Capillary Limitation of Oxygen Distribution in the Isolated Rete Mirabile of the Eel (Anguilla anguilla)

Eugenio A. Rasio and Carl A. Goresky

SUMMARY The presence of a significant barrier at the level of the capillary to the diffusion of oxygen into tissue would have major significance in biology. The rete mirabile of the eel, an organ made up of alternately disposed arterial and venous capillaries, was used to study this question. The two capillary beds were perfused with albumin-containing Krebs-Ringer bicarbonate buffer in an uncoupled countercurrent fashion with equal flows, and the preparation was shielded from atmospheric oxygen with a polyvinylidene chloride film. A constant arterial infusion containing labeled albumin, inulin, and water, and an increased oxygen content, and a venous infusion containing a greatly reduced oxygen content were begun, and from the steady state response to these, capillary permeability-surface products were calculated. Approximately half the labeled water and one-third of the arterial minus venous oxygen increment emerged in the arterial outflow, whereas in the flow-limited case none would be expected. We calculated permeability values for the labeled tracers (albumin, inulin, and water), assuming a surface area of 1 cm²/mg, and found these not to be significantly different from those previously reported (Circ Res 41: 791-798, 1977). The permeability value for oxygen at 25°C was 11.8 ± 1.9 (sd) x 10⁻⁵ cm/sec. The data indicate that the limiting effect of the capillary wall on oxygen transfer is of a magnitude that must be taken into account in describing the factors affecting the distribution of oxygen to tissues. Circ Res 44: 498-503, 1979

THE DISTRIBUTION of oxygen to tissue is the keystone to survival in organs perfused by a vascular system. The processes underlying this distribution have been outlined in a general fashion. Krogh (1919), in his original investigations of the distribution of the respiratory gases to skeletal muscle, formulated the idea that the intercapillary distances were a dominating feature of this distribution. With Erlang, he devised a steady state model in which there was no difference in resistivity (resistance per unit thickness) to the passage of oxygen between the capillary wall and surrounding tissue. The metabolic consumption process was considered to be distributed in a spatially uniform manner in the intercapillary space, so that uniformly decreasing values for oxygen in tissue were predicted with increase in distance from the capillary lumen. The studies of Pappenheimer et al. (1951), in which the conductance of the capillary wall to a group of lipid-insoluble molecules was measured by use of the isogravimetric capillary pressure, led to the general concept that only a small fraction of the capillary wall was available, in the form of water-filled channels, for transfer of these substances across the capillary wall (i.e., that there was an increased resistivity at the level of the capillary wall). The calculated diffusion area appeared too small to account for the observed quantitative rates of transfer of oxygen across the capillary wall. These authors therefore sought an alternate explanation in the lipid solubility of oxygen (its distribution between cottonseed oil and water at 37°C is 5:1 (Lawrence et al., 1946)). They suggested that, unlike the lipid-insoluble molecules, oxygen probably penetrated the whole of the endothelial cell surface. Renkin (1973) subsequently examined the permeability of a group of substances structurally related to antipyrine and found, as expected, that their permeability rapidly increased with lipid solubility. The argument then appeared tenable but not amenable to experimental examination.

Tracer oxygen studies seldom have been carried out, because the half-life of the radioactive forms is too short to work with conveniently, and mass spectrometric methodology has not been widely available for monitoring those stable forms of oxygen that could be used conveniently for these studies. Despite the difficulties associated with the use of the stable isotopes, two sets of data qualitatively different in form have been reported. Chinard et al. (1967) explored renal vein outflow patterns for labeled red cells and ¹⁸O² equilibrated with these red cells prior to renal arterial injection. They found that the bulk of the oxygen tracer emerges with the red cells (this would indicate that little of the tracer had left the capillary circulation). Forster et al.
injected a similar bolus of preequilibrated red cells with indocyanine green into the femoral artery of a dog and analyzed femoral venous blood samples. In this case the bulk of the tissue perfused would have been skeletal muscle. The oxygen tracer was delayed at the outflow with respect to the vascular reference, and the data were interpreted to indicate that tracer oxygen is distributed into some extracapillary pool of oxygen in tissue in a rather free manner (i.e., that most of it had left the capillary circulation). The apparent degree of restriction is quite different in the two studies. The transit times are much larger in resting skeletal muscle than in the kidney, and the difference in the outflow curves may chiefly be a reflection of this. The data indicate that there may be a barrier to oxygen, and, if so, it is of such a magnitude that its effects are easily perceived only when transit times are short.

