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THE HANDLING OF MACROMOLECULES BY THE KIDNEY OF THE RIVER LAMPREY, *LAMPETRA FLUVIATILIS*

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SUMMARY

Micropuncture and renal clearance techniques have been used to investigate the effect of molecular size on glomerular permeability in the river lamprey, *Lampetra fluviatilis*, and to investigate tubular absorption of native and foreign macromolecules.

Polyvinyl pyrrolidone (PVP) molecules of 16 Å in molecular radius were freely filterable but there was significant retention of 37 Å molecules. The estimated sieving coefficients for PVP of 160000 and 360000 in molecular weight (molecular radii > 100 Å) were 0·06 and 0·01 respectively, indicating that the glomerular barrier is relatively leaky.

Up to 50% of microinjected protein was absorbed by the nephron and the results with bovine serum albumin demonstrated that protein uptake occurs chiefly in the proximal brush border segment. The relative rates of uptake for different proteins and different concentrations of injected solutions indicated that the uptake mechanism is easily saturated and unable to distinguish between native and foreign proteins of high molecular weight.

Most lamprey plasma proteins are > 100000 in molecular weight. This, together with other factors such as molecular charge and tubular reabsorption, could account for the low urinary protein concentration of 0·05 mg/ml.

INTRODUCTION

It is well known that small but significant quantities of macromolecules such as albumin and immunoglobulin cross the glomerular barrier of mammals into the ultrafiltrate (e.g. Hardwicke *et al.* 1970); most of these molecules are subsequently reabsorbed by the proximal segment of the nephron (Maunsbach, 1976). There is relatively little published information on these two important aspects of kidney function in lower vertebrates, although a number of anatomical investigations such as those of Morris (1954) and Youson & McMillan (1970*b*) have indicated that the proximal segment of teleosts and cyclostomes is actively engaged in endocytosis. Many ultrastructural features of the proximal epithelium in the river lamprey kidney are consistent with the theory that macromolecules such as plasma proteins are absorbed and transported by the lysosomal pathway (Logan & Morris, 1981). It has

also been shown that the nephron of the sea lamprey, *Petromyzon marinus*, is capable of absorbing exogenous proteins such as horseradish peroxidase (Youson, 1975*b*) but the physiological significance of this is not known.

Glomerular permeability in higher vertebrates has been studied using inert polymers such as dextran and polyvinyl pyrrolidone (PVP) (Brenner, Hostetter & Humes, 1978). A range of PVP molecules was therefore chosen to assess the effect of molecular size on transport through the lamprey glomerulus. Since the ultimate aim of these investigations was to determine the degree of protein leakage through the glomerular barrier, the present study has included an analysis of the size distribution of lamprey plasma proteins.

The second goal of these investigations was to determine the likely fate of any macromolecules which leak into the kidney ultrafiltrate and enter the tubular lumen. The anatomy of the lamprey kidney makes it particularly suitable for micropuncture (Logan *et al.* 1980*a*) therefore a microinjection technique was designed to provide direct evidence of tubular protein uptake and information on the selectivity of the uptake mechanism.

METHODS

River lampreys were trapped during their spawning migration up the River Severn, transported to Nottingham in tanks of cooled aerated water and held in large outdoor concrete tanks suppled with running water. When necessary. lampreys were adapted in cold rooms to the experimental temperature of 12 °C.

Blood sampling and treatment of plasma

Lampreys were anaesthetized in a solution of methane tricaine sulphonate (ethyl-m-aminobenzoate methane sulphonic acid salt, Sigma, 0·01 g/l) and blood was collected either by severing the tail or puncturing the caudal vein with heparinized syringes. Blood was centrifuged immediately; the plasma was separated and either stored under refrigeration or deep frozen.

Three ml lamprey plasma was diluted with 2 ml buffered (Tris/HCl, pH 7·2) saline and applied on a 100 × 3·5 cm column of Sephadex G200 (coarse grade). The eluent was 0·9% saline in Tris/HCl, pH 7·2. Absorption at 280 nm was monitored with an LKB Uvicord and 30 min fractions were collected in an LKB Ultrorac fraction collector. Protein standards (Combithek calibration proteins II, Boehringer Mannheim, GmbH) used for molecular weight calibration of the column were cytochrome *c* (molecular weight 12500), chymotrypsinogen (25000), ovalbumin (45000), bovine serum albumin (BSA) (68000), catalase (240000) and ferritin (450000).

