

## A STUDY OF SOME BIOLOGICAL SURFACTANTS

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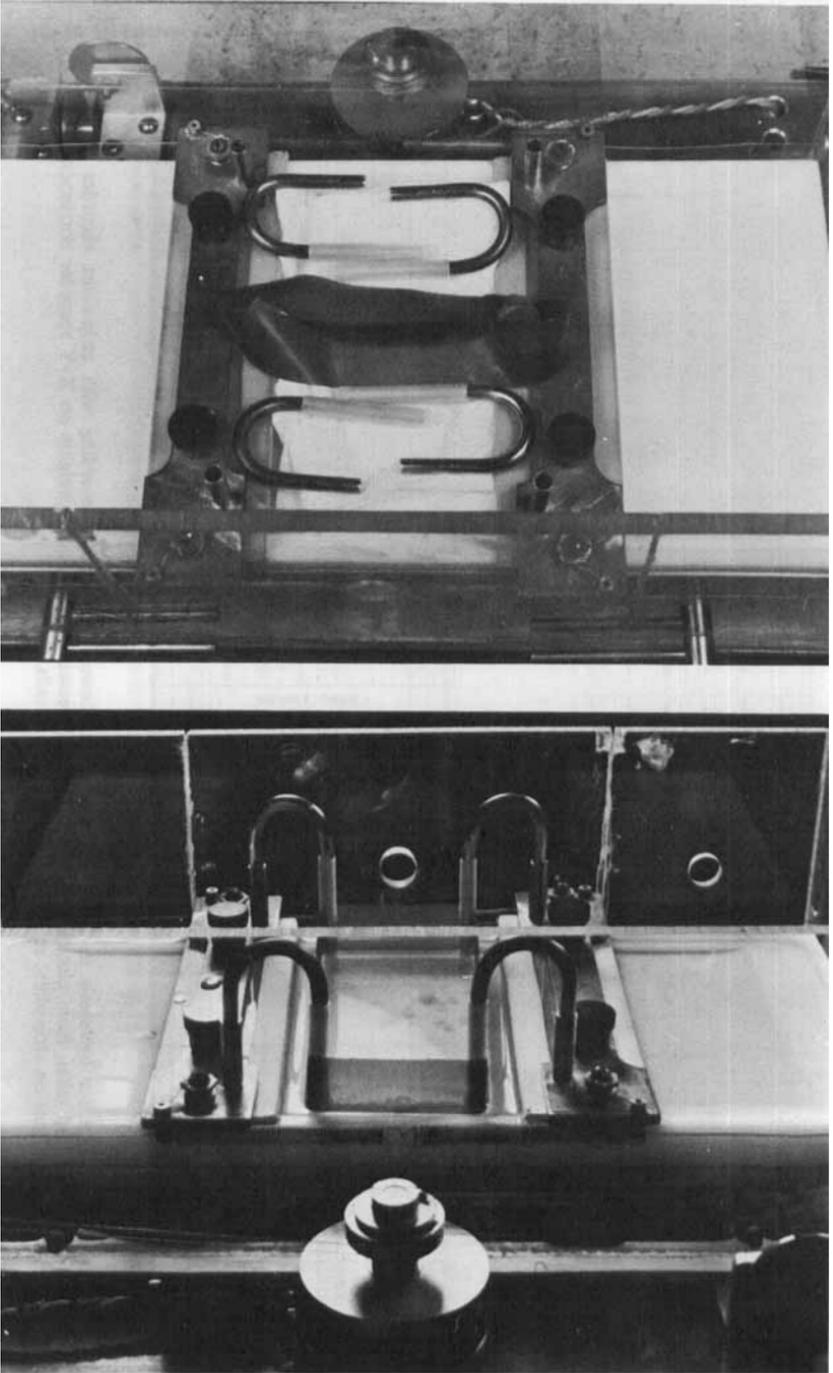
A vertical pull surface balance was used in two different ways to develop comparisons between static and dynamic surface tensions ( $\gamma_s$ ) of lung alveolar surfactant (LAS)<sup>1,2</sup> and several other biological surfactants. The first method permitted each surfactant a certain freedom to spread beyond the confines of the barriers of the surface balance at low  $\gamma$ .<sup>3,4</sup> The second method inhibited such spreading. The results are applied to the role of LAS in lung mechanics and help to reconcile the difference in static  $\gamma$  of LAS obtained with the surface balance and those reported for bubbles derived from the same material.<sup>5</sup> The marked spreading tendency and local surface turbulence shown by surface films of LAS could provide a mechanism for transport at surfaces of this type of mucus. Evidence is given which indicates that films of LAS may be shown capable of converting chemical to mechanical energy.

### METHODS

The surface balance described elsewhere<sup>6</sup> was used to provide stationary and moving areas at the air-water interface. Under dynamic conditions, area was varied cyclically from a maximum to a minimum and back to a maximum at a rate of 10 cycles/min. For static conditions, the same free cycling rate was used, but the change of surface area was arrested periodically for equal intervals of time during the course of a single complete cycle.<sup>4</sup> The downward pull on a plate touching the hypophase and attached by glass fibers to an electric balance was a measure of  $\gamma$  at the air-water interface. It was recorded with a null type X-Y recorder and sometimes also with a strip chart recorder and/or a recording X-Y oscilloscope.

The modified balance has not been described previously (FIGURE 1). A post, sleeved with polytetrafluoroethylene, was placed at each end of the 2 brass supports for the barriers of the surface balance, to form the 4 corners of a rectangle. Dental dam was cut into strips 2 cm wide and the ends glued together with rubber cement. The length of the closed rubber strip was such that it fitted over the four posts, forming the four edges of the rectangle, with a minimum tension when the area enclosed by it was at a minimum. The rubber adhered sufficiently to the polytetrafluoroethylene sleeves so as to slide over them only slightly, resulting in stretch of the rubber mainly in the direction of motion of the barriers. At minimum area the rubber barrier dipped 5 mm into the hypophase and to 3 mm of the bottom of the trough, which was cut to a depth of 8 mm. The two former distances became 4 mm each at maximum area. The glued rubber barrier and supporting sleeves did not alter the  $\gamma$  of distilled water during several half-hour test periods of cycling.

Results of some of the experiments demanded special attention to the possibility of hysteresis in the electronic equipment. Accordingly, the electrical components of the entire system were operated as in a routine experiment. Instead of the plate, however, the chain mechanism of a chain balance was so placed that its free end hung from the glass fibers which otherwise connected the plate to the electric balance. Weight was then supplied smoothly in equal increments and decrements to the electric balance and recorded with the X-Y and strip chart



