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Surface Properties in Relation to Atelectasis and Hyaline Membrane Disease

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Recent observations suggest that a low surface tension may be an important attribute of the lining of the air passages of the lung.[1-4] The purpose of this paper is to present evidence that the material responsible for such a low surface tension is absent in the lungs of infants under 1,100-1,200 gm. and in those dying with hyaline membrane disease. The role of this deficiency in the pathogenesis of the disease is considered.

Surface tension operates so as to minimize the area of the surface. In the lungs, where the internal surface (the alveolar lining) is curved concave to the airway, the tendency of the surface to become smaller promotes collapse. Although the forces not only of surface tension but also of the elastic tissue tend to collapse the lungs, their behavior differs in one important respect. When the lung contains only a small volume of air, the elastic recoil of the tissue is diminished, that is, the less the tissues are stretched, the less are the elastic stresses. In contrast, the contribution of surface tension to the retractive force of the lung is increased. Thus, as the air spaces become smaller and more sharply curved, the "mechanical advantage" of surface tension may be thought of as increasing, promoting the tendency to collapse. Since the air spaces are not uniform in size and are all connected to the airway, the smaller, more sharply curved ones tend to empty their contents into the larger. A high surface tension would favor this phenomenon and predispose to atelectasis, whereas a low surface tension would be a stabilizing influence, diminishing the tendency to collapse. For example, if an alveolus can be thought of as a partial sphere with a radius of 40μ and a surface tension equal to that of plasma (55 dynes/cm.), pressure difference would be 20.5 mm. Hg between the inside and outside of the sphere.* This is the pressure tending to collapse the alveolus. If, however, it had the same radius but a surface tension of only 5 dynes/cm., the pressure tending to collapse it would be 1.86 mm. Hg.

Pattle, and more recently Clements and Brown have focused their attention on the magnitude of the surface tension within the lung. Pattle,[1,2] noting the stability of foam and bubbles arising from the lung, concluded that their surface tension must

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* This is in accord with the LaPlace relationship, $P=2T/r$, where P is the pressure across the wall of the sphere; T is surface tension, and r is the radius of the sphere.

be extremely low. On the basis of measurements which showed that these bubbles were more stable than those produced from plasma or transudates, he deduced that the bubbles from the lung were lined by a material which he thought was derived from the internal surface of the lung. He suggested that absence of this material in the lungs of premature infants might play a role in atelectasis neonatorum and hyaline membrane disease.

Clements [3] and Brown [4] demonstrated that the tension of a surface film derived from the lung was not a constant value; when the surface was stretched the tension was relatively high (40 dynes/cm.), but when the surface area was decreased the tension fell to 10 dynes/cm. These workers first pointed out that such a reduction in surface tension during deflation of the lung would tend to stabilize the air spaces by permitting them to remain open at low lung volumes.

It must be noted that the measurements made by Pattle and Clements and Brown were on material derived from the lung, and not on the alveolar surface itself. Pattle's assumption was that the material lining the internal surface of the lung would also cover small bubbles expressed from its cut surface. Clements and Brown assumed that if a portion of lung were cut in small pieces and stirred with saline, the most surface-active material in the mixture would seek the surface where its tension could be measured. None of these workers knows the precise chemical nature of the surface film studied. However, the observation that the films can be altered by incubation with trypsin and pancreatin suggests that they are at least in part protein.

For the study of the surface behavior of proteins, the classical methods employing a capillary tube or a platinum ring are inadequate since they record only a single value. The surface tension of protein films changes when the area of the surface is changed. Film balances such as the Langmuir-Wilhelmy type used by Clements permit measurements of surface tension as a function of changes in surface area.[5] The dependency of tension on area is an important elastic-like property of protein films. In surface films obtained from lungs the change in tension is not a constant value, but continues to change in time. It is presumed that in addition to elastic behavior there is a time-dependent viscous component, which produces this lag in response, termed hysteresis.[6] Thus the films derived from the lung behave as if they were viscoelastic entities.

