Analysis of the double occlusion which provides four pressure gradients

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ABSTRACT: The arterial, double and venous occlusions are used to partition pulmonary vascular resistance into four segments. In this study, we tested whether the same can be accomplished from one double occlusion.

In an isolated canine lung left lower lobe perfused with blood (flow rate=500 mL·min^{-1}), the pulmonary arterial and venous pressures (Pa and Pv, respectively) were measured directly. Arterial, double, and venous occlusions were performed and analysed as usual (Method 1) to measure pressures in small arteries and small veins (Pa' and Pv', respectively) and capillary pressure (Pc). Alternatively, one double occlusion was analysed (Method 2), not only for Pa, Pv and Pc, but also as independent arterial and venous occlusions to measure Pa' and Pv'.

Method 1 yielded Pa, Pa', Pc, Pv', and Pv (Baseline) of 14.2±1.7, 10.8±1.6, 8.9±1.9, 7.3±1.5 and 1.3±0.6 mmHg, respectively (1 mmHg=0.133 kPa). Method 2 yielded values for the same five pressures equal to 14.7±2.1, 11.0±2.2, 8.9±1.9, 7.3±1.3 and 1.3±0.6 mmHg, respectively. There was no significant difference in the pressure profile obtained using the two methods, nor were there differences during hypoxia and angiotensin infusion.

These results suggest that a more thorough analysis of the double occlusion can provide the same information about distribution of vascular resistance as provided by a combination of the three occlusions. The advantage of the new approach is that fewer occlusions are needed and resistance distribution can be assessed during a transient response. Because all pressures are derived from one occlusion, the pressures would be more accurate relative to each other.
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The arterial and venous occlusion technique [1–3] allows partitioning of the pulmonary vasculature into three segments (arterial, middle and venous), while the double occlusion technique has been used to partition the vasculature into two segments upstream and downstream from a mid-capillary point [4]. The double occlusion technique, however, is not used to determine whether changes in resistance occur within small or large vessels. By combining the arterial, double and venous occlusion techniques, it is possible to partition the vasculature into four segments, and determine whether the site of constriction is in small or in large arteries or veins [2, 5]. In this study, we show how the double occlusion can be analysed to provide four pressure gradients without performing arterial or venous occlusions independently. Thus, by performing one double occlusion manoeuvre lasting 3–4 s it would be possible to measure resistance of four vascular segments. This new approach has the advantage of being able to partition the vasculature into four segments when the vascular resistance is not in a steady-state.

Materials and methods

Isolated lung preparation

Ten mongrel dogs of either sex, weighing 20.3±3.5 (SD) kg, were anaesthetized with sodium pentobarbital (30 mg·kg^{-1} body weight), intubated and ventilated mechanically (Harvard Animal Ventilator). Following administration of heparin (700 U·kg^{-1}), the animals were exsanguinated *via* a catheter in the carotid artery. The procedure for perfusing the left lower lobe *in situ* has been described previously [1–3]. The left upper and middle lobes were tied-off and excised through a left thoracotomy. Cannulae were placed in the left lower lobe artery and vein. The arterial and venous cannulae were connected to a perfusion system. The recirculating perfusion system was filled with autologous blood (400 mL, haematocrit (Hct) ~40%), and consisted of a reservoir, a pump (Masterflex 7015), and a filter, which also acted as a bubble trap and as a damper. Twelve milligrams of indomethacin in sodium bicarbonate (Sigma Chemical Co.) was mixed slowly into the reservoir prior to initiation of the perfusion. The lobe was perfused with blood (400–600 mL·min^{-1}) from the reservoir, and the blood was allowed to drain back passively into the reservoir.