**FIGURE 1.** Equipment for adding rubber dam enclosure to surface balance (top). Enclosure in use (bottom).

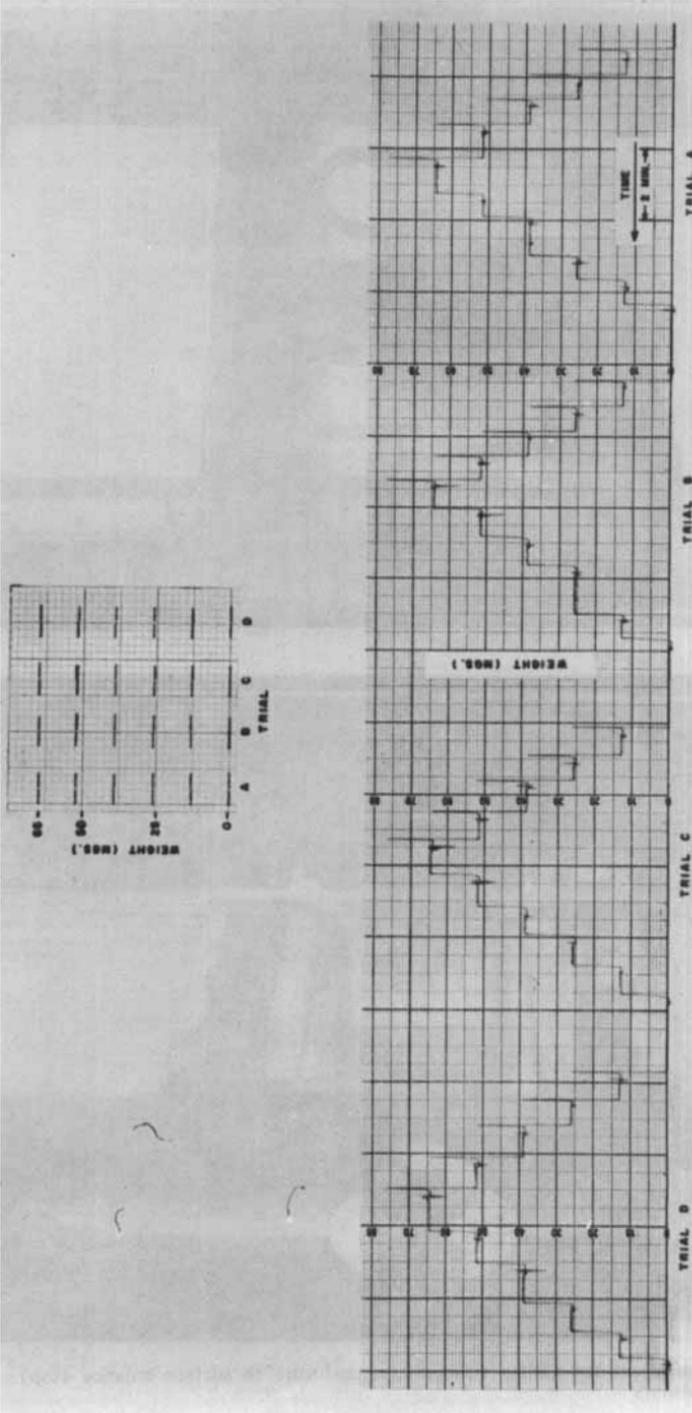


FIGURE 2A. Test for hysteresis in electronic equipment. Lower: continuous recording with strip-chart recorder of weight added to and taken from electric balance. Upper: increased and decreased weights on X-Y recorder corresponding to horizontal lines on strip-chart recording. See text for details.

recorders 4 times successively (FIGURE 2A). Each addition of weight was greater than the final increment, but each subtraction was made directly to the desired amount to prevent apparent hysteresis in the mechanism for adding and subtracting chain to the balance. To allow the chain and glass fibers to hang vertically from the balance, the two branches of the hanging chain had to form an angle of nearly  $360^\circ$ . Irregular movement of chain links through the resultant arc produced a noticeable hysteresis in apparent weights. The artifact, and its elimination by tapping the chain, were recorded on the strip chart. Recording equipment was tested for hysteresis by simultaneous use of 3 different types to record  $\gamma$ -area changes producible with LAS (FIGURE 2B).

During protracted experiments under static conditions, it was necessary to ensure a contact angle of zero between the plate and the meniscus formed on it at the moment of measurement of  $\gamma$ . To do this, the surface balance trough was lowered on its supporting jack until the plate broke away from the surface. It was then raised slowly until the lower edge of the plate touched the surface again, and a fiducial mark was placed upon the frame of the jack opposite a marked link on the jack-chain which actuated the four jack-screws.<sup>6</sup> Following this, the lower edge of the plate was immersed .5 cm into the hypophase, then brought just in contact with the surface again by re-aligning the marks on the frame and the chain. Measurements with a cathetometer showed that the slide could be positioned reliably by this technique, with no special precautions, to within a vertical distance of 40 micra, leading to a maximum error due to buoyancy of about 0.03 dynes/cm in the measured  $\gamma$ .

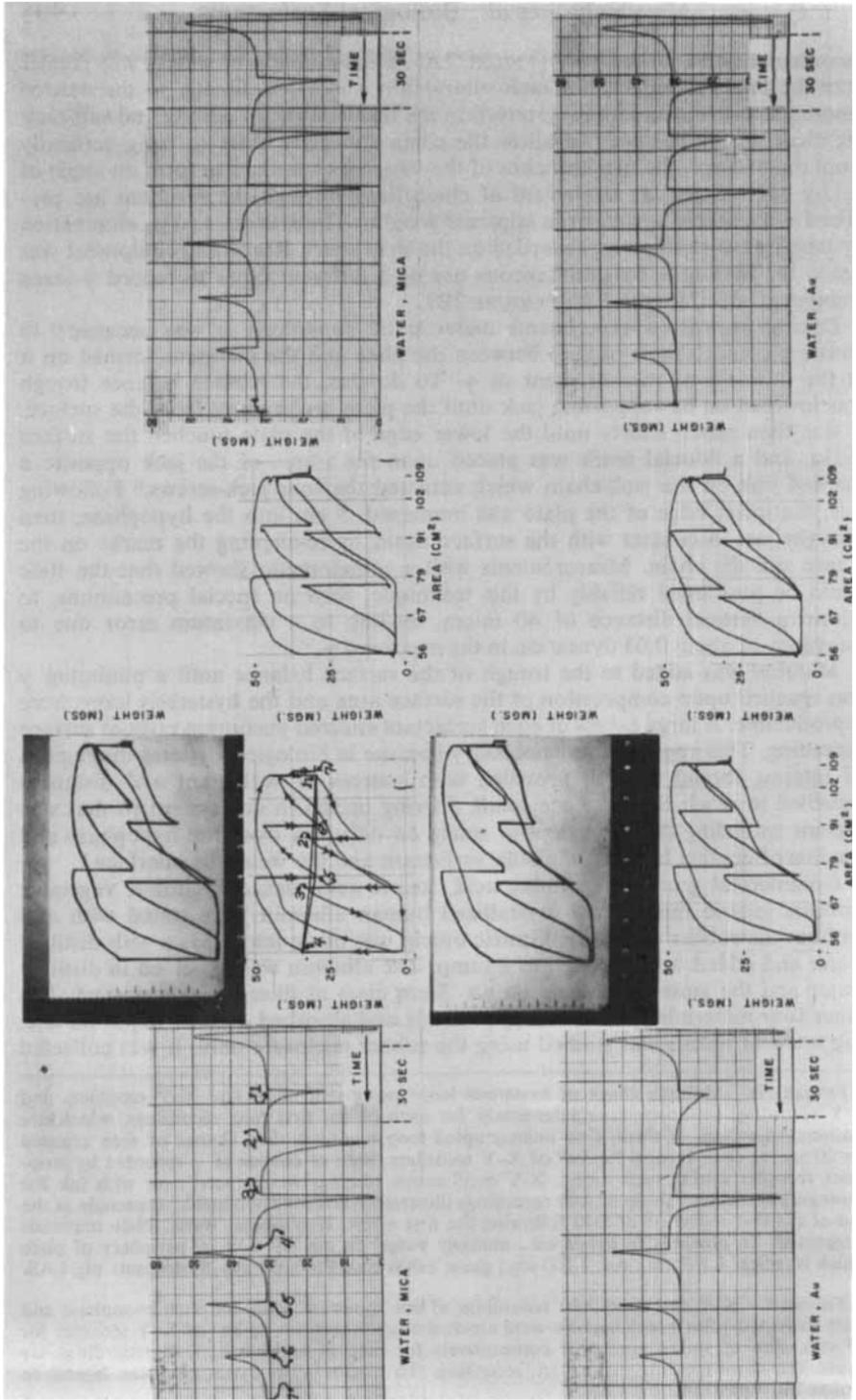
Material was added to the trough of the surface balance until a minimum  $\gamma$  was reached upon compression of the surface area and the hysteresis loops were reproducible. A large excess of each surfactant ensured maximum rates of surface spreading. This approach was necessary because in biological systems the regions of interest should be well provided with sources of surfactant and distances travelled to reach the interface small. Stirring occurs in our system so that surfactant spreading does not depend solely on diffusion from the hypophase and the line of contact between the bulk surfactant and the water-air interface.<sup>7</sup>

