altered fibers was characterized by the appearance of many neurofilaments and by a corresponding decrease in the number of mictotubules. Increased adhesion of the membranes was noted, with the appearance of submembranous aggregates and their association with nearby membranes. On the 10th day of stimulation a similar picture was observed. The ultrastructure of preparations obtained 10 days after the end of stimulation was similar to the control, except that in individual nerve fibers a slight degree of edema of the glioplasm still remained in the region of the nodes and clefts, and the vesicles remained rather more numerous than in the control. At the end of stimulation, a gradual return of the ultrastructure of the nerve fibers to its original state evidently takes place.

The nerve fiber is known to respond to any stimulation by a combination of structural changes, which are not specific in character, i.e., they follow a similar course irrespective of the character of stimulation [6]. The response of the neuron to stimulating agents and to pathological states also was investigated at the ultrastructural level. The changes developing under these circumstances also were considered to be nonspecific [1]. Reactive changes affecting both the axon and the glial cell were manifested, first, as destruction, of certain ultrastructures, principally mitocohodria, microtubules, and the endoplasmic reticulum, and second, as increased formation of others, manifested as a marked increase in the number of vesicles, lysosomes, and membrane bodies. Responses of the neuron and glia discovered in this investigation are also, evidently, nonspecific to stimulation by the acupuncture needle.

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EFFECT OF COLCHICINE AND PILOCARPINE ON SECRETORY ACTIVITY OF TYPE II ALVEOLOCYTES IN THE RAT LUNG

M. S. Pokrovskaya

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Under physiological conditions secretion of alveolar surfactant is of the merocrine type and takes place by exocytosis from the apical surface of type II alveolocytes (AII) [6]. Under the influence of colchichine, secretory activity from the apical surface of the cells is reduced and a basal type of surfactant secretion appears, i.e., the release of osmiophilic material from the basal region of the cell into the interstices [1]. Meanwhile, we know that pilocarpine, as a parasynpathomimetic, stimulates alveolar surfactant secretion from the apical surface of AII into the lumen of the alveoli [4, 5, 7].

With these considerations is mind it was decided to study how the simultaneous action of colchicine and pilocarpine affects the character of secretion of alveolar surfactant.

Laboratory of Pulmonology, Research Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow.

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TABLE 1. Number and Relative Bulk Density of CPL in AII of Control Animals and During Injection of Colchicine and Pilocarpine (M ± m)

Experimental conditions	Number of cells examined	Number of CPL per cell	Relative bulk density of CPL in cell, %
Control seven injections of physiological saline	33	10,12±0,82	10,6±1,1
Experiment 1. six injections of colchicine and one injection of physiological saline	61	10,00±0,65	14,0±0,8*
Experiment 2: Six injections of colchicine and one injection of pilocarpine	56	11,39±0,86	15,0±0,96**

Legend. *P < 0.05, **P < 0.01 compared with control. Differences between experiments not significant.

TABLE 2. Fraction (in %) of AII with Signs of Secretory Activity in Control Animals, in Animals Receiving Colchicine, and in Animals Receiving both Pilocarpine and Colchicine (M ± m)

Experimental conditions	Number of cells examined	Signs of apical secretion		Overall index of apical secretion	Signs of basal secretion		Overall index of basal secretion
		initial phase of apical secretion	outflow of osmiophilic material from apical surface of cell		outflow of osmiophilic material from basal surface of cell	arrangement of osmiophilic material alongside cell in interstitial space of alveolar septum	
Control: seven injections of physiological saline	33	9±5	9±5	18±7	0	0	0
Experiment 1: six injections of colchicine and one injection of physiological saline	61	5±3	0	5±3*	15±5*	37±6*	51±7*
Experiment 2: six injections of colchicine and one injection of pilocarpine	56	9±4	0	9±4	20±5*	27±6*	46±7*