The blood flow rate (Q't) through the lobe was initially adjusted to provide an arterial pressure of 10–15 mmHg. Venous pressure was set at 1–2 mmHg by adjusting the level of the reservoir. The reservoir was immersed in a waterbath to maintain blood temperature at 37°C. The pulmonary arterial and venous pressures, Pa and Pv, respectively, were measured from

side-ports in the cannulae using two pressure transducers (PdI23B) relative to the top of the lobe, and recorded on an 8-channel recorder (Hewlett-Packard 8261). The lobe was ventilated with a gas mixture of 5% CO_2, 35% O_2, and 60% N_2. The airway pressure was also monitored from a side-port in the tracheal tube. End-expiratory pressure was set at 1–2 mmHg and inspiratory pressure was kept below 10 mmHg by adjusting the tidal volume. All measurements reported in this study were obtained with the ventilator turned off in expiration and the lungs were in zone 3 conditions. These ventilatory settings provided a carbon dioxide tension (PCO$_2$)=4.7±0.3 kPa (35.6±2.3 mmHg), oxygen tension (PO$_2$) 28.6±4.1 kPa (215±31 mmHg), and pH 7.37± 0.03 (SD) in the blood. During hypoxic gas breathing (5% CO_2, 3% O_2, and 92% N_2), these values were 4.6±1.0 kPa (34.7±7.4 mmHg), 5.6±1.4 kPa (42.2±10.7 mmHg), and pH 7.39±0.09, respectively (Radiometer IIC, Copenhagen).

Experimental protocol

When steady-state was reached, as indicated by a constant Pa and normal blood gas tensions and pH, the experimental protocol was carried out as follows. The ventilator was turned off during expiration and arterial, venous and double occlusions (AO, VO, and DO, respectively) were performed, and the signals were sampled at 200 Hz and stored in a computer. Arterial occlusion was accomplished using a three-way solenoid valve; venous occlusion was accomplished by rapidly clamping the outflow tubing with a large haemostat; and double occlusion was accomplished by activating the solenoid and clamping the outflow simultaneously. Arterial occlusion lasted for 10 s, while venous and double occlusion lasted 2–4 s. Once this was completed, perfusion was re-established and ventilation of the lobe was switched to a hypoxic gas. Because flow was constant, Pa gradually rose during hypoxia, and stabilized after rising an average of 20 mmHg. AO, VO and DO were repeated. Flow was re-established to ensure that hypoxic vasoconstriction was maintained. Normoxic ventilation was then re-established and Pa returned to baseline value. Angiotensin solution (5 µg·mL^{-1}, Sigma Chemical) was then infused continuously at a rate of 0.26 mL·min^{-1} into the arterial cannula until the Pa rose 5–10 mmHg and stabilized. Ventilation was turned off in expiration, and AO, DO and VO were performed. Angiotensin infusion was stopped and the Pa was allowed to recover.

Data analysis

The occlusion curves were analysed using two different methods.

Method 1. Arterial, venous, and double occlusion tracings were displayed and analysed independently in the usual manner [1–3, 5]. Figure 1 illustrates such analyses. The arterial occlusion was displayed and analysed (fig. 1a). Briefly, a stretch of data on the arterial pressure between 0.2–2.0 s following arterial occlusion was fitted to a single exponential and extrapolated back

