Biochemistry and Surface Tension Properties of the Lung Alveolar Surfactant Obtained by Micropuncture

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The micropuncture technique (MPT, Reifenrath R, Zimmermann I: Resp Physiol 18:238-244, 1973) enables one to obtain fluid from the alveolar walls of isolated lungs. Using this method, average sample volumes of $2 \times 10^{-9}$ liters were obtained. Analysis of the MPT material showed the occasional presence of some albumin (method: micro-discelectrophoresis; detection limit: $0.3 \times 10^{-6}$ g). There was, however, no correlation between the sample volume and the amount of albumin. One may thus conclude that in the lung alveolar fluid, and therefore in the surfactant, there is no plasma protein.

The MPT sample of the largest volume (ca $5 \times 10^{-9}$ liters) contained in the postalbumin region a nonplasma protein (NPP). A lipid analysis of the MPT material was not carried out.

Larger quantities of the material were obtained when alveolar puncture with a rinsing technique were used (micropuncture rinsing technique = MPRT; Reifenrath R, Zimmermann I: Resp Physiol 18:238-244, 1973). Besides containing the NPP in the postalbumin region, the MPRT material also contained four further NPP in the prealbumin region. The only lipids that could be detected in the MPRT material were lecithin and cholesterol in the proportions of 4:1 (w/w) (method: micro-thinlayer-chromatography; detection limits: lecithin $25 \times 10^{-9}$ g, cholesterol $20 \times 10^{-9}$ g).

With a bubble method (Slama H, et al: Pfugers Archiv 322:355-363, 1971) area-surface tension diagrams (ASD) of the MPT and MPRT materials were recorded (hypophase: Ringer's solution; 37°C; relative area variation 75 percent; period duration 3 sec). The minimum surface tension (ymin) was about 18 dynes/cm. The removal of proteins of MPRT material did not change the properties of the ASD.

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ASD’s of the mixed films from synthetic DL-a-dipalmitoyl-lecithin and cholesterol in the proportion of 7:1 (w/w) and 4:1 (w/w) were very similar to those of the MPRT material. The ASD’s from two lipid fractions of the MPRT material were similar to the ASD’s of synthetic lecithin and cholesterol films ($v$ min of the lecithin films $< 1$ dyne/cm and of cholesterol films ca 18 dynes/cm).

With small area variations there were slight differences between lecithin-cholesterol mixed films and pure cholesterol films.

Electron microscopic investigations of MPT and MPRT samples showed a lipid rich material (Gil J, Weibel, ER; personal communication) with a structure and periodicity characteristic for lecithin.

In summary, one can say that the surface tension properties of the lung alveolar surfactant are determined by the lipids lecithin and cholesterol. The minimum surface tension is about 18 dynes/cm and the difference between maximum and minimum surface tension at area variations of 6 percent and 12 percent is less than 10 dynes/cm.

These findings, therefore, necessitate a modification of previous concepts regarding the physiology and pathophysiology of respiratory mechanics.

Discussion

Dr. Reiss: Do you have any idea of the thickness of the wall of the bubble you are working with, or of the concentration of lipids at the interface?

Dr. Reifenrath: We have not measured the wall thickness. We did not find any dependence of the dynamic surface tension behavior of the bubble on concentration.

Dr. Klipper: With this method were you able to assess any absorptive or diffusion process?

Dr. Reifenrath: We have used two kinds of applications. The first is to place the substance directly into the hypophase. After about 200-300 oscillations the so-called final area-surface tension diagram (ASD) can be observed. In the second method, we inject surfactant into the surface of the bubble via a microcapillary. In this case, we can observe the loss of the surfactant into the hypophase. The final ASD is the same in both cases.

Dr. Tierney: It is well known that substances such as cholesterol and oleic acid may be present in lung wash.
samples and many inhibit the relatively sensitive surface tension activity of the surfactant. I think you have improved on the Wilhelmy balance technique, which often homogenizes the inhibitors and surface active fraction, but I don’t believe you have improved on the Pattle technique, which shows that the surface tension does indeed reach zero at physiologic conditions. The Pattle results can be justified in view of physiologic PV studies. When we observe a minimum tension well above zero, as you have done, we attribute this to the presence of contaminants which act to inhibit surface activity. Furthermore, a normal minimum tension of 20 dynes/cm does not explain why the lungs collapse in RDS.

Dr. Reifenrath: I do not think that a minimum surface tension of 20 dynes/cm is too high for physiologic breathing. Part of the problem is due to the application of the LaPlace equation to isolated alveoli. The problem of the high retraction pressure (20 dynes/cm) could be reconciled with the physiologic situation if all the mechanical forces that interplay in the alveoli are included.

Mechanical Regulation of Alveolar Surfactant in Adult Cats: the Effects of Hyperventilation and End-Expiratory Pressure in Vivo*

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Hyperventilation in excised dog lungs or in vivo in guinea pigs results in a shift to the right of the deflation limb of the static pressure-volume curve, consistent with an increase in alveolar surface tension. The shift in the pressure volume curve is prevented to a significant degree by constant end-expiratory pressure during the period of hyperventilation in excised dog lungs. Whether hyperventilation prevents release of surfactant from the alveolar type 2 cell or inactivates alveolar surfactant is not known. Similarly, the mechanism by which end-expiratory pressure prevents the deleterious effects of hyperventilation is unknown. The present study was designed to answer these questions.

We have studied the effects of hyperventilation in paralyzed, anesthetized and artificially-ventilated adult cats, on pulmonary pressure-volume deflation characteristics and surface tension properties of lung extracts. In addition, changes in the specific activities of 3H glycerol and 14C palmitate in lecithins, were used to investigate the distribution of lecithins in endobronchial lavage and lung tissue after hyperventilation.

METHODS

Adult cats were anesthetized with intraperitoneal sodium pentobarbital and a tracheotomy was performed. The animals were paralyzed with gallamine, the thorax widely opened, and the lungs mechanically ventilated with air in a positive pressure respirator at 32 breaths/min for three hours. Each animal was assigned to one of three groups: (A) control ventilation with tidal volumes \( V_T \) equal to 15-20 percent of total lung capacity (TLC); (B) hyperventilation with \( V_T = 65 \) percent TLC; (C) hyperventilation with \( V_T = 65 \) percent TLC with end-expiratory pressure (EEP) of 2.5 cm H2O. At the end of the ventilation period, the animals were sacrificed, the trachea, heart and lungs removed and the lungs degassed in a vacuum jar at 40 mm Hg. Each degassed lung was inflated with air to 15 cm H2O. The pressures, during the deflation maneuvers, were maintained up to one minute, to allow volumes to stabilize. All P-V measurements were performed at room temperature.

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