For ion-exchange chromatography, fresh plasma was dialysed in Visking 18/32 tubing against the starting buffer (Tris/HCl, pH 7·2, 0·05, M-NaCl). Five ml of this dialysed plasma was applied to a 15 × 2·5 column of DEAE Sephacel (Pharmacia) which had been equilibrated with starting buffer. The column was eluted with a step-wise gradient of buffered 0·05, 0·1, 0·15, 0·2 and 0·5 M-NaCl. Absorption at 280 nm was monitored with an LKB Uvicord and 80 drop fractions were collected. Those fractions containing the first peak were pooled, concentrated with Aquacide (Calbiochem-Behring Corp.) and finally dialysed against 0·8% saline.

Identification of isolated plasma protein

The protein fraction obtained by ion-exchange chromatography was subjected to electrophoresis on polyacrylamide gel columns (Davis, 1964) and gave a single protein band, corresponding to one of the 'slowest' components of lamprey plasma protein. The molecular weight of this protein, determined on a calibrated 50 × 2·5 cm column of Sephadex G200 (Superfine grade), was 77620. Its relative abundance, molecular weight and electrophoretic mobility indicated that it was lamprey transferrin, previously isolated from sea lamprey serum by Webster & Pollara (1969).

Urinary protein concentration

The protein concentration of lamprey urine was determined by the Folin-Ciocalteau method (Lowry *et al.* 1951).

Isotopes, iodination procedure and radioactive counting

Iodine-125 (specific activity 16·6 mCi/μg) and [^{125}I]polyvinyl pyrrolidone (PVP, mol. wt. 30000–40000; specific activity 46·4 μCi/mg) were obtained from the Radiochemical Centre, Amersham. Polyvinyl pyrrolidones (Fluka, A.G.) of various molecular weights (10000, 160000, 360000) were labelled with ^{125}I according to the method of Gordon (1958). One mCi ^{125}I was used for each 0·1 g PVP to give the required specific activity. Each [^{125}I]PVP solution was dialysed against 0·9% saline for 24 h before use. To determine the amount of unbound label, samples of PVP solution were subjected to cellulose acetate electrophoresis; each membrane was then cut, parallel to the line of origin, to give a series of strips of the same size. The radioactivity in each strip was then determined.

Bovine serum albumin (BSA) (Sigma), whole lamprey plasm and lamprey transferrin were iodinated by the electrolytic method of Rosa *et al.* (1964). Protein concentrations were determined by the Folin–Ciocalteau method. The approximate specific activities of labelled proteins were: BSA, 200 μCi/μmol and 6 mCi/μmol; lamprey plasma protein, 12 μCi/mg; lamprey transferrin, 3 mCi/μmol.

The proportion of radioactive free iodine was determined by adding excess 10% trichlor-acetic acid to the protein sample, centrifuging at 2000 *g* for 15 min and counting the precipitate and the supernatant separately.

Iodine-125 was counted in a well-type sodium iodide crystal scintillation counter (Packard model 3375 or Mini-Instruments type 6.20).

Gel filtration of labelled PVP molecules

Saline, 0·9%, 2·5 ml, containing 5 mg cytochrome *c*, 0·5 mg ferritin and sufficient [^{125}I]PVP to allow detection in the effluent was applied on a 50 × 2·5 cm column of Sephadex G200 (superfine grade) or Sephacryl S300. The eluent was 0·9% saline. Absorption at 280 nm was monitored with an LKB Uvicord. Forty-drop fractions were collected in an LKB Ultrorac fraction collector and the radioactivity in each fraction was determined. From the elution volume (V_e) of each test molecule it was possible to determine the constant, K_{av}. Bed volume, V_t, was given by the elution

volume of a sample of inorganic radioactive iodine. The void volume (V_0) of each column was determined from the elution volume of Dextran Blue 2000 (Pharmacia). For each gel,

$$K_{av} = \frac{V_e - V_0}{V_t - V_0}$$ (see 'Gel Filtration, Theory and Practice', Pharmacia Fine Chemicals),

K_{av} represents the fraction of stationary gel volume which is available for diffusion of a particular solute. It is independent of bed dimensions and variations in packing. There is a good correlation between K_{av} and molecular radius (Laurent & Killander, 1964). The calibration standards were cytochrome *c* (molecular radius 16 Å), chymotrypsinogen (22 Å), ovalbumin (27 Å), BSA (36 Å), immunoglobulin G (56 Å), ferritin (61 Å) and fibrinogen (107 Å). The figures for molecular radii were taken from Laurent & Killander (1964) and Allen, Hill & Stokes (1977).

