

A MICROPUNCTURE STUDY OF KIDNEY FUNCTION IN THE RIVER LAMPREY, *LAMPETRA FLUVIATILIS*, ADAPTED TO FRESH WATER

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SUMMARY

Micropuncture techniques have been used to investigate the role of each nephron segment in the river lamprey. The proximal segment reabsorbed no more than 10% of filtered water and tubular fluid here was iso-osmotic to plasma, at about 250 m-osmol. Further water reabsorption occurred in later nephron segments so that 44% of all filtered water was reabsorbed. Dilution of tubular fluid began in the ascending limb of the nephron loop and continued in the distal and collecting segments, so that 96% and 97.5% of filtered sodium and chloride, respectively, was reabsorbed by the kidney. Lampreys produce 337 ml kg⁻¹ day⁻¹ of a dilute urine (31.2 m-osmol) and the urinary ducts appear not to modify the composition of this urine.

INTRODUCTION

During the freshwater phase of its life-cycle, body fluids of the lamprey are hyperosmotic to the external environment. Lampreys must therefore be able to counteract the tendency towards salt loss and influx of water across the gills and other permeable surfaces. The osmoregulatory mechanisms involved appear remarkably similar to those in teleosts. This is illustrated, for example, by the presence in both groups of 'chloride cells' which have been implicated in salt uptake (Morris, 1972).

The freshwater lamprey kidney, like that of teleosts, produces a copious dilute urine (Morris, 1972) and there have been a number of attempts at measuring urine flow rates (Wikgren, 1953; Hardisty, 1956; Morris, 1956; Bentley & Follett, 1963) but the estimates vary from 156 to 362 ml kg⁻¹ day⁻¹ and seem to have been affected by the collection technique. Salt concentrations are very low so that the freezing point depression of urine, at 0.077 °C (Morris, 1972) compares with 0.457 °C for plasma (Pickering & Morris, 1970). The role of each nephron segment in urine formation has been inferred from histological studies (Vinnichenko, 1966; Youson & McMillan, 1970, 1971a; Morris, 1972) but direct evidence of the kind provided by kidney micropuncture is lacking.

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It has been shown that much of the lamprey nephron is accessible for micropuncture (Logan, Moriarty, Morris & Rankin, 1979) and samples of tubular fluid can be collected from various points along the nephron. Inulin clearance has been used to estimate single nephron filtration rate (Moriarty, Logan & Rankin, 1978) and this substance can also be used to estimate fluid reabsorption by the nephron. In addition, electrolyte concentrations in samples of tubular fluid can be measured by X-ray microanalysis, which is now a widely used technique in many fields of physiology (Lechene, 1977).

It is hoped, in this way, to link nephron structure with function.

MATERIALS AND METHODS

River lampreys were caught during their spawning migration up the river Severn, transported to the laboratory as described by Logan *et al.* (1979) and held in tanks of fresh water at 12 °C. All experiments were carried out at this temperature, which was sufficiently high to allow rapid urine production (Wikgren, 1953) and yet low enough to reduce the risk of *Saprolegnia* infection (Larsen, 1965). Infected lampreys and those damaged in any other way were not used for osmoregulatory studies.

Preparation of micropipettes

Micropipettes were manufactured in the manner described by Moriarty *et al.* (1978). Pipettes with a tip diameter of 5–8 μm were used for puncturing the narrowest nephron segments but proximal tubules were large enough for pipette tips of 8–12 μm . Urinary ducts were punctured easily with pipettes of about 30 μm .

Constriction micropipettes were used to deliver a constant volume of tubular fluid, plasma or urine. The constriction was made, at a suitable distance from the pipette tip, with a de Fonbrune microforge. Some of these constriction pipettes were used to measure the volume of tubular fluid samples. They were calibrated by filling to the constriction with Iodine-125 solution of known activity and counting the whole pipette.

Measuring inulin clearance

(a) Administration of isotope and radioactive counting

To measure unulin clearance, 50 μCi [^3H]inulin ($> 300 \text{ mCi mmol}^{-1}$: Radiochemical Centre, Amersham), in 0.2 ml distilled water, was injected intraperitoneally approximately 15 h before each experiment. The inulin dose was increased to 250 μCi in 0.3 ml water for single nephron determinations, giving approximately 100 counts per min above background in kidney tubular fluid samples.

