THE INFLUENCE OF SWIMMING ACTIVITY ON SODIUM AND WATER BALANCE IN THE RAINBOW TROUT (SALMO GAIRDNERI)

by

CHRIS M. WOOD

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Department of Zoology

The University of British Columbia
Vancouver 8, Canada

Date January 27th, 1971

ABSTRACT

The permeability of the teleost branchial exchanger to oxygen and carbon dioxide is apparently enhanced during exercise by increased blood perfusion of thin walled high surface area pathways in the gills, the secondary lamellae. However this augmented permeability to respiratory gases may well be accompanied by unfavourable elevations of water and electrolyte fluxes between internal and external environments. The object of the present study was to investigate the effect of imposed swimming activity on sodium and water regulation in the fresh water adapted rainbow trout, Salmo gairdneri.

Radiotracer methods were used to measure unidirectional components of branchial sodium exchange in fish at rest, during one hour of swimming, and during one hour of recovery from this exercise condition. Sodium fluxes during extended exercise (up to 8 hours) were quantified by similar techniques in a second series of experiments. These long term swimming trials provided flux rate data at a wide range of external sodium concentrations; analysis of these results helped to elucidate the relative importance of different mechanisms of branchial sodium transfer in the rainbow trout. Finally, determinations of urine flows and body weight changes under controlled exercise conditions in a swimming respirometer permitted an analysis of water regulation and direct measurement of renal electrolyte losses during activity.

The sodium uptake system of Salmo gairdneri in the present study had an extremely high affinity for the ion (half saturation concentration = .014 mEq Na^+/L). Both unidirectional flux rates at the gills of rainbow trout were greater than those reported for any other fresh water teleost of comparable size, despite external sodium levels much lower than those used by other workers. The presence of an exchange diffusion mechanism for sodium in the trout gill was strongly indicated but not confirmed.. Branchial transport of the electrolyte was tentatively divided into a large exchange diff-

usion component, and smaller active influx and simple diffusional efflux elements.

In resting animals, branchial sodium influx and efflux rates were equal. However short term activity (1 hour) was associated with a 70% increase in efflux of sodium across the gills, creating a net sodium deficit. This effect was quickly reversed (within 5 minutes) upon the termination of swimming. As influx did not vary, these phenomena probably represented changes in the simple diffusional efflux component without disturbance of carrier mediated sodium transport mechanisms. Branchial water entry was also greatly elevated at the start of exercise. These results were interpreted in terms of augmented passive movements of sodium and water caused by increased blood perfusion of the high permeability respiratory pathways of the gills during swimming.

The extended exercise experiments revealed that the high sodium efflux rate of the first hour of activity diminished during the second hour, and had returned to resting levels by the third and subsequent hours of swimming; influx again remained unchanged. The initial high branchial water entry was also apparently curtailed, but over a shorter time interval (15 - 60 minutes after the onset of activity). These reductions in branchial permeability to water and sodium were interpreted as compensations to decrease the osmotic penalty of exercise.

As water entry through the gills declined, urinary output was augmented; an elevated renal sodium loss accompanied the diuresis. However sodium efflux through the kidney remained small relative to the efflux of this electrolyte through the gills. A final equilibrium between branchial entry and renal excretion of water was attained, but at a higher turnover rate than during rest. Before this balance, however, urinary elimination had over-compensated for the initial water gain. The resulting net water

deficit reduced the blood space below resting volume, causing a slight increase in plasma sodium levels despite enhanced branchial and renal losses of the ion. An ischemia of "white" muscle may also have accompanied the haemoconcentration.

In summary, the results indicated that an initial osmoregulatory disturbance was associated with a redistribution of blood flow through the gills during swimming, but that both branchial hydromineral permeability and the functioning of other systems could be modified by compensations necessary to maintain sodium and water balance during extended exercise.

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GENERAL INTRODUCTION

In all higher organisms, the respiratory surface represents the point of closest proximity between the internal and external environments. As such, the structure of this area presents minimal resistance to the diffusion of oxygen and carbon dioxide down activity gradients, while its situation relative to the flows of exchanging fluids optimizes conditions for gas transfer. However a system designed for the effective diffusional transport of one molecular species may equally well serve the flux of another molecular species if appropriate gradients for the latter exist across the surface of the exchanger. The movement of substances across the exchange area which are extraneous to its respiratory purpose may be detrimental to the organism; the validity of this statement is easily demonstrated by the effect of artificial elevation to abnormal levels of a common component of the ventilatory medium (e.g. carbon monoxide).

