Longitudinal Gradients in Periarteriolar Oxygen Tension
A POSSIBLE MECHANISM FOR THE PARTICIPATION OF OXYGEN IN LOCAL REGULATION OF BLOOD FLOW
By Brian R. Duling and Robert M. Berne

ABSTRACT
The oxygen tension (P_{O_2}) on the external surface of arterioles between 8 and 100 μm in diameter was measured with oxygen microcathodes (2 to 6 μm diameter) in the suffused cheek pouch of hamsters and in the cremaster muscle of hamsters and rats anesthetized with pentobarbital and urethane. Intravascular P_{O_2} was measured in 10 vessels and compared with extravascular P_{O_2}. Good agreement was found, with a mean difference of 1.4 ± 0.8 (SEM) mm Hg. Significant longitudinal gradients were observed in periarteriolar P_{O_2}. Oxygen tension fell from 35 ± 4 mm Hg on the small arteries (ca. 80 μm diameter) to 20 ± 3 mm Hg at the end of the terminal arterioles. These measurements were obtained with a suffusion solution P_{O_2} of 39 ± 8 mm Hg, a tissue P_{O_2} of 8 ± 2 mm Hg and femoral arterial blood P_{O_2} of 69 mm Hg. When the suffusion solution P_{O_2} was raised to 79 mm Hg, the resultant measurements were 42 ± 3 on the small arteries and 21 ± 3 mm Hg at the end of the terminal arterioles. Similar experiments were carried out while animals were breathing 95% oxygen and the P_{O_2} of the cheek pouch suffusion solution was 39 mm Hg. Under these conditions, small artery P_{O_2} was 152 ± 13 mm Hg and terminal arteriolar P_{O_2} was 37 ± 9 mm Hg. Femoral artery blood P_{O_2} was 427 ± 12 mm Hg. These data are consistent with the hypothesis that oxygen diffuses from the precapillary vessels and that intravascular P_{O_2} falls progressively along the resistance vessels. This finding suggests a possible mechanism for the involvement of O_{2} in local regulation of blood flow.

ADDITIONAL KEY WORDS blood flow control oxygen electrode rat and hamster cremaster muscle hamster cheek pouch microcirculation vascular oxygen tension

In addition to having an indirect effect on vascular tone, by way of altered parenchymal tissue metabolism, oxygen may be directly involved in local blood flow regulation through an effect on the smooth muscle of the terminal precapillary vasculature (1-4). If oxygen is to act as a blood flow regulator, the oxygen tension at the vascular smooth muscle of the microcirculation must change in the appropriate direction with changes in flow and tissue activity, and the contractile force exerted by vascular smooth muscle must be determined, at least in part, by P_{O_2} at levels that normally exist in the tissue. Neither of these requirements has been demonstrated unequivocally in the microcirculation. The vascular smooth muscle of the arterioles is separated from the blood by a single layer of endothelial cells and thus the distance between what is assumed to be a high intravascular P_{O_2} and the smooth muscle is about 1 μm. With such a short diffusion distance between lumen and smooth muscle, it can be calculated from cylindrical diffusion equations that only a few millimeters of mercury pressure difference would exist be-
ween intravascular Po\(_2\) and the effective smooth muscle Po\(_2\), unless there is an exceptionally large diffusion barrier in the arteriolar wall or the oxygen consumption of the vascular smooth muscle is very high. Changes in tissue metabolic rate would exert minor effects on the Po\(_2\) of the vascular smooth muscle.

We will present the results of experiments which demonstrate how local control of arteriolar resistance, mediated by a direct effect of oxygen on vascular smooth muscle, can be accomplished with existing vascular geometry. The basic hypothesis is that the relatively large ratio of surface area to volume of arterioles, combined with the high lipid solubility of oxygen, will allow significant amounts of oxygen to diffuse across the walls of the resistance vessels during the passage of blood through the tissues, thus lowering intravascular Po\(_2\) below the value in the large systemic arteries and concomitantly reducing smooth muscle Po\(_2\).

Methods

Experiments were carried out primarily on the microcirculation of the suffused hamster cheek pouch, but also on the transilluminated cremaster muscle of the hamster and of the rat. Animals were anesthetized with 60 mg/kg pentobarbital ip and this was supplemented with urethane as required. A single-layered pouch preparation was suffused on both sides with Tris-buffered Ringer's solution at 38°C and pH 7.35. The microvasculature was observed with a Leitz Labolux microscope using long working distance objectives between 10X and 40X magnification.

The preparation provided access to the microvasculature with vessels of all classifications on the surface of the tissue. The vessels were usually covered by a layer of loose connective tissue about 50µ thick which was easily penetrated by the electrodes.

