

FACTORS AFFECTING GLOMERULAR FUNCTION IN THE PACIFIC HAGFISH *EPTATRETUS STOUTI* (LOCKINGTON)

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SUMMARY

Single glomerulus filtration rate in *Eptatretus stouti* averaged 20.3 ± 2.13 (S.E.M.) nl min^{-1} . Single glomerulus glomerular filtration rate (GFR) could be correlated with arterial pressure when arterial pressure exceeded about 4 cm H_2O . Glomerular filtration was affected by postglomerular resistance brought about by alteration of the volume of urinary spaces. Filtration undoubtedly plays a role in glomerular function. However, average colloid osmotic pressure (COP) of the plasma is almost double the average hydrostatic pressure in the segmental arteries serving the glomeruli. The COP of glomerular fluid is essentially nil, therefore it is difficult to see how pressure filtration alone can account for primary urine formation.

When single glomeruli were perfused with colloid-containing Ringer at pressures within the normal range of blood pressures, GFR was within the normal range. GFR was related inversely to the colloid osmotic pressure of the perfusion Ringer. Colloid entered the urine during perfusion. However, in only a few instances did this result in conditions favourable to pressure filtration.

To assess the role of active processes in glomerular filtration, chemical inhibitors were added to the perfusion Ringer. Amiloride, acetazolamide, cyanide, 2-deoxy-D-glucose, iodoacetate and ethacrynic acid were without marked effect on glomerular filtration. Ouabain and dinitrophenol markedly reduced GFR; inhibition was probably not due to indirect effects upon the renal vasculature.

INTRODUCTION

The hagfish mesonephric kidney consists of but two epithelial elements of the vertebrate pattern: segmentally arranged renal corpuscles which empty via short neck segments into a larger ureter. The renal corpuscles are very similar to those of other vertebrates (Heath-Eves & McMillan, 1974; Kühn, Stolte & Reale, 1975). The epithelium of the ureter wall consists of cords of columnar cells between which are bunched cuboidal cells (Conel, 1917; Ericsson, 1967; Heath-Eves & McMillan, 1974). The columnar cells bear a superficial resemblance to cells of the proximal tubule of other vertebrates. Analyses of urine collected from the ureter suggest that secretion and reabsorption of ions occurs (Munz & McFarland, 1964; Rall & Burger, 1967; McInerey, 1974) as does reabsorption of glucose (Eisenbach *et al.* 1971). However, some functions assigned to the proximal tubule appear not to occur. Phenol red is not

concentrated (Fänge & Krog, 1963; Rall & Burger, 1967). Furthermore, inulin is not concentrated by the kidney, so apparently no water reabsorption occurs during elaboration of the urine (Munz & McFarland, 1964; Rall & Burger, 1967).

MATERIALS AND METHODS

The studies reported here were made on the Pacific hagfish *Epatretus stouti* carried out at the Hopkins Marine Station of Stanford University in Pacific Grove, California, U.S.A. Animals were trapped in Monterey Bay adjacent to the marine station. They were kept, unfed, in a shaded concrete tank through which circulated chilled (6–10 °C) sea water. A fresh stock of animals was trapped at about bimonthly intervals. All experiments were carried out at temperatures between 6 and 8 °C.

Measurements of urine flow

A hagfish was weighed and placed in 1 l of chilled sea water to which a small amount of MS 222 (Sandoz, Basel, Switzerland) was added. After relaxation was complete, the body cavity was exposed by a ventral midline incision. The animal was placed dorsal side down in a V-shaped perspex trough which had been immersed previously in a sea water-filled, temperature-controlled bath. An amount of MS 222 was added to the bath sufficient to sustain relaxation for several hours.

Whilst observing with a stereomicroscope mounted over the bath, connective tissue and fat bodies overlying a short section of the kidney were removed. A catheter was tied into the ureter adjacent to the glomerulus chosen for study. Catheters were made from polythene tubing pulled in a microflame to an average internal diameter of about 0.5 mm. Where urine flow was to be measured by collection of urine formed over a timed period, short lengths of catheter partially filled with liquid paraffin were tied into the ureter. Urine was collected from the catheter by inserting a micropipette through the liquid-paraffin seal. Urine droplets were deposited under liquid paraffin on the waxed surface of a Petri dish resting on the stage of a stereomicroscope. The volume of the urine droplets was estimated from measurements of their diameter made at $\times 40$ to $\times 80$ with an ocular graticule.

The foregoing procedure created variable backpressure in the ureter. In some studies it was desired either to study or to minimize the effects of ureter backpressure on GFR. In these studies the catheter was connected to a reservoir and pressure transducer which were filled with liquid paraffin. Urine volume was estimated from measurements of fluid advance in the catheter made at $\times 40$ with an ocular graticule. Strands of ligature *ca.* 5 μm in diameter tied around the catheter at intervals of 3–5 mm provided fixed points of reference for lining up the ocular graticule and catheter.

Estimations of ion concentrations and colloid osmotic pressure

Urine was collected as described above. Blood was collected from the caudal subdermal sinus (Germain & Gagnon, 1968). About 2 ml of blood was mixed rapidly with dry heparin in a prechilled centrifuge tube to a final concentration of *ca.* 500 i.u./ml (Fänge & Gidholm, 1973). Blood was centrifuged for 10 min at 0 °C and *ca.* 2000 rev/min. To obtain plasma samples from animals upon whom experiments had been performed, blood was collected by puncture of the dorsal aorta with centrifugible pipettes (Riegel, 1968).

Colloid osmotic pressure of urine and plasma samples was measured using a comparative method devised for use on small samples of fluid. The details of this method will be published elsewhere, but a brief description follows. A small disc of Millipore 'Pellicon' ultrafiltration membrane (25 000/45 000 mol. wt cutoff) was wetted with hagfish Ringer in its centre, and on one side was deposited a drop (2–5 μ l) of standard colloid solution. The disc was fixed in place in the liquid-paraffin bath of an apparatus which allowed the upper surface of the disc to be observed from the side at a magnification of about $\times 100$. Droplets of standard colloid solutions, plasma and urine, 10–20 nl in volume, were deposited, one at a time, on the upper surface of the disc. Using a calibrated graticule in the eyepiece of the viewing microscope, the rate of shrinkage of each of the droplets was measured over a short interval (usually 3–5 min.). The COP of unknown droplets was obtained by interpolation from a standard curve constructed using the shrinkage rates of droplets of standard colloid. The method appears to be accurate only to about 2 cm H₂O, but that accuracy is sufficient for the present purpose.

