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Measurements of capillary dimensions and blood volume in rapidly frozen lungs

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GLAZIER, J. B., J. M. B. HUGHES, J. E. MALONEY, AND J. B. WEST. *Measurements of capillary dimensions and blood volume in rapidly frozen lungs.* J. Appl. Physiol. 26(1): 65–76. 1969.— Isolated perfused dog lungs were rapidly frozen with liquid Freon. Measurements of the number of red blood cells per 10 μ of alveolar septum, the mean width of capillaries, and the percent of septum occupied by red blood cells were made in freeze-dried sections. The effects of changing vascular and alveolar pressures were studied, with particular attention to the relationship of downstream pressure to alveolar pressure; that is venous pressure was set lower than alveolar pressure (zone II) or higher (zone III). When alveolar pressure exceeded arterial pressure there were less than 0.5 red blood cells per 10 μ of septum and mean capillary width was 2.3 μ. As capillary pressure increased the number of red blood cells per 10 μ of septum increased to a maximum of 2.8 at a capillary pressure of 50 cm H_2O. Mean capillary width increased to 6.5 μ. No further changes occurred in either measurement at pressures up to 100 cm H_2O. At high lung volumes both red blood cell density and capillary width were greatly reduced. Recruitment of new vessels was the predominant mechanism to account for the increase in red blood cell concentration down zone II, whereas expansion of already open capillaries was more important in zone III.

pulmonary capillary morphology; changing vascular and alveolar pressures; capillary red blood cell volume; capillary distention; capillary recruitment

LARGE REGIONAL DIFFERENCES in pulmonary blood flow have been demonstrated in man (2, 25). The nonuniformity of perfusion in the vertical lung is due to the effect of hydrostatic forces on the pulmonary arterial and venous pressures (3, 19, 27). It is logical that capillary pressure must also vary widely in the erect lung and that there are probably significant differences in capillary morphology as well as differences in blood flow.

In this paper we report measurements of capillary dimensions and blood volume under different conditions of vascular and alveolar pressures. We used a technique of rapidly freezing isolated greyhound lungs. Histological measurements provided information concerning *1*) changes in capillary dimensions and red blood cell volume, and *2*) the relative importance of distention of capillaries as opposed to the opening of previously closed capillaries in order to account for increases in red blood cell volume down the lung.

METHODS

Greyhound dogs ranging in weight from 21 to 31 kg were anesthetized with pentothal sodium (50 mg/ml), and anesthesia was maintained with Nembutal (60 mg/ml). The dogs were killed by exsanguination and the left lung together with its main stem bronchus, pulmonary artery, and pulmonary veins including the left atrium was then removed. The lung was suspended in a Lucite box, perfused in closed circuit with blood previously removed from the dog, and ventilated with negative pressure. The average time from the death of the dog to perfusion of the isolated lung was 35 min. The isolated lung preparation and perfusing circuit have been described in detail elsewhere (27).

Freezing Procedure

The lung was frozen with Freon 12 cooled to −155 C with liquid nitrogen. A brass tube 1 cm in diameter and 35 cm long pierced at 1-cm intervals by small holes was positioned vertically about 1 cm away from the vertical lung. The tube was connected to a funnel held about 30 cm above the lung. Approximately 2 liters of liquid Freon were poured into the funnel and sprayed out of the brass tube, rapidly freezing to a depth of 3–5 mm a strip of lung approximately 30 cm in length. The duration of the pour was about 25 sec. The lung was rapidly cut free of the clips holding it (5–12 sec) and it fell into the Freon which had run off the lung and collected at the bottom of the box. This Freon was at a temperature of approximately −120 C. The lung was then immediately transferred to a tub of liquid N_2 where it remained for about ½ hr. Prior to the Freon pour the lung was marked with a soft felt pen at 5-cm intervals next to the strip to

be frozen. Thus even if the lung buckled while being cut down after the pour, the original vertical heights of the tissue to be sampled were obvious.

Removal and Preparation of Tissue

Three blocks of tissue were removed from the frozen (pleural) surface of the lung at each 5-cm interval from the apex to base. The blocks, approximately 5 mm thick x 8 mm x 5 mm were cut from the frozen lung with a band saw with a moderately coarse blade. The tissue was never kept out of liquid N_2 long enough for it to thaw, and the blocks were stored in a freezer at −25 C while awaiting processing.

Three blocks of tissue were freeze-dried together (Pearse-Speedivac freeze dryer, Edwards High Vacuum Ltd.) for 18 hr at a temperature of −20 C and pressure of 0.001 mm Hg. The blocks were then double-embedded in 2% celloidin and paraffin wax (Ralwax 1, R. A. Lamb Co.) as previously described (7). Sections were cut to a thickness of 2 μ on a Cambridge rocking microtome with a technique for the cutting of thin sections described by Wigglesworth (28). A specially sharpened razor blade was used to cut the sections rather than the standard microtome knife. Three serial sections from each block were placed on a slide and stained with hematoxylin and eosin. Cellular detail was excellent in these extremely thin sections (Fig. 1).

Physiological Conditions During Freezing

The dog lungs were frozen under the following vascular pressure conditions: *1*) where arterial pressure was less than alveolar pressure and there was no flow (zone I); *2*) where arterial pressure exceeded alveolar pressure but alveolar pressure was greater than venous pressure (zone II); and *3*) where arterial pressure exceeded venous pressure and venous pressure was greater than alveolar pressure (zone III).

After the lung had been suspended in the Lucite box and ventilated with negative pressure for about 10 min, the back of the box was removed and the lung held at an alveolar pressure of 10 cm H_2O. Arterial pressure was then set to the desired level by adjusting the rate of blood flow and venous pressure was set by raising or lowering the venous reservoir. Most lungs were frozen at a transpulmonary pressure of 10 cm H_2O, in which case the lung was expanded to 25 cm H_2O pressure just prior to the freeze and then given five tidal breaths before being held at 10 cm H_2O pressure. Some lungs were expanded to 25 cm H_2O, held and frozen.

Zone I. Three lungs were frozen under zone I conditions at a transpulmonary pressure of 10 cm H_2O. Venous pressure was set below the bottom of the lung, the arterial pump was turned off, and arterial pressure settled near the bottom of the lung. This left 20–25 cm of lung tissue in zone I in each lung. In one of the three lungs, arterial pressure was raised above the top of the lung just before the pump was turned off. The pressure had settled and the lung was frozen 2 min later.

Zone II. Nine lungs were frozen under zone II conditions, four of them at a transpulmonary pressure of 10 cm H_2O. In two of these four experiments, arterial pressure equaled alveolar pressure about 5 cm below the top of the vertical lung and blood flow was 200 ml/min; in the other two lungs arterial pressure equaled alveolar pressure 5 and 12 cm above the top of the lung and blood flow was 1,200 ml/min.

