J. exp. Biol. (1978), 77, 57–69
With 6 figures
Printed in Great Britain

MEASUREMENT OF SINGLE NEPHRON FILTRATION RATE IN THE KIDNEY OF THE RIVER LAMPREY, *LAMPETRA FLUVIATILIS* L.

BY R. J. MORIARTY,* A. G. LOGAN AND J. C. RANKIN
Department of Zoology, University College of North Wales, Bangor, Gwynedd, LL57 2UW, U.K.

(*Received* 1 *February* 1978)

SUMMARY

1. The reliability of [^{3}H]inulin as a reference substance for the measurement of glomerular filtration rate (GFR) in river lampreys was investigated.
2. Simultaneously measured renal clearances of [^{3}H]inulin and [^{14}C]-polyethylene glycol (PEG) were not significantly different.
3. Recoveries of [^{3}H]inulin in the urine following its injection into the proximal tubule and urinary duct averaged $83{\cdot}5 \pm 4{\cdot}0\%$ ($n = 14$) and $93{\cdot}0 \pm 4{\cdot}6\%$ ($n = 9$) respectively.
4. No evidence was obtained to suggest penetration of the tubular wall by [^{3}H]inulin following its introduction into peritubular capillaries.
5. Gel-filtration of lamprey plasma and urine produced no signs of any significant degradation of the inulin to fructose.
6. [^{3}H]inulin recoveries, though significantly below 100% ($P < 0{\cdot}001$), were considered adequate to justify its use after allowing for possible sources of error.
7. A mean single nephron filtration rate (SNGFR) value of $7{\cdot}02 \pm 0{\cdot}27$ nl/min ($n = 89$) was obtained from the kidneys of anaesthetized freshwater lampreys.
8. [^{51}Cr]EDTA was found to be totally unsuitable for the measurement of GFR in this species.

INTRODUCTION

This paper is the first in a series of investigations which we hope will provide considerable information on nephron function in the kidney of the river lamprey, *Lampetra fluviatilis*.

The river lamprey is able to osmoregulate successfully in both fresh- and salt-water environments at different stages during its life-cycle and must therefore have the ability to drastically modify its renal function. The study of an animal exhibiting such versatility should provide information on both the nature and control of tubular function in general. The lamprey kidney is also of particular interest since its structural organization is fairly simple.

An accurate method of measuring filtration rates at both whole kidney (glomerular filtration rate, GFR) and single nephron (single nephron glomerular filtration rate, SNGFR) levels is essential to any quantitative study of kidney function. Inulin, in

* Present address: Department of Physiology, Manchester University, Manchester.

both its native and radioactively labelled form, has for many years maintained its position as the accepted agent for the determination of GFR in vertebrates. It appears to satisfy the criteria established for glomerular indicators by Smith (1951) and micropuncture studies have demonstrated directly that inulin is not reabsorbed by the kidney of the rat (Baumann *et al.* 1965; Gutman, Gottschalk & Lassiter, 1965; Marsh & Frasier, 1965; Maude *et al.* 1965) or *Necturus* (Tanner & Klose, 1966). Measurements of GFR in fish have also been carried out with inulin though its use in this group has never been justified by micropuncture studies. Recent work on certain fish has shown that inulin clearance is significantly lower than the clearance of another mammalian glomerular indicator, polyethylene glycol (PEG), thus suggesting the possibility of inulin reabsorption (Schmidt-Nielsen & Renfro, 1975; Beyenbach & Kirschner, 1976). Degradation of inulin to low-molecular-weight compounds, e.g. fructose, may explain the anomalous behaviour of this polymer (Lahlou, Henderson & Sawyer, 1969; Beyenbach & Kirschner, 1976).

In this paper we report on the renal handling of inulin in the freshwater-adapted adult lamprey and the subsequent SNGFR values which were obtained.

MATERIALS AND METHODS

Adult, fresh-run specimens of the river lamprey were trapped in the River Severn between Gloucester and Tewkesbury during their autumnal and spring spawning migrations. They were transported to Bangor in well-aerated river water. Survival during transportation was 100%. Following arrival they were transferred to large glass-fibre tanks containing recirculating, dechlorinated, copper-free tapwater maintained at 12–14 °C and exposed to a photoperiod of natural daylength. The lampreys lived until April or May, by which time they were sexually mature and died, paralleling the situation in their natural environment.

The following isotopes were obtained from the Radiochemical Centre, Amersham: [^{3}H]inulin (specific activity > 300 mCi/mmol), [^{14}C]polyethylene glycol 4000 (specific activity 10–20 mCi/g), [D-][U-^{14}C]fructose (specific activity 3 mCi/ml), [^{51}Cr]-EDTA (specific activity 0·5–1·5 mCi/mg Cr).

Micropipettes for withdrawing tubular fluid and injecting isotopes into tubules and capillaries were constructed as follows. Soft-glass capillary tubing (1·0 mm O.D., 0·8 mm I.D., Drummond Scientific Company, U.S.A.) was drawn out in a small gas flame, and the thin section thus produced was then pulled to a fine point using a de Fonbrune microforge. Finally, the tip was ground to a chamfered point by resting it on the revolving surface of a hard-grit Arkansas stone (Oilstone Hb4, Norton, U.S.A.) to give a pipette with a final tip diameter of 10–12 μm. Constant volume pipettes were constructed in a similar way except that a constriction was placed in the shank at a suitable distance from the tip.

