Diffusion Permeability of an Isolated Rete Mirabile

EUGENIO A. RASIO, MOISE BENDAYAN, AND CARL A. GORESKY

SUMMARY The rete mirabile of the swimbladder of the eel is a countercurrent exchange organ composed of alternately disposed arterial and venous capillaries. A countercurrent perfusion of these was set up by isolating arterial input and outflow, and venous input and outflow, and perfusing each set of capillaries with oxygenated albumin-containing Krebs-Ringer bicarbonate buffer. Constant arterial infusions containing tracer albumin, inulin, 3-O-methylglucose, urea, and water were begun and tracer concentrations at the outlets of arterial and venous beds were determined. Capillary permeabilities were determined from the resulting steady state values. The method of calculation of permeability values from the data acquired in the rete involves no approximations and appears to yield unequivocal values. For the solutes, the values appear compatible with transcapillary passage by free diffusion. No evidence of increasing restriction (in comparison to the diffusion coefficients) was found over the range of molecular sizes explored. The permeability for labeled water, in relation to the solutes, was too high, and this augmentation was attributed to the larger surface available for its exchange. Despite this larger value, labeled water did not undergo flow-limited exchange. A significant barrier to its distribution is present in this capillary system.

A RETE MIRABILE is a special arrangement of arterial and venous small vessels or capillaries, in close contact, and in which blood flows in opposite directions. By applying countercurrent exchange, the rete provides a highly efficient system by which the organism can conserve heat, oxygen pressure, or solute concentration.1 It has been shown, for instance, that in some arctic animals adapted to severe exposure of the extremities to cold, the rete mirabile traps most of the blood heat and returns it to the trunk. In the swimbladder of deepsea fishes, the countercurrent exchange reaches its highest efficiency: in the eel, the rete is made of many thousands of blood capillaries, closely intermingled and disposed in a checkerboard pattern so as to gain the greatest possible area of exchange. It has been calculated that across a rete 1 cm long, the oxygen pressure is reduced by a factor of more than 3000 from swimbladder to systemic circulation.

The rete in the eel swimbladder is a compact microvascular organ, free of cell contaminants, in which venous and arterial straight capillaries, approximately 1 cm long, alternate in regular fashion and are in contact with one another by their continuous basement membrane.2 The organ can be countercurrent perfused through its feeding vessels; similarly, the respective outputs at the opposite poles can be collected through corresponding vessels. This is a unique situation for the study of diffusion permeability. By maintaining a similar flow in the opposite directions under similar hydrostatic and osmotic pressures and by analyzing the arterial and venous inputs and outputs during steady state conditions, the permeability can be readily determined under ideally controlled conditions. Such a study was carried out using a variety of inert tracer molecules ranging in size from albumin to water.

Methods

Large female eels, weighing 4-6 lb, were trapped in the St. Lawrence river during the summer, on their spawning migration to the ocean. They were kept in large tanks under running tap water and without food. Prior to surgery, blood was drawn by puncture of the dorsal aorta in its caudal segment; the blood glucose level, as determined by a specific enzymatic method, ranged from 60 to 120 mg/100 ml.

Each eel was anesthetized for 20-30 minutes in 1 gallon of water containing 1 g of tricaine methane sulfonate, then removed from the water. During exposure to the air, oxygenation of the blood can proceed satisfactorily because the gills, deep inside the operculae, remain wet for a long period of time. The abdominal wall was incised longitudinally from the cloacum to the liver. The swimbladder, in retroperitoneal position between the right ovaries and the digestive tract, was isolated with its two symmetric red bodies on the ventral surface.

One red body or rete was prepared for the perfusion. A P.50 polyethylene tubing was inserted into the pre-rete artery, a branch of the celiacomesenteric artery; the perfusion medium was immediately allowed to flow through the rete and the swimbladder. An incision was made in the major collecting vein which runs alongside the feeding artery in order to permit the progressive washout of blood from the rete.

At the opposite pole, two P.50 polyethylene tubings were implanted: one in the post-rete artery, for the collection of the arterial output, and one in the pre-rete vein through which the countercurrent venous perfusion was started.

