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Vertical gradient of alveolar size in lungs of dogs frozen intact[1]

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GLAZIER, J. B., J. M. B. HUGHES, J. E. MALONEY, AND J. B. WEST. *Vertical gradient of alveolar size in lungs of dogs frozen intact.* J. Appl. Physiol. 23(5): 694–705. 1967.—Dog lungs were fixed in situ by freezing and alveolar size measured by histologic morphometric techniques. In the erect lung at functional residual capacity the apical alveoli were about four times larger by volume than the basal ones, most of the change in size being over the upper 10 cm of lung. The difference between apex and base increased to 11:1 when the animals were exposed to 3 G on a centrifuge. No difference in size was found when the lungs were expanded by 30 cm H_2O pressure. In horizontal lungs, alveolar size was the same at the apex and base, but the most superior alveoli were larger than the dependent ones. In inverted dogs alveolar size was uniform from apex to base. The differences in alveolar volume can be explained if the transpulmonary pressure at any level is determined by the cross-sectional area of the lung and the weight of the lung below that level. This pressure apparently changes more rapidly in the upper part of the erect lung than in the lower part.

acceleration; transpulmonary pressure; pressure-volume curves; gravity; posture

SEVERAL INVESTIGATORS have reported that intrapleural pressure becomes less negative toward the bottom of the erect lung. Measurements of this pressure change in man (3) and dogs (7, 12, 15, 16) have ranged from 0.21 to 0.93 cm H_2O per centimeter distance down the lung, the pleural pressure at the base of a 30-cm lung therefore being at least 6 cm H_2O less negative than that at the apex. It has been suggested by Wood and his co-workers (21) that this vertical gradient of pleural pressure causes regional differences in alveolar size. Evidence to this effect was provided by Milic-Emili and his colleagues (10) who showed in man that at normal lung volumes, the regional volume of the ventilating units as a fraction of their volume at total lung capacity is less at the bottom of the lung than at the top.

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It is clear that in order to demonstrate any histological differences in alveolar size it is essential to fix the lungs in situ. Most previous measurements have been made after removing the lungs from the chest and reinflating them, but this will abolish the gradient of pleural pressure. We have used the technique of freezing whole dogs and measuring alveolar size by modern morphometric methods.

METHODS

Freezing technique. Greyhound dogs of weight 19–30 kg were used because of the large size of their lungs. Anesthesia was induced in the horizontal position with 4–10 ml Pentothal (50 mg/ml) and maintained with Nembutal. An endotracheal tube or tracheal cannula was inserted, and the dogs were then placed in a box insulated with polystyrene foam of internal dimensions 47 x 10 x 14 inches. Dogs frozen in the vertical position were supported by strapping their forelegs at the wrist joint to a bar 43 inches above the bottom of the box. In the inverted position, the hindlegs and body of the dog formed an inverted U shape over the bar. Ten to twenty milliliters of Nembutal (60 mg/ml) were then injected intravenously into all but three of the animals. Respiration ceased within a minute and heartbeat, as monitored by ECG, within 5 min. A cardiac catheter was advanced into the right ventricle of one vertical dog and 10 ml of 10% KCl injected, death ensuing in 13 sec.

The animals were immediately surrounded by solid carbon dioxide (Cardice) broken into small (1–3 inch) pieces from 20-lb. blocks. Approximately 140 lb. of carbon dioxide were placed in the box. One dog frozen in the vertical position and one frozen in the horizontal position were maintained under anesthesia within the box, circulation continuing for up to 45 min. When death occurred as indicated by cessation of the heartbeat on the ECG, body temperature was 28 C. All dogs remained in the ice for 24–48 hr. The airway was always kept open to atmospheric pressure during freezing except when pressure was applied to the lung.

Rate of freezing. The rate of temperature fall was recorded from a thermistor probe passed down the endo-

tracheal tube into the lung in six of the dogs. Figure 1 shows the results in one of the centrifuged dogs. It can be seen that the temperature fell rapidly at first, then much more slowly when it was around 0 C, then rapidly again. The middle slow phase can be attributed to the latent heat of fusion of tissue. As the periphery of the dog cools and freezes, the innermost part comes into temperature equilibrium with the freezing boundary before the boundary arrives (9). The centrally placed thermocouple thus shows a plateau around 0 C until the tissue gives up its latent heat of fusion, freezes, and then rapidly cools to equilibrium with the ice. The average time taken for the central lung temperature to reach 0 C was about 4 hr.

Removal of lung tissue. After being removed from the ice, transverse sections 0.5–0.75 cm thick were cut through the chest of the dogs at 5-cm intervals from the apex to the base of the lungs using a band saw with a coarse blade. The slab of tissue at each level was immediately plunged into liquid nitrogen, where enough lung tissue was removed with a scalpel to provide four blocks approximately 1 x 1 x 0.5 cm. These blocks of tissue were then kept continually in liquid N_2 or in a deep freeze at −20 C until ready for freeze drying.

Freeze drying. Four blocks of tissue were freeze dried together (Speedivac-Pearce tissue dryer, Edwards High Vacuum Ltd.) for 18 hr at a temperature of −20 C and a pressure of 0.015 mm Hg. Trays of phosphorus pentoxide absorbed the water vapor. After freeze drying the tissue may stand in the air for many weeks without rehydrating.

Wax embedding. Within 24 hr of freeze drying the tissue was embedded in wax blocks. For the dogs frozen in the vertical position, the blocks of tissue from the uppermost 15–20 cm of lung were double embedded using celloidin and paraffin wax as follows. The blocks were placed for 3 hr in a 50% ether alcohol solution, then 12 hr in a 2% celloidin solution, then 1 hr in each of two chloroform solutions. Finally, they were vacuum embedded in paraffin wax for 2 hr (0.5 hr at 5 mm Hg vacuum, 0.5 hr at 10 mm Hg, and 1 hr at 15 mm Hg). For these more distended alveoli this technique was found to provide better support than ordinary embedding with consequently less rupture of the walls when the block was sectioned. The blocks of tissue from the lower 10 cm of lung were embedded immediately in Ralwax 1 (R. A. Lamb, London) for 2 hr using the same pressures as above.

Sectioning. The blocks were cut into sections 8 μ thick using a Cambridge rocking microtome. Since the sections from the celloidin-embedded blocks had already been fixed, they were floated out on water at 50 C for 2 min. The unfixed sections were floated out on 6% gluteraldehyde at 50 C for 2 min. Four slides were prepared from each of the four blocks. Three serial sections were placed on each slide and the next 300 μ of tissue were discarded so that there would be no overlap of alveoli from one slide to the next. All sections were stained with eosin.

