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*J Appl Physiol* 29:340-344, 1970. ;



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Journal of Applied Physiology
Vol. 29, No. 3, September 1970. *Printed in U.S.A.*

# Site of airway closure in excised dog lungs: histologic demonstration

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Hughes, J. M. B., D. Y. Rosenzweig, and P. B. Kivitz. *Site of airway closure in excised dog lungs: histologic demonstration.* J. Appl. Physiol. 29(3): 340–344. 1970.—On deflation expulsion of gas ceases in excised lungs when pleural pressure exceeds airway pressure (negative transpulmonary (Ptp) pressure) by 2–4 cm $H_2O$. The remaining air is trapped in the lung, presumably by closure of airways. We investigated the site of airway closure in two ways. First, we outlined the bronchi with dye or lead dust and took X-rays at Ptp −6 cm $H_2O$ when gas was trapped. Airways down to 1 mm id were patent. Second, we rapidly froze the lung surface with liquid Freon at Ptp −6 cm $H_2O$ and in a cryostat chamber cut sections 20 $\mu$ thick serially through the outer 2–3 mm of lung tissue, photographing the cut surface as we did so. We observed that airway closure occurred in terminal bronchioles (approximately 0.4–0.6 mm id when fully inflated) and we measured the length of the closed segment (0.1–1.0 mm).

bronchial P-D relations; bronchography; bronchioles; lung freezing; minimal lung volume; trapped gas in the lung

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Airway closure in lungs at low volumes has been inferred from several studies (1–3, 10). Nunn et al. (10), for example, found that desaturation occurred in some normal subjects breathing air at tidal volumes near residual volume (RV). In addition, large alveolar-arterial $O_2$ differences occurred when subjects were breathing 100% $O_2$ suggesting atelectasis following absorption of trapped gas. By nitrogen washout Burger and Macklem (1) found that normal subjects trapped an average of 400 ml of gas while breathing air at low lung volumes. With 100% oxygen quasi-static pressure-volume curves on inflation from RV were shifted (relative to air control curves) in the direction of increased pressure for the same volume, suggesting absorption of trapped gas and atelectasis.

In animals with an open thorax, gas trapping and airway closure occurs at negative transpulmonary pressures (pleural pressure exceeding airway pressure) of between −2 and −4 cm $H_2O$ (2). In the course of our investigations (4) into the trapping of gas in perfused dog lungs we became curious about the site of airway closure. We report here the results of two attempts to demonstrate this directly. With bronchography we looked at the caliber of airways down to 1 mm internal diameter (id) at a negative transpulmonary pressure at which gas was trapped. Closure of airways was not seen. Secondly we examined airways less than 0.8 mm id microscopically after rapidly freezing the surface of lung at Ptp −6 cm $H_2O$; with serial sections we followed changes in the caliber of the lumen of bronchioles.

## METHODS

Greyhound dogs were anesthetized with intravenous thiopentone (0.4–0.6 g) and heparinized (2000 IU/kg). After exsanguination the left lungs were removed, perfused, and ventilated as described (4). Lungs were placed horizontally in a Lucite box. Volume changes were recorded on a 2-liter bell spirometer and transpulmonary pressure changes with a water manometer. Airway pressure remained at atmospheric; pleural pressure was changed by altering the pressure in the box. Absolute lung volume was not measured. Unless specifically mentioned the same volume history was used for all measurements. The lung was inflated maximally by lowering the pressure in the box to −24 cm $H_2O$ and then ventilated for 3–4 breaths from 10 to 15 cm $H_2O$ transpulmonary pressure (Ptp). The lung was next slowly inflated from Ptp 5 to 21 cm $H_2O$ and transpulmonary pressure and volume changes were recorded as the lung was deflated to a negative Ptp of 6 cm $H_2O$. Expulsion of gas ceased between Ptp −2 and −4 cm $H_2O$.

Most of the preparations were perfused with blood at 37 C. Pulmonary arterial and venous pressures were measured with saline manometers, and blood flow by collection of timed samples in a cylinder. Flow was controlled by a roller pump. If perfusion was needed for longer than a few minutes a steady flow of venous blood was taken from a mongrel dog to which the blood leaving the isolated lung was returned.

