STATE OF THE SURFACE ACTIVITY OF RAT LUNG SURFACTANT AT VARIOUS TIMES AFTER LEFT-SIDED PNEUMONECTOMY

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The surface activity of seven successive washings from the right lung, determined with the aid of a modified Wilhelmy balance, was the same as in the control until the 5th day after removal of the left lung and also in the late stage after the operation (γ_{min} = 23-24 dynes/cm). Intracellular edema of the components of the air–blood barrier and the escape of edema fluid into the lumen of the alveoli of the "vesicles" were not reflected in the surface-active properties of the lung surfactant. A sharp increase in size of the alveoli on the 5th-7th day after the operation was accompanied by an increase in the surface-active properties of the lung washing (γ_{min} = 11-15 dynes/cm) and by increased secretion of material of the osmiophilic lamellar bodies from the type II alveolar cells into the lumen of the alveoli. The cytological mechanisms of the increased production of surfactants in the hypertrophied alveoli are activation of lipid synthesis in the type II alveolar cells, hypertrophy of those cells, and the appearance of binuclear cells.

KEY WORDS: *left-sided pneumonectomy*; *surfactant*; *type II alveolar cells*.

The surface-active phospholipid film (surfactant) lining the alveoli determines their stability and thus ensures normal functional activity of the lung [12]. The material of the surfactant is synthesized in the large alveolocytes (type II alveolar cells) [7]. A deficiency of surface-active phospholipids in the alveoli may be accompanied by the development of foci of atelectasis and the appearance of acute respiratory failure. At the same time, in some pathological states (edema of the lung caused by vagotomy, experimental emphysema, a sudden postoperative rise of pressure in the pulmonary artery) partial destruction of the "surfactant alveolar complex" takes place and the surface activity of washings from the lungs is altered [1, 2, 5, 8, 10, 11]. The mechanisms of the disturbance of the surface-active properties of the surfactant in the presence of lung pathology and the relationships of cause and effect relating to these phenomena are insufficiently clear.

In this investigation the effect of the development of intracellular edema and the appearance of edema fluid in the lumen of the alveoli, hypertrophy of the alveoli, and an increased functional load on the state of the surfactant were studied.

On the first day after left-sided pneumonectomy in rats, marked intracellular edema of the components of the air–blood barrier [6] is known to arise in the residual lung and on the 7th day there is a stable increase in volume of the alveoli by 50-100% compared with the control [4]. It was accordingly decided to determine the degree of morphological and functional integrity of the alveolar surfactants during compensatory hypertrophy of the lung and to examine the ultrastructural compensatory and adaptive mechanisms aimed at restoring the normal stability of the alveoli of the hypertrophied lung.

EXPERIMENTAL METHOD

The left lung (37% of the total mass of the organ) was removed from noninbred albino

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rats under pentobarbital anesthesia. Seven successive washings were obtained from the lungs of 5 to 10 animals at each time, 6 h and 1, 2, 4-7, 9, 30, and 275 days after the operation and their surface activity was measured with a modified Wilhelmy balance [3]. The value of the minimal surface tension (γ_{min}), the area of the hysteresis loop S_σ, and the index of stability [$IS = 2(\gamma_{max} - \gamma_{min})/(\gamma_{max} + \gamma_{min})$] were used as the criteria of surface activity. For each group of experiments there was an intact control: K_1) a control for the periods between 6 h and 4 days after the operation; K_2) the control for 5-9 days; and K_3) the control for the late stage (275 days) after the operation.

A parallel investigation was made of the utrastructural organization of the surfactants and the character of secretion of osmiophilic bodies from the type II alveolar cells. For electron microscopy the lungs of the control and experimental animals were fixed 1, 3-7, 30 and 275 days after the operation by perfusion with 2.5% glutaraldehyde in 0.1 M cacodylate buffer through the pulmonary artery by the method of Weibel and Gil [14], followed by post-fixation of the tissue fragments with 1% OsO_4. Material was dehydrated either in alcohol or in acetone of increasing concentration and in propylene oxide and embedded in Epon—Araldite. Sections 400-500 Å thick were stained with lead citrate and examined in the JEM-100B electron microscope.

