Passage of Ions and Dextran Molecules across the Rete Mirabile of the Eel

The Effects of Charge

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SUMMARY. The countercurrent-perfused rete mirabile of the eel is a preparation in which capillary permeability values can be determined accurately in broadscale fashion. To provide insight into charge effects on transendothelial passage, the permeability values of small inorganic cations (labeled sodium and rubidium, and stable potassium) and anions (labeled chloride, iodide, sulfate, and ferrocyanide) were compared to those expected for neutral solutes with approximately matched diffusion coefficients, and that of a neutral dextran fraction was compared to that of a negatively charged dextran sulfate with a similar diffusion coefficient. In the small ion experiments, the labeled iodide values were unexpectedly high, apparently due to the contamination of the labeled iodide solution with I^-. The permeabilities of the rest of the ions clustered at a level about 0.5 of the values which would have been expected for neutral solutes with similar diffusion coefficients. The decrement was interpreted to reflect the presence, of both positive and negative charges along the transendothelial pathway, which effectively decrease the dimensions of the limiting part of the pathway for the charged microions relative to that accessible to comparable nonelectrolytes. The larger negatively charged dextran sulfate was also reduced in its passage, in comparison with its matching neutral dextran; this was taken to indicate the presence of a larger scale average net negative charge along its pathway. The data indicate the presence of a staggering of positive and negative charges along the transendothelial pathway accessible to the microions, and a net negative charge in the more restricted part of the pathway available to the dextrans. (Circ Res 56: 74-83, 1985)

BLOOD capillary permeability to small ions has long been known to be a minute fraction of that for water, irrespective of the methods used and tissues investigated. The data have been interpreted as indicating the presence of differing paths for the passage of ions and water across the endothelial wall, the mechanism for the transfer of each usually being thought to be passive [except in certain special areas, such as the tight capillaries of the central nervous system and the eye, where evidence for some active transport of sodium and potassium ions has been presented (Bradbury and Stulcova, 1970; Eisenberg and Suddith, 1979; Betz and Goldstein, 1980)]. It has been expected that appropriately designed studies with ions and charged molecules would provide information concerning the effects of local fixed charges, if these are present in the regions where inorganic ions and other charged materials traverse the capillary wall.

Few capillary beds have been thoroughly examined from this point of view. The glomerular apparatus of the kidney constitutes the one exception. Studies of glomerular filtration of moderately large molecules with different charges and comparable molecular size have provided evidence for a charge effect in this structure. These studies indicate that, although inorganic ions appear to pass this filter freely, the glomerular capillary barrier is negatively charged in the regions where larger molecules traverse the barrier, and that these fixed negative charges exert an effect on the passage of larger charged molecules, varying with the magnitude and sign of the net charge on the probing molecule (Brenner et al., 1978; Venkatachalam and Rennke, 1978; Deen et al., 1979). The barrier in the glomerulus is composed of endothelial cells containing large operculum-free fenestrae, a thick and dense basement membrane, and epithelial foot processes. The locus of the dominant charge effect is not completely clear, but appears to be at the level of the basement membrane. As a result of the charge, in a homologous series of dextrans, for molecules with similar free diffusion coefficients, the passage of the cationic variant (DEAE-dextran) across the glomerulus is facilitated and that of anionic variant (dextran sulfate) is impeded, in relation to the uncharged or neutral species. Albumin, with a net negative charge, is also somewhat impeded in relation to what would otherwise be expected. The finding of proteinuria (and more especially albuminuria) in glomerular disease processes, which occurs in association with demonstrable charge depletion of the barrier (Myers...
et al., 1982), attests to the biological significance of the glomerular charge effect for macromolecules. The microvasculature at other sites has also been surveyed, but the available data have to do chiefly with the passage from blood to lymph of either proteins or a series of homologous probes like the neutral and charged dextrans. Few data are available, bearing on the permeabilities of capillary endothelia to small ions relative to the values expected for small neutral molecules with similar diffusion coefficients. Yipintsoi et al. (1970) indicated that they encountered a tendency in the heart for the ratio of the permeability surface products for sodium or potassium to the permeability surface products for sucrose or glucose to be less than ratios for their free diffusion coefficients in water, but did not provide any details. Ziegler and Goresky (1971b) found the ratio of the permeability surface products for rubidium relative to that for sucrose in the heart to be of the order of 50% of that expected on the basis of their free diffusion coefficients. These data were thought to indicate the presence of a charge along the permeation pathway through the capillary endothelium, retarding cations. On the other hand, Duran and Yudilevich (1978) found a suggestion of decreased permeability for sodium with respect to glucose, relative to their diffusion coefficients, in only some of their experiments. No significant deviation was found, over the entire group. If a fraction of the glucose traverses the endothelial cells, the differences between the cation:sucrose and the cation:glucose data may be understood; at this stage, however, this must be regarded as only hypothesis. Thus, the data indicate that there may be a larger degree of resistance to the transcapillary passage of cations relative to small neutral molecules, but no larger scale or definitive survey has yet been carried out; correspondingly, data for anions are lacking. It therefore appeared to us that the area as a whole was in need of more systematic exploration.

