THE UNIVERSITY OF HULL

SINGLE NEPHRON STRUCTURE AND FUNCTION, AND RENAL EFFECTS OF CATECHOLAMINES IN THE DOGFISH, SCYLIORHINUS CANICULA

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CAROL GREEN, B.Sc.(Birmingham)

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Summary of Thesis submitted for Ph.D. degree

by

CAROL GREEN

on

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3. The nephron was found to be composed of Bowman's capsule, neck segment, first, second and third proximal segments, and distal segment, identified by characteristic cytological features and dimensions.
4. Renal vasculature was examined using scanning electron microscopy of corrosion casts.
5. Morphological evidence of smooth muscle sphincters, intra-arterial cushions and glomerular bypass shunts in renal vasculature was found. The function of these structures is discussed in relation to patterns of renal blood flow, blood pressure and glomerular perfusion.
6. Plasma levels of the catecholamines adrenaline, noradrenaline and dopamine were determined in resting

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7. Infusion of adrenaline increased systolic and diastolic systemic blood pressure in conscious and anaesthetised fishes. Heart rate was elevated in conscious fishes only. Haemodynamic effects of adrenaline were found to be more potent than those of noradrenaline.
8. Adrenaline induced a marked urinary diuresis, and increased glomerular filtration rate. Urine to plasma inulin concentration ratios, relative clearance of osmolytes and relative free water clearance were unchanged by adrenaline.
9. Anaesthesia significantly reduced urinary output.
10. Micropuncture techniques were used to determine single nephron glomerular filtration rates (SNGFR). Infusion of adrenaline increased mean SNGFR, and increased the tubular fluid to plasma inulin concentration ratio. This is discussed in relation to tubular water secretion.
11. Patterns of glomerular perfusion were determined. Adrenaline decreased the proportion of filtering nephrons.
12. The role of circulating catecholamines is discussed in relation to the regulation of renal circulation.

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I. GENERAL INTRODUCTION

A. Evolution and Distribution of the Fishes

Vertebrates originated in the early Palaeozoic era, between 400-500 million years ago. It is generally thought that they arose from a marine chordate ancestor, possibly a free-swimming tunicate larva (Romer, 1967), but it is unknown whether this transition took place in sea water or fresh water (Smith, 1961; Bentley, 1971). The earliest vertebrates to evolve were the jawless ostracoderm fishes, characteristically protected by hard dermal plates. It is unknown whether possession of these dermal plates was for protection against predators (Romer, 1967), or to allow adaptation to a fresh water habitat by reducing dermal permeability (Smith, 1961).

1. Agnatha

The extinct ostracoderm fishes are classified with the contemporary agnathans (lampreys and hagfishes). Lampreys are commonly found in both fresh and sea water habitats. The sea lamprey (Petromyzon marinus) is anadromic, ascending rivers to breed in fresh water. All lampreys, whether fresh water or marine, maintain body fluids between one-quarter to one-third of the osmotic concentration of sea water (Schmidt-Nielsen, 1975). The hagfishes in contrast, are exclusively marine and stenohaline. They are the only true vertebrates in which extracellular fluid is maintained almost in osmotic equilibrium with the environment (Robertson, 1963).

2. Chondrichthyes

The Chondrichthyes (sharks, rays and chimaeroids) are thought to have originated in fresh water and subsequently adapted to a marine environment (Romer, 1955). Contemporary elasmobranchs are most commonly found in sea water, abundantly in tropical and sub-tropical waters but with many species of sharks, skates and rays found in temperate waters (Smith, 1936). Many of the tropical and sub-tropical species have retained the ability to move from sea water to fresh water, ascending rivers beyond the influence of the tides (Smith, 1931a, 1936; Schmidt-Nielsen, 1975). In addition to these euryhaline species, there are several fresh water elasmobranch species which are almost certainly permanently established in fresh water lakes and rivers (Smith, 1931a; Thorson, 1967).

Marine elasmobranch fishes maintain body fluids hyperosmotic to the environment by approximately 50-100 $mosm.l^{-1}$ (Hickman and Trump, 1969). Like most vertebrates, plasma levels of sodium, potassium and chloride ions are maintained at approximately half those of sea water, calcium levels at approximately one-third, with only very small amounts of magnesium and sulphate ions present (Burger, 1967). The hypertonicity of body fluids is achieved by the retention of urea and trimethylamine oxide (TMAO), (Smith, 1931b). Urea is produced in the liver via the ornithine cycle and is, in all other vertebrates except the elasmobranchs and holocephalans (ratfishes), a toxic waste product of metabolism. Approximately 95% of urea filtered at the

glomerulus is reabsorbed by the elasmobranch renal tubule (Forster, 1967). This maintains a plasma urea concentration of 350-400 $mmol.l^{-1}$ (Schmidt-Nielsen and Rabinowitz, 1964; Wong and Chan, 1977). The concentration of TMAO in extracellular fluid is approximately 70 $mmol.l^{-1}$ (Goldstein and Funkhouser, 1972). The origin of TMAO remains unknown (Goldstein, Hartman and Forster, 1967).

The body fluids of fresh water elasmobranchs have a solute composition similar to that of fresh water teleosts (Thorson, Cowan and Watson, 1967), with urea being virtually absent (Pang, Griffith and Atz, 1977). The stingray, Potamotrygon, commonly found in the fresh water of the Amazon and Orinoco rivers, is completely adapted to life in fresh water, and cannot survive even a gradual transfer to sea water (Pang, Griffith and Kahn, 1972).

The euryhaline elasmobranch Raja eglanteria, captured in sea water of varying salinities, shows plasma urea concentration to vary directly with salinity of the environment (Price and Creaser, 1967). Under experimental conditions, Goldstein, Oppelt and Maren (1968) studying the lemon shark (Negaprion brevirostris), and Goldstein and Forster (1971) studying the little skate (Raja erinacea), observed plasma losses of urea, sodium, chloride and TMAO on transfer from full strength to 50% sea water. The lip-shark, Hemiscyllium plagiosum, has also been shown to reduce plasma urea concentration following dilution of the external medium (Wong and Chan, 1977). Branchial loss of urea was found to be diminished as the plasma urea concentration decreased, with

the kidney becoming the principle site of urea excretion. The rate of urea biosynthesis is also thought to be decreased in response to environmental dilution.

3. Osteichthyes

The teleosts are the predominant group of the Osteichthyes (bony fishes). They are thought to have evolved in a fresh water habitat (Romer, 1955), but are now found distributed thoughout the fresh, brackish and marine waters of the world. Most species of teleost are stenohaline, but there are several species which are able to tolerate a wide range of salinities. This euryhaline habit is often associated with reproductive behaviour. The salmon and shad are well known examples of anadromic breeders, while the eel is catadromic, returning to the sea to reproduce. The flounder, killifish and toadfish are estuarine inhabitants, surviving in waters of constantly changing salinity.

Teleosts maintain the osmotic concentration of their body fluids at between one-quarter and one-third that of sea water. Marine fishes are therefore hypo-osmotic to the environment and will tend to lose water across the permeable surfaces of the body. Marine teleosts compensate for this constant loss by drinking sea water (section I.C.4). Fresh water teleosts are hyperosmotic to their environment. Due to the large permeable surface area of the gills, water gain is a major problem of inhabiting a fresh water environment.

B. Homeostasis of Body Fluids

The ability to survive in a constantly changing environment is dependent upon the ability of an organism to remain independent of that environment. The French physiologist Claude Bernard (1865), stated that the constancy of the "milieu intérieur" in contrast to the variability of the "milieu extérieur", was essential to provide a stable medium necessary for the normal functioning of cells of the body.

Bernard's principle has since been overwhelmingly substantiated, and it is now known that the internal environment of vertebrates, the extracellular fluid, is maintained within narrow limits by complex physiological regulatory mechanisms. Cannon (1929) introduced the term "Homeostasis" to describe the coordinated physiological responses involved in maintaining constancy of the internal body fluids.

C. Osmoregulation in the Fishes

The body fluids of fishes are distributed between the intracellular and extracellular compartments. These fluid compartments are separated from each other by the semi-permeable cell membrane, and from the external environment by the integument and epithelium of the gills and gut. Water, ions and molecules pass across these barriers by diffusion, osmosis and by active transport systems. The

kidneys play a vital role in regulating water and electrolyte balance, acid-base status and the concentration of metabolic waste products. In the fishes the gills, gut, rectal gland, skin and bladder are also important organs involved in the homeostasis of body fluids.

1. The Kidney

(a) Development of the Kidney

The functional unit of the kidney in all vertebrates, is the nephron. Embryonic development of the nephros is from mesodermal tissue, the mesomere, which becomes segmented into nephrotomes. Each nephrotome develops into a nephron. The number of nephrons varies with the species but it is usually in the order of 3-5, corresponding to the number of body segments (Torrey and Feduccia, 1979). These first formed nephrons occupy an anterior position in the body cavity and are known collectively as the "head kidney" or pronephros. Pronephric tubules are linked by a common drainage duct, the pronephric duct, which extends posteriorly towards the cloaca.

With the exception of the hagfishes and some teleosts, the pronephros has only a temporary existence. In Chondrichthyes it is present during early embryonic stages only, and has little or no functional significance. In the active, free-swimming larval stage of the lamprey however, the pronephros forms an important excretory organ.

In all fishes, including the hagfish, a second generation of nephrons develops from the remainder of the mesomere,

forming the mesonephros. In most fishes, the mesonephros is composed of long, structurally complex nephrons, tightly packed together to form the functional kidney. They join the pronephric duct and take over the excretory role of the kidney.

In the dogfish, Scyliorhinus canicula, a further number of excretory tubules develop at the posterior end of the peritoneal cavity, and acquire separate ducts which either unite to form a further duct, or open independently into the posterior section of the pronephric duct (Bourne, 1922). This posterior portion of the kidney is called the metanephros, and in the dogfish is the chief excretory organ. The mesonephros and metanephros are collectively known as the "back kidney" or opisthonephros (Torrey and Feduccia, 1979).

(b) Kidney Structure

The kidneys of fishes are paired, elongate structures, lying dorsally in the body cavity. The kidneys, like those of all vertebrates, are composed of a number of nephron units. These are tubular structures originating from a tuft of capillaries (the glomerulus) which invaginate the Bowman's capsule. The number and size of glomeruli, and the nephron length, varies greatly within the fishes (Nash, 1931).

Arterial blood is supplied to the glomerulus by the afferent arteriole. The efferent arteriole, on leaving the glomerulus, divides to form a network of peritubular capillaries, which eventually unite to form the renal veins. A renal portal blood system is found in many species of

fishes, supplying blood from the segmental musculature and posterior regions of the body to the peritubular capillary network (Hickman and Trump, 1969).

The hagfishes are considered to have the most primitive of all vertebrate kidneys, having 30-35 pairs of large, segmentally arranged glomeruli (Riegel, 1978). Each glomerulus has a short tubule leading to the urinary duct. Lampreys, in contrast have a much larger number of glomeruli, estimated to be 2000-3000 in Lampetra fluviatilis (Moriarty, Logan and Rankin, 1978). The glomeruli are arranged, as in the hagfish, in a continuous row running down the length of each kidney. In the lamprey however, the renal tubules are well developed and several distinct segments can be clearly identified.

The nephrons of the elasmobranchs are typically very long and have large glomeruli (Nash, 1931). The tubules are composed of several morphologically distinct tubular segments (Hickman and Trump, 1969). The elasmobranch nephron is described in further detail in section II.A.2.

Nephrons of the teleost fishes are generally much shorter in length than those of the elasmobranchs, and the glomeruli are generally smaller. The relative development of glomeruli varies considerably within the teleosts. Nash (1931) found the glomeruli of fresh water teleost species to be larger and more developed than those of marine species. Glomeruli are absent in at least 23 species of marine teleosts, and in several fresh water species (Hickman and Trump, 1969; Forster, 1974). Kidneys of the aglomerular goosefish, Lophius

americanus, and toadfish, Opsanus tau, are composed of many blind-ending tubules, which are supplied almost entirely by venous blood (Marshall and Grafflin, 1928; Marshall, 1929; Dobbs and DeVries, 1975).

(c) Kidney Function

Modern theories of urine formation were introduced by Bowman (1842). He described the structure of the renal corpuscle in great detail, and from these studies proposed his theory on the formation of urine. Bowman suggested that water was being filtered from the plasma at the glomerular capillaries, and that all other components of urine were added to the water by tubular secretion. Ludwig (1844), following similar anatomical studies, suggested that urine was being formed at the glomerulus by filtration, with only the large plasma proteins being retained by the capillaries.

Many years later, Wearn and Richards (1924) provided the first experimental evidence supporting Ludwig's theory of ultrafiltration and reabsorption. Fluid collected from Bowman's capsule of anaesthetised frogs, Rana pipiens, was shown to be devoid of protein yet in all other respects was similar to plasma, while simultaneously collected samples of bladder urine differed in composition to the capsular fluid. Thus, the formation of urine involves the ultrafiltration of plasma at the glomerulus, reabsorption of solutes and water from the lumen of the tubule, together with secretion of substances by the tubular cells into the tubular fluid.

The hagfishes are the only vertebrates which maintain

their body fluids iso-osmotic to sea water (Alt, Stolte, Eisenbach and Walvig, 1980). The short renal tubule reabsorbs no water from the glomerular ultrafiltrate, and therefore urine flow directly reflects glomerular filtration rate. The tubule is capable of reabsorbing glucose, calcium and magnesium from tubular fluid, but not sodium. Potassium and phosphate are secreted by the renal tubule. Volume regulation of hagfish body fluids may be due to an influx of water causing increased blood volume and pressure, resulting in an increase in filtration rate and urine flow (Alt et al., 1980).

Euryhaline lampreys are able to maintain a constant plasma composition during adaptation to a fresh or sea water environment. The river lamprey, Lampetra fluviatilis, produces a copious, dilute urine in fresh water as a result of osmotic water influx across the gills. Sodium and chloride ions are reabsorbed by the kidney tubule (Logan, Moriarty and Rankin, 1980). Lampreys in brackish water or sea water on the other hand, reduce glomerular filtration rate by reducing single nephron filtration rates, and thus produce a reduced volume of urine, which is slightly hyperosmotic to the plasma and contains a high concentration of magnesium and sulphate ions (Logan, Morris and Rankin, 1980; Rankin, Logan and Moriarty, 1980).

The slight hypertonicity of marine elasmobranchs ensures that just sufficient water enters the body by osmosis at permeable surfaces, to replace losses in urine and rectal gland secretion. The kidneys excrete small volumes of

iso-osmotic urine containing secreted magnesium, sulphate and phosphate ions (Pang, Griffith and Atz, 1977; Stolte, Galaske, Eisenbach, Lechene, Schmidt-Nielsen and Boylan, 1977).

The mechanism involved in renal reabsorption of urea has been investigated but the exact nature of the process is still not fully understood. Boylan (1972) proposed a model for passive urea reabsorption based on the detailed description of the skate nephron by Deetjen and Antkowiak (1970). He suggested that urea diffuses from the tubular fluid through the thin-walled terminal segment of the nephron with low water permeability, to an environment with a low concentration of urea created by proximal loops of the same nephron. Schmidt-Nielsen, Truniger and Rabinowitz (1972) suggested urea reabsorption in the spiny dogfish, Squalus acanthias, was coupled to, or dependent on, the reabsorption of sodium. There is however, evidence that passive urea transport in the skate, Raja erinacea, is related to fluid reabsorption (von Baeyer and Boylan, 1973). Recent studies have shown the reabsorption of urea by the renal tubule to be blocked by the action of pharmacological inhibitors of active transport, suggesting the involvement of an active transport system (Schmidt-Nielsen and Patel, 1972; Hays, Levine, Myers, Heinemann, Kaplan, Franki and Berliner, 1977). Preliminary studies to identify the site of urea reabsorption suggest it to be between the proximal tubule and the collecting duct, that is, in the distal tubule (Schmidt-Nielsen, Ullrich, Rumrich and Long, 1966; Deetjen, Antkowiak and Boylan, 1972).

Teleosts in fresh water are hypertonic to the medium.

Excess water which enters the body due to the osmotic gradient, is eliminated as a copious hypotonic urine. A large proportion of the solutes filtered from the blood at the glomerulus are reabsorbed by the kidney tubule in order to maintain solute balance (Hickman and Trump, 1969). The primary function of the marine teleost kidney is the excretion of divalent ions, particularly magnesium and sulphate, by renal tubular secretory mechanisms.

The kidneys of marine, aglomerular teleosts function by secretory processes only. Urine formation occurs by the secretion of both monovalent and divalent ions across the tubular epithelium, with water following the passage of the ions osmotically (Evans, 1980). Aglomerular teleosts are not entirely restricted to marine environments. The toadfish, Opsanus tau, is an estuarine-inhabiting species, thriving in coastal waters of 10% sea water (Bentley, 1971). In more dilute media, a relatively dilute urine is produced (Lahlou, Henderson and Sawyer, 1969).

2. Gills

The gills of fishes are composed of a series of gill arches. Each gill arch has two rows of filaments, which bear the secondary lamellae. Secondary lamellae are covered by the branchial epithelium over which gaseous exchange occurs. The permeability of the gills to oxygen and carbon dioxide is however, accompanied by a permeability to water and certain solutes. As the gills account for approximately 70% of the total body surface (Maetz, 1970), the osmotic status of the

fish will be influenced by the external environment.

Fresh water fishes tend to accumulate water across their gills by osmosis. Many fresh water teleosts actively transport sodium and chloride ions into the body across the gills, against the concentration gradient. These ions may be exchanged for ammonium or hydrogen, and bicarbonate ions respectively (Maetz and Garcia Romeu, 1964; Evans, 1982). The sodium and chloride ions accumulated in the body of the marine teleost by drinking sea water, are eliminated by the gills. Keys and Willmer (1932) identified specific cells in the gills of the eel and termed them "chloride cells". These cells are thought to be the site of sodium and chloride ion extrusion in marine teleosts (Evans, 1982).

The gills of elasmobranchs have been shown to have very low permeability to solutes and urea, ensuring that the high plasma urea levels are maintained (Boylan, 1967). The gills are however, capable of considerable sodium and chloride efflux, but whether this involves active transport processes remains unknown (Bentley, 1971).

3. Rectal Gland

The rectal gland is a small, finger-shaped organ located at the posterior end of the intestine of marine elasmobranchs. It is supported by a layer of intestinal mesentry and is composed of many secretory tubules tightly packed together, surrounded by a protective capsule of muscle and connective tissues (Doyle, 1977). Blood is supplied to the gland by the rectal gland artery which arises directly from the dorsal

aorta. The rectal gland artery divides to smaller arteries, before forming a series of blood sinuses which bathe the secretory tubules (Doyle, 1977). The individual tubules lead to a central duct which opens directly into the intestine.

The function of the rectal gland of elasmobranchs was first suggested by the studies of Burger and Hess (1960). They showed the rectal gland to be intermittently secreting a concentrated solution of sodium chloride, and therefore to be a site of extra-renal salt extrusion. Further experiments (Burger, 1962) demonstrated that an injection of sodium chloride solution, sufficient to salt-load the fish, stimulated rectal gland secretion. The rectal gland may not however, be the only site of salt extrusion. Experiments involving the removal of the rectal gland have not always significantly elevated plasma osmolarity (Burger, 1965) or plasma sodium concentration (Chan, Phillips and Chester Jones, 1967). However, other studies have shown plasma sodium levels to gradually increase following removal of the gland (Forrest, Silva, Epstein and Epstein, 1973). If another site for salt extrusion does exist, it is thought likely to be located in the gills.

Rectal glands have been identified in fresh water species of elasmobranchs, but they appear to be reduced in size and non-functional (Oguri, 1964).

4. Gut

The gut of the fishes shows considerable variation with respect to both anatomy and osmoregulatory role. The gut may

be a simple tubular structure, as in the Agnatha, or it may be divided into the anatomically and functionally distinct regions of the oesophagus, stomach and intestine of the elasmobranchs and bony fishes.

Marine teleosts drink water to compensate for the osmotic loss of water across the large gill surface (Smith, 1930). In drinking sea water large amounts of salts are also ingested. Recent studies in the sea water eel, Anguilla japonica, have suggested that an initial process of "desalting", that is removal of sodium and chloride ions, occurs in the oesophagus in order to allow absorption of the water from the intestines without further loss of water from the body (Hirano, 1980). Smith (1930) found that whilst monovalent ions were readily absorbed, considerable amounts of the divalent ions magnesium, calcium and sulphate, remained in the intestinal tract, and were excreted in the faeces. Divalent ions which are absorbed are excreted via the kidneys (Bentley, 1971).

5. Skin

The skin of fishes despite being well vascularised, is generally considered to be impermeable to the passage of water and solutes. However, individual species have been shown to exchange water across the skin, for example, the river lamprey, Lampetra fluviatilis, (Bentley, 1971) and Scyliorhinus canicula (Rankin and Bolis, 1984). Permeability of the skin may be reduced by thickening, protection by mucus, and covering by dermal or placoid scales. The scaleless and well-vascularised areas of skin of the marine goby,

Gillichthy s mirabilis, have recently been shown to be involved in the active transport of monovalent ions (Marshall and Bern, 1980).

6. Bladder

The urinary bladder of teleosts is formed by the enlargement of the distal portion of the mesonephric ducts. In vivo studies have revealed that urine is modified in the bladder of the toadfish (Lahlou et al., 1969) and trout (Fossat and Lahlou, 1977). The bladder of the sea water teleost is thought to be capable of absorption of sodium and chloride ions and water, and the fresh water teleost bladder capable of absorption of sodium and chloride ions only (Skadhauge, 1977).

II. ANATOMICAL STUDIES ON THE KIDNEY OF THE DOGFISH, SCYLIORHINUS CANICULA

A. INTRODUCTION

1. Gross Morphology of the Elasmobranch Kidney

The kidneys of the elasmobranch lie dorsally within the body cavity. They are covered ventrally by the peritoneal membrane, and therefore lie outside the coelomic cavity (Bourne, 1922). The paired kidneys are elongated structures in the sharks, extending forwards from the cloaca, separating and narrowing anteriorly. The kidneys of the skates and rays are generally more rod-shaped.

The kidneys of the dogfish differ in extent and relationship to the reproductive organs in the two sexes (Bourne, 1922). The mesonephros of the male is an elongated, glandular structure which extends anteriorly from, and is continuous with, the metanephric kidney. The mesonephric, or Wolffian duct, carries secretions from the gland and spermatozoa from the testes, so functioning as the vas deferens. Each Wolffian duct dilates at the posterior end to form the paired seminal vesicles which combine to form the urogenital sinus, into which the metanephric ducts, or ureters, discharge.

The mesonephros of the female shark is degenerate and functionless. The Wolffian ducts are narrow anteriorly, but posteriorly dilate to form a pair of urinary sinuses. The

metanephric ureters enter at the union of the urinary sinuses. The urogenital sinus opens to a median, prominent urinary papilla situated within the cloaca of both sexes.

Blood is supplied to the kidneys by the renal arteries, which arise as ventral branches of the paired segmental arteries, from the dorsal aorta. After passing through the kidney, blood is collected in the numerous renal veins which empty directly into the posterior cardinal sinuses. The elasmobranch kidney is also supplied by a renal portal blood system (Bourne, 1922; Ghouse, Parsa, Boylan and Brennan, 1968). The caudal vein divides at the posterior end of the kidneys to form the veins of Jacobson, also known as the renal portal veins, which course along the dorso-lateral margin of the kidneys giving rise to numerous branches. The renal portal blood is drained from the kidneys via the renal veins.

The interrenal and chromaffin endocrine bodies of the elasmobranchs are closely associated with the renal tissues. The rod-shaped interrenal bodies are found embedded in the posterior part of the kidney, or lying along the ventro-medial margin (Lutz and Wyman, 1927). The oval-shaped chromaffin tissues are paired bodies, found along the entire length of the kidneys at the bifurcation of the renal and segmental arteries (Lutz and Wyman, 1927; Deetjen, Dlouha, Brennan and Boylan, 1970).

2. The Elasmobranch Nephron

(a) Nephron Length

Anatomical studies of elasmobranch kidneys have revealed the extreme length and complex structural configuration of the nephron (Nash, 1931; Kempton, 1940, 1943, 1962; Borghese, 1966; Ghouse et al., 1968; Deetjen and Antkowiak, 1970; Lacy, Schmidt-Nielsen, Galaske and Stolte, 1975; Lacy, Reale, Schlusselberg, Smith and Woodward, 1985). Nash (1931) measured the length of complete nephrons in the skate, Raja stabulaforis, and 17 species of teleost. The length of the skate nephron was approximately 90 mm, compared to lengths of 1.65 - 9.70 mm in the teleosts, but there was a wide variation within the same kidney. More recent measurements of nephron length of elasmobranchs include 82 mm and 50 mm in Raja erinacea (Lacy et al., 1975; Deetjen and Antkowiak, 1970), 33 mm in Squalus acanthias (Ghouse et al., 1968), and 52 mm in Scyliorhinus canicula (Brown, unpublished observations).

(b) Number and Size of Glomeruli

The kidney of the elasmobranch is composed of many nephron units, each originating from a single glomerulus. Table 1 summarises published estimations of the glomerular population of four species of elasmobranch. Each estimation was made by macerating a complete kidney, or both kidneys in concentrated acid, to form a suspension. Nash (1931) counted the glomeruli in the whole suspension from each kidney, whereas Antkowiak and Boylan (1974) macerated both kidneys of Squalus acanthias, took known fractions of the suspension and calculated the

Table 1

Estimation of glomerular population in the kidneys of *Mustelis canis*, *Raja diaphanes*, *Raja erinacea* and *Squalus acanthias*.

[a], mean value ± standard deviation; number of animals in parentheses.

[b], estimation from total fused renal mass.

Species	Body Weight (g)	Glomerular count (per kidney)	Method	Reference
Mustelis canis	485	4397	Complete count	Nash (1931)
Mustelis canis	760	5248	Complete count	"
Raja diaphanes	1740	1816	Complete count	"
Raja erinacea	1060	1197	Complete count	"
Raja erinacea	-	2260 ± 120 [a] (n=14)	Fractional count	Antkowiak and Boylan (1974)
Squalus acanthias [b]	-	8898 ± 1208 [a] (n=6)	Fractional count	"

total number of glomeruli. This was divided by two, to estimate the glomerular population present in each kidney.

The distribution of glomeruli within the elasmobranch kidneys may vary between species. Glomeruli of the skate, Raja erinacea, were evenly distributed throughout the renal tissues, but in the dogfish Squalus acanthias more than 90% of the total glomeruli were present in the caudal 50% (by length) of the kidney (Antkowiak and Boylan, 1974).

The glomeruli of the elasmobranch kidney are typically large and ovoid in shape, with tufts of widely patent capillaries (Hickman and Trump, 1969). Ghouse et al., (1968) measured the diameter of the glomerular tuft of Squalus acanthias (polar axis - 139 μm, transverse axis - 169 μm), and the internal diameter of the Bowman's capsule (polar axis - 230 μm, transverse axis - 180 μm). His findings suggested that the elasmobranch glomerulus closely resembled the mammalian glomerulus in both dimensions, and in the arrangement of capillary and mesangial tissues. Ultrastructural studies of the filtration barrier of elasmobranchs show the basement membrane to be thickened with a dense mesangial matrix, containing fibrocytic elements between the endothelial and epithelial cell layers (Bargmann and von Hehn, 1971). The podocytes were shown to bear many microvilli protruding into the capsular space.

(c) Nephron Segments

The exact number of segments and their anatomical features are still under investigation. The elasmobranch

nephron is composed of several segments. Segments may be identified by external tubular and luminal diameter, and cytological features (Kempton, 1940; 1962; Ghouse et al., 1968; Deetjen and Antkowiak, 1970; Lacy et al., 1975; Brown, unpublished observations). It is generally accepted that the nephron is composed of the Bowman's capsule, neck, proximal and distal tubular segments, leading to the collecting duct system. A summary of four investigations into the histology of tubular segments in 3 species of dogfish, is given in Table 2. Similar investigations on the renal tubule of the lesser electric ray, Narcine brasiliensis, (Kempton, 1962), and the skate, Raja erinacea, (Deetjen and Antkowiak, 1970; Lacy et al., 1975), have also shown the tubule to be composed of neck, proximal and distal segments, closely following the pattern observed in the dogfish.

In an early comparative study of vertebrate nephrons, Marshall (1934) observed that the elasmobranch nephron appeared to have two segments in addition to those of other vertebrates. These segments were linked with the reabsorption of urea by Marshall (1934) and Smith (1936), and termed "striated segments", due to their apparent striations running from the base to the apex of the cells. With the application of injection techniques, Kempton (1943) showed conclusively that the "special segment" was in fact part of the distal segment, and was not interposed between the neck and proximal segments as had been thought. The striations are now known to be due to the orderly arrangement of mitochondria, and the infolding of the plasmalemma of the epithelial cells

Table 2

Summary of histological features of nephron segments in the kidneys of *Squalus acanthias*, *Scyliorhinus canicula* and *Scyliorhinus stellaris*.

O.D., external diameter of tubular segment.

[a], order of proximal segments unknown.

Species	Neck Segments	Proximal Segments	Distal Segments	Reference
Squalus acanthias	I Low cuboidal epithelium Nuclei central Flagella No brush border	I Columnar epithelium Nuclei central Flagella Brush border	I Cuboidal epithelium Nuclei central No flagella No brush border	Kempton (1940, 1943)
	II Low cuboidal epithelium Nuclei central No flagella No brush border	II Low cuboidal epithelium Nuclei central Flagella Brush border		
Squalus acanthias	I Cuboidal epithelium Nuceli central Flagella Brush border O.D. 30 µm	I Cuboidal epithelium Nuclei apical No flagella Brush border O.D. 40 µm	I Cuboidal epithelium Nuclei central No flagella No brush border O.D. 30 µm Length 0.05-0.15 mm	Ghouse et al. (1968)
	II Low cuboidal epithelium Nuclei central No flagella No brush border O.D. 30 µm Length I+II, 1.52 mm	II Low cuboidal epithelium Nuclei apical Flagella Brush border O.D. 40 µm Length I+II, 10.72 mm		
		III Columnar epithelium Nuclei central Flagella Brush border O.D. 80 µm Length 18.56 mm		

Species	Neck Segments	Proximal Segments	Distal Segments	Reference
Scyliorhinus canicula	I Cuboidal epithelium Nuclei large Flagella No brush border	I Columnar epithelium Flagella Brush border O.D. 50-70 µm Length 6.0 mm	I Low cuboidal epithelium Flagella Length 13.0 mm	Brown (unpublished observations)
	II Cuboidal epithelium No flagella No brush border Length I+II, 2.8 mm	II Columnar epithelium Flagella Brush border O.D. 110 µm Length 27.0 mm		
		III Cuboidal epithelium No flagella Brush border O.D. 50 µm Length 3.8 mm		
Scyliorhinus stellaris (embryo)	I Cuboidal epithelium Flagella No brush border	I[a] Tall columnar epithelium Flagella Brush border	I Low cuboidal epithelium No brush border	Borghese (1966)
		II[a] Low columnar epithelium Flagella Brush border		

(Borghese, 1966).

The distal tubules of the kidney lead into the collecting duct system. The smaller collecting ducts unite to form the primary collecting ducts (the ureters) which empty directly into the urinary sinuses. The urinary sinuses of the dogfish, Scyliorhinus canicula, are a pair of sacs which unite posteriorly, and open into the cloaca by a median aperture, the urinary papilla (Bourne, 1922). Lacy, Schmidt-Nielsen, Swenson and Maren (1975) describe a bilobed urinary bladder in the female skate, Raja erinacea, which empties into the urogenital sinus via a short urethra, prior to discharge to the cloaca.

(d) Ultrastructure of the Nephron

Despite an increasing interest in the segmental structure and physiology of the elasmobranch renal tubule, very few studies have investigated the ultrastructure of the nephron. Ghouse et al. (1968) examined the microanatomy of the nephron of the spiny dogfish, Squalus acanthias, using light and electron microscopy, together with microdissection techniques. Bargmann and von Hehn (1971) studied the ultrastructure of the glomerulus and proximal tubule in several elasmobranch species, whilst Lacy et al. (1975) determined the fine structure of the skate proximal tubule with respect to the secretion of divalent ions (Stolte, Galaske, Schmidt-Nielsen and Lechene, 1975). Many ultrastructural features of both the glomerulus and renal tubule of elasmobranchs remain unknown.

(e) Absence of Juxtaglomerular Cells in the Elasmobranch Kidney

Juxtaglomerular granulated arteriolar cells have been found in the kidneys of all vertebrate groups except the elasmobranchs and cyclostomes (Sokabe and Ogawa, 1974). Many elasmobranch species have been studied, including Raja laevis (Capreol and Sutherland, 1968), Carcharhinus leucas, Carcharhinus limbatus, Dasyatis sabina, Ginglymostoma cirratum, Sphyrna tiburo, Sphyrna zygaena (Oguri, Ogawa and Sokabe, 1970), Dasyatis akajei, Heterodontus japonicus, Triakis scyllia, Orectolobus japonicus (Nishimura, Oguri, Ogawa, Sokabe and Imai, 1970), Pristis perotteti, Pristis pectinatus, Dasyatis guttata and Potamotrygon motoro (Crockett, Gerst and Blankenship, 1973), yet no juxtaglomerular cells have been identified, indicating that this fundamental part of the renin-angiotensin system is almost certainly absent in elasmobranchs. Other parts of this system may however be present in the elasmobranchs (see section III.A.4(b)(i)).

3. The Renal Circulation

The arterial and venous blood system of the kidney of the dogfish, Squalus acanthias, has been described by Ghouse et al. (1968). The renal arteries enter the caudal kidney at the medial border and branch immediately to form several smaller arteries, which run laterally along the dorsal margin of the kidney. These branches give rise to the afferent arterioles

which supply blood to the glomeruli. The glomerular tuft is composed of large capillary loops which unite to form the efferent arteriole on leaving the glomerulus. The efferent arteriole divides to form the peritubular capillary network, which combines with the vessels of the renal portal blood system prior to discharge into the renal veins.

(a) Techniques Used in the Investigation of Renal Circulation

The renal blood vessels of many higher, and some lower vertebrate species have been investigated using a range of anatomical techniques. The morphological features which these studies have revealed may influence the circulation of blood through the kidneys.

(i) Histological, Ultrastructural and Histochemical Techniques

Standard techniques of histology, scanning and electron microscopy have provided valuable information on cellular and vessel wall structure. The ultrastructure of arteries, arterioles, capillaries, venules and veins of many species, for example, the rabbit (Rhodin, 1967) and the spiny dogfish, Squalus acanthias, (Rhodin, 1972) have been determined by electron microscopy. Similar studies have revealed the presence of muscular structures, known as intra-arterial cushions and precapillary sphincters, throughout the circulatory system, and frequently associated with renal blood vessels.

Intra-arterial cushions have been located at the origins

of afferent arterioles supplying juxtamedullary glomeruli, in the kidneys of the rat, cat and dog (Picard, 1951; Picard and Chambost, 1952; Taggart and Rapp, 1969; Fourman and Moffat, 1971; Moffat and Creasey, 1971). The cushions take the form of an elongated, streamlined ridge in the wall of the parent artery. The ridge is perforated at the origin of the afferent arteriole, so that in cross section through the parent artery, it appears that there are a pair of cushions guarding the entrance to the afferent arteriole. The opening of the branching arteriole is therefore projected into the centre of the parent vessel (Fourman and Moffat, 1971). The cushions are composed of longitudinal smooth muscle fibres embedded in a variable amount of connective tissue (Moffat and Creasey, 1971).

Muscular, ring-like constrictions known as preglomerular sphincters, have been found on afferent arterioles in two species of fishes, the Prussian carp (Elger and Hentschel, 1981a) and rainbow trout (Elger and Hentschel, 1981b; Elger, Wahlqvist and Hentschel, 1984). Whilst precapillary sphincters have been described in the microcirculation of many mammalian species (Rhodin, 1967; McCuskey, 1971), there have been no reports of the occurrence of preglomerular sphincters in the kidneys of mammals to date. Functionally, the presence of intra-arterial cushions and preglomerular sphincters may be important in the determination of distribution of arterial blood within the kidney.

The control of circulation within an organ may be by direct autonomic innervation of blood vessels, or by

circulating catecholamines acting on the cells of the vessel walls (Moffat and Creasey, 1970). The development of histochemical fluorescence techniques (Falck, Hillarp, Thieme and Torp, 1962; Corrodi and Jonsson, 1967) has facilitated the demonstration of adrenergic innervation of vessel walls. Nilsson, (1965) using this technique, studied the innervation of renal vessels in the rabbit and rat and located nerves supplying the renal arteries and arterioles, but ending at the glomerulus. More recent studies in the rat, gerbil, guinea pig, rabbit and cat (Ljungqvist and Wagermark, 1970; Fourman, 1970; Knight and Bazer, 1979) indicate widespread adrenergic innervation supplying efferent arterioles and the vasa recta in addition to preglomerular vessels. Ultrastructural studies have confirmed the extent of innervation of vessel walls in the rabbit and rat kidneys (Gosling and Dixon, 1969; Dieterich, 1974). The renal blood vessels of the rainbow trout have been shown to possess a rich adrenergic innervation, with many myoneural junctions along the afferent arteriole and at the preglomerular sphincter (Elger et al., 1984).

(ii) Injection Techniques

The injection of suitable substances into the renal vasculature allows the spacial arrangement of blood vessels, and patterns of vascular perfusion to be visualised. Bowman (1842), in his classic work, demonstrated the structure of the renal corpuscle and its vasculature, by injection of a solution of potassium bichromate, followed by a solution of

lead citrate. The resultant precipitate allowed the renal arteries, arterioles, glomeruli and vasa recta to be clearly identified under the light microscope.

Modern injection techniques make use of synthetic rubbers (silicone rubber, neoprene and latex) and methyl methacrylate resins. These substances are injected into the vasculature in liquid form and allowed to harden to form solid casts. Tissues can then either be cleared to allow examination using the light microscope (Moffat and Fourman, 1963), or digested with concentrated acid or alkali for examination of the vascular casts by scanning electron microscopy.

Early corrosion casting studies of the renal vasculature of mammalian species investigated the arrangement of renal blood vessels (Shonyo and Mann, 1944). More recently the luminal surface features of blood vessels have been studied (Reidy and Levesque, 1977; Kardon, Farley, Heidger and Van Orden, 1982; Casellas, Dupont, Jover and Mimran, 1982). Comparison of corrosion casts of intra-arterial cushions from rat arteries, with cushions from perfusion-fixed arteries, using scanning electron microscopy has indicated that casts produce a reliable image of surface topography and features (Casellas et al., 1982). Ring-like contrictions in the casts at the origins of afferent arterioles, indicating the presence of preglomerular sphincters, have been found in the kidneys of two species of trout (Anderson and Anderson, 1976).

Using ultrastructural techniques, Ljungqvist (1975) demonstrated a vascular connection between the afferent and efferent arterioles of juxtamedullary glomeruli in the rat.

Although the occurrence of preglomerular shunts is still controversial, Casellas and Mimran (1981) have confirmed the presence of glomerular bypass channels in the rat kidney, following a study of corrosion casts of the renal microvasculature. Scanning electron microscopy studies of vascular casts in a range of other vertebrates have however reported an absence of glomerular shunts (Murakami, 1972; Spinelli, Wirz, Brücher and Pehling, 1972; Anderson and Anderson, 1976; Morris and Campbell, 1978).

Murakami, Miyoshi and Fujita (1971) reported the presence of double efferent arterioles leaving the glomerulus in the rat, confirming the findings of Shonyo and Mann (1944) and Moffat and Fourman (1963). Double efferent arterioles appear to be more common in the trout, Salmo gairdneri, (Brown, 1985) than in the rat. This may be a primitive feature as in the hagfishes, which commonly have two to four efferent arterioles (Heath-Eves and McMillan, 1974). Double afferent arterioles are also present in the trout (Brown, 1985) and though rare, in the rat (Murakami, 1976). The physiological significance of double afferent and efferent arterioles however, remains unknown.

Intra-arterial cushions, preglomerular sphincters, preglomerular shunts, and double afferent and efferent arterioles, will influence the circulation of blood within the kidneys. The present study in the dogfish, Scyliorhinus canicula, aims to describe the overall anatomy of the kidney and renal vasculature, and in particular to determine the

occurrence of structures which may be involved in the regulation of renal blood flow.

B. MATERIALS AND METHODS

1. Experimental Animals

Lesser spotted dogfish, Scyliorhinus canicula, of both sexes, weighing 450-1000 g were obtained from the Plymouth Marine Biological Association, Plymouth, England. The animals were transported from Plymouth in large tanks containing well oxygenated sea water. The temperature of the sea water was kept as close as possible to 9°C by the use of ice-packs.

