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The surface activity of the alveolar lining layer of guinea-pig lung following anaphylaxis

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The surface activity and lipid composition of alveolar extracts of guinea-pig lung following anaphylaxis have been studied. The extracts showed a reduced ability to lower surface tension on compression of the surface film which was maximal 30 min after challenge. No significant changes in the lipid content were found but there was a marked increase in the soluble solids 10 min after anaphylactic shock. The significance of these findings is discussed.

It has been shown that phospholipid is lost from guinea-pig lung following anaphylaxis (Smith, 1962; Goadby & Smith, 1962; Grünspan & Rusovici, 1967; Goadby, 1975). Attempts to identify the site of loss by standard microscopical staining methods were unsuccessful (Goadby, 1974a) and it was concluded that the site of loss was a structure rich in phospholipid but not easily visualized by light microscopy. One such structure is the alveolar lining layer (Nasr & Heinemann, 1965; Pattle, 1965) and therefore changes in the properties and composition of this layer following anaphylaxis were investigated.

MATERIALS AND METHODS

Materials

Tripalmitin (99%) was generously donated by Unilever Research Laboratories, Port Sunlight, Cheshire. L-α-Lecithin (95%) was obtained from Koch-Light Laboratories, Colnbrook, Bucks. All other chemical reagents were of analytical grade (Analar, British Drug Houses, Poole, Dorset) as were the solvents which were redistilled before use. Purified paraffin wax (Stanwax) was a gift from Dr. J. T. Pearson, School of Pharmacy, Sunderland Polytechnic.

Extraction of lung lining layer

Two methods were employed. In the method of Bondurant & Miller (1962) the pulmonary vasculature was perfused with 0·9% (w/v) saline whilst the lungs were alternately expanded and compressed. After a few minutes a foaming effluent issued from the trachea and was collected for use in subsequent experiments. In the second method the alveolar surface was washed via the airways with 40 ml kg^{-1} body weight of 0·9% (w/v) saline (King, 1968).

Estimation of lipid content

Samples of lung lining layer extract were freeze-dried, weighed and extracted with 200 ml g^{-1} chloroform–methanol (2:1) and the lipid content determined as described by Goadby & Smith (1962).

Estimation of surface tension lowering capacity

Samples of the tracheal effluent and lung washings were introduced to the surface of 0·9% (w/v) saline and determinations made of the surface tension by a modified du Noüy method during expansion and contraction of the surface area as described by Goadby (1974b).

In some experiments a modified Wilhelmy method (Brown, Johnson & Clements, 1959) was used to measure the surface tension.

Anaphylaxis in guinea-pigs

Guinea-pigs (350–450 g) of either sex were sensitized to egg albumen and standardized by three weekly exposures to an aerosol of 1% egg albumen as described by Goadby (1975). Groups of 8 animals were killed at (a) the onset of dyspnoea and cough (acute), (b) 10 min, (c) 30 min, and (d) 60 min after the start of exposure to aerosolized antigen at least one week later. The lungs were removed,the lung lining layer separated by alveolar wash and the surface activity and lipid content estimated.

RESULTS

When extracts of the guinea-pig lung lining layer were introduced to the surface of 0·9% (w/v) saline, the surface tension at 100 cm^2 was reduced. The surface tension was further reduced when the surface area was decreased to 10 cm^2 and returned to the previous level when the area was expanded to 100 cm^2. The surface tension-area diagram obtained (Fig. 1) shows that the decrease of surface tension during decrease of surface area followed a different pattern from the increase in surface tension with increase in surface area. This is an example of tension-area hysteresis.

Extracts obtained by both methods gave similar results (Table 1) although the mean surface tensions recorded by the modified Wilhelmy method were lower and there was a greater difference between the arms of the hysteresis curve than with the modified du Noüy method.