The majority of Po\(_2\) measurements were made on the external wall of arterioles using Whalen-type micro-oxygen cathodes with tip diameters between 2 and 6µ. Currents were measured with a Keithly 602 picoammeter and averaged on the order of 10^{-11} amp in room air. The electrodes were calibrated before and after measurements with at least three different standard gas mixtures. In most cases, measurements were made at the midpoint of the vessel in the vertical plane with the electrode just indenting the vessel wall. In a few experiments, intravascular Po\(_2\) was determined with sharpened microcathodes. Femoral arterial blood Po\(_2\) was measured with a Beckman model 160 gas analyzer.

The Po\(_2\) of the suffusion solution was established by bubbling solution reservoirs with one of three gas mixtures, 2%, 5%, or 21% oxygen with the remainder nitrogen. Po\(_2\) in the chamber was not equal to that in the reservoirs because of gas exchange in the supply tubing and in the suffusion chamber. Typically, with gases containing less than 21% oxygen, the oxygen profile in the chamber was a function of depth, with a plateau value intermediate between tissue Po\(_2\) and that of air. This plateau value is reported as the solution Po\(_2\); it averaged 39, 79, and 149 mm Hg for 2%, 5%, and 21% oxygen, respectively.

Results

OXYGEN LOSSES IN THE MICROCIRCULATION

Using sharpened oxygen electrodes, ten measurements of intravascular Po\(_2\) were made in four animals. The vessels penetrated were 20 to 40µ outside diameter. Mean difference in Po\(_2\) between intra- and extravascular determinations during suffusion with 5% O\(_2\) was 1.4 ± 0.8 mm Hg with a range of 5 mm Hg. Therefore, the extravascular measurements seem to be a relatively good index of

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td><strong>Oxygen Tensions in the Hamster Cheek Pouch</strong></td>
</tr>
<tr>
<td><strong>Artery</strong> (60-100µ i.d.)</td>
</tr>
<tr>
<td>Arterial</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>35 ± 4 (3)</td>
</tr>
<tr>
<td>42 ± 1 (6)</td>
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<tr>
<td>43 ± 4 (7)</td>
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</tbody>
</table>

All values were obtained from animals breathing room air and are means ± sd. Oxygen tensions are expressed in mm Hg. Number in parentheses is number of determinations. Mean femoral arterial blood Po\(_2\) was 68 ± 4 mm Hg.
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Room Air Inspired
Solution $P_{O_2} = 39$ mmHg

Schematic illustration of periatheriolar $P_{O_2}$ in the hamster cheek pouch. Measured $P_{O_2}$ at the various perivascular sites are shown (mm Hg) and below them are the estimated hemoglobin oxygen saturations for $pH = 7.4$ and $P_{CO_2} = 40$ mm Hg (7). Values are means from data shown in Table 1. Approximate lengths of large vessel segments are indicated between broken lines and the approximate outside vessel diameters are shown.

Intravascular $P_{O_2}$ at least in the size vessels tested and when suffusing with 5% $O_2$. Furthermore, these data indicate that no large diffusion barriers exist in the arteriolar wall.

Table 1 shows the mean $P_{O_2}$ values obtained on the wall of successive segments of the precapillary vasculature with the various suffusion solutions used. The observations have been grouped according to the type of vessel. During suffusion with solution of mean $P_{O_2}$ of 39 and 79 mm Hg, there was clearly a significant gradient ($P < 0.05$) from the small arteries to the capillaries. When the tissue was suffused with 21% $O_2$, there was no significant gradient. The tissue $P_{O_2}$ values, which were measured in areas where there were no visible capillaries, were substantially below those obtained on any of the arterioles during suffusion with a solution $P_{O_2}$ of 39 and 79 mm Hg.

In four animals, the $P_{O_2}$ of femoral arterial blood was determined simultaneously with the periartrial $P_{O_2}$ on the small arteries. In these animals, mean femoral arterial $P_{O_2}$ was $69 \pm 4$ mm Hg and the small artery $P_{O_2}$ was $43 \pm 5$ mm Hg during suffusion with solution equilibrated with 5% $O_2$.

Figure 1 is a schematic illustration of the findings with a suffusion solution $P_{O_2}$ of 39 mm Hg. The hemoglobin saturation was estimated from published values for the hamster oxyhemoglobin dissociation curve (7). The estimated hemoglobin saturations indicate that the total amount of oxygen lost in the arterioles is quantitatively significant since the blood is estimated to be 67% desaturated at the entrance to the capillaries.