The dogfish were kept in well aerated, fresh sea water (860 $mOsm.l^{-1}$) in large tanks (capacity 500 l). Sea water was delivered by tanker from the Marine Zoological Gardens, Skegness, and stored in large tanks beneath the aquarium. The sea water was filtered through a graduated sand-gravel filter-bed, held within an Eheim filter-pump unit. Water temperature was maintained at 9°C by a cooling unit (ethyl glycol cooling coil) held within the central circulating tank, from which water was circulated to all tanks in the aquarium.

The fish were fed regularly on pieces of fresh fish (plaice, flounder and cod). Fish were not used experimentally for a minimum period of three weeks following transfer from Plymouth, to allow time for adaptation to sea water of a slightly lower osmolarity. The dogfish were starved for 7-14 days before experimentation.

2. Surgical Preparation

Dogfish were anaesthetised by immersion in 0.015% (wt/vol) MS222 (Tricaine methane sulphonate, Sandoz Ltd.). Following the onset of anaesthesia, indicated by the loss of the "righting" reflex and the response to painful stimuli, the fish were placed supine on a V-shaped support constructed of expanded polystyrene, held within a perspex trough. The head and gills of the fish were immersed in aerated, cooled (9°C) sea water, containing 0.0067% (wt/vol) MS222, to maintain anaesthesia.

Through a mid-ventral incision, the lieno-gastric artery (Bourne, 1922) was exposed and catheterised (PE 50, Portex), in preparation for further experimental procedures.

3. Experimental Techniques

(a) Fixation of Tissues

The kidneys were fixed *in situ*, by perfusion with 3% glutaraldehyde in dogfish Ringer's solution (NaCl, 280.0 mM; KCl, 7.2 mM; $CaCl_2$, 5.0 mM; $MgCl_2$, 5.0 mM; Na_2SO_4, 2.0 mM; $NaHCO_3$, 4.6 mM; $NaHPO_4$, 0.5 mM; $(NH_2)_2CO$, 360.0 mM; Osmolarity, 836 $mOsm.l^{-1}$; pH, 7.4). The fixative was infused via the lieno-gastric artery using hand pressure. 50 ml of fixative was infused over a period of 20-30 minutes. Drainage cannulae were inserted into the posterior cardinal sinuses to direct the flow of fixative through the renal vasculature.

The pair of kidneys remained *in situ* within the body

cavity for a further 30 minutes prior to excision. Following excision, both kidneys were immersed in fixative overnight to ensure complete fixation of the tissues.

(b) Serial Sections

Following fixation of the tissues, the kidney pair from each animal was separated. One kidney from a male fish and one kidney from a female fish was divided into blocks by transverse cuts. Each block was dehydrated in alcohol, and embedded in Paraplast. Serial transverse sections (6 μm thick) were cut along the entire length of the kidney and stained with Haematoxylin-Eosin. The serial sections were examined by light microscopy (Kyowa Instruments Ltd.), and the relative positions of glomeruli within the sections along the entire kidney length were recorded.

(c) Semi-thin Sections

Following fixation of the kidneys, strips of tissues measuring approximately 2 mm x 2 mm x 10 mm were cut with razor blades, and post-fixed in 1% osmium tetroxide for 30 minutes. The tissue blocks were dehydrated with ethanol and propylene oxide, and embedded in Emix (Emscope). 1 μm semi-thin sections were cut on a LKB Mk 1 Ultratome, lightly stained with Toluidine blue, and examined under a light microscope (Kyowa Instruments Ltd).

(d) Scanning Electron Microscopy

Glutaraldehyde-fixed tissues from 6 fishes (4 female, 2 male), were sliced with razor blades and post-fixed in 1% osmium tetroxide. Following dehydration in acetone, the tissue slices were critical-point dried using liquid carbon dioxide in a Polaron E3000 unit, before being mounted on aluminium specimen stubs. The tissue surface was then coated with gold (Semprep 2, Nanotech Sputter Coater), prior to examination in the scanning electron microscope (Cambridge Stereoscan 600). Measurements of the diameter of the Bowman's capsule, glomerular capillaries and nephron segments were taken from scanning electron micrographs. Dimensions of the Bowman's capsule and glomerulus were recorded across the longest and shortest axes of the ovoid structures.

(e) Corrosion Casts of Renal Vasculature

Corrosion casts of the renal vasculature were prepared from the kidneys of 4 fishes (1 female, 3 male). Following catheterisation of the lieno-gastric artery, the vasculature was perfused with 100 ml 0.1% Lignocaine (Lignavet, C-Vet Ltd.), in heparinised dogfish Ringer's solution, using hand pressure. This relaxed the vasculature and prevented the formation of blood clots. Blood was withdrawn into a syringe via a cardiac puncture at approximately the same rate as the infusion.

Mercox casting material (Japan Vilene Co. Ltd.) was prepared by mixing 20 g of Mercox resin base with 0.2 g of catalyst, immediately prior to injection. The renal

vasculature was filled by injecting 5 ml of the prepared resin by hand, maintaining a moderate pressure. Both the mixing and injection of the resin were performed under a well ventilated extraction hood, because of the hazardous nature of the fumes.

In early experiments, blood pressure within the posterior mesenteric artery, caudal to the injection site, was monitored (refer to section III.B.2(a) for surgical methodology). Pressure in the posterior mesenteric artery was maintained as close to physiological arterial blood pressure as possible during infusion of the resin, by adjustment of resin flow rate into the vasculature.

Polymerisation of the resin occurred within 20 minutes at room temperature, after which the kidneys were removed en masse and placed in 20% potassium hydroxide solution. Complete digestion of the tissues from the casts took 2-3 days at room temperature, with daily changes of potassium hydroxide solution and washing of the casts with distilled water between changes. After a final rinse in distilled water the casts were examined under the binocular microscope (Kyowa Instruments Ltd.).

Complete casts were occasionally partially dissected under the binocular microscope. Whole casts were softened in ethanol prior to microdissection, to prevent shattering of the fragile casts (Murakami, 1972). Complete or partially dissected casts were re-washed in distilled water, dried and mounted on aluminium specimen holders. The casts were coated with gold and viewed in a scanning electron microscope (Cambridge Stereoscan 600).

(f) Macerated Kidney Tissues

Pieces of fresh kidney tissues, and tissues from fishes infused with sodium ferrocyanide (section III.B.2(d)), were macerated in 20% hydrochloric acid at 37°C, for 3-4 hours. The tissues were stored in a dilute solution of ferric chloride (0.2% ferric chloride and 1% acetic acid in tap water). Individual nephrons and nephron segments were dissected from this material under the binocular microscope, using glass teasing needles pulled from glass haematocrit capillary tubing. Observations were made on the anatomy and structural configuration of the nephron.

The length of a complete single nephron, successfully teased from the kidney of a female dogfish, was measured using an Apple IIe microcomputer and graphic plotter tablet.

(g) In Vivo Observation

In vivo observations of kidney tissues were made during micropuncture experiments (section III.B.2(c)), on 12 fishes of both sexes. Organisation of the nephrons, characteristic features of tubular segments and patterns of blood flow were observed and recorded.

C. RESULTS

1. Gross Morphology of the Kidneys

The kidneys of Scyliorhinus canicula lay dorsally within the body cavity, but were separated from the coelomic cavity by a tough layer of peritoneum. Each kidney was surrounded by a thick layer of protective connective tissue, which completely encased the kidney (Figure 1). The connective tissues surrounding the caudal renal mass were fused and held the kidneys together, although each kidney remained a discrete organ. Each kidney was composed of a number of segmentally arranged lobes. The kidney of the female was composed of approximately 7-8 large posterior lobes and 5-6 much smaller anterior lobes, stretching forward to the mid-point of the coelomic cavity. The kidney of the male dogfish had approximately 7-8 large posterior lobes only, which lay under the posterior end of the coelomic cavity.

2. Organisation of the Kidney

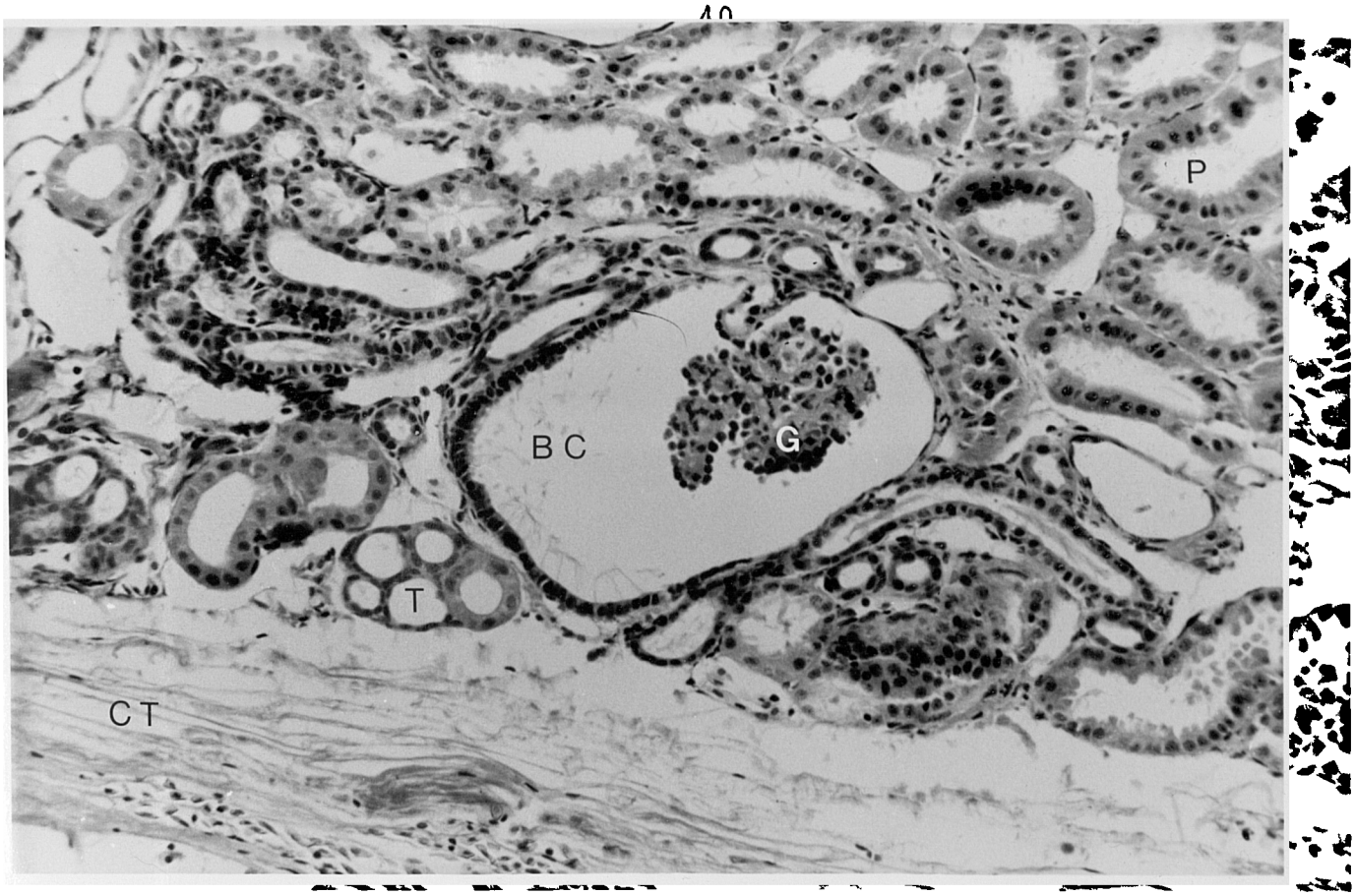
The kidney of Scyliorhinus canicula was composed of many individual nephrons. Figures 1 and 2 show the organisation of nephrons within the large posterior lobes of the kidney. In both sexes, glomeruli were located close to the dorsal margin of the kidney, generally lying beneath bundles of narrow-diameter tubules. Surface glomeruli were only observed very occasionally. Large tubules were found along the lateral

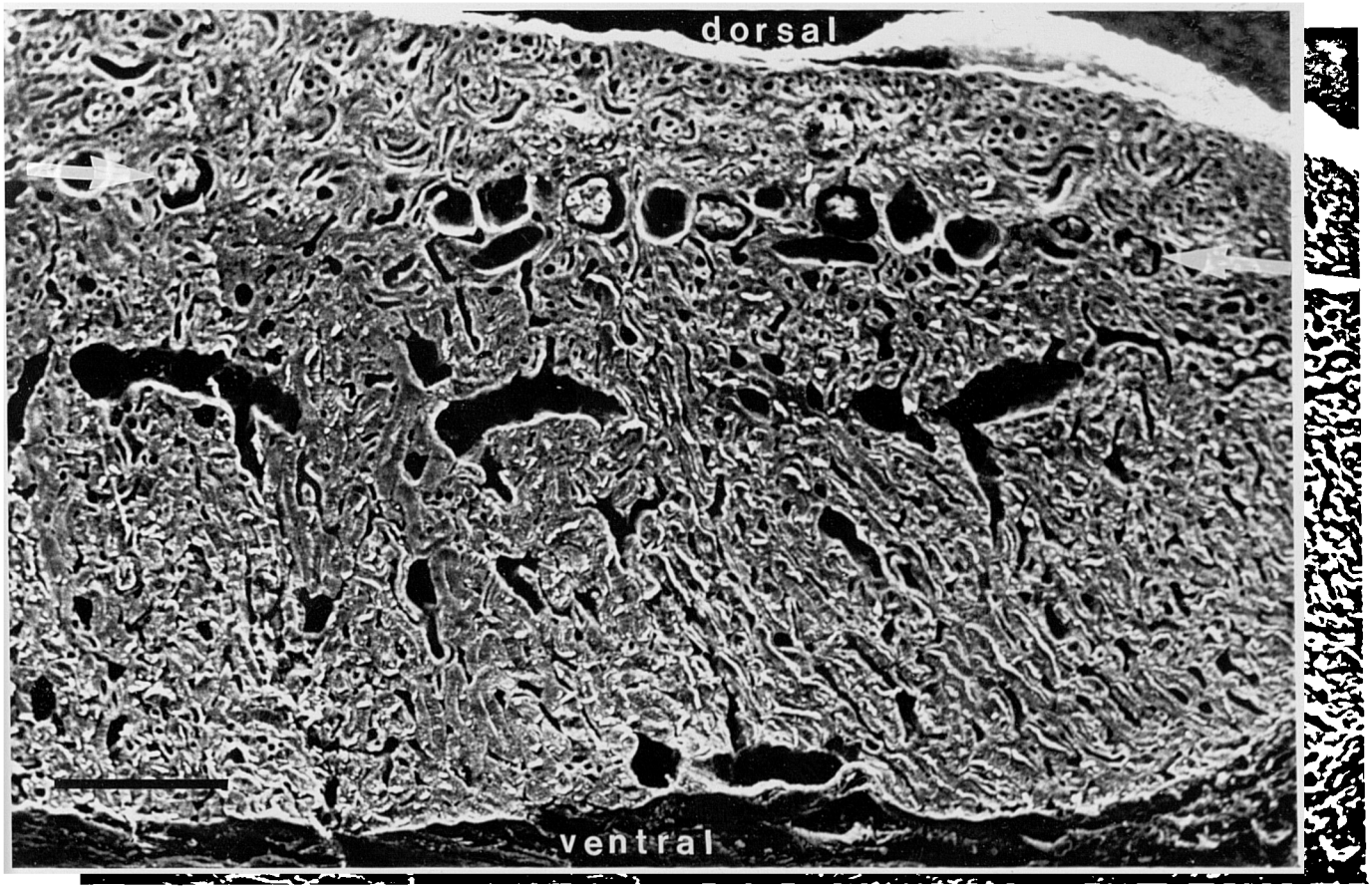
Figure 1

Light micrograph of a section through the dorsal margin of a posterior kidney lobe of Scyliorhinus canicula.
BC, Bowman's capsule; G, glomerulus; T, bundle of 5 tubules; P, proximal tubule; CT, connective tissue. x 100.

Figure 2

Scanning electron micrograph showing the organisation of nephrons in a posterior kidney lobe.
Bowman's capsules (arrows) are located in a row near to the dorsal margin of the kidney. Bar: 400 µm.


P
BC
G
T
CT


dorsal
ventral

and ventral margins of the kidney. The anterior lobes of the female kidney were flattened dorso-ventrally, and showed no consistent patterns of nephron organisation.

3. Glomerular Population

The number of glomeruli within the kidneys of a male and female dogfish is shown in Table 3. The distribution of these glomeruli along the length of the kidney, is represented by Figure 3. The glomeruli of the male kidney were found to be evenly distributed along the length of the kidney, with 44.2% of glomeruli being located in the posterior 50% of kidney by length. The pattern of glomerular distribution in the female kidney was found to be markedly different, as a result of the posterior and anterior lobe structure. 87.7% of glomeruli were located within the posterior 50% of the kidney by length. The remaining 12.3% of glomeruli predominantly occurred in the small anterior lobes of the kidney.

4. The Glomerulus

The glomerulus was found to be an ovoid tuft of capillaries, lying within, and almost completely filling the Bowman's capsule (Figure 4). The external diameter of Bowman's capsules, glomeruli and glomerular capillaries, measured from scanning electron micrographs, are shown in Table 4. External diameter measurements of glomeruli and Bowman's capsules were recorded across the longitudinal

Table 3

Number of glomeruli in the kidneys of a male and female dogfish, *Scyliorhinus canicula*.

Numbers were determined from serial sections of an entire kidney of one male, and one female fish.

	Male	Female
Body Weight (g)	844	684
Kidney Length (cm)	3.42	8.17
Glomerular Count (per Kidney)	1140	1474
Glomeruli / g Body Weight	2.7	4.3
% Glomeruli in posterior 50% kidney Length	44.2	87.7

Figure 3

Distribution of glomeruli in the kidneys of a male and female dogfish, Scyliorhinus canicula.

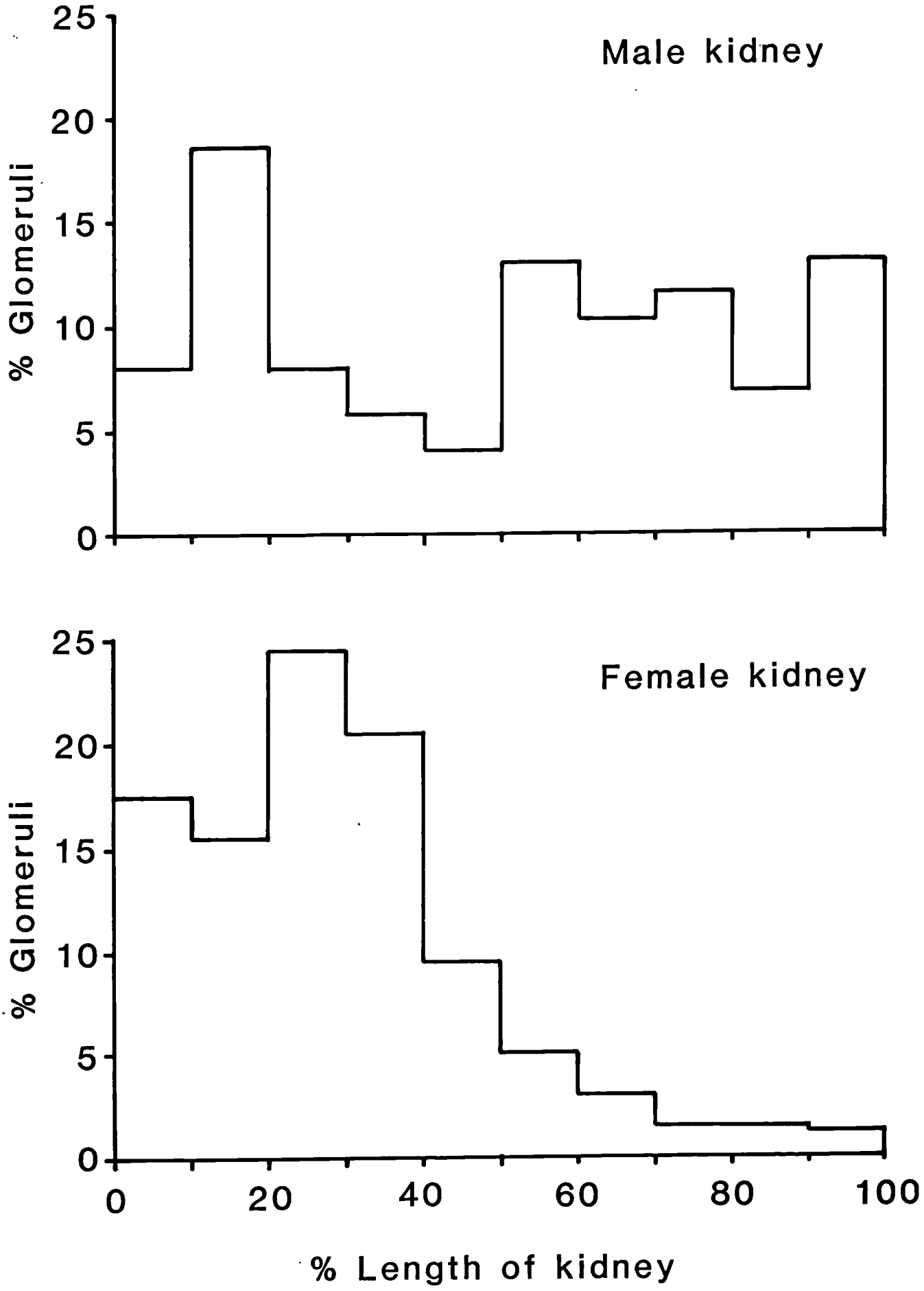

Male kidney
Female kidney
% Glomeruli
% Glomeruli
25
20
15
10
5
0
0
20
40
60
80
100
% Length of kidney

Figure 4

Scanning electron micrograph of a glomerulus.

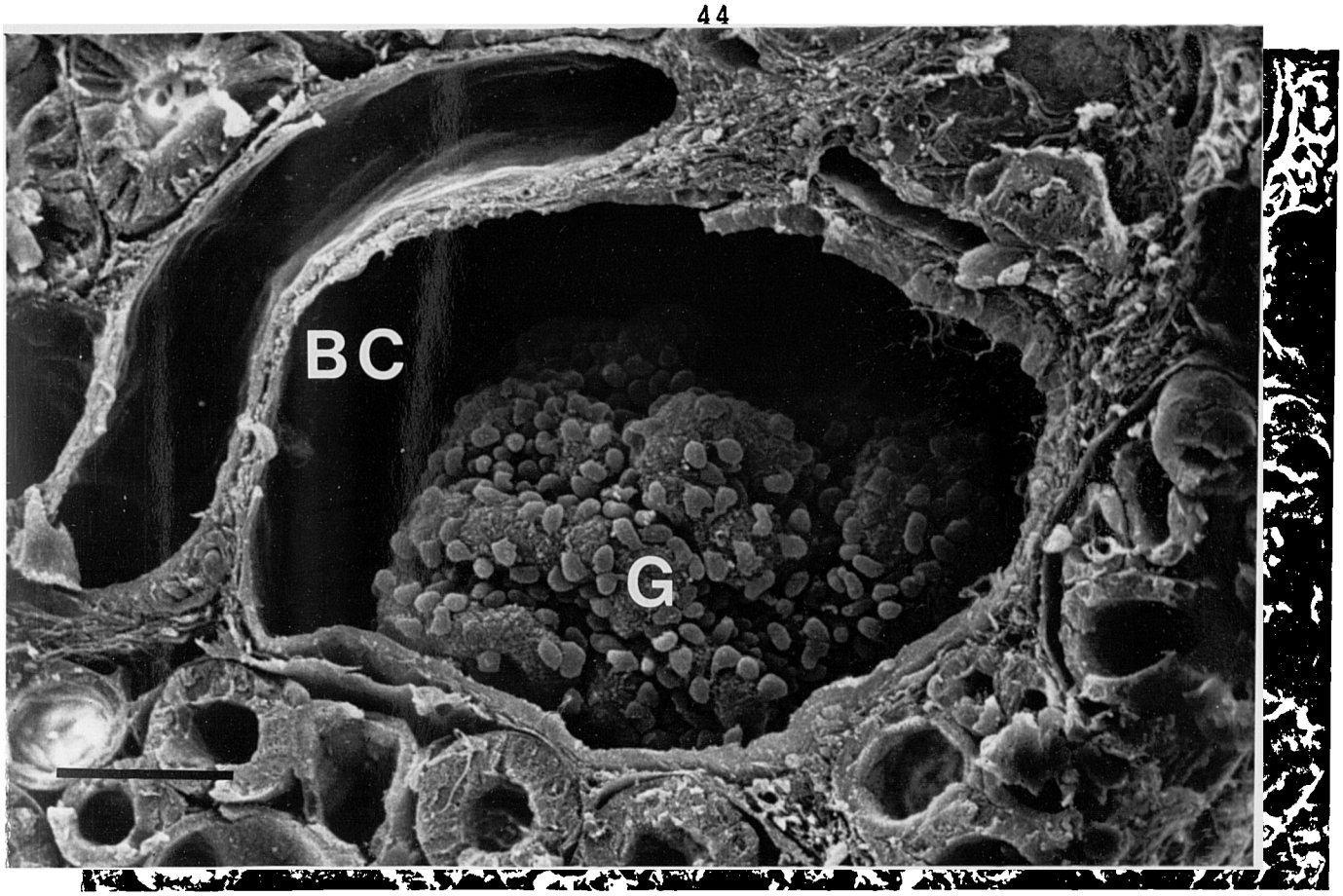
G, glomerulus; BC, Bowman's capsule. Bar: 40 µm.

Figure 5

Scanning electron micrograph of the visceral epithelium of the glomerulus.

Rounded podocytes (P) give rise to primary processes (pp) which branch to form interdigitating pedicels (arrow).

Bar: 4 µm.


BC
G

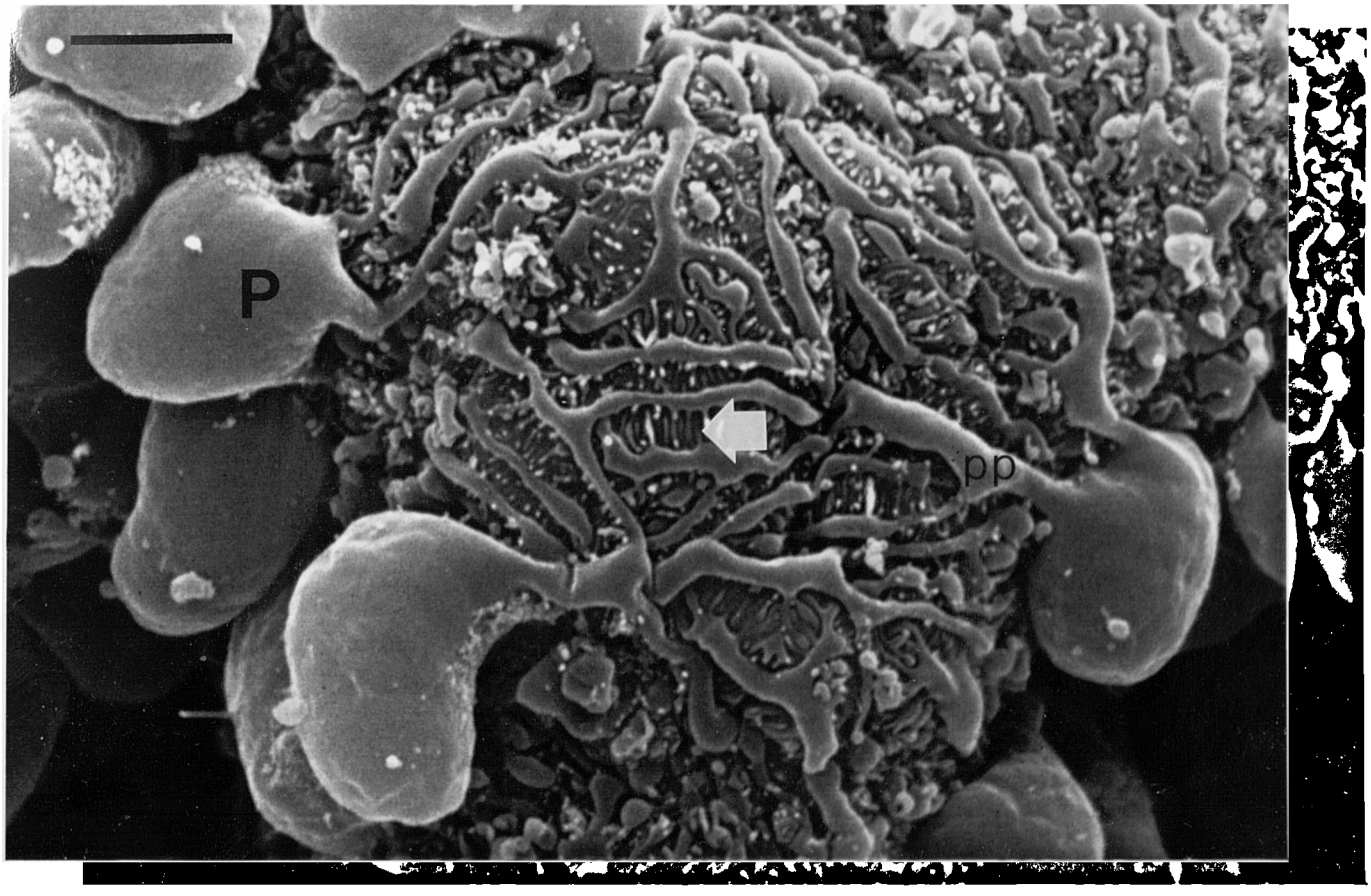

P
pp

Table 4

External diameter of Bowman's capsules, glomeruli and glomerular capillaries of *Scyliorhinus canicula*.

Measurements were taken from scanning electron micrographs of glomeruli and empty Bowman's capsules, sectioned at the vascular pole. Values given are from the kidneys of 6 fishes, and are mean ± standard error. Number of measurements in parentheses.

	Longitudinal Axis (μm)	Transverse Axis (μm)
Bowman's Capsule	164.4 ± 3.8 (40)	129.9 ± 2.9 (40)
Glomerulus	131.2 ± 2.7 (33)	101.7 ± 2.6 (33)
Glomerular Capillary	-	9.0 ± 0.5 (15)

(longest) and transverse (shortest) axes of the ovoid structures. The glomerular hilus, formed by the entry of the afferent arteriole and departure of the efferent arteriole, and the opposing urinary pole, were found to occur at any point of the ellipse. The terms polar and transverse axis which are more commonly referred to, were therefore not used.

The afferent arteriole, on entering the capsule, branched to form the glomerular capillaries. An incomplete layer of irregular-shaped cells, or podocytes, covered the capillaries forming the visceral layer of Bowman's capsule. The podocytes appeared as rounded cells from which primary processes arose (Figure 5). The primary processes branched to form secondary foot processes or pedicels, which interdigitated with the pedicels of neighbouring podocytes.

Morphological variations in the structure of the visceral epithelium of the glomerulus, were commonly seen between glomeruli of the same kidney, and even between adjacent capillary loops of the same glomerulus (Figure 6). Areas of podocytes were often rounded, with the podocyte cell body and epithelial processes, frequently bearing short microvillous projections (Figure 7). Areas of flattened podocytes and processes were also commonly seen (Figure 8). The broadened and flattened primary processes appeared to reduce the area of interdigitating pedicels remaining between the primary processes. Fewer microprojections were present on flattened podocytes and processes.

Figure 6

Scanning electron micrograph of a glomerulus showing morphological variation of the visceral epithelium.
Areas of visceral epithelium show flattening of podocytes and primary processes (arrows), whilst podocytes (P) of adjacent capillary loops are markedly rounded. Bar: 10 μm.

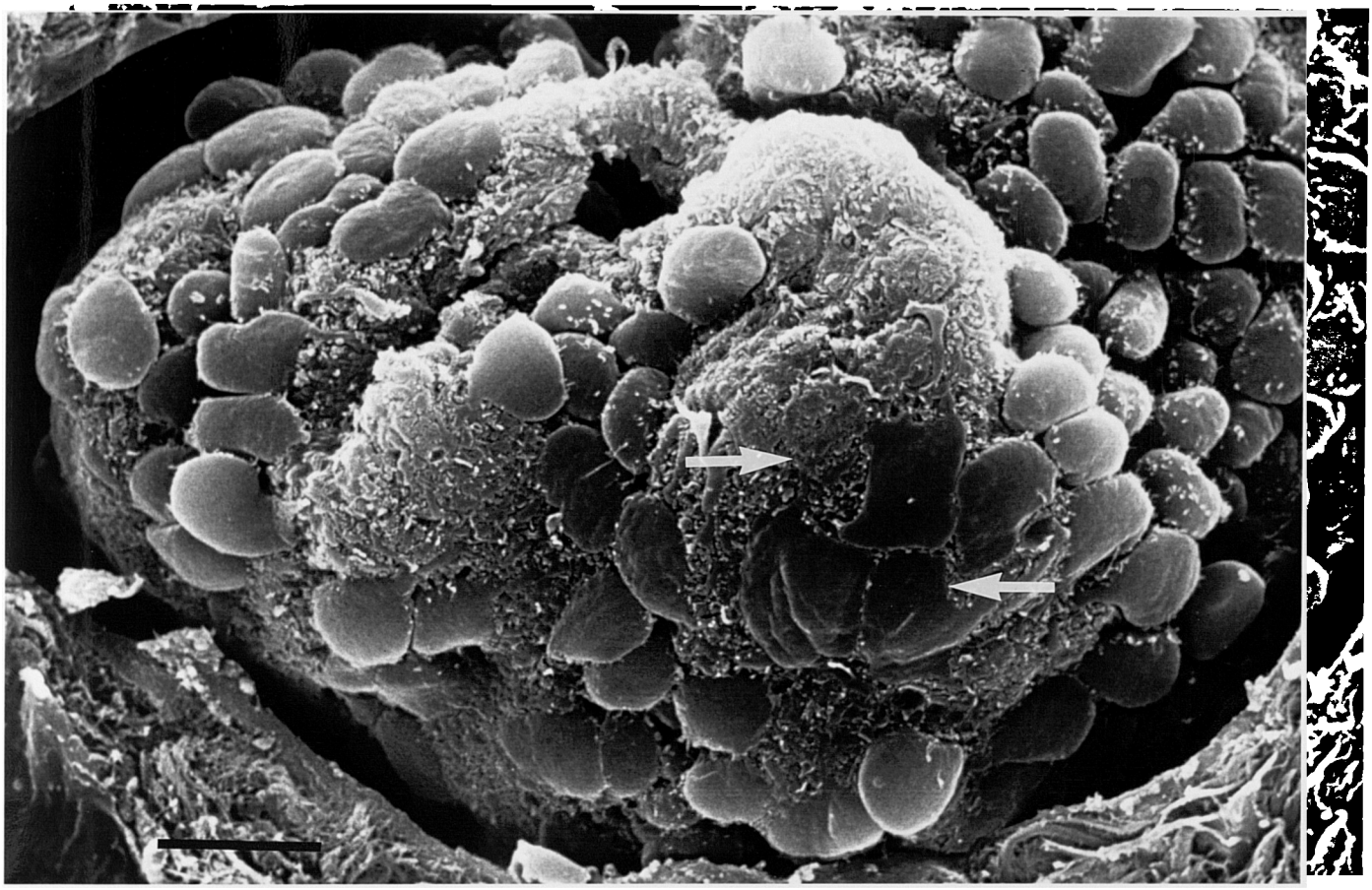
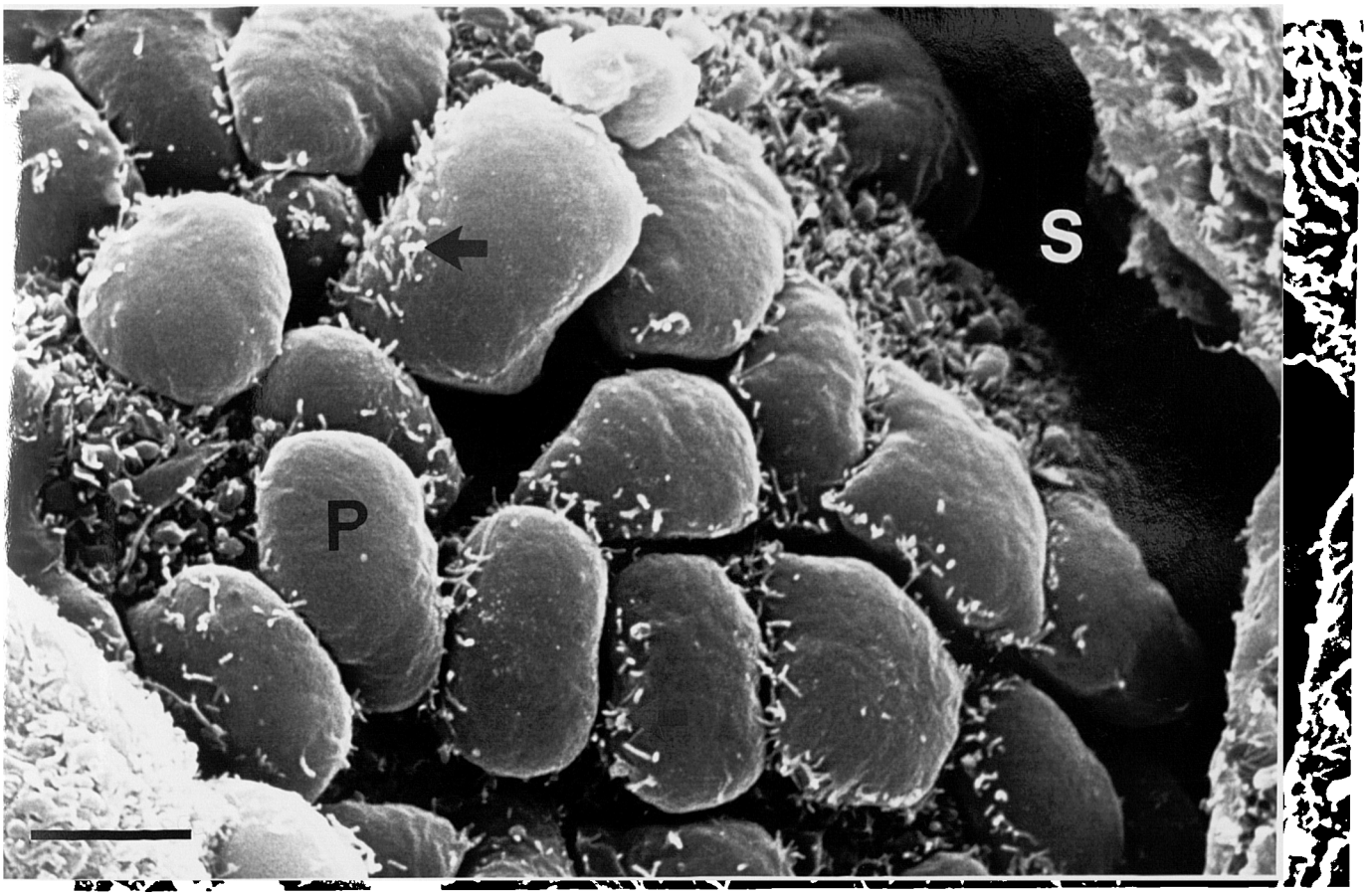


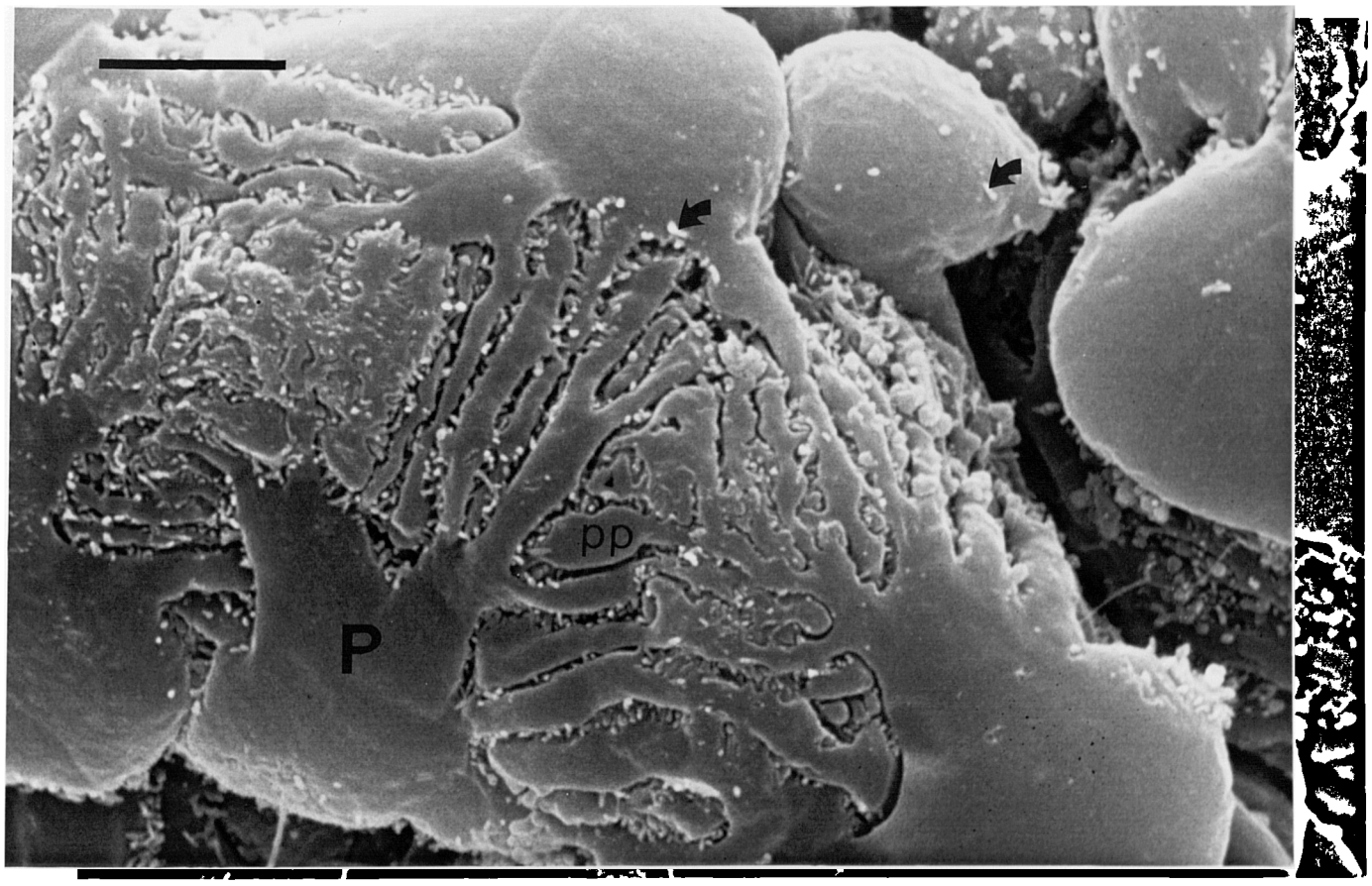
Figure 7

Scanning electron micrograph of visceral epithelium.
Enlargement of Figure 6, showing rounded podocytes (P) and the presence of many microvilli (arrows) projecting into the capsular space (S). Bar: 4 μm.

