smooth muscles can be regarded as optimal evidently because this activity fluctuates during digestion within certain limits: between maximal activity in the period of work and minimal activity in the period of rest outside digestion.

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FUNCTIONAL ROLE OF LUNG SURFACTANTS

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Solutions of surfactants freshly prepared from the rat lung (LS) accelerate absorption of oxygen on the phase boundary. Oxidized solutions of LS have an inhibitory action. It is postulated that the functional role of LS in external respiration is not confined to the lowering of surface tension and stabilization of the alveoli, but also involves participation in the absorption of oxygen and regulation of its transport through the air–blood barrier.

KEY WORDS: lung surfactants; surface-active substances; oxygen transport.

Lung surfactants (LS) play several important functions in external respiration. It has been shown that surfactants lower surface tension and reduce the work required for ventilation of the lungs, stabilize the alveoli, and prevent their atelectasis [6, 9, 12, 13].

The LS consist mainly of lipids, of which 80% are phospholipids [11], which have a higher coefficient of oxygen solubility than aqueous media [8]. Because of this property, the LS film on the surface of the alveoli, like the lipid components of cell membranes, must increase the rate of oxygen absorption from the gaseous phase [1, 10].

To test this hypothesis the effect of a residual film of LS on the kinetics of mass oxygen transfer was studied.

EXPERIMENTAL METHOD

Freshly prepared and oxidized solutions of LS, lecithin, and silicone oil were used as test materials. LS were obtained by the following method: 50 mg rat lung tissue was homogenized and mixed with 25 ml of 0.9% NaCl solution, after which the mixture was centrifuged for 10 min at 900g. The supernatant was recentrifuged at 65,000g for 1 h. The residue was

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transferred to 14 ml of 0.9% NaCl solution and treated on the V-1 shaker for 10 min. To complete the structural organization of the surface film of surfactant the LS solution was allowed to stand for 24 h in order to settle at 0°C. Lecithin was obtained by Tisarowski's method [14] and dissolved in chloroform–methanol mixture (2:1) in the proportion of 1 mg lecithin to 0.1 ml of solution.

A polarographic cell (Fig. 1) 50 mm in diameter, into which 14 ml of 0.9% NaCl solution was poured, was used in the experiments. The electrode was immersed into this volume of fluid to a depth of 0.1 mm. The cell was covered with a lid 5 mm thick with evenly spaced holes 2 mm in diameter (40 holes). To prevent mixing of the fluid under the influence of the gas flow from the inner surface of the lid, filter paper was glued to it. The cell was placed in a chamber the temperature of which was kept constant at 35°C, filled with air. By means of an additional device the gas chamber could be filled with moist oxygen, warmed to 35°C, at the rate of 1 liter/min. The change in the oxygen concentration at a depth of 0.1 mm from the surface of the solution was measured by a stabilized open platinum electrode against an Ag–AgCl half-cell. The diffusion current was recorded on an LP-60 polarograph. By means of this apparatus the kinetics of oxygen transport from the gaseous phase was investigated into 0.9% NaCl solution and into the same solution covered with a layer of freshly prepared and oxidized LS, lecithin, or silicone oil solution.

Before the experiments a layer of the test substance was poured into the cell 30 min before the beginning of measurement. The experiments were conducted at 35°C.

EXPERIMENTAL RESULTS AND DISCUSSION

When the polarographic cell was filled with 0.9% NaCl solution two phase boundaries were created in the system: gas–liquid and liquid–metal of the working electrode. Because of the presence of an oxygen concentration of 4.9 ml/liter [3] in a solution in equilibrium with air under an atmospheric pressure of 754 mm Hg, the electrode of the cell recorded the initial diffusion current, corresponding to the oxygen concentration present (Fig. 2).

