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LACK OF GLOMERULAR INTERMITTENCY IN THE RIVER LAMPREY *LAMPETRA FLUVIATILIS* ACCLIMATED TO SEA WATER AND FOLLOWING ACUTE TRANSFER TO ISO-OSMOTIC BRACKISH WATER

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Summary

Previous studies have suggested that in the lamprey *Lampetra fluviatilis*, in contrast to teleost fish, all glomeruli are actively filtering. In the present study, we have applied the ferrocyanide technique to obtain more definitive values for the population of filtering nephrons in the lamprey under conditions of high (in fresh water) and low (in sea water) glomerular filtration rate (GFR) and when the branchial osmotic gradient was eliminated by acute transfer of freshwater lampreys to iso-osmotic brackish water. These studies demonstrated that the renal antidiuresis in lampreys acclimated to full-strength sea water does not involve any reduction in the filtering population of glomeruli. Transfer to brackish water significantly reduced GFR and thereby urine flow rate (287±23 ml kg^{-1} 24 h^{-1} in fresh water; 6.9±2.5 ml kg^{-1} 24 h^{-1} in brackish water). In four of the eight fish examined, 100 % of glomeruli remained filtering; in the other four fish, non-filtering glomeruli occurred in patches along the kidney, always associated with an absence of vascular perfusion, which implies possible endocrine/neural control of vascular tone. The numbers of non-filtering glomeruli were always small, and these glomeruli do not appear to make a major contribution to the overall decline in urine output. The results provide firm evidence that although lampreys, like teleosts, show considerable variations in urine output, the renal mechanisms by which lampreys and the teleosts achieve this differ fundamentally, with glomerular intermittency playing little or no part in the lamprey.

Key words: lamprey, *Lampetra fluviatilis*, kidney, glomerulus, seawater acclimation, brackish water.

Introduction

Variations in urine output by the vertebrate kidney can be achieved by variations in glomerular filtration rate (GFR) or by an alteration in tubular water reabsorption. While the latter strategy is typical of the mammalian kidney, lower vertebrates usually demonstrate labile filtration rates (Dantzler, 1989). The parallel changes in urine flow rate, GFR and the tubular transport maximum for glucose demonstrated by amphibians and teleost fish have provided indirect evidence of changes in the population of actively filtering nephrons, a phenomenon known as ‘glomerular intermittency’ (Forster et al., 1942; Lahlou, 1966; Brown et al., 1980).

In the rainbow trout *Oncorhynchus mykiss*, the phenomenon of glomerular intermittency has been clearly demonstrated by direct analysis of filtering populations after intravascular injection of concentrated ferrocyanide and subsequent precipitation of visible Prussian Blue (Brown et al., 1980, 1993). At any one time, a relatively large population of glomeruli, approximately half the total population, are actively filtering in freshwater-adapted fish whereas less than 10 % of glomeruli are filtering in the kidneys of seawater-adapted trout.

The river lamprey *Lampetra fluviatilis*, which migrates between fresh water and sea water, can, like euryhaline teleosts, adapt to a range of salinities (depending on seasonal and developmental factors) with profound changes in renal function (Brown et al., 1993; Rankin, 1997). Urine output decreases at high external salinities as a result of variations in GFR (Rankin et al., 1980). In lampreys adapted to full-strength sea water, GFR and urine output are very low (as in marine teleosts), but tubular water reabsorption is also increased (Logan et al., 1980b). Micropuncture studies indicated that changes in GFR result from changes in the rates at which individual glomeruli filter (single-nephron glomerular filtration rate; SNGFR) rather than from changes in the number of glomeruli filtering (Rankin et al., 1980). When freshwater lampreys were transferred to approximately iso-osmotic brackish water, a reduction in effective filtration pressure (EFP) in the glomeruli accounted for the observed drop in SNGFR (McVicar and Rankin, 1985).

Estimated populations of filtering nephrons, calculated by dividing whole-kidney GFR by mean SNGFR, are in approximate agreement with anatomical estimates of

glomerular number (Logan et al., 1980a), suggesting that all glomeruli are filtering in both freshwater-adapted lampreys and lampreys adapted to 50 % sea water (Moriarty et al., 1978; Brown et al., 1993). However, because of the errors inherent in measurements of GFR and SNGFR and in anatomical estimates of glomerular number, these calculations cannot rule out the occurrence of glomerular intermittency. More direct evidence is required for that.

The aim of the present studies was to apply the ferrocyanide technique in lampreys under conditions of high (in fresh water) and low (in sea water) GFR and to repeat the protocol of McVicar and Rankin (1985), which characterized the alterations in filtration dynamics following transfer from fresh water to iso-osmotic brackish water, to determine whether any change in filtering nephron number was involved in the acute reduction in GFR.