Regardless of how far below the bottom of the lung the venous reservoir was set, venous pressure exceeded alveolar pressure 10 cm below the entrance of the pulmonary vein at the hilum because of the Starling resistor effect of the large veins exposed to pleural pressure (26); so that in any one experiment a maximum of 20–25 cm of lung tissue was available in zone II. Furthermore, with the arterial pump at its fastest speed (about 1,200 ml/min), arterial pressure could only be raised 12 cm above the top of the vertical lung in the presence of a very low venous pressure. Thus at a transpulmonary pressure of 10 cm H_2O the tissue available ranged from 1 to 35 cm below the zone I-II junction.

Two lungs were frozen at a transpulmonary pressure of 25 cm H_2O. Tissue was available in each from 1 to 21 cm down zone II. Blood flow was 375 ml/min. Two lungs were frozen at a transpulmonary pressure of 10 cm H_2O during the infusion of serotonin (15 μg/ml). In each case 0.75 ml/min was infused for approximately 2.5 min before the Freon was poured on the lung. At a constant blood flow of 160 ml/min this raised arterial pressure 6 and 7 cm H_2O.

Zone III. Thirteen lungs were frozen in zone III conditions, at a transpulmonary pressure of 10 cm H_2O. In these experiments venous pressure varied from a level just at the top of the vertical lung to a height 75 cm above the top of the lung. Thus, tissue was available up to a distance of 105 cm below the level where venous pressure was equal to alveolar pressure. In three of these lungs arterial pressure was 4 cm H_2O above venous pressure and blood flow was 100–150 ml/min. In six experiments blood flow was turned off about 1 min prior to the freeze and arterial pressure settled to within 1 cm H_2O of venous pressure. In two experiments blood flow was set at a maximum of 1,200 ml/min and the arterial-venous pressure difference averaged 17 cm H_2O. In two other experiments the lung was perfused from vein to artery at a maximum blood flow with an average venous-arterial pressure difference of 15 cm H_2O.

Two lungs were frozen at a transpulmonary pressure of 25 cm H_2O, with an arterial-venous pressure difference of 4 cm H_2O and a blood flow of approximately 100 ml/min. Tissue was available to a distance 35 cm down zone III.

Histological Measurements

Three measurements were made in the 2-μ sections at each level sampled.

Red blood cells per 10 μ septum. The number of red blood cells was counted in each of 30 alveolar septa with a light

microscope and a magnification of 700 ×. The length of each septum was measured with an eyepiece graticule. No red cells were counted that lay within 10 μ of the intersection of two septa and the length of septum available for counting had to be at least 20 μ. The number of red blood cells per 10 μ of septum was calculated for each of the 30 septa and from these figures a mean value and standard error were calculated. By-products of this measurement were the total length of septa counted at each level, the range in length of the septa sampled, and the number of septa at any level in each dog in which there were no red blood cells.

Mean width of capillaries. Approximately 12 microscopic fields at a magnification of 280 × were photographed on 35-mm black and white film, and the developed film strip was projected onto a screen so that the final magnification was 4,500 ×. With a ruler with the axis placed perpendicularly to the septum, the width of the capillaries was measured at 10-μ intervals along the septum. The capillaries were eccentric in shape (Fig. 1*D*), often bulging out into the alveolar space and not necessarily occupying the entire width of the septum. At the magnification used the boundaries of the capillary were obvious, and it was only the width of the capillary that was measured, not the entire thickness of the septum. Measurements were made only if an open capillary was present as indicated by the presence of a red blood cell. At each level 80 measurements of capillary width were recorded.

Percent septum occupied by red blood cells. The microscopic eyepiece graticule graduated in 5-μ intervals was superimposed over each septum in which the number of red blood cells had been counted. The graduations were placed perpendicularly to the septum. Again beginning 10 μ from the intersection of two septa each line of the graticule was noted either to cross or not to cross a red blood cell. The percentage of septum occupied by red cells was calculated in each of the 30 septa and the mean and standard error followed from these figures.

Sampling. The histologic sections were approximately 1 cm long and always included the pleural surface. The width of the rapidly frozen tissue was 4–5 mm. For the first and third measurements which were made with the oil immersion lens, a field containing several septa was selected at one end of the section and 1–2 mm inside the pleural surface. The section was then moved so as to keep the field the same distance from the pleura, and all septa that met the criteria for counting were measured as they came into the field. In order to make the 30 measurements on each section, it was moved through at least three-fourths of its length.

A similar technique was employed in photographing the microscopic fields at the lower magnification for the measurement of capillary width. Beginning at one end of the slide, the section was moved through most of its length in order to photograph 12 separate fields. When the film strip was projected, the 80 measurements of capillary width were made with all septa at least 40 μ in length beginning with the first field.

Measurements were made to determine whether the septa near the pleural surface were systematically different from those a few millimeters deeper in the lung. In one section where there were very few red blood cells (24 cm above the zone I-II junction) and in another section where there were many red blood cells (30 cm below the zone II-III junction), all three measurements were made on tissue within 0.5 mm of the pleural surface and repeated on tissue 3.5–4.5 mm from the pleura. For each of the three measurements the results agreed to within 5%.

Other Measurements

The dimensions of the red blood cells were measured 24 cm above the zone I-II junction and 44 cm below the zone II-III junction with a microscopic eyepiece graticule and a magnification of 700 ×. In each location the length of the axis of the red blood cells perpendicular to the septum and the length of the axis directed along the septum were measured.

The number of vessels open at the junction of two or more alveolar septa (corner vessels) were counted in zones I and III. The criterion for an open vessel was that there were two or more red blood cells located within 10 μ of the junction of the septa. No vessel with a diameter greater than 20 μ was included. One hundred junctions were counted at each level reported in the two zones.

Electron micrographs of capillaries were examined in the following locations: 24 cm above the zone I-II junction, and 50 and 95 cm below the zone II-III junction. Pieces of frozen lung tissue about 1 mm^3 were placed in Palade's buffered osmium tetroxide at 4 C for 1 hr to fix. Standard electron-microscopic techniques were followed to dehydrate and embed the tissue in epon resin. Sections were cut at 500 A and stained with Reynolds lead citrate for 10 min followed by alcoholic uranyl acetate for 15 min.

RESULTS

Appearance of Sections

Figure 1 shows photomicrographs made through the oil immersion lens of the 2-μ sections in zones I, II, and III. It may be seen that the borders of the individual red cells are distinct even low in zone III where their concentration is greatest. The capillary walls are also clearly outlined. Clarity was even better during the actual counting since it was possible to focus up and down on the sections.

