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THE RELATION BETWEEN SURFACE TENSION AND AREA IN THE ALVEOLAR LINING FILM

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SUMMARY

1. The properties of the rat alveolar lining film have been studied by observing the behaviour in a hanging drop, under reduced or increased ambient pressure, of bubbles derived from the lung.

2. When such a bubble, covered by a metastable film of surfactant, is made to shrink, the material displaced from the surface usually remains in a form in which it can be re-adsorbed to the surface and retains its surpellic properties.

3. When an excess of surfactant is available for adsorption to the surface of such a bubble in water, an increase in area to about 1·25 (ϕ) times the metastable area is both necessary and sufficient for additional adsorption to the surface to take place.

4. No significant variation of the ratio ϕ with temperature between 22 and 37° C has been found.

5. It is concluded that during quiet breathing (involving a twofold change in lung volume in the rat) the variation in alveolar surface area is less than 25 %. This finding is compatible with the extant morphometric data, but not with any assumption that the surface area is proportional to the 2/3 power of the gas volume.

6. The behaviour of the bubbles in blood serum is similar to that in water. In a 2 % solution of the detergent Tween 80, further adsorption of surfactant to the bubble surface does not take place.

7. The fact that bubbles obtained from the lung by instillation of a solution of Tween 80 have surfactant linings similar to those of bubbles obtained with water or saline demonstrates that the bubble lining layer consists of the original alveolar lining layer detached.

8. When a metastable bubble is stretched, it sometimes behaves as if some or all of its surfactant had been lost from the surface. The causes of this are unknown.

9. The amount of surfactant associated with metastable bubbles freshly

squeezed from a fragment of lung varies from bubble to bubble; the amounts found have ranged from 1 to 4·5 times (mean, 2) that required to cover the original bubble area with a metastable film. This would be compatible with an alveolar lining film of very uneven thickness.

10. The maximum surface tension reached in the early stages of expansion of a lung bubble from the metastable state is about 34 mN/m.

INTRODUCTION

The lung surfactant is a lipid-protein complex which is secreted by the Type II cells of the alveoli and extruded onto the alveolar lining (Gil & Reiss, 1973) in a form dispersible in saline. It is then adsorbed to the surface to form an insoluble film which can be removed as the lining of bubbles (Pattle, 1954, 1955, 1958). There has been some doubt (Pattle, 1965) as to whether an irreversible change takes place at the formation of the surface film, or on its displacement from the surface.

Mead & Collier (1959) have described a slow decrease in lung compliance, reversible by a single deep breath, in anaesthetised dogs; Bendixen, Hedley-White & Laver (1963) have described similar phenomena in man. Williams, Tierney & Parker (1966) quoted these and many other results, similar to those of their own experiments on rabbits and lambs. All these they explained by postulating that during quiet breathing the surface tension has less than its equilibrium value and that consequently surfactant is slowly lost from the surface; and that during a deep breath the surface is stretched, the surface tension (S.T.) rises above its equilibrium value, and fresh surfactant is adsorbed to the surface from the deeper layers of the alveolar lining. Since then other results susceptible of the same interpretation have appeared. Those of Douglas, Chong & Finlayson (1974) resembled those of Mead & Collier (1959); Herxheimer (1975) found that in a human subject after a deep inspiration the lung volume about which tidal breathing oscillated was raised above its previous value.

To shed light on the processes of adsorption and desorption, the lining of a bubble derived from the lung has been taken as a specimen of alveolar lining material, an assumption further justified during the investigations. Displacement and adsorption have been brought about by changing the size of the bubble, and the increase in area necessary for adsorption of fresh surfactant to the surface has been quantified (Pattle, 1976*b*).

Definitions and symbols

Lung surfactant forms a surface film which, when the surface is compressed, can reduce the surface tension to very low values ($< 0·1$ mN/m). This state is described as 'metastable'. If the area is kept constant, the

S.T. rises to about 30 mN/m (Sutnick & Soloff, 1963). This is described as the 'equilibrium' value. The capacity for forming metastable films of this type is here described by the word 'surpellic' (Latin pellere, to push) (Pattle, 1976*a*). The term 'surface active' is here used in its ordinary physicochemical sense, i.e. it describes any substance which is adsorbed at a surface and lowers the surface tension, whether it is surpellic or not. In specialist lung literature, 'surface active' is sometimes used in the sense of 'surpellic'. In the present work, 'lung surfactant' is retained as a noun.