The advent of the oxygen electrode resulted in a new set of data. Whalen (1971), for instance, showed that both in skeletal muscle and in the heart, very low PO2 values are found within the muscle fibers. The PO2 in venous blood is much higher than that in the tissue. These findings also indicate, despite the earlier hypothesized high rate of passage of oxygen across the capillary wall, that a substantial barrier to oxygen may exist at the level of the capillary wall.

The rete mirabile of the swim bladder of the eel, a countercurrent exchange net composed of alternately disposed arterial and venous capillaries, provides an admirable tool to examine this question. The permeabilities of the barrier between arterial and venous capillaries can be estimated by carrying out steady state infusions of a variety of substances. The manner of calculation of permeability involves no approximations, apart from the use of an estimate of surface area (Rasio et al., 1977). Rasio et al. (1977) previously examined the permeability of the rete mirabile of the swim bladder of the St. Lawrence River eel to both a variety of water-soluble substances and tracer water. They found that labeled water did not undergo flow-limited exchange in this system and that a significant barrier to its distribution was present. We have used this preparation to determine whether there is also a significant barrier to the distribution of oxygen.

**Methods**

The use of the rete mirabile preparation, isolated from the swim bladder of the eel, has been described previously (Rasio et al., 1977). Each rete, weighing approximately 200 mg, was countercurrently perfused through its arterial and venous inputs under similar hydrostatic and osmotic pressures. The experiments were carried out at room temperature (22-25°C), with a constant flow averaging 0.6 ml/min and a constant pressure head of 45 cm H2O. The pressure corresponds to the blood pressure in the swim bladder artery (Stray-Pedersen and Steen, 1975), and the flow is slightly higher than that occurring in the intact preparation (Steen, 1963). The medium was a Krebs-Ringer bicarbonate buffer (pH 7.4) containing glucose, 5 mm, and bovine albumin (Cohn, fraction V), 4 g/100 ml. At the arterial input, the medium was supplemented with tracer concentrations of human albumin-125I (Frost; more than 95% of the labeled iodine was precipitated with 10% trichloroacetic acid), inulin-carboxyl-14C (New England Nuclear; crystalline solid, 1-3 mCi/g; molecular weight, 5000), and tritium-labeled water (New England Nuclear; biological quality, 0.25 mCi/g). Throughout the perfusion, the arterial medium was equilibrated with a gas mixture consisting of 60% O2, 35% N2, and 5% CO2 gassed continuously through the 600-ml reservoir at a rate of 1 liter/min.

The medium infused at the venous input contained no radioactive compounds and was equilibrated with an oxygen-free gas mixture, 95% N2-5% CO2, in a manner similar to that used for the arterial medium. The surface of the rete, the swim bladder, and all of the perfusion tubing systems were wrapped with commercial Saran (polyvinylidene chloride, Dow Chemical Company). This material has an exceedingly low permeability to oxygen (Stannert, 1977) and thus provides a membrane barrier to atmospheric oxygen. Without it, it would have been impossible to carry out these experiments. With these precautions, it was found that the reservoir gas tensions and the tensions of the corresponding arterial and venous solutions at the level of the rete became identical and remained constant throughout the experiment. The partial pressure of oxygen averaged 430 ± 20 (sd) mm Hg at the arterial input and 10 ± 3 mm Hg at the venous input.

