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SINGLE NEPHRON FUNCTION OF THE LESSER SPOTTED DOGFISH, *SCYLIORHINUS CANICULA*, AND THE EFFECTS OF ADRENALINE

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SUMMARY

1. Function of the kidney and the nephron in the lesser spotted dogfish, *Scyliorhinus canicula*, was investigated by clearance, renal tubular micropuncture and ferrocyanide infusion techniques.

2. 70% of the glomerular filtrate was reabsorbed within the renal tubule, producing slightly hypotonic urine.

3. Glomeruli were: perfused and filtering (F); arterially perfused but not filtering (NF); or non-arterially perfused and hence not filtering (NP).

4. Adrenaline reduced the proportion of filtering glomeruli from 94% to 70%. Despite this reduction, a marked overall glomerular diuresis occurred.

5. Single nephron glomerular filtration rates (SNGFRs) ranged from 1·5 to 26 nl min^{-1} with a mean rate of 9·5 nl min^{-1} during control periods. Adrenaline elevated SNGFR to a mean value of 22·9 nl min^{-1} (range 2·4–64·6 nl min^{-1}).

6. Tubular fluid/plasma inulin concentration ratios (TF/P_{in}) indicated reabsorption of around 74% of the glomerular filtrate by the proximal segments. Comparison of TF/P and urine/plasma inulin concentrations (U/P_{in}) strongly suggests tubular secretion of water beyond the point of puncture. Adrenaline infusion appears to increase both proximal water reabsorption and distal tubular secretion.

INTRODUCTION

Elasmobranchs have high concentrations of urea and trimethylamine oxide within the extracellular fluids, which contribute towards making the plasma slightly hypertonic to the surrounding sea water (Stolte & Schmidt Nielsen, 1978). An osmotic influx of water leads to urine flow rates which are more typical of freshwater teleosts than marine teleosts (Evans, 1980). A high filtration rate per nephron is suspected as each glomerulus has a large filtering surface area, but there are, as yet, no published single nephron glomerular filtration rates (SNGFRs) for any elasmobranch species.

The ability to vary urine output is an important aspect of extracellular fluid volume control, and could, in theory, be achieved by modification of tubular reabsorption and/or modification of glomerular filtration rate (GFR). In elasmobranchs, some

Key words: kidney, single nephron, dogfish, *Scyliorhinus canicula*, adrenaline.

studies suggest that control of tubular water permeability is important (Henderson, Brown, Oliver & Haywood, 1978), while others indicate that both GFR and tubular water reabsorption are varied in response to body fluid expansion or changes of external salinity (Goldstein & Forster, 1971; Schmidt Nielsen, Truniger & Rabinowitz, 1972).

Total GFR is influenced by SNGFRs and the number of filtering nephrons (glomerular intermittency). Amongst the lower vertebrates studied so far, with the exception of the agnathan lamprey (Rankin, Logan & Moriarty, 1980; McVicar & Rankin, 1985), GFR is modified by glomerular intermittency (Brown, Oliver, Henderson & Jackson, 1980). A linear relationship between phenol red secretion and GFR in elasmobranchs (Kempton, 1966) suggests glomerular intermittency, but as yet glomerular activity has not been studied directly.

Physiological stressors such as hypoxia, repeated burst swimming and handling have been reported to cause release of the potent vasoactive catecholamines (Mazeaud & Mazeaud, 1981; Butler, Metcalf & Ginley, 1986). These substances can induce a pronounced diuresis (Myers *et al.* 1971; Forster, Goldstein & Rosen, 1972), but the mechanisms of such a renal effect remain unclear.

The present studies are, as far as we are aware, the first direct analysis of single nephron glomerular function of an elasmobranch. SNGFRs were measured by renal tubular micropuncture and the functional state of the glomerular population was studied by a direct approach. Further experiments investigated the influence of adrenaline on glomerular function.

MATERIALS AND METHODS

Fish

Lesser spotted dogfish, *Scyliorhinus canicula*, weighing 450–1000 g were kept in 500-l tanks of aerated, filtered and circulating sea water at 9°C. Experimental fish were starved for 7–14 days before use.

