FUNCTIONS OF THE URINARY BLADDER

IN VIVO IN THE RAINBOW TROUT

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A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Master of Science

McMaster University
Functions of the urinary bladder *in vivo* in the rainbow trout.

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NUMBER OF PAGES: x, 156
ABSTRACT

This thesis examined the function of the urinary bladder in vivo in the freshwater rainbow trout. In the first part of the study two new techniques were developed to examine the possible urine storage and ionoregulatory roles of the bladder in vivo. An indirect approach, using non-catheterized fish, involved "spot sampling" from the bladder to determine urine composition, and measurement of the appearance of $^3$H polyethylene glycol-4000 (a glomerular filtration marker) in surrounding water to quantify urination events. The direct approach employed a new external catheterization technique to collect naturally discharged urine. Both methods demonstrated that resting trout urinate in intermittent bursts at 20 - 30 min intervals, and that natural urine flow rate (U.F.R.) is at least 20% lower and urinary Na$^+$ and Cl$^-$ excretion rates at least 40% lower than determined by the traditional internal bladder catheter technique. The urine is stored for approximately 25 min prior to discharge, and significant reabsorption of water and ions (Na$^+$, Cl$^-$, K$^+$, urea, and possibly other substances) occurs via the bladder epithelium during this period; a small residual volume is likely always maintained.

The second part of the study employed the new external catheter and the traditional internal catheter to quantify the responses of the bladder, relative to those of the kidney, to two experimental disturbances. Chronic (32 h) infusion with 140 mM NaCl produced isosmotic volume loading without a change in plasma [Na$^+$], [Cl$^-$], or acid-base status. The kidney responded with a large increase in glomerular filtration rate (G.F.R.), a smaller increase in U.F.R., and increased reabsorption of water and ions. The bladder responded with a small
increase in urination burst volume, a larger increase in burst frequency, and therefore a decreased urine storage time. Despite this increased throughput, Na\(^+\) and Cl\(^-\) reabsorption rates across the bladder epithelium actually increased. Reabsorption of urea and K\(^+\) remained constant, despite expected decreases due to decreased urine storage time. A similar infusion with 140 mM NaHCO\(_3\) produced isosmotic volume loading together with metabolic alkalosis reflected in increased blood pH, increased plasma [HCO\(_3\)]\(^-\), decreased plasma [Cl\(^-\)], with no change in plasma [Na\(^+\)]. The response of the kidney was similar, though HCO\(_3\)\(^-\) filtration, reabsorption, and excretion rates all increased, while rates for Cl\(^-\) were proportionately lowered; renal Na\(^+\) handling was unaffected. Bladder urination patterns and Na\(^+\) reabsorption were also similar, but there was no evidence of bladder involvement in HCO\(_3\)\(^-\) secretion or reabsorption (ie. in acid-base regulation).

It is concluded that previous studies using internal catheterization have greatly underestimated the ionoregulatory effectiveness of the entire renal system by negating bladder function. The external catheterization technique developed in this thesis provides researchers with a method to collect naturally vented urine, and thereby evaluate the role of the entire renal system, including the bladder, in response to experimental manipulations.
ACKNOWLEDGEMENTS

The undertaking and successful completion of this thesis has not come about through my efforts alone, it has only been possible with the aid of a great many people.

I would first like to thank my supervisor, Dr. Chris Wood, for his tremendous contribution to the planning, compilation, and revision of the thesis.

Secondly, my thanks to the members of the oral examination committee, Drs. Gord McDonald, Colin Nurse, and Mike O’Donnell, for their constructive criticism and evaluation.

I would also like to acknowledge the help of many other people in the McMaster Biology Department. These include the people working in Chris Wood’s lab, who not only provided technical assistance, but also provided a constructive work environment; Greg Goss, Steve Munger, Marj Patrick, Rick Playle, Rod Rhem, Monica Scarabello, Yuxiang Wang, Mike Wilkie, and Rod Wright. Thanks also to those people who provided expertise outside of the realm of fish physiology; Dr. Louise Barber, Brian Hand, and Vandy Thomas. Finally, my thanks to two close friends, Andrew Chek and Terry Geissler, who contributed of their spare time to assist me during some of the long experiments which were undertaken.

My thanks also go to my relatives, both the Curtis and Hampson families, for their encouragement, and positive outlook on my endeavours.

Finally, to my wife Barbara Hampson, for her limitless encouragement, love, and support, as well as for the amount of work she has had to do alone.

To all of you, my thanks and Godspeed.
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CHAPTER 1
General Introduction

Ionoregulation in Freshwater Teleost Fish

Ionoregulation and osmoregulation in freshwater teleosts require the coordination of gills and renal system to reduce energy expenditure to a minimum. Since these fish maintain their internal fluid composition hypertonic to the surrounding environment, water is constantly absorbed along the osmotic gradient through any permeable epithelium. The epidermis is generally considered to be quite impermeable to water, however the respiratory exchange surfaces of the gills are not. At the same time, ions, primarily Na\(^+\) and Cl\(^-\), are lost from the fish across the gills. To compensate for the water uptake, freshwater teleosts produce large quantities of very dilute urine, equal to the volume of absorbed water (Hickman & Trump, 1969), while internal Na\(^+\) and Cl\(^-\) levels are maintained by active transport across the gill epithelium and by reabsorption from the primary urine (Evans, 1979). The branchial portion of the teleost ionoregulatory system has been studied extensively but the renal portion, although equally important, has received relatively less attention.

Renal Blood Supply and Glomerular Filtration

The pair of kidneys in mature rainbow trout (Oncorhynchus mykiss; formerly Salmo gairdneri) are fused and together extend along the length of the body cavity between the swim bladder, ribs, and vertebrae (Anderson & Mitchum, 1974). Blood is directed into the kidneys from the dorsal aorta through renal arteries which subdivide into intrarenal arteries and finally into afferent arterioles supplying the capillary beds of each renal corpuscle. The smaller diameter and
therefore higher resistance of efferent arterioles provides the necessary filtration pressure from the capillary bed, across the glomerular membrane into the Bowman's capsule (Hickman & Trump, 1969). Plasma ultrafiltrate is conducted from the Bowman's capsule into the renal tubule where its composition is altered by active transport across the membranes of surrounding cells. Control of glomerular filtration rate (G.F.R.) remains unclear but is likely a combination of variation in the number of functional renal corpuscles and gradation of filtration in each (Brown et al, 1978, 1980). Blood leaving the renal corpuscle in the efferent arteriole is directed into another capillary bed which surrounds the renal tubule and here it is reconstituted with reabsorbed substances (Anderson & Mitchum, 1974). However, recent work has indicated that capillaries surrounding the proximal segment are instead supplied with blood from the renal portal system (Elger et al, 1984). Blood finally collects in the posterior cardinal vein and is returned to the heart.

Renal Tubule Anatomy and Physiology

The ion transport abilities of the renal tubule are crucial to freshwater survival since the tubules must produce strongly hyposmotic urine at rates of up to 0.4 % body weight·hr⁻¹ (Eddy, 1982) by selective reabsorption, to maintain the internal osmotic balance of fish. The freshwater teleost renal tubule has been divided into 6 functionally distinct segments, each important in urine production. The following description is drawn largely from the classic review of Hickman and Trump (1969) and a more recent review on salmonid excretory function by Wood (1990).

Ultrafiltrate first enters (i) the short, narrow neck segment. The neck
segment is composed of moderately ciliated columnar cells which appear to serve a propulsive function.

From the combined effects of filtration pressure and ciliary propulsion the ultrafiltrate enters (ii) the first segment of the proximal convoluted tubule. The presence of tubules, vacuoles, and vesicles in these tall, columnar cells supports the suggestion of pinocytotic reabsorption of macromolecules in this segment (Anderson & Loewen, 1975). Isosmotic reabsorption of Na\(^+\), Cl\(^-\), and water is thought to occur here, based on the assumption of homology with amphibians. Amino acid and glucose reabsorption, and organic anion secretion are also assumed to occur here (Hickman & Trump, 1969).

The partially refined ultrafiltrate next enters the narrow lumen of (iii) the second segment of the proximal convoluted tubule. This segment comprises approximately 50% of the total nephron length and probably completes the isosmotic NaCl reabsorption. It is also believed to be the site of divalent ion absorption or secretion (Anderson & Loewen, 1975; Evans, 1979); it is the only portion of the proximal tubule found in marine agglomerular teleosts in which the primary function of the kidney is the elimination of divalent ions. Reabsorption or secretion of K\(^+\), H\(^+\), and HCO\(_3^-\) have also been suggested in this segment (Hickman & Trump, 1969).

The ultrafiltrate next enters (iv) the intermediate segment, a very short, ciliated region surrounded by cuboidal epithelial cells. The true function of this region is unknown but it is believed to function primarily in propulsion. Interestingly, the intermediate segment only occurs in freshwater and euryhaline teleosts (Hickman & Trump, 1969) and may be important in controlling the rate of ultrafiltrate movement into the distal convoluted tubule to avoid excessive
water reabsorption in the proximal segments.

The (v) distal convoluted tubule is characterized by a narrower overall diameter accompanied by widening of the lumen itself. This segment is very important in the formation of hyposmotic urine. *In vitro* perfusion experiments have shown that it is virtually impermeable to H$_2$O while being capable of substantial Cl$^{-}$ reabsorption in the presence of Na$^{+}$ (Nishimura et al, 1983). Not surprisingly the distal convoluted tubule is absent in most marine teleost species (Evans, 1979).

At this point urine enters (vi) a collecting duct along with urine from many other nephrons. The cells of the epithelium surrounding this duct are similar in appearance to those of the distal convoluted tubule and further Na$^{+}$ and Cl$^{-}$ reabsorption is thought to occur (Evans, 1979). The diameter of the duct progressively increases as more and more join together until it empties into the mesonephric duct leaving the kidney. It has been suggested that this section of the renal tubule system is the most vital segment for production of hypotonic urine, since it is present in all euryhaline and freshwater teleosts, even those which lack a distal convoluted tubule (Evans, 1979).

The thousands of collecting ducts of each fused hemi-kidney empty into a single mesonephric duct or ureter on each side (the term ureter will be used here for simplicity since mesonephric duct seems to have been used by researchers interchangeably with opisthonephric, archinephric, and wolffian duct (Romer & Parsons, 1977)). The ureters gradually increase in diameter toward the posterior of the kidney as they are required to carry more urine. Their outer surfaces are re-inforced with several layers of smooth muscle and connective tissue. High levels of Na$^{+}$/K$^{+}$ ATPase and oxidative enzymes have been found here, so Na$^{+}$
and Cl⁻ reabsorption are thought to continue in these ducts. The two ureters eventually merge and empty into a dilated portion of the duct, commonly referred to as the urinary bladder (Bell & Bateman, 1960).

*Glomerular Filtration Rate, Urine Flow Rate, and Urine Composition in Freshwater Fish.*

Freshwater fish filter large amounts of plasma through their glomeruli. A rainbow trout can filter up to 37% of its plasma volume every hour based on glomerular filtration rates (G.F.R.) calculated using $^3$H-inulin (Oikari & Rankin, 1985) and plasma volume estimated from haematocrit and total blood volume calculated using radio-labelled erythrocytes (Gingerich *et al.*, 1987). Typical G.F.R. values in freshwater rainbow trout reported over the last 25 years, summarized by Wood (1990), average to approximately 7.5 mlkg⁻¹h⁻¹, over 3 times as high as in seawater adapted rainbow trout.

Urine flow rates (U.F.R.s), however, are significantly lower, averaging 3.6 mlkg⁻¹h⁻¹ in studies where both G.F.R. and U.F.R. were measured (Wood, 1990). Thus, during conduction through the kidney tubules over half of the original filtered water is returned to the plasma at a reabsorption rate of about 3.9 mlkg⁻¹h⁻¹.

At the same time, comparison of plasma and urine ion concentrations indicates much higher relative reabsorption rates for major electrolytes than for water. Based on urine ion concentrations summarized by Wood (1990) and plasma ion concentrations from Wheatley *et al.* (1984), $\text{Na}^+$ reabsorption occurs at about 1100 $\mu$molkg⁻¹h⁻¹ and Cl⁻ at about 1000 $\mu$molkg⁻¹h⁻¹. Thus the concentrations of $\text{Na}^+$ and Cl⁻ in the reabsorbate ($\approx$280 mM) are almost twice
those of plasma (130 to 150 mM), supporting the argument that water passively follows ion reabsorption.

The reabsorption of ions in such a hypertonic solution results in a highly hypotonic product by the time this urine enters the bladder from the ureters. More specifically, Na$^+$ and Cl$^-$ concentrations in ureteral urine are generally less than 10 % of plasma concentrations.

All the lost Na$^+$ and Cl$^-$ must be replaced by active uptake across the gill epithelium from the external freshwater against net ionic concentration gradients of approximately 150 mM for Na$^+$ and 130 mM for Cl$^-$. In contrast, the Na$^+$ and Cl$^-$ reabsorption from the urine by the kidney initially proceeds in the absence of concentration gradients (ie. isosmotic reabsorption in the two segments of the proximal tubule - see iii above). Thereafter the gradients opposing reabsorption must increase, but even by the time the urine reaches the ureters, the maximum ionic gradients encountered are about 140 mM for Na$^+$ and 120 mM for Cl$^-$. Thus, it is metabolically more economical to reabsorb as much Na$^+$ and Cl$^-$ as possible from urine before expulsion, rather than to take up these ions anew from the water at the gills.

It is important to emphasize that all findings on rainbow trout urine ion composition to date have been based on urine collected by internal bladder catheterization. The same is true of virtually all other freshwater fish. This technique collects urine as soon as it enters the bladder from the ureters thereby preventing any possible modification by the urinary bladder which could potentially reduce the osmolality of the urine before expulsion. Wood (1990) has therefore suggested that all previous estimates of the renal ionoregulatory contribution may have underestimated the role of the full renal system, due to
neglect of bladder transport capacity.

**Urinary Bladder: In Vivo Indications of Ion Transport Function**

Until just over 25 years ago it was assumed that the teleost urinary bladder functioned only as a storage organ. However, during an intravascular HCO₃⁻ infusion experiment, Murdaugh, Soteres, Pyron, and Weiss (1963) noted that HCO₃⁻ and inulin appeared in bladder urine of the agglomerular marine teleost *Lophius americanus*. Thus, urinary bladder permeability was tested by comparing HCO₃⁻ and inulin concentrations in ureteral urine and urine incubated in bladders of fish with ligated ureters, during intravascular HCO₃⁻ infusion. Inulin and HCO₃⁻ were found in bladder urine while ureteral urine remained unchanged. However, inulin has since been found in ureteral urine collected from the agglomerular toadfish (Lahlou et al., 1969), and HCO₃⁻ secretion across the renal tubule epithelium has been noted by Hickman and Trump (1969).

The work of Murdaugh et al. (1963) was only reported in an Abstract, and full experimental details were never published. Nonetheless, this abstract seems to have been sufficient to stimulate further study of the subject and four years later the first of only a handful of in vivo studies suggesting active transport by freshwater teleost urinary bladder epithelium was published (Lahlou, 1967).

During a study of the changes in renal function during seawater to freshwater transfer of the European flounder (*Platichthys flesus*), significantly lower Na⁺ and Cl⁻ concentrations were found in the first urine collected from a urinary catheter than in subsequently collected urine. Comparisons of Ca²⁺ and Mg²⁺ showed little or no difference between the two groups. Urine collected initially was thought to have spent at least some time in the urinary bladder while later urine
was collected as soon as it entered from the ureter. Lahlou proposed that the role of the urinary bladder was one of storage and further selective ion reabsorption. He compared his findings with the "laboratory diuresis" of urine from freshly caught marine fish in early studies using bladder catheterization (e.g. Forster & Berglund, 1956), and suggested bladder ion transport as a possible explanation. Similar comparisons of bladder and ureteral urine from the euryhaline toadfish (*Opsanus tau*) in freshwater showed lower Cl\(^-\) but higher Na\(^+\) and K\(^+\) concentrations in bladder urine (Lahlou et al, 1969).

In the only other *in vivo* comparison of freshwater ureteral and bladder urine ion concentrations, *post mortem* bladder urine was collected from brook trout (*Salvelinus fontinalis*; Marshall, 1988) and compared to previously published values for ureteral urine ion concentrations of rainbow trout (Giles, 1984). Na\(^+\), K\(^+\), and Cl\(^-\) concentrations were found to be consistently lower in bladder urine. This comparison was done only as an aside to the main *in vitro* focus of the study, so values for brook trout ureteral urine ion concentrations were not determined. Hence, the usefulness of this comparison remains questionable, because of possible species differences in renal ion handling and urinary flow rates.

Shehadeh and Gordon (1969) have provided the only freshwater rainbow trout bladder urine ion concentrations. However, they did not provide any corresponding ureteral urine ion concentration values since such a comparison was not one of the objectives of their study. It should be noted that this urine had consistently higher Na\(^+\), K\(^+\), and Cl\(^-\) concentrations than rainbow trout ureteral urine of a later study (Giles, 1984).

