Influence of Inhaled Carbon Particulates on Pulmonary Surface Area[1] (36608)

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(Introduced by E. S. Vesell)

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The mammalian lung with its enormous surface area (A_s) for gas exchange represents one of the largest biological membranes in the body (1) and is by far the most extensive surface exposed to the external environment.

Numerous studies have been carried out to determine the effects of inhaled dust on the lung (2, 3). In many cases a wide spectrum of pulmonary damage was produced and often structural characteristics were abnormal to such an extent that there was little difficulty in predicting that extensive damage to A_s would be present. However, during chronic low-level exposure leading to lesser pulmonary damage, changes in A_s, especially at high and low lung volumes, are unknown. Accordingly, we examined chronic low-level effects of particulate on lung A_s. Pulmonary A_s was calculated from the free energy change appearing as the area between liquid and air volume–pressure (V–P) curves and from an assigned surface tension (γ) obtained from lung washings.

Methods. Male Long Evans hooded rats ($N = 8$) were exposed to 545 $\mu g/m^3$ ($\pm$ 193 SD) of carbon particulate for 9 months. The exposure regimen was 24 hr/day, 5 days/week. Mass median diameter (MMD) of the carbon particles was 1.8 μ which is well within the respirable range (4). A qualitative estimate of the fraction deposited in the alveolar region for heterodispersed dusts would be on the order of 40% (5). The carbon was an all gas channel black (Sid Richardson Co., Fort Worth, TX) dispersed by a dry powder aerosol dispenser similar to that described by Crider, Barkley and Strong (6). The exposure system was similar in design to that described by Hinners, Burkart and Punti (7). Control animals ($N = 8$) were placed in identical chambers and received prefiltered air.

Pulmonary A_s was computed from the change in surface free energy at the gas–liquid interface from total collapse to maximum volume. This was accomplished by the basic method used by Radford (8) in which free energy at the alveolar surface can be described by the Helmholtz equation in terms of A_s, γ, and may be obtained from V–P curves in the following way:

$$\int_{V_0}^{V} (P_{\text{air}} - P_{\text{saline}})\, dV = \int_{A_{s_0}}^{A_s} \gamma\, d\, A_s, \quad (1)$$

where V_0 and A_{s_0} represent volume and area of the collapsed lung. Equation (1) thus states the energy at the surface is the product of γ and A_s. Since Eq. (1) contained two unknowns (A_s, γ), and γ is a nonlinear function of A_s, it was necessary to modify the surface tension of the lung without altering its A_s–V relationship. This was accomplished by rinsing the lung with 5% (v/v) polysorbate (Tween) 20 saline mixture (8, 9) and then inflating–deflating with air. For lungs rinsed with Tween 20 γ is constant (γ_K) and

$$\int_{V_0}^{V} (P_{\text{Tween 20}} - P_{\text{saline}})\, dV = \gamma_K \int_{A_{s_0}}^{A_s} d\, A_s. \quad (2)$$

Rearranging and integrating the right side, Eq. (2) becomes:

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$$A_s - A_{s_0} = \frac{1}{\gamma_K} \int_{V_0}^{V} (P_{\text{Tween 20}} - P_{\text{saline}})\, dV, \qquad (3)$$

where $A_{s_0} = A_s$ at zero volume. The initial A_{s_0} of degassed lungs could not be determined directly but Kuno and Staub (10) estimated that at 10% of maximum lung volume A_s was equal to 20% of its maximum value. By trial and error this condition was satisfied by assuming $A_{s_0} = 0$ when $V_0 = 0$. The value γ_K was obtained from subsequent lung washings using a surface tension balance.

V–P apparatus and technique was essentially as described by Frazer, Rhoades and Adams (11). The animals were sacrificed by intraperitoneal sodium pentobarbital (3 mg/kg body wt) injection and rapid exsanguination via a carotid artery and thoracotomy. The heart and lungs were removed *en bloc* and attached to the *V–P* apparatus and inflated–deflated at 1.94 ml/min. All *V–P* measurements were made under constant temperature (22°) and were preceded by degassing the lung under vacuum to standardize initial lung volumes. Volume of the degassed lung served as a zero reference for all *V–P* curves. *V–P* curves were recorded on the second inflation–deflation cycle.

Three separate *V–P* curves were obtained from each set of lungs. First, the lungs were inflated with air to 22 cm H_2O. This was done to standardize both the subsequent saline and Tween 20 *V–P* curves, and to check for leakage. Upon completion of the initial air *V–P* curve, the lungs were degassed and a second *V–P* curve was obtained, inflated–deflated with saline. Inflation with saline was at the same rate and to the same volume as the previous air *V–P* curve. Thereafter, lungs were rinsed with Tween 20, degassed and inflated with air to 22 cm H_2O. Following the Tween 20 *V–P* curve, a lung wash was collected and surface tension measured by methods described previously (12, 13). All *V–P* curves and γ from lung washings were digitized and analyzed on an IBM 360 computer.

