Effect of Temperature Change on the Permeability of Eel Rete Capillaries

Eugenio A. Rasio, Moise Bendayan, and Carl A. Goresky

The changes in the permeability properties of the rete capillaries of the eel in response to temperature shifts were studied during countercurrent perfusion at constant flow and pressure. Tracers and oxygen were added to the arterial perfusate. From the ratio of end concentrations of arterial to venous capillaries divided by surface area, calculated from rete weight, a value for the ratio of permeability to flow, P/F, with dimensions in centimeters^{-2} was estimated. Because flow and surface area are constant, this provides an index of how permeability varies with time. A group of paracellular (albumin, sucrose, and sodium) and cellular (antipyrine, water, and oxygen) probes were used. When the temperature of the perfusate was raised abruptly from 25°C to 35°C, P/F values rose continuously and irreversibly from 0.042±0.009 to 0.281±0.112 cm^{-2} (mean±SEM) for 125I-albumin, from 0.082±0.006 to 1.74±0.070 cm^{-2} for [14C]sucrose, and from 0.32±0.06 to 2.78±0.62 cm^{-2} for 22Na, whereas they were not modified for [14C]antipyrine, [3H]water, and O2. Gradual increase of temperature was accompanied by a smaller rise in sucrose and sodium permeability and no change in albumin permeability; with decrease, the change was reversible. When the temperature was lowered abruptly from 25°C to 5°C, the P/F ratio for sucrose, sodium, and oxygen did not change, while that for [3H]water and [14C]antipyrine decreased to plateau values, from 13.0±3.2 to 9.6±2.6 and from 7.9±2.2 to 4.4±0.4 cm^{-2}, respectively; simultaneously, P/F values for 125I-albumin increased from 0.030±0.007 to 0.063±0.012 cm^{-2}. Thus, increase in rete temperature primarily increases paracellular permeation, whereas decrease in temperature primarily decreases permeability to [14C]antipyrine and [3H]water cellular probes. Oxygen is unaffected throughout. (Circulation Research 1992;70:272–284)

Temperature change has been used in the past as a means either to break the capillary barrier or to characterize its effects on capillary permeability. In general, when heat or cold injury has been induced by rapid change in temperature, a loss of barrier function to macromolecules has been observed. The morphological substratum for this effect has been thought to be an increased vesiculation of the plasma membrane in cold injury and the appearance of occasional gaps between the endothelial cells in heat injury. When progressive rather than sudden change in temperature has been applied to the microvasculature, changes in the hydraulic conductivity of single capillaries and capillary filtration coefficients in isolated organs have appeared reciprocal to changes in water viscosity. In the latter instance, it was concluded that the water pathway across the capillary wall was invariant with temperature. Few studies have addressed the effects of temperature change on the permeability of lipophilic solutes. The question of whether the lipid structure of the endothelial plasmalemma changes with temperature has also been raised. Such a change has been construed to occur in other lipid-containing structures when a sharp break or transition is observed in data relating the logarithm of permeability to the reciprocal of the absolute temperature. In a study seeking to examine this phenomenon, Cua et al. used labeled antipyrine in multiple indicator dilution studies in dog and rat lungs and in an in vitro assessment of the permeability of cultured pulmonary endothelial cells. At normal body temperature, labeled antipyrine was found to penetrate the pulmonary endothelial cells and interstitium in a flow-limited fashion, with barrier-limited cell entry at the epithelial cell surface; however, at temperatures below 21°C, endothelial cell entry also became increas-
ingly barrier limited with decrease in temperature. Labeled water distribution remained low limited at both surfaces over the whole temperature range. Slopes of the logarithm of permeability versus the reciprocal of absolute temperature were the same for cultured endothelial cells over the upper temperature range and for capillary permeability over the lower temperature range, in which it was determinable. A lack of distinct change in the lipid structure of the endothelial cells was inferred.\(^6\) Curry\(^9\) showed that the hydraulic conductivity in single capillaries examined at room temperature and at much lower temperatures changed in a fashion related to the increase in the viscosity of water at the lower temperature. He suggested that the result was compatible with a hydraulic flux principally through an unchanging extracellular rather than transcellular route. In most studies no morphological correlates have been reported.

In the past, we have developed the use of the capillaries of the rete mirabile to provide a general characterization of water and solute capillary permeability during countercurrent perfusion experiments.\(^10\)–\(^12\) The simple measurement of input and output concentrations in this system during steady state allows for the simultaneous estimation of permeability coefficients for a variety of hydrophilic and lipophilic substances. In the present study, we have examined how change in temperature influences the passage across the capillaries of the rete of a variety of substances thought to use paracellular and cellular paths of transfer. The temperature range examined was that between 5°C and 35°C, which is the physiological range for the teleost eel. Changes were applied either abruptly or progressively. Permeability coefficients were measured for albumin, sucrose, sodium, water, antipyrine, and oxygen.

