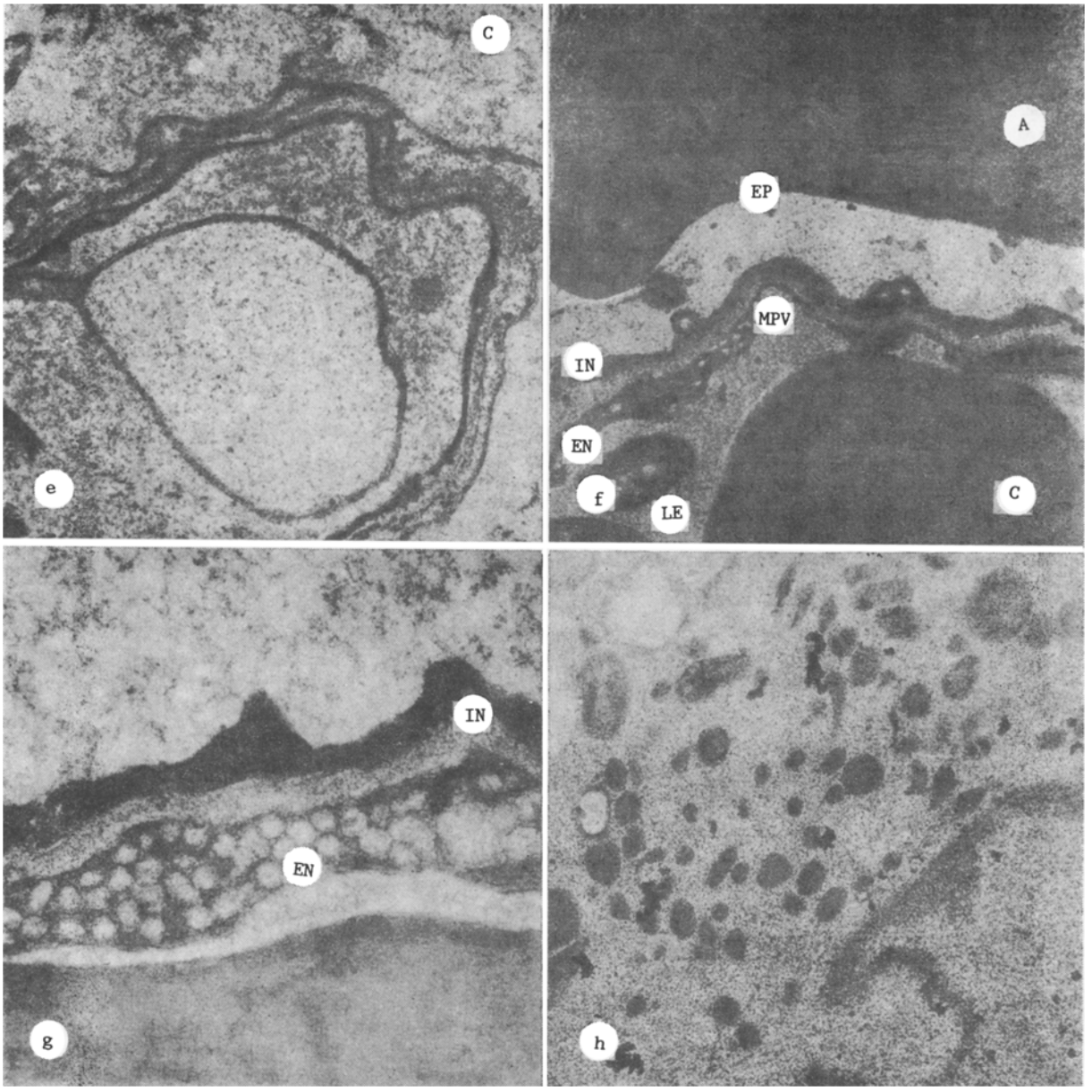

Fig. 1 (continued)

phenomenon is often observed in the presence of other factors, notably hypoxia [6]. The sharp increase in the number of micropinocytotic vesicles led to the appearance of "excessive vesicle formation," [9] in certain areas of the cytoplasmic veils, especially of the endothelial cells, and the number of vesicles to 1 μ in length of the barrier reached 35 or more compared with the normal number of 5-10. The diameter of the micropinocytotic vesicles varied from 0.06 to 0.15 μ. In some cases the vesicles fused together to form larger vacuolar structures. Possibly this activation of pinocytosis may reflect increased transport of structural and energy-producing components from the blood stream and the endoplasmic reticulum of the cells to the damaged alveolar epithelium, where the prospects are best for repair processes to take place. The disturbances noted above in the alveolar apparatus are accompanied by a marked increase in thickness of the ABB, on account of all its layers: the endothelium of the pulmonary capillaries, the interstitial layer, and the alveolar epithelium. On the whole the thickness of ABB was increased about threefold (Table 1). Such a great increase in the thickness of ABB was not observed in pathological states we have studied previously, notably in hypoxic injury to the cells [4]. We are inclined to explain this phenomenon by excessive saturation of the structures of ABB with water. Thus under the influence of stress edema of the lung tissue arises, and in some areas it may lead to the development of intra-alveolar edema.

