Influence of Hematocrit, Blood Gas Tensions, and pH on Pressure-Flow Relations in the Isolated Canine Lung

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ABSTRACT
An isolated perfused canine lung preparation in which determinants of vascular caliber could be individually controlled was developed. The relation of pulmonary arterial (Pa), venous (Pv), and alveolar (P\textsubscript{A}) pressures was such that Pa > P\textsubscript{A} > Pv throughout the whole lung. The addition of isoprenaline to the perfusate abolished vascular reactivity. Once stability was reached, vascular cross-sectional area remained acceptably constant for 2.25 hours as judged by normalized conductance. The influence of perfusate hematocrit, blood gas tensions, and pH on pressure-flow relations was then studied in 15 isolated canine lungs. The hematocrit-vascular conductance relation was derived at constant perfusion pressure. Conductance varied linearly with hematocrit over a range of 16.5 to 89.5%. Mean pulmonary arterial blood gas tensions were: Po\textsubscript{2} = 121 mm Hg, Pco\textsubscript{2} = 28 mm Hg, and pH = 7.46. Acute respiratory acidosis (Po\textsubscript{2} = 30 mm Hg, Pco\textsubscript{2} = 81 mm Hg, pH = 7.17) and lactic acidosis and hypoxemia (Po\textsubscript{2} = 32 mm Hg, Pco\textsubscript{2} = 21 mm Hg, pH = 6.96) did not significantly alter this relation. Transformation of the conductance-hematocrit data indicated that hematocrit was the most important determinant of relative apparent viscosity of the blood. Both acute respiratory and lactic acidosis failed to significantly increase relative viscosity within the range of hematocrit usually found in secondary polycythemia.

Erythrocyte deformability is an important determinant of blood viscosity (1-4) and also of pressure-flow relations in an isolated vascular bed (5). Factors that influence erythrocyte deformability include adenosine triphosphate depletion (6), abnormal hemoglobins (7, 8), heat treatment (9, 10), fixation with acetaldehyde (1), and hypoxemia and acidosis (11-14). Attempts to quantify the importance of hypoxemia and acidosis have yielded conflicting results which can be attributed to methodology. It has been suggested, largely on the basis of in vitro viscometry, that the contribution of blood viscosity to pulmonary vascular resistance has been underestimated, particularly its role in the precipitation of right ventricular failure in acute-on-chronic respiratory insufficiency (15). However, direct hemodynamic measurements suggest that the influence of viscosity is trivial in the genesis of pulmonary hypertension in subjects with emphysema or chronic bronchitis and secondary polycythemia (16, 17). Changes in variables other than viscosity may have led to this conclusion, since pressure-flow relations in the pulmonary vascular bed depend on a complex interrelation of pulmonary arterial, pulmonary venous, interstitial, and alveolar pressures which determines both the transmural pressure and the distribution of blood flow (18, 19).

The object of the present study was to examine the effect of acute respiratory and lactic acidosis over a wide hematocrit range on pressure-flow relations in isolated canine lungs. This approach enabled the determinants of vascular caliber to be more effectively controlled than they are in intact animals and thereby allowed some conclusions to be drawn concerning relative apparent viscosity.

Methods
The results reported in this paper are based on 25 isolated canine lung preparations. Ten preparations served as controls, and the remainder, in groups of 5, provided the pressure-flow data which form the body of this report. Statistical analysis was carried out by standard methods (20).

EXPERIMENTAL PREPARATION
Healthy adult mongrel dogs or greyhounds, weighing 12-35 kg, were anesthetized with sodium thiopental (25 mg/kg, iv). On occasion, additional smaller doses were required to maintain anesthesia. A cuffed endotracheal tube was inserted, and 100% oxygen was administered from an open circuit. After anticoagulation (heparin, 2,000 IU/kg body weight, iv), a large polyethylene catheter was advanced into the right atrium via a femoral vein. Small boluses of isoprenaline hydrochloride (Isuprel) were administered to the dog intravenously immediately preceding and at regular intervals during exsanguination. The total dose was 0.2 mg/500 ml of
blood withdrawn. No isoprenaline was given to the first five control dogs. The blood was collected in nonsiliconized glass containers and allowed to cool to ambient temperature.

