RESPONSE OF THE SURFACTANT SYSTEM AND AIR–BLOOD BARRIER OF THE LUNGS TO ACUTE GENERAL HYPOTHERMIA

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Acute general hypothermia in man and animals can lead to the development of atelectasis and pulmonary edema and it can severely aggravate chronic lung diseases [1-4]. At the same time it has been shown that the elasticity and compliance of the lungs fall gradually during cooling of the body to –20°C [7] and the level of total lung tissue phospholipids is depressed [6].

It can accordingly be postulated that disturbances of the alveolar surfactant system and of the fine structure of the air–blood barrier are among the pathogenetic factors leading to the development of this lung pathology. Meanwhile the ultrastructural basis of lung pathology and of adaptive cellular reactions in the lung during acute general hypothermia have not yet been elucidated.

The aim of this investigation was to discover the morphological substrate of disturbance of permeability of the air–blood barrier and development of atelectasis in acute general hypothermia. Another topic of interest was ultrastructural changes in type 2 alveolocytes, which synthesize and secrete the phospholipids of the surfactant, and also in alveolar macrophages which participate in its utilization and elimination.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 235-350 g were cooled for 4-6 h at a temperature of –20°C. Some rats were killed immediately after cooling, the rest after warming at room temperature for 1-1.5 h. Thoracotomy was performed under deep pentobarbital anesthesia and one lobe of the right lung was removed and immersed in 2.5% glutaraldehyde solution in 0.1 M phosphate buffer, pH 7.4, for 2 h. The peripheral areas of the lobe were cut into small pieces measuring 1-2 mm^3, which were prefixed in 2.5% glutaraldehyde for 1-2 h and then fixed in 1% OsO_4 for 1 h. The material was dehydrated and embedded in Epon-Araldite by the usual method. Ultrathin sections were examined in the IEM-100B electron microscope.

The state of the lung parenchyma was studied in paraffin sections stained with hematoxylin and eosin and also in semithin sections stained with toluidine blue at pH 7.3. To determine functional activity of type 2 alveolocytes they were subjected to morphometry on photographs with a final magnification of 20,000, with the aid of an arbitrary test system (grid), and by calculating the relative bulk density of the mitochondria and osmiophilic lamellar bodies. In addition, the absolute number and dimensions of these organelles were calculated in "central" sections of cells containing the nucleus.

In each case from 150 to 800 alveolar macrophages were studied in semithin sections and the relative percentages of cells in different functional states were determined.

EXPERIMENTAL RESULTS

All six experimental rats subjected to hypothermia survived. The initial rectal temperature, measured by means of a "Temp-60" electric thermometer, was 33.0-36.0°C when the electrode was inserted into the rectum to a depth of 1 cm. During hypothermia it fell to 16.5-28.0°C. After rewarming the rectal temperature rose to 26.0-34.6°C. Hyperthermia and edema of the ears, limbs, and tail were observed.