In the past we utilized the rete mirabile of the eel swimbladder, a countercurrent capillary organ, to gain insight into the general properties of continuous capillary beds (Rasio et al., 1977, 1981; Rasio and Goresky, 1979). This preparation, when countercurrent perfused under steady state conditions of concentration and pressure, yields through simple calculations unequivocal values for permeability surface products. We have previously measured these for various hydrophilic molecules, water, and oxygen (Rasio et al., 1977; Rasio and Goresky, 1979), and these values provide, when related to diffusion coefficients, a baseline picture of capillary function in this preparation. With this past characterization, the countercurrent-perfused rete is ideal for a wide-scale exploration of the effects of charge on transcapillary exchange, across the spectrum ranging from the smaller inorganic ions to larger charged molecules. In the present study, we therefore set out to characterize the permeability surface products for a set of inorganic ions crossing the rete, and to assess from their transcapillary passage in relation to other probes the relative influence of size and charge. We also compared the transcapillary passage of neutral dextran with that of an approximately molecular weight-matched, negatively charged, dextran sulfate fraction, to assess whether charge has an effect on the transcapillary passage of a set of somewhat larger molecules.

Methods

The rete mirabile was isolated from the swimbladder of the St. Lawrence river eel (Anguilla anguilla), and the arterial and venous inputs at the opposite poles were catheterized as described previously (Rasio et al., 1977). The capillary net was countercurrent perfused at room temperature (22–25°C) with a constant flow, the same in each direction, averaging 0.5 ml/min, at a constant pressure head of 45 cm H2O. The average weight of the rete was 120 mg. The medium used for simultaneous perfusion at arterial and venous inputs was a Krebs-Ringer bicarbonate buffer (pH 7.4), containing glucose, 5 mM, and purified bovine albumin, 4 g/100 ml. In a first series of experiments, the following radionuclides (New England Nuclear, >99% purity) were used: 35Cl, 1–5 mCi/g chloride; 125I, 17 Ci/mg iodide; 86Rb, 0.5–10 Ci/g rubidium; 35SO4 (as sodium sulfate), 10–1000 mCi/mmol sulfur; 22Na, carrier free; and sodium 14C-labeled ferrocyanide (New England Nuclear), 10.8 mCi/mmol. These tracers, either alone or in combination, were added to the medium perfusing the arterial input. The medium infused at the venous input did not contain the labeled materials. In experiments in which the permeability surface product for potassium was determined, the medium at the arterial input was the standard Krebs-Ringer bicarbonate buffer with KCl 5 mM, whereas at the venous input, KCl was substituted by equivalent amounts of NaCl. The media infused at both arterial and venous inputs were equilibrated with a gas mixture containing 95% O2 and 5% CO2. Oxygen was supplied to the rete at a rate more than 20 times greater than that required by its respiration (Rasio et al., 1977).

In a second series of experiments, the permeability coefficients of the rete to 22Na and K' were measured in the presence of either of two small molecular weight reference markers with diffusion coefficients of the same order of magnitude. [14C]urea or [3H]mannitol (both from New England Nuclear, 2–10 mCi/mg and 50 mCi/mmol, respectively). This additional approach, in which a non-polar reference material with a reasonably close diffusion coefficient is simultaneously infused, was used in the expectation that with this design significant differences between the charged ions and uncharged reference materials would be more clearly and firmly elucidated.