### Materials and Methods

The rete mirabile, an organ made up exclusively of capillaries, was isolated from the swim bladder of eels previously captured from the St. Lawrence River and adapted to ambient freshwater temperatures of 20–25°C. The rete capillaries were countercurrent perfused with a Krebs-Ringer bicarbonate buffer (pH 7.4) containing 5 mM glucose and 4 g/100 ml bovine albumin (Sigma Chemical Co., St. Louis, Mo.; fraction V powder, 96–99% albumin, remainder globulins) through both arterial and venous feeding vessels, which had been catheterized at the cephalic and caudal poles, respectively. At the various temperatures selected for study, in vitro metabolic experiments were performed to characterize the energy-generating metabolic pathways of the organ, permeability studies were conducted, and morphological examinations were carried out to define any structural changes that could be related to the observed permeability changes.

### In Vitro Metabolic Studies

When the two retia in each eel had been cleared of their blood by the perfusion, they were dissected free of the swim bladder. The capsular and vascular poles were removed, and the remaining capillary organs were cut into small clusters of filaments. Approximately equivalent amounts of capillary tissue (30 mg) were incubated for 2 hours in vials containing 1 ml of the perfusion buffer enriched with \([^{14}C(U)]\)glucose. Six incubations were carried out simultaneously at 5°C, 25°C, and 35°C in closed vials with a mixture of 95% O\(_2\), 5% CO\(_2\) as a gas phase. Vials without capillary tissue served as controls. At the end of the incubations, the media in the closed vials were acidified and the generated carbon dioxide was trapped in a scoop filled with phenylethylamine. Glucose and lactate concentrations in the medium were measured by specific enzymatic techniques. Glucose oxidation to carbon dioxide was calculated by dividing the activity absorbed by phenylethylamine by the specific activity of \([^{14}C(U)]\)glucose in the medium at the start of the incubation. ATP yield was estimated from the stoichiometry of the reactions in which 1 mol glucose produces 2 mol ATP when converted to lactic acid and 38 mol ATP when oxidized through the Krebs cycle.

### Permeability Studies

All perfusions were carried out in situ to avoid damage to the tissue, with a constant flow averaging 0.51±0.01 ml/min (mean±SEM) in the arterial and venous directions and at a constant pressure head of 45 ml H\(_2\)O. The wet weights of the retia averaged 200±14 mg. The medium at the arterial input contained combinations of the following radioactive tracers: human \(_{125}\)I-albumin (Frosst, Montreal, Canada; more than 95% of the labeled iodine was precipitated with 10% trichloroacetic acid), \([^{14}C(U)]\)succrose (New England Nuclear, Boston, Mass.; 1–5 mCi/mmol), \(^{22}\)Na (New England Nuclear; as sodium chloride, 99.9% radionuclidic purity), \([N\text{-methyl-}^{14}C]\)antipyrine (New England Nuclear; 54.7 mCi/mmol, in ethanol), and \([^{3}H]\)water (New England Nuclear; biological quality, 0.1 mCi/g). No tracers were added to the perfusion medium at the venous input.

In perfusion experiments designed to measure oxygen permeability, the medium delivered to the arterial input was continuously equilibrated with a gas mixture of 95% O\(_2\), 5% CO\(_2\) to achieve a partial pressure of oxygen of approximately 400 mm Hg. At the venous input, the medium was partially equilibrated with a gas mixture consisting of 95% N\(_2\), 5% CO\(_2\); with this, its oxygen content was partially desaturated to an average oxygen partial pressure of 100 mm Hg. All tubings carrying the medium to the rete were coated with commercial Saran Wrap (Dow Chemical Co., Indianapolis, Ind.) to minimize oxygen losses. Even with the lowered PO\(_2\) in the venous medium, the capillaries of the rete were supplied with oxygen by the perfusion at a rate many times greater than required by their respiration.
To test the effects of acute temperature change on permeability, the experiments were started with a 60-minute countercurrent perfusion at room temperature (25°C), during which baseline measurements of water and solute permeabilities were carried out. The temperature of the perfusion media was then abruptly increased to 37°C or decreased to 5°C in approximately 1 minute by circulating warm or cold water through the heat exchangers at the inputs; perfusions were then continued for 90 minutes. A total of 20 perfusions were performed to study the effects of acutely warming the rete, while 15 were carried out to test the effects of acute cooling.

To test the reversibility of the effects of acute temperature change on permeability, rete capillaries were first perfused for 60 minutes with media at 35°C or 5°C; the perfusate temperature was then brought to 25°C in approximately 1 minute, and the perfusions were continued for 90 minutes. A total of six and nine perfusions were performed in each group, respectively.

The effects of progressive, rather than abrupt, changes of temperature on permeability and their eventual reversal were tested in six experiments in which perfusate temperature, initially maintained at 25°C for 30 minutes, was increased by approximately 2°C every 10 minutes over the next hour and then reduced at the same rate for another hour.

Rete temperature measurements were recorded with a thermocouple thermometer (08402 series; Cole-Parmer Instrument Co., Chicago, Ill.) from a surface probe positioned under the rete.