Under normal circumstances, significant gradients for readily diffusible constituents of the internal or external media may exist across the respiratory surface. For some such substances, the resultant fluxes may be sufficiently unfavourable to the animal to necessitate limitations of the exchange capacity of the respiratory system. The evolution of the lung in air-breathing tetrapods can be viewed as a gross example of this phenomenon. Loss of body water is the most serious diffusional problem concomitant with the use of air as a respiratory medium. The development of an internal gas

exchanger effectively controlled dehydration but necessitated assumption of the relatively inefficient tidal mode of ventilation, a limitation which the organism could afford because of the high oxygen capacity, large gaseous diffusion coefficients, and low density of air.

A somewhat analagous situation applies in water breathing vertebrates where differential concentrations of ions and water between internal and external milieux cause deleterious hydromineral exchange across the respiratory epithelium. This osmotic deficit cannot be prevented through modification of the mode of ventilation; only the possession of an exchanger with a small functional "size" or permeability will effectively control salt and water fluxes. Thus the respiratory surface area per unit body weight in teleosts (Hughes,1966) is only 1/2 - 1/7 that in mammals (Hildebrandt and Young,1965) while diffusion distances between medium and blood are up to six times greater in the gill (Newstead,1967) than in the lung (Hildebrandt and Young,1965).

However even the possession of a relatively small exchanger has not, by itself, been sufficient to fully control electrolyte and water movements. Tolerance of the osmotic deficit has been effected through the development of secondary compensatory mechanisms with further energetic demand. Active transport apparatus on the gill to pump ions in opposition to their net diffusional fluxes, efficient renal devices to eliminate water gained from a hypo-osmotic environment, and intestinal absorption capabilities to replace water lost to a hyperosmotic external medium have all been necessitated by the use of an aqueous respiratory medium. Comparisons

between fish in normal and isosmotic environments have revealed that these osmoregulatory requirements may account for up to 30% of the organism's total oxygen consumption (Rao,1968; Farmer and Beamish,1969). The low metabolic rate of teleost tissue may thus be at least partially attributable to the osmoregulatory penalty involved in breathing an aqueous medium.

The above considerations indicate that the anatomical development of the teleost respiratory epithelium is limited by the level of ion and water exchange which the animal can tolerate. Yet it is known that fish can elevate their oxygen consumption ten times or more through increases in ventilation, cardiac output, and the oxygen transfer factor of the gills (Randall et al.,1967). The latter expression refers to units of gas transferred per unit gradient and will be affected by the resistance to diffusion of the entire exchanger. Thus it would appear that teleosts have the capacity to vary the effective "size" of the respiratory epithelium within the structural limits described previously. The concept that, at any one time, this branchial permeability must represent a dynamic compromise between respiratory demand and osmotic regulation has been advanced repeatedly (Steen and Kruysse,1964; Randall et al.,1967; Taylor et al.,1968; Kirschner,1969; Randall,1970 a). The origin and development of this hypothesis may be traced to physical, structural, and physiological observations on the teleost gill.

The fundamental unit of branchial structure is the filament; two rows of these flattened tapers are borne on a hemibranch. Parallel rows of leaf-like, lacunar secondary lamellae are situated on both sides of each filament. The

lamellae of adjacent filaments spatially interlock to produce a sieve-like structure (see Hughes,1966); that portion of the ventilatory water flow which is involved in gas, ion, or water exchange passes through the pores of the sieve, thus contacting the walls of the lamellae and the body of the filament. The direction of blood flow in the respiratory leaflets is in opposition to that of water flow, thus creating an efficient counter-current exchange system (Hughes and Shelton, 1962). Substances which move between the internal and external milieux are brought to the exchange surface by the bulk flows of blood and water.

The net passive migration (i.e. by diffusion or osmosis) of any species of particle across the respiratory epithelium may be described by the relation:

$$F = \frac{AD}{X}(Ci - Co)$$

where F = net flux
Ci = activity of particles inside
Co = activity of particles outside
A = surface area across which movement occurs
X = distance through which movement occurs
D = coefficient

D is a measured coefficient quantifying the resistance to movement of the particular substance over the distance implicated. For the purpose of this discussion, Ci and Co must be considered as constants determined by the nature of the external environment and the homeostatic demands of the internal milieu. However variations in the parameters A, D, and X may all be feasible mechanisms for regulating the effective branchial "size". In order to examine how each of these components may operate, it is first necessary to consider present information on the anatomy and physiology of branchial tissue.

