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EFFECT OF COLCHICINE ON SECRETION OF ALVEOLAR SURFACTANT IN THE INTACT AND REGENERATING RAT LUNG

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KEY WORDS: surfactant; secretion; colchicine; regeneration of the lung.

Synthesis of the combination of surface-active substances constituting the surfactant, located on the cellular lining of the alveoli of the lung and responsible for their stability, takes place in type II alveolocytes [6, 7]. It has been shown by electron microscopy that surface-active substances of phospholipid nature are present in the cell as osmiophilic lamellar bodies known as intracellular surfactant [3]. Under ordinary physiological conditions the surfactant is secreted from type II alveolocytes by a merocrine type of mechanism through exocytosis from the apical surface of the cells facing the alveolar lumen [4].

As recent investigations have shown, the level of secretion of surfactant can be modified by the action of various factors. Hyperventilation and other factors stimulate surfactant secretion [6, 8, 9]. Colchicine and vinblastine as a rule depress it [5, 6]. The intracellular mechanisms of regulation of surfactant secretion have not yet been adequately studied. It might be supposed that the restriction or stimulation of surfactant secretion are linked either with changes in the level of metabolism in the type II alveolocytes and the intensity of synthesis of phospholipids or with delay or acceleration of the release of surfactant from these cells into the alveolar lumen.

It was accordingly decided to study changes in the character and level of surfactant secretion under the influence of colchicine, which selectively inactivates the cytoplasmic microtubules that play an important role in the secretory process [10].

EXPERIMENTAL METHOD

Noninbred male rats weighing 220-250 g were used. The animals were divided into four groups. From the rats of group 1 63% of the weight of the lungs (the whole left lung and the diaphragmatic lobe of the right lung) was removed and at 1 p.m. on the 4th-5th day after the operation intramuscular injections of colchicine (from "Merck") began in a dose of 0.1 mg/100 g body weight per injection.

In the course of the 24 h before sacrifice each animal received six injections of colchicine at intervals of 4 h. The total dose of colchicine was 0.6 mg/100 g body weight. From the rats of group 2 63% of the weight of the lung tissue was removed, and physiological saline was injected intramuscularly (0.3 ml per injection). On the rats of group 3 a mock operation was performed and colchicine was injected in the same dose as that given to the rats of group 1. Animals of group 4 underwent the mock operation only. The rats of all groups were

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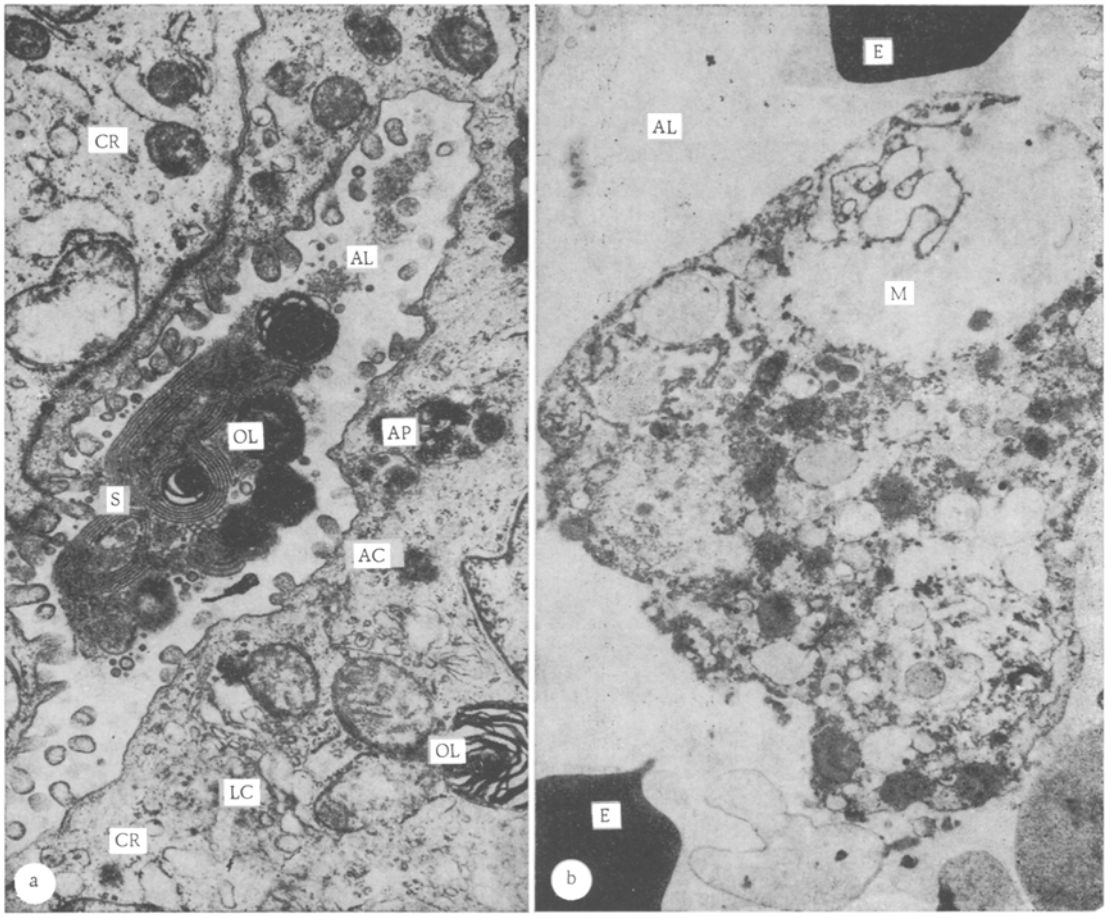