Finally, a fourth catheter, of a larger size, was inserted into the post-rete vein to sample the venous output. All the catheter's tips were secured by knots at approximately 0.5 cm from the rete poles. Additional ligatures were applied on collateral vessels, whenever necessary, to avoid...
seepage. The procedures were completed in approximately 20 minutes. The swimbladder with its isolated rete was then removed from the eel. The capsule surrounding the rete was maintained intact in order to prevent dessication of the outer layer of capillaries. A schematic view of the preparation is shown in Figure 1.

All perfusions were carried out at room temperature (23–25°C) under a constant flow of 0.6 ml/min and a constant pressure of 45 cm H$_2$O. The pressure corresponds to the blood pressure in the swimbladder artery. The medium used for the simultaneous perfusions at the arterial and venous inputs was a Krebs-Ringer bicarbonate buffer (pH 7.4), supplemented with glucose, 5 mm, and bovine albumin (Cohn, fraction V), 4 g/100 ml, and equilibrated with a gas mixture of 95% and 5% CO$_2$. The rete is a glycolytic tissue. More than 95% of the glucose uptake is accounted for by lactate production; its respiration in vitro at 25°C, in a medium of the same composition, averages 115 μl O$_2$ per g wet weight per hour. In our open circuit system, the amount of oxygen dissolved in the medium and supplied to the rete can be estimated to be 20 times greater than that required for its respiration (the average weight of one perfused rete is 150 mg).

After 15 minutes of equilibration, the pre-rete artery was switched to a medium of the same composition, under comparable hydrostatic pressure, and to which various inert radioactive molecules were added in tracer concentrations: human albumin-$^{125}$I (Frost; more than 95% of the labeled iodine was precipitated with 10% trichloroacetic acid), inulin-carboxyl-$^{14}$C [New England Nuclear; crystalline solid, 1–3 mCi/g; molecular weight (M.W.) 5000], 3-O-methyl-$^{14}$C-D-glucose (NEN; ethanol-water solution 7:3 20–55 mCi/mmole; M.W. 194.2), urea-$^{14}$C (NEN; crystalline solid, 2–10 mCi/mmole; M.W. 60.1), and tritium-labeled water (NEN; biological quality, 0.25 mCi/g; M.W. 20).

All labeled compounds were used within days ($^{125}$I) or a few months ($^{3}$H and $^{14}$C) of their purchase. They were stored in liquid solution at −10°C. The albumin-$^{125}$I was added to the medium either alone or in combination with tritium-labeled water and/or a carbon-labeled molecule.

Samples of the medium were simultaneously collected from the arterial and venous outputs, at various time intervals during a 2-hour perfusion. For each sample, two 0.1-ml samples were withdrawn and precipitated with 1 ml of trichloroacetic acid, 10%, in the presence of an excess of KI. The precipitates were washed twice and counted in a Packard gamma scintillation spectrometer (model 3375).

The supernatant fluids were transferred to vials containing 15 ml of Aquasol (Oxford Laboratories) and their radioactivity was determined in a Packard liquid scintillation spectrometer (model 3375).

The activity of the medium at the arterial input and the background activity of the medium at the venous input were similarly determined. All values were corrected for background, quenching and, in multitracer experiments, for cross-over.

At the end of the experiment, heparinized eel blood was injected into the pre-rete artery and then into the pre-rete vein to confirm that no leaks were occurring from the capsule and that there was no patchiness, indicative of poorly perfused areas. The rete was then blotted on filter paper and weighed. In some instances, a small fragment of the perfused rete was fixed by immersion in a glutaraldehyde fixative solution and its structure examined under the electron microscope (Figs. 2 and 3). These show that the structure of the rete remained intact and nonedematous over the period of perfusion.