In a third series of experiments, tritium-labeled neutral and sulfated dextrans with the same molecular radius (25 X 10-6 cm), kindly provided by Dr. B. Brenner, Peter Bent Brigham Hospital, Boston, were utilized. Each was perfused through the same rete for 1 hour, in the sequence neutral:dextran sulfate.

Samples were collected simultaneously from the arterial and venous outputs at 10-minute time intervals during the 2-hour perfusions.

The 125I-labeled albumin radioactivity was measured in a Packard gamma scintillation spectrometer on a 10%
Table 1
Permeability Coefficients of the Rete Mirabile to Various Ions

<table>
<thead>
<tr>
<th>Ionic species</th>
<th>D_25 (10^-5 cm^2/sec)</th>
<th>Source</th>
<th>P_25 (10^-5 cm/sec)</th>
<th>n</th>
<th>P_25/D_25 (per cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>^86Rb</td>
<td>1.96</td>
<td>Mills, 1959</td>
<td>0.37 ± 0.04</td>
<td>9</td>
<td>0.19</td>
</tr>
<tr>
<td>^131I</td>
<td>1.94</td>
<td>Robinson and Stokes, 1959</td>
<td>0.65 ± 0.11</td>
<td>9</td>
<td>0.34</td>
</tr>
<tr>
<td>^36Cl</td>
<td>1.94</td>
<td>Robinson and Stokes, 1959</td>
<td>0.34 ± 0.06</td>
<td>6</td>
<td>0.17</td>
</tr>
<tr>
<td>K^+</td>
<td>1.85</td>
<td>Calculated</td>
<td>0.46 ± 0.10</td>
<td>14</td>
<td>0.25</td>
</tr>
<tr>
<td>^22Na</td>
<td>1.30</td>
<td>Robinson and Stokes, 1959</td>
<td>0.35 ± 0.08</td>
<td>14</td>
<td>0.27</td>
</tr>
<tr>
<td>^35SO_4^-2</td>
<td>1.04</td>
<td>Calculated</td>
<td>0.25 ± 0.05</td>
<td>9</td>
<td>0.24</td>
</tr>
<tr>
<td>Fe(CN)_6^-4</td>
<td>0.72</td>
<td>Calculated</td>
<td>0.23 ± 0.08</td>
<td>8</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± se; n = number of experiments. D_25 = self-diffusion coefficients at 25°C. Experimental values are those corresponding to diffusion in a 0.15 NaCl supporting medium. Calculated values were calculated from these, utilizing the Nernst expression and relative values for the limiting equivalent conductivities of the ions at 25°C (Robinson and Stokes, 1959). P_25 = diffusion permeability coefficient at 25°C.

Results
Table 1 gives the values of the permeability coefficients of the rete to the inorganic cations and anions. The values range from a minimum of 0.23 × 10^-5 cm/sec for ^35SO_4 to a maximum of 0.65 × 10^-5 cm/sec for ^131I. The permeability coefficients for ^22Na and ^39K were studied in 14 paired experiments: that for ^39K was significantly (P < 0.01) higher than that for ^22Na, the average value for the ratio of permeabilities being 1.32 ± 0.04. This ratio compares to the value for the ratio of their self-diffusion coefficients, 1.42. The order of the permeability values for the inorganic cations, in descending order, was K^+ > Rb^+ > Na^+, and that of the anions was I^- > Cl^- > SO_4^-2 > Fe(CN)_6^-4. Table 2 gives permeability and diffusion coefficient data for a set of reference molecules, previously utilized to establish the pattern of transfer of lipid-insoluble materials across the rete mirabile. In Figure 1, these values and the locus of the relation between the permeability coefficient and the diffusion coefficient, for the uncharged lipid-insoluble reference molecules, as defined from the values in Table 2, are displayed. For the inorganic ions, in relation to their diffusion coefficients, the permeability values for K^+, Rb^+, Na^+, Cl^-, SO_4^-2, and Fe(CN)_6^-4 are all substantially lower than the locus predicted for the lipid-insoluble reference molecules. In contrast, the value for I^- is anomalous. It falls close to the locus for the small lipid-insoluble organic molecules.