Figure 8

Scanning electron micrograph showing flattened visceral epithelium.
Podocytes (P) and primary processes (pp) are flattened, and only sparce microvilli (arrows) are present. Bar: 4 μm.


S
P


pp
P

5. The Renal Tubule

(a) Configuration

A combination of histological, ultrastructural and microdissection techniques, together with *in vivo* observations made during micropuncture studies (see section III.B.2(c)), revealed the configuration and characteristics of the various segments of the dogfish nephron. Routine measurement of the overall length of the nephron and individual nephron segments from microdissected nephrons, proved to be impossible due to the length, structural architecture and fragile nature of the partially acid-digested nephron. Figure 9 shows a *camera lucida* drawing of a single nephron, 31.9 mm long, successfully microdissected from a posterior lobe of a female dogfish, and maintained in the configuration of the nephron *in vivo*.

Each nephron was composed of Bowman's capsule, neck segment, proximal and distal segments. Figure 10 shows a scanning electron micrograph of a section through a posterior kidney lobe, illustrating the glomerulus and tubular segments identified in this study. The segments of the nephron were identified by their distinctive cytological characteristics, and differing external and luminal diameters of the tubule (Table 5). The transition from one segment type to another was accompanied by a gradual change in cytological characteristics.

On leaving the Bowman's capsule, the neck segment was seen to course towards the dorsal margin of the kidney, where it turned, and retraced its path back towards the capsule

Figure 9

Camera lucida drawing of a complete nephron of Scyliorhinus canicula.
The nephron, length 31.9 mm, was microdissected from a posterior kidney lobe of a female dogfish.
BC, Bowman's capsule; N, neck segment; PI, proximal segment I; PII, proximal segment II; PIII, proximal segment III; D, distal segment; CD, collecting duct.
Tubules 1-5 form the tubular bundles identified on the dorsal margin of posterior kidney lobes in both sexes.

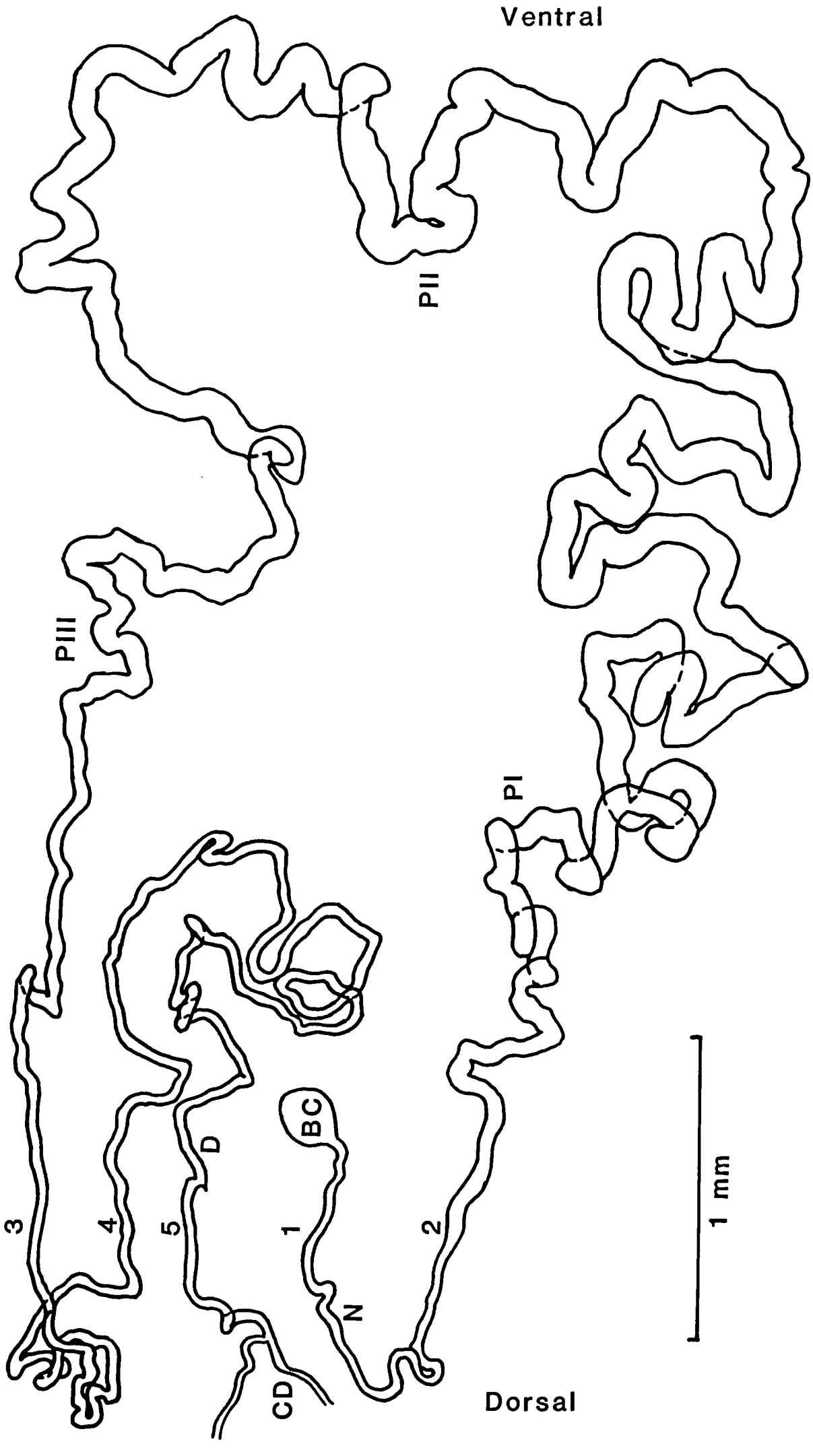

Ventral
PII
PIII
PI
BC
D
CD
N
1
2
3
4
5
1 mm
Dorsal

Figure 10

Scanning electron micrograph of a section through a posterior kidney lobe of *Scyliorhinus canicula*.

The glomerulus (G) is shown lying within Bowman's capsule (C). The origin of the neck segment (N) is shown (arrow). PI, proximal segment I; PII, proximal segment II; PIII, proximal segment III; D, distal segment. Bar: 40 µm.

PIII
PI
PII
C
G
N
D

Table 5

External and luminal diameter of nephron segments of Scyliorhinus canicula.

Measurements were taken from scanning electron micrographs of nephron segments from 6 fishes, displaying characteristic features of that segment. Values given are mean ± standard error. Number of nephron segments in parentheses.

Segment	External Diameter (μm)	Luminal Diameter (μm)
Neck	29.0 ± 0.7 (22)	15.3 ± 0.5 (22)
Proximal I	49.2 ± 0.9 (43)	27.6 ± 0.7 (43)
Proximal II	73.0 ± 1.4 (40)	26.9 ± 1.0 (40)
Proximal III	32.9 ± 0.6 (32)	21.5 ± 0.5 (32)
Distal	22.3 ± 0.5 (37)	12.9 ± 0.3 (37)

(Figure 9). At this point, the tubule widened to lead into the first proximal tubule segment. The early proximal segment was observed to coil repeatedly, tracing a tortuous path towards the ventral margin of the kidney where it widened further to form the large diameter, second proximal segment. This segment was composed of a series of extremely large loops. The narrower, third segment of the proximal tubule originated close to the ventral margin of the kidney. This segment traversed the kidney to the dorsal margin, where it turned and ran back to the centre of the renal mass. Here the distal tubule was initiated, before running past the capsule to the dorsal margin to join the collecting duct system.

The bundles of tubules seen on the dorsal surface of the kidney (Figures 1 and 2) were composed of neck, third proximal and distal tubular segments, tightly bound together by connective tissues (Figures 1 and 9).

(b) Bowman's Capsule

The renal capsule was found to be a large, ovoid structure (Table 4). The parietal layer of the capsule was composed of roughly hexagonally-shaped, squamous epithelial cells, presenting a flat, smooth inner surface (Figure 11). The margins of the cells were lined by short microvilli and a single cilium was located at the centre of each cell.

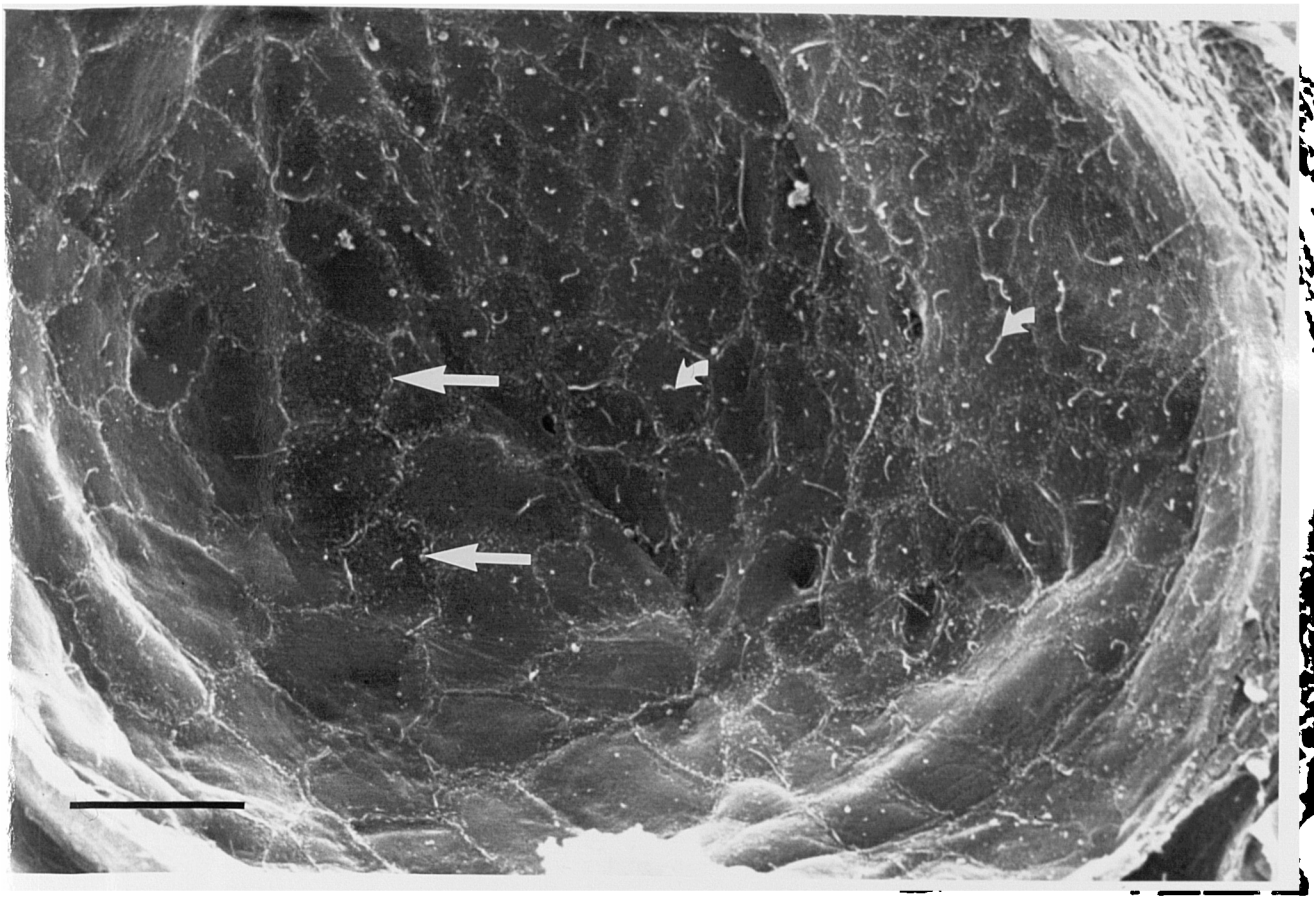
On approaching the urinary pole of the capsule, the parietal squamous epithelium gradually changed into cuboidal epithelium of the neck segment. At this point many groups of long flagella extended from small, specialised epithelial

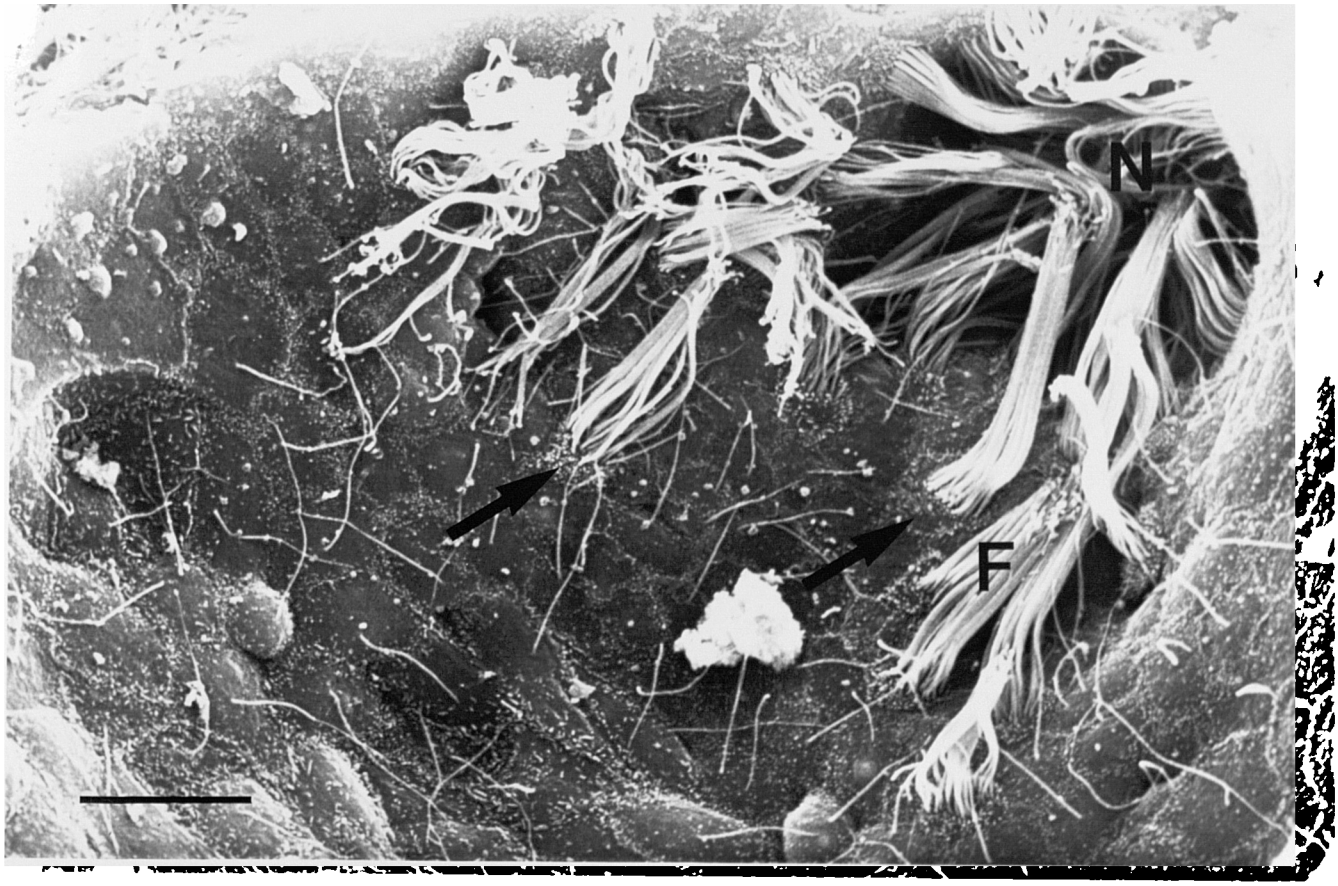
Figure 11

Scanning electron micrograph of Bowman's capsule.
The margins of the hexagonally-shaped cells are lined by microvilli (large arrows), and a single cilium (small arrow) is present at the centre of each cell. Bar: 20 μm.

Figure 12

Scanning electron micrograph of Bowman's capsule showing the origin of the neck segment.
Groups of flagella (F) originate from small specialised epithelial cells (arrows) at the origin of the neck segment (N). The specialised epithelial cells bear increased numbers of microvilli. Bar: 20 μm.




N
F

cells (Figure 12). Increased numbers of microvilli were present on the luminal surface of the specialised cells. These flagella-bearing cells increased in frequency towards the origin of the neck segment of the nephron.

(c) Tubular Segments

(i) Neck Segment

The funnel-shaped neck (Figures 12 and 13) was observed to gradually narrow to form a small diameter tubule (Table 5). The tubule walls were composed of cuboidal epithelial cells with large, basally situated nuclei, and few microvilli projecting from the luminal surface (Figure 13). Many flagella-bearing cells, similar to those of the capsule, continued to be present in the early neck segment. The tufts of long flagella, extending into the tubular lumen in the direction of flow of tubular fluid, appeared to almost fill the lumen of the tubule (Figure 12).

(ii) Proximal Segment I

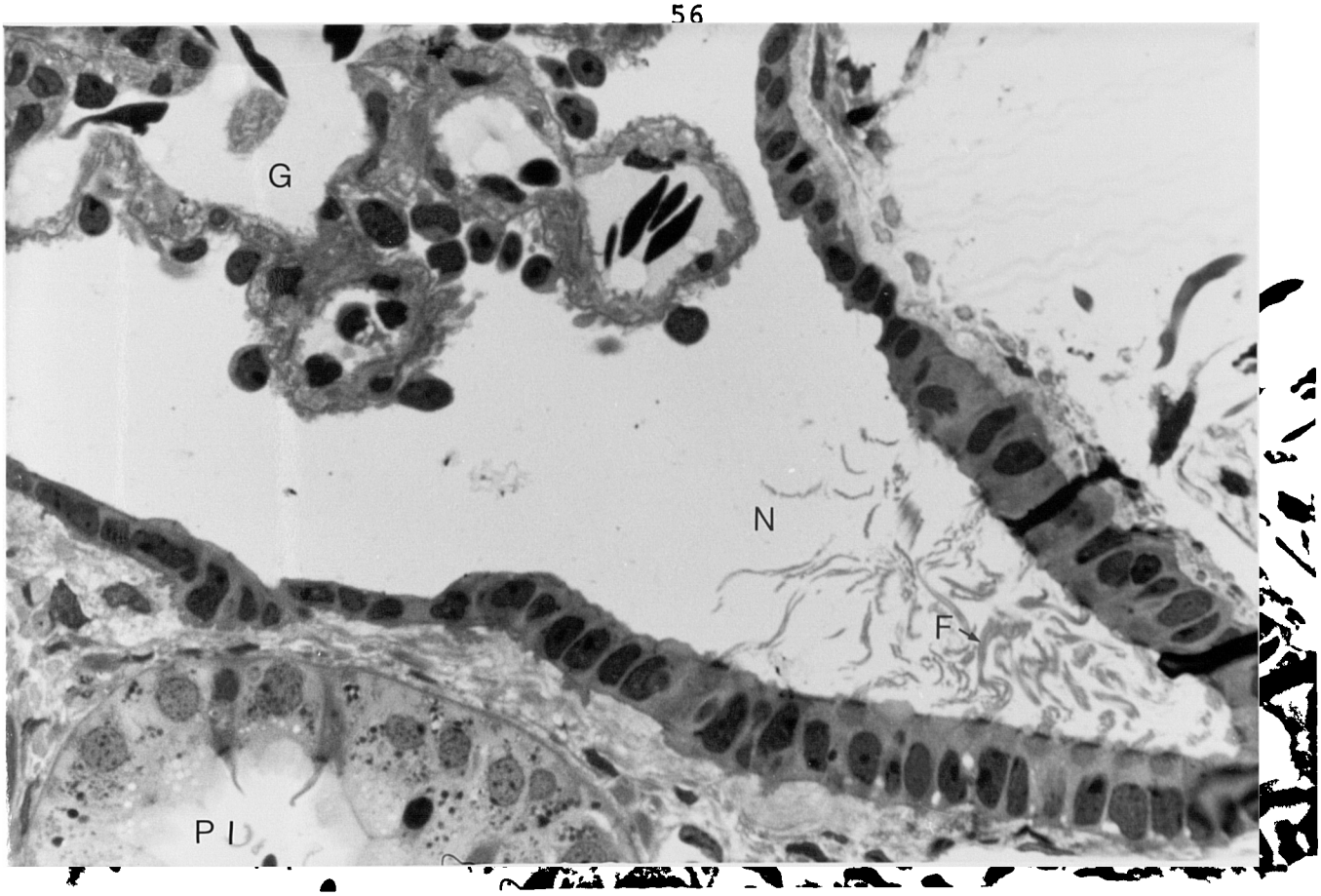
Figures 14 and 15 show photomicrographs of proximal and distal tubular segments identified in this study. Figure 16 shows a scanning electron micrograph of a transverse section of an early proximal segment. Proximal segment I was characteristically wider in diameter than the neck segment, with a large lumen (Table 5). In vivo, the tubule appeared white in colour, and could be identified by its many loops and coils. The tubular wall was found to be composed of columnar

Figure 13

Light micrograph of the glomerulus and neck segment. The glomerular capillaries (G) lie within Bowman's capsule. The origin of the neck segment (N) is funnel-shaped, and many groups of flagella (F) are present. The squamous epithelium of Bowman's capsule is seen to change to cuboidal epithelium at the mouth of the neck segment. PI, proximal segment I. x 400.

Figure 14

Light micrograph of the vascular pole of the glomerulus. G, glomerulus; A, intra-renal artery; a, arteriole; PI, proximal segment I; PII, proximal segment II; D, distal segment. x 400.


G
N
F
P I

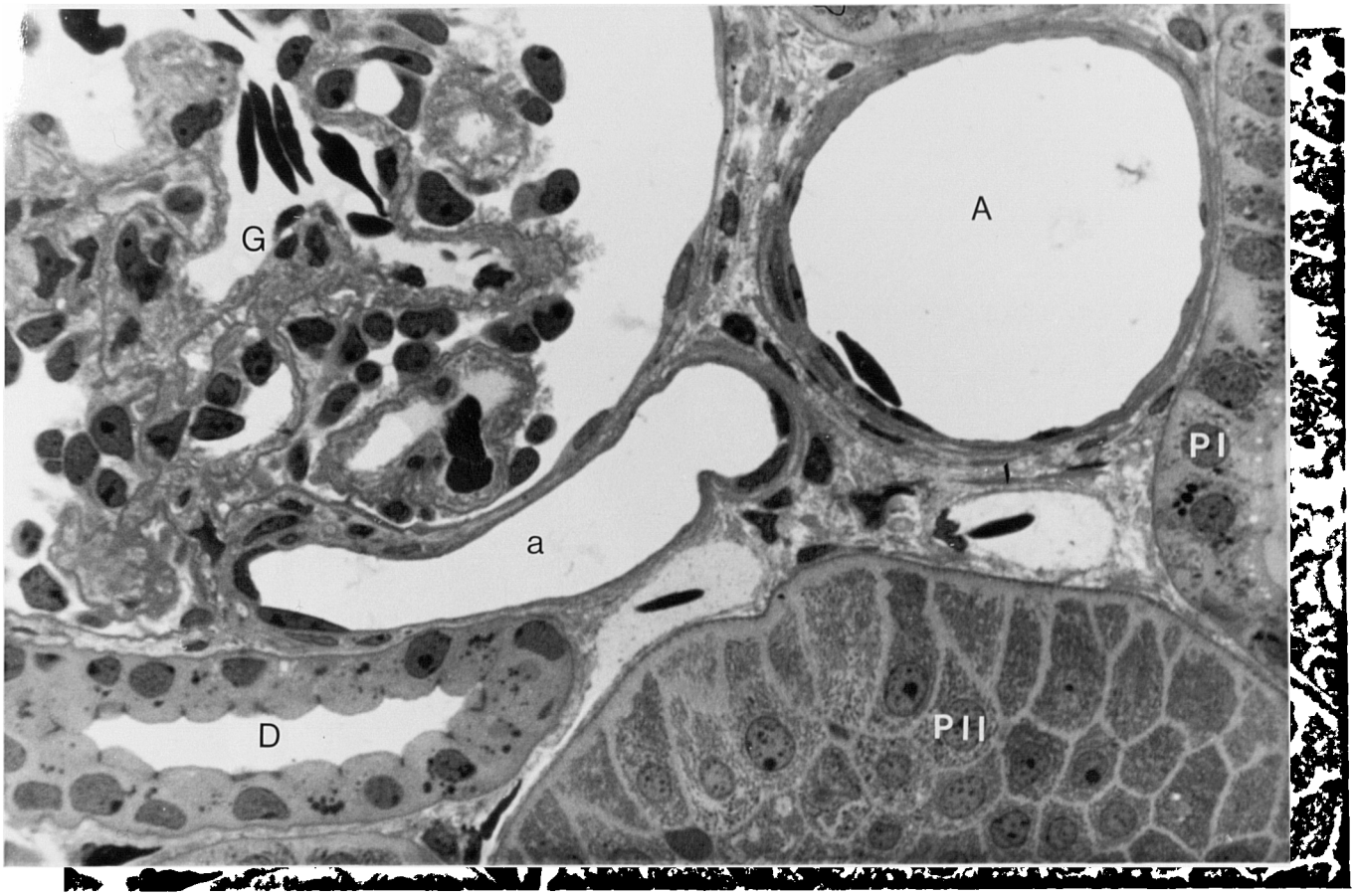
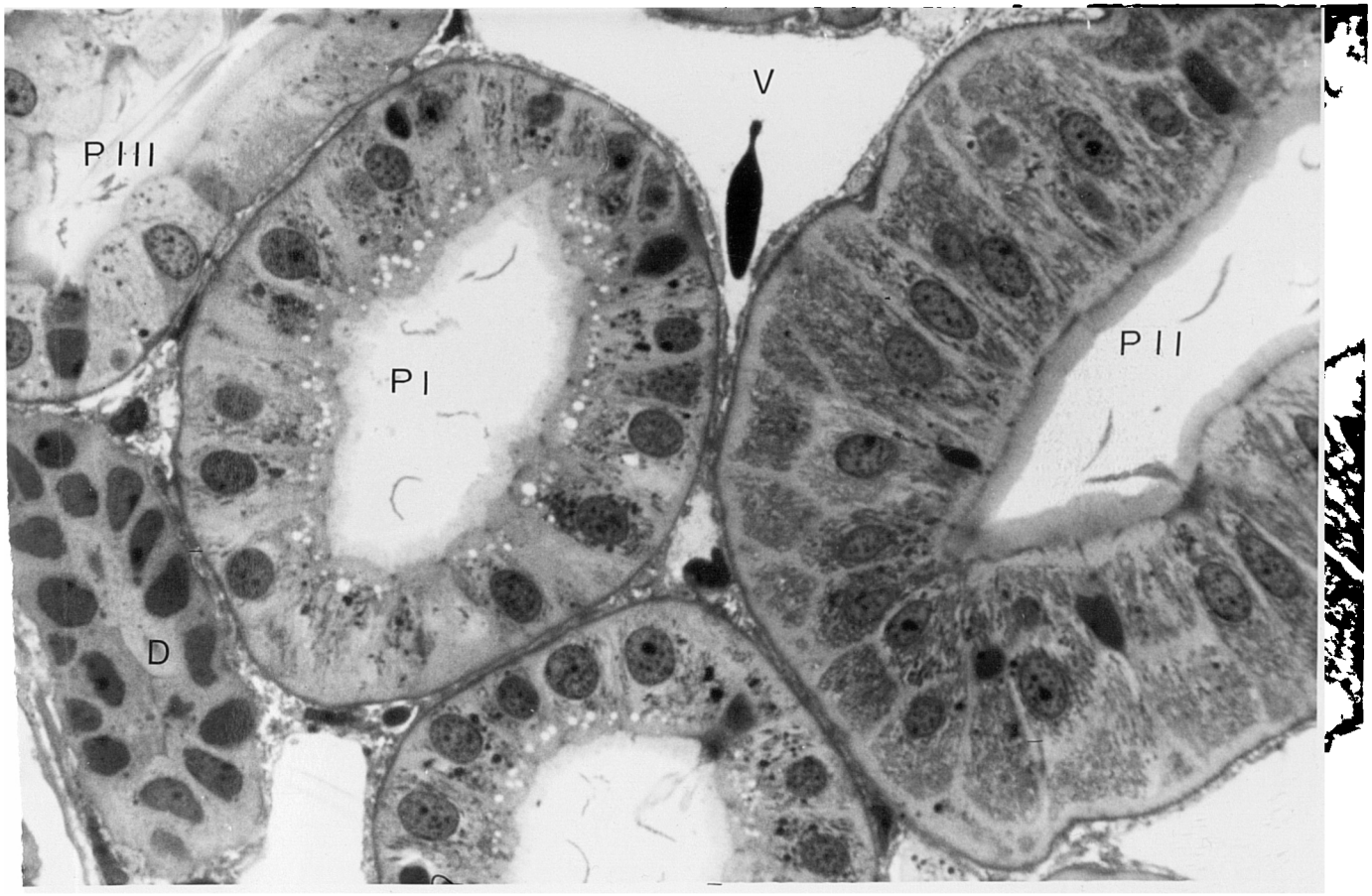

G
A
PI
a
D
PII

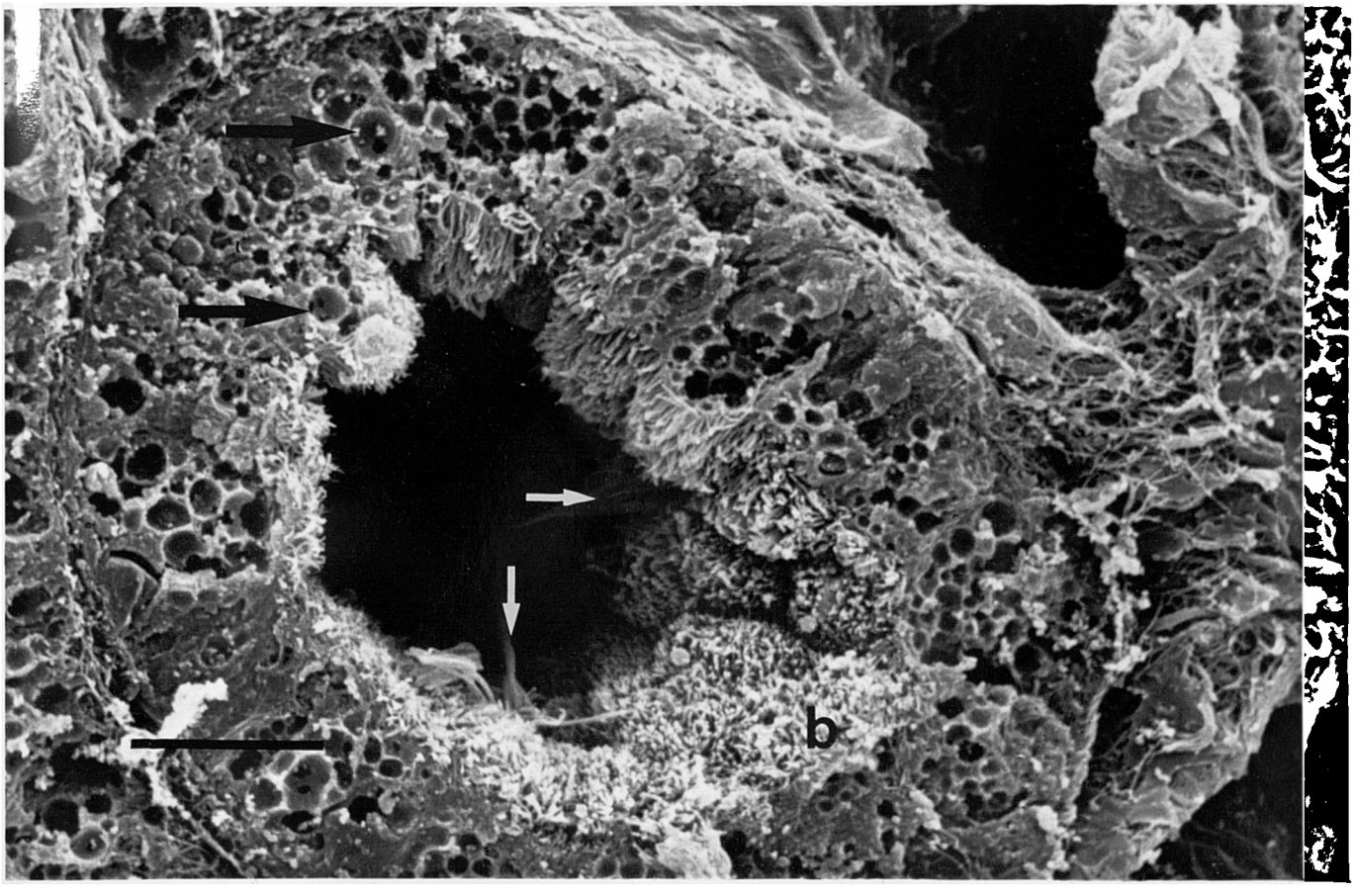
Figure 15

Light micrograph showing proximal and distal segments.
PI, proximal segment I; PII, proximal segment II; PIII, proximal segment III; D, distal segment; V, peritubular capillaries. x 400.

Figure 16

Scanning electron micrograph of proximal segment I.
The columnar epithelial cells contain many large vesicles (large arrows). The luminal surface is covered by a dense brush border (b) of microvilli, and groups of long flagella (small arrows) are present. Bar: 10 μm.


V
P III
P I
P II
D


b

epithelium, containing many large vesicular-structures within the cytoplasm, most frequently occurring in the apical region of the cells (Figures 15 and 16). Nuclei were situated in close proximity to the base of the cell (Figure 15). The luminal surface of the cells was markedly rounded and covered by a dense brush border of short microvilli. Occasional groups of long flagella were present, originating from small cells similar to those of the neck segment (Figure 16).

(iii) Proximal Segment II

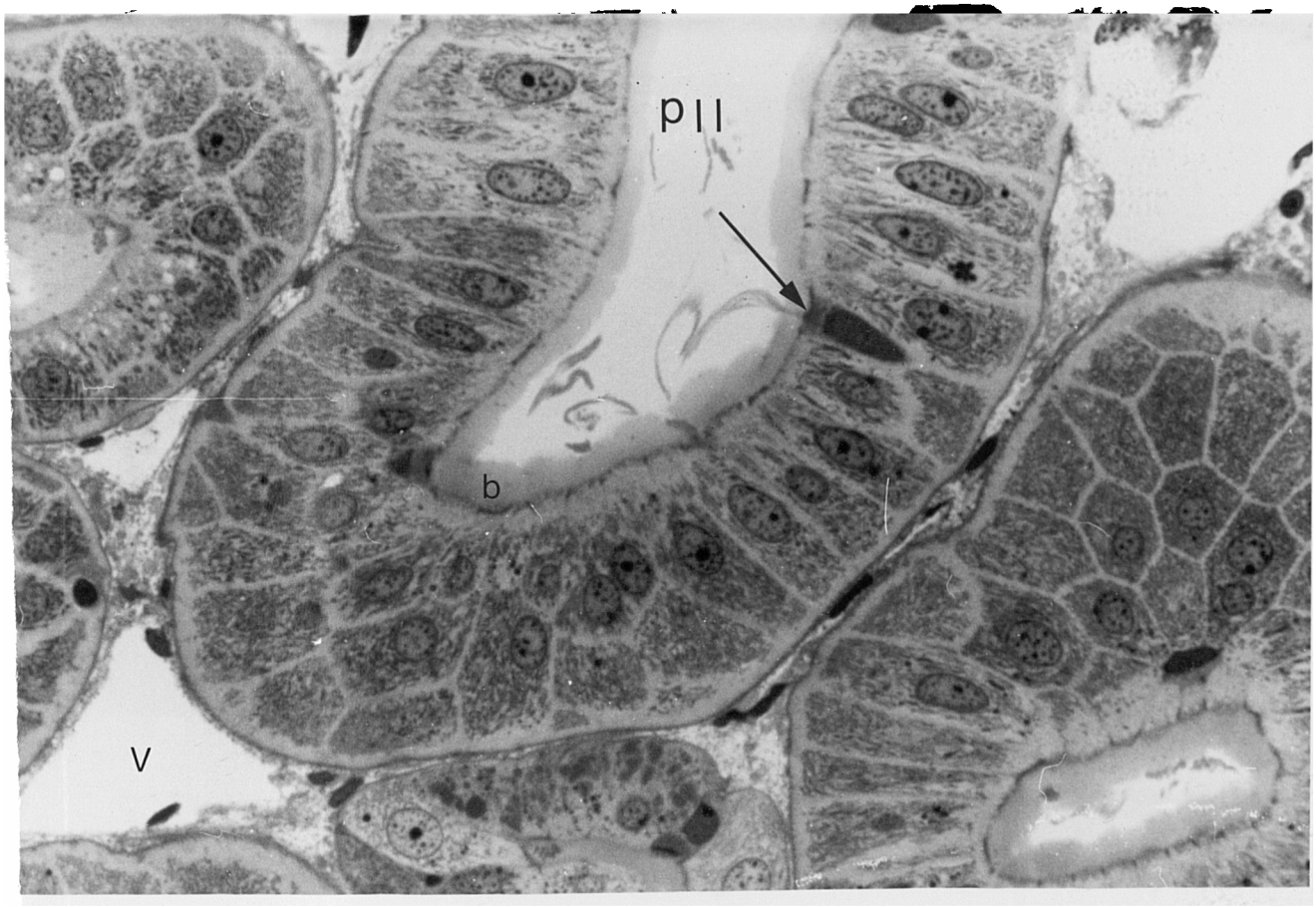
The second proximal segment of the renal tubule was characterised by very tall columnar epithelial cells, with centrally positioned nuclei (Figures 17, 18 and 19), and large external diameter (Table 5). The tubule wall appeared yellow in colouration *in vivo* (section III.B.2(c)), with a distinct, shiny lumen. The luminal surface of the cells was flat, and covered by a dense brush border of short microvilli (Figures 17, 18 and 19). Groups of long flagella periodically arose from specialised, darker epithelial cells (Figure 17), and extended into the lumen. The cytoplasm of the cells appeared dense with many mitochondria, but no vesicles present. Wide intercellular channels were frequently observed, with large spaces close to the basement membrane, and tight junctions at the apex of the cell (Figure 19).