Surgical preparation

Dogfish were anaesthetized by immersion in 0·015 % MS222 and placed on a V-shaped support held within a Perspex trough, with head and gills immersed in aerated 0·0067 % MS222 at 9°C. This level of MS222 maintained anaesthesia without inhibiting spontaneous respiration. Similar anaesthetic levels were used in measuring single nephron glomerular function (see below).

The lienogastric artery and posterior intestinal vein were catheterized (PE 50, Portex) *via* a mid-ventral incision. The body wall and inner peritoneum were sutured separately with a layer of absorbent gelatin sponge between. For experiments on whole kidney function, the urinary papilla of female dogfish was catheterized (PE 50).

Fish were allowed to recover from preparative surgery in 15-l darkened aquaria containing circulating, aerated and cooled (9°C) sea water, for 24 h prior to further

study. During experiments animals were continuously infused intravenously at 5 μl min^{-1} with either dogfish Ringer's solution alone [containing (in mmol l^{-1}) NaCl, 280; KCl, 7·2; $CaCl_2$, 5; $MgCl_2$, 5; Na_2SO_4, 2; $NaHCO_3$, 4·6; NaH_2PO_4, 0·5; urea, 360; pH 7·4] or with adrenaline added so that 1 μg min^{-1} kg^{-1} body mass was administered.

Kidney function

Overall GFR and SNGFR were measured using inulin. Immediately following preparative surgery, a priming injection of 10% inulin in Ringer's solution was administered *via* the posterior intestinal vein, followed by continuous infusion of 2% inulin at 5 μl min^{-1}. For whole kidney studies, timed collections of urine samples into preweighed vials was commenced 24 h later, when plasma inulin concentration was stable at around 1·0–1·5 mg ml^{-1}. A minimum of six, 60-min control renal clearances was collected from each of four dogfish with 500-μl mid-point blood samples taken from the lienogastric artery. A further six clearances were taken during infusion of adrenaline. Blood samples were centrifuged, the plasma was decanted, and erythrocytes were reinjected in 500 μl of Ringer's solution. Urine and plasma samples were stored at 4°C, for analysis of osmolarity, or frozen for later inulin analysis.

Single nephron glomerular filtration rates (SNGFRs)

Micropipettes were pulled from 1 mm external diameter capillary tubing (Clark Electromedical Instruments), forged to a suitably sized tip (De Fonbrune Microforge) and double-bevelled on an air-driven rotating fine grit stone.

A 10-cm ventrolateral incision was made anteriorly from a point caudal to the urinary papilla. Abdominal organs were gently displaced, and an incision in the peritoneal membrane revealed the underlying kidney. Dorsal and lateral edges of the kidney were carefully cleared of connective tissue, and paraffin oil was dropped onto the exposed kidney.

Blood pressure was recorded throughout each experiment *via* a cannula implanted in the lienogastric artery (Washington 400 with PT400 pressure transducer). SNGFRs were measured in 12 anaesthetized fish. Ten animals were used to measure normal SNGFRs. In five of these fish, tubular fluid collections were also made during adrenaline infusion; collections were not commenced until a stable pressor response to adrenaline had been achieved. In a further two animals, collections were only taken during adrenaline infusion. Tubules were punctured with oil-filled micropipettes (paraffin oil dyed with Sudan black) and a block of oil 2–3 times the length of the luminal diameter was injected. Fluid was collected for timed periods of between 5 and 15 min while holding the injected oil block stationary with gentle suction. The samples were sandwiched in paraffin oil, and either analysed immediately or stored overnight at 4°C for later analysis. A 500 μl-blood sample was collected after each tubular fluid collection.

Assessment of the filtering population of glomeruli

Patterns of glomerular perfusion and filtration were examined in seven control animals and five animals infused with adrenaline, by application of the Hanssen ferrocyanide technique (Hanssen, 1958). A 30% solution (mass/volume) of sodium ferrocyanide in Ringer's solution was infused into anaesthetized animals, *via* the lienogastric artery, at 3 ml min^{-1} for 40 s. Renal blood flow was stopped by cutting across the spinal cord, dorsal aorta and posterior cardinal sinuses, anterior to the head kidney. Ferrocyanide infusion had minimal effects on cardiovascular function; systemic blood pressure was only slightly reduced, by a maximum of 10%, and heart rate was unaffected (Green, 1986).

