Effect of Reduced Energy Metabolism and Reperfusion on the Permeability and Morphology of the Capillaries of an Isolated Rete Mirabile

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

The effects of reduction in energy metabolism were explored in the eel rete mirabile, an organ composed predominantly of capillaries. In vitro experiments showed that glycolysis is the major pathway of energy production in this capillary tissue, and that iodoacetate, KCN, and low PO$_2$ in combination markedly reduce its ATP generation. When in situ energy generation was inhibited by this combination during countercurrent perfusion of the arterial and venous capillaries of the rete, an approximate doubling of the intercapillary barrier permeability for human [¹¹I]albumin, [¹⁴C]sucrose, and [¹⁴Na]Na was found. Structural damage was evident, but the intercellular junctions remained intact. The effect of cessation of flow for 30 minutes, followed by reperfusion, was then explored. Stasis alone altered the structure, chiefly of the venous capillary endothelium, but not the permeability of the intercapillary barrier. Stasis with a hypoxic medium containing the inhibitors of energy generation, followed by reperfusion with oxygenated control medium, resulted in a progressive breakdown of the intercapillary barrier, with a threefold to fourfold increase in solute (labeled albumin, sucrose, and sodium) permeability, evolving during early reperfusion, but no change for labeled water permeability. Morphologically, the endothelial cells, especially those in venous capillaries, showed substantial damage; they appeared vacuolated, their cytoplasm was extracted, and cytoplasmic and membrane debris were found in the lumen; intercellular junctions remained intact. Local pericyte detachment with interstitial edema also appeared. Thus, stasis and reperfusion amplified the effects of reduction in energy generation and hypoxia on both permeability and morphological change.

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gen tension in blood or perfusate. Very few investigators have used metabolic inhibitors of energy production, alone or in combination with low oxygen partial pressure. In hypoxia studies, flow has been generally normal or higher than normal. In other types of experiments, hypoxia has been induced by ischemia of various degrees of intensity and duration, and permeability has necessarily been assessed during recirculation through the ischemic tissue, when flow has been varied from small reflow to overperfusion.

In the present study, we have used the rete mirabile of the eel swimbladder to investigate the effects of changes of oxygen supply, cell energy production, and organ flow stoppage on capillary permeability. In a preliminary series of in vitro experiments, we have measured the effects of hypoxia and metabolic inhibitors on substrate utilization and the equivalent yield of ATP by rete capillary endothelial cells. We have then used hypoxia and the metabolic inhibitors in the perfused rete to induce a maximal reduction of energy production under conditions of either continuous flow or arrested flow followed by reperfusion, and have characterized the rete permeability to a set of multiple water soluble tracers. In every set of experiments, we have searched for morphological correlates with changes in capillary function.

Materials and Methods

The rete mirabile was isolated from the swimbladder of the freshwater eel (Anguilla anguilla). The arterial and venous inputs were catheterized at opposite poles, and the arterial and venous capillaries of the rete were simultaneously countercurrent perfused with identical flows of Krebs-Ringer bicarbonate buffer (pH 7.4), containing glucose (5 mM) and purified bovine albumin (4 g/100 ml) and equilibrated with a gas mixture of 95% O2-5% CO2.

In Vitro Metabolic Studies

The rete, cleared of its blood, was removed from the swimbladder and dissected into small clusters of filaments. Approximately 100 mg wet weight of capillary tissue were incubated for two hours at 37°C in vials containing 2 ml of the same buffer.* The in vitro effects of metabolic inhibitors and of lack of oxygen (produced by equilibration with 95% N2-5% CO2) were assessed in paired experiments where control and experimental metabolic vials received equivalent amounts of capillaries from the same eel. The metabolic inhibitors used were sodium iodoacetate, which reacts with thiol groups in many enzymes and blocks glyceraldehyde phosphate dehydrogenase, and potassium cyanide, which suppresses electron flow between the cytochrome oxidase complex and oxygen in the mitochondria. The medium in the closed vial was acidified at the end of the incubation, and the evolved gaseous carbon dioxide was trapped in a scoop filled with phenethylamine. Glucose and lactate concentrations in the medium were measured by specific enzymatic procedures. Glucose conversion into carbon dioxide was calculated by dividing the activity absorbed to phenethylamine by the specific activity of [14C(U)] glucose in the medium at the start of the incubation.