Figure 17

Light micrograph of proximal segment II.
Specialised epithelial cells (arrow) give rise to flagella. A dense brush border (b) of microvilli covers the luminal surface of proximal segment II (PII). V, peritubular capillaries. x 400.

Figure 18

Scanning electron micrograph of proximal segment II.
A dense brush border (b) is seen. Groups of flagella (f) arise from specialised epithelial cells (arrows). Bar: 20 µm.


pII
b
V

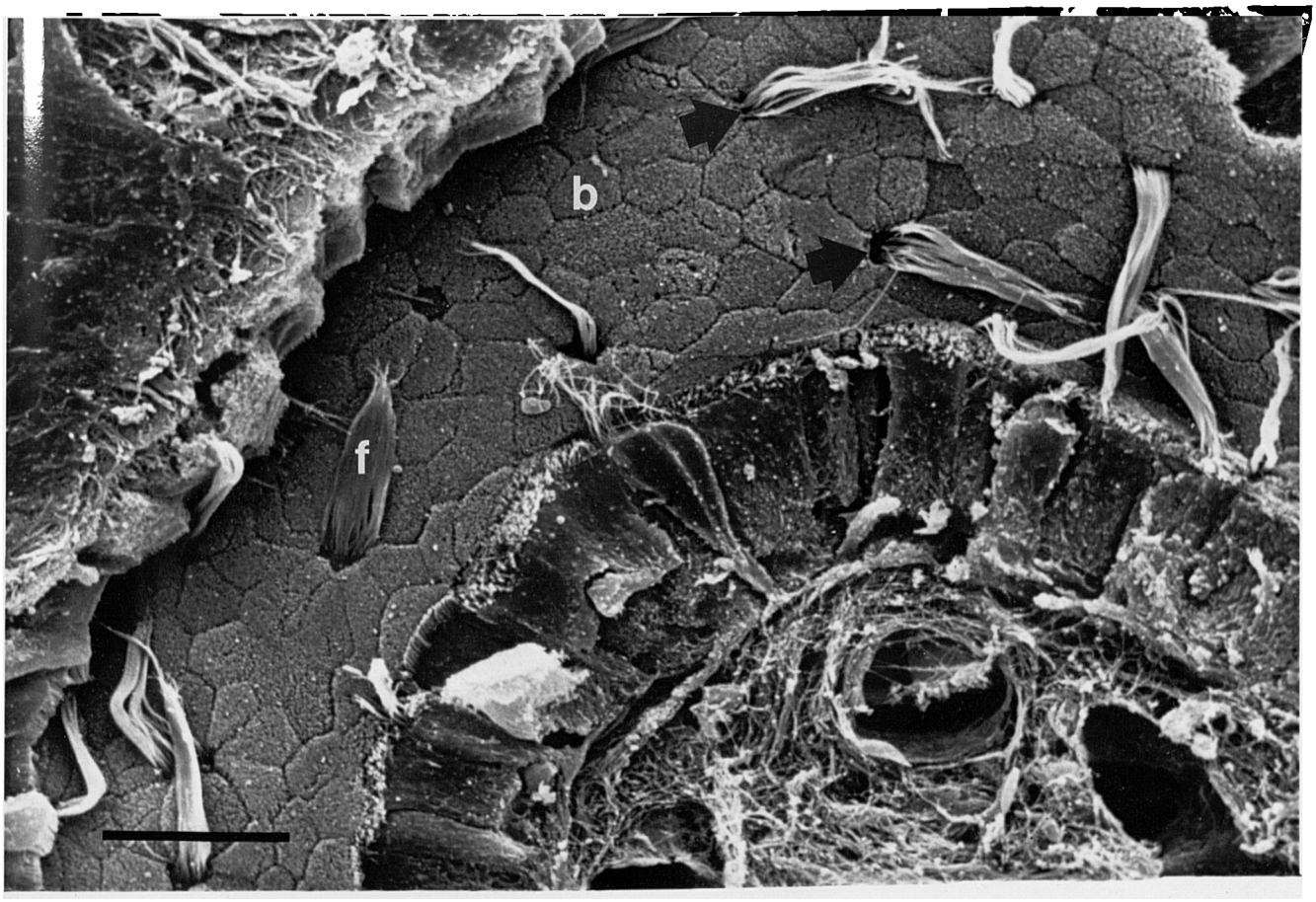
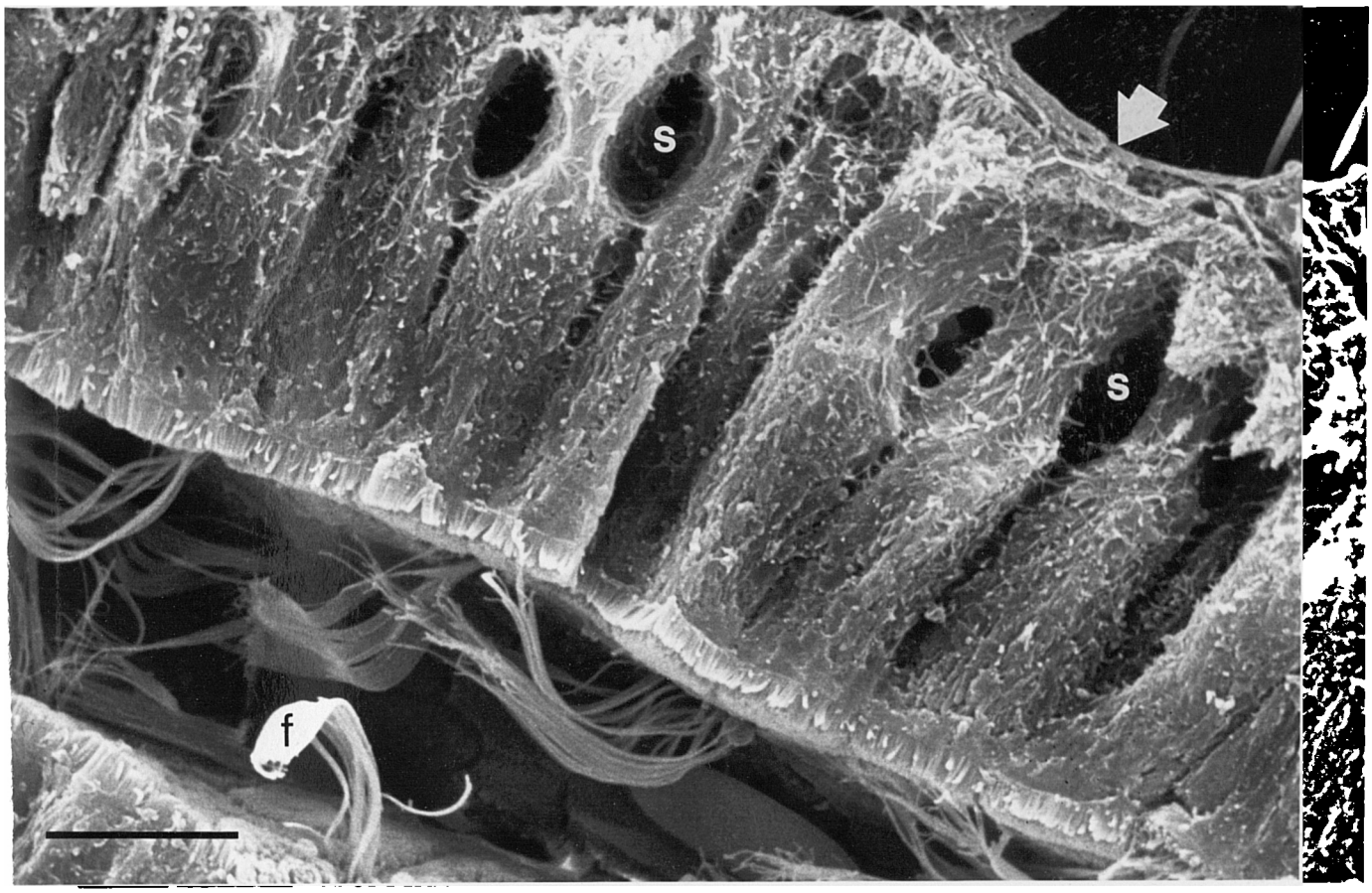

b
f

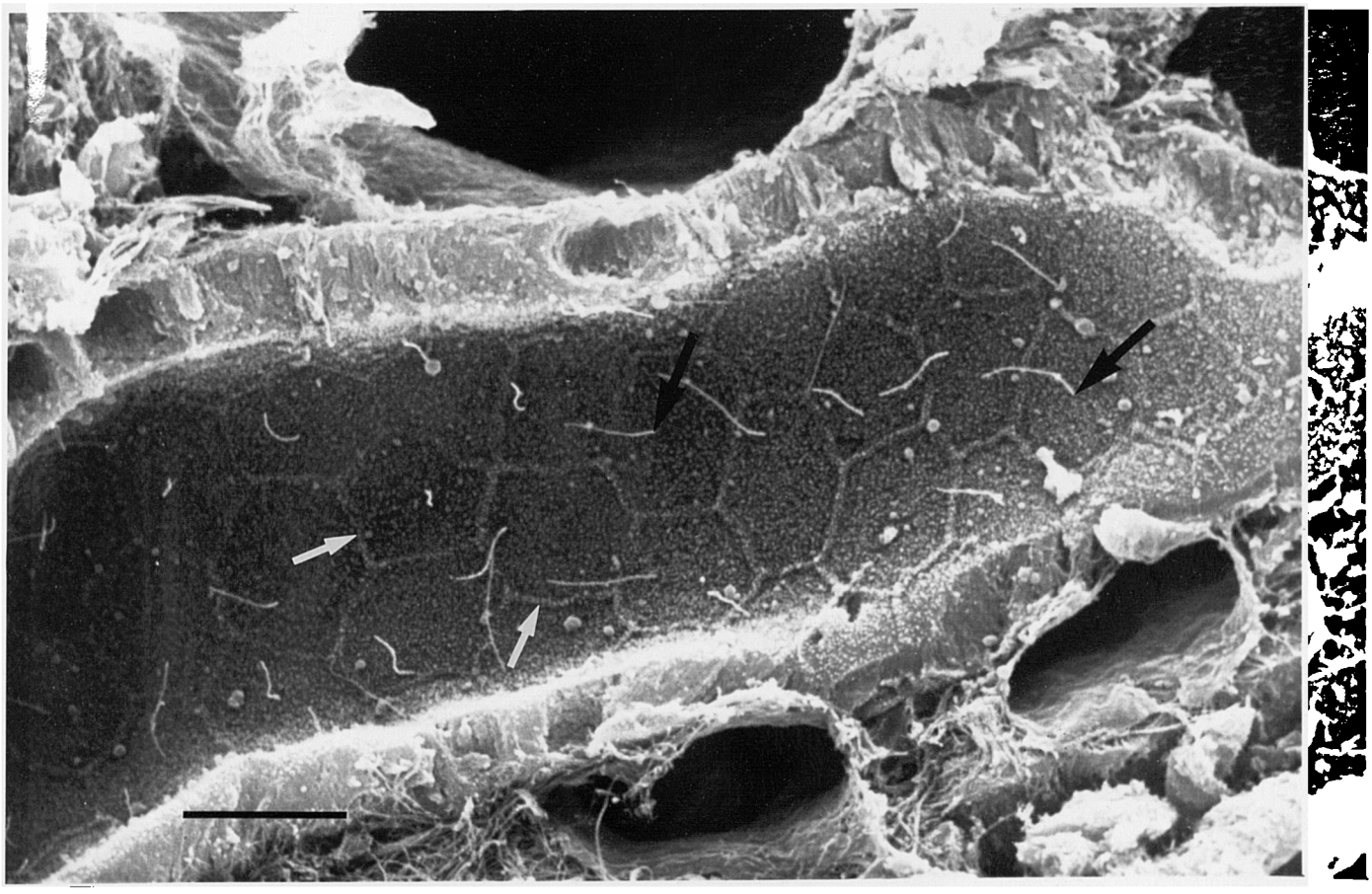
Figure 19

Scanning electron micrograph of the epithelium of proximal segment II.
Intercellular channels and spaces (s) in close proximity to the basement membrane (arrow) are seen between the tall columnar epithelial cells of proximal segment II. Many groups of long flagella (f) are present. Bar: 10 µm.

Figure 20

Scanning electron micrograph of proximal segment III.
The margins of the hexagonally-shaped cells are lined by short microvilli (small arrows), and a single cilium is present at the centre of each cell (large arrow). Bar: 10 µm.


S
S
f



(iv) Proximal Segment III

The third proximal segment was found to be a relatively short segment composed of a small white tubule *in vivo* (section III.B.2(c)), with a relatively large lumen (Table 5). The narrow tubular walls were composed of hexagonally-shaped, cuboidal epithelial cells, presenting a slightly rounded luminal surface (Figures 15 and 20). The cells bore a sparse brush border of short microvilli, and a single central cilium was present on each cell (Figure 20). No intercellular channels or cytoplasmic vesicles were seen in this tubular segment.

(v) Distal Segment

The distal segment was structurally similar to the third proximal segment, differing only in tubular dimensions, position and certain definitive cytological features. It was the smallest tubular segment of the nephron (Table 5; Figure 10). The walls were composed of low cuboidal epithelium with only an occasional microvillous projection. Nuclei were situated at the base of the cells (Figures 14 and 15). The luminal surface of the cells was markedly rounded, with no cilia or flagella present.

6. Renal Vasculature

(a) Renal Arteries

The segmental renal arteries, on entering the kidneys, branched to form several (3-6) smaller intra-renal arteries which in the posterior lobes, coursed laterally over the dorsal aspect of the kidney. Figures 21 and 22 show the intra-renal arteries as they traverse the kidney. Band-like constrictions, thought to be formed by contracted smooth muscle sphincters, were identified on the intra-renal arteries (Figure 21).

Luminal surface features of the intra-renal arteries are clearly visible on the corrosion casts (Figures 21, 22, 23, 24 and 25). Ridges created by the margins of endothelial cells, and nuclear indentations were identified.

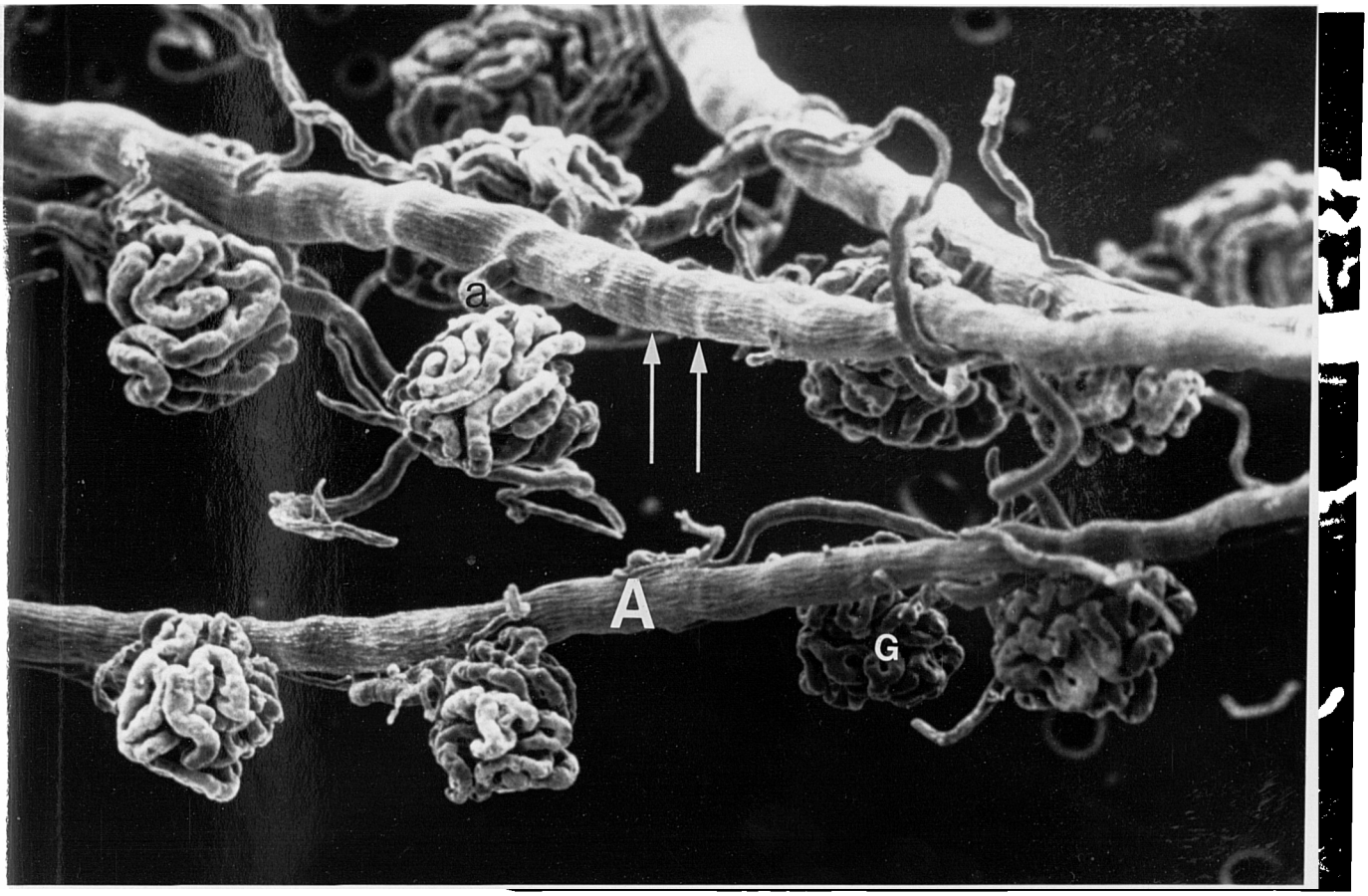
Furrow-like depressions in the parent artery, at the origin of afferent arterioles, were observed in an estimated 50% of all casts (Figures 23, 24, 25, and 27). These furrows took the form of elongated depressions in the casting material, which corresponded to a ridge in the wall of the intra-renal artery. The afferent arteriole arose from within this depression, so that *in vivo*, the orifice must project into the lumen of the parent artery. These depressions appeared to be formed by the presence of intra-arterial cushions.

Figure 21

Scanning electron micrograph of a corrosion cast of intra-renal arteries of Scyliorhinus canicula.
The intra-renal arteries (A) give rise to afferent arterioles (a) and glomeruli (G). Band-like constrictions (arrows) are present on the intra-renal arteries. Bar: 200 μm.

Figure 22

Scanning electron micrograph of a corrosion cast of an intra-renal artery and glomeruli.
Luminal surface features are visible on the intra-renal artery (A). A constriction (arrow) is present at the origin of the afferent arteriole (a). G, glomerulus. Bar: 100 μm.


a
A
G

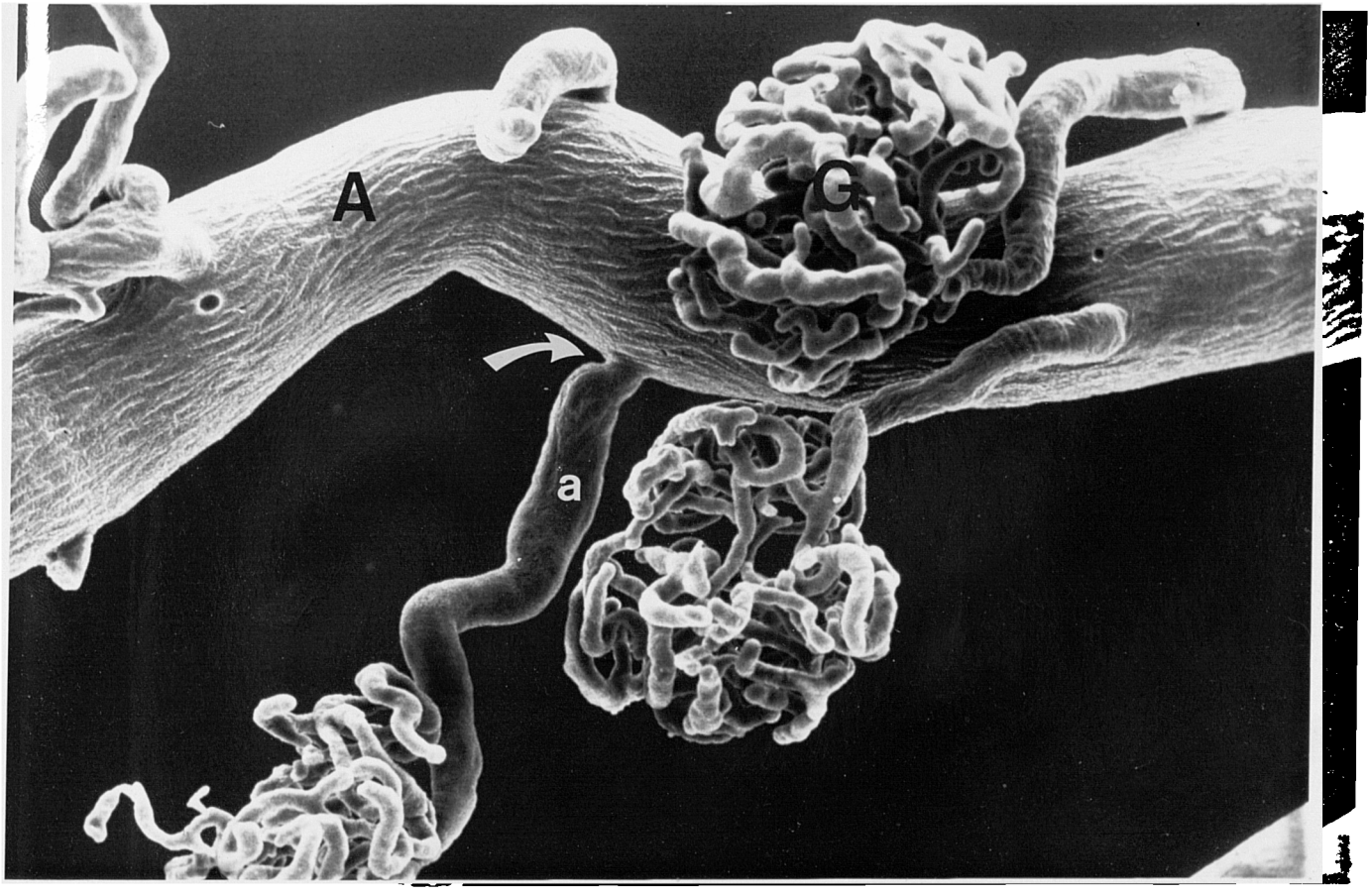
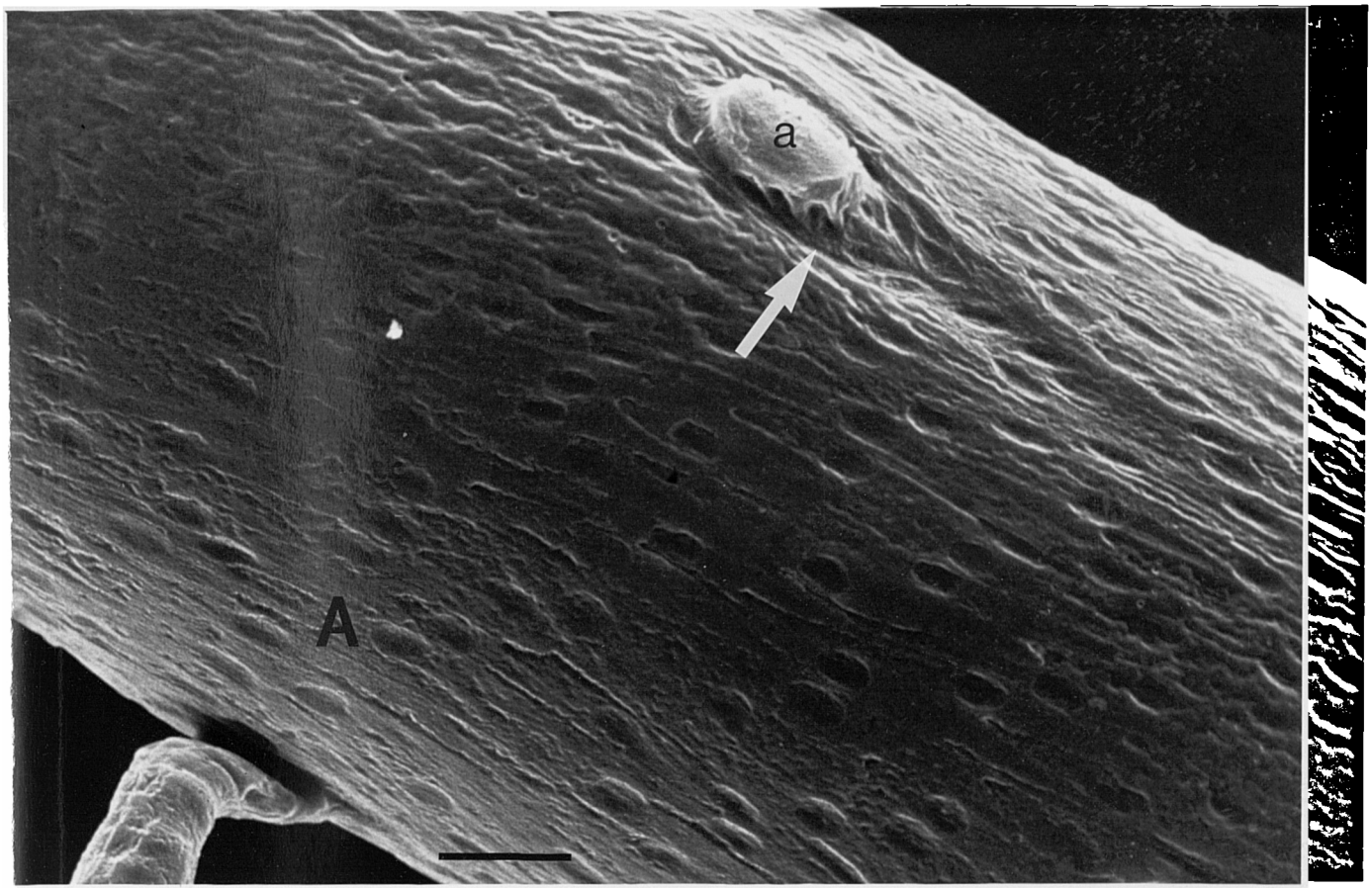

A
G
a

Figure 23

Scanning electron micrograph of a corrosion cast of an intra-renal artery and intra-arterial cushion.
The furrow-shaped depression (arrow) in the intra-renal artery (A), is formed by an intra-arterial cushion at the origin of the afferent arteriole (a). Bar: 40 μm.

Figure 24

Scanning electron micrograph of a corrosion cast of an intra-arterial cushion.
The furrow-shaped depression (arrow) is formed by the intra-arterial cushion. A, intra-renal artery; a, afferent arteriole; G, glomerular capillaries. Bar: 20 μm.


a
A

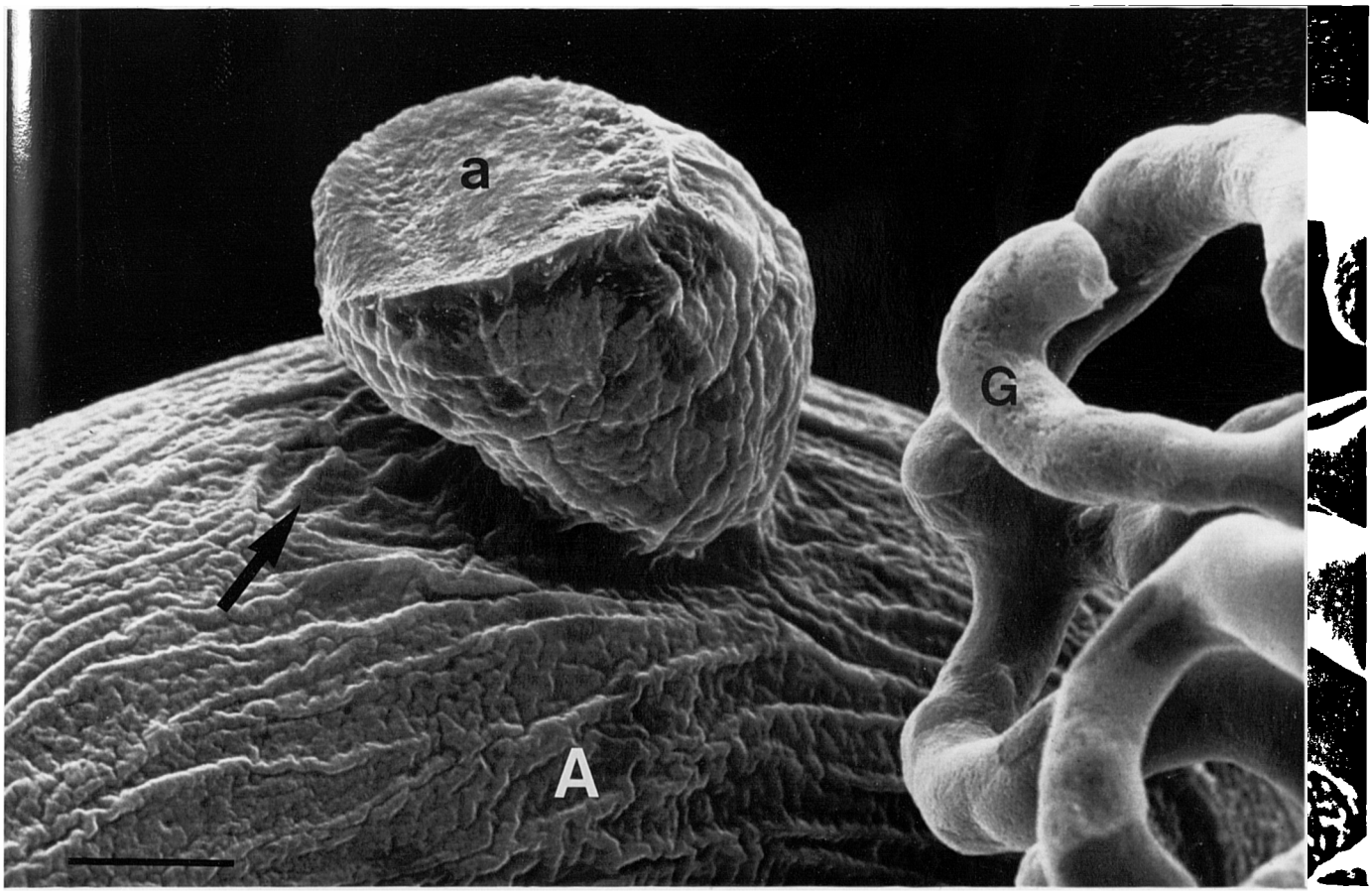

a
G
A

Figure 25

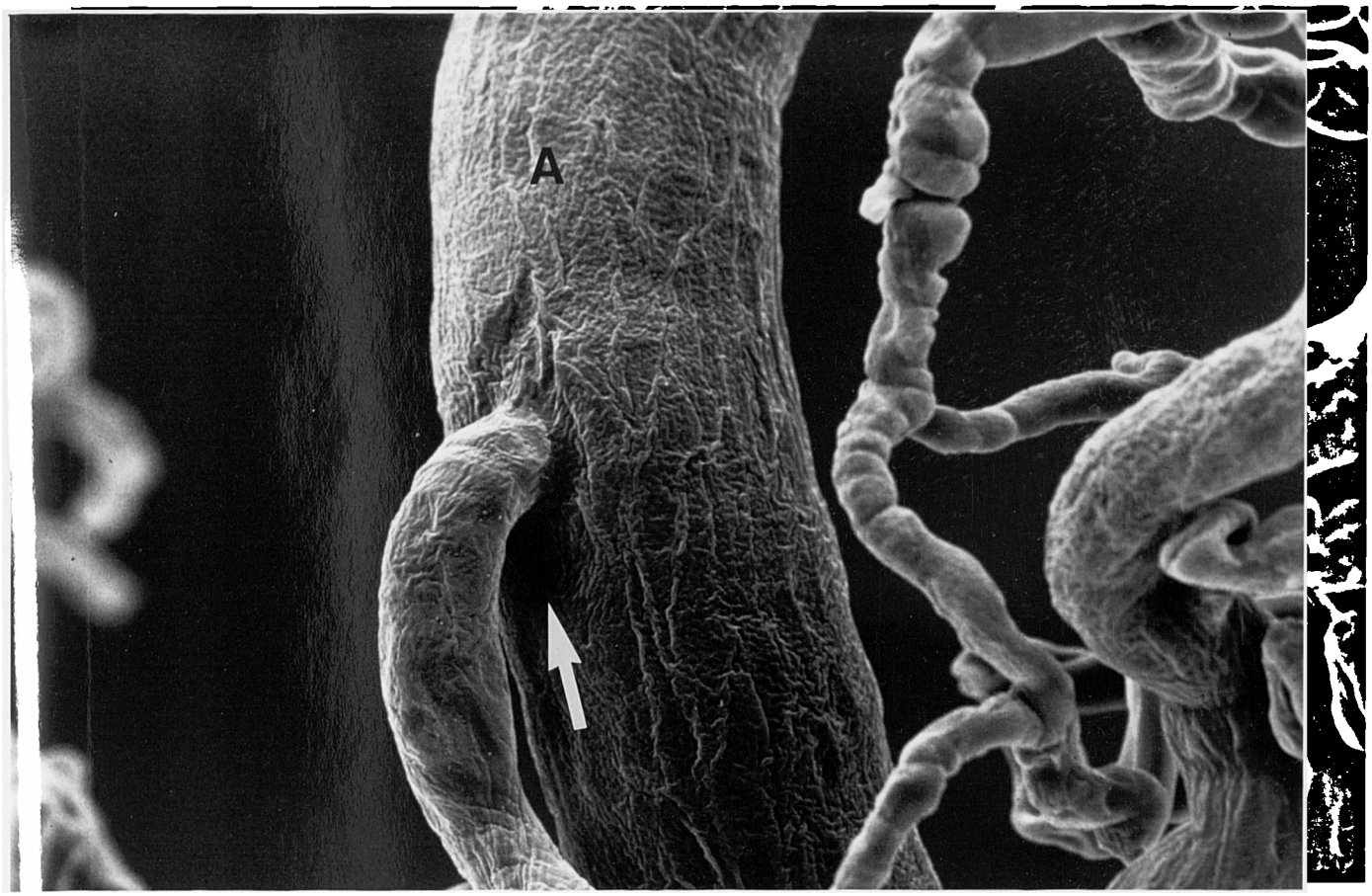
Scanning electron micrograph of a corrosion cast showing the origin of the afferent arteriole from within an intra-arterial cushion.

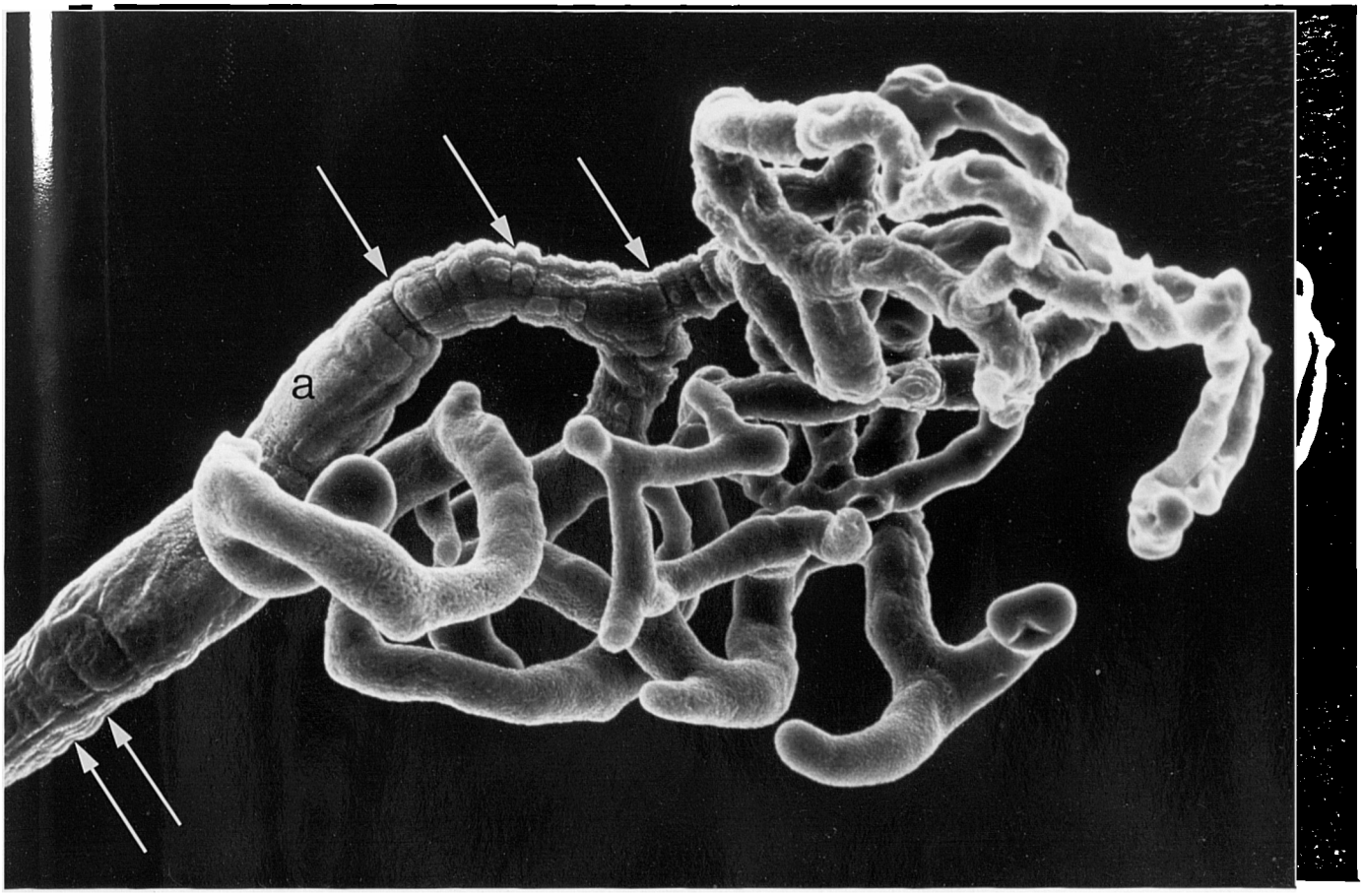
Arrow, furrow formed by intra-arterial cushion; A, intra-renal artery. Bar: 40 μm.

Figure 26

Scanning electron micrograph of a corrosion cast showing a partially filled glomerulus.

Ring-like constrictions (arrows) are present along the length of the afferent arteriole (a), and at the glomerular hilus. The branching of glomerular capillaries to form lobules is seen. Bar: 40 μm.


A


a

(b) Afferent Arteriole

Each intra-renal artery gave rise to numerous short afferent arterioles, each suppling a single glomerulus (Figures 21, and 22). Ring-like constrictions were observed along the length of the afferent arteriole in almost all specimens viewed (Figures 22, 26, 27, 29, and 30). Tight constrictions were seen at the origin of the afferent arteriole (Figure 22), and also at the glomerular hilus (Figure 26).

The afferent arterioles predominantly took the form of a single, short unbranching vessel. In an estimated 25% of all casts viewed, a single, small vessel was seen to branch from the afferent arteriole, and pass alongside the glomerulus (Figures 27, 28, 29 and 30). This glomerular bypass vessel was frequently observed to branch profusely beyond the glomerulus, and join the peritubular capillary network (Figure 30).

There was no evidence indicating the presence of double afferent arterioles, or precocious branching of the afferent arteriole prior to entry into the glomerulus.