When the air was replaced by oxygen in the gas chamber above the test solution to give a pO_2 of about 690 mm Hg, absorption of oxygen by the solution and its diffusion into the liquid began, and the working electrode recorded an increase in oxygen concentration. The rate of rise of the oxygen concentration in the juxtaelectrode region varied under different experimental conditions. For instance, if 0.9% NaCl solution was used without any impurities the oxygen concentration rose exponentially, in agreement with the theoretically expected behavior (Fig. 2, curve 1). If a layer of LS formed a film on the surface of the solution and electrode an increase in the rate of rise of the diffusion current was found (Fig. 2, curve 2). The greatest difference in the rates of rise of the oxygen concentration in all cases was observed during the first 90 sec. For a pure solution of NaCl this rate was 0.1 ml O_2/liter/sec.

Since LS consists of up to 80% of phospholipids, the most important of which is lecithin, the next step was to determine the effect of lecithin itself on the kinetics of oxygen transport in residual membranes. The use of lecithin could also show which boundary was responsible for the observed effect.

When a film of lecithin was formed on the surface of the liquid the rate of rise of the oxygen concentration was the same as that for a pure 0.9% NaCl solution. If, however, the lecithin film was adsorbed on the surface of the electrode (as shown by a reduction in the diffusion current), the rate of rise of the oxygen concentration increased to 0.13 ml O_2/liter/sec. This effect was observed only when freshly prepared lecithin was used. Preparations of oxidized lecithin had no such effect. It was therefore interesting to discover how a film formed from oxidized LS would behave. To obtain an oxidized specimen of LS, a freshly prepared solution of surfactants was allowed to stand in air at room temperature for 24 h.

During the formation of a residual film from oxidized LS the rate of rise of the oxygen concentration at the depth of immersion of the electrode was considerably lower than in the freshly prepared specimen of LS, and lower than in pure 0.9% NaCl solution (Fig. 2, curve 3). This indicates that oxidized LS create a definite resistance to the flow of oxygen, whereas freshly prepared LS accelerate its absorption.

To study the role of the solubility of oxygen in the acceleration of its passage toward the electrode, a solution of silicone oil in chloroform-methanol mixture was used in the same concentration as lecithin. During the formation of a residual film of silicone oil the rate