Permeability Studies

Perfusions were carried out at room temperature (22–25°C), with a constant flow in each direction of 0.5 ml/min, at a constant pressure head of 45 cm H2O. The weight of the rete averaged 200 mg. The medium at the arterial input contained various combinations of the following labeled materials: human [125I]albumin (Frost: more than 95% of the labeled iodine was precipitated with 10% trichloroacetic acid), [14C(U)]sucrose or [14C(U)]urea (both from New England Nuclear, Boston, Massachusetts; 1–5 μCi/mm mol and 2–10 μCi/mm mol, respectively), [3H] water (New England Nuclear; biological quality, 0.1 mCi/g), and 32Na (New England Nuclear; as sodium chloride, 99.9% radionuclidic purity). The medium at the venous input did not contain radioactive tracers.

In a first set of experiments, the effects of metabolic inhibitors and reduced oxygen tension were explored, while the rete was continuously perfused. Perfusion for 1 hour with control medium equilibrated with 95% O2-5% CO2 was followed by a 1-hour perfusion with the same medium where glucose was removed and the metabolic inhibitors iodoacetate 1 mM or potassium cyanide 1 mM were added at both arterial and venous inputs either alone or in combination; in the latter instance, the medium was desaturated to a PO2 of 10±3 mm Hg, by equilibration with a 95% N2-5% CO2 gas mixture.

In a second set of experiments, perfusion of the rete was stopped and then resumed. The protocol was designed to simulate the effects of ischemia, followed by reperfusion. After 30 minutes of perfusion with the control oxygenated medium, the rete was perfused for another 10 minutes with either the same control medium or a medium without glucose, with iodoacetate 1 mM and potassium cyanide 1 mM, and a PO2 of 10 mm Hg. The arterial and venous inputs were then clamped for 30 minutes. Following this, the rete was reperfused with the control oxygenated medium for an additional hour.

Samples were collected simultaneously from the arterial and venous outputs for 5 to 10 minute periods of time throughout the perfusions. There were no significant differences in flow during control and experimental perfusion. The radioactivity of [125I]albumin was measured in a Packard gamma scintillation spectrometer (Packard Instrument Co., Downers Grove, Illinois) on a 10% trichloroacetic acid precipitate washed with excess potassium iodide. The radioactivity of the other tracers, in the protein-free supernatant, was measured with a gamma scintillation spectrometer or with a Packard liquid scintillation spectrometer. All values...
CONTINUOUS PERFUSION: CONTROL

![Graph showing V/A ratio over time](image)

FIGURE 1. Lack of change in the V/A ratio for each permeability probe with time. Changes in permeability-surface area values are reflected by changes in this ratio. Note that a logarithmic ordinate scale has been used. Mean values are given for the data with lines for standard deviations. Number of experiments, 6. Vo and Ao are the simultaneous tracer concentrations at the venous and arterial outputs, respectively. The codes for the curves are 1 for [125I]albumin, 2 for [14C]sucrose, 3 for 22Na, and 4 for [3H]water.

were corrected for background, quenching, and cross-over.

The diffusion capacity (PS) of the rete was calculated when steady state concentrations were achieved, from the equation $PS = (P \times V_o/A_o)$, where $P$ is the permeability coefficient, $S$ is the surface area, $F$ is the flow, and $V_o$ and $A_o$ are the simultaneous concentrations at the venous and arterial outputs. The surface available for capillary exchange is equivalent to $1 \text{ cm}^2/\text{mg}$ wet wt, under normal conditions of hydration. In this study, various degrees of cellular and interstitial space swelling were induced by reduction in the energy metabolism of the rete. Thus, the diffusion capacity (PS) of the rete, rather than its coefficient of permeability ($P$), was followed as a function of time.