Materials and methods

Lampreys

River lampreys *Lampetra fluviatilis* L. were caught in eel traps in the brackish water (440 mosmol kg^{-1}) of Ringkøbing Fjord, west Jutland, in September 1994 and 1995 and, following transport to the Biologisk Institut, Odense, were kept in brackish water (513 mosmol kg^{-1}) at ambient temperature or transferred to fresh water ($[Na^+]$, 1.46 mmol l^{-1}; $[Cl^-]$, 1.39 mmol l^{-1}; $[K^+]$, 0.16 mmol l^{-1}; $[Mg^{2+}]$, 0.64 mmol l^{-1}; $[Ca^{2+}]$, 3.03 mmol l^{-1}; $[NO_3^-]$, 0.028 mmol l^{-1}; $[SO_4^{2-}]$, 1.54 mmol l^{-1}) at 10 °C. After 1 week, the salinity of the brackish water was increased by gradual addition of North Sea water, increasing the osmolality to 895 mosmol kg^{-1} over 40 days. The temperature was gradually reduced to 10 °C during the acclimation period. There was no mortality in the group of fish transferred to sea water. Experiments were carried out on freshwater-acclimated lampreys in fresh water and following acute transfer to 25 % sea water (260 mosmol kg^{-1}) in March of two consecutive years. Further experiments on seawater-acclimated lampreys (sea water at 895 mosmol kg^{-1}) were performed in October.

Experimental procedures

Lampreys (35–77 g) acclimated to either fresh water or North Sea water were anaesthetised (3-aminobenzoic acid ester methanesulphonate, Sigma; 0.065 g l^{-1}, pH in fresh water 7.66) and placed on their backs in a Perspex trough with the head immersed in 800 ml of aerated anaesthetic solution maintained at 10 °C; the rest of the body was covered with damp tissue. Respiratory movements were monitored, and light anaesthesia was achieved by manipulating the concentration of anaesthetic to maintain strong ventilation and thus avoid any reduction in urine flow due to anaesthesia (McVicar and Rankin, 1983). The protocols for the three groups were as follows.

Group A. Freshwater-acclimated lampreys in fresh water. Fish were netted from the stock tank with minimal stress and placed in a bucket containing anaesthetic in fresh water. Once anaesthetised, they were weighed, placed in the trough, and the urinary papilla was catheterized (see below). Urine was collected, generally over 15 min periods, for a total of 1.5–5.5 h, the aim being to ensure that urine was being produced consistently at rates typical of freshwater lampreys, before infusion of ferrocyanide.

Group B. Freshwater-acclimated lampreys transferred to brackish water. Fish were handled as described above and placed initially in fresh water in the trough for periods of 2–3.5 h. The fresh water was then replaced with brackish water (an iso-osmotic dilution of sea water: 259.9±1.2 mosmol kg^{-1}), and the experiment was continued for 4–5 h until consistent low urine flow rates were achieved before the ferrocyanide infusion. In three experiments, GFR was measured. For these experiments, lampreys from a freshwater stock tank were anaesthetized in fresh water and then transferred immediately to brackish water in the trough to follow the decline in GFR and urine flow rate.

Group C. Seawater-acclimated lampreys in sea water. Fish from a seawater stock tank were anaesthetised in sea water, weighed and then placed in a trough with the head immersed in North Sea water (895±2.6 mosmol kg^{-1}) containing anaesthetic. Three fish were maintained under anaesthesia for 5.5–7.5 h, long enough to ensure that they were producing urine and to estimate urine flow rates (see below), before ferrocyanide infusion. Initial and final blood samples were collected (as described below). These samples indicated that plasma osmolality had increased during the long period of anaesthesia. To avoid any potential disturbance of renal function due to this acute rise in blood osmolality, two further seawater-acclimated fish were infused with ferrocyanide as soon as possible after initiation of anaesthesia (60 min and 69 min, which was long enough to ensure stable light anaesthesia, to check plasma osmolality, to obtain a urine sample for analysis and to prepare the fish for infusion).

Blood sampling

Initial blood samples (approximately 0.2 ml) were taken with heparinised 1 ml syringes from a superficial ventral vein in the tail. In some cases, a second blood sample was taken from the caudal vein (which lies deeper) immediately prior to ferrocyanide administration. Syringes were heparinised with ammonium heparin (approximately 20 µl of distilled water containing approximately 40 i.u. of ammonium heparin; Leo Pharmaceutical Products, Denmark) and were either allowed to dry overnight or weighed prior to and after loading so that correction of measured plasma osmolality and ion concentrations could be made.

