Proc. Natl. Acad. Sci. USA
Vol. 73, No. 12, pp. 4698–4702, December 1976
Physiological Sciences

Direct determination of surface tension in the lung

(alveolar surface/spreading of droplets/fluorocarbon fluids)

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Contributed by John A. Clements, September 23, 1976

ABSTRACT We have used the spreading behavior of small drops of several fluorocarbon fluids and silicone oil on air–liquid interfaces to measure the surface tension of lungs *in situ*. The test fluids were calibrated in a surface balance at 37° on monolayers of dipalmitoylphosphatidylcholine. At particular surface tensions characteristic of each fluid used, an increase in the tension of 1 mN/m or less caused the droplets to spread reversibly from a sphere to a lens shape. Using micropipettes we placed such droplets on the alveolar surfaces of excised rat lungs held at functional residual capacity and 37° and found that the surface tension remained below 9 mN/m for at least 30 min. The surface tension–volume relationship was linear for tensions ranging from 9 to 20 mN/m.

Surface tension in the lung has never been measured directly, although estimates have been calculated from *in vitro* experiments with lung extracts or from *in situ* pressure–volume characteristics. In 1955 Pattle (1) reported extraordinary stability of bubbles from surfactant extracts and concluded that this is a function of near zero surface tension produced by surfactant material at the air–liquid interface. He assumed that the film lining the bubbles was the same as the alveolar surface film and thus that the surface tension in the lung was below 1 mN/m.

Clements (2) reported in 1957 the application of the surface balance to studies of lung surfactant. He compressed the expanded films of surfactant periodically and demonstrated that the surface tension varied from 40 to 10 mN/m and that the tension–area characteristic exhibited a large hysteresis. Based on these observations he formulated a theory of alveolar stability (3) which held that as area decreases during deflation, surface tension falls toward zero, thus diminishing the tendency of smaller alveoli to empty into larger ones.

Others have calculated surface tension–area characteristics from pressure–volume data of excised lungs, showing both a large hysteresis and a minimum surface tension below 10 mN/m (4). However, two assumptions were used in deriving the surface tension-area relationship. First, the shape factor K in the area–volume relation, $A = KV^{2/3}$ was assumed not to change with volume and to have the same value for liquid-filled and air-filled lungs. Second, the maximum surface tension at total lung capacity was estimated to be approximately 50 mN/m.

Still others dispute the presence of a low surface tension in the lung. In 1973, Reifenrath and his coworkers withdrew fluid from the alveolar lining layer by a micropuncture technique (5, 6), and used the material to form films for study by a bubble method (7). On the basis of repeated cycling of films from rat alveolar fluid they concluded that the minimum surface tension of alveoli is 18–20 mN/m, a value considered by many authors to be too high to stabilize the lung effectively (8). In addition, papers have recently appeared suggesting that surfactant is not needed to stabilize the lung (9, 10).

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; TLC, total lung capacity; FRC, functional residual capacity.

Since proponents of both low and high surface tension have used indirect means to prove their points, we have sought a direct measure of surface tension within the lung. The results reported below support the contention that alveolar surface tension can indeed reach low levels.

MATERIALS AND METHODS

Control Experiments and Calibration. The method is based on the observation that drops of a variety of nonpolar fluids placed on top of monolayers at the air–water interface change their shape from a sphere to a thin lens as the surface tension is raised past a critical value. The concept of critical surface tension for spreading was originally developed by Zisman (11) from experiments on solid surfaces and was shown to be capable of characterizing the surface properties of liquids (12, 13). Some of the Fluorinert® liquids (3M Co.) are particularly suitable test fluids for studies of lung surfactant monolayers, having surface tensions between 9.5 and 19 mN/m at room temperature. FC 88, at 9.5 mN/m has the lowest surface tension at room temperature, but cannot be used conveniently at 37° since it boils at 30° at one atmosphere ambient pressure.

