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Dipalmitoyl Lecithin and Lung Surfactant Adsorption at an Air–Liquid Interface by Respirable Particles

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The effects of kaolin and flyash on synthetic dipalmitoyl lecithin and lung surfactant from the lavage of excised rat lungs were studied using a Wilhelmy-type surface balance. After control studies with dipalmitoyl lecithin and rat lung surfactant, particles were sprinkled onto the surface at minimum surface area and the surface tensions vs surface areas were recorded. The results showed increased minimum surface tensions and decreased hysteresis areas when the particles were added. More particles were required to change the hysteresis area of lung surfactant than were required for synthetic dipalmitoyl lecithin. The effects of changes in surface areas of alumina and zinc oxide particles on dipalmitoyl lecithin were also studied. The amount of particles required to reduce surface tension–surface area hysteresis increased as the particles' surface area per gram decreased. This study indicates that respirable dusts may adsorb dipalmitoyl lecithin or lung surfactant *in vivo* and, therefore, may prevent surface tension from reaching the low values generally thought to occur *in vivo*. © 1986 Academic Press, Inc.

INTRODUCTION

Since von Neergaard's (1929) demonstration of the effects of surface tension in lungs, many investigators have studied lung surfactants. The proposed physiological advantages of lung surfactant are (1) lowering the surface tension of the alveoli, (2) reducing the work of expanding the alveoli, (3) increasing the stability of the alveoli, and (4) preventing pulmonary edema. Thus, lung surfactant is extremely important for normal lung function. Clements (1957) was the first investigator to study the effects of minced lung extracts on surface tension in a modified Wilhelmy surface balance. Subsequent biochemical studies have shown quantitatively that many chemical species are present in the alveolar lining layer (Galdston *et al.*, 1969; Frosolono *et al.*, 1970; King and Clements, 1972). 1,2-Dipalmitoyl phosphatidylcholine [1,2-dipalmitoyl lecithin (DPL)] is the primary phospholipid constituent of pulmonary surfactant and is probably most responsible for lowering the surface tension of the alveoli. When DPL is spread on an aqueous subphase, a minimum surface tension of 2 dyn/cm is attained with continual compressions (Clements and Tierney, 1965).

Inhalation of dust particles leads to lung responses generally characterized as pneumoconiosis. For example, inhalation of coal dust is known to cause coal workers' penumoconiosis. Kaolin, a well-defined mineral which accounts for about one-half the mineral content of bituminous coal (O'Gorman and Walker, 1972), causes occupational pneumoconiosis comparable to coal workers' pneumoconiosis (Lynch and McIver, 1954; Lapenas *et al.*, 1984; Sheers, 1964). From the general point of view of occupational and environmental health, different par-

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ticulates, when inhaled and deposited in the lung, would be expected to affect the properties of the air–liquid interface. The present study was designed to investigate the possible changes in surface properties of DPL and lung surfactant induced by kaolin dust using a Wilhelmy surface balance. In addition the effects of flyash, which is the solid residue remaining after coal combustion and which is hypothesized to cause a new pneumoconiosis (Golden *et al.*, 1982), were investigated. Since the physiological effects of inhalants are probably determined by the physicochemical properties of the inhaled materials, we also used inorganic dusts of alumina and zinc with known surface areas to study the effects of specific particle surface area on the DPL film at the air–liquid interface.

MATERIALS AND METHODS

Synthetic DPL was obtained from Calbiochem–Behring Corporation. The purity of the phospholipid was checked by thin-layer chromatography on silica gel plates with a developing solvent of chloroform–methanol–water, 65:25:4. Iodine staining of the chromatograms showed one heavy spot so the phospholipid was used without further purification.

Colloidal kaolin was obtained from Fisher Scientific Company. Flyash, obtained from the bag house of the Georgetown fluidized bed combustion project, was a gift from Dr. B. Robenson, Morgantown Energy Technology Center. Alumina (3.04 and 81.4 m^2/g), zinc oxide (0.62 and 3.9 m^2/g), and zinc powder (200 mesh) were obtained from Duke Scientific. Alumina (80–100 mesh) was obtained from Micro Tek Instruments, Inc. All the particles were used without further treatment.