On exsanguination, a thoracotomy was carried out through the left fifth intercostal space, and the lung and the left atrium were removed. The stem bronchus and the pulmonary artery were cannulated, and then an L-shaped, wide-mouthed, glass cannula was tied into the left atrium. The lung was weighed and mounted horizontally, hilum dependent, in a Lucite box. A platform supported the lung. The cannulas, which projected from the front of the box through rubber seals, were connected to the perfusing circuit (Fig. 1). Perfusion was commenced simultaneously with initial lung inflation.

Blood was fed from a reservoir (A) by gravity via a bubble trap (B), a fine nylon mesh filter (C), and a heat exchanger (D) to the pulmonary artery. The flow rate was controlled by a screw clamp (E) on the arterial line. Blood temperature, monitored by a thermometer (F), was kept within the range of 35 to 37°C. Humidification (H) and a similar temperature range, thermostatically determined (K), were achieved in the box by admitting heated, humidified air. Venous blood drained into a small reservoir (Q) with a float-actuated mercury switch (R) which controlled a roller pump (V) (Watson-Marlow, type MHRK). This pump returned the blood to the major reservoir (A). Nontoxic, polyvinylchloride surgical tubing was used to connect the glass items in the circuit. Saline manometers (O and P) were connected close to the venous and arterial cannulas, and the negative inflation pressure in the box was measured by a differential manometer and amplifier (P-amplifier, Godart) (L). The negative inflation pressure could be set at any desired level by adjusting the vacuum pump (J). Ventilation could be effected automatically or manually by cyclically altering the negative box pressure. The circuit allowed the pressures that determine the distribution of blood flow and the overall pressure-flow relations in the lung to be varied at will. Pulmonary arterial pressure was varied by adjusting the screw clamp (E), venous pressure was controlled by altering the height of the reservoir (Q), and alveolar pressure was always atmospheric. Blood flow through the lung was measured by collecting timed samples in a graduated measuring cylinder (U).

The time which elapsed from the death of the donor dog to the start of lung perfusion was between 40 and 105 minutes. The desired blood gas tensions were achieved and maintained by using the isolated lung as a tonometer. The lungs were ventilated with either ambient air or appropriate gas mixtures. The basic experimental protocol was the same once the desired changes in the perfusate had been established; therefore, the control group will be described in some detail, and subsequently only differences will be highlighted.

**CONTROLS**

A static pressure-volume curve was obtained (1,500-ml water-filled spirometer, Palmer) at the commencement and the conclusion of each experiment. The lung was allowed to collapse to minimum air (the volume remaining in the lung at a transpulmonary pressure of 0 cm H₂O) and then inflated by pressure increments of 10 cm H₂O to a peak of 30 cm H₂O at total lung air volume. The

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**FIGURE 1**

*Diagram of the isolated canine lung perfusion circuit. A = main blood reservoir, B = bubble trap, C = filter, D = heat exchanger, E = screw clamp, F = thermometer, G and N = three-way stopcocks, M = Lucite box, H = inlet for humidified air, J = line to vacuum pump, K = thermostat, L = line to differential manometer and amplifier, O and P = saline manometers, Q = venous reservoir, R = float-actuated mercury switch, S and T = venous line clamps, U = graduated cylinder, and V = roller pump.*

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corresponding deflation limb was constructed from pressure decrements of 5 cm H$_2$O. Care was taken to ensure that each point was read under static conditions, a situation which was usually reached within 30 seconds. Static compliance was expressed as the change in volume produced by a defined change in transpulmonary pressure.

The relation of the pulmonary arterial (Pa), venous (Pv), and alveolar (P$_A$) pressures was such that Pa > P$_A$ > Pv throughout the whole lung (18). Hydrostatic pressure variations within the lung were minimized by horizontal mounting. Pulmonary arterial and venous pressures were related to the most dependent part of the lung. A fixed relation was maintained between intravascular and alveolar pressures (21). Pressure-flow curves were obtained alternately under conditions of near-normal blood gas tensions and acute respiratory acidosis. This cycle was repeated over 3 hours. Oxygen tension (PO$_2$), carbon dioxide tension (PCO$_2$) and pH (Beckman physiological gas analyzer, model 160) were determined on pulmonary arterial and venous samples after the completion of each pressure-flow curve. Perfusion pressures ranged from 8 to 28 cm H$_2$O in the 25 experiments. Flow rates were always measured by approaching the perfusion pressures from below when conditions were judged to be stable; each curve was constructed from at least four points. Each pressure-flow curve was preceded by a standard volume history, i.e., the lung was inflated to near total lung air volume at a transpulmonary pressure of 25 cm H$_2$O and held there for 15 seconds before it was deflated to 15 cm H$_2$O. The hematocrit was monitored at suitable time intervals. It was measured in capillary tubes after centrifugation for 5 minutes at 8,944 g (Adams Readacrit, model CT-3400). Subphysiological flow rates were deliberately chosen to diminish the rate of edema formation. At a normal mean canine hematocrit of 45.5% and a perfusion pressure of 15 cm H$_2$O, flows were 26 ± 14 (SD) ml/min kg$^{-1}$ body weight. Plasma isoprenaline levels were estimated before it was deflated to 15 cm H$_2$O. The stability of vascular cross-sectional area of the isolated perfused canine lung preparation during each experiment was evaluated by three different but complementary approaches. Results