From this review of the literature, it is clear that there have been no studies to date on freshwater rainbow trout which directly compare ureteral urine
Figure 1.1. Rainbow trout urinary bladder (UB) epithelium fixed stretched in buffered formalin, cut in 3 μm sections, and stained with Periodic Acid-Schiff and counter-stained with haematoxilin, showing epithelial cells (EC) with large, centrally located nuclei. Note the presence of a large blood vessel (BV). X1200.
Figure 1.2. Rainbow trout urinary duct (UB) between the urinary bladder and the urinary papilla. Bands of striated muscle positioned at an oblique angle (SM) with the direction of the duct and other muscle suggests the presence of a urinary sphincter. (see Figure 1.1 for fixation details) X1200.
Figure 1.3. The urinary bladder (UB) epithelium and associated blood vessels (BV) in the submucosal layer. (see Figure 1.1 for fixation details) X1200.
coordinate contractions of intestinal wall smooth muscle (Leeson et al, 1985), and combined with the presence of a potential sphincter downstream of the bladder, this observation further supports the idea of urine storage.

**Urinary Bladder: In Vitro Indicators of Ion Transport Function**

The most substantial body of evidence favouring urinary bladder ionoregulation comes from *in vitro* studies of several teleost species. Most of these involved monitoring ion concentration, water volume, or electrical potential changes on either side of the bladder wall in an Ussing-type chamber (eg. Marshall, 1986) or in a perfused sac type apparatus (eg. Hirano et al, 1973). Several species of marine and euryhaline fish have been investigated using these techniques and their ion transport mechanisms characterized to greater or lesser extents. Lahlou and Fossat (1984) have presented the most recent review of these studies. The present discussion will be limited to studies on rainbow trout.

The first experiment showing ionoregulation by rainbow trout bladder epithelium utilized the perfused sac type apparatus bathed with isotonic Ringer’s solution on both sides (Lahlou & Fossat, 1971). Net mucosal to serosal Na\(^+\), Cl\(^-\), and H\(_2\)O fluxes, without a change in transepithelial electrical potential, were seen. Comparison of Na\(^+\) and H\(_2\)O fluxes indicated that the concentration of the reabsorbed fluid was 6 times that of the mucosal Ringer’s. Eventually, an equilibrium was attained in which the mucosal Ringer’s was diluted up to 10 times that of the serosal Ringer’s. Active transport of Na\(^+\) and Cl\(^-\) by an electrically silent mechanism was proposed, which accounted for the entire decrease in mucosal osmolality.

A comparative study of ion and water movement across urinary bladder
epithelia of freshwater, marine, and euryhaline fish, including the rainbow trout, also found net reabsorption of Na⁺ and Cl⁻ in the absence of a concentration gradient (Hirano et al., 1973). However, in this study net Na⁺ movement appeared to be greater than Cl⁻.

The presence of Na-K-ATPase has been observed in bladder epithelium from both freshwater and seawater adapted trout (Fossat et al., 1974). Decreases in enzyme activity closely paralleled decreases in Na⁺ transport of fish adapted to water of increasing salinity. Addition of ouabain (a known Na-K-ATPase blocker) to the serosal side of the membrane significantly reduced mucosal to serosal Na⁺ movement, while K⁺ removal from the serosal Ringer’s inhibited Na⁺ transport entirely. From these three sets of results, it was proposed that Na-K exchange pumps located on the serosal side of epithelial cell membranes were vital to Na⁺ transport in freshwater.

The presence of a Na-Cl co-transport pump has been proposed for the mucosal side of the epithelial cell membranes (Fossat & Lahlou, 1979a). Support for this hypothesis stems from the electrical neutrality of NaCl transport across the membrane as occurs in other transporting epithelia using Na-Cl cotransport (Frizzel et al., 1979). Removal of either Na⁺ or Cl⁻ from the mucosal solution stopped any net mucosal to serosal movement of the other ion, even when the replacement was another permeable ion. This suggested interdependence of Na⁺ and Cl⁻ movement. Other experimental tests demonstrated that the Na-Cl co-transport step appeared to be the rate determining step. A Cl/Cl exchange diffusion component was also thought to occur on the mucosal surface.

Although the bladder was classified as a "leaky" epithelium due to its low resistance during voltage clamping experiments, triaminopyrimidine (TAP; a
paracellular cation selective channel blocker) was ineffective in decreasing the permeability of the membrane to cations (Fossat & Lahlou, 1979b). This, coupled with higher Cl⁻ passive permeability measurements (Demarest & Machen, 1982) suggested that a small portion of the Cl⁻ movement occurred by passive diffusion through anion selective, paracellular channels to the serosa along an electrochemical gradient set up by the Na-K exchange pump on the serosal membrane. Net Na⁺ transport across the serosal membrane occurred through the efforts of the Na-K exchange pump, which transported 3 Na⁺ ions out of the cell for every 2 K⁺ ions pumped in.

An overall model of Na⁺ and Cl⁻ transport (Figure 1.4) has been proposed by Fossat & Lahlou (1979a), however the paracellular diffusion component is still questionable since other research has shown bladder epithelium to have a higher resistance (Demarest & Machen, 1982; Marshall, 1988). It has been suggested that at least some of the "leakiness" may be artifactual due to epithelial stretching and edge damage in the Ussing chambers (Lahlou & Fossat, 1984; Marshall, 1988).

Brook trout (Salvelinus fontinalis) urinary bladders, on the other hand, appear to transport Na⁺ and Cl⁻ independently by a fundamentally different mechanism (Marshall, 1986) (Figure 1.4). Removal of Cl⁻ from the mucosal Ringer's did not stop the transport of Na⁺ and in the absence of Cl⁻, increased titratable acidity was observed in the mucosal Ringer's suggesting a Na⁺/H⁺ exchange. Similarly, Cl⁻ transport persisted when the bladder was bathed with Na⁺ and K⁺ free Ringer's on the mucosal side, suggesting Cl⁻/HCO₃⁻ exchange. These conclusions were re-inforced by the use of appropriate anion and cation blockers. This type of independent Na⁺/H⁺ and Cl⁻/HCO₃⁻ exchange is often
Figure 1.4. A schematic representation of freshwater rainbow trout urinary bladder Na\(^+\) and Cl\(^-\) transport. A: based on *in vitro* work on *Salmo gairdneri irideus* (Fossat *et al*, 1984). B: based on *in vitro* work on *Salvelinus fontinalis* (Marshall, 1986, 1988).

* assumed by the authors to occur but not tested for experimentally.
mucosa epithelium serosa

0mV  

Cl⁻ diffusion

-56mV

3Na⁺

2K⁺  

=0mV

Cl⁻ diffusion

0mV  

H⁺  

Na⁺  

K⁺  

OH⁻/HCO₃⁻  

+6.7±1.6 mV
found in acid-base transporting epithelia, such as the freshwater teleost gill (Payan et al, 1984). A limited acid-base regulating capacity has been demonstrated previously in the kidney of the rainbow trout (Wood & Caldwell, 1978; McDonald & Wood, 1981; Wheatley et al, 1984). Marshall (1988) therefore suggested that the bladder may serve to supplement this role. However, it must be emphasized that this type of transport system has not been seen in rainbow trout bladder in vitro, but rather a Na\(^+\)-Cl\(^-\) cotransport system which is not thought to be involved in acid-base transport. It seems curious that two fairly closely related fish, both members of the family Salmonidae, should have fundamentally different transport mechanisms in their bladders.

**Goals of the Present Thesis**

Although Na\(^+\) and Cl\(^-\) transport have been demonstrated in the freshwater rainbow trout urinary bladder in vitro and the probable mechanisms of transport outlined, to date no results have been presented to show that the urinary bladder actually plays a part in osmoregulation in vivo. In vitro studies have not even simulated naturally occurring conditions. Excised bladders have been bathed on both sides with a Ringer’s solution resembling trout plasma, a condition which would occur in vivo only if the renal tubules were non-functional. Moreover, at present nothing is known about the natural storage volume or period, or the natural pattern of urination.

For these reasons, the goals of the first experimental chapter (Chapter 2) were to determine the natural patterns of urination and to measure ion concentrations in this urine to see whether ion transport occurs in vivo. To achieve this, urination patterns were observed using two new techniques. Firstly,
individual urination events were detected and quantified in uncatheterized fish by using the novel technique of injecting $^3$H polyethylene glycol-4000, a known extracellular space marker, into the fish and subsequently monitoring the pattern of its appearance in the surrounding water. Urine composition in uncatheterized fish was also monitored. Secondly, a new external bladder catheterization technique was developed which allowed the fish to urinate voluntarily. This new method was compared with the classical internal catheterization technique to obtain a more accurate picture of the function of the whole renal system. Ion concentrations and urine flow rates collected by the two techniques were compared to estimate the ionoregulatory contribution of the bladder. The work of this chapter is now in press (Curtis & Wood, 1990).

The objective of the second experimental chapter (Chapter 3) was to use this new external catheterization technique to assess the possible role of the urinary bladder in responses to experimentally induced changes in renal function. Two experimental treatments were employed, and in both, responses were compared with those measured by the traditional internal catheterization technique. While the focus of these experiments was on bladder function, considerable new information was learned about the basic function of the kidney in vivo.

The first experimental treatment was a simple chronic infusion with isotonic NaCl to increase both urine flow rate and urinary NaCl excretion, to see how the urination pattern and electrolyte handling might change under these conditions. In nature, such changes may occur as the trout moves through salinity gradients during freshwater-seawater migration, especially if drinking or water ingestion with the food takes place (Shehadeh & Gordon, 1969; Miles 1971;
Figure 2.1. Schematic diagrams of a trout fitted with an External Urinary Bladder Catheter (A) and a trout fitted with an Internal Urinary Bladder Catheter (B).
Catheter Attachment
Stitches

Urinary Bladder

Urinary Sphincter

Intestinal Ligation

Body Wall Incision

Catheter Attachment Stitches

Catheter

Urinary Bladder

Urinary Catheter

Intestinal Ligation

Body Wall Incision

Urinary Papilla Tie

Purse String Ligatures
45 cm of Clay-Adams PE-60 with one end heat-flared to approximately 1.5 times its original diameter. The catheter was initially filled with water to exclude air bubbles. The flared end was inserted into the urogenital papilla and advanced dorsally 1.0-1.5 cm into the urinary bladder. The papilla was tied tightly around the PE-60 tubing twice with 2-0 silk suture. The integrity of the seal was tested by injecting 0.5-1.0 ml water; fish which leaked were discarded. Properly sealed catheters were secured with three purse string ligatures, one just anterior to, and two to the side of the anal fin. Urinary flow rates (U.F.R.; equation 5 below) were monitored over the 48 h recovery period.

External Urinary Catheter This new technique was designed to collect urine as it was naturally discharged from the urogenital papilla (Figure 2.1A). The catheter was made from a 40 cm Bard "All-Purpose Urethral Catheter" (size 12-French; elastic rubber), with the dilated end cut off to form a funnel. This funnel was stitched to the ventral surface of the fish around the urogenital papilla and anus using 16 equally spaced purse string ligatures (2-0 silk). Prior to catheter attachment, the intestine was ligated with 2-0 silk through a longitudinal incision in the ventral surface of the fish immediately anterior to the proposed location of the catheter. This 2 cm incision was subsequently closed using 3 stitches. This ligation served to prevent blockage of the catheter by expelled faecal sacs as occurred in preliminary experiments without ligation. During comparisons of internal and external catheters (series iii, below), the intestines of fish fitted with internal catheters were also ligated.

Once the catheter was secured, the area around the stitches was thoroughly wiped to remove any mucus and a thin film of tissue cement (3M Vetbond) was applied to aid in leakage prevention. The integrity of the seal was tested by
Table 2.1: A comparison of ion concentrations in urine collected by internal bladder catheterization with those in urine obtained by spot sampling in series i. Mean $\pm$ 1SEM (N).

† in this calculation, phosphate mEq/l were calculated based on an assumed urine pH = 7.2 (Wood, 1988) & pK = 6.8 (Wheatly et al, 1984)

* significantly different from internally catheterized value (p $\leq$ 0.05)

Ψ significantly different from zero (p $\leq$ 0.05)
<table>
<thead>
<tr>
<th>Substance</th>
<th>Internally Catheterized</th>
<th>Spot Sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$ (mmoll$^{-1}$)</td>
<td>7.12 ± 0.79 (16)</td>
<td>2.12 ± 0.41 * (15)</td>
</tr>
<tr>
<td>K$^+$ (mmoll$^{-1}$)</td>
<td>0.92 ± 0.09 (16)</td>
<td>0.81 ± 0.06 (15)</td>
</tr>
<tr>
<td>Ammonia (mmoll$^{-1}$)</td>
<td>0.79 ± 0.13 (16)</td>
<td>0.47 ± 0.07 * (16)</td>
</tr>
<tr>
<td>Ca$^{2+}$ (mmoll$^{-1}$)</td>
<td>1.35 ± 0.22 (16)</td>
<td>1.35 ± 0.09 (16)</td>
</tr>
<tr>
<td>Mg$^{2+}$ (mmoll$^{-1}$)</td>
<td>0.50 ± 0.11 (16)</td>
<td>0.79 ± 0.10 (16)</td>
</tr>
<tr>
<td>Cl$^-$ (mmoll$^{-1}$)</td>
<td>5.21 ± 0.34 (16)</td>
<td>3.35 ± 0.23 * (16)</td>
</tr>
<tr>
<td>NO$_3^-$ (mmoll$^{-1}$)</td>
<td>0.05 ± 0.02 (16)</td>
<td>0.09 ± 0.02 (16)</td>
</tr>
<tr>
<td>SO$_4^{2-}$ (mmoll$^{-1}$)</td>
<td>1.08 ± 0.10 (16)</td>
<td>1.24 ± 0.08 (16)</td>
</tr>
<tr>
<td>Phosphate (mmoll$^{-1}$)</td>
<td>0.40 ± 0.23 (16)</td>
<td>0.46 ± 0.23 (16)</td>
</tr>
<tr>
<td>Urea (mmoll$^{-1}$)</td>
<td>0.81 ± 0.16 (15)</td>
<td>0.55 ± 0.10 (15)</td>
</tr>
<tr>
<td>All measured substances (mmoll$^{-1}$)</td>
<td>17.73 ± 1.27 (15)</td>
<td>11.16 ± 0.43 * (14)</td>
</tr>
<tr>
<td>$\Sigma$Cations - $\Sigma$Anions $^+$ (mEq$^{-1}$)</td>
<td>4.34 ± 1.04 $^\gamma$ (16)</td>
<td>0.69 ± 0.57 * (15)</td>
</tr>
</tbody>
</table>
Figure 2.2. A typical record of the appearance of PEG-4000 in the external water of a non-catheterized, naturally urinating trout (series ii) over an 8h period. Sudden "step" increases in water radioactivity, marked by arrows, were interpreted as bursts of voluntary urination.
regular intervals of approximately 30 min (Table 2.2); in 12 trout the range in mean interval was 25.8 - 34.6 min. The average urination volume (V_{burst}; Table 1) was 1.20 mlkg^{-1} (range 0.76 - 1.92). Assuming that all urination occurred in bursts, the calculated U.F.R. for the non-catheterized fish of series ii (2.40 mlkg^{-1} h^{-1}) was significantly lower (by 23 %) than that directly measured in the internally catheterized fish of series i (3.11 mlkg^{-1} h^{-1}; n=19) (Table 2.2). The calculated V_{burst} for naturally urinating fish of 1.20 mlkg^{-1} may be compared with a mean maximum volume of the urinary bladder of 2.20 \pm 0.19 mlkg^{-1} (range 1.50 - 2.80; n=6), and a mean urine volume obtained by spot sampling of 0.52 \pm 0.11 mlkg^{-1} (range 0.13 - 1.67; n=14). The maximum urinary volume ever obtained by spot sampling was 1.67 mlkg^{-1}.

The G.F.R. calculated from plasma PEG-4000 clearance through the urine in non-catheterized fish (4.52 mlkg^{-1} h^{-1}), again assuming that all urination occurred in bursts, was about twice the U.F.R. (Table 2.2). This accounted for about 80 % of the total clearance, the remaining 20 % comprising the E.C.R. If some fraction of urine discharge does not occur in bursts, but rather continuously, then U.F.R. and G.F.R. will have been underestimated, and E.C.R. proportionately overestimated. However, the present figures are supported by the direct measurements of U.F.R., G.F.R., and E.C.R. in the externally catheterized fish of series iii (see below).

*Urinary Excretion Rates in Non-catheterized vs. Internally Catheterized Trout*

Urinary ion excretion rates were estimated for non-catheterized fish by applying the mean U.F.R value obtained from PEG-4000 fluxes in series ii to the
Table 2.2. A comparison of urine flow rates (U.F.R.) measured by internal bladder catheterization (series i) with those measured by the appearance of PEG-4000 in the water from non-catheterized fish (series ii). Glomerular filtration rate (G.F.R.), urination pattern, and extra-renal clearance rate (E.C.R.) of PEG-4000 are also shown for the latter. Means ± 1SEM.