**Morphological Studies**

Fragments of the rete were prepared at the end of perfusions, as described previously. The tissues were fixed by immersion in glutaraldehyde fixative solution, postfixixed with osmium tetroxide, and embedded in Epon. Each eel has two symmetric retia. The rete that was not used in the experiments was removed during the initial preparative procedures and similarly processed for control morphological examinations. Thin sections were cut and, after staining, were examined with an electron microscope (model 410, Philips Electronic Instru-

### Table 1. Effect of Inhibitors of Energy Production on Glucose Utilization by Isolated Rete Capillaries

<table>
<thead>
<tr>
<th>Group</th>
<th>Perfusion</th>
<th>Glucose uptake (μmol/g/hr)</th>
<th>Lactate production (μmol/g/hr)</th>
<th>Glucose conversion to CO2 (% control value)</th>
<th>ATP yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control medium</td>
<td>18.2±2.0</td>
<td>36.6±4.5</td>
<td>0.545±0.047</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>+ KCN (1 mM)</td>
<td>135±20</td>
<td>135±16</td>
<td>27±3</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>95% N2-5% CO2</td>
<td>132±15</td>
<td>125±9</td>
<td>14±7</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>+ Iodoacetate (1 mM)</td>
<td>23±2</td>
<td>0</td>
<td>29±3</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>+ KCN (1 mM)</td>
<td>30±8</td>
<td>33±5</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>Control medium</td>
<td>18.8±2.0</td>
<td>36.3±4.5</td>
<td>0.534±0.047</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>+ KCN (1 mM)</td>
<td>135±20</td>
<td>135±16</td>
<td>27±3</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>95% N2-5% CO2</td>
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<td>11</td>
</tr>
<tr>
<td></td>
<td>+ KCN (1 mM)</td>
<td>30±8</td>
<td>33±5</td>
<td>0</td>
<td>21</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SEM. Number of experiments: 32 for control and eight for each group of metabolic inhibitors. The control medium is a Krebs-Ringer bicarbonate buffer with purified bovine albumin 4 g/100 ml and glucose 5 mM. Incubations were carried out for 2 hours at 37°C. All values for metabolic inhibitors are significantly different from control values: $p<0.01$ (Student’s t test for paired experiments).

### Table 2. Effect of Inhibitors of Energy Production on Rete Diffusion Capacity to Water Soluble Tracers

<table>
<thead>
<tr>
<th></th>
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<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>4.64±2.06</td>
<td></td>
<td>3.09±1.00</td>
<td>98.8±9.7</td>
<td>1,805±184</td>
</tr>
<tr>
<td>Group 2</td>
<td>3.49±0.88</td>
<td>14.9±2.5</td>
<td>5.04±1.09*</td>
<td>98.6±18.4</td>
<td>1,832±260</td>
</tr>
<tr>
<td>Group 3</td>
<td>4.55±1.49</td>
<td>14.0±2.3</td>
<td>10.19±3.01†</td>
<td>43.2±7.6</td>
<td>2,294±328</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SEM. Each tracer was tested in eight paired experiments in groups 1 and 2 except for sucrose and urea in group 2, which were tested in four paired experiments. Each tracer was tested in six paired experiments in group 3. $PS_a$, permeability-surface area product at 25°C. The control medium is a Krebs-Ringer bicarbonate buffer with purified bovine albumin (4 g/100 ml) and glucose (5 mM).

*Value significantly different from control value: $p<0.01$ (Student’s t test for paired experiments).

†Value significantly different from control value: $p<0.05$. 

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CONTINUOUS PERFUSION

CONTROL  DECREASED ATP GENERATION

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>( V_o / A_o )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>90</td>
<td>1000</td>
</tr>
<tr>
<td>120</td>
<td>10000</td>
</tr>
</tbody>
</table>

FIGURE 2. Time course of \( V_o / A_o \) after a steady arterial infusion of tracers beginning at time zero, in a countercurrent perfused rete. Perfusion was initially with an oxygenated control medium. At the end of the first hour, the perfusion was switched to a deoxygenated control medium lacking glucose and containing 1 mM KCN and 1 mM iodoacetate. \( V_o \) and \( A_o \) are simultaneous tracer concentrations at venous and arterial outputs, respectively. Mean values are given for the data, with lines for standard deviations. Number of experiments, 6. *Values significantly higher than averaged control values (p<0.05). 1=\([125I]\)albumin, 2=\([14C]\)sucrose, 3=\(22Na\), and 4=\([3H]\)water.