Lung surfactant not forming part of the surface film, but available for adsorption thereto, is referred to as 'excess surfactant'. Areas are denoted by A, diameters by d. Quantities referring to the metastable state are denoted by an asterisk thus: A^*.

THEORY

In order for surfactant to be adsorbed to the surface, it is necessary for the surface tension to be raised to or above its equilibrium value. Suppose that the area of a metastable film has to be increased by a factor ϕ to raise the S.T. to this value. Then if the area of a metastable bubble is increased by a factor of less than ϕ, and it is then allowed to contract under the influence of its own surface tension, it will return to its original metastable area, whether or not excess surfactant is available for adsorption. Suppose now that the area is increased by a factor of more than ϕ, and that at its maximum A_{m} there is still excess surfactant. The bubble will then be covered by an equilibrium film of area A_{m}, which will, when it is allowed to contract, assume a new metastable area A_2^*, equal to A_{m}/ϕ. The degree of stretch ϕ necessary for adsorption can therefore be measured by observations on bubbles.

Measurements such as these give no information as to the actual surface tensions involved. Suppose however that the hanging drop contains an isolated metastable bubble of diameter d_0^*, and the ambient pressure is suddenly reduced. The gas tension in the drop is at first unaltered, but the hydrostatic pressure around the bubble falls, and gas tends to come out of solution and expand the bubble. How far it expands will depend on whether the difference P, between hydrostatic pressure and gas tension, can be made up by the additional pressure due to the surface tension γ during the early stages of expansion. This additional pressure, according to Laplace's formula, is given by $4\gamma/d$. In practice such a bubble either hardly expands at all, or expands to many times its original size.

If the S.T. is very low at diameter d_0^*, or area $\pi\, d_0^{*2}$, it will rise to its equilibrium value when the area is $\phi\pi\, d_0^{*2}$, and the diameter is $\phi^{\frac{1}{2}}\, d_0^*$.

If the bubble expands only slightly, it will reach a maximum diameter d_m, which can be measured. The value of γ at diameter d_m must be greater than $d_m P/4$; for, if it were less, the bubble would continue to expand. We may therefore suppose that if d_m was no greater than $\phi^{\frac{1}{2}} d_0^*$ (or about 1·118 d_0^*, Pattle, 1976*b*), the surface tension reached a maximum at d_m and did not exceed the equilibrium value γ_0. We then have

$$\gamma_0 > d_m P/4. \tag{1}$$

If the bubble expands beyond $\phi^{\frac{1}{2}} d_0^*$, this shows that the S.T. at diameter $\phi^{\frac{1}{2}} d_0$ was less than $\phi^{\frac{1}{2}} d_0^* P/4$; for, if it had not been, the bubble would not have expanded. The tension at diameter $\phi^{\frac{1}{2}} d_0^*$ cannot have been less than the equilibrium S.T., but may have been more, as the bubble was rapidly expanding. We can therefore put

$$\gamma_0 < \phi^{\frac{1}{2}} d_0 P/4. \tag{2}$$

Eqns. (1) and (2) can thus be used in conjunction with the experimental results to set upper and lower bounds to the equilibrium surface tension γ_0.

METHODS

Bubble observations

The apparatus consisted of a test tube mounted horizontally on a microscope slide. In the upper part of the tube was a hole 10 mm across, which was sealed by a glass disk 0·8 mm thick. From a bung at the open end of the tube a connexion led to a mercury manometer and a mouthpiece via which air could be forced into or out of the apparatus. The tube was first held with the disk downwards and a drop of water or other liquid was placed on the disk. Into this bubbles were squeezed, by means of curved forceps, from a fragment of rat lung. The tube was then inverted and placed on the stage of a microscope fitted with an eyepiece graticule. Bubbles were viewed in the hanging drop and measured with the graticule. Some of the experiments were done with the tube and microscope (except for the eyepiece) in a Perspex box which could be kept at 37° C.