Micropipette construction and counting of microinjectate

Micropipettes were constructed by drawing out soft glass capilary tubing (1 mm O.D., 0·8 mm I.D., Drummond Scientific Company, U.S.A.) on a pipette puller (Model M1, Industrial Science Associates, Inc.) and the fine section produced was ground to a chamfered point on a revolving Arkansas stone (Oilstone Hb 4, Norton, U.S.A.). Micropipettes with a tip diameter of 5–8 μm were used for puncturing the narrowest nephron segments but proximal tubules were large enough to be entered by pipette tips of 8–12 μm. Urinary ducts were punctured easily with pipettes of approximately 30 μm.

Constriction pipettes were used to deliver a known volume of fluid. The constriction was made in a drawn-out capillary with a hand-built microforge. The pipette was then further drawn-out with the microforge and broken at a point which gave approximately the required volume within the constriction. Each pipette held approximately 20 nl and was calibrated by filling it to the constriction with ^{125}I solution of known specific activity which was then emptied into a counting tube containing a small volume of water.

To prepare and measure the radioactivity of injected solutions, a calibrated (20–60 nl) constriction pipette was used to deposit 5 identical drops of dyed (1% lissamine green) injectate under water-saturated liquid paraffin in a watch-glass. One of these droplets was carefully taken up behind a column of oil in a microinjection pipette and the pipette tip was sealed with a small oil droplet ready for injection. The other 4 aliquots of injectate were transferred individually by pipette into counting tubes containing a fixed volume of water. When counted, these gave an indication of the variation in volume delivered.

Urine collection and micropuncture procedure

Lampreys were immobilized and held in a perspex trough, with their ventral surface exposed, as described by Moriarty, Logan & Rankin (1978). To assess glomerular clearance of macromolecules, [^{125}I]PVP solution (50–100 μl) was injected into the caudal vein of each lamprey and a polythene catheter (PP60, Portex Ltd.) inserted into the urinary papilla for 30 min urine collections.

A 1 cm incision was made with a cautery unit so that the ventral wall could be tied back, exposing the gut, gonads and kidneys. Where necessary the gut and gonads were carefully removed and the gut cauterized. Loss of blood was usually negligible. The kidneys were then flooded with liquid paraffin to prevent dessication. The kidney to be used for micropuncture was illuminated with a cold light source (Intralux 150 H) and viewed through a Wild (M 8) operating microscope.

Nephron capsules were easily accessible for micropunture without further surgery. Micropuncture samples were collected in the manner described by Logan, Moriarty & Rankin (1980*b*).

Small blood samples (100 μl or less) were taken from the injection site in the tail at 30, 60 and 90 min after the injection of labelled substances. Radioactivity in blood plasma was determined for the mid-point of each urine and micropuncture sample collection period.

Pooled urine was dialysed in Visking (8/32 or 18/32) tubing to determine the proportion of free iodide, low-molecular-weight breakdown products or impurities.

For microinjections of labelled solutions, the lamprey kidney was exposed as above. Nephron segments on the dorsal kidney surface (see Logan *et al.* 1980*b*) were made accessible by inserting a tapered polyethylene catheter into the urinary duct and carefully turning over the kidney. The flow of blood and tubular fluid through the kidney was unaffected and in these experiments, urine was collected through the catheter into pre-weighed plastic tubes. Otherwise, a catheter was inserted into the urinary papilla. Timed urine collection periods were from 15 to 30 min duration.

Microinjection rates were controlled by hand so that the dyed injectate could be seen entering the tubular lumen in short pulses which cleared rapidly in the stream of tubular fluid. Injections were from 10 to 20 min duration.

RESULTS

Lamprey plasma and urinary protein

There was no dominant low-molecular-weight protein component (Fig. 1) similar to the albumin component of higher vertebrates. The majority of lamprey plasma proteins were large molecules > 100000 mol. wt.

The concentration of protein in urine collected from lampreys shortly before spawning was 0.05 ± 0.01 mg/ml ($n = 7$).

