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ELECTRON-MICROSCOPIC DEMONSTRATION OF CALCIUM IONS AT DIFFERENT STAGES OF SURFACTANT FORMATION IN NORMOTHERMIA AND HYPOTHERMIA

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Calcium participates in the integrative reactions of the body [2, 7, 11, 13, 14], including in phospholipid, protein, and carbohydrate metabolism [1, 5]. Calcium ions in lung tissues regulate the interstitial fluid pressure [9, 12]. The discovery of large numbers of calcium ions in washings from the bronchi and also in the amniotic fluid in the final stages of formation of the fetus [8, 15] probably indicates their active participation in surfactant formation.

The aim of this investigation was to study the localization and intensity of accumulation of calcium ions at the sites of formation of osmiophilic lamellar bodies (OLB) and their release from type II alveolocytes, under both normothermic and hypothermic conditions, because surfactant formation in the alveolocytes is intensified at low temperatures [3, 4].

EXPERIMENTAL METHOD

The lungs of 20 chinchilla rabbits were investigated. Ten rabbits constituted the control group. The remaining animals were cooled during a single session in a "Feurton-3101-01" climatic chamber for 3 h at − −30°C. Calcium ions were revealed in lung tissue (cardiac lobe) by the electron-histochemical method [6, 10], after preliminary perfusion fixation, with a control to verify absence of ammonium oxalate in the medium. Acid mucopolysaccharides were detected with ruthenium red, after immersion of the lung tissue in fixative [11]. A scanning cytophotometer, an improved version of the "Impulse analyzer" attachment for the UEM-100A electron microscope, was used for microscanning. The electrical signal during scanning (magnification (10,000) was led from the photomultiplier to a matching amplifier and integrator, where summation of the values of optical density took place, the result being proportional to the density of that part of the picture being analyzed under the electron microscope. The transformed analog signal was recorded on a "Konsul-254" printer. Eight gradations of density of the test object (from 0 to 7) were used in the investigation, the background of the object corresponding to "7" and the part of the specimen with the highest electron density corresponding to "0." To estimate activity of the histochemical reaction the overall density was calculated over the range from 0 to 4. For volume analysis of the OLB by the dot counting method, a Weibel's grid was used.

EXPERIMENTAL RESULTS

Histochemical reaction products were found in the form of small round electron-dense granules 12-15 nm in diameter, or of an area of dust-like granularity. Within the alveolar lining the granules of the histochemical reaction for calcium ions were found in small numbers in type I alveolocytes. The reaction products were rather more abundant in lung macrophages, especially in those regions of the cytoplasm where phagocytosis was in progress. The reaction for calcium ions took place most intensively in the cytoplasm of the type II alveolocytes (Fig. 1). The distribution of granules of the end products of the histochemical reaction for

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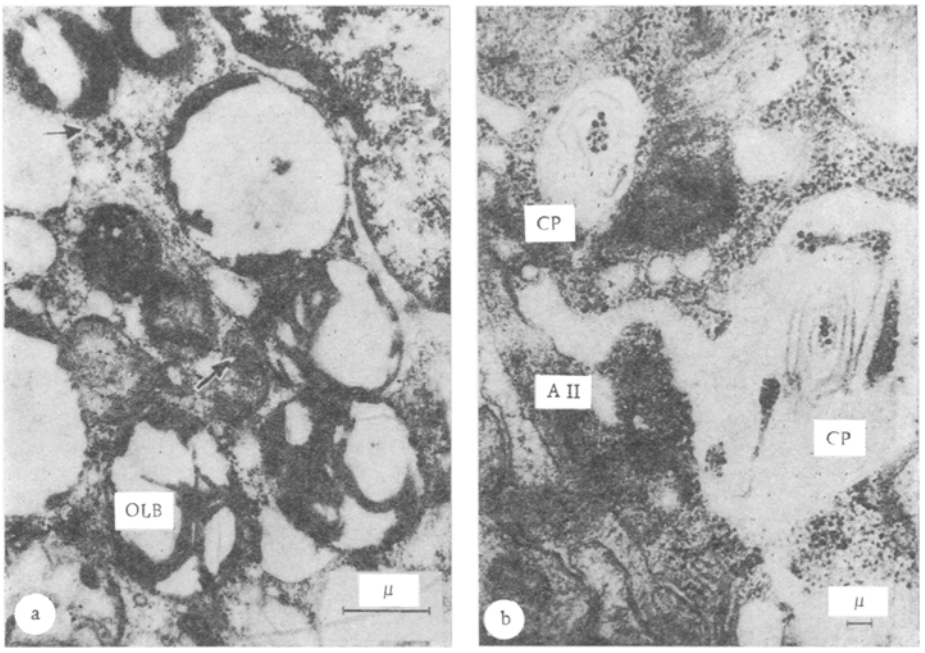

Fig. 1. Reaction for calcium ions in cytoplasm of type II alveolocytes. a) End product of reaction for calcium ions in type II alveolocytes (AII). Electron-dense material present as concentration of granules in cytoplasm, and detectable in OLB (12,000 ×); b) localization of products of reaction for calcium in type II alveolocyte (75,000 ×). CP) Calcium packets; OLB) osmiophilic lamellar bodies.