From the various Fluorinert liquids we chose FC 72, FC 75, and FC 40 as test fluids. Their surface tensions at 37° determined by the Wilhelmy plate method (14) are 9 ± 0.5, 13 ± 0.5, and 16 ± 0.5 mN/m, respectively. Silicone oil (Dow Corning, 1107 fluid) with a surface tension of 20 mN/m at 37° also proved to be a suitable test fluid.

We spread monolayers of dipalmitoylphosphatidylcholine (DPPC) in a film balance on a subphase of 0.9% NaCl from a 1 mg/ml solution in chloroform. The films were confined by an endless Teflon® ribbon attached to a rhombic frame which could be adjusted to change the surface tensions of the films. Surface tension was monitored by a platinum plate-strain-gauge-amplifier system (Statham gold cell). A flexible light pipe provided illumination from below the glass trough. The balance was enclosed in a Plexiglas® box held at 37°. Experiments were done in an atmosphere nearly saturated with the vapor of the particular test fluid.

We placed test fluid droplets of 10–200 μm diameter on top of the DPPC monolayers with a micropipette having a tip diameter of 1–3 μm. We observed that when the surface tension of the test fluid was lower than that of the DPPC film, the spherical drop from the pipette spread out to a thin lens. On the other hand, if the surface tension of the test fluid was greater than or equal to that of the film-covered solution, the droplet remained spherical. For all four test fluids, the transition of the shape from a sphere to a thin lens occurred at a surface tension not measurably different from the surface tension of the test fluid itself. The size of the drops had no influence on the transition. Fig. 1 shows a drop of FC 40 (γ = 16 mN/m) resting on a DPPC film at three different surface tensions. Less than 0.5 mN/m above 16 mN/m, the spherical drop changes its shape to a lens. AT 17 mN/m, the diameter of the lens is approxi-

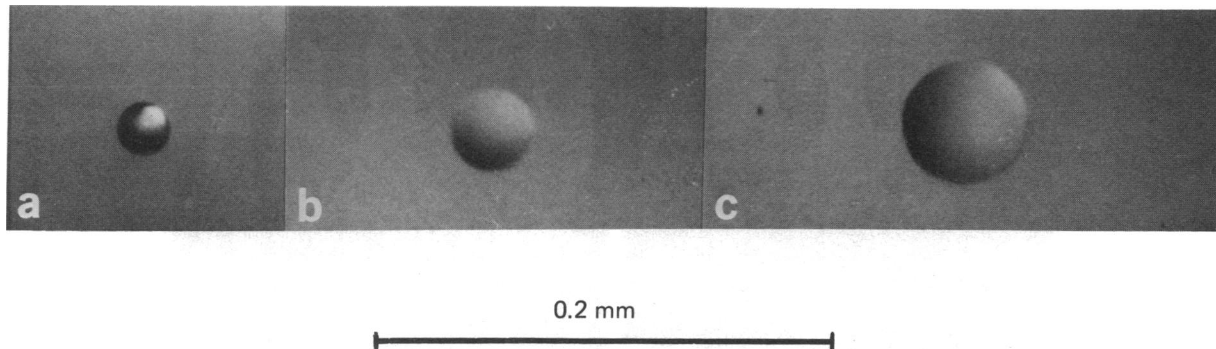

FIG. 1. Drop of FC 40, (γ = 16 mN/m) resting on a DPPC monolayer at three different surface tensions, at 23°. (a) $\gamma \leq$ 16 mN/m, (b) γ = 17 mN/m, (c) γ = 20 mN/m.

mately twice that of the original spherical drop, and at 20 mN/m it is about three times as great.