The stability of vascular cross-sectional area of the isolated perfused canine lung preparation during each experiment was evaluated by three different but complementary approaches. First, a correlation was sought between the percent of lung weight gain (reflecting severity of edema) and the static pulmonary compliance (reflecting the elastic properties of the lung), since a change in the latter can alter vascular geometry. No correlation was found. Preliminary studies indicated that vascular conductance was not consistently diminished until the lung weight had doubled. All lungs which increased their weight by more than an arbitrarily set level of 45% were rejected. Data from another laboratory (23) in which similar preparations have been used indicate that a gain of more than 35% of the original lung weight is required before alveolar edema becomes evident histologically. The mean weight gain of the 25 preparations accepted for analysis was 28.3% with a range of 16.5 to 42.2%.

Second, since the experimental design precluded ready measurement of absolute lung volumes, an index of the lung volume change in relation to a certain transpulmonary pressure was derived. Assuming that at a transpulmonary pressure of 30 cm H$_2$O the total lung air volume is reached (24-26), then, given a constant volume history, a change in the percent of total lung volume at the same transpulmonary pressure on the deflation limb will...
reflect a change in the elastic properties of the lung (Fig. 2). Moreover, the change in percent of total lung volume is a quantitative reflection of alveoli and terminal airways which exhibit a tendency to premature closure. The rightward displacement of the pressure-volume curve did not reach levels of significance at any transpulmonary pressure ($P > 0.05$).

The third and most direct way of monitoring the stability of vascular cross-sectional area was to perfuse the lung with a cell suspension of constant viscosity under conditions for which all of the variables that determine vascular caliber were controlled. Vascular stability was not achieved before a mean of 0.75 hours of perfusion had elapsed. Vasoconstriction in response to a combined stimulus of alveolar and perfusion hypoxia and hypercapnia (the pH was allowed to vary with $P_{CO_2}$) persisted for up to 2 hours (Fig. 3). Isoprenaline hydrochloride abolished significant vascular reactivity for a period of up to 3 hours (Fig. 4). Plasma isoprenaline levels (measured in two experiments) declined rapidly in the first 1.33 hours of perfusion, but thereafter they remained virtually constant at around 20 ng/ml. The initial reduction in plasma isoprenaline concentration was not reflected by a corresponding change in vascular
Conductance or reactivity. Because this method of expressing the data resulted in some smoothing of the resultant mean conductance curves, the coefficient of variation, $G$ (%) in Figures 3 and 4, was calculated from the experimentally obtained points for each hematocrit (20). Clearly, isoproterenol both stabilized vascular conductance and abolished vascular constriction in response to a hypoxic, hypercapnic stimulus.

HEMATOCRIT EFFECT

The results of each experiment were represented by a family of pairs of pressure-flow curves, the characteristics of which changed in a typical manner (Fig. 5). With elevation of hematocrit, the curves became more convex to and were progressively displaced toward the pressure axis. Conductances in all groups were calculated at a perfusion pressure of 15 cm H$_2$O. The relation between vascular conductance at constant perfusion pressure and hematocrit was linear. Although the absolute values varied considerably, when they were expressed as a percent of the conductance at a hematocrit of 20%, the slopes of the lines from the five individual experiments were almost identical. The regression line, fitted by the method of least squares, is shown in Figure 6. The regression equations for the data from this and subsequent sections, together with the group mean pulmonary arterial blood gas tensions and the pH values, are shown in Table 1. Pulmonary venous blood was sampled simultaneously with pulmonary arterial blood, and it did not differ significantly with respect to blood gas tensions, indicating a steady-state situation.