Urine collection

The urinary papilla of each anaesthetised fish was catheterised with a short length of polyethylene tubing (Portex, UK; 0.7 mm internal diameter) with a slight constriction near the tip to allow firm ligaturing into the papilla. The catheters

were initially filled with distilled water and led to pre-weighed microcentrifuge tubes below the lamprey box so that the urinary ducts (lampreys have no bladder) were continually drained by slight suction.

In freshwater fish, high rates of urine production allowed the collection of a series of timed urine samples and gravimetric determination of urine flow rates. Low urine flow rates following transfer to brackish water necessitated collection over prolonged periods, under paraffin oil to avoid evaporative loss. To obtain urine samples from seawater fish, in which the rate of urine production is extremely low, the body cavity was gently squeezed and 'milked' to empty the urinary ducts into an air-filled catheter tied into the urinary papilla. In the three fish in which urine flow rate was estimated, the catheter was sealed at a known time so that urine accumulated in the urinary ducts and, immediately prior to the ferrocyanide experiments, the catheter was unsealed and the ducts 're-milked' to collect the urine for analysis and estimation of flow rate. Urine volumes in these fish were determined by aspiration into a 25 µl Gilson positive displacement pipette.

Urine and plasma analysis

Urine and plasma osmolalities were measured on 10 µl samples using a Wescor 5500 vapour pressure osmometer, except for very dilute urine samples which were measured on a Gonotech Osmomat 030 freezing-point osmometer, which was also used for bath-water samples. Analysis of inorganic anions and cations was carried out by ion chromatography (Dionex 4500I) after sample dilution by 50-fold (freshwater urine) or 200-fold (all other samples) with linear three-point calibrations after each 20 or so samples.

Measurement of glomerular filtration rate

Three freshwater-acclimated lampreys received intraperitoneal injections of 370 kBq (10 µCi) of [^{3}H]inulin (Amersham) in 100 µl of distilled water 18–22 h prior to the experiment. After this time, plasma inulin content varied by 0.7–4.7 % h^{-1}, which permits estimation of GFR from two blood samples. Although far from ideal, this was considered preferable to risking excessive changes in blood volume by taking multiple samples. After being anaesthetized in fresh water, fish were transferred to brackish water in the experimental troughs, and the first blood sample (see above) was taken immediately. Urine was then collected until the second blood sample was taken 5–6.25 h after the first and immediately prior to ferrocyanide infusion. Duplicate 25 µl plasma and urine samples were counted in Packard Ultima Gold LSC cocktail in a Packard 2200 CA tricarb liquid scintillation counter with external standard quench correction. GFR was calculated as UV/P, where U is urine disints min^{-1} μl^{-1}, V is urine flow rate (ml kg^{-1} 24 h^{-1}) and P is the estimated plasma disints min^{-1} μl^{-1} at the calculated midpoint of the urine formation time (after allowing for catheter dead space measured at the end of each experiment).

Ferrocyanide technique for assessment of the filtering population of glomeruli

The filtering population of glomeruli was determined by modification of the ferrocyanide technique described by Brown et al. (1980). The dorsal aorta was exposed by a short mid-ventral incision, just posterior to the liver and well anterior to the kidney. In some fish examined during March, the removal of eggs or a small amount of testis was necessary to expose the aorta. The aorta was punctured with the end of a 23 gauge needle bent to lie parallel to the aortic wall and attached to a length of catheter (Portex 0.58 mm external diameter). Sodium ferrocyanide (20 % w/v) was infused intra-aortically at 80–200 µl min^{-1} depending upon the size of the fish (Sage infusion pump or more approximately by hand) for 0.5–1.5 min, with larger fish receiving longer infusions. Towards the end of the infusion, isopentane, cooled to −160 °C with liquid nitrogen, was poured onto the kidney and into a well between the tissue paper and the posterior of the fish to submerge and snap-freeze the body. The frozen fish was rapidly severed anterior and posterior to the kidney region and dropped into isopentane at −160 °C.

Several minutes later, the ventral half of the frozen body segment was rapidly sawn off, removing the body wall, gut and gonad and almost exposing the kidneys. This was essential to ensure rapid penetration of the −20 °C alcoholic ferric chloride into which the section of fish was immediately transferred. It was then left overnight at −20 °C for the formation of insoluble Prussian Blue (ferric ferrocyanide) precipitate as described previously (Brown et al., 1980; Amer and Brown, 1995).

