STATE OF "GLYCOCALYX" OF RAT LUNG CELLS AFTER LEFT-SIDED PNEUMONECTOMY

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Edema develops in the tissues of the air–blood barrier of the right lung in rats 24 h after left-sided pneumonectomy. Electron-microscopic histochemical investigations (using ruthenium red – RR) showed that the layer of acid muscopolysaccharides of the supraplasmalemmal covering of the alveolar cells and endothelium is thickened, electron-dense masses of reaction products with RR accumulate on the surface of the respiratory alveolocytes and endothelium, and "vesicles," covered with reaction product with RR and connected with the plasmalemma of the alveolar and endothelial cells, appear. These findings point to a role of the acid muscopolysaccharides of the surfactant system of the lungs in the accumulation and elimination of fluid from the edematous tissues of the air–blood barrier.

KEY WORDS: surfactant; air–blood barrier; edema; acid mucopolysaccharides.

In the early stages after unilateral pneumonectomy in rats and dogs edema of all components of the air–blood barrier of the alveoli of the residual lung develops [1, 6, 9]. At the same time, in rabbits with pulmonary edema caused by vagotomy, both the phospholipid and the mucopolysaccharide components ("glycocalyx") of the surfactant system of the organ are destroyed [8]. It is not yet known whether these changes in the surfactant system are specific for pulmonary edema of any particular etiology [2].

The object of the present investigation was to study the state of the mucopolysaccharide component of the surfactant system of the alveoli of the residual lung in the early periods after unilateral pneumonectomy, i.e., in the initial stage of development of edema, and to ascertain its response to changes in water metabolism in cells of the air–blood barrier.

EXPERIMENTAL METHOD

The writers showed previously that 24 h after left-sided pneumonectomy in rats the dry weight of tissue of the residual right lung is altered, the thickness of the air–blood barrier of its alveoli is increased, and evidence of pulmonary edema appears in the cytoplasmic processes of the respiratory alveolocytes (type I cells) and in the capillary endothelium [4, 5]. It was therefore decided to examine the state of the "glycocalyx" of the lung cells at this stage after unilateral pneumonectomy.

Male rats (4) weighing 150-160 g, 24 h after removal of the left lung, were anesthetized with pentobarbital and perfused through the pulmonary artery under the pressure of 25-30 mm Hg with physiological saline followed by a fixing mixture consisting of glutaraldehyde, cacodylate buffer, and ruthenium red (RR). Fixation and subsequent processing of the lung tissue were carried out by the method of Romanova and Boikov [7]. The fixing mixtures were prepared by Luft's formula [10]. Two intact rats were used as the control. After rapid dehydration of the lung tissues in alcohols of increasing concentration and in propylene oxide by Morozov's method [3] the material was embedded in Durcupan. Sections cut to a thickness of 400-

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TABLE 1. Thickness of Electron-Dense Layer (reaction product with RR) on Surface of Cells of Air–Blood Barrier of Lungs of Control and Experimental Rats

Location of electron-dense material	Thickness of electron-dense layer (Å)		
	minimal	maximal	mean
Intact animals			
On plasmalemma of alveolar cells	200	800	450
Experimental animals			
On plasmalemma of respiratory alveolocytes:			
As a continuous layer in close contact with the plasmalemma	510	1,300	830
As a layer covering the vesicles in contact with the cell surface	490	1,450	770
As "masses" in close contact with the cell surface	1,250	5,000	2,650
On plasmalemma of large alveolocytes:			
As a continuous layer in close contact with the plasmalemma	370	1,440	750
As a layer covering the vesicles in contact with the cell surface	375	1,250	725
On surface of alveolar epithelium as masses in zones of contact between cells:			
Surface electron-dense layer	420	1,770	1,100
Zone of average electron density	3,010	11,330	7,900
On plasmalemma of endothelium:			
As a continuous layer in close contact with the plasmalemma	150	760	360
As "masses" in close contact with the cell surface	410	1,830	825
On surface of vesicles lying freely in lumen of alveoli	640	1,045	790