Permeability Studies

Control experiments, shown in Figure 1, demonstrate that the ratio \( V_o / A_o \), which reflects the permeability-surface area product in the presence of constant flow, remains steady for each tracer during a baseline 2-hour perfusion. These control studies indicate that, in the absence of experimental manipulation, a steady baseline can be expected for this period.

The results of the effects of inhibitors of energy production and lack of glucose in the perfusate on the permeability-surface area values of the rete capillaries are shown in Table 2. There were no detectable effects of potassium cyanide on the tracer concentrations and perfusate flows at the arterial and venous outputs. Consequently, the diffusion capacities of the rete for \([125I]\)albumin, \([14C]\)urea, and tritiated water were similar during control and experimental conditions. Addition of iodoacetate to the perfusate increased the permeability-surface area value for albumin by approximately 50% (p<0.01); the effect was established and steady from the 10th minute onward. The permeability-surface product values for \([14C]\)sucrose, \([14C]\)urea, and tritiated water were not modified by the iodoacetate. When the rete was perfused with a mixture of potassium cyanide and iodoacetate at low \( P_{O_2} \) mM is added to the medium or when the oxygen tension is reduced, glucose uptake and lactate output are stimulated by approximately 30% above control values, while oxidation of glucose to \( CO_2 \) is reduced by an average of 80%. The combined effect is that ATP continues to be generated at a normal rate. Iodoacetate, at a concentration of 1 mM, when used alone or in combination with cyanide and low oxygen tension, suppresses or strongly reduces both the anaerobic and aerobic catabolism of glucose, so the total equivalent rate of yield of ATP drops to 10–20% of the control value.
TABLE 3. Effects of Stagnant Hypoxia and Reperfusion on Rete Diffusion Capacity for Water Soluble Tracers

<table>
<thead>
<tr>
<th>Perfusion</th>
<th>$[^{125}]$I Albumin</th>
<th>$[^{14}]$C Sucrose</th>
<th>$^{23}$Na</th>
<th>$[^{3}H]$Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion with control medium</td>
<td>2.43±1.40</td>
<td>15.5±5.7</td>
<td>57.5±13.7</td>
<td>2,643±254</td>
</tr>
<tr>
<td>Stasis for 30 minutes + reperfusion with</td>
<td>3.59±1.64</td>
<td>26.3±10.8</td>
<td>49.2±6.7</td>
<td>2,673±278</td>
</tr>
<tr>
<td>control medium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfusion with control medium</td>
<td>2.26±0.56</td>
<td>16.0±2.2</td>
<td>46.3±6.2</td>
<td>1,817±211</td>
</tr>
<tr>
<td>Stasis for 30 minutes with metabolic</td>
<td>6.27±0.40*</td>
<td>70.2±14.3†</td>
<td>198±44†</td>
<td>2,264±529</td>
</tr>
<tr>
<td>inhibitors + reperfusion with control medium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean±SEM. Each tracer was tested in six paired experiments. PS$_p$, permeability-surface area product at 25°C. The control medium is a Krebs-Ringer bicarbonate buffer with purified bovine albumin (4 g/100 ml) and glucose (5 mM). The medium with metabolic inhibitors has no glucose, contains KCN (1 mM) and iodoacetate (1 mM) and is equilibrated with 95% N$_2$:5% CO$_2$.

*Value significantly different from control value: p<0.01 (Student's t test for paired experiments).
†Value significantly different from control value: p<0.05.

(Figure 2), its diffusion capacity for $[^{125}]$I albumin, $[^{14}]$C sucrose, and $^{23}$Na increased by approximately 100% (p<0.05), while remaining similar for tritiated water. For the group of tracers exhibiting the increased permeability, the change became perceptible within 10 minutes and progressed until plateau values were reached after 30 minutes.