Lung alveolar surfactant (LAS), from male Sprague–Dawley rats, was obtained by lavaging the lungs of anesthetized rats 5 times with a total volume of 30 ml of saline. The total lung wash was centrifuged at 500*g* for 5 min at 4°C to remove the cellular materials. LAS was further purified according to the method of Watkins (1968). The supernatant was then washed twice by vigorous shaking with equal volumes of saline solution. The white foam was removed and stored at −20°C until used.

The surface tension was measured as a function of the surface area on a Wilhelmy balance (Kimray-Greenfield). The apparatus consists of a 5.0 × 11.5-cm Teflon trough and a movable Teflon barrier. The Teflon barrier fits tightly against the walls of the trough and is driven by a constant speed electric motor. Surface tension is measured with a platinum flag suspended from a lever attached to a pneumatic measuring system, the output of which is directly recorded on graph paper. The cycling speed was set at 2.5 min/cycle with the surface area being compressed to a minimum of 15% of the total surface area. Prior to each experiment the Teflon trough, barrier, and platinum flag were thoroughly cleaned with chromic acid and absolute ethanol. The flag was then flamed. Phosphate buffer (0.01 M, pH 7.0) with 10^{-4} M EDTA was used as the subphase except for the flyash experiments where 0.05 M phosphate buffer with 10^{-4} M EDTA at pH 7, 9, and 12 was used as the subphase. In each case, the subphase was cleaned by aspiration. Prior to each experiment the subphase was tested for surface activity by compressing to the minimum surface area. If this test run revealed a constant surface tension of 72 dyn/cm, a DPL or LAS film was spread onto the subphase. The temperature of the subphase was not controlled, but within the range of 22–24°C.

When DPL was spread on the surface of the subphase, 8 μg of DPL in a 1 mg/ml chloroform solution was used. The solvent was allowed to evaporate for 5 min before compression was begun. The surface measurement for LAS was obtained by transferring small amounts of the foam onto the subphase until the maximum surface tension was lowered to about 50 dyn/cm at 100% surface area. The foam on the surface was allowed to spread for 15 min before compression was begun.

In the surface tension–area measurements, the largest changes occurred during the first four cycles and the subsequent cycles showed almost no deviation. The fourth compression–expansion loops were therefore taken as the representative cycle in our experiments. Particles were sprinkled onto either DPL or LAS films after the fourth cycle at the minimum surface area.

RESULTS

Figure 1 shows the fourth cycle of the surface tension–surface area curve for a DPL film spread on a subphase of 0.01 M phosphate buffer, pH 7, with 10^{-4} M EDTA at room temperature. Figure 1 also shows the surface tension–surface area curves of DPL with increasing amounts of kaolin particles, i.e., 0.47, 1.47, and 3.23 mg, respectively, sprinkled on the surface at minimum area. The minimum surface tension for DPL is near zero while the maximum surface tension is about 68 dyn/cm. With increasing amounts of kaolin sprinkled onto the DPL film, the hysteresis of the surface tension–surface area decreased; the maximum surface tension remained approximately constant while the minimum surface tension in-