The following day, the kidney was dissected out, generally as several large pieces, and transferred to ferric chloride wash solution (0.2 % $FeCl_3$ and 1 % acetic acid) for storage at 10 °C until acid maceration (in 20 % HCl at 40 °C for 1.5–2 h) within 1 week. Pieces of macerated kidney were stored in the ferric chloride wash solution until microdissection in glycerine using fine glass needles. During microdissection, the line of glomeruli running along each kidney, with neck segments and attached tubules arising from these, was easily discernible within a segment of kidney. For each kidney, glomeruli with their attached nephrons were microdissected from at least four different pieces of kidney selected at random and classified as (i) filtering (Prussian Blue precipitate in glomerular capillaries and tubule) or (ii) non-filtering (clear glomerular capillaries and tubule). Where both types existed, microdissection was continued until a constant value for the proportion of each type was obtained; in individual preparations, this required microdissection and classification of 49–163 nephrons obtained from 4–13 separate pieces of kidney.

Statistical analyses

Urine flow rates in the three experimental groups were compared using Student's *t*-tests with sequential Bonferroni rejection tests to exclude type 1 errors (Rice, 1989).

Table 1. *Urine flow rates and population of filtering nephrons (%) in individual freshwater-acclimated and seawater-acclimated lampreys and in individual fish transferred to brackish water*

Fresh water		Sea water		Transfer to brackish water	
V (ml kg^{-1} 24 h^{-1})	Filtering (%)	*V* (ml kg^{-1} 24 h^{-1})	Filtering (%)	*V* (ml kg^{-1} 24 h^{-1})	Filtering (%)
374	100 (19)	2.0	100 (56)	2.3	92.7 (55)
305	96 (26)	2.0	100 (66)	4.2	63.8 (163)
285	100 (23)	4.5	100 (50)	14.2	100 (87)
290	100 (53)	–	100 (65)	17.9	100 (67)
200	100 (61)	–	100 (81)	–	100 (92)
267	100 (50)			1.1	100 (49)
				5.6	88.0 (69)
				2.8	98.9 (90)
Mean 286.8±23.0		2.83±0.83***		6.87±2.46***	

V, urine flow rate.

The numbers of nephrons microdissected and classified are given for each fish in parentheses.

Urine flow in seawater-acclimated fish and in fish transferred to brackish water compared with freshwater fish: ***P<0.001 (Student's *t*-test with Bonferroni sequential rejection; Rice, 1989).

Results

Lampreys in fresh water demonstrated high urine flow rates (Table 1). Lampreys transferred from fresh water to iso-osmotic brackish water showed a rapid decline in urine flow rates stabilising within 3–4 h at levels approximately 2.5 % of those in freshwater fish (286.8±23.0 ml kg^{-1} body mass 24 h^{-1} in freshwater lampreys, 6.9±2.5 ml kg^{-1} body mass 24 h^{-1} in lampreys transferred to brackish water; Fig. 1; Table 1). This reflected a declining GFR (Fig. 1). On the basis of urine/plasma inulin concentration ratios, calculated tubular water reabsorption increased from 36.5±3.4 % to 70.1±4.8 % (means ± S.E.M.) in the three fish in which inulin was administered. The osmotic and ionic compositions of plasma samples taken before or immediately after transfer were typical of freshwater-acclimated lampreys (Table 2). In three fish in which initial and final blood samples were taken (for counting), there was no change in plasma osmolality during the experiment (–3 %, 0 %, +2 %). Most of the small urine samples were used for counting, but final urine (catheter contents) was analysed in two fish, and osmolality was measured in one final and one late (4 h to 5 h collection period) urine sample from a further two fish. These analyses indicated that urine was still typical of very dilute freshwater urine in all four fish.

The ferrocyanide technique demonstrated that virtually 100 % of nephrons were filtering in freshwater-acclimated lampreys (Fig. 2A–D; Table 1). Only a single nephron in a single fish appeared to be non-filtering. After transfer to brackish water in four of the eight fish studied, 100 % of glomeruli were filtering (Table 1); in the other four fish, a small population of glomeruli (mean 14 %) were non-filtering (Fig. 3A–D). The non-filtering glomeruli occurred in patches along the kidney (Fig. 3A,B) and were usually associated with an apparent lack of complete vascular perfusion of the renal artery, afferent arteriole and glomerular capillaries (Fig. 3A–D). Taking the mean value for all eight fish (which is probably not very meaningful), only 7 % of glomeruli in fish transferred to brackish water were non-filtering.