Figure 3 shows the results of the combined effects of stasis and reperfusion on the permeability-surface area values of the rete for water soluble tracers. In the left-hand panel, the results pertain to experiments where the flow of an oxygenated energy-rich medium was discontinued and then resumed. The time course of the tracer output concentrations
from arterial and venous capillaries was not altered by the procedure; the illustrated $V_o/A_o$ value, which reflects the permeability-surface area values (Table 3), were steady for each tracer following reperfusion. In the right-hand panel, the results are displayed for the experiments in which both flow and energy generation were arrested: the inhibitors iodoacetate and KCN were introduced in a low Po medium before flow stoppage, and normal conditions of flow and energy were then restored by reperfusion with an oxygenated energy-rich medium. The $V_o/A_o$ concentration ratio values for $[^{125}]$albumin, $[^{14}]$sucrose, and $^{22}Na$ begin to rise after 5 to 10 minutes following resumption of the perfusion, and attained plateau levels at the 30th minute. The corresponding arterial output concentrations decreased and then leveled out, pari passu with the venous output concentrations. There was no significant change with time in the $V_o/A_o$ ratio for tritiated water. The permeability-surface area values, calculated from averaged steady-state tracer concentrations during the initial 30 minutes of control perfusion and the last 30 minutes of reperfusion, showed a threefold to fourfold increase for $[^{125}]$albumin, $[^{14}]$sucrose, and $^{22}Na$, while for tritiated water they were not modified (Table 3).

**Morphological Studies**

Normal rete capillaries are shown in Figures 4 and 5. The endothelial cells in the arterial capillary were high, and their cytoplasm contained a well-developed vesicular-tubular system. The endothelial cells in the venous capillaries were thin and fenestrated; their fenestrae were closed by a diaphragm. Mitochondria were present. The intercellular junctions were tight, and the basal laminae, continuous. Pericytes were abundant and lined the outer aspects of the endothelial cells. The intercapillary extracellular space was small and filled with bundles of collagen fibers.

When the rete was perfused with potassium cyanide or iodoacetate and no glucose, the mitochondria in the endothelial cells and pericytes became swollen and vacuolated, and their cristae were reduced or even absent (Figure 6). However, the intercellular junctions were tight and the venous capillary fenestrations remained closed by their diaphragms. In the series of experiments in which the perfusate contained the metabolic inhibitors iodoacetate and KCN, and no glucose, and input oxygen tension was kept low but flow was maintained, consistent and widespread lesions were
observed in both arterial and venous endothelia (Figure 7). The luminal membrane of the endothelial cells was swollen and formed large vacuoles that protruded into the lumen. These large vacuoles were outlined by layers of concentric membranes, displaying myelin-like profiles; some vacuoles were eventually released into the lumen. The mitochondria were vacuolated. Intercellular junctions and fenestrae, however, were intact.

Figures 8 and 9 illustrate the lesions that were seen when flow was arrested for 30 minutes and then resumed for 1 hour. The lesions were similar whether or not the medium stagnating in the rete was enriched with metabolic inhibitors and deprived of glucose and most of its oxygen. The endothelial cells of the venous capillaries were vacuolated and their cytosol was extracted; both cytoplasmic and membrane debris were found in the lumen. The endothelial cells of the arterial capillaries were better preserved. Without metabolic inhibitors, the interstitial space remained intact (Figure 8). With metabolic inhibitors, patches of edema appeared in the interstitial space at sites where adjacent pericytes were detached from their endothelial cells (Figure 9).