The media were collected simultaneously from arterial and venous outputs for 10-minute periods of time throughout the perfusion. The flow was maintained constant and identical in each direction during control and experimental conditions. The radioactivity of 125I-albumin was measured on a 10% trichloroacetic acid precipitate washed with excess potassium iodide by use of a gamma spectrometer (Packard Instrument Co., Inc., Meriden, Conn.). The other tracers were measured in the protein-free supernatant with a Packard liquid scintillation spectrometer. Values were corrected for background and crossover. The partial pressure of oxygen was measured with an in-line oxygen tension measuring system with a dual oxygen electrode amplifier (model 203, Instech Laboratories, Plymouth Meeting, Pa.).

Where no tracer was introduced in the venous perfusion, permeability coefficients P (centimeters per second) for tracers were calculated from the equation \( P = \frac{F \times V_o}{(A_e \times S)} \), where F is the flow (milliliters per second), \( V_o \) and \( A_e \) are the concurrent concentrations at the venous and arterial outputs, and \( S \) is the surface area available for capillary exchange (1 cm²/mg wet wt). The permeability coefficient for oxygen was calculated by use of the same equation after subtracting the value of oxygen pressure at the venous input from \( V_o \) and \( A_e \). Recoveries of the test substances from the rete outputs were within 2% of the inputs.

The ratio values \( V_o/A_e \) for tracers not entering at the venous input and the corresponding ratio for oxygen provide an estimate of the ratio PS/F, the ratio of the permeability surface area product to flow for each tracer. To obtain an index of permeability directly from the plotted data, the ratio \( V_o/A_e \) was multiplied by 1/S, where S was calculated by multiplying the weight of the rete (which can be obtained only at the end of the study) by the standard surface area estimate (1 cm²/mg wet wt). The product \( V_o/A_e \times 1/S \) is, then, \( P/F \), where F is the standardized flow in the system, 0.51 ml/min, and the dimensions of \( V_o/A_e \times 1/S \) or \( P/F \) are in centimeters⁻². Permeability will be numerically about 0.51 times the ordinate value of \( V_o/A_e \times 1/S \), when expressed in centimeters per minute and 0.51/60 or 0.0085 times this value when expressed in centimeters per second. Because flow F is maintained constant in this system and the surface area S can be presumed not to vary under these circumstances, change in the output rete concentration ratio \( V_o/A_e \) can be taken to reflect alterations in the permeability of the system. In the present study, we have used this ratio to track the change in permeability with time.

The equations developed for calculating permeability apply to a steady state, which often is not established in these experiments. On the other hand, because half the time for outflow response to change in input concentration is on the order of 1 minute and the rate of change in the outflow value for \( V_o/A_e \times 1/S \) or \( P/F \) is usually much less than this, calculated values will be approximately correct. They will be expected to provide, moreover, a good index of the pattern and rate of change of permeability.

Morphological Studies

Each eel has two symmetrical retia. Both were used when morphology was assessed. In each experiment, one rete was perfused for the permeability studies, while the other was removed for control morphological examination. For this examination, both the control and experimental retia were fixed by immersion in a 1% glutaraldehyde–0.1 M phosphate buffer solution and processed for electron microscopy as described in detail previously. Thin sections were cut, mounted on nickel grids, stained, and examined with a Philips 410 SL electron microscope. For morphometric evaluations of the vesicular system of the arterial endothelial cells, electron micrographs of the capillary walls were recorded and enlarged to a final magnification of 36,000. The volume density of the vesicular system was evaluated on control and experimental tissues by direct planimetry on the photomicrographs with the use of a Videoplan 2 digital image analysis system (Carl Zeiss, Toronto, Canada) in reference to the cellular volume (excluding nuclei profiles). Ten cross-sectioned capillary profiles were recorded for each control and each experimental rete. In addition, the size of the plasmalemmal vesicles from control and experimental tissues was measured on the photomicrographs and
statistically analyzed (Student's *t* test). The size of 100 vesicles was analyzed for each control and each experimental rete. Six retia at raised temperature with their corresponding control retia and six retia at low temperature with their controls were examined.

**Results**

*Metabolic Studies In Vitro*

The effect of temperature on glucose uptake, lactate production, and glucose conversion to CO$_2$ by isolated rete capillaries is shown in Table 1. Glucose utilization increases with temperature with a step-up of ATP generation between 25°C and 35°C, which is accounted for by both glycolysis and glucose oxidation. At 5°C and 25°C, more than 95% of the energy produced is from glycolysis. At 37°C, glycolysis accounts for two thirds of the ATP yield and the Krebs cycle for the other one third.

**Permeability Studies**

Figure 1 shows the raw experimental data for a representative example from the first set of experiments. The stability of the data over the baseline period of observation at 25°C is evident, together with patterns of change after the temperature was abruptly raised from 25°C to 35°C. Calculation of permeability values from the data for aggregated control periods at 25°C results in the set of data given in Table 2 and illustrated in Figure 2. In a plot of permeability at 25°C ($P_25$) versus diffusion coefficient at the corresponding temperature ($D_25$), a line passing through the labeled albumin, sucrose, and $^{22}$Na values would correspond approximately to that previously established from albumin through inulin, 3-O-methyl-D-glucose, and urea and thought to represent the manner in which permeability is related to the diffusion coefficient for substances passing through the intercapillary barrier via a pathway presumed to be extracellular. In contrast, the values for water, oxygen, and antipyrine, which are expected also to pass through the whole of the cell surface, lie substantially above what would be the extrapolated upper end of the paracellular line.