Analyses of ions in plasma and urine were made with a Perkin Elmer heated graphite atomizer (HGA 2100).

Measurements of hydrostatic pressure

Hydrostatic pressures were measured in the dorsal aorta, segmental artery, left posterior cardinal vein and ureter. One port of a blood-pressure transducer was connected to a perfusion pump and the other port was connected to a small cannula. Cannulae were made of 2 mm diameter capillary that had been pulled to a tip diameter of *ca.* 0.05 mm and sharpened. Placement of the cannulae was facilitated by use of a Narishige micromanipulator.

All pressure measurements were made with Elcomatic type EM 750 transducers coupled to a George Washington recorder (MD 400/2) through full-bridge strain-gauge preamplifiers. The sensitivity of the recorder amplifier was set in most cases so that pressure changes of 0.25 cm H₂O produced a deflexion of 1 mm on the recording chart. Pressure was usually read to the nearest mm, but small pressure changes were read to the nearest 0.5 mm.

Perfusion studies

Ureters of relaxed hagfishes were catheterized as described earlier. Loose ligatures were placed around the dorsal and ventral branches of the segmental artery (Fig. 1). GFR was measured until a consistent rate was established with blood perfusing the glomerulus. The segmental artery was then cannulated, the pressure in the segmental artery recorded briefly, and perfusion begun. At this time, the loose ligatures were tightened. The composition of the perfusion fluid is shown in Table 1.

Ficoll 70 (Pharmacia (GB) Ltd.), a polymer whose molecular weight averaged 67 000, served as a colloid and glucose served as a metabolizable substrate. Perfusion fluid was made up each day by adding weighed amounts of glucose and Ficoll 70 to stock buffered Ringer. Each batch of perfusion fluid was filtered through a Millipore filter of 1.2 μ m average pore diameter.

In one series of experiments the following chemical inhibitors were used: acetazolamide (Diamox), 2,4-dinitrophenol, 2-deoxy-D-glucose, ouabain (Strophanthin

Table 1. *The composition of fluid used to perfuse isolated renal segments of hagfishes*

(A buffered Ringer (upper part of the table) was added to the organic constituents listed in the lower part of the Table. The Ringer composition was based on analyses of plasma ions made by Munz & McFarland (1964); the sodium concentration was reduced to compensate for Tris.)

Substance	g ml ⁻¹ × 500	mm l ⁻¹
NaCl	15.5	530
KCl	0.2611	7
CaCl ₂ · 2H ₂ O	0.3308	4.5
MgCl ₂ · 6H ₂ O	1.2198	12
Na ₂ SO ₄ · 10H ₂ O	0.1870	0.85
Tris	1.5145	25
pH	Adjusted to 7.6 with HCl	
	g ml ⁻¹ × 10	
Glucose	0.0032	
Ficoll 70		
6 cm H ₂ O	0.1746	
12 cm H ₂ O	0.3492	
24 cm H ₂ O	0.6984	

G), iodoacetic acid, sodium cyanide, amiloride and ethacrynic acid. The latter two chemicals were supplied through the courtesy of Dr G. M. Fanelli of Merck, Sharp and Dohm, West Point, Pennsylvania, U.S.A.

In experiments involving chemical inhibitors a dual perfusion arrangement was used. One of two identical channels of a perfusion pump could be selected by simultaneously turning 3-way stopcocks at the cannula and pump. Both channels were connected to blood-pressure transducers and pressure in the channel perfusing isolated renal segments was recorded continuously.

RESULTS

Fig. 1. depicts the renal structures found in one of the 30 or so body segments which bear glomeruli. The kidney is served by renal arteries which are said to supply the glomerulus exclusively (Grodziński, 1926; Heath-Eves & McMillan, 1974). In Fig. 1 the renal artery is shown as a branch of a segmental artery, but renal arteries also arise directly from the dorsal aorta or as a branch of a mesenteric artery (Grodziński, 1926). Efferent to the glomerulus are 1-3 vessels said by Heath-Eves & McMillan (1974) to be arterioles. These vessels supply the extensive capillary network of the ureter wall. The ureter wall is also supplied by blood vessels which branch directly off the segmental arteries (personal observation).

Shown in the upper part of Fig. 1 is the configuration of the renal corpuscle and ureter in a freshly dissected hagfish. After some hours of functioning with a catheter tied into the ureter (and blocking it), the renal capsule and ureter usually became grossly distended. This is shown in the lower part of Fig. 1. Distension of the renal segment caused the closely packed cords of columnar epithelial cells seen in the collapsed ureter to move apart. Commonly also blood flow in the postglomerular vasculature was slowed or halted, and the glomerular capillary tuft became collapsed.