Administration of isotopes

In those experiments involving comparison of the clearances of the three different glomerular indicators the following doses were administered to the fish via intraperitoneal injection:

(1) [^{3}H]inulin, 50 μCi in 0·2 ml distilled water;

(2) [^{14}C]PEG, 5 μCi in 0·1 ml distilled water;

(3) [^{51}Cr]EDTA, 10 μCi in 0·1 ml solution as supplied.

Preliminary experiments showed that plasma isotope levels reached optimum values for sampling 24 h after administration.

To obtain accurately detectable quantities of [^{3}H]inulin in tubular fluid samples, 150–250 μCi [^{3}H]inulin (according to fish size, and contained in 0·6 ml distilled water) was administered to fish used for the determination of SNGFR values.

Radioactive counting

[^{3}H]inulin, [^{14}C]PEG and [^{14}C]fructose were counted by liquid scintillation (Nuclear Chicago Unlilux III) using Aquasol (New England Nuclear) as scintillant. Windows were adjusted to discriminate between the two isotopes and corrections made for the amount of [^{14}C] appearing in the [^{3}H] channel. When the combination [^{3}H]inulin:[^{51}Cr]EDTA was used, gamma activity of the latter was counted in an automatic (Panax) sodium iodide scintillation counter and its proportional beta-emission subtracted from the liquid scintillation counts of both [^{3}H]inulin and [^{51}Cr]EDTA. Counting efficiencies were calculated from external standard ratios and found not to vary between any of the samples used. All results are therefore expressed in counts per minute (cpm). Tubular fluid samples taken for SNGFR determinations were approximately 100 cpm above background. Therefore vials were counted for 100 min before and after addition of the sample.

General preparation

Lampreys were initially anaesthetized by immersion in a 0·01 % solution of MS 222 (methane tricaine sulphonate, Sandoz Ltd). Following immobilization the animal was weighed and then laid on its back in a Perspex trough (Fig. 1) with the head and gill regions submerged in a well-aerated, circulating 0·005 % solution of MS 222 to maintain anaesthesia during the subsequent operation. Some of the solution was allowed to pass over the animal's flanks, and exposed surfaces of the fish were covered in water-soaked tissue paper. The temperature of the solution was kept at 12–14 °C, and the strength of the MS 222 was adjusted where necessary to ensure that the fish maintained strong, rhythmical ventilatory movements.

Body fluid sampling

Blood samples (100 μl) were taken at hourly intervals during the experiment from the caudal vein. Following centrifugation, plasma samples (50 μl) were analysed for the different indicator concentrations by radioactive counting. A polyethylene catheter (PP60, Portex Ltd, Hythe, Kent) was inserted to a depth of 5 mm into the urinary papilla. The papilla contracted around the catheter forming an effective seal. Urine was collected at half-hourly intervals under paraffin oil into pre-weighed plastic vials and 25 μl aliquots were taken for radioactive counting.

Micropuncture experiments

A mid-line ventral incision was made using cautery (Thackray Cautery Unit) from a point 2 cm in front of the urinary papilla, extending forwards approximately 2 cm.

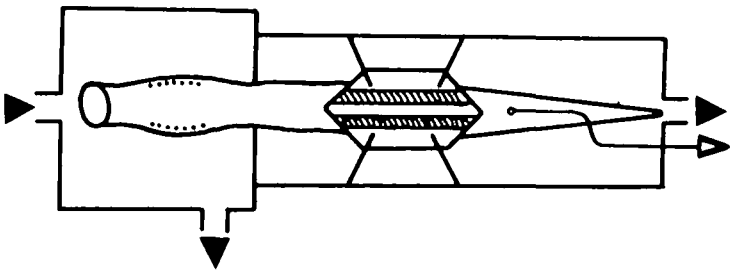

Fig. 1. Diagram of a completed lamprey preparation viewed from the position normally occupied by the microscope. The cut edges of the body wall are held apart by four ties. The kidneys (cross-hatched) are shown exposed on the dorsal body wall. The solid arrows indicate the direction of circulating water flow. The open arrow indicates the point of urine outflow.

The incision was held open using two ties attached to each side of the cut body wall and anchored to the sides of the trough. The exposed section of intestine was removed and any gonad obscuring the kidneys was gently freed from the dorsal suspending mesenteries and removed. Excess body fluid was drained and the kidneys covered with paraffin oil to prevent desiccation and aid visibility. The kidney surface was illuminated with a powerful cold light source (Rank optics Division; fibre optic system) and viewed using a Zeiss OPMI 6 operating microscope. Micropipettes were held by a de Fonbrune series A micromanipulator.

The pipettes used in all microinjection studies held a fixed volume of injectate. The actual number of counts injected was determined as the mean count for at least five samples expelled directly into Aquasol (or water in the case of [^{51}Cr]EDTA microinjections). The maximum variation between samples from the same pipette was less than 3 %.

