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The Permeability of the Microvasculature of the Perfused Rat Testis to Small Hydrophilic Substances

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ABSTRACT: The permeability-surface area products (PS) for sodium, Cr-EDTA, and cyanocobalamine (CoB_{12}) have been determined in isolated perfused rat testes, using the single-passage multiple tracer technique, with albumin as the reference tracer. The validity of using albumin was established from its recovery in the perfusate leaving the testis, which was 98.73 ± 0.48% of that for Cr-labeled red cells. The PS values obtained for Na, Cr-EDTA, and CoB_{12} were correlated with perfusate flow, both below and above levels that were equivalent to normal rates of blood flow in the testis (0.3 mL/[g·min]). The values found at the highest flow rates obtained (between 2.7 and 3.5 mL/[g·min]) were 2230 ± 240 μL/(g·min) (n = 8) for sodium, 1460 ± 140 μL/(g·min) (n = 7) for Cr-EDTA, and 850 ± 80 μL/(g·min) (n = 7) for CoB_{12}. These values are similar to those reported at equivalent flow rates for heart muscle and greater than those reported for skeletal muscle, both of which have unfenestrated capillaries similar to testis, but are less than the values for pancreas and salivary gland, which have fenestrated capillaries and are similar to most other endocrine tissues. However, the permeability coefficients for these markers in the testis (calculated using published values for the surface area of the testicular microvasculature) appear to be considerably greater than for any other tissue studied thus far. By extrapolating extraction values, either linearly or logarithmically, to obtain maximal values for PS for Cr-EDTA and CoB_{12}, and comparing the ratio of these PS area values with the ratio of the diffusion coefficients of these molecules, it can be calculated that the equivalent pore radius for the testicular endothelium is between 5 and 6 nm, comparable to those calculated for other nonfenestrated endothelia.

Key words: Sodium, Cr-EDTA, vitamin B-12, capillary, albumin.
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Lymph flow from the testis is appreciably higher than from many other tissues; the protein concentration of the lymph is higher than that from most other sites in the body; and large, water-soluble marker molecules appear to pass relatively rapidly from the bloodstream into the lymph. It is therefore often assumed that permeability of the microvasculature of the testis must be high (Setchell, 1986, 1990; Setchell et al, 1994). This seems logical because the cells in the testis are stimulated by luteinizing hormone (LH) and follicle-stimulating hormone, peptides that have a molecular weight of about 30 kd. LH in particular is released into the bloodstream in pulses. Nevertheless, doubts have been expressed as to how much of each LH pulse the Leydig cells actually receive (Setchell, 1994; Turner and Rhoades, 1995). However, the endothelium of the capillaries in the rat testis is continuous, not fenestrated as in other endocrine tissues, and it has been suggested that the endothelial cells may restrict the entry of some dyes into the interstitial tissue as they do in the brain (see Ploen and Setchell, 1992). Evidence exists that there are significantly more junctional clefts between endothelial cells in the testis than in the brain and, although none of these clefts was expanded in the brain, about 15% were in the testis (Holash et al, 1993), a value comparable to that in the brains of fetal and new-born animals (Stewart and Hayakama, 1987) and muscle (Coomber et al,

Estimations of the permeability-surface area product (PS) or clearance for radioactive albumin and gammaglobulin in the testes of anesthetized rats suggest that the entry of these proteins into the testis is more rapid than into many other tissues (Haraldsson et al, 1987; Setchell et al, 1988; Pollanen and Setchell, 1989), but these values may be influenced more by the nature of the interstitial tissue than the characteristics of the blood vessel wall (Haraldsson, 1986). It therefore seemed appropriate to report in full measurements of the permeability of the microvasculature of the perfused rat testis using the single-passage, multiple-tracer dilution technique for some small hydrophilic molecules because comparable data are now available for these markers for other tissues. A preliminary report of these findings was presented to the Physiological Society (Bustamante and Setchell, 1981).