[^3H]Inulin was counted by liquid scintillation (Nuclear Chicago Unilux III), using Aquasol or water-diluted Biofluor (New England Nuclear) as scintillant. Counting efficiencies were determined by the external standard ratio method (Peng, 1977). There were no significant differences in quenching between any sample vials. All results are expressed in counts per minute. Each sample was counted for sufficient time to accumulate 4000–10000 counts and corrected according to the background count for each vial.

(b) Blood sampling, urine collection and micropuncture procedure

For inulin clearance studies, 150 μ l blood was taken from the caudal vein prior to the first urine collection period. Further blood samples were taken at 2 h intervals. For analytical purposes, 1–2 ml blood was taken in the same way.

All blood samples were centrifuged immediately and the plasma removed for analysis or radioactive counting. Plasma inulin counts were plotted against time, so that plasma counts at the mid-point of each urine collection period could be determined from the graph.

A polyethylene catheter (PP60, Portex Ltd., Hythe, Kent) was inserted into the urinary papilla and urine collected under oil in plastic tubes. Timed urine collection periods were from 0.5 to 1 h.

[³H]Inulin was counted in plasma and urine samples delivered into the scintillant by 100 nl calibrated micropipette.

Lampreys were prepared for kidney micropuncture in the manner described by Moriarty *et al.* (1978). Tubular fluid samples of 20 nl or less were sufficient for osmolarity measurements and electrolyte analyses. The orderly arrangement of lamprey nephrons (Morris, 1972; Logan *et al.* 1979) meant that segments being punctured could be easily identified.

For the calculation of tubular fluid:plasma inulin ratios, a calibrated micropipette was used to determine each sample volume, by repeated aspiration to the constriction, before transferring into scintillant for counting.

Nephron segments on the dorsal kidney surface (see Logan *et al.* 1979) were made accessible for micropuncture in the following way. A 5 mm length of fine, polyethylene tubing was inserted, through a transverse incision, into a urinary duct and a length of nylon thread tied around the tubing so that the kidney could be carefully turned-over and held in place by the line. The flow of renal blood and tubular fluid appeared to be unaffected, but urine was lost through the incision in the duct and could not be collected.

Analytical procedures

Electrolyte concentrations in suitable dilutions of plasma and urine were measured by emission (sodium) or atomic absorption (K, Ca, Mg) spectroscopy (EEL Atomic Absorption Spectrophotometer, 240). Chloride concentrations were determined by titration with an Aminco chloride meter (American Instruments Co.). Osmolarities of all samples were measured in a nanolitre osmometer (Clifton Technical Physics, N.Y.).

X-ray microanalysis

The polished surface of a block of beryllium (Metals Res. Ltd.) was covered in water-saturated paraffin oil. A 0.3 nl constriction pipette was used to deposit five identical drops of each sample on to the polished surface. This process was repeated for a range of standard solutions containing each element under investigation. Samples of urine and dialysed plasma were also deposited on the block using the same pipette, making a total of 100–200 drops per block.

Excess oil on the surface was drawn off into a glass capillary, before immersing the

block in chloroform for approximately 30 s to remove the remaining oil. The block was then pressed on to dry ice for 20 s to freeze the samples and transferred to a desiccator packed in ice, connected to a high vacuum pump. After 2–3 min, the vacuum pump was disconnected and the sealed desiccator left in a freezer at -20°C overnight. On removal from the freezer, the desiccator was warmed up to room temperature, and the block examined.

Freeze-drying of 0.3 nl samples generally gave uniform circular deposits of fine crystals which were analysed in a JEOL JXA₃A microprobe using the wavelength dispersive system. The electron beam current was 0.5 μA and the accelerating voltage was 15 kV. The beam was defocused to cover the largest crystal deposit, which was approximately 130 μm diameter. Two X-ray spectrometers were used so that two elements in each sample deposit were counted simultaneously.

The diffraction crystals used for each element were: Na, Mg, P, S: potassium hydrogen phthalate (K.A.P.); Ca, K: SiO_2 ; Cl: Mica.

Counting times were from 10 to 100 s, so that at least 1000 counts were accumulated for each element. Background corrections were obtained by directing the beam onto random points on the block, away from the crystal samples, and counting for 100 s. Concentrations of each element in a sample deposit were obtained by referring to the appropriate standard curve.