THE PERMEABILITY OF THE BARRIER BETWEEN THE ARTERIAL AND VENOUS CAPILLARIES

Consider fluid flowing with a velocity $W_1$ in an arterial capillary of cross-sectional area $A_1$, and fluid flowing countercurrent with the velocity $W_2$ in a venous capillary of cross-sectional area $A_2$. Then if the concentration of the tracer solute in the arterial limb is $u(x,t)$ and that in the venous limb is $v(x,t)$, we find the following equation of conservation:

$$A_1 \left( \frac{\partial u}{\partial t} + W_1 \frac{\partial u}{\partial x} \right) + A_2 \left( \frac{\partial v}{\partial t} - W_2 \frac{\partial v}{\partial x} \right) = 0. \tag{1}$$

Now, if we neglect the capacity of the interstitial space, and assume that the permeability-surface or PS product of the combined capillary barrier interstitial space can be described by a single value (which presumably corresponds most closely to that of the least permeable element, the arterial capillary$^{2,3}$), we find

$$A_1 \left( \frac{\partial u}{\partial t} + W_1 \frac{\partial u}{\partial x} \right) = \frac{PS}{L} (v - u) \tag{2}$$
$$A_2 \left( \frac{\partial v}{\partial t} - W_2 \frac{\partial v}{\partial x} \right) = \frac{PS}{L} (u - v) \tag{3}$$

where $L$ is the length of the barrier.

In continuing this development we may use either of these last two equations, since their sum gives back Equation 1.

When the flows in each direction have been set equal,

$$F_1 = W_1 A_1 = W_2 A_2 = F_2. \tag{4}$$

When the countercurrent loop is disconnected and a steady infusion of tracer in unit concentration is begun in the arterial limb, whereas the countercurrent infusion in the venous limb contains no tracer,

$$u(0, t) = S(0) \tag{5}$$

and

$$v(L, t) = 0 \tag{6}$$

where $S(0)$ is a unit step function at $x = 0$.  

---

**Figure 1**  
Schematic view of the countercurrent perfusion system in the rete mirabile. $A_{in}$ and $A_{out}$ correspond to arterial input and output, and $V_{in}$ and $V_{out}$, to venous input and output.
FIGURE 2  Low power electron micrograph of capillaries of a rete examined at the end of a 2-hour perfusion. The arterial (A) and venous (V) capillaries are intact. Their structure is normal with the exception of the moderately vacuolar aspect of the endoplasmic reticulum. 8,000 ×.
Figure 3 High magnification of the capillary wall at the end of a 2-hour perfusion. The fenestrated endothelium in the venous capillary (V), the continuous but vesiculated endothelium in the arterial capillary (A), and the pericyte (Per) have a normal structure. The mitochondria, the nucleus, and the microtubular system are well preserved. The intercellular junctions (J) remain tight. The basement membranes (LB) between adjacent capillaries maintain their normal filamentous structure. 75,000 x.
The equations can then be solved for the transient behavior of the tracer concentration, in response to the steady arterial infusion begun at $t = 0$. The experimental data can usefully be analyzed in toto only if the group of tracers infused contains one substance which does not pass the barrier in either direction, so that it can be considered to be a nondiffusible reference substance. To our surprise, even labeled albumin was found to traverse the barrier between arterial and venous capillaries in measurable concentrations, in the present experiments. Since we therefore did not have a nondiffusible reference substance, we decided to utilize the later part of the response to characterize the effects of the barrier (the data acquired at long time, when the time-dependent part of the behavior is completed and the steady state response is established). Under these conditions the partial derivatives with respect to time may be set equal to zero, and the resulting simpler equations may then be solved.

If we use the transformation $y = x/L$, so that distances are expressed in terms of fractions of the length ($0 < y < 1$), and if we let the parameter $R = PS/A_2W_2 = PS/F$, we find that the steady state solutions are

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

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

where in each case $0 < y < 1$. Three characteristics of the system bear emphasis:

1. The gradient along the length is constant in each channel, and the drop in concentration across the barrier is a constant $u(y) - v(y) = \frac{1}{1 + R}$.

The changes expected in the profiles with permeability increase are shown in Figure 4. It should be noted that we have implicitly assumed that $P$ is constant along the length.