The kidneys were rapidly removed, snap-frozen by immersion in isopentane at about −160 °C and cut into small pieces for freeze-substitution with ferric chloride. Following precipitation of Prussian blue, the kidney was macerated in 20% HCl at 37 °C for 4 h, and stored in dilute ferric chloride (Hanssen, 1958). Glomeruli and attached neck segments were microdissected.

Analyses

Osmolarity of urine or plasma was determined in a Wescor 5100C vapour pressure osmometer. Inulin concentrations of plasma and urine were determined by the resorcinol method (Schreiner, 1950), scaled down to use 50-μl samples of plasma and 5-μl samples of urine. Standard solutions of inulin (0·5–4 mg 100 ml^{-1}) were run in triplicate. In preliminary studies, peak absorbance occurred at 410 nm and this wavelength was used throughout. Plasma blanks gave consistently low absorbance readings (0·006 ± 0·003).

The mean renal clearances for control and experimental periods were taken for each fish. Data for the group of animals were expressed as mean ± standard error and analysed by paired *t*-tests.

The volumes of tubular fluid samples were calculated from the diameters of the fluid droplets ejected into water-equilibrated oil. Tubular fluid inulin concentrations were assayed by a fluorometric technique (Vurek & Pegram, 1966; Green, 1986) using a Perkin Elmer LS5 (405 nm excitation, 472 nm emission).

Single nephron glomerular filtration rates were not normally distributed and were therefore analysed by the non-parametric Wilcoxon two-sample test. Differences in tubular fluid/plasma inulin concentration ratios (TF/P_{in}) were analysed by the confidence interval test (Scheer, 1986). Patterns of glomerular perfusion were analysed by testing the equality of two percentiles (Sokal & Rohlf, 1969).

RESULTS

Renal function of conscious, resting dogfish is summarized in Table 1. Long clearance collections (60 min) overcame variations in urine output caused by periodic discharge from the large urinary sinuses during periods of spontaneous activity. The mean urine/plasma inulin concentration ratio (U/P_{in}) indicates that a net 70% of the

Table 1. *Renal function of conscious, resting dogfish*

	$\dot{V}$	U/P_{in}	GFR	U/P_{osm}	C_{osm}/GFR	C_{H_2O}/GFR
Control	0·17 ± 0·02	3·5 ± 0·4	0·58 ± 0·04	0·98 ± 0·01	0·29 ± 0·03	0·006 ± 0·002
Adrenaline	0·50 ± 0·11*	3·3 ± 0·4	1·59 ± 0·31*	0·98 ± 0·01	0·32 ± 0·05	0·007 ± 0·002

Values are mean ± S.E.M. from four fish infused with dogfish Ringer's solution during control periods followed by 1 $\mu g\,min^{-1}\,kg^{-1}$ adrenaline.
$\dot{V}$, urine flow rate ($ml\,h^{-1}\,kg^{-1}$); U/P_{in}, urine/plasma inulin concentration ratio; GFR, glomerular filtration rate ($ml\,h^{-1}\,kg^{-1}$); U/P_{osm}, urine/plasma osmolarity ratio; C_{osm}/GFR, relative clearance of osmolytes; C_{H_2O}/GFR, relative free water clearance.
* Values significantly different from controls, $P < 0·05$.

