J. Physiol. (1979), **297**, *pp.* 217–227
With 2 text-figures
Printed in Great Britain

SURFACE TENSION INDUCED BY DIPALMITOYL LECITHIN *IN VITRO* UNDER PHYSIOLOGICAL CONDITIONS

BY R. E. BARROW AND B. A. HILLS*

From the Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550, U.S.A.

(*Received 10 April 1979*)

SUMMARY

1. The surface tension of 161 films of DPL have been measured on a Langmuir trough using a Wilhelmy balance under conditions controlled to simulate the state of the alveolar lining *in vivo*.

2. The parameters controlled were temperature (maintained at 37 °C), humidity (100% at 37 °C), surfactant concentrations (encompassing the best available estimates), area changes (consistent with normal respiration), frequency adaptation to continuous cycling and composition and pH of the aqueous hypophase.

3. Simultaneously maintaining all of these parameters within the best estimates of physiological limits, the relationships between surface tension and surface area showed appreciable differences from previous studies, our results showing higher minimum values of surface tension, appreciably less change in surface tension with compression and far less hysteresis between surface tension and surface area.

4. The higher minimum values are consistent with original estimates of alveolar surface tension made by von Neergaard, namely 35–41 dyne cm^{-1}.

5. Although appreciably smaller than hitherto reported, the change in surface tension with change in area is still adequate to impart alveolar stability.

6. The reversibility between surface tension and surface area under physiological conditions is discussed in connection with compliance hysteresis which is considered to be more dependent upon geometric irreversibility of the alveolar surface than upon any intrinsic property of the surfactant.

INTRODUCTION

Since von Neergaard (1929) performed his original experiments upon excised lungs, the surface tension of the alveolar–air interface has attracted much attention as a major factor in pulmonary mechanics. The subsequent demonstration of surfactants in the lung (Pattle, 1955, 1958) and their likely influence upon the properties of this interface, has led to many *in vitro* studies of surfaces modified with lung extracts or with one of the most surface-active components – α-dipalmitoyl lecithin (DPL) (Brown, 1964; Pattle, 1965). In many of these studies from which new concepts of alveolar mechanics have been derived, especially those related to alveolar stability

* Present address: Department of Community and Occupational Medicine, University of Dundee, Dundee.

0022-3751/79/6330-0151 $01.50 © 1979 The Physiological Society

and compliance hysteresis, the aqueous hypophase lining the airways of the lung has been simulated by the pool of a Langmuir trough (Clements, 1957). The surfactant under investigation is applied to the surface of the pool and the barrier is cycled back and forth to simulate changes in alveolar surface area induced by ventilation; while surface tension is usually monitored continuously by means of a Wilhelmy balance.

This balance has been used in conjunction with the Langmuir trough in several hundred studies investigating the effect of DPL upon the surface tension of films deposited upon aqueous hypophases under various conditions. Various parameters have been investigated individually or maintained at physiological values. These include temperature, pH, surface area, frequency of cycling that area, frequency adaptation, surfactant concentration and composition of the hypophase.

The effect of change of temperature has been investigated in several studies with varying results (Avery & Mead, 1959; Tierney & Johnson, 1965) while, in numerous others, surface tension has been measured at 37 °C. The influence of change in pH of the aqueous hypophase has also been investigated by the same authors, and others (Gladston & Shah, 1967) with negative findings. Composition of the aqueous hypophase has been shown to influence surface tension, there being a distinct difference between water, saline and various electrolyte solutions (Scarpelli, Gabbay & Kochen, 1965; Tierney & Johnson, 1965); although few studies could be found which employ Ringer solution (Reifenrath & Zimmermann, 1973). Surface area has been varied between 100 and 15–30% (Gladston & Shah, 1967) of initial area in most studies since these are the limits of one of the more popular surface balances available commercially and described later; although some studies have employed lesser excursions (Reifenrath & Zimmermann, 1973). These investigators have also cycled the surface area at frequencies more akin to normal ventilation. The third or subsequent cycles of the barrier have been selected as the most relevant since these are almost reproduced by subsequent tracings (Clements *et al.* 1961; Kuenzig, Hamilton & Peltier, 1965) and thus allow for adaptation of the film to the cyclic nature of the area changes induced by ventilation. Surfactant concentration at the alveolar–air interface is difficult to estimate, but the effect of DPL concentration has been demonstrated in several studies, one of the widest ranges covering 0·3–33 $\mu g\ cm^{-2}$ (Fujiwara, Adams & Scudder, 1964). No record, however, could be found where the atmosphere above the trough had been humidified to simulate the state of the air in the alveoli and terminal airways.