Red Blood Cells per 10 μ of Septum

Since alveolar size does not change with distance down this isolated lung preparation (7), this is a measurement of the number of capillary red blood cells per unit volume of lung parenchyma. If hematocrit is constant this will reflect regional capillary blood volume. Very little is known about hematocrit in small blood vessels although

a recent publication suggests that there may be a rise in blood viscosity and therefore in hematocrit in capillaries less than 5 μ in diameter (5). As shown below mean capillary width ranged from 2.5 to 6.5 μ as capillary pressure increased. The hematocrit in the perfusing circuit was measured in 10 of the 25 experiments and ranged from 43 to 65%, with a mean of 52%.

Zone I. Figure 2 shows that there were very few red

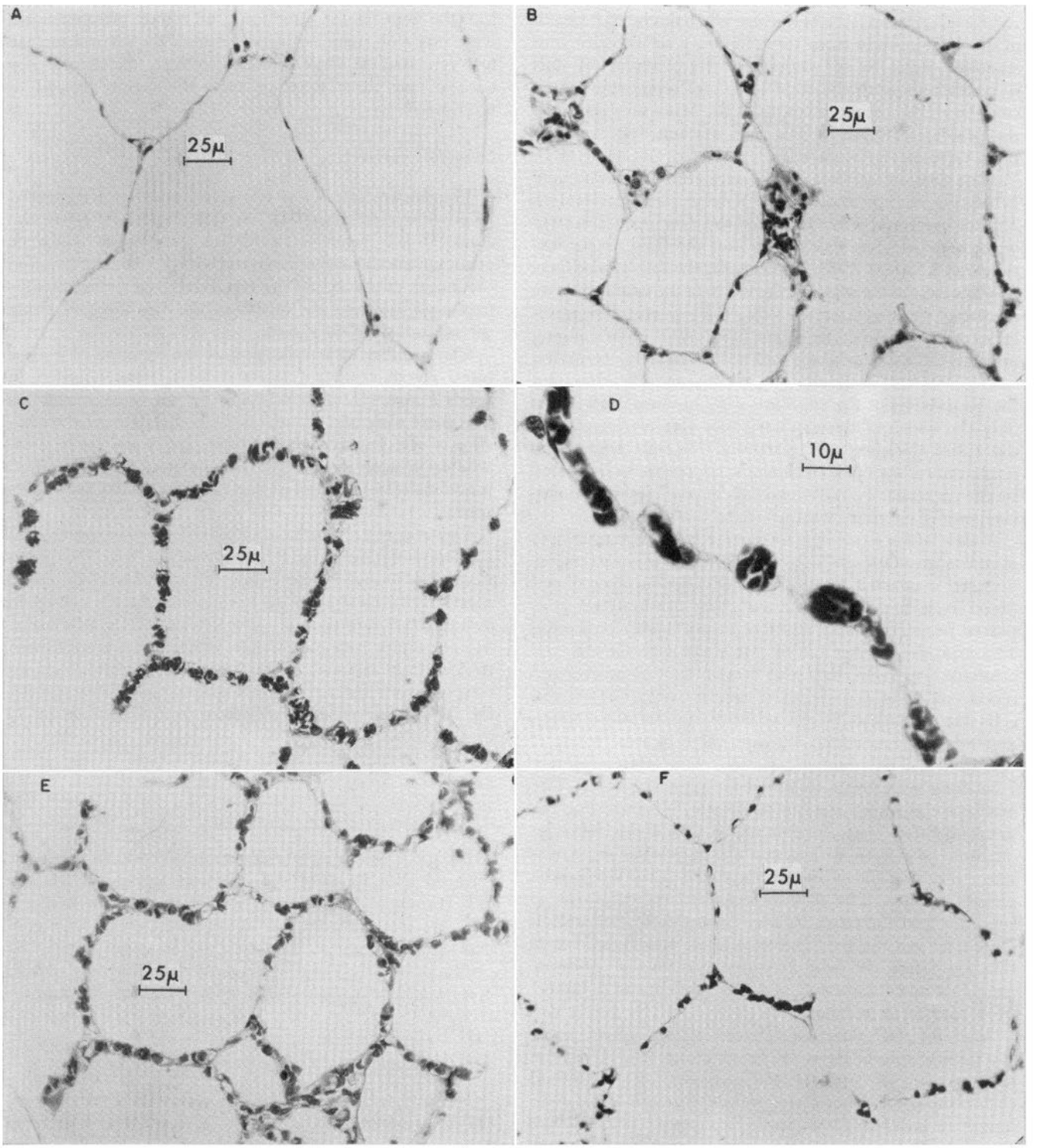

FIG. 1. Histological sections, thickness 2 μ: *A*: 24 cm above zone I-II junction; *B*: 5 cm below zone I–II junction; *C*: 44 cm below; and *D*: 88 cm below zone II–III junction; *E*: 32 cm below zone II–III junction, transpulmonary pressure 10 cm H_2O; *F*: 35 cm below zone II–III junction, transpulmonary pressure 25 cm H_2O. Note clarity of red blood cell outline even when their concentration is greatest (*C* and *D*) and the striking reduction in red blood cell density at transpulmonary pressure 25 cm H_2O (*F*) as compared to transpulmonary pressure 10 cm H_2O (*E*) when hydrostatic pressure gradients are comparable.

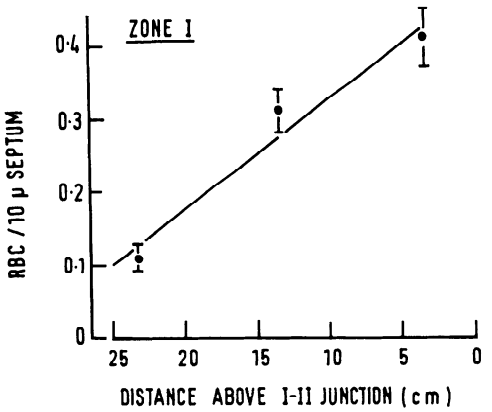

FIG. 2. Number of red blood cells per 10 μ septum plotted against distance up lung above zone I–II junction. There is no blood flow in this zone where alveolar pressure exceeds pulmonary arterial pressure and the capillaries are compressed (Fig. 1*A*). Each point is the mean and standard error of 30 measurements made on each of three dogs, i.e., 90 measurements in all.