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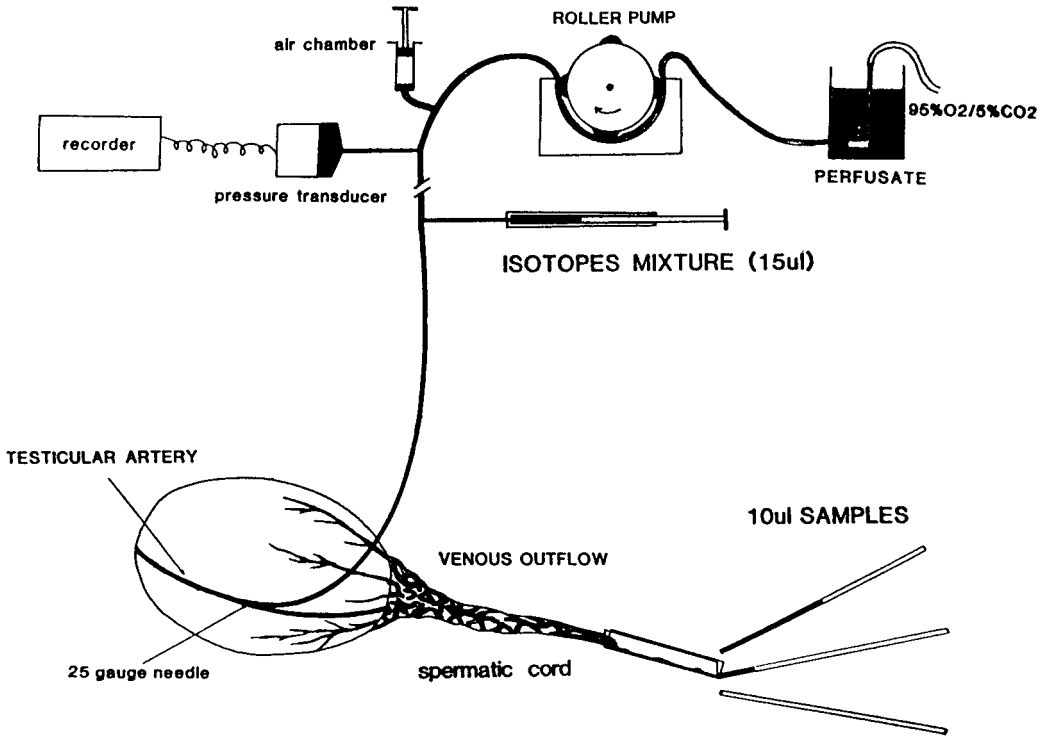

Figure 1. A schematic diagram of the isolated perfused testis of the rat. Except for the collecting trough at the venous outlet of the organ and the syringe containing the mixture of isotopes, the whole perfusion system was contained in a chamber that was kept at 32.5°C, normal testis temperature in this species (Setchell et al, 1994). The volume (5–15 μL) and the number of samples (7–15) collected at the spermatic cord with glass capillary tubes varied according to the experimental conditions.

Materials and Methods

Animal Preparation

Twenty-five male rats of the Wistar strain were used, each weighed between 250 and 400 grams. They were anesthetized with an intraperitoneal injection of pentobarbitone sodium (Sagatal, May and Baker, Dagenham, United Kingdom; 35mg/kg body weight); a single injection maintained adequate anesthesia for establishing the perfusion, and the rats were then killed with an overdose of anesthetic. The left testis and spermatic cord were exposed by cutting the scrotal tissue and tunica vaginalis with small scissors. The spermatic cord was tied with a ligature about 2 cm above the testis, which interrupted the circulation of blood. The testicular artery on the surface of the testis was immediately pierced with a sterile 0.5-mm hypodermic needle and the artery was cannulated with a blunted piece of the same type of needle tubing filled with perfusion fluid and attached to a length of polythene tubing, with an inside diameter of 0.5 mm. The metal cannula was inserted into the artery to a distance of between 5 and 10 mm and, initially, was held in place with a suture, but this proved to be unnecessary, and in most of the perfusions, no suture was used. The spermatic cord was then severed a few millimeters below the suture and, usually within 20 seconds of ligation of the cord, perfusion was begun with a Krebs-Ringer solution containing 118 mM NaCl, 27 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, 5.6 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 11 mM glucose with 10 g/L bovine serum albumin (Cohn fraction V), and 1 mg/L cyanocobalamin (vitamin B_{12}), delivered from a peristaltic pump (Minipuls II, Gilson Medical Electronics, France). The perfusion fluid was kept in a covered beaker and gassed with a mixture of 95% O_2 and 5% CO_2 to keep the pH between 7.3 and 7.4. Bubble formation was prevented by applying a small amount of a silicone polymer-based antifoam agent (Antifoam A, Sigma Chemical Company, St Louis, Mo) to the surface of the liquid. The pressure oscillations from the pump were dampened out with a length of highly flexible polyurethane tubing and an air damper. Perfusion pressure was recorded with a strain gauge transducer, and did not exceed 160 mm Hg. A T-piece near the testis allowed mixtures of isotopes to be injected into the arterial catheter; the dead space between the injection site and the testis was about 40 μL. The venous outflow from the testis ran from the severed end of the spermatic cord along a sloped foil trough from which samples could be collected or flow measured by timed collections (Figure 1). Flow remained constant (±10%) for at least 60 minutes. No attempt was made pharmacologically to dilate the vascular bed.