towards time of occlusion. The breakpoint between the rapid and slow phases was then used as a pressure in the small arteries (Pa'). The double occlusion tracings were then displayed and capillary pressure (Pc) was determined by averaging the arterial and venous pressures between 2–4 s after the double occlusion (fig. 1b). The venous occlusion tracing was then displayed (fig. 1c), and a stretch of data (0.2–2.0 s) on the venous
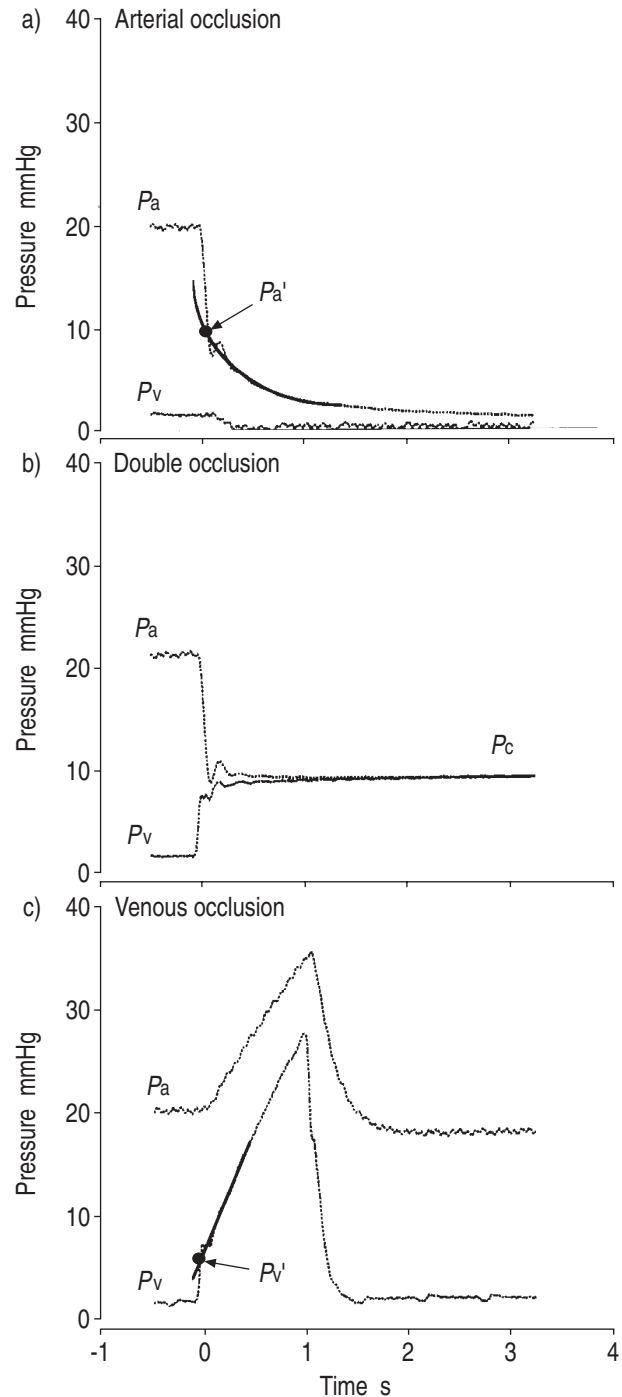

Fig. 1. – Typical examples of the shape of pressure tracings during: a) an arterial occlusion; b) double occlusion; and c) venous occlusion. The method of analysis of each occlusion is illustrated by the thick lines (see text). Pa: arterial pressure; Pa': arterial occlusion pressure; Pc: double occlusion pressure (capillary pressure); Pv: venous pressure; Pv': venous occlusion pressure. (1 mmHg=0.133 kPa).

pressure was fitted to a straight line and extrapolated back towards time of occlusion. The breakpoint between the rapid and slow phase was used as a pressure in the small veins (Pv').

Method 2. Alternatively, only the double occlusion tracings were displayed and analysed more thoroughly, as follows (fig. 2). The arterial pressure was first analysed to calculate Pa', then the venous pressure was analysed to calculate Pv'. Because the pressures stabilized quickly during a double occlusion, a shorter stretch of data between 0.2–1.5 s (instead of 2.0 s) was selected and fitted to a monoexponetial. The fitted curve was extrapolated back towards time of occlusion and the breakpoint pressure between the rapid and slow phase was used as Pa'. Likewise, data between 0.2–1.5 s on the venous pressure tracing was fitted to an exponential and extrapolated back towards time of occlusion. The breakpoint between the rapid and slow phase was used as Pv'. The average of Pa and Pv between 3–4 s after the occlusion was used, as before, to represent capillary pressure (Pc).