Studies on isolated perfused gill preparations have demonstrated that the vascular resistance of the branchial capillary network is sensitive to several vaso-active agents (Krawkow,1913; Keys and Bateman,1932; Ostlund and Fange,1962; Randall *et al.*,1969; Richards and Fromm,1969,1970); in particular catecholamines increase, while acetyl choline decreases, the rate of fluid flow through the preparation. Data collected on the *in vivo* effects of these drugs has so far been limited to their actions on blood pressure, effects which are ultimately dependent on cardiac output and the resistance of the entire circulatory system, and therefore difficult to interpret with respect to the branchial area alone (Mott,1951; Steen and Kruysse,1964; Randall and Stevens,1967; Kirschner, 1969). However, at least for adrenaline and noradrenaline, the results seem to agree qualitatively with the observations on excised preparations.

Mediation of these resistance shifts is probably effected by the double nature of the circulation in a typical gill filament (Riess,1881). Steen and Kruysse (1964) have demonstrated that at any one time, blood can flow from the afferent to efferent filamental arteries either through the lacunae of the respiratory lamellae or through the body of the filament. In the latter pathway, passage to the excurrent artery is possible via either a large connective tissue space, the central "lymphatic" sinus, or direct vascular connections at the tip of the filament. Microscopic examination of single excised filaments indicated that in the presence of adrenaline, blood moved solely through the lamellae; conversely addition of acetyl choline restricted flow entirely to the

filamental routes. More sophisticated studies (Richards and Fromm,1969, 1970) supported these observations but revealed that the division between lamellar and filamental flow was a graded rather than an absolute response. These workers concluded that significant adjustments in branchial perfusion patterns are mediated adrenergically with minor tonic cholinergic control.

The factors operating these vascular shunts constitute, however, a matter of considerable dispute at present. The collagen bearing pillar cells which form the skeleton of the respiratory lamellae have long been suspected of possessing innate contractility (Plehn,1901). The discovery, through electron microscopy, of cytoplasmic filaments in these cells similar to the myofilaments of smooth muscle (Rhodin,1964; Hughes and Grimstone,1965; Newstead,1967) has lent considerable support to this belief. This finding, in combination with an apparent absence of structural contractile elements in afferent or efferent arterial walls, has led some workers to propose that blood distribution is regulated exclusively by the pillar cells (Hughes and Grimstone,1965; Newstead,1967; Datta Munshi and Singh,1968). Changes in the cross-sectional area of the respiratory lacunae through contraction or expansion of the skeletal cells would presumably affect the resistance of all three shunt pathways. However Richards and Fromm (1969) reject this theory on the grounds that, if the lamellar volume is the only rheostat in the circuit of three parallel flow routes, then it must be capable of an almost infinite range of resistance to account for the observed variations in blood distribution among the shunts. In addition they have demon-

strated muscular elements or similar thickenings in the walls of lamellar and central sinus vessels, and argue that the control of gill blood distribution involves some combination of afferent and efferent lamellar arterioles and efferent sinus vessels. Recent infrared photographic observations on live gill tissues in this laboratory (Davis and Randall - personal communication) reveal that lamellae located distal to the hemibranch on a filament are not normally filled with blood but may be opened by intravascular administration of adrenaline. Circumstantial evidence indicates that this distribution of blood may be related in part to blood pressure effects, individual lamellae having specific opening pressures. Muir (1970) has discovered valve-like flaps on branches of afferent filamental arteries in the tuna which may occlude or direct flow to whole groups of respiratory lamellae; such structures have not been observed in other teleosts. Whatever the operant mechanisms, the fact remains that dynamic regulation of blood distribution between the high volume respiratory lamellae and low volume filamental channels provides a possible explanation for the measured resistance changes across the branchial network.

Histological and cytological investigations of gill tissue have determined gross differences between the structure of the lamellae and that of the filaments. The typical respiratory lamella is a delicate flattened structure, about 10 u thick, supported by the aforementioned pillar cells; these cell bodies enclose the vascular area. The blood/water barrier, with which an erythrocyte makes intimate contact, comprises a narrow epithelium, a basal lamina, and the cytoplasmic flanges

of the pillar cells; total width of the boundary is 1 - 5 u (Hughes and Grimstone,1965; Newstead,1967). The blood space accounts for about 70% of the total lamellar volume (Hughes and Grimstone,1965). A branchial filament, however, is a long, more cylindrical process covered by an interlamellae filamental epithelium ("salt secretory epithelium") of 2 - 8 cell layers (Conte,1969). Beneath this layer is the central sinus, lined with endothelial cells and filled with a network of anastomosing connective tissue (Steen and Kruysse,1964). The thickness of this blood/water barrier is 10 - 30 times greater than the distance between the lacunae of the respiratory lamellae and the external medium.