Leakage of polyvinyl pyrrolidone

Each iodinated PVP solution gave a single peak in radioactivity when eluted from a column of Sephadex G200 or Sephacryl S300. PVP of 160000 and 360000 molecular weight was excluded from both gels. The mean molecular radii for PVP of 10000 and 30000–40000 molecular weight were 16 and 37 Å respectively. Only 2.5%, or less, of the radioactivity in each solution of PVP was lost through dialysis tubing. Cellulose acetate electrophoresis of some 30000–40000 molecular weight PVP solution indicated that 2.5% of the radioactivity was in the form of unbound ^{125}I.

After intravenous injection of PVP solution, radioactivity in the blood of lampreys

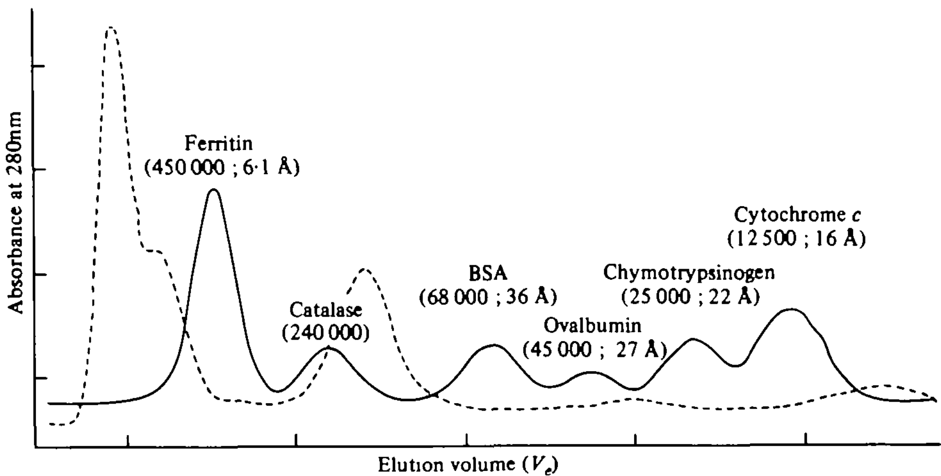

Fig. 1. Molecular size distribution of lamprey plasma proteins (---) determined on a calibrated (——) 100 × 3·5 cm column of Sephadex G200.

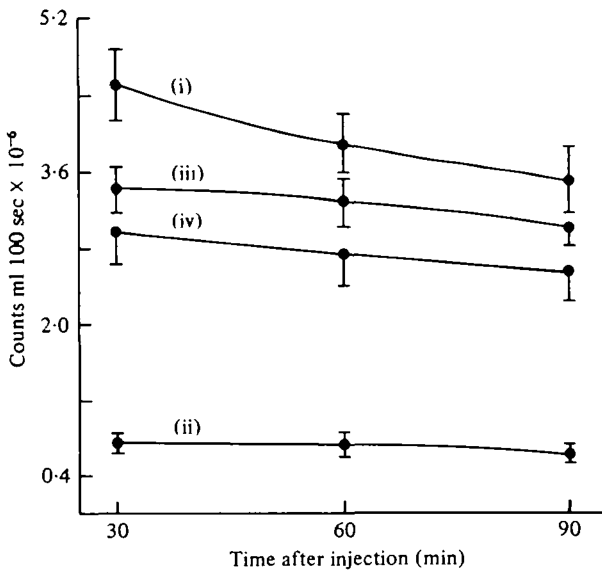

Fig. 2. Levels of radioactivity (mean ± S.E.M. from five fish) in blood plasma of lampreys after intravenous injection of labelled PVP of 10000 (i), 30000–40000 (ii), 160000 (iii) and 360000 (iv) in molecular weight.

fell very slowly for the first 90 min (Fig. 2) although plasma counts fell rather more rapidly in those lampreys given 10000 mol. wt. PVP.

Mean urine/plasma counts after injections of 10000, 30000–40000, 160000 and 360000 molecular weight PVP were 1·22, 0·29, 0·12 and 0·026 respectively (Table 1). The ratio of counts in glomerular filtrate to plasma counts after a single intravenous dose of 10000 mol. wt. PVP was 1·16 ± 0·17 (n = 9).