Fig. 2 is a plot of sphere-to-lens transition surface tensions versus the corresponding surface tensions of the pure test components themselves, measured with the Wilhelmy method. Drops of test fluids were placed onto the DPPC film at a surface tension of approximately 30 mN/m. The fluid lenses were observed through a stereo microscope while the surface tension was decreased by reducing the film area slowly. At the transition point, when the lens took a spherical shape, the surface tension was noted. The precision was better than ±0.5 mN/m and the surface tensions of the test fluids could be determined with standard errors smaller than 0.5 mN/m by this method. Thus, the surface tension of an underlying phase is lower than or equal to that of the test fluid if the spherical drop keeps its shape, or higher if spreading to a lens occurs.

Films of distearoyl and other disaturated phosphatidylcholines, of sphingomyelin (palmitoyl), and also of lung surfactant were used and gave equivalent results. We further demonstrated that the change of shape of the droplets was not dependent on the depth of the subphase in the surface balance by slowly raising a block of 0.5% agar below the monolayer until all the visible water was drained from the space between the monolayer and the agar surface. By adding sucrose or polysaccharides to the subphase we could also show that the viscosity of the subphase had no influence on the surface tension at which the transition of the shape of the test fluid droplets occurred.

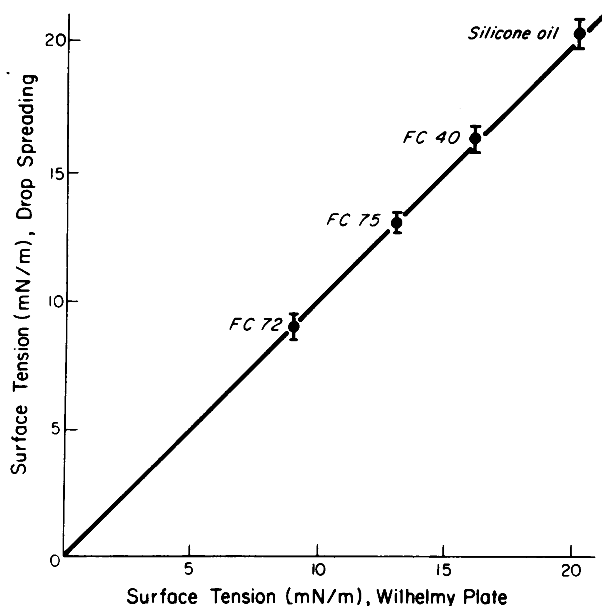

FIG. 2. Plot of surface tensions at sphere-to-lens transition versus the corresponding surface tensions of the pure test fluid components themselves, measured by the Wilhemy method. Temperature is 37 ± 1°. The points represent mean ± standard error and the line is the line of identity.

***In Situ* Studies.** Rats, 300–500 g, were anesthetized with 30–60 mg of intraperitoneal sodium pentobarbital. The lungs were excised, degassed, and put in a temperature-controlled chamber on a plastic dish which was partially filled with 0.9% NaCl solution. We connected the trachea to a constant volume manometer and a syringe, and allowed the lungs to warm up to 37° for 10–15 min. The lungs were then inflated stepwise with air from the syringe until the transpulmonary pressure was 25 cm H_2O (1 cm H_2O = 98 Pa). Two minutes was allowed for equilibration at each step. Total lung capacity (TLC) at 25 cm H_2O pressure was recorded and the functional residual capacity (FRC) was taken as 40% TLC. For each measurement during deflation 3–4 ml of air was withdrawn and an equilibration time of 2 min was allowed. At FRC, the pressure recorded was usually between 3.5 and 4.5 cm H_2O. If the pressure at FRC was above 4.5 cm H_2O, that particular lung was discarded. We then degassed the lungs and reinflated them to TLC using air almost saturated with the vapor of the test fluid. After equilibration for 2 min at TLC the lung was considered ready for the surface tension measurements during deflation. Using four lungs, we compared the pressure–volume curves obtained by air inflation and by inflation with air almost saturated with the vapor of the test fluid and noted no measurable difference (t-test, $P <$ 0.005).