Although the precise anatomical correlates of these pressures are not known, the evidence in dog [2] and lamb lungs [6, 7] suggests that the arterial and venous segments represent vessels larger than 100 µm in diameter. The small arterial and venous segments, therefore, represent vessels smaller than 100 µm in diameter. Vascular resistance of each of the four segments can be calculated from the pressure gradient of each segment divided by the mean flow rate. For presentation of the data, the mean of each pressure from each method was calculated. A one-way analysis of variance (ANOVA) for repeated measures and a Dunnett t-test were used to determine the significance of changes in each segment under each condition. A paired Student's t-test was used to compare the mean pressures obtained with the two methods of analyses. A p-value of less than 0.05 was considered significant. Agreements between Pa' and Pv' derived from the two methods were tested further using two other tests [8, 9], as described in the results.
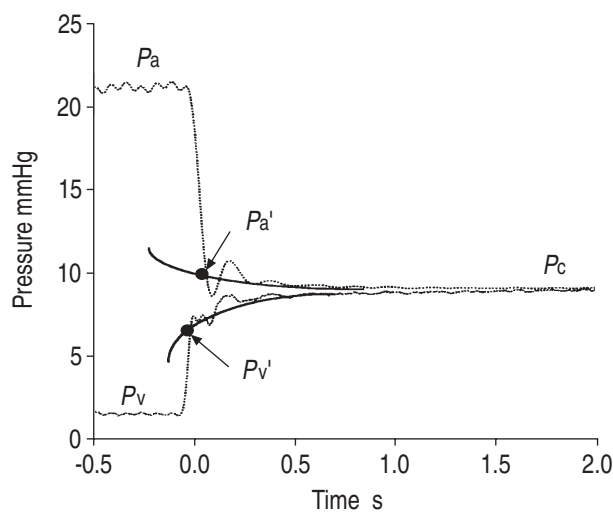

Fig. 2. – Tracings of pressures during a double occlusion (same one as shown in figure 1). Complete analysis of the tracings to derive Pa, Pa', Pc, Pv', and Pv is illustrated by the thick lines (see text). For definitions see legend to figure 1. (1 mmHg=0.133 kPa).

Results

Figure 3 illustrates three double occlusions during the 2 s immediately following the occlusion, for normoxia, hypoxia, and angiotensin infusion. Clearly, both arterial and venous pressures had an initial rapid phase followed by a second slow phase, during which time the two
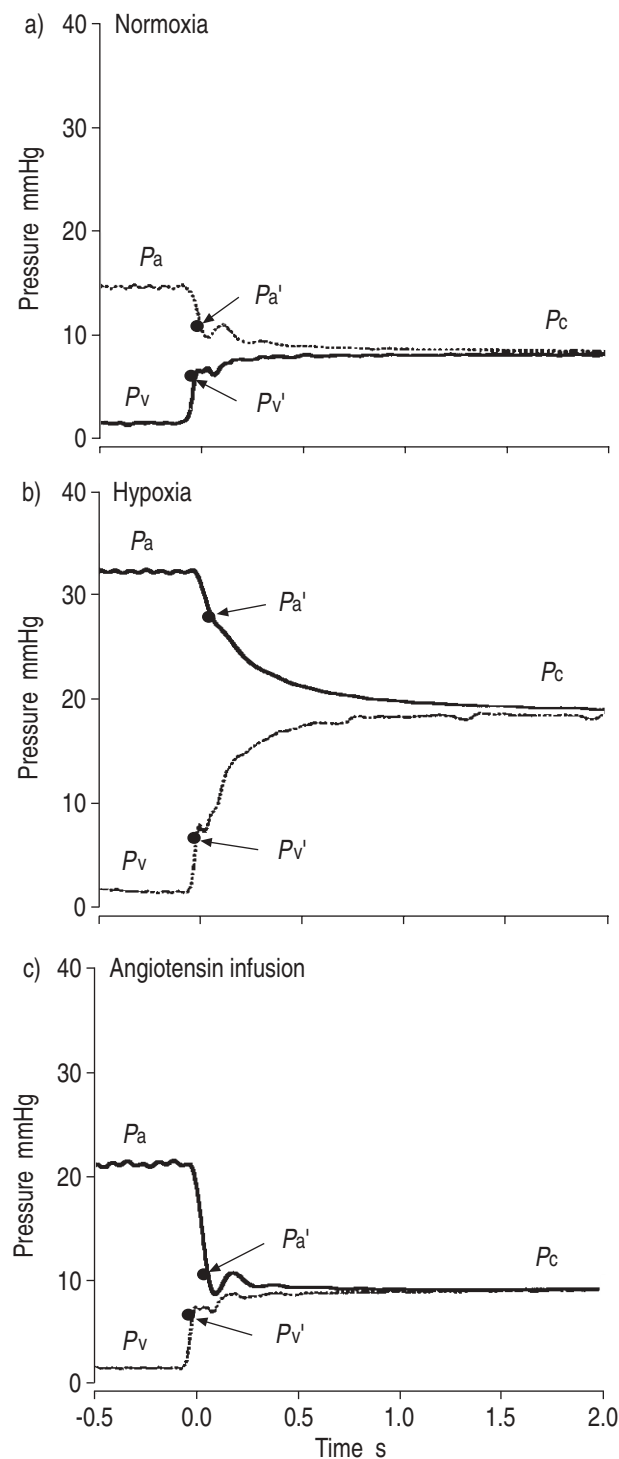