Nephrons in the five seawater lampreys investigated were consistently filtering. This applied both to the three lampreys that had been in anaesthetic for 6–7.4 h (as in brackish-water transfer experiments), so that urine flow could be estimated (Table 1) and the urine analysed (Table 2), and to those that had been in anaesthetic for approximately 1 h. In contrast to the transferred fish, the three fish held in sea water for some time showed increases in plasma osmolality over this period of 4.5 % h^{-1}, 6.0 % h^{-1} and 5.1 % h^{-1}, rising to 300, 341 and

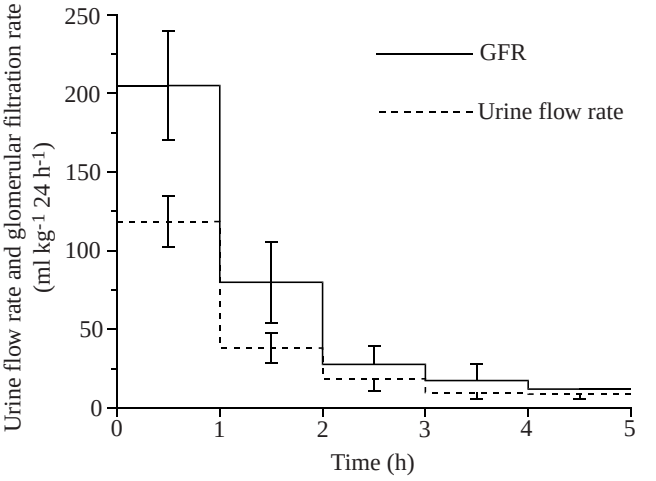

Fig. 1. Urine flow rates and glomerular filtration rates (GFR) of lampreys transferred from fresh water to iso-osmotic brackish water, prior to ferrocyanide experiments. Values (means ± S.E.M.) for GFR are from three fish; values for urine flow are from eight fish including those for which GFR was measured.

Table 2. *Urine and plasma osmolality and ionic concentrations in urine and plasma samples from the acute-transfer and seawater-acclimated groups*

	Osmolality (mosmol kg^{-1})	$[Na^+]$ (mmol l^{-1})	$[K^+]$ (mmol l^{-1})	$[Mg^{2+}]$ (mmol l^{-1})	$[Ca^{2+}]$ (mmol l^{-1})	$[Cl^-]$ (mmol l^{-1})	$[SO_4^{2-}]$ (mmol l^{-1})
Acute transfer							
Initial plasma (*N*=6)	274.5±8.0	129.5±4.7	2.50±0.89	0.76±0.13	3.91±2.50	117.6±1.3	1.63±0.05
Final urine (*N*=2+2)	18	4.5	<0.04	2.5	2.1	6.4	0.67
Sea-water acclimated							
Initial plasma (*N*=8)	269.0±6.8	144.2±5.0	2.86±0.95	1.44±0.50	2.62±0.38	118.2±3.1	2.43±0.18
Urine (*N*=4)	367.5±12.9	6.9±2.1	<0.13	184.5±9.7	6.25±1.68	135.4±24.6	135.2±0.2

Values are means ± S.E.M.

319 mosmol kg^{-1} from starting values of 243, 248 and 259 mosmol kg^{-1} respectively. For these fish, urine flow rates were one-third to two-thirds of the mean rate seen in fish acutely transferred to isotonic brackish water (Table 1). Urine was hyperosmotic in all four of the seawater fish studied. Dionex analyses showed that urinary Na^+ concentration was extremely low and K^+ was undetectable in most samples, but there was a high concentration of Mg^{2+} and SO_4^{2-} (Table 2).

Discussion

Previous studies have suggested that glomerular intermittency, a phenomenon well known in teleosts (Brown et al., 1980, 1993), does not occur in lampreys despite the apparent similarities at a gross level between the renal adaptations to salinity shown by these groups. Thus, estimations of the filtering population of glomeruli based on whole GFR and SNGFR suggested values in line with anatomical estimates of the total population of glomeruli (Logan et al., 1980a; Brown et al., 1993). However, because of the errors inherent in measurement of both GFR and SNGFR and in estimating the glomerular number, direct evidence for the absence of glomerular intermittency was sought. The present studies provide this evidence and indicate a distinct absence of any change in glomerular population in circumstances where urine flow and GFR are profoundly reduced, namely acclimation to sea water and following acute transfer to iso-osmotic brackish water.

In each of the seawater-acclimated lampreys, urine was hyperosmotic to the initial plasma sample. This agrees with previous reports that the lamprey is able to produce hyperosmotic urine (Logan et al., 1980b). Dionex analyses showed that urinary $[Na^+]$ was extremely low (6.9 mmol l^{-1}) and $[K^+]$ too low to be measured accurately at the dilution used. A much higher urinary Na^+ concentration (70±19 mmol l^{-1}; mean ± S.E.M., *N*=9) was measured by Rankin (1997), but this was for lampreys kept in sea water until the spring, although they would normally have migrated to fresh water in the autumn. The production of urine with a Na^+ concentration lower than that of plasma cannot assist in the elimination of excess Na^+ in a seawater fish because all water lost has to be replaced by drinking sea water, from which virtually all the Na^+ (99.3 %; Rankin, 1997) must be absorbed to get the water into the body. All elimination of excess Na^+ occurs across the gills. Urine is the route of Mg^{2+} and SO_4^{2-} excretion which, given that maximum urine osmolality is less than 100 mosmol kg^{-1} greater than plasma osmolality, can be achieved with minimal water loss by producing urine almost devoid of Na^+. The urine contained high levels of divalent ions, predominantly Mg^{2+} and SO_4^{2-}, at concentrations similar to those previously reported (Logan et al., 1980b; Rankin, 1997).