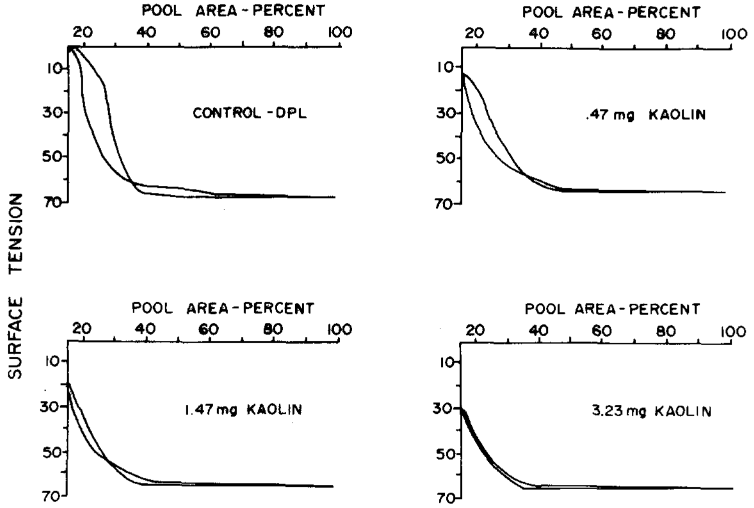

FIG. 1. Surface tension as a function of pool area during the fourth continuous compression–expansion cycle. Control was obtained when 8 μg DPL was spread on the subphase. Different amounts of kaolin were sprinkled onto the DPL film in the other panels.

creased. Figure 2 shows the change in hysteresis area as a function of the amount of kaolin added to the DPL film. The hysteresis area essentially disappeared when about 4 mg of kaolin was added.

Figure 3 shows the results obtained when foam obtained from rat lung lavage fluid was spread onto the aqueous surface of the Wilhelmy balance. Compared to Figure 1, the minimum surface tension was higher and the maximum surface tension was lower than the results obtained with the synthetic DPL. The hysteresis area is, however, larger than that produced by DPL. Figure 3 also shows the results when increasing amounts of kaolin were sprinkled onto the surface. Kaolin caused the hysteresis area of the lung surfactant to decrease. The percentage hysteresis area decrease was plotted as a function of the amount of kaolin used and is shown in Figure 4. The results of adding kaolin to lung surfactant are similar to those found with DPL. However, the variability was greater and much more kaolin was needed for the same percentage decrease in hysteresis. The minimum surface tension increased with both lung surfactant and synthetic DPL. Table 1 shows the effect of kaolin on the minimum and the maximum surface tensions of DPL and lung surfactant.

Figure 5 shows the results of sprinkling flyash onto a DPL film when the aqueous subphase was buffered at pH 7, 9, or 12 with 0.05 M phosphate and 10^{-4} M EDTA. The subphase required buffering because flyash particles themselves would change the pH of the subphase. About the same amount of flyash (~14 mg) was required to eliminate the hysteresis at each pH. As shown, the results with flyash are similar to those using kaolin.

Table 2 shows the results of the effect of flyash on lung surfactant films, with 0.05 M phosphate buffer and 10^{-4} M EDTA used as the subphase. The hysteresis of DPL disappeared when ~14 mg of flyash was added; however, it took approxi-

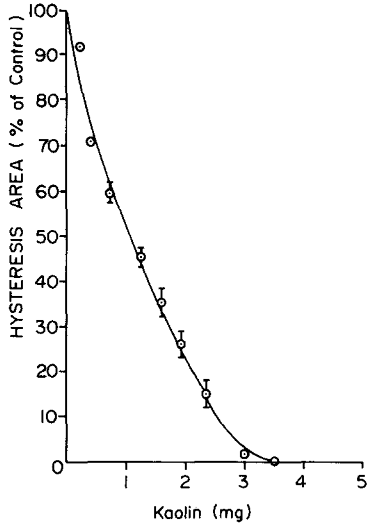

FIG. 2. Plot of the percentage hysteresis area of a DPL film as a function of the amount of kaolin sprinkled onto the film at minimum surface area. Bars represent SEM, $N = 6$.