2. With flow equal in the two directions, conservation dictates that the sum of the concentrations of the two steady state outputs, $u(1)$ and $v(0)$, is equal to the sum of the two steady state input concentrations. In this case, with the one unit input, $u(1) + v(0) = 1$. Experimentally, when steady state conditions were attained, the sums of the averaged radioactivity at the arterial and venous outputs were found to fall between 98% and 102% of the averaged radioactivity concentration at the arterial input.

3. From the arterial and venous outputs, values for the parameter $R$ or $PS/F$ can be derived. We find

$$R = \frac{v(0)}{u(1)} = \frac{PS}{F}$$

and, if we know the values for flow and surface area,

$$P = F \frac{v(0)}{S u(1)}$$

It is appropriate to note that this expression, unlike that utilized by Crone and by Renkin, is exact. No approximations are involved.

Figure 4. The effect of increase of the permeability and hence of the parameter $R$ ($PSIF$) on the vascular profiles. The solid line corresponds to concentrations along the arterial capillaries; and the dashed line to those along the venous capillaries.

In the experiments reported in the present paper, the average flow in each direction is 0.6 ml/min, for a rete of average weight 150 mg, and the average area $S$ is in the range of 150 cm$^2$ (calculating it on the basis of 1 cm$^2$/mg wet weight). In utilizing Equation 9, the concentrations were averaged at each output over the steady state period of observation, from 10 to 120 minutes.

Results

Figure 5 shows a typical experiment in which three tracers were used: $\text{I}^3$-labeled albumin, $\text{C}^{14}$-labeled urea, and tritium-enriched water. The concentrations at the arterial and venous outputs are expressed as a proportion of the unit concentration arterial input. The concentrations of the arterial and venous outputs add up to unity for each indicator, as expected, when the tracer has reached its steady state concentration.

The steady state values from experimental data of this sort, the measured value for flow, and an appropriate estimate of the surface area of the capillaries were inserted in Equation 9, to give a set of values for the permeability of the intercapillary barrier. The average values obtained from the present series of experiments are listed in Table 1.

The relation between the coefficients of free diffusion in water at 25°C for the substances studied and the observed coefficients of permeability in the rete are displayed in Figure 6. For molecules other than labeled water, the permeability appears to increase on the log-log plot in a linear fashion with the diffusion coefficient. For labeled water, the permeability value is disproportionately
The time course of the output arterial and venous concentrations of $^{125}$I-labeled albumin, $^{14}$C-labeled urea, and tritium-enriched water, after beginning a constant arterial infusion, in a countercurrent-perfused rete. $A_{in}$: concentration at the arterial input = 1.0; 1 and 1': $^{125}$I-labeled albumin; 2 and 2': $^{14}$C-labeled urea; 3 and 3': tritium-enriched water. The solid line corresponds to concentrations at the arterial output ($A_{out}$); and the dashed line, to concentrations at the venous output ($V_{out}$).

**Discussion**

The various techniques by which the exchange processes in blood capillaries can be estimated rely on many assumptions which cannot be directly verified. With the exception of studies by Landis in single capillaries where the permeability was measured from filtration data obtained by the osmotic transient method, the diffusion permeability has generally been determined on capillary beds in whole organs. For these investigations, it has usually been presumed that all capillaries in an organ are similar with respect to length, surface of exchange, blood flow, and pressure, so as to be assimilated into a single capillary model. Of the various methods available for the study of capillary permeability, the osmotic transient, the single injection, and the continuous infusion techniques provide outflow observations from which events at the level of the capillary are deduced by use of a model, which is usually utilized in such a fashion that it provides approximate but fairly good estimates of capillary permeability. These estimates have been best for substances to which the capillaries are not highly permeable. The tissue clearance and tissue injection techniques are other approaches, but these can also present problems due to inhomogeneities and unstirred layer effects in the interstitium.

The rete mirabile of the eel swimbladder presents a set of advantages for the study of diffusion permeability not present in other preparations. Structurally, the system is remarkably homogeneous. Apart from the slightly greater size of the venous capillaries, the system is perfectly symmetrical. All capillaries are straight, unbranched, of similar length, and perfused at the same flow rate under the same hydrostatic and osmotic pressures. In steady state conditions, the concentration gradient is linear and potentially could be measured directly. Furthermore, any loss of indicator molecules can readily be detected. The rete thus provides an unusual combination of factors favorable for the measurement of capillary permeability.