Samples of the media were collected simultaneously from the arterial and venous outputs at various time intervals during a 2-hour perfusion. The samples were analyzed for their radioactivity contents by the methods previously published (Rasio et al., 1977). Precautions were taken in particular to assay only washed trichloroacetic acid-precipitated 125I-albumin activity in samples and standards. Samples for measurement of the partial pressure of oxygen were collected into siliconized glass syringes, kept on ice, and immediately analyzed with a Clark electrode 229 in a BMS radiometer. Appropriate standardizations with gas mixtures of known composition were carried out.

**Estimation of the Permeability of the Barrier between the Arterial and Venous Capillaries**

In the steady state, with uncoupled countercurrent perfusion of the arterial and venous capillary beds with equal flows in either direction, the outflow concentrations from the arterial and venous systems can be used to calculate the permeability of the rete barrier between the two flows (Rasio et
al., 1977; Stray-Pedersen and Steen, 1975). In the present experiments, two different initial conditions were used.

1. The case in which the venous reservoir contains none of the material being examined, and the material being studied is being steadily infused at the arterial input: In our experiments the arterial reservoir contained labeled albumin, inulin, and water. The steady input of each of these will be described as a unit concentration step function. If the parameter $R = PS/F$, where $PS$ is the permeability-surface product describing the barrier and $F$ is the steady flow of perfusion medium in each direction; $x$ is the distance from the arterial input along the capillaries of length $L$, and $y = x/L$, the fractional distance along the rete; and $u(y)$ and $v(y)$ are the concentrations in the arterial and venous capillaries, then we find (Rasio et al., 1977):

$$u(y) = 1 - \frac{R}{1 + R}y,$$

$$v(y) = \frac{R}{1 + R}(1 - y).$$

When $y = 1$:

$u(1) = 1 - \frac{R}{1 + R},$
$v(1) = \frac{R}{1 + R}$,

Whereas, when $y = 0$:

$u(0) = 1$, and
$v(0) = \frac{R}{1 + R}$.

The sum of the outputs $F[u(1) + v(0)]$ is again equal to the sum of the inputs $F[u(0) + v(1)]$. In this case we find:

$$[u(0) - u(1)]/[u(0) - v(0)] = R = PS/F.$$

The expression needed to calculate a value for $R$ and hence that for permeability differs from that used in the first case.

In each of these two cases the arterial and venous capillary concentrations fall linearly along the length, and there is a constant difference between arterial and venous capillaries at each point along the length. The differences in concentrations are $1/(1 + R)$ and $(1 - v_1)/(1 + R)$, respectively, in the two cases.

To calculate the value for the permeability $P$ from the expression $PS/F$, the measured value for flow $F$ (cm$^3$ sec$^{-1}$ mg$^{-1}$) was used in each case, and the surface area was taken to be 1 cm$^2$/mg wet weight (Krogh, 1922). The concentrations appropriate to the calculations were averaged over the steady state period of observation, from 30 to 120 minutes.

2. The case in which the venous reservoir contains a small concentration of the substance being infused on the venous side: In the case of oxygen we were unable to bring the levels of the venous reservoir down precisely to zero. The oxygen tensions in the venous perfusate were small but finite. In this case let $v(1)$ be a concentration step function of magnitude $v_1$, expressed as a fraction of the concentration in the arterial input. We then find:

$$u(y) = 1 - (R + v_1)/(1 + R)y + v_1 y$$

and

$$v(y) = \frac{R}{1 + R}(1 - y) + v_1 y.$$

In this case, when $y = 1$,

$u(1) = 1 - R/(1 + R) + v_1[R/(1 + R)]$
$v(1) = v_1$

and when $y = 0$,

$u(0) = 1$
$v(0) = R/(1 + R) + v_1[1/(1 + R)].$

The results

We previously have reported permeability values for labeled albumin, inulin, and water. Those previous studies were carried out in the early summer. The present studies were carried out in the late fall and early winter. Both because we did not know whether seasonal variation would occur (since the eels would normally be at sea at this time of the year), and to find out how reproducible our observations were, we measured the permeability of these substances once again, while studying the permeability of the rete to oxygen.