ACIDOSIS

Acute respiratory acidosis and lactic acidosis both failed to significantly alter the conductance-hematocrit relation from that found with normal blood gas tensions ($P > 0.90$) (Table 1). Although the quantity of trapped plasma at a given hematocrit was probably greater in the acidosis series than it was in the normal blood gas tension series (27, 28), failure to correct for this difference does not invalidate the comparison of pressure-flow curves between the three groups. The pulmonary vasculature “sees” the measured or effective hematocrit.

Discussion

All designs of a roller pump produce hemolysis by mechanically damaging the erythrocytes. Plasma hemoglobin was estimated in three experiments and found to reach a peak of 155 mg/100 ml after 2 hours and 47 minutes of perfusion, having risen from a base-line value of 3.65 mg/100 ml. Considerable variability was detected in both the base line and the peak values, suggesting that the extent of hemolysis was not a simple function of perfusion time. The low peak concentration of plasma hemo
globin per se does not influence blood rheological properties (29) and, hence, pressure-flow relations in the isolated lung.

The low resistance of the pulmonary vascular bed dictates that added resistances of all of the connections be known or be negligible. The arterial cannula has a significant resistance (30), but this value was not taken into account; thus, the results presented pertain to the vasculature of the isolated lung in series with the arterial cannula.

**HEMODYNAMIC CONSIDERATIONS**

Before any change in vascular conductance can be attributed to a change in the characteristics of the perfusate, vascular geometry must be shown to be constant. In these experiments, great care was taken to ensure that the many, not necessarily independent, physiological variables which determine vascular caliber were either controlled or measured. Thus, pressure-flow measurements were obtained after a constant volume history at a specified transpulmonary pressure with the entire lung in zone 2 conditions. Perfusion was continued until the influence of vasoactive substances in the perfusate had disappeared or become constant. Isoprenaline abolished active vasoconstriction in response to hypoxia, hypercapnia, and increased hydrogen ion concentration.

The inevitable development of pulmonary edema in this type of preparation may have resulted in a progressive rise in pulmonary vascular resistance. Although compliance, measured over the tidal volume range, falls progressively with increasingly severe pulmonary edema, the deflation limbs virtually coincide before and after the onset of edema provided total lung capacity is reached (24). Furthermore, the total volume of the severely edematous lung is nearly as large (94% of control) as that of the normal lung at a transpulmonary pressure of 30 cm H2O (24). The rightward displacement of the deflation pressure-volume curve may be the result of groups of alveoli which tend to deflate at abnormally high distending pressures or of increased elastic recoil due to interstitial edema. The relative contribution of these two influences to any change in vascular conductance is not known.

It was shown that, under the conditions of the present measurements, the total effective vascular cross-sectional area remained constant at a given perfusion pressure if the nature of the perfusate was kept constant (Fig. 4). Other investigators have inferred this fact from a variety of isolated lung preparations (31-34) and preparations from the systemic vascular bed (35-37). Once perfusate characteristics are changed, constancy of vascular cross-sectional area can no longer be assumed in constant-perfusion pressure experiments. For example, as hematocrit is increased, the intraluminal pressure will fall in all parts of the vasculature due to viscous and inertial (38) forces during steady flow. In the presence of a constant extravascular pressure, transmural pressure will decrease, and, unless the vessels are rigid, luminal narrowing may result. Pulmonary vascular interdependence (39) is likely to minimize any tendency for vessels to narrow or close.

This study demonstrated quite clearly that, in the presence of a constant perfusion pressure, hematocrit is an important determinant of vascular conductance. Normalization for lung size permits evaluation of the effect of a change in perfusate characteristics on a group of lungs of different size over a given range of hematocrit. The slope of the conductance-hematocrit line (Fig. 6) may represent the combined influence of progressively diminishing vascular cross-sectional area and progressively increasing perfusate viscosity. Experimental design can only minimize but never obviate this criticism. That acute respiratory and lactic acidosis failed to alter the slope of the conductance-hematocrit line (Table 1) is highly significant. It implies that any change in perfusate characteristics, such as decreased erythrocyte