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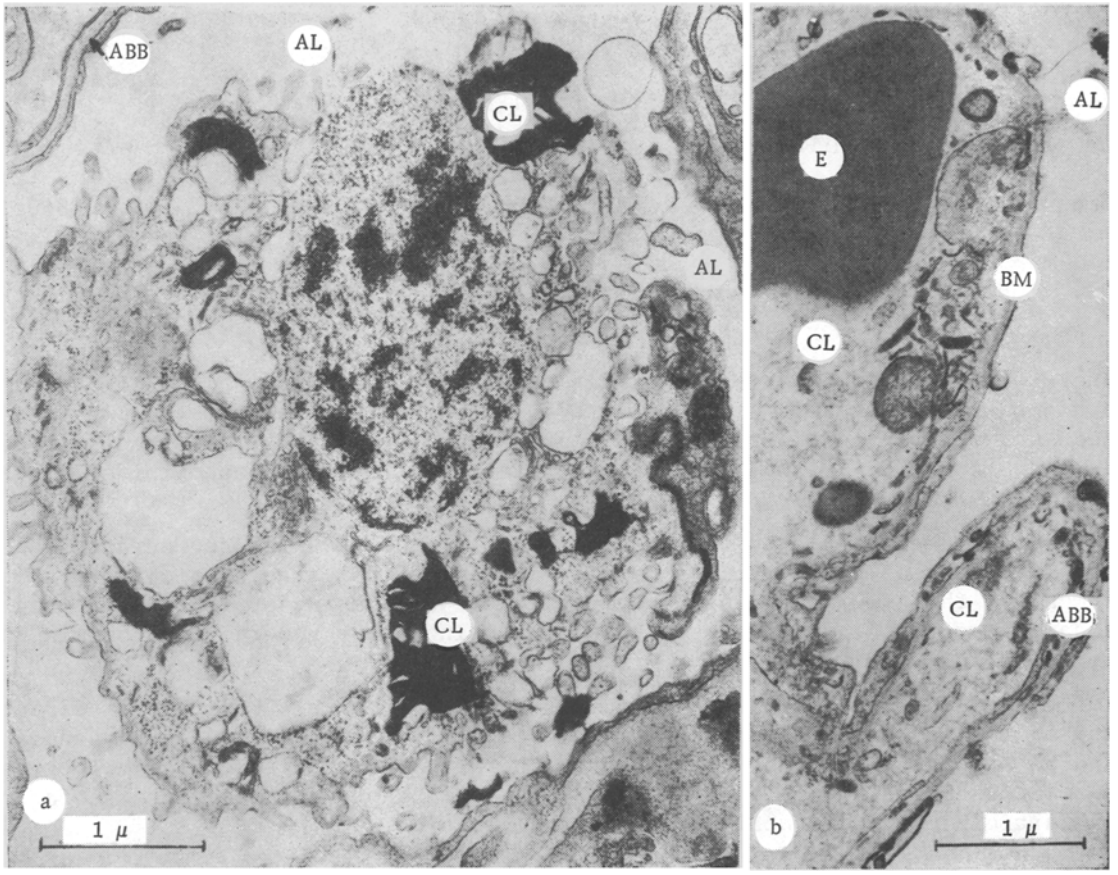

Fig. 1. Lung of rat in acute general hypothermia. a) Type 2 alveolocyte with signs of hydropic degeneration in lumen of alveolus. 27,000 ×; b) air–blood barrier, destruction of type 1 alveolocyte and endotheliocytes with denudation of basement membrane. 24,000 ×. AL) Alveolar lumen, CL) capillary lumen, ABB) air–blood barrier, BM) basement membrane, A2) type 2 alveolocyte, CL) cytophospholiposomes, E) erythrocyte.

TABLE 1. Number of Osmiophilic Lamellar Bodies and Mitochondria and Their Relative Bulk Density in Type 2 Alveolocytes of Lungs of Control and Hypothermic Rats

Experimental conditions	Osmiophilic lamellar bodies		Mitochondria	
	number per cell	relative bulk density in cell, %	number per cell	relative bulk density in cell, %
Control (n = 19)	10,5±1,2	8,8±1,3	10,4±1,2	11,9±1,9
Acute hypothermia:				
"air" parenchyma (n = 13)	7,9±1,0	4,6±1,0*	6,5±1,0	9,4±1,6
zones of dys- and atelectasis (n = 30)	14,9±2,2*	7,7±1,2	10,1±1,2	10,2±1,3

Legend. Here and in Table 2, asterisk indicates significant differences from control.

Comparatively large round and wedge-shaped areas of atelectasis, local hemorrhages, and interstitial and intra-alveolar edema appeared in the lungs both of the hypothermic rats and of animals rewarmed after hypothermia. Marked hydropic degeneration and destruction of some type 1 alveolocytes and endotheliocytes of the air–blood barrier were distinctly visible (Fig. 1b), and these changes constituted the ultrastructural basis for development of pulmonary edema.