In the first three seawater fish investigated, plasma osmolality increased by 5.5 % h^{-1} between the first blood sample collected soon after initiation of anaesthesia and the second blood sample collected some 4–6 h later. This effect is likely to reflect an inhibition of drinking in anaesthetised fish (Rankin, 1997), which would have led to a progressively increasing level of dehydration. Two fish were therefore investigated more rapidly, without attempting to measure urine flow rate. Once again, the results from both these fish indicated that the entire population of glomeruli was actively filtering.

In fish acutely transferred to brackish water, the change in overall glomerular filtration rate was not associated with a comparable decline in the filtering population of glomeruli, although in some fish there was evidence of some non-filtering glomeruli. Whether intermittency is part of any normal physiological adjustments of renal function during acute exposure to increasing environmental salinity is not clear at this stage. It is clear that any contribution of intermittent glomerular function to renal acclimation to salinity would appear to be at most limited and was non-existent in many individuals. In those fish where non-filtering glomeruli were apparent, this was always linked to a lack of vascular perfusion, with no evidence of perfused but non-filtering glomeruli, as occur in teleosts (Brown et al., 1980).

The use of ferrocyanide allowed easy visualisation of the neatly arranged vasculature, particularly the central dorsal aorta, and the series of renal arteries, each supplying a group of glomeruli. The question as to whether lamprey glomeruli are separate or fused has been the subject of much discussion (Youson, 1981). The kidney of the adult sea lamprey *Petromyzon marinus* has been described as a pair of nephric folds each containing ‘a single elongated renal corpuscle composed of a glomus (compound glomerulus) and many nephric capsules which extend between the capillary loops of

the glomus' (Youson, 1982). In the present studies, although individual glomeruli were very difficult to separate, this was possible (see Figs 2, 3). In teleost fish, after partial acid digestion, microdissection of entire nephrons including the glomerulus is much easier, and where glomeruli do lie close to one another, these can be readily separated (Brown et al., 1980). In contrast, the large glomeruli of the river lamprey were very closely attached and separation was much more difficult. The capsules appeared to be extremely thin and fragile, except towards the neck of the tubule, and often broke during microdissection, so that the neck and initial proximal segment with a partial capsule was usually detached from its glomerular capillary knot, which tended to stay firmly attached to its vascular pole. The glomerular capillary knot, at least a partial capsule, neck and proximal segment remained intact in only a relatively few cases (see examples in Figs 2, 3). Nevertheless, during the process of microdissection, the arrangement of individual glomerular capillary knots supplying individual nephrons was discerned. This is entirely consistent with the picture obtained from vascular and nephron casts (Logan et al., 1980a).

From microscopic examination of the whole kidney, as well as microdissection, it was clear that non-filtering glomeruli occurred in patches associated with a restricted blood flow (see Fig. 3). How the implied regulation of vascular tone in the lamprey was achieved is not apparent from the present studies, but the evidence for endocrine/paracrine control of renal function in teleost kidneys may provide some clues. In teleosts, the neurohypophysial hormone arginine vasotocin is antidiuretic at physiological concentrations, regulating the filtering population of glomeruli (Amer and Brown, 1995). However, only a diuretic action is apparent in the lamprey (Uchiyama and Murakami, 1994), so that there is no evidence for any role in control of glomerular function during acute seawater acclimation. An alternative means of control could be *via* the actions of the renin–angiotensin system. In teleosts, the vasoconstrictor peptide angiotensin II has a glomerular