Shrinkage. Using a dissecting microscope, measurements

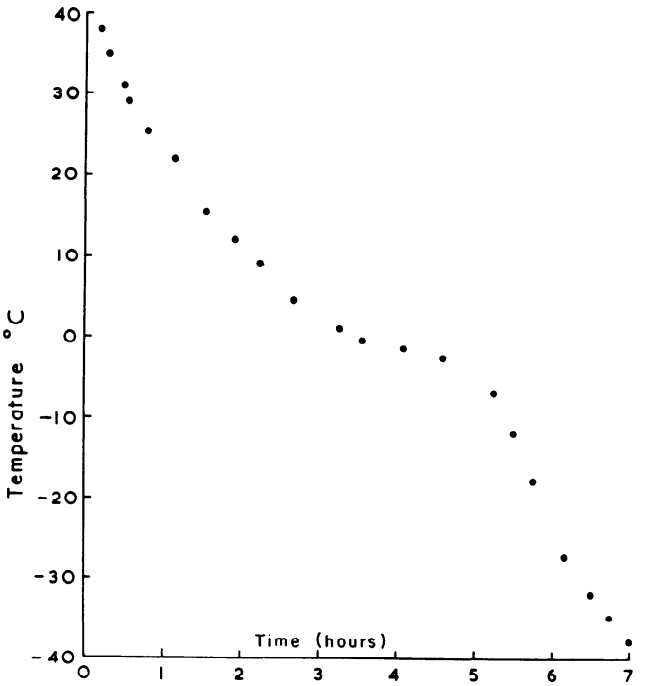

FIG. 1. Temperature fall in a vertical dog at 3 G acceleration as monitored by a thermistor in a main bronchus. Note the plateau in the middle of the tracing caused by the latent heat of fusion.

were made of the length and width of several blocks of tissue from the apical and basal areas of the lungs of dogs frozen in the vertical position. The blocks were measured while immersed in liquid N_2 immediately after removal from the dog, after freeze drying, after wax embedding, and finally when mounted on slides. After freeze drying, the blocks had decreased an average of 8% in width and 6% in length from their dimensions in liquid N_2. A further 1–2% shrinkage in length and width occurred after wax embedding. After microtoming, the length of the section measured on the slide was unchanged, but the width had decreased a further 14%, due to the compressing action of the microtome during sectioning. Any obvious compression of alveoli that was visible on the slides occurred at the edges, and these fields were not included in the counting. No systematic differences between blocks from the upper and lower regions of the lung were found. Note that a 5% reduction in linear dimensions all around will cause about a 15% reduction in volume. The results as reported below have not been corrected for shrinkage.

Counting methods. Alveolar size was determined by morphometric techniques as described by Weibel and his colleagues (17, 19). A microscopic field was projected onto a screen 30 cm^2 using a Leitz Prado microscope slide projector and a grid (Fig. 2) superimposed on the field. The grid consists of 21 test lines equivalent to 100 μ long at the magnification used, with one point located at the ends of each line (see Fig. 5A, reference 19).

The relative volume of the lung occupied by alveoli is equal to the percentage of points falling within alveoli (alveolar hits). To decide whether a point lay within an

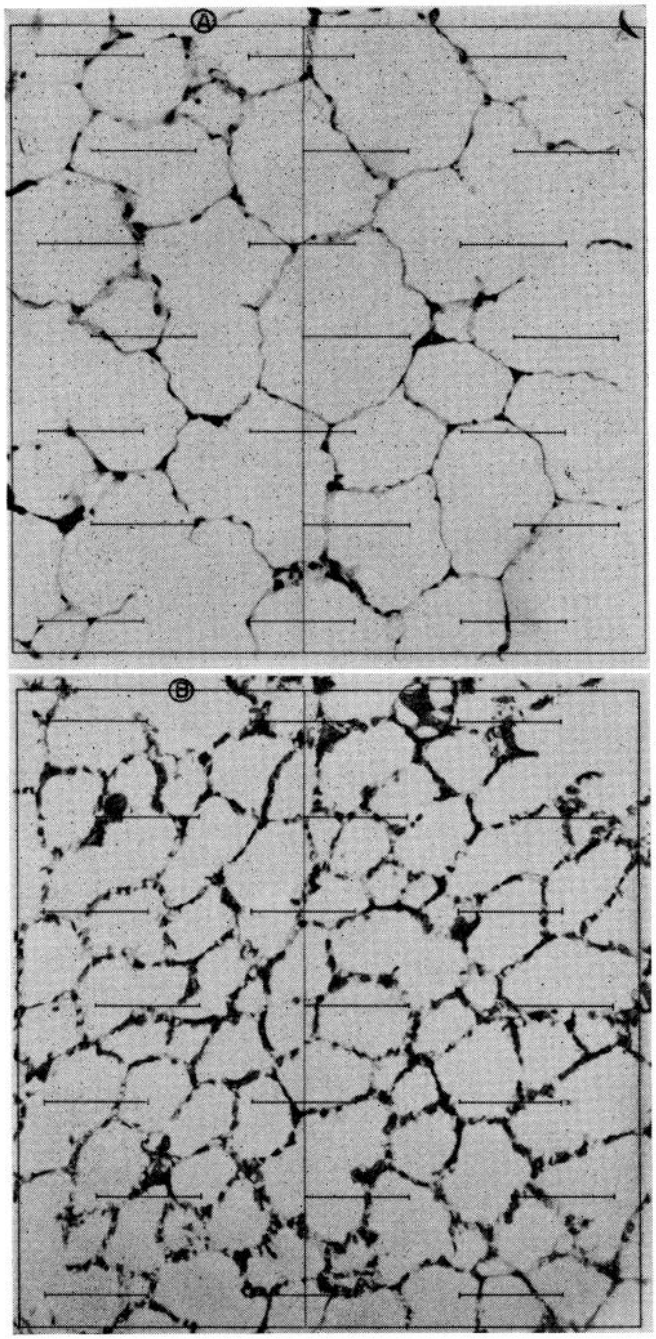

FIG. 2. Sections of lung from *A* apex and *B* 20 cm lower down the lung of a greyhound dog frozen in the vertical position. The grid used for determining alveolar size is superimposed on the fields. Each of the test lines is equivalent to 100 μ.

alveolus or not, an imaginary line was drawn across the open mouth and any point lying within this area was regarded as an alveolar hit. The surface area of the alveoli is proportional to the number of times the test line intersects the alveolar walls, and inversely proportional to the length of the lines. The volume-to-surface (V/S) ratio of the alveoli may then be calculated from:

$$V/S = \frac{\text{length of test lines} \times \text{no. of alveolar hits}}{4 \times \text{alveolar wall intersections}}$$

Two intersections are recorded each time the test line crosses an alveolar septum because this includes two alveolar walls.

Since volume is proportional to the cube of a linear dimension (radius) and surface to its square, V/S is proportional to the radius (r) of the alveolus. Thus $(V/S)^3$ is proportional to volume and alveolar volume may therefore be compared at different levels in the lung.

It is not possible to determine the absolute volume of the alveoli from V/S because the shape of the alveoli is not accurately known. If the alveoli were spherical, V/S would equal $\frac{4}{3}\pi r^3/4\pi r^2 = r/3$. Alveolar volume would then be given by $\frac{4}{3}\pi r^3$, or $\frac{4}{3}\pi(3V/S)^3$, or $36\pi(V/S)^3$. Thus for spherical alveoli, the alveolar volume could be obtained in absolute units.

However, as the shape of the alveolus is not spherical, it is not possible to present the volumes in absolute units. The relationship between V/S and alveolar volume depends on an unknown shape factor. Nevertheless if alveolar shape is the same from animal to animal, comparisons of alveolar size from one to another are valid.

The comparison of alveolar volumes down the lung is also only valid if the shape of the alveoli remains the same. In RESULTS, we show that upper and lower zone alveoli are isotropic; that is, equally expanded in all directions, and this shows that alveolar shape does not change by flattening. Furthermore, inspection of sections from different parts of the lung fails to show any difference in shape.

In two of the dogs frozen in the vertical and two frozen in the horizontal position, alveolar size was also compared by counting the number of alveolar transections (that is, each whole alveolus as cut in a plane) within the grid. Knowing the grid area, the number of alveoli per cubic millimeter of lung parenchyma could be calculated (17) from:

$$\text{No. of alveoli} = \frac{1}{k} \times \frac{(\text{no. of transections in unit area parenchyma})^{3/2}}{(\text{volumetric fraction parenchyma occupied by alveoli})^{1/2}}$$

where k is a shape factor which cancels out when the alveoli at one level are compared with those in another.

Agreement between the two methods was reasonably good (4) and increased our confidence in both. However, the transection method is more liable to subjective error than the first and all data reported here are therefore based on the linear intersection method.