Although all of the above parameters except the last have been studied individually, while one or two others may have been maintained within physiological limits, we could find no study where all had been simultaneously deployed at the values believed to apply *in vivo*. This study has been designed to measure the surface tension induced by DPL at aqueous surfaces when all seven parameters listed above are maintained at physiological levels or varied to encompass the best available estimates. The emphasis is one of conforming to those constraints *simultaneously* rather than investigating each as an individual variable in the system.

METHODS

Materials

The surfactant used was DL-α phosphatidyl chloline dipalmitoyl (lot 38C-002) supplied by the Sigma Chemical Company and kept refrigerated. This was dissolved in chloroform in concentrations of 10·0, 2·0 and 0·4 g/l.

The pool used to simulate the aqueous hypophase of the lung was 32 ml. of either Ringer solution or dog serum covering an area of 52·5 cm^2.

Apparatus

The pool was contained within a Langmuir-type trough fitted with a movable barrier all constructed of Teflon. The barrier was programmed to cycle between 100 and 87·5 %, 75 or 62·5 % of initial area at its maximum rate of 1 % of pool area per second.

The surface tension of the film was measured continuously by means of a Wilhelmy balance (Kimray–Greenfield surfactometer) whose platinum flag was suspended partially immersed in the pool. The flag, trough and fluid were maintained at 37 $\pm$ 0·5 °C within a Perspex box which was continuously flushed with air saturated with water vapour at 37 °C to simulate alveolar temperature and humidity. Temperature and pH of the bath were monitored by means of a thermistor and an electrode respectively, both totally immersed in the pool.

Procedure

The trough and all surfaces were kept particularly clean with special attention being paid to the platinum surfaces of the Wilhelmy flag which were cleaned by scouring, rinsing in acetone and flaming before each run. Surface films were applied by 'touching off' onto the pool 0·01 ml. of one of the three DPL solutions described above and then allowing the volatile, water-immiscible chloroform to evaporate, evenly depositing the surfactant over the surface. After allowing 5 min for the solvent to evaporate, the surface film was compressed to either 62·5, 75 or 87·5 % of its original area and then returned to 100 % at the same rate. These degrees of compression were selected to bracket the true area change occurring *in vivo*. The procedure was repeated until the relationship between surface tension and pool area did not change appreciably with further cycling. This was intended to simulate not only the change in alveolar surface area with ventilation but the repetitive nature of such changes in normal respiration. Each run was repeated for at least six freshly prepared surface films. For all three area excursions the procedure was repeated for surface concentrations of 1·90 and 0·08 $\mu g\ cm^{-2}$, i.e. fivefold either side of the concentration of 0·38 $\mu g\ cm^{-2}$ estimated as the physiological level at the alveoli (Brown, 1964; Fujiwara *et al.* 1964) and used in the first series of experiments.

Each series was performed using both Ringer solution and dog serum as the pool fluid in order to encompass the composition of the aqueous hypophase believed to line the alveoli. Surface tension *versus* surface area loops were also recorded for serum alone.

RESULTS

The surface tension (γ) *versus* surface area was recorded for a total of 161 individual surface films. Mean values ($\bar{\gamma}$) for surface concentrations of 0·8, 0·38 and 1·90 μg DPL cm^{-2} for each of three area excursions of 100–62·5, 75 and 87·5 % are given in Fig. 1 for both Ringer solution and dog serum as the pool fluid. To indicate the reproducibility of each loop, the mean surface tension ($\bar{\gamma}$) is given in Tables 1–6, with the standard error of the mean (S.E. of mean), for each combination of fluid composition, area excursion and surface concentrations of DPL. Values for serum alone are included in Tables 4–6 as zero DPL concentrations. No contact angle at the platinum flag could be observed on any run.