Two 150 W light sources connected to flexible light pipes provided transmitted illumination from below the lung tissue, and a water layer of about 1 cm thickness below the lung served to decrease heat input to the lung. Particular care was taken to measure and control lung temperature. Three lungs were used solely to monitor the temperature change due to illumination. A thermistor needle probe was introduced into the lung tissue near the edge of a lobe and the change of temperature with time was determined for a given temperature of the lung environment. During observation the light sources were used only for short periods of time, up to 30 sec, so that the temperature at the point of observation in the lung could be kept at 37 ± 1°.

Micropipettes. Before the pipettes were pulled, the glass tubes were filled to capacity with glass fibers of 60–70 μm diameter (Radio Shack). (Micropipette Puller: Industrial Science Assoc., Ridgewood, N.Y., glass tubing: Corning 7740). The glass fibers had two functions: first, to facilitate filling of the pipettes with a test fluid; second, to provide enough resistance to flow so that controlled quantities of fluid could be squeezed out and flooding of the alveoli avoided. The pipettes had tip diameters of 1–3 μm and were beveled for easier penetration through the pleural surface. The pipettes were connected with Teflon

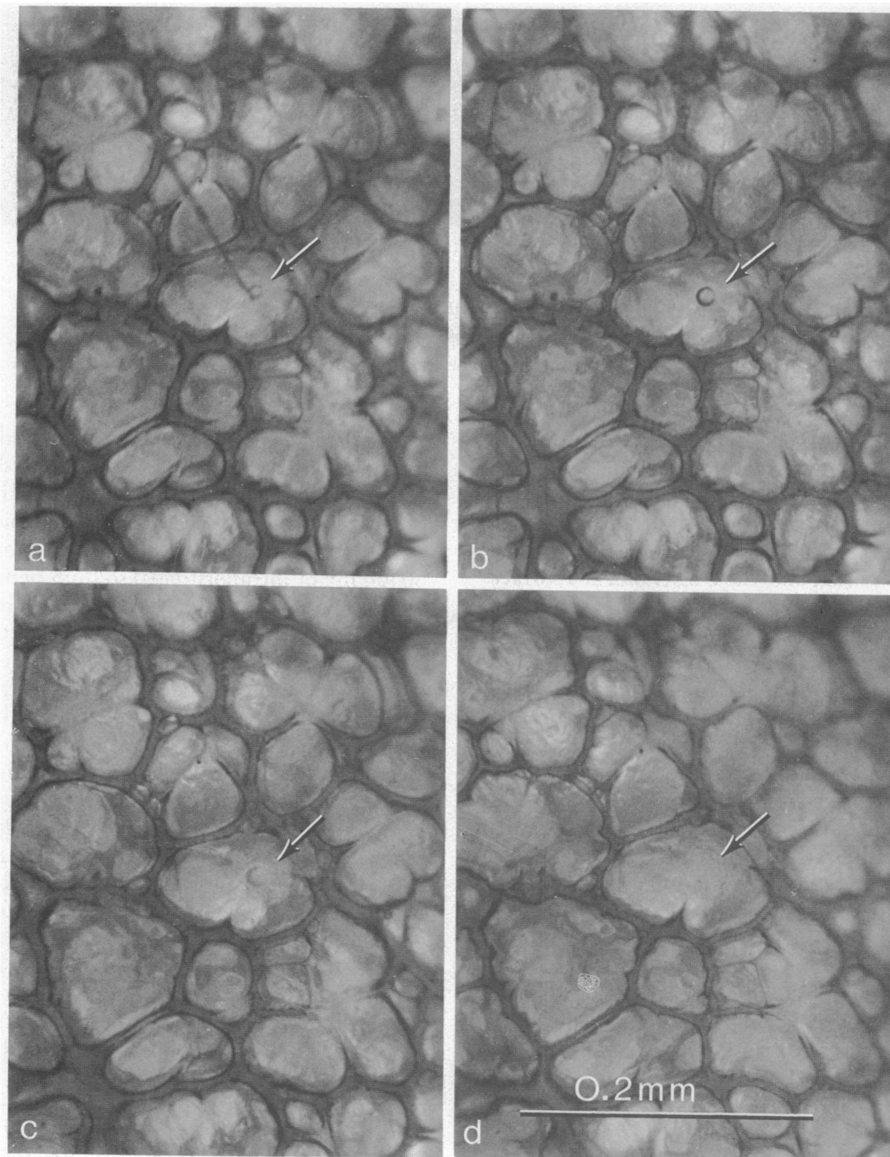

FIG. 3. Typical experiment performed on a cat lung at 23°. Test fluid FC 40 (γ = 16 mN/m). Same magnification in all photographs. (a) Micropipette, its tip in an alveolar space with a drop prior to deposition. (b) Same drop on the alveolar surface adjacent to the pleural surface. Since the drop did not spread upon deposition, the surface tension was taken as $\leq$ 16 mN/m. Lung volume was approximately 70% TLC. (c) Same drop at an alveolar surface tension greater than 16 mN/m. Volume was about 80% TLC. (d) Lung at 90% TLC. Lens is no longer visible.

tubing to a precision graduated syringe and mounted on a micromanipulator. They reached the interior of the temperature-controlled chamber through a rubber membrane.