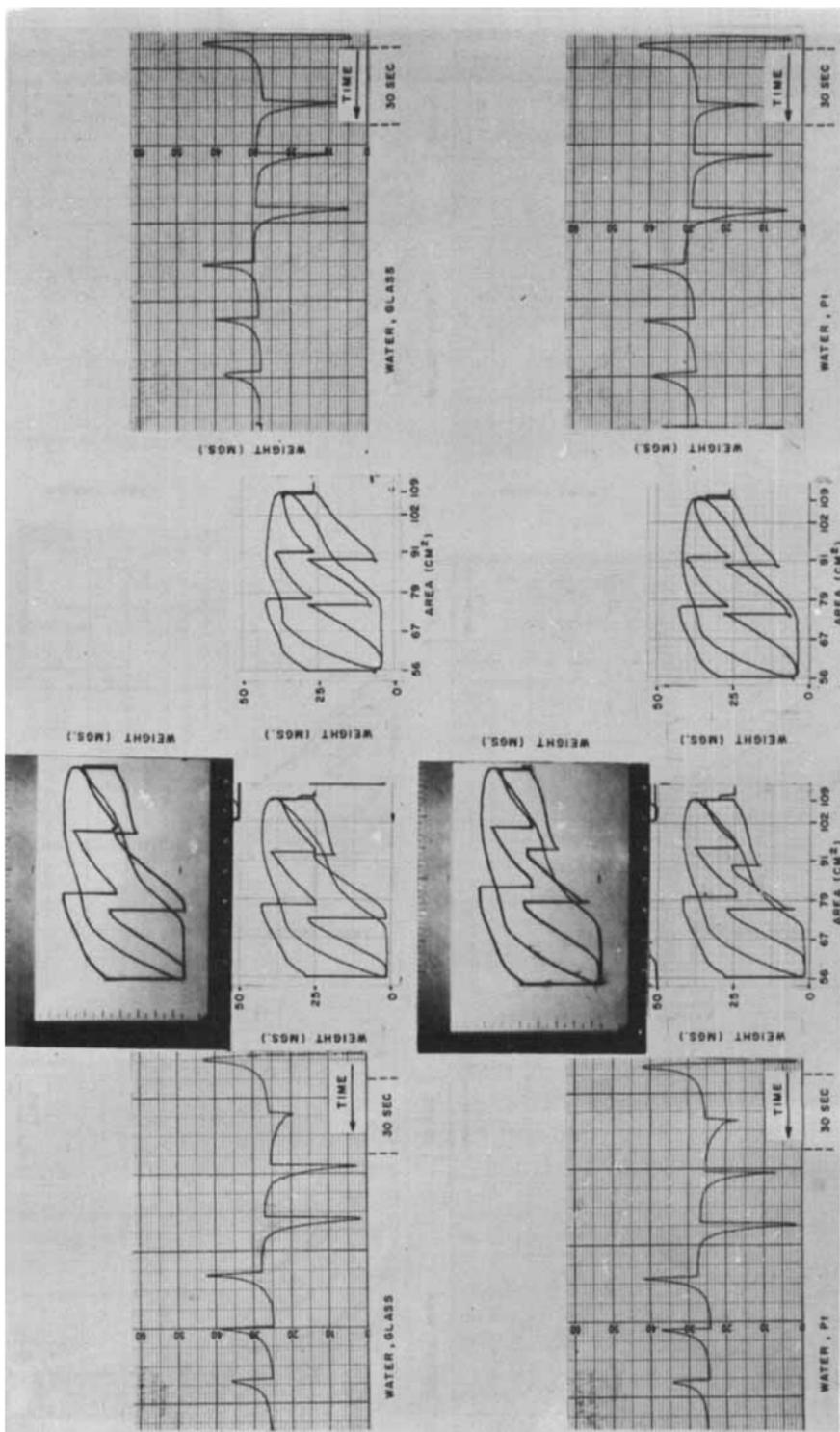
Commercial grades of palmitic acid, stearic acid, hexadecylamine, vegetable lecithin, gastric mucin, and crystallized human albumin were tested with and without the rubber enclosure. Gastric mucin was made into a paste with distilled water and added to the trough as a lump. The albumin was dissolved in distilled water and the amount retained on six .5 cm discs of filter paper was used. The other four materials were dissolved in EtOH and absorbed on filter paper for use. Pig tracheal mucus was studied using the rubber enclosure only. It was collected

