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A THERMAL SURFACE PHENOMENON IN THE RABBIT LUNG: POSSIBLE BASIS FOR THE CONVERSION OF HEAT INTO WORK

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SUMMARY

1. Surfactant and fluid expressed from rabbit lungs has been used to form films on a simple Maxwell frame having a ratio of area-to-thermal capacity comparable to that existing *in vivo*.

2. When the area was increased by 2:1, the temperature fell by 5·6 °C and returned to 37 °C upon contraction as recorded by an infra-red thermometer with no thermal capacity.

3. The experiment was repeated upon thin lung sections when the temperature fall was 2·4 °C and was again reversed upon contraction.

4. When surfactant was removed from the surface of those sections, the temperature changed in the opposite direction, indicating that surfactant was responsible for the changes described above.

5. This surface energy phenomenon is discussed in relation to the common assumption that the lung operates under isothermal conditions when it may explain some of the discrepancies between studies of lung mechanics over different time scales.

6. Since the results are compatible with the inversion of hysteresis loops for surface tension *versus* the area of surfactant monolayers cycled to steady state in previous studies, it is speculated that heat, e.g. waste metabolic heat, might be the energy source for this surface 'engine' if, indeed, it is contributing to the work available for breathing.

INTRODUCTION

It has been universally accepted that the work of breathing is provided entirely by the muscles of the chest wall and diaphragm with the lungs playing a purely passive role in the ventilation process. An appreciable proportion of the work needed to overcome frictional resistances has been attributed to surface forces since the large hysteresis characteristic of quasi-static volume:pressure ($V:P$) loops obtained from excised lungs is largely eliminated upon liquid filling (Radford, 1957).

Although this interfacial hysteresis is much less if the lungs remain *in situ* (Hills & Barrow, 1986), it is usually explained by the large hysteresis in surface tension (γ) *versus* area (A) found when monolayers of lung surfactant or its most active component, dipalmitoyl lecithin (DPL), are cycled on a Langmuir trough. The

graphical areas represented by both PV and γA have the same dimensions of work and, in those studies, the handedness of rotation of the cycles showed that both represented energy *lost* as heat in *doing* work against friction and any other opposing forces.

In most studies of the relationship between γ and A of DPL films, on the Langmuir trough, the first or third cycles are normally recorded (Clements, Hustead, Johnson & Gribetz, 1961) but, as Notter & Morrow (1975) point out, they continue to shift and this is important because ventilation is a continuous process. These γ:A loops are much thinner when performed under more physiological conditions including 100% humidification with area changes corresponding to normal ventilation (Barrow & Hills, 1979*a*). Moreover, upon repeating the process over hundreds of cycles at respiratory frequency, the DPL film reaches the same dynamic steady state from whichever direction it is approached (Hills & Barrow, 1984). the surprising feature of this dynamic steady state is that the hysteresis loop displays a significant degree of inversion which is not artifact due to such parameters as contact angle at the interface with the device used to monitor surface tension (Barrow & Hills, 1979*b*). Thus the surface tension during compression exceeds that for expansion at the corresponding area. This inversion implies that, in place of work being lost as heat, work is being gained from another source of energy. This does not imply a perpetual motion machine because it would not function without the primary drive being provided by the barrier of the Langmuir trough or, in the lung, by the muscles of the chest wall and diaphragm. This is analogous to the manner in which a refrigerator can transmit heat from a colder to a hotter body – but only when the motor is running.

A surface contribution to the work available for breathing could be significant if it approaches the maximum of 23% of the work needed for breathing as estimated theoretically from γ:A loops cycled to steady state by Hills & Barrow (1984). However, for this contribution to be recognized as a reality in pulmonary function, it requires some demonstration in the lung and, in particular, some indication of the source of energy using lung tissue in addition to fluid films. The first has been afforded by a novel method for liquid-filling lungs *in situ* which avoids the large geometric irreversibility introduced by excision from the thoracic cavity (Hills, 1971). Although V:P curves for both air- and liquid-filled lungs and chest wall cycle in the direction of hysteresis, i.e. an *input* of work, the loop for the difference representing the interfacial component cycles in the opposite direction (Hills & Barrow, 1984), i.e. it displays inversion or work *output*.

