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BUBBLES LINED WITH LUNG ALVEOLAR SURFACTANT

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Abstract. A field of bubbles expressed from lung alveoli of mice was restrained in water under a flat surface. The bubbles were expanded by reducing the ambient pressure, then compressed by increasing this pressure to one atmosphere. Compression resulted in rapid, reciprocal changes in polar and equatorial diameters of the bubbles. Moving pictures of the process were taken. Measurements, from the pictures, of polar and equatorial diameters showed: (1) the size of the bubbles did not necessarily diminish after a series of compressions, (2) the shapes of the bubbles might have been determined by forces contributed by a variably mobile structure in addition to forces of surface tension and gravity. The suggestion is made that the rapid oscillation of the bubble diameters, as described, was produced by Thomson-Marangoni effects.

Lung alveolar surfactant

Introduction

The surface of a bubble obtained from a lung alveolus of a mammal consists of highly surface active material termed lung alveolar surfactant (LAS). When such bubbles are placed in a chamber filled with water, they rise and are restrained by the inner and upper surface of the chamber. Under these experimental conditions, and using deaerated water, such bubbles have been observed to become alternately flattened upwards slowly, then rounded again rapidly in a cyclic process, while becoming smaller continually as gas diffused from their interiors to the surrounding water (PATTLE, 1958).

The process, termed "clicking" (PATTLE, 1960), has been explained by assuming that the shape of a bubble is determined by its surface tension (γ) and gravity and that part of its insoluble surface is somehow shed while the bubble is flattened. At this moment its γ rises resulting in a more rounded shape. Continued further compression and shedding of part of the surface produces continued "clicking" (PATTLE, 1958).

We have observed bubbles lined with LAS in a similar physical environment, but have used a different technique to produce "clicking" of the bubbles. Repeated experiments using our technique indicated that somewhat different conclusions could be

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drawn regarding the events and forces which govern movement and shape of these bubbles. An experiment was designed, therefore, to help quantitate what we had observed repeatedly in a qualitative manner, and the results may be of value when attempts are made to assign functions to the LAS in lung mechanics.

Materials and methods

A small piece of fresh mouse lung was placed in a square cuvet which was filled with air-saturated water (24 °C) then attached to a vacuum pump by tubing with a hole in it. Pressure in the cuvet was reduced to $\frac{1}{2} - \frac{1}{3}$ atmosphere as indicated in fig. 1 (at Frames No. 0 and 270) by partial obstruction of the hole in the vacuum line while the vacuum pump was running. A large field of smooth, silvery bubbles, without visible excess material attached to them emanated from the lung tissue at this time, whereupon the pressure in the cuvet was allowed to rise to one atmosphere by opening fully the hole in the vacuum line.

The bubbles, in close proximity to one another, were observed continuously, in side view, through a stereoscopic microscope. Illumination was kept at a minimum to minimize temperature changes. A 16 mm moving picture camera was fixed to the third ocular of the microscope. The camera was set to record at 64 frames/sec at 1/160 second exposure time per frame.

To obtain data, the bubbles were expanded by reducing the surrounding pressure as described. Photography, with extra illumination, was begun after the bubbles were expanded and continued while the pressure was allowed to rise. The bubbles "clicked" actively while contracting at mutually independent and unpredictable rates. Photography, and the extra illumination required for it, were stopped when atmospheric pressure was reached and the bubbles stopped "clicking". The entire process of expansion, requiring about 1 sec, and contraction, requiring about 3.5 sec, is termed a cycle. A total of 36 consecutive cycles was achieved during the experiment and photographs were taken as described between the limits of full expansion and just after the

TABLE 1

Effect of compressions on diameters of a lung alveolar bubble.*

Cycle	Polar diameter (micra)	F ratio (Polar diameters)	Equatorial diameter (micra)	F ratio (Equatorial diameters)
	Mean ± Standard deviation		Mean ± Standard deviation	
1, 6, 11			282.9 ± 12.5	0.98
1, 6, 11, 16	250.4 ± 5.6	1.52	279.5 ± 13.7	3.59**
16			269.3 ± 12.5†	

*Diameters measured at the end of the 1st, 6th, 11th and 16th compression cycles. First series of recorded observations. (See text.)