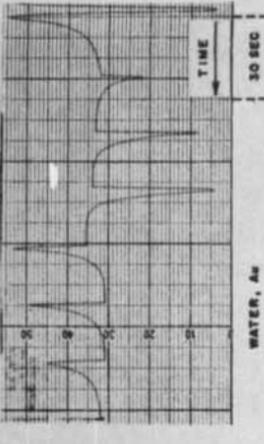
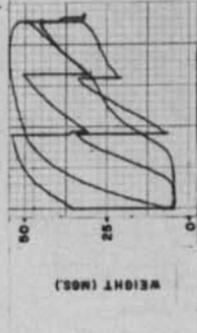
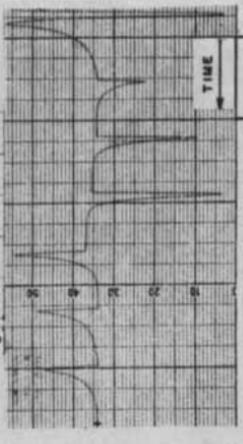
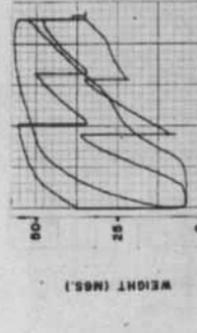
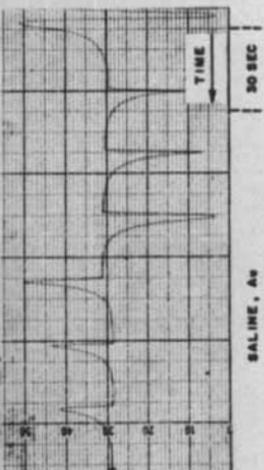
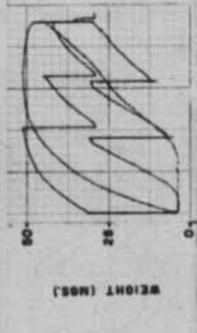
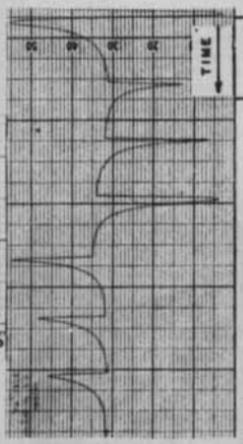
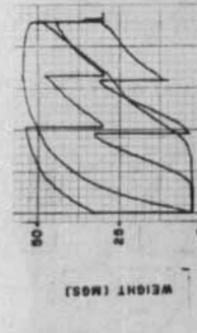
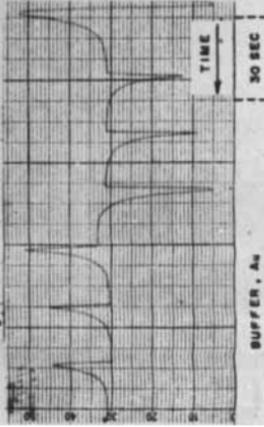
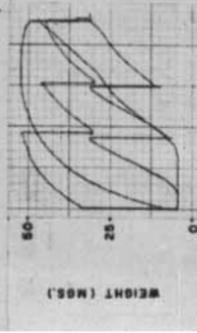
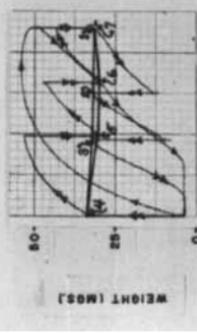
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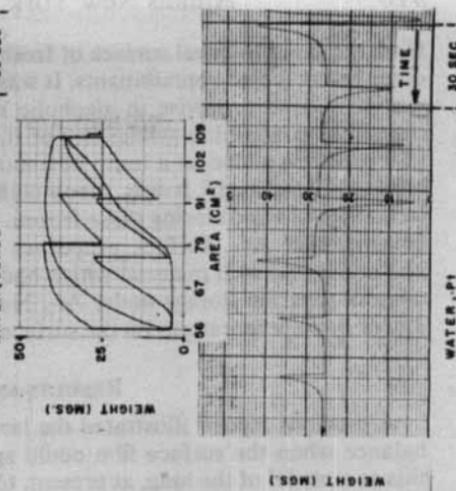
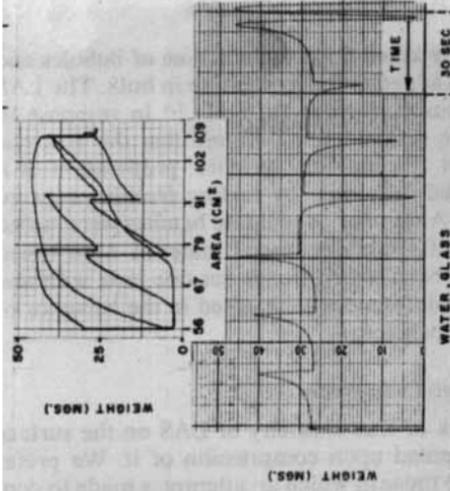
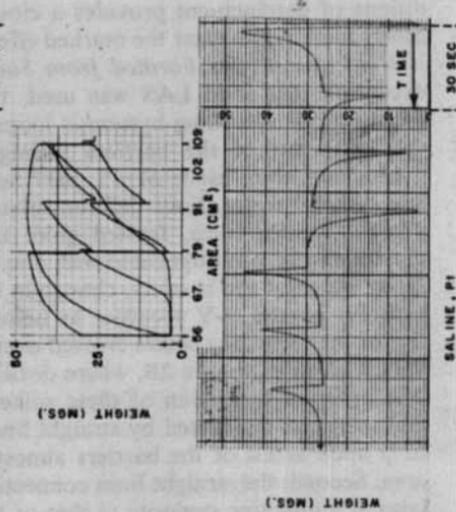
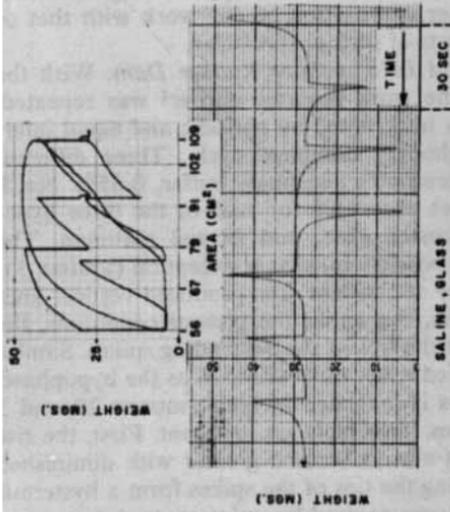
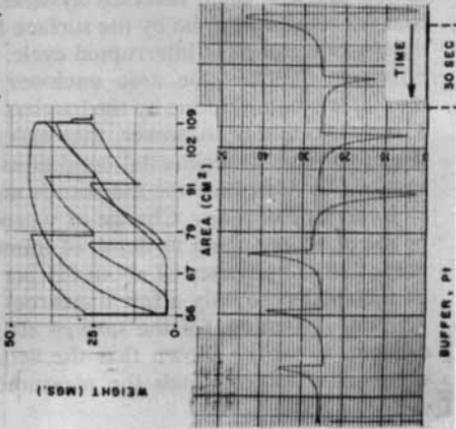
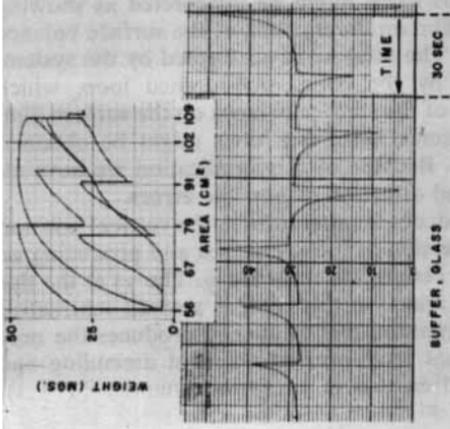
FIGURE 2B. Multiple check of hysteresis loops using strip-chart and X-Y recorders and X-Y recording oscilloscope simultaneously for each of the first four recordings, which are clustered in groups of three. One uninterrupted loop recorded, then change of area arrested for 20 sec. at each vertical "spike" of X-Y recorders. Rate of change of  $\gamma$  recorded by strip-chart recorder during each arrest. X-Y oscilloscope tracings were traced over with ink for photography. Second group of four recordings illustrates variations obtainable, especially at the end of the first reduction of area following the first arrest. Hypophase: water. Plate materials designated. To obtain  $\gamma$  in dynes/cm., multiply weight in mg. by  $0.59 \div$  periphery of plate which is: mica, 1.168 cm.; Au, 1.100 cm.; glass, 1.006 cm.; Pt, 0.950 cm. Surfactant: pig LAS.

FIGURE 3. X-Y and strip-chart recordings of one hysteresis loop, for each hypophase and plate indicated, after which barriers were arrested at each vertical "spike" of X-Y recorder for 20 secs. Tips of spikes numbered consecutively for data at upper left. X-Y recordings are above the corresponding strip-chart recording. To obtain  $\gamma$  in dynes/cm., see legend to FIGURE 2B. Surfactant: pig LAS.









from the inner tracheal surface of freshly killed hogs and was free of bubbles and other macroscopic contaminants. It was added to the hypophase in bulk. The LAS was not added dropwise in alcoholic suspensions as formerly,<sup>3,4</sup> in response to objections concerning artificiality of the system.<sup>8</sup> It was found that this material also could be added as a lump of about 100 mg (dry weight); preparation of it has been described.<sup>3</sup> It was accumulated ordinarily by adding freshly prepared batches to old and storing these frozen. A number of separate batches were stored frozen under  $\text{Ar}_2$ . Surface properties of freshly prepared material were tested and compared with material which had been stored frozen for one year with and without separate storage under  $\text{Ar}_2$ . No differences were noted in the behavior of any of these preparations on the surface balance.

### RESULTS AND DISCUSSION

A previous report<sup>4</sup> illustrated the lack of true elasticity of LAS on the surface balance when the surface film could spread upon compression of it. We prefer this as a model of the lung, at present, to those in which an attempt is made to confine the surface within rigidly defined areas. Nonetheless, approaching such conditions of confinement provides a closer comparison of our work with that of others, and makes clear the marked effects of surface spreading.

*Hysteresis Loops Formed from Solid LAS without Rubber Dam.* With the exception that solid LAS was used, the work reported earlier<sup>4</sup> was repeated, consisting of recording hysteresis loops interrupted by periodic and equal intervals of arrest of the barriers throughout a complete cycle. Three different hypophases were used: M/15, pH7 Sorenson's phosphate buffer, 0.85% NaCl, and distilled water. Four different plates were used for each of the three hypophases: frosted mica, frosted gold, frosted glass, and frosted platinum. The previous work was duplicated with one, possibly important exception (FIGURE 3). Upon arrest of the barriers, change in  $\gamma$  at constant area produced vertical lines (spikes) on the X-Y recorder as before, but under the present conditions, the tips of the ascending spikes crossed over the tips of the descending spikes. Similar data is given in FIGURE 2B, where distilled water only was used as the hypophase. The order of formation of these spikes is indicated in both FIGURES 2B and 3 and their tips connected by straight lines. Two facts are apparent. First, the rise in  $\gamma$  upon arrest of the barriers almost always became greater with diminished area. Second, the straight lines connecting the tips of the spikes form a hysteresis loop in a direction opposite to that of loops formed by uninterrupted movement of the barriers. Such reversed hysteresis loops might be interpreted as showing that net work was done by the surface film on the barriers of the surface balance during one complete interrupted cycle. The total work performed by the system, however, includes the area enclosed by the entire saw-toothed loop, which measures net work done by the barriers of the surface balance on the surface film. The results imply, however, that exergonic hysteresis loops might be obtained under suitable experimental conditions. Because of this implication we have attempted to determine the magnitude and direction of possible errors.

*Effect of Buoyancy.* Change of  $\gamma$  produces an alteration in the vertical position of the plate, changing its depth of immersion as the  $\gamma$  changes and producing an error due to buoyancy of about 0.5 per cent in the recorded  $\gamma$ . The  $\gamma$ s at the tips of the spikes are only a few dynes/cm apart, so this error is a small subtractive artifact. Assuming, for the sake of estimation, that buoyancy produces the only artifact, it can be shown that the actual cross-over of adjacent ascending and descending spikes equals the measured cross-over times the quantity  $(1 + \epsilon)$ ,

where  $\epsilon$  is the buoyant force upon the slide per unit depth of its immersion. Unit depth of immersion refers to the change in distance the slide is immersed in the hypophase for a change of 1 dyne/cm of force applied to it in a direction normal to the surface of the hypophase.