<table>
<thead>
<tr>
<th></th>
<th>Internally Catheterized (n=19)</th>
<th>Non Catheterized (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>320.8 ± 17.4</td>
<td>324.4 ± 9.6</td>
</tr>
<tr>
<td>U.F.R. (mlkg⁻¹hr⁻¹)</td>
<td>3.11 ± 0.22</td>
<td>2.40 ± 0.29 *</td>
</tr>
<tr>
<td>G.F.R. (mlkg⁻¹hr⁻¹)</td>
<td>-</td>
<td>4.52 ± 0.50</td>
</tr>
<tr>
<td>Burst Volume (mlkg⁻¹)</td>
<td>-</td>
<td>1.20 ± 0.14</td>
</tr>
<tr>
<td>Interval (min)</td>
<td>-</td>
<td>29.82 ± 0.90</td>
</tr>
<tr>
<td>E.C.R. (mlkg⁻¹hr⁻¹)</td>
<td>-</td>
<td>1.15 ± 0.20</td>
</tr>
</tbody>
</table>

* significantly different from internally catheterized value (p ≤ 0.05).
measured ion concentrations of urine obtained by spot sampling in series i. The resulting estimates were compared to the directly measured excretion rates for internally catheterized fish in series i (Figure 2.3). This analysis demonstrated that the urinary excretion rates were significantly lower in non-catheterized fish for Na\(^+\) (by 79 %), K\(^+\) (by 35 %), ammonia (by 58 %), Cl\(^-\) (by 51 %), and urea (by 43 %). The total excretion rate of all measured substances in non-catheterized trout (26.8 ± 1.0 \(\mu\)molkg\(^{-1}\)hr\(^{-1}\); n=14) was therefore less than half (47 %) that (57.5 ± 6.9 \(\mu\)molkg\(^{-1}\)hr\(^{-1}\); n=15) in fish fitted with internal bladder catheters despite only a 23 % difference in U.F.R. The urinary bladder clearly plays a significant role in reabsorption \textit{in vivo}.

\textit{Urine Composition in Externally Catheterized vs. Internally Catheterized Trout}

In series iii, external catheters were employed to collect "naturally" vented urine in one group, while urine was collected by internal bladder catheterization in a second group which were otherwise treated identically (including intestinal ligation). In agreement with series i (cf. Table 2.1), urinary Cl\(^-\) concentration was significantly lower in externally catheterized fish (Table 2.3), though the difference (~25 %) was somewhat smaller than seen previously (~35 %). However in contrast to series i, the reduction in urinary Na\(^+\) was not statistically significant (0.10 > p >0.05), and there were also no significant differences in ammonia, the total concentration of measured substances, and \(\Sigma\)cations - \(\Sigma\)anions in the urine. Except for a lower NO\(_3\)^{2-} concentration, the urine composition of the internally catheterized fish of series iii was virtually identical to that of the comparable group in series i (cf. Tables 2.1 & 2.3), indicating that the intestinal
Figure 2.3. A comparison of the measured rates of urinary ion excretion from fish fitted with internal bladder catheters (n=16; series i) with the estimated rates for non-catheterized fish (n=16; data from series i & ii combined; see text for details). Means ± 1SEM. * = significantly different (p ≤ 0.05)
Internally Catheterized
Non-Catheterized

Rate (umol/kg/hr)

Sodium  Ammonia  Magnesium  Nitrate  Phosphate
Potassium  Calcium  Chloride  Sulphate  Urea

* Indicates significant difference
Table 2.3. A comparison of ion concentrations in urine collected by internal bladder catheterization with those in urine obtained by external catheterization in series iii. Means ± 1SEM (N).

* significantly different from internal value (p ≤ 0.05)

† in this calculation, phosphate mEq l⁻¹ were calculated based on assumed urine pH = 7.2 (Wood, 1988) and pK = 6.8 (Wheatly et al, 1984).
<table>
<thead>
<tr>
<th>Substance</th>
<th>Internally Catheterized</th>
<th>Externally Catheterized</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Na⁺</strong> (mmoll⁻¹)</td>
<td>6.73 ± 0.66 (9)</td>
<td>4.84 ± 0.74 (9)</td>
</tr>
<tr>
<td><strong>K⁺</strong> (mmoll⁻¹)</td>
<td>0.69 ± 0.09 (10)</td>
<td>0.56 ± 0.08 (9)</td>
</tr>
<tr>
<td>Ammonia (mmoll⁻¹)</td>
<td>0.72 ± 0.13 (10)</td>
<td>0.67 ± 0.07 (9)</td>
</tr>
<tr>
<td><strong>Ca²⁺</strong> (mmoll⁻¹)</td>
<td>1.09 ± 0.13 (10)</td>
<td>1.45 ± 0.13 (8)</td>
</tr>
<tr>
<td><strong>Mg²⁺</strong> (mmoll⁻¹)</td>
<td>0.48 ± 0.11 (10)</td>
<td>0.46 ± 0.04 (9)</td>
</tr>
<tr>
<td><strong>Cl⁻</strong> (mmoll⁻¹)</td>
<td>6.46 ± 0.38 (9)</td>
<td>4.79 ± 0.44 (10)</td>
</tr>
<tr>
<td><strong>NO₃⁻</strong> (mmoll⁻¹)</td>
<td>0.28 ± 0.04 (9)</td>
<td>0.23 ± 0.03 (10)</td>
</tr>
<tr>
<td><strong>SO₄²⁻</strong> (mmoll⁻¹)</td>
<td>0.99 ± 0.11 (9)</td>
<td>0.92 ± 0.07 (10)</td>
</tr>
<tr>
<td>Phosphate (mmoll⁻¹)</td>
<td>0.25 ± 0.05 (9)</td>
<td>0.17 ± 0.05 (10)</td>
</tr>
<tr>
<td><strong>Urea</strong> (mmoll⁻¹)</td>
<td>0.92 ± 0.08 (10)</td>
<td>0.92 ± 0.06 (9)</td>
</tr>
<tr>
<td>All measured substances (mmoll⁻¹)</td>
<td>18.61 ± 1.34 (9)</td>
<td>15.72 ± 1.08 (8)</td>
</tr>
<tr>
<td><strong>ΣCations - ΣAnions</strong> (mEq⁻¹)</td>
<td>1.90 ± 0.63 (9)</td>
<td>2.27 ± 0.60 (8)</td>
</tr>
</tbody>
</table>
ligation and other differences in procedure between series i and iii did not confound the results.

**Urination Patterns of Externally vs. Internally Catheterized Fish**

The U.F.R. (2.01 mlkg\(^{-1}\)h\(^{-1}\)) of externally catheterized trout in series iii was significantly lower (by 21 %) than that (2.53 mlkg\(^{-1}\)h\(^{-1}\)) of fish fitted with internal catheters (Table 2.4). This difference compared well with data from non-catheterized fish vs. internally catheterized fish in series i and ii (Table 2.2). G.F.R. (4.0 - 4.5 mlkg\(^{-1}\)h\(^{-1}\)) was not significantly different between externally and internally catheterized trout in series iii (Table 2.4) and comparable to the value measured in non-catheterized trout in series ii (Table 2.2). Thus differences in U.F.R. between groups were not due to different rates of filtration at the glomeruli. G.F.R. accounted for 86 % of the total PEG-4000 clearance in externally catheterized fish, not significantly different from 88 % in internally catheterized fish (Table 2.4) and 80 % in non-catheterized trout (Table 2.2). E.C.R values were therefore comparable between groups. This indicates that leakage from the external catheter was not a problem in series iii. Furthermore it suggests that the assumption adopted in series ii (that all natural urination in non-catheterized trout occurs in identifiable bursts) was not unreasonable.

Figure 2.4 displays typical records of urination pattern from internally and externally catheterized trout of series iii, as directly recorded via infra-red optical switches. Based on the criterion (see Methods) that a series of drops released at a rate greater than 1 per sec represented a \(V_{burst}\), only 12 % (0 - 10 % in most fish) of the total U.F.R. occurred in bursts in internally catheterized trout (Table
Table 2.4. A comparison of urine flow rates (U.F.R.), glomerular filtration rate (G.F.R.), urination pattern, and extra-renal clearance rate (E.C.R.) of PEG-4000 measured in internally catheterized and externally catheterized fish of series iii. Means ± 1SEM.

<table>
<thead>
<tr>
<th></th>
<th>Internally Catheterized</th>
<th>Externally Catheterized</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight (g)</strong></td>
<td>340.0 ± 18.7 (11)</td>
<td>330.0 ± 14.4 (10)</td>
</tr>
<tr>
<td><strong>U.F.R. (mlkg⁻¹hr⁻¹)</strong></td>
<td>2.53 ± 0.12 (11)</td>
<td>2.01 ± 0.17 * (10)</td>
</tr>
<tr>
<td><strong>G.F.R. (mlkg⁻¹hr⁻¹)</strong></td>
<td>4.40 ± 0.27 (8)</td>
<td>4.05 ± 0.48 (7)</td>
</tr>
<tr>
<td><strong>Burst Volume (mlkg⁻¹)</strong></td>
<td>0.45 ± 0.06 (10)</td>
<td></td>
</tr>
<tr>
<td><strong>Burst %</strong></td>
<td>11.8 ± 4.5 (11)</td>
<td>64.7 ± 5.6 * (10)</td>
</tr>
<tr>
<td><strong>Interval (min)</strong></td>
<td></td>
<td>21.0 ± 2.4 (10)</td>
</tr>
<tr>
<td><strong>E.C.R. (mlkg⁻¹hr⁻¹)</strong></td>
<td>0.61 ± 0.17 (8)</td>
<td>0.68 ± 0.16 (6)</td>
</tr>
</tbody>
</table>

* significantly different from internally catheterized value (p ≤ 0.05)
Figure 2.4. A typical record of the urination pattern of an externally catheterized fish (A) and an internally catheterized fish (B). Each drop of urine was recorded as a spike on the recording (see text for details).
For the great majority of the time, urine simply dripped from the catheter at an even rate. In contrast, 65% (range 35 - 92% in 10 trout) of the total U.F.R. occurred in discrete bursts in externally catheterized trout. As in series ii, these bursts were asynchronous in different trout run at the same time. Average $V_{burst}$ was 0.45 mlkg$^{-1}$ (range 0.25 - 0.85), considerably lower than that (1.20 mlkg$^{-1}$; Table 2.2) estimated in non-catheterized fish of series ii. The mean interval was about 21 min (range 9.7 - 31.3), again significantly lower than the 30 min measured in series ii. Thus relative to the PEG-4000 flux approach, the external catheterization technique suggested that urination occurred more frequently, in smaller $V_{burst}$'s, with some non-burst urination.

**Urinary Excretion Rates in Externally Catheterized vs. Internally Catheterized Trout**

Directly measured urinary excretion rates in externally catheterized trout of series iii were significantly lower for both Na$^+$ (by 45%) and Cl$^-$ (by 43%) (Figure 2.5). Other substances were not significantly affected. The total excretion rate of all measured substances in externally catheterized fish ($33.3 \pm 2.8$ $\mu$molkg$^{-1}$hr$^{-1}$; n=8) was only about 75% that ($44.3 \pm 3.3$ $\mu$molkg$^{-1}$hr$^{-1}$; n=9) of internally catheterized fish. Qualitatively, these results are in broad agreement with those of series i and ii (cf. Figure 2.3) and reinforce the conclusion that the urinary bladder plays a significant role in reabsorption in vivo.
Figure 2.5. A comparison of the measured rates of urinary ion excretion from fish fitted with internal bladder catheters (n=10) with those from fish fitted with external catheters (n=9) in series iii. Means ± 1SEM. * = significantly different (p ≤ 0.05)
Internally Catheterized
Externally Catheterized

Rate (umol/kg/hr)

Sodium
Potassium
Ammonia
Calcium
Magnesium
Chloride
Nitrate
Sulphate
Phosphate
Urea
Discussion

Internal Catheter Measurements

Direct recording of the urination pattern in fish fitted with the traditional internal bladder catheter indicated that urine was drained more or less continuously, and only occasionally appeared in bursts (Figure 2.4 & Table 2.4). In general, the present urinary flow values (Tables 2.2 & 2.4) and composition (Tables 2.1 & 2.3) determined from rainbow trout fitted with internal catheters compared favourably with the results of previous researchers using this method (Holmes & Stainer, 1966; Hunn, 1969; Hunn & Willford, 1970; Hofmann & Butler, 1979; McDonald & Wood, 1981; Elger & Hentschel, 1983; Giles, 1984; Wheatly et al. 1984; Oikari & Rankin, 1985; Elger et al. 1986; Erickson & Gingerich, 1986; Wood, 1988). One exception was $\text{SO}_4^{2-}$ with a concentration of about 1 mmoll$^{-1}$ compared to a reported value of $2.9 \pm 0.4$ mmoll$^{-1}$ by Oikari & Rankin (1985). However the fish used in their study were starved for between 4 and 6 days. Fish used in the present study were starved for at least 7 days, allowing a longer time for excretion of residual $\text{SO}_4^{2-}$ presumably obtained from their food.

PEG-4000 as a G.F.R. Marker

PEG-4000 was found to be highly reliable as a G.F.R. marker in the present study on freshwater trout, thereby supporting the findings of Beyenbach & Kirschner (1976) which were obtained on seawater trout. This reliability was reflected in the high proportion of PEG excreted through the urinary system (at least 86 % in internally and externally catheterized fish); the figure was almost as high (80 %) in non-catheterized fish. The reliability was further demonstrated by
the finding of almost identical G.F.R values in internally, externally, and non-catheterized fish (Tables 2.2 & 2.4); PEG-4000 was likely neither reabsorbed or secreted by the bladder epithelium. The present mean G.F.R values (4.40 ± 0.27 mlkg⁻¹h⁻¹) were generally lower than those (5 - 11 mlkg⁻¹h⁻¹) recorded in previous studies on freshwater rainbow trout using a variety of different G.F.R. markers (Holmes & Stainer, 1966; Elger & Hentschel, 1983; Oikari & Rankin, 1985; Elger et al. 1986; Erickson & Gingerich, 1986). Only the carboxyl-inulin value of Hofmann & Butler (1979) was comparable to the present G.F.R, but this was obtained at a much lower temperature (8 °C versus 15 °C).

The only previous G.F.R. value obtained with PEG-4000 in freshwater rainbow trout, by Erickson & Gingerich (1986), was much higher (8.0 ± 1.1 mlkg⁻¹h⁻¹). In their study, fish were allowed a 24 h recovery period after insertion of a dorsal aortic catheter. In the present experiments, aortic catheters were not used and fish were allowed to recover for at least 48 h from MS-222 anaesthesia. Anaesthetization using MS-222 has been shown to significantly increase U.F.R. up to 3-fold (Hunn & Willford, 1970). In the experiments of Chapter 3, slightly higher U.F.R. and G.F.R. values were recorded from trout fitted with dorsal aortic catheters, but otherwise treated identically to those of the present study. It was therefore concluded that the lower values presented here likely reflect relatively lower levels of stress, as a result of generally longer recovery times from surgery and an absence of arterial catheterization.

**Indirect and Direct Measurements of Natural Urination**

While the two methods used to assess the volume, pattern, and composition of naturally vented urine produced different absolute values for many
parameters, they were in broad general agreement overall. Both approaches demonstrated that rainbow trout urinate intermittently in bursts at 20 to 30 min intervals, that the natural U.F.R. is at least 20% lower than that determined by the traditional internal catheter method, and that the natural urinary excretion rates of Na\(^+\) and Cl\(^-\) are at least 40% lower than determined by the internal catheter technique. Thus urine is clearly stored in the bladder for some time prior to discharge, and significant reabsorption occurs during this period. As the relative reduction in ion excretion rates is larger than that in U.F.R., the effectiveness of the entire renal system in freshwater ionoregulation is improved when the bladder is allowed to function normally.

The indirect approach (combination of spot sampling and PEG-4000 appearance in the water in different batches of non-catheterized fish; Figure 2.3) indicated a greater effectiveness of the bladder in ion conservation than did the direct approach (the new external catheterization method; Figure 2.5). It is problematical which of the two techniques produced the more accurate picture of the natural situation. However, the results of Chapter 3 make the picture more clear.

On the one hand, small bursts or slow seepage of urine may have been missed in the non-catheterized fish, leading to an underestimate of U.F.R. and ion excretion rates. This error would have been due to the detection limits of the technique, resulting in overestimates of burst intervals, overestimates of average \(V_{\text{burst}}\), and slight overestimates of extra-renal clearance rate (E.C.R.). Nevertheless, estimated U.F.R. was certainly not lower in non-catheterized fish than externally catheterized fish, and the larger average \(V_{\text{burst}}\) appeared more reasonable relative to other indices of bladder storage capacity (see below). On
the other hand, the use of a constant slight negative pressure of about -3 cm H$_2$O (ie. siphon) applied to the external catheter may have interfered with the competence of the urinary sphincters, perhaps causing them to open at a lower internal bladder pressure. If this occurred, it would explain the 35 % of U.F.R. which did not occur in bursts, the much smaller average $V_{burst}$, and shorter interval seen in externally catheterized fish. The net effect would be to over-estimate U.F.R. and urinary ion excretion rates by reducing mean residence time for urine in the bladder.