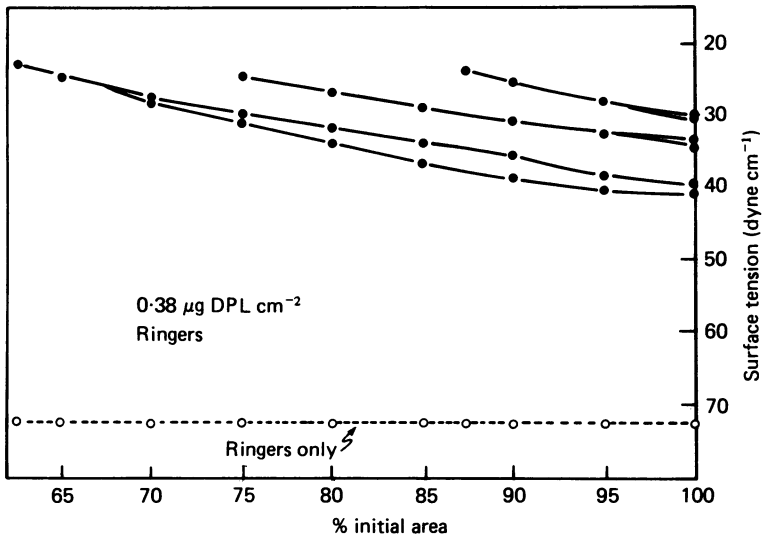

Fig. 1. Surface tension versus surface area for films of 0·38 μg DPL cm^{-2} deposited upon an aqueous hypophase of Ringer solution when each film is cycled between 100 and 87·5 %, 75 or 62·5 % of initial area for the third time. Each loop represents the average compiled from the results for at least ten freshly prepared films.

TABLE 1. Mean surface tension ($\bar{\gamma}$) recorded at various stages of the third cycle of a 12·5 % area change in DPL films of various concentrations deposited upon Ringer solution

	Concentration of DPL (μg cm^{-2})								
	0·08			0·38			1·90		
Initial area (%)	$\bar{\gamma}$	S.E. of mean	n	$\bar{\gamma}$	S.E. of mean	n	$\bar{\gamma}$	S.E. of mean	n
100	69·2	0·9	6	30·0	1·0	10	26·5	1·1	6
95	67·8	1·1	6	28·0	0·9	10	26·2	1·1	6
90	66·0	1·2	6	25·1	0·9	10	24·3	0·8	6
87·5	64·6	1·2	6	23·7	0·8	10	23·2	0·7	6
90	66·0	1·2	6	25·2	0·7	10	24·2	0·8	6
95	67·8	1·1	6	27·9	0·7	10	26·3	1·1	6
100	69·2	0·9	6	30·3	1·1	10	26·8	1·2	6

n, number of films.

DISCUSSION

Before discussing the differences between the above results and those of previous studies in which fewer parameters were maintained within physiological limits, it seems imperative to establish the degree to which this study simulated actual conditions at the alveolar surface. There can be little question concerning the desirability of working at (37 °C) and maintaining 100 % humidity at body temperature as generally assumed in the alveoli since it even applies in the upper airways (Ingelstedt,

TABLE 2. Mean surface tension ($\bar{\gamma}$) recorded at various stages of the third cycle of a 25 % area change in DPL films of various concentrations deposited upon Ringer solution

	Concentration of DPL (μg cm^{-2})								
	0·08			0·38			1·90		
Initial area (%)	$\bar{\gamma}$	S.E. of mean	n	$\bar{\gamma}$	S.E. of mean	n	$\bar{\gamma}$	S.E. of mean	n
100	69·3	0·9	6	33·1	1·9	10	30·5	1·4	8
95	68·7	1·2	6	32·5	1·9	10	30·1	1·3	8
90	67·3	1·2	6	30·8	1·6	10	28·9	1·3	8
85	65·2	1·2	6	28·7	1·3	10	27·0	1·0	8
80	63·0	1·3	6	26·5	0·8	10	25·5	0·9	8
75	60·9	1·5	6	24·3	0·7	10	22·2	1·0	8
80	63·0	1·4	6	26·6	0·6	10	25·5	1·0	8
85	65·5	1·4	6	28·8	1·0	10	27·1	1·0	8
90	67·9	1·3	6	30·5	1·3	10	28·9	1·2	8
95	69·0	1·1	6	32·5	1·7	10	30·1	1·3	8
100	69·3	0·9	6	34·4	2·1	10	32·5	1·4	8

n, number of films.

