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HYDROSTATIC PRESSURES IN GLOMERULI AND RENAL VASCULATURE OF THE HAGFISH, *EPTATRETUS STOUTI*

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SUMMARY

Hydrostatic pressures in the renal vasculature of hagfish have been studied. Estimates of the blood colloid osmotic pressure (COP) have been made. In blood vessels supplying the renal corpuscles, the average hydrostatic pressure is about 1 kPa. The average hydrostatic pressure falls to a value of 0·04 kPa within the postcardinal vein efferent to the renal corpuscle. Within the glomerular capillaries the hydrostatic pressure averages 0·21 kPa. Since the blood COP averages about 1·4 kPa, it is clear that glomerular filtration in the hagfish is not underlain by the hydrostatic pressure of the arterial pulse.

In some blood vessels efferent to the renal corpuscles, hydrostatic pressure may be as high as in the afferent supply. Evidence is presented that the glomerular capillaries are shunted by this high pressure vascular pathway.

INTRODUCTION

Studies made of the glomeruli of hagfish mesonephric kidneys demonstrate their close identity with glomeruli in the kidneys of other vertebrates. This identity is true for the gross anatomy (e.g. Heath-Eves & McMillan, 1974) and the fine structure (Kühn, Stolte & Reale, 1975). The primary urine produced by hagfish glomeruli differs little from a colloid-free filtrate of the blood plasma (Eisenbach *et al.* 1971; Riegel, 1978; Alt, Stolte, Eisenbach & Walvig, 1981). Furthermore, the renal test substance, inulin, enters the glomerular fluid at a concentration equal to the inulin concentration in the plasma (Munz & McFarland, 1964; Rall & Burger, 1967; Stolte & Eisenbach, 1973; Alt *et al.* 1981). For the foregoing reasons it would be expected that hagfish glomeruli should function in the same way as do glomeruli of other vertebrates. It was hypothesized by Starling that filtration of fluid outwards across the walls of the capillaries is due to an excess of hydrostatic pressure of the arterial pulse over the opposing COP of the plasma. This hypothesis is supported by an abundance of experimental evidence accumulated over several decades (Renkin & Gilmore, 1973; see also Arendshorst & Gottschalk, 1985). Consequently, it would be

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expected that pressure filtration should underlie the formation of primary urine by hagfish glomeruli.

The results of a study of urine formation by isolated glomeruli have shown that conditions necessary for pressure filtration apparently do not exist in the hagfish. The COP of the blood was found to be more than double the hydrostatic pressure in the blood vessels (segmental arteries) that supply the microvasculature of the kidney. Despite the unfavourable pressure gradient, glomeruli produce urine at appreciable rates (Riegel, 1978).

The results presented here derive from attempts to discover if there are conditions detectable within hagfish glomeruli which mitigate the adverse pressure relationship seen in the general blood circulation.

MATERIALS AND METHODS

Specimens of the Pacific hagfish *Eptatretus stouti* (Lockington) were studied at the Hopkins Marine Station of Stanford University in Pacific Grove, California, USA. Hagfish were caught, maintained in captivity and prepared for study in ways which were closely similar to those described earlier (Riegel, 1978). All experiments were conducted at a temperature of 5 °C.

Measurement of hydrostatic pressure

Use was made of a servo-nulling pressure microtransducer like that first described by Wiederhielm, Woodbury & Rushmer (1964) and improved by Intaglietta, Pawula & Tompkins (1970). Pressure-sensing micropipettes were made from 2 mm o.d. borosilicate self-filling capillaries (Clarke Electromedical Supplies, Reading, England). The capillaries were pulled and the tip was ground on two or three sides to produce a sharp, bevelled tip. A grinder like that described by Chang (1975) was used. The diameter of the micropipette tips, measured at the widest part of the opening, ranged from approx. 2–5 μm, with an electrical resistance to a.c. of between approx. 0·8 and 2·5 MΩ. Micropipettes were filled just prior to use with 1 or 2 mol l^{-1} NaCl to which a quantity of the dye, Lissamine Green, was added. The electrolyte was boiled and filtered twice through a Millipore filter (Millipore Ltd, Harrow, England) which had an average pore diameter of 0·8 μm.