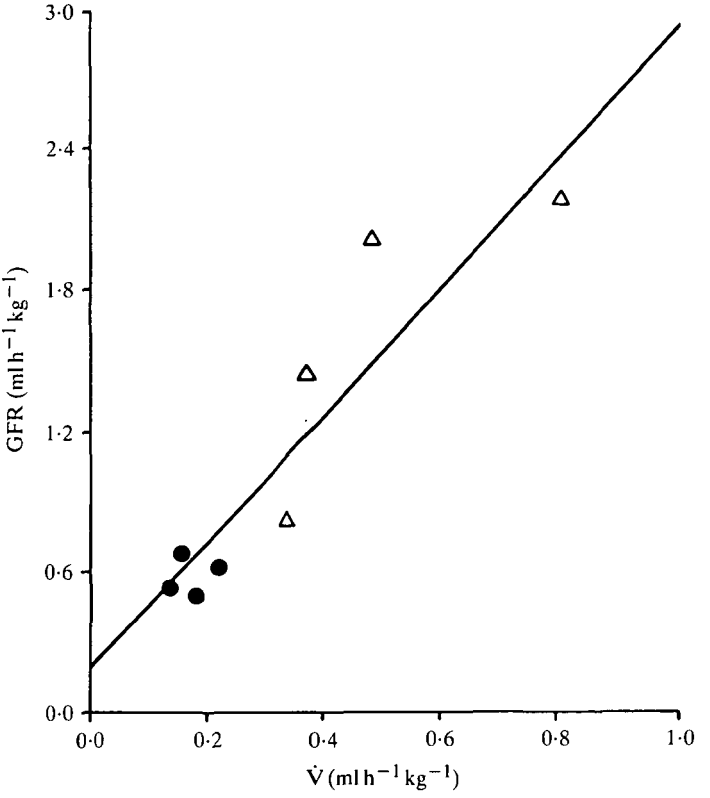


Fig. 1. Relationship between glomerular filtration (GFR) and urine flow rate ($\dot{V}$) in the spotted dogfish, *Scyliorhinus canicula*. Values are mean rates during infusion with dogfish Ringer's solution (●) and subsequent infusion of adrenaline at 1 μg min^{-1} kg^{-1} (△); $r = 0{\cdot}92$; $N = 8$; $P < 0{\cdot}001$.

filtered volume was reabsorbed by the renal tubule. Urine was slightly hypotonic relative to plasma with a mean U/P osmolarity ratio of 0·98. Hence, there was a small relative free water clearance of less than 1 %, whereas approximately 30 % of filtered osmolytes were excreted.

Infusion of adrenaline produced a marked though variable degree of glomerular diuresis (Table 1). There was a clear linear relationship between glomerular filtration and urine flow rates when data from both control and adrenaline-infused animals were included (Fig. 1). U/P_{in} was unchanged, indicating that adrenaline had no net effect on water reabsorption. Relative free water and osmolyte clearances were also unchanged by infusion of adrenaline (Table 1).

The distribution of SNGFRs of control and adrenaline-infused dogfish are shown in Fig. 2. In control animals SNGFR ranged from 1·5 to 26·0 nl min^{-1} ($N = 26$), with a mean value of $9{\cdot}5 \pm 1{\cdot}4$ nl min^{-1}. During infusion of adrenaline, some glomeruli appeared to continue to filter at normal rates, but in others the SNGFR was clearly elevated giving a wide range of values (2·4–64·6 nl min^{-1}). The mean SNGFR of $22{\cdot}9 \pm 3{\cdot}6$ was significantly elevated ($P < 0{\cdot}01$).

The mean TF/P_{in} ratio of micropuncture samples from control fish, $3{\cdot}8 \pm 0{\cdot}3$, differed significantly ($P < 0{\cdot}001$) from the mean during adrenaline infusion, $7{\cdot}1 \pm 0{\cdot}6$. The distribution of the values is shown in Fig. 3. The mean ratio in the controls indicates a mean net reabsorption of 74% of the filtered fluid, while the value in adrenaline-infused fish indicates 86% net reabsorption.

The SNGFRs reported here are values for those nephrons in which there was fluid flow along the tubule. In some punctured tubules the injected oil did not move. The Hanssen ferrocyanide technique showed that glomeruli were in one of three different functional states (Fig. 4): perfused and filtering (F); arterially perfused but non-filtering (NF); or non-arterially perfused and hence non-filtering (NP). In control animals, almost all glomeruli ($93{\cdot}8 \pm 2{\cdot}0\%$) were filtering. A small proportion, $4{\cdot}4 \pm 1{\cdot}9\%$ were arterially perfused but not filtering, with only $1{\cdot}8 \pm 0{\cdot}6\%$ not arterially perfused (Fig. 5). During adrenaline infusion there was a reduction in the proportion of F nephrons to $69{\cdot}5 \pm 2{\cdot}4\%$, reflecting a doubling of the proportion of NF nephrons ($9{\cdot}0 \pm 2{\cdot}5\%$) plus a dramatic increase in the number of NP nephrons ($21{\cdot}5 \pm 1{\cdot}7\%$; Fig. 5). Thus adrenaline reduced the number of filtering nephrons while significantly increasing the number of non-filtering (NF and NP) nephrons within the kidney ($P < 0{\cdot}001$).