Micropuncture of Alveoli. The edge of the lobe to be punctured was viewed through a stereo microscope (Zeiss) with a magnification up to 100×. At low magnification the micropipette was advanced to the pleural surface and the magnification was gradually increased. Although the very tip could not be seen because of its small diameter, the alveolus to be punctured could easily be recognized, since the pleural surface adjacent to the alveolus was slightly dimpled prior to penetration. We then punctured the alveolus and pulled back the micropipette until the external surface distortion disappeared. A drop of fluid was then squeezed out and remained hanging on the pipette when the tip was in the alveolar space. By moving the micromanipulator gently, we could move the spherical drop on the pipette back and forth in the alveolar space. The micropipette was then gently withdrawn or advanced until the drop was deposited onto an alveolar surface. As soon as the drop touched the alveolar surface, it either spread out to a barely visible lens or stayed spherical with no observable change in diameter. In this latter case our *in vitro* studies indicated that the surface tension in the alveolus was lower than or equal to the surface tension of the test fluid. When spreading occurred, the surface tension in the alveolus was therefore taken to be higher than that of the fluid. Observations were done in alveoli of the top two layers adjacent to the pleural surface and 1–3 mm from the edge of a lobe. The fluid droplet diameter was approximately 10 μm for rat lung alveoli. For one measurement, observations in at least ten different alveoli were made. If the surface tension in an alveolus was lower than the surface tension of the test fluid, three typical events occurred: (*i*) the drop could be deposited on a horizontal surface where it remained after the pipette was completely withdrawn; (*ii*) the drop would move slowly due to gravity without changing its diameter appreciably and come to rest still visible somewhere in the al-

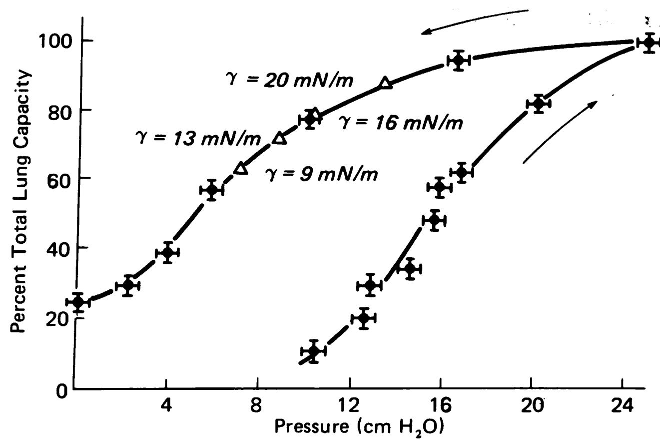

FIG. 4. Typical pressure–volume characteristic of rat lungs at 37°. Surface tension values found in the spreading experiments have been added on the deflation limb. Error bars represent the estimated precision of the pressure and volume readings.

veolus; or (*iii*) the drop would move out of sight so fast that no clear observation was possible.