Table 1. *Mean urine/plasma counts at* 30, 60 *and* 90 *min after intravenous injection of iodinated PVP solution*

Consecutive urine samples after injection of:		Lamprey no. 1	2	3	4	5	6
10000 PVP, overall mean 1·22±0·36	30 min	0·33	0·83	0·55	1·30	1·04	0·86
	60 min	1·57	1·10	0·70	1·33	1·21	0·95
	90 min	1·70	1·35	0·91	2·42	1·64	1·15
	Mean	1·53	1·09	0·72	1·68	1·30	0·99
30000–40000 PVP, overall mean 0·29±0·01	30 min	0·29	0·33	0·26	0·30	0·25	
	60 min	0·27	0·26	0·26	0·29	0·29	
	90 min	0·32	0·28	0·23	0·31	0·35	
	Mean	0·29	0·29	0·25	0·30	0·30	
160000 PVP, overall mean 0·12±0·02	30 min	0·14	0·09	0·11	0·14	0·11	
	60 min	0·19	0·08	0·10	0·11	0·14	
	90 min	0·22	0·12	0·10	0·10	0·12	
	Mean	0·18	0·10	0·10	0·12	0·12	
360000 PVP, overall mean 0·026±0·002	30 min	0·021	0·030	0·025	0·029	0·021	
	60 min	0·020	0·031	0·027	0·027	0·024	
	90 min	0·021	0·031	0·028	0·029	0·025	
	Mean	0·021	0·031	0·027	0·028	0·023	

Dialysis of pooled urine samples from lampreys given 30000–40000 PVP and 160000 PVP indicated that 1·7% and 12·6% respectively of the radioactivity was in the form of free iodine or bound to PVP molecules of less than 5000 mol. wt.

Macromolecular absorption

Inorganic ^{125}I appeared to be readily absorbed by the nephron (Table 2) and this underlined the need to determine the amount of unbound iodine in each solution used for microinjection.

Only 1·2% and 3·3% of the counts in BSA and transferrin injectate, respectively, were non-TCA precipitable – that is, either in the form of inorganic iodine or as molecules with a sedimentation coefficient < 3S (Morris & Morris, 1979). The non-TCA precipitable fraction was higher in both plasma protein preparations (7·7 and 9·2%) and it is thought this was due mostly to labelled proteins of low molecular weight.

The manufacturers (Radiochemical Centre, Amersham) claimed there was less than 5% free iodine in the PVP solution and dialysis using 8/32 Visking tubing gave a figure of 2·1%. When PVP was injected directly into the urinary ducts the percentage recovery in the urine was almost complete, at 98·3 ± 1·7 (n = 8).

Significant amounts of ^{125}I-labelled PVP, BSA, lamprey plasma protein and lamprey

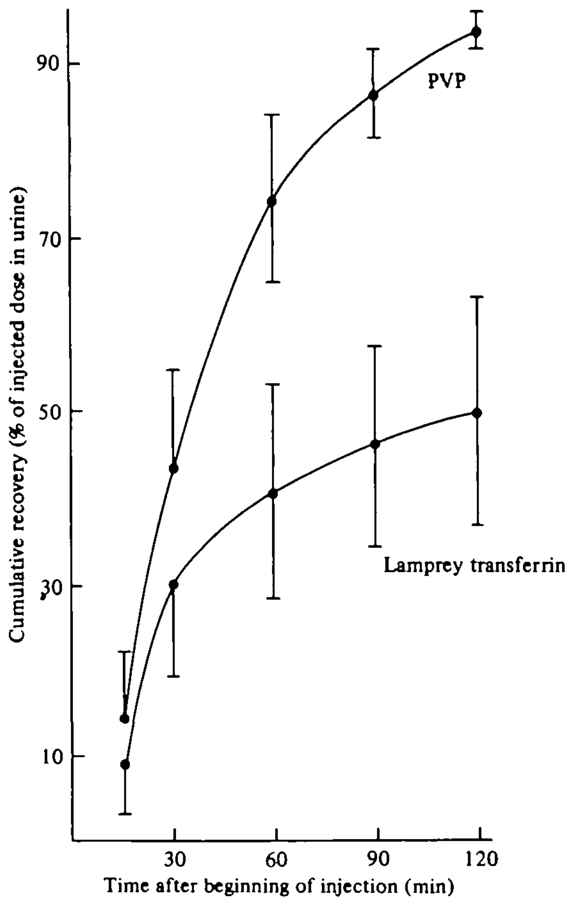

Fig. 3. Cumulative recovery (% of injected dose, mean ± S.E.M.) in the urine of ^{125}I-labelled PVP and lamprey transferrin after injection into the nephron capsule.