EXPERIMENTAL RESULTS

The minimal surface tension of washings of the hypertrophied lung remained almost unchanged for the first 4 days after the operation (Fig. 1). No significant differences likewise were found between the values of the area of the hysteresis loop and index of stability in the control and experimental series. However, on the 5th day after the operation there was a sharp increase in the surface activity of the washings, as shown by a significant ($P < 0.01$) decrease in the mean value of γ_{min} to 11.3 ± 3 dynes/cm (in K_2, γ_{min} = 21.7 ± 0.62 dynes/cm), an increase in the area of the hysteresis loop to 1100 ± 80 conventional units (in K_2 S_σ = 730 ± 20), and an increase in the index of stability to 1.4 ± 0.13 (in K_2 IS = 0.87 ± 0.04). The increase in the surface activity of the washing on the fifth to sixth day after left-sided pneumonectomy was also repeated in a special series of experiments, confirming the reliability of this phenomenon. These continued on the 7th day after the operation although they were less marked in character. On the ninth day and 275 days after left-sided pneumonectomy the mean indices of surface activity of washings from the hypertrophied lung were close to the control values, indicating normalization of the surface tension of the surfactant in the enlarged alveoli.

Electron microscopy showed that in the early period (up to 7 days) after the operation areas of low electron density, free from cell organelles, were found in the cytoplasm of most respiratory alveolocytes (type I alveolar cells) and endothelial cells of the pulmonary capillaries, evidence of the presence of intracellular edema. The intracellular edema and the outflow of fluid in the "vesicles" into the lumen of the alveoli were most marked on the first day after the operation [6]. Evidence of edema was absent in the cytoplasm of the type II alveolar cells.

The type II alveolar cells of the lungs of the experimental animals were heterogeneous in composition. Mononuclear cells with a normal volume of cytoplasm, and hypertrophied and binuclear cells could be distinguished among them. In most of the type II alveolar cells the quantity of membranes of the granular cytoplasmic reticulum and of the lamellar complex was increased by the third day after the operation, and the mitochondria, multivesicular bodies, and polysomes were more numerous. Evidence of increased lipid synthesis in the type II alveolar cells was given by the appearance of many intermediate forms between multivesicular and osmiophilic bodies in them. Several workers have stated that osmiophilic lamellar bodies can be formed from multivesicular bodies in the type II alveolar cells of intact animals [7]. In the hypertrophied and binuclear cells the number of mitochondria was doubled and the total number of osmiophilic lamellar bodies was almost three times greater than in the control. The cytoplasm of the hypertrophied cells as a rule was more electron-dense, as a result of which the cell appeared "dark" against the background of the surrounding "pale" lung tissue (Fig. 2a).

In serial sections through the type II alveolar cells the successive course of discharge (exocytosis) of the contents of the same osmiophilic lamellar body into the lumen of the alveolus could be traced (Fig. 2b, c, d). Initially one or more evaginations appeared on the

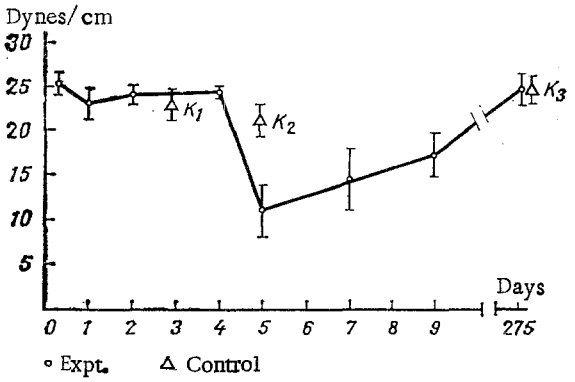

Fig. 1. Surface tension of washings (γ_{min}, in dynes/cm) from lungs of intact and experimental rats at various times after operation. Ordinate, γ_{min} (in dynes/cm); abscissa, days after operation.

