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Direct determination of volume- and time-dependence of alveolar surface tension in excised lungs

(micropuncture/dipalmitoyl phosphatidylcholine/lung surfactant)

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ABSTRACT **We measured alveolar surface tension directly by observing the spreading properties of fluid droplets placed by micropipette within individual alveoli. Alveolar surface tension in excised rat lungs at total lung capacity and 37° was 29.7 ± 1.4 mN·m^{-1}. In lungs held at functional residual capacity, surface tension changed slowly, rising to 9 mN·m^{-1} at 30 min with a subsequent approximately linear rise to 16 mN·m^{-1} at 115 min. Thereafter it rose more rapidly (20 mN·m^{-1} at 140 min), suggesting that it was not asymptotically approaching an equilibrium value.**

Alveolar surface tension has previously been estimated from lung extracts and broncho-alveolar lavage liquid in surface balances (see ref. 1 for a review) or with bubble methods (2, 3). The assumption in these studies was that alveolar surfaces have physicochemical properties similar to those of extracts and lavage liquid. Other authors have used pressure–volume measurements on excised lungs to calculate alveolar surface tension (4–6), assuming both a relationship between lung surface area and lung volume and a value for maximum surface tension. The latter was estimated from *in vitro* surface balance studies (7, 8) and was usually taken as 50 mN·m^{-1}.

Recently we reported (9) the use of fluorocarbon and silicone liquids to measure alveolar surface tension directly in excised lungs at 37°. The method is based on the observation that when droplets of such a liquid are placed on a lipid monolayer at an air–water interface, they spread to thin lenses as the tension of the monolayer-covered surface is raised past a critical value. We found that surface tension (γ) in rat lung at 40% total lung capacity was initially less than 9 mN·m^{-1}, and it rose to this tension when the lungs were held at constant volume for about 30 min. The relationship between lung volume and surface tension during stepwise deflation from 85 to 60% total lung capacity was linear, and surface tension ranged from 20 to 9 mN·m^{-1}.

In the present study we measured surface tension directly within alveoli at total lung capacity (TLC) with new test liquids suitable for tensions above 20 mN·m^{-1}. We also determined the rate of rise of surface tension in lungs held for prolonged periods at functional residual capacity (FRC).

MATERIALS AND METHODS

Our method is similar to one that has been reported (9) and is based on the observation that a droplet of a test liquid resting on a monolayer-covered aqueous subphase begins to spread as the interfacial tension rises beyond a value characteristic of the test liquid. We again employed the fluorocarbon test liquids (Fluorinert®, 3M Co.) and silicone oil (1107, Dow Corning) we had used previously for the surface tension range of 9–20 mN·m^{-1}. We were faced with a new problem when attempting to find liquids suitable for measuring higher surface tensions. Whereas the droplet-to-lens transition was sharp for liquids in the lower range of surface tension, there was a more gradual change for all the liquids we tried at surface tensions above 20 mN·m^{-1}. After preliminary experiments with both cat and rat lungs we chose a test liquid (DMP/O) consisting of dimethylphthalate (Matheson, Coleman and Bell) and normal octanol (Fisher, reagent) in a 4:1 (vol/vol) mixture. This was doped with crystal violet (Sigma) at 4 mg/ml to aid visualization, because the differences in refractive index between these new test liquids and water were not as great as those between the fluorocarbons and water.