Despite the lack of direct measurements of surface tension at the alveolar-air interface, the low values obtained by Pattle and Clements and Brown with indirect methods would account for the stability of an alveolus at end-expiration. If then the prevention of atelectasis depends on the presence of a material with a very low surface tension lining the air spaces, it seemed attractive to examine the lungs of small premature infants and those dying with hyaline membrane disease for this material. In these infants there is always some atelectasis. The absence of a low surface tension in extracts of their lungs would support the theory put forward by Pattle and Clements and Brown, and at the same time explain the predisposition of these infants to atelectasis.

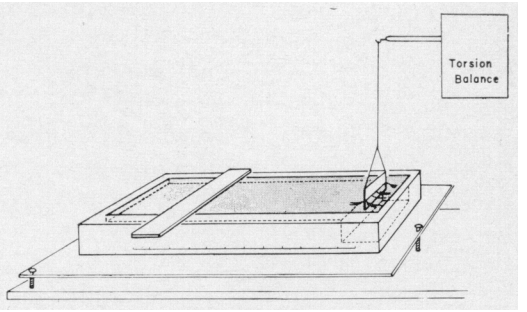


Fig. 1.—The dimensions of the trough are 15×7.5×1.7 cm. outside, 11.8×5×1 cm. inside. At one end is a well 5×1.5×1.3 cm. to permit submersion of the stirrup for a zero reference point. The trough is filled so that barrier touches the surface (65 ml.). A centimeter scale is attached to one side to permit measurement of the area where the barrier is moved. The metal plate under the trough is supported by three screws to permit leveling.

Methods

The method to be described is similar to that used by Clements. The film balance is shown in Figure 1. The trough is constructed of a single block of polytetrafluoroethylene (Teflon).† (This has the advantage over paraffin-coated troughs in that it is less wettable than paraffin, chemically inert, and provides a surface which is easy to clean.) A thin, frosted platinum strip or "stirrup" is partially submerged in the fluid. The force of surface tension, pulling down on the wettable stirrup,‡ is measured on a torsion balance with attached transducer through a direct-writing oscillograph.

Four grams of lung was cut into pieces approximately 2 by 5 mm. and diluted with 65 ml. of 0.85% saline. The mixture was stirred vigorously for about five minutes, filtered through gauze, and poured into the trough. The surface was "aged" one hour before testing. To change the area of the surface, a Teflon strip (11.3 cm. × 2.2 cm. × 0.3 cm.) under a heavy brass bar used as a barrier, was moved once a minute in 1 cm. steps, starting from the end of the trough opposite the stirrup and approaching 0.5 cm. from the stirrup (15% of the original area). The precedure was reversed to extend the film.

A change in surface area was promptly followed by a maximal change in tension, which decreased with time. By the end of one minute at tensions above 20 to 30 dynes per centimeter about 90% of the change has taken place.

At lower tensions the surface appeared irregular and occasionally had whitish linear streaks parallel to the barrier. This easily recognizable change was considered a "gelling" of the film. When this occurred the initial and one-minute readings were nearly the same, and the tension remained constant even on further compression of the surface. Thus there seemed to be a lower limiting tension, often about 5 dynes/cm. At lower tensions when the film did not "gel," the surface tended to creep over the edge of the trough, gradually extending the area so that the one-minute readings had no meaning in terms of tension at a given area. When this happened only the initial value was recorded. (The initial readings at all areas are called dynamic values. The one-minute readings are called quasistatic values.)

The possible influence of concentration of tissue on the results was studied by using 0.5 gm. of lung per 65 ml. of saline and 20 gm. of lung per 65 ml. of saline. The highest and lowest tensions recorded were the same. No attempt was made to establish the minimal amount of tissue needed. Four grams per 65 ml. was the concentration used in these experiments because of convenience in handling this amount.

The possibility that the age of the tissue after death would alter the surface behavior was investigated because the samples of human lungs were obtained at different times post mortem. Therefore samples of dog lung were studied immediately after the animal was killed, and after either refrigeration or freezing for as long as six days. Within these limits there was no significant change in the results obtained.

Temperature changes, within the range of 70 to 101 F and changes in pH of the substrate by addition of HCl and NaOH to a range of pH 1-pH 11 did not influence the surface tension-area relationship. Most of the measurements of the human lungs were made at temperatures between 75 and 85 F, while the pH of the filtered lung extracts was usually 6.5 to 7.0.