Retrograde injections into proximal tubules were avoided by observing the movement of a small oil droplet introduced into the tubular lumen prior to delivery of the injectate. The flow rate of injectate was controlled at approximately 3 nl/min and about 5000 cpm of [^{3}H]inulin were injected in a total volume of 30 nl of 0·9 % saline. Urinary ducts and peritubular capillaries were injected in a similar manner though a greater quantity of [^{3}H]inulin (25000 cpm in 150 nl 0·9 % saline at a rate of 150 nl/min) was injected into each capillary. The urine from each kidney was collected by catheterization of both urinary ducts using PP 60 polyethylene tubing. Tubular fluid samples for the determination of SNGFR were collected in the following way. A proximal tubule was punctured with an oil-filled pipette and a column of oil at least 3–4 times the tubule diameter injected into the lumen. Tubular fluid entered the pipette by capillarity and if necessary slight suction was applied to control the rate of collection and keep the oil-block stationary. Each collection period was taken as the time between the start of the aspiration and the removal of the pipette tip from the tubule.

Pipettes, holding on average 70 nl of tubular fluid, were emptied into accurately backgrounded scintillation vials containing Aquasol prior to counting. As many as ten such samples were taken from each fish.

Calculations

The clearance of [^{3}H]inulin, [^{14}C]PEG or [^{51}Cr]EDTA during each urine collection period was given by:

$$C_X = \frac{U\dot{V}}{P} \text{ ml/min},$$

where U = urine cpm/ml, $\dot{V}$ = urine flow rate (ml/min), P = plasma cpm/ml at urine mid-point collection time. SNGFR values were calculated according to the equation:

$$\text{SNGFR} = \left[\frac{\text{TF}}{P} \times \frac{1}{t}\right] \text{ nl/min},$$

where TF = tubular fluid inulin cpm, P = plasma inulin cpm/ml, t = collection time (min).

If it is assumed that all nephrons are filtering in the freshwater-adapted lamprey and that the tubules punctured are a representative sample, then the number of nephrons (N) in both kidneys will be given by

$$N = \frac{\text{GFR (nl/min)}}{\text{mean SNGFR (nl/min)}}.$$

The urine collection delay time was corrected by the method of Hickman (1968). The Student's t test was used for evaluating the difference between paired observations (inulin:PEG or inulin:EDTA).

Gel filtration

Samples of injectate and of urine and plasma from lampreys previously injected with [^{3}H]inulin for GFR determinations were eluted down a 50 × 1·5 cm column of Sephadex G25 in 0·9 % saline. 0·01 μCi of [^{14}C]fructose was added to each sample prior to elution down the column. Serial 5 minute fractions were collected directly into vials containing scintillant.

RESULTS

Clearance ratios

Results of experiments in which the renal clearance rates of [^{3}H]inulin and [^{14}C]-PEG were measured simultaneously are shown in Fig. 2. Data has been pooled from several fish and a wide range of filtration rates covered. Pooled values for all clearances gave a mean value of $16{\cdot}2 \pm 0{\cdot}96$ ml kg^{-1} h^{-1} ($n = 54$) for inulin and $17{\cdot}2 \pm 1{\cdot}03$ ml kg^{-1} h^{-1} ($n = 54$) for PEG, not significantly different ($P > 0{\cdot}1$). Similar experiments involving comparison of [^{51}Cr]EDTA and [^{3}H]inulin as glomerular indicators showed that EDTA clearances consistently underestimated those of inulin. Mean clearances were $12{\cdot}1 \pm 0{\cdot}90$ ml kg^{-1} h^{-1} ($n = 31$) for EDTA and $19{\cdot}8 \pm 1{\cdot}6$ ml kg^{-1} h^{-1} ($n = 31$) for inulin, a highly significant difference ($P < 0{\cdot}001$).

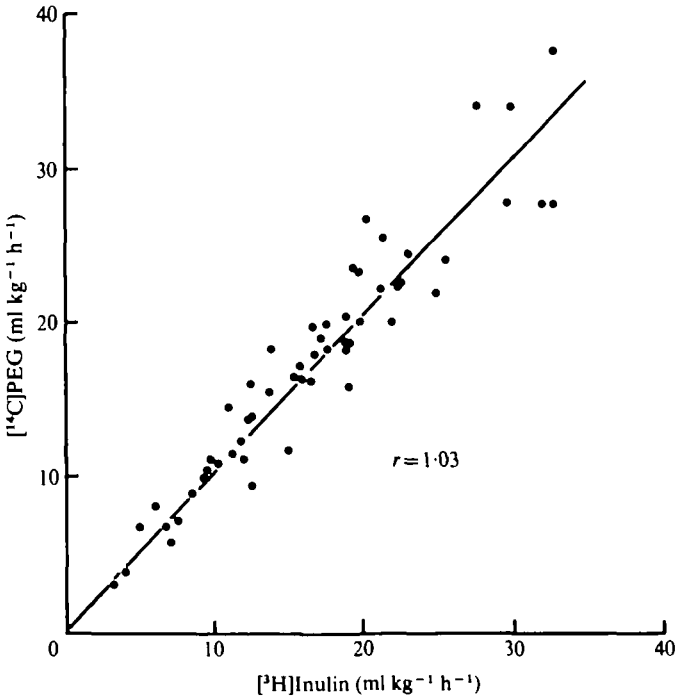

Fig. 2. Relationship between simultaneously measured renal clearance rates of [^{3}H]inulin and [^{14}C]PEG in lampreys. Low values obtained by exposure to 20% sea water.