*X-ray measurements.* In 10 experiments 2–4 ml of contrast medium (oily or aqueous Dionosil) was instilled into the airways through the bronchial cannula. In three other experiments finely particulate lead was introduced into the airways as described by Leopold and Gough (7). After inflation to Ptp 21 cm $H_2O$, the lung was deflated slowly over about 5 min to Ptp −6 cm $H_2O$. Radiographs were taken at Ptp 21, 10, 5, 0, and −6 cm $H_2O$. A nonscreen film and a 0.3-mm focal spot was used. The distance from the X-ray source to the lung was 96 cm, and from the lung to the film was 5–7 cm.

The radiographs were magnified 10 times by projection onto a screen and the diameters of airways ranging from 17 mm to 0.8 mm id were measured. The airways selected initially were used in all subsequent measurements throughout the experiment.

*Histological measurements.* In 11 experiments the lung was rapidly frozen at Ptp −6 cm $H_2O$ by submerging it in liquid Freon (Arcton 12, ICI). The lungs were placed horizontally in a metal box with a Lucite top. Above the lung was a brass tube, perforated by many small holes, which connected with tubing outside the box in the shape of a Y. The tubing was filled with Freon (cooled with liquid nitrogen to −150 C) and a clamp at its base was immediately released so that Freon ran into the box and over the lung. The level of Freon in the tubing was maintained at about 50 cm and 4 liters were poured over the lung in 20–30 sec until it was submerged. Box pressure was maintained at +6 cm $H_2O$ during this time.

The lung was transferred to liquid nitrogen and stored in a deep freeze at −20 C. Small blocks of the frozen tissue (2 x 3 cm across and 1 cm thick) were placed in a cryostat containing a microtome (Slee, London). The frozen surfaces were examined with a steroscopic microscope (Nikon SM7-2, Japan) mounted on a specially constructed frame. Sections, 20 μ in thickness, were cut away and photographs taken of the exposed surface with a camera attached to one of the eyepieces. Initially (Fig. 4) photographs were taken with an Exa 1A camera, not specifically designed for photomicrography; later, we used a Nikon PFM (Fig. 3). The size of the airways to be photographed was measured by means of a graticule in the other eyepiece. Illumination was by reflected light from three photoflood bulbs. A thermometer within the cryostat chamber measured the temperature which was kept below −10 C.

## RESULTS

The volumes of gas expelled as lungs were deflated from Ptp 21 cm $H_2O$ to −6 cm $H_2O$ were comparable to those found in the previous study (4), averaging 1,216 ml (SE ± 70 ml) in 12 lungs. Intermediate volume changes were similar also; the volume expired from Ptp 0 cm $H_2O$ to −6 cm $H_2O$ was 103 ml (SE ± 16 ml).

*X-ray measurements.* Satisfactory bronchograms were obtained in seven preparations. Three of the lungs were unperfused. In the perfused lungs the vascular pressures and blood flows were very similar. Referred to the level of the hilum the average pulmonary arterial pressure was 10 cm $H_2O$ and pulmonary venous pressure −5 cm $H_2O$. Flow averaged 155 ml/min.

A lung with the airways outlined with lead dust is illustrated in Fig. 1. The lower lobe bronchus and its branches are shown at full inflation (Ptp 21 cm $H_2O$) and after deflation to a negative transpulmonary pressure (−6 cm $H_2O$). There is considerable shortening and narrowing of all the bronchial segments at the latter pressure but no evidence of closure of airways. Neither of the contrast media caused any detectable change in the mechanical properties of the lung as judged by the deflation pressure-volume relations or by dynamic compliance, or in the vascular resistance. We compared bronchial diameter measurements obtained with the liquid and dust media and found that they were similar except for airways of 1.5 mm id or less. In such small airways we found that at Ptp −6 cm $H_2O$ the diameter had decreased by 40% relative to that at Ptp 21 cm $H_2O$ in the dust outlined lungs but by only 5% in the liquid-filled lungs. We have rejected measurements of airways less than 1.5 mm id in lungs containing the dye since we presume they were blocked by the liquid.