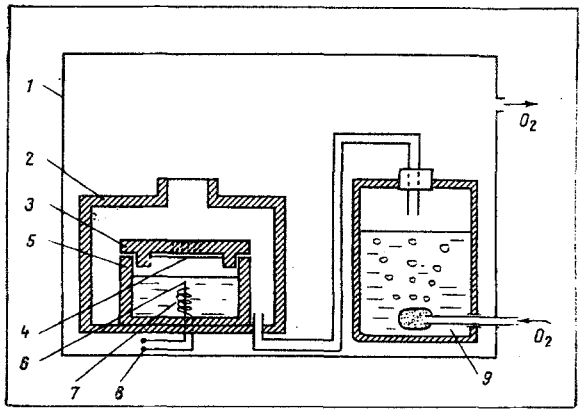

Fig. 1

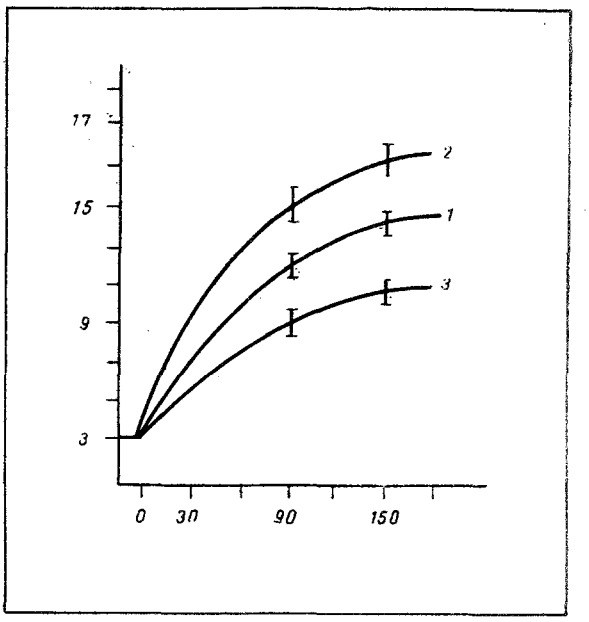

Fig. 2

Fig.1. Diagram of polarographic cell for simulating oxygen transport through phase boundary. 1) Thermostat; 2) gas chamber; 3) lid of cell; 4) filter paper; 5) cell proper; 6) platinum electrode; 7) Ag—AgCl electrode; 8) LP-60 polarograph; 9) humidifying chamber. Cell is shown enlarged.

Fig. 2. Changes in rate of rise of oxygen concentration in juxtaelectrode layer under different experimental conditions: 1) in 0.9% NaCl solution; 2) in solution covered by layer of freshly prepared LS; 3) in solution covered by layer of oxidized LS. Ordinate, oxygen concentration in solution (in ml O_2/liter); abscissa, time of measurement (in sec).

of rise of the oxygen concentration also was higher than in pure NaCl solution (Fig. 3). This is evidence that high solubility plays the leading role in the genesis of the effect. Acceleration of the passage of oxygen to the surface of the electrode utilizing oxygen took place as a result of the fact that a film made from any substance with a high absorption coefficient can act as an oxygen concentrator.

These results suggest that a thin layer of LS and also, possibly, other phospholipid membranes located immediately by the oxygen consumer, may lower the diffusion resistance of oxygen and lead to an increase in its mass transfer.

Comparison of results obtained on freshly prepared films of LS and after their preliminary oxidation (and also in the case of the use of reduced and oxidized lecithin) leads to the logical conclusion that the ratio between the concentration of reduced and oxidized forms in the composition of surfactants may affect the kinetics of mass transfer of oxygen and may act as a regulator of this process. It will be noted that the role of the physicochemical properties of the medium in which diffusion of the gas takes place has been inadequately investigated so far as biological strucutres are concerned. At the same time, it is known that carbon dioxide and oxygen diffuse through a porous membrane at about equal rates, but during passage through a water film, because of reaction with the medium and its high coefficient of absorption, carbon dioxide diffuses 25 times faster than oxygen. Similar differences arise also during diffusion through rubber films. Lipid components of biomembranes, with a high oxygen absorption coefficient, must behave in relation to oxygen in the same way as the aqueous components which accelerate carbon dioxide transport by diffusion.

The surface of surfactant films is known to be an organized monolayer structure of phospholipid molecules [9, 12], whereas the underlying layer — the hypophase — consists of an aqueous solution of coiled micelles of surfactants. Meanwhile, it has been shown by electron microscopy [5] that phospholipid bands up to 10 μ in diameter are present in the structure of

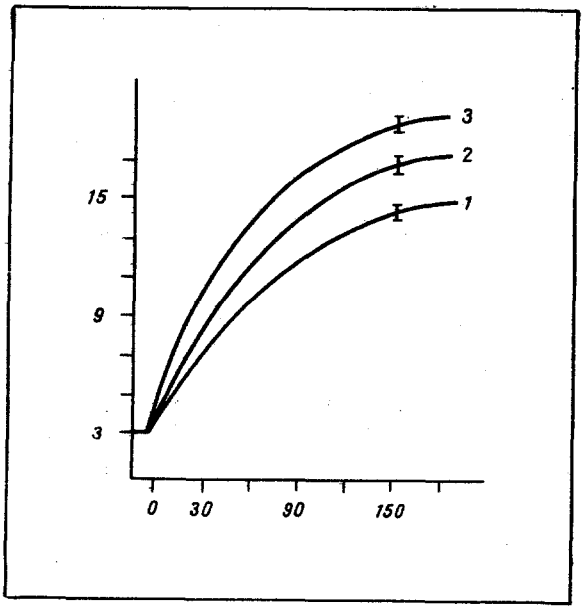

Fig. 3. Changes in rate of rise of oxygen concentration in juxtaelectrode layer in presence of substances with different coefficient of oxygen absorption: 1) in 0.9% NaCl solution; 2) in lecithin solution; 3) in solution of silicone oil. Remainder of legend as in Fig. 2.

the hypophase and create a definite continuity of the phospholipids as far as the cytoplasmic membrane of the alveolar epithelium.