Microinjection studies

Mean recoveries for tubular and urinary duct [^{3}H]inulin microinjections were $83{\cdot}5 \pm 4{\cdot}0\%$ ($n = 14$) and $93{\cdot}0 \pm 4{\cdot}6\%$ ($n = 9$) respectively, the former value being significantly below 100% ($P < 0{\cdot}001$: Student's t test).

Approximately 90% of the [^{3}H]inulin injected into each proximal tubule was recovered in the urine of the same kidney within 1 h of injection (Fig. 3). A small amount of inulin (4%) was also detected in the urine of the non-injected kidney.

[^{3}H]inulin was excreted at the same rate by each kidney after microinjection into a peritubular capillary. Its recovery following such an injection is outlined in Fig. 4. The more rapid appearance of [^{3}H]inulin in the urine of the non-injected kidney is thought to be of no particular significance. Within 1 h of injection inulin concentrations of the urine of both kidneys were similar.

The mean recovery of [^{51}Cr]EDTA following tubular microinjection was $90{\cdot}2 \pm 5{\cdot}5\%$ ($n = 9$).

Gel filtration

The elution patterns of inulin injectate and five lamprey plasma and urine samples gave no indication of any fructose peaks or indeed of any significant breakdown products. The recovery profiles of an injectate and plasma sample are given in Fig. 5.

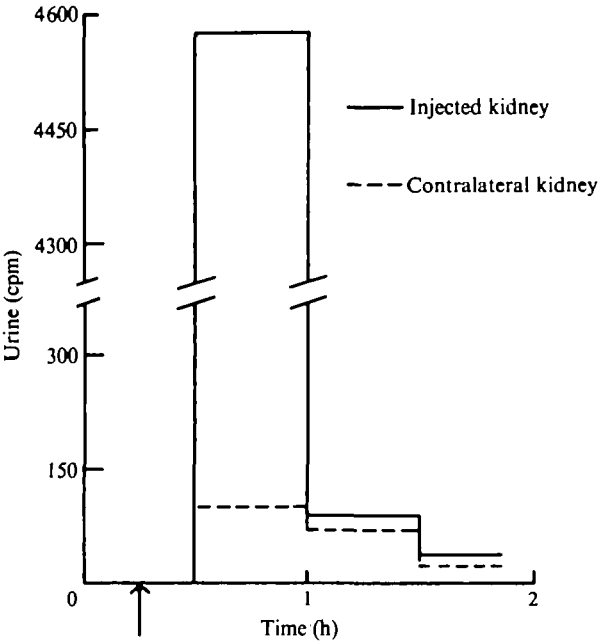

Fig. 3. The pattern of recovery of [^{3}H]inulin in the urine of the two kidneys of a lamprey following the tubular microinjection of 4950 cpm of the isotope into one kidney. The arrow marks the time of injection.

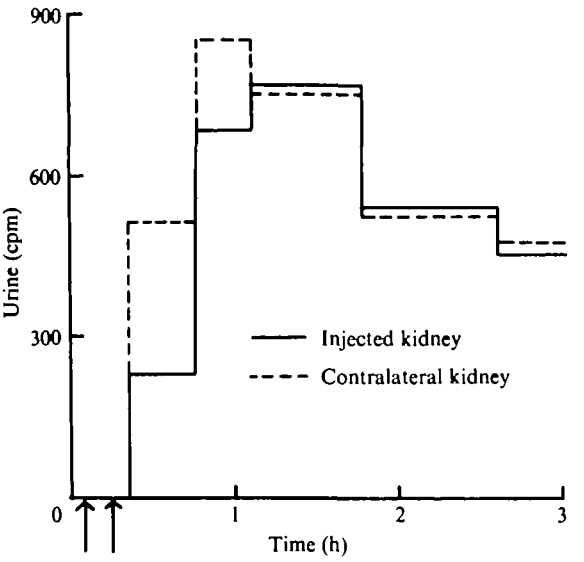

Fig. 4. The pattern of recovery of [^{3}H]inulin in the urine of the two kidneys of a lamprey following the microinjection of 25000 cpm into a peritubular capillary of one kidney. The arrows mark the duration of the injection.