*Effect of Contact Angle.* Another source of error is often associated with differences in the contact angle between the meniscus and plate as  $\gamma$  changes. It is thought to be produced by vertical-pull surface balances because the plate height varies inversely as  $\gamma$ . As the plate moves deeper into the hypophase, the line of wetting between the meniscus and dry plate above it is said to advance up the plate producing a contact angle greater than zero, so that the vertical, or measured, component of force is diminished.<sup>9</sup> With decreasing  $\gamma$ , the meniscus is said to recede down the wetted and rising plate, producing a zero contact angle and a correct measurement of  $\gamma$ . In our experiments, when  $\gamma$ s were taken under static conditions, changes in contact angle would not affect readings for the descending spikes, but would produce spuriously low readings for the ascending spikes, reducing the measured cross-over with respect to the actual cross-over. It must be emphasized that the contact angle in our experiments with the LAS nearly always appeared to be zero because the line of wetting of the frosted glass plate was as far above the upper part of the bulk of the meniscus as this part was above the flat surface of the hypophase. This uppermost line of wetting did not change as  $\gamma$  changed, except as described later. The meniscus can be described as follows: It "fills in" with rising  $\gamma$ , and "collapses" against the plate with falling  $\gamma$ . Pictorially, the change in shape of our menisci with change in  $\gamma$  could be obtained if one end of a limp rope were attached to a wall near the ceiling and the other end moved alternately away from and toward the wall near the floor. In side view, a good actual imitation of a cross-section of our menisci would be obtained at high and low  $\gamma$ , respectively. If the rope could recede into a vertical groove in the wall beneath a recessed point of attachment, then, when seen in side view, a moving point of contact between the rope and wall would be observed. This corresponds to the visible side view of our menisci, because the layer of wetting of the plate is too thin to be seen. This could be misinterpreted to mean that the menisci advance and recede with rising and falling  $\gamma$ , but when seen frontally, the error is discerned quickly.

*Summary of Artifacts.* Hysteresis in the electronic equipment was shown to be negligible (FIGURE 2), so that the cross-over of the spikes appears to be unassociated with artifacts due to the electronic equipment, contact angle, or buoyancy. It might be attributable, obscurely, to spreading of the surface film on compression.

*Comparison with Other Work.* The process of producing the cross-over spikes is a logical extension of Pattle's description<sup>8</sup> of data obtained by Sutnick and Soloff.<sup>10</sup> We are unable to obtain such information from the data, but Pattle's description implies that compression of a film of LAS bounded by a polytetrafluoroethylene ribbon to .2 its initial area produced, by storing energy, a  $\gamma$  greater than that obtained by spontaneous spreading. The same phenomenon has been used by Pattle to explain his "clicking" bubbles. Bubbles squeezed from a cut lung surface and trapped in de-aerated water under a shelf alternately flatten toward the shelf and return suddenly to a more spherical shape. In becoming round, they do work against gravity. Pattle suggested that the source of energy is compressional energy stored in the surface of each bubble as gas diffuses out of it and it becomes smaller, giving a diminished total surface energy but an increased energy per unit area.

*Surface Properties of LAS Surface Films Enclosed by Rubber Dam*

**Long-term Experiment.** Further data on this subject was obtained. After full compression of LAS within the rubber enclosure (FIGURE 4A), the area was held constant and readings of  $\gamma$  taken at timed intervals. A  $\gamma$  of 23.5 dynes/cm was reached in 5 $\frac{3}{4}$  hours, and 28 dynes/cm in 20 hours. The area was then increased and held for 2 hours at each of the spikes of FIGURE 4B, resulting repeatedly in a diminution of  $\gamma$  to about 27 dynes/cm. If the  $\gamma$  reached at the end of each 2-hour period of arrest corresponded to that of a spontaneously formed film, then there was a slight increase in ultimate  $\gamma$  upon compression of the film. This experiment again does not prove a net work capacity of LAS films for the reasons given earlier and requires further investigation.

The proposal that spreading of the compressed film produced the cross-over spikes becomes more tenuous for the long-term experiment since equilibrium conditions should have been reached sufficiently slowly to have prevented an overshoot.

**Spreading on Frosted Glass.** The LAS exhibits another phenomenon which, to our knowledge, has not been reported. Liquid often climbed to the top of the plate, about 1.4 cm above its level in the trough, on initial compression of the surface area. The plate appeared more translucent on subsequent compression than on expansion, indicating that compression increased, and expansion decreased, the thickness of liquid on the plate. The plate gradually assumed its normal frosted appearance with continued cycling. The entire process could be repeated by three different manipulations: (1) the cycling process could be interrupted for about 15 minutes; (2) the plate could be removed from and retouched to the surface; (3) the edge of contact between the meniscus and plate could be agitated by scraping the sides of the plate with a clean spatula in a downward direction through the meniscus.

**Effect of  $\gamma$  Differentials.** It is difficult to account for the phenomenon by invoking the principles usually used to explain the spreading of liquids on clean solids, the primary forces being considered to be produced by differences in  $\gamma$  at the air-liquid, liquid-solid, and air-solid boundary. If the liquid spreads and deposits a layer of its molecules on the solid, then the liquid film may recede due to the new and reduced set of  $\gamma$  differences. The LAS formed a film upon the plate which should have reduced greatly the  $\gamma$  there. However,  $\gamma$  might still have been greater than that of a compressed film of the same substance and so pro-

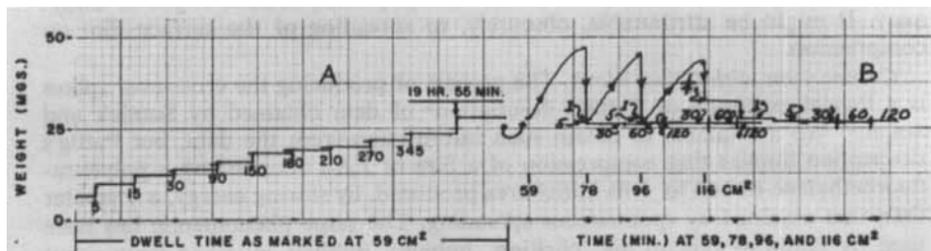


FIGURE 4. Surfactant: pig LAS. (A.) Change of area arrested at minimum area (59 cm.<sup>2</sup>). Vertical line at left records rise in  $\gamma$  during first 5 min. Each succeeding vertical line, connected to the previous one by a horizontal line, records the rise in  $\gamma$  during the marked interval (min.). The short horizontal lines projecting to the left of some vertical lines record  $\gamma$  at the time interval shown, but before correction for change in contact angle (see text). (B.) Begins at 19 hours, 55 min. at 59 cm.<sup>2</sup> Area increased progressively and arrested at each vertical line. Minutes marked on vertical and horizontal lines.

duced the rewetting, although it occurred as rapidly as the original wetting when the  $\gamma$  differentials should have been relatively enormous.

*Effect of Capillarity.* The other major cause of spreading of liquids on solids is that of capillary effects due to surface scratches. The height of rise of a liquid which wets a given capillary is directly proportional to the  $\gamma$  of the liquid, so if capillary effects were the cause of spreading up the plate, then it would occur with the high  $\gamma$  at full expansion of the surface. In our experiments the spreading occurred at low  $\gamma$ .

*Effect of Surface Structure.* For these reasons, we believe that the surface film was mostly pushed up the plate mechanically rather than being motivated by  $\gamma$  differentials and/or capillary forces. For this to happen, the surfactant film would require some structural rigidity, at least momentarily. Avery and Mead<sup>11</sup> reported evidence for formation of a solid film of their LAS in the form of "whitish linear streaks" parallel to the barrier of their surface balance. Although we have not witnessed this phenomenon with our LAS, we have observed solid films produced by other surfactants, as discussed shortly, which also produced very low  $\gamma$  on compression, but did not produce a wetting of the plate as did LAS. The process is undoubtedly complex and serves to illustrate the difficulty of confining surface films of this material within definite boundaries upon diminution of the confined area.