Table 2
Permeability of the Rete Mirabile to Reference Materials

<table>
<thead>
<tr>
<th>Reference substance</th>
<th>D_25 (10^-5 cm^2/sec)</th>
<th>P_25 (10^-5 cm/sec)</th>
<th>n</th>
<th>P_25/D_25 (per cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>0.066</td>
<td>0.079 ± 0.011</td>
<td>41</td>
<td>1.20</td>
</tr>
<tr>
<td>Inulin</td>
<td>0.17</td>
<td>0.162 ± 0.040</td>
<td>16</td>
<td>0.95</td>
</tr>
<tr>
<td>3-O-methylglucose</td>
<td>0.67</td>
<td>0.397 ± 0.069</td>
<td>11</td>
<td>0.59</td>
</tr>
<tr>
<td>Urea</td>
<td>1.38</td>
<td>0.739 ± 0.088</td>
<td>18</td>
<td>0.54</td>
</tr>
<tr>
<td>Water</td>
<td>2.44</td>
<td>7.880 ± 1.039</td>
<td>33</td>
<td>3.23</td>
</tr>
</tbody>
</table>

D_25 = Diffusion coefficients at 25°C. P_25 = Permeability coefficients at 25°C. The values given are the overall means ± se for observations aggregated from the present study, from Rasio et al. (1977), and from Rasio and Goresky (1979).
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TABLE 3
Paired Inorganic Ion-Reference Material Experiments

<table>
<thead>
<tr>
<th>Ionic species</th>
<th>D_{25} (10^{-5} \text{ cm}^2/\text{sec})</th>
<th>P_{25} (10^{-5} \text{ cm/sec})</th>
<th>n</th>
<th>P_{25}/D_{25} (per cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\text{Simultaneous sodium and potassium-urea data}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\text{Na} \text{ }\text{^2}</td>
<td>1.30</td>
<td>0.403 ± 0.148</td>
<td>7</td>
<td>0.311</td>
</tr>
<tr>
<td>K \text{ }\text{^+}</td>
<td>1.85</td>
<td>0.499 ± 0.183</td>
<td>7</td>
<td>0.270</td>
</tr>
<tr>
<td>\text{^14} \text{C-urea}</td>
<td>1.38</td>
<td>0.716 ± 0.158</td>
<td>7</td>
<td>0.519</td>
</tr>
<tr>
<td>\text{Simultaneous sodium and potassium-mannitol data}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\text{Na} \text{ }\text{^2}</td>
<td>1.30</td>
<td>0.409 ± 0.133</td>
<td>10</td>
<td>0.316</td>
</tr>
<tr>
<td>K \text{ }\text{^+}</td>
<td>1.85</td>
<td>0.534 ± 0.154</td>
<td>10</td>
<td>0.289</td>
</tr>
<tr>
<td>\text{[3H]Mannitol}</td>
<td>0.68</td>
<td>0.305 ± 0.130</td>
<td>10</td>
<td>0.447</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE; n = number of experiments. The difference between the sodium and urea permeabilities is highly significant at the P < 0.0005 level, and that between potassium and urea, at the P < 0.005 level. Similarly, the difference between the sodium and mannitol permeabilities is highly significant at the P < 0.0005 level, and that between potassium and mannitol, at the P < 0.0005 level. D_{25} = self-diffusion coefficients at 25°C. That for mannitol was obtained from the CRC Handbook of Chemistry and Physics, 64th edition, 1983. P_{25} = diffusion permeability coefficient at 25°C.

the group of inorganic cations and anions, excluding \( I \), the displacement from the locus is largely independent of the sign of the charge, although there is some tendency for it to be somewhat larger for the negatively charged than the positively charged group. It appears to be chiefly dependent on the presence of charge, whether positive or negative. The group of anions and cations, excluding \( I \), have permeabilities in relation to their diffusion coefficients which are of the order of 0.5 times those expected for uncharged molecules with the same diffusion coefficients. They are substantially lower than would be predicted for a neutral or uncharged species with the same diffusion coefficient.