Fig. 1. Regenerating lung of rat receiving colchicine. Region of partial atelectasis: a) structure of type II alveolocyte and of intracellular surfactant released from cell into alveolar lumen (22,000 ×); b) erythrocytes and fragment of destroyed alveolar macrophage in lumen of alveolus (10,000 ×). AL) Alveolar lumen; S) surfactant; AC) type II alveolocyte; OL) osmiophilic lamellar bodies; LC) lamellar complex; AP) autophagosomes; M) macrophage; E) erythrocyte; CR) cytoplasmic reticulum.

killed at 10 a.m.-1 p.m. on the 5th-6th day after the operation. For electron microscopy, the lungs taken from the animals (three from each group) under pentobarbital anesthesia were fixed by perfusion with 2.5% glutaraldehyde solution in 0.1 M cacodylate buffer, pH 7.4, through the pulmonary artery under a pressure of 20-25 mm Hg [1]. Pieces of tissue measuring 1 × 1 × 1 mm were postfixed in 1% OsO_4 solution. The material was dehydrated in acetones of increasing strengths and the specimens were embedded in Epon-Araldite. Sections 40-50 nm thick were stained with lead citrate and examined in the TEM-100B microscope. The number of osmiophilic lamellar bodies was counted in the type II alveolocytes (50 cells in each group were examined) and their mean number per section through the cell in the region of the nucleus was determined.

EXPERIMENTAL RESULTS

At the time of sacrifice all rats in the experiments of groups 3 and 4 were still alive; of the eight rats of group 2, two died 24 h after the operation; of the six rats of group 1, three also died before sacrifice, i.e., immediately after injection of colchicine in a dose of 0.6 mg/100 g body weight. This suggests that combined action of colchicine, in the above dose, and the increased functional loads arising after extensive resection of the lungs proved to be incompatible with life in half of the experimental animals. The residual lung after extensive resection increased sharply in volume, evidence of the marked compensatory growth which was subjected to detailed quantitative analysis previously [1]. Against the background of hypertrophied alveolar passages and alveoli there were solitary small regions of partial and total atelectasis. In the regenerating lung under the influence of colchicine zones with collapsed alveoli were found more frequently; individual alveoli contained erythrocytes and homogeneous masses distributed as half-moons on the cell lining.