Figure 2 shows the changes of diameter in airways of different sizes as transpulmonary pressure decreases from 21 cm to −6 cm $H_2O$. These represent values from seven lungs of measurements made at the beginning of the experiment. The dye and lead dust results have been combined except for the 0.8–1.0 mm id airways. The pressure-diameter relations for airways of different sizes are approxi-

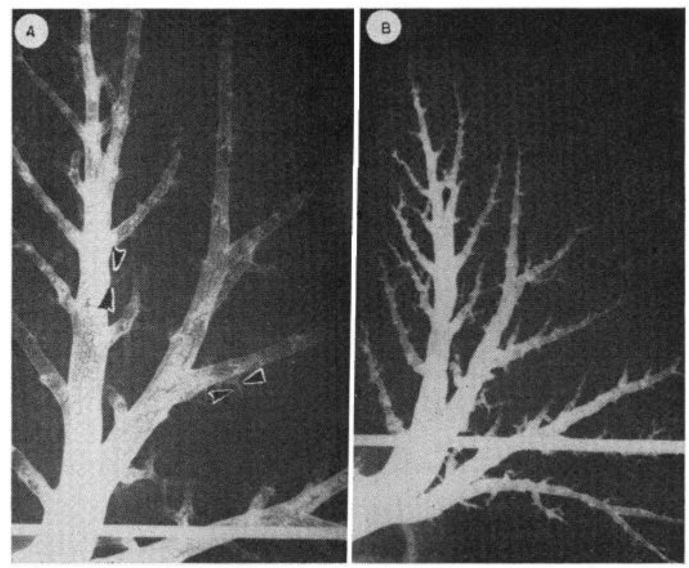


FIG. 1. X-ray of an isolated lung with airways of lower and middle lobe outlined with lead dust. Left-hand picture shows lung at nearly full inflation (Ptp 21 cm $H_2O$) compared with maximal deflation (Ptp −6 cm $H_2O$) in the right-hand X-ray. Arrows at Ptp 21 cm $H_2O$ mark airways of 1.5 and 0.5 mm diameter.

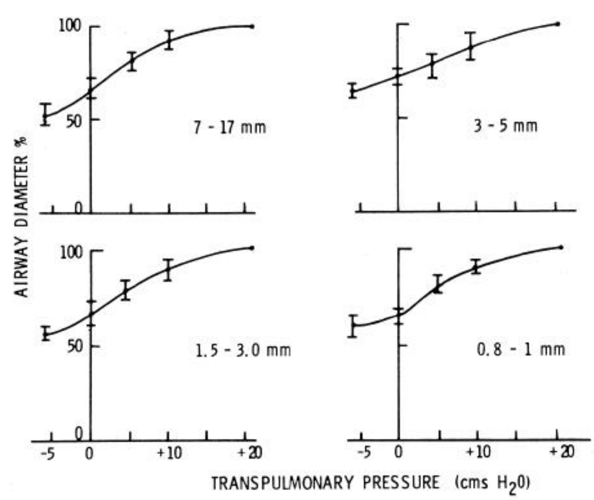


FIG. 2. Diameter of airways of different sizes (as a percentage of their diameter at Ptp 21 cm $H_2O$) plotted against transpulmonary pressure on deflation. Mean values with standard deviation of the mean from bronchograms in 7 lungs are shown (4 outlined with liquid dye and 3 with lead dust). Measurements of the 0.8–1 mm airways were taken from lead dust lungs only. On average at minimal volumes (Ptp − 6 cm $H_2O$) airways have a diameter 55% of maximum.

mately similar. The diameter at minimal volume (Ptp −6 cm $H_2O$) ranged from 51 to 63% of their size at Ptp 21 cm $H_2O$. It is of interest that the shape of our airway pressure-diameter curves differ to some extent from those published for dog lungs by Hyatt and Flath (6). In their studies also on deflation bronchi generally remained at their maximum diameter until transpulmonary pressures of 5–6 cm $H_2O$ were reached. Differences of tone or volume history may explain this discrepancy.