Alveolar washing extracted a greater total weight of solids which contained a greater proportion of cholesterol than the tracheal effluent although the difference was

Table 1. *Surface activity and lipid content of the lung lining layer of the guinea-pig.*

	Method of obtaining lining layer	
	Tracheal effluent	Alveolar wash
Surface tension at 100 cm^2 (dynes cm^{-1})		
modified Du Noüy method	45·4 ± 2·4	49·3 ± 2·2
modified Wilhelmy method	43·9 ± 3·8	44·8 ± 2·8
Surface tension at 10 cm^2 (dynes cm^{-1})		
modified Du Noüy method	16·8 ± 2·3	18·6 ± 2·1
modified Wilhelmy method	15·5 ± 1·8	17·2 ± 2·0
Tension—Area hysteresis	Present	Present
Volume of extract (ml)	14·0 ± 2·5	32·8 ± 2·5
Dry weight of extract (mg)	42·3 ± 9·1	61·8 ± 11·7
* Cholesterol (mg g^{-1})	7·7 ± 0·9	25·0 ± 3·8
* Glyceride (mg g^{-1} as tripalmitin)	48·0 ± 9·6	45·1 ± 3·2
* Phospholipid (mg g^{-1} as lecithin)	336·0 ± 60·8	272·4 ± 29·8

Results are expressed as mean of eight samples ± standard error of the mean.
* Lipid contents are expressed as mg of lipid per g dry weight of extract.

not significant ($P > 0{\cdot}05$). However, the weight of solids per ml of extract was higher for the tracheal effluent which may explain its surface activity. Following anaphylaxis many lungs were emphysematous and the tracheal effluent method failed. Consequently, the lung washing technique was employed in all subsequent experiments.

When compared to extracts from sensitized, standardized, control animals, samples from animals subjected to anaphylaxis showed a reduced ability to lower surface tension (Table 2). The minimum surface tension of the extracts obtained 30 min after anaphylaxis was significantly greater than that of the controls ($P < 0{\cdot}05$).

Table 2. *Surface activity and lipid content of the lung lining layer of the guinea-pig following anaphylaxis induced by aerosolized antigen.*

	Control	Acute	Time after anaphylaxis (min) 10	30	60
Surface tension at 100 cm^2 (dynes cm^{-1})	45·4 ± 2·5	43·9 ± 1·8	44·8 ± 2·9	48·5 ± 3·3	50·0 ± 3·2
Surface tension at 10 cm^2 (dynes cm^{-1})	18·9 ± 1·8	20·0 ± 2·2	19·5 ± 2·6	28·1† ± 1·7	26·9 ± 2·0
Weight of freeze-dried extract (mg)	48·5 ± 7·0	68·3 ± 16·5	149·0† ± 49·4	77·7 ± 23·5	64·5 ± 22·4
Cholesterol* (mg g^{-1})	23·4 ± 2·8	20·9 ± 2·7	15·6 ± 2·4	19·8 ± 2·2	22·2 ± 2·4
Glyceride* (mg g^{-1} as tripalmitin)	32·2 ± 3·3	46·2† ± 2·7	32·6 ± 1·9	36·6 ± 2·8	34·6 ± 3·3
Phospholipid* (mg g^{-1} as lecithin)	242·6 ± 22·3	276·8 ± 26·5	192·0 ± 23·7	257·6 ± 29·1	298·8 ± 21·5

Results are expressed as the mean of eight samples ± standard error of the mean.
* Lipid results are shown as mg of lipid per g dry weight of extract.
† Significantly different from controls ($P > 0{\cdot}05$).
Samples obtained by alveolar wash (King, 1968) and surface tension determined by a modified du Noüy method (Goadby, 1974b).

There was an increase in the weight of extracted solids 10 min after anaphylaxis and, although the proportion of lipid was lower in these samples, this reduction was not significant in 8 animals ($P > 0{\cdot}05$). The glyceride content of extracts obtained from animals in acute anaphylaxis, was greater than that of control animals, although the actual content was similar to that found in unsensitized animals (Table 1) and, because of the wide variation in lung glyceride content of guinea-pigs in this laboratory (Goadby, 1974a), the result is viewed with caution.