(c) Glomerular Capillaries

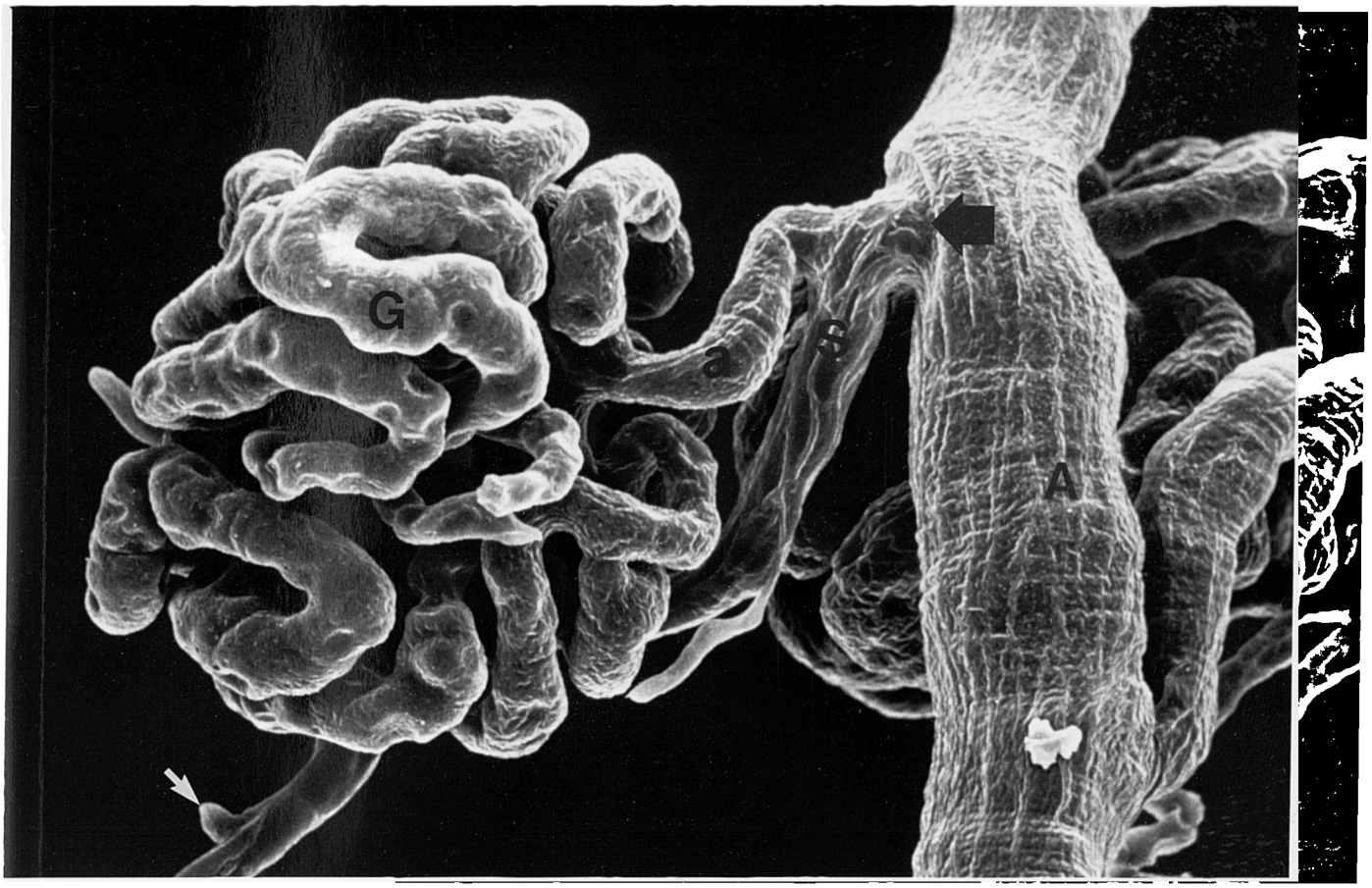
The glomerular capillaries formed a large, ovoid tuft of vessels (Figures 21, 22, 27, 28, 29, and 30). An incompletely-filled cast of the glomerular capillaries illustrates the branching network (Figure 26). The afferent arteriole was found to branch initially to form two to four vessels, leading to lobules of branching capillaries.

Figure 27

Scanning electron micrograph of a corrosion cast of an afferent arteriole and glomerular bypass vessel.
The glomerular bypass vessel (S) arises from the afferent arteriole (a) and passes adjacent to the glomerulus (G). The vessel branches (small arrow) into the peritubular capillary network beyond the glomerulus. A, intra-renal artery; large arrow, intra-arterial cushion. Bar: 40 μm.

Figure 28

Scanning electron micrograph of a corrosion cast of a glomerular bypass vessel.
The glomerular bypass vessel (S) arises from the afferent arteriole (a) and travels beyond the glomerulus (large arrow). Constrictions (small arrows) are present on the afferent arteriole in advance of, and following branching of the shunt vessel. Bar: 40 μm.


G
a
A

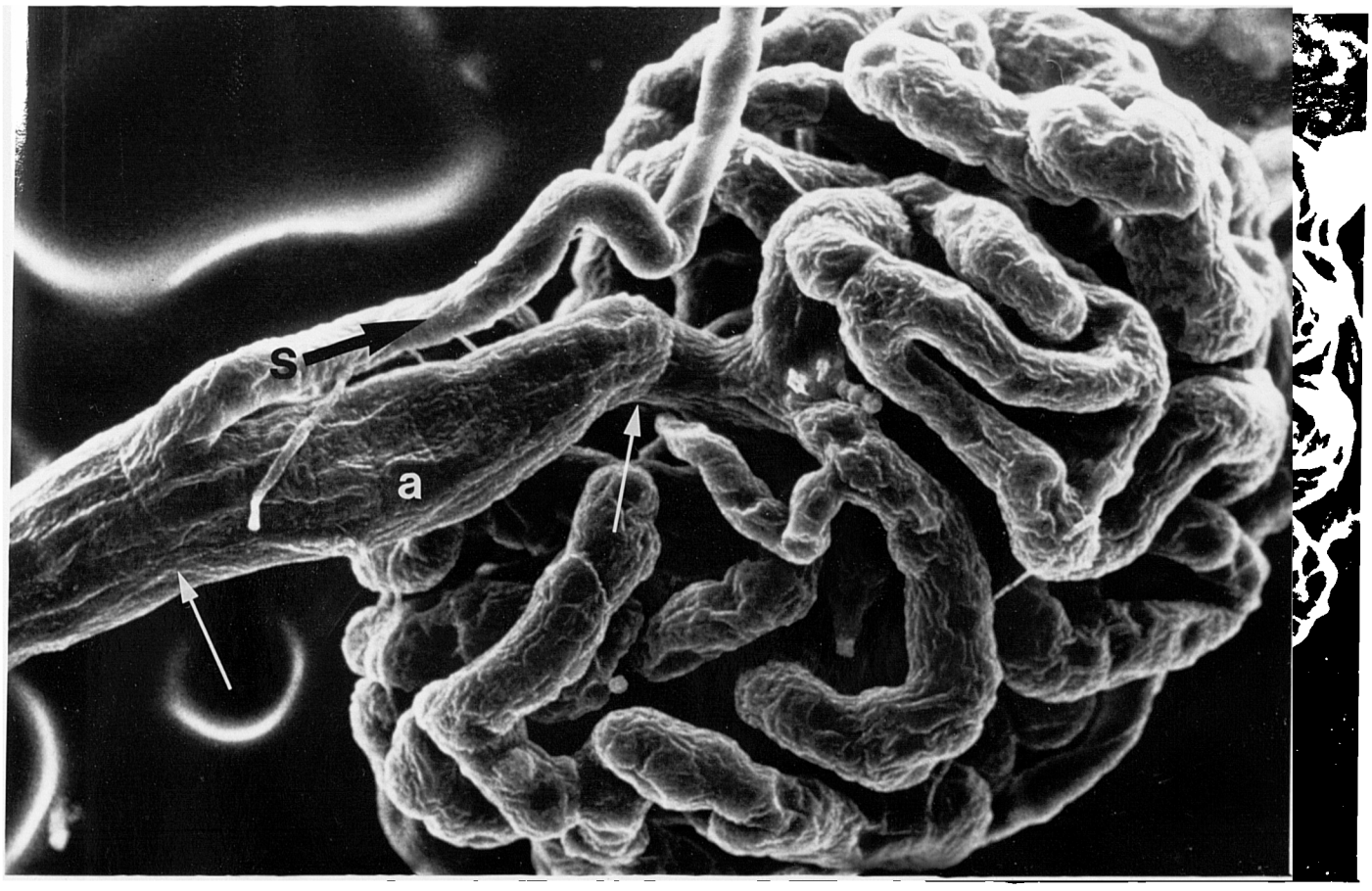
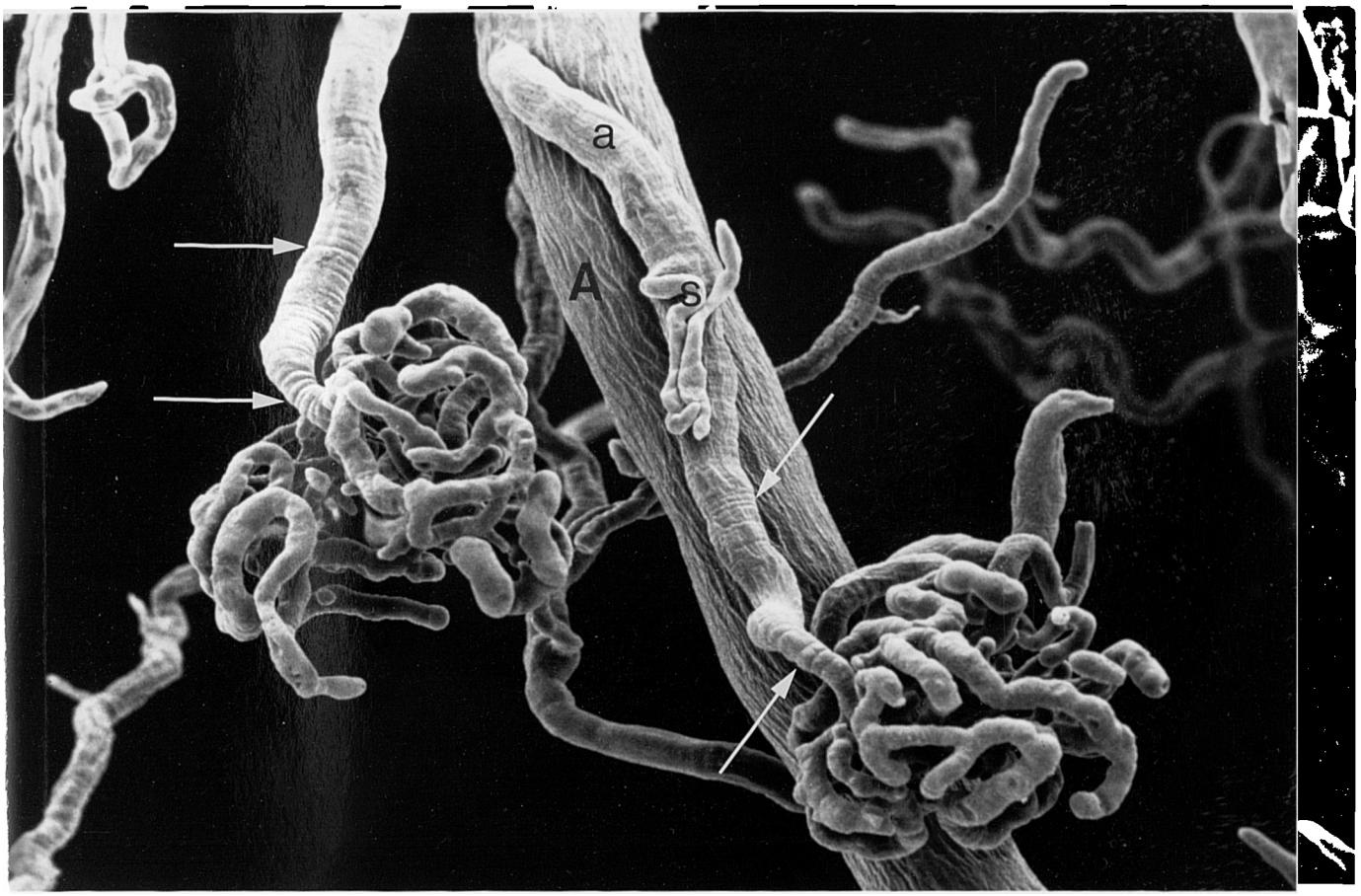

S
a

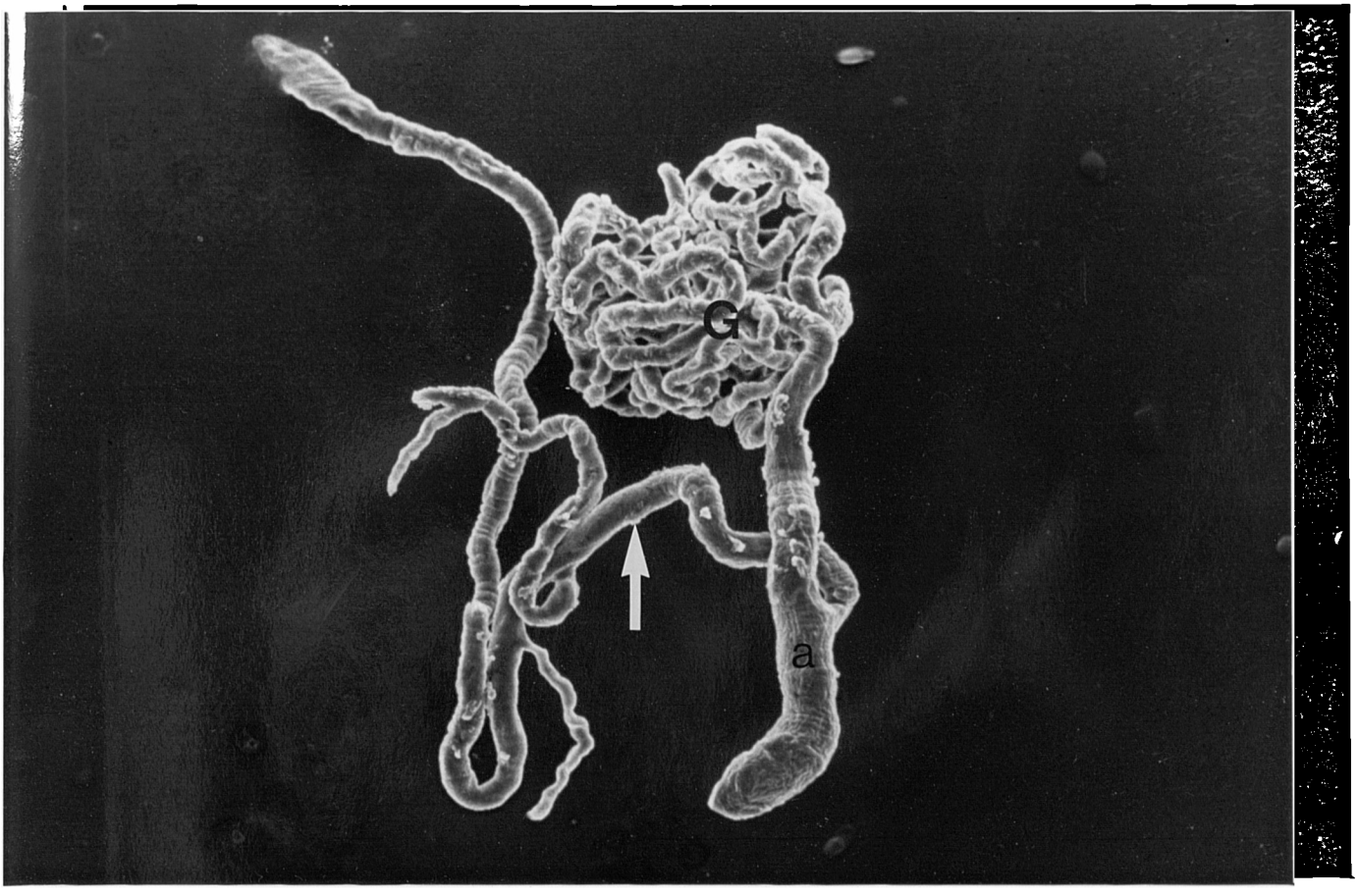
Figure 29

Scanning electron micrograph of a corrosion cast showing the origin of the glomerular bypass vessel.
Ring-like constrictions (arrows) are present on the afferent arterioles (a). A, intra-renal artery; S, glomerular bypass vessel. Bar: 100 μm.

Figure 30

Scanning electron micrograph of a corrosion cast of an isolated afferent arteriole, glomerulus and glomerular bypass vessel.
The bypass vessel (arrow) branches to form the peritubular capillaries. G, glomerulus; a, afferent arteriole.
Bar: 100 μm.


a
A
s


G
a

(d) Efferent Vasculature

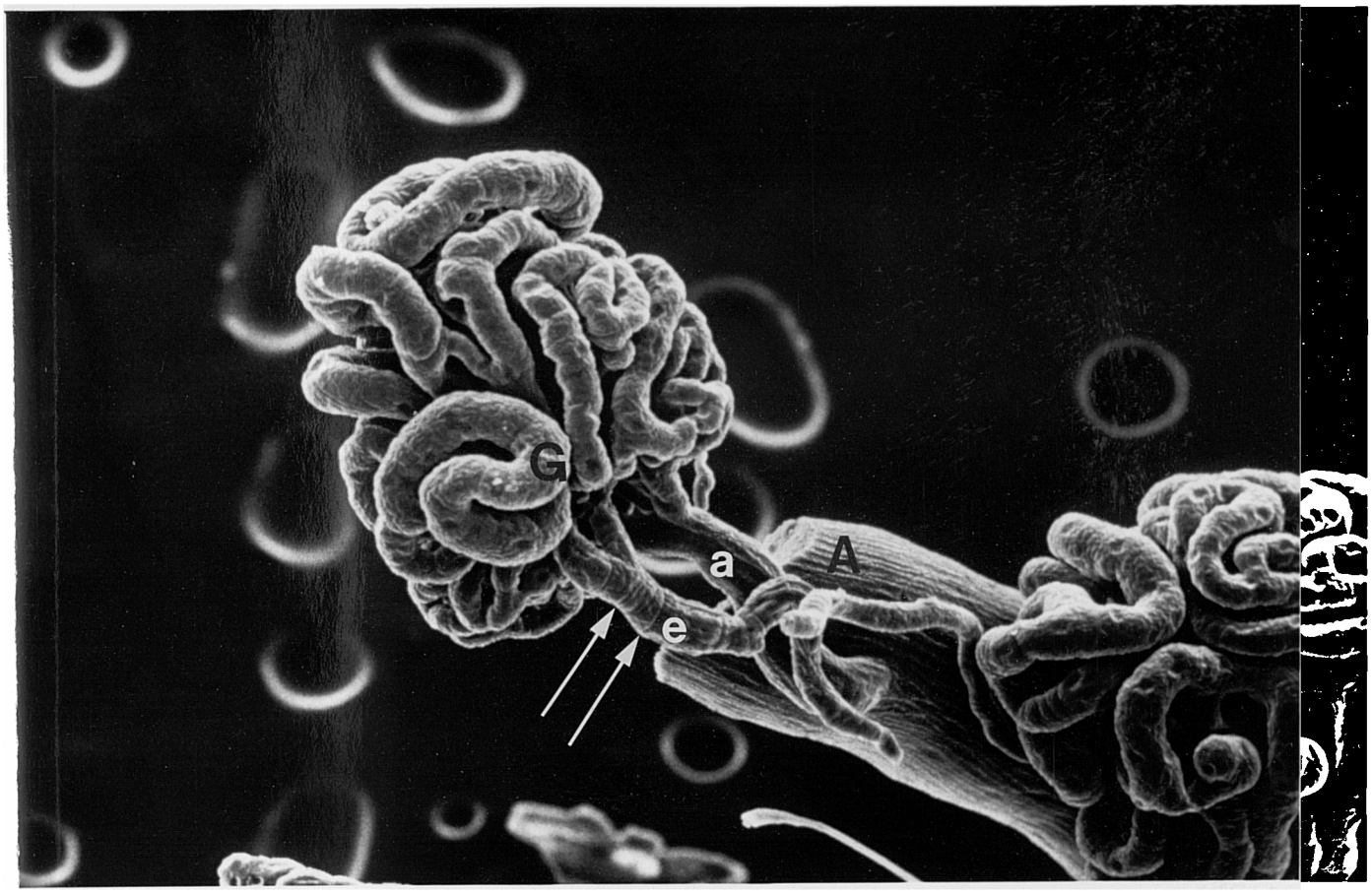
Efferent arterioles were of similar diameter to afferent arterioles (Figure 31). Ring-like constrictions were observed on the casts of efferent arterioles, most frequently at the point of departure from the glomerulus (Figure 31). The efferent arteriole drained into the peritubular capillary network (Figure 32). There was no evidence of double efferent vessels. The peritubular vessels formed a network of anastomosing vessels surrounding the tubules, resembling a network of small blood sinuses (Figure 32).

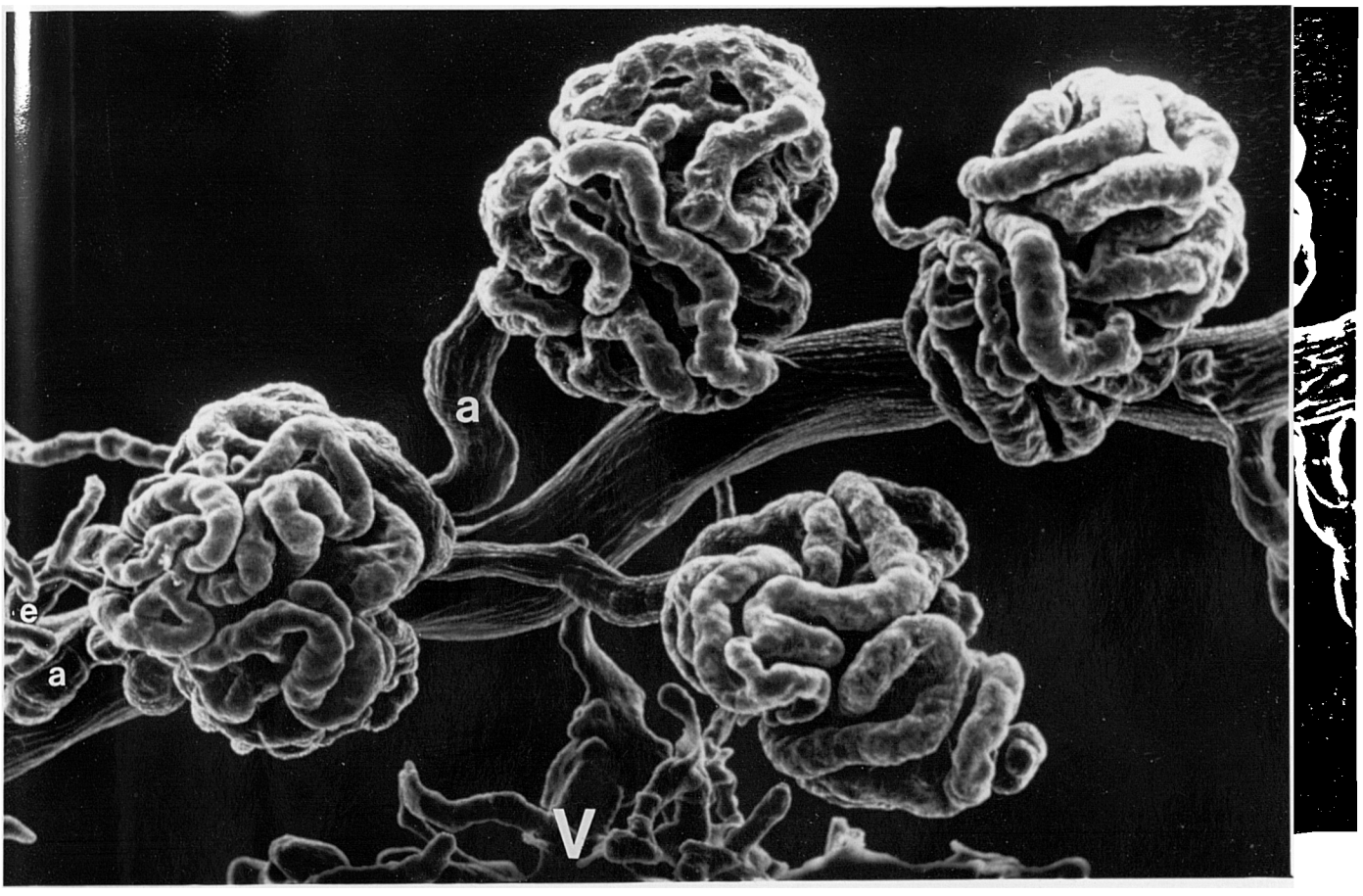
Figure 31

Scanning electron micrograph of a corrosion cast of a glomerulus and afferent and efferent arterioles.
The afferent arteriole (a) arises from the intra-renal artery (A). The glomerular capillaries (G) reunite at the vascular pole and form the efferent arteriole (e). Constrictions (arrows) are present on the efferent arteriole. Bar: 100 µm.

Figure 32

Scanning electron micrograph of a corrosion cast of the arterial tree and peritubular capillary network.
V, peritubular capillaries; a, afferent arteriole; e, efferent arteriole. Bar: 100 µm.


G
a
A
e


a
e
a
V

D. DISCUSSION

The anatomical heterogeneity of renal organs in the two sexes of dogfish (Bourne, 1922) has been confirmed in this study. The distribution of glomeruli along the length of the kidney reflects the different lobular arrangement of the kidneys of male and female dogfish. The anterior metanephric lobes of the female are considerably smaller than the posterior kidney tissues, and contain relatively few glomeruli. Antkowiak and Boylan (1974) reported a comparable glomerular distribution in the kidney of the female dogfish, Squalus acanthias, with 300 glomeruli per gram of tissue in the thin cephalic lobes, compared to 3000 glomeruli per gram of caudal renal tissue. It is unfortunate that the weight of posterior and anterior lobes was not recorded in the present study, so that anterior and posterior lobe glomerular densities cannot be compared. However, observations made during the microdissection of macerated tissues (section II.B.3(e)), suggested no difference in glomerular density, tubular length or in the amount of peri-tubular connective tissues. It is possible that the low glomerular density in anterior kidney lobes reported by Antkowiak and Boylan (1974), may be accounted for by the inclusion of the large chromaffin bodies, which in dogfish species are embedded within the anterior kidney tissues (Lutz and Wyman, 1927).

The estimated total number of glomeruli per gram of body weight was found to be greater in the female dogfish than in

the male. This is thought to be due to the possession of additional anterior kidney lobes in female fishes.

The numbers of glomeruli present in the kidneys of Scyliorhinus canicula, were at variance with counts made in previous studies of other dogfish species (Nash, 1931; Antkowiak and Boylan, 1974). The numbers of glomeruli per gram of body weight were considerably higher in Mustelis canis (Nash, 1931) than in Scyliorhinus canicula, while values for the skates Raja erinacea, and Raja diaphanes (Nash, 1931) were of a similar magnitude to those seen in the present study. Glomerular counts in Squalus acanthias (Antkowiak and Boylan, 1974) appear to be much higher than those of Scyliorhinus canicula, but as the body weight of specimens of Squalus acanthias was not recorded, a meaningful comparison cannot be drawn.

All previous studies have determined glomerular counts by the acid-maceration technique. Whilst the determination of glomerular populations from serial sections is time-consuming, the results are very accurate, and valuable information on the organisation of nephron units is obtained. Rytand (1938) found the variation in glomerular counts obtained by acid-maceration of mammalian kidneys, to be between 10% and 15%. In elasmobranch species, errors may be even higher. In the present studies, the elasmobranch renal capsule was found to be a large, delicate structure, easily damaged by the mechanical trauma of acid-maceration and microdissection. Thus, considerable errors in the estimation of glomerular populations by the acid-maceration technique may be introduced

in both the identification of partially-digested glomeruli, and in the subsequent calculation of the population from sample fractions.

These investigations have provided the first scanning electron micrographs of the renal capsule, glomerulus and nephron of Scyliorhinus canicula. The ultrastructure of the visceral epithelium conformed to the typical vertebrate pattern (Andrews and Porter, 1974; Spinelli, 1974), though there were fewer capillary loops than in mammalian species. The visceral epithelium was composed of rounded podocytes, from which the primary processes arose, and further gave rise to the interdigitating pedicels.

Morphological variations in the structure of the visceral layer were frequently observed in glomeruli of the same kidney, kidney lobe or even on adjacent capillary loops of the same glomerulus. Podocytes were observed to be markedly rounded, or to show varying degrees of flattening, with an apparent reduction in the numbers of pedicels and the degree of interdigitation. Similar flattening of podocytes and broadening of processes has been observed in the rainbow trout following adaptation to sea water (Brown, Taylor and Gray, 1983), and has been suggested to reduce the number of functional slit diaphragms. The passage of water across the glomerular capillaries is thought to be via the endothelial fenestrae, basement membrane, slit diaphragms and slit pores (Karnovsky, 1979). A reduction in the number of functional slit diaphragms will affect water permeability (k_f) and therefore the ultrafiltration coefficient (K_f; the product of

permeability and surface area) of the glomerular capillaries. Individual glomeruli, or capillary loops of the dogfish, may therefore possess different ultrafiltration coefficients or water permeabilities, suggesting variable filtration rates of individual glomeruli of a kidney.

The occurrence of epithelial microprojections varied considerably in the dogfish glomeruli. Rounded podocytes appeared to bear a greater number of microprojections than areas of flattened podocytes and processes. An increase in cytoplasmic microvilli and blebs was found to be associated with the transfer of the fresh water rainbow trout, Salmo gairdneri, to a marine environment (Brown et al., 1983). Microprojections have been suggested to be involved in the uptake of nutrients from the capsular fluid (Andrews, 1981).

A relationship between the degree of glomerular development and the habitat of an animal was first suggested by Marshall and Smith (1930). Nash (1931) investigated this relationship by comparing the number and size of glomeruli in different fish species, recognising that the total filtering surface area was dependent on the number of glomeruli, size and degree of branching of the capillary tuft. He concluded that glomerular development was greater in fresh water teleosts than in marine teleosts.

Changes in glomerular dimensions and number have also been associated with the transfer of euryhaline fish species from fresh to sea water. Glomeruli of the stickleback, eel, Prussian carp, goldfish and trout (Olivereau and Olivereau, 1977; de Ruiter, 1980; Elger and Hentschel, 1981a; Brown et

al., 1983) have been found to be reduced in size following adaptation to sea water, whilst a reduction in the number of glomeruli has been observed in Pacific pink salmon fry reared in sea water (Ford, 1958).

It is known that many species of elasmobranchs are able to tolerate a reduction in environmental salinity (section I.A.2). The dogfish, Scyliorhinus canicula, is a marine coastal inhabitant. Most of the year is spent in deep waters, but the female migrates to shallow waters in early winter to lay eggs. In April or May the male fishes join the females in shallow water, where they remain until late summer when they move back to deeper waters (Wheeler, 1969). It is conceivable that dogfish encounter waters of differing salinities during migration in coastal waters, and that structural modification of glomerular vessels may occur in response to changing osmoregulatory status. The unavoidably low osmolarity of the aquarium sea water (860 $mOsm.l^{-1}$) may thus have caused structural modifications, which in itself requires further investigation.

The parietal layer of Bowman's capsule is composed of cells possessing a single, central cilium. This pattern is typical of higher vertebrates (Andrews and Porter, 1974) through to the fishes (Brown et al., 1983; Zuasti, Aguelleiro and Hernandez, 1983) and lampreys (Miyoshi, 1978).

The elasmobranch nephron is composed of several tubular segments. The exact number, and cytological features of individual segments in different elasmobranch species, is still under investigation. There has been a paucity of

studies investigating ultrastructural elements of the glomerulus and renal tubule.

The configuration of the elasmobranch nephron has been more widely studied. The findings of this study confirm the general pattern observed in other elasmobranch species (Kempton, 1940; 1943, 1962; Deetjen and Antkowiak, 1970; Lacy et al., 1975; 1985). Glomeruli were located on, or near to the dorsal margin of the kidney, lying beneath bundles of parallel tubules. The ventral margin was mostly composed of the large proximal segment II. The dorsal tubular bundles, which are common to all species of elasmobranchs investigated to date, were found to be composed of neck, late proximal and distal segments of the same nephron, surrounded by connective tissues. Recently, Lacy et al. (1985), have shown the five tubules to be tightly wrapped in a peritubular sheath, which also encloses a capillary network. The parallel arrangement of tubules and capillaries has been suggested to have the potential to operate as a countercurrent multiplier system (Deetjen and Antkowiak, 1970; Lacy et al., 1985).

Identification of individual nephron segments of Scyliorhinus canicula, have broadly confirmed the preliminary findings of Brown (unpublished observations). The nephron was found to be composed of neck, three proximal and distal tubular segments. The neck segment was well provided with extremely long flagella, and the first and second proximal tubular segments also bore flagella. Marshall (1934) observed that large numbers of flagella were present in the neck segments of lower vertebrates only, and concluded that the higher blood pressure of birds and mammals rendered possession

of such structures unnecessary. The function of the flagella is thought to be to propel the tubular fluid down the extremely long tubule preventing, a build up of back pressure, and thereby increasing the effective filtration pressure at the glomerulus (Kempton, 1943, 1964).

The cells of the first proximal tubular segment contained many vesicular structures within the cytoplasm. Similar supra-nuclear vacuoles have been identified in the early proximal segments of Squalus acanthias, and other elasmobranch species (Ghouse et al., 1968; Bargmann and von Hehn, 1971). Numerous large endocytic vacuoles and lysosomes, together with many mitochondria were found in the first and second proximal tubular segment of Sparus auratus (Zuasti et al., 1983), indicating that these segments of the fish nephron are involved in the active reabsorption from, and secretion of substances into the tubular fluid.

The present studies represent the first detailed examination of anatomical features of the renal vasculature of Scyliorhinus canicula. Scanning electron microscopy of corrosion casts of blood vessels permits detailed and accurate study of the microvasculature. Pressure within the vasculature proximal to the resin infusion site was monitored in early experiments, to maintain as close to physiological pressure as was possible. Moffat and Fourman (1963) however, argued that the pressure of the injection medium on entering the body, bears little relationship to the actual pressure in the vascular bed under investigation. In these studies, there was no apparent damage to the vasculature, such as might occur

with excessive injection pressures. During polymerisation of Mercox resin, some shrinkage is known to occur (Morris and Campbell, 1978; Brown, 1985), however this does not affect the accurate representation of luminal surface features of the vasculature (Casellas et al., 1982).

The renal vascular pattern revealed in these studies, broadly conformed to the overall description of the arterial and venous blood vessels of the kidney of Squalus acanthias (Ghouse et al., 1968), but interestingly, both vascular features and new vascular pathways of potential functional importance were identified.

Band-like constrictions, thought to represent smooth muscle sphincters, were identified on casts of intra-renal arteries, at the origin of afferent arterioles, on afferent arterioles, at the glomerular hilus of the afferent arteriole and on efferent arterioles. Smooth muscle sphincters have been located at the origin of the afferent arteriole and at the glomerulus in the rainbow trout, but not in the Prussian carp (Elger et al., 1984).

Intra-arterial cushions were present at the origins of afferent arterioles in an estimated 50% of all casts studied. Further studies to analyse the distribution of these intra-arterial cushions within the kidney tissues would be interesting. There have been no published descriptions of intra-arterial cushions in other fish species, however preliminary findings suggest they may occur, though infrequently, in the rainbow trout (Brown, personal communication).

The demonstration of a glomerular bypass vessel in Scyliorhinus canicula, is the first report of a renal vascular shunt in a fish species. The relatively common occurrence of the glomerular shunts in the dogfish (estimated to be present in approximately 25% of all afferent arterioles studied) suggests their participation in an important functional role.

The renal circulation is likely to be controlled by both extra- and intra-renal feedback mechanisms, involving both hormonal and neuronal systems. The renal vessels of the rainbow trout have been shown to be well innervated by adrenergic nerves, with extensive innervation of the afferent arterioles and preglomerular sphincters (Elger et al., 1984). It remains unknown whether the dogfish kidney is innervated in this manner, or whether control of the preglomerular and post-glomerular sphincters is mediated by the release of catecholamines into the blood stream. The close proximity of the segmental chromaffin bodies to the renal tissues (Deetjen et al., 1970) suggests that circulating catecholamines may play an important role in the regulation of renal circulation. Control of vascular sphincters in the pre- and post-glomerular circulation will modify blood pressure and blood flow within the glomerular capillaries, and so regulate filtration (see section III.A.4(a)(viii)).

The function of intra-arterial cushions has been related to the rheological properties of blood flowing in the microcirculation. Blood flowing through a small artery has been shown to separate to some extent, to give a cell-rich axial stream, and a relatively cell-poor peripheral zone. The

term "plasma skimming" was introduced by Krogh (1929) to describe the phenomonen of a branch leaving the parent artery at approximately right angles, drawing its blood supply from the peripheral zone. This phenomenon has been confirmed in studies in vitro, and in vivo (Kinter and Pappenheimer, 1956; Pappenheimer, 1958). Intra-arterial cushions are streamlined, and project into the lumen of the parent artery. Fourman and Moffat (1961) have suggested that their function may be to abolish the effect of the plasma skimming, or even to produce the opposite effect, producing a cell-rich blood supply within the branching artery.

According to Poiseulle's law of fluid mechanics, an increase in viscosity of a fluid flowing through a pipe of uniform diameter at a constant pressure, will result in a reduction in flow rate within that pipe (Caro, Pedley, Schroter and Seed, 1978). Thus, there will be variability in the viscosity of blood within individual afferent arterioles as a result of plasma skimming or cell concentration, which will affect blood flow in the glomerular capillaries, and hence affect single nephron filtration rates. The ability of the smooth muscle of the vessel walls to determine the functional diameter of the vessel, coupled with the closure of preglomerular sphincters to determine blood pressure within the glomerular capillaries, illustrates the potential control of effective filtration pressure of individual nephrons within the kidney.

The presence of glomerular bypass shunts in Scyliorhinus canicula, leading from the afferent arteriole to the

post-glomerular circulation, indicates further potential control over the renal circulation. Constriction of smooth muscle sphincters at the glomerular hilus may divert blood into the post-glomerular circulation without passage through glomerular capillaries, or vary the rate of blood flow in the glomerulus, hence providing a further mechanism by which the filtration rate of individual nephrons may be controlled.

In summary, the kidney of Scyliorhinus canicula possesses anatomical structures identified in this study, which may be used to determine patterns of glomerular perfusion, blood flow and blood pressure within the renal circulation. The control mechanisms regulating the renal circulation in the elasmobranchs remain unknown.

Future studies are required in order to investigate the innervation of the dogfish kidney, particularly in relation to smooth muscle sphincters and their functional role in the control of blood flow and blood pressure within the renal vasculature.

III. RENAL FUNCTION OF THE DOGFISH, SCYLIORHINUS CANICULA

A. INTRODUCTION

1. Physiological Measurement of Renal Function

The kidneys play a vital role in the homeostasis of body fluids, accurately maintaining the composition of the internal environment. The formation of urine begins with the ultrafiltration of plasma at the glomerulus. As the ultrafiltrate passes down the renal tubule, solutes and water are reabsorbed from the tubular lumen, and various substances are secreted into the tubular fluid by the tubular cells. Each of these processes can be studied, using many different techniques, some of which will be considered below.

(a) Glomerular Filtration Rate

The functioning of the glomerular kidney is dependent on the initial filtration of the plasma. Measurement of glomerular filtration rate is made by determining the clearance of a substance.

The term clearance was first used by Möller, McIntosh and van Slyke (1929), when attempting to derive a mathematical formula to compare the filtration of urea from the blood, in healthy and diseased kidneys. Clearance of a substance (Cx) may be defined as the volume of plasma which is totally cleared of that substance in unit time, and can be represented by:

$$Cx = \frac{Ux \cdot \dot{V}}{Px}$$

Where: Ux = concentration of x in the urine

Px = concentration of x in the plasma

$\dot{V}$ = urine flow

The clearance of a substance is a theoretical concept, as plasma is not completely cleared of any substance during its passage through the glomerulus. Glomerular filtration rate (GFR) is equal to the clearance of a substance, provided that the following criteria are fulfilled (Smith, 1951):

1. The substance is freely filtered at the glomerulus
2. It is neither reabsorbed, nor secreted by the renal tubule
3. It is biologically inert, so does not affect renal function in any way.

Inulin is a substance, frequently used in clearance studies to measure GFR, which appears to broadly fulfill the above criteria. It is a polysaccharide sugar with a molecular weight of approximately 5500, which is not normally found in the body. Micropuncture studies in the rat have established that inulin is not reabsorbed in significant quantities by the renal tubule, and so is a suitable substance for the measurement of GFR in this species (Maude, Scott, Shehadeh and

Solomon, 1965). Similar studies in the river lamprey, Lampetra fluviatilis, have validated the use of inulin in this species (Moriarty et al., 1978). The renal clearance of inulin has however, been shown to be significantly lower than polyethylene glycol in the eel, suggesting that tubular reabsorption of inulin may occur in this species (Schmidt-Nielsen and Renfro, 1975). Further, inulin has been demonstrated in the urine of aglomerular fish species following intra-venous injection (Murdaugh, Robin, Malvin, Soteres, Pyron and Weiss, 1962; Lahlou, Henderson and Sawyer, 1969). The bladder of Lophius americanus (Murdaugh et al., 1962), and renal tubules or ureters of Opsanus tau (Lahlou et al., 1969) have been shown to be permeable to inulin. Whilst there have been no micropuncture experiments to validate the use of inulin in dogfish species, early experiments by Shannon (1934) suggested that inulin was a suitable substance for the measurement of GFR in Squalus acanthias.