We have previously reported that, for tracer studies of permeability during countercurrent perfusion, the ratio $V_o/A_o$, which we are using to track permeability change with time, remained steady in studies at 25°C for the 3 hours over which it was tested. A similar stability was observed in random control experiments carried out during the current period (data not shown). The stability of the $V_o/A_o$ ratio indicates that, in the absence of experimental manipulation, the permeability of the rete capillaries is time independent.

Figure 3 shows the results of experiments in which the perfusate temperature was sharply raised from 25°C to 35°C. These are the same experiments as shown in Figure 1, but the ordinate is now proportional to the estimated permeability. The permeability of the arterial to venous capillary barrier to $^{125}$I-albumin, $[^{14}C]$sucrose, and $^{22}$Na rose significantly.

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**Table 1. Effect of Temperature on Glucose Utilization by Isolated Rete Capillaries**

<table>
<thead>
<tr>
<th>Medium Temperature (°C)</th>
<th>Glucose Uptake (μmol/g/hour)</th>
<th>Lactate Production (μmol/g/hour)</th>
<th>Glucose Conversion to CO$_2$ (μmol/g/hour)</th>
<th>ATP Yield (μmol/g/hour)</th>
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</thead>
<tbody>
<tr>
<td>5</td>
<td>0.67±0.20</td>
<td>1.45±0.25</td>
<td>0.003±0.002</td>
<td>1.5</td>
</tr>
<tr>
<td>25</td>
<td>3.71±0.31</td>
<td>8.99±0.46</td>
<td>0.013±0.004</td>
<td>9.5</td>
</tr>
<tr>
<td>35</td>
<td>11.67±1.01</td>
<td>27.25±1.00</td>
<td>0.389±0.159</td>
<td>41.0</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=6). For each aspect of glucose utilization, values are significantly different from each other: *p* < 0.001 (Student's *t* test for paired experiments).

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**FIGURE 1.** Average values (±SEM) for the time courses of output arterial (solid lines) and venous (dashed lines) concentrations of $^{125}$I-albumin, $[^{14}C]$sucrose, and $^{22}$Na and for a second group, $[^{14}C]$antipyrine, $[^{3}H]$water, and oxygen. In the case of oxygen, venous input values are subtracted from arterial and venous output values before plotting. $A_o$ is the steady reference input concentration value of 1.0 for all probes. Steady countercurrent perfusion at 25°C was begun at time zero; from 60 minutes onward, the temperature of the medium was raised to 35°C. 1 and 1', $^{125}$I-albumin; 2 and 2', $[^{14}C]$sucrose; 3 and 3', $^{22}$Na; 4 and 4', $[^{14}C]$antipyrine; 5 and 5', $[^{3}H]$water; 6 and 6', oxygen. In each instance, n=7 to 17.
whereas that to [14C]antipyrine, [3H]water, and oxygen was not modified. A significant temperature-induced increase in permeability for [125I]-albumin became apparent only after 40 minutes but was seen within 10 minutes for [14C]sucrose and [22Na]. For all three of these tracers the rise in the V0/Ao×1/S parameter or P/F ratio continued with time until the end of the experiment, when it reached seven, 20, and nine times baseline values, respectively (that is, from 0.042±0.009 to 0.281±0.112 cm−2 for [125I]-albumin, from 0.082±0.006 to 1.74±0.070 cm−2 for [14C]sucrose, and from 0.32±0.06 to 2.78±0.62 cm−2 for [22Na]). The permeability increase for the three tracers thought to permeate through paracellular paths continued over the period during which the temperature was elevated. No final asymptotic unchanging steady state was attained.

Figure 4 shows the results of the experiments in which the perfusate temperature was sharply reduced from 25°C to 5°C. The V0/Ao×1/S parameter or analogue permeability index P/F values for oxygen, [22Na], and [14C]sucrose were not modified throughout the experiments. After the perfusate was cooled, the P/F ratios for [125I]-albumin rose continuously to twice the average baseline values at the end of the experiments. The P/F ratios for [14C]antipyrine