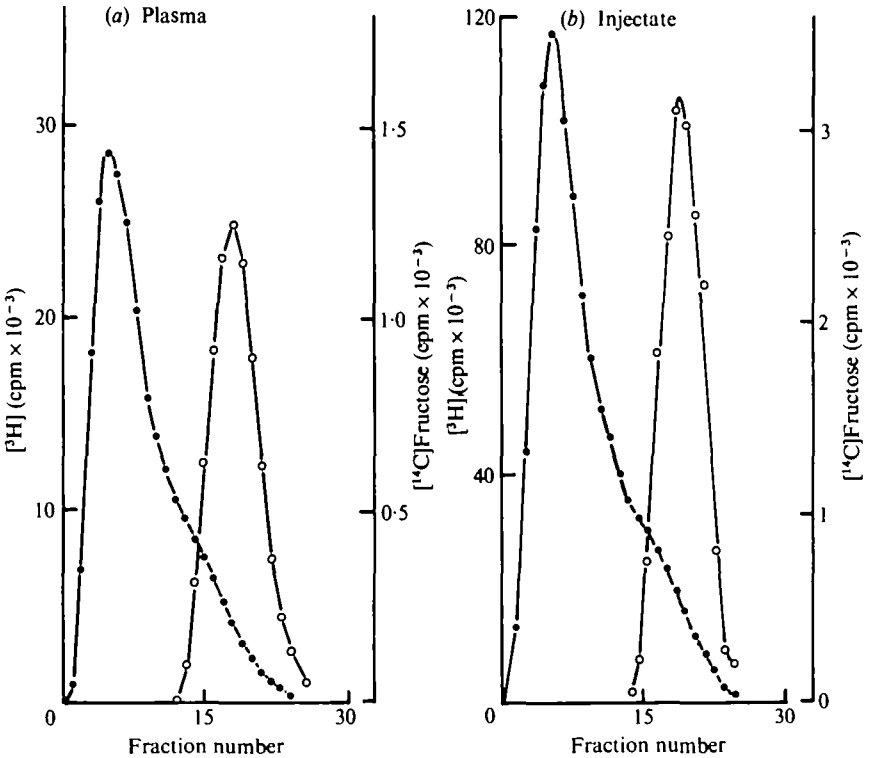

Fig. 5. Chromatograms of a Sephadex G25 column of (*a*) [^{3}H]inulin injectate and (*b*) lamprey plasma. ●, Radioactivity in column eluates. ○, Radioactivity from [^{14}C]fructose which was added to the sample prior to chromatography.

SNGFR determinations

The mean SNGFR value from 54 determinations in 9 lampreys was 7·35 ± 0·37 nl/min. Individual results together with GFR, urine flow rate and N values, are presented in Table 1. A further 35 SNGFR determinations were carried out on fish in which the GFR was not recorded. The combined estimates of SNGFR show a near normal distribution (Fig. 6) with a peak at 6–8 (nl/min). The overall mean value was 7·02 ± 0·27 nl/min (n = 89), which falls within this peak class size.

DISCUSSION

The unreliability of inulin as a glomerular indicator in fish has been suggested by several workers. Beyenbach & Kirschner (1976) compared renal clearances of [^{3}H]-methoxy inulin, [^{14}C]PEG and [^{125}I]iothalamate (glofil) in seawater-adapted rainbow trout. They showed that ureteral clearances of [^{14}C]PEG were approximately 22% higher than those of [^{3}H]inulin and glofil and provided direct evidence that the three macromolecules were absorbed by the urinary bladder and possibly the renal tubules and ureters of this species. Schmidt-Nielsen & Renfro (1975) cast doubt on the use of inulin when they showed that the ratio of [^{14}C]PEG to [^{3}H]inulin clearance was greater than unity in both fresh- and seawater eels. Evidence has also been provided for the accumulation of inulin in the kidneys of the winter flounder (Hickman, New-

Table 1. *Summary of SNGFR, GFR, urine flow rate and N (number of functional nephrons) values in 9 freshwater-adapted lampreys*

(Values are means ± S.E. *n* = no. of determinations in each case.)

Weight (g)	(*n*)	Mean SNGFR (nl/min)	Mean GFR (μl/min)	Mean urine flow rate (μl/min)	*N*
45·6	(6)	7·11 ± 1·43	8·95 ± 0·12	7·02 ± 0·81	1545 ± 379
21·0	(7)	3·29 ± 0·26	8·11 ± 0·63	4·53 ± 0·90	2595 ± 308
36·4	(8)	8·50 ± 0·46	23·00 ± 1·75	13·0 ± 1·71	2718 ± 160
32·3	(6)	9·09 ± 1·11	19·67 ± 1·71	10·11 ± 1·90	2201 ± 97
34·9	(7)	6·87 ± 0·49	17·00 ± 1·31	11·00 ± 1·43	2503 ± 166
23·0	(9)	7·24 ± 0·53	16·30 ± 0·08	7·13 ± 1·10	2353 ± 183
40·4	(4)	7·60 ± 0·91	10·80 ± 2·50	4·80 ± 2·23	1397 ± 246
32·5	(3)	7·83 ± 2·07	18·1 ± 3·20	8·25 ± 2·30	2409 ± 237
39·7	(7)	8·60 ± 0·82	25·62 ± 1·09	12·80 ± 1·71	3178 ± 364
			Overall mean values		
35·6 ± 2·39 (9)		7·35 ± 0·57 (57)	16·36 ± 2·04 (57)	8·74 ± 1·06 (57)	2322 ± 185 (57)