Fig. 3. – Tracings of pressures during double occlusions under three experimental conditions: a) normoxia; b) hypoxia; c) angiotensin infusion. The approximate location of Pa' and Pv' are indicated by a large dot. Pc is the average pressure after equilibration. For definitions see legend to figure 1. (1 mmHg=0.133 kPa).

Table 1. – Mean pressure profiles calculated either from analysis of arterial, venous, and double occlusion tracings (Method 1) or from more thorough analysis of one double occlusion tracing (Method 2)

	Method	P_a	P_a'	P_c	P_v'	P_v
Normoxia	1	14.2±1.7	10.8±1.6	8.9±1.9	7.3±1.5	1.3±0.6
	2	14.7±2.1	11.0±2.2	8.9±1.9	7.3±1.3	1.3±0.6
Hypoxia	1	31.8±6.4	27.0±5.8	17.7±4.3	9.8±4.1	1.3±0.6
	2	32.2±5.7	27.2±5.7	17.7±4.3	9.6±3.4	1.3±0.6
Angiotensin	1	23.3±4.4	13.1±5.2	11.3±2.6	8.8±2.7	1.4±0.5
	2	24.2±5.0	14.9±5.9	11.3±2.6	9.2±2.0	1.4±0.5

The values are presented as mean±SD from 10 animals. P_a: arterial pressure; P_a': arterial occlusion pressure; P_c: double occlusion pressure (capillary pressure); P_v': venous occlusion pressure; P_v: venous pressure. Mean flow rate in the 10 animals was 493±60 mL·min^{-1}. There was no difference between values calculated from the two methods.

pressures equilibrate with capillary pressure. The shape of the curves are more visible during hypoxia, when the resistance of the small vessels is elevated. The two methods of analysis were used to analyse the occlusion tracings under all three conditions. Table 1 shows the mean data for each condition. The first line for each condition represents the mean pressure values obtained with Method 1, as described previously [1–3, 6, 10–13]; P_a' was obtained from an arterial occlusion, P_c was obtained from a double occlusion, and P_v' was obtained from a venous occlusion. P_a and P_v were averaged from the three values prior to each occlusion manoeuvre. The five values, P_a, P_a', P_c, P_v', and P_v, which were derived from three occlusion manoeuvres, were then combined into one pressure profile.

The second line for each condition in table 1 presents the mean data from Method 2, whereby all five pressures, P_a, P_a', P_c, P_v', and P_v, were obtained from a single double occlusion manoeuvre. The agreement between the two methods was tested in three different ways. Firstly, the mean values of each pressure with the two methods were compared using a simple Student's t-test. There was no significant difference between the mean values for P_a, P_a', P_c, P_v', and P_v derived using the two methods under all conditions.

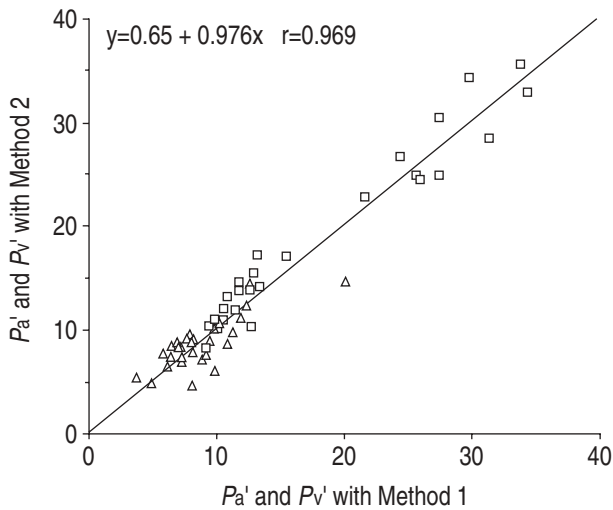The agreement between the two methods was further tested by using regression analysis. A scattergram of all data points for P_a' and P_v' from the two methods is shown in figure 4. All the data points fell close to the line of identity. The slope of linear regression was 0.957, with a 95% confidence interval of 0.960–0.976, based on Fisher's z-transformation analysis [8]. The regression analysis suggested an excellent agreement between the two methods.