Most of the experiments were conducted with excess surfactant available for adsorption at the bubble surface. To ensure this, the pressure in the tube was lowered at the beginning of the experiment to 100–300 mmHg (13–40 kPa) below atmospheric and kept at that level for 20 min. A metastable bubble, say 60 μm across (area A_0^*) was then found. Atmospheric pressure was then restored; the bubble contracted in accordance with Boyle's law, and then started to dissolve, the water in the drop being now undersaturated at atmospheric pressure. The degree to which the bubble dissolved depended strongly on the size of the drop; a drop 1 mm across and 0·5 mm thick rapidly came into equilibrium with the air in the tube, and only the Boyle contraction was important, while with a drop 3 mm across and 2 mm deep the bubble might dissolve completely unless the pressure was lowered again.

Let us suppose that either contraction ceased at a new metastable area A_1^* (diameter say 30 μm), or that the contraction was reversed, by lowering the pressure, at a minimum area A_1^* (the presence of a metastable film being highly probable). It was then assumed that, provided the area did not again exceed A_0^*, excess surfactant, enough to provide a metastable film of area A_0^*, was present. The bubble was then expanded by lowering the pressure, so that it reached a maximum area

A_m, less than A_0^*. The time taken to reach A_m ranged from a few seconds in the smallest drops to 2 min in the largest, and the degree of expansion varied correspondingly. The maximum diameter, A_m, was measured and the bubble then contracted under the influence of its surface tension, usually to a new metastable area A_2^* which was also measured. The whole process could then be repeated on the same bubble. If A_2^* was greater than A_1^*, this showed that during the expansion to A_m fresh surfactant had been adsorbed to the bubble surface. The deductions which may be made from these measurements are described in the Results section.

In experiments to determine the equilibrium surface tension, a small bubble (of diameter d_0^* 10–25 μm) in a fairly large drop was measured. The quantity $4\gamma/d_0^*$, with γ set at 30–40 mN/m, was then calculated; this is around the maximum pressure which S.T. might produce if a bubble of this diameter were to expand. The ambient pressure was then lowered by about this amount, so that expansion might or might not be limited by surface tension. If the bubble expanded only a little, its maximum diameter was measured. If it expanded to a much greater extent, the fact was noted. Limiting values for the equilibrium surface tension γ_0 were obtained from eqns. (1) and (2).

Accuracy of measurements

The diameters were measured with an eyepiece graticule one division of which was equivalent to 3·3 μm when the × 40 objective was used. Measurements were recorded to one tenth of a division, but no greater accuracy than 0·6 μm can really be claimed. In experiments on area ratios, the minimum diameter used at A_m or A_2^* was 25 μm. Errors of up to 10 % in the area ratios might therefore sometimes be expected. The recorded results actually varied rather less than this, perhaps because of subjective bias caused by knowledge of previous experiments. The bubbles used in the experiments to determine surface tension were often smaller, and errors of 6 % in measurements of diameter might be expected, but only one measurement occurs in each estimate of S.T.

In all the bubble experiments measurements of the diameter were made when it was at a maximum or minimum, or was steady in the metastable state. They are thus more accurate than measurements of a rapidly changing diameter would be.

The methods described above were those finally adopted. In other experiments other methods of manipulating bubble size, such as increasing the pressure above atmospheric, or introducing desaturated water into the hanging drop, were used. In others the presence of excess surfactant was not ensured. The results of these experiments were wholly compatible with those of the more recent ones, and will not be further referred to.

RESULTS

Some of these are displayed in Table 1 and Figs. 1 and 2.

Non-adsorption with small increases in area

When a metastable bubble, at room temperature (22–25° C), in the presence of excess surfactant, was expanded (by lowering the pressure) by less than 25 % in area, and was then allowed to contract, its new metastable area A_2^*, was very close to its initial metastable area A_1^*. Thus if A_m is the maximum area attained, and A_0^* is the original metastable area beyond which the bubble is not subsequently stretched,

$$A_m < A_0^*, \quad A_m/A_1^* < 1{\cdot}25, \quad A_2^* \sim A_1^*.$$

In seven experiments, in which A_m/A_1^* ranged from 1·15 to 1·26, A_2^*/A_1^* ranged from 0·98 to 1·02 (mean 1·00). These are represented by the symbols ● to the left of the line $A_m/A_1^* = 1{\cdot}26$ in Fig. 1. Similar results were obtained at 37° C.