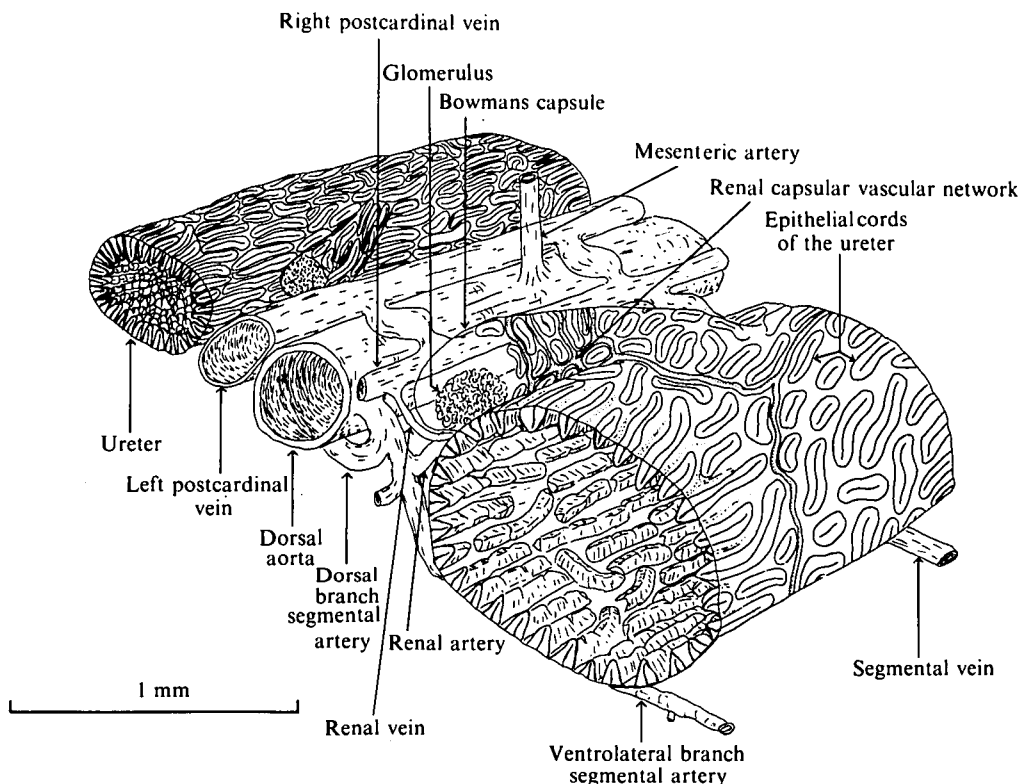


Fig. 1. Kidney and associated vasculature in one body segment from the ventrolateral aspect with the animal lying on its back. The overlying peritoneum and associated fat bodies have been removed. The upper part of the figure shows the freshly dissected kidney. The lower part of the figure shows the kidney after several hours of function with the ureter blocked.

The rate of urine formation

In the following discussion the rate of fluid movement into ureter catheters will be called 'glomerular filtration rate' (GFR). The justification for this rests on two observations. Firstly, the ionic composition of fluid collected from the ureter catheter more nearly resembles plasma than does final urine (Munz & McFarland, 1964; Stolte & Eisenbach, 1973). Secondly, water apparently is not reabsorbed from the urine in hagfishes (Munz & McFarland, 1964; Rall & Burger, 1967). Therefore, fluid collected in the ureter catheter should be isovolumetric with glomerular fluid.

Seventy-one measurements of maximum GFR made in 58 animals averaged 20.3 ± 2.13 (S.E.M.) nl min⁻¹. In the vast majority of the estimates ureter pressure was approximately atmospheric. There seemed to be no practical way of independently assessing the accuracy of measurements of GFR. However, on average, advance of fluid in the ureter catheter of one graticule division (= 0.05 mm) represented a volume of *ca.* 10 nl (i.e. $\pi r^2 h = 3.14 (0.25)^2 (0.05) = 0.00981$ mm³). Fluid advance in the catheter usually was read to a graticule division, although one-half division was easily estimated. Except when GFR was very slow, readings were made after fluid had advanced 10 divisions or so in the catheter. Therefore the accuracy of estimating fluid advance was at least 10%; the accuracy of estimating GFR probably was

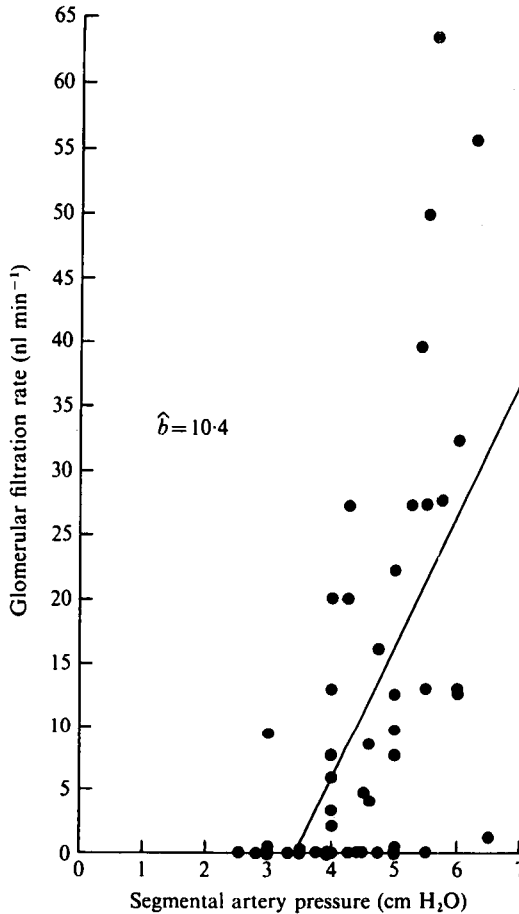


Fig. 2. Relationship between segmental artery pressure and GFR of the adjacent glomerulus. The line was plotted by least squares; its slope is significantly different from zero ($P \ll 0.01$).

similar. No GFR was measurable in 25 animals, although the blood flow through the renal vasculature was apparently normal.

Measurements of hydrostatic pressure in the segmental artery serving the catheterized ureter segment were made in 48 animals. In 29 such animals GFR was measurable. Fig. 2 illustrates the relationship between GFR and segmental artery pressure. The slope of the regression line (plotted by least squares) is statistically different from zero ($P \ll 0.01$). As shown in Fig. 2, GFR appears to be dependent upon arterial pressure when arterial pressure exceeds about 4 cm H₂O. The average pressure measured in segmental arteries of urine-producing glomeruli was 4.94 ± 0.16 (S.E.M.) cm H₂O. The average pressure measured in segmental arteries of hagfishes in which GFR was not measurable was 3.93 ± 0.20 (S.E.M.) cm H₂O.

Isolation of single glomeruli possibly could have an adverse or enhancing affect on GFR. To check this, measurements were made of urine produced by the entire kidney on one side of the body. A catheter was tied into the ureter well posterior to the glomerulus-bearing portion of the kidney. Six successful estimations of urine flow were made. These averaged $0.956 \mu\text{l min}^{-1}$ (range = 0.687 to $1.22 \mu\text{l min}^{-1}$). Three

Table 2. *Averages of the estimations of concentrations of major cations in plasma and urine of seven hagfishes and three samples of habitat water*

(Also shown are averages of the urine: plasma concentration ratios.)