A hagfish was prepared for study as described previously (Riegel, 1978). A pressure-sensing micropipette was brought adjacent to the kidney using a micromanipulator, and the pressure microtransducer was balanced electrically and hydraulically. The vessel or tissue space under study was penetrated; the location of the tip of the pressure-sensing micropipette usually could be visualized by expelling a bolus of dyed electrolyte from the micropipette by pressurizing the hydraulic system of the microtransducer. Output from the microtransducer was displayed on an oscillographic recorder (MD 400/2, Bioscience Ltd, Sheerness, England).

The servo-nulling pressure microtransducer gave a nearly linear response over the range of pressures from 0 to approx. 1·5 kPa (Fig. 1). This was determined by inserting the tip of a pressure-sensing micropipette into Ringer solution in a 'test'

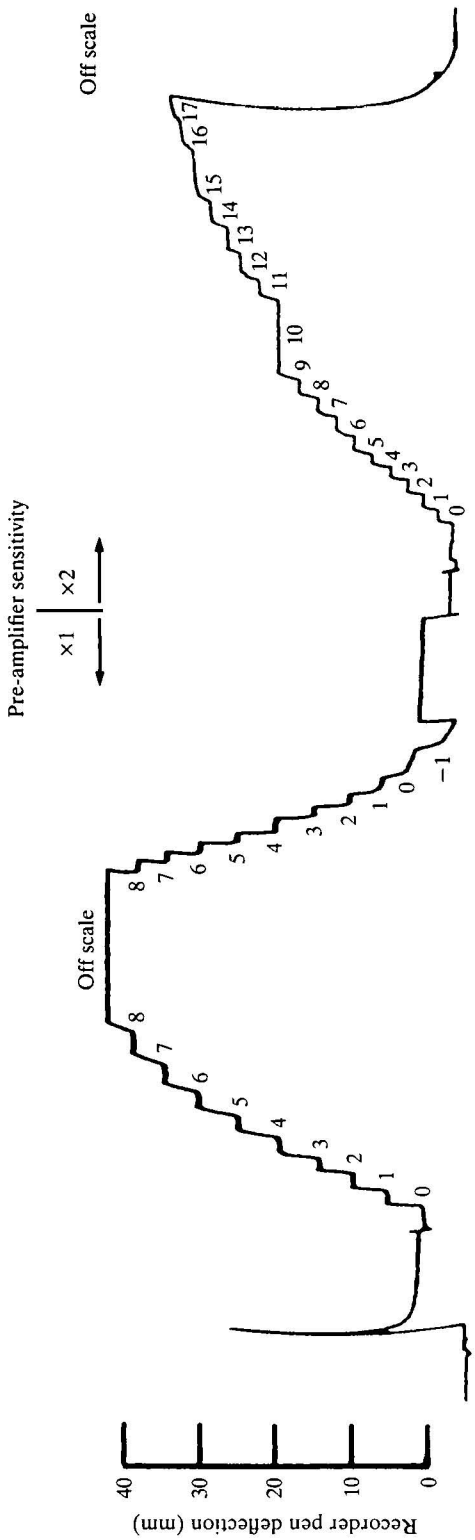

Fig. 1. Oscillograph traces showing the output of a servo-nulling pressure microtransducer in response to pressures applied with a syringe to a test chamber into which the pressure-sensing micropipette part of the microtransducer was inserted. Pressure in the test chamber was read off from a Ringer-filled manometer which communicated with the Ringer-filled bottom portion of the test chamber into which the micropipette tip was inserted. The sensitivity of the recorder was adjusted so that full scale deflection was about 0·8 kPa (left trace) or 1·6 kPa (right trace).

chamber. Pressure applied to the chamber with a syringe could be read off on a water manometer which communicated with the Ringer-filled part of the chamber. The sensitivity of the oscillographic recorder preamplifier was adjusted so that 0·1 kPa caused a pen displacement of either 2·5 or 5 mm. The more sensitive setting was used for recording pressures less than approx. 0·8 kPa. The calibration of the pressure microtransducer and oscillographic recorder was checked from time to time, and this remained remarkably constant over the period of pressure measurements.