Results. Figure 1A shows air, saline, Tween 20 *V–P* curves obtained from a pair of lungs. Air and saline *V–P* curves show the typical characteristics for excised lungs. After rinsing the lung with Tween 20, the air inflation curve followed a path similar to the air *V–P* curve; however, during deflation the volume for each corresponding pressure of the Tween 20 curve was markedly lower than the air curve. These findings have been well documented by others (8, 9). Figure 1B

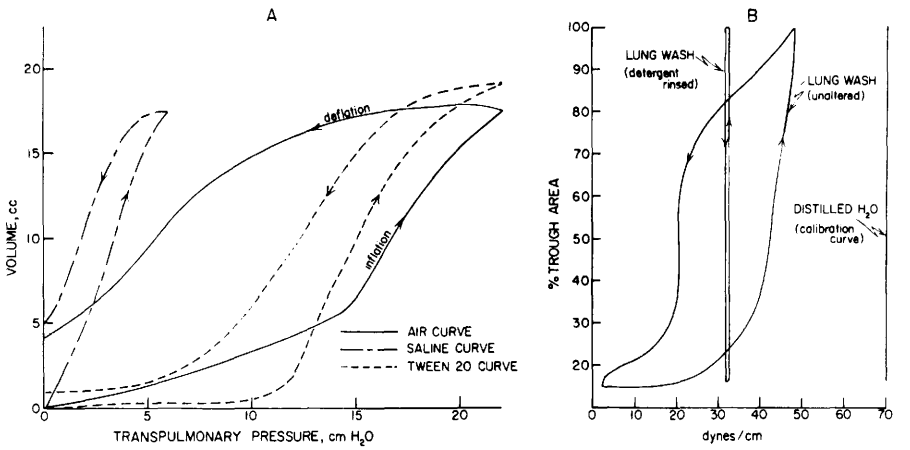

FIG. 1A. Set of *V–P* curves on an excised rat lung (left). Sequence for recording curves was as follows: (a) inflated–deflated with air; (b) inflated–deflated with saline; (c) rinsed with 5% Tween 20 mixture and inflated and deflated with air. Each *V–P* curve was preceded by degassing. (B) Surface tension–area curves of an unaltered lung wash and an altered wash (lung rinsed with Tween 20).

TABLE I. Effect of Carbon Particulate on Internal Surface Area (A_s/V_{max}) of Rat Lungs.[a]

	Relative lung volume (V/V_{max})				
	0.20	0.40	0.60	0.80	1.00
Controls $N = 8$	0.025 ± 0.003	0.074 ± 0.009	0.153 ± 0.010	0.229 ± 0.012	0.324 ± 0.018
Carbon exposed $N = 8$	0.009 ± 0.004[b]	0.044 ± 0.007[b]	0.122 ± 0.010[b]	0.195 ± 0.011[b]	0.275 ± 0.015[b]
Decrease (%) from controls	64	41	20	15	15

[a] Values are expressed in square meters and are averages, ± SE.

[b] Statistically significant from controls at the 5% level.

shows tension–area characterististics of an unaltered and altered wash. The unaltered lung wash gave a typical surface tension–area curve upon cyclic compression and expansion. The altered wash (lungs previously rinsed with Tween 20) gave a small hysteresis loop with the expansion limb giving a constant γ between 100 to 20% trough area. Surface tension during film expansion averaged 32.9 dyn/cm (± 1.25 SD) for all altered lung washings.

Rats exposed to carbon particulate tended to have lower body weights and lower lung volumes for the air inflation–deflation V–P curves. However, none of these were statistically significant ($p > 0.05$) from controls. Pulmonary A_s calculated from Eq. (3) is shown in Table I. A_s was divided by maximum lung volume (V_{max}) to correct for differences in lung volumes. At V_{max}, A_s for the control group averaged 0.324 m^2 and decreased as a function of relative lung volume. The calculated surface area is in good agreement with literature values (14). The carbon exposed lungs showed a significant decrease in A_s at all relative lung volumes measured. The percentage decrease in A_s [(A_s control $-A_s$ carbon)/A_s control] was much greater at lower lung volumes reaching a maximum of 64% decrease at 20% total lung volume.

Discussion. The pulmonary surface is comprised of an air–liquid interface having surface active properties (15). At near static conditions in which pulmonary flow resistance is eliminated, the energy expended in inflating the lungs by creating a large air–liquid interface and by stretching the elastic fibers is converted into potential energy. During deflation this potential energy is released. By recording V–P curves of lungs filled with air and then with saline, both the elastic and total free energy can be calculated. The differences between these two energies is the surface free energy component described by Radford (8) and was the means used to determine pulmonary A_s.