Multiple Tracer Dilution Experiments

For each measurement of vascular permeability, a 15-μL bolus of medium containing a mixture of radioactive tracers was injected rapidly, usually within less than 1 second, into the T piece, using a gas-tight syringe. Sequential collections of 7 to 15 samples each of 5 to 15 μL of the outflow fluid from as near as possible to the end of the spermatic cord were collected within 10 to 40 seconds, starting immediately after the injection, using a fan of calibrated glass capillary tubes that were held together

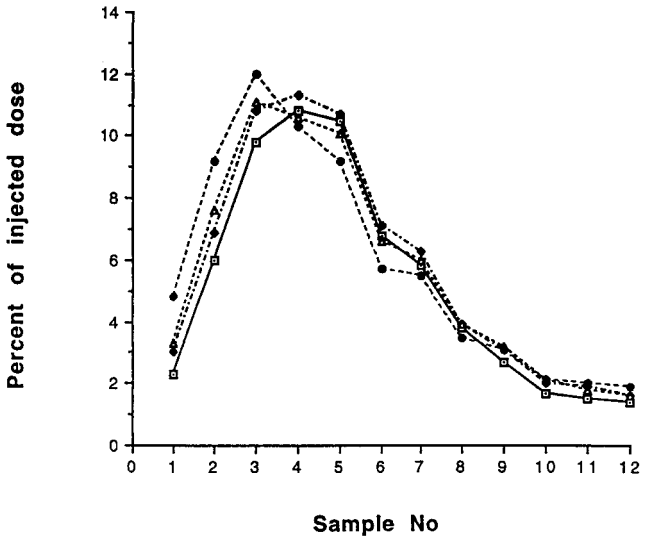

Figure 2. A typical simultaneous venous dilution of 4 radioactive tracers injected close-arterially after a single passage through the testicular circulation. The 12 samples were collected over a period of 11 seconds. The overall recovery of the tracers was 69.6% of the injected dose for ^{125}I-albumin, 68.9% for ^{51}Cr-EDTA, 68.5% for $^{57}CoB_{12}$, and 64.4% for ^{22}Na. The perfusate flow in this experiment was 2960 μL/(g·min). ♦ indicates ^{125}I-albumin; Δ, $^{57}CoB_{12}$; ◆, ^{51}Cr-EDTA ; and ⊡, ^{22}Na.

with a block of plasticine. The dead space of the collection system was less than 10 μL. The exact volume of each sample was unimportant because the total radioactivity in each sample was related to either sample number (Figures 2 and 3A) or to Tau, the accumulated percentage of the reference tracer that was recovered (Figure 3B). The tracers used were [^{125}I]-labeled human serum albumin, [^{57}Co]-labeled cyanocobalamin, [^{51}Cr]-labeled chromium salt of ethylenediamine tetraacetic acid (EDTA), and [^{22}Na]-NaCl, all obtained from Radiochemical Centre (Amersham, United Kingdom). The molecular weights of the tracers were 69 000, 1353, 357, and 58, respectively; their free diffusion coefficients were 0.085, 0.39, 0.70, and 1.80 × 10^{-5} cm^2/s, respectively; and their molecular radii were 3.6, 0.84, 0.47, and 0.23 nm, respectively (Landis and Pappenheimer, 1963; Paaske 1977). Each injection contained about 0.1, 0.3, 0.03, and 0.01 μCi, respectively of the 4 tracers. The outflow samples were transferred to polythene tubes and the radioactivity was counted in an automatic 1-channel gamma spectrometer (Model 80.000, Wallac-LKB, Cambridge, United Kingdom) with windows set to discriminate between the 4 isotopes with minimal cross-channel interference. The amount of each isotope in each sample was expressed as a percentage of the injected dose, and a fractional extraction (E) in relation to albumin was calculated as

$$E = 1 - (\%\ \text{injected test tracer} / \%\ \text{injected } [^{125}\text{I}]\text{-albumin}).$$