Apart from all of these, the rete possesses another tremendous advantage. In the steady state, with counter-current perfusion, outflow concentrations can be used directly to calculate values for capillary permeability, with no approximations, and the approach is as valid for highly

**Table 1** Diffusion Permeability Coefficients of the Rete to Water-Soluble Substances

<table>
<thead>
<tr>
<th></th>
<th>M.W.</th>
<th>$r$</th>
<th>$D_m$</th>
<th>$P_m$</th>
<th>$P/D$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin-$^{125}$I</td>
<td>69000</td>
<td>36.7</td>
<td>0.066</td>
<td>0.063 ± 0.011</td>
<td>0.95</td>
<td>12</td>
</tr>
<tr>
<td>Inulin-carboxyl-$^{14}$C</td>
<td>3000</td>
<td>12.0</td>
<td>0.17</td>
<td>0.119 ± 0.028</td>
<td>0.70</td>
<td>8</td>
</tr>
<tr>
<td>2-O-methyl-$^{14}$C-glucose</td>
<td>124</td>
<td>3.7*</td>
<td>0.67*</td>
<td>0.397 ± 0.069</td>
<td>0.59</td>
<td>11</td>
</tr>
<tr>
<td>Urea-$^{14}$C</td>
<td>60</td>
<td>2.6</td>
<td>1.38</td>
<td>0.781 ± 0.106</td>
<td>0.57</td>
<td>11</td>
</tr>
<tr>
<td>Water-$^3$H</td>
<td>20</td>
<td>1.5</td>
<td>2.44</td>
<td>7.366 ± 1.368</td>
<td>3.02</td>
<td>18</td>
</tr>
</tbody>
</table>


* Calculated from tabulated glucose values, as suggested by Edwards.
diffusible molecules as it is for poorly diffusible molecules. One can therefore approach the estimates derived from the rete with a fair degree of confidence, even in the case of the highly diffusible molecules.

The values of the coefficients of permeability obtained in this study do not apply to a single capillary wall, but to two contiguous endothelial layers with their respective basement membranes. From the point of view of the structure, the major resistance would be expected to be situated in the arterial capillary and, since this will be limiting, the value derived will be expected to describe this more closely than it will the venous capillary.

The linear relationship between diffusion coefficient and permeability coefficient for albumin, inulin, 3-O-methyl-glucose, and urea in the rete (Fig. 6) indicates that there is no significant restriction imposed upon the passage of water-soluble tracer molecules with a molecular weight ranging from 69,000 to 60. The ratio of the calculated capillary permeability in the rete to the free diffusion coefficient of the tracer is in the same range of magnitude for albumin, inulin, 3-O-methyl-glucose, and urea. In the heart, Alvarez and Yudilevich have noted that their single injection studies show that the permeability-surface product ratio for sucrose to inulin is equal to the ratio of their free diffusion coefficients in water. In skeletal muscle Crone similarly found no evidence of restriction, whereas other studies suggest that it may be present.

The coefficients of permeability of the rete to the various inert molecules which were utilized in this study are of the same order of magnitude as those reported by Stray-Pedersen and Steen. These authors used a similar preparation, but the arterial and venous systems were perfused in a concurrent fashion using the vessels at the heart pole of the rete as input vessels. The value calculated for the permeability of the capillary barrier to tritiated water in their experiments was $3.3 \times 10^{-5}$ cm sec$^{-1}$. The value arising from our experiments was similar but smaller, $7.4 \times 10^{-5}$ cm sec$^{-1}$. In the species which we studied, Anguilla anguilla, the arterial capillaries, when fixed in situ by perfusion at physiological pressures, are found to be lined by a high endothelium 2–4 $\mu$m thick; whereas, in the species studied by Stray-Pedersen and Steen, Anguilla vulgaris, the arterial capillaries are lined by a low flat endothelium 0.3–1 $\mu$m thick. The difference in structure appears to account for the difference in the estimates. For $^{14}$C-labeled urea, Stray-Pedersen and Steen found a permeability of $0.87 \times 10^{-5}$ cm sec$^{-1}$; in the present investigation, the value found was $0.78 \times 10^{-5}$ cm sec$^{-1}$.