*Surface Turbulence.* Compression of a surface film of LAS within the rubber barrier produced surface turbulence which appeared like small water spiders skating rapidly and randomly over the surface. The moving rubber barrier stirred the hypophase, producing variations in surfactant concentration on the surface and resulting in local surface movements. They would seem too large to occur, say, in lung alveoli. However, we do have evidence which indicates that Pattle's clicking bubbles, which are about the same size as lung alveoli, are a demonstration of interfacial turbulence due to the escape from the structured bubble lining of highly surface active, water soluble,  $\text{Ca}^{++}$  precipitable molecules (from the authors' unpublished notes).

#### *Surface Properties of Some Other Biological Surfactants*

*Palmitic Acid, Stearic Acid, and Hexadecylamine.* The behavior of each of the other substances tested with and without the rubber barrier was not influenced markedly by that enclosure (FIGURE 5). The low  $\gamma$  values obtainable upon compression of films of palmitic acid (7.5 dynes/cm), stearic acid (6 dynes/cm), and hexadecylamine (5 dynes/cm), compare to that obtained with the LAS. These films did not tend to spread beyond the confines of the full trough in the absence of the rubber barrier. This may be attributed to the visible scum which they formed upon the water surface which possibly had sufficient structural strength to prevent free spread of the film on compression. Change of area of films of these materials produced changes in  $\gamma$  which could be almost instantaneous (FIGURE 5). Resumption of change of surface area produced a reversal in change of magnitude of  $\gamma$  so abruptly that the recorder pen retraced part of each spike, indicating the presence of solid condensed films as judged by their surface compressional moduli.<sup>12</sup> Continued compression resulted in an alteration of the surface film to the liquid condensed state as judged by the same criterion.

*Gastric Mucin, Human Albumin, and Vegetable Lecithin.* Surface films of these substances produced small hysteresis loops. Reproducibility is shown by the five consecutive uninterrupted loops recorded for albumin. Irregularities in

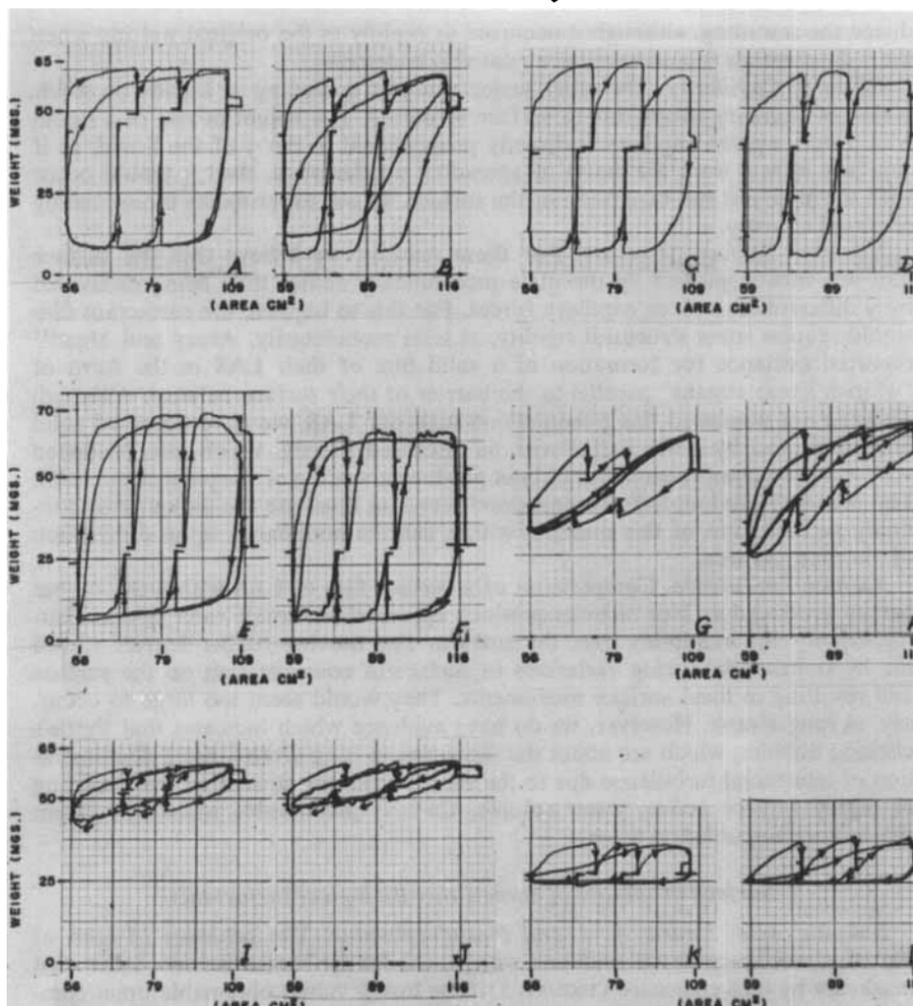


FIGURE 5. (A-L.) Hysteresis loops in groups of two. One loop recorded, except for C & D, then change of area arrested for 300 sec. at each vertical "spike." (Spikes of I and J represent 600 sec. arrests.) Water hypophase, glass plate throughout. Letters designate: A palmitic acid, no dam (see text); B palmitic acid, dam; C stearic acid, no dam; D stearic acid, dam; E hexadecylamine, no dam; F hexadecylamine, dam; G gastric mucin, no dam; H gastric mucin, dam; I human albumin, no dam; J human albumin, dam; K vegetable lecithin, no dam; L vegetable lecithin, dam. Short horizontal lines protruding from rising or falling spikes record  $\gamma$  at 5 sec. after arrest of area change for each recording.

these loops are not ascribed to buckling of the film, since once initiated, it should continue with continued compression, but did not. Similar irregularities occurred upon expansion, so that abrupt, reversible, and reproducible changes in surface structure would be a more apparent explanation.

*Pig Tracheal Mucus.* This substance formed a surface film capable of reaching a  $\gamma$  of about 24 dynes/cm, which rose to only 29 dynes/cm after 5 minutes in the compressed state (FIGURE 6). Its presence delayed but did not prevent the usual results obtained with solid LAS at dynamic equilibrium.

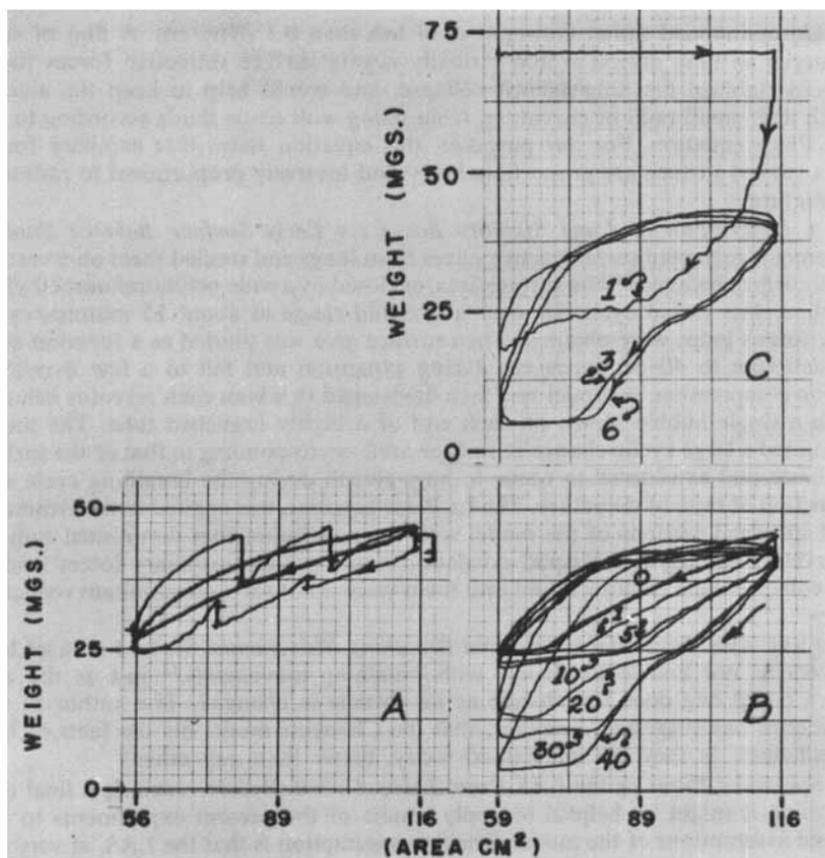


FIGURE 6. (A.) Pig tracheal mucus. Five consecutive loops followed by periodic 300 sec. arrest of area change, enclosed by rubber dam. Short horizontal lines protruding from apikes record  $\gamma$  reached at end of 60 sec. (B.) Curve O records five consecutive loops for pig tracheal mucus at the end of which a lump of pig LAS was added to the trough giving curve 1. Recordings taken at the 5th, 10th, 20th, 30th, and 40th loop as indicated. (C.) Recording begun at minimum area, with no surfactant added. At maximum area, lump of pig LAS added and 1st, 3rd, and 6th loops recorded. Water hypophase, glass plate, rubber dam throughout.