The results of the analysis of the data from the paired sodium, potassium-nonpolar reference infusion studies are listed in Table 3. In the studies in which \( \text{[14C]urea} \) was used as a reference, the calculated permeability values found for \( \text{Na} \), \( \text{K} \), and \( \text{[14C]urea} \) are quite similar to those found in the previous studies listed in Tables 1 and 2. In studies in which \( \text{[3H]mannitol} \) was used as the uncharged reference material, the calculated permeability values for \( \text{Na} \) and \( \text{K} \) are again similar to previously obtained values, and the permeability value for \( \text{[3H]mannitol} \) falls close to the average locus illustrated in Figure 1 for the previously studied materials. The behavior of labeled urea, as well as of mannitol, appears to correspond to that of the group of probes which permeate the capillary without entering the endothelial cells at any significant rate. For urea, this is what is expected, in view of previous observations of restricted tracer urea permeation of capillaries in the heart (Ziegler and Goresky, 1971a) and the lungs (Chinard, 1969), and of its large induced osmotic transient in the hind leg (Renkin, 1952). The paired sodium, potassium-nonpolar reference studies thus confirm the conclusions arising from the nonpaired studies previously outlined. In the paired studies, the charged ions are again found to penetrate the capillary less well than would have been expected, on the basis of their diffusion coefficients.

Table 4 gives the values obtained for the perme-
amination is therefore the question of why the ra-

The Small-Ion Permeability Values

Relative Magnitude of the Values

All of the permeability values for the ions illustrated in Figure 1, except those for iodide, are substantially less than expected on the basis of the relation drawn through the set of data for the reference substances, and especially relative to the values for the small neutral molecules mannitol, 3-O-methylglucose, and urea, for which the diffusion coefficients are close to the cluster of values for the microions. The first facet of the data needing examination is therefore the question of why the ra-

Discussion

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ated with these. These surface polymers, as might have been expected, exert an excluded volume effect. Larger molecules are excluded from a larger part of the polymer layer associated with the cell surface (Bojesen, 1982; Polefska et al., 1984).

**Bulk Polyelectrolyte Effects**

The effects of neutral polymers and of polymeric polyelectrolytes on diffusion coefficients have been investigated extensively. In a neutral polymeric gel, the first and greatest effect on the diffusion coefficient is an obstruction effect, a decrease in the diffusion coefficient due to a network of obstructions. Mackie and Meares (1955) developed an expression for this effect in an infinite medium,

$$D = D_0 \left(1 - \frac{1 - \phi}{1 + \phi}\right)^2$$

where $\phi$ is the excluded volume fraction, $D_0$ is the free diffusion coefficient, and $D$ is the restricted diffusion coefficient. For molecules of similar size and excluded volume, one would expect an identical decrease in diffusion coefficient. Thus, in vitro, in a hydrated neutral cellulose gel, Brown and Chitumbo (1975a) found that, for both small molecules and microions, the network perturbation of the diffusion process could be represented by a single common factor, reducing diffusion coefficients in common fashion for both uncharged and charged species; for larger molecules, the excluded volume effect was, of course, larger. In the absence of charge on the gel, no differential effect on small neutral and small charged molecules was found.

When the polymer is charged, charge interactions will be expected to modify the behavior of the microions. If there is a predominance of one charge or the other, a corresponding Donnan effect will be expected, one which increases the concentration of the counterion in the polymer phase and decreases that of the coion. The relative lack of preponderance of effect on microions of one charge vs. the other in our data appears to indicate that the transcapillary path for these must include both negative and positive charges, in almost equal amounts. Under these circumstances, the Donnan effect will not be expected to contribute differentially to the observed diffusional slowing.

The major effect known to cause a differential slowing in the diffusion of a microion through a charged polyelectrolyte is the binding of oppositely charged mobile counterions. Ordinarily, investigations demonstrating this effect have been carried out in a homogeneous medium, and the effect has been found to be confined to ions of one specific charge. Thus, in a polar negatively charged polycrylamide gel, Brown and Chitumbo (1975b) found the diffusion coefficients of the alkali metal cations to be much reduced in relation to those of small uncharged molecules. Similarly, Pikal and Boyd (1973), in their examination of the diffusion of ions in polystyrenesulfonate ion exchange gels, found the diffusion coefficients of alkali metal counterions to be much reduced, whereas those of negatively charged mobile cations, in relation to labeled water, were of the order expected. The data were, in each case, interpreted to reflect a fixed charge effect on diffusion.

The decrease in the diffusion coefficients in gels of counterions, relative to small uncharged molecules in gels, has been interpreted to result from binding to the relatively immobile oppositely charged polymeric chains (Pikal and Boyd, 1973). The phenomenon is presumably a reflection in part of the Debye-Huckel double-layer effect, around the fixed charges. If the approximately equivalent reductions in the permeability of cations and anions are interpreted to result from phenomena associated with the surface polymer, our data must indicate a heterogeneity of fixed charges in the surface polymer, which the microions must penetrate. Both positive and negative charges must be distributed along this part of the pathway, in distributed microdomains, in either shared or sequential fashion.