TABLE 3. Mean surface tension ($\bar{\gamma}$) recorded at various stages of the third cycle of a 37·5 % area change in DPL films of various concentrations deposited upon Ringer solution

	Concentration of DPL (μg cm^{-2})								
	0·08			0·38			1·90		
Initial area (%)	$\bar{\gamma}$	S.E. of mean	n	$\bar{\gamma}$	S.E. of mean	n	$\bar{\gamma}$	S.E. of mean	n
100	70·4	0·7	9	39·3	2·4	12	38·1	2·0	8
95	70·0	0·8	9	38·1	2·2	12	37·4	1·9	8
90	68·7	0·9	9	35·4	1·9	12	35·3	1·4	8
85	67·2	1·0	9	33·8	1·4	12	33·2	1·2	8
80	65·0	1·2	9	31·5	1·0	12	31·4	1·1	8
75	63·2	1·3	9	29·4	0·8	12	29·3	1·1	8
70	61·1	1·5	9	27·3	0·8	12	26·2	1·5	8
65	58·5	1·6	9	24·3	1·3	12	27·1	2·3	8
62·5	57·3	1·5	9	22·8	1·6	12	20·7	2·5	8
65	58·2	1·6	9	24·4	1·6	12	22·3	2·4	8
70	61·2	1·5	9	28·0	1·1	12	26·1	2·2	8
75	63·9	1·4	9	30·7	0·9	12	28·9	2·4	8
80	65·9	1·3	9	33·8	0·9	12	31·1	2·4	8
85	67·9	1·1	9	36·5	1·3	12	34·0	2·4	8
90	69·2	1·0	9	38·9	1·8	12	36·9	2·3	8
95	70·2	0·8	9	40·1	2·0	12	39·3	2·2	8
100	70·4	0·7	9	41·0	2·3	12	41·3	2·4	8

n, number of films.

TABLE 4. Mean surface tension ($\bar{\gamma}$) recorded at various stages of the third cycle of a 12·5 % area change in DPL films of various concentrations deposited upon dog serum

	Concentration of DPL (μg cm^{-2})											
	0*			0·08			0·38			1·90		
Initial area (%)	$\bar{\gamma}$	S.E. of mean	n	$\bar{\gamma}$	S.E. of mean	n	$\bar{\gamma}$	S.E. of mean	n	$\bar{\gamma}$	S.E. of mean	n
100	46·7	0·7	8	44·1	0·5	6	41·7	1·1	6	40·0	0·8	6
95	46·3	0·7	8	42·4	0·8	6	39·8	1·6	6	37·5	0·9	6
90	45·2	0·7	8	40·4	1·1	6	37·3	1·8	6	34·7	0·9	6
87·5	44·4	0·6	8	39·5	1·2	6	36·0	1·9	6	33·2	1·1	6
90	45·3	0·7	8	40·4	1·1	6	37·4	1·8	6	34·8	0·9	6
95	46·3	0·7	8	42·4	0·8	6	41·1	1·7	6	38·3	0·9	6
100	46·8	0·7	8	44·2	0·5	6	42·7	0·8	6	40·7	0·7	6

n, number of films.
* Serum only.

TABLE 5. Mean surface tension ($\bar{\gamma}$) recorded at various stages during the third cycle of a 25 % area change in DPL films of various concentrations deposited upon dog serum