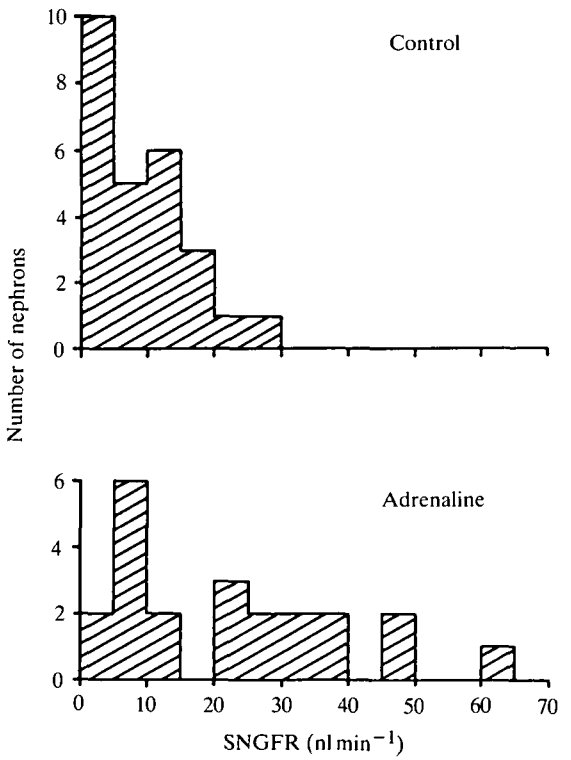


Fig. 2. Distribution of single nephron glomerular filtration rates (SNGFR) in control animals infused with Ringer's solution and during infusion of adrenaline ($1\ \mu g\ min^{-1}\ kg^{-1}$).

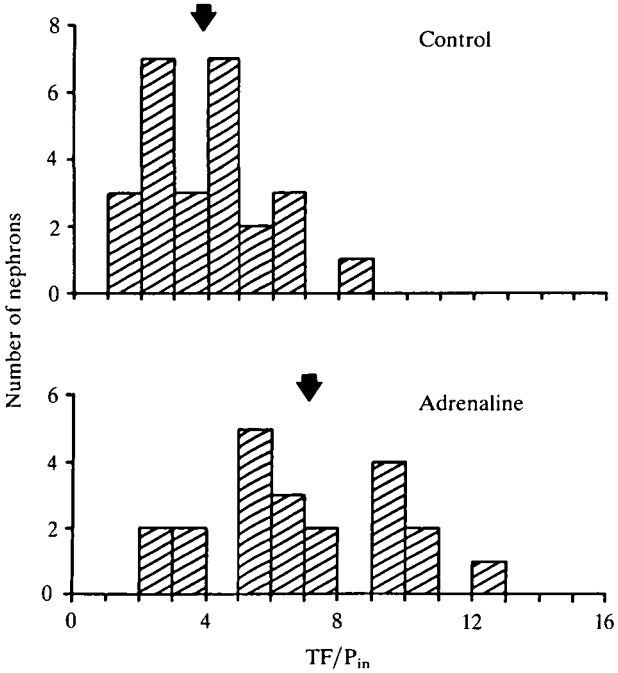


Fig. 3. Distribution of tubular fluid/inulin concentration ratios (TP/P_{in}) in control animals infused with Ringer's solution and during infusion of adrenaline ($1\,\mu g\,min^{-1}\,kg^{-1}$). Arrows show mean ratios which differ significantly ($P < 0{\cdot}001$).