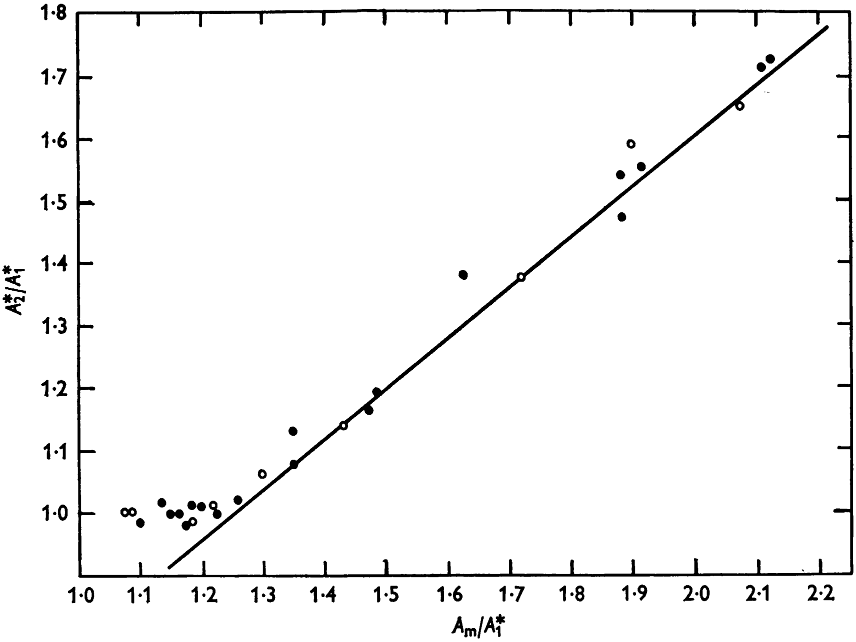

Fig. 1. Behaviour of rat lung bubbles in presence of excess surfactant, on forced expansion from A_1^* to A_m and spontaneous contraction to A_2^*. ●, in water at room temperature; ○ in water at 37° C. Line indicates $A_m/A_2^* = 1{\cdot}25$.

Adsorption with larger increases in area

When, in the presence of excess surfactant, a bubble was expanded by more than 25 % in area, and then allowed to contract, the new metastable area was about 0·8 times the maximum reached. Then

$$A_m < A_0^*, \quad A_m/A_1^* > 1{\cdot}25, \quad A_m/A_2^* \sim 1{\cdot}25.$$

In ten experiments at room temperature in which A_m/A_1^* ranged from 1·47 to 5·11, A_m/A_2^* ranged from 1·23 to 1·33 (mean 1·259). In ten experiments at 37° C, the values of A_m/A_2^* ranged from 1·19 to 1·28 (mean 1·235). It would not be justifiable to deduce any real variation with temperature, and for purposes of discussion a value of 1·25 will be adopted for ϕ. These results are shown by the symbols ● and ○ to the right of the line $A_m/A_1^* = 1{\cdot}26$ in Figs. 1 and 2.

Adsorption in solutions of Tween 80 and in serum

When bubbles were squeezed into a drop of horse blood serum at 37° C, instead of into water, the results were similar to those obtained in water (Table 1, expt. 3; mean ϕ, 1·26). By contrast, in a drop of 2 % (v/v) Tween 80 (a detergent liquid containing sorbitan polyoxyethylene monooleate), a bubble, when expanded, always returned to its original (Table 1, expt. 6), or occasionally to a smaller (Table 1, expt. 7) size. The results with Tween 80 are displayed by ■ in Fig. 2.