RESULTS

Fig. 3 demonstrates a typical experiment performed on a cat lung at room temperature. Photographs of rat lungs were less clear because of small alveolar size, although visual observations were easily made. Fig. 3a shows the micropipette, its tip in an alveolar space with a drop prior to deposition. The test fluid was FC 40 (γ = 16 mN/m, temperature 25°). Fig. 3b shows the spherical drop following deposition on the alveolar surface adjacent to the pleural surface. Since the drop did not spread upon deposition, the surface tension in Fig. 3b was taken as ≤ 16 mN/m. The pressure was 8 cm H_2O, corresponding to a volume of approximately 70% TLC. Fig. 3c shows the same drop at an alveolar surface tension greater than 16 mN/m. The lung volume was about 80% TLC. In Fig. 3d, at a volume of about 90% TLC, the lens is no longer visible. In many cases the original spherical shape of the drop could be regenerated merely by deflation of the lung, showing the measuring process to be a reversible one.

Surface Tension Rise with Time at FRC. The lungs were deflated from TLC for 3–4 min until the pressure reached a value corresponding to FRC. Timing was started after a 2 min equilibration at this pressure. At least ten alveoli were punctured per lung, and droplets of FC 72 (γ = 9 mN/m) were placed onto alveolar surfaces. In a series of 10 punctures usually between two and three droplets could be placed onto horizontal surfaces where they stayed if the micropipette was withdrawn very carefully. The shapes of these drops were then observed continuously until the original spherical droplet had increased its diameter approximately 50%, indicating an increase of the surface tension of 0.5–1.0 mN/m. In a series of 10 rat lungs with a total of 25 observations, we found that it took 30 ± 2 min (Mean ± SEM) for the surface tension to rise to 9 ± 1 mN/m, at 37° and FRC. Observations over longer periods of time revealed that droplet spreading occurred slowly beyond that point, another 15–30 min passing before the diameter of the drops increased 100%: i.e., the surface tension had risen to 10–11 mN/m. Nine mN/m was not reached simultaneously in all of the punctured alveoli of a particular lung. We observed time differences of up to 15 min between alveoli.

Surface Tension at Different Volumes. The lungs were deflated from TLC for 3–4 min until the pressure reached its FRC value, after which two min were allowed for equilibration. Several alveoli were then punctured and droplets of a particular test fluid were placed onto alveolar surfaces. Transpulmonary pressure was then increased slowly until the originally spherical droplets clearly began spreading. The pressure at which spreading started was recorded and the related volume was determined from the pressure–volume characteristic. This method of observing the droplets on horizontal surfaces was successful in only 30% of the trials because deflation or inflation often caused the lungs to move and test fluid droplets to roll out of view. We therefore adopted an additional method of observation. Starting at TLC, the lungs were deflated to a pressure of about 15 cm H_2O, 10 different alveoli were punctured, and the behavior of the droplets was recorded. Pressure was further reduced 2 cm H_2O and again 10 alveoli were punctured and droplets were deposited. This procedure was repeated until the drops kept their spherical shape upon placement on alveolar surfaces, indicating a surface tension less than or equal to that of the test fluid. The lungs were then reinflated stepwise by increasing the pressure in intervals of 1 cm H_2O. After each step 10 alveoli were punctured and the behavior of the droplets was observed. The pressure at which spreading occurred was recorded, and the midpoint of the last pressure interval was taken as the transition pressure at which the surface tension in the alveoli was equal to that of the test fluid. At this particular pressure the related volume was determined from the pressure–volume characteristic.