Legend. P* < 0.01 compared with control. For all parameters differences between experiments not significant.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 180-200 g, divided into four groups. Rats of group 1 (n = 5) were given an intramuscular injection of colchicine (Merck, West Germany) in a dose of 0.1 mg/100 g body weight six times a day, at intervals of 4 h (starting at 1 p.m.). The animals were given an intraperitoneal injection of pilocarpine in a dose of 8 mg/100 g 30 min before sacrifice. Rats of group 2 (n = 4) were given colchicine at the same time and in the same dose, but an intraperitoneal injection of physiological saline 30 min before sacrifice. Rats of group 3 (n = 5) were given intramuscular injections of physiological saline at the same times as colchicine and pilocaprine, and in the same volume. Rats of group 4 (n = 3) were given six injections of physiological saline followed by pilocarpine, 30 min before sacrifice, in the dose mentioned above. For electron-microscopic investigation the lungs of the control and experimental animals were fixed in a 2.5% solution of glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) and postfixed in 1% OsO_4 solution. Ultrathin sections were examined under the JEM-100B and JEM-100S electron microscopes. The number and relative bulk density of osmiophilic lamellar bodies (cytophospholiposomes — CPL) were determined in sections through AII containing the nucleus and apical microvilli [2]. The number of cells showing signs of apical and basal secretion of surfactant material was counted. The results were subjected to statistical analysis by the Fisher–Student test.

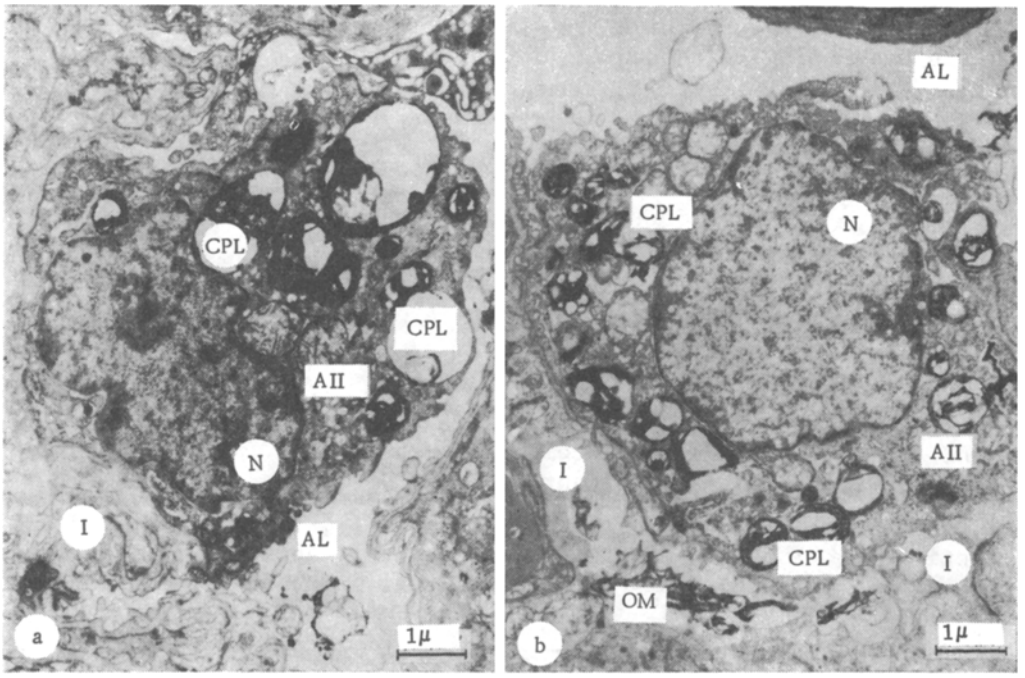

Fig. 1. AII in lung of rat receiving colchicine: a) enlarged CPL can be seen in cytoplasm of AII (100,000 ×); b) concentrations of osmiophilic material the result of basal secretion of AII, can be seen in the interstitial space of the alveolar septum (8000 ×). AL) Alveolar lumen; I) interstitial space of alveolar septum; N) nucleus; OM) osmiophilic material.

EXPERIMENTAL RESULTS

The mortality of the animals receiving six injections of colchicine at intervals of 4 h was 29.4% (five of the 17 animals used in the experiments died). Against the background of colchicine, whether given with or without pilocarpine, local edema of the type I alveolocytes and of the endothelium of the blood capillaries of the air-blood barrier was oberved, but the integrity of the cells was unimpaired.

The number of CPL in AII of the control animals (receiving seven injections of physiological saline) averaged 10-12, and their relative bulk density was 10.6% (Table 1). AII contained large CPL appeared in the animals receiving colchicine, and in some cells their fusion was observed (Fig. 1a). The number of CPL per AII was almost unchanged by the action of colchicine compared with the control (Table 1). However, their relative bulk density increased significantly by 32% compared with the control, evidence of hypertrophy of CPL and delayed secretion of surfactant material from the cell under the influence of colchicine. The number and relative bulk density of CPL per AII under the influence of colchicine and pilocarpine simultaneously did not differ significantly from values obtained with colchicine alone.