Calibration curves in our previous study were performed in an ordinary Teflon® (polytetrafluorethylene) surface balance using dipalmitoyl phosphatidylcholine (DPPC) monolayers. In the present experiments we attempted to mimic the alveolar situation more closely by placing a small block of 0.5% agar (Difco) within the trough and lowering the subphase surface until it was actually 0.5 mm lower than the top of the block itself. Platinum sensors attached to a strain gauge (Statham gold cell) or an electrobalance (Cahn) recorded surface tension outside the block area and within a small well cut into the top of the block. Calibrating liquid drops were placed on top of the agar or, for measurements of interfacial tension, were placed on the surface over the well. In this latter case, droplets were larger than the agar well over which they were placed and thus were kept in contact with the platinum sensor. We observed that spherical drops of nonspreading liquids increased about 26% in diameter after deposition on the agar, corresponding to a sphere-to-hemisphere transition. On viewing drops of FC 40 fluorocarbon test liquid (γ = 16 mN·m^{-1} 37°) tangentially with a stereo microscope (Zeiss), we observed a hemispherical shape at monolayer tensions below 16 mN·m^{-1}. Above this value the droplets increased in diameter but maintained the segment-of-a-sphere shape that minimized their air–interfacial areas. As surface tension of the DPPC-covered subphase was varied, tensions measured at the well accurately followed those recorded outside the agar, indicating that a continuous DPPC monolayer covered the block. These changes in droplet configuration with increasing tension were associated with decreases in the contact angle of the drop with the monolayer (Fig. 1) and were compatible with Young's equation:

$$\gamma_{A/W} = \gamma_{L/W} + \gamma_{A/L} \cos \theta.$$

Here $\gamma_{A/W}$ is the surface tension at the air/water interface as

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Abbreviations: DPPC, dipalmitoylphosphatidylcholine; TLC, total lung capacity; FRC, functional residual capacity; DMP/O, dimethylphthalate and normal octanol, 4:1 (vol/vol).

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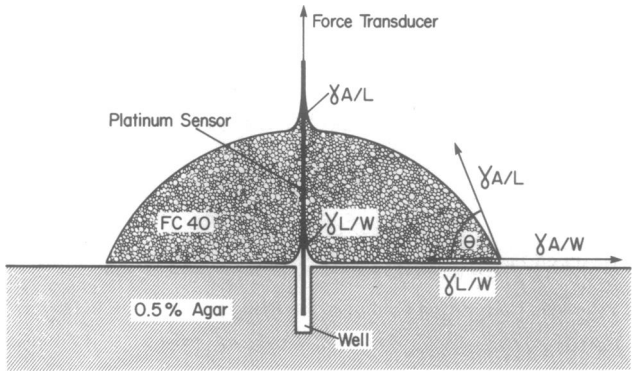

FIG. 1. Drop of FC 40, (γ = 16 mN·m^{-1}) resting on a DPPC monolayer. The monolayer and the adjacent subphase are supported by a block of 0.5% agar. $\gamma_{A/W}$, surface tension at the air/water interface as modified by the film; $\gamma_{L/W}$, tension at the test liquid/water interface in the presence of a monolayer; $\gamma_{A/L}$, surface tension at the free, air/test liquid interface; θ, contact angle.

modified by the film, $\gamma_{L/W}$ is the tension at the test liquid/water interface in the presence of a monolayer; and $\gamma_{A/L}$ is the tension at the free, air/test liquid interface. Only rough estimates of the contact angle, θ, were made.

To test further the applicability of Young's equation in this system, we placed drops of FC 40 onto the monolayer lying over the agar well so that they were intercepted by the dipping platinum sensor. By penetrating one or both interfaces with the sensor (Fig. 1) and being particularly careful to maintain zero contact angle with the platinum surface we directly measured $\gamma_{A/L}$ or the sum of $\gamma_{A/L}$ and $\gamma_{L/W}$ while varying $\gamma_{A/W}$. Fig. 2 reports our results. $\gamma_{A/L}$ remained constant throughout at its previously measured value of 16 mN·m^{-1}, indicating that the air/test liquid interface remained free of DPPC. $\gamma_{L/W}$, calculated from the above two measurements, remained constant at 8 mN·m^{-1} as $\gamma_{A/W}$ was varied from 10 to 20 mN·m^{-1}; above this value $\gamma_{L/W}$ increased approximately linearly with $\gamma_{A/W}$.

