Method for Estimating Respiratory Surface Area of Mammalian Lungs from Their Physical Characteristics.* (21284)

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The surface area available for gas exchange in the lungs is an important factor in gas transfer between the alveoli and the blood(1,2). Present estimates of this area are based on measurements of alveolar dimensions taken from fixed histologic specimens(3-5). These estimates of total surface area are made by measuring or calculating the area/volume ratio for a small portion of lung tissue and applying this ratio to an assumed total lung volume. The volume which must be assumed is uncertain because the degree of inflation represented in the particular specimen is difficult to determine, especially if the lungs are allowed to collapse before fixation is complete. Moreover, distortion of lung architecture inevitably occurs with standard histologic methods involving dessication and embedding, and such lobular subdivisions as the air-sacs and alveolar ducts, which contribute only slightly to the respiratory surface area, have in the inflated state a much larger volume than is generally realized(6). For these reasons calculations by anatomic methods tend to result in overestimating the number of alveoli and the total lung surface.

The method of estimating lung surface described in this report is based on physical measurements of the lungs and avoids any assumption of size or configuration of respiratory structures. The calculation depends on consideration of the changes in free energy of the lungs as they are deflated. During inflation, energy is stored by deforming elastic elements, and by the creation of a large air-liquid interface. If the lungs are deflated by allowing volume equilibrium at successive pressure decrements, the stored energy released during deflation can be measured. As the volume of air in the lungs decreases from a volume V_1 to the totally collapsed volume V_0, the free energy recovered (or work done by the lungs) is given by

$$\int_{V_0}^{V_1} P dV$$

where P is the pressure relative to atmospheric. As will be shown below, it is possible to estimate the contribution of elastic energy to the total free energy and obtain the surface free energy by difference.

At equilibrium in a 2-phase system of several components, the surface area A_s is related thermodynamically to the (Helmholtz) free energy of the surface, F_s; for a plane interface (assuming a dividing layer of negligible thickness):

$$\frac{dF_s}{dA_s} = \gamma - S_s \frac{dT}{dA_s} + \sum_r \mu_r \frac{dn_r}{dA_s} \qquad (1)$$

where γ denotes the surface tension coefficient, S_s surface entropy, T ambient temperature, and the summation term includes the chemical potential μ and number of moles n for each component r. If the temperature is constant and the quantity and total composition of the associated bulk phases is independent of area, the last two terms are zero. Equation 1 may be applied to curved surfaces when the radii of curvature are very much greater than molecular dimensions(7). The radii of curvature of lung air-liquid surfaces undoubtedly exceed 10^{-4} cm and this condition is easily met. Subject to the assumptions implied above, equation 1, as applied to the lungs, may be written in its usual form

$$\frac{dF_s}{dA_s} = \gamma.$$

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For a determination of the surface area from experimental estimates of F_s, the relationship between γ and A_s must be known for the lungs. Because no evidence is as yet available on this point, γ will be assumed constant, which would be true for an aqueous interface. With this added assumption,

$$\int_{V_0}^{V_1} dF_s = \gamma \int_{V_0}^{V_1} dA_s$$

which states that the work done by the surface as the volume of air in the lungs decreases from V_1 to V_0 is represented by the product of the surface tension coefficient and the decrease in surface area. In our experiments V_0 is nearly zero, and the surface free energy given up by deflation of the lung from a particular volume is a measure of the total air-liquid surface which existed at that volume; that is, $As = Fs/\gamma$, where F_s and A_s refer to a particular lung volume.