Results

The relationship of surface tension to area as measured on the film balance, is shown in Figure 2. Here the path of changing tension with decreasing area is on the left, and the tension with increasing area is on the right side of each plot. The solid lines connect the points obtained immediately after moving the barrier. The inner dotted lines connect the points recorded after one minute at the same area.

These curves differ from the one published by Clements in that they show a steeper slope at the beginning of compres-

† Dupont registered trade-mark.

‡ One correction necessary when using a partially submerged stirrup is for buoyancy. With a very thin platinum strip, this is almost negligible. It can be measured by recording the tension of a known solution with the stirrup at different depths. If subsequent measurements are made at a given depth, the contribution of buoyancy is known.

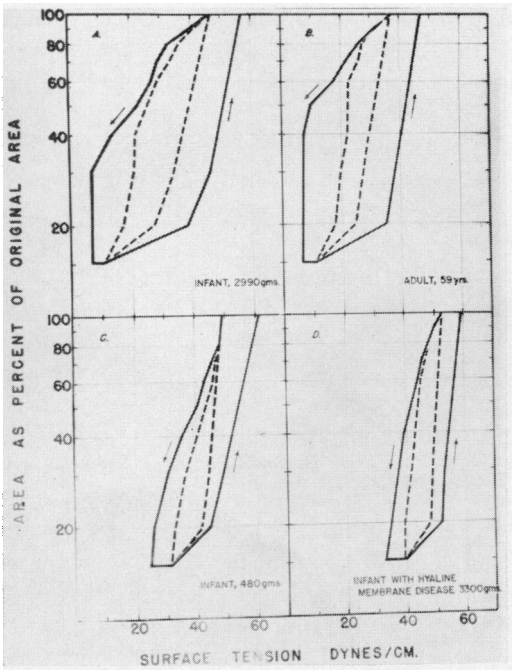


Fig. 2.—Illustrative force-area diagrams. The outer solid line connects the initial values for surface tension obtained when the area was changed. The inner dotted lines connect the values for surface tension after one minute at the given area.

sion of the film. The slope, which is the coefficient of compressibility, is variable, depending on the age of the surface, the number of times cycled and unknown factors. In our experience the least variable values were the lowest and highest tensions recorded. For comparison of the surface behavior of lung extracts from infants of different birth weights, the highest tension reached on extension of the surface, and the lowest tension reached on compression with the means and deviation from the mean are presented in the Table. There is considerable variation in the upper tensions and no pattern is evident. However, a definite pattern appears in the lowest tensions recorded (Fig. 3). The lowest values in the lung extracts of infants under 1,100-1,200 gm. are 20-30 dynes/cm. The lowest tensions in the extracts of heavier infants, older children and adults are under 20 dynes/cm. and usually 5-7 dynes/cm. The only exceptions to this are the lowest tensions measured in the lung extracts of infants dying with hyaline membrane dis-

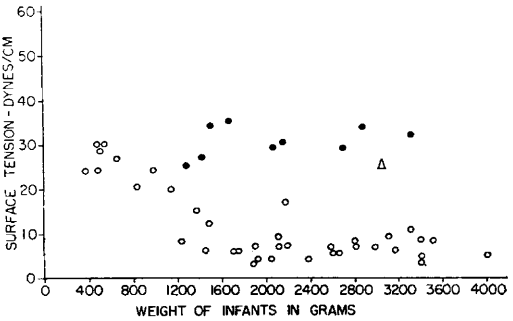
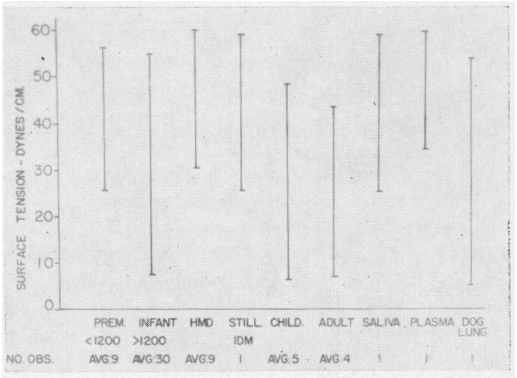


Fig. 3.—Lowest surface tension measured on compression of the surface. Open circles=infants dying from causes other than hyaline membrane disease. Closed circles=infants dying with hyaline membrane disease. Triangle=stillborn infant of a diabetic mother.

ease. In these, the corresponding figures are all above 20 dynes/cm. and most are above 30 dynes/cm. even though the infants were in the weight group where values below 20 dynes/cm. would be expected. This was true in all nine of the infants with hyaline membrane disease studied. The only infant who did not have the disease whose lung had a surface tension higher than expected was a stillborn infant of a diabetic mother.