Measurements of single glomerulus filtration rate (SGFR)*

Soda glass microcannulae were used to measure SGFR by direct puncture of renal corpuscles. Glass capillaries of approx. 1·5 mm o.d. were pulled and sharpened on two sides using the Chang grinder fitted with a coarser alumina surface than that used to grind pressure-sensing micropipettes. This surface was made of alumina of 50 μm average particle size embedded in an adhesive-backed plastic film (A. H. Thomas Co., Philadelphia, PA, USA). Sharpening widened the microcannulae tips to approx. 50–80 μm, measured at the widest part of the opening. Microcannulae were mounted in a pressure-tight holder which was held in the object clamp of a micromanipulator. The holder was connected by a three-way stopcock to a syringe and to a reservoir. The whole of the system was filled with liquid paraffin coloured with Sudan Black. Pressure in the system could be altered either with the syringe or by varying the height of the reservoir.

Renal corpuscles were prepared for cannulation by removing the overlying peritoneum and any fat bodies obscuring vision. Ligatures were placed around the adjacent ureter at points sufficiently anterior and posterior to the renal corpuscle under study so as not obviously to interfere with the glomerular blood supply when tightened. The renal capsule was penetrated and liquid paraffin was forced into the neck segment and adjacent ureter; the ligatures around the ureter were then tightened. The result of this procedure was a renal corpuscle isolated with a short segment of ureter which was filled with liquid paraffin. Pressure in the microcannula was adjusted to a level slightly below atmospheric initially to promote movement of fluid into its tapered portion. Pressure was adjusted to that of the atmosphere after fluid had advanced to the non-tapered portion of the microcannula.

Glomerular filtration rate was determined by the rate of fluid advance in the microcannula. This was observed at ×40 with a stereomicroscope with an eyepiece fitted with a calibrated graticule. The graticule was aligned with markings made on the microcannula with a diamond-tipped marker. The internal diameter of the microcannula was determined by taking the average of several readings made along the length of the untapered portion with the eyepiece graticule. The rate of advance of the glomerular fluid was then measured at timed intervals by noting the change in position of the interface between glomerular fluid and the liquid paraffin filling the microcannula. The volume of the glomerular fluid entering the microcannula during the timed interval was calculated using the formula for the volume of a cylinder.

* Although this has been designated single *nephron* glomerular filtration rate (i.e. SNGFR), that term hardly seems appropriate for a kidney that lacks nephron tubules.

After completion of SGFR measurements, the contents of the microcannula were discharged into a liquid-paraffin-filled tube and stored in a freezer until COP analyses could be made. This method of storage did not alter the COP of either plasma samples or Ficoll 70 containing standard solutions. It was therefore assumed that the COP of glomerular fluid samples was not altered by freezing. This assumption was confirmed as true in the case of two samples whose COP values were determined on the same day as collection and found to be nil.

Measurement of colloid osmotic pressure

The COP of glomerular fluid and samples of blood and plasma was measured with membrane electro-osmometers. The details of construction and use of these is reported elsewhere (Riegel, 1986), but a brief description of the principles of their operation will be given here. Devices were constructed in which a disc of ultrafiltration membrane is held in such a way as to separate a sample chamber from a Ringer-filled reference chamber. The reference chamber communicates with the sensitive surface of an electronic pressure transducer. One of the devices makes use of a miniature pressure transducer and is suitable for samples as small as approx. 0·5 μl. The other device makes use of an ordinary blood-pressure transducer and is suitable for samples of approx. 5 μl or larger.

When a fluid sample containing colloid is placed in the sample chamber of one of the devices, fluid is drawn from the reference chamber, lowering the hydrostatic pressure there. If the ultrafiltration membrane used is completely impermeable to the colloid in the sample, the fall in hydrostatic pressure in the reference chamber is directly proportional to the COP of the sample. The ultrafiltration membrane used in these studies was a Millipore PTGC (Millipore Ltd, Harrow, England) which had a nominal molecular weight limit of 10 000 Da. The membrane was calibrated both with a water manometer and with 'standard' colloid solutions containing known amounts of Ficoll 70 (Pharmacia Ltd, Milton Keynes, England). The concentration of Ficoll 70 was related to its COP using a formula derived by Gamble (1983).