**Charge Effects in the Transendothelial Channel**

In the foregoing, the phenomena considered have been those known to influence diffusion in a continuous medium. It is generally thought, however, that the limiting phenomenon in transcapillary diffusion is a size-dependent one, that the dimensions of the transcapillary pathway produce the overall restriction characteristics of the process (Crone and Levitt, 1984). In a structure of limiting dimensions not filled with polymer, the superimposition of fixed charges has been expected to reduce the effective cross-sectional area available to ions of the same charge and, with this, to reduce the permeability of the membrane to such ions (Curry, 1984). The presence of a charged surface polymer filling the limiting structure might then be expected to result in two different effects: the decreased access for entry of ions of the same charge, and the binding to the polymer and slowing of diffusion of ions of the opposite charge, again presumably mediated by the Debye-Huckel double-layer effect. The two effects acting together might be expected to reduce the rate of passage across the membrane of both positive and negative ions. The effects would be expected to be especially prominent for small ions, in view of the dimensions across which the effect will be expected to operate. At the ionic strength of plasma, the double-layer width will be expected to be about 0.8 nm. Since the width of carbohydrate chains is of the order of 1 nm, the local charge effect will not only have the effect of immobilizing ions of the opposite charge but will also potentially reduce the proportion of the area of a glycoprotein-filled pore available to ions of the same charge. The heterogeneity of charges added to be present in the domains traversed by the microions is nevertheless probably also still present. Charge heterogeneity of
microdomains on endothelial cell membranes has been demonstrated morphologically (Simionescu et al., 1981a, 1981b). A structural correlate to the postulated fixed charge staggering therefore exists.

Our present reconstruction is, of course, an incompletely formulated description. It does not appear possible to be more precise, with the data we have available. The Curry (1980, 1981) elaboration of the gel matrix hypothesis indicates that a network of fibrous molecules could produce the kind of limitations observed in transcapillary exchange, especially if this produces or is superimposed on a small dimension-limiting structure. To evaluate its role in the microion effects, it will be necessary to add to it those general elements necessary to account for the observed restrictions of cation and anion permeability.

Comparison with Other Values

Stray-Pedersen and Steen (1975) previously explored potassium permeability in relation to that of urea in the rete mirabile of the European eel (Anguilla vulgaris) and noted that potassium did not penetrate the capillary barrier as well as expected, in relation to the ratio of its diffusion coefficient to that of urea. They hypothesized that this resulted from urea permeation through endothelial cells. In view of the restricted permeation of tracer urea through cardiac and lung capillaries (Chinard, 1969; Ziegler and Goresky, 1971a) and of our present findings in the simultaneous microion-nonpolar reference experiments, where, with the use of either mannitol or urea as a nonpolar reference, there were similar findings, we would interpret their experiments to indicate in that species of eel, as well, a decreased permeability of the rete capillaries for potassium in relation to urea.

It also now becomes appropriate to review the data from the heart. Whereas Ziegler and Goresky (1971b) observed a major relative reduction in the rate of transcapillary passage of rubidium ions relative to sucrose, corresponding to what we have recorded above in the rete of the eel, Duran and Yudilevich (1978) found no relative restriction in the passage of sodium ions relative to glucose. Apart from the entry of glucose into endothelial cells, there are two other potential explanations for the apparent discrepancy in the data. In their preparation, Duran and Yudilevich (1978) used blood diluted approximately 1:5 with buffer for perfusion. With the resulting plasma dilution, the differential permeability characteristics of the membrane may have been altered, since plasma proteins are known to be required to maintain the integrity of size-limiting structures of the capillary wall, both in the mammal (Curry and Huxley, 1982) and also in the eel (Myhr and Steen, 1976). With this alteration, charge effects at the wall also may have been altered. It is also possible that the use by Duran and Yudilevich of the Crone approximation—rather than a more exact approach for the estimation of permeability, allowing for both return of tracer to the capillary and heterogeneity of capillary transit times—accounts for the discrepancy. The Crone approximation values for the higher permeability sodium ion will be expected to be less diminished in relation to real values than would those for glucose (Bassingthwaighte and Goresky, 1984). The sodium:glucose ratio approximation of Crone values will therefore be expected to overestimate the real value. On the other hand, there may be a difference between the capillaries of the heart and the rete, with respect to the charge effect. The matter bears further investigation.