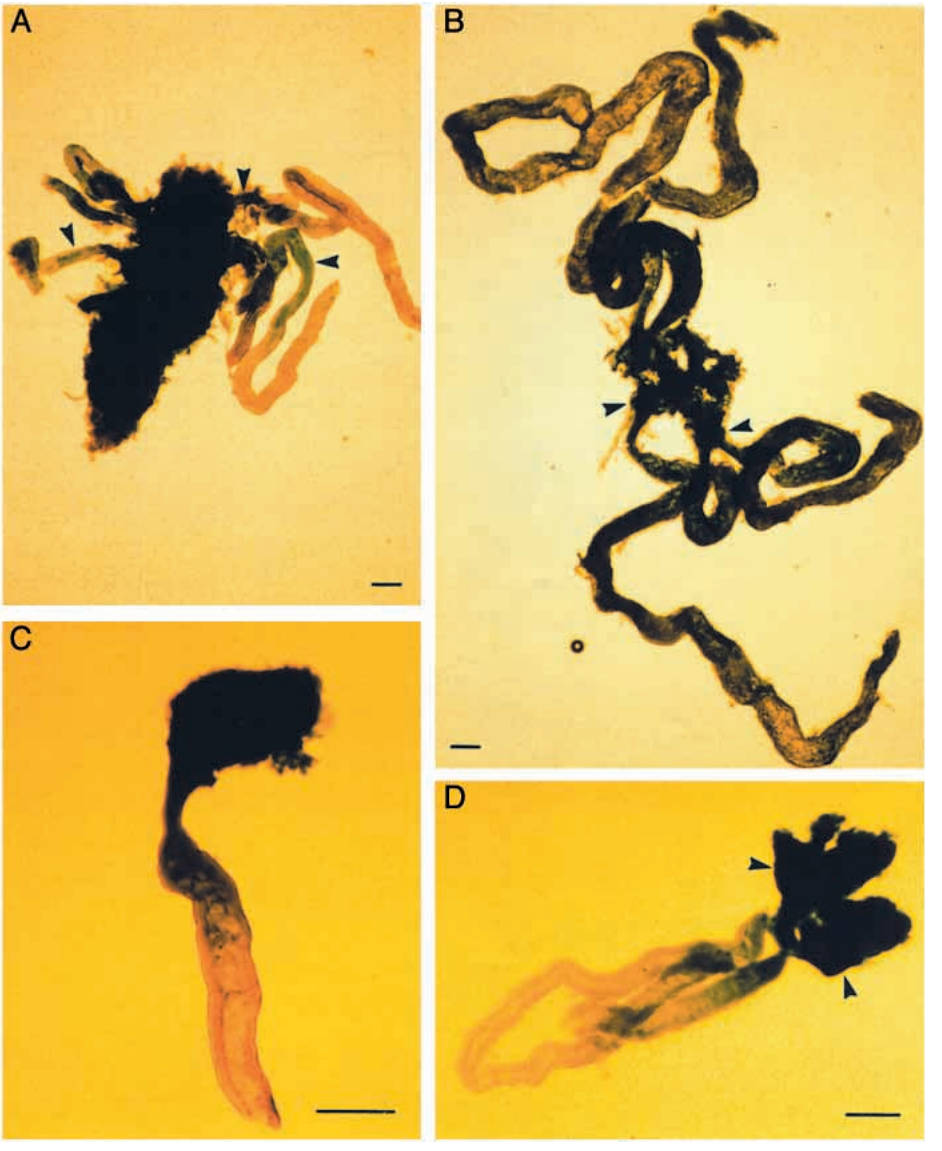

Fig. 2. Examples of microdissected filtering glomeruli of the lamprey with attached renal tubules, illustrating various stages in microdissection. (A) Short lengths of glomeruli (part of the longitudinal 'glomus'; Youson, 1981; see Discussion) running along the length of the kidney containing Prussian Blue precipitate. Most nephrons have been dissected away, but several short lengths of individual filtering nephrons containing Prussian Blue are distinct (arrowheads). (B) Nephrons with individual filtering glomeruli (arrowheads) separated from the glomus but incompletely microdissected. (C) A completely separated filtering glomerulus with precipitated Prussian Blue in the tubular lumen. (D) A pair of filtering glomeruli (arrowheads) with attached tubules. Scale bars, 100 μm.