Signs of apical secretion were observed much less frequently in AII under the influence of colchicine than in the control. For instance, the outflow of osmiophilic material from the apical surface of the cell was not observed in any of 61 cells in the experiment (Fig. 2a; Table 2). The initial stage of secretion (formation of an evagination of CPL on the apical surface of the cell) was observed 1.8 times more often in the control than under the influence of colchicine (Fig. 2b). The over-all indices of apical secretion (Table 2) are evidence that colchicine depresses the level of apical secretion of surfactant in AII. These data point to the participation of cytoplasmic microtubules in extrusion of the secretion.

Under the simultaneous influence of colchicine and pilocarpine a tendency was observed for the number of AII with signs of apical secretion to increase compared with the experiment in which colchicine alone was given (Table 2). Pilocarpine thus potentiates a little the initial phase of apical secretion, despite the inhibitory action of colchicine. Quantitative analysis of the scale of basal secretion of AII showed that under the influence of colchicine 51% of the cells had direct and indirect evidence of basal secretion (Table 2). By

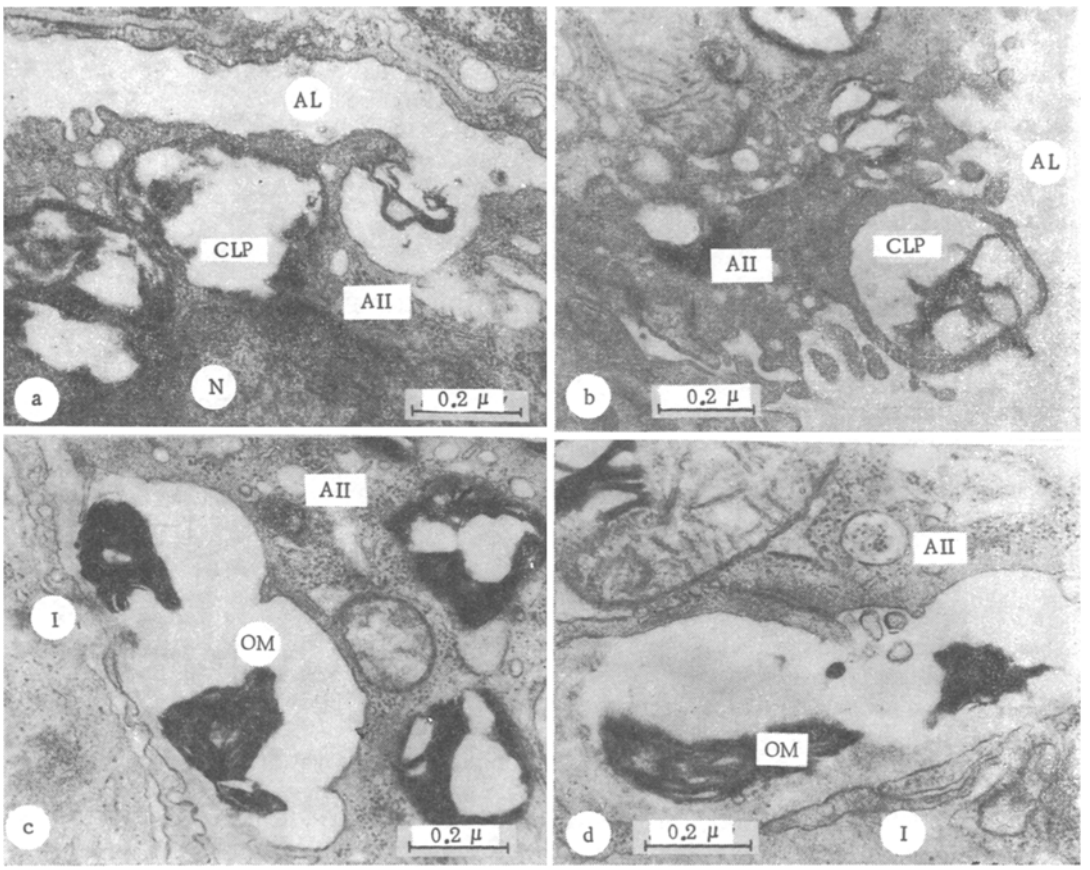

Fig. 2. Signs of secretory activity in AII: a) extrusion of surfactant into alveolar lumen from apical surface of AII (70,000 ×); b) initial stage of secretion — formation of evagination of CPL on apical surface of AII (70,000 ×); c) outflow of osmiophilic surfactant material from basal surface of AII into interstitial space of alveolar septum (85,000 ×); d) arrangement of osmiophilic material in interstitial space alongside plasmalemma of AII (85.000 ×). Remainder of legend as to Fig. 1.