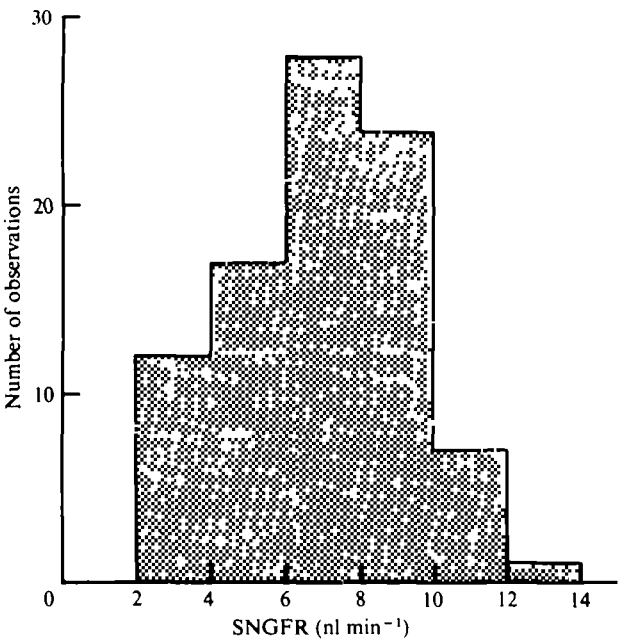

Fig. 6. Distribution of single nephron glomerular filtration rates (SNGFR) measured in 16 freshwater-adapted lampreys.

comb & Kinter, 1972) the rainbow trout and the southern flounder (Schmidt-Nielsen, Renfro & Benos, 1972). Balanced against these findings are the results of Williams, Chen & Huang (1973, 1974), who found little difference between the clearances of [^{3}H]inulin and [^{14}C]PEG in the winter flounder. The likelihood of these two structurally dissimilar molecules being reabsorbed to the same extent seems remote. The

same can be said for the results of Babiker & Rankin (1975), who showed similar clearances of [^{51}Cr]EDTA and [^{3}H]inulin in the eel and African lungfish.

In the lamprey we found renal clearances of [^{3}H]inulin and [^{14}C]PEG to be virtually identical over a wide range of filtration rates. However, the mean recovery of [^{3}H]inulin following tubular microinjections was significantly below 100 %, suggesting the possibility of reabsorption of this substance. The recovery of significant quantities of tritium in the urine of the non-injected kidney (Fig. 3) was also consistent with this hypothesis since it must gain access to the general body circulation prior to filtration at the glomeruli and appearance in the urine.

It is of interest to compare the results in the lamprey with those in higher vertebrates. Maude *et al.* (1965) recovered 93·7 % of the [^{14}C]inulin injected into proximal tubules of the rat. This compared with a mean value of 95 % reported by Baumann *et al.* (1965). Both groups recognized that most sources of error tended to increase the apparent absorption of inulin and considered their results fully justified its use as a reference substance with which to measure GFR. The low recoveries (mean $83{\cdot}5 \pm 4{\cdot}0$ %) found in the present study may be due to several causes. Leakage of injectate around the point of puncture and/or retrograde flow of injectate to the glomerulus are possible sources of error. Dye was added to the injectate to enable easier recognition of such loss though a small amount of leakage was still conceivable.

It has been suggested previously that metabolic breakdown of inulin could occur in the body (Lahlou *et al.* 1969; Beyenbach & Kirschner, 1976). This of course would not explain the low recoveries following microinjections of inulin into tubules but could give rise to falsely lowered GFR values. However, there was no evidence for this in the lamprey since recovery profiles for inulin injectate and plasma were very similar and, most important of all, showed no real signs of the presence of any fructose peaks (Fig. 5). Nevertheless great care should be taken in the storage of [^{3}H]-inulin. It is supplied as a freeze-dried powder sealed under nitrogen. The Radiochemical Centre, Amersham, do not recommend storage at −20 °C for more than 6 months, and once in solution its lifetime is obviously much more limited. Lahlou *et al.* (1969) also found that the recovery profile of toadfish plasma containing [^{14}C]-inulin was identical to that of the inulin injected but they found breakdown products in the urine which we did not.

There was no leakage of [^{3}H]inulin into the tubular fluid or urine following its injection into a peritubular capillary. After a slight delay, similar levels of inulin appeared in the urine of both kidneys (Fig. 4). Thus inulin did not diffuse into the tubular lumen down its concentration gradient. It is therefore unlikely that it would be reabsorbed by simple diffusion from the tubule into the blood. Possibly pinocytotic reabsorption of filtered protein involves some uptake of fluid containing inulin. Thus we have shown that small but significant quantities of [^{3}H]inulin do enter the blood of the lamprey following microinjection into the proximal renal tubules. The small quantities involved make it difficult to decide whether this is the result of reabsorption or experimental error. If, as suggested by microinjection recoveries, 16·5 % of [^{3}H]inulin is reabsorbed then the mean GFR and N values become 19·7 μl/min and 2798 respectively. However, in view of its similar clearance values when compared with PEG, we are inclined to believe that [^{3}H]inulin is a satisfactory substance to use as a glomerular indicator in this species. We were unable to measure

the recoveries of [^{14}C]PEG following tubular injection since supplies of this substance of sufficiently high specific activity are not commercially available. It is unlikely that inulin reabsorption, if it did occur, would markedly affect SNGFR results since these are calculated from the inulin concentrations of tubular fluid taken from the early proximal tubule.

Renal clearances of [^{51}Cr]EDTA were significantly lower than simultaneously measured [^{3}H]inulin clearances ($P < 0{\cdot}001$) indicating that the former substance was unsuitable for use as a glomerular indicator in the lamprey. The mean recovery of [^{51}Cr]EDTA following its tubular injection was not lower than the corresponding inulin value suggesting that the discrepancy in the clearances of these two substances was due to restricted filtration of [^{51}Cr]EDTA rather than greater reabsorption.