Calculations

Inulin clearance was calculated in a similar manner to Moriarty *et al.* (1978).

Percentage fluid reabsorption was given by:

$$\left(1 - \frac{U \text{ or } TF}{P}\right) \times 100,$$

where U = urine inulin cpm ml^{-1} , TF = tubular fluid inulin cpm ml^{-1} , P = plasma inulin cpm ml^{-1} at mid-point of urine collection period.

Net reabsorption of filtered electrolyte x was given by:

$$\left(1 - \frac{Ux \text{ or } TFx}{Px} \times \frac{P}{U \text{ or } TF}\right) \times 100.$$

A negative result indicates net secretion of x , where Ux = concentration (mM) of x in urine, TFx = concentration (mM) of x in tubular fluid, Px = concentration (mM) of x in plasma, U, P, TF = as above.

No correction was made for any Gibbs–Donnan effect across the glomerular barrier as no data were available on the magnitude of such effects in lampreys.

RESULTS

Concentrations of sodium, chloride and potassium in plasma and urine samples of freshwater river lampreys are given in Table 1. Calcium and magnesium concentrations were measured only in plasma samples.

Inulin clearance in 19 fish was $601.7 \pm 55.7 \text{ ml kg}^{-1} \text{ day}^{-1}$ (Table 2). Water reabsorption by the kidney, calculated on the assumption that C_{in} equals GFR, amounted to $44.3 \pm 2.3\%$. There was considerable variation between fish and in the same fish over different collection periods.

Table 1. *Electrolyte concentrations (mM) in plasma and urine of freshwater lampreys*

	Na ⁺	Cl ⁻	K ⁺	Ca ²⁺	Mg ²⁺
Plasma	122.1 ± 2.9 (27)	108.0 ± 4.3 (28)	3.2 ± 0.7 (17)	2.4 ± 0.2 (20)	1.1 ± 0.2 (20)
Urine	16.5 ± 1.8 (20)	11.0 ± 3.5 (19)	2.2 ± 0.6 (13)	—	—

Results are given as means ± S.E. The number of estimates are given in parentheses.

Table 2. *Urine flow rate and inulin clearance in freshwater lampreys*

Urine flow rate (ml kg ⁻¹ day ⁻¹)	Urine/plasma inulin	Inulin clearance (ml kg ⁻¹ day ⁻¹)	Water reabsorption (%)
337.1 ± 29.1 (19)	1.80 ± 0.08 (19)	601.7 ± 55.7 (19)	44.4 ± 2.3 (19)

Results are given as means ± S.E. The number of estimates are given in parentheses.

Table 3. *Water reabsorption by different segments of the freshwater lamprey nephron*

	Tubular fluid/plasma inulin	% water reabsorption (cumulative)
Proximal	1.10 ± 0.06 NS (26)	9.1
Nephron loop		
Desc. proximal	1.05 ± 0.03 NS (8)	4.8
Asc. distal	1.11 ± 0.03* (6)	9.9
Coll. duct	1.40 ± 0.06*** (10)	28.6
U. duct	1.62 ± 0.08** (5)	38.3
Urine	1.68 ± 0.05*** (12)	40.5

See Fig. 1 for description of nephron segmentation. Results are given as means ± S.E. The number of estimates are given in parentheses. Significance of differences from *TF/P* of 1.00 are given: NS, not significant; **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

No more than 10% of filtered water was reabsorbed by the proximal segment and descending limb of the nephron loop (Table 3). The six samples of distal fluid were collected near the bend in the loop. Most water reabsorption occurred further along the distal segment and in the collecting ducts, so that 38.3% of filtered water was reabsorbed before reaching the urinary ducts. There was no significant difference between this value and that for final urine (44.3%), which indicates there is little or no water reabsorption in the urinary ducts. There was also no significant difference (Student's *t* test) in osmolarity and electrolyte composition between urinary duct fluid and final urine (Tables 4, 5).