The small-sample (0·5 μl or larger) osmometer was capable of measuring the COP of samples of known composition with an error not greater than $\pm 10\%$. The error of the larger-sample device in measuring the COP of samples of known composition was no greater than $\pm 8{\cdot}5\%$ (Riegel, 1986). Therefore, it was assumed that the error in making measurements of the COP of hagfish blood, plasma and glomerular fluid, whose composition is unknown, was no greater than the values stated.

RESULTS

Hydrostatic pressure measurements

In most cases it was not possible to make serial measurements of blood pressures in single animals. Heavy connective tissue invests the walls of most of the renal blood vessels, and the blood itself clots very rapidly when the vessel walls are damaged. Small blood vessels usually would become blocked with a clot a few minutes after

Table 1. *Hydrostatic pressure in the urinary space and various vascular elements associated with the hagfish kidney*

Pressure	Dorsal aorta	Segmental artery	Renal artery	Glomerular capillary	Urinary space	LPEV	HPEV	Postcardinal vein
Average	0·90	1·0	0·78	0·21	0·10	0·14	0·77	0·04
S.D.	0·22	0·20	0·35	0·10	0·05	0·10	0·14	0·02
N	17	20	5	13	11	14	20	9
Range	0·52–1·3	0·58–1·4	0·31–1·2	0·12–0·41	0·03–0·15	0·03–0·33	0·55–1·2	0·02–0·10

Pressures are expressed in kiloPascals (kPa).
LPEV, low-pressure efferent vessel; HPEV, high-pressure efferent vessel.

penetration with a pressure-measuring micropipette, rendering them useless for further pressure recording. Siliconing the micropipettes and reducing the concentrations of NaCl in the micropipettes had no beneficial effect. As a consequence of these and another difficulty which will be discussed below, pressures had to be measured randomly in the various blood vessels of a large number of animals.

Table 1 summarizes the reliable data obtained from measurements of the hydrostatic pressures in the urinary space or in one or more blood vessels of a total of 47 hagfish. Not shown are three recordings from large vessels, which, because of their location on the preglomerular side of the renal corpuscle, may have been afferent arterioles. However, the wide range of the pressures recorded (i.e. 0·12, 0·12 and 0·9 kPa) suggests that recordings were made in two kinds of vessels; it is not possible to decide which of these, if either, was the afferent arteriole.

The data of Table 1 reveal that quite possibly there are two vascular pathways through the hagfish renal corpuscle. This is shown by pressure measurements in vessels efferent to the renal capsule which fall into two distinct groups. In one group the blood pressure averaged 0·14 kPa; these vessels drained into the postcardinal vein directly or *via* another vessel. The second group consisted of vessels in which the hydrostatic pressure averaged 0·77 kPa, a value approximating to the average pressure in the renal artery. Vessels of the second group usually were single and always went onto the ventral surface of the ureter where they usually joined an artery arising from an adjacent segmental artery (see Fig. 2). This vessel appeared to break up into a system of capillaries and small blood vessels that serve the ureter wall. Both high-pressure efferent vessels (designated HPEV) and low-pressure efferent vessels (LPEV) may arise from within the renal corpuscle. This was ascertained where possible by following the movement of dyed electrolyte expelled from a pressure-measuring pipette lodged in an intracorpuscular vessel.