Creatinine clearance is often used as a measure of GFR clinically, since it is a normal product of muscle metabolism. It has however, been shown to be secreted by the renal tubule in some species, including man (Pitts, 1968).

(b) Relative Osmolar Clearance and Free Water Clearance

If the body becomes dehydrated, the kidneys reabsorb osmotically-free water from the tubules, and conversely if the body fluids are hypotonic, the kidneys will excrete excess osmotically-free water in a dilute urine. If the kidney is producing a urine iso-tonic to plasma, then the

osmotically-active constituents of urine are being excreted in a volume of water sufficient to keep the solutes at the same osmotic pressure as the plasma. This volume of water is equivalent to the rate at which osmotically-active substances are cleared from the plasma, i.e. the osmolar clearance.

Osmolar clearance is defined as:

$$Cosm = \frac{Uosm \cdot \dot{V}}{Posm}$$

Where: Uosm = osmolarity of urine

Posm = osmolarity of plasma

$\dot{V}$ = urine flow.

If the urine to plasma osmolarity is less than unity, a dilute urine is being produced, i.e. an iso-tonic urine (Cosm) with an additional volume of free-water (C_{H_2O}), so:

$$\dot{V} = Cosm + C_{H_2O}$$

or, $$C_{H_2O} = \dot{V} - Cosm$$

If the urine : plasma osmolarity ratio is greater than unity, a hypertonic urine is produced, i.e. osmotically free-water is reabsorbed. Lower vertebrates are generally unable to produce a urine more concentrated than plasma. Only the birds and mammals have evolved the specialised loop of Henle, interposed between the proximal and distal tubule segments, which allows

the production of urine hyperosmotic to the body fluids. It has been suggested however, that a counter-current loop exists in the kidney of the lamprey, and this may allow the production of a slightly hypertonic urine in sea water adapted animals (Logan et al., 1980).

(c) Renal Tubular Micropuncture Techniques

Micropuncture techniques allow the measurement of individual nephron filtration rates, and the localization and analysis of renal tubule transport processes. Following the successful collection of fluid from single nephrons in the frog, to gain insight into the formation of urine (Wearn and Richards, 1924; Richards and Schmidt, 1924), micropuncture techniques have become more advanced and widely used (Windhager, 1968; Lang, Greger and Lechene, 1978). Several of the principle micropuncture techniques employed are described below.

(i) Free-Flow Micropuncture

This remains the most widely used micropuncture technique. It allows the collection of fluid from defined sites in the nephron. The samples of tubular fluid may then be analysed for volume and concentration of given substances. Using this technique, single nephron glomerular filtration rates (SNGFR) may be determined. Substances used to determine SNGFR must obviously obey the criteria for measurement of overall GFR (see section III.B.1(a) above). Following the introduction of an oil-block into the nephron, timed

collections of tubular fluid are made. The concentration of the glomerular filtration marker is then determined, and the SNGFR may be calculated according to the equation:

$$SNGFR = \frac{TFx \cdot \dot{V}}{Px}$$

Where: TFx = concentration of x in the tubular fluid

Px = concentration of x in the plasma

$\dot{V}$ = tubular fluid flow

There are essentially two types of suitable substances which may be used. These are the polysaccharides which are analysed chemically, and radioactively labelled molecules, analysed by scintillation counting. Due to the very small volumes of tubular fluid obtained, analysis of very small quantities must be possible.

(ii) Microinjection and Microinfusion

In the microinjection technique, a small volume of ^{3}H- or ^{14}C-labelled inulin and labelled tracer of a substance under investigation, is injected into segments of superficial nephrons. The urinary recovery of the tracer is compared to the recovery of the inulin, to estimate the fractional reabsorption of the injected substance. Microinfusion is a development of this technique, where the inulin and tracer are infused into the tubule by a microperfusion pump.

(iii) Microperfusion

Continuous microperfusion is used to measure an increase or decrease in concentration of a substance in tubular fluid, indicating tubular secretion or reabsorption. An oil-filled pipette is inserted into a tubule, and an oil-block injected to identify subsequent loops of that same tubule. The microperfusion pipette containing the perfusate solution, is inserted beyond the first pipette, and a second oil-filled pipette is inserted at the last accessible point of the tubule. Oil-blocks are inserted from both oil pipettes, and then perfusion of the tubule from the first pipette, and continuous collection by the second oil pipette is made.

(iv) Stationary Microperfusion and Shrinking Droplet

The stationary microperfusion technique again allows the collection and analysis of perfusate, to study tubular transport processes. The technique involves the injection of a block of perfusate into the centre of an oil-block, and the subsequent recollection of that perfusate.

In the shrinking droplet technique, a double-barrelled micropuncture pipette is used for essentially the same technique. An oil-block is divided by a block of test-solution. The shrinkage of the droplet indicates fluid reabsorption.

(d) Use of Sodium Ferrocyanide

The value of sodium ferrocyanide as an indicator of glomerular filtration was first realised by Marshall (1930). In comparing the function of glomerular and aglomerular kidneys of various species of fishes, amphibians, reptiles and birds, he showed that sodium ferrocyanide injected intra-muscularly into glomerular species was eliminated renally, whilst none was detected in the urine of aglomerular species.

(i) Evaluation of Glomerular Filtration

Hanssen (1958) developed a histochemical method enabling excreted sodium ferrocyanide to be visualised in isolated kidney tubules of the mouse. Following the intravenous injection of a bolus of sodium ferrocyanide, the kidneys were removed and the ferrocyanide precipitated as Prussian blue. The nephrons which had received filtered blood were identified by the presence of Prussian blue precipitate contained within the tubular lumen.

(ii) Measurement of SNGFR

A modification of the Hanssen (1958) technique allows the measurement of SNGFR (Hanssen, 1963) without the use of micropuncture techniques. This method allows the measurement of SNGFR from nephrons which are inaccessible by micropuncture techniques, and so enables a more realistic population of nephrons to be studied.

The technique involves the infusion of a labelled

solution of sodium ferrocyanide, followed by a pulse of non-radioactive sodium ferrocyanide. SNGFR is calculated by dividing the radioactivity in the tubule filtered in unit time, by the radioactivity in unit volume of plasma. Filtration rates of individual nephrons have been calculated using this method, in mammalian species (Rouffignac and Bonvalet, 1972; Bankir and Rouffignac, 1976), and in the rainbow trout Salmo gairdneri, (Brown, Jackson, Oliver and Henderson, 1978).

2. Renal Function of Elasmobranchs

Early experiments on renal function in elasmobranchs measured urine flow, and glomerular filtration rate using clearance techniques (Shannon, 1934; Smith, 1939a; Kempton, 1966; Burger, 1967). Urinary flow rates and glomerular filtration rates of marine elasmobranchs are similar to those of freshwater rather than seawater teleosts (Hickman and Trump, 1969; Evans, 1980). Glomerular filtration rate is variable, ranging from 0.2 to 12.0 $ml.hr^{-1}.Kg^{-1}$ (Hickman and Trump, 1969). Urine flow and GFR have been shown to fluctuate in response to handling or catheterisation, and may cease altogether (Clarke and Smith, 1932). This is in contrast to teleosts, which typically show a marked diuresis following handling. Table 6 summarises the composition of plasma and urine of the marine spiny dogfish, Squalus acanthias.

Much of the water filtered at the glomerulus is reabsorbed by the renal tubule (Kempton, 1953; Schmidt-Nielsen

Table 6

Composition of plasma and urine of the spiny dogfish, Squalus acanthias, in sea water.

GFR	[d]	3.50 $ml.hr^{-1}.Kg^{-1}$
$\dot{V}$	[d]	1.15 $ml.hr^{-1}.Kg^{-1}$

All values from Burger (1967), except as noted:

[a] Maren (1967)

[b] Clarke and Smith (1932)

[c] Kempton (1953)

[d] Shannon (1940)

Solute	Plasma concentration (mM)	Urine concentration (mM)	U:P Ratio
Sodium	250.00	240	0.96
Potassium	4.00	2	0.50
Calcium	3.50	3	0.86
Magnesium	1.20	40	33.30
Chloride	240.00	240	1.00
Sulphate	0.50[a]	70	140.00
Phosphate[b]	0.97	33	34.00
Glucose[c]	14.00	3	0.21
Urea	350.00	100	0.29
TMAO	70.00	10	0.14
Osmolarity	1000	800	0.80
pH	7.48	5.80	-

and Rabinowitz, 1964). The urine of elasmobranchs is invariably acidic (Smith, 1939b; Hodler, Heinemann, Fishman and Smith, 1955; Burger, 1967). Injection of a pH indicator, phenol red, into the glomerular capsule and renal tubule, located the site of acidification of the neutral ultrafiltrate of plasma, as being in the proximal tubule (Kempton, 1940b). The rate of acidification of the tubular fluid has subsequently been determined using the split-drop micropuncture technique (Deetjen and Maren, 1974).

Micropuncture techniques have been used to study the reabsorption of urea from the renal ultrafiltrate of elasmobranchs (see section I.C.1(c)), (Schmidt-Nielsen et al., 1966; Deetjen et al., 1972; von Baeyer and Boylan, 1973). The principle sites of ion reabsorption in the renal tubule of the skate, Raja erinacea, have been identified using free-flow micropuncture techniques (Stolte, Eisenbach, Antkowiak and Boylan, 1971; Stolte et al., 1977). The proximal tubule was found to be the principle site for the secretion of magnesium, phosphate and sulphate, and for the reabsorption of sodium and chloride. The distal tubular segment and collecting ducts have been shown to be the primary sites of urinary dilution (Thurau and Acquisto, 1969; Stolte et al., 1971). The ability to produce a dilute urine is an important mechanism, allowing fishes to adapt to environments of lowered salinity.

3. Regulation of Renal Function

Measurements of renal function, particularly in non-mammalian vertebrate species, show considerable variation both under natural and laboratory conditions. The ability to regulate kidney function to meet changing osmoregulatory requirements is an important evolutionary adaptation, though little is known of the regulatory mechanisms involved. Kidney function may be regulated by changes in glomerular filtration rate, or by changes in the tubular reabsorption-secretion processes (Bentley, 1971). Changes in the overall glomerular filtration rate of an animal could theoretically result from:

A. Changes in SNGFR.

B. Changes in the number of filtering glomeruli, reflecting glomerular intermittency.

These modifications may reflect:

1. Composition of plasma. Variation in the concentration of cations, anions, glucose, urea and particularly plasma proteins, will create variations in osmotic pressure of the blood, which will directly affect hydrostatic pressure and the effective filtration pressure within the glomerular capillaries. An increase in osmotic pressure of the blood will reduce the effective filtration pressure, thus reducing SNGFR.

2. Haemodynamic effects. Changes in arterial blood pressure and patterns of blood flow within the kidney, are likely to influence the hydrostatic pressure within the glomerular capillaries and thus affect SNGFR, and the number

of filtering glomeruli. There is little evidence of autoregulation of renal blood flow within lower vertebrates (Hickman and Trump, 1969; Rankin, Wahlqvist and Wallace, 1984). In higher vertebrates, autoregulation maintains a stable glomerular filtration rate despite changes in systemic blood pressure, by mechanisms which are not yet fully understood.

3. Ultrafiltration coefficient of the glomerulus (K_f). This is the product of the filtering surface area and the permeability of the glomerular capillaries. Morphological changes in the structure of the filtration barrier could increase or decrease the permeability of the glomerular capillaries, altering K_f and thus SNGFR, and the population of filtering nephrons.

Changes in the reabsorption-secretion rates of the tubule may reflect changes in the composition of plasma, haemodynamic effects, changes in the permeability of the tubular epithelium or changes in transport mechanisms.

4. Control of Renal Function in Fishes

The direct linear relationship between inulin clearance and phenol red secretion in elasmobranchs, has been taken as evidence for a changing population of filtering glomeruli, or glomerular intermittency (Shannon, 1940; Kempton, 1966). Additionally, the direct relationship between inulin clearance and urine flow in elasmobranchs (Kempton, 1953, 1966) provides

further evidence in support of this theory. Smith (1951) reported that only approximately 2% of renal plasma blood flow (RPF) was filtered at the glomeruli in the dogfish, Squalus acanthias, while less than 1.5% RPF was filtered in the skate, Raja erinacea (Forster and Goldstein, 1969). These very low filtration fractions, compared to values of approximately 20% in mammals, have suggested that as in teleosts (Forster, 1953; Lahlou, 1970; Brown, Oliver, Henderson and Jackson, 1980), a low proportion of nephrons normally filter, and that glomeruli of elasmobranchs may be capable of intermittent filtration. However, an alternative explanation may be that both elasmobranchs and teleosts have a low ultrafiltration coefficient.

Large increases in urine flow and GFR following environmental dilution (Foster and Goldstein, 1969; Goldstein and Foster, 1971), and in response to the administration of adrenaline (see section III.A.4(a)(vii)), suggest that normal filtration rates of marine elasmobranchs are considerably lower than the potential maximum, and furthermore, increases in the population of filtering glomeruli have been suggested to accompany these diureses (Deetjen and Boylan, 1968).

The regulation of elasmobranch kidney function is likely to involve both neural and hormonal factors.

(a) Autonomic Nervous System

(i) Anatomy

The autonomic nervous system is composed of all efferent nervous pathways having a ganglionic synapse outside the central nervous system (Campbell, 1970). The autonomic nervous system may be divided into the cranial autonomic system, supplying the effector organs in the head and anterior part of the trunk including the heart via a cardiac branch of the vagus, the spinal autonomic system supplying the posterior part of the body, and the enteric system consisting of neurons supplying the gastro-intestinal tract (Nilsson, 1983).

Autonomic nerves extend to almost every part of the vertebrate body including the smooth muscle of blood vessels, cardiac muscle, gills and chromaffin tissues (Nilsson, Holmgren and Fänge, 1983). They are intrinsically involved in the homeostasis of body fluids, and may exert a direct influence on the kidney, by constriction and relaxation of vasculature smooth muscle, and indirectly by the control of the levels of circulating humoral factors.

In 1933, J. Z. Young made a detailed study of the autonomic nervous system of selachians. The spinal autonomic nerves of the elasmobranch, form two chains of segmentally arranged paravertebral ganglia. The most anterior pair of these ganglia form the axillary bodies, or chromaffin bodies, situated within the posterior cardinal venous sinuses (Lutz and Wyman, 1927; Gannon, Campbell and Satchell, 1972).

(ii) Chromaffin Tissues

The axillary bodies are composed of ganglion cells and masses of catecholamine storing chromaffin cells. Lutz and Wyman (1927) studied the relative positions of chromaffin tissues and inter-renal bodies (together forming the adrenal glands of higher vertebrates) in several species of elasmobranchs, and found that extracts of the chromaffin tissues produced a mydriatic dilator response in the frog, confirming the presence of catecholamines.

The catecholamines adrenaline and noradrenaline are biosynthesised and stored in the chromaffin cells of the axillary bodies. The relative proportions of stored catecholamines varies in different species of fish (von Euler and Fänge, 1961), and even within the elasmobranchs where approximately 70% of stored catecholamine is noradrenaline, and 30% is adrenaline (Shepherd, West and Erspamer, 1953). Small quantities of the catecholamine dopamine (approximately 0.74% of catecholamines) have also been found in the chromaffin tissues of Scyliorhinus canicula (Dalmaz and Peyrin, 1978).

(iii) Circulating Catecholamines

The release of catecholamines is thought to be mediated by the preganglionic-cholinergic innervation of the chromaffin cells, directly into the posterior cardinal sinuses (Gannon et al., 1972; Abrahamsson, 1979; Mazeaud and Mazeaud, 1981).

The biosynthesis of catecholamines, rate of turnover and

degradation has been reveiwed by Mazeaud and Mazeaud (1981). Catecholamines are released in response to "stress" (Mazeaud and Mazeaud, 1981), which may be induced by strenuous swimming, hypoxia, wounding, capture, handling and confinement.

(iv) Cardiovascular Effects of Circulating Catecholamines

Circulating catecholamines have a profound effect on the entire cardiovascular system of fishes. The effects of noradrenaline do not parallel those of adrenaline. The difference in physiological action of the catecholamines may be due to the existence of multiple receptor sites in the target tissues (Insel and Snavely, 1981; Nilsson et al., 1983).

The anatomy and physiology of the cardiovascular system of fishes have been studied extensively (Randall, 1970; Satchell, 1971). The effects of circulating catecholamines on cardiac function, and branchial and systemic vasculature have been investigated in the elasmobranchs. Early experiments by Lyon (1926), monitored changes in branchial and systemic arterial blood pressure, heart rate and respiratory frequency following induced stress (wounding), and the administration of adrenaline in the sand shark, Charcharias. The overall result of inducing stress or catecholamine perfusion of the vasculature, was a rise in aortic blood pressure (Lyon, 1926; MacKay, 1931; Wyman and Lutz, 1932; Peirce, Kent, Peirce and Mumford, 1970; Opdyke, Carroll and Keller, 1982).

(v) Cardiac Effects of Circulating Catecholamines

The elasmobranch heart has only a sparce adrenergic innervation within the sinus venosus, compared to the intense innervation of the teleostean heart (Gannon et al., 1972). However, the liberation of catecholamines from the chromaffin bodies in the posterior cardinal sinuses results in rapid aspiration into the heart. Both adrenaline and noradrenaline increase myocardial contractility (positive inotropic effect) and heart rate (positive chronotropic effect) in elasmobranchs (Gannon et al., 1972; Forster, Hannafin, Shiffrin and Morad, 1978; Randall, 1982). Circulating catecholamines released from the head kidney of the teleost Gadus morhua, appear to maintain an adrenergic "tonus", affecting both heart rate and blood pressure (Holmgren, 1977; Wahlqvist and Nilsson, 1977).

A marked rise in plasma levels of both adrenaline and noradrenaline, and a rise in blood pressure have been observed following periods of induced swimming in Squalus acanthias (Opdyke et al., 1982), while spontaneous bursts of swimming in Scyliorhinus canicula were associated with an increased cardiac rate and cardiac output (Piiper, Meyer, Worth and Willmer, 1977). Thus exercise in elasmobranchs appears to increase the level of circulating catecholamines, which directly affects cardiac function.

(vi) Branchial Effects of Circulating Catecholamines

In teleosts, branchial vascular resistance is lowered by the action of catecholamines on beta-adrenoreceptors located within the gills (Keys and Bateman, 1932; Ostlund and Fänge,

1962; Randall and Stevens, 1967; Wahlqvist, 1980). Similarly, isolated gill preparations from Scyliorhinus canicula and Squalus acanthias, have shown a beta-adrenergic dilatory response to physiological levels of adrenaline and noradrenaline (Rankin and Davies, 1972; Davies and Rankin, 1973; Capra and Satchell, 1974).

Vasodilation of branchial vasculature, coupled with an acceleration of blood perfusion rate resulting from cardiac effects of circulating catecholamines, cause disturbances in both the normal respiratory uptake of oxygen and carbon dioxide excretion (Jones and Randall, 1978), and osmoregulatory mechanisms operating in the gills (Capra and Satchell, 1977; Mazeaud and Mazeaud, 1981). Circulating catecholamines have been found to increase gill permeability to water (Rankin and Maetz, 1971), and affect ionic regulation of the branchial tissues in teleosts (Mazeaud and Mazeaud, 1981).

(vii) Systemic Effects of Circulating Catecholamines

The dominant effect of both adrenaline and noradrenaline on the systemic vasculature is vasoconstriction in both teleosts and elasmobranchs. This effect is mediated by the action of alpha-adrenergic receptors, and results in an increase in systemic blood pressure. It remains uncertain whether the adrenergic control of the systemic vasculature in teleosts is predominantly neuronal, or humoral via circulating catecholamines, and it may prove to be species dependent (Wahlqvist and Nilsson, 1977; Smith, 1978). In elasmobranchs,

arterial vasomotor tone of the systemic circulation has been suggested to be controlled by circulating catecholamines, the concentration of which may be controlled by peripheral neurogenic activity (Holcombe, Wilde and Opdyke, 1980).

(viii) Renal Effects of Adrenergic Innervation and Circulating Catecholamines

The relative importance of neuronal and humoral factors in the control of vascular resistance in renal vessels is not fully understood. Using an *in situ* perfused kidney of the rainbow trout, *Salmo gairdneri*, stimulation of the sympathetic nervous system was found to have little effect on GFR or urine flow (Rankin *et al.*, 1984). Both circulating adrenaline and noradrenaline however, produced a dose-dependent antidiuresis, suggesting that the predominant control of renal vascular resistance in the perfused kidney preparation, was humoral. However, widespread adrenergic innervation of the renal arteries, afferent arterioles and preglomerular sphincters has been demonstrated in the sole, *Parophrys vetulus*, and rainbow trout (Bulger and Trump, 1968; Elger *et al.*, 1984). Whole animal studies in the rainbow trout using Bretylium, an adrenergic neuron blocking agent, have suggested neural control of renal haemodynamics and function (Elger and Hentschel, 1983). Unfortunately, no comparable studies have as yet been attempted in the elasmobranchs.

Stress is reported to cause a diuresis in teleosts (Hickman and Trump, 1969). Toth (1939) investigated the effects of adrenaline on urine flow in the glomerular puffer

fish, Spheriodes maculatus, and the aglomerular toadfish, Opsanus tau. He observed a diuresis in the glomerular fish only and concluded that adrenaline was acting on the glomerular circulation in some manner. The known pressor effects of adrenaline and the apparent lack of autoregulation in teleost kidneys, may suggest that diuresis is a secondary effect of the systemic action of adrenaline. However, while pressor doses of catecholamines are diuretic in the eel, they are antidiuretic in the cod and trout (Rankin and Babiker, 1981; Rankin et al., 1984). This suggests there may be renal vasoconstriction due to circulating catecholamines, which in the cod and trout, predominates over the pressor effects of the catecholamines.

In elasmobranchs, adrenaline has consistently been reported to induce a glomerular diuresis (McCrory, Biggs, Boyarsky, Rieck and Soley, 1956; Silverman, Gerstein and Boylan, 1966; Forster, Schweickert and Goldstein, 1969).

(b) Renal Effects of other Hormones

(i) Renin-Angiotensin System

The role of the renin-angiotensin system has been extensively studied. It is known to influence blood pressure, adrenocortical function, renal function and fluid balance (Sokabe and Ogawa, 1974; Taylor, 1977; Henderson, Oliver, McKeever and Hazon, 1980). The renin-angiotensin system has been shown to be present in all vertebrate groups except the elasmobranchs and cyclostomes. In elasmobranchs, there is an

apparent lack of granular epithelioid cells secreting renin (section II.A.2(e)), but renin-like material which generates pressor activity in the rat has been extracted from the dogfish kidney (Henderson, Oliver, McKeever and Hazon, 1980).

The enzyme renin is released from granular epithelioid cells of the juxtaglomerular apparatus, and acts on circulating angiotensinogen to form angiotensin I. The biologically active hormone angiotensin II, is formed from angiotensin I by the action of a converting enzyme. The term juxtaglomerular is however, inappropriate to many lower vertebrate species possessing renin-containing epithelioid cells. The location of these cells varies, and may be within the afferent arteriole, at a considerable distance from the glomerulus (Henderson et al., 1980), and further, aglomerular teleosts are known to possess granular epithelioid cells (Sokabe and Ogawa, 1974).

Angiotensin II has a vasopressor action in teleosts. In the eel, Anguilla rostrata, angiotensin II produces an increase in dorsal aortic blood pressure and a urinary diuresis (Nishimura and Sawyer, 1976), but in the rainbow trout, Salmo gairdneri, despite pressor activity angiotensin II induces a glomerular antidiuresis (Henderson, Brown, Oliver and Haywood, 1978; Brown et al., 1980; Gray, 1985).

A marked pressor effect in response to angiotensin II has been reported in the spiny dogfish, Squalus acanthias (Opdyke and Holcombe, 1976), but this effect was completely abolished by treatment with the adrenergic blocking agent,

phentolamine. More recent studies (Opdyke, Carroll, Keller and Taylor, 1979; Carroll, 1981) suggest that the the pressor response is entirely attributable to the release of catecholamines, mediated by the action of angiotensin II. This indicates that receptors sensitive to angiotensin II must exist in the chromaffin tissue, and that receptors mediating the actions of angiotensin II may have evolved before other components of the renin-angiotensin system. Interestingly, an equipotent pressor response to angiotensin I was also demonstrated (Opdyke and Holcombe, 1976) suggesting that a converting enzyme exists in the dogfish, Squalus acanthias. Addition of an angiotensin I converting enzyme inhibitor blocked the pressor response to angiotensin I, providing further evidence that a converting enzyme is present in the dogfish (Opdyke and Holcombe, 1976).

(ii) Arginine Vasotocin

The neurohypophysial hormones arginine vasopressin (AVP) of mammals, and arginine vasotocin (AVT), found in all other vertebrates, are thought to be involved in the control of renal function.

Whilst the injection of high, possibly pharmacological doses of AVT into the freshwater eel, Anguilla anguilla, produces a dose-dependent glomerular diuresis, low doses have been shown to produce a glomerular antidiuresis (Henderson and Wales, 1974; Babiker and Rankin, 1978). AVT is antidiuretic at all doses in the rainbow trout (Rankin et al., 1984). An AVT induced glomerular diuresis has however, been

reported in the freshwater lamprey (Rankin, McVicar and Babiker, 1983). The effects of AVT on osmoregulation in the elasmobranch fishes has not been studied.

The precise role of AVT in the osmoregulation of body fluids of fresh and marine fishes however, remains unknown.

(iii) Prolactin

The anterior pituitary hormone prolactin has been shown to be secreted in teleosts, on adaptation to a dilute environment (Rankin, Henderson and Brown, 1983). Prolactin influences ion and water permeability in the kidney, urinary bladder and gills. It is also thought to have a direct effect on the teleost kidney, either by increasing GFR or reducing tubular water reabsorption, or a combination of both (Bentley, 1982).

Little is known of the action of prolactin in non-teleostean fish species. Water uptake across the gills is reduced following hypophysectomy in elasmobranchs, and restored by injection of prolactin (Payan and Maetz, 1971). It is not known whether prolactin has a direct effect on the kidney of elasmobranchs (Bentley, 1982).

(iv) Corticosteroids

Corticosteroid hormones are secreted from the interrenal tissues of fishes, released under the control of pituitary adrenocorticotrophic hormone (Bentley, 1982). The corticosteroids present in the plasma of elasmobranchs have been identified, and 1α-hydroxycorticosterone found to be the

principal corticosteroid present (Bern, deRoos and Biglieri, 1962; Truscott and Idler, 1972; Hazon, 1983).

Following adaptation to a hypo-osmotic environment an increase in plasma 1α-hydroxycorticosterone levels have been reported in the dogfish, Scyliorhinus canicula (Hazon, 1983). This suggests that this hormone is secreted in response to environmental dilution, and may contribute to the regulation of plasma composition (Hazon, 1983). There is a reported increase in corticosteroid activity associated with adaptation of euryhaline teleosts to hyperosmotic environments (Henderson et al., 1978).

The elasmobranch kidney plays a vital role in the homeostasis of body fluids. The ability of the kidney to osmoregulate in a changing environment may be regulated by neural and hormonal control mechanisms, which are not as yet fully understood. This study aims to investigate overall renal, and single nephron function of the dogfish, Scyliorhinus canicula, and to study the role of catecholamines in the regulation of renal function.

B. MATERIALS AND METHODS

1. Experimental Animals

Lesser spotted dogfish, Scyliorhinus canicula, of both sexes, weighing 450-1000g were used in the experiments (see section II.B.1). The animals were kept in large tanks of well aerated sea water at 9°C. They were starved for 7-14 days before experimentation.

2. Surgical Preparation

Dogfish were anaesthetised by immersion in 0.015% (wt/vol) MS222. After the onset of anaesthesia, each fish was placed supine on a V-shaped support constructed of expanded polystyrene, held within a perspex trough. The head and gills were immersed in aerated and cooled (9°C) 0.0067% (wt/vol) MS222 in sea water. This level of MS222 maintained anaesthesia, without inhibiting spontaneous respiration.

Through a mid-ventral incision, the lieno-gastric artery and posterior intestinal vein (Bourne, 1922) were catheterised (PE 50, Portex). The body wall and inner peritoneum were sutured separately, with a layer of absorbent gelatin sponge (Steriospon, Allen and Hanburys Ltd.), placed between to encourage haemostasis in damaged capillaries and healing of the wound.

The urinary papilla of female dogfish was catheterised (PE 50) for the collection of urine. The tip of the urinary

cannula was flared by heat, and held in position by a single suture. In male dogfish, urine is mixed with secretions from the Wolffian gland in the urogenital sinus (see section II.A.1). Male fish were therefore only used in experiments where urine was not collected.

Following preparative surgery, each fish was placed in a darkened recovery tank (65 x 16 x 18 cm) containing 15 litres of aerated, cooled (9°C) sea water, for 24 hours prior to further study.

3. Experimental Techniques

(a) Measurement of Haemodynamic Response to Catecholamines

Blood pressure and heart rate were monitored using a Washington 400 MD2C oscillograph (Bioscience), with FC137 strain gauge coupler and PT400 pressure transducer (Bioscience). Pressure was recorded from the lieno-gastric artery, which arises from the dorsal aorta at a point just anterior to the head kidney and segmental arteries (from which the renal arteries arise). This blood vessel was connected to the pressure transducer by a short length of polyethylene tubing (PE 50), filled with heparinised dogfish Ringer's solution (section II.B.3(a)). A mercury manometer was used to calibrate the system.

Infusions of catecholamines were administered via the posterior intestinal vein, in conscious and anaesthetised fishes. The catecholamines used were adrenaline bitartrate (Sigma Chemical Co.), and L-noradrenaline-L-tartrate

(Levophed, Winthrop Laboratories), diluted in dogfish Ringer's solution. All infusions were expressed as $\mu g.min^{-1}.Kg^{-1}$ of catecholamine, infused at 5 $\mu l.min^{-1}$ (Sage infusion pump, Model 352).

(b) Overall Kidney Function Measurements

Urine flow was measured by timed collection of urine, via the catheter implanted in the urinary papilla of female dogfish. Urine was collected in pre-weighed polyethylene vials.

Overall glomerular filtration rate was assessed by clearance of inulin from the kidney. Immediately post preparative surgery, a priming injection of 10% (wt/vol) inulin (Sigma Chemical Co.) in dogfish Ringer's solution, was administered via the posterior intestinal vein. The dose was calculated to give a plasma inulin concentration of 1 $mg.ml^{-1}$, assuming an inulin space of 27% of body weight. A continuous intravenous infusion of 2% (wt/vol) inulin was commenced following the priming injection, at a rate of 5 $\mu l.min^{-1}$ (Sage infusion pump, Model 352). After 24 hours, the plasma inulin level was stable at a concentration of 1.0-1.5 $mg.ml^{-1}$.

Throughout the experiment, 60 minute urine collections were taken together with 500 μl mid-point blood samples, via the lieno-gastric artery. Blood samples were immediately centrifuged, and the plasma decanted. The remaining blood cells were resuspended in 500 μl dogfish Ringer's solution and reinjected into the fish. Urine and plasma samples were stored at 4°C for analysis of osmolarity, and at -20°C for

subsequent analysis of inulin concentration.

At least six control collections were taken during the infusion of 5 $\mu l.min^{-1}$ dogfish Ringer's solution. In some animals, control clearance periods were followed by at least six clearance periods during which adrenaline (1 $\mu g.min^{-1}.Kg^{-1}$) was added to the Ringer's solution. Both arterial and venous catheters were found to remain patent without the use of heparin. Blood pressure and heart rate were monitored throughout the experiments (section III.B.3(a)).

(c) Determination of Single Nephron Glomerular Filtration Rate

(i) Preparation of Micropipettes

Micropipettes were made from 1 mm external diameter capillary tubing (Clark Electromedical Instruments), and pulled on a vertical pipette puller (Department of Zoology, University of Hull) using a 0.26 mm diameter platinum filament, heated electrically. Pulled pipettes were forged (De Fonbrune Microforge) to produce pipettes with a sharply tapering shank, but a gently tapering tip. This shape was found to have strength to resist the elastic nature of the tubule wall, yet flexibility to allow grinding of the tip.

Following forging, the tip of the pipette was broken cleanly using watchmakers forceps, to give an external diameter of 5-25 μm. Pipette tips were ground on an air-driven rotating grit-stone, superfused with water, at an

angle of 30°. After grinding for 10 minutes, the pipette was rotated by approximately 90° and grinding repeated for a further minute, to produce a double bevelled tip (Chang, 1975). The pipettes were finally washed with acetone, the tip size remeasured, and stored for use.

(ii) Instrumentation

To minimise the transmission of vibrations from the operating bench to the micropipette, all instruments were fixed to a heavy metal baseplate (Leitz). The kidney surface was viewed through a binocular microscope (Kyowa Instruments Ltd.), illuminated by two flexible fibre optic lights (Fort) giving a high intensity, cold light source. Micropipettes were held in position by a Leitz instrument holder, connected by polyethylene tubing to a 10 ml syringe. The system was air-filled as this was found to be generally more reliable and more controllable than an oil-filled system. The micropipette holder was held in position in a Leitz micromanipulator, which allowed both coarse and fine movement of the micropipette in all directions.

(iii) Preparative Surgery and Exposure of the Kidney

Single nephron glomerular filtration rates were recorded in 12 fishes of both sexes. Preparative surgery involved the catheterisation of the lieno-gastric artery and posterior intestinal vein as described above (section III.B.2). Plasma inulin levels were raised to 1.0-1.5 $mg.ml^{-1}$ as described above.

Each fish was reanaesthetised by addition of 0.0067% MS222 to the recovery tank. After transfer to the operating support, a blood sample was taken to estimate haematocrit level. On rare occasions, a fish was observed to have a low haematocrit, suggesting substantial blood loss following preparative surgery; these fishes were discarded. Blood pressure and heart rate were monitored throughout the experiment (section III.B.3(a)).

An incision was made 0.5 cm ventrally to the distinctive lateral line, from a point caudal to the urinary papilla extending anteriorly by approximately 10 cm. After cauterising the segmental arteries of the body wall, the abdominal organs were gently displaced to reveal the peritoneal membrane overlying the urogenital organs. An incision was made in the peritoneal membrane, revealing the kidney below. The dorsal and lateral edges of the kidney were carefully cleared of connective tissue using watchmakers forceps, to facilitate micropuncture studies. Water-equilibrated paraffin oil (prepared 7 days in advance by shaking equal volumes of water and paraffin oil together), was dropped onto the exposed kidney to prevent surface evaporation.

(iv) Collection of Tubular Fluid and Plasma Samples

Micropipettes were filled with water-equilibrated paraffin oil, dyed with Sudan Black (Gurr). After selection of a suitable tubule on the kidney surface for micropuncture, the micropipette was advanced towards the tubule at an angle

of approximately 45°. Using the fine movement control of the micromanipulator, the pipette tip was gently thrust at the tubule, piercing the wall. Once positioned in the lumen of the tubule, a block of oil, approximately two times the length of the luminal diameter, was ejected and its passage down the tubule observed.

In functional tubules, a second oil block was placed in the tubule before collecting tubular fluid. Timed collections were made holding this oil block in a stationary position, by applying gentle, controlled suction to the syringe and collecting tubular fluid for approximately 10 minutes (range 5-15 minutes). The aspiration of tubular fluid was continually observed to ensure that the oil block was held in a stationary position, there was no leakage of fluid at the puncture site, and that the tip of the pipette remained within the tubular lumen.

On withdrawing the micropipette from the tubule, a small volume of paraffin oil from the kidney surface was drawn up the pipette, to sandwich the sample between water-equilibrated oil, thus preventing evaporation or contamination of the sample. Pipettes were either stored overnight at 4°C for analysis of inulin content the following day, or were analysed immediately. Following each collection of tubular fluid, a 500 µl blood sample was taken as described previously (section III.B.3(b)) for the analysis of plasma inulin concentration. The blood cells were resupended in a volume of dogfish Ringer's solution equal to that of the blood sample, and reinjected as before.

Samples of tubular fluid and plasma were collected from control animals, infused with dogfish Ringer's solution (5 $\mu l.min^{-1}$), and animals having adrenaline (1 $\mu g.min^{-1}.Kg^{-1}$) added to the infusion of Ringer's solution.

(d) Determination of Patterns of Glomerular Perfusion

Patterns of glomerular perfusion were determined in 12 fishes of both sexes, using a modification of the Hanssen (1958) technique. Anaesthesia was induced as described above (section III.B.3(c)(iii)). Blood pressure and heart rate were recorded from the lieno-gastric artery until the infusion of sodium ferrocyanide was commenced.

A freshly prepared solution of 30% (wt/vol) sodium ferrocyanide in dogfish Ringer's solution, was infused via the lieno-gastric artery (Sage infusion pump, Model 351) at a rate of 3 $ml.min^{-1}$ for 40 seconds. Preliminary studies had established this concentration and rate of infusion gave an optimum density of Prussian blue precipitate in the glomerular capillaries and renal tubule.

Immediately following infusion of the sodium ferrocyanide, the animal was sacrificed by severing across the spinal cord, dorsal aorta and posterior cardinal sinuses, at a point anterior to the head kidney. This instantly halted blood flow within the kidney.

The kidneys were rapidly removed and snap-frozen by immersion in iso-pentane, cooled to approximately -160°C by liquid nitrogen. Whilst still frozen, the kidneys were divided into 6 sections (taking 3 each from posterior and

anterior kidney lobes of the female, see section II.C.1), thus ensuring nephrons from all regions of the kidney were sampled. These sections were cut into small pieces and placed in a solution of ferric chloride (60g hydrated ferric chloride dissolved in 95 ml ethanol and 5 ml concentrated hydrochloric acid), pre-cooled to -20°C. The freeze-substitution reaction (conversion of ferrocyanide to Prussian blue precipitate by ferric chloride) was allowed to proceed for 18 hours at -20°C.

Following precipitation of Prussian blue within the kidney, the tissues were macerated in 20% hydrochloric acid at 37°C for 4 hours, to facilitate microdissection of the nephrons. Tissues were stored in a dilute solution of ferric chloride (0.2% ferric chloride and 1% acetic acid in tapwater), which softened the tissues made brittle by the maceration process.

Microdissection of nephrons was performed in glycerol under the binocular microscope (Kyowa Instuments Ltd.), using glass teasing needles pulled from capillary tubing in a bunsen flame. Small pieces of tissue (approximately 1 mm^3) were repeatedly sampled from each section. Individual glomeruli complete with a section of renal tubule, were dissected free and categorised. Three categories of glomerular perfusion were defined:

1. Perfused and filtering (P + F)
2. Perfused but non-filtering (P + NF)
3. Non-perfused and non-filtering (NP)

Table 7 summarises the criteria used to categorise individual glomeruli.

Patterns of glomerular perfusion were determined in control fishes, infused with 5 $\mu l.min^{-1}$ dogfish Ringer's solution, and in a further group of animals to which adrenaline (1 $\mu g.min^{-1}.Kg^{-1}$) was added to the infusion. Adrenaline was infused for at least 30 minutes prior to the infusion of sodium ferrocyanide solution, to ensure a maximum and stable haemodynamic effect.

Studies on two female dogfish indicated that the infusion of sodium ferrocyanide used in these studies, had little effect on cardiovascular parameters. Systemic blood pressure was gradually lowered by approximately 10%, with no effect on heart rate during the infusion.

(e) Determination of Circulating Catecholamine Levels

Plasma levels of the circulating catecholamines adrenaline, noradrenaline and dopamine were analysed in fishes of both sexes. One ml blood samples were taken via the lieno-gastric artery, and centrifuged immediately. Plasma samples (500 μl) were decanted, frozen immediately, and stored at -20°C. Analysis of catecholamines was performed within 2-3 days.

Plasma samples were obtained from 3 groups of dogfish, to study the levels of catecholamines in resting animals, and the effects of anaesthesia and surgery on those levels. The 3 groups were:

Table 7

Categories of glomerular perfusion.

Glomeruli were categorised by the presence or absence of Prussian blue precipitate in the afferent arteriole (where present), glomerular capillaries and neck segment of isolated nephrons.

P + F, perfused and filtering; P + NF, perfused but non-filtering;

NP, non-perfused and non-filtering; +, precipitate present; -, precipitate absent.