**Significantly different from all other groups in its series by the F test ($P < 0.05$).

†4.8 ± 2.8% smaller than equatorial diameter for combined cycles 1, 6 and 11.

TABLE 2

Effect of compressions on diameters of lung alveolar bubble after combination with another lung alveolar bubble.*

Cycle	Polar diameter (micra)	F ratio (Polar diameters)	Equatorial diameter (micra)	F ratio (Equatorial diameters)
	Mean ± Standard deviation		Mean ± Standard deviation	
21, 26, 31	304.9 ± 11.5	1.79		
21, 26, 31, 36	299.6 ± 14.1	11.6**	309.2 ± 12.2	0.55
36	283.8 ± 8.0†			

*Polar and equatorial diameters of the same mouse lung alveolar bubble given in table 1, but after enlargement due to combination with another bubble originating from the same lung. Diameters measured at the end of the 21st, 26th, 31st, and 36th cycles. Second series of recorded observations. (See text.)

** Significantly different from all other groups in its series by the F test ($P < 0.05$).

†6.9 ± 1.9% smaller than polar diameter for combined cycles 21, 26 and 31.

last "click" for the 1st, 6th, 11th, 16th, 21st, 26th, 31st and 36th cycles.

One of the bubbles survived without apparent coalescence with any other bubble for the first 16 cycles which we term the first series of recorded observations (table 1). During the 17th cycle this bubble combined with another bubble and the resultant larger bubble survived without apparent coalescence with any other bubble through the 36th cycle. The second series of recorded observations began with the 21st cycle and ended with the 36th cycle (table 2), which terminated the experiment.

The resultant moving picture was projected at a total magnification of 42.5 × on a frosted glass screen at 7.29 frames/sec. The equatorial diameters (ED)s and polar diameters (PD)s of the original bubble (first series, table 1) and the combined bubble (second series, table 2) were measured from the projected images at the end of each recorded cycle when the external pressure was 1 atmosphere (cycles 1, 6, 11, 16, 21, 26, 31 and 36).

Since single estimates seemed inadvisable, the measurements were repeated 10 times on 10 separate projections of the same film. The hypothesis was formulated that, at 1 atmosphere external pressure, the surface area of the bubble, as a function of its PD and ED, did not change during the first or second series of recorded observations. The hypothesis was tested by analysis of variance methods at the 95 percent confidence level (tables 1 and 2).

Direct computation of bubble area is unnecessary to arrive at a conclusion concerning the likelihood of change in its area. Measurement of the governing dimensions of a bubble was accepted as a suitable indicator.

The rate of "clicking" and number of "clicks"/cycle could not be estimated from direct observation. However, estimates were secured from projections of the film at 7.29 frames/sec. Rate of "clicking" = (64 frames/sec) (No. of "clicks" in the "clicking" phase of a cycle) ÷ (No. of frames in the "clicking" phase of a cycle).

Too many photographs/cycle were taken to permit of their publication, so a substitute was devised. 570 consecutive frames of the film strip, embracing two complete cycles were developed as 4″ × 5″ enlargements. Single measurements of the PD, ED, and polar radius (PR) of each of three separate bubbles were taken from each of the developed enlargements and plotted against frame number (fig. 1).

A separate experiment was performed to quantitate possible temperature changes due to the light used during the study. A thermometer replaced the vacuum line to the cuvet, the bulb of the thermometer being placed near the wall of the cuvet facing the light. The thermometer bulb and the immersing water were illuminated for 5 min with the dim light used for focusing the camera and microscope and for continuous observation of the bubbles between recorded observations. A rise in temperature of 0.3 °C (23.3–23.6 °C) was produced. The bright light necessary for photography was