Figure 1 shows results of a typical experiment, in which all four substances were studied ($^{125}$I-labeled albumin, $^{14}$C-labeled inulin, $^3$H-enriched water, and oxygen). The outflow concentrations of each test substance reach their steady state of response soon after infusion is begun, and thereafter change little. In the steady state the sums of the averaged radioactivity or oxygen pressures at the arterial and venous outputs were within the range of 96–102% of the corresponding input values.

In the present calculations the consumption of oxygen by the rete has been neglected. The rete is primarily a glycolytic tissue, more than 95% of its glucose uptake being accounted for by lactate production (Rasio, 1973). In vitro, at 25°C, its oxygen consumption averages 100 μl of O$_2$/g wet weight per hour (Rasio, 1973). In the present experiments this uptake will account for less than 4% of the oxygen being delivered to the rete.

It is appropriate to take note of the manner in which the outflow concentration will be expected to change as the permeability increases. In the case in which there is no tracer in the venous reservoir, the asymptotic response as the permeability becomes...
Figure 1 The time course of the output concentrations from arterial and venous capillary beds of $^{125}$I-labeled albumin, $^{14}$C-labeled inulin, $^3$H-enriched water, and oxygen, after beginning a constant arterial infusion, in the countercurrent-perfused rete. None of the tracer material was present in the venous reservoir, whereas in the case of oxygen, the $P_{O_2}$ in the venous reservoir was 0.023 of that in the arterial reservoir. $A_0$: concentration at the arterial input = 1.0; 1 and 1': $^{125}$I-labeled albumin; 2 and 2': $^{14}$C-labeled inulin; 3 and 3': tritium-enriched water; and 4 and 4': oxygen levels. The solid line corresponds to concentrations at the arterial output and the broken line to concentrations at the venous output.

Infinite (the flow-limited case) is one in which all of the tracer emerges in the venous outflow and none reaches the arterial outflow. In the case of tracer water, Figure 1 shows that approximately half of the tracer emerges in the venous outflow and half in the arterial outflow. There is a substantial barrier to the movement of tracer water in the rete. In the case of oxygen, approximately one-third of the arterial input emerges in the outflow from the arterial capillaries, and two-thirds in the outflow from the venous capillaries. The permeability of the barrier to oxygen is larger than that to water, but not substantially so. A significant barrier to the movement of both substances exists.

The average permeability values from the present data are displayed in Table 1. To assess whether the values for $^{125}$I-labeled albumin, $^{14}$C-labeled inulin, and $^3$H-enriched water had been drawn from a population identical to that previously studied by Rasio et al. (1977), a Student's $t$-test of the null hypothesis was carried out. The difference between the means was found not to be significantly ($P < 0.001$) different from zero. In the table the values for the earlier and later data have therefore been consolidated, and the averages from the total group are also given.

For the substances studied, the relation between the permeability values and the coefficient of free diffusion in water at $25°C$ are plotted (Fig. 2). In addition, the values for urea and 3-O-methylglucose from the studies of Rasio et al. (1977) are displayed. For the water-soluble substances there is a linear increase in permeability with diffusion coefficient, on the log-log plot. A sharp break in the locus occurs, both for labeled water and for oxygen, the permeability values for both being disproportionately high. This is what would have been expected if both labeled water and oxygen permeated the endothelial cells themselves, as well as traversing the water-filled pores accessible to the other substances. The value for oxygen deviates from the primary locus to an even larger degree than does that for labeled water.

Discussion

We believe our observations to be the first quantitative determinations of the permeability to oxygen of a set of systemic capillaries. In the exceedingly long (8-mm) capillaries of the rete, a substantial proportion of the oxygen entering the arterial circuit passes through, to emerge in the effluent.
The relation between the diffusion coefficients ($D$) and the average permeability coefficients ($P$) from various molecules in the isolated perfused rete at 25°C. The standard errors of the permeability measurements are indicated. The values taken from Rasio et al. (1977) for 3-O-methylglucose and urea are represented by unfilled circles.