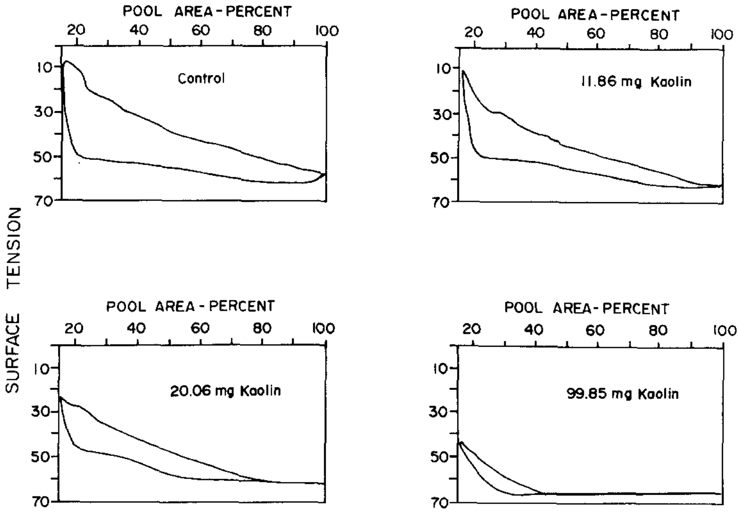

FIG. 3. Surface tension as a function of pool area during the fourth continuous compression–expansion cycle for the control curve using a film of lung surfactant obtained from rat lung lavage and spread on the aqueous subphase with 0.01 M phosphate buffer and 10^{-4} M EDTA, pH 7. Differing amounts of kaolin were added in the other panels.

mately 60 mg of flyash to diminish the hysteresis of lung surfactant. The effect of flyash on the minimum and maximum surface tensions for both DPL and lung surfactant are summarized in Table 3.

Different size particles of alumina and zinc oxide were used to study the effects of surface area of the particles on the surface activity of DPL when 0.01 M phosphate buffer, pH 7.0, with 10^{-4} M EDTA was used as the aqueous subphase. Table 4 gives the values of the minimum surface tensions (γ_{min}) with different sizes of

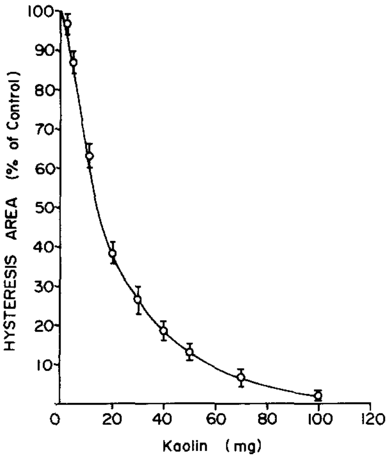

FIG. 4. Percentage hysteresis area of the lung surfactant film is plotted as a function of the amount of kaolin sprinkled onto the film. Notice the increased variability and the increased amount of kaolin needed to reduce the hysteresis. Bars represent SEM, $N = 6$.

TABLE 1
EFFECT OF KAOLIN ON THE MINIMUM AND MAXIMUM SURFACE TENSION OF DIPALMITOYL LECITHIN AND LUNG SURFACTANT[a]

	Control[b]		Experimental[c]	
	γ_{min}	γ_{max}	γ_{min}	γ_{max}
I. Dipalmitoyl lecithin	1.2 ± 0.6	64.8 ± 1.9	24.8 ± 1.1	60.2 ± 2.2
II. Lung surfactant	6.57 ± 1.6	56.1 ± 3.1	52 ± 2.1	62.9 ± 2.5

[a] Values are means ± SEM; $N = 6$.
[b] Without kaolin.
[c] With kaolin.

particles. When 1 mg of alumina or zinc oxide was added to the DPL film, γ_{min} increased to a higher extent when the particles with larger surface area were used. In each case the final γ_{min} remained about the same (~24 dyn/cm).

Figure 6 shows the effect of 0.62 and 3.9 m^2/g zinc oxide and 200-mesh zinc powder on the hysteresis area of DPL films. The hysteresis area decreases faster as the surface area per gram of the particle increases. The effects of different sizes of alumina (3.04 m^2/g, 81.4 m^2/g, and 80–100 mesh) on DPL films are plotted in Figure 7. With these particles also the hysteresis decreased faster as the surface area per gram of particle increased.