The osmolarity of lamprey plasma was 248.7 ± 9.5 m-osmol (Table 5, Fig. 1) and tubular fluid was iso-osmotic as far as the end of the proximal segment. There was some dilution of fluid in the ascending limb of the nephron loop and osmolarity

Table 4. *Electrolyte concentrations (mM) in urinary duct fluid and urine of freshwater lampreys*

	Na ⁺	K ⁺	Cl ⁻
Urinary duct fluid	13.0 ± 2.3 (8)	2.2 ± 0.3 (8)	14.7 ± 2.9 (8)
Urine	14.4 ± 1.9 (8)	1.8 ± 0.4 (8)	12.7 ± 3.4 (8)

Results are given as means ± s.e. The number of estimates are given in parentheses.

Table 5. *The osmolarity (m-osmol) of freshwater lamprey plasma and of samples taken from different nephron segments*

	Osmolarity
Plasma	248.7 ± 9.5 (14)
Proximal	249.6 ± 8.9 NS (18)
Desc. prox.	248.3 ± 3.5 NS (37)
Asc. distal†	217.1 ± 10.3** (11)
Coll. duct	44.9 ± 3.6*** (54)
U. duct	31.6 ± 6.4*** (12)
Urine	31.2 ± 5.9*** (9)

Results are given as means ± s.e. The number of estimates are given in parentheses. Significance of differences from plasma are given: NS, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

† Samples taken only from first third of this section.

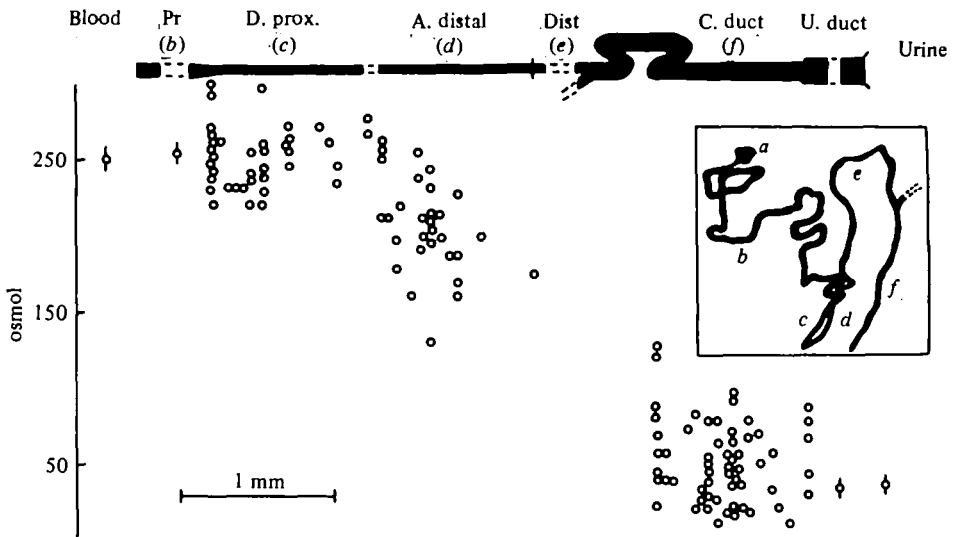


Fig. 1. Osmolarity (m-osmol) of fluid samples taken from various accessible points along the nephron of freshwater lampreys. Mean values ± s.e. are given for blood, proximal segment, urinary duct and urine. Drawing of a whole nephron shows relative lengths of each nephron segment: (a) capsule; (b) proximal segment; (c) descending proximal limb of nephron loop; (d) ascending distal limb; (e) distal segment; (f) collecting duct.

Table 6. Electrolyte concentrations (mM) and TF/P_{10n} ratios, obtained by X-ray microanalysis of fluid samples taken from the freshwater lamprey kidney

	Na ⁺	Cl ⁻	K ⁺	Mg ²⁺
Plasma	125.6 ± 4.9 (8)	122.8 ± 5.6 (9)	2.6 ± 0.2 (6)	1.5 ± 0.2 (6)
Proximal	120.5 ± 2.6 NS (27)	117.9 ± 2.9 NS (24)	1.9 ± 0.2 NS (22)	1.0 ± 0.1* (10)
Descending proximal	121.4 ± 4.4 NS (12)	119.8 ± 4.2 NS (12)	1.9 ± 0.1* (9)	1.0 ± 0.1* (5)
Ascending distal	120.5 ± 6.3 NS (7)	119.3 ± 5.8 NS (8)	1.9 ± 0.3* (9)	1.7 ± 0.1 NS (5)
Collecting duct	14.3 ± 2.7*** (12)	12.0 ± 2.9*** (12)	0.7 ± 0.2*** (11)	0.6 ± 0.2** (7)
Urinary duct	7.6 ± 2.2*** (9)	5.0 ± 1.5*** (9)	0.8 ± 0.1*** (8)	1.0 ± 0.2* (8)