Returning now to the diffusion equation presented on page 4, it can be seen how variations in branchial blood distribution through vascular shunting will change some components of the relationship, thereby modifying effective gill permeability to gases, ions, and water. The mean diffusion distance, X, will obviously be greatly reduced by increases in lamellar blood flow because of the above differences in width of the blood/water barrier. There exist no reports relating the total areas of filamental and lamellar surfaces; however it has been possible, assuming a mean filament depth ¼ the average length of the lamellae (from the micrographs of Hughes and Grimstone,(1965)) to calculate these values from the data given by Hughes (1966). For a 175 g specimen of Salmo trutta (Hughes,1966, p.180), the entire lamellar area is approximately 6 times the total filamental surface (59,222 mm^2 vs. 10,575 mm^2). The area parameter A of the equation is therefore extremely labile to relative blood flows through the

two regions. Making further suppositions of a lamellar thickness equal to the diameter of an erythrocyte and an accessible blood space of 70% total volume in both the lamellae and the filaments, then the maximal vascular volume of the former is 1.7 x that of the filament bodies (290 mm^3 vs. 173 mm^3). More importantly, the surface area to blood volume ratio of the lamellar shunts is 3.3 times greater than that of the filamental routes (203 mm^{-1} vs. 61 mm^{-1}). This difference is especially important for gas exchange which is significantly restricted by the diffusion distance in plasma and cytoplasm as well as by that through the cell membrane (Krogh,1941; Steen and Kruysse,1964).

Conclusions as to the effects of differential blood flow through the various routes on D, the diffusion or osmotic coefficient, can only be hypothetical as no measurements of this parameter for the substances of interest have been made in teleost tissue. However it seems likely that the simple epithelium of the lamellar wall provides less diffusional resistance than the densely reticulated and mitochondria packed cells of the interlamellae filamental epithelium. The impermeability of the plasma membrane to lipid insoluble ions and to a lesser extent water, is so severe that the movement rate of these substances is determined solely by the membrane (Davson,1964; Woodbury,1965). However diffusion coefficients for the respiratory gases are relatively similar in water and tissue (Krogh,1941) as their high partition coefficients permit migration directly through the phospholipid plasma membrane, rather than through the restrictive leaks, cell junctions, pores, or vesicles which are usually postulated

for electrolyte and water movement (Pappenheimer,1953; Woodbury 1965).

All these considerations indicate that the lamellar blood pathway will be most effective in promoting gas exchange and least effective in preventing osmotic exchange; conversely, the central filamental shunts will be virtually non-functional in respiration but highly efficient in preventing passive fluxes of salts and water between plasma and external medium. Evidence that teleosts can, in vivo, dynamically adjust the blood perfusion of different branchial pathways as in isolated preparations is provided by the large increases in oxygen consumption observed during exercise in salmonids without significant variations in the partial pressure gradient for diffusion (Stevens and Randall,1967 b). The accompanying rise in the oxygen transfer factor (Randall et al.,1967) reflects this effect and indicates that the gill permeability must increase through diversion of more blood to the respiratory lamellae. Increased lamellar flow can be related to the significant elevation of circulatory catecholamines during activity (Nakano and Tomlinson,1967). The eel, unlike the trout, does not always fully saturate blood leaving the gills; Steen and Kruysse (1964) found that dorsal aortic PO_2's could be significantly increased by intravascular adrenaline infusions. Calculations of metabolic rate based on estimated diffusion coefficients and known gradients and distances, and the anatomical area of the secondary lamellae, yield values greatly exceeding resting rates (Saunders,1962; Steen and Kruysse,1964; Hughes,1966). Conversely, Randall (1970a)has computed the functional lamellar area for gas exchange in trout from known

resting state oxygen consumption; the area implicated at rest is only 20% of the entire anatomical lamellar surface reported by Hughes (1966). Thus quiescent fish must perfuse only a small number of their respiratory lamellae with blood, or else only a small portion of each lamella.