The averaging of data, as in Table 1, obscures conditions that exist when flow is vigorous in all parts of the hagfish blood circulation. Consequently, Fig. 2 has been made to illustrate the pressures and the shape of the pressure pulses in the blood circulation and urinary space under conditions of vigorous blood flow. The data are derived from measurements made on only two animals. One animal (120) contributed recordings from the segmental artery and postcardinal vein. The other

animal (160) contributed the remainder of the recordings shown in Fig. 2. It is clear that during vigorous blood flow, there is a sharp pressure drop between the renal artery, glomerular capillaries, LPEV and the postcardinal vein. However, pressure

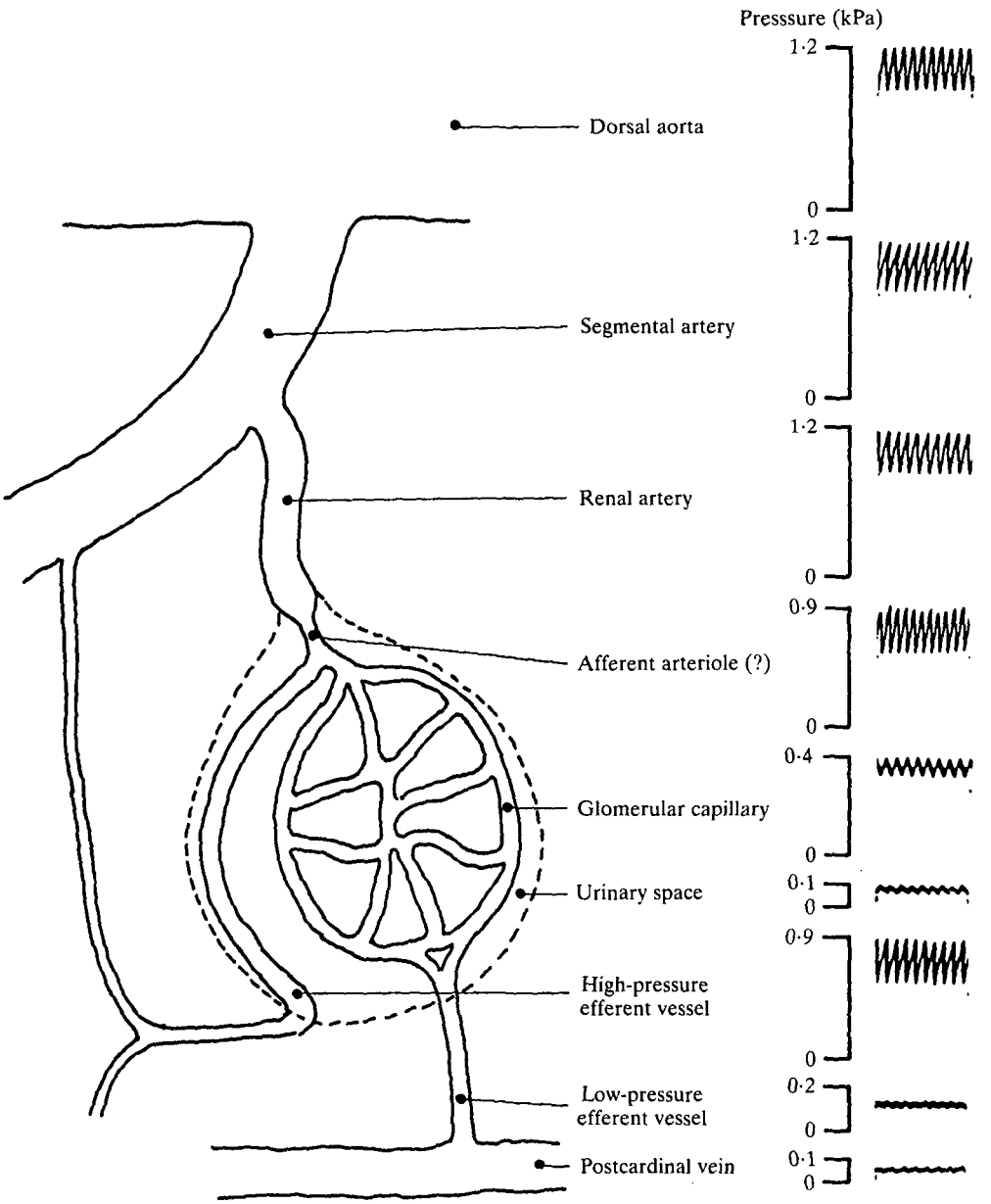

Fig. 2. One-minute segments of pressure recordings in the urinary space and blood vessels of two hagfish in which blood flow in all parts of the vasculature was vigorous. Recordings were taken from studies of animals (120, 160) in which blood pressures and heartbeat rates were comparable.