The extractions were then plotted, either against sample number, collection time, or Tau, the fraction of the total reference tracer recovered. Each perfusion lasted up to 45 minutes and, during this time, up to 6 measurements, usually 3 or 4, were made, in 9 experiments with different flows between 60 and 500 μL/min for each measurement; and in 16 experiments, with the same flow between 500 and 3500 μL/min throughout the experiment.

In 3 experiments, the extraction of albumin in relation to

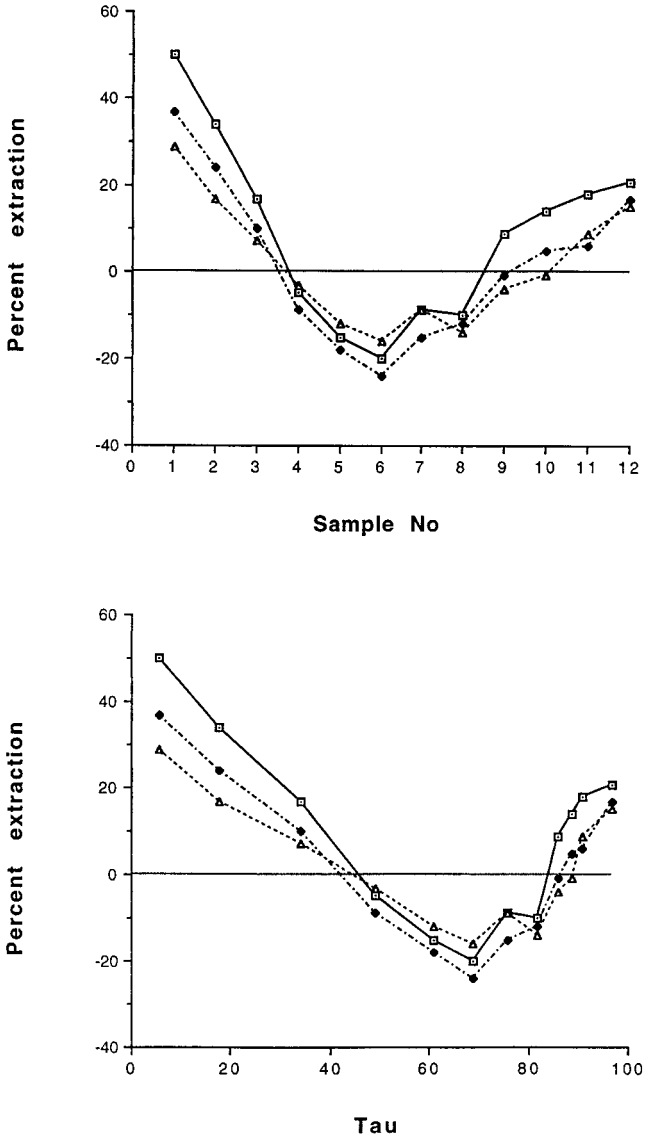

Figure 3. The extractions, in a single passage though the testicular microvasculature of the test tracers measured against the reference tracer ^{125}I-albumin. The extractions were calculated from the values shown in Figure 2 as $E(\%) = (1\text{-}C_t/C_r) \cdot 100$, where C_t and C_r refer to the venous concentrations of the test and reference markers, respectively. Extractions are plotted against sample number and against Tau, which represents the accumulated percentage of the recovered reference tracer. The falling and negative extractions are the result of back flux of the test tracers from the extravascular space into the circulation. The initial extraction was obtained by retropolation to time 0 of the extraction versus Tau plot. ⊡ indicates ^{22}Na; ◆, ^{51}Cr-EDTA; and Δ, $^{57}CoB_{12}$.