The behavior of extracts of lungs of dogs, cats, rabbits, and guinea pigs was much the same as that of human lungs. Washings from the tracheal-bronchial tree had the same surface-tension values as lung extracts. However, other tissues, including plasma, whole blood, gastric juice,

Fig. 4.—The horizontal lines connect the highest and lowest tensions measured in extracts of lungs and other tissues. HMD=hyaline membrane disease; still. IDM=stillborn infant of a diabetic mother.



Highest and Lowest Surface Tension of Lung Extracts

Wt., Gm.	Infants Live-born or Stillborn	Highest Tension	Lowest Tension
390	S	49	24.5
470	S	58.2	30.6
480	L	61.5	24.5
500	S	57.5	29
520	L	61	30.5
680	L	56	27
830	L	55	20.8
970	L	59	24.5
1,150	S	49	20
		m=56.2	m=25.7
		± 4.34	± 3.65
1,220	L	52.5	8.6
1,390	L	48	15.2
1,430	L	54	6.6
1,460	L	56	12.2
1,700	L	55	6.1
1,740	L	54	6.1
1,870	L	60	3.6
1,900	S	59	7.3
1,940	S	56	4.9
2,100	S	51.5	9.8
2,125	L	56	7.3
2,180	S	56	17.1
2,180	S	58	7.6
2,390	L	55.5	4.4
2,495	L	61.5	6.8
2,500	L	53	6.1
2,640	L	58	6.1
2,670	L	53.5	6.1
2,800	L	61	8.5
2,800	S	51	7.4
2,990	S	58.2	7.3
3,100	S	61.3	9.8
3,170	S	47	6.1
3,300	S	39.2	11
3,400	L	51	4.9
3,400	L	57.5	8.6
3,400	L	60	3.5
3,515	L	57.5	8.6
4,000	L	52.5	5.4
		m=55	m= 7.6
		± 4.67	± 3.05

Hyaline Membrane Disease

Wt., Gm.	Highest Tension	Lowest Tension
1,260	58.8	25.7
1,420	61	27
1,500	60	34.4
1,650	63.5	35.5
2,050	59	29.4
2,150	58	30.5
2,700	62.3	29.5
2,860	59	34.4
3,300	59	32.3
	m=60	m=30.4
	± 1.41	± 3.12

Children

Age	Highest Tension	Lowest Tension
9 wk.	54	6.1
3 mo.	51	5.4
8 mo.	51.4	4.9
23 mo.	35.5	7.4
4 yr.	50.6	9.8
	m=48.5	m= 6.7
	± 7.4	± 1.96

Adults

Age, Yr.	Highest Tension	Lowest Tension
37	40	9.3
44	41.5	5.4
56	47	7.3
59	46.5	6.8
	m=43.8	m= 7.2
	± 3.53	± 1.61

saliva, synovial fluid, liver, and muscle had surface properties very different from normal lung (Fig. 4).

Comment

The results show that without exception the surface behavior of lung extracts of the nine infants with hyaline membrane disease was different from that of infants dying from other causes and the same as that of infants smaller than 1,200 gm. This suggests that the disease is associated with the absence or delayed appearance of some substance which in the normal subject renders the internal surface capable of attaining a low surface tension when lung volume is decreased.