Our studies show chronic low-level exposure to carbon dust results in a significant reduction of A_s in the lung and is more pronounced at lower lung volumes. The proportionally greater reduction in A_s at lower lung volumes assumes physiologic significance since alveolar ventilation under resting conditions also occurs at these volumes.

The manner in which carbon dust reduces A_s is not known. Since carbon is relatively "inert" compared to other particulates such as silica, the material would not be intrinsically toxic and attack lung parenchyma directly. The work of Nau and associates (16) using the same type of carbon particles supports this view. During chronic exposure to varying concentrations of carbon dust, they observed no significant structural damage to the alveolar region.

High surface tension could theoretically result in a reduction of pulmonary A_s (17). However, there is no evidence that such a phenomenon occurs from chronic low-level exposures to relatively "inert" particulates. Acute studies (18) do not support a surface tension effect. One consistent finding from histological examination was terminal airway

blockage (unpublished data). This blockage could have been brought about by airway constriction (19) or by the deposition of carbon particulates leading to the proliferation of cellular and acellular material and hence to the formation of aggregates in the airways. Regardless of the mechanism, airway obstruction would explain the reduction in A_s at low lung volumes. For example, at high lung volumes the greater pressure would open partially blocked airways whereas at lower lung volumes with concomitant lower pressure, the blocked airway would remain closed. Additional studies examining the degree and severity of airway obstruction are required for firm support of these findings. Nevertheless, a high degree of airway obstruction would result in uneven gas distribution which would ultimately lead to a ventilation perfusion imbalance. Such an imbalance would explain the increased alveolar arterial O_2 differences often reported in workers suffering from pneumoconiosis (20, 21).

Summary. Pulmonary surface area was significantly reduced in rats chronically exposed to low levels of carbon particulates (545 $\mu g/m^3$, $\pm$ 193 SD) for 9 months. The decrease in surface area was much more pronounced at lower lung volumes. The suggestion is made that the reduction in lung surface area is related to terminal airway obstruction.

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1. Heinemann, H. O., and Fishman, A. P., Physiol. Rev. **49**, 1 (1969).

2. Blake, M. R., "Handbook of Physiology Respiration," Sect. 3, Vol. 2, p. 1601. Amer. Physiol. Soc., Washington, DC (1965).

3. Davies, C. N., "Inhaled Particles and Vapours", Vol. 2, 605 pp. Pergamon, London (1967).

4. Boren, H. G., Yale J. Biol. Med. **40**, 364 (1968).

5. Task Group on Lung Dynamics, Health Phys. **12**, 173 (1966).

6. Crider, W. L., Barkley, N. P., and Strong, A. A., Rev. Sci. Instrum. **39**, 152 (1968).

7. Hinners, R. G., Burkart, S. K., and Punti, C. L., Arch. Environ. Health **16**, 194 (1968).

8. Radford, E. P., Jr., Proc. Soc. Exp. Biol. Med. **87**, 58 (1954).

9. Clements, J. A., Hustead, R. F., Johnson, R. P., and Gribetz, I., J. Appl. Physiol. **16**, 444 (1961).

10. Kuno, K., and Staub, N. C., J. Appl. Physiol. **24**, 83 (1968).

11. Frazer, D. G., Rhoades, R. A., and Adams, W. S., J. Appl. Physiol. **29**, 534 (1970).

12. Godish, D., and Rhoades, R. A., Resp. Physiol. **10**, 396 (1970).

13. Williams, R. A., Rhoades, R. A., and Adams, W. S., Med. Biol. Eng. **9**, 729 (1971).

14. Tenney, S. M., and Remmers, J. E., Nature (London) **197**, 54 (1963).

15. Pattle, R. E., Physiol. Rev. **46**, 48 (1965).

16. Nau, C. A., Neal, J., Stembridge, V. A., and Cooley, R. N., Arch. Environ. Health **4**, 45 (1962).

17. Scarpelli, E. M., "The Surfactant System of the Lung," p. 231. Lea and Febiger, Philadelphia (1968).

18. Rhoades, R. A., Life Sci. **11**, 33 (1972).

19. Widdicome, J. G., Kent, D. C., and Nadel, J. A., J. Appl. Physiol. **17**, 613 (1962).

20. Becklake, M. R., DuPreez, L., and Lutz, W., Amer. Rev. Tuberc. Pulm. Dis. **77**, 400 (1957).

21. Caplan, A., Brit. J. Ind. Med. **19**, 171 (1962).

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