The oxygen losses are substantially larger than expected in the precapillary vessels, and since the suffusion solution was carbon dioxide-free, we believed that the observed fall in $P_{O_2}$ might be the result of a leftward shift in the oxyhemoglobin dissociation curve. For this reason, experiments were done using...
Effect of increased $P_{CO_2}$ of solution on the periarteriolar $P_{O_2}$ gradient. Upper pair of frames are from successive segments of a vascular network when suffused with fluid equilibrated with 2% $O_2$ and 0% $CO_2$. Lower pair of frames are from the same segments during suffusion with solution equilibrated with 2% $O_2$ and 5% $CO_2$.

Figure 2 illustrates the results of one of these experiments. The upper panels show the $P_{O_2}$ measured at two successive points of a vessel network during suffusion with a carbon dioxide-free solution. The lower panels show the same vessels during suffusion with a solution equilibrated with 5% $CO_2$. There was a 10 mm Hg increase in $P_{O_2}$ at both points. However, the precapillary gradient persisted in the presence of carbon dioxide, and therefore the reduction in $P_{O_2}$ observed in the precapillary vessels cannot be attributed solely to loss of dissolved oxygen as the result of a leftward shift in the oxyhemoglobin dissociation curve.

Another possible reason for the considerable precapillary loss is that during suffusion with solutions of very low $P_{O_2}$, an essentially infinite sink for oxygen is provided by the suffusion solution. Hence, very large losses in oxygen might be expected from the diffusion boundary conditions. However, in the case of suffusion with solution equilibrated with 5% $O_2$, the solution $P_{O_2}$ was higher than at any point measured in the tissue; consequently the suffusion solution acted, if anything, as an oxygen source. Furthermore, suffusion with solutions equilibrated with room air produced a measurable decrease in periarteriolar $P_{O_2}$ along the distribution pathway in approximately half of the animals. Therefore, a large port of the measured loss in oxygen was to the
GRADIENTS IN PERIARTERIOLAR OXYGEN TENSION

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TABLE 2

<table>
<thead>
<tr>
<th>Inhale</th>
<th>Artery</th>
<th>Arteriole</th>
<th>Arterial capillary</th>
<th>Suffusion solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>% O₂</td>
<td>(60-100μ a.d.)</td>
<td>(10-20μ a.d.)</td>
<td>(5-10μ a.d.)</td>
<td>Solution</td>
</tr>
<tr>
<td>95</td>
<td>152 ± 13 (2)</td>
<td>59 ± 0 (3)</td>
<td>47 ± 9 (7)</td>
<td>37 ± 9 (7)</td>
</tr>
<tr>
<td>95</td>
<td>39 ± 8 (3)</td>
<td>24 ± 3 (11)</td>
<td>47 ± 9 (7)</td>
<td>37 ± 9 (7)</td>
</tr>
</tbody>
</table>

All values were obtained from tissues suffused with 2% O₂ solution and are means ± SE. Oxygen tensions are expressed in mm Hg. Numbers in parentheses represent numbers of determinations. Mean femoral arterial P₀₂ during inhalation of 95% O₂ was 427 ± 12 mm Hg.

Schematic illustration of P₀₂ and estimated saturation during respiration with 95% O₂. Numbers refer to same variables as in Figure 1.

Schematic illustration of P₀₂ and estimated saturation during respiration with 95% O₂. Numbers refer to same variables as in Figure 1.

Femoral arterial PO₂ of the hamsters was low (69 mm Hg), and to ensure that the oxygen losses observed were not the result of a low initial arterial PO₂, measurements were made on animals ventilated with 95% O₂. Table 2 presents the data from vessel PO₂ measurements made during suffusion of the tissue with a solution with a mean PO₂ of 39 mm Hg. When the hamster breathed 95% O₂, the mean arterial PO₂ of femoral blood was 427 mm Hg, whereas the perivascular PO₂ of the 60 to 100μ artery in the cheek pouch was 152 mm Hg. The precapillary gradient in perivascular PO₂ in the pouch persisted during 95% O₂ inhalation with a fall in PO₂ from 152 mm Hg to 37 mm Hg.
Comparison of microvessel $P_O_2$ from hamster cremaster muscle and rat cremaster muscle. Oxyhemoglobin dissociation curves are taken from Ulrich et al. (7) and the Biological Handbook (8). Pairs of points connected by broken lines are from top to bottom: arterioles, terminal arterioles, and arterial capillaries. Horizontal bars represent standard errors. Data are means from six hamsters and five rats.