The permeability values for the rete, over the lower molecular weight range, for both uncharged small solutes and ions, are quite low. They are of the order of $4 \times 10^{-6}$ cm/sec, a value which is of the order of one-fifth to one-tenth of indicator dilution estimates of capillary permeability for corresponding solutes in the mammalian heart (Alvarez and Yudilevich, 1969; Ziegler and Goresky, 1971a; Duran et al., 1973; Guller et al., 1975; Rose and Goresky, 1976) and limbs (Trap-Jensen and Lassen, 1970). The eel rete arterial capillaries are much thicker than continuous non-neural mammalian capillaries, but they contain an approximately equivalent density of vesicles (Rasio et al., 1981). The rete permeability values approximate the same order of magnitude as those estimated by tracer dilution for isolated dog lungs perfused by high rates of plasma flow (Tancredi et al., 1980); and the rete values, in turn, are approximately 10 times higher than the permeability values of brain capillaries, measured with sensitive microelectrodes placed in the interstitium (Crone, 1975; Hansen et al., 1977).

The data do not elucidate which of the potential pathways underlies the transcapillary passage of tracer microions. The transcapillary route for small ions has commonly been considered to include the interendothelial cell junctions (Ohori, 1963), transendothelial channels formed by vesicle fusion (Simionescu et al., 1975), or both, but more likely predominantly the former.

The Neutral and Negatively Charged Dextran Data

Whereas the microions, with their accompanying clusters of water molecules, have been expected to sense effects at a smaller scale, larger charged molecules, such as the dextran sulfate, have been expected to sense net charge effects in the region where their transcapillary passage is occurring. The ability of large probes to detect local charge in the transendothelial pathway will be expected to be dictated not only by the presence of the charge on the probe, but also by the density of the charge on the probe. Some idea of this can be gained from past electrophoretic mobility measurements. For the dextran sulfate, a polyglucosyl containing, in this
instance, 2.3 sulfate groups per glucosyl residue, the present 25 Å fraction has been shown to have a net molecular charge at physiological pH of —6.1 (Deen et al., 1980). On the other hand, our two large molecular weight reference materials are also charged. The carboxyl-inulin, a smaller polyfructosyl (with molecular weight about 5000 daltons), has a terminal carboxyl, and so its maximum charge could be only —1. There also does not appear to be a large charge effect in its behavior. Albumin, on the other hand, with an effective radius of about 34 Å, has, when purified, a net charge of —19 at pH 7.4 (Deen and Satvat, 1981). This may be somewhat smaller in plasma, where it binds a variety of materials, but it is clear that one would expect it to behave as a charged probe. We have no uncharged material with which to compare its behavior, and so we have not been able either to perceive or record the effect of the charge on this larger molecule.

In the present study, the negatively charged dextran sulfate molecules were found to penetrate the rete permeability barrier less well than the essentially equivalent neutral dextran molecules. One would therefore infer that there is probably a net negative charge at some limiting site in this barrier, which is made up of the arterial capillary endothelial cell layer, a layer of interstitial material, and the fenestrated venous capillary cell layer. The site of the additional resistance to the negatively charged dextran is not clear, but one might expect it to be at the site of restriction in the major permeability barrier, the arterial capillary endothelial cell layer. If this is so, the capillary layer would appear to have a net negative charge in the region across which the molecules penetrate (the interendothelial cell junctions, the vesicle chains, or both). The sensing of a net negative charge on this macromolecular scale indicates that the corresponding apparently slightly larger restriction for negatively charged microions may be real. However, one must, at the same time, remember that the larger dextran molecules will be excluded from the interior of much of the surface glycoproteins, and, hence, the charge effects affecting these will be those related to the glycoprotein levels which the dextran molecules contact, rather than the whole glycoprotein structure (which would, of course, be permeated by the microions). The fixed charges encountered by the dextran molecules and the microions will therefore differ, in part.