	Concentration of DPL (μg cm^{-2})											
	0*			0·08			0·38			1·90		
Initial area (%)	$\bar{\gamma}$	S.E. of mean	n	$\bar{\gamma}$	S.E. of mean	n	$\bar{\gamma}$	S.E. of mean	n	$\bar{\gamma}$	S.E. of mean	n
100	47·3	0·4	8	45·3	0·9	6	43·8	1·4	6	41·6	0·8	8
95	47·1	0·4	8	45·0	0·8	6	42·5	1·3	6	38·9	1·3	8
90	46·5	0·3	8	44·3	0·7	6	41·0	1·1	6	36·7	1·1	8
85	45·5	0·4	8	43·0	0·7	6	38·7	0·8	6	34·4	1·2	8
80	44·6	0·4	8	39·9	1·2	6	36·3	0·9	6	32·0	1·3	8
75	43·8	0·3	8	36·3	1·5	6	33·7	1·3	6	29·8	1·4	8
80	44·7	0·4	8	39·9	1·2	6	38·3	1·3	6	34·2	1·4	8
85	45·9	0·3	8	43·0	0·7	6	41·8	1·0	6	38·6	1·3	8
90	46·8	0·3	8	44·3	0·7	6	44·3	0·7	6	41·6	1·3	8
95	47·4	0·3	8	45·3	0·7	6	45·0	0·9	6	42·9	1·2	8
100	47·3	0·4	8	45·5	0·7	6	44·5	1·3	6	42·7	1·3	8

n, number of films.
* Serum only.

TABLE 6. Mean surface tension ($\bar{\gamma}$) recorded at various stages during the third cycle of a 37·5% area change in DPL films of various concentrations deposited upon dog serum

Initial area (%)	Concentration of DPL (μg cm^{-2}) 0* $\bar{\gamma}$	S.E. of mean	n	0·08 $\bar{\gamma}$	S.E. of mean	n	0·38 $\bar{\gamma}$	S.E. of mean	n	1·90 $\bar{\gamma}$	S.E. of mean	n
100	48·9	0·5	12	48·3	0·5	6	43·9	1·1	6	41·3	0·9	8
95	48·6	0·4	12	48·3	0·5	6	42·8	1·1	6	41·5	1·4	8
90	47·8	0·3	12	47·6	0·4	6	41·6	0·9	6	40·5	1·4	8
85	46·8	0·3	12	46·9	0·5	6	40·3	0·9	6	38·9	1·4	8
80	46·1	0·3	12	46·0	0·4	6	38·8	0·9	6	36·8	1·2	8
75	45·1	0·3	12	44·5	0·5	6	37·3	1·0	6	35·1	1·2	8
70	43·7	0·3	12	42·9	0·5	6	36·3	1·0	6	33·1	1·1	8
65	42·4	0·3	12	40·5	0·7	6	34·5	1·3	6	30·7	1·1	8
62·5	41·7	0·3	12	38·3	1·1	6	34·0	1·2	6	29·5	1·0	8
65	42·5	0·3	12	40·5	0·7	6	36·3	1·6	6	32·7	1·1	8
70	44·5	0·3	12	43·5	0·5	6	41·5	0·9	6	38·8	1·1	8
75	45·9	0·4	12	45·6	0·6	6	44·3	0·3	6	42·7	0·8	8
80	47·0	0·6	12	46·9	0·8	6	46·3	0·3	6	44·7	0·6	8
85	48·2	0·7	12	48·0	0·7	6	46·8	0·8	6	45·5	0·6	8
90	49·0	0·7	12	48·9	0·7	6	46·8	0·9	6	45·8	0·8	8
95	49·3	0·7	12	48·8	0·5	6	46·0	0·7	6	45·3	0·9	8
100	49·3	0·6	12	48·5	0·7	6	44·8	0·8	6	44·5	0·9	8

n, number of films
* Serum only.

1956; Negus, 1952, 1956). No reference could be found in the literature to any surfactant studies where both of these conditions had been maintained *in vitro*.

The emphasis upon the third cycle in allowing for the repetitive nature of ventilation has been well accepted since subsequent cycles show almost no deviation (Clements, Hustead, Johnson & Gribetz, 1961; Kuenzig *et al.* 1965), a point further substantiated in this study.

In view of the influence of area change upon surface tension, it becomes highly desirable to select the best estimates for physiological limits for the excursion of the barrier. Assuming that the relationship between lung volume and surface area follows the two thirds relationship often invoked (Clements, 1957), a subject with a tidal volume of 500 ml. and functional residual capacity of 2 l. would then experience an alveolar surface area change of the order of 25 %. Pattle (1977) has emphasized that it would be no more than this value in the resting subject and much lower than the 70–85 % excursions employed in most studies using the Langmuir trough and Wilhelmy balance (Clements, 1957; Brown, Johnson & Clements, 1959). It is conceivable that the actual area change in the lungs may be one half of the above estimate while, for exercise, it could be larger. Hence surface tension measurements were included for excursions of 12·5 and 37·5 in addition to the estimated value of 25 %.