	Na	K	Ca	Mg
Plasma concentration (mM)	506	6.88	9.86	12.2
Urine concentration (mM)	519	7.74	8.37	20.6
Seawater (mM)	496	12.5	11.4	50.5
Urine:plasma concentration ratio	1.02	1.29	0.89	1.07

Table 3. *Average hydrostatic pressures (cm H₂O) measured in the vasculature and ureter of hagfishes*

	Dorsal aorta	Segmental artery	Posterior Cardinal vein (left)	Blocked ureter	Ureter segment
Pressure	5.31	4.64	0.1	1.3	1.1
Range	4.25-6.90	2.80-5.75	0-0.4	0.5-2.75	0-3
Number	13	13	7	13	8

animals in which a count of glomeruli was made had a GFR per glomerulus of 34.3, 31.3 and 23.3 nl min⁻¹. These values fall well within the range of maximum GFR measured in single glomeruli (Fig. 2).

Estimates of COP and concentrations of ions

The average COP of the plasma of nine animals was 10.5 cm H₂O; blood samples from the caudal sinus appeared to be identical with blood samples taken from the dorsal aorta. The average COP of urine of four animals was 0.4 cm H₂O.

Estimations of the concentrations of major cations in plasma and urine are summarized in Table 2. Also shown are the average urine:plasma concentration ratios and the average concentrations of major cations in habitat water. Limits of error in analyses using the HGA 2100 were not studied systematically. However, the variation of individual estimations suggests that, except for calcium, the limits of error certainly were within 10% and probably closer to 5%. Calcium analyses were at times so difficult they were abandoned. It seems likely that except for potassium, concentrations of cations in the primary urine approximate to the plasma cation concentrations.

Measurements of hydrostatic pressure

Hydrostatic pressure measurements are summarized in Table 3. On average, the dorsal aorta pressure was higher than the pressure in the adjacent segmental artery. However, withdrawal of the pressure-detecting cannula from the dorsal aorta usually led to extensive haemorrhage. For this reason pressure recordings in the dorsal aorta were made at the end of experiments. The average difference may reflect only the time interval (generally 5-6 h) between the two measurements. Hydrostatic pressure in the left posterior cardinal vein was negligible.

The procedure adopted for isolating renal segments blocked the ureter. As shown in Table 3, hydrostatic pressures in blocked ureters could rise to quite appreciable levels. This was reflected by the degree of distension of the ureters.

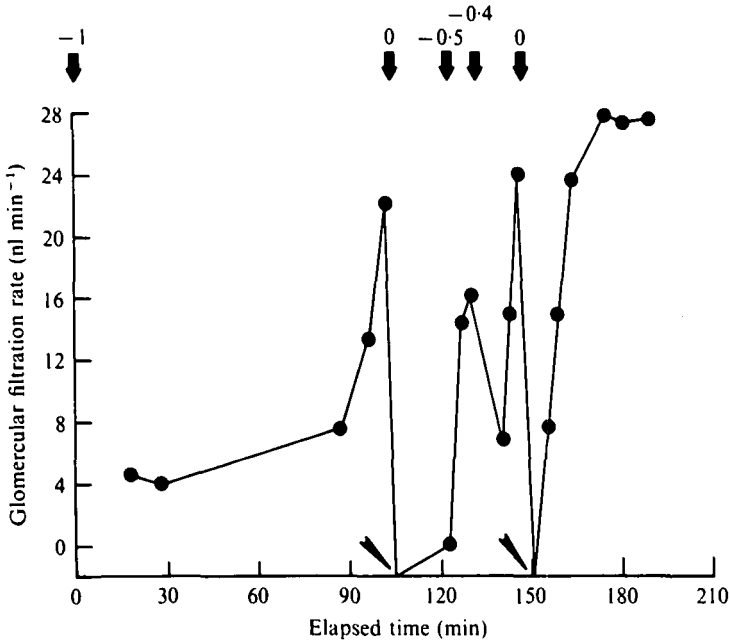


Fig. 3. Relationship between GFR and backpressure applied to the ureter (upper arrows). Lower (narrow) arrows indicate times at which backpressure caused fluid to move from catheter to ureter.

Effects of ureteral backpressure on GFR

Ureter distension accompanied the slowing and (ultimately) complete cessation of blood flow in the ureteral and glomerular capillaries. A gradual rise in GFR usually accompanied ureter distension. Obviously, ureter distension resulted in increased hydrostatic pressure in the ureter lumen (Table 3). Two questions arose from these observations: (1) Does luminal pressure represent a backpressure on the glomerulus? (2) Is GFR directly affected by luminal pressure? Experiments of the kind illustrated in Fig. 3 provided at least partial answers to these two questions. The ureter pressure was adjusted initially to minus one centimetre of water, and the GFR was determined for about $1\frac{1}{2}$ h. At this point the ureter pressure was adjusted to atmospheric, causing fluid to move from catheter into ureter (narrow arrow at the bottom of the figure). After some minutes during which no GFR was measurable, the ureter pressure was lowered and GFR became measurable again. A slight elevation of ureter pressure at this time slowed GFR, but only momentarily. Return of the ureter pressure to atmospheric caused fluid to move rapidly out of the catheter again, but recovery was fairly rapid and GFR rose to a high value quickly.