It is of interest to attempt to relate the results obtained to the pathogenesis of the disease. In all lungs with the first breath, large pressures are necessary to create an air-liquid interface (Table). In this respect the normal lung would not differ from the lung without the surface-active material since surface tension on extension of the surface is similar in both cases. Thereafter, during expiration, the alveolar surface of the normal lung would have diminished tension (Fig. 2), thus reducing the tendency of the air spaces to collapse. On the other

hand, in a lung lacking this lining material, surface tension would tend to remain high during expiration; the air spaces would be unstable, and some would collapse. Once a sufficient number had closed, others would remain open inasmuch as the interpleural pressure at end-expiration would be sufficiently negative to prevent further closure. The net mechanical effect would be a lower than normal interpleural pressure, both at end-expiration and, more particularly, at end-inspiration. This is in accord with the measurements of Cook et al. on living infants with the disease in whom the interpleural pressure at end-inspiration can be calculated to be at least -15 cm. H_2O, about a threefold increase over normal.[7]

As a result of an increased mean pressure difference between the thorax and the rest of the body, intrathoracic blood volume would be increased. In atelectatic regions, and for that matter in air-containing regions as well, presumably the pulmonary capillaries would be influenced by the more negative interpleural pressure and would therefore share in the congestion. The evidence presented by Gitlin and Craig [8] that the membranes contain fibrin, derived from the pulmonary circulation as fibrinogen, indicates that transudation occurs in hyaline membrane disease. There is no evidence that the congestion resulting from the increased body to thorax pressure difference would be sufficient to account for this transudation. It is possible that surface forces may produce highly localized distention and leakage of capillaries, although it is probably true that Pattle's estimate of these effects is an oversimplification.[1]

It is of interest that atelectasis, of the type seen in hyaline membrane disease, but without any membrane, has been described.[9,10] Potter suggests that it is an infrequent occurrence seen in infants with a clinical course compatible with the disease.[11] If the primary event is atelectasis with the membrane being formed later, it would be anticipated that some might die before the membrane had developed.

Certain clinical features of hyaline membrane disease could be explained if the disease results from the absence of a surface-active material:

1. The disease has not been described in stillborn infants. The surface forces at an air-fluid interface could not operate before the first breath.

2. The symptoms may begin within the first few minutes after birth, but often do not become severe until several hours later; death or recovery usually ensues in 4 to 72 hours. Although a normal initial expansion of the lungs would be expected, it would take time for the subsequent mechanical difficulties to be evident. If maturation of the lung lining occurred in the first few days of extrauterine life, recovery would be expected.

3. The disease is more common the more premature the infant.[12] Since our data suggest the normal surface behavior usually appears in infants of about 1,100-1,200 gm., its absence from the lungs of certain infants weighing more than this could be an instance of delayed appearance of the normal lung lining material, and more likely the more premature the infant. One could ask if the absence of a specific surface-active material in the lung predisposes to hyaline membrane disease, why do not all infants under 1,100-1,200 gm. (lacking the material) have the disease? The nine such infants thus far studied showed surface tensions similar to those from infants with the disease, but four of the nine were stillborn and two lived only minutes (Fig. 3). In the three who lived more than four hours, long enough to have signs of the disease, the lungs were indeed atelectatic, although there were no membranes. In any case, one cannot expect every very small premature infant to have the disease, without assurance that 1,100-1,200 gm. is a sharp zone of demarcation before which surface-active material never appears.

Among the unexplained features of this disease is the high incidence in infants of diabetic mothers. Whether the resemblance of this group of infants to premature in-

fants is sufficient to assign a similar pathogenesis of the disease remains to be seen.

Finally, the hypothesis presented here that the lack of a normal lining material in the lungs of infants would contribute to the atelectasis seen in hyaline membrane disease does not preclude the possible importance of other factors in the pathogenesis. Immaturity of the lung lining may be associated with immaturity in other respects.[13] A combination of deficiencies or external insults may be required for the complete syndrome. Moreover, other properties or functions of the lung-lining layer deserve investigation.

Summary

Recent observations suggest that a low surface tension in the lining of the lung may permit stability of the alveoli at end-expiration. Lacking such a material, the lung would be predisposed to collapse.

Measurements of the surface tension of lung extracts confirm the presence of a very surface-active substance in lungs of infants over 1,100-1,200 gm. and in children and adults. In lung extracts of very small premature infants and infants dying with hyaline membrane disease the surface tension is higher than expected, suggesting that the surface active material is deficient.

The possible role of this deficiency in the pathogenesis of hyaline membrane disease is discussed.

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