Sampling. A transparent grid 1 ft square consisting of 100 lines in each of the vertical and horizontal directions was laid over the projection screen and a low-power magnification ($\frac{1}{7}$ that usual for counting) of the slide

was projected. Fields to be counted were selected from a four-digit table of random numbers, the first two corresponding to the vertical axis of the grid and the second two to the horizontal axis. The area to be counted was moved to the center of the grid and the magnification increased for counting. A field was not used if it fell within five lines of the edge of the slide, if it contained a blood vessel or bronchus greater than 100 μ in diameter, or if more than 25 % of the field was occupied by artifact. Twenty-five fields were counted at each level with six fields coming from each of four blocks of tissue.

Statistical analysis. Alveolar volume was computed for each of the 25 fields counted and from these the mean alveolar volume at that level was calculated. Using 25 fields, the standard error of the mean ranged from 4 to 10% with the greater variation occurring at the apex where fewer intersections were counted because the alveoli were larger. The data from the five vertical dogs were combined at similar levels. Thus 125 fields were analyzed at the apex, 150 at the base (measurements made in one dog at 24 and 28 cm below the apex were both included), and 75 fields at 5, 10, 15, and 20 cm below the apex.

The Cochran modification of Student's t test was used to compare alveolar size at one level of the vertical dogs with a similar level in another dog when the variances of the populations were very different (13). For other comparisons Student's t test was used.

Postures and conditions during freezing. A total of 24 dogs were studied. Five were frozen in the vertical (head up) position at FRC. In three of these, tissue was taken for analysis at 5-cm intervals from apex to base, and in the other two only apical and basal sections were counted. Three dogs were frozen in the horizontal position with the right side lowermost (right lateral), one dog was supine, and one prone. The lung volume for each dog was that at the end of a normal expiration. Tissue was taken from apex and base in the same horizontal plane. Other sections were taken from the most dependent area of the lung and from an area 8–13 cm superior to it.

Two dogs were frozen in the inverted position (head down) at FRC and one was frozen inverted with 10 cm H_2O positive pressure applied to the trachea through a tracheostomy. The constant pressure was obtained from a compressed air tank and a line provided with a T piece immersed to the appropriate depth in water. The pressure was maintained for 24–48 hr which was long after the lung had frozen. Four dogs were frozen in the vertical position with positive inflation pressures of 5, 10, 20, and 30 cm H_2O applied to the lung. Two vertical dogs were subjected to a negative tracheal pressure of 10 and 20 cm H_2O, respectively. One dog was frozen in the vertical position after a 6-inch-wide abdominal bandage had been placed around the abdomen to simulate the effect of a pressure G suit. Two vertical dogs were bled to death from a femoral artery or vein before freezing to reduce the density of the lung tissue.

Two dogs were exposed to headward acceleration

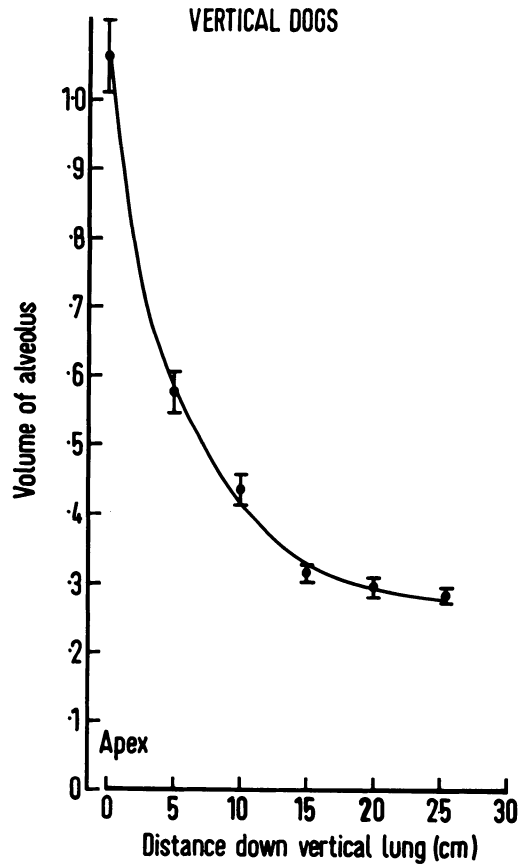

FIG. 3. Average volume of the individual alveoli at 5-cm intervals from the apex to base of the vertical (head up) lung. Measurements are from five dogs. The mean values with their standard deviations are shown.

($+3G_z$) on the human centrifuge (radius 30 ft) at the Royal Air Force Institute of Aviation Medicine, Farnborough. One of these dogs had an abdominal binder applied. The anesthetized dogs were placed in the insulated box and killed with Nembutal. The box and dog were weighed and then positioned in the car of the centrifuge. A weighed quantity of Cardice was then added to fill the box and the centrifuge started. The times from death to the beginning of acceleration were 35 and 55 min, and the times from adding the Cardice to acceleration were 15 and 5 min. The dogs were centrifuged for 8 hr by which time the temperature of the thermistors in the lung had fallen to -38 and -17 C, respectively. The weight of the Cardice in the box decreased 54 lb. in one experiment and 48 lb. in the other.

Lung volume. In 11 of the 24 dogs, chest radiographs were taken in order to measure any changes in lung volume which occurred after death or during freezing. In most dogs, anteroposterior (AP) and lateral radiographs were taken of the anesthetized animal at the end of a normal expiration. The dog was then killed with Nembutal and more radiographs were taken. A third set was taken after freezing.

Radiologic chest volume (RCV) was determined by

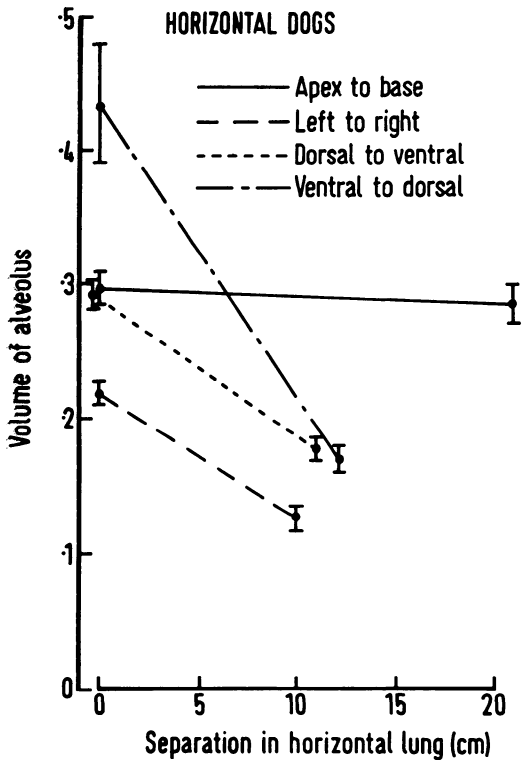

FIG. 4. Alveolar volume at the apex and base and superior and dependent regions from five horizontal lungs. Mean volumes from four dogs for apex to base measurements, from three dogs for left to right measurements, and one each for the other two.

the technique of Cobb et al. (2). Although this procedure has only been validated for the human lung, it is reasonable to expect it to demonstrate changes in lung volume in the dog. A line was drawn around the lungs including the mediastinum on the AP view and the area measured with a planimeter, duplicate determinations agreeing within 2%. This area was multiplied by the largest AP diameter on the lateral view to give RCV. In the six dogs not subjected to abnormal pressures, the average increase in RCV after death was +8% (range −1 to +15%). There was no further systematic change after freezing, the average decrease being 1% (range −7 to +3%).

Chest circumference was also measured at three levels immediately after death and again after freezing. No systematic differences were found and most of the measurements agreed to within 1 cm.

Alveolar size in isolated lung. On two occasions, the left lung was removed from a greyhound, suspended in a Lucite box, ventilated with negative pressure, and perfused with its own blood. The outside of the box was then removed and ventilation continued with positive pressure.