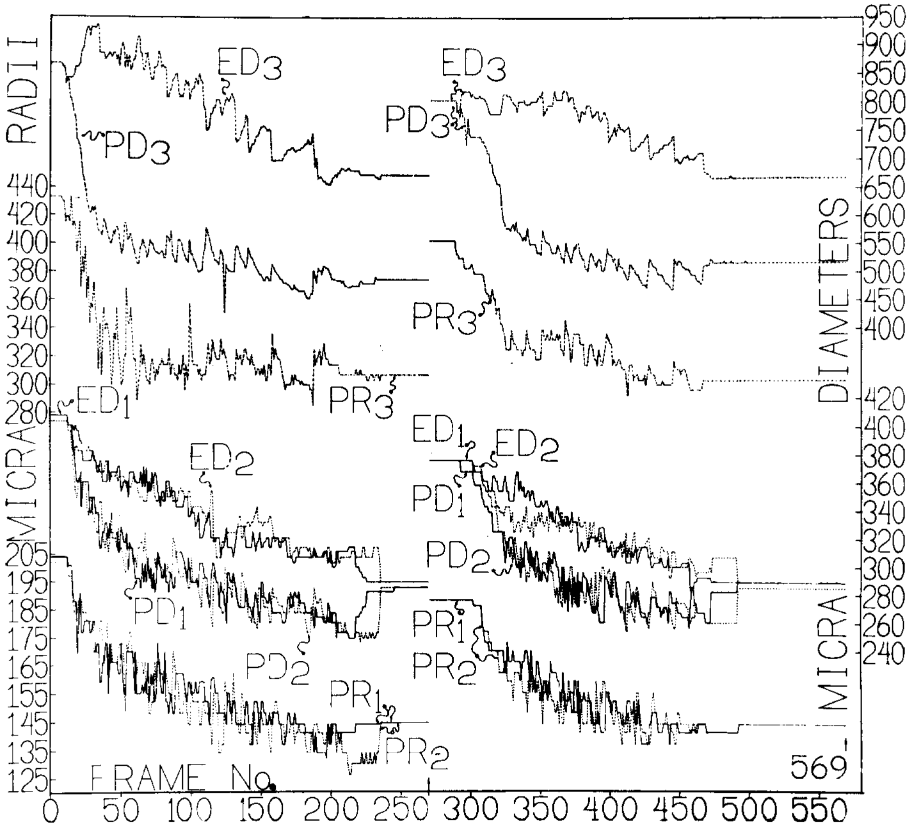

Fig. 1. Polar radius (PR), polar diameter (PD), and equatorial diameter (ED) of three lung alveolar bubbles (subscripts 1, 2 and 3), plotted against Frame No. for 570 consecutive moving picture frames which were taken at 64 frames/sec and 1/160 sec exposure time/frame. Dimensions on the left ordinate are for bubble radii; on the right, for bubble diameters. The sudden break in the data for each bubble at Frame No. 269 (indicated by an arrow), marks the time when one cycle was ended and the bubbles were re-expanded to begin a new cycle. (See text.)

then used in five second bursts for additional illumination. Use of this strong illumination was repeated eight times, with 15 sec intervals wherein only the dim light burned. This corresponded to the conditions of the actual experiment with a generous surplus of luminous flux and produced a further rise in temperature of 0.4 °C (23.6–24.0 °C) which stabilized during the last 45 sec. The temperature rise of 0.4 °C corresponded to the period of experimentation with the bubbles during which data on them were taken. Assuming no restriction by the bubble film it amounts, in terms of volume increment of gas in the bubbles to about 0.15 percent of initial volume, or 0.05 percent of initial principal radii of curvature and will be neglected.

Results

The results in table 1 indicate that, for the first 11 cycles, the bubble returned to its original surface area each time the external pressure returned to 1 atmosphere. However, when the external pressure had returned to 1 atmosphere at the end of the 16th cycle, a slight diminution of area was indicated by a 5% reduction in ED, without a change in PD. Similarly, the new bubble (table 2) also appears to have retained its original area, from the 21st through the 31st cycles, then decreased about 7 percent in PD, but not significantly in ED during the next five cycles.

For the first series of recorded observations (cycles 1 through 16, table 1), the average number of "clicks" was estimated to be 34/cycle for a total of 544 "clicks". For the 1st through the 11th cycle, the corresponding data were 34 and 374. For the second series of recorded observations (cycles 21 through 36, table 2), 28 "clicks"/cycle were estimated, or 448 "clicks" total, and for cycles 21 through 31, 25 "clicks"/cycle, or 275 "clicks" total. The average rate of "clicking" throughout was estimated at about 17 "clicks"/second.

A total of about 374 "clicks" occurred during the 1st through the 11th cycles with no measured change in the final dimensions of the bubble (table 1). A total of about 275 "clicks" occurred during the 21st through the 31st cycles with no measured change (table 2).