Other parameters which could influence the surface tension of the DPL films are the pH and chemical constitution of the pool fluid in general. It is virtually impossible to know the exact composition of the alveolar hypophase, but it can be argued that it should be intermediate between those for Ringer solution and for serum. Hence both of these have been used as the pool fluid in this study.

Although true alveolar conditions can never be reproduced exactly on the surface of the Langmuir trough, those employed in this study are probably appreciably closer to the situation *in vivo* than in previous studies. Hence it is a serious matter when our results show surface tension *versus* surface area loops which differ widely from previous studies (Clements, 1957; Clements, Brown & Johnson, 1958; Brown *et al.* 1959) on which several basic concepts of alveolar mechanics have been derived. Two major points of difference are the much smaller change in surface tension found in this study over the area cycle and the more reversible nature of that change, i.e. the much thinner loop, indicating appreciably less surface hysteresis.

Considering the first difference, our results indicate a reduction of only 8·8 dyne cm^{-1} (27 %) for Ringer solution or 10·1 dyne cm^{-2} (23 %) for serum for the 25 % compression likely *in vivo* at the approximate physiological concentration of 0·38 μg cm^{-2}. This is considerably smaller than indicated in previous studies and, therefore, raises the question concerning whether the changes observed are adequate to satisfy the surfactant theory of alveolar stability (Clements, 1957; Clements *et al.* 1958, 1961). That theory is based upon the argument that, if any two bubbles with the same surface tension (γ) are connected, then the larger will grow at the expense of the smaller since its gas will have a lower internal pressure (ΔP) induced by surface curvature (radius r) in accordance with the Laplace equation:

$$\Delta P = 4\gamma/r. \tag{1}$$

Thus any system of interconnected unequal bubbles is potentially unstable. Hence, it is generally accepted that if the decrease in surface area with contraction of the

smaller bubble greatly reduces surface tension, then this decrease in γ in eqn. (1) can offset the decrease in r and so alleviate the potentially unstable situation expressed by the Laplace equation. The decrease in γ of the order 23–27 % found in this study is still ample to outweigh the 13 % decrease in r in eqn. (1) which would be expected for a 25 % decrease in area, assuming a spherical system. Hence, quite apart from any restoring forces imparted by the parenchymal tissue (Mead, Whittenburger & Radford, 1970), the surface tension changes measured under physiological conditions would still be roughly twice the minimum required for alveolar stability to be provided by surfactant alone.

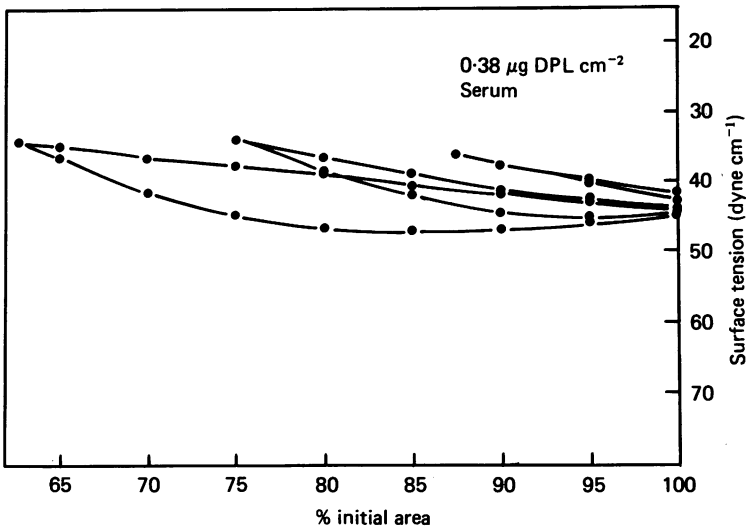

Fig. 2. Surface tension *versus* surface area for films of 0·38 μg DPL cm^{-2} deposited upon an aqueous hypophase of dog serum when each film is cycled between 100 and 87·5 %, 75 or 62·5 % of initial area for the third time. Each loop represents the average compiled from at least six freshly prepared films.