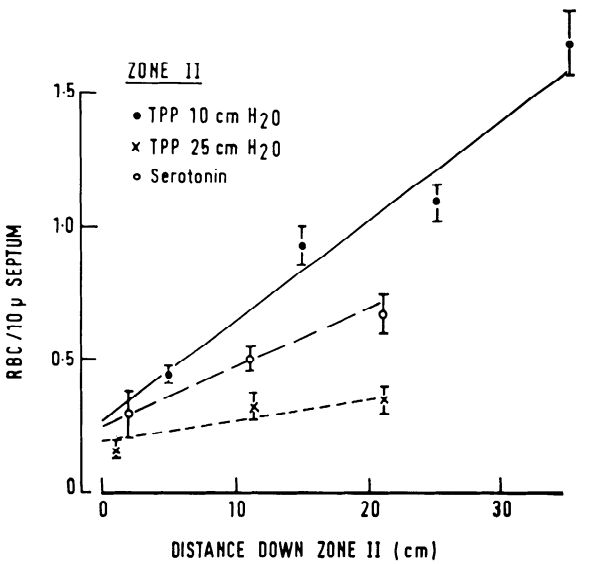

FIG. 3. Red blood cells per 10 μ septum down zone II measured at transpulmonary pressure 10 cm H_2O, transpulmonary pressure 25 cm H_2O, and transpulmonary pressure 10 cm H_2O during infusion of serotonin. Note the striking reduction in red blood cell density at the high lung volume. The total number of measurements for each point are as follows. Closed circles: 5 cm, 150; 15 cm, 120; 25 cm, 60; 35 cm, 30. Open circles: 2 cm, 60; 11 cm, 90; 21 cm, 60. Crosses: each, 60.

blood cells above the level where arterial pressure equaled alveolar pressure. The average septal length at 10 cm H_2O transpulmonary pressure was 50 μ with a range of 20–135 μ. Note therefore that 25 cm above the junction between zones I and II there was only 1 red blood cell per 100 μ of septum.

It was interesting that there were fewer red cells higher up than low down in this zone of no blood flow. This was probably due to a more rapid drainage of red blood cells from the higher part of the zone with the consequent trapping of fewer cells when the arterial pressure was reduced. There was no significant difference found in the measurements made at comparable levels between the one lung perfused with the arterial pressure above the top of the lung just before being frozen and the other two lungs which were frozen some minutes after the arterial pressure had been reduced.

Zone II. Figure 3 shows the change in red blood cells per 10 μ septum down zone II measured at transpulmonary pressures of 10 and 25 cm H_2O and at 10 cm H_2O during the infusion of serotonin.

At a transpulmonary pressure of 10 cm H_2O the increase in red cells was nearly linear with distance down zone II ($r = 0.78$, $P < 0.01$). Note that high in zone II and low in zone I the concentrations of red blood cells were similar. At approximately 20 cm down zone II red blood cell concentration had increased to 1 red blood cell per 10 μ septum or approximately 5 red blood cells per septum. This increase in red cells presumably reflects the increase in capillary pressure down zone II, for arterial pressure (driving pressure) is increasing 1 cm H_2O for each centimeter of distance below the level where arterial equals alveolar pressure.

At a transpulmonary pressure of 25 cm H_2O there was a striking reduction in the number of red blood cells at any level down the length of zone II. The points at 1 and 21 cm differed significantly ($P < 0.01$) but the increase in red blood cells was very small over this distance. The number of red blood cells 19 cm down the zone was equivalent to the number 2 cm down zone II at a transpulmonary pressure of 10 cm H_2O. The smaller number of red blood cells in the capillaries must be due to compression of these vessels by stretching of the alveolar septa or an increase in the vascular resistance of vessels upstream, or both, which causes a reduction of capillary pressure.

It may be seen that the two lines of best fit at the different alveolar volumes are not parallel. This can be explained by the increase in blood flow with distance down zone II. If the vascular resistance of vessels upstream is increased by tension in the alveolar septa the corresponding pressure drop will be greater as distance increases down zone II. Thus the separation of the slopes at 10 and 25 cm H_2O transpulmonary pressure becomes greater with distance down zone II indicating that the upstream vessels contribute to the fall in capillary pressure. This is in contrast to what happens under zone III conditions (see below).

Infusion of serotonin caused a decrease in red blood cell concentration, but not to the extent observed at the transpulmonary pressure of 25 cm H_2O. In the lungs receiving serotonin, capillary pressures must have been lower at comparable levels than in the lungs not receiving the drug. This is most likely due to an increased resistance in the muscular vessels upstream from the capillaries. The number of red cells seen 20 cm down the zone during serotonin infusion was comparable to the number 11 cm down zone II in the other lungs at a transpulmonary pressure of 10 cm H_2O.

Zone III. Figure 4 shows the change in red blood cell concentration down zone III at transpulmonary pressures of 10 and 25 cm H_2O. The data shown in Fig. 4 were obtained with an arterial-venous pressure difference of 4

cm H_2O in five experiments and less than 1 cm H_2O in the six others. The abscissa shows the distance below the level at which the pressure halfway between arterial and venous pressures was equal to PA. This is not strictly the distance down zone III (which is the distance below the level where Pv = PA) but the discrepancy must be less than 2 cm. The advantage of plotting the data in this way is that the abscissa now gives true capillary pressure with a possible error of only 1–2 cm H_2O. Thus the number of red blood cells is a manometer of capillary pressure.

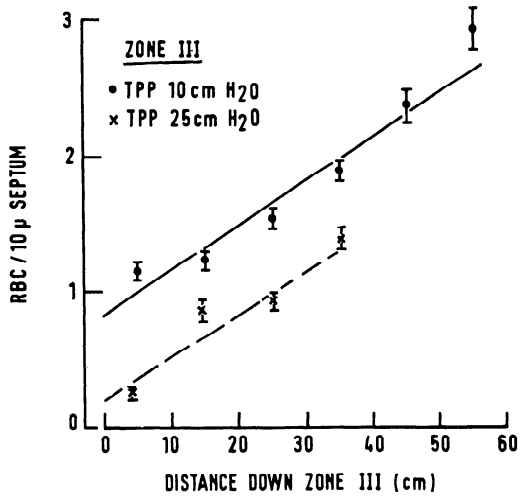

FIG. 4. Red blood cell concentration down zone III at transpulmonary pressures of 10 and 25 cm H_2O. The abscissa represents true capillary pressure (see text). The slope at transpulmonary pressure 10 cm H_2O is not so steep as that in zone II at the same inflating pressure. At a transpulmonary pressure of 25 cm H_2O the number of red blood cells at any level is the same as that at a capillary pressure of 20 cm H_2O less at the lower transpulmonary pressure. The number of measurements from which the mean and standard error at each point were calculated are as follows. Closed circles: 5 cm, 150; 15 cm, 120; 25 cm, 120; 35 cm, 120; 45 cm, 60; 55 cm, 60. Crosses: each, 60.

At the transpulmonary pressure of 10 cm H_2O a linear regression line has been put through the means (r = 0.83, $P < 0.05$). This seems to be a reasonable fit, but there is a questionable region near the top of the zone where a linear relationship may not hold true.

Figure 5 shows that no further increase occurs in the number of red blood cells at capillary pressures greater than 55 cm H_2O. The mean ± standard error of all these measurements from 50 to 105 cm, 2.8 ± 0.1 red blood cells per 10 μ septum, represents the maximum capillary red blood cell concentration as vascular pressures are raised, i.e., about 14 red blood cells per average septum.