The second task of determining the source of energy is more difficult but the options are virtually limited to thermal and chemical with the latter being unlikely since the reproducibility of γ:A loops indicated that a steady state had been attained in the studies described above. Teleologically, it would make good sense for the body to re-use some of the waste metabolic heat otherwise 'dumped' into the atmosphere at the lungs. For heat to be the source of energy, however, the mechanism must satisfy a basic principle of thermodynamics that any engine converting heat into work must operate over a range of temperature (see Reynolds, 1965). Hence this study has been designed to detect any change in temperature with the area of films

of surfactant expressed from lungs and to estimate the magnitude of those changes. This has been extended to lung tissue slices with and without removal of the surfactant to establish its relevance to the lung and to gain insight into the adiabatic properties of lungs hitherto studied under conditions that were assumed to be isothermal.

METHODS

Principle

Temperature changes essential to any 'engine' function of surfactant would be minimized by a large thermal capacity of the system studied, i.e. any heat liberated by a surfactant film would be taken up before it could register as a rise in temperature. To combat this limitation, surfactant can be studied as a thin two-sided film stretched on a wire frame as used by Maxwell (1890) and other physicists during the last century. This provides the most favourable relationship between surface area and film thickness in attempting to attain adiabatic conditions. It also offers the best simulation of the lung which has a very high surface-to-weight ratio. Heat dissipation is further reduced in this study by avoiding any physical contact between the film and the thermoprobe by using an infra-red thermometer which records the instant temperature of the film from the radiation it emits (Shellock, Rubin & Everest, 1984). The instrument has an accuracy of 0·5 °C and a response time of 50 ms. This study has been extended to sections of lung tissue cut in the manner previously used by Lulich, Mitchell & Sparrow (1976) for mechanical studies relating force to length with the exception that surface temperature is recorded while the tissue is cycled at respiratory frequency. This experiment can then be repeated after removing surfactant with acidified aspirin solution. This reagent is needed to remove any surfactant demonstrated by Cavagna, Velasquez, Wetton & DuBois (1967) and by Ueda *et al.* (1985) to be directly absorbed to epithelial cells just as it desorbs similar phospholipids lining the gastric mucosa (Hills, Butler & Lichtenberger, 1983).

Materials

The source of both surfactant and lung tissue was New Zealand White rabbits (2–2·5 kg). Each animal was anaesthetized with sodium thiamylal (I.V.) and, after tracheal cannulation, was killed by exsanguination. Blood volume in the lung was further minimized by several large inflations after excision from the chest.

Surfactant was harvested by cutting the tissue and gently expressing fluid from the exposed areas as described by Pattle (1966). Tissue sections were obtained by dissecting strips of parenchyma from the peripheral margins of lobe taking care not to include any large airways as described by Lulich *et al.* (1976).

Surfactant films

Surfactant expressed from the lungs as described above was placed in a small trough in which it covered the bottom of a vertical glass 'U' frame. A horizontal glass bar held by a vertical glass drive rod was then held firmly against the vertical limbs of the 'U' frame to form a Maxwell frame. When the rod was dipped into the surfactant and then raised, it formed a double-sided film whose area could be varied by moving the drive rod. The overall apparatus is shown in Fig. 1.