The results of Chapter 3 more closely resemble the results obtained using the indirect approach. Average burst interval and $V_{burst}$ were closer to values estimated in non-catheterized fish of the present study, and non-burst urination was reduced to 10 % indicating that very little urine seepage occurs which might be missed in non-catheterized fish. It was felt that the external catheter placement in Chapter 3 was improved, due to practice, and the use of slightly less negative pressure at the catheter tip (-2 cm H$_2$O), and as a result the urinary bladder and sphincter were not stressed during the experimental period. In light of these findings, it appears that the indirect approach was quantitatively more realistic in the present study.

Ion Reabsorption by the Urinary Bladder

In agreement with the present in vivo results, net mucosal to serosal transport of Na$^+$ and Cl$^-$ has been demonstrated in vitro in bladder preparations from both European and North American strains of rainbow trout (see Introduction for references). While there is some controversy over the passive Na$^+$ permeability and electrical resistance of the epithelium (Marshall, 1988), the
active transport mechanism has been characterized as an electrically silent NaCl cotransport system ("secondarily active Na\(^+\)-dependent Cl\(^-\) transport"; Figure 1.4) which is typical of many transporting epithelia. However the studies on which this conclusion are based all employed the unphysiological situation of isotonic saline on both mucosal and serosal surfaces. In vivo, the mucosal surface is normally bathed with urine in which Na\(^+\) and Cl\(^-\) levels are less than 10% of those in blood plasma (eg. Tables 2.1 & 2.3). In the frog skin, replacement of isotonic saline with a "physiological" solution (freshwater) on the mucosal surface resulted in a fundamental re-interpretation of the transport mechanism; coupled NaCl transport was replaced by independent Na\(^+\)/H\(^+\) and Cl\(^-\)/HCO\(_3^-\) exchanges (see Kirschner, 1983). In an in vitro bladder preparation of the brook trout (Salvelinus fontinalis), Marshall (1986) has recently identified the latter as the major transport mechanism. Clearly, there is a need to re-assess the situation in the rainbow trout bladder using physiological conditions (artificial urine) on the mucosal side.

If Na\(^+\)/H\(^+\) and Cl\(^-\)/HCO\(_3^-\) exchange mechanisms are identified in the rainbow trout bladder, then it is conceivable that bladder transport could also contribute to systemic acid-base balance in the rainbow trout, as suggested by Marshall (1986) for the brook trout. In this regard it is interesting that an apparent cation-anion imbalance, suggestive of the presence of HCO\(_3^-\), was seen in the urine of internally catheterized fish of series i, but not in the urine of spot-sampled fish (Table 2.1). This question is pursued in Chapter 3.

The net rates of Na\(^+\) and Cl\(^-\) reabsorption by the bladder in vivo calculated using data from the indirect (Figure 2.3) and direct approaches (Figure 2.5) were approximately 19, 8, 7, and 5 μmolkg\(^-1\)h\(^-1\) respectively. Assuming a
bladder surface area of about 7.5 cm²kg⁻¹ (based on a filled volume of about 2 mlkg⁻¹), these rates would translate to 0.7 - 2.5 μmolcm⁻²h⁻¹. *In vitro*, reported net mucosal to serosal Na⁺ and Cl⁻ transport rates (with isotonic saline on both sides) were very similar, ranging from 1.5 to 2.8 μmolcm⁻²h⁻¹ (Fossat *et al.* 1974; Fossat and Lahlou, 1979a; Marshall, 1988). Thus despite the large urine to plasma concentration gradients opposing transport, reabsorption *in vivo* proceeds just as effectively as in the absence of gradients *in vitro*. The question of whether these reabsorption rates change with volume loading and NaCl loading (ie. greater rates of urine throughput through the bladder) is pursued experimentally in Chapter 3.

In addition to Na⁺ and Cl⁻, the indirect method suggested that water, urea, ammonia, and K⁺ were also reabsorbed by the bladder (Table 2.3; Figure 2.3). Water movement undoubtedly follows active Na⁺ and Cl⁻ transport, as demonstrated in a number of *in vitro* studies on rainbow trout bladder (eg. Lahlou & Fossat, 1971; Hirano *et al.* 1973; Fossat *et al.* 1974). Based on the relative fluxes of Na⁺, Cl⁻, and water, the reabsorbed fluid would be hypertonic to urine but hypotonic to blood plasma. The neutral urea molecule would likely move passively with water reabsorption. Ammonia could diffuse as either NH₃ or NH₄⁺, in view of measured ammonia levels in urine (Table 2.1) which were 3-10 fold higher than reported plasma levels (Wright & Wood, 1988). K⁺ movement would probably be active, because urinary K⁺ concentrations (Table 2.1) were much lower than typical plasma levels (Wheatly *et al.* 1984). The only relevant *in vitro* study for trout bladder (Harvey & Lahlou, 1986) suggested that K⁺ was secreted, rather than reabsorbed by the epithelial cells. However, interpretation was again complicated by the use of isotonic saline on the mucosal surface, as
well as by the possibility of $K^+$ leakage from the epithelial cells due to stretch-induced damage.

**Urine Residence Time in the Bladder**

Earlier, it was demonstrated that the net rates of $Na^+$ and $Cl^-$ reabsorption by the bladder estimated *in vivo* compared favourably with values measured *in vitro*. The significance of the mean residence time of urine in the bladder ($T_r$) lies in the kinetics of the transport mechanism. In a static system, clearly the longer the $T_r$, the greater the net reabsorption which can be achieved. However the bladder is a dynamic system, with continual entry of new urine from the ureteral ducts, and periodic discharge of processed urine. Here the advantages of longer $T_r$ are more subtle. The greater the $T_r$, the larger will be the mean operating volume of the bladder, and therefore the more surface area involved in transport. Furthermore, the greater the $T_r$, the more chance all the urine has of contacting the transport sites if they are not homogeneously distributed over the bladder surface as indicated by Nagahama *et al* (1975) in *Gillichthys mirabilis* (see Chapter 1). For a given net reabsorption rate, the longer the $T_r$, the lower the fluctuation in concentration to which the transport sites are exposed, and therefore presumably the more efficient is the system.

It is possible to estimate $T_r$ assuming a constant flow rate of urine down the mesonephric ducts, assuming that all discharge of urine from the bladder occurs in bursts, and using average values for burst volume ($V_{burst}$) and interval between urinations ($T_i$):

$$T_r = \frac{\frac{T_i}{2} \times \frac{(V_r + V_t)}{(V_{burst})}}$$

(9)
where $V_r$ is the average residual volume left in the bladder after a bout of urination, and $V_t (= V_r + V_{burst})$ is the average total volume in the bladder immediately before each bout.

While the present study provides measurements of $T_i$ and $V_{burst}$ by both indirect (Table 2.2) and direct methods (Table 2.4), the difficulty lies in determining the correct value for $V_r$ and/or $V_t$. However the measurements of maximum urinary bladder volume ($2.20 \text{ mlkg}^{-1}$) and volumes obtained by spot-sampling ($0.52 \text{ mlkg}^{-1}$; range $= 0.13 - 1.67$) do provide some guidance. $V_t$ must clearly be less than $2.20 \text{ mlkg}^{-1}$, for it is unlikely that this maximum possible distension would ever occur in vivo. A more reasonable estimate would be $1.67 \text{ mlkg}^{-1}$ (the largest volume obtained by spot sampling); the difference between this figure and $V_{burst}$ measured by the indirect method ($1.20 \text{ mlkg}^{-1}$; Table 2) would yield $V_r = 0.47 \text{ mlkg}^{-1}$. Applying these figures, plus values for $T_i$ and $V_{burst}$ obtained by the indirect method (Table 2.2), to equation 9 yields an estimate of $26.6 \text{ min}$ for $T_r$. This suggests that mean residence time is approximately the same as the burst interval ($29.8 \text{ min}$). If the values obtained by the direct method from externally catheterized fish (Table 2.4) were used instead, $T_r$ would be much longer, $67.4 \text{ min}$, but this seems unreasonable in light of the results of Chapter 3, and because the small $V_{burst}$ would result in a value for $V_r$ of $1.22 \text{ mlkg}^{-1}$, considerably greater than the mean volume obtained by spot sampling ($0.52 \text{ mlkg}^{-1}$).

The Significance of Urinary Bladder Function In Vivo

To conclude, in resting freshwater rainbow trout, the urinary bladder stores
urine for approximately 25-30 min prior to discharge in comparably timed bursts, and likely always maintains at least a small residual volume. Periodic, rather than continuous discharge of urine may be advantageous in avoiding olfactory signals to potential predators. Intermittent urination clearly also plays a significant role in normal ionoregulation. During the holding period, the bladder actively reabsorbs \( \text{Na}^+ \) and \( \text{Cl}^- \) and possibly other substances, thereby aiding the function of the kidney in salt conservation. On a quantitative basis, this reabsorption by the bladder \( (< 20 \, \mu\text{molkg}^{-1}\text{h}^{-1}) \) amounts to only a few percent of the total \( \text{NaCl} \) reabsorption by the kidney \( (500 - 1000 \, \mu\text{molkg}^{-1}\text{h}^{-1}) \). Nevertheless, the net result of this final "scavenging" is to cut urinary \( \text{NaCl} \) losses by at least 40%. In turn this will reduce by a comparable amount the need for net \( \text{Na}^+ \) and \( \text{Cl}^- \) uptake from the water at the gills, where the gradients opposing uptake, and therefore the costs, are undoubtedly greater than in the bladder.

By negating the function of the urinary bladder, previous studies using the traditional internal catheter method have underestimated the ionoregulatory effectiveness of the entire renal system. Now that this ionoregulatory potential of the urinary bladder has been established in a control situation, the next step will be to explore its role in a dynamic situation (e.g. salt and volume loading, acid-base disturbance) where it might play an even more important part in homeostasis. These experiments are the focus of Chapter 3.
CHAPTER 3

Renal Responses to Isosmotic NaCl and NaHCO₃ Loading: Inclusion of the Urinary Bladder for the Complete Picture

Chronic plasma volume loading in teleost fishes has been examined in only a few studies (eg. Vermette & Perry, 1987; Goss & Wood, 1990b). Nevertheless it is a situation which is probably encountered routinely in migrating salmonids during their transfer from seawater to freshwater, especially if drinking or water ingestion with the food occurs (Shehadeh & Gordon, 1969; Miles, 1971; Talbot et al, 1989). This results in significantly elevated urinary flow rates (U.F.R.) which must place increased importance on renal reabsorption mechanisms to maintain plasma ion balance. The effects of chronic isotonic plasma volume loading on branchial ion transport have been studied (Vermette & Perry, 1987; Goss & Wood, 1990b). However the effects on renal ion handling were limited to measurements of U.F.R. and urine composition via the traditional internal bladder catheter.

In the present study, trout have been chronically infused with 140 mM NaCl (approximately isosmotic) for 32 h at a rate calculated to approximately double their normal resting U.F.R. Blood responses (plasma Na⁺ and Cl⁻, acid-base status) and renal responses (G.F.R., U.F.R., urination pattern, urinary concentrations, and excretion rates) have been evaluated using both the traditional internal bladder catheter technique, and the new external catheter technique (cf. Chapter 2) which allows the bladder to function normally. Comparison of responses with the two techniques has identified and quantified the important specific roles of the urinary bladder in addition to the kidney itself in these responses.
A role for the bladder in acid-base regulation was first suggested by the observations of Murdaugh et al. (1963) on \( \text{HCO}_3^- \) movement across the urinary bladder of *Lophius americanus*. More recently, evidence of \( \text{Na}^+/\text{H}^+ \) and \( \text{Cl}^-/\text{HCO}_3^- \) exchanges in an *in vitro* bladder preparation of the brook trout, *Salvelinus fontinalis* (Marshall, 1986) has also pointed to this conclusion. Charge balance discrepancies in urine collected by internal catheterization (Table 2.1) but not by spot sampling (Table 2.1) or external catheterization (Table 2.3) in Chapter 2 could also indicate an acid-base involvement.

To investigate this possibility, a chronic infusion with 140 mM NaHCO_3, for 32 h, comparable to that for 140 mM NaCl, has been carried out, designed to promote an elevation in \( [\text{HCO}_3^-] \) ("metabolic alkalosis") in the blood plasma of the fish. In previous studies, plasma \( \text{HCO}_3^- \) buildup has been identified as a mechanism employed by salmonids to compensate for decreased plasma pH during respiratory acidosis (i.e. increased \( \text{PCO}_2 \)) due to environmental hypercapnia (Lloyd & White, 1967; Eddy et al., 1977; Perry et al., 1987a) or hyperoxia (Hobe et al., 1984; Goss & Wood, 1990a). The contribution of branchial transport mechanisms in regulating elevated plasma \( \text{HCO}_3^- \) levels during these exposures is now well understood (Cameron, 1976; Wood et al., 1984; Perry et al., 1987a; Goss & Wood, 1990a). Branchial transport processes have also been recently studied during chronic infusion with 140 mM NaHCO_3, but again evaluation of the renal responses was limited to measurements of U.F.R. and urine composition via the traditional internal bladder catheter (Goss & Wood, 1990b).

Indeed, the rationale behind the use of isosmotic NaHCO_3 infusion in the present study was based on previous observations in our laboratory. Differences in arterial blood plasma composition were noticed between groups of NaHCO_3
infused fish, one fitted with the traditional internal urinary bladder catheter, and the other not catheterized (G. Goss, R.S. Munger, & C.M. Wood, personal communication). During intravascular NaHCO$_3$ infusion, consistently lower plasma pH, lower plasma HCO$_3^-$, and higher plasma Cl$^-$ concentrations were observed in uncatheterized fish. Since the internal bladder catheter collects urine as soon as it enters the bladder, one explanation of this difference could be HCO$_3^-$ secretion by the urinary bladder epithelium, perhaps in exchange for Cl$^-$. In the present study, comparisons of blood and renal responses to NaHCO$_3$ infusion between internally and externally catheterized fish has separated the responses of the urinary bladder from the overall responses of the kidney itself. In addition, comparisons between comparably catheterized groups loaded with isosmotic NaCl versus isosmotic NaHCO$_3$ has allowed examination of Cl$^-$-specific and HCO$_3^-$-specific regulatory processes.
MATERIALS AND METHODS

Experimental Animals

Adult rainbow trout (*Oncorhynchus mykiss*), weighing 230-320 g, were acclimated at 15 ± 1 °C in Hamilton tapwater for at least 7 days prior to experimentation. Unless otherwise stated, methods were the same as in Chapter 2.

To allow for infusion and blood collection during the experiments trout were anaesthetized in MS-222 (100mg l⁻¹) and then fitted with dorsal aortic catheters filled with heparinized Cortland saline (Wolf, 1963; 50 iu ml⁻¹ sodium heparin; Sigma), according to the method of Soivio et al. (1972). Fish were then fitted with either the traditional internal bladder catheter or the newly developed external urinary catheter as described in Chapter 2 (cf. Figure 2.1). A slight difference in placement technique was used for the internal catheters in comparison to Chapter 2 (see Results).

Once recovered from anaesthesia, trout were placed in darkened Perspex boxes and allowed to recover for 6 - 12 h, at which time each fish was given a bolus injection of ³H PEG-4000 (17 µCi in 0.66 ml of Cortland saline) through the dorsal aortic catheter. This was followed by 0.3 ml of heparinized Cortland saline (50 iu ml⁻¹ sodium heparin; Sigma) to push the entire ³H PEG-4000 bolus into the bloodstream and to prevent clotting in the catheter. A further 20 h recovery period was allowed prior to the start of experiments to allow equilibration of the label throughout the extra-cellular space.

Experimental Series

Series iv The fourth series of experiments was designed to directly compare the
patterns of urination, U.F.R.s, G.F.R.s, urine compositions and urinary ion excretion rates between trout fitted with internal (n=6) and external (n=7) bladder catheters during infusion of 140 mM NaCl, approximately isosmotic to trout plasma. The osmotic pressure of 140 mM NaCl is 276 mOsmkg\(^{-1}\) measured on a vapour pressure osmometer (Wescor 5100C) compared to 282 mOsmkg\(^{-1}\) in trout plasma (Wheatly et al, 1984). This allowed observation of urinary dynamic changes during plasma volume loading without a change in osmolality.

At 20 h after \(^{3}\)H PEG-4000 injection, a 300 μl blood sample ('control') was taken and replaced with 300 μl of Cortland saline. Whole blood pH was immediately measured, after which total CO\(_2\) was measured in the plasma extracted by centrifugation in a sealed haematocrit tube (see below). The remaining whole blood was then centrifuged and the plasma decanted and frozen for analysis of \(^{3}\)H PEG-4000, Na\(^{+}\), and Cl\(^{-}\) concentrations.