The transpulmonary pressure of 25 mm H_2O caused a marked reduction in the number of red blood cells (see also Fig. 1*F*). At any level the effect of stretching of the alveolar septa was to reduce the red blood cell count as if the capillary pressure had been reduced 20 cm H_2O. This reduction in the volume of the capillaries for a comparable capillary pressure at a high transpulmonary pressure is due to a fall in compliance of the vessels. It can be explained by distortion of the vessels as the tension in the alveolar septa rises. The volume of a capillary segment depends on both the transmural pressure which acts across the wall of the segment and the tension in the wall. An increase in the tension of the wall by stretching leads to a decrease in capillary volume if the transmural pressure is constant. In zone III (Fig. 4) the transmural pressure at a given distance down the lung is identical at both transpulmonary pressures.

In the vascular pressure conditions of zone III, unlike zone II, it is possible to say that the reduction in red blood cells at any level was due solely to distortion of the

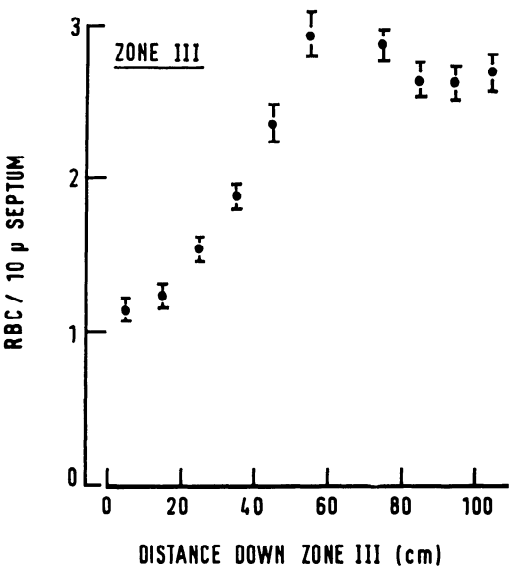

FIG. 5. Change in red blood cell concentration as capillary pressure increases to 105 cm H_2O. There is no further increase in red blood cells at pressures greater than 55 cm H_2O. Each point from 55 to 105 cm H_2O comprised 60–120 measurements.

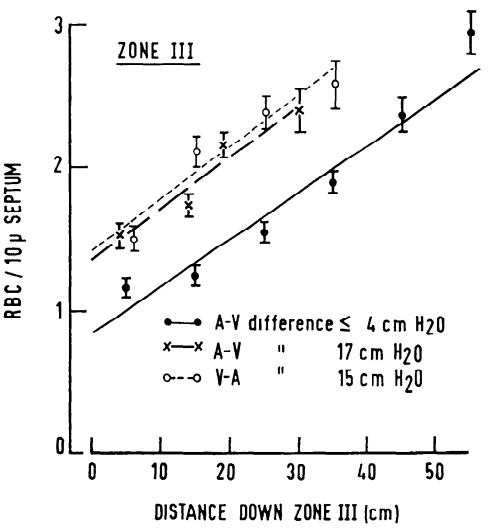

FIG. 6. The effect of a large arterial-venous and venous-arterial pressure difference on the concentration of red blood cells in zone III. Red blood cell density at any level is the same as that found 17 cm lower down zone III when the arterial-venous difference was very narrow (0–4 cm H_2O). The capillaries appear to be seeing a pressure which is close to that of the upstream pressure.

capillary vessels; for with the very narrow arterial-venous pressure difference the greatest pressure drop at the upstream end of the capillary could only have been 1–2 cm H_2O. On the other hand, at the high transpulmonary pressure in zone II the vessels upstream of the capillaries do seem to be contributing to the increased vascular resistance. Thus 20 cm down zone II there has been only a minimal increase in capillary pressure, as judged by the increased number of red cells, whereas in zone III the change in capillary pressure parallels that at the lower transpulmonary pressure.

Figure 6 compares the change in red blood cell concentration down zone III at a transpulmonary pressure of 10 cm H_2O when the lung was perfused with an arterial-venous pressure difference of 17 cm H_2O and back perfused with a venous-arterial pressure difference of 15 cm H_2O with the results shown in Fig. 4. In this figure the abscissa for the arterial-venous pressure differences of 17 and 15 cm H_2O shows the distance below the level at which venous pressure was equal to alveolar pressure. In both situations the red cell concentration 10 cm down the zone was the same as that 17 cm lower at the very small arterial-venous pressure difference. Since the slope of the red blood cell concentration at the very narrow arterial-venous pressure difference serves as a manometer of capillary pressure, we are able to say that capillary pressure at any level (at the high arterial-venous pressure difference) is 17 cm H_2O higher than in the lung with the low arterial-venous pressure difference. Thus the capillaries appeared to be seeing the upstream pressure. This indicates that the muscular precapillary vessels were contributing very little to the vascular resistance, and this finding is in agreement with that of Fowler et al. (6) who showed that much of the pulmonary vascular resistance lay in vessels exposed to alveolar pressure.

Capillary Width

This measurement gives mean capillary width with respect to distance along the septum. This value is less than that which would be obtained by measuring the maximum diameter of many vessels and taking the average.

Zone I. The capillary widths ± standard errors above the zone I-II junction were: 4 cm, 2.8 ± 0.14 μ; 14 cm, 2.0 ± 0.08 μ; and 24 cm, 1.99 ± 0.08 μ. There were 80 measurements in each case. The widths at 4 and 24 cm were significantly different ($P < 0.001$).

Zone II. Figure 7 shows the change in capillary width down zone II at transpulmonary pressures of 10 and 25 cm H_2O. There was an approximately linear increase in width at the lower inflating pressure ($r = 0.90$, $P < 0.01$). At 25 cm H_2O transpulmonary pressure, capillaries were significantly wider at 22 cm than at 2 cm ($P < 0.01$) below the zone I-II junction but the difference in width was very small (3.67 μ compared to 3.15 μ).

Zone III. Figure 8 shows the increase in capillary width down zone III at high and low alveolar inflating pressures. At the transpulmonary pressure of 10 cm H_2O

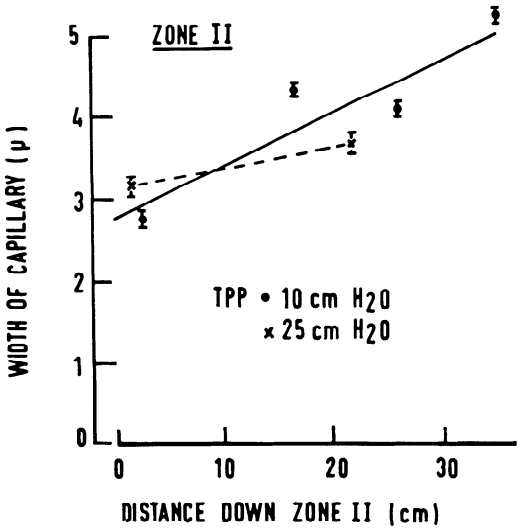

FIG. 7. Capillary width plotted against distance down zone II at two different inflating pressures.