Finally, the limits of agreement between the two methods were tested according to the analysis of BLAND and ALTMAN [9]. The difference between the P_a' or P_v'
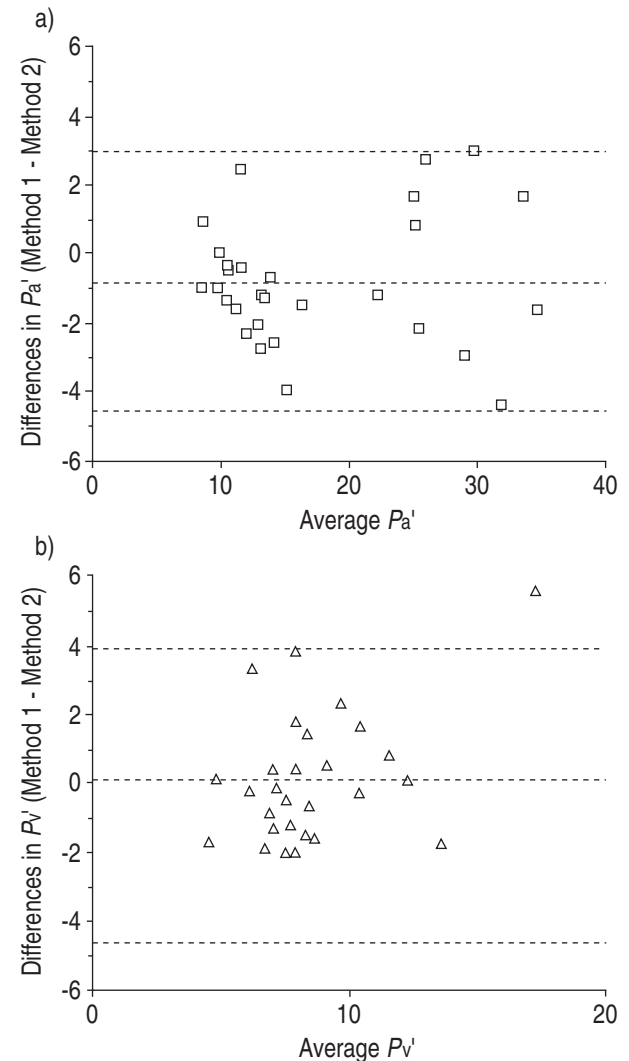
a)

b)

Fig. 5. – Difference in a) P_a' and b) P_v' between the two methods against the average of the two methods. The mean difference ±2 SD is indicated by the dotted lines. For definitions see legend to figure 1.

Fig. 4. – Relationship of P_a' (□) and P_v' (△) from the two methods. The line of identity is drawn. The equation for linear regression is also shown. For definitions see legend to figure 1.

y=0.65 + 0.976x   r=0.969

values from the two methods was plotted against the average of the two methods (fig. 5). The figure indicates that the mean difference is not significantly different from zero and that all differences fell within ±2 SD. An estimate of reliability of agreement (intraclass regression) between the two methods was calculated from mean squares among (M_a) and within (M_w) the values, $(M_a-M_w)/(M_a+M_w)$ [8]. An estimate of 1 suggests perfect agreement, and 0 suggests no agreement between the two methods. Estimate of reliability for P_a' was 0.97 and for P_v' was 0.78. This test further supports the excellent agreement between the two methods.