### Table 2. Permeability Coefficients of the Rete to the Substances Studied at 25°C

<table>
<thead>
<tr>
<th>Substance</th>
<th>MW</th>
<th>D25 (10⁻⁵ cm²/sec)</th>
<th>P25 (10⁻⁵ cm²/sec)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>[125I]-Albumin</td>
<td>69,000</td>
<td>0.066</td>
<td>0.0317±0.0053</td>
<td>28</td>
</tr>
<tr>
<td>[14C]Sucrose</td>
<td>342</td>
<td>0.52</td>
<td>0.104±0.018</td>
<td>13</td>
</tr>
<tr>
<td>[22Na]</td>
<td>22</td>
<td>1.30</td>
<td>0.327±0.041</td>
<td>28</td>
</tr>
<tr>
<td>[14C]Antipyrine</td>
<td>188</td>
<td>0.91*</td>
<td>6.45±0.88</td>
<td>20</td>
</tr>
<tr>
<td>[3H]Water</td>
<td>20</td>
<td>2.44</td>
<td>10.82±1.50</td>
<td>31</td>
</tr>
<tr>
<td>Oxygen</td>
<td>16</td>
<td>2.11</td>
<td>13.44±2.99</td>
<td>13</td>
</tr>
</tbody>
</table>

Values for P25 are mean±SEM. MW, molecular or atomic weight; D25, diffusion coefficient at 25°C; P25, diffusion permeability coefficient at 25°C; n, number of experiments.

*Personal communication from Drs. R.A. Garrick and F.P. Chinard.

**Figure 2.** Relation between the permeability coefficients (P25) and diffusion coefficients (D25) for the various substances derived from the rete perfused in a countercurrent fashion at the baseline temperature of 25°C. Values are mean±SEM. For each substance, n=13 to 28.

**Figure 3.** Time course of the ratio of the concurrent concentrations at the venous and arterial outputs (V0/Ao) per milligram wet weight after a steady arterial infusion of tracers and oxygen beginning at time zero in a countercurrent-perfused rete. Values are mean±SEM. *Values significantly higher than averaged control values (p<0.05). 1, [125I]-Albumin; 2, [14C]sucrose; 3, [22Na]; 4, [14C]antipyrine; 5, [3H]water; 6, oxygen. For each substance, n=7 to 17. From 0 to 60 minutes, a control perfusion was carried out at 25°C. From 60 minutes onward, the temperature of the medium was raised to 35°C. S, surface area available for capillary exchange; P/F, ratio of permeability to flow.
and [3H]water were reduced significantly and reached plateau values within 30 minutes of cooling of 0.56 and 0.74 times the corresponding baseline values. The effect on antipyrine is significantly greater than that on water (p<0.05). With the drop in temperature, the viscosity of water increases.\textsuperscript{14} If the [3H]water permeation were occurring in a bulk water phase, it would have been expected to decrease to 0.62 times its rate at 25°C. The change for both water and antipyrine is of this order, even though the major part of the pathway is likely through the cell surface. In contrast, for the previously outlined increase in temperature from 25°C to 35°C, no significant change in the permeability to labeled water and antipyrine was documented, even though on the basis of the viscosity change for water an increase of the order of 1.32 might have been expected.

Figure 5 shows the results of the experiments in which the rete was first perfused with a medium heated to 35°C and then with medium at the control temperature of 25°C. The $V_o/A_o \times 1/S$ or P/F values for oxygen, [3H]water, and [14C]antipyrine were indifferent to the temperature change. The steady rise for [125I]-albumin, [14C]sucrose, and 22Na observed at 35°C was arrested within 10 minutes of the perfusion at 25°C but did not revert to previous levels; high plateau values were found to be maintained thereafter for these three tracers, until the end of the experiment.

Figure 6 shows the results of the experiments in which the rete was first perfused with medium cooled to 5°C and then with medium at the control temperature of 25°C. Small increments of borderline statistical significance were observed in the $V_o/A_o \times 1/S$ or P/F ratios for [3H]water, [14C]antipyrine, 22Na, and [14C]sucrose.

Figure 7 shows the results of experiments in which the rete was perfused with medium, the temperature of which was progressively increased from 25°C to 35°C and then progressively decreased to 25°C. The $V_o/A_o \times 1/S$ or P/F values for 125I-albumin were not modified by the temperature excursion, whereas for [14C]sucrose and 22Na they rose and then decreased, following the temperature change. These effects on permeability are
in contrast to those occurring with more rapid temperature change in that the magnitude of the increase in permeability for sucrose and sodium is much reduced and is reversible, and there is no perceptible variation in the albumin permeability.