A measurable decrease in the final ED at 1 atmosphere pressure did occur during cycles 12–16 (cycle 16, table 1), and similarly in the final PD during cycles 32–36 (cycle 36, table 2). An "order of magnitude" estimate of reduction in area/"click", assuming an approximately spherical shape, and using a compound decrement formula, yielded a maximum of about 0.03 percent reduction in area/"click" for both instances.

Fig. 1 gives the results of measurements of ED, PD, and PR for three bubbles (subscripts 1, 2 and 3) plotted against time (Frame No.) over two complete cycles. These results are qualitative in comparison to those given in tables 1 and 2.

Discussion

We have used an intentionally different process from PATTLE's (1958, 1960) to produce the "clicking" phenomenon and to estimate the overall reduction in area/"click". Our estimate is based on the total number of "clicks" produced over many cycles of expansion and contraction of the bubbles and is the logical procedure required as a critical test of our hypothesis.

The "clicking" process, as described by PATTLE (1958, 1960) involved the shedding of an insoluble "ghost" or skin which on one occasion was reinflated. His conclusions could not have deviated greatly from those deduced from his information. His bubbles went through one compression only and evidence was present for the shedding of an insoluble skin. We have not seen a shedding of a surface layer in any of thousands of "clicking" bubbles. If our "clicking" process is associated with shedding of a skin, then this skin would have to strip off and disappear. If the amount of skin available closely approximates the amount required to form a single surface layer (PATTLE, 1958, p. 222), then with loss of bubble lining with each "click", the bubble would, perforce, become smaller at the end of each compression phase of our "clicking" cycles or else the bubble would be incompletely covered at the air:water interface. If the latter did occur, one would expect to find irregularly shaped bubbles due to local variations in γ. We did not observe this. Our estimate, therefore, of the amount of area lost/"click" is an estimate of the maximum area of skin which could have been shed invisibly at each "click" without compromising the integrity of the surface remaining on the bubble.

The small diminution in surface area of the bubble at the end of each recorded series (cycle 16, table 1; cycle 36, table 2) cannot be explained on the basis of desaturation of the water with respect to air because, if this were true, the second series (from cycle 21 onward, table 2) should have shown a steady diminution in size, the point of sufficient desaturation having been indicated during the 16th cycle (table 1). A different micro-environment about the bubble during the second recorded series does not seem to suggest an appropriate explanation since the great activity of "clicking" of it and other bubbles nearby produced considerable local mixing of the immersing liquid.

A possible explanation for change in bubble size at one atmosphere ambient pressure, when it did occur, could make use of the following two facts: (1) We have observed from other experiments (MENDENHALL, SUN and MENDENHALL, 1967) that LAS is composed of several highly surface active components at least one of which is soluble in distilled water. The water soluble component forms a white precipitate upon addition of Ca^{++}. Two other components are separable by centrifugation. The heavier of these turns brick-red on drying, and the lighter is a light jade-green, wet or dry; (2) the "clicking" bubbles, as we have described them, ultimately lose the capacity to "click".

Applying these facts, we might consider that the surface of the bubbles was at first a comparatively thick structure under the conditions of the experiment, but transfer outward of a water-soluble component proceeded until the structure, perhaps hydrated, was reduced markedly in thickness due to loss of this component. After the end of the first series, new water soluble material was added to the bubble under observation by coalescence with a fresh bubble so that the process could be repeated. This would require an initial wall thickness of several micra which might seem large for its thickness *in vivo*. The tendency of the material which forms the walls of these bubbles to take up water has been demonstrated (MENDENHALL and SUN, 1964).

All previous work with surface balances indicates that the material which forms the surfaces of these bubbles must be compressed continuously in order for it to reach and maintain a low γ. If such information is applicable to the study of bubbles we would conclude that a lung alveolar bubble should continue to flatten while its surface was being compressed continually by continually increasing ambient pressure. The bubble could begin to become round when compression of it ceased since further diminution of area has ceased, and its γ could begin to rise (MENDENHALL and MENDENHALL, 1964, 1966; SUTNICK and SOLOFF, 1963). Actually the bubbles become flattened and rounded cyclically while being compressed, which would correspond to an undulating relation between γ and area on the surface balance. Evidence for such a relationship has not yet been published although an initial decrease of γ followed by an increase during the same compression of a film of LAS on the surface balance has been observed at low rates of compression (MENDENHALL, 1963).