TABLE 1. Total Number of Extrusions of OLB in Type II Alveolocytes of Rabbit Lungs in Normothermia (A) and Hypothermia (B) (mean data for 100 cells)

Parameter	A	B	P
Number of OLB in one type II alveolocyte	19,0±2,0	23±2,0	0,05
Number of extrusions of OLB in one type II alveolocyte	0,2±0,04	0,4±0,06	0,05

Ca^{++} ions was heterogeneous. In some places concentrations of 30 to 40 granules had formed, and around them the membrane of the endoplasmic reticulum had begun to fold concentrically. These structural formations attained a length of 1 μ and a thickness of up to 0.1-0.2 μ. They were conventionally called "calcium packets" (CP). As a rule they began to form close to the nuclear membrane. In this zone they were small and the reaction product for Ca^{++} ions was most frequently seen as pale dust-like granules. The increase in the volume of CP took place on account of growth around the osmiophilic membranes, in concentric layers one above the others. High activity of the histochemical reaction for calcium ions was observed in the newly formed OLB (especially in the "nucleus" of the formation). The following rule was noted: If the "nucleus" of the OLB lies close to its common membrane, calcium oxalate will be present in the form of large electron-dense granules. The further from its common membrane the "nucleus" of the OLB lies, the smaller the granules of reaction product. With an increase in the size of OLB the histochemical reaction products began to be concentrated more often beneath the common membrane, forming a distinctive band below the membrane, consisting of small calcium granules 40-50 nm thick. Highest activity of the histochemical reaction for calcium ions was found to be characteristic of OLB with a volume of over 5 μ^3 (17.5 conventional units), bodies up to 3 μ^3 in volume (15.0 conventional units) were less active, and no reaction product for calcium ions was present in OLB with a volume of under 3 μ^3 (Table 1). The greater the diameter of the body, the wider the band of the histochemical reaction for calcium ions. Meanwhile acid glycosaminoglycans were discovered in these areas by means of

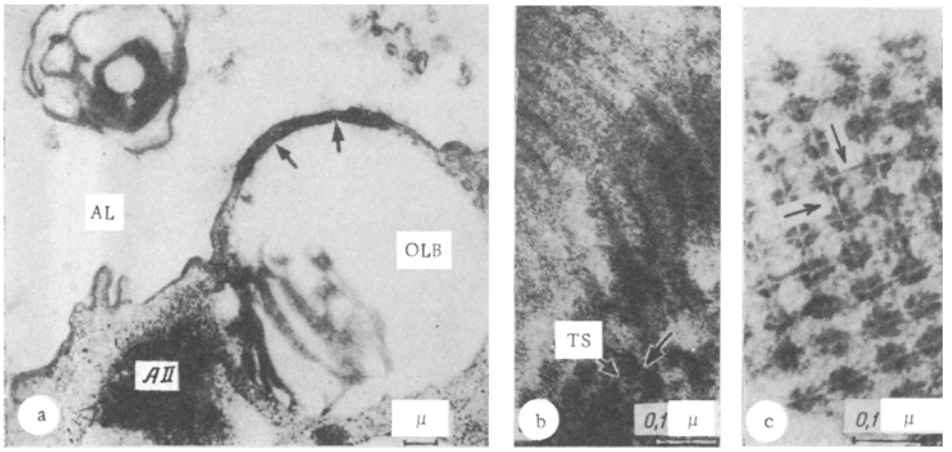

Fig. 2. Distribution of products of reaction for calcium ions and for mucopolysaccharides in cytoplasm of type II alveolocytes. a) Site of extrusion of OLB into alveolar lumen (AL), which contains finely granular product of reaction for calcium (55,000 ×). b) Fragment of tubular surfactant (TS), electron-dense material revealed in corners and in center of lattice-squares (100,000 ×); c) lattice-squares of TS are filled with mucopolysaccharides (revealed by ruthenium red, 125,000 ×).