Table 2. *Changes in the average pressure and direction of flow in high-pressure efferent vessels (HPEV) after ligation of the renal artery*

Animal number	Average pressure (kPa) Before ligation	After ligation	Flow in HPEV
133	0·55	0·22	reversed
136	0·65	0·33	reversed
137	0·69	0·65	reversed
138	0·37	0·22	reversed
141	0·71	0·24	stopped

in HPEV diminishes relatively little. These values, as well as those summarized in Table 1, suggest that the LPEV are efferent to the glomerular capillaries and are part of the vascular pathway that drains into the postcardinal vein. Similarly, the HPEV must form a shunt around the low-pressure vascular system (see Fig. 2).

The existence not only of high pressure, but also an arterial pulse (Fig. 2), in blood vessels apparently efferent to the glomerulus clearly required further study. The renal arteries of 12 renal corpuscles were loosely ligated and the effect on flow in the HPEV was observed as the ligature was tightened. In nine examples, the direction of flow reversed. This could occur only if the pressure in the HPEV fell relative to the pressure in the direct branch off the segmental artery to which the HPEV joined (see Fig. 2). This was a clear indication that blood in the HPEV is derived from the renal artery and has a common source with blood in the glomerular capillaries. It was possible to obtain reliable pressure recordings in only five of the renal corpuscles whose renal arteries were ligated. The commonest cause of failure was complete or partial displacement of the pressure-sensing micropipette during the act of ligation. The data from the five successful recordings are shown in Table 2. In four of the recordings there was a marked fall in blood pressure in the HPEV with a corresponding diminution of the pressure pulse. In one case there was only a slight fall in pressure. The change in pressure at the time of ligation is illustrated by the oscillographic recording shown in Fig. 3.

These results indicate that, in a high proportion of cases, blood flowing in the HPEV is derived from blood entering the renal corpuscle by the renal artery. Since the renal artery also supplies the glomerular capillaries, the conclusion seems inescapable that the glomerular capillaries are shunted by a high-pressure vascular pathway. Whether or not this pathway is functionally linked with flow through the glomerular capillaries remains to be demonstrated (see below).

As shown by the range of values under each heading in Table 1, there appears to be considerable variation in the blood pressures of different hagfish. With respect to the dorsal aorta and segmental artery, the variation may reflect variations in the state of anaesthesia. The average pressures measured are more than double the average values obtained by direct cannulation of those vessels (Riegel, 1978). That this discrepancy is not due to the methods used to measure pressure is shown by recent perfusion studies (to be reported elsewhere), in which segmental arteries were cannulated and pressures recorded as in Riegel (1978). However, the pressures

recorded in the more recent studies fell within the range of values recorded using the servo-nulling microtransducer.

In the previous study, MS 222 was used as an anaesthetic at a dosage which supported active flow in the major blood vessels and created a relatively stable preparation. Nevertheless, it was noted that flow in the renal vasculature commonly either did not commence at once or was intermittent for some hours after induction of anaesthesia. In an effort to correct this when studies using the servo-nulling microtransducer were commenced, the MS 222 dosage was reduced progressively. The final dose levels used in the present studies were less than one-third those used earlier. Lowering the MS 222 dose brought about a greater consistency in the observation of vigorous blood flow in the major blood vessels. However, observations of improved flow in the renal vasculature were less consistent. Nevertheless, a concomitant of the reduction of the MS 222 dose was an increase in the pressures measured in the major blood vessels. Therefore, it seems likely that MS 222 acts to depress blood pressure in the hagfish. Whether or not the anaesthetic is responsible for the intermittent or static flow in the renal vasculature remains to be demonstrated. However, such an effect has been attributed to MS 222 in studies of the mud puppy, *Necturus maculatus* (H. J. Schatzman, quoted in *MS 222*, a publication of Sandoz Pharmaceutical Co., Basel, Switzerland). Furthermore, McVicar & Rankin (1983) have stated that high doses of MS 222 diminish the glomerular filtration rate of the river lamprey, *Lampetra fluviatilis*.