Also at 20 h post-injection (ie. after about 30 h of recovery) the first urine collection period was started. Urine was allowed to pass through the infra-red optical drop counting apparatus (described in Chapter 2) to measure urination dynamics and was then collected in vials. In order to minimize the effects of any negative pressure on the external urinary catheters, the catheter tips were placed 2 cm below the surface of the water (in contrast to 3 cm in Chapter 2). This pressure difference was the very minimum necessary in order to allow the urine to pass through the drop counter without inducing any back pressure on the catheter. Urination dynamics were recorded over a 2 h period, whereas urine was collected over 4 h to ensure sufficient volume for analysis. One ml of urine was decanted, stored at 4 °C, and analysed within 24 h of collection for titratable acidity minus bicarbonate (TA-HCO\(_3\)). The remaining urine was stored frozen
Figure 3.1. Plasma concentrations of Na\(^+\) (A) and Cl\(^-\) (B) in fish fitted with external and internal bladder catheters during NaCl and NaHCO\(_3\) infusion. Means ± 1 SEM.

* denotes a significant difference from the control value by a paired t-test (p ≤ 0.05).

♦ denotes a significant difference of the bicarbonate infused fish from the corresponding NaCl infused fish (ie. at the same time interval) by an unpaired t-test (p ≤ 0.05).
**A**

- ○ - Internal & NaCl
- ● - External & NaCl
- ▲ - Internal & NaHCO₃
- ▲ ▲ - External & NaHCO₃

**B**

- ○ - Internal & NaCl
- ● - External & NaCl
- ▲ - Internal & NaHCO₃
- ▲ ▲ - External & NaHCO₃

[Graph showing changes in [Na]ₚ and [Cl]ₚ over time (h) with different conditions.]
Figure 3.2. Blood pH (A), plasma HCO$_3^-$ concentrations (B), and plasma Pco$_2$ (C) in fish fitted with external and internal bladder catheters during NaCl and NaHCO$_3$ infusion. Means ± 1 SEM.

* denotes a significant difference from the control value by a paired t-test (p ≤ 0.05).

◊ denotes a significant difference of the bicarbonate infused fish from the corresponding NaCl infused fish (ie. at the same time interval) by an unpaired t-test (p ≤ 0.05).
were caused entirely by significantly elevated plasma $\text{HCO}_3^-$ to about 17 mM (Figure 3.2B) since arterial $\text{Paco}_2$ levels remained unchanged during the infusion period (Figure 3.2C). The increase in plasma $\text{HCO}_3^-$ was approximately equivalent to the decrease in plasma $\text{Cl}^-$ concentration on an absolute basis (Figure 3.1B)

Arterial pH’s were significantly higher in NaHCO$_3$ than NaCl infused fish throughout the infusion period (Figure 3.2A) due entirely to higher plasma $\text{HCO}_3^-$ levels in the NaHCO$_3$ infused fish (Figure 3.2B).

No differences in blood pH, plasma $\text{HCO}_3^-$ concentration, or plasma $\text{Pco}_2$, at the same sample times, appeared between fish fitted with internal catheters and those fitted with external bladder catheters during either NaCl or NaHCO$_3^-$ infusion (Figure 3.2).

In all groups haematorcrits started at about 28 %, falling gradually to 12 % after 32 h due to repetitive blood sampling. There were no significant differences between groups.

*Urinary Dynamic Responses.*

Glomerular filtration rates (G.F.R.), initially about 5.3 mlkg$^{-1}$h$^{-1}$, increased significantly in internally and externally catheterized fish infused with both NaCl and NaHCO$_3$ (figure 3.3A). The final increases were slightly more than 4 mlkg$^{-1}$h$^{-1}$, which was greater than the infusion rate of 3 mlkg$^{-1}$h$^{-1}$. G.F.R.s were equal between experimental groups in both the controls and during infusions indicating no effect of either catheter type or base infusion.

In parallel to G.F.R., urinary flow rates (U.F.R.) increased significantly during the infusion period in both internally and externally catheterized fish
Figure 3.3. Glomerular filtration rates (A) and urinary flow rates (B) in fish fitted with external and internal bladder catheters during NaCl and NaHCO₃ infusion. Means ± 1 SEM.

* denotes a significant difference from the control value by a paired t-test (p ≤ 0.05).

• denotes a significant difference of a fish fitted with an external urinary catheter from a fish fitted with an internal bladder catheter by an unpaired t-test (p ≤ 0.05).
(Figure 3.3B), though the absolute changes were only about half those in G.F.R. (Figure 3.3A). The increases were similar in both NaCl and NaHCO$_3$ infused fish, amounting to about 2.6 ml kg$^{-1}$ h$^{-1}$ in internally catheterized and 2.2 ml kg$^{-1}$ h$^{-1}$ in externally catheterized fish. The elevations in U.F.R. were lower than the infusion rates, suggesting that fluid was being retained within the fish.

During the control period, U.F.R.s were significantly lower in externally catheterized fish, which excreted 75% as much urine as internally catheterized fish. This difference persisted throughout the infusion period as the absolute U.F.R.'s of both groups increased. At 24-32 h, externally catheterized fish were excreting 79% as much urine as internally catheterized fish.

All fish fitted with internal bladder catheters exhibited similar urination patterns, with urine simply dripping from the catheters in a continuous manner. No internally catheterized trout showed any clumping of drips to indicate bursts of urination, in contrast to Chapter 2, where up to 12% of U.F.R. occurred in bursts in such fish. This is attributed to a simple difference in technique between Chapters 2 and 3. In both chapters the polyethylene catheters were inserted as far into the urinary bladder as possible; hitting the ureteral end of the bladder wall was used as an indication of successful location of the bladder. In Chapter 2 catheters were then immediately tied. In the present chapter, catheters were pulled back about 0.5 cm from the end of the urinary bladder before tying to the urinary papillae. This seems to have reduced blocking of the catheter tips by the urinary bladder epithelium. It was thought that this blocking led to occasional burst-type urination patterns in internally catheterized fish of Chapter 2. During infusion with NaCl or NaHCO$_3$, the drip rate increased, but there was no evidence of any patterning.
Fish fitted with external bladder catheters, on the other hand, urinated in an extremely uniform pattern of 0.9 mlkg\(^{-1}\) bursts (Figure 3.4B) at about 25 min intervals (Figure 3.4A) during the control period. 90 % (range 85 - 97 %; n=14) of the total U.F.R. occurred in discrete bursts in the present externally catheterized trout, relative to 65 % (range 35 - 92 %) in Chapter 2. This difference is attributed to the more exact placement of the catheter tips in the optical devices relative to the surface of the water (see Methods) in the present chapter.

Interestingly, during infusion the intervals between bursts decreased while average burst volumes increased (Figure 3.4). For the most part, only NaHCO\(_3\) infused fish showed significant differences from controls but this was apparently due to higher variation between fish in the NaCl infusion group, since no significant differences occurred between fish infused with NaCl and those infused with NaHCO\(_3\).

**Urinary Acid-Base Fluxes**

During NaCl infusion no change in urine pH (approximately 7.3; Figure 3.5B) or renal excretion of TA-HCO\(_3^-\) (very slightly negative at about -2 \(\mu\)molkg\(^{-1}\)h\(^{-1}\); Figure 3.5A) occurred. These responses were to be expected since NaCl infusion caused no significant change in blood acid-base status (Figure 3.2).

At the same time, renal ammonia excretion rates (+1 \(\mu\)molkg\(^{-1}\)h\(^{-1}\)) remained unchanged during NaCl infusion (Figure 3.6B) and consequently net H\(^+\) excretion rates remained constant at -1 \(\mu\)molkg\(^{-1}\)h\(^{-1}\) (Figure 3.6A).

NaHCO\(_3\) infusion though, produced a highly significant increase in urine pH (Figure 3.5B) starting at control values of 7.3 and eventually appearing to
Figure 3.4. Intervals between bursts of urination (A) and average urination burst volumes (B) in fish fitted with external bladder catheters during NaCl and NaHCO₃ infusion. Means + 1 SEM.

* denotes a significant difference from the control value by a paired t-test (p ≤ 0.05).
Figure 3.5. Renal TA-HCO$_3^-$ excretion rates (A) and urine pH (B) in fish fitted with external and internal bladder catheters during NaCl and NaHCO$_3$ infusion. Means ± 1 SEM.

* denotes a significant difference from the control value by a paired t-test (p < 0.05).

♦ denotes a significant difference of a NaHCO$_3$ infused fish from the corresponding NaCl infused fish (ie. at the same time) by an unpaired t-test (p ≤ 0.05).
Figure 3.6. Renal net H\(^+\) excretion rates (A) and net ammonia excretion rates (B) in fish fitted with external and internal bladder catheters during NaCl and NaHCO\(_3\) infusion. Means ± 1 SEM.

* denotes a significant difference from the control value by a paired t-test (p \(\leq 0.05\)).

\(\odot\) denotes a significant difference of a NaHCO\(_3\) infused fish from the corresponding NaCl infused fish (ie. at the same time) by an unpaired t-test (p \(\leq 0.05\)).
Figure 3.7. Renal Cl⁻ excretion rates (A) and renal Na⁺ excretion rates (B) in fish fitted with external and internal bladder catheters during NaCl and NaHCO₃ infusion. Means ± 1 SEM.

* denotes a significant difference from the control value by a paired t-test (p ≤ 0.05).

• denotes a significant difference of a NaHCO₃ infused fish from the corresponding NaCl infused fish (ie. at the same time) by an unpaired t-test (p ≤ 0.05).

◇ denotes a significant difference of a fish fitted with an external urinary catheter from a fish fitted with an internal bladder catheter by an unpaired t-test (p ≤ 0.05).
Table 3.1. A comparison of urine ion concentrations before and in the interval of 24 to 32 hours after the start of infusions of 140 mM NaCl, from fish fitted with internal (n=6) and external (n=7) bladder catheters. Means ± 1SEM.

* significantly different from the control value (p ≤ 0.05).

♀ significantly different from the internally catheterized value (p ≤ 0.05)

♀♀ significantly different from zero (p ≤ 0.05)
<table>
<thead>
<tr>
<th>Substance</th>
<th>Internally Catheterized</th>
<th>Externally Catheterized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 24-32 h</td>
<td>Control 24-32 h</td>
</tr>
<tr>
<td>Na⁺ (mM)</td>
<td>7.28±1.50 6.98±0.68</td>
<td>4.62±0.48 4.33±0.53</td>
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<tr>
<td>K⁺ (mM)</td>
<td>0.89±0.14 0.87±0.13</td>
<td>0.72±0.11 0.82±0.09</td>
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<td>Ammonia (mM)</td>
<td>0.36±0.05 0.29±0.06</td>
<td>0.31±0.04 0.27±0.06</td>
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<tr>
<td>Ca²⁺ (mM)</td>
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<td>1.54±0.17 1.24±0.14</td>
</tr>
<tr>
<td>Mg²⁺ (mM)</td>
<td>0.79±0.22 0.28±0.06</td>
<td>0.63±0.10 0.22±0.05 *</td>
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<tr>
<td>Cl⁻ (mM)</td>
<td>6.41±0.78 6.45±0.57</td>
<td>4.67±0.61 4.58±0.33</td>
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<tr>
<td>NO₃⁻ (mM)</td>
<td>0.18±0.09 0.26±0.14</td>
<td>0.16±0.11 0.31±0.13</td>
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<tr>
<td>SO₄²⁻ (mM)</td>
<td>0.96±0.30 0.45±0.14</td>
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<tr>
<td>Phosphate (mM)</td>
<td>0.05±0.02 0.09±0.07</td>
<td>0.17±0.08 0.20±0.08</td>
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<td>TA-HCO₃⁻ (mM)</td>
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<td>-0.50±0.24 -0.17±0.12</td>
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<tr>
<td>Urea (mM)</td>
<td>0.90±0.06 0.83±0.13</td>
<td>0.87±0.13 0.82±0.13</td>
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<td>All measured substances (mM)</td>
<td>19.18±0.62 17.47±0.58</td>
<td>14.47±0.44 12.73±0.38</td>
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<tr>
<td>ΣCations-ΣAnions (mN)</td>
<td>3.50±0.40 2.91±0.37</td>
<td>2.02±0.34 1.53±0.23</td>
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Table 3.2. A comparison of urine ion concentrations before and in the interval of 24 to 32 hours after the start of infusions of 140 mM NaHCO₃, from fish fitted with internal (n=7) and external (n=7) bladder catheters. Means ± 1SEM.

* significantly different from the control value (p ≤ 0.05)

♀ significantly different from internally catheterized values (p ≤ 0.05)

♂ significantlly different from zero (p ≤ 0.05)

† significantly different from NaCl infused fish (p ≤ 0.05)
<table>
<thead>
<tr>
<th>Substance</th>
<th>Internally Catheterized Control</th>
<th>Internally Catheterized 24-32 h</th>
<th>Externally Catheterized Control</th>
<th>Externally Catheterized 24-32 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺ (mM)</td>
<td>6.97±0.50</td>
<td>6.84±0.52</td>
<td>4.50±0.30</td>
<td>4.51±0.12</td>
</tr>
<tr>
<td>K⁺ (mM)</td>
<td>1.08±0.19</td>
<td>0.93±0.13</td>
<td>0.67±0.09</td>
<td>0.71±0.14</td>
</tr>
<tr>
<td>Ammonia (mM)</td>
<td>0.33±0.07</td>
<td>0.25±0.05</td>
<td>0.32±0.06</td>
<td>0.28±0.08</td>
</tr>
<tr>
<td>Ca²⁺ (mM)</td>
<td>1.62±0.18</td>
<td>1.27±0.10</td>
<td>1.64±0.15</td>
<td>1.24±0.10</td>
</tr>
<tr>
<td>Mg²⁺ (mM)</td>
<td>0.79±0.14</td>
<td>0.22±0.06</td>
<td>0.75±0.22</td>
<td>0.20±0.06</td>
</tr>
<tr>
<td>Cl⁻ (mM)</td>
<td>6.52±0.57</td>
<td>3.99±0.45</td>
<td>4.62±0.65</td>
<td>2.68±0.48</td>
</tr>
<tr>
<td>NO₃⁻ (mM)</td>
<td>0.09±0.08</td>
<td>0.12±0.07</td>
<td>0.04±0.03</td>
<td>0.06±0.05</td>
</tr>
<tr>
<td>SO₄²⁻ (mM)</td>
<td>0.99±0.26</td>
<td>0.39±0.09</td>
<td>1.04±0.22</td>
<td>0.40±0.10</td>
</tr>
<tr>
<td>Phosphate (mM)</td>
<td>0.15±0.07</td>
<td>0.16±0.05</td>
<td>0.10±0.09</td>
<td>0.24±0.13</td>
</tr>
<tr>
<td>TA-HCO₃⁻ (mM)</td>
<td>-0.47±0.16</td>
<td>-6.26±1.01</td>
<td>-0.83±0.33</td>
<td>-8.18±1.61</td>
</tr>
<tr>
<td>Urea (mM)</td>
<td>0.87±0.03</td>
<td>0.83±0.06</td>
<td>0.89±0.09</td>
<td>0.83±0.08</td>
</tr>
<tr>
<td>All measured substances (mM)</td>
<td>19.00±0.40</td>
<td>20.43±0.94</td>
<td>14.52±0.51</td>
<td>18.51±0.81</td>
</tr>
<tr>
<td>ΣCations-ΣAnions (mM)</td>
<td>3.80±0.39</td>
<td>-0.56±1.06</td>
<td>2.49±0.39</td>
<td>-3.91±0.76</td>
</tr>
</tbody>
</table>
Cl transport by the bladder epithelium clearly did not diminish during the infusion period.

Renal K⁺ excretion rates increased equally in both NaCl and NaHCO₃ infused fish (Figure 3.8A), becoming significantly higher after 8 h of infusion. Again, these increases were entirely due to higher U.F.R.s without any accompanying change in urine K⁺ concentrations (Tables 3.1 & 3.2).

When control K⁺ excretion rates (ie. pre-infusion values) for the two externally catheterized treatments were grouped (≈1.7 μmolkg⁻¹h⁻¹), they were significantly lower (by 44 %) than those in the similarly grouped internally catheterized fish (≈3.2 μmolkg⁻¹h⁻¹; Figure 3.8A). This finding supports the results from series i and ii of Chapter 2 (cf. Figure 2.3). This difference continued during infusion, however it was not significant and the difference did not increase over the infusion period. Infusion of NaCl or NaHCO₃ did not have a differential effect on internally catheterized fish versus externally catheterized fish.

Urea excretion rates also increased from control values during NaCl and NaHCO₃ infusions (Figure 3.8B), becoming significantly higher after 4 h of infusion. At no times were the urea excretion rates significantly different between NaCl and NaHCO₃ infused fish. Once again, increased renal urea excretion was due entirely to increased U.F.R. since urea concentrations in the urine did not change significantly during infusion (Tables 3.1 & 3.2). In fact, concentrations were slightly, though not significantly, lower in samples collected 24 h after the start of infusion than control values (Tables 3.1 & 3.2).