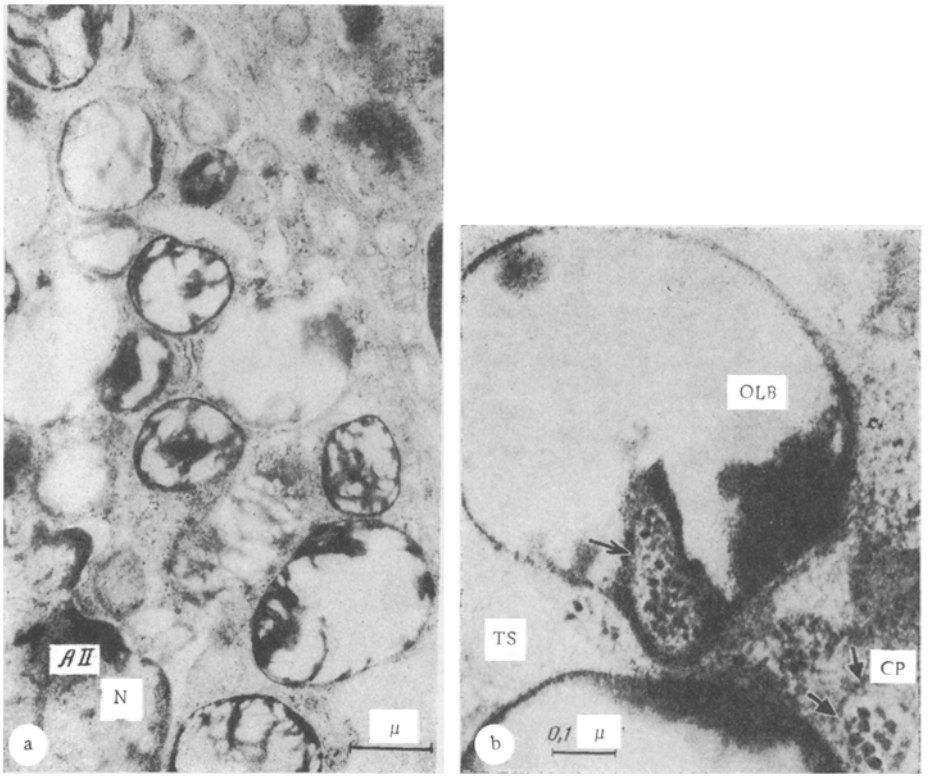

Fig. 3. Distribution of granules of end product of reaction for calcium ions in type II alveolocytes (AII) in hypothermia. a) Number of OLB labeled with electron-dense material in type II alveolocyte is increased (18,000 ×); b) increase in number of CP with calcium oxalate granules (75,000 ×). N) Nucleus.

ruthenium red. Not all myelin bodies showed an intense reaction for calcium ions. OLB of different diameters and "calcium-free" OLB were found. The closer the OLB to the apical surface of the type II alveolocytes, the larger its membranes band consisting of products of the histochemical reaction for Ca^{++} ions, especially at the point of contact with the cell membrane of the type II alveolcytes (Fig. 2a).

TABLE 2. Results of Scanning Cytophotometry of Calcium Ions (in conventional units) in OLB of Type II Alveolocytes in Rabbit Lungs during Normothermia (A) and Hypothermia (B)

Volume of OLB, μ^3	Calcium ions		
	A	B	Control
Over 5.0	17,5±3,2	83,3±4,0	0
From 3.0 to 5.0	15,0±1,3	16,34±7,2	0
Under 3.0		1,0±0,3	0

Legend. Density of histochemical reaction products for calcium ions measured on scanning cytophotometer of UÉM-100A electron microscope.

Calcium ions probably bind phospholipids of the cell membrane and OLB in this area. The OLB, projecting above the general surface of the type II alveolocyte, lifts the cell membrane, which is thinner in this area, after which it breaks, and extrusion of the OLB into the lumen of the alveolus takes place. After release of the OLB from the type II alveolocytes and the formation of tubular surfactant (TS), the participation of calcium ions in this process also can be observed. The dust-like granules in the cross section of TS, the product of the histochemical reaction for calcium ions, form "crossing lines" connecting the phospholipid walls diagonally in each lattice-square (Fig. 2B). The lattice squares are filled with acid mucopolysaccharides, clearly distinguishable with the aid of ruthenium red on electron-microscopic analysis, which was carried out on the same material (Fig. 2c). The hydroxyl groups of the glycosaminoglycans evidently interact strictly regularly with calcium ions to form the crossing lines of TS.

In hypothermia the intensity of formation of OLB, labeled with calcium oxalate, in the type II alveolocytes increased (Fig. 3). The number of sites of CP formation and extrusions of OLB became much greater under these conditions (Table 1). The quantity of products of the histochemical reaction for calcium in OLB increased. For instance, in bodies with a volume of over 5 μ^3 the intensity of the reaction was 83.3 conventional units (c.u.), for bodies between 3 and 5 μ^3 in volume it was 16.34 c.u., and in those under 3 μ^3 in volume the calcium oxalate granules had a density of under 1 c.u. (Table 2). In the control experiments, when ammonium oxalate was not present in the incubation medium, no end product of the reaction was detected.

Histochemical demonstration of calcium ions in the respiratory compartment of the rabbit lung thus showed that granules of the end product of the histochemical reaction are mainly located in type II alveolocytes. During hypothermia the intensity of the reaction increased. Calcium ions were found most clearly at sites of formation and extrusion of OLB, and also in sites of TS formation.

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