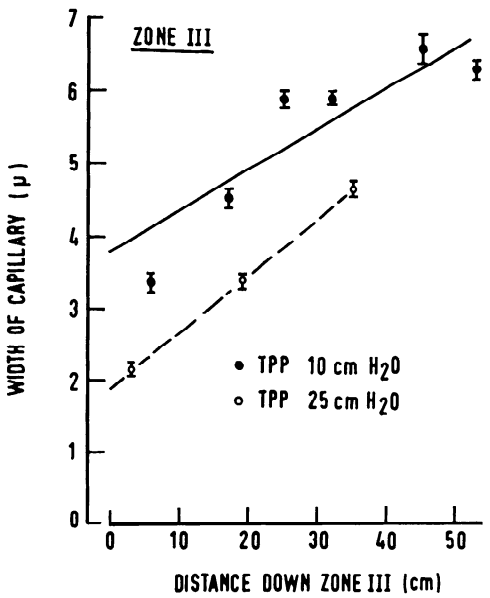

FIG. 8. Change in capillary width down zone III at transpulmonary pressures of 10 cm H_2O and 25 cm H_2O. At the lower transpulmonary pressure, capillary width almost doubles as capillary pressure is increased to 50 cm H_2O.

capillary width almost doubled as capillary pressure was increased to 50 cm H_2O. There was no further measured increase in width at pressures of 50–100 cm H_2O (Fig. 9). Although the greatest mean capillary width was 6.5 μ, the maximum measured capillary diameter was appreciably larger. Values above 10 μ were frequently encountered, the highest measured diameter being 13 μ. Blood cells lying 2 or 3 abreast were often seen (Fig. 1*D*).

At the transpulmonary pressure of 25 cm H_2O. capillary width at any level in the lung was comparable to that measured 22 cm higher up the zone at the lower inflating pressure. This is in good agreement with the measurements of red blood cell concentration in zone III

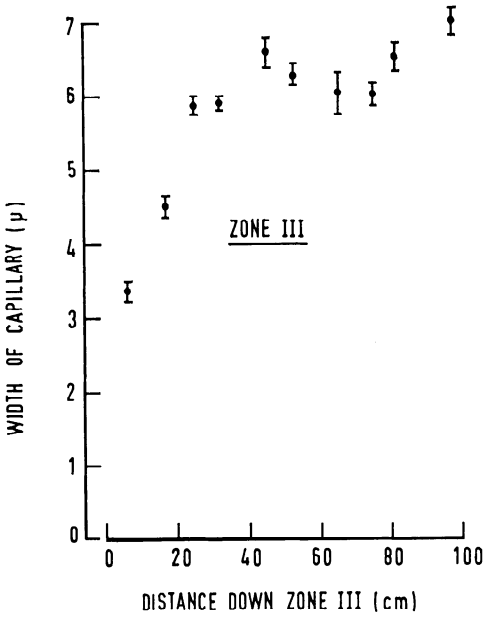

FIG. 9. Capillary width plotted against distance down zone III. Note that at capillary pressures more than 50 cm H_2O there is no consistent change in width.

at a transpulmonary pressure of 25 cm H_2O in which the red blood cell density at any location in the lung was reduced as if capillary pressure were 20 cm H_2O lower (Fig. 4).

Percent Septum Occupied by Red Blood Cells

This measurement indicates the proportion of the alveolar septum in which open capillaries containing red blood cells are present. An increase in this measurement may occur either when a previously closed capillary opens or when an already open capillary distends along the septum.

Zone I. The percent septum occupied by red blood cells ± standard errors above the zone I-II junction were: 4 cm, 16 ± 1 %; 14 cm, 12 ± 1 %; 23 cm, 5 ± 1 %. The slope of the line connecting these points is similar to the slope describing changes in red blood cell volume in zone I (Fig. 2).

Zones II and II. Figure 10 shows the changes in this measurement down zones II and III. The slopes of the lines are similar to those depicting the red blood cell concentration (Figs. 3 and 4). When capillary pressure was increased from 50 to 100 cm H_2O there was no further increase in the percent of septum occupied by red blood cells (compare Figs. 5 and 9).

Number of Septa Without Red Blood Cells

Figure 11 shows the percentage of the total number of septa at each level under the different vascular pressure conditions that did not contain any red blood cells. It is of considerable interest that some septa in zone III conditions required a capillary pressure between 15 and 25 cm H_2O before they contained red blood cells.

Corner Vessels

Measurements made at 3, 13, and 24 cm above the zone I-II junction showed that about 30 % of the corners at each level contained open vessels with red blood cells. The percentages were 31, 31, and 34 %, respectively. Since there was no blood flow in zone I and the capillaries in the alveolar septa were collapsed, this is evidence that the corner vessels were exposed to different stresses. Presumably they were held open to some extent by the alveolar septa and possibly by surface forces.

In zone III about 65 % of the corners contained vessels with red blood cells 3 cm down the zone and the figure was about 80 % 40 cm lower.

Dimensions of Red Blood Cells

At 24 cm above the zone I-II junction the average length of the red blood cells directed along the alveolar septum was 5.39 ± 0.12 μ. The width of the red blood cells was too narrow to measure accurately but was less than 2 μ. At 44 cm down zone III the length of the red blood cells oriented along the septum measured 3.16 ± 0.11 μ, whereas the axis of the red blood cells measured perpendicularly to the septum was 4.42 ± 0.08 μ ($P < 0.001$).

These measurements suggest that in zone III the red blood cells tend to move through the capillaries with their long axes directed perpendicularly to the alveolar septum. In zone I the red blood cells must be compressed and elongated since their length measured along the septum is now not only greater than their width, but this length is greater than any measured dimension of the red blood cells in zone III.

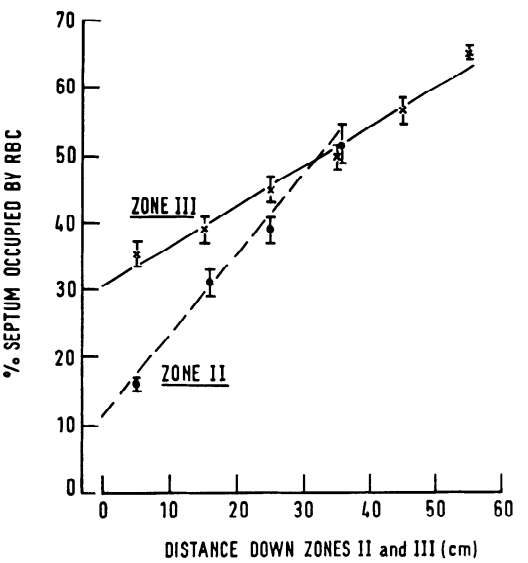

FIG. 10. Percent septum occupied by red blood cells plotted against distance down zones II and III. Steepness of slope predominantly influenced by rate of opening of previously closed vessels. Steeper slope in zone II suggests that recruitment of vessels occurs to a greater extent in this zone than in zone III.