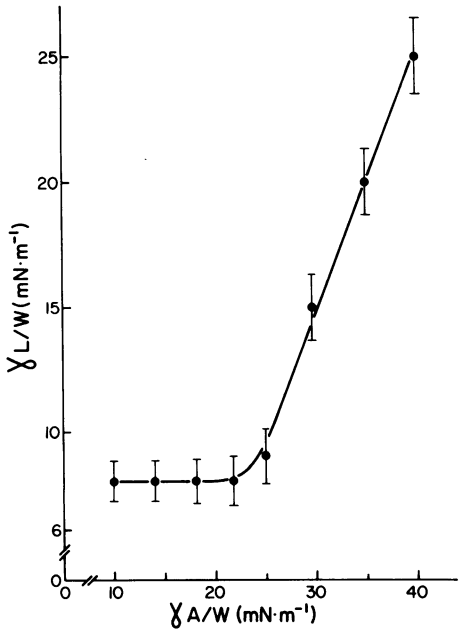

FIG. 2. Tension $\gamma_{L/W}$ at the FC 40/water interface in the presence of a DPPC monolayer vs. surface tension $\gamma_{A/W}$ at the air/water interface as modified by the DPPC film. Error bars indicate ± one standard error of the mean.

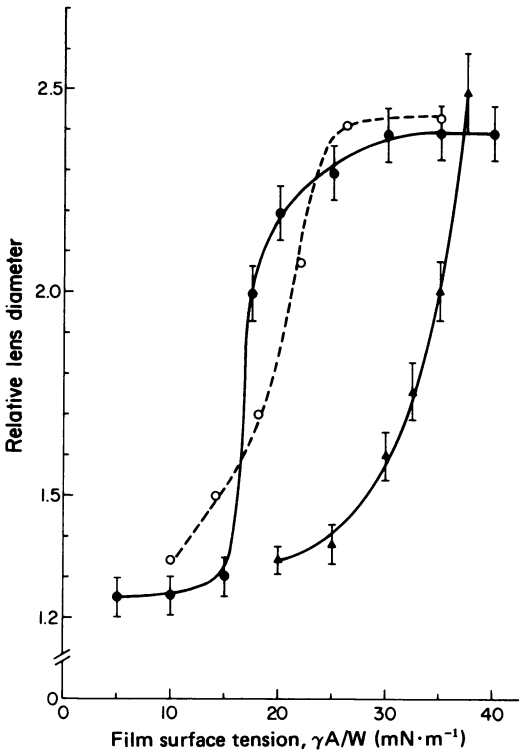

FIG. 3. ●—●, Relative diameter of a droplet of FC 40 fluorocarbon liquid vs. the surface tension of a DPPC-covered subphase over a 0.5% agar block; O- - -O, calculated drop diameter using $\gamma_{L/W}$ and $\gamma_{A/W}$ data from Fig. 2 and Young's equation; ▲—▲, relative diameter of a droplet of DMP/O mixture vs. the surface tension of a DPPC-covered subphase over a 0.5% agar block.

In Fig. 3 the solid curve (filled circles) is a plot of the relative diameter of a droplet of FC 40 fluorocarbon liquid versus the tension of a DPPC-covered subphase over a 0.5% agar block. The ordinate is normalized to the diameter of the spherical droplet prior to deposition. The dashed line (open circles) represents calculated drop diameter using $\gamma_{L/W}$ and $\gamma_{A/W}$ data from Fig. 2 and Young's equation to determine the intermediate variable, θ. The near identity of the two curves lends support to the application of Young's equation in this experimental situation. Note that the steepness of the transition made it a simple matter to read surface tension at the point of the first discernible diameter change.

Test liquids suitable for use at higher film tension presented a different picture. In this situation we had to measure carefully the drop diameter shortly after deposition, because its change with $\gamma_{A/W}$ was more gradual. Fig. 4 shows a sessile drop of DMP/O resting on a DPPC monolayer at surface tensions of 25 and 33 mN·m^{-1}. The monolayer was supported by a block of 0.5% agar. Fig. 3 (triangles) gives the calibration curve for this test liquid, which was used at volumes near TLC. Identical curves were obtained by using monolayers prepared from purified lung surface active material (gift of Bradley J. Benson),

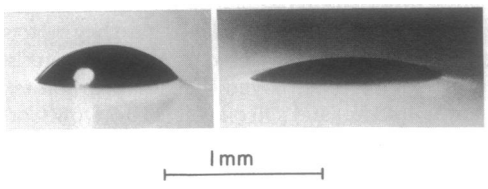

FIG. 4. Sessile drop of DMP/O resting on a DPPC monolayer at surface tensions of 25 mN·m^{-1} (*Left*) and 33 mN·m^{-1} (*Right*). The monolayer was supported by a block of 0.5% agar.

and the results were independent of initial drop diameter from 0.01 to 1 mm for $\gamma_{A/W} < 35$ mN·m^{-1}. Measurements for each calibration curve were made at 37° ± 0.5°.