There is as yet, however, very little evidence for the osmotic deficit which, by the above arguments, should be concomitant with increased gas exchange. Farmer and Beamish (1969) found non-significant differences in the osmolarity of plasma between exercised and resting Tilapia nilotica; in sea water, plasma concentrations exceeded those of unexercised fish; the trend was reversed in fresh water. Stevens (1968 a) postulated that observed changes in haemoglobin and plasma protein concentrations of rainbow trout blood during severe activity were associated with increased water fluxes. Activity and catecholamine administration increased the release of injected radiosodium from the gills of trout in fresh water (Randall et al.,1969). However, these experiments did not reveal whether any net efflux of sodium occurred. This latter study points up a complexity in considering the ion exchange/gas exchange compromise at the branchial surface which previous workers have not considered. Histological and cytological evidence now assigns active electrolyte transport function only to the interlamellae filamental epithelium which overlies the central sinus blood pathway (see Conte,1969) and not to the walls of the respiratory lamellae. Radiotracer techniques developed by Maetz and his co-workers (e.g. Maetz,1956; Motais et al.,1966; Motais,1967; Maetz,1969) have revealed that both active transport and exchange diffusion of ions occur in the

gills. Observed net fluxes may represent only a small proportion of the total salt exchange, while measurements of unidirectional fluxes alone cannot detect an ionic deficit. It seems reasonable that branchial blood shunting could affect carrier mediated ionic transport processes as well as exerting simple permeability effects. Increased blood flow through the central filament could unmask or activate more transport sites, while greater perfusion of the respiratory pathway could decrease the number of carrier sites in operation. Wood and Randall (1971) have recently provided some support for this hypothesis.

The purpose of the present study was to examine that component of the respiratory/osmoregulatory compromise in the teleost gill which has previously received considerable speculation but little experimental attention, ion and water exchange. An intensive programme of research on the fresh water adapted rainbow trout, Salmo gairdneri, in this laboratory over the past six years has produced an understanding of respiratory and cardiovascular function in the species and a wealth of experimental techniques. These factors, combined with the fish's availability in uniform size and condition, made it an excellent experimental animal on which the majority of the study was performed. The same problem, with a slightly different emphasis, was examined in the sea water adapted euryhaline southern flounder, Paralichthys lethostigma, in somewhat lesser detail; these results have been reported elsewhere (Wood and Randall, 1971).

The general investigational approach utilized in the present study was to treat gas exchange as the independent

variable and electrolyte and water balance as the dependent variable. Thus conditions known to modify oxygen uptake, and therefore gill blood distribution, were imposed on the animal and the associated sodium and water fluxes quantified. Exercise was utilized as a tool to change the gas exchange factor in branchial "size"; extensive data on the cardiovascular and respiratory effects of swimming in salmonids (Brett,1964; Stevens and Randall,1967 a, b; Randall et al., 1967; Smith et al.,1967; Stevens,1968 b; Davis,1968) were available for comparison. Variations in activity are probably the primary cause for redistribution of blood flow in the gills under natural circumstances. The overall aim of the study was to evaluate the effects of changing the respiratory/osmoregulatory branchial adjustment on sodium and water regulation in rainbow trout, and to determine whether compensatory mechanisms are invoked to alter the dynamics of this compromise.

SECTION I

THE EFFECT OF EXERCISE (ONE HOUR) ON SODIUM BALANCE

INTRODUCTION I

The present lack of information on the osmoregulatory component of the postulated respiratory/hydroelectrolyte compromise in the teleost gill during exercise is largely attributable to the experimental difficulties inherent in attacking the subject. A variety of satisfactory techniques have long been available for the investigation of gas exchange during activity in fish (see Fry,1957; Brett,1964; Klontz and Smith,1968). However all such swimming procedures have involved the recirculation of relatively large volumes of water in relation to the size of the experimental animal; under such conditions, the uptake or release of an electrolyte by the fish from or to the external medium is undetectable due to massive dilution both by water and by the mass of ions of the same species in the medium. The only possibility for detecting changes in osmoregulatory functions associated with swimming under these circumstances lies in sampling of the animal's internal environment. This approach has been taken by Rao (1968) and Farmer and Beamish (1969), but has proven relatively uninformative because of the large natural variability in concentration of teleost plasma constitutents (Wedemeyer and Chatterton,1970) and their lability to the stress involved in

sampling procedures (Houston, personal communication). Even when such methods have been able to detect significant differences (e.g. Toews,1969), the results can shed little light on the mechanisms involved; it is theoretically possible for an animal to greatly elevate or depress its salt turnover rate without variation in steady state internal concentrations.