**Morphological Studies**

The morphological features of the normal eel rete capillaries are illustrated in Figures 8a and 9a. The endothelial cells of the arterial capillaries are high and have a well-developed vesicular system. This is formed by membrane-bound vesicles and tubules, some of which open directly into the luminal and abluminal plasma membranes. The fenestrated venous capillaries, on the other hand, are formed by low endothelial cells with fewer vesicles and numerous fenestrations closed by a well-defined diaphragm. Intercellular tight junctions are present between endothelial cells. Both types of capillaries are lined with their respective basement membranes. Bundles of collagen fibers and pericytes are found in the interstitial space. When the temperature was raised from 25°C to 35°C (Figure 8b) or when it was reduced from 25°C to 5°C (Figure 9b), little immediate evident morphological changes were detected in the capillary wall. Indeed, the capillaries were intact, no edema was found in the interstitial space or in the endothelial cells, and the junctions remained tight, sealing the capillary lumina with no evidence of leakage. In the venous capillaries, the endothelial fenestrations were still present with their diaphragms. In both experimental protocols, the vesicular system of the arterial endothelial cells appeared less developed. This was confirmed by the morphometric analysis, which demonstrated that, when compared with the corresponding controls, the experimental tissues showed a significant reduction in the volume density of the vesicular system (Table 3). This is probably due to a decrease in the number of vesicles. Indeed, both in control and experimental conditions, the average diameter of the vesicles remained constant (Table 3) and no apparent edema was detected in the arterial endothelial cells.
FIGURE 8. Electron micrograph of arterial (AC) and venous (VC) capillaries of a control rete (panel a) and a rete for which the temperature of the perfusion medium was raised abruptly from 25°C to 35°C (panel b). The morphological features are similar in control and warmed retia: vesicular profiles (V), intercellular junctions (J), basement membrane or basal lamina (BL), and pericytes (P). Bars, 1 μm.
Figure 9. Electron micrograph of arterial (AC) and venous (VC) capillaries of a control rete (panel a) and a rete for which the temperature of the perfusion medium was lowered abruptly from 25°C to 5°C (panel b). The morphological features are similar in control and cooled retia: vesicles (V), intercellular junctions (J), basement membrane (BL), pericytes (P), and fenestrations in venous capillaries (f). Bars, 1 μm.
Discussion

The purpose of this study was to determine to what extent the permeability of a capillary barrier to a variety of solutes is sensitive to changes in temperature. In these experiments, solutes with differing size and lipid solubility were used simultaneously to characterize the aqueous and lipid pathways that participate in their passage across the endothelium. This approach was made possible by virtue of the relative simplicity of determination of permeability in the countercurrent-perfused rete system. A second characteristic of the study is that we were able to use, in this instance, a physiological range of temperatures from 5°C to 35°C, as these encompass the normal adaptive capacities of the freshwater eel to the ambient milieu. Finally, because there are two symmetrical retia per eel, we were able to search for possible morphological correlates of the temperature-induced changes.

General Effects of Temperature

The experiments as a whole provide results that indicate the presence of selective responses in the pathways underlying intercapillary transport. Clearly, the responsiveness of the rete capillary barrier to a given temperature change varies considerably with the probe substance and the direction and rate of temperature change. The behavior of a single tracer is not by itself indicative of the permeability status of the rete barrier. This is true whichever the direction (warming or cooling) or the modalities (rapid or progressive) of the temperature change. Thus, [125I]-albumin, [14C]sucrose, and 22Na are very sensitive indicators of functional changes induced by a rapid warming of the perfusate from 25°C to 35°C, but [14C]antipyrine, [1H]water, and oxygen are not. The changes induced by rapid cooling of the perfusate, on the other hand, are functionally detected with [14C]antipyrine, [1H]water, and [125I]-albumin but not with other tracers.

For any given probe, the response to warming or cooling varies with the temperature range. Thus, when the temperature of the medium is raised, the passage of 22Na across the rete capillaries is temperature insensitive between 5°C and 25°C but becomes highly responsive between 25°C and 35°C. When the perfusate is cooled from 35°C to 25°C, the transcapillary passage of [14C]antipyrine is not modified, whereas it diminishes significantly on cooling the medium from 25°C to 5°C.

Finally, a similar qualitative effect on permeability was observed for labeled albumin when rapid temperature changes were applied in either direction. The permeability of the rete capillary barrier to 125I-albumin was increased both by warming the perfusate from 25°C to 35°C and by cooling it from 25°C to 5°C.

Permeability of the Rete at 25°C

We previously measured the permeability coefficient of the rete to a variety of solutes in experiments ordinarily conducted with perfusate at 25°C. In this new series of experiments, when data were pooled from control perfusions at 25°C, permeability coefficients for oxygen, [1H]water, 22Na, and 125I-albumin approximated values already reported.10-12 The values for oxygen and water indicate that their passage across the rete capillaries is barrier limited. The permeability values for albumin in our perfusions of the rete have been consistently larger than those expected if restricted diffusion were to take place.15 The nature of the processes underlying the difference is not evident. Ordinary charge effects are unlikely to enhance albumin movement because we have shown that the permeability of a dextran sulfate fraction is lower than that of a neutral dextran fraction of matched diffusion coefficient.12 It is possible that albumin also participates in a special pathway, such as a vesicular transport mechanism, and we have indeed demonstrated the presence of albumin in the vesicular system of the rete arterial capillaries.16 We have used [14C]antipyrine and [14C]sucrose for the first time as additional tracers for lipid and paracellular aqueous pathways, respectively. Table 2 shows that the mean $P_{25}$ value for [14C]antipyrine is 6.45×10⁻⁵ cm/sec, while that for [14C]sucrose is 0.10×10⁻⁵ cm/sec. The large discrepancy between the $P_{25}$ values of these two tracers of relatively close molecular weights is most likely related to the difference in their oil/water partition coefficients. The lipid solubility of antipyrine, with an oil/water solubility coefficient of 0.032, as contrasted to a value of 0.0007 for water,17 should favor its transcellular, membrane-associated passage. In contrast, sucrose is a water-soluble extracellular tracer, and its transport across the endothelium is expected to be through paracellular aqueous pathways. Cua et al17 have derived from experiments in rats with the indicator dilution technique a permeability value for antipyrine in pulmonary epithelium of 8×10⁻⁵ cm/sec and from probe studies with cultured pulmonary endothelial cells a