Discussion

Studies of respiration and energy metabolism of blood capillary endothelial cells are very scarce. One of us has reported that glucose is the major energy substrate of the capillaries and that the activity of the glycolytic pathway is very high. In the present study, we confirm and complete these data with incubation media of the same composition as those that we used to perfuse the rete during permeability experiments. The results apply to the mixed population of the cells: arterial and venous endothelial cells, and pericytes. The relative contributions of each cell type to the overall metabolic measurements is not known. In control media, two thirds of ATP regeneration was through glycolysis and one third was through mitochondrial respiration. Cyanide and low oxygen tension reduced respiration, as expected. However, glycolysis was stimulated, and as a result, the ATP yield remained comparable to that obtained with the control oxygenated medium. Iodoacetate (1 mM) significantly reduced the flow of glucose through glycolysis and the Krebs cycle. No additional decrease in the rate of ATP yield could be elicited by the addition of
cyanide and low oxygen tension. Under the conditions of maximally inhibited glucose utilization, the yield of ATP averaged 6 to 12 μmol/g/hr, compared with 57 μmol/g/hr in the control medium. As previously shown,4 the rete capillaries contain glycogen at a concentration of 0.4 g/100 g wet wt; this represents 22 μmol of glucose equivalent, per gram of wet weight, which would be sufficient to sustain ATP regeneration for more than 1 hour in the absence of extracellular glucose. In considering our experimental design, we therefore concluded that the most efficient way to assess the effects of energy deprivation on the permeability of the rete was to use a perfusion medium deprived of glucose and most of its oxygen and enriched with metabolic inhibitors.

The effects of hypoxia and ischemia on capillary permeability remain controversial. While many reports have indicated a lack of effect of hypoxia on the capillary permeability of isolated organs,12-16 there is also a wealth of data demonstrating the opposite.2,3,17-21 In our first series of permeability experiments, the intent was to induce a decrease in cell oxidative metabolism without a loss of flow. We observed that the permeability response varied with the metabolic inhibitors added to perfusate and with the tracer substances used (Table 2). The lack of effect of potassium cyanide on the diffusion capacity of the rete to albumin, urea, and water could be ascribed to an apparently adequate availability of ATP through the compensatory effects of glycogenolysis (Table 1). In conditions of more stringent inhibition of energy production such as induced by iodoacetate alone or in combination with KCN and decreased oxygen, significant increases of permeability-surface values for albumin, sucrose, and sodium were observed, indicating a possible involvement of ATP in maintaining endothelial cell permeability. The process regulating the permeability, if any, does not, however, seem to make a large demand on the ATP supply. The diffusion capacity of the rete to water was unchanged, as would be expected if water were to diffuse in large part through the general surface of the endothelium rather than through selective pathways used by the other tracers. Electron microscopic examination demon-
FIGURE 8. Electron photomicrograph of capillary tissues at the end of perfusion experiments in which flow was arrested for 30 minutes. Stagnant control medium. The endothelial cells of the venous capillaries (VC) show signs of degeneration; vacuoles protrude into the lumen of the capillaries. The interstitial space remains compact. AC, arterial capillaries; J, intercellular junctions; P, pericytes; f, fenestration. Magnification, x20,000.

strated swelling of the endothelial cells but did not reveal disruption or widening of the intercellular junctions (Figure 7); major alterations were detected in mitochondria, reflecting modifications of the energy-generating machinery, and in endothelial cell membranes that formed vacuoles with myelin-like structures, some of which were shed intraluminally. Endothelial swelling has also been described in human muscle cell capillaries as a sequel to prolonged ischemia.

The biochemical and physiological factors underlying capillary permeability changes during anoxia or ischemia or during recirculation of ischemic areas are only partially known, and their relative importance is conjectural. One can speculate that anoxia increases permeability by modifying the contractility of the cytoskeleton, the shape of the endothelial cell, and the tightness of the intercellular junction, through restructure or loss of actin filaments brought about by low ATP generation. There is evidence that oxygen-derived free radicals produced in the mitochondrial electron transport chain or along the pathways for adenine nucleotide degradation are toxic and are capable of directly damaging the cell membrane by peroxidation of the unsaturated fatty acids of its phospholipid component. The localization of xanthine dehydrogenase and the important content and high turnover of intracellular adenine nucleotides in the microvascular endothelium of the heart, have been thought to provide a basis for understanding the induction of lesions by ischemia followed by reperfusion.