Table 2. *Recovery in the urine of inorganic ^{125}I and various labelled macromolecules after microinjection ioto the nephron capsule or proximal segment*

	Concentration of injectate	Recovery (% of injected dose)
Inorganic ^{125}I	7.9×10^{-7} gm atom l^{-1}	44.5 ± 12.0** (7)
[^{125}I]PVP	8.6×10^{-5} M†	92.3 ± 0.7*** (18)
[^{125}I]BSA	3.0×10^{-4} M	87.3 ± 2.3** (6)
	4.4×10^{-6} M	49.2 ± 7.3*** (6)
[^{125}I]lamprey plasma protein	5.4 mg ml^{-1}	81.1 ± 2.2*** (17)
[^{125}I]lamprey transferrin	3.4×10^{-5} M	58.1 ± 6.4** (6)

† Assumes mean molecular weight of 35000. Significance of differences from 100 are given: *** $P < 0.001$, ** $P < 0.01$.

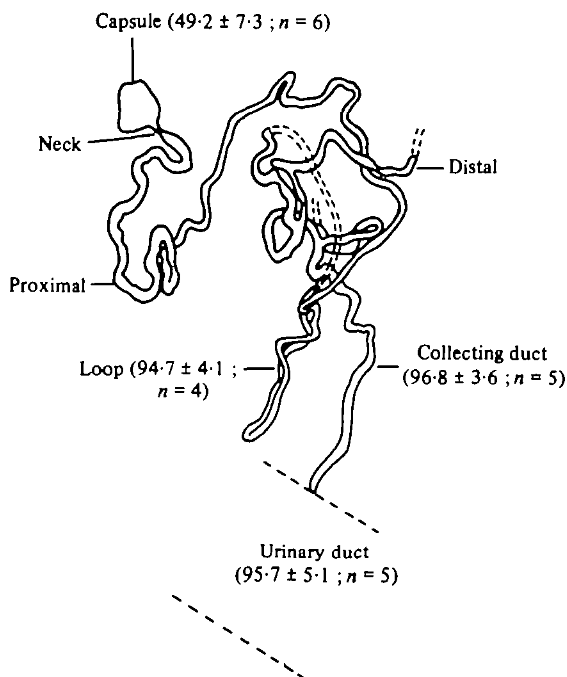

Fig. 4. Recovery (% of injected dose, mean ± S.E.M.) in the urine of ^{125}I-labelled BSA after injection into different nephron segments. (For detail of kidney anatomy, see Logan *et al.* 1980*a*.)

transferrin were not recovered in the urine after microinjection into the nephron lumen (Table 2) and some of each labelled compound was presumed to have been absorbed.

Radioactivity in the urine was highest within 30–60 min of injection (Fig. 3) and generally returned to background level within 2 h of injection.

Since only 3000–9000 counts/100 s were injected, the apparent absorbed fraction in most cases amounted to less than 1000 counts/100 s. Some of these counts were detectable in the injected kidney at the end of the experiment (up to 20% of the injected counts in the case of transferrin), but the fate of most of the absorbed macromolecules could not be determined.

When urine was collected simultaneously from both ducts after transferrin injection, no radioactivity was detected in the urine from the non-injected kidney.

It was found that when the protein concentration of the injectate was reduced (and specific activity increased to give adequate counts in each dose), this increased the apparent fractional absorption. Thus for BSA, a reduction from 3×10^{-4} to 4.4×10^{-6} M gave a reduction in urinary recovery (Table 2) and an increase in apparent tubular absorption from approximately 13% to 51%. At the end of each microinjection experiment, up to 50% of the injected counts were detected in a small piece of kidney containing the injected nephron. The remainder of each kidney gave only background counts.

There was almost complete urinary recovery of labelled BSA injected into th lamprey nephron beyond the proximal segment (Fig. 4) and at the end of each experiment, pieces of kidney containing the injected tubules gave only background counts.

DISCUSSION

(a) *Permeability of the glomerulus*

As expected, the lamprey glomerulus discriminated between molecules according to size. It was assumed that other factors such as charge and molecular shape were the same for each PVP preparation. PVP molecules whose molecular radii were 16 Å appeared to pass freely through the glomerulus (glomerular filtrate: plasma was 1·16). Hardwicke *et al.* (1970) found that PVP molecules of 25 Å were filtered across the human glomerulus as readily as creatinine whilst neutral dextrans of 20 Å or less were freely filterable across the rat glomerulus (Chang *et al.* 1975*b*). Unfortunately, our investigations were limited to PVP preparations of 16 and 37 Å but the results indicated that the 'cut off' point was between these molecular sizes.