#### *Application of Results to Lung Mechanics*

It should be worthwhile to apply the present findings to a specific biologic field of interest. Attempts to relate LAS to lung physiology constitute a pertinent field in which the authors have had the most experience and involve interpretation of results obtained by model systems, as discussed elsewhere.<sup>4</sup>

The concept that surface active material exists in lung alveoli was considered 36 years ago.<sup>13</sup> The hypothesis that such material controls the stability of lung tissues recently has been developed extensively by Pattle<sup>1,5,8</sup> and Clements.<sup>2,14</sup>

*A Description of Lung Stability Based on Surface Properties of Bubbles Lined with LAS.* Pattle obtained bubbles from the lungs of animals. These bubbles were similar in size to their alveolar source. He concluded from physical measurements on them that their surfaces in contact with water on the outside and air on the

inside maintained rather constant  $\gamma$ s of less than 0.1 dyne/cm. A film of such material in lung alveoli would virtually negate surface retractive forces there, would stabilize the lung against collapse, and would help to keep the alveoli, with their small radii of curvature, from filling with tissue fluids according to the La Place equation. For our purposes, this equation states that capillary forces at a curved surface are proportional to  $\gamma$  and inversely proportional to radius of curvature.

*A Description of Lung Stability Based on Early Surface Balance Studies.* Clements extracted surface-active juices from lungs and studied them on a vertical pull surface balance.<sup>14</sup> The surface area, enclosed by a wide polytetrafluoroethylene ribbon, was varied cyclically over a five-fold range at about 15 minutes/cycle. Hysteresis loops were obtained when surface area was plotted as a function of  $\gamma$ , which rose to 40–50 dynes/cm during expansion and fell to a few dynes/cm upon compression. A model was then envisioned in which each alveolus behaved like a single bubble blown on each end of a highly branched tube. The model assumed a large cyclic change in surface area, corresponding to that of the surface balance and considered to occur in lung alveoli during the breathing cycle as a function of radii of curvature. The La Place equation was applied to the symmetrical spherical sections of the model with the conclusion that diminished radii of curvature of lung alveoli upon exhalation would produce capillary forces tending to collapse them or draw liquid into them were it not for a concomitant reduction in  $\gamma$ .

*Lung Alveolar Surface Area and Breathing Movements.* Surface area of lung alveoli is not known to change with breathing movements,<sup>15</sup> just as the area of a paper bag does not change as its volume is changed. The author of this criticism has suggested, however, that the Clements model fits the facts of lung mechanics, as they are understood today, better than any other.<sup>8</sup>

*Spread of Films of the LAS From Lung Alveoli.* Before reaching a final conclusion, it might be helpful to apply results of the present experiments to two basic assumptions of the model. The first assumption is that the LAS, at very low  $\gamma$ , remains in the alveoli and disregards the manifold phenomena of surface spreading known as the Thomson-Marangoni effects.<sup>16</sup> If the  $\gamma$  in lung alveoli became much less than that of adjacent and distal surfaces, a more or less rapid exodus of the alveolar mucus should occur. This material might combine with tracheal mucous secretions and become difficult to recognize as alveolar mucus. An immediate result of spreading from the alveoli would be to negate the effect of compression with a resultant rise of  $\gamma$ .

*Factors Inhibiting Spread of LAS.* However, the postulate is not defenseless. Spreading of a surface film is resisted by the presence of other surfactants and by the viscosity of the hypophase. The equilibrium  $\gamma$  of substances such as hexadecylamine (27–32 dynes/cm), stearic acid (36–38 dynes/cm), and palmitic acid (43–52 dynes/cm) are substantially higher than that of the LAS. However, their surface compressional moduli are so great that a given expansion of the LAS against them would produce a very rapidly rising resistance to further expansion. The lowest  $\gamma$  reached by substances such as gastric mucin, human albumin, and vegetable lecithin would be insufficient to prevent expansion of the LAS.

The retarding force of viscosity relates to the layer of hypophase carried with a spreading film. This layer has been measured at an equivalent thickness of about 30 micra in an experiment using oleic acid spreading over a water surface at 5 cm/sec and increased linearly with respect to the viscosity of the hypophase.<sup>17</sup> Rate of spreading under a given driving force approximates an inverse proportion

to the viscosity of the hypophase,<sup>18</sup> making significant the viscosity of the mucous lining adjacent and distal to the alveoli.

The pig tracheal mucus perhaps affords the best example of what might be found in the lung itself to resist movement of the LAS out of the alveoli. It produced hysteresis loops considerably different from those formed by the LAS, but did not prevent ultimate formation of normal dynamic hysteresis loops by the latter (FIGURE 6). These facts might help to discount the proposition that tracheal mucus is contaminated heavily by LAS as a result of copious spreading from the lung alveoli.

*Spread of Films of the LAS from Alveolus to Alveolus.* The second assumption is that alveolar  $\gamma$  is inversely proportional to alveolar size, thus reducing its capacity to collapse the lung. To accomplish this, areas of different alveoli must change at different relative rates inversely according to their size. If breathing were interrupted, the existing momentary differences in  $\gamma$  would have to be maintained under static conditions. Requisite to this would be some positive barrier to flow of the mucous film from smaller to larger alveoli of the air sacs which form a single primary lobule. Without such a barrier, which is not known to exist, flow of surfactant from small to large alveoli would produce a rise in  $\gamma$  in the former, causing failure of the proposed stabilizing mechanism. The effect would arise even with a surplus of LAS in each alveolus, because a large reduction of the equilibrium static  $\gamma$  is reached only upon compression of the open surfactant surface. A reduction of  $\gamma$ -differentials, brought about by surface spreading in the primary lobules, would help to prevent the spreading of LAS against the tracheal mucus and so help to confine the LAS to the primary lobules.

*Surface Movements as a Clearance Mechanism.* A surface movement of the sort just suggested would provide a basis for Macklin's proposed clearance mechanism,<sup>19</sup> wherein an alveolar mucoid film could entrap and move some foreign substances toward phagocytic cells in the alveoli. Macklin also suggested that the alveolar mucoid material consists of two parts; in his terminology, an aqueous mucoid fluid which drains through the lymphatics and a (more solid) mucoid fraction which combines with the mucus of the goblet cells and is carried out of the lung by ciliary action.

*Barach's Chamber.* Another question concerning the role of LAS in lung stability is raised by use of Barach's chamber, which is so designed that adequate external respiration is provided for human subjects without detectable respiratory movements.<sup>20</sup> The volume, and presumably the surface area of the lung alveoli do not change in Barach's chamber after respiratory movements cease.

The  $\gamma$ s of all alveoli would then be expected to arrive at a static equilibrium value of about 25 dynes/cm, and the protective mechanisms thought to be provided by low  $\gamma$  again would tend to vanish. A  $\gamma$  on an open surface of 25 dynes/cm is still lower than those produced by most other biological surfactants, including tracheal mucus, in which case a drift of mucus from the alveoli outward should still occur in Barach's chamber.