Our second series of experiments was designed to test the effects of hypoxia and decreased energy generation during flow stoppage followed by reperfusion on the diffusion capacity of the rete. Stasis and reperfusion induced extensive damage of the venous endothelial cells while the arterial endothelial cells were merely injured (Figures 8 and 9). Striking differences in permeability-surface area values were observed depending on the composition of the medium bathing the rete capillaries during the 30-minute period of stasis (Table 3). With the medium initially well oxygenated and supplemented with glucose and no inhibitors, permeability-surface area values for all tracers were similar...
FIGURE 9. Electron photomicrograph of capillary tissues at the end of perfusion experiments in which flow was arrested for 30 minutes. Stagnant medium with metabolic poisons. In addition to the damage described above, the interstitial space (IS) is edematous, and the pericytes (P) are separated from their adjacent endothelial cells. The intercellular junctions (J) appear normal. AC, arterial capillaries; VC, venous capillaries; f, fenestration. Magnification, ×15,000.

during control and recirculation conditions. The concurrent important evolution of structural lesions in the venous capillaries indicates that, as previously suspected, the arterial endothelial cell sheet is the rate limiting factor in the rete barrier permeability. With a medium containing inhibitors of energy metabolism, no glucose, and little oxygen during the period of stasis, permeability-surface area values for albumin, sucrose, and sodium during reperfusion were initially unchanged and then increased progressively to reach levels three to four times higher than during the control period. Concurrent morphological examination detected the presence of significant patchy interstitial edema, together with separation of some of the pericytes from adjacent endothelial cells. There may be a role for the pericytes in this regulation of capillary permeability. Their cytoplasmic projections contain bundles of filamentous actin and extend to the edges of intercellular junctions and fenestrations of the endothelium. The speculation is that the width of these pathways may vary with the degree of tension on the actin filaments.\textsuperscript{30,31} The initial lack of change in permeability and subsequent evolution during reperfusion support the idea that a major part of the alterations observed is due to the events put into motion with and occurring during the reperfusion. One possible explanation is that free radical-mediated oxidative injury of the endothelial cells (and, potentially, pericytes) occurs during reoxygenation of the hypoxic rete and produces the damage. This remains to be tested.

It is of interest that in our series of experiments, whenever solute permeability is increased by hypoxia, labeled water permeation has not been modified. During hyperosmolar infusions, we also observed an increase in solute permeability in the absence of change in water permeability.\textsuperscript{32} We have also reported that the permeability coefficient for water in the rete capillary is higher than expected from the log-log linear relation between permeability and diffusion coefficients for hydrophilic molecules and have inferred that the surface available for tracer water transfer is larger than that for solutes. Thus, their respective pathways for diffusion would not be expected to be modified similarly by experimental maneuvers.
The observation that solute permeability is increased by hypoxia while the interendothelial junctions remain intact is intriguing. For large solutes, such as albumin, we have shown in the rete,\(^3\) and others have confirmed in the murine myocardium,\(^4\) that there is entry into plasmalemmal vesicles; transport across the endothelium could take place through these vesicles. For sodium and sucrose, however, the commonly held view is that the major path for diffusion is the interendothelial junction. This space is likely to be filled by a matrix of fibrous molecules, with associated charge effects.\(^5\) Our observation that ionic transport is reduced in the rete capillaries by comparison with neutral solutes with similar diffusion coefficients\(^6\) is consonant with this idea. Alterations of the matrix density or charge during hypoxia could potentially account for changes in small solute permeability without visible widening of interendothelial junctions.

In conclusion, we have shown that the microvascular endothelial cells of the rete have an important energy metabolism mostly accounted for by glycolysis. When ATP generation is inhibited, structural injury and selective permeability increases are observed. Stasis with an oxygenated energy-rich medium damages the venous capillary endothelium, but the lesions of the arterial capillary endothelium are not so severe as to alter its permeability. On the other hand, stasis, together with hypoxia and inhibition of energy generation, results in a progressive breakdown of the capillary barrier to the passage of albumin, sucrose, and sodium, which evolves during the reperfusion, while the diffusion capacity for water remains unchanged.

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