## Figure 2

<table>
<thead>
<tr>
<th>Substance</th>
<th>$D_{25}$ (cm$^2$/sec)</th>
<th>$P_{25}$ (cm$^2$/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urea</strong></td>
<td>1.62 $\times 10^{-5}$</td>
<td>1.31 $\times 10^{-4}$</td>
</tr>
<tr>
<td><strong>3-O-Methylglucose</strong></td>
<td>0.03 $\times 10^{-7}$</td>
<td>0.13 $\times 10^{-7}$</td>
</tr>
<tr>
<td><strong>Albumin</strong></td>
<td>2.11 $\times 10^{-5}$</td>
<td>1.23 $\times 10^{-4}$</td>
</tr>
</tbody>
</table>

from the arterial capillary bed. This indicates that, although the permeability of these capillaries to oxygen is great, it is not so great that the effect of the barrier on the diffusion of oxygen from the capillary lumen into tissue can be neglected. The limiting effect of the capillary wall on oxygen transfer is very real and must be taken into account in formulating any comprehensive description of the factors affecting the distribution of oxygen in tissue. In addition, it must be considered in a concrete fashion in attempts to explain why, in the heart and in skeletal muscle, venous oxygen tensions remain relatively high in the face of very low tissue values.

In characterizing our data we have assumed that the most useful approach would be to quantify the permeability of the barrier to oxygen transfer. This kind of analysis originally arose because of an appreciation of the anisotropy of tissue and, in particular, because of the large barrier effect found at the level of capillaries. Alternatively, we can gain useful insight by using the more classical approach, that of characterizing the diffusion constant for oxygen in the barrier. In this system the rate of diffusion across the barrier is known (it is equal to the amount lost by the arterial system), and the difference in oxygen concentration between arterial and venous streams at each point is both constant and known (it is equal to the concentration difference at either pole). The mean width of the barrier between arterial and venous channels is 3 μm (Bendayan et al., 1975). If we use the same estimate for surface area as that utilized above, 1 cm$^2$/mg, we can calculate a parameter with the dimensions of a diffusion coefficient, $D$. The value arising from our data is $0.3 \times 10^{-7}$ cm$^2$/sec. The values for oxygen diffusing in distilled water and normal plasma are $2.11 \times 10^{-5}$ and $1.62 \times 10^{-5}$ cm$^2$/sec, respectively (Goldstick et al., 1976), and that through a slab of muscle is of the order of 50% of that in distilled water (Krogh, 1922). The much smaller value found for diffusion through the barrier in the rete as compared to that for diffusion through plasma or a muscle slab (where intercapillary volume is considerable) emphasizes the substantial restriction imposed by the capillary walls. The arterial capillary would be expected to provide the bulk of the resistance, since it is thick-walled, whereas the venous capillary is thin-walled and fenestrated.

In our experiments the uncoupled rete is functioning as a countercurrent exchanger, and the oxygen gas tension decreases along the length as the distance from the arterial input increases. In vivo, where the arterial rete blood circulates to the swim bladder and returns, the oxygen gas tension increases along the length of the arterial capillaries with increase in distance from the arterial source when oxygen secretion into the bladder is taking place. The secretory epithelium of the swimbladder produces lactic acid and carbon dioxide and adds these to the venous blood, which subsequently enters the venous capillary system of the rete (Steen, 1963). The acid diffuses through the intercapillary barrier of the rete and acidifies the arterial blood, with rapid increase (half-time of 50 msec) of the arterial capillary oxygen tension due to the Root effect. In the venous blood, the oxygen capacity increase secondary to the loss of lactic acid and carbon dioxide has a half-time of 10–20 seconds. This asymmetry then results in a countercurrent multiplication effect which increases the $P_O_2$ values along the arterial capillary system. The whole depends on the properties of the hemoglobin in the red cells rather than on an asymmetry at the level of the capillary barrier (Berg and Steen, 1968).

