DETECTION OF THE MUCOPOLYSACCHARIDE COMPONENT OF THE SUPRAPLASMALEMMAL COVERING OF CELLS LINING THE ALVEOLI OF THE RAT LUNG

L. K. Romanova and A. K. Boikov

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The mucopolysaccharide component of the supraplasmalemmal covering (glycocalyx) of the alveolar epithelial cells was detected by electron microscopy of the lung fixed with solutions containing ruthenium red (RR) via the pulmonary artery. This glycocalyx consists of an electron-dense layer extending over the whole of the alveolar epithelium on the side of the alveolar air space. The thickness and configuration of the glycocalyx vary in different cells and on the surface of different sides of the same cell. The mucopolysaccharide component of the supraplasmalemmal covering of the type I alveolar cells participates in the active transmembrane transfer of substances through the air-blood barrier. No hypocalyx could be detected on the surface of the alveolar capillary endothelium or within the pinocytotic vesicles found in the cytoplasm of these cells, or on the surface of the plasmalemma of the alveolar epithelial cells facing their basal plate; this was due either to its absence or to its exceedingly small thickness.

It has been shown by a combination of histochemical methods and electron microscopy that a covering consisting of glycoproteins and mucopolysaccharides, described by Bennet as the glycocalyx [1, 4, 7, 11, 12], exists on the plasmalemma of most cells.

According to some workers' observations [8, 14], the supraplasmalemmal covering of the cells lining the alveoli of the mammalian lungs are complex and consist of two component parts: 1) a monomolecular phospholipid surface-active film or surfactant, in contact with the gaseous medium of the air space of the alveoli and determining their surface tension; 2) a hydrophilic layer or hypophase, in close contact on one side with the cell membrane of the alveolar cells and on the other side with the surfactant. This layer is actually the glycocalyx of the alveolar cells of the lung. Despite the comparatively many investigations of the electron-microscopic and biochemical characteristics of the surfactant, information on the layer in direct contact with the plasmalemma of the alveolar cells is scanty [3, 5, 6, 9, 10]. Much regarding the character of the localization, structure, and function of this layer remains unexplained.

The objects of the investigation described below were to detect a mucopolysaccharide component of the supraplasmalemmal covering of the alveolar cells of the lung and to examine its role in transmembrane transport through the air-blood barrier.

EXPERIMENTAL METHOD

The dye ruthenium red (RR) is known to bind with acid-substituted biopolymers, including the acid mucopolysaccharides [11]. Admittedly, the specificity of the reaction between RR and the acid mucopolysaccharides is only relative, as was shown by a special investigation of this problem by Luft [11]. However, the use of additional controls and preliminary treatment of the material with various enzymes (cho-

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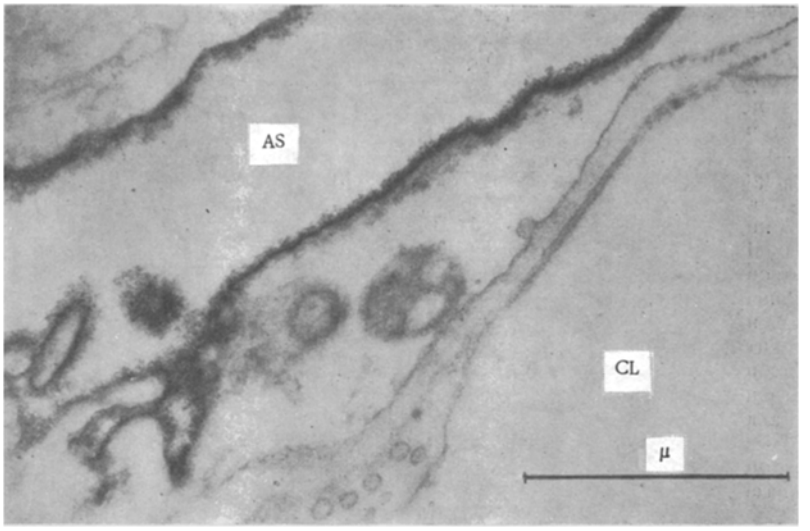

Fig. 1. Air-blood barrier. Reaction product with RR located on plasmalemma of cytoplasmic processes of type I alveolar cells. AS) alveolar air space; CL) capillary lumen, 55,000×.