Returning to the second major difference from previous studies, appreciably thinner loops recorded in Figs. 1 and 2 and almost total reversibility of surface tension with surface area for many combinations of likely physiological conditions raises another question concerning the role of surfactant in compliance hysteresis. The popular theory that pressure–volume hysteresis in the excised lung can be largely attributed to the hysteresis between surface tension (γ) and alveolar surface area (A) has been based upon studies performed on the Langmuir trough under less physiological conditions (Clements, 1957). If, however, there is a negligible area in the γ/A loop under physiological conditions, as indicated by the results shown in Figs. 1 and 2, then it is difficult to attribute compliance hysteresis to the surfactant. Compliance hysteresis can still be attributed to the alveolar–air interface as originally deduced from the very much thinner pressure–volume loops obtained when air is replaced by saline as the medium used to inflate the lung (Mead *et al.* 1957). The source of the irreversibility is more likely to be geometric rather than derived from any inherent property of the surfactant itself. In excised lung studies, inflation pressure is plotted against lung volume whereas, in surfactant studies, surface tension is plotted

against surface area. Hence, it has been pointed out (Hills, 1971) that, in attributing hysteresis in one to hysteresis in the other, the implicit assumption is effectively made that the relationship between lung volume and surface area is totally reversible. However the gross dimensions do not reverse between inflation and deflation (Hills, 1971) nor does the sequence of dimensional changes observed at the alveolar level (Daly, Parks, Edmonds, Hibbs & Norman, 1975). Hence it would appear that surfactant is likely to make an appreciably smaller contribution to compliance hysteresis than geometric irreversibility of which a portion must be represented by the failure of recruitment of alveoli during inflation to be retraced by derecruitment during deflation (Radford, 1964).

Another feature of our results is the different magnitude of the surface tension values obtained under simulated physiological conditions compared to previous studies using the Wilhelmy balance in which very low values of the order of 0–15 dynes cm^{-1} were common (Clements, 1957; Brown *et al.* 1959; Radford, 1964). By comparison, for a probable DPL surface concentration of 0·38 $\mu g\ cm^{-2}$ deposited on serum, the minimum γ values for 12·5, 25 and 37·5% excursions in Tables 1–6 lie within the range 33·7–36·0 dyne cm^{-1}. This implies that surfactant is exerting less influence than generally believed in reducing inflation pressure of the lung. In fact it is interesting to note that, in applying eqn. (1) to his original experiments on excised lungs, von Neergaard (1929) estimated alveolar surface tension to be about 35–41 dynes cm^{-1}, a range which is close to our values.

It is difficult to determine which parameter or set of conditions are responsible for the differences from previous studies using the Wilhelmy balance since this would involve varying some twenty-eight possible combinations. One other possibility involves the contact angle observed at the surface of the Wilhelmy flag when making no effort to follow physiological conditions comprehensively (Barrow & Hills, 1979). This could partially explain the unduly low readings recorded in earlier work. No contact angle was observed in this study. However, these results emphasize the need for simulating as many physiological conditions as possible when undertaking surface tension studies *in vitro*, since the conclusions to be derived can place a different perspective upon the role of the alveolar–air interface in pulmonary mechanics.

The authors wish to thank Mrs Laura Huddleston for her assistance in preparing this manuscript. The research reported here has been supported under the Office of Naval Research (contract N00014-75-C-1035) with funds provided by the Naval Research and Development Command.

REFERENCES

AVERY, M. E. & MEAD, J. (1959). Surface properties in relation to atelectasis and hyaline membrane disease. *Am. J. Dis. Childh.* **97**, 517–523.

BARROW, R. E. & HILLS, B. A. (1979). A critical assessment of the Wilhelmy method in studying lung surfactants. *J. Physiol.* **295**, 217–227.

BROWN, E. S. (1964). Isolation and assay of dipalmitoyl lecithin in lung extracts. *Am. J. Physiol.* **207**, 402–406.