Figure 3 is a schematic diagram illustrating perivascular $P_O_2$ and estimated hemoglobin saturation during respiration with 95% O2. The $P_O_2$ values along the entire precapillary tree were higher than during respiration with 21% O2. However, estimation of oxygen loss, ignoring the Bohr effect, yielded values that were quite similar; 0.040 ml O2/ml blood with 95% O2 in the inspired air and 0.048 ml O2/ml blood with 21% O2 in the inspired air, assuming a blood hemoglobin level of 0.15 g/ml.

Comparable determinations to those just described have also been made on skeletal muscle vessels. These studies were done in the hamster cremaster muscle; values similar to those in the cheek pouch were recorded in the various sections of the precapillary vasculature. Mean values (±SE) for four animals during sulfusion with 5% O2 solution were 33 ±1, 22 ±1, and 18 ±5 mm Hg for arterioles, terminal arterioles, and arterial capillaries, respectively.

If the observations reported here truly represent losses in oxygen from hemoglobin in the blood, then the measured $P_O_2$ values would be expected to be a function of the characteristics of the oxyhemoglobin dissociation curve. Rat hemoglobin has an oxygen dissociation curve substantially different from that of the hamster and, assuming a comparable ratio of metabolic rate to flow for the two species, one would predict that the different characteristics of rat hemoglobin should result in substantially different $P_O_2$ values in comparable vessels. Figure 4 shows that this is indeed the case. Oxyhemoglobin dissociation curves for the hamster (7) and the rat (8) were drawn, and on these the measured $P_O_2$ values for equivalent microvessels in the cremaster muscles of the two animals have been plotted and connected by broken lines (Fig. 4). The lower pair of points represents data taken from measurements on the capillary bed, the middle pair of points data from terminal arterioles, and the upper pair of points data from feeding arterioles. The geometry of the cremaster muscle made it impossible to obtain reliable values on the small arteries. There are very substantial differences between the data for the rat and the hamster, and in general they tend to follow the pattern which would be expected from the different oxyhemoglobin dissociation curves. In each case, the values for the vessels of the rat cremaster muscle are significantly higher than those for the hamster.

**OXYGEN LOSSES IN THE MACROCIRCULATION**

Oxygen tensions on the largest vessels available for study in the cheek pouch were on the order of 30 mm Hg lower than those measured in the femoral arterial blood. An effort was made to determine the site of this decrease in oxygen tension. $P_O_2$ was measured on the surface of the larger vessels using the same type of electrodes as in the previous study. Oxygen tensions were measured in three animals on the carotid artery, the major supply artery to the pouch, the major branch artery supplying the arterial vessels (small artery of Tables 1 and 2), and the jugular vein. Mean oxygen tensions were: 69.3 mm
Hg on the carotid, 60.3 mm Hg on the supply artery, 50.7 mm Hg on the branch artery, and 35.8 mm Hg on the jugular vein. Most of the fall in Po2 occurred over the length of the supply artery, which in the cheek pouch is quite long (approximately 2 cm) and has a relatively small diameter over its entire length (approximately 100 μ).

**VASCULAR DIAMETER CHANGES**

A thorough statistical analysis of the relationship between vessel diameter and oxygen tension has not been accomplished. However, changes in vascular diameter in the appropriate direction in general accompanied changes in both solution Po2 and inspired gas Po2. An example of this is shown in Figure 5. The upper pair of panels shows the pouch vessels during suffusion with a solution equilibrated with 5% oxygen. The solution plateau Po2 was 37 mm Hg. The lower panels show the effect of changing to a solution equilibrated with room air. A marked constriction can be observed in most vessels, associated with the increase in suffusion fluid Po2.

**Discussion**

The close agreement between intravascular and extravascular Po2 found in these experiments indicates that the smooth muscle Po2 is very close to intravascular Po2 and that oxygen tension of the vascular smooth muscle at any given location is determined primarily by the Po2 of the luminal blood at that site. However, the longitudinal gradient in Po2 observed in the arterioles suggests a mechanism whereby oxygen can be a determinant of local blood flow. Assuming conservation of...
mass and cylindrical diffusion geometry, the longitudinal gradient in $P_{O_2}$ will be directly proportional to the ratio of metabolic rate to blood velocity and inversely proportional to the square of the vessel radius. On the basis of these relations, we propose the following mechanism. As blood passes through the arterioles, total oxygen loss at any point is a function of the combined effects of blood velocity and the diffusion gradient into the tissues. A reduction in velocity would lower intravascular $P_{O_2}$ and thus the $P_{O_2}$ of the vascular smooth muscle. If arteriolar smooth muscle is sensitive to decreased $P_{O_2}$ at levels normally found in the microcirculation, then the reduced $P_{O_2}$ would produce dilation of the arterioles and an increase in the velocity of flow. A new steady state would then be achieved at a larger arteriolar diameter. Similarly, an increase in metabolic rate of the tissue would produce dilatation of the arterioles and a relaxation of vascular smooth muscle. This formulation is similar to one proposed for precapillary sphincters by Guyton (9).