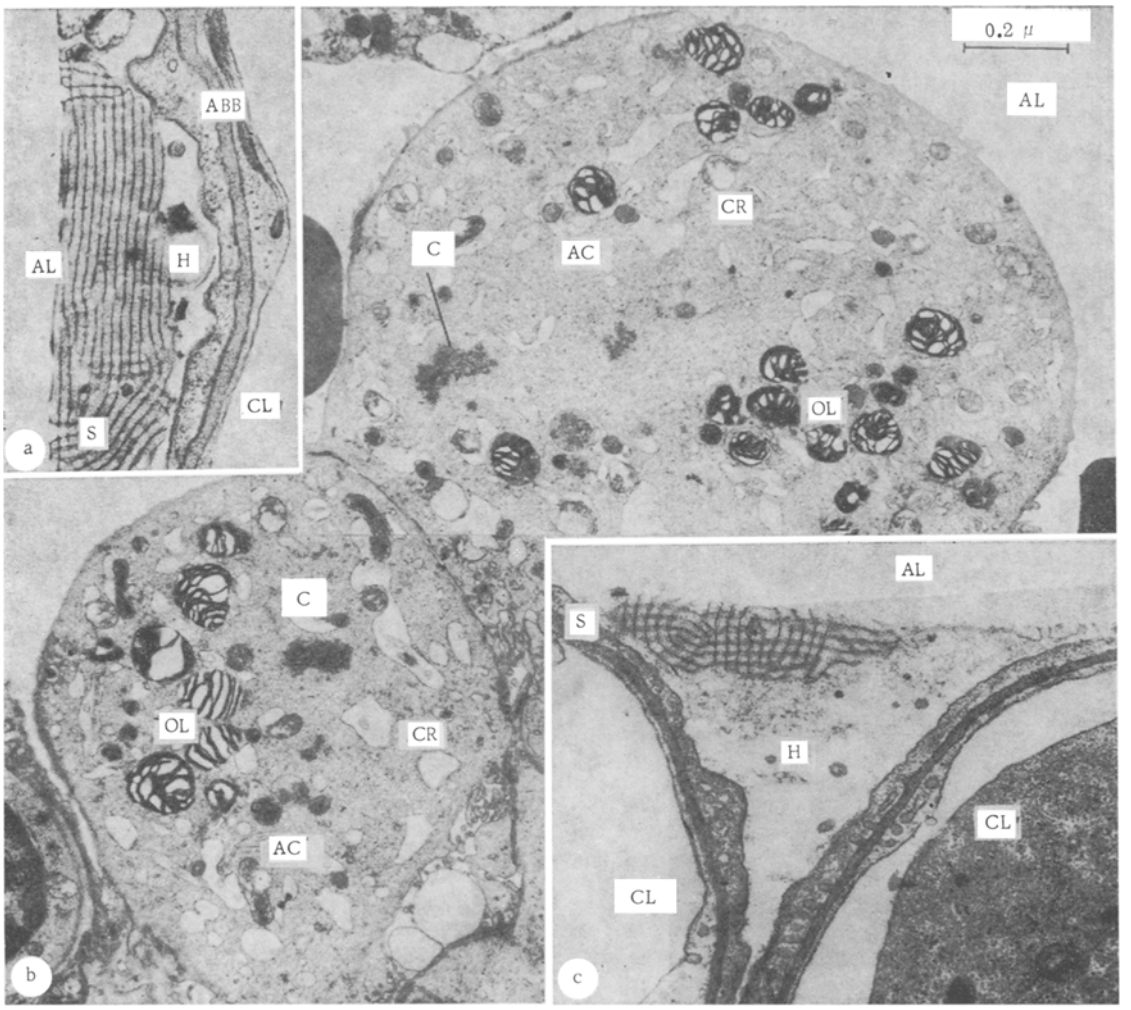

Fig. 2. Regenerating lungs of rat receiving colchicine. a, c) Structure of surfactant alveolar complex on surface of well-expanded alveoli, under magnifications of 37,500 and 34,500 respectively; b) type II alveolocyte in state of mitosis (8000×. CL) Capillary lumen; ABB) air–blood barrier; H) hypophase; C) chromosomes. Remainder of legend as in Fig. 1.

Over a wide area of the surface of the partially or totally collapsed alveoli membranous structures of intracellular surfactant could not be seen. In the slit-like lumen of these alveoli membranous formations with a succession of osmiophilic lamellar bodies, loose granular material of the hypophase, and alveolar macrophages at various stages of degeneration were occasionally seen (Fig. 1).

Extracellular surfactant covering the surface of most of the well-expanded and air-filled alveoli of the lungs of all the animals, including those receiving colchichine, were indistinguishable in their ultrastructural organization from the surfactant of normal lung the structure of which has been described elsewhere [2, 3, 4]. Extracellular surfactant could be seen both in the niches between the cells and in the region of "working" zones of the air–blood barrier. It consisted either of regularly oriented membranous formations or of a thin osmiophilic layer including one or several tightly packed membranes (Fig. 2).