Table 1. Lung volume–alveolar surface tension relationship

No. of lungs	No. of observations	% TLC, mean ± SEM	Surface tension (mN/m)	Test fluid
6	25	62 ± 1.2	9	FC 72
7	31	71 ± 1.0	13	FC 75
5	21	78 ± 1.6	16	FC 40
4	18	87 ± 1.3	20	Silicone oil

We made two to eight measurements in each of four to seven rat lungs for each fluid tested. The measurements from a particular lung were treated as a sample. An analysis of variance at the 5% level of significance showed that all of the samples were taken from the same population, and hence the results from different lungs were pooled. Table 1 summarizes our findings. Fig. 4 gives a typical pressure–volume characteristic of rat lungs. Surface tension values found in the spreading experiments have been added on the deflation limb. Fig. 5 shows the average surface tension–volume relationship.

DISCUSSION

Our measurements confirm in a direct way what many surface balance and pressure–volume studies have suggested: alveolar surface tension does reach very low values during deflation of the lungs. In rat lungs, at FRC and 37° we found surface tension to be ≤9 mN/m. Unfortunately we have found no convenient test fluid for even lower values. Alveolar surface tension at FRC remained below 9 mN/m for about 30 min at 37°. This time is much longer than that expected from measurement of surfactant in the surface balance, (15–18) or with a bubble method (19). However, Horie *et al.* (20) calculated from pressure–volume curves of excised cat lungs that the surface tension rose only 1–2 mN/m during 20 min at 40% TLC and room temperature. The low surface tension at FRC and its high stability

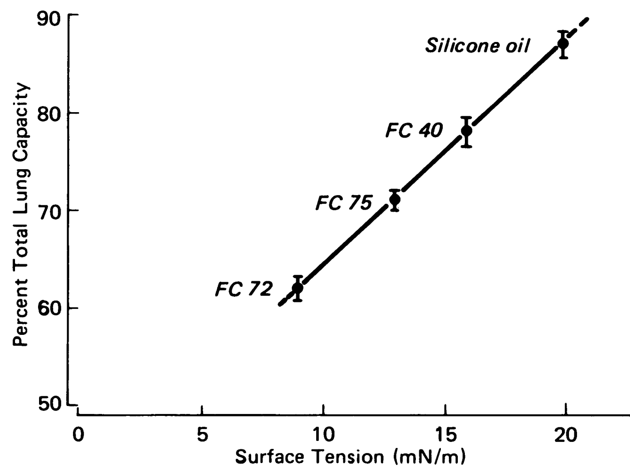

FIG. 5. Volume-surface tension relationship for rat lungs at 37°. (See Table 1.) The volume corresponding to a particular surface tension was found by sphere-to-lens transition of test fluid droplets in alveoli.

with time suggest that the lipids in the alveolar surface film are present in different proportions from those in films formed in a surface balance from the complex mixture of lipids present in lung surfactant. The alveolar film is probably greatly enriched in fully saturated phosphatidylcholine, especially DPPC. Preliminary results in our laboratory suggest that films of pure DPPC or mixed films with relatively large quantities of DPPC are able to produce very stable, low surface tensions ot 37° (21). Some selection process, not yet fully understood, may produce a film rich in DPPC.

By direct determination of the alveolar surface tension at various volumes, we find a linear relationship between surface tension and volume during deflation of rat lung from approximately 85–60% TLC at 37°. We hope to find suitable test fluids for surface tensions above 20 mN/m to allow the study of lung surface tension at volumes greater than 85% TLC.

We are grateful to Mr. James Noel of the 3M Company for supplying samples of the Fluorinert liquids used in our pilot studies, and to Dr. Donald M. McDonald for valuable help with photography. S.S. is a Julius H. Comroe, Jr., Fellow. J.G. is supported in part by Grant HL-06285 from the National Heart, Lung, and Blood Institute. J.A.C. is a Career Investigator, American Heart Association.