BROWN, E. S., JOHNSON, R. P. & CLEMENTS, J. A. (1959). Pulmonary surface tension. *J. appl. Physiol.* **14**, 717–720.

CLEMENTS, J. A. (1957). Surface tension of lung extracts. *Proc. Soc. exp. Biol. Med.* **95**, 170–172.

CLEMENTS, J. A., BROWN, E. S. & JOHNSON, R. P. (1958). Pulmonary surface tension and the mucus lining of the lungs: some theoretical considerations. *J. appl. Physiol.* **12**, 262–268.

CLEMENTS, J. A., HUSTEAD, R. F., JOHNSON, R. P. & GRIBETZ, I. (1961). Pulmonary surface tension and alveolar stability. *J. appl. Physiol.* **16**, 444–450.

DALY, B. D. T., PARKS, G. E., EDMONDS, C. H., HIBBS, C. W. & NORMAN, J. C. (1975). Dynamic alveolar mechanics as studied by videomicroscopy. *Resp. Physiol.* **24**, 217–232.

FUJIWARA, R., ADAMS, F. H. & SCUDDER, A. (1964). Fetal lamb amniotic fluid: relationship of lipid composition to surface tension. *J. Pediat.* **65**, 824–830.

GLADSTON, M. & SHAH, D. O. (1967). Surface properties and hysteresis of dipalmitoyllecithin in relation to the alveolar lining layer. *Biochim. biophys. Acta* **137**, 255–267.

HILLS, B. A. (1971). Geometric irreversibility and compliance hysteresis in the lung. *Resp. Physiol.* **13**, 50–61.

INGELSTEDT, S. (1956). Studies on the conditioning of air in the respiratory tract. *Acta oto-lar.* (Suppl.) **131**, 1–80.

KUENZIG, M. C., HAMILTON, R. W. & PELTIER, L. F. (1965). Dipalmitoyl lechithin: studies on surface properties. *J. appl. Physiol.* **20**, 779–782.

MEAD, J., TAKISHIMA, T. & LEITH, D. (1970). Stress distribution in lungs: a model of pulmonary elasticity. *J. appl. Physiol.* **28**, 596–608.

MEAD, J., WHITTENBURGER, J. L. & RADFORD, E. P. (1957). Surface tension as a factor in pulmonary volume-pressure hysteresis. *J. appl. Physiol.* **10**, 191–196.

NEERGAARD, K. von (1929). Neue Auffasungen über einen Grundbegriff der Atemmechanik. Die Retraktionskraft der Lunge, abhängig von der Oberflachenspannung in den Alveolen. *Z. ges. exptl. Med.* **66**, 373–394.

NEGUS, V. E. (1952). Humidification of the air passages. *Thorax* **7**, 148–151.

NEGUS, V. E. (1956). The air-conditioning mechanism of the nose. *Br. med. J.* **1**, 367–371.

PATTLE, R. E. (1955). Properties, function and origin of the alveolar lining layer. *Nature, Lond.* **175**, 1125–6.

PATTLE, R. E. (1958). Properties, function and origin of the alveolar lining layer. *Proc. R. Soc.* B **148**, 217–240.

PATTLE, R. E. (1965). Surface lining of lung alveoli. *Physiol. Rev.* **45**, 48–79.

PATTLE, R. E. (1977). The relation between surface tension and area in the alveolar lining film. *J. Physiol.* **269**, 591–604.

RADFORD, E. P. (1964). Static mechanical properties of mammalian lungs. In *Handbook of Physiology*, sect. 3, Respiration, vol. 1, ed. FENN, W. O. & RAHN, H., pp. 429–449. Washington, D.C.: Am. Physiol. Soc.

REIFENRATH, R. & ZIMMERMANN, I. (1973). Surface tension properties of lung alveolar surfactant obtained by alveolar micropuncture. *Resp. Physiol.* **19**, 369–393.

SCARPELLI, E. M., GABBAY, K. H. & KOCHEN, J. A. (1965). Lung surfactants, counterions and hysteresis. *Science, N.Y.* **148**, 1607–9.

TIERNEY, D. F. & JOHNSON, R. P. (1965). Altered surface tension of lung extracts and mechanics. *J. appl. Physiol.* **20**, 1253–1260.