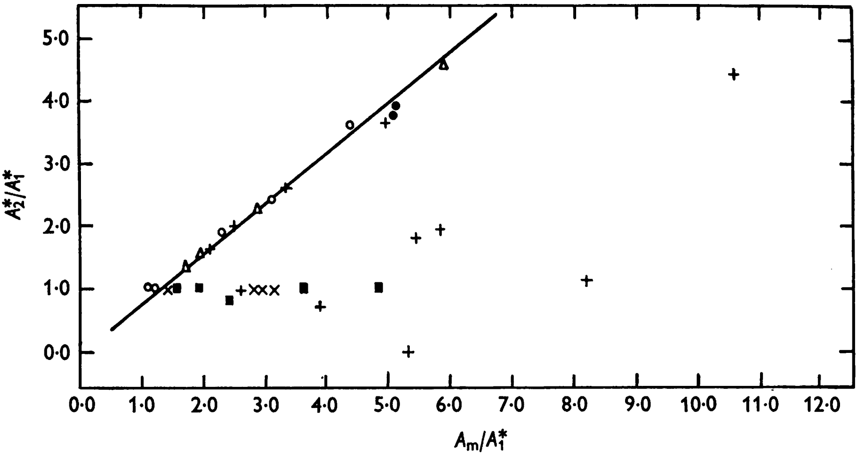

Fig. 2. Behaviour of rat lung bubbles in presence of excess surfactant, on forced expansion from A_1* to A_m and spontaneous contraction to A_2*: ●, ○ as in Fig. 1; △, in blood serum at 37° C; ■, in 2 % Tween 80 at room temperature. Bubbles direct from lung, excess surfactant not always present: +, in water at room temperature; ×, in 2 % Tween 80 at room temperature. Note scatter of + symbols. Line indicates A_m/A_2* = 1·25.

Properties of bubbles squeezed directly from lung

Bubbles were freshly squeezed from a fragment of lung into water, and, without being taken to A_0* or any other treatment, were allowed to become metastable at area A_1*. They were then expanded and allowed to contract; the ratio of the maximum Area A_m to the final metastable area A_2* was often greater than 1·25. This showed that the bubble had just enough surfactant to cover a metastable area A_2*. The ratio A_2*/A_1* is therefore a measure of the amount of surfactant carried by the original bubble. This ratio varied greatly (Table 1, expt. 8; mean ratio 2·23). The bubbles thus had more than enough surfactant attached to them, on an average, to cover their metastable area twice over. In two cases (Table 1, expt. 9), however, the bubbles failed to become metastable after expansion and disappeared. These results are shown as + in Fig. 2. When such bubbles were expanded in 2 % Tween 80, the final metastable area was always very close to the initial area, showing that the Tween 80 had prevented the adsorption to the surface of the excess surfactant usually present (Table 1, expt. 10; × in Fig. 2).

TABLE 1. Relations of initial (A_1^* or A_1), maximum (A_m), and final (A_2^*) diameters of lung bubbles; n observations under each set of conditions; room temperature unless otherwise stated

Line no. and symbol in Figures		Conditions (expansion etc.)	n	Range A_m^*/A_1^*	Range A_m/A_2^*	Mean A_m/A_2^*
		Excess surfactant proved present				
1	●	Expd. in water	10	1·47–5·11	1·23–1·33	1·259
2	○	Expd. in water, 37° C	10	1·31–4·39	1·19–1·28	1·235
3	△	Expd. in serum, 37° C	4	1·69–5·90	1·23–1·28	1·260
				A_m^*/A_1^*	A_2^*/A_1^*	
4	●	Expd. in water	7	1·15–1·26	0·98–1·02	1·00
5	○	Expd. in water, 37° C	4	1·08–1·24	0·98–1·02	1·00
6	■	Expd. in 2 % Tween 80	4	1·54–4·84	0·98–1·03	1·01
7			1	2·45	0·79	—
		Bubbles squeezed direct from lung fragments				
8	+	Expd. once only in water	7†	2·07–10·6	0·98–4·46	2·23
9			3	5·3–84·8	0·0	0·0
10	×	Expd. only once in 2 % Tween 80	5	1·38–3·13	0·98–1·03	1·01
				A_m/A_1	A_2^*/A_1 (s.r.)	
11	—	Not expd., in water	30	1·0	0·2–1·0	0·82
12	—	Not expd., in 2 % Tween 80	30	1·0	0·0–1·0	0·785
		Obtained from airways after liquid instilled; not expanded				
13	—	Excised lung, saline	10	1·0	0·8–1·0	0·96
14	—	Excised lung, 2 % Tween 80	10	1·0	0·8–1·0	0·98
15	—	*In vivo*, saline	10	1·0	0·8–1·0	0·88
16	—	*In vivo*, 2 % Tween 80	10	1·0	0·8–1·0	0·95

† Line 8: A_m/A_2^* range 1·33–7·09.