A circular brass tube 28 cm long and 0.75 cm in diameter, pierced by 1-mm holes at 0.5-cm intervals from top to bottom, was positioned vertically about 1 cm away from the lung. The tube was connected to a funnel above it and 2 liters of liquid Freon 12 at −145 C were then poured into the funnel over a period of 30 sec. In this way, a 30-cm strip of lung was rapidly frozen to a depth of 3–5 mm.

The whole lung was then plunged into liquid N_2 and pieces of the frozen strip removed at 5-cm intervals. These blocks of tissue were then processed as described above. The two lung strips were frozen at transpulmonary pressures of 10 and 23 cm H_2O, respectively, and alveolar size determined at 5-cm intervals from apex to base.

RESULTS

Vertical lungs. Figure 3 depicts the volume change at 5-cm intervals down the lung. The apical alveoli were, on the average, 3.7 times larger than the basal alveoli. The major part (over 80%) of the gradient of alveolar size lay in the region between the apex and 10 cm lower, with only a relatively small change over the next 15 cm. In the two dogs in which alveolar volume was measured by the transection method, the average gradient from apex to base was 3.4:1.

Horizontal lungs. Figure 4 shows the results of measurements made on the five dogs frozen in the horizontal position. Alveolar size was similar at the apex (0.295) and base (0.284), but in the superior region was significantly greater ($P < 0.001$) than in the dependent region whether the dog was supine, prone, or with the right side lowermost. Note that the gradient of alveolar size was greatest (2.6:1) in dogs in the supine position where the vertical separation of the sections was the largest. In the two horizontal dogs in which alveolar transections were counted, the average gradient from apex to base was 1.01:1.

Isolated lungs. In order to determine whether a vertical gradient of alveolar size exists when the lung is everywhere exposed to the same transpulmonary pressure, measurements were made on two isolated lungs at transpulmonary pressures of 10 and 23 cm H_2O after they had been rapidly frozen as described above. After being inflated by a negative pressure of −23 cm H_2O, the lungs were ventilated by a negative pressure between −6 and −10 cm H_2O. One lung was then held at a pressure of −10 cm H_2O and immediately frozen; the second was inflated to a negative pressure of −23 cm H_2O, held, and frozen. It was found that alveolar size did not differ significantly from apex to base in either lung.

At a transpulmonary pressure of 10 cm H_2O, alveolar volume was 0.366 ± 0.036 at the apex and 0.343 ± 0.024 at the base. At a transpulmonary pressure of 23 cm H_2O, the volumes were 0.948 ± 0.093 at the apex and 0.981 ± 0.084 at the base. The latter volume is similar to that obtained at the apex of the intact vertical dogs and is evidence that these apical alveoli are virtually fully expanded (see Fig. 10).

Reduced lung volume. In the two dogs subjected to negative alveolar pressures of 10 and 20 cm H_2O, 360 ml and 900 ml, respectively, of gas were removed from the lungs below FRC. In the dog exposed to 20 cm

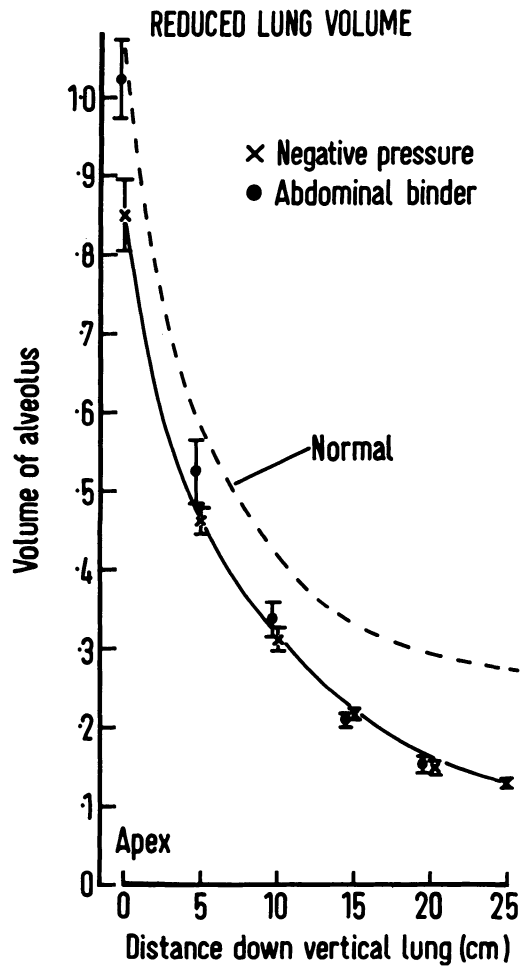

FIG. 5. Alveolar volume in two dogs frozen while subjected to −10 and −20 cm H_2O alveolar pressure. The results at each level were pooled (shown by x) and a line of best fit was drawn. The results obtained in the dog wearing an abdominal binder are also shown. The results were not significantly different except at the apex and 5-cm levels.

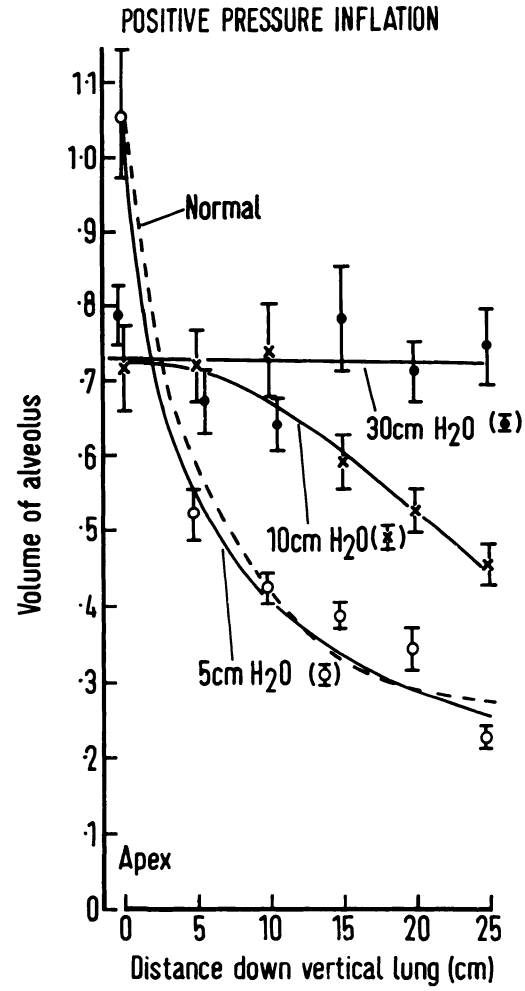

FIG. 6. Alveolar volume in dogs frozen while subjected to 5 cm (○), 10 cm (×), and 30 cm H_2O (●) positive-pressure inflation. In this last dog, there was no significant difference in volume between apex and base.

H_2O negative pressure, radiologic chest volume (RCV) decreased 22% below FRC when the negative pressure was applied, and this volume was maintained after freezing. Alveolar size at comparable levels was similar in both dogs and the measurements were combined (Fig. 5). The alveoli were smaller than in the normal lungs at every level (apex and 5 cm below the apex, $P < 0.005$; 10–25 cm below the apex, $P < 0.001$). The basal alveoli (25 cm below the apex) were still 1.5 times larger than the basal alveoli in the centrifuged dog with abdominal binder (see Fig. 8).