The magnitude of F_s is estimated experimentally from lung pressure-volume curves in the following manner. (Details of methods and results will be published elsewhere.) Gas-free animal lungs are prepared by allowing the pulmonary blood flow to absorb the alveolar gases(8), after initially hyperventilating the lungs with pure oxygen. These completely deflated lungs are removed, filled with isotonic NaCl solution while suspended in a saline bath, and the "elastic" curve is obtained during deflation by allowing the volume to reach equilibrium at each pressure. This curve is then compared with the deflation curve subsequently obtained in the same way after air inflation at the same temperature. Typical saline and air curves for rat, cat, and dog lungs are shown in Fig. 1. The volume ordinates for the 3 species are different, but the pressure scales are the same. If we assume that the curve from the saline run gives a reliable estimate of the work done by elastic elements during deflation in air, the area between the 2 curves represents the free energy of the surface recovered as the area decreases. The surface energy is roughly ⅔ of the total for the rat, ½ for the cat, and ¼ for the dog. This species difference has been a consistent finding. The surface energy has been obtained for a specific air volume by direct measurement of the shaded areas. The particular volumes selected are approximately those the animals would have *in vivo*. The results for the three animals in Fig. 1 are: Rat, 1.23×10^4 ergs for air vol. of 5 cm^3; Cat, 12.4×10^4 ergs for air volume of 100 cm^3; Dog, 26.7×10^4 ergs for air vol. of 400 cm^3. The air volumes are less than the total volumes shown in Fig. 1 because of residual saline solution remaining in the lungs.

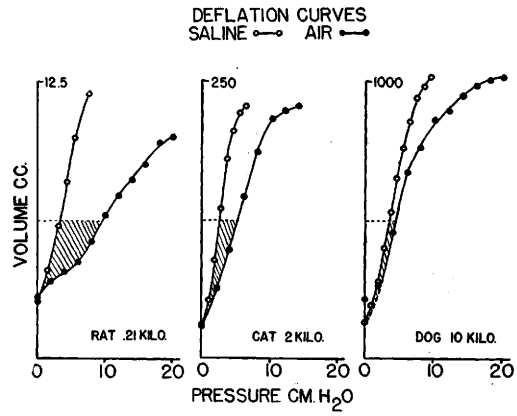

FIG. 1. Static pressure-volume curves from excised rat, cat, and dog lungs. Volumes are relative to initial volume of completely deflated lungs taken from the animal. Trapping of air due to production of a stable foam occurred in the dog experiment and lower portion of the air curve has been corrected by calculating surface energy remaining in the foam.

Aside from technical difficulties such as leaks and foaming, these values may be in error for two additional reasons. First, the "elastic" curves obtained in saline may not be strictly applicable to air-filled lungs. Although gross dimensions of the lungs are very similar during the saline and air experiments, differences in elastic strain at the alveolar level may result from the presence of an air-liquid interface. Second, theoretical considerations relating to serial closing of respiratory units during deflation(9) suggests that part of the total surface energy may be dissipated in frictional losses as closure occurs. The first source of error would result in overestimating the surface energy while the second would result in underestimating it; thus, the two tend to nullify each other. The second factor is probably the more important of the two at low levels of

inflation, and the free energy estimated by integration may therefore be too small at moderate lung volume. There is no reason to believe, however, that these sources of error are very significant.

Only the surface tension coefficient remains to be considered in order to calculate the surface area. No direct measurements of γ in the lungs have been made. An attempt at estimating γ for animal lungs was made by von Neergaard(10) who measured the surface tension coefficient of lung tissue extracts, using the method of Lenard *et al.*(11). His results varied from 35 to 41 dynes/cm; in view of errors of the method(12) and the presence of intracellular substances, these values cannot be applied to intact lungs. von Neergaard found no conclusive evidence for a surface-active film in the lung, and concluded that the interfacial layer is probably an aqueous solution having a surface tension coefficient near that of serum. Human blood serum has a static surface tension coefficient of about 50 dynes/cm(13), and this value will be used for the lungs until better data are available. The figure of 50 may be too low to apply to our experiments, since the lungs have been flushed with saline ($\gamma = 72$), but colloidal solutions such as serum maintain their static surface tension even with high dilution(14).

The lung surface areas for the 3 animals of Fig. 1, as calculated from the formula $A_s=F_s/50$, are: rat, 246 cm^2; cat, 2480 cm^2; dog, 5340 cm^2. These results apply to the particular volumes at which F_s was measured.