Properties of bubbles obtained from the lung in the presence or absence of Tween 80

Bubbles were squeezed from fragments of lung into hanging drops of water, or of a 2 % (v/v) solution of Tween 80 at room temperature (28° C). The ratios of the metastable to the initial surface area (known as the stability ratio, s.r.; Pattle, 1958) were measured. The mean s.r.s of batches of ten bubbles were: in water, 0·85, 0·84, 0·77 (mean,0·82); and in Tween 80 solution, 0·80, 0·84, 0·615 (mean, 0·785). In both liquids Brownian motion of the smallest bubbles was observed. When the bubbles dissolved in deaerated water, a rhythmical flattening and sudden return to spherical shape, appearing from above as a slow expansion and sudden contraction, ('clicking', Pattle, 1958) was prominent. These facts demonstrate that the bubbles, into whichever liquid they had been squeezed, were lined with normal films of surfactant. The initial areas, not being metastable, are denoted by A_1, not A_1*, in Table 1.

After water or 2 % Tween 80 solution had been forced into the main bronchus of an excised lung and released, copious froth welled out. The s.r.s of batches of ten bubbles from this were: with water, 0·96; with Tween 80 solution, 0·98. When dissolving, the bubbles 'clicked' vigorously and regularly. In neither case was the froth affected by silicone anti-foam 'A'. Froth formed directly from Tween 80 solution was rapidly destroyed by the anti-foam. Bubbles formed directly from the Tween 80 solution had zero stability ratio; when dissolving in de-aerated solution, they sometimes lost their spherical shape, but did not 'click'. Brownian motion was not observed.

Similar results were obtained *in vivo*, when saline or Tween 80 solution was injected into the trachea of an anaesthetized rat. Copious froth, resistant to anti-foam, was obtained in both cases. The s.r.s of batches of ten bubbles were: with saline, 0·88; with Tween 80 solution, 0·95. In both cases vigorous 'clicking' was found.

The contrast between these experiments, in which normal bubbles were obtained from lung irrespective of the presence or absence of Tween 80, and the capacity of Tween 80, as shown in Table 1 and Fig. 2, completely to prevent the adsorption of surfactant to the surface of a bubble, is discussed later.

Experiments on expanded bubbles to determine the equilibrium surface tension

In these experiments an isolated small bubble, 10–25 μm in diameter, was measured. The quantity $4\gamma/d_0$ was calculated for an S.T. of 30 mN/m, and the ambient pressure was reduced by approximately this amount. Some bubbles expanded only a little, and then contracted again. In these cases the maximum diameter d_m was measured. If d_m was less than $\phi^{\frac{1}{2}}$ (1·118) times the original diameter d_0 a lower bound for the value of γ_0 was calculated from eqn. (1). The bubble could then be used again by lowering the pressure by another step. Other bubbles expanded much more, and eqn. (2) was used to calculate an upper bound for γ_0.

In eleven such experiments at 37° C the highest lower bounds for γ_0 were 29·2, 33·7, and 33·8 mN/m, and the lowest upper bounds were 34·2, 34·7, and 36·0 mN/m. These values suggest a value of about 34 mN/m for the surface tension of a bubble expanded to ϕ times its metastable area.

Proof of re-adsorption to the surface of previously displaced surfactant

The experiments described above suggest that surfactant displaced from the surface can, in the absence of any interfering substance, be re-adsorbed thereto; they do not, however, prove it conclusively, as the surfactant adsorbed during any experiment might have been present as an excess at the beginning.