As shown in Fig. 3, pressure in the ureter lumen does seem to exert a backpressure on the glomerulus because GFR slowed. However, the effect of increased ureter backpressure on GFR seemed to diminish the longer the experiment continued. For example, the only obvious difference between conditions at *ca.* 104 and 145 min elapsed time was the greater ureter distension visible at the latter time. These observations suggest that when the ureter is distended, the resulting backpressure in the lumen has a tendency to slow GFR. However, if the ureter wall is well distended

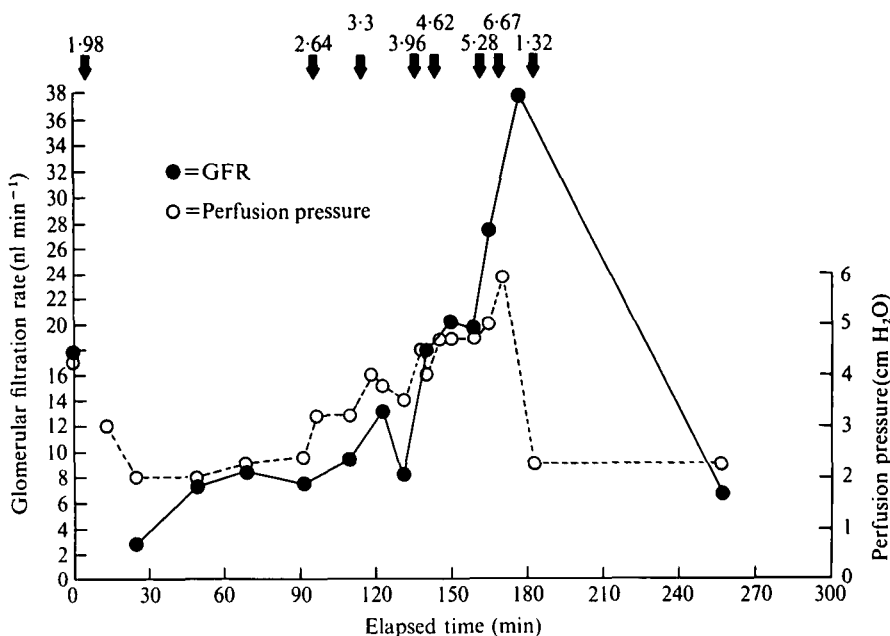


Fig. 4. Results typical of perfusions of isolated renal segments with 6 cm colloid Ringer. Arrows indicate the times at which perfusion at the rates indicated (in $\mu\text{l min}^{-1}$) was begun. Perfusion pressure and GFR indicated on the left-hand ordinate are those measured just prior to cannulation of the segmental artery.

the luminal backpressure may collapse the capillaries of the ureter wall, increasing postglomerular vascular resistance and increasing GFR. That the compliance of the ureter wall is related to blood pressure may be shown by the following observation. In some experiments there was a rapid deterioration in the blood circulation leading to a slowing or cessation of GFR; occasionally, urine moved from catheter into ureter. Assuming that a fall in blood pressure accompanied a slowing of circulation, then re-entry of urine into the ureter probably was caused by expansion of the ureter wall. This observation raises the possibility that blood vessels of the ureter wall control the compliance of that structure (and vice versa).

Perfusion studies

Perfusion studies produced few definitive results. These are of interest if only to illustrate that glomerular filtration may be a more complex phenomenon than believed generally.

Effects on GFR of colloid in the perfusion Ringer

Preliminary estimates of the COP of hagfish plasma suggested that a normal value was *ca.* 12 cm H_2O . To assess the influence of COP on GFR, glomeruli were perfused with Ringer whose calculated COP was adjusted to one-half normal, normal, and twice normal for plasma (i.e. 6, 12 and 24 cm H_2O).

Isolated renal segments perfused with Ringer whose calculated COP was 6 cm H_2O (6 cm colloid Ringer) produced urine in a manner most like blood-perfused renal segments. The perfusion rate was varied between $0.08 \mu\text{l min}^{-1}$ and $6.67 \mu\text{l min}^{-1}$;

Table 4. *Summary of perfusion rates, hydrostatic pressures and glomerular filtration rates during perfusion of renal segments with Ringer having colloid osmotic pressures (COP) of 6 and 12 cm H₂O*

Perfusion fluid COP	Average urine flow rate (nl min ⁻¹)	Average perfusion pressure (cm H ₂ O)	Average perfusion rate (μl min ⁻¹)	N
6 cm H ₂ O	10.7	3.0	1.51	36
12 cm H ₂ O	9.48	8.2	3.40	39

the perfusion pressure varied from < 1 to *ca.* 6 cm H₂O. At the lowest perfusion rate and pressure at which measurable GFR occurred, namely 0.33 μl min⁻¹ and *ca.* 2 cm H₂O, GFR averaged 0.09 nl min⁻¹ over several hours. However, appreciable GFR occurred only at perfusion rates greater than 1 μl min⁻¹.

Fig. 4 illustrates results typical of experiments in which isolated renal segments were perfused with 6 cm colloid Ringer. At the time of cannulation the segmental artery pressure was 4.25 cm H₂O and GFR was 17.8 nl min⁻¹ (left-hand ordinate). Perfusion was begun at the rate of 1.98 μl min⁻¹ and a pressure of *ca.* 2 cm H₂O. Glomerular filtration rate fell initially, but it recovered to about 8 nl min⁻¹. The perfusion rate was increased stepwise to a value of 6.67 μl min⁻¹; each increase led to an increased GFR. When the perfusion pressure approximated to the arterial pressure measured at the time of cannulation, GFR was comparable to the GFR measured at that time. This suggested that 6 cm colloid Ringer was adequate to support the function of isolated glomeruli. Furthermore, when the perfusion pressure approached the calculated COP of the perfusion fluid, GFR became very rapid.

Results of the perfusion of isolated renal segments with 12 cm colloid Ringer were more equivocal than the foregoing. Although close to the measured COP of hagfish plasma, 12 cm colloid Ringer seemed to increase the vascular resistance of the preparation. The ureter wall usually became shrunken and sometimes urine moved back into the ureter from the catheter when perfusion with 12 cm colloid Ringer was begun. To achieve roughly comparable GFR, isolated renal segments had to be perfused with 12 cm colloid Ringer at a pressure over twice that prevailing during perfusions with 6 cm colloid Ringer (Table 4). In some experiments perfusion at pressures approaching the COP of the perfusion fluid caused a rapid GFR, as in perfusions with 6 cm colloid Ringer; in other experiments this did not occur.

Perfusion of isolated renal segments with 24 cm colloid Ringer produced erratic results. The preparations suffered extensive shrinking and usually began 'leaking' perfusion fluid. Because of the generally unsatisfactory nature of these experiments they will not be considered further.