The temperature of the film was monitored by a infra-red thermometer (Everest-model 310) with a sensitivity of 0·01 °C connected to a recorder with an overall delay of only 0·1 s. The film was viewed horizontally against a matt black background through windows in a Perspex chamber used to maintain 100% humidity at 37 °C around the film. The infra-red thermometer was carefully calibrated by changing the film temperature by means of the thermostat setting for a static film. Having calibrated the infra-red thermometer for film temperatures around mean values of 25 °C and 37 °C, the variation of temperature for a 2:1 expansion (followed by compression) of the film was recorded for each of these starting temperatures. The time interval for the whole cycle was 4 s, corresponding to a respiratory frequency of 15 min^{-1}. The experiment was repeated for a 2:1 compression followed by expansion. Both experiments were repeated for surfactant expressed from ten rabbits.

Ancillary experiment

Since the above experiment had been designed to minimize the thermal capacity of the film and probe for temperature measurement in attempting to stimulate the area:thermal capacity of the lung, it was important to try to estimate film thickness. This was achieved by blowing a bubble of known volume through a small ring and then catching the bubble and weighing the residue after it had burst. One bubble was measured in this way for each of the ten surfactant films.

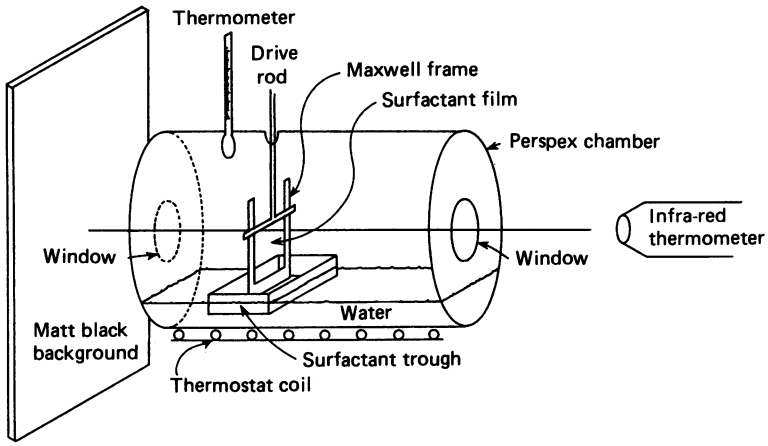

Fig. 1. Depicting the apparatus in which a film of rabbit surfactant is extended over a Maxwell frame in which the area can be changed by means of the drive rod. The film is viewed by an infra-red thermometer (to minimize heat losses) through windows in the Perspex chamber used to maintain 100% humidity at 37 °C.

Tissue experiments

In a second series of experiments, sections of lung tissue were mounted horizontally in a humidified chamber at 37 °C and the infra-red thermometer mounted vertically and focused upon the centre of the sample. No special calibration for background was needed since the sample was now opaque to infra-red. The tissue was stretched to twice its length and back in 4 s, again simulating respiratory frequency, while surface temperature was monitored continuously.

Each tissue sample was then incubated in a solution of 20 mM-acetylsalicyclic acid (aspirin) and 10 mM-HCl in 100 mM-NaCl (for isotonicity) for 15 min to desorb surfactant located at both liquid–air and epithelial surfaces. This procedure is described in more detail by Hills, Butler & Lichtenberger (1983). The incubated tissue was then lightly blotted with filter paper and returned to the frame where it was stretched in the field of view of the infra-red thermometer and the stretch experiment repeated as described above.

In an ancillary experiment the tissue was held extended until it had equilibrated to ambient conditions and then it was allowed to contract as surface temperature was recorded. Tissue samples were checked by electron microscopy to ensure that epithelial cells were intact after aspirin incubation.