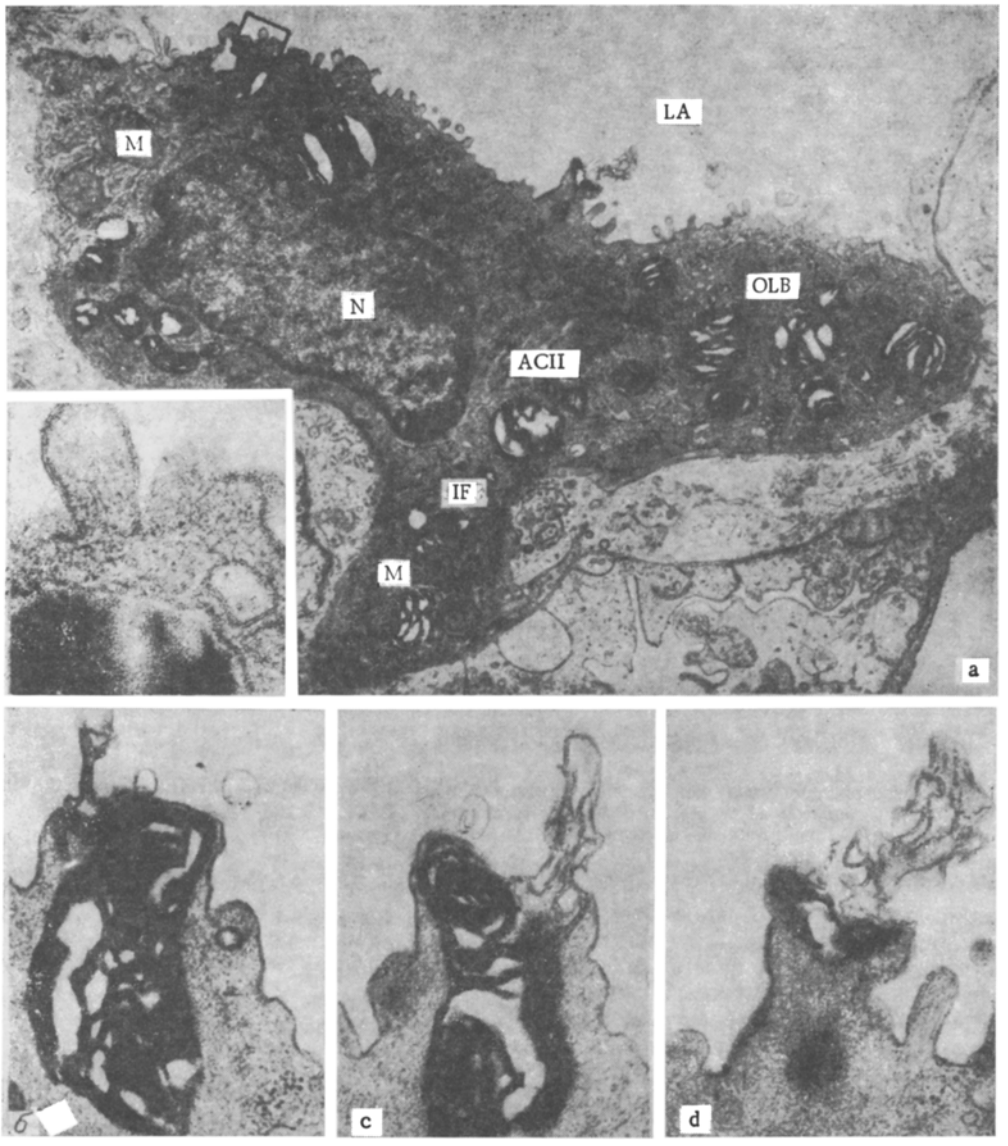

Fig. 2. Hypertrophied "dark" type II aveolar cell from right lung of rat 5 days after left-sided pneumonectomy (a: magnification 12,000 and 108,000 ×; successive discharge of contents of osmiophilic lamellar body into lumen of alveolus (b, c, d; 41,000 ×). Here and in Fig. 3: LA) lumen of alveolus; ACII) type II alveolar cell; OLB) osmiophilic lamellar body; N) nucleus; M) mitochondrion; IF) intermediate form of transformation of multivesicular body into osmiophilic.