DISCUSSION

Our results show that the effects of particulate on synthetic DPL and lung surfactant surface properties can be successfully studied in a simple *in vitro* system such as the Wilhelmy balance. Barrow and Hills (1979) have investigated DPL

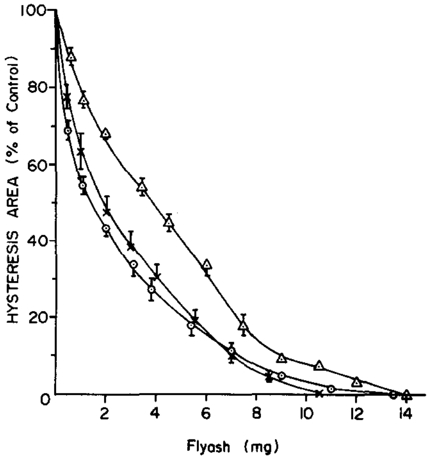

FIG. 5. Effect of flyash particles on the percentage hysteresis area of DPL films with 0.05 M phosphate buffer and 10^{-4} M EDTA as the subphase. ×, pH 7: △, pH 9; ○, pH 12. Bars represent SEM, $N = 6$.

TABLE 2

EFFECTS OF FLYASH ON THE PERCENTAGE HYSTERESIS FROM FILMS OF LUNG SURFACTANT WITH 0.05 M PHOSPHATE BUFFER AND 10^{-4} M EDTA AS SUBPHASE[a]

Flyash (mg)	Flyash hysteresis area (cm^2)		
	pH 7 (N = 6)	pH 9 (N = 5)	pH 12 (N = 6)
5	77.4 ± 5.6	71.3 ± 4.8	71.4 ± 4.4
10	54.0 ± 8.7	53.2 ± 5.8	59.0 ± 5.8
15	37.0 ± 9.2	41.7 ± 6.1	50.4 ± 6.9
20	26.2 ± 7.4	26.4 ± 3.7	36.0 ± 5.1
30	10.5 ± 5.0	15.8 ± 2.8	24.8 ± 5.6
40	5.7 ± 3.9	8.4 ± 1.8	16.8 ± 3.5
50	2.6 ± 1.6	3.4 ± 1.6	10.8 ± 3.5
60	1.3 ± 0.8	0.5 ± 0.3	5.9 ± 2.4
80	—	—	1.3 ± 1.3

[a] Values are means ± SEM.

films on distilled water using two different surface balance methods simultaneously, the Wilhelmy balance and the Du Nuoy ring method. They found appreciably lower surface tension values for DPL using the balance than when using the ring method. They suggested that the difference could be explained on the basis of a significant contact angle, which they showed to vary with pool area, a relationship exhibiting hysteresis. The hysteresis on the higher surface tension portion in our surface tension area loop of DPL can be explained by this contact angle hysteresis. The contact angle between the flag and the liquid surface is a problem when measuring high surface tensions with the Wilhelmy balance but does not interfere at lower surface tensions.

TABLE 3

EFFECT OF FLYASH ON THE MINIMUM AND MAXIMUM SURFACE TENSION OF DIPALMITOYL LECITHIN AND LUNG SURFACTANT[a]

	Control[b]		Experimental[c]	
	γ_{min}	γ_{max}	γ_{min}	γ_{max}
I. Dipalmitoyl lecithin				
pH 7	3.0 ± 1.5	64.7 ± 1.5	31.0 ± 3.0	63.3 ± 3.5 (10 mg)[d]
pH 9	1.5 ± 0.5	64.2 ± 1.6	24.0 ± 3.7	65.2 ± 0.9 (10 mg)[d]
pH 12	1.0 ± 0.7	64.2 ± 0.9	30.0 ± 0.8	63.7 ± 1.5 (10 mg)[d]
II. Lung surfactant				
pH 7	5.3 ± 2.0	54.2 ± 2.0	34.2 ± 3.2	57.0 ± 2.0 (35 mg)[d]
pH 9	10.0 ± 2.0	55.2 ± 1.5	39.2 ± 5.2	60.2 ± 2.0 (50 mg)[d]
pH 12	9.3 ± 1.9	51.2 ± 2.4	30.2 ± 1.8	59.3 ± 1.6 (50 mg)[d]

[a] Values are means ± SEM.