Fluorocarbon and silicone oil test liquids did not measurably displace or dissolve the calibrating DPPC film, because the addition of many large drops to the surface neither changed $\gamma_{A/W}$ nor displaced talc "markers" previously dusted onto the film. However, when DMP/O was used at $\gamma_{A/W} > 35$ mN·m^{-1}, surface tension began to fall slowly, presumably as a result of the spreading of the lower surface tension droplet. Because our measurements at TLC indicated an alveolar tension less than this value, we assume that our results were not affected by this potential artifact.

All observations of alveolar surface tension were made at 37° in lungs excised from 200- to 400-g male Sprague–Dawley rats as previously described (9). TLC was taken as the lung volume corresponding to 25 cm H_2O transpulmonary pressure, and FRC was assumed to be 40% of TLC. Distending pressure at FRC was usually between 3.6 and 4.5 cm H_2O, and those lungs requiring higher pressures were discarded. About 20 min elapsed between removal of the lung from each experimental animal and the first measurements.

Droplets of different diameters (5–20 μm) were placed in adjacent alveoli. They were observed to spread simultaneously, indicating that the estimation of alveolar surface tension did not depend on droplet size.

Measurement of Alveolar Surface Tension at TLC. Lungs were held at 25 cm H_2O for 3 min before puncture at TLC. For surface tension measurements at TLC, DMP/O droplet size was measured through a calibrated eyepiece (Wild, ×20) at ×80 total magnification, before and after deposition. Relative diameter was related to alveolar surface tension through the calibration curve (Fig. 3). In general, micropuncture was more easily performed at TLC than FRC because alveoli were larger and illumination better at the larger lung volume.

For each lung we took 10–20 readings in alveoli with diameters ≥60 μm. Except for size selection, the alveoli were punctured at random over a period of 30–60 min in a 2-mm strip along the edge of a lobe. We used DMP/O in eight lungs, and an analysis of variance at the 5% level of significance allowed us to pool the measurements of droplet diameter from the different lungs. We then used the calibration curve of Fig. 3 to determine the surface tension at the calculated mean diameter.

In three additional lungs we used a second test liquid as well. After making 10–20 measurements in each lung with DMP/O, the lungs were degassed again, then inflated to TLC. Ten to 20 droplets 4:1 (vol/vol) diethyloxalate (Matheson, Coleman and Bell)/normal octanol were placed in alveoli of a lobe that had not been punctured. We prepared a separate calibration curve for this liquid.

Measurement of Alveolar Surface Tension at FRC. Stability of surface tension at FRC was assessed using the fluorocarbon and silicone liquids. Because of the complexity of the experiments and the necessity for completing measurements prior to deterioration of the alveolar surface after excision of the lungs, we were usually able to use droplets of only a single kind of liquid in each lung.

Lungs in this series were deflated from TLC to FRC. After 3–4 min of equilibration at FRC, the pressure was recorded and timing was started. In two to three regions of a lung lobe, up to 2 mm from the edge of the lobe, we chose an area of approximately 4 mm^2, where we punctured at random alveoli of 40 to 80 μm in diameter. In each area we kept depositing droplets until at least five remained on alveolar surfaces adjacent to the pleural surface. For fluorocarbons FC 72 and FC 75 we checked the droplets at 5-min intervals, to see whether they had flattened to thin lenses. For FC 40 and silicone oil we chose 10-min intervals. We also recorded the transpulmonary pressure at each interval.

Table 1. Surface tension rise with time for excised rat lung at constant volume (FRC) and 37°.