The urine/plasma concentration ratio for 37 Å PVP was 0·29. Inulin clearance was not measured in the same collection periods but Logan *et al.* (1980*b*) found that water reabsorption by the lamprey kidney was fairly constant in the short-term and mean urine/plasma for inulin was 1·8. Combination of these figures gave a filtration factor (ultrafiltrate/plasma concentration) or sieving coefficient for 37 Å PVP of 0·16. This was almost one half that of similar PVP preparations crossing the human glomerulus (Hardwicke *et al.* 1970), but approximately the same as that of neutral dextran, of the same molecular size, in the rat glomerulus (Brenner *et al.* 1977).

There was a surprisingly high count-rate in the urine of lampreys following injection of 160000 molecular weight PVP (molecular radius > 100 Å). Neutral dextrans > 42 Å were almost totally excluded from the ultrafiltrate of rats (Chang *et al.* 1975*a*; Brenner, Hostetter & Humes, 1978) and PVP molecules > 60 Å were completely excluded from the ultrafiltrate of human kidneys (Hulme & Hardwicke, 1966). PVP molecules of 70 Å in molecular radius were detected, however, in the urine of patients with diseased kidneys. Unfortunately, there was insufficient radioactivity in the urine of lampreys given PVP of 160000 and 360000 in molecular weight to permit gel filtration of this urine on Sephadex but diaylsis through Visking tubing revealed that only 12·6% of the radioactivity in the urine was due to free iodine or molecules less than 5000 in molecular weight. The solutions of 160000 and 360000 mol. wt. PVP gave single sharp peaks when eluted from Sephadex G200, but there may have been trace amounts of molecules which were small enough to cross the glomerular barrier. The true sieving coefficients for these large PVP molecules may therefore have been less than those indicated by the results (0·06 and 0·01 for 160000 and 360000 mol. wt. PVP, respectively). Tubular reabsorption, if operating, would have had the opposite effect on apparent sieving coefficients.

The glomerulus of the lamprey appears to be rather more permeable than that of higher vertebrates to large PVP molecules. Hulme & Hardwicke (1966) observed a difference in PVP clearance between normal and diseased human kidneys. In patients with heavy proteinuria, there was increased permeability to large PVP molecules and

reduction in permeability to small PVP molecules. The relatively wide, fibrous basement membrane of the lamprey glomerulus (Youson & McMillan, 1970*a*; Logan & Morris, 1980*b*) is certainly reminiscent of some pathological conditions in mammals which cause proteinuria (Simon & Chatelanat, 1969). Nevertheless, without direct measurement of protein concentration in lamprey kidney ultrafiltrate, it is not possible to conclude that there is more or less protein leakage than in higher vertebrates.

There are a number of other factors, including the unusually large size of lamprey plasma proteins, which must affect transport through the glomerulus. It is unlikely that the lamprey glomerulus discriminates according to molecular size alone. The clearance of albumin in the mammalian kidney was much lower than expected on the basis of size alone (Gaizutis, Pesce & Lewy, 1972; Eisenbach, van Liew & Boylan, 1975). Anionic molecules were inhibited by some component of the glomerular wall (Chang *et al.* 1975*a*) and positively charged molecules crossed the barrier more readily than neutral ones of a similar size (Bohrer *et al.* 1978). Anionic sites were identified in the glomerular basement membrane (Caulfield & Farquhar, 1976; Kanwar & Farquhar, 1979), suggesting that the basement membrane selected macromolecules according to molecular size and charge.

The location of the main barrier to filtration of macromolecules within the lamprey glomerulus is unknown. Schaffner & Rodewald (1978) found that the glomerular basement membrane of the bullfrog kidney was permeable to ferritin (molecular radius 61 Å) but passage into the ultrafiltrate was prevented by the slit diaphragms. Youson (1975*a*) was unable to identify the main barrier to macromolecular filtration in the sea lamprey kidney, but the slit diaphragms retarded movement of ferritin. It may be that during the evolution of an efficient glomerular barrier there has been a trend towards a less permeable basement membrane (Schaffner & Rodewald, 1978) and that the lamprey glomerulus illustrates the 'primitive' condition.

(*b*) *Macromolecular absorption*

It has been shown that inorganic ^{125}I was readily absorbed by the lamprey kidney. A similar proportion of injected iodide was absorbed by the proximal segment of the rat nephron (Cortney, Sawin & Weiss, 1970). There was no noticeable uptake of iodide following microinjection into the distal rat nephron, therefore it seems unlikely that iodine was simply diffusing into the blood.