In one vertical dog the effects of abdominal compression on alveolar size were determined by wrapping a 6-inch cloth bandage as tightly as possible around the abdomen, beginning at the upper border of the pelvis and extending to the lower border of the rib cage. This maneuver caused an appreciable rise in the level of the diaphragm and RCV averaged 14% below FRC. The volume was maintained during freezing. Alveolar size (Fig. 5) was decreased in all levels except at the apex and 5-cm level where the volume of the alveoli was not significantly different from normal. The alveoli were significantly smaller at 10 cm below the apex ($P < 0.005$) and 15–20 cm below the apex ($P < 0.001$). Except at the apex and 5 cm below this, alveolar size was similar to that in the dogs exposed to negative alveolar pressure.

Positive-pressure inflation. In four dogs exposed to positive alveolar pressures of 5, 10, 20, and 30 cm H_2O during freezing, alveolar size became progressively more uniform. With 5 cm H_2O positive pressure, 250 ml entered the lung and radiologic chest volume (RCV) barely changed (+7% above FRC); with 30 cm H_2O positive pressure, 1,400 ml entered the lung and RCV increased by 69% above FRC.

Alveolar szie was not significantly different from normal at 5 cm H_2O inflation (Fig. 6), but at 10 cm H_2O inflation, the vertical gradient of alveolar size had decreased from the normal value of 3.7:1 to 1.7:1 ($P < 0.001$). At 20 cm H_2O inflation, the gradient had decreased to about 1.3:1 although the measurements showed a large amount of scatter. At 30 cm H_2O the

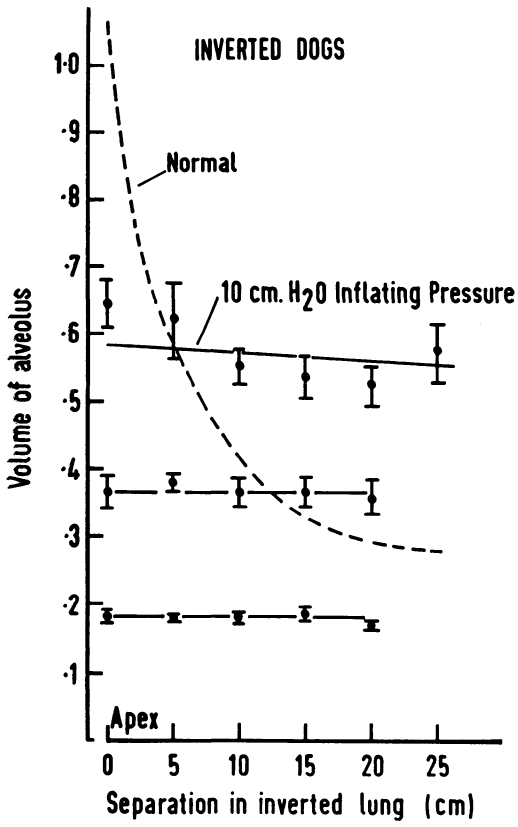

FIG. 7. Alveolar volume in three dogs frozen in the inverted (head down) position. 10 cm H_2O positive inflating pressure was applied to the trachea of one of the dogs during freezing. Note that there were no differences in alveolar size down the lung.

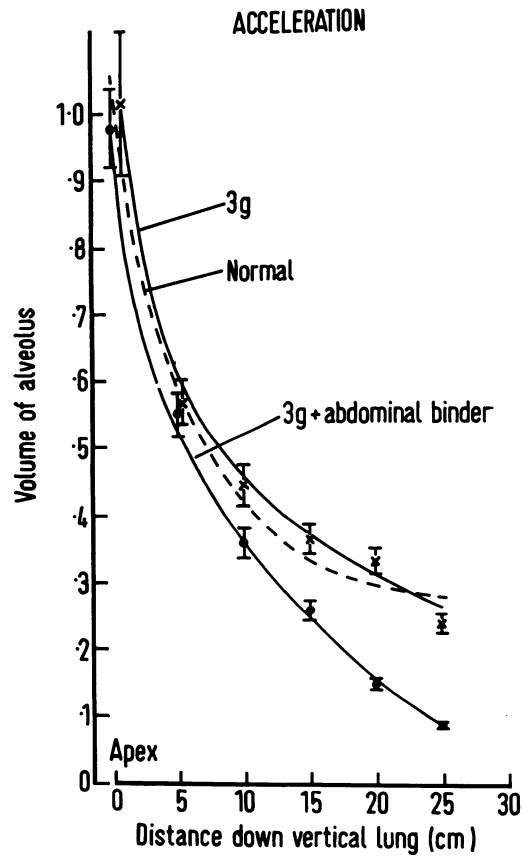

FIG. 8. Alveolar size in two dogs frozen during 3 G headward acceleration ($+3G_z$). One dog was wearing an abdominal binder to simulate the effect of a pressure G suit.

gradient was abolished, there being no significant difference between the alveoli at the apex and base.

A puzzling finding was that the volume of the extreme apical alveoli became significantly smaller with positive-pressure inflation. This presumably means that the pattern of lung expansion produced by active contraction of the respiratory muscles is different from that occurring when the lung and chest wall are passively distended by positive internal pressure. Certainly, the alteration in the shape of the chest wall under the two conditions of expansion may be different (1). A possible explanation of the reduction in volume of the apical alveoli is given later.

Inverted lungs. In the three dogs frozen in the inverted position, alveolar size was uniform from apex to base of the lung (Fig. 7). The RCV at end expiration of the dog with the smallest alveoli was 77% of the average RCV of the upright vertical dogs; their weights were comparable. In this inverted dog, the alveoli were smaller than those at the 25-cm level in the normal vertical dogs but larger than the alveoli at the 25-cm level in the dogs exposed to negative pressure or accelerated on the centrifuge. It is unlikely therefore that their size was uniform because they were at minimal volume. With 10 cm H_2O inflating pressure, the alveoli were of comparable size to those at the 5-cm level in the normal upright dogs.

Effect of increased G. Figure 8 shows the alveolar size in the lungs of two dogs exposed to a headward acceleration of 3 G during freezing. The mean values for the volume of the alveoli in the accelerated dog (without the abdominal binder) were larger than those in the normal vertical dogs at all levels except the apex and 25 cm below the apex, but the differences were not significant. Radiographs showed that there was descent of the diaphragm as a result of the acceleration with a consequent increase in lung volume. Since a larger lung volume itself reduces the differences in alveolar size (Fig. 6), it is probable that any tendency of the acceleration to exaggerate the vertical gradient was nullified by this increase in lung volume.

In the dog with an abdominal binder in place (analogous to a G suit), downward descent of the diaphragm was prevented and a striking exaggeration of the normal differences in alveolar size was observed. Alveolar volume at the base was approximately 11 times smaller than that at the apex, compared with the normal gradient of 1:3.7. The volume of the apical alveoli and those 5 cm below did not change significantly compared with the normal vertical dog, but volumes were significantly smaller at

the 10- and 15-cm levels ($P < 0.025$), and 20- and 25-cm levels ($P < 0.001$). The alveoli at the 25-cm level were the smallest found in any experiment, and consideration of the pressure-volume relationships of isolated dog lungs suggests that they were at their minimal volume (see DISCUSSION).

Exsanguinated lungs. In order to study the effect of changing lung density on alveolar size, two dogs were bled before freezing, 2,150 and 1,500 ml of blood being removed. Figure 9 shows that the alveoli were significantly larger than those of the normal vertical dogs at the 15- and 20-cm levels ($P < 0.005$) and 25-cm level ($P < 0.025$).

Measurements of the density of the frozen tissue confirmed that this had been reduced by bleeding the dogs. Transverse sections approximately 3 cm thick were taken at 5-cm intervals from apex to base and a rectangular block of tissue was cut from each and weighed. Its dimensions were then measured with a caliper. A second measurement of the volume of the block was also obtained by pouring cold, fine grain sand into the space left by the block. The two volumes thus obtained agreed within 10% and the two density measurements obtained were averaged.