[^{51}Cr]-labeled erythrocytes was determined in the same way. A rat was anesthetized as described earlier and a blood sample was removed from a catheter in 1 jugular vein. The red cells were labeled by mixing 2 mL of the blood with 2 mL of heparin-saline (30 IU/mL) and adding 30 μCi of [^{51}Cr]-labeled sodium chromate to the cell suspension, which was then kept at 36°C for 30 minutes. The red cells were separated by gentle centrifugation and washed by resuspension in heparin-saline until the supernatant contained negligible amounts of radioactivity. Equal

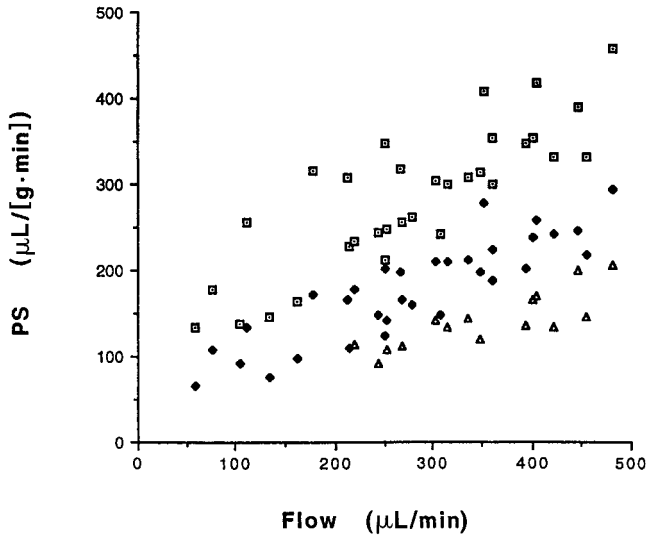

Figure 4. The calculated permeability-surface area product (PS) of the microvasculature of the perfused rat testis plotted against perfusate flow for experiments in which the latter ranged between 50 and 500 μL/min, and where several estimates were made at different flows in a single perfusion experiment. ⊡ indicates ^{22}Na; ♦, ^{51}Cr-EDTA; and Δ, $^{57}CoB_{12}$.

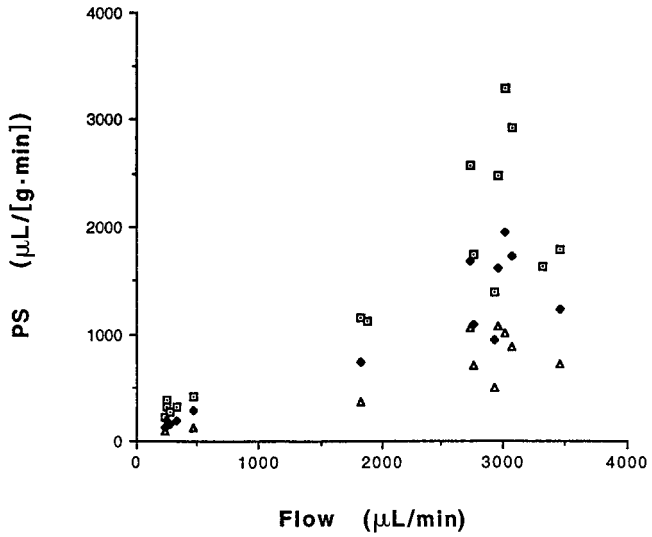

Figure 5. The calculated permeability-surface area product (PS) of the microvasculature of the perfused rat testis plotted against perfusate flow for experiments in which the latter ranged between 250 and 3460 μL/min, and where several estimates were made at the same flow in a single perfusion experiment. ⊡ indicates ^{22}Na; ♦, ^{51}Cr-EDTA; and Δ, $^{57}CoB_{12}$.

volumes of resuspended red cells, perfusate, and radioactive human serum albumin were mixed for injection into the testis of the same rat, which, in the meantime, had been prepared for perfusion.

Results

A representative simultaneous dilution curve is shown in Figure 2. In the first samples, the intravascular reference tracer has a higher recovery than the other substances because the smaller molecules more readily pass into the extravascular space in the tissue during the passage of the injection bolus through the vascular bed. The calculated extractions of the 3 tracers, relative to albumin, plotted against sample number or Tau, the fraction of the reference tracer recovered, are shown in Figure 3. The extractions fell with sample number up to 6, or increasing Tau up to about 70%, and then increased again. This is presumably the result of a back flux of the smaller tracers from the interstitial tissue into the vasculature after the bolus has passed through the vascular bed and, consequently, retropolation to Tau = 0 was used to determine an initial extraction. This was then used to calculate PS area products from the formula

$$PS = -Q/\ln(1 - E_0),$$

where Q is the perfusate flow and E_0 is the fractional extraction at time 0 (Renkin, 1959).