Discussion

The method of analysis of arterial and venous occlusion tracings and the anatomical correlates of the arterial, venous and middle segment has been the subject of other papers by our group and by others [1, 6, 7, 13, 14]. Analysis of the double occlusion has been less controversial. The validity of pressures derived using the occlusion technique was verified by comparing the occlusion measurements with direct measurements using small catheters or by micropuncture [2, 6]. Criteria of the analysis, and the influence of small variations in the number of data points and the selection of time zero has also been discussed previously [1]. This study uses such published analytical criteria, and presents new information which can be derived from the double occlusion. The double occlusion is a simultaneous arterial and venous occlusion, and the shape of the arterial and venous pressure tracings appear very similar to those recorded during a single arterial and a single venous occlusion. Therefore, it occurred to us that the analysis which is used for arterial and venous occlusion could also be applied to the tracings during a double occlusion. The purpose of this study was, therefore, to determine whether the pressures which are derived from the arterial and venous occlusion can also be derived from a double occlusion.

The results show that, indeed, one double occlusion can be analysed more thoroughly to partition the pulmonary vasculature into four segments. The pressures derived from analysis of one double occlusion were not significantly different from those obtained from analyses of arterial, double, and venous occlusions. Therefore, the double occlusion can differentiate not only whether the site of resistance is upstream or downstream from a mid-capillary point, but with this thorough analysis it provides information about whether the resistance had changed in the large or in the small vessels. The data from previous studies suggest that P_a' and P_v' represent pressures in arteries and veins <0.1 mm in diameter [2, 6, 7]. Thus, the occlusion technique partitions resistance into four segments: large arteries (>0.1 mm in diameter); small arteries (<0.1 mm); small veins (<0.1 mm); and large veins (>0.1 mm). The increase in resistance in the small vessels can be due to vasoconstriction, or due to mechanical factors, such as an increase in alveolar pressure or an increase in blood viscosity [2, 12].

The results of the present study are consistent with previous findings, namely that hypoxia increases the resistance of the small vessels, while vasoactive agents increased the resistance of the large vessels [5]. The hypoxic response in these animals was much larger than in a previous study because the animals were pretreated with indomethacin to enhance the hypoxic vasoconstriction [10, 15]. Addition of indomethacin to the blood, however, had caused an increase in venous resistance during baseline conditions, as reported previously [16]. Therefore, the relative contribution of the venous resistance in this study was larger than previously reported by us in the same animal preparation.

The two methods for partitioning of pulmonary vascular resistance into four segments agreed strongly with each other. Partitioning of the resistance into four segments from one double occlusion with Method 2 has some advantages. One obvious advantage is time-saving during the experiment, since only one occlusion is necessary instead of three. More importantly, however, if the pulmonary arterial pressure is not stable, the double occlusion analysis is very suitable. When three occlusions are being utilized, it is necessary to wait for the pressure to return to a steady value before the next occlusion can be performed. This can create problems because vascular constriction is not always perfectly stable. The lack of stability may lead to measurement of a capillary pressure that is larger or smaller than the P_a' or P_v', respectively. This problem is totally eliminated if one utilizes one double occlusion to derive all five pressures. Lack of a stable P_a becomes a problem, in particular, if one is evaluating a transient response, such as during injection of drugs as a bolus, whereby the pressure rises and falls without stabilizing. Under such conditions, the double occlusion is useful and can be performed at any point during the transient response.

Conceptually, there may be an advantage in analysing the double occlusion as described in Method 2. While a shorter segment of data becomes available for the analysis, such segments may provide more accurate data because the shape of the arterial and venous pressures are determined primarily by the upstream and downstream segments, respectively. Thus, the shape of the arterial pressure during a double occlusion may be more reflective of the arterial tree, while the shape of the venous pressure may be more reflective of the venous tree, because the two pressures equilibrate with capillary pressure. Consequently, calculation of P_a' and P_v' may be more accurate or, at worst, not significantly different from values obtained from an arterial and a venous occlusion.

One disadvantage of Method 2 is the shorter segments of data which are recorded before the pressure reaches a steady-state. This may conceal the shape of the pressure curves and may make them more difficult to analyse. This problem can be resolved by recording the pressure on an expanded scale, so that the shape of the curve remains visible. Acquisition of data using a computer is also helpful in this regard. Although we performed exponential fitting to the curves, in many instances it would have been possible to fit a straight line to the data in order to extrapolate back to time zero.

In summary, we found that the double occlusion can be analysed more thoroughly to partition the pulmonary vasculature into four segments, thus providing more information than previously suggested.

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