In a normal vertical dog, lung density (g/ml) was found to increase from apex to base, the values being 0.10–0.12 from 3–10 cm below the apex, 0.17–0.18 from 13–20 cm below the apex, and 0.22 from 25–57 cm down. By contrast, lung density was nearly uniform down the lung of an exsanguinated dog being 0.10 over the distance 3–20 cm and 0.13 at 25–27 cm below the apex. The measured densities in the normal vertical lung are presumably lower than during life because blood drains from the lung after death. In one normal anesthetized vertical dog, we measured the mean pulmonary artery pressure before and after death. The maximum height of the lung was approximately 28 cm, and pulmonary artery pressure referred to the lowermost aspect of the lung was 31 cm H_2O before death. It fell to -5 cm H_2O after death. By contrast, in one of the inverted dogs (lung height 22 cm), pulmonary artery pressure referred to the lowermost aspect of the lung (apex) was 14 cm H_2O before death rising to 22.5 cm H_2O after death, presumably because of drainage of blood down into the heart.

Comparison of hilar and peripheral tissue at a given level. Because it has been suggested from time to time that hilar and peripheral regions of the lung expand to different degrees during ventilation (6), we separately removed tissue from the hilar and peripheral regions of lung slices in one normal vertical dog and from a second vertical dog inflated by 10 cm H_2O positive pressure. No significant differences in the size of the alveoli were found. For example, in the normal vertical dog at the 20-cm level alveolar volume at the hilar region was 0.379 ± 0.022 and at the periphery of the lung it was 0.376 ± 0.022. At the 10-cm level of the lungs inflated by positive pressure, alveolar volume in the hilar region

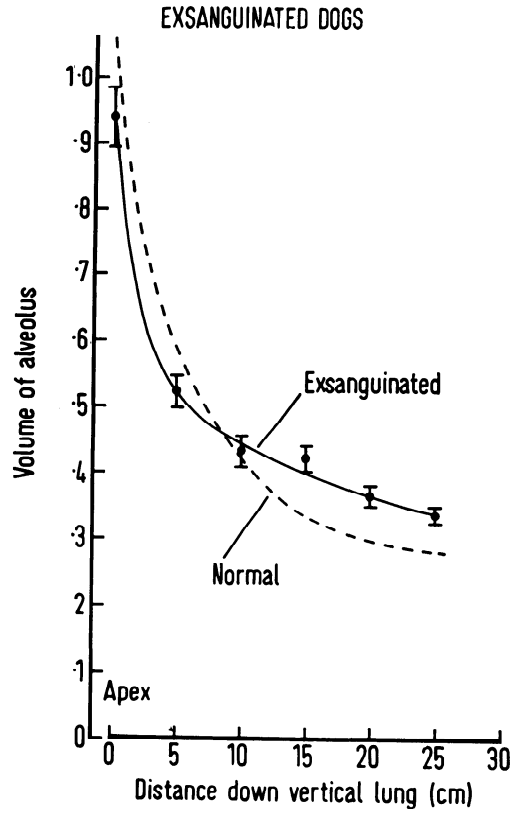

FIG. 9. Alveolar volume in two dogs frozen after being bled to death in the vertical position. The lung density was reduced by bleeding and the vertical gradient of alveolar size was less than in normal dogs (compare Fig. 3).

was 0.744 ± 0.068 and at the periphery, the volume was 0.740 ± 0.062.

Shape of alveoli. In order to determine whether the alveoli were expanded equally in all three dimensions, wax-embedded blocks of tissue taken 5 cm and 25 cm below the apex of a vertical dog were sectioned in two planes at right angles to one another. Fifty fields were counted, twenty-five with test lines running across the field (as in Fig. 2) and twenty-five after rotating the grid 90° so that the test lines were vertical. In this way, any asymmetry of alveolar shape (anisotropicity), such as squashing, would be disclosed.

The results showed no evidence of any anisotropicity. At the 5 cm below the apex level, the four means of the measurements of alveolar volume (with their standard errors) were 0.648 ± 0.038, 0.643 ± 0.030, 0.657 ± 0.030, and 0.630 ± 0.029. At the 25-cm level the measurements were 0.271 ± 0.014, 0.272 ± 0.010, 0.273 ± 0.014, and 0.276 ± 0.012.

A test for homogeneity using an analysis of variance showed that at each level there was no significant difference between the four populations of alveoli. In addition, inspection of the slides of lung taken at different levels showed no evidence of change of alveolar shape. We concluded that the alveoli were expanded equally in all planes and were symmetrical in shape.

Relative expansion of alveolar ducts and alveoli. In order to

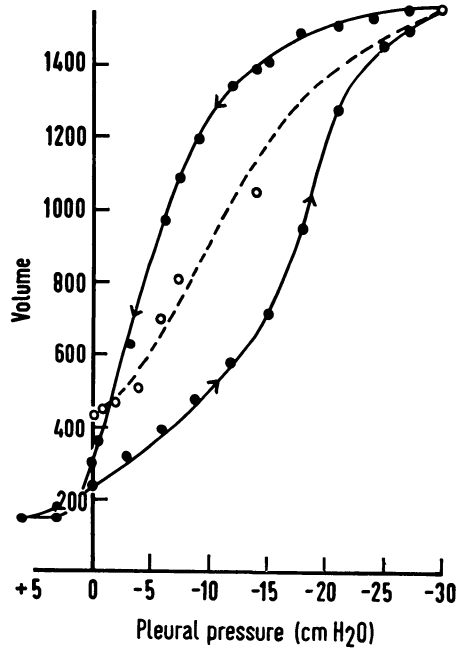

FIG. 10. Pressure-volume diagram of the left lung of a greyhound dog. Inflation and deflation limbs are shown. The broken line shows the computed in vivo pressure-volume relationship within the lung. This was obtained from the observed differences in alveolar size at various levels and the calculated differences in pleural pressure.

determine whether the vertical gradient of alveolar size was accompanied by a similar change in the size of alveolar ducts, we measured the relative volume of lung tissue occupied by alveoli and alveolar ducts at the apex and 25 cm below this in two normal vertical dogs. Relative volume was determined by the point-counting technique in 25 fields at each level magnification being 40% of that used for the measurements of volume-to-surface ratio. Three fields were counted on each slide. The ratio of alveolar ducts-to-alveoli at the apex and 25-cm level in one dog was 10.78 $\pm$ 1.01% and 11.57 $\pm$ 1.10%. At comparable levels in the other dog, the figures were 8.87 $\pm$ 0.08% and 9.30 $\pm$ 0.07%. Since the volume of lung parenchyma occupied by alveolar ducts and alveoli did not differ significantly from apex to base in either dog, we conclude that the alveolar ducts expanded at the same rate as the alveoli. This confirms earlier measurements of Storey and Staub (14). The proportion of lung parenchyma occupied by alveolar ducts is lower than that reported for man by Weibel and Gomez (18). The reason for this discrepancy is not clear, but it may be that some spaces which were alveolar ducts were thought to be artifacts during counting and were therefore not included in the measurements.

DISCUSSION

Historical. One of the earliest recorded investigations of alveolar size was made on the calf's lung by Hales in 1731 (5). He reported that a middle-sized alveolus had a diameter of $\frac{1}{100}$th part of an inch and that the total alveolar surface area was 40,000 square inches. Numerous determinations have been made since then without mention of a systematic regional difference in size. Macklin and Hartroft (8) noted no difference in size when peripheral and central alveoli from small animal lungs appeared on the same slide, but speculated that "in large animals at least, alveoli may differ in size at various depths within the lung." The lungs previously examined have, for the most part, been fixed while exposed to atmospheric pressure; under these conditions, alveolar volume would be expected to be uniform, as it is in the isolated dog lung after rapid freezing.