The blood pressures measured in the present study may more closely resemble the pressures prevailing in conscious hagfish than do the pressures measured previously. However, the lower anaesthetic dose resulted in an experimental preparation which was less stable than is desirable. Usually, the animals were irritable and exhibited movement soon after the start of the experiment. This movement contributed generously to the failure to make pressure measurements in more than a few blood vessels in most animals.

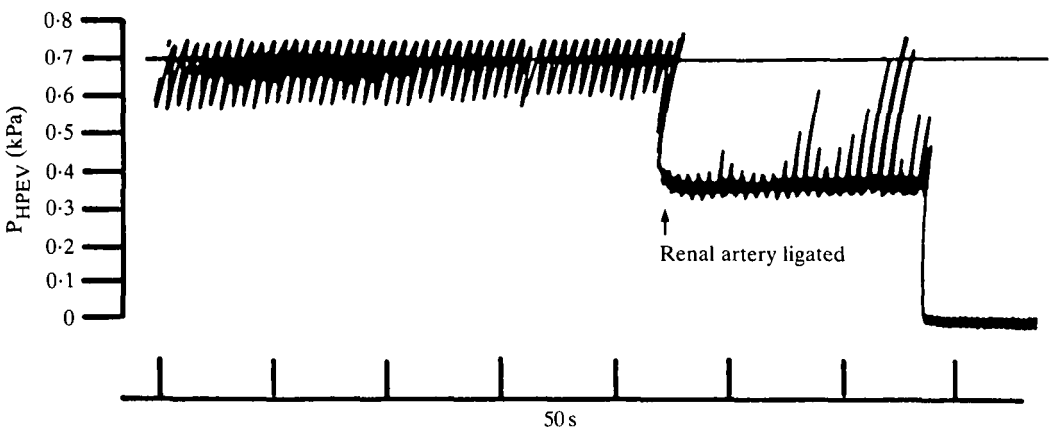

Fig. 3. Oscillograph recording of pressure change in a high-pressure efferent vessel (HPEV) during ligation of the renal artery (animal 136) (see Table 2).

Measurement of single glomerulus filtration rate

The average value of SGFR measured in nine animals was $58{\cdot}2 \pm 43{\cdot}1$ nl min^{-1} ($\pm$ S.D.) (range = 6–118 nl min^{-1}). It was not possible to measure hydrostatic pressures in glomerular capillaries of cannulated renal corpuscles. Efforts to penetrate the renal capsule with a pressure-sensing micropipette always dislodged the microcannula.

Three additional measurements of SGFR were made in animals before and after ligation of the renal artery. Before ligation SGFR was 88, 53 and 26 nl min^{-1}; after ligation those values fell to 2·6, 0·96 and 0·66 nl min^{-1}, respectively, measured over periods of 45 min to 3·5 h. In all three ligated glomeruli whose SGFR was measured, the direction of blood flow in the HPEV reversed. However, apparently this did not result in resumed flow through the glomerular capillaries, since post-ligation SGFR was negligible.

Measurements of colloid osmotic pressure

Only two out of eight glomerular fluid samples had a measurable COP, and those values were 0·01 and 0·05 kPa. Measurements of the COP of 45 heparinized whole blood and plasma samples taken from 12 hagfish averaged $1{\cdot}4 \pm 0{\cdot}26$ kPa. The COP values of plasma samples were not different from the COP values of whole blood samples taken from the same animal.