1. Pattle, R. E. (1955) "Properties, function and origin of the alveolar lining layer," *Nature* **175**, 1125–1126.
2. Clements, J. A. (1957) "Surface tension of lung extracts," *Proc. Exp. Biol. Med.* **95**, 170–172.
3. Clements, J. A., Hustead, R. F., Johnson, R. P. & Gribetz, I. (1961) "Pulmonary surface tension and alveolar stability," *J. Appl. Physiol.* **16**, 444–450.
4. Bachofen, H., Hildebrandt, J. & Bachofen, M. (1970) "Pressure-volume curves of air- and liquid-filled excised lungs—surface tension *in situ*," *J. Appl. Physiol.* **29**, 422–431.
5. Reifenrath, R. & Zimmermann, I. (1973) "Blood plasma contamination of the lung alveolar surfactant obtained by various sampling techniques," *Respir. Physiol.* **18**, 238–248.
6. Reifenrath, R. & Zimmermann, I. (1973) "Surface tension properties of lung alveolar surfactant obtained by alveolar micropuncture," *Respir. Physiol.* **19**, 369–393.
7. Slama, D., Schoedel, W. & Hansen, E. (1971) "Bestimmung der Oberflächeneigenschaften von Stoffen aus den Lungenalveolen mit einer Blasenmethode," *Pfluegers Arch.* **322**, 355–363.
8. Goerke, R. J. (1975) "Lung surfactant," *Biochim. Biophys. Acta* **344**, 241–261.
9. Fung, Y. (1975) "Does the surface tension make the lung inherently unstable?," *Circulation Res.* **37**, 497–502.
10. Reifenrath, R. (1975) "The significance of alveolar geometry and surface tension in the respiratory mechanics of the lung," *Respir. Physiol.* **24**, 115–137.
11. Zisman, W. A. (1964) in *Contact Angle*, Advances in Chemistry Series 43, ed. Gould, R. F. (American Chemical Society, Washington, D.C.), pp. 1–51.
12. Jarvis, N. L. & Zisman, W. A. (1959) "Surface activity of fluorinated organic compounds at organic liquid-air interfaces. I. Surface tension, parachor and spreadability," *J. Phys. Chem.* **63**, 727–734.
13. Shafrin, E. G. & Zisman, J. A. (1967) "Critical surface tension for spreading on a liquid substrate," *J. Phys. Chem.* **71**, 1309–1316.
14. Goerke, J., Harper, H. H. & Borowitz, M. (1970) "The interaction of calcium with monolayers of stearic and oleic acid," in *Surface Chemistry of Biological Systems*, ed. Blank, M. (Plenum Press, New York), pp. 23–36.
15. Tierney, D. F., & Johnson, R. P. (1965) "Altered surface tension of lung extracts and lung mechanics," *J. Appl. Physiol.* **20**, 1253–1260.
16. Tierney, D. F. (1965) "Pulmonary surfactant in health and disease," *Dis. Chest* **47**, 247–253.
17. Clements, J. A. (1967) in *Development of the Lung*, a Ciba Foundation Symposium, eds. De. Reuck, A. V. S. & Porter, R. (J. and A. Churchill, London), pp. 202–221.
18. Schoedel, W., Slama, H. & Hansen, E. (1969) "Zeitabhängige Veränderungen des Filmdruckes alveolärer Oberflächenfilme im Langmuir-Trog," *Pfluegers Arch.* **306**, 20–32.
19. Schoedel, W., Slama, H. & Hansen, E. (1971) "Zeitabhängiges Verhalten von Filmen von oberflächenaktivem Material aus Lungenalveolen," *Pfluegers Arch.* **322**, 336–346.
20. Horie, T. & Hildebrandt, J. (1971) "Dynamic compliance, limit cycles, and static equilibria of excised cat lung," *J. Appl. Physiol.* **31**, 423–430.
21. Goerke, J. & Clements, J. A. (1973) "Relative stability of air/water films of palmitoyl, myristoyl- and dipalmitoyl-phosphatidylcholine at 37°C," *Physiologist* **16**, 323.