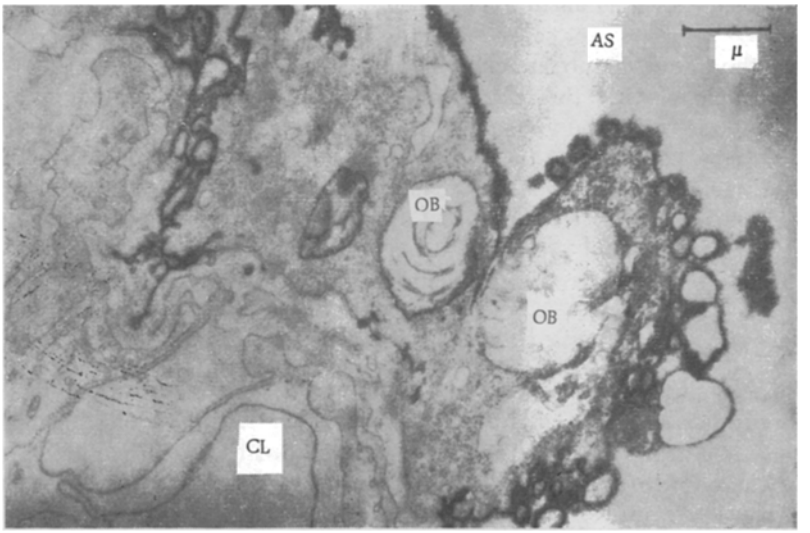

Fig. 2. Localization of reaction product with RR on surface of type II alveolar cell. OB) osmiophilic bodies, 18,200×. Remainder of legend as in Fig. 1.

lagenase, hyaluronidase, neuraminidase), incorporated in certain investigations [7, 11, 13], demonstrate conclusively that the reaction product with RR is nothing more than acid mucopolysaccharides with some contamination by sialomucin, so that this dye can be used to detect these substances during electron microscopy.

The lung tissues of four noninbred male rats weighing 150-180 g were treated successively with solutions of glutaraldehyde with RR (mixture No. 1) and OsO_4 with RR (mixture No. 2), prepared by Luft's formula [11]. Two different methods of fixation were tested. 1) Under deep pentobarbital anesthesia the heart-lung preparation was isolated. Pieces of lung tissue measuring 1-2 mm were fixed in accordance with Luft's scheme [11]. The tissue was dehydrated in alcohols of increasing concentration and propylene oxide. Rapid embedding of the material in Durcupan was carried out by Morozov's method [2].

2) According to Luft RR does not penetrate through the undamaged cell plasmalemma. Knowing the size of the RR molecule (11.3 Å), it was decided to use it as an appropriate marker for determining the permeability of the membranous structures of the air-blood barrier of the normal lung. To test whether

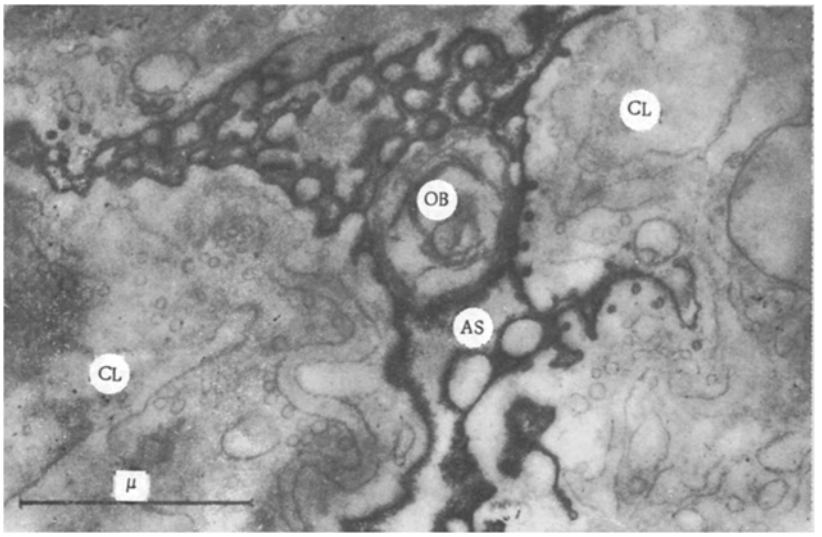

Fig. 3. Localization of reaction product with RR in pinocytotic vesicles and invaginations of plasmalemma of type I alveolar cells on surface of two neighboring cells. Osmiophilic body in lumen of alveolus. Legend as in Fig. 1, 48,400 ×.

RR could pass through the intact membranous components of the barrier it was injected into the blood stream of the organ. In animals anesthetized with pentobarbital and with the trachea ligated in inspiration, mixture No. 1 was injected through the vessels of the pulmonary arterial system under a pressure of 25-35 cm Hg for 10 min. From 20-25 ml of the fixative was used to perfuse the lungs of each animal. The heart-lung preparation was then immersed for 45-50 min in mixture No. 1 at room temperature. Pieces of tissue (1-2 mm) of these lungs were treated and embedded in Durcupan in the same way as during fixation by the first method. Sections 400-500 Å in thickness were cut on a Reichert's microtome and examined without staining in UEMV-100V electron microscope.