In attempting to explain the sites of deposition of pleural pigment in tuberculosis, Orsos (11) in 1912 predicted that with respiration the uppermost alveoli would stretch to a much greater extent than the lower ones. He assumed a homogeneous elastic lung model having a smaller cross-sectional area at the apex than the base. The forces stretching the lung in the vertical direction therefore caused more elongation in the uppermost tissue. In essence, this model anticipates our explanation below for the vertical gradient of alveolar size.

Errors in the method. Ideally, any measurement of alveolar size should be carried out on the lung during life. Failing this, the lung should be fixed in situ during life or as short a time after death as possible. In the present series of experiments, freezing occurred some 4–6 hr after death and the possibility that the gradient of alveolar size was affected by the mode of death or the effect of cold on the lung after death should be examined.

The mode of death appears to be unimportant because changes in this did not affect the results. Thus in one dog, the heart was stopped within 13 sec of the injection of 10 ml of 10% potassium chloride through a cardiac catheter in the right ventricle and respiration also stopped within seconds of this. Two other dogs were maintained under anesthesia while surrounded by Cardice with the endotracheal tube brought outside the box. Circulation continued for up to 45 min and body temperature fell to 28 C before death occurred. In all of these animals, the results of the measurements of alveolar size were very similar to those from the dogs who died following intravenous barbiturate.

In order to determine whether the cooling process itself affected alveolar size, we compared the behavior of two excised lung lobes each suspended by a glass cannula tied into the bronchus. Each lobe was inflated by a constant airway pressure of 10 cm H_2O; one was surrounded by room air at a temperature of 20 C, while the other was surrounded by air kept at -70 C by means of a box of Cardice. The outside dimensions of each lobe were measured with calipers for up to 12 hr by which time the cold lobe had frozen solid. All dimensions showed a decrease of approximately 3%; there was no systematic difference between the warm and cold lobes. We concluded from these experiments that the volume of lung held at a constant pressure does not change during slow freezing.

The histological sections (Fig. 2) show that the alveo-

lar architecture was well maintained during the slow freezing though there was some damage at the cellular level. This is caused by ice crystal formation which inevitably accompanies slow freezing. Comparison of these sections with others obtained by rapidly freezing isolated, perfused lung by pouring liquid Freon gas cooled to −145 C over it shows general agreement. Thus the alveolar septa are of similar thickness at comparable heights in the lung, being very thin at the apex where there are few red cells in the capillaries and thicker at the base of the lung where the capillaries are engorged.

Gradient of alveolar size. Further confidence in the measurement of alveolar size is obtained if we compare the results from the slowly frozen dogs with observations on quick-frozen isolated lungs and the pressure-volume characteristics of excised lungs. It will be shown that our hypothesis to account for the vertical gradient of alveolar size predicts a transpulmonary pressure of some 30 cm H_2O at the apex of the vertical dog lung. In this region, the mean volume of the alveoli was 1.063 ± 0.052 and the mean volume measured from an isolated lung expanded by a transpulmonary pressure of 23 cm H_2O was 0.948 ± 0.093 at the apex and 0.981 ± 0.084 at the base. Bearing in mind the shallow slope of the pressure-volume curve at these high transpulmonary pressures (Fig. 10), the agreement is very good.

The volume of the alveoli at the base of the lung from the dog exposed to 3 G while wearing an abdominal binder fits well with the minimal volume of alveoli in a freshly excised dog lung. Figure 10 shows a typical pressure-volume curve obtained on the left lung of a greyhound dog suspended vertically in a Lucite box and perfused with its own blood. The alveolar pressure remained at atmospheric and the pleural pressure was initially + 6 cm H_2O above atmospheric pressure. It was then reduced 3 cm H_2O at a time to −30 cm H_2O and then returned in 3-cm H_2O steps to +6 cm H_2O. Each pressure was held for 3 min.

When no more air was expelled from the lung by increasing the pressure around it, the trachea was clamped and the volume of air plus tissue was measured by water displacement. The lung was then degassed in a vacuum jar and the volume of the tissue again measured by water displacement. The difference between the two measurements was 150 ml which was taken to be the minimal gas volume. The maximum volume (at a transpulmonary pressure of 30 cm H_2O) was 1,565 ml so that the ratio of maximum-to-minimum volumes was 10.4:1. In the centrifuged dog (with abdominal binder) the ratio was 11.3:1 so that the agreement is good. Thus the maximum and minimum volumes for alveoli found in the slow-frozen dogs agree well with the extremes of volume found in excised dog lungs.

The measurements reported here confirm those of Milic-Emili and his colleagues (10) who showed that the regional volume of the ventilating units as a percentage of their volume at TLC (Vr/TLC_r) is greater at the top than at the bottom of the lung in man. These investigators found that in man at FRC, Vr/TLC_r was 2.1 times greater in the upper regions of the lung compared with the lower. If the upper counters were 3–4 cm below the top of the lung, this result would fit well with our measurements on the vertical dogs (Fig. 3). However, our results show that the vertical gradient of alveolar size is very alinear rather than linear with distance as may be inferred from the measurements of Vr/TLC_r.

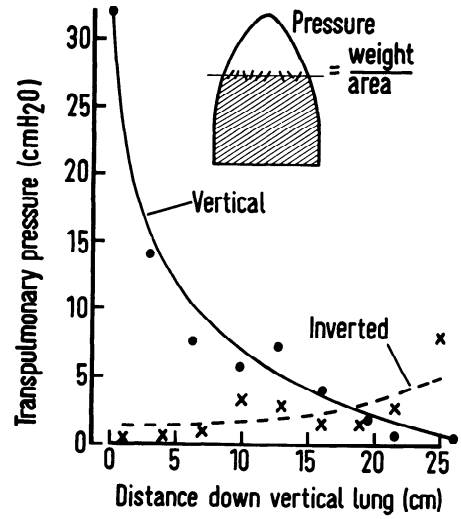

FIG. 11. Transpulmonary pressure from apex to base of an upright (●) and an inverted (×) lung as calculated from measurements of weight/area. Note that in the upright lung the vertical gradient of pressure is very large, whereas in the inverted lung the regional differences are small.

Cause of the difference in alveolar size. To account for the alinear gradient of alveolar volume down the vertical lung, we have chosen a model which divides the lung into horizontal planes, each one supporting the weight of the lung below it (inset of Fig. 11). If there are n fibers in a plane and each transmits a force f, the total force is F = fn or W the weight. The corresponding alveolar expanding pressure is F/area or W/A. At the apex, for example, where the cross-sectional area is very small but weight of the lung below is greatest, W/A and hence the expanding pressure will be large. In order to test this hypothesis, we measured the cross-sectional area of the lung at various levels and the weight of the lung below each level on one vertical and one inverted dog. Transverse sections approximately 3 cm thick were cut through the chest from the apex to the base of the lung. The cross-sectional area at each level was measured by tracing over the lung outline and measuring the area with a planimeter. The frozen lung was then shelled out of the 3-cm cross section with a scalpel and weighed. The total weight of the lung below each cross-sectional area at the various heights was thus recorded.

From the measurements of cross-sectional area and the weight of the lung below it, we calculated W/A in g/cm^2 at approximately 3-cm intervals from apex to base of the vertical and inverted lungs. W/A in g/cm^2

was taken to be equal to the transpulmonary pressure in cm of H_2O and the transpulmonary pressure obtained in this way is plotted against distance down the lung in Fig. 11 for both postures.

If these regional differences in transpulmonary pressure are responsible for the differences in alveolar size, it should be possible to predict the change in size knowing the pressure-volume relationships of the lung. Unfortunately it is difficult to know what pressure-volume curve to take since this depends very much on the previous volume history (Fig. 10). We have therefore taken the observed differences in alveolar size and related them to the calculated vertical gradient of transpulmonary pressure to derive the in vivo pressure-volume curve of the lung. The largest alveoli were taken to be maximally expanded, see *Isolated lung* in RESULTS section. The results of these calculations are shown by the open circles and interrupted line in Fig. 10, and it can be seen that it lies about midway between the inflation and deflation pressure-volume curves measured in an excised lobe. We conclude from this that the weight/area hypothesis to explain regional transpulmonary pressure is compatible with the observed differences in alveolar size in the upright vertical lung.