Experiments were therefore conducted in which a metastable bubble squeezed directly from the lung, say 40 μm in diameter (area $A_1'^*$), was expanded, by lowering the pressure, to a maximum A_m', 2–4 times $A_1'^*$, and watched contracting under its own S.T. to a metastable area $A_2'^*$ (occasionally metastability was not attained and the bubble disappeared). If A_m' was clearly more than $\phi\, A_2'^*$ ($\phi = 1{\cdot}25$), this showed that at A_m' the film was stretched beyond the equilibrium area, and that therefore at $A_2'^*$ only enough surfactant was present to provide a metastable film covering the bubble, without any excess. The bubble was then made to shrink to an area A_1^*, and then re-expanded to near $A_2'^*$ and allowed to contract to a new metastable area A_2^*. Of the surfactant on the surface at this new metastable area, a fraction $(A_2^* - A_1^*)/A_2^*$ must have been on the surface at area $A_2'^*$, have been displaced during contraction to A_1^*, and have been re-adsorbed during the subsequent expansion, without losing its surpellic properties. In practice $(A_2^* - A_1^*)/A_2^*$ depended on how far the bubble could be made to contract without becoming too small to re-expand when the pressure was lowered. A typical value was 0·79.

DISCUSSION

Direct origin of bubble lining film from alveolar lining film

When bubbles were expanded in a 2 % solution of Tween 80, lung surfactant, though available, was never adsorbed to the surface. This shows that Tween 80 inhibits the adsorption of lung surfactant; the detergent is more surface-active, but less surpellic, than lung surfactant. Clements, Hustead, Johnson, & Gribetz (1961) obtained comparable results using lung extracts on the Wilhelmy trough and the laurate analogue Tween 20.

When however bubbles were obtained by squeezing lung fragments into a hanging drop, or instilling liquids into the airways *in vivo* or *in vitro*, the bubbles obtained had the same properties whether or not Tween 80 was present; a surfactant layer was present on the bubbles in all cases.

As Tween 80 prevents adsorption of lung surfactant, the surface film cannot have been formed by this process and therefore must have been present at the alveolar surface before the Tween 80 solution arrived in the alveoli. This confirms the deduction of Pattle (1958) that the bubble lining is in fact the original alveolar lining detached, and justifies arguments deducing the properties of the alveolar lining substance from those of the bubble lining. These findings are incompatible with the hypothesis of Brooks (1971), who suggested that the surfactant is only released from the Type II cells after the arrival of bulk liquid in the alveoli.

The ease with which the alveolar lining is detached supports the view (see Weibel, 1973) that the surfactant layer is underlain by a thin layer of mobile liquid. The variability of the amount of surfactant found on metastable bubbles squeezed from the lung is in accord with their variable behaviour towards organic vapours (Pattle, Schock & Battensby, 1972) and with such pictorial evidence as is available (cf. Dermer, 1969); the alveolar lining film appears to be of very uneven thickness.

Relation of lung volume to alveolar surface area

If we accept the hypothesis of Williams *et al.* (1966) that surfactant is slowly lost from the alveolar surface during quiet breathing, and restored by a deep breath, we can use the results obtained from bubbles to calculate limits to the change in surface area of the alveoli with changes in air volume; this matter is at present somewhat obscure.

Suppose that A_3 is the alveolar surface area at end-expiration. If the surface area increases to $A_3\,\phi\,(1+x)$, x being positive, at end-inspiration at each breath, then surfactant whose metastable area is at least xA_3 must be adsorbed to the surface at each breath. Suppose that after p quiet breaths the compliance falls to a fraction q of its initial value; we may suppose that the loss of surfactant from the surface is of the same order of magnitude. An increase in x given approximately by

$$x = (1-q)/p$$

would then have been sufficient to prevent the loss of surfactant from the surface. In one of the experiments of Williams *et al.* (1966) $q = 0{\cdot}2$, $p = 500$, giving $x \sim 0{\cdot}0016$. To put the argument another way, a very small adsorption of surfactant to the surface at each breath would suffice to prevent the slow desorption which actually occurs. We may therefore suppose that the fractional increase in surface area during a quiet breath cannot be significantly in excess of ϕ, or 1·25. This would not be incompatible with the morphometric results of Gil & Weibel (1972), which show a slow increase in area with volume at lower volumes and a much more rapid increase at higher volumes.