When control urea excretion rates (ie. pre-infusion values) for the two externally catheterized treatments were grouped and compared to grouped values for internally catheterized fish (as for K⁺), urea excretion rates from externally


Figure 3.8. Renal K⁺ excretion rates (A) and renal urea excretion rates (B) in fish fitted with external and internal bladder catheters during NaCl and NaHCO₃ infusion. Means ± 1 SEM.

* denotes a significant difference from the control value by a paired t-test (p ≤ 0.05).

\( \ast \) denotes a significant difference of fish fitted with external urinary catheters from fish fitted with internal bladder catheters by an unpaired t-test (p ≤ 0.05).

\( \ast \) denotes a significant difference of fish fitted with external catheters from fish fitted with internal bladder catheters using grouped data of NaCl and NaHCO₃, infused fish, by an unpaired t-test (p ≤ 0.05).
catheterized fish ($\approx 2.2 \mu \text{mol kg}^{-1} \text{h}^{-1}$) were significantly lower than from internally catheterized fish ($\approx 2.9 \mu \text{mol kg}^{-1} \text{h}^{-1}$). This finding again supports the results from series i and ii of Chapter 2 (cf. Figure 2.3).

During infusions of both NaCl and NaHCO$_3$, renal Ca$^{2+}$ excretion rates of both internally and externally catheterized fish increased significantly from control values of about 4.5 $\mu$mol kg$^{-1}$ h$^{-1}$ to about 6.5 $\mu$mol kg$^{-1}$ h$^{-1}$ after 24-32 h (Figure 3.9A). Once again, this was due to increased U.F.R. since no significant change in urinary Ca$^{2+}$ concentrations were seen throughout the infusion period (Tables 3.1 & 3.2). There did not appear to be any difference between fish infused with NaCl and those infused with NaHCO$_3$. During the pre-infusion period no significant difference was found between fish fitted with internal catheters and those fitted with external catheters, even when values from similarly fitted fish of different infusion groups were combined. Ca$^{2+}$ excretion rates were generally higher throughout the infusion period in the internally catheterized fish, though none of the differences were significant.

Renal Mg$^{2+}$ excretion rates ($\approx 2 \mu$mol kg$^{-1}$ h$^{-1}$) were about half those of Ca$^{2+}$ under control conditions. Furthermore, in contrast to Ca$^{2+}$, renal Mg$^{2+}$ excretion rates decreased significantly from control values over the infusion period (Figure 3.9B). Urine samples collected at 24-32 h contained significantly lower Mg$^{2+}$ concentrations than control samples (Tables 3.1 & 3.2). No significant difference between internally and externally catheterized fish was seen either during the pre-infusion period or the experimental period.

SO$_4^{2-}$ excretion rates ($\approx 3 \mu$mol kg$^{-1}$ h$^{-1}$ under control conditions) did not change significantly in either NaCl or NaHCO$_3$ infused fish (Figure 3.9C). However, a slight decrease appeared in all groups during the infusion period.
Figure 3.9. Renal Ca\(^+\) excretion rates (A), renal Mg\(^{2+}\) (B), and renal SO\(_4\)\(^{2-}\) excretion rates (C) in fish fitted with external and internal bladder catheters during NaCl and NaHCO\(_3\) infusion. Means ± 1 SEM.

* denotes a significant difference from the control value by a paired t-test (p ≤ 0.05).
Renal Ca Excretion Rate (umol/kg-1.h-1)

A

Renal Mg Excretion Rate (umol/kg-1.h-1)

B

Renal SO4 Excretion Rate (umol/kg-1.h-1)

C

Control 0-4 4-8 8-12 12-16 24-32

Time (h)
similar to that for Mg$^{2+}$. Concentrations in urine collected at 24-32 h were significantly lower than controls (Tables 3.1 & 3.2). Again, the type of catheterization had no influence on the response at any time during the experiment.

Renal NO$_3^-$ excretion rates were low ($\approx 0.5 \, \mu$mol kg$^{-1}$ h$^{-1}$) and rather variable (Figure 3.10A). No significant change occurred in the NO$_3^-$ excretion rate during NaCl or NaHCO$_3$ infusion in internally or externally catheterized fish. Values for NaCl infused fish were not significantly different from values from NaHCO$_3$ infused fish throughout the infusion. Similarly NO$_3^-$ excretion rates were similar for both internally and externally catheterized fish (Figure 3.10A).

Renal phosphate excretion rates for all experimental groups were also very low ($\approx 0.5 \, \mu$mol kg$^{-1}$ h$^{-1}$) and increased slightly over the infusion period (only one significant increase occurred at 24-32 h, from externally catheterized fish infused with NaCl; Figure 3.10B). Excretion rates for NaCl infused fish did not differ significantly from excretion rates for NaHCO$_3$ infused fish at any time. Excretion rates of internally catheterized fish were not significantly different from excretion rates of externally catheterized fish during either the control or experimental periods.

Comparisons of total positively charged ion concentrations and total negatively charged ion concentrations in the urine showed slightly more positive than negative charges in the control samples (Tables 3.1 & 3.2), as in Chapter 2 (Tables 2.1 & 2.3). Note however, that TA-HCO$_3^-$ was taken into account in the present experiments, in contrast to Chapter 2. After 24-32 h of NaCl infusion, the difference was slightly lower but remained positive in both externally and internally catheterized fish, presumably due to lower ion concentrations overall.
Figure 3.10. Renal $\text{NO}_3^-$ excretion rates (A) and renal phosphate excretion rates (B) in fish fitted with external and internal bladder catheters during NaCl and NaHCO$_3$ infusion. Means $\pm$ 1 SEM.

* denotes a significant difference from the control value by a paired t-test ($p \leq 0.05$).
Renal NO₃ Excretion Rate (μmol·kg⁻¹·h⁻¹)

Renal PO₄ Excretion Rate (μmol·kg⁻¹·h⁻¹)

Time (h)
(see Table 3.1). However, at the end of NaHCO$_3$ infusion the charge balance was negative in both internally and externally catheterized fish (Table 3.2), suggesting that the HCO$_3^-$ was being excreted in the presence of a cation which was not measured. The difference was particularly pronounced in the externally catheterized fish. During the control period externally catheterized fish had lower charge imbalances than internally catheterized fish, but this difference was not significant (Tables 3.1 & 3.2). However after 24-32 h of NaHCO$_3$ infusion, externally catheterized fish had a more negative charge discrepancy than internally catheterized fish (Table 3.2).

Finally, the sums of all measured substances in the urine were compared between control samples and samples taken after 24-32 h of infusion (Tables 3.1 & 3.2). NaCl infused fish showed slightly lower overall urine concentrations after 24-32 h of infusion, however these decreases were not significant. NaHCO$_3$ infused fish, on the other hand, showed higher overall concentrations at the end of infusion (table 3.2), primarily due to increased HCO$_3^-$ excretion. In support of Chapter 2, the overall concentration of urine from externally catheterized fish was consistently lower than urine from internally catheterized fish.

The total excretion rate of all measured substances in externally catheterized fish during the control period was only 59% of the rate in internally catheterized fish (Table 3.3). The relative increases in excretion rates from the control period to 24-32 h of infusion were similar in both internally and externally catheterized fish (39% for NaCl infused fish & 49% for NaHCO$_3$ infused fish), however the absolute increases were lower in externally catheterized than internally catheterized fish (23.1 $\mu$molkg$^{-1}$h$^{-1}$ and 35.6 $\mu$molkg$^{-1}$h$^{-1}$ for externally catheterized fish infused with NaCl and NaHCO$_3$ respectively vs. 39.3 $\mu$molkg$^{-1}$h$^{-1}$...
1 and 58.7 $\mu$mol kg$^{-1}$ h$^{-1}$ for internally catheterized fish infused with NaCl and NaHCO$_3$ respectively; Table 3.3).
Discussion

Blood Electrolytes

The lack of any marked change in either plasma Na\(^+\) or Cl\(^-\) concentrations during chronic NaCl infusion is in agreement with several previous investigations. Studies in which fish received injections of 120 mM NaCl (7.0 ml kg\(^{-1}\); Wood & Caldwell, 1978; Kobayashi & Wood, 1980) found no significant changes in plasma Na\(^+\) or Cl\(^-\) concentrations. Similarly, infusion with an artificial teleost plasma saline solution containing Na\(^+\) at approximately 128 mM and Cl\(^-\) at 120 - 129 mM (Perry & Vermette, 1987) resulted in no significant alteration of plasma Na\(^+\) and Cl\(^-\) levels. Infusion of 140 mM NaCl resulted in a significant increase in plasma Na\(^+\) concentrations (Goss & Wood, 1990b), but it should be noted that control plasma Na\(^+\) concentrations were lower than those found in the present study. These results suggest that changes in G.F.R., U.F.R., and urination patterns in the present study were due entirely to plasma volume loading and not due to any disturbance of blood ion concentrations.

Present results resemble those found in migrating salmon. During migration from seawater to freshwater, U.F.R. increases significantly, while plasma Na\(^+\) and Cl\(^-\) levels drop to typical freshwater values from higher seawater concentrations (*Oncorhynchus kisutch*, Miles, 1971; *Salmo salar*, Talbot *et al.*, 1989). Similarly, smolting juvenile *Salmo salar* exhibited increased U.F.R.s in preparation for migration from freshwater to seawater (Eddy & Talbot, 1985), although plasma electrolytes were not measured. Plasma electrolytes in smolting rainbow trout have been measured, however, and these did not change significantly (Holmes & Stainer, 1966). Since plasma Na\(^+\) and Cl\(^-\) concentrations did not drop below freshwater values, during the actual migrations, it appears
that, as with NaCl infusion in the present experiment, volume loading resulted from increased intestinal water permeability (Eddy & Talbot, 1985) combined with drinking (cf. Shehadeh & Gordon, 1969). The volume expansion itself was likely the determining factor in causing increased G.F.R. and U.F.R..

Excretion of excess Na\(^+\) and Cl\(^-\) added to the plasma during NaCl infusion is almost entirely the result of loss at the gills. Branchial Na\(^+\) and Cl\(^-\) net flux rates changed from a net uptake of approximately +20 \(\mu\)molkg\(^{-1}\)h\(^{-1}\) to a net excretion of approximately -200 \(\mu\)molkg\(^{-1}\)h\(^{-1}\) after 16 h of 140 mM NaCl infusion (Goss & Wood, 1990b). Renal Na\(^+\) and Cl\(^-\) excretion rates increased by only 8 - 15 \(\mu\)molkg\(^{-1}\)h\(^{-1}\) (Figure 3.7). Renal Na\(^+\) and Cl\(^-\) reabsorption rates, on the other hand, doubled after 24-32 h of NaCl infusion (from \(\approx\)700 \(\mu\)molkg\(^{-1}\)h\(^{-1}\) during the control period to 1400 \(\mu\)molkg\(^{-1}\)h\(^{-1}\); Figures 3.11 & 3.12) to effectively reabsorb almost all of the Na\(^+\) and Cl\(^-\) filtered by the glomeruli. Bladder reabsorption also increased over the same interval (Figures 3.11 & 3.12).

Excretion of excess Na\(^+\) added to the plasma during chronic NaHCO\(_3\) infusion is again almost entirely due to loss at the gills (Goss & Wood, 1990b) at similar rates to those during NaCl infusion (see above). Again, renal Na\(^+\) excretion rate increased by only 8 - 15 \(\mu\)molkg\(^{-1}\)h\(^{-1}\) (Figure 3.7) because renal Na\(^+\) reabsorption increased greatly so that virtually all the glomerular filtered Na\(^+\) was reabsorbed (Figure 3.11).

On the other hand, NaHCO\(_3\) infusion produced a significant decrease in plasma Cl\(^-\) concentrations. The decrease of Cl\(^-\) seems to have been replaced by an increase of HCO\(_3\)\(^-\) in almost equimolar amounts (Figures 3.1 & 3.2). This observation is in agreement with a previous NaHCO\(_3\) infusion study which showed direct equimolar replacement of plasma Cl\(^-\) by HCO\(_3\)\(^-\) (Goss & Wood, 1990b). A
Figure 3.11

A. Net glomerular filtration of $\text{Na}^+$ (o) and net renal tubular reabsorption of $\text{Na}^+$ (●) during NaCl infusion.

B. Net bladder reabsorption of $\text{Na}^+$ during NaCl infusion.

C. Net glomerular filtration of $\text{Na}^+$ (o) and net renal tubular reabsorption of $\text{Na}^+$ (●) during $\text{NaHCO}_3$ infusion.

D. Net bladder reabsorption of $\text{Na}^+$ during $\text{NaHCO}_3$ infusion.

Note the difference in scales between panels A & C versus B & D
Figure 3.12

A. Net glomerular filtration of Cl⁻ (○) and net renal tubular reabsorption of Cl⁻ (●) during NaCl infusion.

B. Net bladder reabsorption of Cl⁻ during NaCl infusion.

C. Net glomerular filtration of Cl⁻ (○) and net renal tubular reabsorption of Cl⁻ (●) during NaHCO₃ infusion.

D. Net bladder reabsorption of Cl⁻ during NaHCO₃ infusion.

Note the difference in scales between panels A & C versus B & D.
similar situation has occurred in numerous studies of trout during adaptation to environmental hypercapnia (Lloyd & White, 1967; Eddy et al, 1977; Perry et al, 1987a), and environmental hyperoxia (Hobe et al, 1984; Wheatley et al, 1984).

Plasma Cl' concentrations decreased despite increased renal reabsorption (Figure 3.12), unchanged renal excretion rates (Figure 3.7), and significantly increased Cl' uptake across the gills (Goss & Wood, 1990b). In response to an acute infusion of HCO₃⁻, McDonald and Prior (1988) also found a small increase in branchial Cl' uptake. This decrease in plasma Cl' concentrations, despite a lack of increased excretion, suggests some sort of Cl' redistribution within the trout. It is possible that Cl' is transported into the intracellular compartments of the body in exchange for HCO₃⁻ in an effort to maintain acid-base balance on a cellular level. During experiments on the adaptation of rainbow trout to varying levels of salinity, Eddy and Bath (1979) found that Cl' was transported into muscle cells in an effort to control increasing plasma Cl' levels in dilute seawater. It is possible that a similar mechanism is used to control intracellular HCO₃⁻.

**Blood Acid-Base Status**

The acid-base status of the blood is different during NaHCO₃ infusion than during either the natural compensation of hypercapnia or hyperoxia. In simple terms, the blood pH is set by the ratio of [HCO₃⁻] to dissolved CO₂ (as represented by Pco₂) according to the Henderson-Hasselbalch relationship:

\[
\text{pH} = pK' + \log \frac{[\text{HCO}_3^-]}{\alpha \text{CO}_2 \times \text{P} \text{CO}_2}
\]

where pK' and αCO₂ have the same meaning as in equation (10). During environmental hypercapnia, the fish has greater difficulty in ridding itself of CO₂.
than during normocapnia, and this results in an increase in $P_{\text{CO}_2}$ which creates an initial drop in blood pH (Perry et al, 1987a). During hyperoxia, the ventilation of the fish decreases, and hence blood $P_{\text{CO}_2}$ again increases. This causes a similar shift of the equilibrium, lowering the pH (Hobe et al, 1984). By increasing $\text{HCO}_3^-$ concentrations in the blood while eliminating $\text{H}^+$ (at constant $P_{\text{CO}_2}$), the $[\text{HCO}_3^-]/P_{\text{CO}_2}$ ratio can be returned towards normal, and hence pH is restored. It is now known that fish return blood pH toward normal during hypercapnia and hyperoxia by increasing $\text{H}^+$ efflux at the gills and excretion through the kidney, while simultaneously increasing $\text{HCO}_3^-$ influx at the gills and reabsorption from the kidney tubules (Wheatly et al, 1984; Wood et al, 1984; Perry et al, 1987a, b). The gill component is by far the more important (80 - 95 %).

In contrast, NaHCO$_3$ infusion produces a net alkalosis of the blood by increasing the plasma $\text{HCO}_3^-$ concentrations without altering $P_{\text{CO}_2}$, thereby increasing the $[\text{HCO}_3^-]/P_{\text{CO}_2}$ ratio, and consequently pH, above normal levels. However, during the initial period of return to normoxia from either hypercapnia (Perry et al, 1987a) or hyperoxia (Hobe et al, 1984), the blood becomes alkalotic since the $P_{\text{CO}_2}$ drops quickly while $\text{HCO}_3^-$ drops more slowly. This is the natural situation which $\text{HCO}_3^-$ infusion most closely resembles. Thus, it should be kept in mind that although hypercapnia, hyperoxia, and NaHCO$_3$ infusion all produce elevated plasma $\text{HCO}_3^-$ concentrations, the blood acid-base status in the two former cases is quite different from the latter. In the two former cases the fish attempts to retain $\text{HCO}_3^-$, while in the latter case the fish tries to remove $\text{HCO}_3^-$ from the body (Goss & Wood, 1990a).