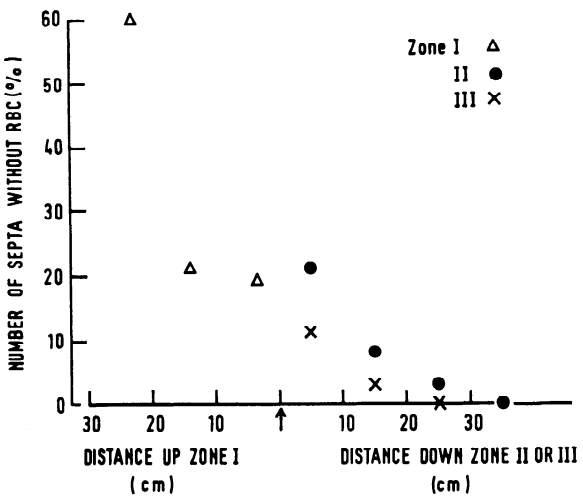

FIG. 11. Number of septa at various levels in all three zones that contained no red blood cells. Septa averaged 50 μ in length. Note systematic difference in zones II and III which is confirmatory evidence that capillary pressure differs in these zones even though upstream pressure is the same.

Electron Micrographs

The electron micrographs confirmed the appearance of the capillaries seen on light microscopy. In sections 24 cm above the zone I-II junction the red blood cells were flattened and elongated. The sections made from tissue 50 and 95 cm down zone III showed that the capillaries frequently bulged out into the alveolar space. In addition they often did not occupy the entire width of the septum.

DISCUSSION

Many earlier investigators (8, 18, 20, 24) who studied changes in capillary morphology under physiological conditions examined blood flow on the surface of the transilluminated lung. However, little quantitative data were obtained in this way and there was some disagreement about what was seen through the microscope. Hall (8), Olkon and Joannides (18), and Ramos (20) noted decreased filling and flattening of capillaries as the alveoli were inflated, whereas Wearn et al. (23) saw no change. Most authors agreed that flow was intermittent through the capillaries although opinion was not unanimous (8).

In 1960 Staub and Storey (22) showed that lung tissue could be frozen so rapidly that its true dimensions were preserved. The tissue was fixed without ever allowing it to thaw, and thus it was possible to examine structural relations as they existed during life. With this technique undistorted tissue is available to a depth of 4 mm below the pleural surface, and the estimated freezing time of this thickness of tissue varies from 500 msec 1 mm below the pleura to just under 10 sec 3 mm deeper. As already mentioned we compared tissue at 1 mm and 4 mm below the pleura and were unable to find any differences in histological appearances.

The question arises as to whether the alveolar capillary bed just below the pleural surface is representative of that deeper in the lung. Miller (15) thought that the alveolar capillary mesh contained fewer capillary segments where it was in direct contact with the pleural surface or other connective tissue, and this is in accord with von Hayek's (10) observation that it is only after two alveolar walls have fused during embryological development that the adult number of capillary segments are present in the alveolar septum. The alveoli on which we made measurements were not in contact with the pleural surface, and we are not aware of other data which suggest that peripheral lung tissue differs from that more centrally located. Some evidence that peripheral and hilar lung parenchyma is similar comes from measurements in lungs fixed in situ by freezing (7). We found that although there are large regional differences in alveolar size in the upright lungs of intact dogs, there are no differences in alveolar size from just below the pleura to 4 cm deeper in the lung at the same vertical height (7).

Mechanisms for increase in capillary red blood cell volume in zones II and III. It is possible to use the three principal measurements—red blood cells per 10 μ septum, capillary width, percent of septum occupied by red blood cells—to evaluate the roles of distention of already opened capillaries and recruitment of unopened capillaries in the increase in blood volume as capillary pressure is raised. By distention we mean an increase in the cross-sectional area of the capillaries. We do not necessarily imply any increase in the perimeter of the vessel by stretching of the vessel wall. The first measurement is an indication of the total capillary red blood cell volume. The second measurement depends solely on the expansion of capillaries, while the third is related partly to the opening of new capillaries and partly to the distention of existing capillaries in so far as they distend along the septum. In Table 1 we show the increase in each of the three measurements down zones II and III. The values at the top of the zone are shown as 1.0. We have squared the measured mean capillary width at each level in the two zones in order to show the increase in capillary area that occurs solely from the expansion of already open capillaries, on the assumption that the capillaries ex-

TABLE 1. *Relative value of measurements*

Distance Down Zone, cm	RBC/10 μ Septum	Capillary Width, μ	(Capillary Width)^2	Septum Occupied by RBC, %
Zone II				
0	1.0	1.0	1.0	1.0
10	2.6	1.2	1.5	2.0
20	4.0	1.4	2.1	3.0
30	5.6	1.7	2.8	4.0
Zone III				
0	1.0	1.0	1.0	1.0
10	1.4	1.2	1.3	1.2
20	1.8	1.3	1.7	1.4
30	2.1	1.5	2.1	1.6
40	2.5	1.6	2.6	1.8

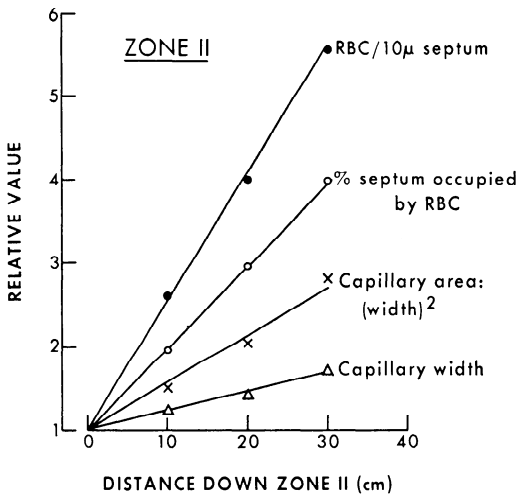

FIG. 12. Increase in the three measurements down zone II. Capillary width has been squared to indicate the increase in capillary area due to expansion of already open vessels. If the line for capillary width is subtracted from the line for percent septum occupied by red blood cells (see text) the difference is the increase in capillary area due to recruitment of new vessels. The total increase in area (expansion + recruitment) $\cong$ the increase in red blood cell volume (Table 1). In zone II approximately one-half of the increase in red blood cell volume is due to recruitment of new vessels.