The results of these calculations are shown in Figure 4 for those experiments in which flows of up to 500 μL/min were varied in the same testis, and in Figure 5 for experiments in which flows of between 500 and 3500 μL/min was kept constant in each testis. With the lower flows, it was possible to change the flow and to obtain sufficiently stable conditions to allow the required number of measurements to be completed within a 45-minute experiment, but with higher flows it was not possible to change the flow and obtain stable conditions quickly enough. Therefore, at higher flows, a number of measurements were made in each testis at the same flow.

The PS for sodium was higher than it was for Cr-EDTA, which in turn, was higher than for CoB_{12}. PS increased with flow for all tracers, and even with the highest flows obtainable, maximum values did not appear to have been reached.

Because particulate markers such as labeled red cells have a faster transit time through a capillary bed than dissolved markers do (Rapaport et al, 1956; Goldsmith, 1967), it is not valid to calculate PS area from initial extractions for albumin against chromium-labeled red cells. Instead, the uptake of albumin from the circulation into the tissue relative to Cr-labeled red cells in a single passage can be calculated from the areas under the curves. In 3 experiments we made 8 estimations, giving a mean uptake of 1.27 ± 0.48%, from which a PS of 2.86 μL/(g·min) can be calculated.

Discussion

Our results show that the permeability-surface area product (PS) of the microvasculature of the testis to small

Table 1. *Values for permeability-surface area product (PS; μL/[g·min]) for sodium, Cr-EDTA, and Co-cyanocobalamine at high flow rates in various tissues*

Tissue and species	PS for Na	PS for CrEDTA	PS for Co-B_{12}	Flow, mLg^{-1} min^{-1}	Reference
Testis					
Rat	2230 ± 240 (8)	1462 ± 140 (7)	850 ± 80 (7)	3–3.5	This paper
Heart					
Rabbit	2710	1060	620	2.97	Mann, 1981
Dog	1140			1.5–2.5	Alvarez and Yudelivich, 1969
Dog		1800*		3.5	Cousineau et al, 1986
Rat		5350	2200	29	Wahlander et al, 1993
Muscle					
Cat		450	220	2.98	Paaske, 1977
Rat		129	51	extrapolated to infinity	Haraldsson and Rippe, 1986
Pancreas					
Cat	4000	2000	1000	3–4.5	Sweiry and Mann, 1991
Pig		1230		3	Haraldsson et al, 1982
Salivary gland					
Cat		2300	1400	2–4	Mann et al, 1979

* Marker used was sucrose, which has a similar molecular radius to Cr-EDTA (0.481 cf 0.438 nm; Paaske and Sejrsen, 1989).

hydrophilic molecules is higher than that of the nonfenestrated capillaries of skeletal muscle, comparable to that of heart, and appreciably lower than that for the fenestrated capillaries of the pancreas and salivary glands (Table 1). As in many other tissues (Haraldsson and Rippe, 1986; Sweiry and Mann, 1991; Wahlander et al, 1993), PS in the testis depends on flow, particularly at lower flow rates, but the testis is unusual in that maximal values were not reached even at the highest flows achievable without causing edema or damage to the tissue. At the flow rates normally found in the testis (about 0.3 mL/min; Galil and Setchell, 1988), PS was considerably less than the highest values recorded. This is probably the result of heterogeneity of 2 types among the capillaries (Wahlander et al, 1993).

It should be remembered that the surface area of the vascular bed of the testis is only 2700 mm^2/g (Damber et al, 1985); a similar value can be calculated from the 3-minute volume of distribution of albumin in the rat testis parenchyma (10 μL/g; Setchell et al, 1988) and the diameter of the testicular capillaries (10–12 μm, Fawcett et al, 1969; Murakami et al, 1989). This is considerably less than in other tissues (60 000 mm^2/g for heart muscle, Turek et al, 1972; 7000 mm^2/g for skeletal muscle, Pappenheimer et al, 1951; and 18 200 mm^2/g for exocrine pancreas, Vetterlein et al, 1987). Therefore, when permeability constants are calculated by dividing PS by the surface area of the vascular bed, the vessels in the testis appear to be considerably more permeable to small hydrophilic molecules per unit surface area than any vascular bed ever studied (Table 2). Furthermore, the values suggested for the surface area of the testis microvasculature is likely to be maximal, so the difference in permeability would be even greater under normal physiological conditions if the surface area of the blood vessels is usually less than maximal.