*Histologic measurements.* Bronchographic measurements showed that at Ptp −6 cm $H_2O$ the diameter of airways down to about 1 mm had narrowed to approximately 50% of that at full inflation. We presumed therefore that the site of airway closure at Ptp −6 cm $H_2O$ lay in more peripheral structures. By rapidly freezing the surface of the lung at this transpulmonary pressure we were able to examine airways of 500 μ diameter and less. We have confined our observations to the freshly excised lung where the trapped gas volume at Ptp −6 cm $H_2O$ is low. In preliminary experiments we found that at the high trapped gas volumes induced by edema (4) an insufficient number of airways could be found within 3 mm of the pleural surface. The first eight experiments were preliminary; the observations reported here came from the three remaining. The results for these three lungs were similar although the conditions of ventilation and perfusion differed slightly in each case. The two lungs illustrated in Figs. 3 and 4 were allowed to collapse passively on opening the thorax after death of the animal; they were removed from the chest and placed in the box. The lungs were compressed by raising box pressure to +6 cm $H_2O$; the airway was connected to a spirometer. The lungs were then rapidly frozen as described. The lung in Fig. 3 was perfused with blood; at the time of freezing the pressures relative to the height of the hilum were, pulmonary arterial pressure 26.8 cm $H_2O$, and venous pressure −3.5 cm $H_2O$; flow was 168 ml/min. The lung in Fig. 4 was unperfused. In the other case (not illustrated) the lung was reexpanded after removal from the thorax, deflated to Ptp −6 cm $H_2O$ and perfused until frozen. Flow was stopped at the moment of freezing.

Figure 3 shows a series of photomicrographs from the lower lobe of a lung rapidly frozen with liquid Freon at a transpulmonary pressure of −6 cm $H_2O$. Serial sections through the lung have been made from the hilum towards the periphery, and the same field has been photographed at appropriate intervals. Two airways can be seen closed initially but open nearer the periphery of the lung. If cuts are made at 20-μ intervals, with the microscope it is possible to tell whether the airways are being sectioned across or obliquely. Serial sections in another lung (Fig. 4) show an airway closed, open, and closed again. It is most unlikely that this could occur as a result of cutting into an airway longitudinally. This bronchiole and its neighbors were fol-