EXPERIMENTAL RESULTS

Electron microscopy of the lung tissue fixed in pieces, i.e., by the first method, revealed the reaction product with RR only in a few sections, close to the surface of the block, because of the comparatively shallow penetration of RR into the tissue. Consequently, the ordinary method of fixation of the lung when treated with RR for electron microscopy has certain limitations, and these must be taken into account if it is used.

After perfusion of the lung with fixing solutions containing RR, the reaction product with it could be clearly seen over the whole extent of the alveolar epithelium as an electron-dense layer lying immediately on the surface of the plasma membrane of the types I and II alveolar cells. The configuration and thickness of this layer differed in different parts of the alveolar epithelium.

On the plasmalemma of the type I alveolar cells and their cytoplasmic processes, facing the lumen of the alveoli, except at the points of intercellular contact, the outlines of the electron-dense layer were either smooth or wavelike; i.e., they reflected the character of the outlines of the plasmalemma of those cells. At sites of invagination of the plasmalemma and in the pinocytotic vesicles in contact with it or lying freely in the cytoplasm of the type I alveolar cells a substance reacting with RR also was present (Fig. 1). Sometimes single round vacuoles, surrounded by a double membrane and an adjacent electron-dense layer, were found directly on the surface of the type I alveolar cells in the lumen of the alveolus. As a rule the connection between this layer and the main electron-dense layer covering the plasmalemma of the cells could be identified. There is therefore good reason to suppose that the vacuoles observed are nothing more than transverse or oblique sections through the cytoplasmic processes of the type I alveolar cells.

The reaction product with RR located on the surface of the type II alveolar cells reproduced exactly the course of the plasmalemma of these cells, reflecting the outlines of transversely and obliquely cut microvilli which are numerous on these cells (Fig. 2). The osmiophilic lamellar bodies located both in

the cytoplasm of the type II alveolar cells and in the lumen of the alveoli had a distinctive appearance: only the outlines of the particles remained and their characteristic osmiophilic lamellae were absent; within the particles only shapeless zones of an amorphous substance of low electron density remained. These findings indicate that the saturated phospholipids, the principal component of the lamellae of the osmiophilic bodies, were eluted by fixation under the conditions adopted.

The localization of the reaction product with RR in the intercellular spaces of the alveolar epithelium clearly reflected the configuration of these zones, the pattern of which sometimes resembled a network of merging cytoplasmic processes of the type I alveolar cells or microvilli of two adjacent type II alveolar cells. A tangential section through the microvilli of type II alveolar cells, the number of which is somewhat increased in the region of intercellular contact, is illustrated in Fig. 3. As a rule the electron-dense masses did not reach the basal plate in the region of two neighboring cells of the alveolar epithelium, suggesting that the glycocalyx is absent on the plasmalemma of the basal portions of the lateral surfaces of these cells.

No reaction product with RR could be detected on the plasmalemma of the alveolar capillary endothelium, inside the pinocytotic vesicles present in the cytoplasm of these cells, or on the surface of the alveolar epithelial cells facing their basal plate.

Fixation of the lung by perfusion through the pulmonary arterial system with solutions containing RR, in conjunction with electron microscopy, thus provides a sufficiently complete picture of the topography of the reaction product with this dye and reveals a mucopolysaccharide component (glycocalyx) on the surface of the plasmalemma of the alveolar cells. The continuity of the glycocalyx over the whole extent of the alveolar epithelium on the side of the alveolar air space is evidence that RR molecules can diffuse through the intact membranous structures of the air-blood barrier. Considering that RR is a hexavalent cation which diffuses with difficulty through the pores of the cytoplasmic membrane, which carry a negative charge, its active transport through all the membranous components of the barrier can be postulated.

The thickness of the glycocalyx on the surface of the alveolar epithelial cells varies from 200 to 600 Å, its predominant thickness being about 300-400 Å. It can differ on the surface of different sides of the same cell. These observations agree with those made by other workers [8, 12, 13].

Under high power of the microscope three zones composing the glycocalyx of the alveolar epithelial cells can be distinguished: 1) an electron-dense homogeneous layer lying next to the plasmalemma (30-40 Å); 2) an electron-dense granular layer (≈150-180 Å), and 3) an amorphous layer of average electron density (≈120-150 Å).

The appearance of the reaction product with RR in pinocytotic vesicles of the type I alveolar cells and its absence from the pinocytotic vesicles of the endothelium of the lung capillaries suggest the selective role of the supraplasmalemmal mucopolysaccharides in transmembrane transport, the degree of which is evidently determined primarily by the specific functional features of the organ, the histophysiological characteristics of its cells, and the physicochemical properties of the substance which has to pass through the plasmalemma into the cell.

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