The ratios of the PS of various markers can also be used to determine whether any of these substances crosses the endothelium by a mechanism other than simple diffusion. Although PS is usually determined as described earlier, it can also be defined as:

$$PS = A_p/D/\Delta_x^{-1}$$

where A_p is the capillary area of exchange for the marker, D is the molecular free diffusion coefficient of the marker in water, and Δ_x is the distance separating the intravascular and extravascular compartments. It is assumed that A_p and Δ_x are the same for the 2 molecules and that either the vessel wall in a homoporous membrane with cylindrical pores of uniform size and length, or the exchange of the markers, is entirely through the same pore population in a heteroporous membrane. The latter is a more likely situation.

If the ratio PS_S/PS_L is greater than the ratio D_S/D_L (where the subscripts S and L refer to the smaller and larger markers, respectively), then the larger molecule does not freely diffuse through the membrane. The ratio PS_{Na}/PS_{EDTA} is 1.53 ± 0.02, and 1.56 ± 0.09 over the flow ranges of 0.3 to 0.5 and 1.8 to 3.5 mL/min, respectively; and the ratio PS_{EDTA}/PS_{CoB12} is 1.51, and 1.76, respectively over the same flow ranges. These values are thus less than the ratios of the diffusion coefficients, 2.57 and 1.79, respectively, which suggests that all 3 markers are crossing the endothelium by simple diffusion at the

Table 2. *A comparison of the characteristics of the microvasculature of the rat testis obtained by linear extrapolation of extractions to obtain maximal permeability-surface area product (PS) values with maximal values for other tissues obtained at flow rates at which PS was flow-independent*

Variable	Testis	Pancreas	Muscle	Heart
PS (μL/[g·min])				
Cr-EDTA	2240	2000	129	5590
Co-B_{12}	920	1000	51	2200
Pore radius (nM)	5.8	6 to 11	5.3	5.1
Surface area of vessels ($mm^2\ g^{-1}$)	2700	18 200	7000	60 000
Permeability constant (10^{-4}mm sec^{-1})				
Cr-EDTA	93	22	3.1	15
Co-B_{12}	43	11	1.2	6.1
Reference	This study	Sweiry and Mann, 1991	Haraldsson and Rippe, 1986*	Wahlander et al, 1993

* See also Paaske and Sejrsen (1989).

flow rates studied, but that the estimate of PS_{Na} is probably a considerable underestimate.

However, if the PS values had reached maximal values at high flow rates, then the PS ratio can be used to calculated apparent pore size using the formula

$$[P_S/P_L]/[D_S/D_L] = [(1 - a_S/r)^2(1 - 2.104(a_S/r) + 2.09(a_S/r)^3 - 0.95(a_S/r)^5)] \div [(1 - a_L/r)^2(1 - 2.104(a_L/r) + 2.09(a_L/r)^3 - 0.95(a_L/r)^5)],$$

where P is permeability, D is the diffusion coefficient in water, a is the molecular radius of the smaller (S) and larger (L) markers, and r is the apparent pore radius (Renkin, 1954).

Although the experimentally obtained PS values did not appear to have reached flow-independent maxima, it was possible to calculate through the following equations maximal PS values by extrapolating the extraction values according the observed relationship between flow (Q) over the flow range of 300 to 3500 μL/min and extraction (E):

$$\%E_{EDTA} = 45.09 - (2.579 \times 10^{-3}) \times Q \quad (r = -0.49,\ P < .02,\ n = 25)$$

$$\%E_{CoB_{12}} = 32.72 - (3.182 \times 10^{-3}) \times Q \quad (r = -0.62,\ P < .005,\ n = 20)$$

$$\%E_{Na} = 60.37 - (3.696 \times 10^{-3}) \times Q \quad (r = -0.54,\ P < .005,\ n = 27).$$