If we now turn to the calculated gradient of transpulmonary pressure for the inverted lung, we see that with the exception of one point at the extreme base of the lung, the pressure everywhere is less than 4 cm H_2O. This clearly predicts very little difference in alveolar size down the lung which is precisely what we observe (Fig. 7). Thus the weight/area hypothesis not only fits the surprisingly large gradient of alveolar size in the vertical lung but also the unexpected absence of a gradient in the inverted lung.

The hypothesis also explains the effects of exsanguination and acceleration. Thus if lung density is reduced by bleeding, the gradient of transpulmonary pressure is less marked and there are smaller differences in alveolar volume down the lung (Fig. 9). By contrast, headward acceleration increases the effective weight of the lung and thus the difference in alveolar size, as long as descent of the diaphragm and the corresponding increase in lung volume are prevented by the abdominal binder.

It should be noted that in the calculations of the gradient of transpulmonary pressure on the weight/area hypothesis, we have assumed that the lung is supported only from above. In fact, it is likely that under some conditions, support is provided from below with a corresponding reduction in transpulmonary pressure at the apex. It is probable that this situation occurs during headward acceleration when the descent of the diaphragm is prevented so that the pleural pressure at the base of the lung exceeds atmospheric pressure. This would explain why the basal alveoli are at their minimal gas volume. By contrast, the basal alveoli in the normal vertical lung are about three times their minimal volume. The dependent alveoli in the horizontal lung are somewhat smaller (Fig. 4). There is evidence that the lung is not supported by the hilum inside the chest. Observations of the shape of the lung of a rhesus monkey inside and outside the chest have shown that the main stem bronchus does not take any of the weight of the lung in situ (20).

It is possible that the upward support afforded by the diaphragm under some circumstances explains the apparently analogous finding of the reduction in volume of apical alveoli in the vertical lung when positive pressure is applied to the airways (Fig. 6). It could be argued that with expansion of the lower regions of the lung against the diaphragm some parenchyma is pushed up into the extreme 2–3 cm of the apex of the thoracic cavity. In any event, it seems certain that the mode of expansion of the lung under these conditions of passive distension is different from that produced by active contraction of the chest wall muscles.

The calculated gradient of intrapleural pressure (Fig. 11) is very alinear and is consistent with Turner's (16) observation that the pleural pressure gradient is more marked over the upper two-thirds of the lung. However, Krueger and his colleagues (7) found a steeper gradient of pleural pressure in the lower regions than in the upper regions of the lung of the vertical dog. The total change in pressure reported here is approximately 30 cm H_2O over 25-cm distance or 1.2 cm H_2O/cm distance down the lung. However, the gradient calculated from a point about 5 cm below the apex to the base is 0.55 cm H_2O/cm distance which is similar to the direct measurements of Rutishauser (12). The exact gradient in these studies would depend on how close to the apex the manometer was actually positioned. It should be remembered that the vertical dog lung is much narrower than the human lung where the same weight/area hypothesis would result in a less marked gradient of transpulmonary pressure.

There are many pathophysiological implications of the vertical gradient of alveolar size. Thus the small alveoli in the dependent regions of the lung will be unstable and therefore the first to collapse during prolonged shallow breathing or general anesthesia. Since the bulk of the perfusion goes to these dependent areas, gas exchange would then be seriously impaired. Moreover the expanding forces on the apical alveoli are large so that any parenchymal damage by disease may well cause cavitation in that region.

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REFERENCES

1. AGOSTONI, E., P. MOGNONI, G. TORRI, AND A. F. AGOSTONI. Static features of the passive rib cage and abdomen-diaphragm. *J. Appl. Physiol.* 20: 1187–1193, 1965.

2. COBB, S., D. J. BLODGETT, K. B. OLSON, AND A. STRANAHAN. Determination of total lung capacity in disease from routine chest roetgenograms. *Am. J. Med.* 16: 39–54, 1954.

3. DALY, J. W., AND S. BONDURANT. Direct measurement of respiratory pleural pressure changes in normal man. *J. Appl. Physiol.* 18: 513–518, 1963.
4. GLAZIER, J. B., J. M. B. HUGHES, J. E. MALONEY, M. C. F. PAIN, AND J. B. WEST. Decreasing alveolar size from apex to base in the upright lung. *Lancet* 2: 203–204, 1966.
5. HALES, S. *Vegetable Staticks.* London, 731, p. 241.
6. KEITH, A. The mechanism of respiration in man. In: *Further Advances in Physiology*, edited by L. Hill, London: Arnold, 1909.
7. KRUEGER, J. J., T. BAIN, AND J. L. PATTERSON. Elevation gradient of intrathoracic pressure. *J. Appl. Physiol.* 16: 465–468, 1961.
8. MACKLIN, C. C., AND W. S. HARTROFT. *Size of Pulmonic Alveoli Based on Measurements in 25 Micro Sections of Human and Common Laboratory Animal Lungs Fixed in State of Expansion.* Canada Sub-Committee on Physiological Aspects of Chemical Warfare, 1943.
9. MERYMAN, H. T. Mechanics of freezing in living cells and tissues. *Science* 124: 515–521, 1956.
10. MILIC-EMILI, J., J. A. M. HENDERSON, M. B. DOLOVICH, D. TROP, AND K. KANEKO. Regional distribution of inspired gas in the lung. *J. Appl. Physiol.* 21: 749–759, 1966.
11. ORSOS. Die Pigmentverteilung der Pleura pulmonalis und ihre Beziehung zum Atmungsmechanismus und zur generellen mechanischen Disposition der Lungenspitzen fur die Tuberkulose. *Verhand. Deut. Pathol. Ges.* 15: 136–149, 1912.
12. RUTISHAUSER, W. J., N. BANCHERO, A. S. TSAKIRIS, A. C. EDMUNDOWICZ, AND E. H. WOOD. Pleural pressures at dorsal and ventral sites in supine and prone body positions. *J. Appl. Physiol.* 21: 1500–1510, 1966.
13. SNEDECOR, G. W. *Statistical Methods.* Ames: Iowa State Univ. Press, 1956, p. 98.
14. STOREY, W. F., AND N. C. STAUB. Ventilation of terminal air units. *J. Appl. Physiol.* 17: 391–397, 1962.
15. SURPRENANT, E. L., AND S. RODBARD. A hydrostatic pressure gradient in the pleural sac. *Am. Heart J.* 66: 215–220, 1963.
16. TURNER, J. M. Distribution of lung surface pressure as a function of posture in dogs. *Physiologist* 5: 223, 1962.
17. WEIBEL, E. R. *Morphometry of the Human Lung.* Berlin: Springer, 1963, p. 9–39.
18. WEIBEL, E. R., AND D. M. GOMEZ. Architecture of the human lung. *Science* 137: 577–585, 1962.
19. WEIBEL, E. R., G. S. KISTLER, AND W. F. SCHERLE. Practical stereological methods for morphometric cytology. *J. Cell. Biol.* 30: 23–37, 1966.
20. WEST, J. B. Physiological consequences of the apposition of blood and gas in the lung. In: *Development of the Lung*, edited by A. V. S. De Reuck and R. Porter. London: Churchill, 1967, p. 176–194.
21. WOOD, E. H., A. C. NOLAN, D. E. DONALD, AND L. CRONIN. Influence of acceleration on pulmonary physiology. *Federation Proc.* 22: 1024–1034, 1963.