Although approximately 430 $\mu\text{molkg}^{-1}\text{h}^{-1}$ of NaHCO$_3$ was infused into the trout over 32 hours (ie. 14 mmolkg$^{-1}$), plasma $\text{HCO}_3^-$ concentrations rose to just
over 16 mM, where they levelled off (Figure 3.2). The extracellular fluid volume is about 250 mlkg⁻¹ (Milligan & Wood, 1982), so plasma levels over 50 mM would occur if there were no regulation. Similarly, Goss and Wood (1990b) produced an increase of plasma HCO₃⁻ to approximately 20 mM during an infusion of 420
reabsorption rates. The measurements made in the present study allow these to be calculated separately for the kidney (ie. tubule system; T.R.), and the urinary bladder (B.R.). Reabsorption rates were calculated by means of the following formulae using mean values for G.F.R., excretion rates from internally catheterized fish (I.E.R.), and excretion rates from externally catheterized fish (E.E.R.):

\[ T.R. = G.F.R. - I.E.R. \]  \hspace{1cm} (13)  
\[ B.R. = I.E.R. - E.E.R. \]  \hspace{1cm} (14)  
\[ G.F.R. = G.F.R. \times [X]_p \]  \hspace{1cm} (15)  

\([X]_p\) represents the concentration of the substance \( X \) in the plasma. T.R. and G.F.R. could be calculated directly only for \( \text{H}_2\text{O}, \text{Na}^+, \text{Cl}^- \), and \( \text{HCO}_3^- \); because only these substances were measured in blood plasma. However B.R. could be calculated for all measured substances, as these were common to both methods of catheterization.

Increases in net tubular reabsorption of \( \text{H}_2\text{O} \) did not keep pace with increases in G.F.R. in either NaCl or NaNHCO$_3$ infused fish (Figure 3.13). This, in combination with much greater relative increases in tubular reabsorption of \( \text{Na}^+ \) and \( \text{Cl}^- \) (Figures 3.11 & 3.12) resulted in the production of an increased volume of dilute urine. This elevated U.F.R. compensated largely but not completely for the volume loading induced by the infusion. U.F.R.s increased by 2.6 and 2.2 mlkg$^{-1}$h$^{-1}$ in internally and externally catheterized fish respectively after 24-32 h, which would have eliminated between 74 and 84 % of all infused water at that time period. However, U.F.R.s did not increase to these values immediately at the start of infusion (Figure 3.3) and hence the elimination over the entire time period would be somewhat lower. In fact a total of 26.8 mlkg$^{-1}$ and 23.6 mlkg$^{-1}$
Figure 3.13

A. Glomerular filtration rate (○) and net tubular H₂O reabsorption (●) during NaCl infusion.
B. Net bladder H₂O reabsorption during NaCl infusion.
C. Glomerular filtration rate (○) and net tubular H₂O reabsorption (●) during NaHCO₃ infusion.
D. Net bladder H₂O reabsorption during NaHCO₃ infusion.

Note the difference in scales between panels A & C versus B & D.
were not excreted through the renal system (ie. retained internally or excreted elsewhere) of externally and internally catheterized fish respectively during the infusion period from 0 to 16 h when U.F.R.s had not yet peaked, amounting to a 10 % increase in extracellular fluid volume. Goss & Wood (1990b) reported similar figures. The 10 % increase in extracellular fluid volume argues for some sort of a fluid shift into the intracellular compartment to compensate, or else efflux through other routes.

Since the increases in G.F.R. and consequently U.F.R. were similar in both NaCl and NaHCO₃ infused fish, and NaCl infusion produced no change in plasma Na⁺ or Cl⁻ concentrations, then changes in G.F.R. and U.F.R. must have been due entirely to blood volume loading. G.F.R. increases were over 40 % higher than infusion rate, however H₂O reabsorption in the kidney reduced U.F.R. to only about 80 % of the infusion rate (see above). Increases in G.F.R. may have been due to increased rates of filtration at all functional glomeruli or through glomerular recruitment (see Chapter 1). How these changes are mediated in the fish kidney is not well understood at present.

It is possible that purely physical factors were wholly responsible. Dilution of plasma protein concentration probably decreased plasma oncotic pressure, and this, coupled with increased blood pressure due to increased plasma volume, undoubtedly contributed to the increased G.F.R. through increased net filtration pressure on the glomerular membrane. Exercise, which also produces significant increases in blood pressure (Stevens & Randall, 1967), has been shown to increase G.F.R. and U.F.R. (Wood & Randall, 1973; Hofmann & Butler, 1979) in rainbow trout.

However, the question of whether physical factors alone can account for
increased G.F.R., or whether other mediators are involved is unresolved. The renin-angiotensin system would appear to play no role during volume loading since infusion of angiotensin II has been shown to reduce whole-kidney G.F.R. in rainbow trout (Brown et al, 1980). Prolactin may be a mediator, since it has been shown to increase both glomerular filtration rate and number of filtering glomeruli in teleosts (Bern, 1975) and is important during seawater to freshwater migration (Nishimura & Imai, 1982). Direct neural input may also be important during glomerular recruitment, as the presence of adrenergic innervation of pre-glomerular sphincters on the afferent arterioles has been shown (Elger et al, 1984). Adrenergic neuron blockade reduced but did not abolish changes in U.F.R. and G.F.R. occurring during adaptation of rainbow trout to altered salinities (Elger & Hentschel, 1983). Further investigation is needed to determine the factor(s) controlling increased G.F.R. during isosmotic volume loading.

Renal Electrolyte Handling

Interestingly, renal tubular Na⁺ and Cl⁻ reabsorption kept pace with increased G.F.R. (Figure 3.11 & Figure 3.12), so that virtually all the extra Na⁺ and Cl⁻ filtered was removed from the urine. This occurred despite the constancy of plasma Na⁺ and Cl⁻ concentrations. Urinary bladder Na⁺ and Cl⁻ reabsorption also increased during infusion (Figure 3.11 & 3.12). Similar patterns were seen in fish infused with NaHCO₃ (Figure 3.11 & 3.12). Again, prolactin may be the mediator of increased reabsorption (Bern, 1975).

By virtue of this increased renal reabsorption, renal Na⁺ and Cl⁻ excretion rates increased by only a relatively small amount during NaCl infusion, while only
Na⁺ excretion rate increased slightly during NaHCO₃ infusion. Similar excretion rate findings were reported by Goss & Wood (1990b). Most notably, Vermette and Perry (1987) found decreased Na⁺ and Cl⁻ excretion rates during intravascular saline infusion. Using the results of the present study, it is estimated that if renal tubular reabsorption had not increased during isosmotic volume loading, urinary Na⁺ and Cl⁻ excretion rates would have increased by approximately 700 µmol kg⁻¹ h⁻¹ in all these studies (cf. Figures 3.11 & 3.12). Clearly then, despite a lack of renal reabsorption rate data in the previous studies, they both support the present finding of substantial increases.

Using the present results then, it appears that the freshwater teleost kidney is a simple organ, designed to reabsorb as much Na⁺ and Cl⁻ as possible from the urine. During isosmotic volume loading, this results in H₂O retention in the body due to increased passive H₂O reabsorption back into the blood (Figure 3.13) following Na⁺ and Cl⁻ across the renal tubule epithelium (Figures 3.11 & 3.12). Indeed the calculated Na⁺ and Cl⁻ "concentrations" of the reabsorbed fluid remained constant over the NaCl infusion period (Control = 356 mM, 24-32 h = 355 mM).

It is interesting to note that Na⁺ and Cl⁻ reabsorption do not appear to be completely coupled in the renal tubule. During NaCl infusion, increases in Na⁺ and Cl⁻ reabsorption were approximately equal (Figures 3.11A & 3.12A). However during NaHCO₃ infusion, increases in the rate of Na⁺ reabsorption were not directly paralleled by increases in Cl⁻; Na⁺ reabsorption increased to a greater extent than Cl⁻ reabsorption (increases in Cl⁻ reabsorption were only 79 % of increases in Na⁺, compare Figures 3.11 & 3.12). Increased HCO₃⁻ reabsorption only made up for half of this difference. Nishimura et al (1983) have proposed
that coupled Na\(^+\)-Cl\(^-\) co-transport occurs in the distal tubules of rainbow trout
based on transport blocking \textit{in vitro} by furosemide, ouabain, or replacement of
either Na\(^+\) or Cl\(^-\) in the perfusate. Ion transport in the proximal tubules has not
been characterized in teleosts, however, partially uncoupled Na\(^+\) and Cl\(^-\) transport
may occur, based on comparison to amphibians which seem to utilize active Na\(^+\)
transport and accompanying passive Cl\(^-\) movement (Dantzler, 1984).

If plasma concentrations of all the other substances measured in the urine
but not in the plasma (ie. K\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), ammonia, SO\(_4^{2-}\), NO\(_3^-\), phosphate, and
urea) are assumed not to have changed significantly during either the NaCl or
NaHCO\(_3\) infusion periods, then filtration rate increases would be equal to the
increase in G.F.R. multiplied by the plasma concentration of the substances.
Using plasma concentration estimates from the literature (see Figure 4.1, Ca\(^{2+}\)
and Mg\(^{2+}\) corrected for protein binding) and a G.F.R. increase of 4.3 mlkg\(^{-1}\)h\(^{-1}\)
(Figure 3.3), expected filtration rate increases can easily be estimated (K\(^+\)=18,
Mg\(^{2+}\)=7, Ca\(^{2+}\)=14, ammonia=0.5, SO\(_4^{2-}\)=4, NO\(_3^-\)=8, phosphate=5, and urea=11
\mu\text{molkg}^{-1}\text{h}^{-1}). Since, apart from ammonia, none of the measured excretion rates
even approached these values (indeed most were less than half), increased
tubular reabsorption of K\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), SO\(_4^{2-}\), NO\(_3^-\), phosphate, and urea likely all
occurred during isosmotic volume loading to compensate for increased filtration.
Renal ammonia reabsorption was not expected, since it is normally secreted into
the urine across the renal tubular epithelium (Figure 4.1). Clearly however, the
reabsorptive system was more efficient for some substances (Mg\(^{2+}\) and SO\(_4^{2-}\),
Figure 3.9B, C) than for others (K\(^+\), Ca\(^{2+}\), NO\(_3^-\), phosphate, and urea, Figures
3.8A, B, 3.9A, 3.10A, B).
Renal Acid-Base Balance

The contribution of the kidney to acid-base balance during NaHCO₃ infusion appeared to be entirely through increased HCO₃⁻ excretion. During NaCl infusion, renal tubule HCO₃⁻ reabsorption closely matched glomerular filtration. However during NaHCO₃ infusion, filtration greatly increased and tubular reabsorption, while increasing more than during NaCl infusion, did not keep up (Figure 3.14). The net effect was greatly increased HCO₃⁻ excretion. During the return to normoxia after hyperoxia, a situation in which plasma acid-base balance is similar to during NaHCO₃ infusion (see above), Wheatly et al (1984) found a very similar response, with elevated HCO₃⁻ filtration exceeding elevated HCO₃⁻ reabsorption.

It is interesting to compare this pattern with that reported during the compensation of hyperoxia (Wheatly et al, 1984) or hypercapnia (Perry et al, 1987b), where the fish is accumulating HCO₃⁻ to compensate acidotic blood pH, rather than excreting HCO₃⁻ to compensate alkalotic pH. Under these circumstances, net renal HCO₃⁻ reabsorption exceeds net filtration (ie. net H⁺ excretion occurs) coincident with increased NH₄⁺ and TA (as phosphate) excretion via the urine. In both cases, plasma [HCO₃⁻] and therefore HCO₃⁻ filtration rate is greatly elevated, but during acidosis, reabsorption exceeds filtration, while during alkalosis, reabsorption is less than filtration. From this it would appear that the HCO₃⁻ reabsorption rate is modulated by blood pH.

Quantitatively, the kidney remains much less important than the gills in the acid-base response. Based on the results of Goss and Wood (1990b) for gill responses during NaHCO₃ infusion and the present renal measurements, the kidney accounts for about 1 % of HCO₃⁻ excretion during control conditions,
Figure 3.14

A. Net glomerular filtration of HCO$_3^-$ (o) and net renal tubular reabsorption of HCO$_3^-$ (•) during NaCl infusion.

B. Net bladder reabsorption of HCO$_3^-$ during NaCl infusion.

C. Net glomerular filtration of HCO$_3^-$ (o) and net renal tubular reabsorption of HCO$_3^-$ (•) during NaHCO$_3$ infusion.

D. Net bladder reabsorption of HCO$_3^-$ during NaHCO$_3$ infusion.

Note the difference in scales between panels A & C versus B & D.
increasing to 6 - 10 % during NaHCO$_3$ infusion. These percentages are very similar to those reported by Wheatly et al (1984) and Perry et al (1987b).

*Urinary Bladder Dynamics*

During the control period, urination patterns in externally catheterized fish were much closer to those of the uncatheterized fish of Chapter 2, than to those of the externally catheterized group of Chapter 2. Intervals between bursts (25 min) and average burst volumes ($\approx 0.9$ mlkg$^{-1}$; Figure 3.4) in the present study fell between the values for uncatheterized fish (29.8 min, 1.2 mlkg$^{-1}$; Table 2.2) and those originally found in externally catheterized fish (21.0 min, 0.5 mlkg$^{-1}$; Table 2.4). It is believed that this result reflects an improved placement of the catheter tips in the optical recording devices (see Methods) which prevented siphoning. This suggests that the urination patterns seen in the uncatheterized fish of Chapter 2 were realistic. External catheterization appears to be an effective way of collecting naturally vented urine as long as the catheter tips are positioned correctly.

Average interval between bursts decreased equally in both NaCl and NaHCO$_3$ infused fish, and average burst volumes increased equally (Figure 3.4). The increase in burst frequency ($\approx 40 \%$) was greater than the increase in burst volume ($\approx 20 \%$) which suggests that, as in higher vertebrates (eg. humans - Guyton, 1986), the act of urination is triggered by a critical filling volume or pressure in the bladder (ie. via stretch receptors). Using the urinary bladder residence time equation (9) and a residual volume of 0.47 mlkg$^{-1}$, both from Chapter 2, it can be calculated that the average residence time decreased from $\approx 25$ min during the control period to $\approx 17$ min after 24 h, assuming that the
residual volume remained constant throughout the infusion period. At the same time, average urine volume in the bladder increased from 0.9 to 1.0 ml kg\(^{-1}\).

**Urinary Bladder Electrolyte Handling**

H\(_2\)O reabsorption from the urinary bladder increased during the infusion of both NaCl and NaHCO\(_3\) (Figure 3.13B & D). Increases in Na\(^+\) reabsorption during NaCl and NaHCO\(_3\) infusion (Figure 3.11B & D) and Cl\(^-\) reabsorption during NaCl infusion (Figure 3.12B) were in proportion to the increase of H\(_2\)O reabsorption so that the "concentration" of the reabsorbed fluid stayed approximately constant at the control level of about 14 mM throughout the infusion period.

From the above calculations of residence time, it appears that urinary bladder Na\(^+\) and Cl\(^-\) reabsorption became much more efficient during volume loading. Na\(^+\) and Cl\(^-\) reabsorption rates increased on an absolute basis (Figures 3.11B & D, and 3.12B) despite the fact that the length of time that urine was in contact with the ion transporting epithelial cells was shorter during the infusion period. Prolactin, earlier suggested as a possible mediator of increased G.F.R. and NaCl reabsorption in the renal tubule, may have also contributed to increased urinary bladder Na\(^+\) and Cl\(^-\) reabsorption. Although no studies have been done on rainbow trout, injection of prolactin into seawater adapted *Platichthys stellatus* (Utida *et al*, 1974) or *Platichthys flesus* (Hirano, 1975) stimulated urinary bladder Na\(^+\) and Cl\(^-\) reabsorption, simulating seawater to freshwater transfer.

For all measured urinary constituents other than Na\(^+\), Cl\(^-\), and HCO\(_3\)^- (discussed subsequently), bladder reabsorption rates (B.R.) were almost identical.
in the NaCl and NaHCO₃ infusion treatments. Therefore overall averages of the
groups are summarized in Figure 3.15.

Urea reabsorption occurred in the urinary bladder during the control
period. This agrees well with the results of non-catheterized fish from Chapter 2
(Figure 2.3). There do not seem to have been any previous studies which have
looked at urea transport across the teleost urinary bladder epithelium. It is
generally believed that urea passively accompanies water movement across the
teleost renal tubule epithelium (Hickman & Trump, 1969). Interestingly, urea
reabsorption did not decrease over the infusion period, even though epithelium
contact time was reduced (Figure 3.15B).