Number of lungs	Time for spreading to occur (min), mean ± SEM	Surface tension, mN·m^{-1}	Test liquid
9	30.1 ± 2.6	9	FC 72
8	75.6 ± 8.5	13	FC 75
8	115.6 ± 10.3	16	FC 40
7	141.4 ± 10.9	20	Silicone oil

RESULTS

Surface Tension at TLC. By using DMP/O to measure surface tension at TLC in rat lungs held at 37°, we found a value of 29.5 ± 1.5 mN·m^{-1} (mean ± SEM). By using diethyloxalate/octanol-1, we found the alveolar surface tension to be 30.3 ± 1.3 mN·m^{-1}, which was not significantly different from that determined by DMP/O ($P > 0.5$, unpaired t test). We therefore pooled all these measurements, obtaining 29.7 ± 1.4 mN·m^{-1} for the surface tension at TLC and 37°.

Three other lungs were studied with DMP/O at TLC, deflated to FRC for 3 min, and then reinflated to TLC. After a further 3-min equilibration, a second series of 10 to 20 readings was obtained with DMP/O. Again, we found no significant difference due to this inflation history ($P > 0.1$, unpaired t test).

Surface Tension Rise with Time at FRC (Constant Volume). In measuring the rise of surface tension with time in nonadjacent alveoli we found that droplets of a given test liquid did not all spread at exactly the same moment. For example, we found differences of as much as 20 min for FC 72 and FC 75 and 30 min for FC 40 and silicone oil. The time recorded for a given test liquid was therefore taken by arbitrary convention to be the point at which 60% of the droplets had enlarged by 50% over the original spherical drop size. According to our *in vitro* calibration such an increase in diameter indicated that the tension of the subphase surface was approximately 1 mN·m^{-1} greater than that of the test liquid.

Table 1 summarizes our findings. Surface tension increased throughout the observation period, which lasted for over 2 hr. The range of readings for any test liquid reflects not only variations between lungs but also the within-lung variation mentioned above and experimental error. These data appear graphically in Fig. 5 (open circles) and suggest that the increase in tension with time is accelerating after 2 hr at FRC.

Fig. 5 also shows a plot of transpulmonary pressure versus time for eight lungs held at FRC (filled circles). Four of these lungs were being simultaneously monitored with FC 40 as a test liquid, and four were being tested with droplets of silicone oil. Again the pattern of accelerated change seen in the surface tension–time relation was observed: a faster than linear increase in pressure occurred at constant volume.

DISCUSSION

Surface Tension at TLC. We found that alveolar surface tension at TLC was 29.7 ± 1.4 mN·m^{-1} at 37°. This value is close to the equilibrium surface tension of about 25 mN·m^{-1} for extracts containing pulmonary surfactant (10, 11) or for a