direct evidence of basal secretion we imply the outflow of osmiophilic material from the basal or lateral surface of the cells (Fig. 2c), and by indirect — the arrangement of osmiophilic material in the interstitial space of the alveolar septum, in the immediate vicinity of the plasmalemma of AII (Fig. 2d).

Basal surfactant secretion is exhibited when the body is exposed to certain extremal factors, notably acute general hypo- and hyperthermia. The mechanism of the basal secretion is not clear. It is evidently not directly connected with the apparatus of the cytoplasmic microtubules, which are damaged by the action of colchicine [8] and of low temperatures [3].

The level of basal secretion under the influence of a combination of pilocarpine and colchicine did not differ significantly from the level observed with colchicine alone (Table 2).

When the cytoplasmic microtubules are destroyed through the action of colchicine, a "switching" of surfactant secretion from the apical to the basal type thus takes place in AII. Pilocarpine, which stimulates apical secretion, if injected after colchicine potentiates the initial phase of apical secretion somewhat, but does not activate basal secretion. It can therefore be tentatively suggested that the basal type of secretion in AII and, to some extent also, the apical type is not mediated entirely through the function of the microtubules.

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ULTRASTRUCTURE OF ALVEOLAR MACROPHAGES OF THE LUNGS DURING PERORAL ADMINISTRATION OF NITROSODIMETHYLAMINE

V. V. Fetisov, N. N. Litvinov, and Z. M. Gasimova

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KEY WORDS: alveloar macrophages; nitrosodimethylamine; lavage; cellular destruction

The alveolar macrophages of the lungs (AML) constitute a barrier system of the body, maintaining its resistance to unfavorable environmental factors and, in particular, the action of inhaled substances. Their role in reactions of the body to toxic chemicals assimilated via the gastrointestinal tract has received less study. Experiments on rats have shown that during peroral administration of nitrosodimethylamine (NDMA) the functional state not only of the intracellular organelles of the liver — the target organ [3], but also of AML is disturbed [2, 5]. The number of AML is reduced, their viability is impaired, their surface architectonics and their ability to adhere and to spread out in a layer are modified. The study of AML in recent times has increasingly involved the use of lavage (flushing out AML from the air passages of the lungs), both in experiments on animals [6, 7, 10] and in the practive of pulmonology, for diagnostic and therapeutic purposes [1, 8, 9]. A mass of cytological data on the cell composition and morphology of cells flushed out of the human lungs, mainly of patients, and from the lungs of experimental animals of different species, is accumulating.

The aim of this investigation was to study changes in the ultrastructure of AML obtained by lavage from intact rats and rats receiving NDMA by gastric tube.

EXPERIMENTAL METHOD

AML were obtained by flushing out the lungs of two groups of noninbred male albino rats — one intact, the other receiving a single dose of NDMA by gastric tube in a concentration of 30 mg/kg body weight 3, 12, and 24 h beforehand. Under pentobarbital anesthesia 5 ml of physiological saline was injected by means of a syringe through a tracheostomy into the lungs. The liquid was aspirated from the lungs 15 min later and a second injection of the same volume of physiological saline was given. The procedure was repeated six times. The washings, the final volume of which was 25-30 ml, were centrifuged for 10 min at 1500 rpm. The residue was dehydrated in alcohols of increasing concentration and embedded in polyethylene capsules in a mixture of Epon and Araldite. Ultrathin sections were cut on an LKB-III Ultrotome, stained with lead and uranium salts, and examined in the Hitachi H-300 microscope (Japan).

EXPERIMENTAL RESULTS

Great polymorphism of the AML was discovered in the intact animals. The cells differed in size (their diameter varied from 6 to 25 μ), the abundance of their cytoplasmic processes and intercellular organelles, the electron density of their cytoplasm, and the configuration

A. N. Sysin Research Institute of General and Communal Hygiene, Academy of Medican Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. I. Sidorenko.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 1, pp. 114-117, January, 1987. Original article submitted May 16, 1986.

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