Results are given as means ± s.e. The number of estimates are given in parentheses. Significance of differences from plasma are given: NS, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

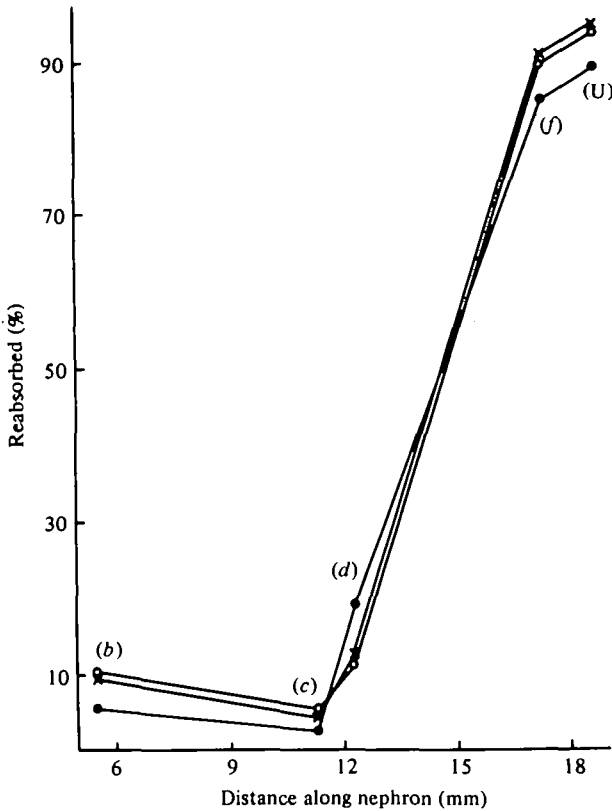


Fig. 2. Reabsorption of sodium (O), chloride (x) and total electrolytes (●) by different parts of the lamprey kidney. Estimates of reabsorption by each segment have been pooled and the mean value plotted against distance between glomerulus and mid-point of the corresponding segment. See Fig. 1 for explanation of labelling used. U represents urinary duct samples.

correlated well ($r = -0.42$; $P < 0.05$) with relative positions of the puncture sites along this section. The equation of the regression was:

$$y_{\text{osmol}} = 235.6 - 12.6x$$

(x represents distance along the ascending distal segment, as a % of segment length). Further dilution occurred in the distal tubule so that tubular fluid near the end of the collecting duct, at 44.9 ± 3.6 m-osmol, was close to the osmolarity of final urine (31.2 ± 5.9 m-osmol).

Plasma sodium, potassium and magnesium concentrations obtained by X-ray microanalysis were 125.6, 2.6 and 1.5 mM, respectively (Table 6). These are not significantly different ($P > 1$) from the estimates, given in Table 1, obtained by standard analytical methods. X-ray microanalysis gave a plasma chloride value of 122.8 mM, which was significantly higher ($P < 0.05$) than that obtained by the use of a chloride meter (108.0 mM). There is evidence to suggest that chloride ions in standard solutions, but not tubular fluid samples, are lost in the microprobe as chlorine gas (Roinel, 1975; Garland, Brown & Henderson, 1978). This would effectively elevate the chloride estimates in tubular fluid. Absolute values for chloride may therefore be suspect, but comparisons between different microprobe analyses should be consistent and valid.

Changes in absolute concentration and in tubular fluid:plasma (TF/P) ratios of sodium, chloride, potassium and magnesium along the nephron are given in Table 6. Approximately 96% of filtered sodium and 97.5% of filtered chloride was reabsorbed by the kidney (Fig. 2).

DISCUSSION

The physiological data obtained from anaesthetized lampreys during a micro-puncture experiment compare favourably with estimates of the same parameters in unanaesthetized fish. Concentrations of the principal plasma and urinary electrolytes (Table 1) are similar to those given by Robertson (1954) and Morris (1972), respectively. Moriarty *et al.* (1978) concluded that anaesthesia had no drastic effect on renal function in the lamprey although they accepted that anaesthesia and/or trauma from the operational procedure may cause slight diuresis, but this makes no difference to the validity of conclusions drawn in the present paper.