**TABLE 1**

*Regression Equations of Conductance and Hematocrit in Experimental Groups*

<table>
<thead>
<tr>
<th>Group</th>
<th>Regression equation</th>
<th>se</th>
<th>r</th>
<th>P0.4 ± sd (mm Hg)</th>
<th>PCO2 ± sd (mm Hg)</th>
<th>pH ± sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>G₃₄ = -124.19 - 1.21H</td>
<td>5.27</td>
<td>-.98</td>
<td>121 ± 21</td>
<td>28 ± 4</td>
<td>7.46 ± 0.03</td>
</tr>
<tr>
<td>2</td>
<td>G₃₄ = 123.85 - 1.21H</td>
<td>6.52</td>
<td>-.97</td>
<td>30 ± 6</td>
<td>81 ± 7</td>
<td>7.17 ± 0.07</td>
</tr>
<tr>
<td>3</td>
<td>G₃₄ = 125.26 - 1.26H</td>
<td>4.59</td>
<td>-.99</td>
<td>32 ± 3</td>
<td>21 ± 2</td>
<td>6.96 ± 0.07</td>
</tr>
</tbody>
</table>

Group 1 = hematocrit effect, group 2 = respiratory acidosis, and group 3 = lactic acidosis. G₃₄ = conductance as a percent of that at a hematocrit of 25% (the subscript denotes the perfusion pressure of 15 cm H2O), and H = hematocrit (%). se = standard error of the regression estimate.
deformability, was hemodynamically unimportant. Reduction in total vascular cross-sectional area and progressively diminishing shear rate in a relatively high resistance system would contribute to a positive artifact.

Whittaker and Winton (35) were the first to show that the "apparent viscosity" of blood flowing in the vessels of an isolated canine hind-limb preparation differs significantly from that measured, under otherwise identical conditions, in a high-velocity glass tube viscometer. Other investigators using similar (36) and different (37) approaches have confirmed the findings of Whittaker and Winton. Only recently have comparable data become available for the pulmonary circulation (31-34). The data shown in Figure 6 can be transformed to yield an index of relative apparent viscosity (Fig. 7). Extrapolation of conductance (%) to a hematocrit of 0% prior to transformation enables derivation of apparent viscosity relative to plasma. The present results can then be compared with the results of "biological viscometry" obtained in a number of different vascular beds and tube viscometers (Fig. 8). The differences so demonstrated are attributable to experimental design and to the properties peculiar to each type of viscometer, e.g., totally dissimilar vasculature between the lung and the

hind-limb (40), Fahraeus-Lindqvist effect (41), differences in shear rate, and inertial pressure losses (38). On the other hand, the relative apparent viscosity obtained from a model based on the alveolar sheet (42) agrees quite well with the results reported in the present paper and with those reported by others in a variety of lung preparations (31, 33).

In vitro viscometric data indicate that raising the hydrogen ion concentration results in increased viscosity (11-14, 43-45). The magnitude of the viscosity increase is disputed; some investigators claim that large increases result at all shear rates but particularly at high hematocrits (12, 45) but others suggest that any increase is minimum (13) or detectable only at low shear rates (44). The majority of investigators attribute this increased viscosity to changes in cell morphological characteristics (11, 13, 14, 43, 44), but some think that an increase in erythrocyte internal viscosity is paramount (12, 45). The variability of the reported viscosity increases is attributable in part to differences in methodology and in part to failure to
assess independently the different interacting variables.

Granted the generally lower viscosity values obtained by biological viscometry, it is surprising that determinants of viscosity other than hematocrit have received so little attention. Levy and Share (36) rendered their hind-limb preparation totally ischemic for 10 minutes and therefore severely hypoxic and acidotic, with a view to inducing maximum vasodilation. The oxygen tension of the donor blood was said to have been considerably lowered, but no measurements of arterial \( P_{O_2} \), \( P_{CO_2} \), or \( pH \) were made. Any change in the erythrocyte characteristics may have been obscured by the consequences of the marked change in hind-limb vascular dimensions.

It is concluded that the conductance-hematocrit relation in the isolated lung is not changed when mean blood gas tensions similar to those encountered in severe acute respiratory and lactic acidosis are imposed. This fact implies that any changes which acidosis and hypoxemia induce in canine erythrocytes must be slight with little effect on their deformability. The results suggest that viscosity factors play a relatively unimportant role in the genesis of acutely increased pulmonary vascular resistance and right ventricular failure in subjects with chronic obstructive airway disease and acute-on-chronic respiratory failure.

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