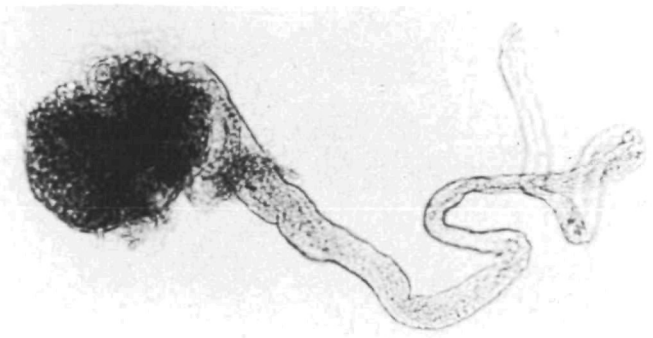
DISCUSSION

The use of inulin to measure glomerular filtration rates and study tubular handling of water depends upon the assumption that inulin is freely filtered at the glomerulus and neither reabsorbed nor secreted by the renal tubule. The spiny dogfish, *Squalus acanthias*, was one of the first animals in which inulin was used in this way (Shannon, 1934). More recently, its application has been thoroughly assessed in the rat where it appears to be a satisfactory marker for GFR (Maude, Scott, Shehadeh & Solomon, 1965). In lower vertebrates the picture is less clear. There have been no detailed studies of the handling of inulin by elasmobranch renal tubules or the urinary sinus. Slight tubular reabsorption has been reported to occur in the river lamprey (Moriarty, Logan & Rankin, 1978) and the urinary bladder and tubules of teleost fish are slightly permeable to inulin (Beyenbach & Kirschner, 1976). In the light of these studies, the present measurements may slightly underestimate GFR. Any errors in the SNGFR measurements are likely to be less significant, as tubular fluid was collected from proximal segments.

Infusion of *Scyliorhinus canicula* with adrenaline resulted in a marked diuresis. Adrenaline has been reported to cause a glomerular diuresis (Myers *et al.* 1971) but

Fig. 4. Glomerular types showing precipitated Prussian blue. (A) Arterially-perfused and filtering (F) with dark precipitate in glomerular capillaries and neck segment; (B) arterially perfused but not filtering (NF) with precipitate in glomerulus but not neck segment; (C) non-perfused (NP) with no associated Prussian blue. Scale bar, 200 μm.

4 A



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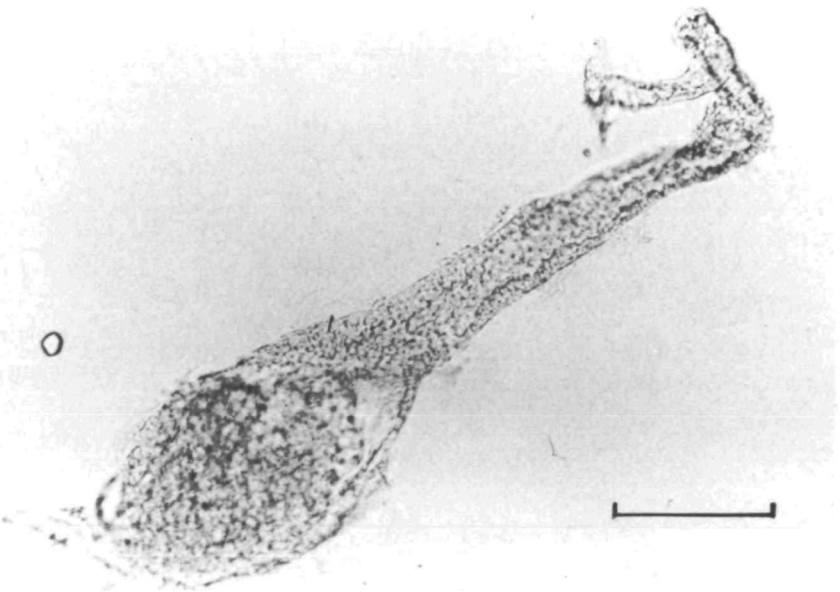