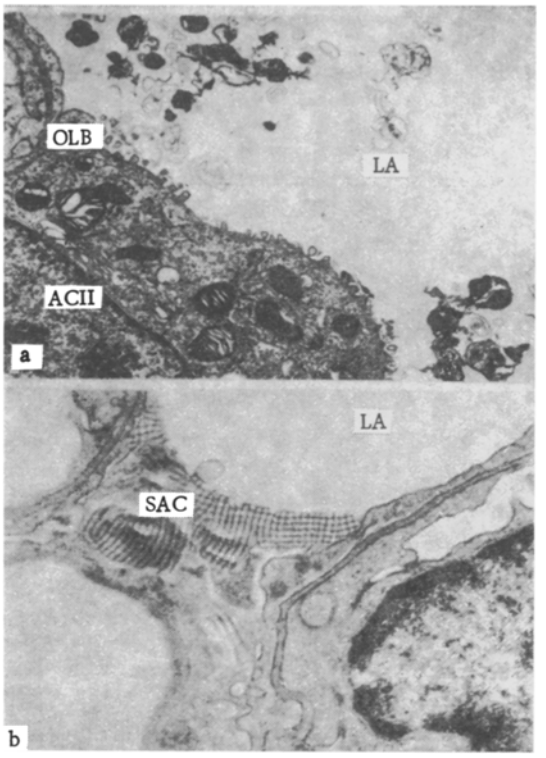

Fig. 3. Content of osmiophilic lamellar bodies of type II alveolar cells in lumen of alveolus (a); surfactant alveolar complex in niche between cells of alveolar epithelium (b). SAC) surfactant alveolar complex; 12,000×.

apical surface of the body, lying close to the plasmalemma (Fig. 2b). Under high power they could be seen to be bounded by a double membrane (Fig. 2a). The outer layer of the membrane bounding the evagination of the osmiophilic lamellar body comes into contact with the inner layer of the cell plasmalemma and fuses with it. In turn, the inner layer of the membrane of the evagination of the osmiophilic lamellar body fused with the outer layer of the cell plasmalemma, to form a hole. Through the hole the contents of the body escape into the lumen of the alveolus (Fig. 2c, d). Material destined for surfactant formation thus was discharged from the type II alveolar cells into the lumen of the alveoli as in the merocrine type of secretion, the possibility of which has been stated previously [9, 13]. The secreted material consists of double membranes, bound into a coil.

At the end of the first postoperative week osmiophilic material similar in structure to the contents of the osmiophilic lamellar bodies could be seen in the lumen of the alveoli more often than in the control (Fig. 3a). In those areas of the alveoli of the hypertrophied lung where the "surfactant alveolar complex" still remained, no difference in its structure from the control could be detected (Fig. 3b).

The alveolar surfactant of the hypertrophied rat lung in both the early and the late stages after removal of 37% of the functioning tissue thus remained relatively intact morphologically and functionally. Intracellular edema of the components of the air–blood barrier and the discharge of edema fluid into the lumen of the alveoli, which were most marked 24 h after left-sided pneumonectomy, were not reflected in the surface-active properties of washings from the lungs. The sharp increase in size of the alveoli observable on the fifth to seventh day after the operation was accompanied by an increase in the surface-active properties of the lung washings and increased secretion of material of the osmiophilic lamellar bodies of the type II alveolar cells into the lumen of the alveoli. In emphysema produced in rats by inhalation of a papain aerosol, a marked decrease in the surface tension of the washings also is observed [11]. It can accordingly be postulated that mechanical stretching of the alveolus and of its cellular lining is one of the stimuli which activates secretion of the intracellular surfactant from the type II alveolar cells. The cytological mechanisms

responsible for the increased output of surfactants consists of activation of lipid synthesis in the large alveolocytes, hypertrophy of these cells, and the appearance of binuclear cells.

The appearance of binuclear and enlarged (possibly polyploid) large alveolocytes during compensatory hypertrophy of the lung reflects a compensatory-reparative response of these cells to increased functional demands.

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