It seems desirable to attempt to explain "clicking" of the bubbles by a mechanism which does not require shedding or puckering of their surfaces. If a bubble surface were composed of a single thin layer, or of lamellae, too thin for observation by our methods, then it or these might, under undefined conditions, slide over one another unnoticed. Electron micrographs of material *in vitro* (MENDENHALL and SUN, 1964) and *in situ*, in dead specimens (TYLER and PANGBORN, 1964) exist for support of this speculation in that they demonstrate the tendency of LAS or similar material to form lamellae.

We suggest another mechanism in the form of interfacial turbulence. An example of this phenomenon which seems appropriate is given by DAVIES and RIDEAL (1963), wherein a drop of water is allowed to form on the tip of a pipet, the tip being immersed in a solution of acetone (4 percent) in toluene. Local regions of high concentration of acetone at the surface of the drop cause the acetone to adsorb rapidly at the water: toluene interface. The corresponding rapid reduction in γ, spreading over the surface causes the drop to "kick" quickly. The acetone diffuses from the surface and into the interior of the drop of water so that the surface is prepared for a repetition of the process. "Kicking" ceases when the equilibrium concentrations of acetone between water and toluene is reached. Such phenomena are known collectively as Thomson-Marangoni effects (SCRIVEN and STERNLING, 1960), which are considered to be due to local gradients in γ produced by local variations in surface concentrations of surfactants.

Applying this concept to the "clicking" bubbles, we call attention to the water-soluble material which is washed in quantity by distilled water from a water-insoluble constituent of the bubbles' surface. In addition, capacity of the bubbles to "click" is ultimately lost. These 3 facts suggest the possibility that compression of the lining film of a bubble promotes passage of a highly surface active water-soluble component from within the lining film to the interface between its insoluble elastic surface (DANIELLI, 1936, p. 401) and water. The highly surface active component reduces γ

at the designated interface more rapidly than it passes into the water and so permits a flattening of the bubble by the buoyant force of air in the bubble. The bubble becomes rounded when the water-soluble component desorbs into the water from the surface of the insoluble layer. The process is repeated upon further compression of the bubble. "Clicking" ceases when the accountable molecular species is exhausted to the aqueous phase. Possible details of this mechanism are enumerated by MENDENHALL *et al.* (1967). It is not considered necessary that the events occur at surface energies above the equilibrium surface energies of the responsible surfactants. More important are the instantaneous differentials between surface energies of a water-insoluble "skin" and a water soluble species at this "skin": water interface.

The information gained concerning the effect of "clicking" on bubble size was anticipated from prior extensive observations. The following information confirms repeated observations also, but is of a highly individual nature and such a comparison between any two bubbles cannot be predicted qualitatively or quantitatively. The larger bubble (table 2) was approximately spherical at the end of the "clicking" process from the 21st through the 31st cycles, and when this bubble was reduced in size, it was due to reduction of the PD by about 7 percent. The smaller bubble (table 1) maintained an ED larger than the PD throughout its life, although it was the ED which decreased by about 5 percent when this bubble became smaller. After reduction in PD of the larger bubble, and ED of the smaller bubble, the ratio of ED to PD was, in each case, about the same (about 1.08). Before these events, the larger was more spherical and the smaller one was more flattened, the PD of the latter from the 1st through the 11th cycles being 11.4 ± 1.5 percent smaller than the ED.

These observations may be explained simply on the basis that the γs of the bubbles were below their equilibrium values, hence momentary differences need not seem odd. An alternate explanation which is not eliminated by our evidence, arises from a possible independence between shape of the bubbles, their γs, and the buoyant force of gas contained in them. This independence could arise through the possession of structure by the bubble wall (MENDENHALL and SUN, 1964). The work of TYLER and PANGBORN (1964), indicates the possibility for existence of a "lamellar" structure at the air-liquid interface which was also the disposition of the bubble lining layer under the physical conditions of the experiments reported here.

Data such as is given in fig. 1 present the opportunity to study changes in γ during "clicking" based on (PR) $\div$ (equatorial radius) according to PORTER (1933). We have refrained from doing this because the method applies to cases where γ and gravity are the only active forces, so that we have been unsuccessful in convincing ourselves that the method is applicable to our data.

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