The structure of the type 2 alveolocytes, alveolar macrophages, and alveolar surfactant complex in the straightened out alveoli differed from that in zones of dys- and atelectasis (Tables 1 and 2). The number and relative bulk density of the mitochondria were reduced in

TABLE 2. Relative Percentages of Alveolar Macrophages with Different Levels of Functional Activity in Lungs of Control and Hypothermic Rats

Experimental conditions	Number of alveolar macrophages, %			
	containing primary and single secondary lysosomes	containing many primary and secondary lysosomes	containing many lipid granules	with signs of destruction
Control (n = 307)	31,8	58,5	9,7	0,0
Acute hypothermia:				
"air" parenchyma (n = 56)	32,3	57,4	9,7	0,6
zones of dys- and atelectasis (n = 1692)	26,8	48,7	23,9*	0,6

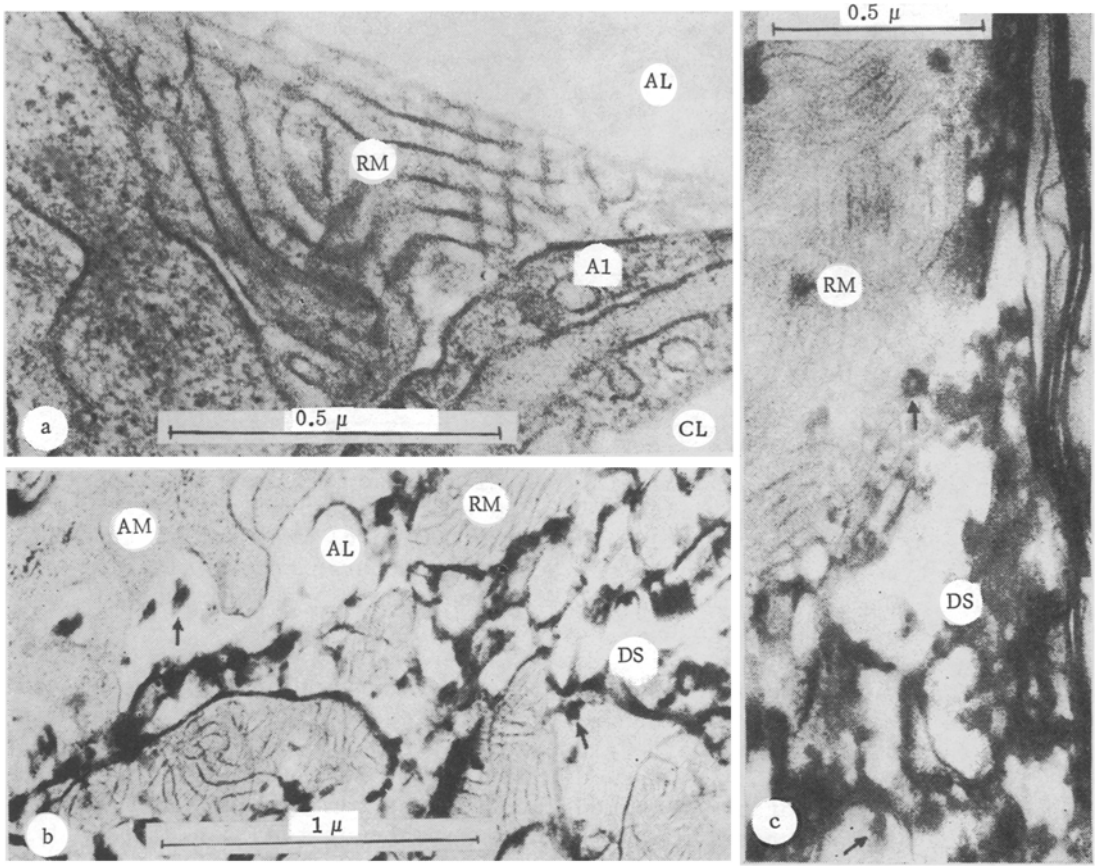

Fig. 2. Ultrastructural organization of alveolar surfactant in rat lung: a) control. 100,000 ×; b, c) acute general hypothermia: disintegration and aggregation of surfactant membranes; disturbance of spatial organization of surfactant and appearance of liposome-like structures can be seen (arrow). b) 49,000 ×; c) 58,000 ×. RM) Regular surfactant membranes, Al) type 1 alveolocyte, AM) alveolar macrophage, DS) disintegrated surfactant. Remainder of legend as to Fig. 1.