These experiments in which colloid was perfused through isolated renal segments seemed to confirm the conclusion based on the earlier measurements of urine and plasma COP, namely that glomerular filtration can occur against an apparently unfavourable thermodynamic gradient. The possibility remained that the effective COP of the perfusion fluid was not that calculated from the Ficoll 70 content. During perfusions with 24 cm colloid Ringer it was obvious that there was escape of perfusion fluid from renal capillaries. Escape of fluid was not obvious during perfusions with 6 and 12 cm colloid Ringer, but the possibility could not be excluded. Therefore, the

Table 5. *Estimates of the colloid osmotic pressure (COP) of perfusion fluid and urine produced during perfusions of single glomeruli*

(Values are expressed as the average with the range of data in parentheses.)

Perfusion fluid	Perfusion fluid COP (cm H ₂ O)	Urine COP (cm H ₂ O)	N
6 cm colloid Ringer	6.3 (5.8-7.5)	1.2 (0-1.7)	4
12 cm colloid Ringer	13.2 (12.4-14.2)	7.1 (3.9-9.9)	6

COP of samples of urine produced during perfusion of renal segments with 6 and 12 cm colloid Ringer was measured. Table 5 shows that when 12 cm colloid Ringer perfused the glomeruli appreciable amounts of colloid entered the urine. Three of four samples of urine produced by glomeruli perfused with 6 cm colloid Ringer also had a slight but measurable COP. However, in only two of the examples shown in Table 5 was the COP of the urine sufficiently high to permit the possibility of a pressure gradient favourable to filtration.

Effects of chemical inhibitors on GFR of perfused glomeruli

Numerous studies have shown that infusion of inhibitors into the general circulation of intact animals causes a reduction in the GFR (e.g. Lie, Johanneson & Kiil, 1973, 1975). This effect is generally attributed to an indirect influence of inhibitors on plasma volume or renal vasculature. In the present studies it was hoped that by perfusing individual renal segments specific effects of inhibitors upon the glomeruli could be separated from generalized effects.

Although 6 cm colloid Ringer has a COP somewhat less than the average of hagfish plasma, of the Ringers tried it seemed to be the most suitable as a perfusion medium. Consequently it was used as the standard perfusion fluid during studies of the effects of chemical inhibitors on GFR.

The variability of the GFR of isolated renal segments is large (Fig. 2). For this reason, each preparation served as its own control. A renal segment was perfused with 6 cm colloid Ringer which lacked inhibitor (control channel) for 1 h or more. Then switchover was made to 6 cm colloid Ringer containing inhibitor (experimental channel). Switchover resulted in a momentary lowering of pressure in the perfusion channel, usually with a brief slowing of GFR. However, it must be emphasized that a prolonged lowering of GFR followed by subsequent recovery (such as seen in Figs. 5 and 6) was never observed during control perfusions. This observation supports the view expressed below that lowering of GFR was a specific effect of chemical inhibitors rather than a random effect.

After it was established whether or not perfusion with inhibitor affected GFR, perfusion was switched to the control channel. At each switchover it took 14-40 min before the alternative fluid perfused the preparation. This was due to a 'dead space' between the 3-way stopcock and canula tip whose volume was estimated for each canula. The stippled arrows in Figs. 5 and 6 indicate the shortest and longest possible 'lag' times. Table 6 summarizes the results of perfusions with inhibitors. Only ouabain and dinitrophenol (DNP) had a consistently observed effect on the GFR of renal segments.

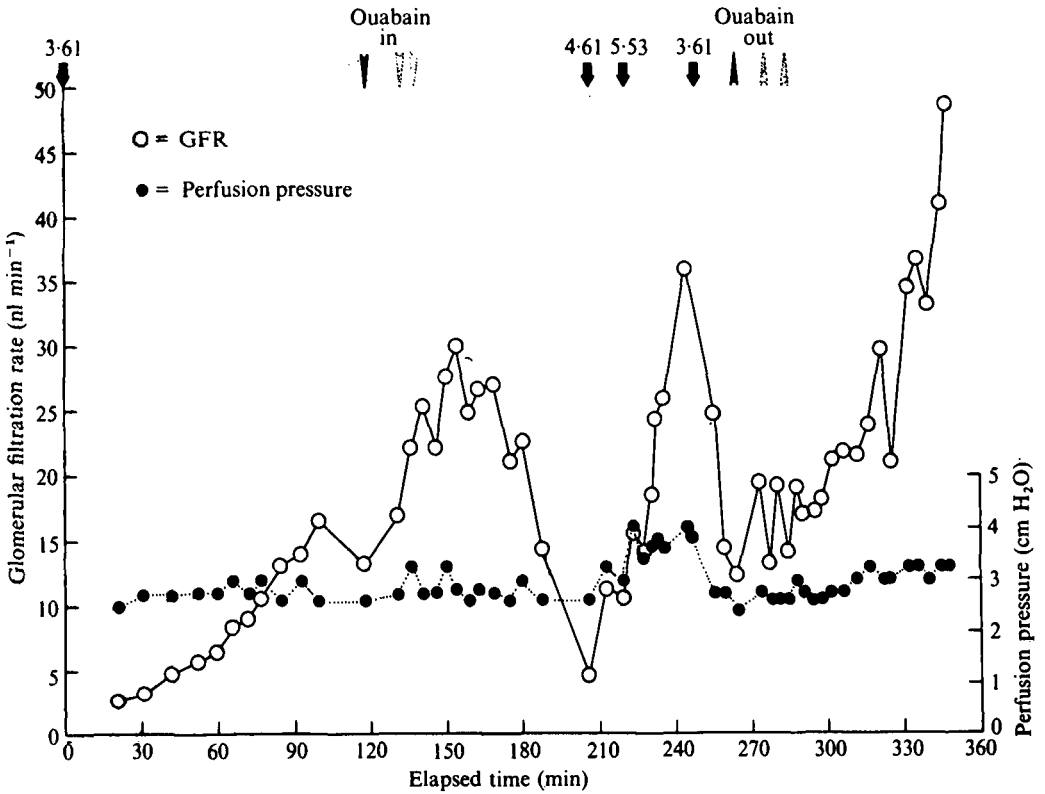


Fig. 5. The effect of ouabain on GFR of perfused renal segments. Perfusion was changed from control to experimental channel at 'ouabain in' and from experimental to control channel at 'ouabain out'. The stippled arrows indicate the shortest and longest time interval between the changing of perfusion channel and perfusion with the alternative solution. Solid arrows indicate times at which perfusion at the rate indicated (in $\mu\text{l min}^{-1}$) was begun.