	Afferent arteriole	Glomerular capillaries	Neck segment
P + F	+	+	+
P + NF	+	+	-
NP	+/-	-	-

1. Resting, conscious fishes (control group). Blood samples were obtained from fishes at rest, in darkened recovery tanks (section III.B.2), more than 24 hours post-anaesthesia and catheterisation.

2. Anaesthetised fishes (0.0067% (wt/vol) MS222), immediately following catheterisation of the lieno-gastric artery and subsequent suturing (section III.B.2).

3. Anaesthetised fishes (0.0067% (wt/vol) MS222). Samples were taken from fishes having been reanaesthetised more than 24 hours after initial catheterisation. Anaesthesia was maintained for at least one hour prior to samples being taken.

4. Analytical Techniques

(a) Catecholamines

Plasma samples were transported over solid carbon dioxide in a Thermos flask, to the Department of Zoology and Comparative Physiology, University of Birmingham, for analysis with Dr. J. Metcalfe.

Catecholamines (adrenaline, noradrenaline and dopamine) were extracted from the plasma samples by adsorption onto acid-washed alumina (Bioanalytical Systems, Anachem) in alkaline conditions (pH 8.6). Following washing of the alumina, the catecholamines were eluted from the alumina in acid ($HClO_4$) prior to injection into a high pressure liquid chromatography (HPLC) column (Ultrasphere I.P.). Plasma adrenaline, noradrenaline and dopamine levels of the samples

were recorded and compared against peak heights of standard solutions of catecholamines (range 1-100 pmol).

(b) Inulin

(i) Plasma and Urine Samples

Plasma and urinary inulin concentrations were determined by the method of Schreiner (1950). Plasma proteins were precipitated from 50 µl aliquots of plasma by the addition of 150 µl of cadmium sulphate solution (34.67g $3CdSO_4.8H_2O$ in 169 ml 0.5M H_2SO_4 made up to 1000 ml with distilled water), and 50 µl of sodium hydroxide (1.1M), to prevent inaccuracies due to the turbidity of plasma proteins. After centrifugation, 100 µl aliquots of the supernatant were diluted with 900 µl of distilled water.

Five µl samples of urine were diluted with 995 µl of distilled water. To each sample was added 1 ml of freshly prepared resorcinol (0.1% (wt/vol) in 95% alcohol), and 3 ml of 30% sulphuric acid, before being placed in a water bath at 80°C for 25 minutes. The inulin within the samples was hydrolysed to fructose by the acid, prior to the development of colour by the action of resorcinol on the fructose.

Standard solutions of inulin (range 0.5-4.0 mg/100 ml distilled water) were also processed as described above. Standards and samples were read in a spectrophotometer (CE303 grating spectrophotometer, Cecil Instruments Ltd.), at a wavelength of 410 nm.

(ii) Tubular fluid samples

Due to the very small quantities of tubular fluid collected during micropuncture experiments, tubular fluid inulin concentrations were determined by modification of the fluorometric method of Vurek and Pegram (1966). The handling of tubular fluid samples, and the preparation of microcuvettes was achieved by the use of a micromanipulator (Leitz) and binocular microscope (Kyowa instruments Ltd.).

The tubular fluid samples were individually ejected from their micropipettes into water-equilibrated paraffin oil, held within a siliconised glass dish. The diameter of each sphere of tubular fluid was measured using a calibrated micrometer scale within the eye-piece of the binocular microscope, and the volume calculated.

Microcuvettes were constructed from 100 µl Microcap glass tubing (Drummond), cut in half and cleansed thoroughly. A mouth-operated suction pipette was used to introduce 2 µl of freshly prepared dimedone solution (1% (wt/vol) (Sigma Chemical Co.) in ortho-phosphoric acid) into each microcuvette. Known volumes of tubular fluid (range 2-6 nl) were introduced into each microcuvette using a constant volume constriction micropipette (Garland, Brown and Henderson, 1978). The free end of the microcuvette was sealed in a bunsen flame, prior to centrifugation of the sample to allow the second end of the microcuvette to be sealed. Identical volumes of samples and standard solutions of inulin (range 1.5-15.0 ng/nl distilled water) were processed as described above. Tubular fluid samples were prepared in

duplicate or triplicate, and standards in triplicate.

The microcuvettes were placed in a polyurethane foam support and lowered into boiling water for 10 minutes. This allowed the dimedone to react with the inulin producing a fluorescent product. Following rapid cooling in cold water, the microcuvettes were placed in a modified cuvette holder and the fluorescence, which was directly proportional to the concentration of inulin, was measured in a Perkin Elmer LS-5 luminescence spectrophotometer at wavelengths of 405 nm (excitation) and 472 nm (emission).

(c) Osmolarity

Osmolarities of plasma and urine samples were analysed in a Wescor 5100C vapour pressure osmometer using 8 μl samples. Relative osmolyte clearances and relative free water clearances were calculated.

(d) Statistical Analysis

Experimental data were expressed as mean $\pm$ standard error, and analysed by Student's t-test, or paired t-test (refer to individual analyses). Single nephron glomerular filtration rates, and tubular fluid to plasma inulin concentration ratios for control and adrenaline-infused animals, were analysed by a non-parametric method, the Wilcoxon two-sample test (Sokal and Rohlf, 1969). The patterns of glomerular perfusion in control and adrenaline-infused animals were analysed by testing the equality of two percentiles (Sokal and Rohlf, 1969).

C. RESULTS

1. Plasma Catecholamine Levels

Plasma levels of the catecholamines adrenaline, noradrenaline and dopamine, for fishes in three experimental groups, are shown in Figure 33.

Plasma catecholamine levels of resting, conscious fishes (control group), more than 24 hours post-anaesthesia and surgery, showed very low levels of both adrenaline (1.9 $\pm$ 0.9 $pmol.ml^{-1}$), and noradrenaline (4.9 $\pm$ 0.7 $pmol.ml^{-1}$), with no detectable dopamine present. Noradrenaline levels were significantly greater than adrenaline levels in this group (Student's t-test, $P < 0.05$).

Fishes having blood samples taken during anaesthesia and immediately post surgery (Group 2), showed dramatically elevated plasma catecholamine levels. In this group, in contrast to group 1, adrenaline was the predominant circulating catecholamine (Student's t-test, $P < 0.05$), with a plasma level of 559.9 $\pm$ 64.1 $pmol.ml^{-1}$, an increase of more than 293 fold. Noradrenaline plasma levels were also elevated, to 357.4 $\pm$ 11.1 $pmol.ml^{-1}$, a 72 fold increase. Circulating dopamine levels were found to be 5.9 $\pm$ 1.1 $pmol.ml^{-1}$.

Plasma catecholamine levels of fishes during sustained anaesthesia (Group 3), appeared to show noradrenaline to be the predominant circulating catecholamine, but levels were very variable and not significantly different from those of

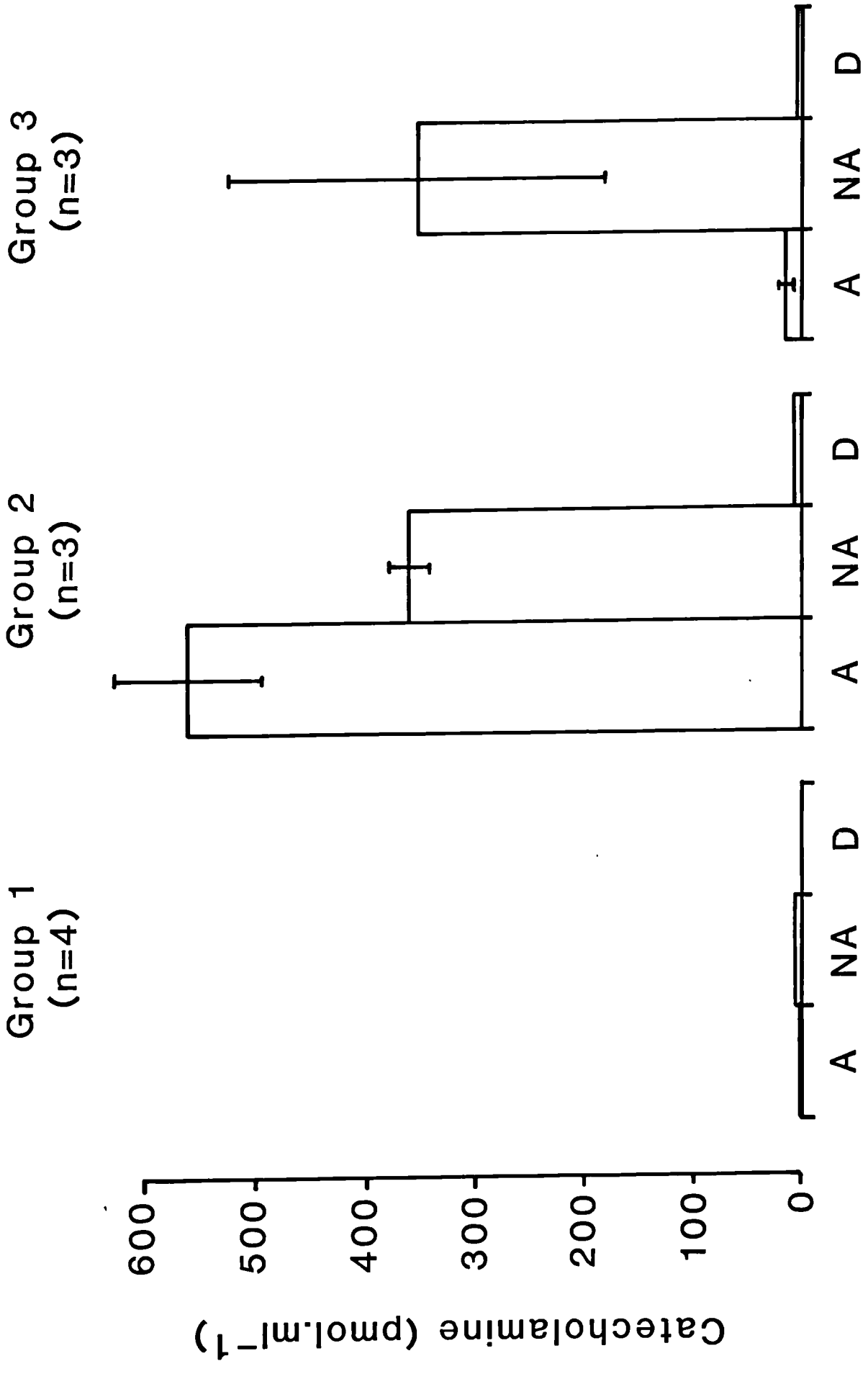
Figure 33

Plasma catecholamine levels in Scyliorhinus canicula.

Group 1, control animals; Group 2, anaesthetised animals following catheterisation surgery; Group 3, anaesthetised animals, (refer to section III.B.3(e)).

A, adrenaline; NA, noradrenaline; D, dopamine.

Values given are mean ± standard error. Number of animals in parentheses.


Group 1
(n=4)
Group 2
(n=3)
Group 3
(n=3)
A
NA
D
Catecholamine (pmol.ml^-1)
0
100
200
300
400
500
600

group 2 (Student's t-test). The mean value of noradrenaline was 351.2 ± 171.2 $pmol.ml^{-1}$. Adrenaline levels were low (14.4 ± 4.2 $pmol.ml^{-1}$) compared to group 2, where anaesthesia was accompanied by surgery. Dopamine levels of group 3 fishes were elevated in comparison to the control group (2.8 ± 1.4 $pmol.ml^{-1}$), but were not significantly lower than those of group 2 fishes (Student's t-test).

2. Haemodynamic Effects of Adrenaline and Noradrenaline

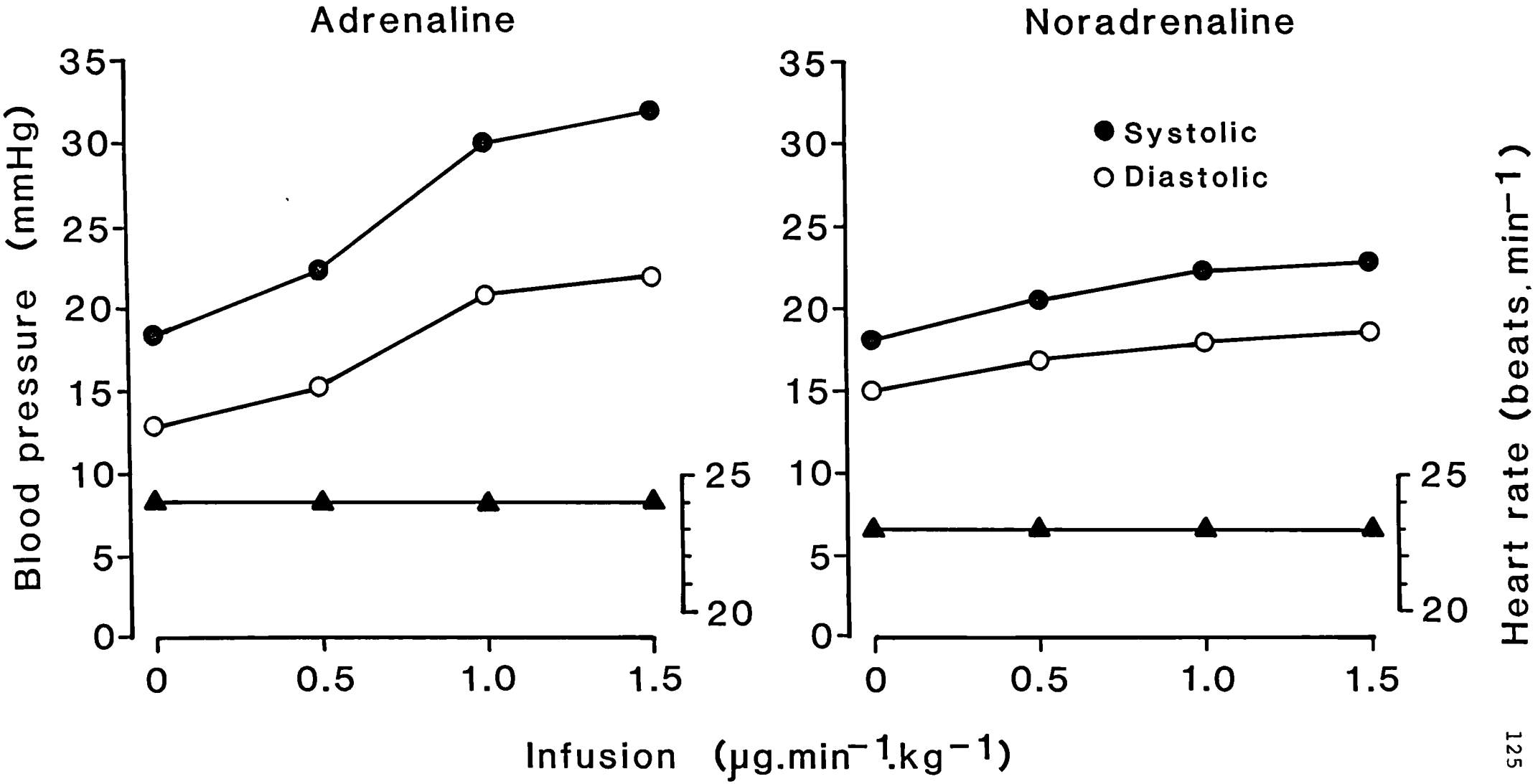
Figure 34 shows typical effects of infusions of adrenaline and noradrenaline, on the systolic and diastolic blood pressure and heart rate in two anaesthetised dogfish.

For each concentration infused, the effect on both systolic and diastolic blood pressure was seen to be greater in the adrenaline-infused fish than in the noradrenaline-infused fish. During infusion of 1.5 $\mu g.min^{-1}.Kg^{-1}$ adrenaline, the maximum dose employed, systolic blood pressure was raised from 18.2 to 31.9 mm Hg, an increase of 75.0%, whilst diastolic pressure was elevated from 12.9 to 22.0 mm Hg, a rise of 70.5%. However, the same dose of noradrenaline only produced a 27.1% increase in systolic pressure (18.1 to 23.0 mm Hg), and a 23.8% increase in diastolic pressure (15.1 to 18.7 mm Hg). Pulse pressure (systolic - diastolic pressure) was also elevated to a greater extent at each concentration in the adrenaline-infused fish than in the noradrenaline-infused fish. For example, at a concentration of 1.5 $\mu g.min^{-1}.Kg^{-1}$, pulse pressure was

Figure 34

Typical cardiovascular effects of intravenous infusions of adrenaline and noradrenaline in Scyliorhinus canicula.

Infusions of adrenaline and noradrenaline (0.5, 1.0 and 1.5 $\mu g.min^{-1}.Kg^{-1}$) in dogfish Ringer's solution, were infused at 5 $\mu l.min^{-1}$. Animals were anaesthetised in 0.0067% (wt/vol) MS222.


Adrenaline
Noradrenaline
Systolic
Diastolic
Blood pressure (mmHg)
Heart rate (beats.min⁻¹)
Infusion (μg.min⁻¹.kg⁻¹)
35
30
25
20
15
10
5
0
25
20
0
0.5
1.0
1.5

elevated by 86.8% during infusion of adrenaline, yet by only 41.9% during infusion of noradrenaline.

Heart rate was unaltered by infusion of either adrenaline or noradrenaline.

Infusions of adrenaline were found to consistently produce greater elevation of blood pressure and pulse pressure than noradrenaline infusions of the same concentration. For this reason, and in view of adrenaline being the predominant circulating catecholamine released in response to severe stress (section III.C.1), adrenaline alone was used in subsequent experiments.

An infusion of 1.0 $\mu g.min^{-1}.Kg^{-1}$ adrenaline in dogfish Ringer's solution was used in the following experiments, infused at a rate of 5 $\mu l.min^{-1}$. This infusion was calculated to give a plasma adrenaline load of approximately 500 $pmol.ml^{-1}$, assuming an adrenaline space of 10%, and a life of approximately 10 minutes in plasma prior to catabolism (Ungell and Nilsson, 1983).

Table 8 summarises the effects of adrenaline on systemic blood pressure and heart rate in both the conscious and anaesthetised (0.0067% MS222) dogfish. The effects of anaesthesia alone on systolic and diastolic blood pressure and heart rate were very dramatic, causing a 42.1% decrease in systolic pressure (Student's t-test, $P < 0.001$), and a 47.7% decrease in diastolic pressure (Student's t-test, $P < 0.001$). Heart rate was significantly elevated by anaesthesia, showing

Table 8

Effects of adrenaline on systemic blood pressure and heart rate in *Scyliorhinus canicula*.

1 $\mu g.min^{-1}.Kg^{-1}$ adrenaline was infused intravenously in dogfish Ringer's solution at a rate of 5 $\mu l.min^{-1}$, following a control infusion of Ringer's solution only. Values given are mean $\pm$ standard error. Number of animals in parentheses. Statistical analysis using paired t-test.

***, $P < 0.001$

**, $P < 0.01$

*, $P < 0.05$

NS, Not significant

	Conscious Fishes		Anaesthetised Fishes	
	Control -infused	Adrenaline -infused	Control -infused	Adrenaline -infused
Systolic blood pressure (mm Hg)	28.0 ± 2.4 (5)	49.9 ± 2.8** (5)	16.2 ± 0.6 (11)	26.3 ± 1.4*** (11)
Diastolic blood pressure (mm Hg)	24.3 ± 2.2 (5)	40.8 ± 1.5** (5)	12.7 ± 0.4 (11)	19.9 ± 0.8*** (11)
Heart rate ($beats.min^{-1}$)	20.4 ± 0.7 (5)	23.5 ± 0.7* (5)	24.6 ± 0.6 (11)	24.7 ± 0.7NS (11)

a 20.6% increase in rate (Student's t-test, $P < 0.001$).

In the conscious fish, adrenaline significantly elevated blood pressure (Table 8). Systolic pressure increased by 78.2%, and diastolic pressure increased by 67.9% ($P < 0.01$). Heart rate also showed a significant elevation of 15.2% ($P < 0.05$).

Blood pressure of the anaesthetised fish was also raised by the action of adrenaline, but to a much lesser degree than that shown by the conscious fish. Systolic pressure was increased by 62.3%, and diastolic pressure by 56.7% ($P < 0.001$). Heart rate, already elevated by the action of the anaesthetic, remained stable on addition of adrenaline to the control infusion.

Figure 35 shows typical effects of infusion of adrenaline on blood pressure and heart rate in the conscious and anaesthetised fish. A maximum response to the infusion was typically reached within 25 minutes, with the response being sustained for the duration of the infusion. Following withdrawal of the infusion of adrenaline, control values of blood pressure and heart rate in both conscious and anaesthetised fishes, were typically reattained within 25 minutes (Figure 36).

Figure 35

Typical cardiovascular effects of adrenaline in the conscious and anaesthetised dogfish.

Intravenous infusion of 1 $\mu g.min^{-1}.Kg^{-1}$ adrenaline in dogfish Ringer's solution, was commenced at the arrow, at a rate of 5 $\mu l.min^{-1}$, following a control infusion of Ringer's solution only. Anaesthesia was maintained by 0.0067% (wt/vol) MS222.

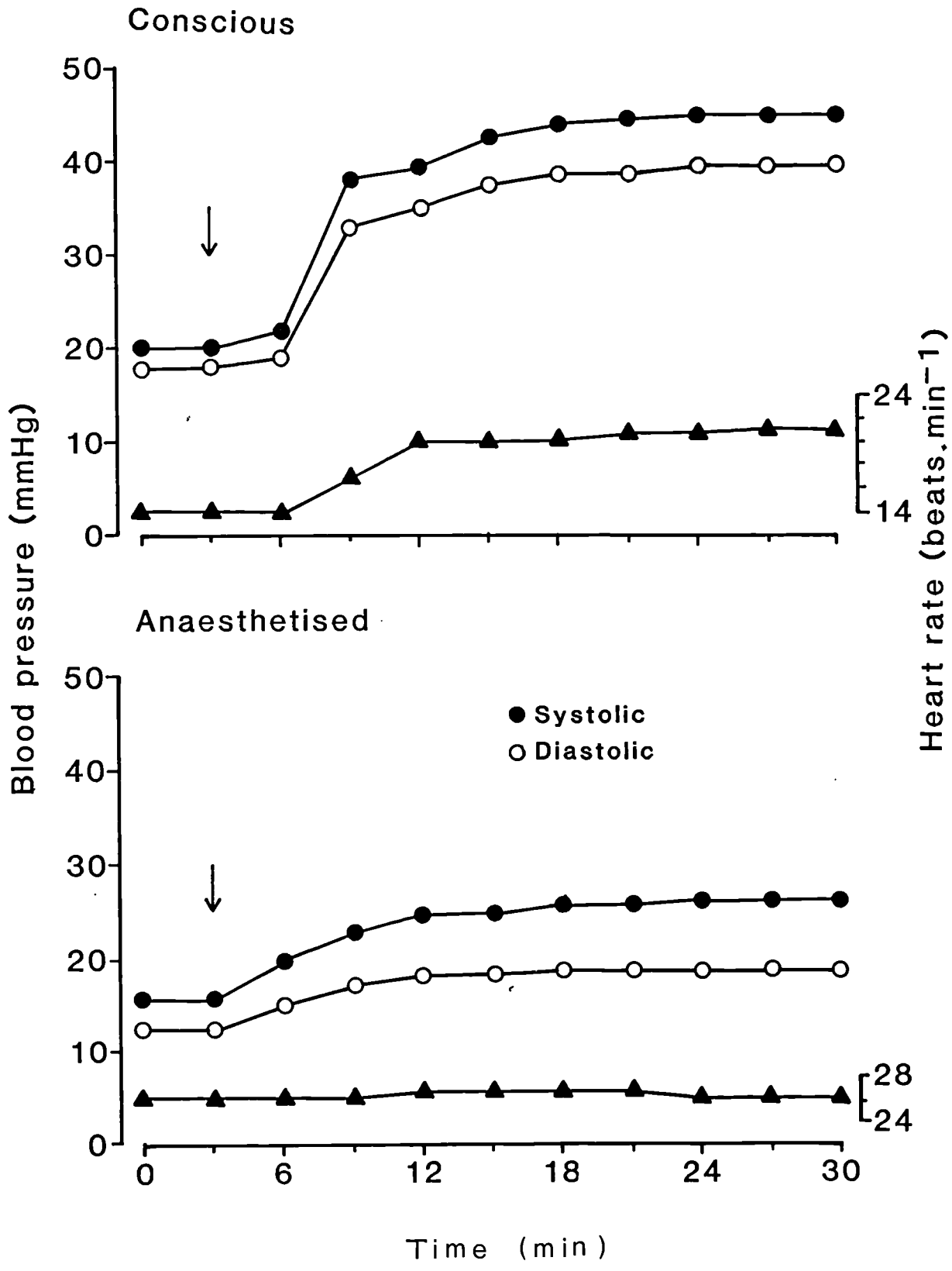
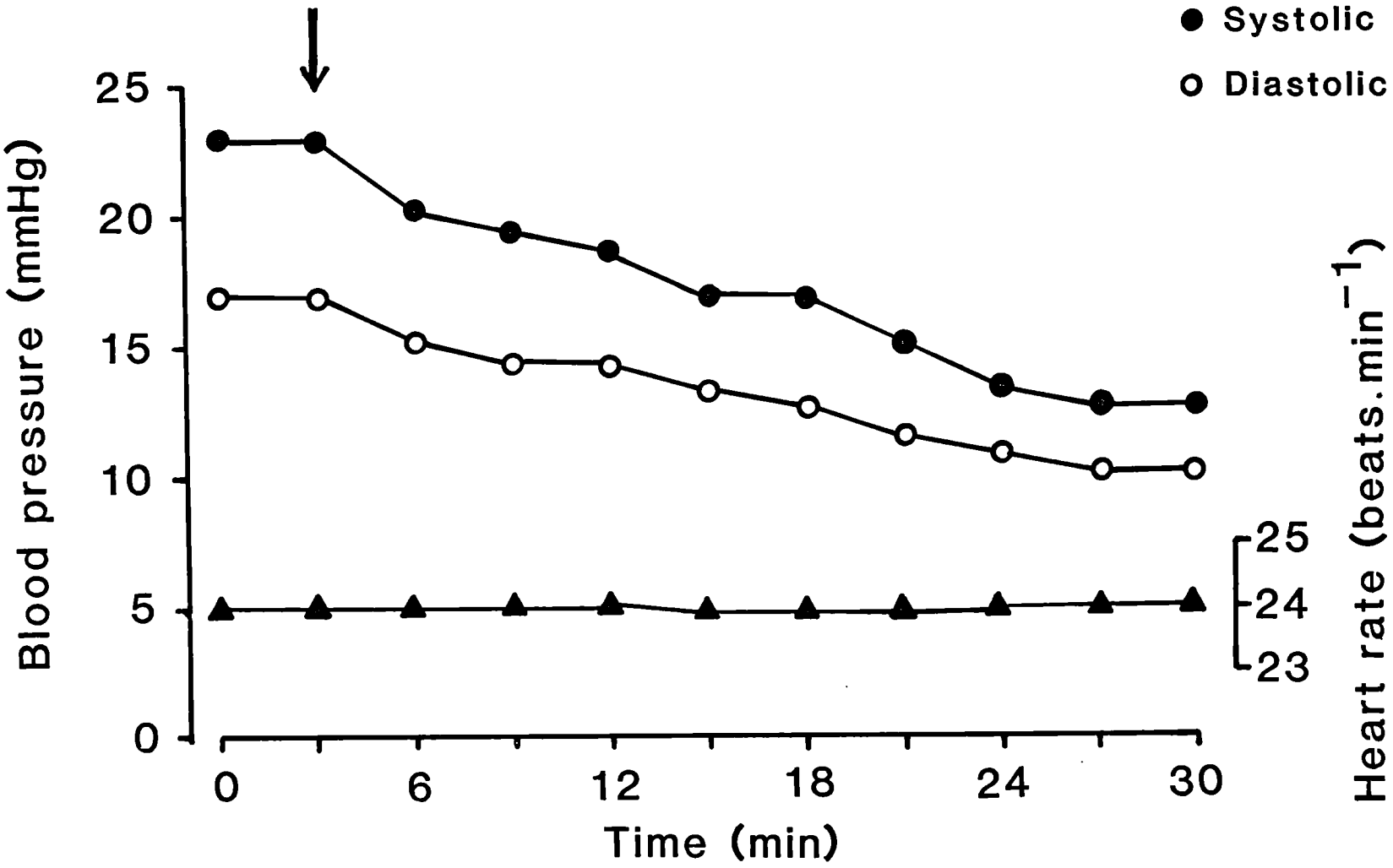

Conscious
Anaesthetised
Blood pressure (mmHg)
Heart rate (beats.min⁻¹)
Systolic
Diastolic
50
40
30
20
10
0
24
14
28
24
0
6
12
18
24
30
Time (min)

Figure 36

Typical cardiovascular effects of cessation of adrenaline infusion in the anaesthetised dogfish.
Infusion of 1 $\mu g.min^{-1}.Kg^{-1}$ adrenaline was discontinued at arrow. Infusion of 5 $\mu l.min^{-1}$ dogfish Ringer's solution was maintained throughout.


Systolic
Diastolic
Blood pressure (mmHg)
25
20
15
10
5
0
0
6
12
18
24
30
Time (min)
25
24
23
Heart rate (beats.min^{-1})

3. Renal Function

(a) Conscious Animals

Urine output was found to be influenced by the activity of the conscious fish, and therefore was very variable. Under normal circumstances, urine was discharged from the large urinary sinuses in spurts, during periods of spontaneous swimming. Long clearance periods (60 minutes) were therefore taken to overcome the intermittent output of urine.

Figure 37 shows the typical effect of adrenaline on urine flow in the conscious dogfish. Mean urine flow was increased by more than 2.7 fold.

Renal function of conscious, resting dogfish is summarised in Table 9. Infusion of adrenaline produced a marked urinary diuresis. This significant increase in urine flow ($P < 0.01$) was not associated with a change in the urine to plasma inulin concentration ratio (Table 9). An increase in glomerular filtration rate ($P < 0.01$) was therefore responsible for the observed diuresis. Figure 38 shows the linear relationship between glomerular filtration rate and urine flow in fishes infused with dogfish Ringer's solution and adrenaline.

The measurement of urine and plasma osmolarities showed the dogfish to be producing urine slightly hypotonic to body fluids. The mean ratio of urine to plasma osmolarity was less than unity (0.98 ± 0.01) and was unchanged by the infusion of adrenaline. The relative clearance of osmolytes by the kidney was not significantly altered by adrenaline, nor was the

Figure 37

Effect of adrenaline on urine flow in the conscious dogfish, Scyliorhinus canicula. 1 $\mu g.min^{-1}.Kg^{-1}$ adrenaline was added to the control infusion of 5 $\mu l.min^{-1}$ dogfish Ringer's solution at arrow.

$\dot{V}$, urine flow.

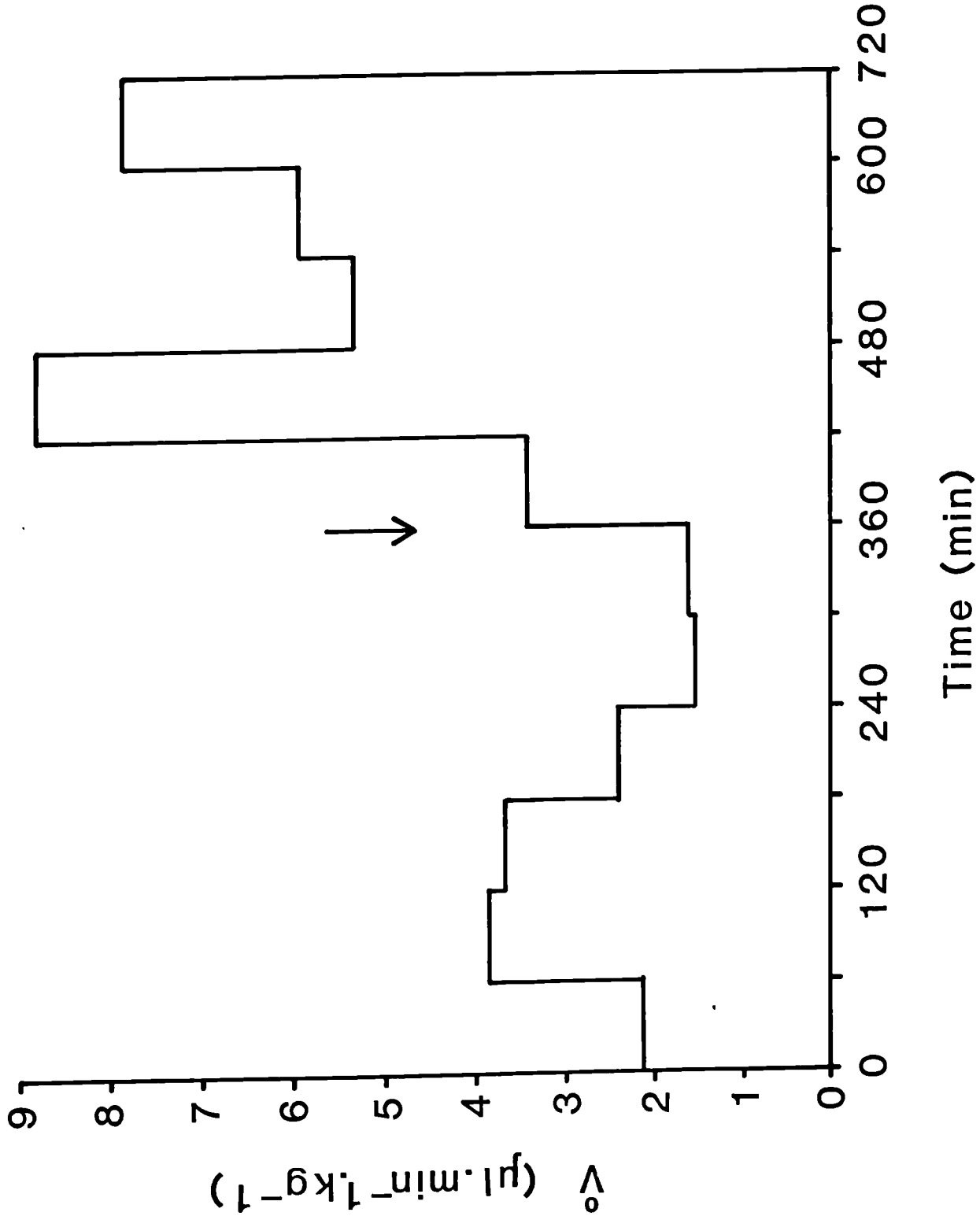

$\dot{V}_o$ (µl.min^{-1}.kg^{-1})
0
1
2
3
4
5
6
7
8
9
0
120
240
360
480
600
720
Time (min)

Table 9

Renal function of Scyliorhinus canicula.

Control animals were infused with dogfish Ringer's solution at a rate of 5 $\mu l.min^{-1}$. 1 $\mu g.min^{-1}.Kg^{-1}$ adrenaline was added to the control infusion in adrenaline-infused animals.

$\dot{V}$, urine flow ($\mu l.min^{-1}.Kg^{-1}$); $U:P_{In}$, urine to plasma inulin concentration ratio; GFR, glomerular filtration rate ($\mu l.min^{-1}.Kg^{-1}$); $U:P_{osm}$, urine to plasma osmolar ratio; C_{osm}/GFR, relative clearance of osmolytes (%); C_{H_2O}/GFR, relative free water clearance (%).

Values given are mean $\pm$ standard error. Number of animals in parentheses. Statistical analysis using Student's t-test.

**, $P < 0.01$

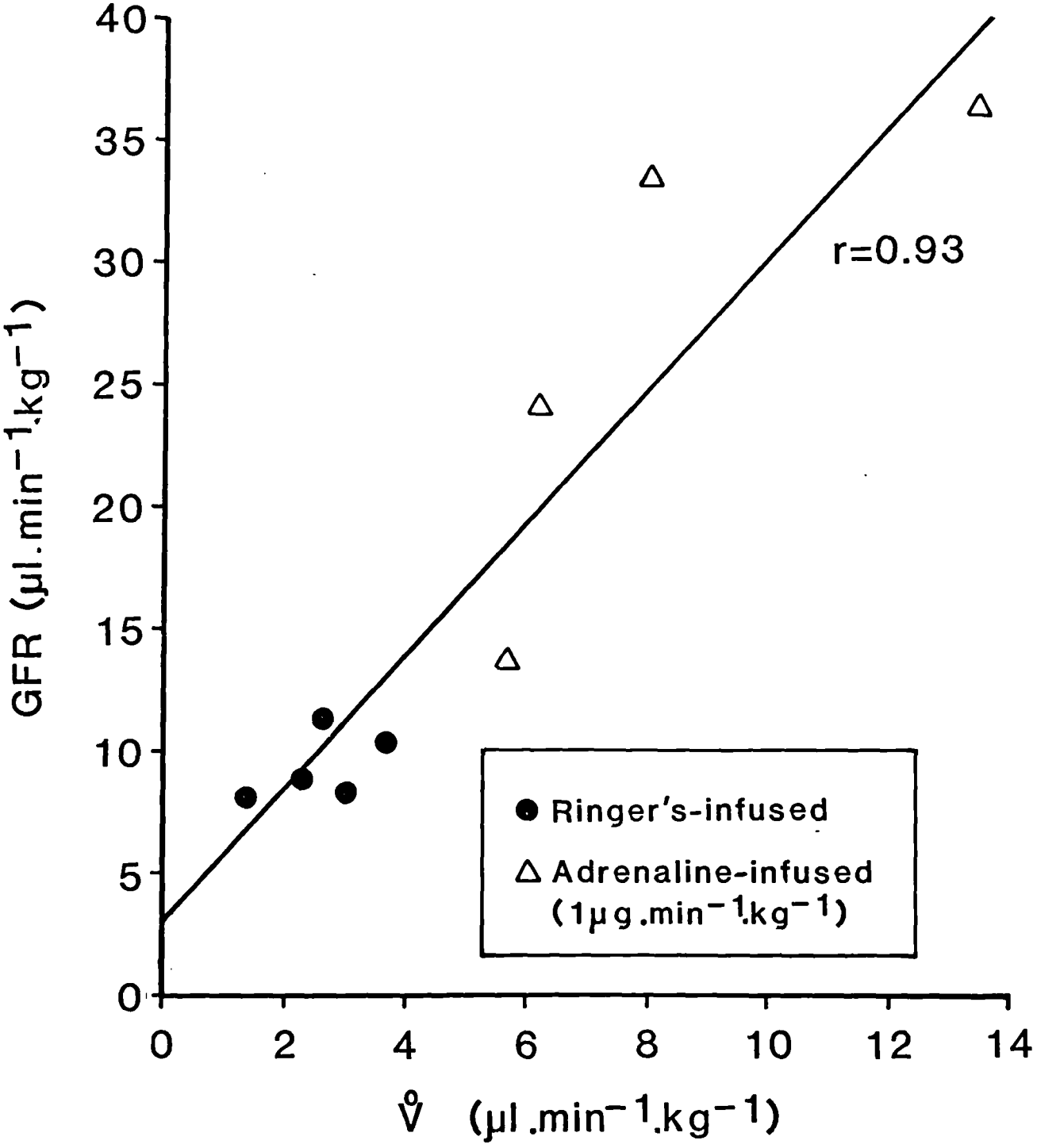
NS, Not significant

	Control-infused	Adrenaline-infused	
$\dot{V}$	2.33 ± 0.23 (16)	8.77 ± 1.45 (5)	**
$U:P_{In}$	3.38 ± 0.26 (6)	3.25 ± 0.44 (4)	NS
GFR	9.45 ± 0.52 (6)	26.57 ± 5.21 (4)	**
$U:P_{osm}$	0.98 ± 0.01 (4)	0.98 ± 0.01 (4)	NS
C_{osm}/GFR	29.43 ± 3.37 (4)	31.94 ± 4.52 (4)	NS
C_{H_2O}/GFR	0.60 ± 0.19 (4)	0.74 ± 0.22 (4)	NS

Figure 38

Relationship between glomerular filtration rate and urine flow in Scyliorhinus canicula.

The significant value of r (0.93), calculated by regression analysis, indicates a linear correlation between glomerular filtration rate (GFR) and urine flow ($\dot{V}$), in control and adrenaline-infused animals.


40
35
30
25
20
15
10
5
0
0
2
4
6
8
10
12
14
GFR (µl.min⁻¹.kg⁻¹)
V̊ (µl.min⁻¹.kg⁻¹)
r=0.93
Ringer's-infused
Adrenaline-infused
(1µg.min⁻¹.kg⁻¹)

relative free water clearance, indicating that adrenaline did not affect net renal tubular function in the dogfish.

Figure 39 shows measurements of renal function in five female dogfish, and the effects of adrenaline on these parameters.