DISCUSSION

The function and morphology of vascular pathways through the hagfish renal corpuscle have not yet been studied in any systematic way. Whether or not there is a discrete dichotomy into the high-pressure and low-pressure pathways, as depicted in Fig. 2, remains to be demonstrated morphologically. However, further physiological evidence for the existence of the two pathways is found in the results of perfusion studies. Stolte & Eisenbach (1973) perfused isolated glomeruli of hagfish with Ringer solution. When perfusion pressure was elevated from the 'normal' value of approx. 0·5 kPa to approx 1·6 kPa, average SGFR rose from 23 to 159 nl min^{-1}. However, when pressure in the postcardinal vein was elevated from a value near zero to approx. 0·5 kPa, SGFR rose to an average value of 306 nl min^{-1}. The investigators concluded that elevation of the postcardinal vein pressure diminished the pressure drop in the glomerular capillaries, thus increasing the capillary surface available for filtration. It could be argued also that pressure applied through the postcardinal vein has a greater effect on SGFR because the postcardinal vein and glomerular capillaries are contiguous; pressure applied through the afferent supply to the glomerulus may be subject to reduction through the shunt pathway.

If it exists, a complex pattern of flow through the renal corpuscles would not be unique to the hagfish. Studies of other vertebrates reveal pathways within the glomerulus which open or close depending upon the rapidity of blood flow or the application of certain drugs. These studies reveal also the existence of direct shunts

(anastomoses) between afferent and efferent arterioles in glomeruli of some vertebrates (see Barger & Hird, 1973).

The present studies provide no firm evidence for a mechanism other than pressure filtration which can account for the formation of primary urine in hagfish. Nevertheless, such a mechanism must exist. Earlier studies (Riegel, 1978) have shown that SGFR of perfused glomeruli of hagfish is inhibited reversibly by ouabain and 2,4-dinitrophenol, with no detectable effect on perfusion pressure. Consequently, there is a possibility that active transport of solute plays a role in SGFR. The perfusion studies of Stolte & Eisenbach (1973) and the writer (1978, unpublished data) indicate that SGFR may vary with the area of glomerular capillaries exposed to fluid flow. This is consistent with active transport; it would be expected that fluid transport would be limited by the volume of fluid flowing through the transport site. The area of glomerular capillaries exposed would also limit an arterial-pressure-based mechanism. However, the absence of an effective filtration pressure would rule out that mechanism. The existence of a glomerular–capillary shunt complicates the interpretation of the effects of the inhibitors. However, it seems unlikely that the inhibitors would have a differential effect on the vasomotor tone of the vasculature so as to shunt flow away from the glomerular capillaries.

The composition of hagfish glomerular fluid conforms closely to a colloid-free plasma filtrate (Eisenbach *et al.* 1971; Riegel, 1978, this paper). Consequently, there is justification for continuing to describe the process that gives rise to the primary urine as 'glomerular filtration'. Since it is unlikely that arterial pressure is responsible, some other source of energy must be sought. The most likely source of this energy is an osmotic pressure gradient established by an active transport mechanism. In fluid-transporting tissues where there is no obvious transepithelial hydrostatic pressure gradient, it is thought that osmotic gradients established by active solute transport give rise to fluid movement. Although fluid-secreting tissues are thought to have a low passive permeability, especially to large molecules, this is not necessarily true. For example, some arthropod Malpighian tubules are relatively permeable to molecules as large as inulin (Farquharson, 1974; Maddrell & Gardiner, 1974; Dalton & Windmill, 1981). Furthermore, the permeability characteristics are such as to suggest that the large molecules are filtered.

In the glomeruli of all other vertebrates studied so far, conditions are conducive to pressure filtration (Persson, 1981; Arendshorst & Gottschalk, 1985; McVicar & Rankin, 1985). In some animals, glomerular filtration may become dependent upon flow, but this is considered to involve a different set of circumstances to that seen in hagfish. The forces responsible for pressure filtration may come to equilibrium at some point along a glomerular capillary between its afferent and efferent ends. When that happens filtration by the glomerulus as a whole depends upon the total proportion of glomerular capillaries affected. That is, the higher the rate of flow, the smaller the proportion of glomerular-capillary area affected by pressure equilibrium and the greater the glomerular filtration rate. The lower the rate of flow, the greater the proportion of glomerular-capillary area affected by pressure equilibrium and the smaller the glomerular filtration rate (Brenner, Baylis & Deen, 1976).

Why hagfish should be apparently so different from other vertebrates is unclear. Possibly glomerular function has become adapted through evolution to cope with low blood pressures that are characteristic of the animal.

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