Fig. 5 shows the results of an experiment in which 10^{-5} M ouabain was perfused through an isolated renal segment. The initial perfusion rate was $3.61 \mu\text{l min}^{-1}$; the pressure varied between about 2.5 and 3 $\text{cm H}_2\text{O}$. After about 2 h of perfusion through the control channel GFR rose to *ca.* 30 nl min^{-1} . Perfusion was changed then to the experimental channel (solid arrow). Fourteen to 20 minutes later ouabain solution was perfusing the renal segment. Twenty to 30 min after ouabain reached the glomerulus GFR began to fall. The rate slowed to a low value of *ca.* 4 nl min^{-1} over a period of about 1 h. As can be seen in Fig. 5, there was no significant change in the perfusion pressure during the period of falling GFR. After a very low GFR was attained, the perfusion rate was increased stepwise to $5.53 \mu\text{l min}^{-1}$, resulting in an increase of GFR at each step. This indicated that GFR was not completely inhibited by ouabain. The perfusion rate was returned to $3.61 \mu\text{l min}^{-1}$, and perfusion was continued through the control channel. After about 30 min GFR began to recover and rose to a very high value despite only modest rises in perfusion pressure.

In no instance did ouabain (or DNP) completely inhibit GFR. Furthermore, some perfused renal segments were not noticeably inhibited by ouabain even at a

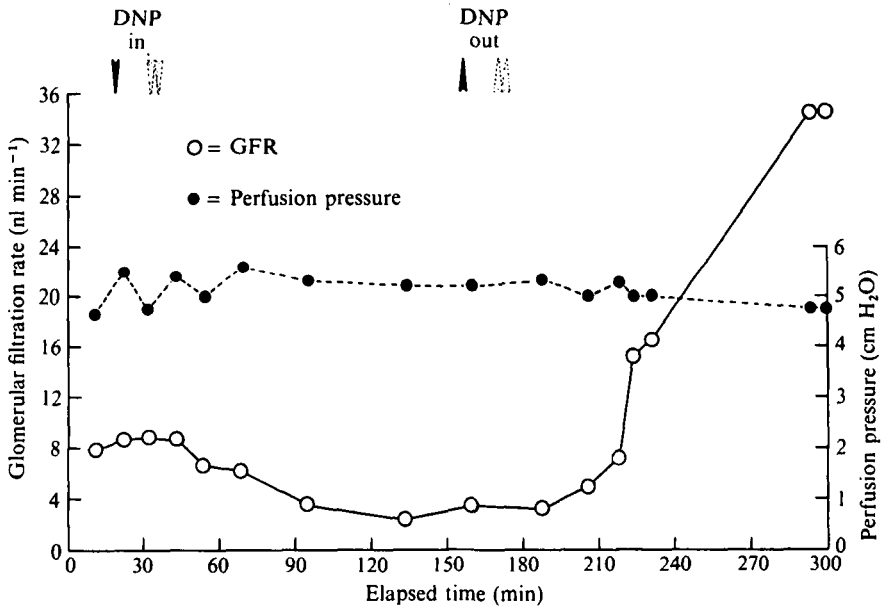


Fig. 6. Effect of dinitrophenol on GFR. Solid arrows indicate the times at which perfusion was changed from control to experimental channel and vice versa. Stippled arrows indicate shortest and longest periods between the change of perfusion channel and time that the alternative perfusion fluid entered the preparation. Perfusion rate = $3.5 \mu\text{l min}^{-1}$.

Table 6. Summary of the effects of chemical inhibitors on GFR of perfused hagfish glomeruli

(Concentrations relate to the concentration of the inhibitor in the perfusion fluid.)

Inhibitor	Concentration (M)	Effect
Amiloride	10^{-3} , 10^{-4}	None apparent*
Acetazolamide	10^{-3}	None apparent
Cyanide	10^{-2} , 5×10^{-3}	None apparent†
	10^{-3}	None apparent†
Cyanide + Iodoacetate	5×10^{-3} each	None apparent†
2-Deoxy-D-glucose	10^{-2}	None apparent
2,4-dinitrophenol	10^{-3}	GFR reduced
	10^{-4} , 5×510^{-5}	None apparent
Ethacrynic acid	10^{-5}	None apparent*
Iodoacetate	10^{-2}	None apparent†
Ouabain	10^{-5} , 10^{-3}	GFR reduced‡

* Amiloride and ethacrynic acid were so insoluble in hagfish Ringer that the validity of these results is doubtful.

† GFR occasionally slowed, but no profound effects.

‡ Reduction of GFR was not always observed.

concentration of 10^{-3} M. In such experiments, however, relatively high perfusion pressures (4–5 cm H_2O) prevailed.

Dinitrophenol did not inhibit GFR except at a concentration of 10^{-3} M, which always caused inhibition. A typical experiment is shown in Fig. 6. The GFR reached a maximum value of 9 nl min^{-1} during perfusion through the control channel. After DNP began perfusing the renal segment, GFR was affected relatively rapidly. Similarly, after perfusion with control Ringer was recommenced, recovery of the GFR was relatively rapid.

Although the data in Table 6 suggest that only ouabain and DNP affect GFR of perfused renal segments, this conclusion might be misleading. The poor solubility of amiloride and ethacrynic acid in hagfish Ringer makes it possible that very little of those compounds remained in solution in the perfusion fluid. Furthermore, GFR was reduced during some perfusions with cyanide and iodoacetate, but the reduction was never large and the effect was not observed consistently.