The significantly lower urinary K⁺ excretion rates (Figure 3.8) in externally
catheterized fish again supports the findings from non-catheterized fish of
Chapter 2 (Figure 2.3). As with urea, K⁺ reabsorption did not decrease during
the infusion period, suggesting higher efficiency of the transport sites during
volume loading (Figure 3.15A). The mechanisms of K⁺ transport in the renal
tubule have not been worked out, but the fact that the clearance ratio is highly
variable suggests that K⁺ can be either reabsorbed or secreted (Wood, 1990). It
is possible that Na⁺-K⁺-2Cl⁻ co-transport may be interchangeable with Na⁺-Cl⁻ co-
transport, based on Na⁺ and K⁺ availability (cf. Geck et al, 1980; Palfrey & Rao,
1983), thereby providing a mechanism for K⁺ reabsorption. Previous in vitro
studies of bladder ion transport have shown only K⁺ secretion, and not
reabsorption, as discussed in Chapter 2. Harvey and Lahlou (1986) demonstrated
the presence of Na⁺-Cl⁻ co-transport in the trout bladder in vitro, but saw no
involvement of K⁺, possibly because of their use of isotonic Ringer’s solution in
which Na⁺ was abundantly available relative to K⁺. Use of an artificial urine in
Figure 3.15. Since reabsorption rates of the substances presented in this table were not significantly different between NaCl and NaHCO$_3$ infused fish, average reabsorption rates of the two groups are presented.

A. Net bladder reabsorption of K$^+$ during NaCl and NaHCO$_3$ infusion.
B. Net bladder reabsorption of urea during NaCl and NaHCO$_3$ infusion.
C. Net bladder reabsorption of Mg$^{2+}$ during NaCl and NaHCO$_3$ infusion.
D. Net bladder reabsorption of SO$_4^{2-}$ during NaCl and NaHCO$_3$ infusion.
E. Net bladder reabsorption of Ca$^{2+}$ during NaCl and NaHCO$_3$ infusion.
F. Net bladder reabsorption of NO$_3^-$ during NaCl and NaHCO$_3$ infusion.
G. Net bladder reabsorption of NH$_4^+$ during NaCl and NaHCO$_3$ infusion.
H. Net bladder reabsorption of phosphate during NaCl and NaHCO$_3$ infusion.
which Na\textsuperscript{+} concentrations are much lower may establish this mechanism.

For all other urinary electrolytes except phosphate, the B.R.\textsubscript{x} calculation indicated that the ions were subject to net reabsorption (Figure 3.15C, D, E, F, G, & H). However, in all these cases, the calculation was based on excretion rates which were generally not significantly different, so the results must be interpreted cautiously. Certainly, the role of the urinary bladder in Mg\textsuperscript{2+}, Ca\textsuperscript{2+}, and SO\textsubscript{4}\textsuperscript{2-} secretion is negligible in freshwater (Figures 3.9 & 3.15C, D, & E). However it may become important in this regard in seawater where concentrations are present in toxic concentrations. In seawater the primary function of the kidney is the production of urine with high concentrations of Ca\textsuperscript{2+}, Mg\textsuperscript{2+}, and SO\textsubscript{4}\textsuperscript{2-} while retaining as much water as possible (Wood, 1990).

The only paper to date addressing the question of the contribution of the rainbow trout urinary bladder to divalent excretion in seawater measured only Mg\textsuperscript{2+} (Beyenbach & Kirschner, 1975). Higher concentrations of Mg\textsuperscript{2+} and lower concentrations of Na\textsuperscript{+} were found in samples collected by spot sampling than in urine collected by internal bladder catheterization. Although the authors suggested that these results were produced by Na\textsuperscript{+} and H\textsubscript{2}O reabsorption resulting in concentrated Mg\textsuperscript{2+}, it is possible that some Mg\textsuperscript{2+} secretion also occurred.

During the control period, as well as during NaCl infusion, it appears that more positive, than negatively charged ions were being excreted (Tables 3.1 & 3.2), which may be explained by the presence of unmeasured negatively charged organic acids (eg. lactate, pyruvate). A similar imbalance was present in the results of Wheatly \textit{et al} (1984). However, after 24 h of NaHCO\textsubscript{3} infusion there appeared to be more negative ions excreted than positive, in the urine of
externally catheterized fish, whereas approximate charge balance was achieved in the internally catheterized group. This suggests that there may be a positively charged ion secreted across the urinary bladder which was not measured, though its identity is difficult to predict.

The Role of the Urinary Bladder in Acid-Base Regulation

The present results show conclusively that the role of the urinary bladder in acid-base regulation during NaHCO₃ infusion is negligible. HCO₃⁻ reabsorption rates in the bladder were effectively zero (Figure 3.14B & D), and therefore (negative) net H⁺ excretion rates through the renal system were identical in internally and externally catheterized fish (Figure 3.6). Thus, HCO₃⁻ transport across the urinary bladder epithelium does not explain the plasma HCO₃⁻ concentration differences seen between fish fitted with internal bladder catheters and uncatheterized fish in previous work in this laboratory (see Introduction). This discrepancy must be the result of different batches of fish or as yet unknown methodological differences. Nor does this same mechanism explain the net positive charge balance of the sum of all the measured ions in urine collected by internal bladder catheterization (series ii, Table 2.1). The missing negative charge was likely made up of unmeasured anions such as organic acids, rather than HCO₃⁻.

This study lends some support to the proposal of Na⁺ and Cl⁻ reabsorption in rainbow trout by the separate Na⁺/H⁺, Cl⁻/HCO₃⁻ exchange mechanism found in the bladder of the brook trout (Marshall, 1986, 1988). During NaHCO₃ infusion urinary bladder Na⁺ reabsorption increased by almost 10 μmolkg⁻¹h⁻¹ (Figure 3.11) while Cl⁻ reabsorption increased by only about 2 μmolkg⁻¹h⁻¹ (Figure
3.12), showing that Na\(^+\) reabsorption is not entirely coupled to Cl\(^-\) reabsorption. Similarly, Hirano et al (1973) found higher Na\(^+\) than Cl\(^-\) reabsorption rates in vitro (see Chapter 1). This result is not easily explained by the proposed coupled Na-Cl co-transport mechanism of Fossat and Lahlou (1979a; see Chapter 1).

It is still possible that the bladder could contribute to acid-base regulation during hyperoxia or hypercapnia, when acidification of the plasma occurs (Wheatly et al, 1984; Perry et al, 1987). Net HCO\(_3^–\) reabsorption in the renal tubules has been shown in both cases and is thought to be vital to the maintenance of elevated plasma HCO\(_3^–\) levels (Wheatly et al, 1984; Perry et al, 1987b). Since the renal system seems to play a bigger part in acid-base balance during acidosis (eg. McDonald & Wood, 1981; Wood, 1988), it is more likely that the contribution of the urinary bladder is also important. HPO\(_4^{2–}\) (titrated to H\(_2\)PO\(_4^–\)) is thought to be the main component of urine titratable acidity (Hills, 1973; Wheatly et al, 1984), and although its concentration was negligible in urine in the present study, during plasma acidification, HPO\(_4^{2–}\) secretion into the bladder across the epithelium from the blood may be very interesting. Indeed, a slight net phosphate secretion into the bladder is shown in Figure 3.15H. Previous work has shown significantly elevated ureteral urine total phosphate concentrations during acidic conditions (McDonald & Wood, 1981; Wheatly et al, 1984).

**Summary: the Role of the Urinary Bladder in Renal Responses to Plasma Volume Loading and Acid-Base Disturbance**

In addition to confirming the basic conclusions of Chapter 2, the present results provide some unique insights into the response of the renal system in
general, and the urinary bladder specifically, to plasma volume loading and acid-base disturbance.

During volume loading, the interval time between urinations decreases from approximately 25 min during the control period to about 18 min after 24-32 h of infusion. However, urination burst size increases only slightly from 0.9 mlkg$^{-1}$ to 1.1 mlkg$^{-1}$, suggesting that the stimulus for urination is based on bladder volume and not residence time.

During isosmotic volume loading, G.F.R. increases greatly, probably due to increased blood pressure and decreased plasma oncotic pressure, and possibly mediated by hormonal and/or neural mechanisms. Na$^+$ and Cl$^-$ reabsorption rates almost double to reabsorb virtually all the Na$^+$ and Cl$^-$ from the urine while increases in H$_2$O reabsorption did not keep up with increased filtration. The net effect is that U.F.R. increases to excrete 80% of the infused fluid with only a very small rise in Na$^+$ and Cl$^-$ excretion rates. The urinary bladder also increases Na$^+$, Cl$^-$, and H$_2$O reabsorption, thereby further reducing renal Na$^+$ and Cl$^-$ losses but slightly exacerbating fluid retention.

These results suggest that the freshwater rainbow trout kidney and urinary bladder are simple organs, well adapted to produce large quantities of hyposmotic urine, even during isosmotic volume loading when it would be more effective to decrease reabsorption to rid the blood of extra NaCl. Instead the infused Na$^+$ and Cl$^-$ are largely dealt with by extra-renal mechanisms (i.e. excretion at the gills). It would be interesting to investigate whether renal Na$^+$ and Cl$^-$ reabsorption would be inhibited if plasma Na$^+$ and Cl$^-$ concentrations increased as a result of hyperosmotic infusion. Increased plasma Na$^+$ and Cl$^-$ concentrations would simulate the changes which occur during freshwater to
seawater transition, in which renal tubule Na\(^+\) and Cl\(^-\) reabsorption decreases from freshwater values (Hickman & Trump, 1969), and the release of prolactin into the blood-stream is inhibited (Nagahama et al, 1975). This might also help determine whether prolactin has played a role in the present results.

During NaHCO\(_3\) loading, G.F.R. and U.F.R. also increase, and kidney tubular reabsorption changes in a similar fashion to that seen during isosmotic volume loading. The one exception is HCO\(_3^-\), for which increases in tubular reabsorption did not keep up with increased glomerular filtration, producing an increase in excretion. The possibility for some degree of Cl\(^-\) / HCO\(_3^-\) reciprocity exists in the renal tubules, where HCO\(_3^-\) reabsorption may substitute for Cl\(^-\) reabsorption in the absence of Cl\(^-\). Na\(^+\) reabsorption increased to a higher rate than Cl\(^-\), or than the sum of Cl\(^-\) plus HCO\(_3^-\) reabsorption, both in the renal tubule and across the urinary bladder epithelium. This suggests that their transport across both epithelia is at least partially uncoupled. HCO\(_3^-\) reabsorption from or secretion into the urinary bladder was not apparent during NaHCO\(_3\) infusion.

Thus, the present study also shows that the kidney plays a role in maintaining plasma acid-base balance during alkalosis caused by NaHCO\(_3\) infusion by increased HCO\(_3^-\) excretion, but that the urinary bladder does not. Further study of urinary bladder ion transport during plasma acidosis may still reveal a role for the urinary bladder in acid-base regulation.
CHAPTER 4
General Summary and Conclusions

This thesis has examined the contribution of the urinary bladder to overall ionoregulation in the freshwater teleost rainbow trout under normal resting conditions and during plasma volume loading and plasma NaHCO₃ loading. A more exact estimate of the normal in vivo role of the entire renal system, including the urinary bladder, can now be made.

Employing new techniques, it has been found that urination occurs in discrete bursts of 0.9 - 1.0 mlkg⁻¹ at intervals of 25 - 30 min, providing sufficient time for ion reabsorption to occur in the bladder. After 24-32 h of isosmotic NaCl or NaHCO₃ infusion, the burst volume is increased slightly to ≈1.1 mlkg⁻¹, while the average interval between bursts decreases to 18 min, suggesting that bladder volume, rather than urine residence time, controls urination. During this time Na⁺, K⁺, Cl⁻, urea, and H₂O reabsorption all occur across the epithelium from the urine. Na⁺ reabsorption, previously thought to be entirely coupled to Cl⁻ reabsorption (Fossat & Lahlou, 1979a), appears to be capable of uncoupled uptake when Cl⁻ concentrations in the urine are decreased (eg. during NaHCO₃ infusion, when plasma and hence urine Cl⁻ concentrations fell significantly). It is therefore possible that there are two mechanisms for Na⁺ uptake in vivo, one coupled to Cl⁻ and the other uncoupled. The uncoupled portion may employ Na⁺ / H⁺ exchange seen in brook trout (Marshall, 1986). Urea and H₂O likely move passively, following the gradient set up by active Na⁺ and Cl⁻ transport.

The entire renal system can now be modelled to provide a new picture of the renal contribution to ionoregulation which includes the important role of the bladder (Figure 4.1). At the glomeruli, plasma is filtered at a high rate (up to 37
Figure 4.1. A schematic representation of the role of the entire renal system, including the urinary bladder, in ionoregulation in the freshwater rainbow trout. Arrows indicate direction of net fluxes of the ions indicated. All rates were calculated from values measured in the present study with the exception of plasma $K^+$, $Ca^{2+}$, $Mg^{2+}$, and phosphate (taken from Hille, 1982), plasma $SO_4^{2-}$ (taken from Höbe, 1987), ammonia, and urea (taken from M. Wilkie, personal communication). Urine $HCO_3^-$ was calculated from $TA-HCO_3^-$ by assuming that titratable acidity (TA) was entirely composed of phosphate. Filtration rates of $Ca^{2+}$ and $Mg^{2+}$ take into account plasma protein binding (Björnsson & Haux, 1985). Locations of ion transport in the renal tubule were taken from Hickman & Trump (1969).
Glomerular Filtration Rates (μmolkg⁻¹h⁻¹)

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</table>

Burst Interval=25 min
Burst Volume=0.9 mlkg⁻¹

pH=7.9

Net Tubular Reabsorption (μmolkg⁻¹h⁻¹)

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H₂O=1.6 mlkg⁻¹

Net Bladder Reabsorption (μmolkg⁻¹h⁻¹)

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<tr>
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<td>SO₄²⁻</td>
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<tr>
<td>HCO₃⁻</td>
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<tr>
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<td>urea</td>
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H₂O=0.6 mlkg⁻¹

Urinary Excretion Rates (μmolkg⁻¹h⁻¹)

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</tr>
<tr>
<td>urea</td>
<td>1.8</td>
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H₂O=2.3 mlkg⁻¹
pH=7.3
% of the plasma volume per hour (see Chapter 1), although in the present study it was closer to 9 %), providing an ultrafiltrate containing ions in approximately equal concentrations to those in plasma. In the renal tubules Na\(^+\), K\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), ammonia, Cl\(^-\), SO\(_4^{2-}\), HCO\(_3^-\), phosphate, and urea are reabsorbed, along with approximately 35 % of the filtered water. Thus, by the time the urine reaches the urinary bladder, it is highly hypotonic to blood, and contains Na\(^+\) and Cl\(^-\) at concentrations of less than 5 % of plasma levels. In the urinary bladder, Na\(^+\) and Cl\(^-\) reabsorption continues, along with passive urea and H\(_2\)O reabsorption. This final "scavenging" of Na\(^+\) and Cl\(^-\) via the bladder effectively reduces their excretion rates by 50 %. At the same time, only about 25 % of the H\(_2\)O entering the urinary bladder is reabsorbed, and thus the osmolality of the urine is further reduced before release. K\(^+\) and urea reabsorption also occurs across the bladder epithelium.

The bladder, by cutting renal Na\(^+\) and Cl\(^-\) excretion rates by half, achieves a 50 % reduction in the necessary net Na\(^+\) and Cl\(^-\) uptake across the gill, since net gill uptake must approximately equal Na\(^+\) and Cl\(^-\) excretion in the urine (see Discussion, Chapter 2). Reabsorption of Na\(^+\) and Cl\(^-\) from urine in the urinary bladder is metabolically less costly, as the plasma to urine ionic gradient is lower than the plasma to external water gradient. Thus, the extra reabsorption of the bladder saves energy for the fish by reducing the work of NaCl uptake in the gills.

The present investigation clearly establishes the ionoregulatory role in vivo in the freshwater rainbow trout of the entire renal system, including the urinary bladder. This role is the production of an extremely hypotonic urine, primarily through high rates of Na\(^+\) and Cl\(^-\) reabsorption, while keeping H\(_2\)O reabsorption
to a minimum. Reabsorption of most other filtered substances also occurs (Figure 4.1) in order to conserve them in the blood. During isosmotic volume loading, reabsorption rates increase overall, but the primary function of the renal system does not appear to change. Thus the system rids the body of most of the excess water, but not the excess NaCl.

The role of the renal system in response to plasma alkalosis induced by NaHCO₃ infusion has also been established. This role is primarily increased excretion of HCO₃⁻. The response is brought about by increased HCO₃⁻ filtration at the glomeruli, accompanied by increases in tubular reabsorption which do not match the increased filtration rate. The urinary bladder appears to play no role in acid-base regulation during plasma alkalosis.

Although the present work has clearly established the ionoregulatory role of the freshwater rainbow trout urinary bladder *in vivo*, many questions remain unanswered. The exact transport mechanisms for Na⁺, Cl⁻, and K⁺ are still subject to question, in the bladder as well as in the renal tubules. It remains possible that the bladder plays a regulatory role in other types of acid-base disturbance. Further study of the urinary bladder epithelial morphology and cellular ultrastructure may also provide valuable information. Research using methods from the present work, and utilizing seawater adapted rainbow trout may also reveal an interesting role for the bladder in hyposmotic ion regulation.
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