[b] Without flyash.

[c] With flyash.

[d] Amount of flyash added.

TABLE 4
EFFECTS OF ALUMINA AND ZINC OXIDE PARTICLES ON THE MINIMUM SURFACE TENSIONS OF DIPALMITOYL LECITHIN[a]

Particle type and size	γ_{min}	
	With 1-mg particle ($N = 6$)	Final ($N = 6$)
Alumina (81.4 m^2/g)[b]	16.2 ± 1.7	22.0 ± 2.5 (2 mg)[c]
Alumina (3.04 m^2/g)[b]	9.7 ± 1.9	21.0 ± 3.0 (6 mg)[c]
Alumina (80–100 mesh)[b]	3.0 ± 1.1	28.0 ± 1.7 (50 mg)[c]
Zinc oxide (3.9 m^2/g)[b]	10.7 ± 1.0	24.3 ± 1.8 (7 mg)[c]
Zinc oxide (.62 m^2/g)[b]	7.0 ± 1.8	24.2 ± 2.0 (34 mg)[c]
Zinc (200 mesh)[b]	1.8 ± 1.0	23.2 ± 1.5 (140 mg)[c]

[a] Values are means ± SEM.

[b] Surface area of the particle.

[c] Amount of particles used in the experiment.

Lung surfactant produced a minimum surface tension of 10 dyn/cm which may be the minimum surface tension required to stabilize the lung (Abrams, 1966). The hysteresis loops for lung surfactant exhibit a plateau around 25 dyn/cm in the compression phase. The plateau was explained by Watkins (1968) either as a rapid "squeeze-out" of some of the components of the lung surfactant or perhaps by a comformational change within the choline moieties. Although some protein associated with crude lung extracts was removed when we rinsed the pulmonary foam with saline solution, proteins and some unsaturated lipids could be the components squeezed out of the surface of the film.

Wallace *et al.* (1975) have reported that when kaolin was suspended in DPL dispersion, about 18% of the DPL was adsorbed by kaolin particles. Present results have shown that the minimum surface tension increased and the hysteresis area decreased when kaolin was placed on the DPL films at the air–liquid interface. The adsorption of the surface active materials to the particles is probably responsible for these effects. Particles of propyliodone and tantalum have been shown to reduce surface activity of films from lung surfactant and DPL in surface balance experiments by Schurch and Roach (1976). More kaolin particles were required to change the hysteresis area of lung surfactant than to change the hysteresis area of a DPL film. This is probably due to the complex composition of the lung surfactant. Also, it may be that the different components of the lung surfactant might compete with DPL for adsorption to the particles and thus preserve the intact air–liquid interface of the alveoli *in vivo*.

When flyash, alumina, zinc, and zinc oxide particles were placed on the DPL films, an effect similar to kaolin particles was obtained. The results indicate that these particles also adsorb DPL from the air–liquid interface. When the same amount of particles of different surface areas was used, the hysteresis area decreased and the minimum surface tension increased as the surface area per gram of the particles was increased. This clearly shows that the amount of surface active material adsorbed is dependent on the surface area of the particles used.