DISCUSSION

The results reported here are roughly comparable to those obtained by others. Compared to plasma ion analyses made by Munz & McFarland (1964), present results exhibit considerably lower sodium and somewhat higher magnesium concentrations. However, plasma ion concentrations (especially sodium) seem to be very variable in hagfishes, possibly reflecting conditions under which the animals are kept in captivity (McInerey, 1974). Eisenbach *et al.* (1971) measured osmolality and concentrations of sodium and potassium in plasma and glomerular fluid of hagfishes. Sodium and potassium concentrations of glomerular fluid approximated to plasma concentrations of those ions. Curiously, the osmolality of glomerular fluid was significantly less than the osmolality of the plasma.

Average 'whole animal' urine-flow rate of specimens of *Eptatretus stouti* calculated from single glomerulus GFR was 15 ml kg^{-1} per day. This is greater than the average value (5.4 ml kg^{-1} per day) obtained by Morris (1965) by collection of urine from specimens of *Myxine glutinosa*. However, it is comparable to the maximum value (10 ml kg^{-1} per day) measured by Munz & McFarland (1964) on a single specimen of *E. stouti*.

The primary purpose of the present experiments was to elucidate the process of glomerular filtration in the hagfish. The present state of knowledge makes it hazardous to extrapolate the results to conditions which may exist in conscious, intact hagfishes.

Effects of colloid on renal hydrodynamics

The results of the present studies of colloid effects appear to contrast with some of those obtained in studies of mammalian kidneys. Perfusion of hagfish renal segments with colloid-containing solutions led to an apparent increase in the resistance to flow. Colloidal solutions cause an increased flow in mammalian kidneys, implying a decreased intrarenal resistance. It can be surmised from the data shown in Table 4 that in the hagfish, filtration fraction (GFR \div perfusion rate) varies inversely with the COP of the perfusion fluid. The same relationship holds true for mammalian kidneys (Navar *et al.* 1971; Bowman & Maack, 1974; Little & Cohen, 1974). Therefore both

in mammals and hagfishes the specific effect of colloid appears to be a reduction of flow in the glomerular capillaries. Work of Piene (1976) on the vascular bed of the cat's lung suggests an explanation of the apparent paradox. In the cat lung small and medium-sized arteries may react to perfused colloid by vasoconstriction, whilst large arteries dilate. Dilatation of larger arteries may mask the constriction of smaller arteries and arterioles. Since the vasculature of the hagfish kidney consists preponderantly of vessels smaller than $100\ \mu\text{m}$, the constriction of these vessels may not be masked as perhaps occurs in mammalian kidneys.

Effects of colloid on glomerular filtration rate

Aside from its effects on the renal vasculature, colloid has further effects on GFR which are also difficult to interpret. Ficoll 70 enters the urine and it would be expected that small amounts of colloid of the molecular weight of this compound would enter the glomerular filtrate. However, the actual amounts which did so seem excessive and probably are not due entirely to filtration. Entry of perfused or infused colloid into the urine is observed commonly. If leakage of Ficoll 70 occurred across glomerular capillaries, it is puzzling that GFR was so little affected in most cases. Glomerular filtration rate increased rapidly only after perfusion pressure exceeded the calculated COP of the perfusion fluid.

Effects of backpressure on glomerular filtration rate

Stolte & Eisenbach (1973) perfused kidney segments of specimens of *Myxine glutinosa* using a technique similar to the one used here, except that their Ringer lacked colloid. When isolated glomeruli were perfused at pressures normally recorded in the dorsal aorta (i.e. 5–6 cm H_2O), the GFR averaged *ca.* 23 nl min^{-1} . When perfusion pressures were increased above the physiological range (up to 17 cm H_2O), GFR rose to an average value of 159 nl min^{-1} . Backpressure applied to the renal vasculature through the posterior cardinal vein caused GFR to be elevated enormously. For example, when the postcardinal vein pressure was 4.8–5.3 cm H_2O and the arterial perfusion pressure was 5–6.2 cm H_2O , GFR averaged 306 nl min^{-1} ! Stolte & Eisenbach concluded that the glomeruli of the hagfish exhibit a degree of autoregulation of the filtration rate. Results of the present studies are in accord with this conclusion and suggest that autoregulation is due primarily to changes in the postglomerular resistance. However, the results of Stolte & Eisenbach imply that backpressure in the postglomerular vasculature has no influence on preglomerular pressures. This finding is not in accord with the present studies.

Effects of drugs on the glomerular filtration rate

The effects of inhibitors on GFR probably are not susceptible to simple interpretation. Although ouabain and DNP have well known and fairly specific effects, it is difficult to decide how these effects might affect GFR. That is, whether or not the inhibitors affect GFR directly by some action upon the glomerular epithelial cells or indirectly by acting upon the cells of the renal vasculature. It was the consistent observation in the present studies that any change in postglomerular resistance of perfused renal segments was reflected in the perfusion (preglomerular) pressure as well as GFR. However, Stolte & Eisenbach have demonstrated that GFR can be

markedly affected without apparent change in perfusion (preglomerular) pressure. Therefore, it is possible that ouabain and DNP have a differential effect on glomerular and postglomerular vasculature. Certainly there is a precedent for this in frog kidneys: cyanide and oxygen lack reduce GFR by perfused frog kidneys with no apparent effect on perfusate flow. Beck, Kempton & Richards (1938) demonstrated that glomerular and non-glomerular arterioles react differently to anoxia: glomerular arterioles constrict, non-glomerular arterioles dilate. Unlike the frog kidney, however, there do not appear to be blood vessels derived from the renal artery (and therefore perfused in the present experiments) which shunt the glomerulus (Grodziński, 1926; Heath-Eves & McMillan, 1974). Therefore, the mechanism whereby collapse of one resistance (glomerular capillaries) could be compensated by altering a resistance in series (postglomerular arterioles or venules) remains obscure.

It is equally difficult to interpret the results of these studies with respect to possible direct effects of drugs upon glomerular epithelia. That the inhibitors which have a pronounced effect on GFR are also those which block phosphorylation/dephosphorylation reactions may be of significance. However, it must be admitted that the present data do not permit of even a good guess as to the significance of inhibitor effects and point to the obvious need for further experimentation.

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