the type 2 alveolocytes of the straightened out alveoli, evidently on account of their destruction and lysis. This phenomenon can be attributed to the hypoxia which arises during hypothermia. The number of osmiophilic lamellar bodies or, as we call them, "cytophospholiposomes," and their relative bulk density were significantly reduced, and this can be regarded as a sign of active secretion of alveolar surfactant. Secretion of surfactant is not only merocrine, but also apocrine, or even holocrine in character. The type 2 alveolocytes, located within the lumen of the alveoli, showed signs of hydropic degeneration and destruction (Fig. 1a).

Phospholipid synthesis in type 2 alveolocytes was appreciably activated in the zones of atelectasis, as shown by an increase in the number and relative bulk density of the

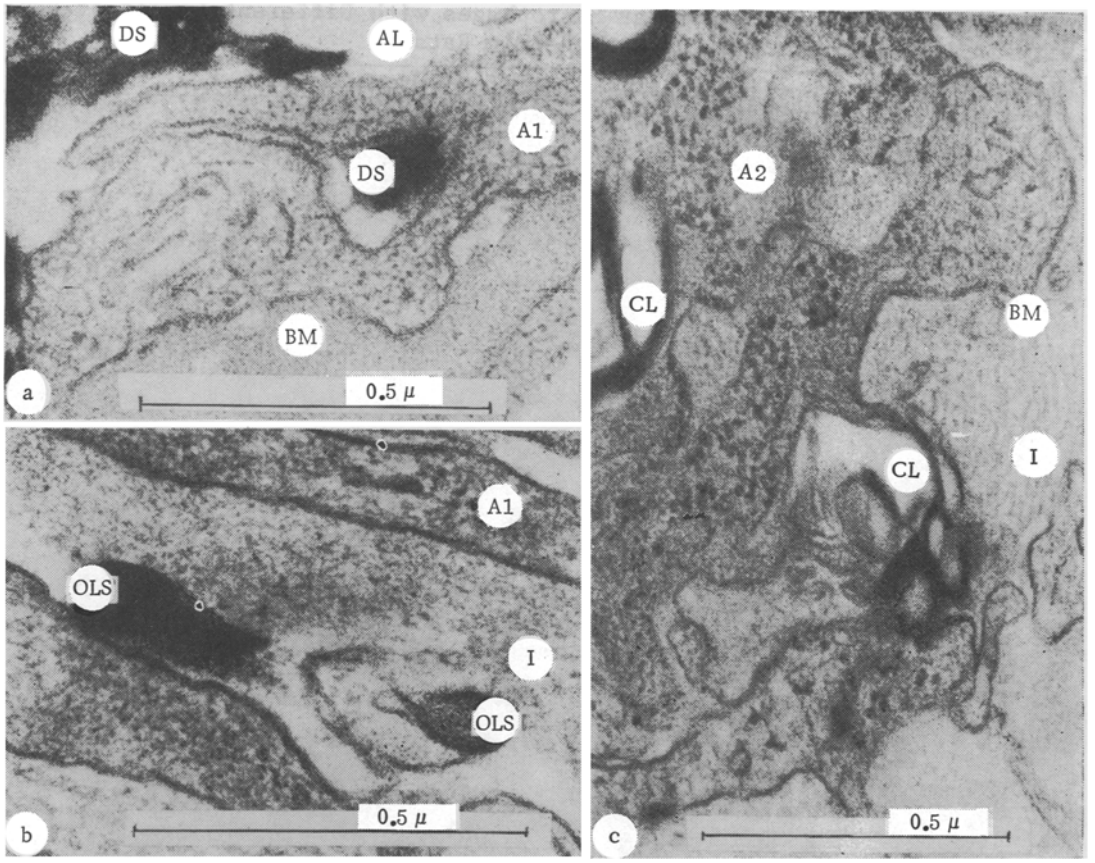

Fig. 3. Lung in acute general hypothermia. a) Elimination of disintegrated alveolar surfactant by type 1 alveolocyte. 115,000 ×; b) osmiophilic lamellar structures in interstices of alveolar septum. 130,000 ×; c) fragment of type 2 alveolocyte, basal type of secretion of surfactant in interstices of alveolar septum. 100,000 ×. OLS) Osmiophilic lamellar structures; I) interstices of alveolar septum. Remainder of legend as to Figs. 1 and 2.