### Table 3. Volume Density of the Vesicular System and Average Diameter of the Vesicles in Arterial Endothelial Cells

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Control rete</th>
<th>Experimental rete</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Volume density</td>
<td>Vesicle diameter</td>
</tr>
<tr>
<td>25–35°C</td>
<td>0.147±0.010</td>
<td>103.07±3.32</td>
</tr>
<tr>
<td>25–5°C</td>
<td>0.246±0.025</td>
<td>98.43±3.20</td>
</tr>
</tbody>
</table>

Values are mean±SEM. For each type of experiment, six control retia and six corresponding experimental retia were examined. In each rete, 10 cross-sectioned capillary profiles were observed. The diameter of 100 vesicles was measured for each control and experimental sample.
value of approximately $95 \times 10^{-5}$ cm/sec at 25°C. A value of $67.5 \times 10^{-5}$ cm/sec was reported by Curry in individually perfused frog capillaries of the mesentry, at the same temperature, with a procedure involving responses to solute concentration gradients. The value for the pulmonary epithelial cells is close to our value of $6.45 \times 10^{-5}$ cm/sec for the rete intercapillary barrier and indicates that the permeability of the thick-walled arterial capillaries, the dominant part of the rete capillary barrier, is of the same order as that of the epithelial cells of the perfused rat lung. The capillary endothelium of the rat lung is much more permeable to antipyrine than is the epithelium, demonstrating barrier-limited behavior and therefore measurable permeability values during perfusion only at temperatures lower than 25°C. The $P_{35}$ value for $^{14}$C-antipyrine in the rete is consistently lower than the $P_{35}$ value for $[^3H]$water. This observation has been reported by others both for capillaries and cells, especially at lower temperatures, and indicates that antipyrine will be a valid tracer for water only when both behave in a barrier-limited fashion.

**Effects of Rapid Temperature Change on Rete Permeability**

For the sake of convenience, we have labeled as a control period of observation the initial time during which the rete is perfused with medium at 25°C. This is indeed the average temperature of the water in the tanks where the eels are kept before their use. It is also the average room temperature of the laboratory where rete perfusion is carried out. From this control value of 25°C, the temperature of the medium was rapidly raised to 35°C or lowered to 5°C. Although the eel is perfectly capable of living at these extreme temperatures, and even beyond them, it only does so ordinarily by adaptation to stepwise changes of water temperature. The abrupt changes of temperature induced in our study, although within the range compatible with life, would probably be pathological for the whole eel. For the capillary tissue, however, respiration and energy production are measurable at 5°C, 25°C, and 35°C, and the morphological features do not indicate structural cell damage or anomalies in junction morphology. It is therefore reasonable to presume that, in our experimental conditions, the permeability effects of the temperature changes are the result of a physiological change with no detectable morphological correlate.

When the temperature of the perfusate is raised from 25°C to 35°C (Figure 3), there is a very rapid and important activation of the path of transport of $^{22}$Na and $[^3H]$sucrose. These tracers are both presumed to cross capillary endothelial barriers through the intercellular junctions, which would account for their similar behavior. The temperature effect on these tracers considerably exceeds that which could be accounted for by a mere decrease in water viscosity, indicating that more complex phenomena than changes in passive diffusion through water-filled pores are taking place. The nature of the change is not evident, but it likely involves a change in the molecular mechanisms forming linkages between the endothelial cells.

The passage of $^{125}$I-albumin is also concomitantly increased, although the temperature effect is delayed with respect to that observed for $^{22}$Na and $[^3H]$sucrose. The delay in the change in albumin permeability and its increasingly steep slope at the end of the observation period appear to indicate quite a different mechanism from that available for sodium and sucrose. The morphological substratum for the passage of albumin across the capillary wall remains controversial. With respect to the hypothesis of vesicular transport, we observed a diminution of vesicles at 35°C in comparison with 25°C. However, vesicular activation could be induced with the temperature rise. This would require energy, and our metabolic studies indeed demonstrate a considerable increment of ATP yield at 35°C. If vesicular transport were a major component of the albumin pathway across the capillary endothelium, one would expect inhibition at lower temperatures, when the respiration of the tissue is considerably reduced. We have found the opposite. It is more plausible, therefore, that at 35°C, albumin, sodium, and sucrose share to a great extent the same interendothelial path, functionally enlarged.