RESULTS

Surfactant films

In all cases film temperature was found to fall upon expansion and to rise upon compression. The relationship between film area and temperature shows some hysteresis and the composite of ten films is shown in Fig. 2. The maximum drop in temperature was $5{\cdot}43 \pm 0{\cdot}24$ °C (mean $\pm$ S.E.M.). When starting at 100% area and compressing the film by 50%, the maximum rise in temperature was $4{\cdot}12 \pm 0{\cdot}31$ °C

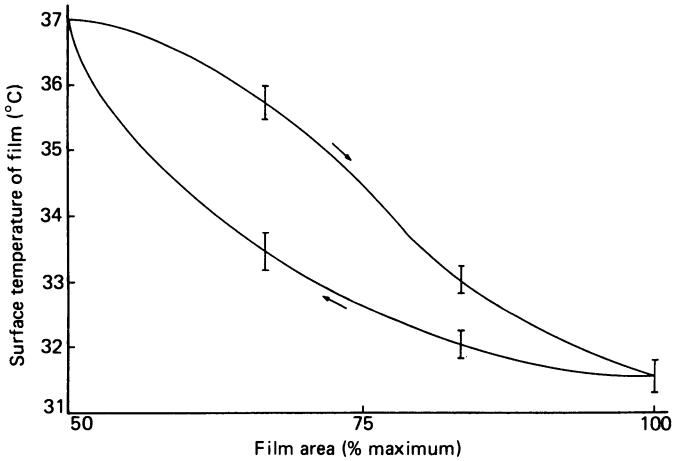

Fig. 2. A composite of ten runs for as many thin films of surfactant expressed from rabbit lungs, showing how temperature falls upon expansion and rises upon compression.

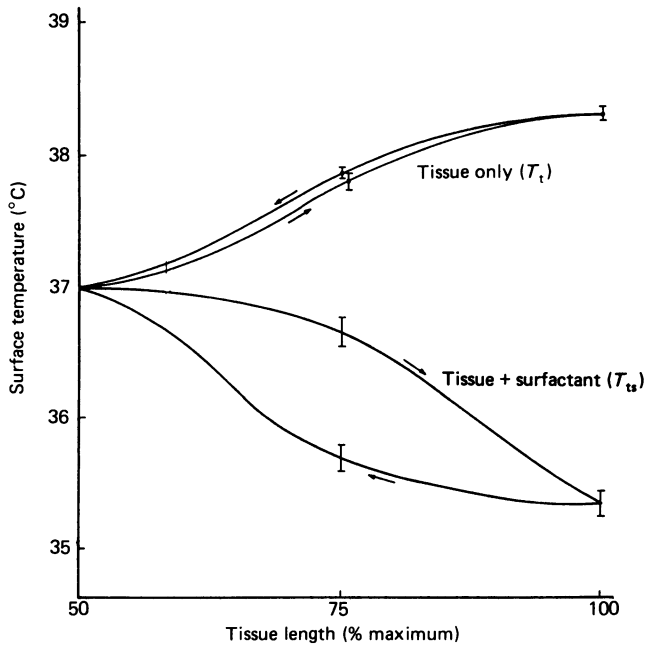

Fig. 3. A composite of ten runs for as many sections cut from lung parenchymal tissue showing how the temperature for tissue + surfactant (T_{ts}) decreases upon expansion and increases upon compression. Also shown are the corresponding values for tissue alone (T_t), i.e. after removal of the surfactant. Note the 'rubber band' effect which is the reverse of T_{ts} and the liquid films (Fig. 2), strongly suggesting that the fall in temperature upon expansion of the intact (untreated) tissue is related to the surfactant.

($N = 10$). When starting at room temperature, the drop upon 2:1 expansion was $5{\cdot}64 \pm 0{\cdot}25$ °C while the rise upon 2:1 compression was $3{\cdot}94 \pm 0{\cdot}20$ °C ($N = 10$). The average thickness of the films in the bubble (average 11·4 mm diameter) was 160 ± 23 μm.

Tissue sections

In all cases it was found that when tissue sections were extended, the surface temperature fell whereas the temperature rose upon return to its resting length. The relationship between temperature (T_{ts}) and sample length and, hence, overall tissue area is shown in Fig. 3 as a composite of ten samples. The maximum fall in T_{ts}, corresponding to 2:1 extension, was $2{\cdot}42 \pm 0{\cdot}11$ °C. When the tissue was held extended, equilibrated at 37 °C and then allowed to return to its original length, the temperature rose by $2{\cdot}18 \pm 0{\cdot}15$ °C ($N = 10$).