The possibility of direct oxidation of surfactants in the presence of a high oxygen concentration in the inhaled gas mixture is not disputed. In some cases, however, it is found only 66 h after the beginning of oxygen inhalation [4]. This could be the result of adequate reserves of LS in the second-order pneumocytes, but it could also be the result of the fact that most workers, when determining surface tension, used excessively high concentrations of LS, masking the initial changes on account of the excess of surfactants in the hypophase. The use of small samples of lung tissue (10 mg) reveals changes in surface tension as early as 30 min after the beginning of oxygen administration [2].

Workers who have studied oxygen transport regard the diffusion coefficients as constants pertaining to a given tissue. Because of the lability of the relations between the different components of biological barriers and membranes, the presence of structural transitions and conformational changes affecting oxygen absorption, it can be tentatively suggested that diffusion coefficients may have certain limits of variation, which change under different physiological conditions.

In conclusion, the physiological role of LS is evidently not confined to lowering the surface tension and stabilizing the alveoli, but also includes participation in the absorption of oxygen and regulation of its transport through the air–blood barrier.

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ACTIVITY IN MYELINATED CUTANEOUS NERVE FIBERS IN RESPONSE TO COOLING IN CATS

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By means of the colliding impulses method and methods improving the signal to noise ratio in antidromic action potentials recorded from a cutaneous nerve, afferent impulses in its fibers were analyzed in response to cooling in cats. Fibers of group $A\delta_1$ and $A\delta_2$ were shown to conduct impulses during cooling of the skin receptors. A small group of fibers with conduction velocities of 13.0-7.5 m/sec showed inhibition of activity in response to cooling. A group of "mixed" fibers mainly responded by inhibition of activity, and only a few fibers of this group responded by excitation to cooling of the skin receptors.

KEY WORDS: myelinated nerve fibers; afferent impulsation; cooling of the skin.

Afferent activity in response to cooling has been adequately studied in cutaneous nerves of animals of different species [8]. Whereas in primates thin myelinated fibers have been shown to participate in the transmission of excitation to cooling of the skin [9], in rats and cats their activity has been confirmed only in the case of cooling the skin of the scrotum and the dorsum of the nose [10, 11]. The writers found only one reference in which the response of mechanoreceptors with myelinated fibers of the hairy skin to cooling is described in cats [5]. In these experiments myelinated fibers gave a very short spike discharge with low frequency (10-20 spikes/sec). Among authors who have studied temperature reception there is no general agreement as regards the degree of participation of myelinated fibers of the hairy skin in the transmission of information about cooling. Some workers consider that myelinated fibers in general are not excited during cooling, whereas others have shown that these fibers, which are mechanoreceptors, give a very small discharge during cooling [6], and on that basis they are dubious of their role in the perception of temperature sensation. However, there is indirect evidence that the temperature sensitivity of the hairy skin in cats is equal to the sensitivity of the skin in primates [7].

The object of this investigation was to determine the degree of participation of thin myelinated fibers in the transmission of information about cooling of the whole receptor field of the hairy skin in cats.

EXPERIMENTAL METHOD

Experiments were carried out on nine adult cats anesthetized with hexobarbital. The common trunk of the saphenus nerve was divided in the region of the inguinal fold and placed on stimulating platinum electrodes. Conduction velocity along the nerve was disturbed proximally to the stimulating electrodes. The lateral cutaneous branch of the saphenus nerve was

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