The inference of the foregoing is that the physical hypotheses developed to provide size estimates, on the basis of the permeation of a variety of molecules through a clean pore, cannot be applied to a passage filled with biopolymer without modification. Use of permeability data to derive a dimension for such a passage could be expected to provide a valid estimate only if there were knowledge of the characteristics of the polymer, especially since the smaller molecules will have access to a larger proportion of the passage than the larger ones.

Analogous large molecular effects are well documented in the rat glomerulus. Negatively charged exogenous polymers are filtered to a much lesser extent than neutral or positively charged materials of equivalent molecular radius (Brenner et al., 1978; Venkatachalam and Rennke, 1978; Bohrer et al., 1979; Rennke and Venkatachalam, 1979; Deen et al., 1980). The data indicate that, just as in the rete, there is across this permeability barrier a net negative charge. Anionic ruthenium red-staining sites are richly evident in the basement membrane of the glomerulus (Charonis and Wissig, 1983), the major permeability barrier in this structure. These sites have been partially characterized by enzyme digestion with heparin, heparan sulfate, and proteoglycans of mixed chemical nature (Kanwar and Farquhar, 1979; Parsarathy and Spiro, 1982).

For capillaries lined by an ordinary continuous endothelial lining, the picture is less clear, however. The rete data indicate that there is a net negative charge along the transendothelial pathways in the rete capillaries. Garby and Areekul (1970) showed that the reflection coefficient in rabbit ear capillaries for negatively charged dextran sulfate was, similarly, much larger than that for neutral dextran with similar molecular weight. The expectation has usually been that this phenomenon would be general. Observations from other organs have, however, shown some disparate results. Whiteside and Silverman (1984) have shown with labeled probes that the renal postglomerular capillaries have a charge polarity opposite that of the glomerulus. Their permeability-surface products for dextran sulfate are larger than those for neutral dextrans of the same molecular size. Pietra et al. (1982) have found, in the lungs of conscious sheep, that the movement of anionic dextran sulfate from blood to lymph is facilitated with regard to that of molecular weight-matched neutral dextrans. This would imply that the pulmonary capillary bed, with its rich detoxifying function, also has an obverse or positive charge. Taylor and Granger (1983) have found, in complementary fashion, in the lungs, and Perry et al. (1983), in the fenestrated small intestinal capillary bed, that the appearance in the corresponding lymphatics of cationic macromolecules is reduced and that of negatively charged or anionic molecules, enhanced. Ultrastructural studies in these organs have provided additional data. In the lungs, polycationized ferritin, a substance whose passage would be expected to be diminished, binds heavily to the clefts leading to the interendothelial cell junctions but rarely enters endothelial cell vesicles (Pietra et al., 1982). In the intestinal capillaries, which are characterized by apertured fenestrae, domains binding cationized ferritin are again evident; high concentrations are found over the opercula of the fenestrae and the plasmalemmal proper, but there is no binding within plasmalemmal vesicles, transendothelial channels, or their stomata (Simionescu et al., 1982).
In the capillaries of the epididymal fat pad, which might be assumed to conform to the expected pattern with the opposite polarity, whereas transferrin is found ultrastructurally in increased concentrations in the vesicles (Wagner et al., 1983), albumin is generally excluded (Wagner et al., 1980). If one presumes that vesicle exclusion or concentration of proteins reflects relative capability to move across capillary walls, order comes into the data. The morphological and physiological data correspond. The renal postglomerular, lung, and small intestinal capillaries differ from the expected pattern; they appear to have net positive charges in their surface layers in the region where transcapillary passage of larger molecules takes place.

The Peritoneum

The peritoneal surface has permeability properties which are, in many ways, analogous to those of the endothelial surface of an ordinary continuous capillary. These have been explored and defined during peritoneal dialysis in patients (Boen, 1961). In relation to uncharged materials, these investigations have shown a relative resistance to electrolyte transfer from the peritoneum to the dialysis fluid (Nolph, 1979). These explorations have shown, for example, that potassium, which has a diffusion coefficient 1.35 times that for urea, enters the dialysate from the blood less well than urea rather than better, as would be expected from their diffusion coefficients (Boen, 1961; Robson et al., 1978). The finding of a relative resistance to electrolyte transfer in the eel rete is therefore not unique; it is also present at the peritoneal surface. Therefore a systematic exploration of whether similar properties characterize continuous mammalian capillaries is needed.

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