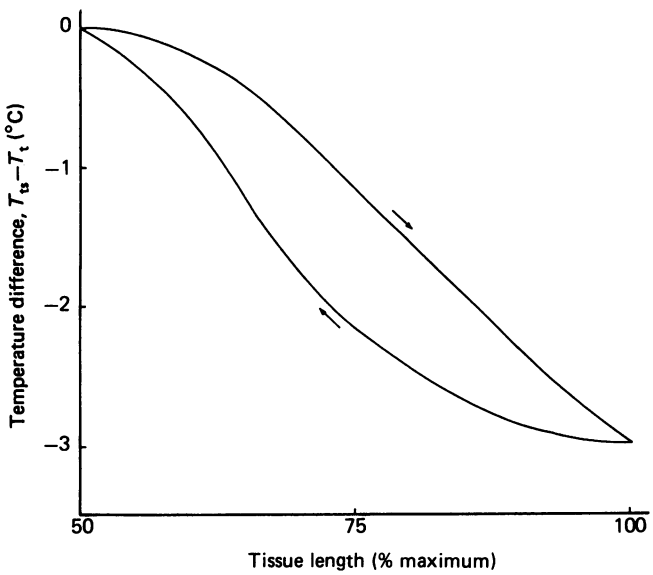

Fig. 4. Depicting the net difference ($T_{ts}-T_t$) in the temperature for tissue with its surfactant (T_{ts}) and tissue after removal of surfactant (T_t) as drawn from data shown in Fig. 3. Note the similarity between this surfactant curve and that for surfactant films shown in Fig. 2.

After removing the surfactant with acidified aspirin, the reverse trends were recorded on all ten sections. The temperature of the tissue alone (T_t) rose upon expansion and dropped upon return to the original length, there being less hysteresis than with the untreated tissue. The composite of the ten films for tissue only, i.e. after incubation with acidified aspirin, is also shown in Fig. 3. The maximum rise in temperature with expansion was $1{\cdot}32 \pm 0{\cdot}06$ °C.

The surfactant component is shown in Fig. 4 as the difference in temperature ($T_{ts}-T_t$) between tissue + surfactant (T_{ts}) and tissue alone (T_t) as a composite for all ten samples. The maximum change in the surfactant component ($T_{ts}-T_t$) was a drop of 3·74 °C upon expansion. Statistical analysis showed that all temperature changes

were significant to a level of $P < 0{\cdot}005$ or better. Student's t test gave $t = 3{\cdot}26$ for $N = 10$.

DISCUSSION

The results of the surfactant films leave no doubt that the temperature falls as the area is increased and rises as it is decreased at a rate too fast to allow any significant recruitment or decruitment of molecules from the surface. This is consistent with standard theory of surfactant treated as a two-dimensional gas phase and with the heat output and uptake for the phase transitions known to occur in phospholipid monolayers upon changing the area per molecule (Chapman, 1973). It establishes one very important point that the temperature of the surface of an aqueous hypophase can change *in vivo*. Moreover, the surfactant system as expressed from the lung itself has the ability to take up and give out heat, this capability being a fundamental prerequisite for any heat engine (Reynolds, 1965).

In the true thermodynamic sense of the name, a heat 'engine' takes up heat (H) from a hotter source (at T_1, say) converts part of it into work (W) and gives out the remainder at a lower temperature (T_2). The maximum (thermodynamic) efficiency is dictated by the second law of thermodynamics (Reynolds, 1965) as:

$$W/H < (T_1 - T_2)/T_1. \quad (1)$$

Thus the maximum efficiency for a surface engine displaying the temperature difference $(T_1 - T_2) = 5{\cdot}43$ °C found in the liquid films (Fig. 2) is $100 \times 5{\cdot}43/310 = 1{\cdot}75\%$ since $T_1 = 273 + 37 = 310$ K.