Even when uptake of free iodide by the lamprey kidney was taken into account there must have been significant absorption of native and foreign macromolecules (Table 2). It is unlikely that leakage from the injection site or some other form of experimental error could account for the apparent uptake since results from only the technically perfect experiments were included. As others have pointed out (Maude *et al.* 1965), most sources of error in such experiments would lead to over-estimates of tubular reabsorption. It has been shown clearly, for example, that increased intratubular pressure, such as that caused by excessive perfusion pressure or injection rate, can cause leakage of macromolecules into the intracellular spaces (Ottosen, 1976) and presumably from there into the blood. This was avoided in the lamprey by injecting dyed solutions only in small pulses which were rapidly diluted and cleared by the flow of tubular fluid. The usual injection rate was 1–2 nl/min, compared with a mean

single nephron filtration rate of 7 nl/min (Moriarty, Logan & Rankin, 1978). Injection rates used in the rat were much higher in relation to tubular flow rate (Cortney *et al.* 1970).

There was almost complete recovery in the urine of [^{125}I]PVP and [^{125}I]BSA after injection into the urinary duct, therefore it is unlikely that the wall of the duct is normally involved in protein uptake. Youson (1975*c*) came to the same conclusion after electron microscopic tracer studies in the sea lamprey..

Recoveries in the urine of [^{125}I]BSA injected into different nephron segments (Fig. 4) indicated that only the proximal segment was significantly involved in protein uptake. The cells of the proximal segment had a pronounced brush border, below which were numerous endocytic vesicles and lysosomes (Logan & Morris, 1981), as might be expected of cells which were actively engaged in endocytosis (Maunsbach, 1976).

The decrease in fractional reabsorption of BSA by lamprey proximal cells following an increase in injected load (from data in Table 1) suggested that the uptake mechanism was easily saturated. Cortney *et al.* (1970) injected different concentrations of various proteins into the rat nephron and found that the fractional absorption of each protein was independent of the concentration of the injectate. They calculated, for example, that only 22% of labelled albumin (20, 40 or 100 mg/100 ml) was reabsorbed by the proximal segment and argued that this was the proportion of filtered albumin normally reabsorbed by the kidney. It has been shown, however, that the absorptive mechanism of the lamprey nephron is more efficient than this and Galaske, Baldamus & Stolte (1978) have shown recently that 92% of filtered albumin was reabsorbed by the rat nephron and the transport maximum was almost reached in the normal animal. An increase in filtered load of albumin simply resulted in reduced fractional absorption, as in the lamprey.

Fractional absorption of [^{125}I]lamprey transferrin (approximately 40% of injected dose) was similar to that expected for [^{125}I]BSA of the same molecular concentration. It therefore appears that, as in the rat (Galaske *et al.* 1978), the uptake mechanism did not distinguish between different proteins of high molecular weights.

Fractional absorption of PVP by the lamprey kidney was considerably less than that of both foreign and native protein (Table 1). Allowing for 2% free iodine in the injectate, absorption of PVP was only approximately 6%. Moriarty *et al.* (1978) found that recovery in the urine of [^{3}H]inulin and [^{51}Cr]EDTA after tubular microinjection was 83·5 and 90·2% respectively. It was likely that experimental error accounted for some of the 'lost' inulin and it is reasonable to assume that PVP, inulin and EDTA were taken up by the same mechanism. The relative rates of uptake indicated that this was a less-efficient process than that for protein.

PVP was taken up by endocytic vesicles in the rat yolk sac (Williams *et al.* 1975*a*) but albumin was adsorbed on to the plasma membrane and taken up much more quickly than PVP (Williams *et al.* 1975*b*). It has been shown that, probably as the first step in pinocytosis, proteins were bound to isolated renal brush border membranes (Just & Habermann, 1973) and the degree of binding was dependent on the isoelectric point of the protein. The binding process demonstrated saturation kinetics and this could explain the different rates of uptake for proteins and neutral molecules such as PVP.

It is not known whether the lamprey kidney has a significant role in the catabolism of plasma protein. This study has shown that the lamprey glomerulus may be rather more permeable to large macromolecules than that of higher vertebrates, but lamprey plasma proteins are unusually large molecules to that the protein load to the proximal absorptive cells may be no greater than in other vertebrates.

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