cytophospholiposomes (Table 1). Meanwhile the secretion of surfactant was intensified, and the amount of it in the lumen of the collapsed alveoli increased. However, whereas in straightened alveoli the surfactant preserved its usual structural plan characteristic of the normal lung, in atelectatic areas it was modified (Fig. 2a-c). More disintegrated surfactant was present in the lungs of the rewarmed rats. Consequently, in acute general hypothermia the appearance of atelectases in the lungs was due, not to a deficiency of alveolar surfactant, as is generally considered, but mainly to a change in its quality, expressed at the ultrastructural level by disintegration, aggregation, and lysis of membranes.

The disintegrated surfactant was actively ingested by alveolar macrophages, among which cells containing many secondary lysosomes and lipid granules began to predominate (Table 2). Cholesterol crystals appeared in the cytoplasm of individual alveolar macrophages. All these findings indicate increased functional activity of the alveolar macrophages under conditions of acute general hypothermia.

Disintegrated surfactant could also be eliminated by endocytosis by type 1 alveolocytes (Fig. 3a). As a result of this process material of phospholipid nature (osmiophilic lamellar formations, fragments of membranes, liposome-like structures) were found in the interstices of the interalveolar septa (Fig. 3b). Another way in which phospholipids could penetrate into the interstices was by secretion of surfactant through the base of type 2 alveolocytes (Fig. 3c), which in the writers' opinion is connected with partial inactivation of cytoplasmic microtubules of the apical compartment of these cells. This hypothesis is not without foundation because it has been shown that microtubules of various cells when cultured at low temperatures (0-4°C) are destroyed [5]. At the same time the basal type of surfactant secretion also was discovered by the present writers in the rat lung after exogenous introduction of colchicine, which blocked the outflow of phospholipids by exocytosis from the apical

surface of type 2 alveolocytes. It can accordingly be supposed that activation of the basal type of secretion of alveolar surfactant in the lung of the hypothermic animal is based on a similar mechanism, for the necessary conditions are present for its realization: the presence of active synthesis of phospholipids in type 2 alveolocytes and partial limitation of the secretion of these phospholipids from the apical surface of the cells. Liberation of phospholipids from the interstices evidently took place through the lymphatic system of the lung and the general lymphatic circulation.

Changes in the lung in hypothermia are thus characterized by a local disturbance of integrity of the air–blood barrier, disintegration, aggregation, and lysis of membranes of the alveolar surfactant, activation of synthesis and secretion of surfactant by type 2 alveolocytes, and the accumulation of surface-active material in the interstices of the alveolar septa, which in the writers' opinion, promotes destruction of their collagen and elastic fibers and also an increase in functional activity of the alveolar macrophages, accompanied by destruction of some cells.

Activation of synthesis and secretion of alveolar surfactant, and intensive utilization of disintegrated surfactant by alveolar macrophages and by type 1 alveolocytes are an expression of compensatory and adaptive reactions against the background of disturbance of ventilation, the hemodynamics, and also of lipid metabolism in the lungs, due to acute general hypothermia.

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ULTRASTRUCTURAL MANIFESTATIONS OF RUBOMYCIN-INDUCED ABNORMAL SYNTHESIS OF CONTRACTILE PROTEINS BY RAT CARDIOMYOCYTES

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Toxic injury to the myocardium by antibiotics of the anthracycline series disturbs the synthesis of specific contractile proteins in cardiomyocytes and leads to the development of heart failure [11, 12]. Administration of these antibiotics, especially rubomycin, to animals can be used as an experimental model of chronic cardiac pathology [7, 11, 13].

The present investigation was carried out with the aim of studying the time course of ultrastructural changes arising in the cardiomyocytes of albino rats in response to administration of rubomycin.

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