For an adult with a metabolic heat production (H) of 5210 J min^{-1} (75 kcal/h^{-1}) (Rowlands & Dudrick, 1981), an efficiency as low as 1·75% could give a work output (W) of 91 J min^{-1} (1·31 kcal h^{-1}). This is far in excess of the maximum work contribution by the surfactant system of 0·95 J min^{-1} estimated from surface tension data by Hills & Barrow (1984) and indicates that the mechanical efficiency need be only 10·4% i.e. an order of magnitude smaller than their estimated maximum surface contribution of 23% of the work of breathing. This is based upon a figure of 0·05 kg ml^{-1} ventilation (Otis, 1965) or 4 J min^{-1} for a minute volume of 8 l at rest. Expressed in another way, a heat 'engine' operating at the thermodynamic efficiency of 1·75% would need only one-tenth of the temperature change actually recorded in the film experiments (Fig. 2) in order to provide the estimated surface contribution to the work of breathing from 're-use' of waste metabolic heat.

While the temperature change of surfactant films *in vitro* and their magnitude may indicate that a surface engine is feasible, it does not prove that it actually occurs in lung tissue. However the same phenomenon seems to occur in lung parenchyma since similar loops were found in the untreated tissue samples (see Fig. 3). Moreover the effect is due to surfactant since its removal by acidified aspirin reverses the whole direction of the temperature effect (see the tissue loop in Fig. 3). Parenchymal tissue is then behaving just like a rubber band, or most plastics (Flory, 1953), in warming upon stretching and cooling upon relaxing. Hence there is little doubt that the reverse trend, i.e. cooling upon stretching of the untreated tissue, is due to surfactant. If the effect of surfactant alone is taken as the difference $(T_{ts} - T_t)$

depicted in Fig. 4, the loop is similar in form and magnitude to that for the fluid film (Fig. 2). The difference in the absolute values of the temperature limits can be attributed to different values in the relationship between heat generated and the heat sink, i.e. area-to-mass ratio.

For a blood-filled human lung with a weight of 600 g (Cander & Forster, 1956) and surface area of 90 m^2 (Weibel, 1964) the weight : area ratio is $6{\cdot}7 \times 10^{-4}$ gm cm^{-2}. Thus the low thermal capacity relative to area is ideal for promoting the 'surface engine' phenomenon. The ratio would be higher in the exsanguinated lung used in compliance studies, indicating large temperature changes, and this may explain the wide discrepancy in studies of the effect of temperature pointed out by Inoue, Inoue & Hildebrandt (1982) since different protocols can lead to different compromises between isothermal and adiabatic conditions. The area : weight ratio would be lower in the oedematous lung which could compromise the 'engine' contribution.

The general finding that liquid layers and tissue sections give similar results largely avoids the controversy concerning the location of surfactant, that is whether it functions as a monolayer (Clements *et al.* 1961) or a solid 'raft' (Morley, Bangham, Johnson, Thorburn & Jenkin, 1978) at the air interface with an aqueous hypophase assumed to line the alveolus, or whether it is directly adsorbed to alveolar epithelium or both (Hills, 1982). A liquid lining is, perhaps, more relevant to the practical implications of the temperature phenomenon since it is undoubtedly present in the newborn and in cases of alveolar flooding. It is tempting to speculate that the 'engine' contribution could affect the delicate balance between the work *available* and the work *needed* for ventilation where any respiratory distress is witnessed, i.e. in the infant and adult forms of the syndrome which these words describe, viz. respiratory distress syndrome (RDS).

Although it would be unnecessary for the lungs to derive work from heat under normal physiological conditions, the phenomenon would afford an ingenious means of re-using a little of the waste metabolic heat off-loaded at the alveolar surface.

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