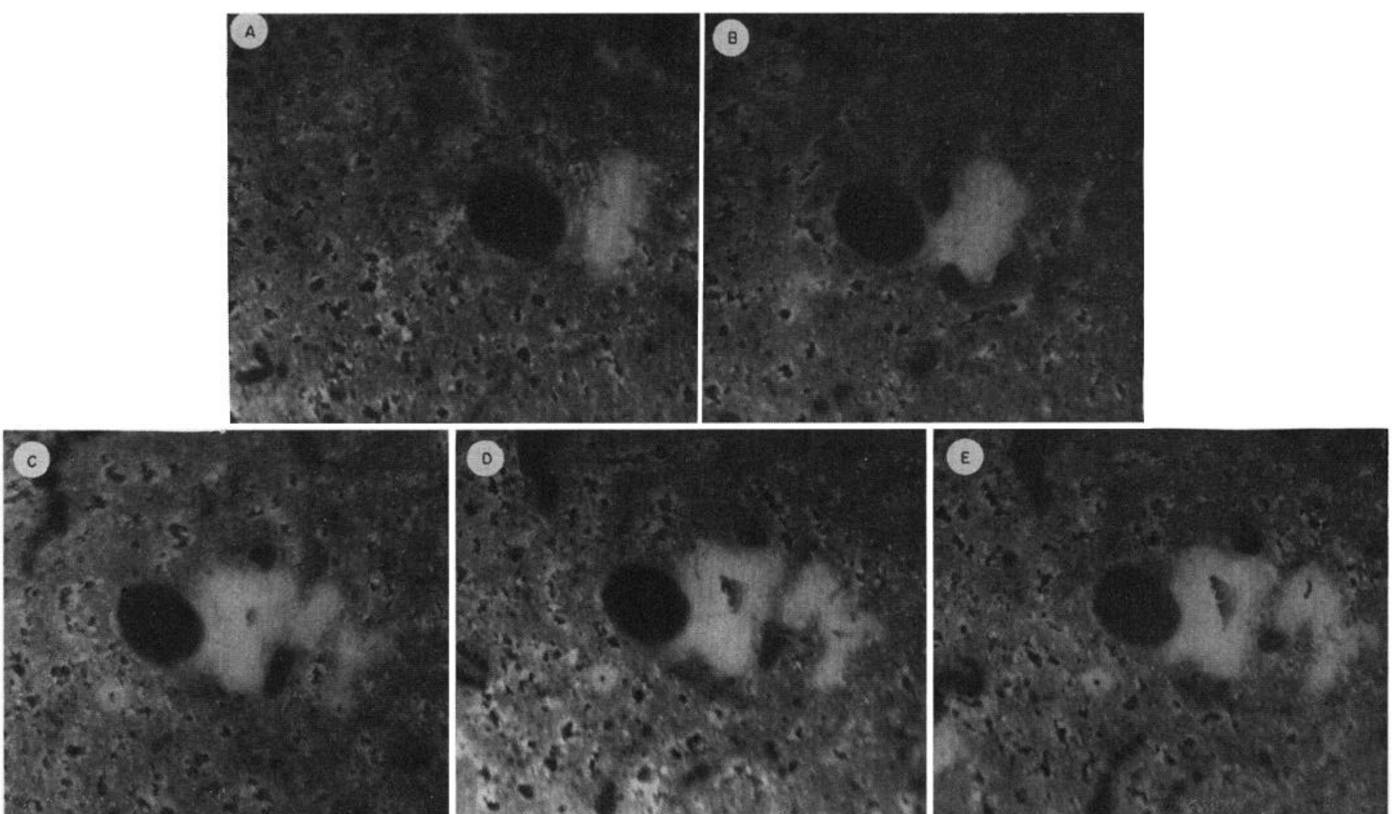


FIG. 3. Photomicrographs of the lower lobe of an isolated lung rapidly frozen at minimal volume (Ptp − 6 cm $H_2O$). Magnification ×30. Distance from pleura 1.7 mm. Serial sections of the same field (*A* being nearest the hilum and *E* the most peripheral) show a blood vessel in the center accompanied by several airways. Lumen of larger airway is initially closed (*A*) and gradually opens (*B–E*); similar changes can be seen in a smaller accompanying airway (*C–E*). Diameter of the central blood vessel (in *E*) is 370 μ and maximal internal diameter of airway next to it is 170 μ. Distance between sections: *A–B* 0.2 mm; *B–C* 0.44 mm; *C–D* 0.26 mm; *D–E* 0.06 mm.