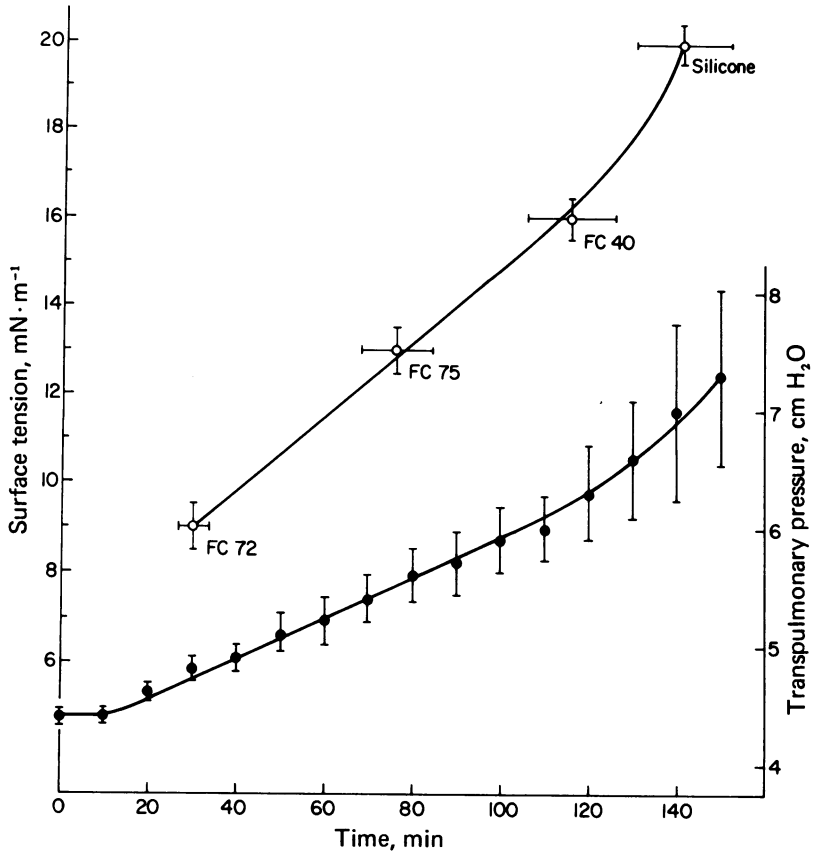

FIG. 5. O, Surface tension–time relationship for rat lung at 37° (see Table 1); ●, transpulmonary pressure–time relationship recorded simultaneously. Error bars indicate ± 1 SEM.

0.15 M sodium chloride solution covered with a DPPC monolayer (10). We made our measurements under quasi-static conditions after equilibration for at least 2 min at TLC.

Surface balance studies performed by others with lung extracts have suggested a maximal surface tension between 31 and 50 mN·m^{-1} (7, 8, 12). However, Goerke (13) and Bachofen *et al.* (5) have pointed out that deducing values for surface tension in the alveoli from such observations is uncertain, because the actual concentration of surface-active agents at the alveolar surface is not known. The value of 29.7 mN·m^{-1} that we observed under quasi-static conditions is lower than that commonly found for lung extracts in surface balances and suggests that the concentrations of surface-active molecules in alveoli at maximal expansion might be greater than those at the lung extract surface. It is possible that the alveolar surface tension is instantaneously higher and decreases within a few seconds to an equilibrium value (10) before we can measure it by the micropuncture technique.

Surface-Tension Increase with Time at Constant Volume (FRC). Two approaches to the measurement of alveolar film stability have been taken in the past: lung surface-active material or its most active component, DPPC, has been studied in a surface balance, or alternatively, film behavior has been calculated from pressure/volume plots of intact lungs.

In surface balance experiments using lung extracts, the monolayer which spontaneously forms at the air/water interface was compressed to 20 or 25% of its maximum area (10, 12). At this point, area was held constant and surface tension increased as film collapse or desorption occurred. At room temperature, surface tension increased from 5 to 20 mN·m^{-1} in 40 min and thence to 36 mN·m^{-1} by 180 min. At 37° the increase was faster, going from 5 to 20 mN·m^{-1} in 20 min (14). In such experiments surface tension tended towards an asymptotic high value between 24 and 30 mN·m^{-1} (14, 15), and its instantaneous rate of increase was nearly directly proportional to the difference between the observed tension and its asymptotic value. Such a kinetic process could be described by an exponential function:

$$\gamma_t = \gamma_{eq}[1 - \exp(-kt + C)]$$

in which γ_t and γ_{eq} are the instantaneous and equilibrium surface tensions, respectively, k is the rate constant, and C is a constant.

In similar experiments at 35–38° (10), surface tension increased from 10 to 20 mN·m^{-1} in 5.3 min. By using a bubble method to measure surface tension, other investigators (16) found that the surface tensions of solutions of partially purified lung surfactant increased from 2 to 20 mN·m^{-1} in less than 1 sec at 37°.

Monolayers of pure DPPC, on the other hand, are far more stable (13, 16). In experiments which have been confirmed in our own laboratory and elsewhere, Watkins (11) found that DPPC monolayers, compressed to a surface tension of 1 mN·m^{-1} at 25°, collapsed at approximately 0.1 mN·m^{-1}·hr^{-1}.

The stability of excised lungs, as judged by pressure/volume diagrams (17), is more in keeping with surface balance studies of DPPC than with those using whole surface-active material. Horie and Hildebrandt (6) calculated from room-temperature pressure/volume diagrams of excised rat lungs held at 40% TLC that surface tension increased by only 1 to 2 mN·m^{-1} in 20 min.