Secretion of surfactant was increased in the aerated regions of the regenerating lung of rats not receiving colchicine: Osmiophilic lamellar bodies were frequently found in the alveolar lumen, on the surface of the cellular lining, and in the hypophase. Colchicine inhibited the release of intracellular surfactant from the type II alveolocytes to the alveolar lumen of both the intact and the regenerating lung. In the latter case the inhibitory action of colchicine was more clearly defined. Indirect evidence that surfactant secretion was depressed was given by the total or almost total absence of osmiophilic lamellar bodies in most phagosomes of the alveolar macrophages in the lungs of animals receiving colchicine (Fig. 1).

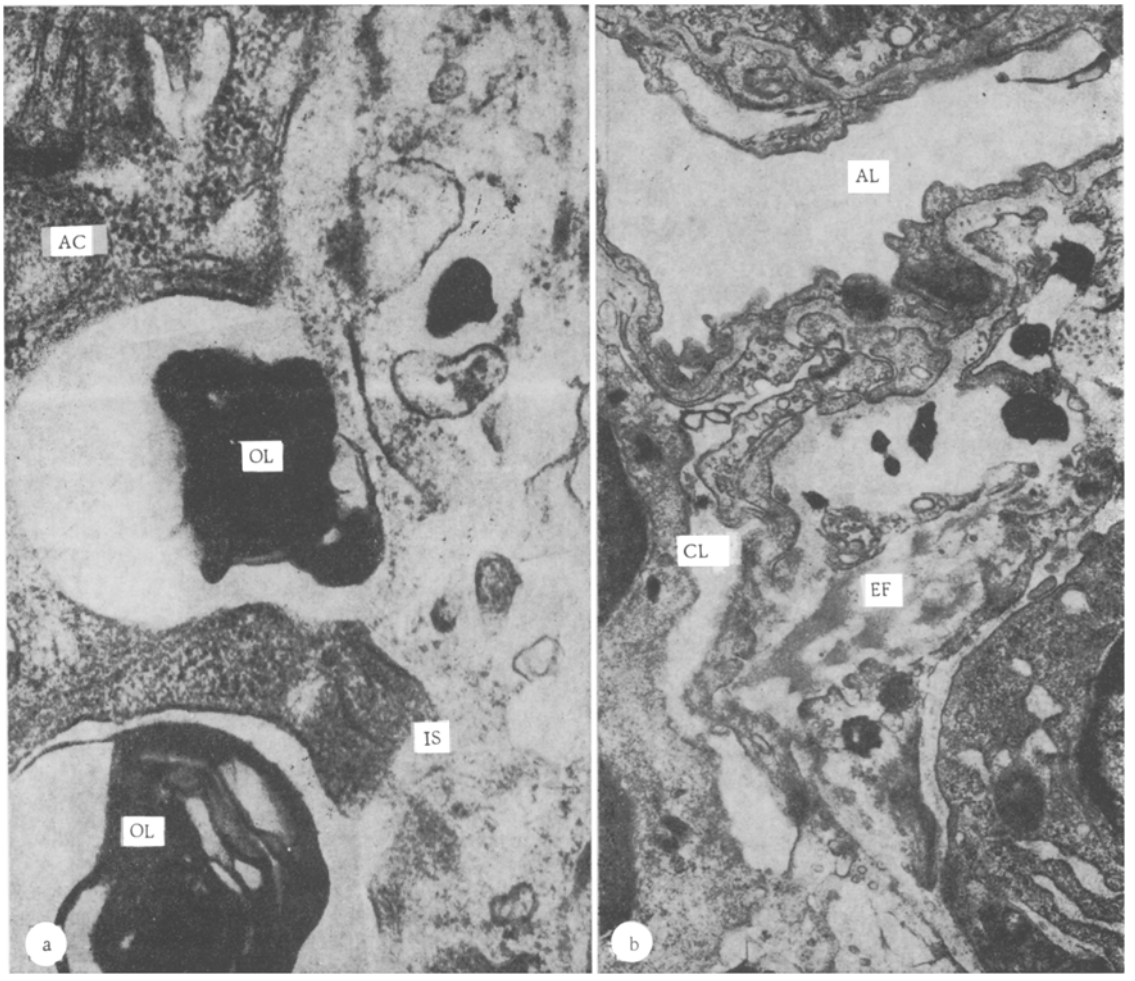

Fig. 3. Lung of control rat receiving colchicine: a) secretion of intracellular surfactant from basal part of type II alveolocyte into interstitial space (75,000 ×); b) osmiophilic lamellar material located in interstitial space and lumen of alveolar capillary (25,000 ×). IS) Interstitial space of alveolus; EF) elastic fibers. Remainder of legend as in Figs. 1 and 2.

Against the background of reduced surfactant secretion from the apical surface of the type II alveolocytes, release of osmiophilic lamellar material was observed from the basal part of these cells toward the interstitial space of the alveoli, where its presence was revealed by empty spaces due to traces of disintegration of collagen and elastic fibers of the alveolar septa (Fig. 3).

The ultrastructure of most type II alveolocytes was modified by the action of colchicine: The number of microvilli on the apical surface was reduced, the cisterns of the cytoplasmic reticulum were greatly dilated, vacuoles of the lamellar complex were enlarged, bundles of microfilaments appeared beneath the apical plasmalemma, and heterochromatinization of the nucleus was observed. However, the number of osmiophilic lamellar bodies (8-12 per section through the part of the cell containing the nucleus) in the lungs of all groups of animals varied within similar limits. Meanwhile, giant osmiophilic lamellar bodies, or autophagosomes, containing material of lipid nature were seen in some of the type II alveolocytes; cisterns of the cytoplasmic reticulum were filled with homogeneous osmiophilic material. These findings indicate changes in lipid metabolism in these cells. In the type II alveolocytes in a state of mitotic division, blocked by colchicine, there were a fairly large number (up to 20) of osmiophilic lamellar bodies, which can be regarded as specific markers of this type of cell (Fig. 2).