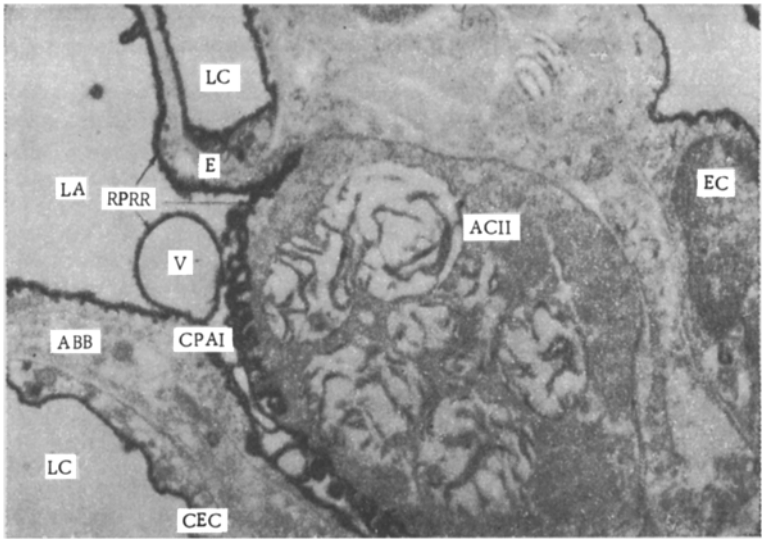

Fig. 1. State of layer of acid mucopolysaccharides of surfactant system of right lung of a rat 24 h after left-sided pneumonectomy (14,000 ×). Here and in Figs. 2 and 3: LA) lumen of alveola, LC) lumen of capillary, ACII) large alveolocyte (type II alveolar cell), ABB) thinnest part of air–blood barrier, EC) endothelial cell, E) edema, CPAI) cytoplasmic process of respiratory alveolocytes (type I alveolar cell), CEC) cytoplasm of endothelial cell, V) "vesicle," RPRR) reaction product with RR.

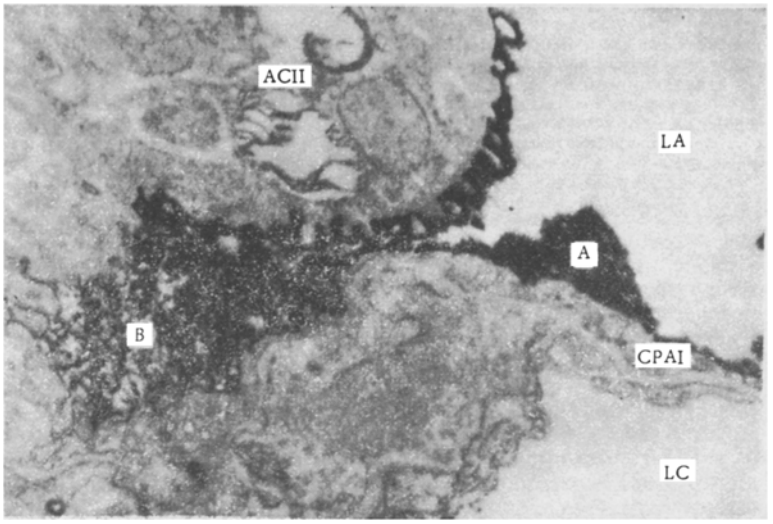

Fig. 2. Accumulation of reaction product with RR between alveolar cells (B) and on surface of cytoplasmic process of respiratory alveolocyte (A) (14,000×).

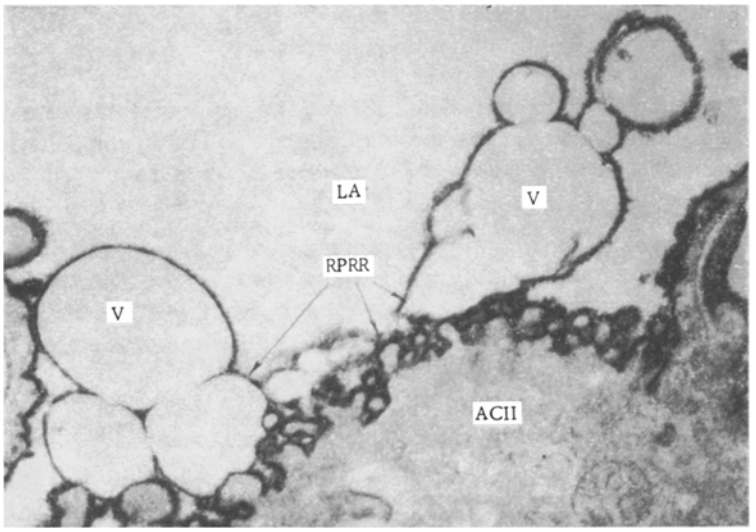

Fig. 3. Formation of "vesicles" on surface of large alveolocyte in lung of experimental rats (13,000 ×).