The data of our own study, although performed at 37°, also support the concept of a very stable DPPC-like alveolar monolayer. Surface tension increased in 30 min to 9 mN·m^{-1}

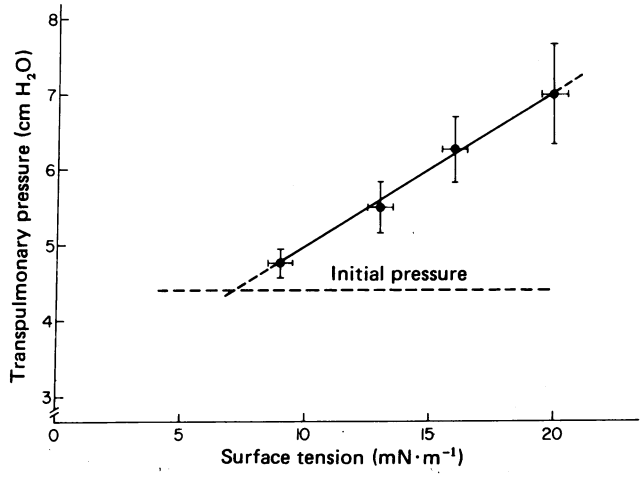

FIG. 6. Transpulmonary pressure versus surface tension in rat lungs at 37°. The initial pressure was 4.4 cm H_2O at FRC, corresponding to a surface tension of 7 mN·m^{-1} (extrapolated).

at 40% TLC and in more than 2 hr, to 20 mN·m^{-1}. Transpulmonary pressure, recorded simultaneously, increased from 4.4 to 7.3 cm H_2O in 160 min.

It is also possible to estimate from our data the lowest surface tension reached when lung volume is decreased abruptly to FRC. The plot of surface tension versus transpulmonary pressure is linear (Fig. 6) at constant volume. This would be expected from the equation relating pressure, surface tension, area, and volume:

$$\Delta P_s = \gamma dA/dV$$

where ΔP_s is the component of transpulmonary pressure due to surface tension; γ, the surface tension; A, the area; and V, the volume of an alveolar space. If alveolar size and shape is assumed to remain constant at constant volume, one can extrapolate the γ–ΔP plot to the ΔP measured at FRC and read a value for γ of approximately 7 mN·m^{-1}.

On the other hand, extrapolating the linear volume versus surface tension curve of our previous paper (9) to FRC yields a value of zero for surface tension. The reason for this discrepancy may be that near and below FRC, lung alveoli can be reduced in volume while surface area remains nearly constant (18, 19). This would tend to keep surface tension constant, because the tension decreases in response to surface compression, but would require that the additional forces balancing the tendency to collapse be supplied by tissue. Thus, extrapolating the volume–tension plot below FRC may be incorrect.

Neither the γ–t nor the ΔP–t plot (Fig. 5) exhibits first order kinetics during the initial 2–3 hr, and, in fact, γ and ΔP increase faster at later times. Several possible explanations for these observations come to mind. First, a shift of gas from smaller alveoli into larger ones would increase mean alveolar radius, particularly if collapse of some of the smaller units occurred. The remaining larger alveoli would be expected to have greater surface tensions. Our inability to measure surface tension in alveoli <40 μm in diameter might also have produced falsely high readings at such small volumes. Second, if film compressibility were lower at tensions of 15 to 20 than at 5 to 10 mN·m^{-1}, tension would increase more rapidly, even if the rate of loss of material per unit area were constant. Third, DPPC might undergo enzymatic degradation within the monolayer. The resulting lysophosphatidylcholine, fatty acid, dipalmitin, or other products would be expected to increase surface tension (unpublished observation) and also might be rapidly absorbed by the epithelium. Fourthly, DPPC might be removed from the surface by nonenzymatic processes which have greater velocities at greater surface tension—i.e., at lower film pressure and looser molecular packing. The information we have at present does not permit us to make a definite choice among these possible explanations.

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