In the same series of experiments, the passage of oxygen, $[^3H]$water, and $[^14]$C-antipyrine was not influenced by the rise of temperature of the perfusate from 25°C to 35°C. This set of substances, with permeability coefficients higher than expected from their molecular size, can be considered to use a preferential area of transport represented by the entire membrane surface on the endothelium, with water and lipids as integral parts of the membrane. Thus, the transcellular water and lipid pathways, whether distinct or shared, apparently operate at full capacity at 25°C. This likely obscures any temperature effect that may occur for these substances at the intercellular junctions, because the surface of these is trivial compared with the cell surface.

When the temperature of the perfusate is decreased from 25°C to 5°C (Figure 4), the passage of $[^14]$C-antipyrine and $[^3H]$water, but not that of oxygen, is reduced. The effect is seen within 10 minutes. $V_A/S$ values for $[^14]$C-antipyrine and $[^3H]$water reach an equilibrium at a lower level; the decrease for $[^3H]$water is of the order of that expected from the increase in viscosity of water with the decrease in temperature, whereas that for $[^14]$C-antipyrine is significantly larger. The disproportionately large change in the behavior of antipyrine has been observed in other capillary systems. In the isolated cat hind leg, antipyrine induces no osmotic transient at 36°C, whereas at 8°C, a significant transient is observed. The behavior of tracer antipyrine in dog lungs parallels this. Although the tracer is flow limited at the endothelial cell surface and barrier limited at the epithelial cell surface at 37°C, it becomes barrier
limited at both surfaces when the temperature is reduced to 8°C.7 The change in behavior is particularly striking when outflow dilution curves for [14C]antipyrine are compared with those of [3H]water. At 38°C, these are quite close, whereas at 8°C they exhibit a major divergence, the bulk of the [14C]antipyrine emerging much earlier.7 The present data, which indicate a more substantial change in the [14C]antipyrine than [3H]water exchange on cooling to 5°C, correspond to the above findings, and all can be interpreted to indicate that changes in membrane lipids at the lower temperature underlie the experimental findings. The lack of a commonality of behavior with oxygen suggests that, for this, a predominantly different pathway is used, likely a lipid pathway. The suggestion is reinforced by the much higher lipid solubility of oxygen. The distribution ratio between cottonseed oil and water at 37°C is 5:1.21 One might infer that oxygen likely penetrates the whole endothelial surface. Probes such as methyl antipyrine or iodoantipyrine, which are more lipid soluble than antipyrine, may behave more like oxygen during rapid cooling.

For sucrose and sodium, the intercellular path of transport is remarkably cold insensitive. In striking contrast is the increased permeability to 125I-albumin. The discrepancy between the sodium and sucrose lack of response to low temperature and the stimulation of albumin transport indicates that the pathway of albumin transport is not entirely through the interendothelial junction. Cryogenic injury in cerebral and peripheral nerve capillaries induces a leakage of albumin from the vascular to the extravascular space, by stimulation of endocytosis and focal opening of endothelial tight junctions.22 In our experiments, perfusion of the capillaries with a cold medium increased the permeability to albumin without visible cellular lesions, junctional openings, or interstitial edema.

Reversibility of the Temperature Effects

Temperature and osmolality changes have been used to break capillary barriers in the past to either explore mechanisms of molecular transport or gain temporary access to tissues for otherwise impermeant therapeutic agents. The latter feat requires that the breakdown of the microvascular barrier be reversible. We have explored with the rete capillaries the reversibility of the functional alterations induced by rapid warming of the rete to 35°C, followed by rapid cooling of the perfusate to the baseline temperature of 25°C (Figure 5). At 35°C, the V0/Ao×1/S values for 125I-albumin, [14C]sucrose, and 22Na were found to rise continuously and then on abrupt return to 25°C were rapidly stabilized but did not decrease to expected levels. The functional lesions created by warming were thus arrested in their progression but not reversed. A more complete reversal was achieved in perfusions in which the temperature was changed in a more gradual manner (Figure 7). The permeability effect associated with the gradual temperature change was smaller; it was evidently associated with a greater degree of preservation of the integrity of the 125I-albumin pathway of transport.

When the temperature of the perfusate was brought up rapidly from 5°C to 25°C (Figure 6), plateau values were obtained at 5°C for all the substances tested, contrary to the non–steady-state changes observed for some tracers at 35°C. The sudden 20°C jump in temperature had little impact on the V0/Ao ratios. These rose slightly for [14C]sucrose, 22Na, [14C]antipyrine, and [3H]water in a fashion compatible with a low activation energy for the diffusion process.

Conclusions

The findings show characteristic changes in permeability when the temperature is raised or lowered from the ambient temperature to which the eels are adapted. Raising the temperature results in an increased permeation of sucrose and sodium, which are characteristic paracellular labels. The increment in permeability is smaller and reversible with stepwise change, but larger and irreversible with sudden change. Lowering the temperature, in contrast, results in a reversible decrease in the permeability of the capillaries to labeled water and antipyrine, more or less proportional to the decrease in the viscosity of water, but no change in the permeability for sucrose and sodium. These are the characteristic changes. Increased permeation of albumin occurs in either direction, but only with rapid change in temperature. The lack of parallelism with changes in the permeation of sodium and sucrose with drop in temperature suggests some separation in pathways over that range.

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References


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