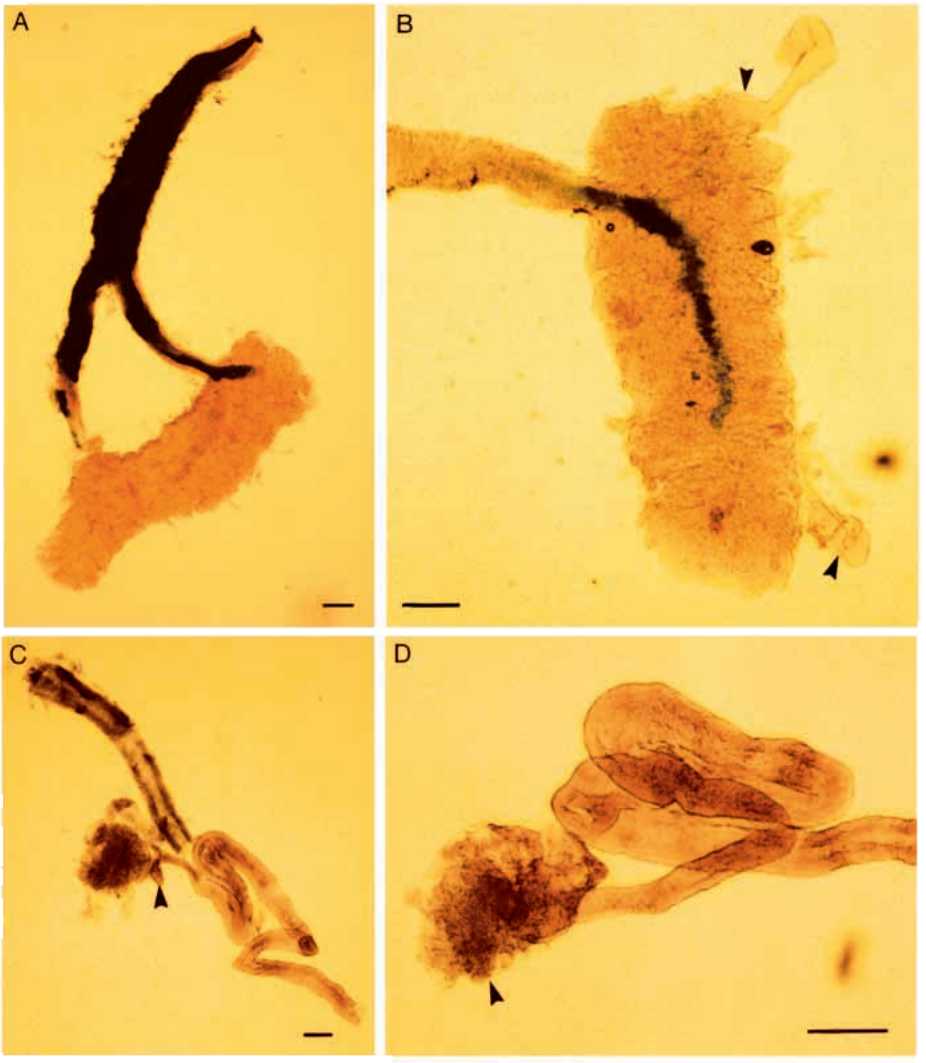

Fig. 3. Examples of microdissected non-filtering glomeruli from the lamprey kidney. (A,B) Groups of clear glomeruli within the 'glomus'; renal arteries containing Prussian Blue precipitate; tubules of two non-filtering nephrons, one with part of the capsule (arrowheads), are visible. (C) A non-filtering glomerulus with a partial capsule attached (arrowhead), neck and proximal tubule; the afferent arteriole is clear, but Prussian Blue precipitate is visible in the renal artery. (D) A non-filtering clear glomerulus (arrow) with attached renal tubule. There is no evidence of Prussian Blue precipitate. Scale bars, 100 μm.

antidiuretic action, reducing the filtering population of glomeruli (Brown et al., 1980; Henderson et al., 1993). In view of the new evidence that the lamprey does possess a renin–angiotensin system, also with antidiuretic actions (Y. Takei, C. L. Broadhead and J. C. Rankin, unpublished observations; see Rankin, 1997), angiotensin II may play a role during acute regulation of the vascular supply to the glomeruli, but any role in acute salinity transfers remains to be investigated.

In summary, application of the direct ferrocyanide method for assessment of the filtering population of glomeruli has demonstrated that renal acclimation of the lamprey *Lampetra fluviatilis* to full-strength sea water is not associated with any reduction in the filtering population of glomeruli, despite a declining overall GFR. Instead, a low rate of urine production is mainly achieved by a decrease in SNGFR, with increased tubular reabsorption of water also contributing (Brown et al., 1993). The greatly reduced urine output after transfer to iso-osmotic brackish water could only be accounted for by a reduced SNGFR and the observed doubling of water reabsorption. Although small patches of non-filtering glomeruli were seen in some fish, this could only have accounted for a small fraction of the antidiuresis in these individuals, and half the fish managed to achieve very low urine flow rates with no reduction in the number of filtering glomeruli.

There is an obvious anatomical reason why lampreys should not reduce or close down glomerular perfusion. They lack a renal portal system which, in teleosts, enables tubular secretion to continue in the absence of glomerular perfusion. In the extreme case of aglomerular teleosts, all urine is produced by tubular secretion from renal portal-derived blood. Glomerular vascular bypass shunts, as found in the hagfish *Myxine glutinosa* (Brown, 1988), could also provide an alternative supply to the tubules in the absence of perfusion of glomerular capillaries. However, no evidence of such shunts was seen in lampreys following vascular casting with Microfil (Logan et al., 1980a), and no perfused nonfiltering glomeruli were observed in the present study. Vascular corrosion casting of lamprey glomeruli, which would give a more detailed picture, has not been attempted.

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