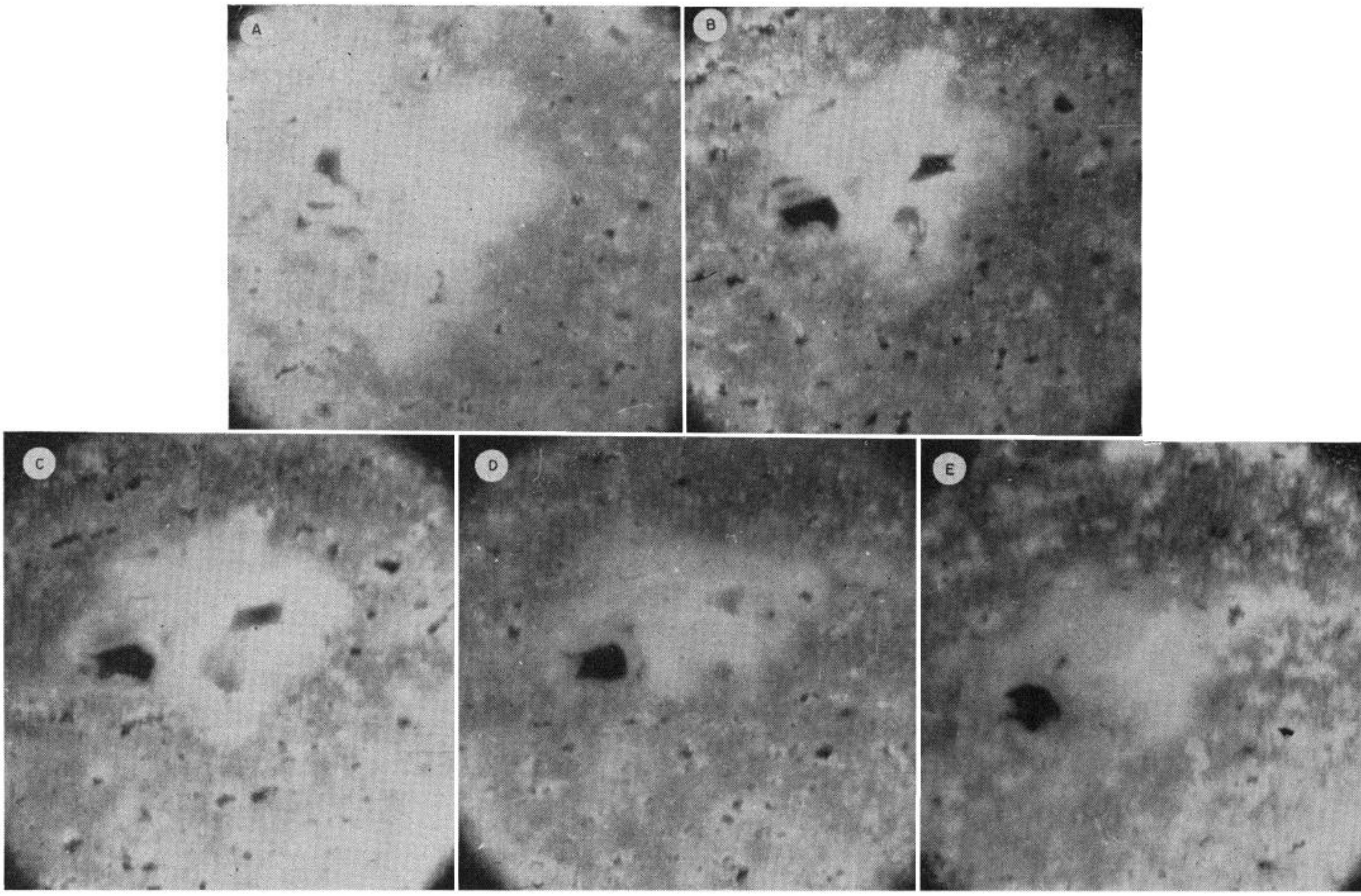


FIG. 4. Photomicrographs of lower lobe of an isolated dog lung rapidly frozen at minimal volume (Ptp − 6 cm $H_2O$). Magnification ×20. Distance from pleura 3.3 mm. Serial sections of same field (as in Fig. 3) show on the left a blood vessel (0.25 mm id in *C*) and bronchial tissue in the center. In *B* an airway opens (dark rectangular shadow); it is joined by a branch in *C* and shuts again at *E*. Airway lumen in *D* is 150 μ. Distance between sections: *A–B* 0.12 mm; *B–C* 0.1 mm; *C–D* 0.24 mm; *D–E* 0.28 mm.

lowed over a distance of 3.5 mm, involving 172 photographs at 20-μ intervals.

In the three preparations where photographs were obtained, we recorded with the serial sectioning technique about 20 examples of airways where the lumen was closed at one point but open when sectioned near or further from the hilum.

The maximal diameter of the lumen in the vicinity of the site of closure ranged from 40 to 500 μ with a mean in 17 measurements of 180 μ. We cannot be sure of the diameter these structures would have had at Ptp 21 cm $H_2O$ but our pressure-diameter curves for bronchi down to 1 mm (Fig. 2) suggest that diameters at Ptp −6 cm $H_2O$ are about half that at maximal inflation. If smaller airways behave similarly we would put the maximal diameter of the closed airways as somewhere between 0.4–0.6 mm id, i.e., terminal bronchioles.

*Errors introduced by freezing*. The biophysics of freezing the lung surface with liquid propane (Freon is similar) has been investigated by Staub and Storey (12). They showed that the outer 2 mm freezes in 2–3 sec. Normally, in the inflated lung there are few if any bronchioles within 3 mm of the lung surface, but at the low volumes induced by negative transpulmonary pressures we found many airways up to 1 mm diameter in this region. Our experimental design meant that the superficial parts of the lung contained gas that was trapped distal to closed airways and thus isolated from the main bronchus and spirometer. As the trapped gas cools at constant pressure it will shrink in volume and distort the surrounding tissue. Once the temperature of the tissue has fallen to approximately 0 C it will for the most part resist further deformation; consequently the gas contained in it will continue to cool at constant volume by changing its pressure. From 0 to −100 C gas pressure may fall by 250 mm Hg. A fall of alveolar pressure occurring in this manner, if transmitted to parts of the lung still unfrozen and pliant, might result in collapse of airways and air spaces. This fall of pressure would not be transmitted from the alveolar to the bronchial side of the site of airway closure, and airways from the bronchus down to the site of airway closure would be unaffected. In fact we only examined the outer 2–3 mm where the rate of freezing was sufficiently rapid to minimize this effect.