(a) *Function of the proximal segment*

Micro-puncture samples from various points along the lamprey proximal segment indicate that iso-osmotic reabsorption accounts for no more than 10% of filtered water (Table 3). This compares with up to 50% in the proximal tubule of *Necturus* (Bott, 1962; Garland, Henderson & Brown, 1975) and at least 67% in the rat (Gottschalk, 1961; Litchfield & Bott, 1962). Goncharevskaya (1976) also found that the fluid reabsorptive activity of the lamprey proximal segment is much lower than that of the rat.

The present study has shown that ion transport by the proximal segment in fresh-water lampreys is insignificant compared with that in later segments. This is consistent with evidence showing that the level of Na-K-ATPase activity in the lamprey proximal segment is much lower than in the distal segment and also lower than in the rat proximal segment (Natochin, 1972).

The structural similarity of lamprey proximal cells to those in the urinary duct of *Myxine glutinosa* (Ericsson & Seljelid, 1968), proximal segments of glomerular teleosts (for example: Bulger & Trump, 1968; Anderson & Loewen, 1975; Hentschel, 1977) and higher vertebrates (for example: Graham & Karnovsky, 1966; Maunsbach, 1969), indicates that their main function is reabsorption of filtered macromolecules. Reabsorbed protein may be transported intact (Youson, 1975) as seen in the flounder (Bulger & Trump, 1969), or digested by lysosomes.

(b) Distal segment and collecting tubules

Apart from possible slight reabsorption in the proximal segment, most water reabsorption by the freshwater lamprey kidney occurs between the beginning of the distal tubule and the end of the collecting duct (Table 3). Unfortunately, most of the distal tubule and half of the collecting duct is inaccessible for micropuncture, but samples from points along the straight section of the collecting duct indicate that water reabsorption is certainly not restricted to the distal tubule (Table 3).

Given that the main function of the freshwater lamprey kidney is to eliminate free water, it may appear strange that any filtered water should be reabsorbed.

TF/P_{osmol} in the late distal and collecting segments may however be lower than 0.2 and some passive water movement across the tubular epithelium may be unavoidable.

The main function of the distal tubule in freshwater lampreys, as in freshwater teleosts (Hickman, 1965), is salt reabsorption, with consequent dilution of tubular fluid. Reabsorption of Na^+ and Cl^- ions accounts for the change in osmolarity (Fig. 2), and the rapid fall in osmolarity along the ascending limb of the loop (Fig. 1) suggests that Na^+ and Cl^- reabsorption occurs largely in the distal tubule. There seems to be little change along the straight section of the collecting duct, though the inaccessible part of this segment may well be involved in salt reabsorption.

Histological evidence also points to the involvement of the distal segment in urinary dilution. The system of tubular endoplasmic reticulum and numerous mitochondria described in distal tubule cells of *Entosphenus japonicus* (Miyoshi, 1970), *Petromyzon marinus* (Youson & McMillan, 1971a) and *Lampetra fluviatilis* (Vinnichenko, 1966) is similar to that in cells known to be involved in ion transport, particularly fish 'chloride cells' (e.g. Conte, 1969; Pickering & Morris, 1977).

(c) Significance of the nephron loop

In the lamprey, each nephron segment occupies a specific region of the kidney. Microfil injection experiments (Logan *et al.* 1979) have confirmed Morris's (1972) plan of the spatial arrangement of nephrons in river lamprey kidneys. Ion concentrations and osmolarities of fluid samples taken from the descending proximal limb of the loop indicate there is no significant modification of fluid in this region. The descending proximal limb is simply the end of the proximal segment and is not a functionally distinct structure.

The nephron loop in lampreys has been compared with the loop of Henle in higher vertebrates (Youson & McMillan, 1971a; Natochin, 1977a) and it has even been suggested that it may be involved in the setting up of an osmotic gradient (Youson & McMillan, 1971b). Natochin (1977b) suggested however that the ability to produce a

hyperosmotic urine required a higher rate of blood flow through the kidneys than that found in lampreys.

Although no apparent function for the nephron loop was found in the freshwater lamprey, its resemblance to the mammalian loop of Henle raises the possibility that it may be important in some circumstances, possibly during the marine phase of the life-cycle.

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