DISCUSSION

The lungs have been described as "an intercommunicating system of alveoli with an inherent tendency to collapse" (Harlan, Margraf & Said, 1966). The system is stabilized by a lining layer or film which has the property of drastically lowering the surface tension of the sharply curved inner surface of the alveoli, thus preventing the development of a negative pressure which would cause transudation from the blood

vessels with consequent alveolar collapse (Pattle, 1965). The characteristic properties of the lining film are its ability to lower surface tension and the tension-area hysteresis exhibited as the surface area is reduced and expanded. Both properties were present in the samples studied (Fig. 1). A similar hysteresis is shown by pressure-volume diagrams for air-inflated lungs (Brown & others, 1959).

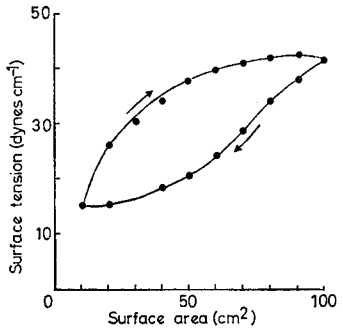

FIG. 1. Surface tension—area diagram of alveolar wash fluid from guinea-pig lung. Sample =31 ml 0·9% (w/v) saline wash from alveoli of unsensitized guinea-pig lung. Surface tension measured by a modified du Noüy method (Goadby, 1974b).

The surfactant material of the lung lining layer has been described as a lecithin-protein complex (Pattle & Thomas, 1961) and the results presented here are consistent with this description. Klaus, Clements & Havel (1961) found 50–60% lipids in the surfactant layer of beef lungs, whereas, in this study, guinea-pig lung surfactant contained only 35–40% lipids. This may be a species difference or, alternatively, it may be due to the different method of extraction of the lipids. Klaus & others (1961) used hot extraction for 12 h in a Soxhlet apparatus with ethanol and acetone, whilst the method reported here involved cold extraction with chloroform–methanol. The proportions of the lipid components were similar in both studies: Klaus & others reported 74% phospholipid, 10% glycerides, 8% cholesterol and 8% fatty acids, and in the present study the relevant percentages were 80% phospholipids, 13% glycerides and 7·5% cholesterol. The lipid content of the extract would appear to influence its ability to reduce surface tension as, in the current experiments, the lowest surface tension was 12·5 dynes cm^{-1} whereas Klaus & others found that their powder, with its greater lipid content, reduced surface tension to less than 10 dynes cm^{-1}.

The extracts obtained 30 and 60 min after anaphylaxis showed less ability to reduce tension on compression of the surface film than did the controls. An impaired ability of the lung lining layer to reduce surface tension is rare. Fujiwara, Adams & Seto (1964) and Bondurant & Smith (1962) were unable to show any change in the surface activity of lung surfactant in severe oxygen toxicity despite the presence of considerable oedema. Pattle & Burgess (1961) examined several pathological conditions of lung and found the surface activity of the alveolar lining layer was unimpaired. They found that inflammation of long standing, long exposure to cadmium oxide and the intratracheal administration of surface-active agents were the only conditions which reduced the surface activity of lung surfactant. An examination of their results showed that impairment of the surface properties of the lining film required a minimum of 4 h to develop in contrast to the rapidly developed effect reported here. This

study has revealed a small reversible impairment of the surface activity of the lung lining layer of a type not previously detected in other pathological conditions of the lung.

The increased weight of solids in the alveolar extract 10 min after shock is similar to the findings of Hemingway & Williams (1952) and Fujiwara & others (1964) in oxygen poisoning, which they attributed to pulmonary oedema.

There were no notable changes in the lipid composition of the extracts obtained after anaphylaxis, even in those samples which showed reduced surface activity. The percentage lipid content of extracts obtained 10 min after anaphylaxis was, however, almost 10% less than that of the other extracts, which indicated that the increase in solids was not due to lipid.

The results suggest that anaphylaxis causes pulmonary oedema which results in an increase in soluble solids in the alveoli although the surface properties of the lung lining layer are, at this time, unimpaired. Later, there is a transient reduction in the ability of the lining film to reduce surface tension on compression of the surface film. The effects of such a change on the physiological function of the lung remain to be determined.

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