Certain workers (Bachofen, Hildebrandt & Bachofen, 1970; Hills, 1974) have assumed, without morphometric proof, that the alveolar surface area is proportional to the 2/3 power of the volume of the contained gas, as the area of an inflated rubber balloon would be. If the surface area changes by no more than ϕ ($=1{\cdot}25$) in quiet breathing (and it may well change by a smaller factor), the two thirds power law would require that the volume should change by a factor of no more than $\phi^{\frac{3}{2}}$, or 1·40. Crossfill & Widdicombe (1962) found, however, that in anaesthetized rats weighing 250 g, the mean FRC was 1·55 ml. and the mean tidal volume 1·55 ml., giving a ratio of end-inspiration volume to FRC of 2·0. The present findings are thus incompatible with the two thirds power law, which also fails to fit the findings of Gil & Weibel (1972). The present results therefore suggest that during quiet breathing the lung behaves rather more like a concertina than a rubber balloon. Teleological arguments, too, would lead to a similar expectation. A large change in surface area would not only prevent the surfactant from using its capacity to lower the S.T. far below that of ordinary body fluids; it would also lead to a great deal of work being done against elastic forces.

Quantitative aspects of surface tension

The experiments do not tell us the actual values of surface tension during quiet breathing. The value of 34 mN/m probably represents the S.T. at the end of a deep inspiration, when surfactant has been adsorbed to the surface. During a subsequent expiration the film will probably be compressed by more than 20 %, and at end-expiration the S.T. will probably be low. It is however unlikely that in the alveolus as low an S.T. can be maintained as in a bubble, whose configuration favours even compression of the film. Between these extremes it is not possible to predict the S.T. without a better knowledge of the variation of alveolar surface area with volume and of S.T. with the rate of stretching or compression of the surface; the latter variation being a usual property of colloidal films.

During quiet breathing a number of interacting effects, leading to a fall in compliance, are to be expected. They include loss of surfactant, decreased surface area, decreased end-expiratory volume (as noted by Herxheimer, 1975), increased surface tension, and (possibly) collapse of complete alveoli and airways. Williams *et al.* (1966) found evidence of alveolar atelectasis, but not of airway collapse, in rabbits. The present findings give little help in distinguishing between these effects. The balance between them probably depends on the elastic properties of the lung and chest wall, as well as on the surface properties of the lung; the former, but not the latter, vary widely between species. Thus von Neergard (1929) studying air-filled and liquid-filled lungs, concluded that the elastic recoil of pig, dog, sheep, and human lungs fell to zero about F.R.C., the further contraction of air-filled lungs being due to surface tension. By contrast, recent work on rat lung (R. E. Pattle and M. T. Gorton, unpublished) has found that for a 250 g rat (FRC 11·55 ml.) the volume at zero transtracheal pressure after inflation with liquid is less (0·6–0·8 ml.) than that after inflation with air (0·9–1·0 ml.). This suggests air trapping. There are many sources of error in this field, and more work is required.

Although the film formed on the Wilhelmy trough by lung extracts is less directly derived from the lung lining than is the bubble lining film, it shows a similar increase in S.T. with increase in area. Thus in the tension-area diagram of Scarpelli (1968) an increase from 20 % to 25 % of total trough area raises the S.T. from 5 mN/m to 28 mN/m.

The finding of an S.T. as high as 34 mN/m in an expanded bubble fails to support the suggestion of Copeland (1969) that a substance resembling lung surfactant assists in the generation of intracellular bubbles which, according to one hypothesis, are liberated into the swimbladders of fish.

Re-adsorption of previously desorbed surfactant

The present experiments show that surfactant can be desorbed from and re-adsorbed to the surface without loss of surpellic properties. The water-dispersible surfactant has been exhaustively analysed by King & Clements (1972); the present work suggests that the insoluble bubble lining, partially analysed by Pattle & Thomas (1961) and by Klaus, Clements & Havel (1961), is formed from the water-dispersible substance without chemical change. It does not, however, show that the process can be repeated indefinitely; collapse of the lung after every breath, such as occurs in the respiratory distress syndrome of the new-born, may well give rise to an increased rate of consumption of the surfactant (Avery, 1973). The occasional complete disappearance of an originally metastable bubble after expansion may perhaps be due to the surfactant decomposing with some components spreading more than others.

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