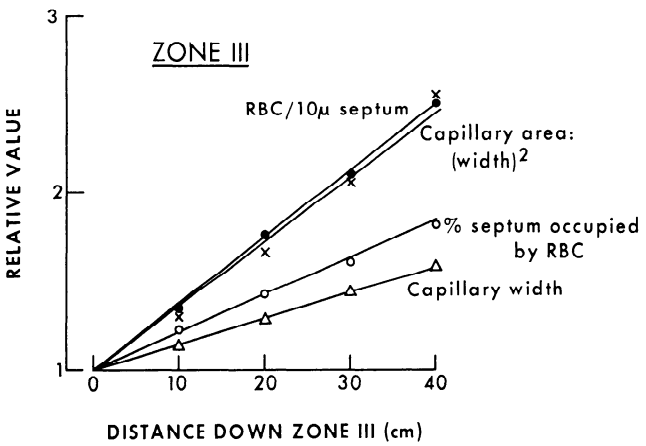

FIG. 13. An analysis similar to that in Fig. 12. The increase in capillary red blood cell volume down zone III is due almost entirely to distention of already open vessels.

pand along the septa to the same extent as they expand at right angles to it.

These results are presented in Figs. 12 and 13. The magnitude of the increase in red blood cell concentration depends on the increase in total capillary area. If all of the increase in capillary area could be explained by expansion of already open vessels the line for capillary area (width^2) would overlie the line for red blood cells per 10 μ septum; whereas if there were no expansion of already open vessels, the line for width^2 would overlie the abscissa. Furthermore, on the assumption that already open vessels expand along the alveolar septum to the same extent as into the alveolar space, we may subtract the line for capillary width from the line for percent septum occupied by red blood cells to indicate how much of the increase in percent septum occupied by red blood cells is due solely to the opening up of previously closed capillaries.

In zone II (Fig. 12) the increase in width^2, i.e., capillary distention, accounts for approximately one-half of the total increase in capillary area while the percent septum occupied by red blood cells minus capillary width, i.e., capillary recruitment, accounts for the remaining increase in area. In zone III (Fig. 13) the situation is strikingly different. Here capillary distention, i.e., width^2, accounts for virtually the whole of the increase in red blood cells per 10 μ septum. Furthermore the increase in percentage septum occupied by red blood cells is almost completely explained by the increase in capillary width. Thus expansion of already open vessels is the principal mechanism to account for the increase in red cell volume as capillary pressure increases. This analysis suggests that most capillaries are already open in zone III, whereas recruitment of new vessels is an important mechanism under zone II conditions.

This conclusion might have been anticipated from the data in Fig. 10, which shows a considerably steeper slope in zone II than in zone III for the percent septum occupied by red blood cells. The increase in this measurement is small when a vessel distends by even 50–100% compared to the increase in proportion of septum occupied by red blood cells which occurs with the opening up of a new vessel.

These data throw some light on the way in which the pulmonary microcirculation operates as a Starling resistor (zone II), that is when alveolar pressure exceeds venous pressure. In the past, the collapse point has usually been assumed to occur at the extreme downstream end of the collapsible vessels (27) because this is where the collapse initially occurs in rubber tube models operating under similar conditions. However, the present results provide evidence that collapse occurs throughout much of the capillary mesh. Figure 10 for example implies large differences in the number of capillaries closed high in zone II compared with high in zone III. Since the only difference between the two situations is whether the venous pressure is below or above alveolar pressure, we conclude that merely raising venous pressure above alveolar pressure under these conditions is sufficient to open up many capillaries.

Gas exchange. Changes in capillary red blood cell volume (Vc) have important implications for gas exchange, especially for the amount of O_2 transferred across the alveolar capillary membrane. The evidence that capillary red blood cell volume increases is consistent with the increasing diffusing capacity (D_{LCO}) measured in athletes exercising up to a $\dot{V}O_2$ of 4 liters/min (16). The fact that diffusing capacity was still increasing with O_2 consumption at these high levels of exercise suggests that the maximum blood volume of the capillary bed had not been reached.

Our data suggest that a maximum diffusing capacity will not be attainable in human subjects since the capillary red blood cell volume may continue to increase up

to a pressure of about 50 cm H_2O. Therefore, for all the capillaries at the apex of the vertical lung to attain their maximal red blood cell volume, capillary pressure at the lung base would be approximately 80 cm H_2O, and alveolar edema would develop.

In measurements of diffusing capacity on dog lungs, Karp et al. (13) demonstrated that DL increased rapidly with increases in pulmonary artery pressure when left atrial pressure was low (i.e., zone II), whereas with each increment in left atrial pressure (i.e., as more of the lung entered zone III) a similar plot of DL against increasing pulmonary artery pressure showed a more shallow slope. The explanation for this finding probably lies in the different slopes for red blood cell concentration in zones II and III (Figs. 2 and 3); that is, capillary red blood cell volume increases faster with perfusing pressure in zone II conditions where the principal mechanism for the increase in capillary volume is opening up of new vessels rather than distention of those already open. It seems likely, at least in the greyhound lung, that most of the capillaries are open at a perfusing pressure of about 25 cm H_2O (Fig. 11 and explanation in text) and further increases in red blood cell volume at higher pressures must be more gradual and due primarily to distention of capillaries.

There has been much conflicting evidence concerning the normal volume of the pulmonary capillary bed at rest as well as the direction of the change in Vc when lung volume is increased. Roughton and Forster (21) and others (4, 14) measured resting Vc near full lung volume and obtained values between 50 and 100 ml. Weibel (24) calculated the anatomic volume of the capillary network in postmortem lungs and found it to be of the order of 100–200 ml. With the single-breath measurement of diffusing capacity (DL), Vc has been reported to stay the same (17), increase (1), and decrease (9) as lung volume is increased.

The measurements reported here show the importance of vascular pressures in determining the volume of the pulmonary capillary bed. In particular the relations between alveolar and venous pressures are important. Thus the effect of lung inflation on Vc will depend very much on what happens to the vascular pressures. In addition the present results show a direct effect of lung inflation in reducing the size of the capillary bed when alveolar pressure is constant in relation to vascular pressures. As far as we are aware this is the first direct demonstration of this effect. Howell et al. (11) found compression of the capillaries by lung inflation but only when alveolar pressure was raised in relation to vascular pressures.

Finally it must be borne in mind that our measurements were made on an isolated lung with uniform alveolar size. In the lung in vivo the capillary blood volume will increase down the lung not only because of the hydrostatic forces discussed here but also because of the regional differences in alveolar size; that is, there are approximately four times as many alveoli per ml of lung parenchyma at the lung base as at the apex. Furthermore, capillary volume itself will also be affected by the degree of lung inflation. Thus at the apex of the upright lung where the alveoli are at a high volume the capillaries will be compressed by tension in the alveolar septa.

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