In this study, it is found that labeled water exchanges more rapidly than would be expected, in terms of its molecular size. This observation is in keeping with data reported in the literature for most types of capillaries or biological barriers in series. It has been implied that there is an area in the capillary wall that allows transcapillary exchange of labeled water but not of small tracers such as sodium or urea. The preferential area for passage of water is represented by the entire histological surface of the capillary endothelium, whereas solutes appear to have access chiefly to pores of definite dimensions (these previously were thought to be represented only by the interendothelial cell junctions but now are also considered to be represented by multivesicular transendothelial channels, when the endothelium contains many vesicles, as it does in the arterial capillaries of the rete). The barrier itself is known to be highly permeable to lipid-soluble materials, and the organic amides and diols have been found to approach the permeability characteristics of this group of substances as their carbon chain length increases. On the basis of these data it has been suggested that water is an integral part of the barrier membrane and that the high or low permeability of a solute at the continuous surface of the membrane depends on its interaction with the structure of water as well as its molecular volume. With this in mind, one would expect lower permeability coefficients for urea and saccharides such as 3-O-methyl-glucose and inulin, whereas water itself would be expected to cross the barrier more readily. Our data on the permeability of the rete, which show a set of permeability values for our probing solutes which are lower in terms of their molecular size, and higher for water, conform to these expectations. In the absence of evident restriction on diffusional loss of the tracer solutes, it is inappropriate to try to derive an estimate of an approximate “pore size” for the channels conducting the solutes. However, it is evident that it is fairly large and the data would be compatible with the hypothesis that the rather larger vesicular channels may be involved in the transcapillary exchange of a variety of solutes.

Some years ago it was hypothesized that the exchange of water across capillaries would be flow-limited. The absence of total exchange of labeled water between the arterial and venous capillaries of the countercurrent perfused rete indicates that this is not true, that the exchange is barrier-limited. The observations fit well with more modern data. Limitations in the distribution of labeled water have been found both at the blood-brain barrier and blood-heart interfaces in mammals. The best estimate of the permeability of the cerebral capillaries to labeled water appears to be that of Bolwig and Lassen, in the rat, $4 \times 10^{-5}$ cm sec$^{-1}$. These capillaries lack endothelial vesicles and the interendothelial slits are sealed by tight junctions. The value which we found for the rete at 25°C was $7.4 \times 10^{-5}$ cm sec$^{-1}$. If we had carried out our experiments at 37°C, we would expect the observed permeability to have been higher, approximately $11 \times 10^{-5}$ cm sec$^{-1}$. In the rete studied by Stray-Pedersen and Steen, where the arterial capillary endothelium is composed of flattened rather than cuboidal cells, the expected value at 37°C would have been $40 \times 10^{-5}$ cm sec$^{-1}$. By comparison, the value found in the dog heart by Rose et al. (after a rather complex analysis of their data, in contrast to the present rather simple process of calculation) was $60 \times 10^{-5}$ cm sec$^{-1}$. This endothelium is also flat and multivesiculated. The difference between these estimates and their order thus appears sensible. The data from this variety of sources fit together and indicate that limitation in the distribution of labeled water at the capillary barrier should be an expected phenomenon, when one considers its kinetics within an organ, under physiological circumstances.
Acknowledgments

We express our appreciation to Marie-Paule Dea for her skillful technical assistance, and thank Margaret Mulherin for typing this manuscript.

References

Diffusion permeability of an isolated rete mirabile.
E A Rasio, M Bendayan and C A Goresky

Circ Res. 1977;41:791-798
doi: 10.1161/01.RES.41.6.791

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/41/6/791

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://cicrres.ahajournals.org/subscriptions/