TABLE 1. Changes in Arithmetic Mean (τ) and Harmonic Mean (τ_h) Thickness of ABB of Lungs and its Individual Layers during Immobilization Stress

Conditions	Total thickness of ABB, μ		Epithelial layer, μ		Interstitial layer, μ		Endothelial layer, μ	
	τ	τ_h	τ	τ_h	τ	τ_h	τ	τ_h
Control ($n = 39$)	0,163±0,008	0,155±0,009	0,071±0,005	0,065±0,007	0,049±0,003	0,046±0,003	0,063±0,007	0,050±0,008
Immobilization stress ($n = 31$)	0,496±0,052	0,429±0,029	0,143±0,016	0,126±0,022	0,115±0,012	0,107±0,010	0,174±0,015	0,142±0,022

Legend. In all cases $p < 0.001$.

It is an interesting fact that edema of ABB tissues is accompanied by the formation of particular type of clefts in the intercellular junctions between both endothelial and epithelial cells, commensurate in size with the thickness of the barrier, or in some cases actually exceeding it, reaching 0.5-0.6 μ. Under these circumstances, a local decrease in its thickness takes place in these areas of the barrier, and this may evidently promote optimization of the passage of O_2 from the air into the blood. A similar response of the cellular structures is characteristic of exposure to hypoxia [10].

Another noteworthy fact is the almost complete absence of both intracellular (reserve) and extracellular (active) surfactant in the lungs of animals exposed to stress, evidence of inhibition of activity of the lung surfactant system. This result is consistent with the close relationship between the number and activity of β-adrenoreceptors of the lungs, located on type II pneumocytes, and the synthesis and secretion of lung surfactant [13]. Since during stress, including immobilization stress, the number of β-adrenoreceptors in several organs and tissues is reduced [11, 12], and this may be considered to be the situation in the lungs also, a reduction in the total content of surfactant can be more easily explained.

The considerable increase in the number of primary and secondary lysosomes in the cellular structures of the lungs must evidently be taken into account also, for it may also indicate an increase in the contribution of destructive processes in the cell to the development of the stress reaction.

The combination of ultrastructural changes which we found in the lungs after a single exposure to severe stress is made up of edema and a sharp increase in the thickness of ABB and of all its components, an edematous-hemorrhagic syndrome, damage to the alveolar epithelium, and a disturbance of the surfactant system of the lungs. This combination of changes, which we describe as "stress lung," can very probably disturb oxygen transport from the alveoli into the lung capillaries. The results thus suggest that stress-induced damage can substantially disturb the function of external respiration and provoke or aggravate diseases of the lungs.

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INCORPORATION OF ^{3}H-THYMIDINE BY INTERSTITIAL CELLS OF THE RAT MYOCARDIUM AFTER A SINGLE INJECTION OF THE LABEL

E. V. Zamaraeva

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An important role in cardiac function is played by interaction between cardiomyocytes and connective tissue, for the latter not only forms a supportive carcass, but also mediates the metabolic processes of the cardiomyocytes. It is accordingly interesting to study the character of renewal of connective-tissue cells in the heart. We know that if a single injection of ^{3}H-thymidine is given to an animal, the precursors of different types of connective-tissue cells require 2-4 days in order to divide, migrate into the organ, and specialize [4, 5]. The kinetics of connective-tissue cells incorporating ^{3}H-thymidine was found to be similar for different organs. Maximal incorporation of label is observed on the 4th day after a single injection, and the level of labeling thereafter falls for 1-2 weeks [7, 15]. However, despite the similar time course of labeled connective-tissue cells, the highest percentage of them in different organs and under different conditions of testing, differed considerably, possible evidence of a difference in the degree of renewal of the connective-tissue, and a sign of organ specificity. The heart has not been investigated deliberately from this point of view, and the investigation described below as accordingly carried out for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on 20 male Wistar rats weighing 60-115 g. The animals were given a single intraperitoneal injection of ^{3}H-thymidine in a dose of 1 μCi/g (specific activity 1 TBq/mmole). Thoracotomy was performed on the animals under pentobarbital anesthesia (0.05 mg/g) 1, 2, 3, 4, 5, 6, 7, 14, and 21 days later and the heart was perfused with Hanks' solution and then with 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 10-15 min. The heart was then removed. A lamina 1 mm thick was excised from the middle third of the left ventricle, perpendicularly to its axis. The laminae were fixed in 1% osmium tetroxide solution in phosphate buffer, and them embedded in Epon by the standard method. Three blocks 0.5 mm wide were excised from the middle third of the lamina and semithin sections cut from them through the whole thickness of the wall of the left ventricle. The sections were covered with type M emulsion and exposed at 4°C for 2 weeks. After development, the sections were stained with methylene blue. Cells were considered to be labeled if there were 7 grains of silver or more above the nucleus. The background level was 1-4 grains per cell. The percentage of labeled cells on the whole surface of the section and separately for each layer of the myocardium was counted. The number of cells counted each time was 2500-6000. The criterion for isolation of a layer was the direction of the muscle fibers and the connective-tissue bands containing large blood vessels. According to these features three layers were distinguished: subendocardial, subepicardial, and middle. The numerical results were subjected to statistical analysis [8].

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