(b) Anaesthetised Animals

Light anaesthesia (0.0067% (wt/ vol) MS222) markedly affected renal function in the dogfish. Figure 40 shows the decrease in urine output which occurred during anaesthesia. In the five fish under study in this series of experiments, mean urine output decreased from 2.13 $\pm$ 0.45 to 0.46 $\pm$ 0.09 $\mu l.min^{-1}.Kg^{-1}$, (paired t-test, $P < 0.05$).

4. Single Nephron Studies

(a) Single Nephron Glomerular Filtration Rates

The distribution of single nephron glomerular filtration rates (SNGFR) in control and adrenaline-infused dogfish are shown in Figure 41.

SNGFR values of fishes receiving a 5 $\mu l.min^{-1}$ infusion of dogfish Ringer's solution (control infusion) ranged from 1.54-26.00 $nl.min^{-1}$, with a mean value of 9.46 $\pm$ 1.39, (n = 26). Values for adrenaline-infused fishes extended over a much greater range, of 2.40-64.63 $nl.min^{-1}$, with a mean value of 22.94 $\pm$ 3.60, (n = 22). Non-parametric statistical analysis (Wilcoxon two-sample test) showed SNGFR values for control-infused and adrenaline-infused fishes to be

Figure 39

Effects of adrenaline on renal function of Scyliorhinus canicula.

The effects of infusing 1 $\mu g.min^{-1}.Kg^{-1}$ adrenaline in dogfish Ringer's solution at 5 $\mu l.min^{-1}$, following control infusion, are shown for individual animals.

$\dot{V}$, urine flow ($\mu l.min^{-1}.Kg^{-1}$); GFR, glomerular filtration rate ($\mu l.min^{-1}.Kg^{-1}$); $U:P_{In}$, urine to plasma inulin concentration ratio; $U:P_{osm}$, urine to plasma osmolar ratio; C, control infusion; A, adrenaline infusion.

Statistical analysis using paired t-test.

*, $P < 0.05$

NS, Not significant

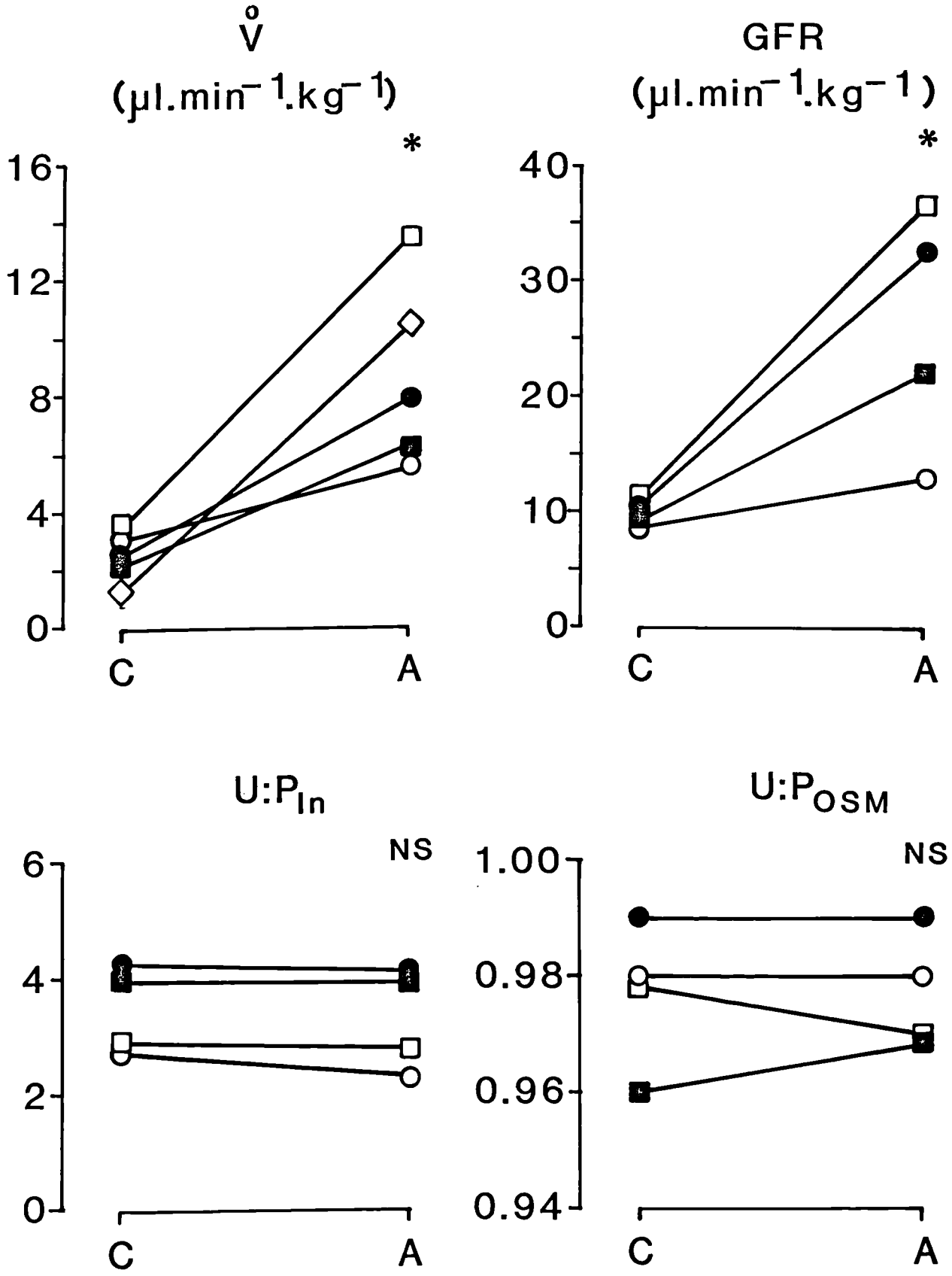

$\dot{V}$
(µl.min^{-1}.kg^{-1})
*
16
12
8
4
0
C
A
GFR
(µl.min^{-1}.kg^{-1})
*
40
30
20
10
0
C
A
U:P_{In}
NS
6
4
2
0
C
A
U:P_{OSM}
NS
1.00
0.98
0.96
0.94
C
A

Figure 40

Effects of anaesthesia on urine output of *Scyliorhinus canicula*.

Urine output prior to, and during MS222 (0.0067% wt/vol) anaesthesia is shown for 5 fishes.

Mean values ± standard error are shown.

Statistical analysis using paired t-test.

*, $P < 0.05$

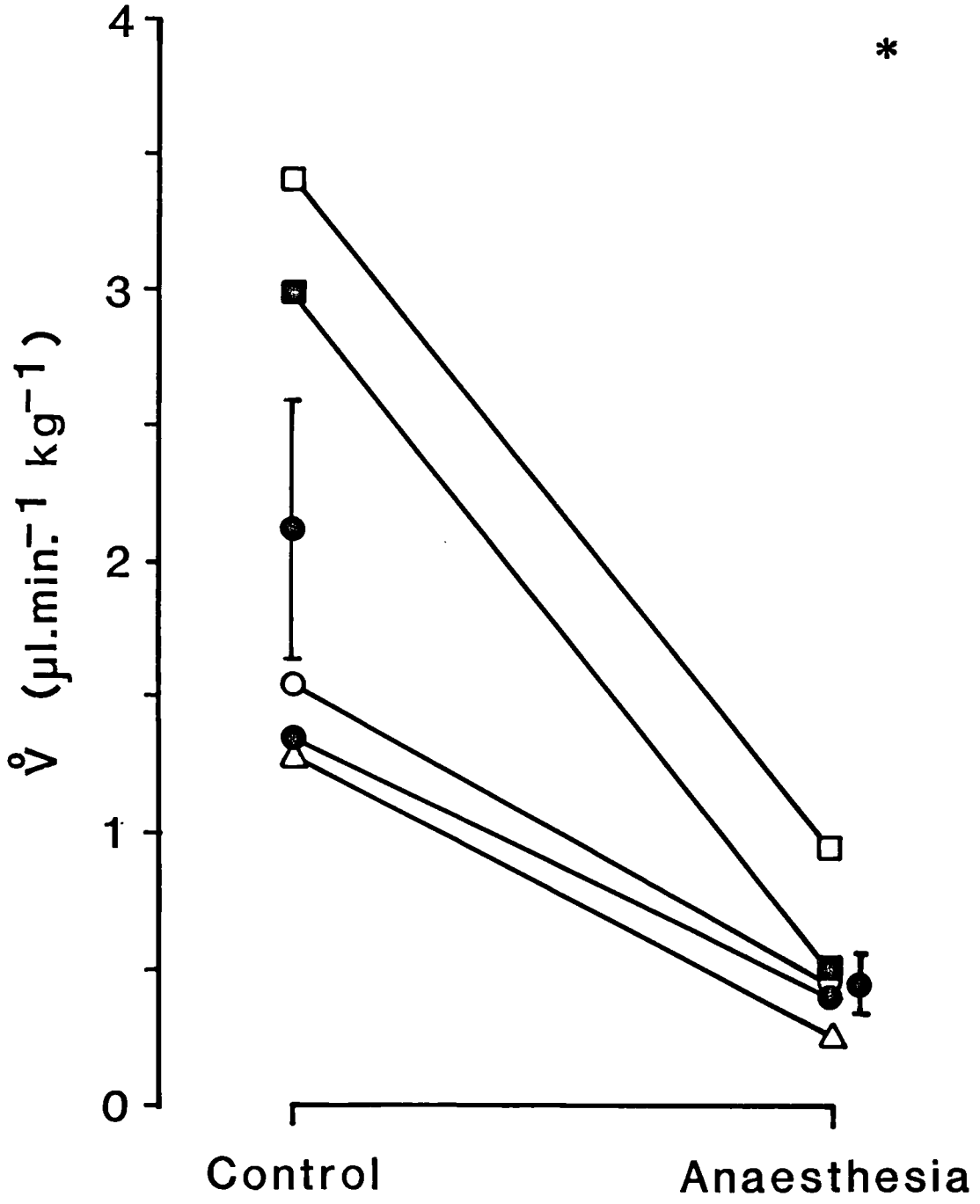

4
3
2
1
0
*
$\dot{V}$ (µl.min.$^{-1}$ kg^{-1})
Control
Anaesthesia

Figure 41

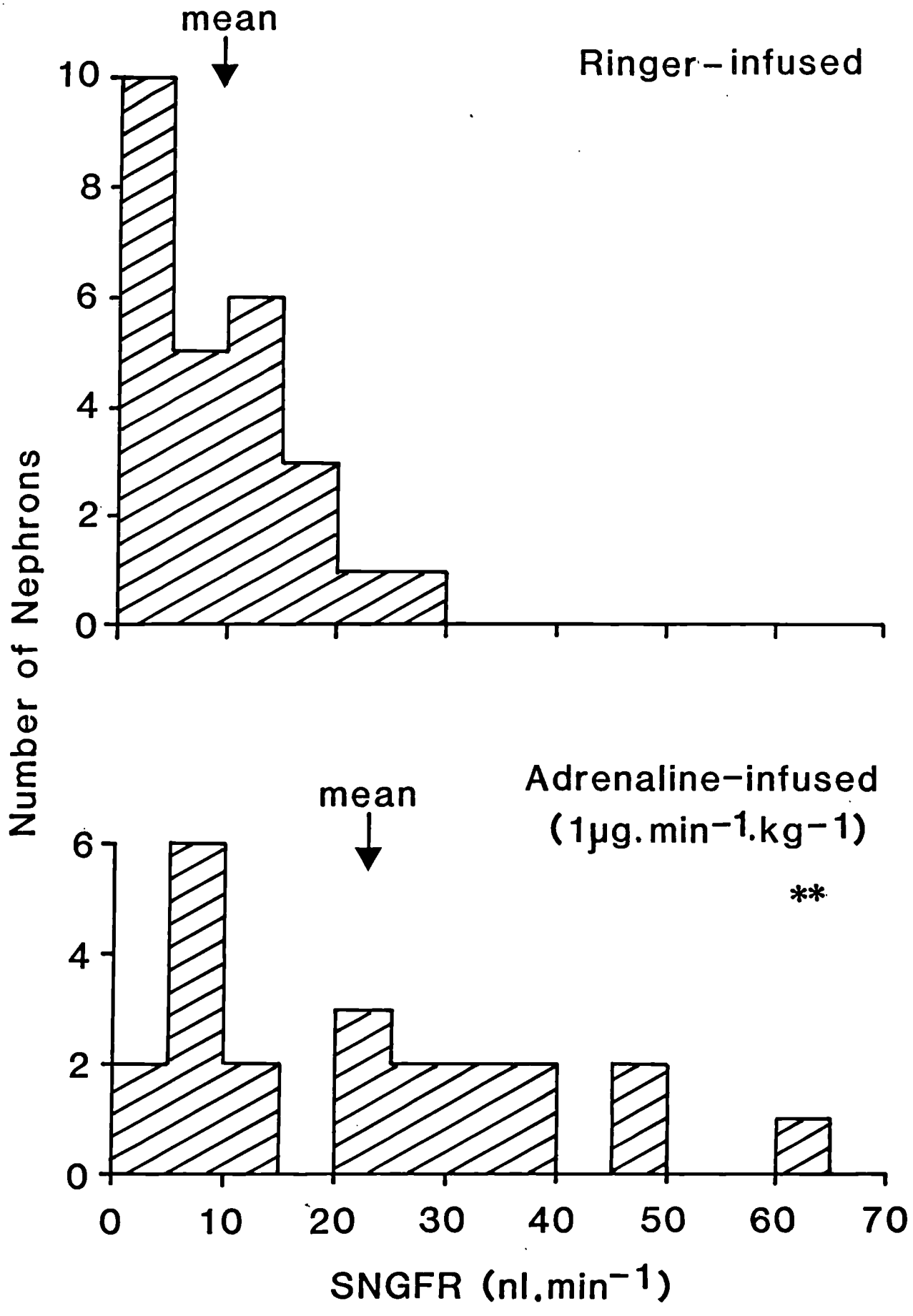
Distribution of single nephron glomerular filtration rates in Scyliorhinus canicula.

Control animals were infused with 5 $\mu l.min^{-1}$ dogfish Ringer's solution. 1 $\mu g.min^{-1}.Kg^{-1}$ adrenaline was added to the control infusion in adrenaline-infused animals.

SNGFR, single nephron glomerular filtration rate.

Statistical analysis using Wilcoxon two-sample test.

**, $P < 0.01$


mean
Ringer-infused
10
8
6
4
2
0
Number of Nephrons
mean
Adrenaline-infused
(1µg.min-1.kg-1)
**
6
4
2
0
0
10
20
30
40
50
60
70
SNGFR (nl.min-1)

significantly different ($P < 0.01$).

The distributions of SNGFR shown in Figure 41 represent only those nephrons which were filtering at the time of tubular puncture, and therefore permitted measurement of SNGFR. However, it should be noted that a proportion of punctured tubules showed no movement of the introduced oil block, and hence appeared to be non-functional (see section III.C.5). Observation of these tubules over a period of time often revealed subsequent movement of the oil block, indicating recommencement of filtration.

The mean tubular fluid to plasma inulin concentration ratio for control-infused fishes (3.84 ± 0.34, $n = 26$) was found to be similar to the mean urine to plasma inulin concentration ratio value obtained from both control and adrenaline-infused conscious fishes (Table 9), but significantly less (Wilcoxon two-sample test, $P < 0.001$) than the mean tubular fluid to plasma inulin concentration ratio for adrenaline-infused fishes (7.10 ± 0.61, $n = 22$).

(b) Puncture Site

Due to the extreme length and complex configurational structure of the dogfish nephron (section II.C.5(a)), it was not possible to accurately identify the position of the puncture site along the length of the nephron *in vivo*. Tubular fluid samples were predominantly obtained from the third proximal segment. This segment was located on the lateral and dorsal margins of the posterior kidney lobes, and was easily identified by its white tubular wall composed of

cuboidal epithelium, enclosing a relatively large lumen (section II.C.5(c)(iv)). Some collections were made from the very large, characteristically yellow-coloured second proximal tubular segment (section II.C.5(c)(iii)), but due to the very tall columnar cells of this segment, great resistance was encountered in penetrating the tubular wall.

5. Patterns of Glomerular Perfusion

Figure 42 summarises the patterns of glomerular perfusion found within the dogfish kidney, in control and adrenaline-infused animals. The percentages of nephrons within the three categories of glomerular perfusion were found to be similar for fishes within the same experimental group. Fishes receiving the control infusion of 5 $\mu l.min^{-1}$ dogfish Ringer's solution, showed a range of 84.5-100.0% perfused and filtering nephrons, with a mean value of 93.8% $\pm$ 2.0 (n = 7); a range of 0-13.0% perfused but non-filtering nephrons, with a mean value of 4.4% $\pm$ 1.9 (n = 7); and a range of 0-3.8% non-perfused nephrons, with a mean value of 1.8% $\pm$ 0.6 (n = 7).

Fishes having received the infusion of adrenaline showed a range of 63.0-75.6% perfused and filtering nephrons, with a mean value of 69.5% $\pm$ 2.4 (n = 5); a range of 3.0-17.7% perfused but non-filtering nephrons, with a mean value of 9.0% $\pm$ 2.5 (n = 5); and a range of 19.0-28.0% non-filtering nephrons, with a mean value of 21.5% $\pm$ 1.7 (n = 5).

Thus, the infusion of adrenaline caused a change in the

Figure 42

Effects of adrenaline on patterns of glomerular perfusion in Scyliorhinus canicula.

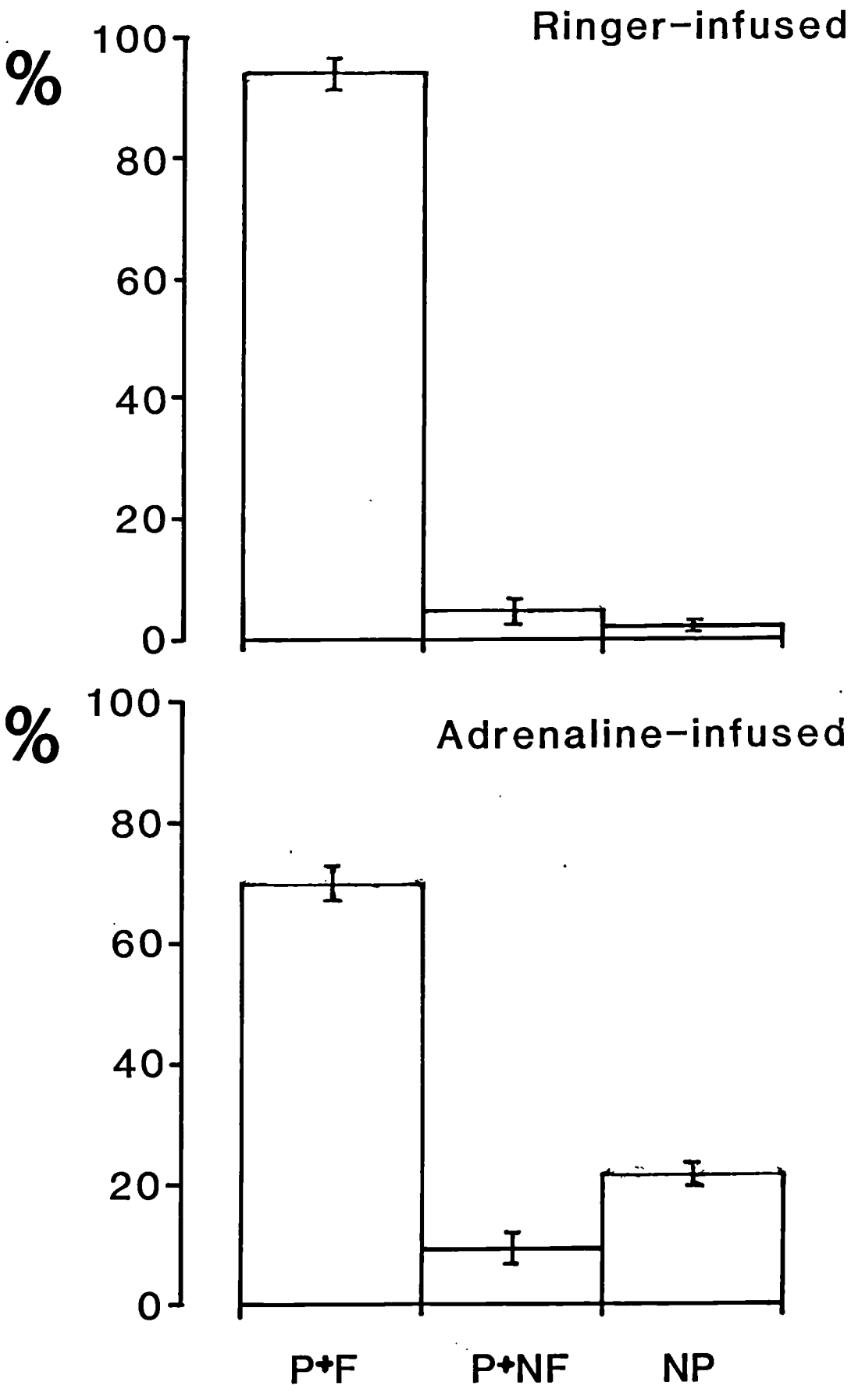
Control group animals were infused with dogfish Ringer's solution at 5 $\mu l.min^{-1}$. 1 $\mu g.min^{-1}.Kg^{-1}$ adrenaline was added to the control infusion in adrenaline-infused animals.

Mean values $\pm$ standard error are shown.

Control group, 1224 nephrons sampled,

Adrenaline group, 1215 nephrons sampled.

Categories of glomerular perfusion (refer to section III.B.3(d)): P + F, perfused and filtering; P + NF, perfused but non-filtering; NP, non-perfused and non-filtering.


Ringer-infused
%
100
80
60
40
20
0
Adrenaline-infused
%
100
80
60
40
20
0
P+F
P+NF
NP

patterns of glomerular perfusion in the dogfish. The major effect of adrenaline was to reduce the number of filtering nephrons, and hence to increase the proportion of non-filtering nephrons within the kidney ($P < 0.001$, testing the equality of two percentiles).

Non-filtering nephrons included both those arterially perfused but non-filtering, and non-perfused nephrons. Nephrons in which the glomerular capillary tuft was perfused, yet they were not filtering, accounted for only 4.4% of nephrons in the control fishes, and 9.0% in adrenaline-infused fishes. Non-perfused nephrons accounted for only 1.8% of nephrons in control fishes, but occurred more commonly in adrenaline-infused fishes, in 21.5% of nephrons.

D. DISCUSSION

The use of general anaesthetics in fishes has been reviewed by the Fisheries Research Board of Canada (1964). In anaesthetising any animal, certain physiological parameters are unavoidably altered. Campbell and Davies (1963) observed the effects of high doses of MS222 (Tricaine methane sulphonate) on the cardiac and respiratory systems of three marine elasmobranch species. They reported a bradycardia and fall in respiratory rate. Low doses of MS222, comparable with those used in the present study, were found to either increase, or have no effect on the heart rate of Squalus acanthias, and to reduce stroke volume, cardiac output and blood pressure (Peirce, Peirce and Peirce, 1967). In the present study, light MS222 anaesthesia was found to increase heart rate slightly, and depress blood pressure.

Urine output was found to be significantly reduced by light MS222 anaesthesia. The depression of both blood pressure and urine output during anaesthesia suggests a lack of autoregulation of renal blood flow in the dogfish. Urine output was however, clearly influenced by the activity of the conscious fish, with discharge of urine from the urinary sinuses occurring during periods of spontaneous activity. The anaesthetised fish was obviously inactive, and urine was observed to accumulate in the large urinary sinuses during experiments involving laparotomy.

Catecholamines are released in response to stress in many species of fishes. Levels of adrenaline, noradrenaline and

dopamine have been shown to be elevated within minutes of the onset of stress, and may be maintained at high levels for several hours (Opdyke et al., 1982; Mazeaud and Mazeaud, 1981).

The removal of catecholamines from the circulation of elasmobranchs has been shown to occur in two phases (Ungell and Nilsson, 1983). Initially, there is a rapid loss of catecholamines from the plasma into the tissues (probably including storage in adrenergic neurons), representing approximately 80% of catecholamines removed within 10 minutes. Complete degradation of the catecholamines within the tissues, and excretion of catabolites via the kidneys occurs over a period of several days. A similar pattern of degradation and excretion of catecholamines has been demonstrated in the Atlantic cod (Ungell and Nilsson, 1979).

Circulating catecholamines have been shown to play an important role in the control of the vascular system of elasmobranchs (Davies and Rankin, 1973; Nilsson, Holmgren and Grove, 1975). The rapid disappearance of catecholamines from plasma is therefore of great importance to the functioning of this hormonal system.

Plasma levels of adrenaline and noradrenaline in the resting, unrestrained dogfish, Scyliorhinus canicula, were found to be very low, with no dopamine detected. These results are in agreement with resting levels in Squalus acanthias (Abrahamsson, 1979), but are much lower than previously recorded values in Scyliorhinus canicula (Butler, Taylor, Capra and Davison, 1978; Mazeaud and Mazeaud, 1981).

The possibility of degradation of catecholamines during storage was considered, but as plasma catecholamine levels of samples taken from stressed fishes at the same time, and stored under the same conditions, were very high, it appeared that the low plasma catecholamine levels of resting fishes reflected the unstressed state. The great variation in reported resting levels of catecholamines, suggests that elasmobranchs may be very easily stressed.

The stressful effects of surgical trauma during MS222 anaesthesia, were shown by the dramatically elevated levels of both adrenaline and noradrenaline, with adrenaline being the predominant catecholamine. Levels of the catecholamines under these conditions were found to be in the same range as values recorded under severe hypoxic conditions in Scyliorhinus canicula (Butler et al., 1978; Mazeaud and Mazeaud, 1981), although here noradrenaline predominated. Dopamine levels were relatively low, as found in previous studies (Butler et al., 1978). It is thought that dopamine, found in the chromaffin tissues, may serve exclusively as a precursor of noradrenaline (Dalmaz and Peyrin, 1978).

Submersion in MS222 anaesthetic alone, perhaps not suprisingly, appeared to be much less stressful than when accompanied by surgery. Adrenaline levels were only slightly raised, while noradrenaline levels increased significantly, to levels similar to those found in surgically stressed fish. Butler, Taylor and Davison (1979) recorded a similar increase in the level of noradrenaline only, in Scyliorhinus canicula, during mild hypoxia. It is probable that release of

catecholamines in response to stress, is dependent upon both the nature and the degree of stress imposed.

The secretion of variable levels of adrenaline and noradrenaline under differing stressful circumstances, may be physiologically important in view of the differing circulatory responses to the two catecholamines. For example, in Squalus acanthias, adrenaline has been shown to increase central venous pressure and caudal vein pressure, whilst noradrenaline decreases pressure in these veins. Stroke volume is increased by adrenaline but decreased following administration of noradrenaline, and noradrenaline causes dilatation of cutaneous blood vessels whilst adrenaline had no effect (Capra and Satchell, 1977).

In these studies, infusion of adrenaline increased arterial blood pressure and pulse pressure in both conscious and anaesthetised animals, and increased the heart rate of conscious fishes. The effects of adrenaline were found to be more potent than those of noradrenaline. Circulating catecholamines therefore appear to exert influence over the whole circulatory system of the elasmobranch, and may control the distribution of blood from the heart to the gills and systemic vasculature.

Infusion of adrenaline in Scyliorhinus canicula produced a marked urinary diuresis. The mean urine to plasma inulin concentration ratio was however, unchanged by the administration of adrenaline, indicating that the diuresis must have resulted entirely from the observed increase in glomerular filtration rate. Catecholamines have been shown to

induce a urinary diuresis in the eel, but in contrast, are antidiuretic in the cod (Rankin and Babiker, 1981). Recent studies have also indicated a dose-dependent anti-diuresis in the perfused rainbow trout kidney (Rankin et al., 1984).

Most teleostean species show a linear relationship between urine flow and glomerular filtration rate, indicating that water excretion rates are primarily determined by filtration rates (Hickman and Trump, 1969). This relationship does not however, exist in the eel (Schmidt-Nielsen and Renfro, 1975) and is not typical of the higher vertebrates, where urine output is primarily regulated by changes in tubular water reabsorption. In the dogfish, the clear relationship between urine flow and glomerular filtration rate appears to indicate the typical lower vertebrate pattern of control of water excretion.

There have been no attempts made to measure intra-renal blood pressure in elasmobranchs, and so although the systemic pressor effect of adrenaline is well established, the effect on the renal vasculature remains unknown. In the mammalian kidney, glomerular filtration is generally autoregulated, and is independent of changes in systemic blood pressure or cardiac output. There is still a great amount of controversy concerning the mechanisms governing autoregulation, but it is thought that changes in vascular tone may regulate blood flow. Maintenance of a relatively stable SNGFR is therefore the primary concern of the renal circulation in the mammalian kidney (Brenner, 1981). This contrasts with the situation seen in euryhaline teleosts where there is little evidence of

autoregulation (Hickman and Trump, 1969). In the perfused trout kidney, urine production and glomerular filtration rate appear to be directly related to vascular perfusion pressure (Rankin et al., 1984). It is not known whether elasmobranchs show autoregulation of renal blood flow. The lowering of systemic blood pressure and concomitant reduction in urine output during light anaesthesia, suggests that the general lower vertebrate pattern of systemic blood pressure directly affecting renal function, is present in the dogfish. In addition, the highly variable filtration rate of the dogfish under differing experimental conditions, suggests only limited, if any autoregulation.

Changes in glomerular filtration rate and urine flow in teleosts, accompany acclimation to and from environments of differing salinity (Hickman and Trump, 1969). The changes in GFR have been found to result from changes in the population of filtering glomeruli, a process known as glomerular intermittency (Brown et al., 1980). During micropuncture of single nephrons of Scyliorhinus canicula, oil-blocks introduced into renal tubules occasionally remained stationary. Following a variable period of time, the stationary oil-blocks were often observed to spontaneously move down the tubule. Thus nephrons changed between the functional, filtering state, and the non-filtering state. This phenomenon was confirmed by the microscopic examination of superficial glomeruli in vivo, in which the visible flow of erythrocytes through capillaries engorged with blood was often followed by a period in which blood flow ceased,

suggesting that the glomerulus had been temporarily inactive.

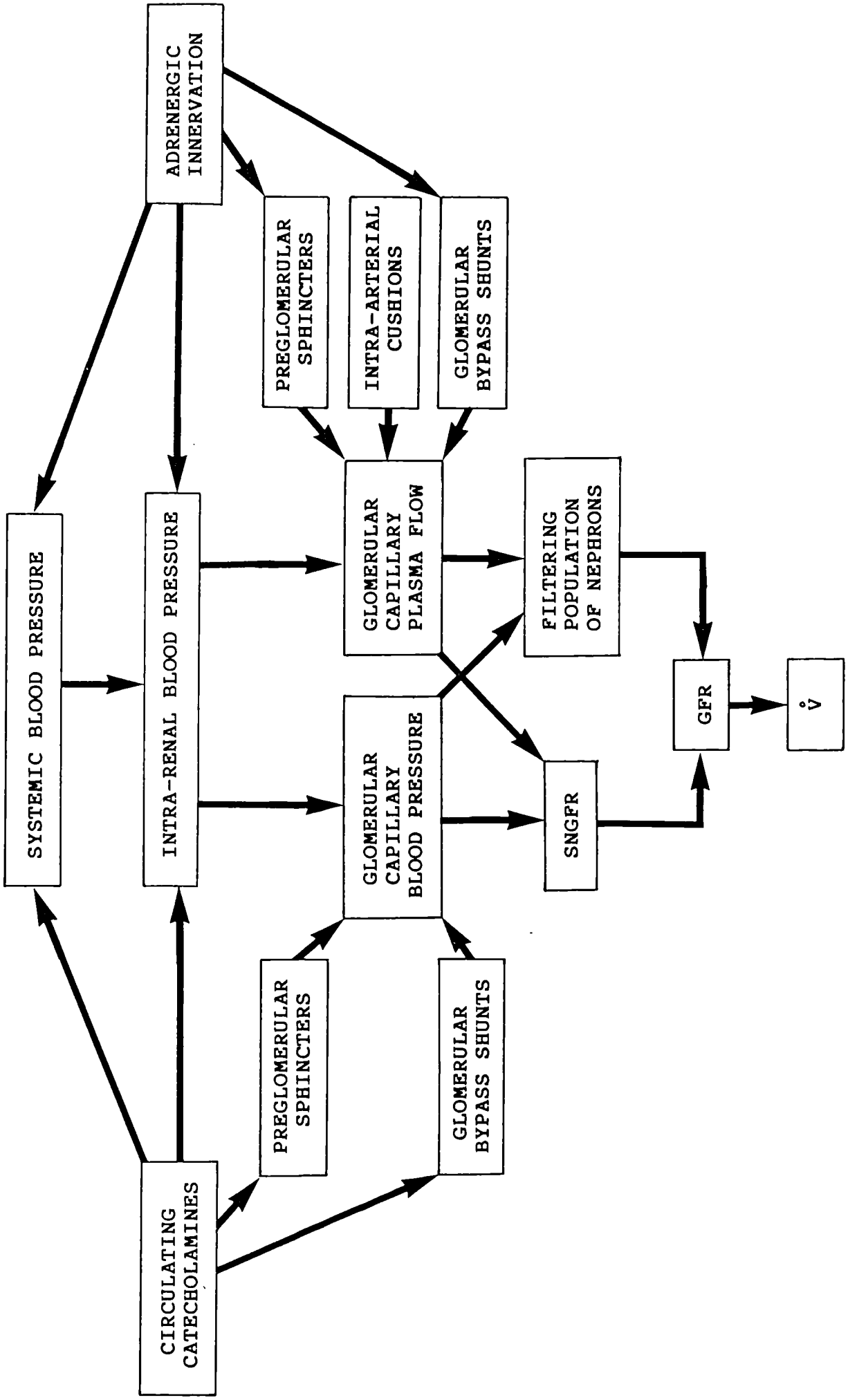
These qualitative observations were confirmed by the quantitative analysis of the patterns of glomerular perfusion in unconscious dogfish. At any time, a small percentage of glomeruli were either completely unperfused with blood, or the glomeruli were perfused but for some reason, possibly inadequate effective filtration pressure or a low coefficient of ultrafiltration, filtration was not occurring. Infusion of adrenaline into the vasculature significantly decreased the percentage of filtering glomeruli. This observation conflicts with previous suggestions of an adrenaline-induced diuresis resulting from an increased population of filtering nephrons (Deetjen and Boylan, 1968).

It followed, from the observation of a reduced filtering population of nephrons in adrenaline-infused fishes, that the observed diuresis must result from elevated filtration rates of individual nephrons, and single nephron filtration rates of functional nephrons were indeed found to be significantly higher following the administration of adrenaline. There are a number of possible causes of such an effect, but given the vascular action of adrenaline and the suggested lack of autoregulation of glomerular function in lower vertebrates, increased glomerular capillary pressure or plasma flow would be suspected (Figure 43).

Measurement of glomerular capillary pressures would be feasible in the dogfish, as large surface glomeruli with clearly visible capillary loops are present on the dorsal margin of the posterior kidney lobes. Observations of

Figure 43

Interrelationships between the effects of circulating catecholamines and adrenergic innervation on systemic and renal vasculature, and the regulation of urine flow in the dogfish, Scyliorhinus canicula. SNGFR, single nephron glomerular filtration rate; GFR, overall glomerular filtration rate; $\dot{V}$, urine flow.


ADRENERGIC INNERVATION
PREGLOMERULAR SPHINCTERS
INTRA-ARTERIAL CUSHIONS
GLOMERULAR BYPASS SHUNTS
SYSTEMIC BLOOD PRESSURE
INTRA-RENAL BLOOD PRESSURE
GLOMERULAR CAPILLARY PLASMA FLOW
FILTERING POPULATION OF NEPHRONS
GLOMERULAR CAPILLARY BLOOD PRESSURE
SNGFR
GFR
V̊
CIRCULATING CATECHOLAMINES
PREGLOMERULAR SPHINCTERS
GLOMERULAR BYPASS SHUNTS

erythrocyte flow in glomerular capillary loops during micropuncture experiments suggested that increased capillary blood flow contributed to the increased SNGFR. These observations should however, be investigated further by the measurement of blood flow in glomerular capillaries, for example, by application of the microsphere technique (Lameire, Chuang, Osgood and Stein, 1978).

Single nephron studies revealed a significant increase in the tubular fluid to plasma inulin concentration ratios in adrenaline-infused fishes, compared to animals infused with dogfish Ringer's solution. This was suprising in view of the unchanged overall urine to plasma inulin concentration ratios. This suggests that adrenaline may profoundly alter tubular water flux. Water may be reabsorbed from the ultrafiltrate of plasma in early tubular segments proximal to the micropuncture collection site, and replaced by tubular secretion in more distal segments.

The distal tubular segment and collecting ducts have been suggested to be the site of urinary dilution in the elasmobranchs (Thurau and Acquisto, 1969; Stolte *et al.*, 1971). In teleosts, tubular water secretion has been implied from whole animal studies (Schmidt-Nielsen and Renfro, 1975), and clearly demonstrated by the *in vitro* perfusion of isolated renal tubules by Beyenbach (1982). More recently, a secretory chloride transport mechanism has been demonstrated in isolated renal proximal tubules of the dogfish, *Squalus acanthias* (Beyenbach and Frömter, 1985). Further studies, using the isolated proximal tubule preparation of *Squalus acanthias*,

have demonstrated a net fluid secretion, thought to be driven by the secretion of sodium and chloride ions (Sawyer and Beyenbach, 1985). The demonstration of renal tubular fluid secretion in both teleosts and elasmobranchs, suggests that this process may be more widespread, at least amongst the fishes, than was previously appreciated.

Renal haemodynamics are predominantly regulated by the alpha-adrenergic vasoconstriction of smooth muscle elements of vessel walls, however specialised muscular structures identified in these studies (section II.C.6) must be considered in the interpretation of both the patterns of renal blood flow and glomerular haemodynamics.

The presence of smooth muscle sphincters in the renal vasculature of Scyliorhinus canicula (section II.C.6) and the rainbow trout (Elger and Hentschel, 1981a; Elger et al., 1984) are thought to determine patterns of glomerular perfusion within the kidney. Sphincters could also control the distribution of preglomerular blood flow between the afferent arteriole and glomerular bypass vessels, observed in this study (section II.C.6(b)). These sphincters may be controlled by circulating catecholamines (Moffat and Creasey, 1970), although the possible involvement of adrenergic nerves of the autonomic nervous system cannot be ruled out. During the transfer of rainbow trout from fresh to sea water, both bretylium, an adrenergic neuron blocker, and phentolamine, an alpha-adrenergic receptor blocking agent, independently reduced the expected reduction in both GFR and urine flow (Elger and Hentschel, 1983), suggesting that in teleosts at

least, the adrenergic system is involved in the control of filtration. Innervation of the smooth muscle of collecting tubules has recently been reported in teleosts (Tsuneki, Kobayashi and Pang, 1984). Neurally-induced contraction of this smooth muscle sheath could influence glomerular filtration by increasing intra-tubular pressure, or by increasing efferent arteriolar resistance (Elger et al., 1984). Whether such control mechanisms exist in the dogfish remains unknown. The potential role of the adrenergic nervous system can only be a subject of conjecture until the necessary anatomical and pharmacological investigations have been carried out.

The renin-angiotensin system has also been implicated in the control of glomerular activity in teleostean fishes (Henderson et al., 1978; Brown et al., 1980), although the apparent absence of renin-secreting granules in the elasmobranch fishes suggests alternative control mechanisms may exist. There appears to be a close inter-relationship between the renin-angiotensin system and the catecholamines. Catecholamines are known to influence release of renin in mammalian species (Insel and Snavely, 1981), whilst angiotensin has been shown to stimulate adrenal medullary catecholamine secretion (Peach, 1971). Furthermore, angiotensin has been shown to produce a pressor effect in Squalus acanthias, mediated by the release of catecholamines (Opdyke and Holcombe, 1976).

In summary, circulating catecholamines play a major role in the regulation of renal circulation in Scyliorhinus canicula. Their release in response to stress, or to modulate renal function to meet osmoregulatory requirements of a constantly changing environment, would have profound effects on single nephron filtration rates and the patterns of glomerular perfusion, thus influencing urine flow (Figure 43). The regulation of smooth muscle vascular elements of renal vessels, and hence the regulation of renal blood flow distribution and pressure, is likely to be mediated by the action of circulating catecholamines, adrenergic sympathetic innervation and other hormonal systems, as yet unidentified in the elasmobranchs.

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