#### *Further Indications of the Spreading Tendency of Films of LAS*

*Stability of surface films of LAS and their geometric configuration.* Compressed films of LAS wet polytetrafluoroethylene and tend to leave a trough made of it.<sup>3</sup> Avery and Mead interpreted this occurrence as vitiating their results, so they did not record  $\gamma$  values at such times.<sup>11</sup> We have interpreted such effects as demonstrating a mechanism for clearance of foreign particles from lung alveoli<sup>9</sup> and inability to exhibit true elasticity in the lung.<sup>4</sup> Sutnick and Soloff<sup>10</sup> were able to

slow down a rise in  $\gamma$  with a wide barrier of polytetrafluoroethylene. The enclosed surface of our experiments also was intended to mimic the closed surface of a bubble, where the surface has nowhere to escape, except by dissolving or by folding over itself. There is, however, an enormous ratio between the area of a film on a surface balance and the same film on a bubble of a few hundred micra diameter, which would furnish greater opportunity for collapse on the larger area.<sup>8</sup> However, the *ratio* of collapsible areas might not be different, constituting a pertinent point concerning relative stability of  $\gamma$ .

*Effect of Wall Thickness on Stability of Lung Bubbles.* Pattle considered the wall thickness of his bubbles to be about 50 Å.<sup>5</sup> Goldacre has found that thin layers frequently produce folds with diameters approximately equal to 1000 times their thickness,<sup>21</sup> providing space for about 120 points for folding about one periphery of a bubble 200 micra in diameter with a wall 50 Å thick. While these folds could not exist simultaneously, there would be considerable space for folding prior to local collapse. The bubbles are apparently large enough so that wall thickness becomes a crucial factor when considering stability due to local folding. To prevent local collapse by folding (with a fold diameter equal to the bubble radius) the bubbles would require walls about 0.1 micron thick, or some 20 times the thickness estimated previously. On this basis, surface spreading on the surface balance would explain the difference in apparent  $\gamma$ s between LAS on the surface of a balance and a bubble.

*A Possible Artifact in Measuring the  $\gamma$  of Lung Bubbles.* The comparison should not ignore possible effects of a structured bubble wall on the methods used for measuring  $\gamma$ . The ratio of polar to equatorial radii of bubbles trapped under a shelf in water were measured; by applying Porter's tables,<sup>22</sup> an estimate of the  $\gamma$ s of such bubbles was secured.<sup>5</sup> Porter's methods do not consider possible structural strength of a surface film but apply to drops acted upon by gravitational and capillary forces only. Even though such measurements, applied to lung bubbles, might be too low, their  $\gamma$ s appear to be stable for much longer periods than have been achieved with the same material on a surface balance. For a possible answer to this we look again to the spreading tendency of LAS on open surfaces at reduced  $\gamma$ .

#### GENERAL APPLICATIONS

Although we have stressed the surface properties of a perhaps rather specialized form of mucus, the same phenomena should exist to a variable degree at biological interfaces generally, including other mucous surfaces.

The role of surface-active agents in biological processes has been considered to be a vital one for several generations.<sup>23</sup> The details of such speculations are difficult to uncover due not only to the immensely complex mixtures of surfactants which exist everywhere in biology but also to the ways which these substances have of forming surface structures. Hill, for example,<sup>24</sup> considered theories of muscular contraction based on changes in  $\gamma$ .<sup>25</sup> His most penetrating objection to such theories was that the surface area of muscle tissue was thought, in 1925, to be insufficient to permit the demonstrable forces of contraction by reasonable changes in  $\gamma$ . It would seem now, however, that Hill's conclusions might be reconsidered in view of the vast interfacial areas of muscle tissue revealed by electron microscopy.

Kavanau has expanded on such concepts to formulate a generalized theory of bioenergetics.<sup>26</sup> There seem to be no pertinent reasons, at present, to exclude the possibility that phenomena based on identical principles are at work at certain mucous interfaces.

## REFERENCES

1. PATTLE, R. E. 1955. Properties, function and origin of the alveolar lining layer. *Nature* **175**: 1125-1126.
2. CLEMENTS, J. A. 1956. Dependence of pressure-volume characteristics of lungs on intrinsic surface-active material. *Am. J. Physiol.* **187**: 592.
3. MENDENHALL, R. M. 1963. Pulmonary mechanics. Some physical and biochemical factors. *Arch. Environ. Health* **6**: 74-79.
4. MENDENHALL, R. M. & A. L. MENDENHALL, JR. 1964. Lung alveolar surfactant, lung elasticity, and lung stability. *Nature* **204**: 747-748.
5. PATTLE, R. E. 1958. Properties, function, and origin of the alveolar lining layer. *Proc. Roy. Soc. London* **148(B)**: 217-240.
6. MENDENHALL, R. M. & A. L. MENDENHALL, JR. 1963. Surface balance for production of rapid changes of surface area with continuous measurement of surface tension. *Rev. Sci. Instr.* **34**: 1350-1352.
7. DAVIES, J. T. & E. K. RIDEAL. 1963. *Interfacial Phenomena*. 2nd Ed. : 29. Academic Press, New York, N. Y.
8. PATTLE, R. E. 1965. Surface lining of lung alveoli. *Physiol. Revs.* **45**: 48-79.
9. HARKINS, W. D. & T. F. ANDERSON. 1937. I. A simple accurate film balance of the vertical type for biological and chemical work, and a theoretical and experimental comparison with the horizontal type. II. Tight packing of a monolayer by ions. *J. Am. Chem. Soc.* **59**: 2189-2197.
10. SUTNICK, A. I. & L. A. SOLOFF. 1963. Surface tension reducing activity in the normal and atelectatic human lung. *Amer. J. Med.* **35**: 31-36.
11. AVERY, M. E. & J. MEAD. 1959. Surface properties in relation to atelectasis and hyaline membrane disease. *Am. J. Dis. Child.* **97**: 517-523.
12. DAVIES, J. T. & E. K. RIDEAL. 1963. *Interfacial Phenomena*. 2nd Ed. : 265. Academic Press, New York, N. Y.
13. VON NEERGAARD, K. 1929. Neue Auffassungen über einem Grundbegriff der Atemmechanik. Die retraktionskraft der Lunge, abhängig von der Oberflächenspannung in den Alveolen. *Ztschr. ges. exper. Med.* **66**: 373-394.
14. CLEMENTS, J. A., E. S. BROWN & R. P. JOHNSON. 1958. Pulmonary surface tension and the mucous lining of the lungs: some theoretical considerations. *J. Appl. Physiol.* **12**: 262-268.
15. PATTLE, R. E. 1963. The lining layer of the lung alveoli. *Brit. Med. Bull.* **19**: 41-44.
16. SCRIVEN, L. E. & C. V. STERNLING. 1960. The Marangoni effects. *Nature* **187**: 186-188.
17. SCHULMAN, J. H. & T. TEORELL. 1938. On the boundary layer at membrane and monolayer interfaces. *Trans. Farad. Soc.* **34**: 1337-1342.
18. DAVIES, J. T. & E. K. RIDEAL. 1963. *Interfacial Phenomena*. 2nd Ed. : 25-28. Academic Press, New York, N. Y.
19. MACKLIN, C. C. 1954. The pulmonary alveolar mucoid film and the pneumocytes. *Lancet* **266**: 1099-1104.
20. BARACH, A. L. 1947. Immobilization of both lungs produced by the equalizing pressure chamber with results of treatment in pulmonary tuberculosis. *Ann. Int. Med.* **26**: 687-703.
21. GOLDACRE, R. J. 1958. Surface films, their collapse on compression, the shapes and sizes of cells and the origin of life. *In* *Surface Phenomena in Chemistry and Biology*. J. F. Danielli, K. G. A. Pankhurst & A. C. Riddiford, Eds. : 278-298. Pergamon Press, New York, N. Y.
22. PORTER, A. W. 1933. The calculation of surface tensions from experiment. Part I. Sessile drops. *Phil. Mag.* **15(7)**: 163-170.
23. THOMPSON, D'ARCY W. 1951, 1952. *On Growth and Form*. I and 2. Cambridge University Press, New York, N. Y.
24. HILL, A. V. 1925. The surface tension theory of muscular contraction. *Proc. Roy. Soc.* **98(B)**: 506-515.
25. BAYLISS, W. M. 1927. *Principles of General Physiology* : 61, 448. Longmans, Green & Co. London, England, and New York, N. Y.
26. KAVANAU, J. L. 1963. Structure and functions of biological membranes. *Nature* **198**: 525-530.