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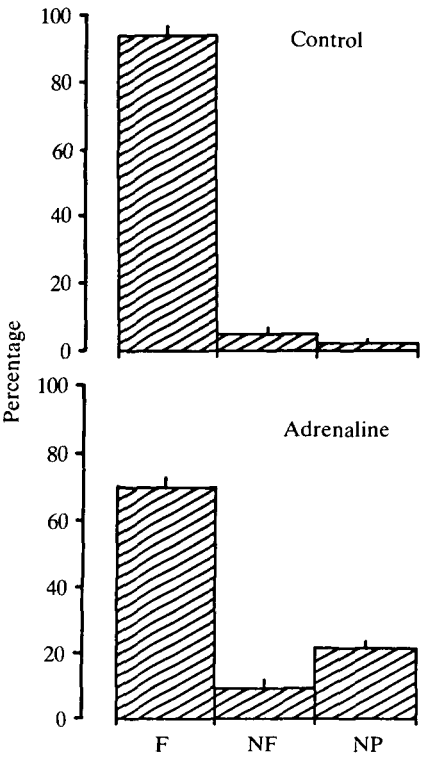

Fig. 5. Distribution of three types of glomeruli occurring in the dogfish kidney; arterially-perfused and filtering (F), arterially perfused but non-filtering (NF) and non-arterially perfused (NP). Values are mean percentages ± S.E. in control animals infused with Ringer's solution and fish infused with adrenaline.

other studies have suggested a tubular response may occur (Forster *et al.* 1972). In the present experiments, the observed degree of diuresis was variable but resulted entirely from increases in GFR with no detectable change in net tubular water reabsorption.

Most teleost fish show linear relationships between urine flow and glomerular filtration rates suggesting that extracellular fluid volume is principally regulated by changes in glomerular filtration rates (Brown *et al.* 1980; Rankin, Henderson & Brown, 1983). In the dogfish, in our experimental conditions, there appears to be a similar pattern for control of water balance.

During the micropuncture experiments, oil blocks introduced into renal tubules occasionally remained stationary. After a variable period of time such blocks sometimes moved down the tubule, suggesting recommencement of filtration. Glomerular intermittency was also suggested by microscopic examination of superficial glomeruli *in vivo*, where visible flow of erythrocytes through the capillary loops was sometimes followed by an apparent cessation of flow or *vice versa*. Intermittent activity was confirmed by analysis of the patterns of glomerular perfusion. At any time, a small percentage of glomeruli was either completely

unperfused or was perfused but failed to filter. The differences between these physiological states within the glomerular population are unclear but differing filtration pressures or ultrafiltration coefficients within the glomerular population must be suspected. These glomerular states also exist in teleosts where modification to the pattern of glomerular activity can occur (Brown *et al.* 1980).

It is particularly interesting that in the control dogfish most of the glomeruli appeared to be filtering. In the freshwater-adapted rainbow trout 45 % of glomeruli filter, but this is reduced to 5 % in seawater-adapted fish (Brown *et al.* 1980). In the agnathan lamprey, however, all glomeruli are thought to be filtering (Moriarty *et al.* 1978). The reduction in the population of filtering glomeruli observed during infusion of adrenaline conflicts with earlier speculation that adrenaline-induced diureses may result from an increased population of filtering nephrons (Deetjen & Boylan, 1968). It followed from our observations of decreased numbers of filtering nephrons, during an overall glomerular diuresis, that SNGFR was likely to be elevated. Our micropuncture studies confirmed this notion but measurements can only be made on nephrons with superficial tubular segments. Fortunately, the nephron configuration within the elasmobranch kidney suggests that many nephrons have surface loops in both the dorsal 'bundle' region and the ventral 'sinus' region (Lacy & Reale, 1985; Lacy *et al.* 1985).

The observed SNGFRs indicate an extremely heterogeneous population of glomeruli. Rates in control animals are high compared to those of the seawater-adapted rainbow trout (Brown, Jackson, Oliver & Henderson, 1978) or the lamprey in brackish water (Rankin *et al.* 1980; McVicar & Rankin, 1985). This may reflect the large filtering surface area of the dogfish glomerulus, or differences in the haemodynamics of filtration.