A critical point in this hypothesis is that arteriolar vascular smooth muscle be sensitive to changes in $P_{O_2}$ at levels existing in situ. Whereas the oxygen sensitivity of vascular smooth muscle from the larger vessels has been demonstrated (10-12), no such relation has to date been shown unequivocally for the microcirculation. Nicoll and Webb (13) have reported that the frequency and duration of closure of precapillary sphincters in the bat wing are increased by oxygen inhalation, but their experiments did not eliminate the possibility that local neural influences or altered parenchymal tissue metabolism contributed to the response. In our own experiments, as shown in Figure 5, vascular diameter was altered by changes in solution oxygen tension. However, it is not possible to separate the direct effects of oxygen on vascular smooth muscle from indirect effects, such as vasodilator metabolite release due to the decrease in $P_{O_2}$ of the parenchymal cells. Further studies involving changes in $P_{O_2}$ restricted specifically to the microvessels are necessary to resolve this question.

The arteriolar losses in oxygen, which were calculated from the observed oxygen tensions, appear to be surprisingly large. It should be pointed out, however, that these are only calculated losses and are totally dependent on the estimation of oxygen saturation from published hemoglobin dissociation curves and on the accuracy of our measurements of oxygen tension. To what extent these are in error can only be determined by direct measurement of hemoglobin saturation in the arterioles.

As was pointed out previously, the arteriolar losses in oxygen will be proportional to the ratio of tissue metabolic rate to blood flow velocity and either a very high metabolic rate or a low flow velocity would markedly increase the precapillary oxygen losses. Neither of these parameters has been measured directly in our experiments, but we have no reason to believe that either is markedly different from other tissues. The flow in arterioles was sufficiently rapid to make it impossible to distinguish individual red blood cells, and in vitro the oxygen consumption of the cheek pouch is in the range of that reported for skeletal muscle ($6 \times 10^{-4}$ ml $O_2 \cdot \text{min}^{-1} \cdot \text{g}^{-1}$; Duling, unpublished observations). Furthermore, blood flow in the pouch, as determined by the fractional extraction of rubidium, is relatively higher than that reported for skeletal muscles ($0.13 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$; Duling and Weiner, unpublished observations). These facts would support the contention that neither the blood flow nor the metabolic rate of the cheek pouch is exceptional and responsible for the large changes in oxygen tension observed.

The accuracy of measurements of oxygen tension with the oxygen microelectrode cannot be assessed completely, as it was impossible to calibrate the electrodes while they were in the tissue. However, when suffusing with a solution equilibrated with air, repeated measurements of the $P_{O_2}$ in the solution over the pouch usually yielded good agreement with the calibration values ($\pm 5\%)$. 

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It is important that more direct measurements of oxygen exchange sites be made as our data are consistent with the hypothesis that a significant fraction of the total tissue oxygen supply may be by diffusion from precapillary vessels. There are other data which suggest, but do not prove, that this may be the case. In frozen sections of pulmonary tissue, Staub has reported that there is a visible change in hemoglobin color in precapillary vessels. He interpreted the color change as indicating that there was some oxygen exchange in these thin-walled pulmonary vessels, but did not attach any quantitative significance to the observations (14). Other investigators have measured relatively high Po2 levels immediately outside large arterial vessels of the microcirculation (15, 16), which would imply some loss of oxygen from the blood in these vessels. Neither of these observations, however, demonstrates how much of the total exchange might occur in precapillary vessels of the microcirculation.

On the assumption that oxygen exchange occurs exclusively in capillaries, various investigators have calculated that only a few millimeters difference in mercury pressure would be required to supply all the necessary oxygen to meet the demands of most tissues (17-19). If these calculations are correct, then it would follow that even the arterioles with their smaller ratio of surface area to volume and larger intervascular distances might be able to supply significant amounts of oxygen to the tissues. This is particularly true if the higher intravascular Po2 of the arterioles, and thus the larger diffusion gradient, is considered.

Neither these studies nor our experiments demonstrate unequivocally that there is significant precapillary transport of oxygen. Furthermore, definite conclusions as to the general applicability of our findings are not possible because of the special circumstances of the suffused tissue and because hemoglobin saturation was not determined directly. Also the lack of knowledge of the relative values of flow and metabolism in the cheek pouch and cremaster muscle make extrapolations to other tissues tenuous. However, there is sufficient evidence to warrant further studies on the exchange site for oxygen.

References


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