The surface area of human lungs may also be estimated by this method. On the assumption that the pressure-volume relationship is linear and that the transmural pressure is 6 cm-of-water for a lung volume of 3500 cm^3, the total work done, F_T, during complete deflation is $F_T=\frac{1}{2}PV=\frac{1}{2} \times 6 \times 980 \times 3500=10.29 \times 10^6$ ergs. If one-fourth of this energy is surface energy (as is approximately the case for the dog of Fig. 1), then $F_s=\frac{1}{4}F_T=2.57 \times 10^6$ ergs. Assuming $\gamma=50$ dynes/cm, $A_s=F_s/\gamma=5 \times 10^4$ cm^2 or 5 m^2 at a lung volume of 3.5 liters. Since the total surface energy may be somewhat larger than estimated above (assuming an error of 100 per cent which is very liberal), the true surface area may lie between 5 and 10 m^2.

The striking difference between the estimate for human lungs of 5 to 10 m^2 by the method discussed in this report and the estimates of 50 to 100 m^2 obtained from histologic measurements, deserves additional comment. None of the assumptions on which the surface energy method depends could account for this difference unless the lung surface were a semi-solid phase or consisted of a highly surface-active substance. Neither of these possibilities is at all likely. On the other hand, some of the problems in interpreting dimensions taken from fixed lung specimens have already been mentioned. For example, Willson(4) and Gertz(5) used dimensions taken from collapsed lungs in their calculations; to obtain the total area they assumed lung volumes of 3000 to 3500 cm^3. These values are much too large—a better range would be 600 to 1000 cm^3(15). If the latter figures had been used, their calculations would have yielded 7 to 20 m^2. Errors in estimates from anatomic measurements may also have arisen because in collapsed lungs the size of the air-sacs and alveolar ducts is grossly distorted, and the alveoli appear to occupy most of the lung volume. The results obtained by the method given in this report are consistent with the idea that in inflated lungs the alveoli are actually sections of spheres superimposed on large air-sacs and alveolar ducts, the volume of the alveoli representing only a small part of the total lung volume.

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Metabolism of Benzoic Acid in Normal and Irradiated Rats.*† (21285)

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In a previous communication, it has been shown that the "free glycine pool" increases in size after rats have been exposed to total body x-radiation(1). These conclusions were based on determinations, before and after irradiation, of the isotope concentration of the urinary hippuric acid excreted after injection of C-14 labeled glycine and benzoic acid. The experimental technic was modified somewhat to test the ability of irradiated rats to conjugate labeled benzoic acid. The conjugation process may be looked upon as a 2-step process involving coenzyme A (Co A) and transesterification at the S-acyl bond(2).

Benzoic acid + acetyl — Co A→Benzoyl-Co A + acetic acid(1)

Benzoyl-Co A + glycine→Benzoyl glycine + Co A(2).

Studies with benzoic acid-carboxyl-C^{14} revealed that radiation did not interfere with hippuric acid synthesis. It was noted also that a marked dilution of the specific activity of urinary hippuric acid occurred in starved animals and this suggested that there must be an endogenous source of benzoic acid. The findings to be reported in this paper indicate that benzoic acid may be the end product of an as yet little explored metabolic pathway of one of the aromatic amino acids, *e.g.*, phenylalanine.

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Experimental. Four of 8 Wistar-type rats, all weighing 150-175 g, were exposed to 700 roentgens of x-rays. Immediately after the irradiation procedure, all were given an intraperitoneal injection of sodium benzoate-carboxyl-C^{14} followed by 2 more injections at 24-hour intervals. The injected solutions contained 18.3 mg of sodium benzoate. The specific activity of the material used in the initial dose was 22,100 c.p.m. per mg carbon and that used in each of the 2 succeeding doses was 18,000 c.p.m. per mg carbon. The rats were placed in confining metabolism cages and urine (without fecal contamination) was collected at daily intervals. The animals were not fed during the 3-day period of study and were not given water except for the 10 ml of saline in which each daily dose of sodium benzoate was dissolved. A description of the animals and irradiation procedure used is given elsewhere(1). The total amount and the specific C^{14} activity of the urinary hippuric acid was determined according to the method of Gaffney and coworkers(3). The C^{14}-activity of the unconjugated urinary benzoic acid was determined by measuring the radioactivity of the appropriate spot on the paper chromatograms used for the assay of hippuric acid. The total quantity of the excreted benzoic acid was not determined chemically.

Results. The data obtained are presented in Table I. Average values as well as ranges