Finally, it is appropriate to emphasize the tremendous advantage of using the uncoupled countercurrent system of the rete to obtain values for capillary permeability. The values obtained can be calculated directly, with an estimate of surface area, and values for capillary permeability can be obtained even for very highly permeable substances.

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## References

THE OCCURRENCE of left ventricular dysfunction shortly following the onset of a myocardial infarction is well established (Swan et al., 1972; Kupper et al., 1977; Rahimtoola et al., 1972; Kumar et al., 1970). Indeed, in studies of ventricular performance in dogs with healed myocardial infarctions, recovery is so complete that the hemodynamic measurements are either entirely normal (Hood, 1970) or demonstrate only a minor elevation (31-48%) inns of baseline hemodynamics or peak indices of pumping and pressure-generating ability when compared to the sham-operated, noninfarcted rats. Rats with moderate (31-48%) infarctions had normal baseline hemodynamics but reduced peak flow indices and developed pressure. Rats with infarctions greater than 48% had congestive heart failure, with elevated filling pressures, reduced cardiac output, and a minimal capacity to respond to pre- and afterload stresses. The entire spectrum of postinfarction ventricular function was observed, from no detectable impairment to congestive failure. In this model of histologically healed myocardial infarction, the impairment of left ventricular function was directly related to the loss of myocardium. Circ Res 44: 503-512, 1979

### Myocardial Infarct Size and Ventricular Function in Rats

**MARC A. PFIFEFER, JANICE M. PFIFEFER, MICHAEL C. FISHBEIN, PETER J. FLETCHER, JOEL SPADARO, ROBERT A. KLONER, AND EUGENE BRAUNWALD**

**SUMMARY** To define the relationship between infarct size and ventricular performance, we performed hemodynamic studies in rats 21 days after left coronary artery occlusion. Ventricular performance was assessed under ether anesthesia by measurements of baseline hemodynamics and stressed performance as determined by the peak cardiac output and stroke volume obtained during intravenous volume loading and by the peak left ventricular developed pressure obtained during occlusion of the ascending aorta. Infarct size was determined by planimetry of the endocardial circumference of each of four histological slices of the left ventricle. Rats with small (4-30%) myocardial infarctions had no discernible impairment in either baseline hemodynamics or peak indices of pumping and pressure-generating ability when compared to the sham-operated, noninfarcted rats. Rats with moderate (31-48%) infarctions had normal baseline hemodynamics but reduced peak flow indices and developed pressure. Rats with infarctions greater than 48% had congestive heart failure, with elevated filling pressures, reduced cardiac output, and a minimal capacity to respond to pre- and afterload stresses. The entire spectrum of postinfarction ventricular function was observed, from no detectable impairment to congestive failure. In this model of histologically healed myocardial infarction, the impairment of left ventricular function was directly related to the loss of myocardium. Circ Res 44: 503-512, 1979

**THE OCCURRENCE of left ventricular dysfunction shortly following the onset of a myocardial infarction is well established (Swan et al., 1972; Weber et al., 1978; Page et al., 1971). After this...**

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**Berg T, Steen JB The mechanism of oxygen concentration in the swimbladder J Physiol (Lond) 196: 631-638, 1968**


**Krogh A: The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissue. J Physiol (Lond) 52: 409-415, 1919**

**Krogh A: The Anatomy and Physiology of Capillaries. New Haven, Yale University Press, 1922**

**Lawrence JH, Loomis WF, Tobias CA, Turpin FH: Preliminary observations of the narcotic effect of xenon with a review of values for solubilities of gases in water and oils. J Physiol (Lond) 105: 197-204, 1946**


**Stray-Pedersen S, Steen JB: The capillary permeability of the rete mirabile of the eel, Anguilla vulgaris L. Acta Physiol Scand 94: 401-422, 1975**

**Whalen WJ: Intracellular Po2 in heart and skeletal muscle. Physiologist 14: 69-82, 1971**
Capillary limitation of oxygen distribution in the isolated rete mirabile of the eel (Anguilla anguilla).

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