Logarithmic transformations of the extraction values also gave extrapolations with the same levels of significance:

$$\ln(\%E_{EDTA}) = 3.80 - (6.957 \times 10^{-5}) \times Q \quad (r = -0.49,\ P < .02,\ n = 25)$$

$$\ln(\%E_{CoB_{12}}) = 3.48 - (1.248 \times 10^{-4}) \times Q \quad (r = -0.61,\ P < .005,\ n = 20)$$

$$\ln(\%E_{Na}) = 4.11 - (7.411 \times 10^{-5}) \times Q \quad (r = -0.56,\ P < .005,\ n = 27).$$

Maximal PS values for EDTA, Co_{B12}, and Na of 2244, 920, and 3000 μL/(g·min), respectively, were obtained by linear extrapolation to flows of 8200, 7200, and 4800 μL/min, respectively. By extrapolation of the logarithms of the flows, maximal values of PS for EDTA, CoB_{12}, and Na of 2617, 1028, and 3470 μL/(g·min), respectively, were obtained at extrapolated flows of 13 000, 7500, and 12 000 μL/min, respectively. The ratios of maximal PS_{EDTA}/PS_{CoB12} were thus 2.429 and 2.545, which are 1.36 and 1.42 times greater, respectively, than the ratio of their diffusion coefficients (0.70 and 0.39 × 10^{-4} mm^2/s, ratio 1.79). These values correspond to apparent pore radii of 5.8 and 5.2 nm, which are similar to values reported for other unfenestrated capillaries (Haraldsson and Rippe, 1986, 1991; Wahlander et al, 1993). Similar apparent pore sizes could be calculated from maximal PS values obtained by extrapolating extraction values obtained over the flow range of 300 to 500 μL/min (data not shown), although the correlation coefficients of these extrapolations were much lower and were obtained with many fewer data. However, a similar calculation made with the maximal PS values for Na and EDTA gives a ratio that is still considerably less than the ratio of the diffusion coefficients; thus, these conclusions must be treated with caution.

The extraction of albumin relative to Cr-labeled red blood cells is low (1.27 ± 0.48%), which suggests that

little albumin is escaping from the vessels during 1 passage of the blood through the vascular bed of the testis. Furthermore, considerable errors are introduced into any calculation based on this value, which is similar to that reported for pancreas (1.4 ± 3%; Sweiry and Mann 1991), whereas more albumin than red blood cells was recovered in the perfusate from salivary gland (ie, the extraction was apparently negative; Mann et al, 1979). Nevertheless, the PS for albumin in the rat testis can be calculated using the earlier formula to be about 3 μL/(g·min), a value considerably greater than that of about 0.3 μL/(g·min) obtained from the rate of accumulation of marker in the tissue in the hours following injection into the circulation (Haraldsson et al, 1987; Setchell et al, 1988; Pollanen and Setchell, 1989). Not too much emphasis can be placed on this difference because of possible experimental errors and the importance of serum proteins other than albumin, which were not present in the perfusate, in determining the permeability to macromolecules (Haraldsson and Rippe, 1987). Nevertheless, the difference suggests that testis interstitial tissue is an important factor in determining the rate of entry of albumin into the tissue, or that the rate of fluid filtration, rather than permeability, determines the rate at which albumin and, presumably, other proteins such as LH, enter the tissue. It has also been suggested (Ghinea et al, 1994) that human chorionic gonadotropin (hCG) is transported across the endothelial cell layer in the testis by transcytosis involving specific LH/hCG receptors in these cells. Further experiments will be necessary to decide whether this mechanism can explain the access of LH to the Leydig cells, or whether convective transport of LH with other proteins through large pores is involved (Rippe and Haraldsson, 1994).

One factor that was not taken into account in our experiments is the possible role of vasomotion, or the rhythmic fluctuations in blood flow through the testis (Damber et al, 1982, 1986; Setchell et al, 1995) in determining the filtration from, and reabsorption of fluid into, the vasculature. This would probably not affect our measurements of PS area, especially those made at high flow rates, but could have an important influence on the movement of macromolecules such as albumin and the gonadotrophins between the circulation and the tissue. It is not known whether vasomotion persists in the isolated perfused testis. At high flows this would appear to be unlikely, and the extension of our results to an in vivo situation requires caution. The comparison with other tissues is valid, however, because all estimates were made under comparable conditions with perfusates containing sufficient albumin to maintain normal permeability to small solutes and water. The additional serum proteins that are necessary for normal macromolecular exchange do not appear to be important for small, water-soluble markers (Wahlander et al, 1993).

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