To sum up, the following conclusions can be drawn from the results. Colchicine lowers the level of surfactant secretion from the apical surface of the type II alveolocytes of the intact and regenerating lung and does not prevent the release of intracellular surfactant by exocytosis from the basal part of the cell. The basal type of surfactant secretion is evidently due to inactivation of the microtubules of the apical parts of the cytoplasm

and "overloading" of the type II alveolocytes by phospholipids, the rate and pathways of synthesis of which are modified under the influence of colchicine, but evidently only to an insignificant degree. These results indicate both a varied role of the cytoplasmic microtubules in the migration and secretion of surfactant in different compartments of the cell, and also the important role of the microtubules in the release of secretion from the apical surface of the type II alveolocytes.

The results described above suggest that lung pathology connected with the action of factors inactivating the cytoplasmic microtubules is based on a disturbance of the character and level of secretion of surfactant by type II alveolocytes.

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MECHANISMS OF NEUROTROPHIC DISTURBANCES IN SKELETAL MUSCLES CAUSED BY BOTULINUS TOXIN

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KEY WORDS: muscle cell; electrogenesis; neurotrophic regulation.

In botulism, disturbances of the electrogenic properties of striated skeletal muscles arise without any structural or ultrastructural changes in the neuromuscular synapses [4, 7]. Meanwhile in the fibers of muscles paralyzed by botulinus toxin (BT), histological investigations have revealed an increase in the number of cell nuclei [3, 5]. It was decided to study whether any causal relationship exists between changes in electrogenesis of fibers of skeletal muscles paralyzed by BT and the increase in the number of their cell nuclei under these conditions.

EXPERIMENTAL METHODS

Experiments were carried out on 20 male Wistar rats weighing 120-130 g. Local botulinus paralysis was induced by intramuscular injection of a sublethal dose of type A BT (0.05 mg/100 g body weight; 1 mouse MLD = 0.00005 mg) into the right (experimental) leg. The animals' left limbs served as the control. Inhibition of DNA synthesis in the muscle tissue was produced by daily (starting 24 h before intramuscular injection of BT) intraperitoneal injections of fluouracil, which blocks DNA synthesis, into the rats in a dose of 50 mg/kg [2] starting the day before intramuscular injection of BT. The experimental animals were divided into two groups. Only

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