SNGFRs and the functional state of glomeruli were studied in anaesthetized animals, while GFR and whole kidney function were only examined in the conscious, resting animal. Unfortunately, the lack of movement during anaesthesia precludes the reliable collection of free-flow urine. GFR of control anaesthetized animals calculated from SNGFRs and the number of filtering nephrons is high ($1{\cdot}2\,ml\,h^{-1}\,kg^{-1}$) compared to the range observed in conscious animals ($0{\cdot}34$–$0{\cdot}81\,ml\,h^{-1}\,kg^{-1}$) while estimated GFR in adrenaline-infused, anaesthetized fish ($2{\cdot}1\,ml\,h^{-1}\,kg^{-1}$) is within the range for conscious, adrenaline-infused animals ($0{\cdot}8$–$2{\cdot}2\,ml\,h^{-1}\,kg^{-1}$). This discrepancy may be the result of adrenaline release. Circulating levels of adrenaline are elevated during MS222 anaesthesia ($1{\cdot}9 \pm 0{\cdot}9\,pmol\,ml^{-1}$ conscious resting fish, $14{\cdot}4 \pm 4{\cdot}2\,pmol\,ml^{-1}$ during anaesthesia; Green, 1986). It is therefore likely that the single nephron effects of adrenaline will be unavoidably underestimated in the present studies.

There are a number of possible causes of the adrenaline-induced changes in single nephron function. The pressor action of adrenaline (Capra & Satchell, 1977) may elevate glomerular capillary blood pressure and/or increase glomerular capillary blood flow (SNGBF) and thus elevate SNGFR. Direct observations of glomeruli during micropuncture experiments suggested an increased SNGBF. Dilation of

glomerular bypass shunts located between the afferent arteriole and the peritubular capillary network (Green, 1986) may be behind the concomitant increase in the proportion of non-arterially perfused nephrons.

The extreme length and complex configuration of the elasmobranch nephron (Lacy *et al.* 1985) precludes the determination of an exact site of micropuncture. Some tubular fluid samples were taken from the large diameter, ventrally situated, yellow convoluted proximal segment which makes up the majority of the nephron. Most samples, however, were from a more distally situated and narrower proximal segment, located on the dorsal and lateral margins of the posterior kidney.

In our studies, adrenaline infusion had no effect on net tubular water reabsorption (U/P_{in}) but fluid reabsorption by the proximal tubule appeared to be elevated. This does not appear to be due to a glomerulo-tubular balance as TF/P_{in} and SNGFR in animals infused with adrenaline were not correlated.

Urine/plasma inulin concentration ratios of conscious fish are lower than proximal TF/P_{in} ratios of anaesthetized fish, which suggests tubular water secretion beyond the point of puncture. Infusion of adrenaline appears to elevate this more distal secretion. The implied tubular effects of adrenaline on proximal water reabsorption and distal water secretion may be due to a more generalized permeability effect; adrenaline increases the permeability of other epithelia such as fish gills and frog skin (Isaia, 1984).

From the mean values of SNGFR, TF/P_{in} and U/P_{in} we can estimate that a minimum of $0{\cdot}2\,nl\,min^{-1}$ in control animals and $4\,nl\,min^{-1}$ in adrenaline-infused animals was secreted by the distal segments or collecting duct beyond the point of micropuncture. Water reabsorption in the distal segment and collecting duct seems probable so that secretion is likely to exceed these estimates. Admittedly these predictions involve combining data from conscious and anaesthetized fish, but they do nevertheless provide some guide as to the possible extent of distal water secretion. An *in vitro* technique recently demonstrated tubular water secretion of $29\,pl\,min^{-1}\,mm^{-1}$ by the large-diameter second proximal segments of *Squalus acanthias* nephrons (Sawyer & Beyenbach, 1985). The length of this convoluted segment in *Scyliorhinus canicula*, based on a reconstruction of an entire nephron is about 27 mm (J. A. Brown, unpublished observations) so that proximal secretion of around $0{\cdot}8\,nl\,min^{-1}$ may occur. The present studies imply that water secretion is more widespread within the elasmobranch nephron than was previously appreciated. It would seem to be important to investigate the intense secretion of water apparently occurring in the more distal segments of the spotted dogfish nephron, especially during adrenaline infusion.

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