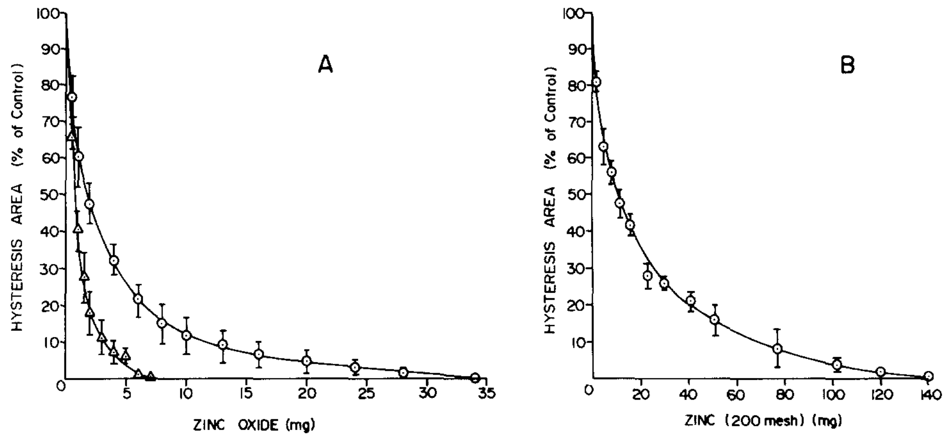

FIG. 6. Effect of zinc oxide particles with surface areas of (A) 0.62 m^2/g (⊙) and 3.9 m^2/g (△), and (B) 200-mesh zinc powder on the DPL film with 0.01 M phosphate buffer, pH 7, and 10^{-4} M EDTA as subphase. Bars represent SEM, $N = 6$.

When particles were sprinkled onto the surface films, the minimum surface tension increased from near zero to a maximum value of about 30 dyn/cm. These results show that the minimum surface tension produced by dynamic compression–expansion of a film is sensitive to the contamination by particles. Since the air–liquid interface of the alveolar membrane is an environmental interface, materials which are deposited on the outermost layer of this surface would appear to have the maximum probability of biological interaction. Although the results of this simple *in vitro* system indicate that respirable dusts may adsorb surface active material *in vivo* and thus might lead to a decrease in lung compliance or increase in atelectasis or edema, additional experiments will be required to prove the hypothesis. There is some published evidence using smoke particles which already supports the hypothesis (Nieman *et al.*, 1980).

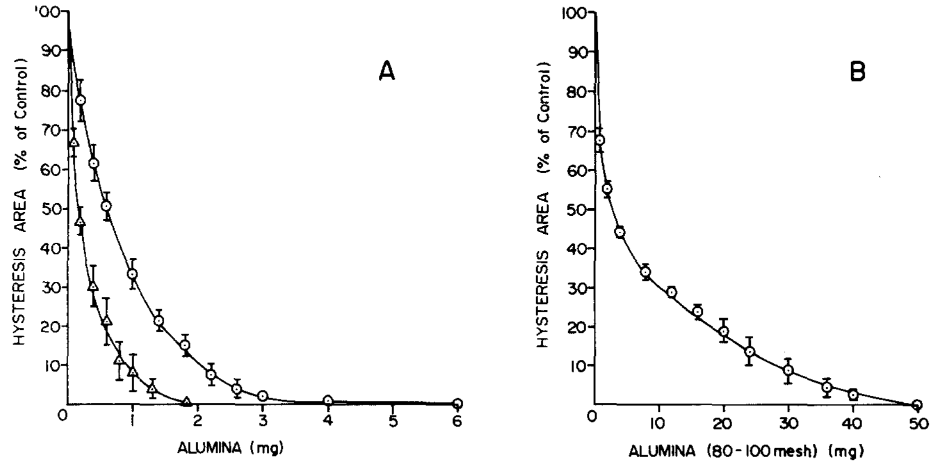

FIG. 7. Effect of alumina particles with surface areas of (A) 3.04 m^2/g (⊙) and 81.4 m^2/g (△), and (B) 80–100 mesh on the DPL film with 0.01 M phosphate buffer, pH 7, and 10^{-4} M EDTA as subphase. Bars represent SEM, $N = 6$.

The proposed subtle lung changes may be the initial steps in the development of lung pneumoconiosis. When the lung responds to some inhaled particles with an increase in surfactant production (Miller and Bondurant, 1962; Heppleston and Young, 1972) it may be trying to prevent the decrease in lung hysteresis and increase in minimum surface tension which may occur within the lung periphery as particles were deposited.

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