It is difficult to compare urine flow-rate values obtained in the present study with those present in the literature since the latter exhibit considerable variation. Mean values from conscious freshwater lampreys have ranged from 156 ± 99 ml kg^{-1} day^{-1} (Morris, 1956) up to 329 ± 65 ml kg^{-1} day^{-1} (Bentley & Follett, 1963) and may reflect the method of urine collection used. The mean urine flow rate found in the present study, when recalculated, gives a value of 353 ± 93 ml^{-1} kg^{-1} day. This slightly higher value may indicate a diuretic effect of the anaesthesia or general trauma of the operational procedure. Few workers have measured GFR values in lampreys. Bentley & Follett (1963) took the endogenous clearance of creatinine as a measure of GFR but this substance has since been shown to be reabsorbed by the kidney of the Great Lakes lamprey, *Petromyzon marinus* (Malvin *et al.* 1970). The latter workers found urine:plasma inulin ratios of 1·76 and 2·53 at 4 °C and 26 °C respectively, which compare with our mean value of 1·87 recorded at 13 °C.

In view of the similarity between our data and that appearing in the literature it would seem that anaesthesia is not having any drastic effect on renal function in the lamprey. This is also supported by our values for urinary ion concentrations and osmolarity. The mean urine sodium and chloride concentrations were 12·3 and 7·4 mM respectively and urine osmolarity was 22·3 m-osmol/l. These values compare favourably with those previously recorded for this species (for review see Morris, 1972).

The accurate measurement of SNGFR is fundamental to any quantitative investigation of tubular reabsorptive or secretory transport processes. It also provides valuable information as to the degree of heterogeneity of nephron function. Fish have largely been ignored by the renal micropuncturist since the problems imposed by their mode of life and kidney structure have been difficult to overcome. It is only comparatively recently that the successful application of micropuncture methods to this group has been reported (e.g. Thurau & Aquisto, 1969; Moss, Moriarty & Rankin, 1974; Stollte *et al.* 1977). Consequently there is a lack of SNGFR data in the literature with which to compare the results of the present study.

Jackson *et al.* (1977), using a modified version of the ferrocyanide method (Hanssen, 1963), recorded a mean SNGFR of $1{\cdot}31 \pm 0{\cdot}2$ nl/min in the freshwater-adapted rainbow trout which rose to $3{\cdot}4 \pm 1{\cdot}1$ nl/min in sea water. The only other recorded values for SNGFR in fish are those calculated by dividing total GFR by the estimated number of nephrons. These give SNGFR values of 2·56 and 0·55 nl/min for the freshwater sawfish, *Pristis microdon* (Smith, 1931), and catfish, *Ameurius nebulosus* (Smith, 1951), respectively. The mean value of $7{\cdot}02 \pm 0{\cdot}27$ nl/min ($n = 89$)

for lampreys may seem in comparison rather high but it must be remembered that lampreys have urine flow rates which are generally greater than most freshwater teleosts that have been studied (Morris, 1972). Values for SNGFR in adult rats, for comparison, are generally found to be around 30 nl/min for superficial nephrons (e.g. Glabman, Aynedijian & Bank, 1965; Lewy & Windhager, 1968). This value is a lot higher than that found in the lamprey; a fact readily explained by the rat's higher blood pressure.

Mean SNGFR values recorded from different fish were, with one exception, very similar to one another. There was, however, some variation in SNGFR values recorded from individual fish and this is reflected in the shape of the histogram in Fig. 6. This variation is more likely to be the result of small changes in the general condition of the fish rather than any inherent heterogeneity of nephron function. Two basic theories, that of graded filtration and glomerular intermittency, have been invoked previously to explain the regulation of GFR in lower vertebrates. Data from studies such as this where SNGFR's are measured in individual fish should help to distinguish between these two possibilities.

This work was supported in part by SRC research grant GR/A/1820.7. R. J. Moriarty was supported by a SRC research studentship and A. G. Logan by a UCNW research studentship. We wish to thank Mr P. J. Gaskins of Tirley for supplying us with lampreys through two difficult 'lamprey seasons' and Miss V. Griffiths for technical assistance.

REFERENCES

BABIKER, M. M. & RANKIN, J. C. (1975). Rationale for the use of [^{51}Cr]EDTA for estimation of glomerular filtration rate in fish. *Comp. Biochem. Physiol.* **50** A, 177–179.

BAUMANN, K., OELERT, H., RUMRICH, G. & ULLRICH, K. J. (1965). Ist Inulin zur messung des glomerulum-filtrates beim Warmblutter geeignet. *Pflügers Arch. ges. Physiol.* **282**, 238–241.

BENTLEY, P. J. & FOLLETT, B. K. (1963). Kidney function in a primitive vertebrate, the cyclostome *Lampetra fluviatilis*. *J. Physiol.* **169**, 902–918.

BEYENBACH, K. W. & KIRSCHNER, L. B. (1976). The unreliability of mammalian glomerular markers in teleostean renal studies. *J. exp. Biol.* **64**, 369–378.

GLABMAN, S., AYNEDJIAN, H. S. & BANK, N. (1965). Micropuncture study of the effect of acute reductions in glomerular filtration rate on sodium and water reabsorption by the proximal tubules of the rat. *J. Clin. Invest.* **44**, 1410–1416.