Although the thermal properties of gas and tissue are different, most investigators have assumed that because of the intimate arrangement of air and tissue thermal changes will occur in both almost simultaneously. Our measurements of lung structure before and after freezing indicate that any alterations in shape that occur are small. In eight preparations we measured the external dimensions of the lung before and after freezing at Ptp −6 cm $H_2O$, but we did not detect any decrease. However, the accuracy of these measurements was not great and an 18% change in lung volume could result from a 6% decrease of length in each direction (0.3–1.3 cm measured change). No change in lung volume occurred as monitored by the spirometer attached to the bronchial cannula. In three preparations bronchograms were taken immediately before and after freezing at Ptp −6 cm $H_2O$. Airways from 0.5 to 15 mm were measured; the mean decrease in diameter was 10.4% (range 4–17%). If we assume no changes in length this would correspond to a decrease in bronchial volume of 20%. At constant pressure a volume of gas will contract by 17% as the temperature is lowered from 37 C to zero, and by 47% as it cools from 37 C to −100 C. We have already estimated that the bronchial volume (from main bronchus to terminal bronchioles) at Ptp −6 cm $H_2O$ is about 2–3% of lung volume (360 ml), i.e., 10 ml. A 20% decrease in this volume would be 2.0 ml. Since our spirometer does not record step changes of volume of less than 10 ml, we did not record this change. The spirometer measurements also suggest that parenchyma of the lung is isolated from the larger airways by airway closure. A change of parenchymal volume of 20%, i.e., 60 ml, would have been seen easily.

We conclude that during rapid freezing of the lung under the conditions of our experiment there is a decrease in bronchial and probably parenchymal volume of about 20%. This is consistent with gas and tissue cooling at equal rates and at constant pressure until a temperature of approximately 0 C is reached. Changes in volume as the temperature falls further do not seem to occur. It is therefore probable that we are underestimating bronchial diameter by 10%. On the other hand, comparable changes in dimensions occur during freeze-drying, processing and staining which our method avoids.

## DISCUSSION

Under these conditions, in freshly excised lungs the site of closure appeared to be in very small airways, i.e., bronchioles 0.5 mm or less in diameter (Figs. 3 and 4). These results do not conflict with what is already known of the behavior of the larger pulmonary airways. For example, our bronchographic measurements (Fig. 2) showed that Ptp −6 cm $H_2O$ reduced the caliber of airways (17 to 0.8 mm diameter) by 50% but did not close them. Other measurements in excised lungs (5) showed that bronchi 2–6 mm in diameter decreased about 30% from their maximum size at zero Ptp and 60% at Ptp −6 cm $H_2O$. In isolated 1–2 mm bronchial segments Olsen et al. (11) found the volume at Ptp −6 cm $H_2O$ to be 23% of that at Ptp 20 cm $H_2O$. They showed that zero volume only occurred at Ptp −25 cm $H_2O$. In earlier studies on excised segments (9) transmural pressures of 30 cm saline were necessary to collapse bronchi of 5 mm diameter and 10 cm saline for 2-mm bronchi.

Two other considerations suggest to us that the site of airway closure in this and similar preparations must be in very small airways lacking the protective armor of cartilage. First, we observed during these and similar (4) experiments that wedges of tissue removed from the periphery of a lung where gas trapping was prominent remained inflated without clamping or fixation of any kind. Second, it would appear that the transpulmonary pressure sufficient to cause airway closure in open-chest animals (−2 to −4 cm $H_2O$) is of the same order as that which collapses alveoli (2).

An alternative site of airway closure has been suggested by Burger and Macklem (1) to explain the airway opening pressures of 4.5 cm $H_2O$ they had observed in man. They postulated that a hemispherical meniscus of fluid forms across a closed airway. From the Laplace relationship and assuming a surface tension for the liquid of 50 dynes/cm, they calculated that an opening pressure of 4.5 cm $H_2O$ would be consistent with a meniscus forming across an airway of 0.9 mm id. In this preparation, airway opening pressures on reinflation of the lung from TPP −6 cm $H_2O$, are about 6 cm $H_2O$ (4, Fig. 5). This suggests from the previous calculation that the airways which close would have a diameter of about 0.66 mm. It is also possible (P. T. Macklem, personal communication) that the airway wall is recoiling outward at low volumes, thus reducing the opening pressure to a value lower than that determined by surface forces alone.

We are grateful to Professor R. E. Steiner and Dr. J. B. West for their encouragement, and to Mrs. R. Stevenson, Mr. G. Kingaby, Mr. M. Dickens, and Mr. D. Woodland for valuable technical assistance.

D. Y. Rosenzweig is the recipient of a special fellowship from the National Heart Institute.

Received for publication 10 April 1969.

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