500 Å on the LKB ultratome were examined without further staining in the JEM-7A and JEM-100B electron microscopes. Besides the ordinary morphological identification of the "glycocalyx" of the alveolar cells an attempt was made to estimate its thickness quantitatively (Table 1). For this purpose, the thickness of the electron-dense layer (reaction product with RR) was determined in the control and experimental series in photographs measuring 18 × 24 mm (magnification 80,000 to 96,000 ×) every 5 mm over the whole area of the alveolar lining. The thickness of the electron-dense supraplasmalemmal covering on the endothelium of the pulmonary capillaries also was measured.

EXPERIMENTAL RESULTS

The reaction product with RR on the epithelial lining of the alveoli in the experimental series consisted of a continuous electron-dense layer in close contact with the apical plasmalemma of the alveolar cells and following the outlines of their surface exactly (Fig. 1). Small translucent areas, indicating developing postoperative edema of the organ, could be seen in the cytoplasm of a few respiratory alveolocytes. Collections of pinocytotic vesicles, many of them containing reaction product with RR, were located in the thickened areas of the cytoplasmic processes of these cells. Frequently compact electron-dense masses

with irregular outlines and a fibrous structure could be seen on the surface of the cytoplasmic processes of the respiratory alveolocytes (Fig. 2A). Structures of this sort were virtually never seen in the control. Here also, i.e., in the thinnest parts of the barrier, single "vesicles" of various sizes, covered with a continuous layer of reaction product with RR, were sometimes seen (Fig. 1). The lumen of the vesicles was "colorless."

The cytoplasm of the large alveolocytes (type II alveolar cells) was indistinguishable in its ultrastructure from the cells of the intact lung (Fig. 1). "Vesicles" consisting of masses of curious shape (Fig. 3), were seen on the surface of these cells. No such patterns were found in the control series.

Particular attention must be paid to the accumulation of "loose" material of irregular electron density filling the intercellular spaces of the alveolar lining (Fig. 2B). The varied electron density of the material of the intercellular aggregations is evidently due to differences in the functional state of the acid mucopolysaccharides of the surfactant system.

The electron-dense layer of reaction product with RR appeared regularly in nearly all capillaries in the lungs of the experimental rats, whereas in the intact animals it was rarely seen on the surface of the endothelial lining. The electron-dense layer was continuous and in close contact with the plasmalemma of the endothelial cells and their cytoplasmic processes (Fig. 1). Sometimes small collections of electron-dense material or single small "vesicles" could be seen on the surface of individual endothelial cells. In their shape and structure they resembled the corresponding formations on the surface of the thin areas of the cytoplasmic processes of the respiratory alveolocytes.

The experiments thus showed that 24 h after left-sided pneumonectomy the following changes took place against the background of developing edema of the air–blood barrier of the residual lung: 1) an increase in thickness of the supraplasmalemmal covering of the alveolar cells and endothelium of the capillaries (Table 1), 2) the formation of electron-dense accumulations of reaction product with RR on the surface of the respiratory alveolocytes and endothelium, 3) the appearance of "vesicles," covered with reaction product with RR and connected with the plasmalemma of the alveolar and endothelial cells, 4) an increase in the frequency of occurrence of intercellular masses of reaction product with RR of irregular electron density.

These findings are evidence of functional stress in the surfactant system of the alveoli. Acid mucopolysaccharides can evidently accumulate fluid from the cells of the air–blood barrier and thus participate in the regulation of their water metabolism. The appearance of vesicles bounded by acid nucopolysaccharides on the surface of the alveolar and endothelial cells reflects the mechanism of elimination of fluid into the lumen of the alveoli or capillaries.

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