GUTMAN, Y., GOTTSCHALK, C. W. & LASSITER, W. E. (1965). Micropuncture study of inulin absorption in the rat kidney. *Science, N.Y.* **147**, 753–754.

HANSSEN, O. E. (1963). Method for comparison of glomerular filtration in individual rat nephrons. *2nd Int. Congr. Nephrol.* (Abstracts) **1**, 527.

HICKMAN, C. P. (1968). Glomerular filtration and urine flow in the euryhaline southern flounder *Paralicthys lethostigma* in sea-water. *Can. J. Zool.* **46**, 427–438.

HICKMAN, C. P., NEWCOMB, E. W. & KINTER, W. B. (1972). Anomalous behaviour of [^{3}H]methoxy and ^{14}C-labelled inulin and ^{3}H-labelled polyethylene glycol in incubated kidney tissue of the winter flounder *Pseudopleuronectes americanus*. *Bull. Mt Desert Isl. biol. Lab.* **23**, 47–50.

JACKSON, B. A., BROWN, J. A., OLIVER, J. A. & HENDERSON, I. W. (1977). Actions of angiotensin on single nephron filtration rates of trout, *Salmo gairdneri*, adapted to fresh- and sea-water environments. *J. Endocr.* **75**, 32*P*–33*P*.

LAHLOU, B., HENDERSON, I. W. & SAWYER, W. H. (1969). Renal adaptation of *Opsanus tau*, a euryhaline aglomerular teleost to dilute media. *Am. J. Physiol.* **216**, 1266–1272.

LEWY, J. E. & WINDHAGER, E. E. (1968). Peritubular control of proximal tubular fluid reabsorption in the rat kidney. *Am. J. Physiol.* **214**, 943–954.

MALVIN, R. L., CARLSON, E., LEGAN, S. & CHURCHILL, P. (1970). Creatinine reabsorption and renal function in the freshwater lamprey. *Am. J. Physiol.* **218**, 1506–1509.

MARSH, D. & FRASIER, C. (1965). Reliability of inulin for determining volume flow in rat renal cortical tubules. *Am. J. Physiol.* **209**, 283–286.

MAUDE, D. L., SCOTT, W. N., SHEHADEH, I. & SOLOMON, A. K. (1965). Further studies on the behaviour of inulin and serum albumin in rat kidney tubule. *Pflügers Arch. ges. Physiol.* **285**, 313–316.

MORRIS, R. (1956). The osmoregulatory ability of the lampern (*Lampetra fluviatilis* L.) in sea water during the course of its spawning migration. *J. exp. Biol.* **33**, 235–248.

MORRIS, R. (1972). Osmoregulation. In *The Biology of Lampreys*, vol. II (M. W. Hardistry and I. C. Potter), pp. 193–240. London, New York: Academic Press.

MOSS, N. G., MORIARTY, R. J. & RANKIN, J. C. (1974). The investigation of individual nephron function in the euryhaline teleost, *Salmo gairdneri*. A new approach using micropuncture techniques. INSERM Colloque Européen de Physiologie renale, 30. *Physiologie du Nephron: Mécanismes et regulation*, p. 180. Paris.

SCHMIDT-NIELSEN, B. & RENFRO, J. L. (1975). Kidney function of the American eel, *Anguilla rostrata*. *Am. J. Physiol.* **228**, 420–431.

SCHMIDT-NIELSEN, B., RENFRO, J. L. & BENOS, D. (1972). Estimation of extracellular space and intracellular ion concentrations in osmoconformers, hypo and hyperosmoconformers. *Bull. Mt Desert Isl. biol. Lab.* **12**, 99–104.

SMITH, H. W. (1931). The absorption and excretion of water and salts by the elasmobranch fishes. I. Freshwater elasmobranchs. *Am. J. Physiol.* **98**, 279–295.

SMITH, H. W. (1951). *The Kidney: Structure and Function in Health and Disease*. New York: Oxford University Press.

STOLLTE, H., GALASKE, R. G., EISENBACH, G. M., LECHENE, C., SCHMIDT-NIELSEN, B. & BOYLAN, J. W. (1977). Renal tubule ion transport and collecting duct function in the elasmobranch little skate, *Raja erinacea*. *J. exp. Zool.* **199**, 403–410.

TANNER, G. A. & KLOSE, R. M. (1966). Micropuncture studies of inulin reabsorption in *Necturus* kidney. *Am. J. Physiol.* **211**, 1036–1038.

THURAU, K. & ACQUISTO, P. (1969). Localisation of the diluting segment in the dogfish nephron – a micropuncture study. *Bull. Mt Desert Isl. biol. Lab.* **9**, 60–63.

WILLIAMS, W. M., CHEN, T. S. T. & HUANG, K. L. (1973). Comparison of renal clearance of several glomerular markers in winter flounder (*Pseudopleuronectes americanus*). *Bull. Mt Desert Isl. biol. Lab.* **13**, 128–131.

WILLIAMS, W. M., CHEN, T. S. T. & HUANG, K. C. (1974). Renal handling of aromatic amino acids, sugar and standard glomerular markers in winter flounder. *Am. J. Physiol.* **227**, 1380–1384.