Worthwhile investigation of ionic balance during exercise therefore demanded the use of techniques capable of detecting and quantifying the dynamic processes involved. Radiotracer methods have been developed for this purpose (Maetz,1956; Maetz and Garcia-Romeu,1964) which reveal the influx, efflux, and net flux components of electrolyte transfer between the fish and its external environment. However, these methods necessitate the use of water volumes only a few times greater than that of the fish to accurately quantify fluxes. At the inception of the present study, attempts were made to exercise fish in very small volume swimming respirometers but failed due to problems in obtaining laminar flow and temperature regulation in the apparatus. Consequently it became necessary to modify original plans for simultaneous determination of both oxygen uptake and branchial sodium exchange in favour of the more important objective of directly measuring only the latter during swimming in trout. Exercise was imposed on the fish through manual chasing in a small aquarium, and flux rates ascertained during activity compared with values determined on resting and post-exercise animals. Collection of urine outside the tank prevented renal contribution to measured ionic exchange. The quantity of external water used was approximately 3 times greater than that desirable for

optimal precision in flux rate measurements, but was the minimum volume which would support swimming activity in chased fish yet still yield reasonably accurate radiotracer determinations.

The use of such a system did not permit direct evaluation of the exercise level imposed through measurement of swimming speed or metabolic rate. However procedures for cannulation of the dorsal aorta (Smith and Bell,1964) and branchial chambers (Saunders,1962) have recently been adopted to measure cardio-respiratory changes in salmonids subjected to swimming respirometry (Stevens and Randall,1967 a, b; Smith et al.,1967; Davis,1968). Application of these techniques to trout in the present study permitted an evaluation of the physiological significance of the chasing procedure through comparison with the cardio-respiratory changes observed under more controlled swimming conditions by other workers. A further advantage of vascular catheterization was the provision of a means for serial blood sampling without disturbance to the animal; assay of plasma aliquots from these samples permitted determination of internal sodium levels and distribution throughout the experiment.

Radiotracer techniques of this type have not previously been applied to intact rainbow trout, Salmo gairdneri, and little has been reported about the processes of sodium regulation in the fresh water adapted form of this species. In order to interpret possible changes in sodium balance associated with readjustment of the respiratory/osmoregulatory compromise in the gill, it was necessary to learn as much as possible about both the steady state and dynamic distribution

of the ion within the organism. Thus a number of factors related to this topic were measured in addition to branchial flux rates.

The object of this section was therefore twofold:
(i) to describe sodium "metabolism" in the fresh water adapted rainbow trout
(ii) to examine the effect of exercise and recovery from exercise on the processes involved in sodium regulation in this teleost.

METHODS I

1. Experimental Animals

All fish used in this study were sexually mature rainbow trout (Salmo gairdneri) obtained from a commercial supplier, Sun Valley Trout Farm, Port Coquitlam, B.C. The trout weighed between 180 and 350 g. The animals were held at the University of British Columbia in large outdoor concrete or galvanized steel tanks supplied with dechlorinated fresh water at seasonal temperatures. During this time, the fish were fed weekly with commercial trout pellets. Experiments were performed during the period from May to December, 1969.

Prior to experimentation, the trout were transferred to one of two indoor 160 gallon concrete tanks and held at $14.5 \pm 1.5^{\circ}C$. for 1 - 4 weeks. Each tank was supplied with a glass cooling coil and two 250 watt aquarium heaters. During November and December, lights situated in the covers of the tanks were utilized as supplemental heat sources to maintain the acclimation temperature. The dechlorinated fresh water in each tank was continually aerated and recirculated through an activated charcoal and Fiberglas filter, and partially replaced at weekly intervals. Periodic determinations of sodium concentration in the acclimation facility water yielded values from 1.0 - 4.0 ug/ml.

At the time of transfer, most trout were lightly anaesthetized with MS 222 and labelled with coloured cotton threads sewn into the dorsal and caudal fins to separate

different acclimation batches. No more than 20 fish were held in each tank at any one time. During the acclimation period, the animals were not fed in order to avoid faecal contamination of the external water in subsequent experiments.

2. Operating Procedures and Cannulations

Dorsal aortic, buccal, and urinary cannulae were implanted in all experimental fish. Trout were anaesthetized in water containing 1/15,000 MS 222 and then placed ventral side up on an operating table (Smith and Bell,1964). The table was so constructed that either an anaesthetic solution (1/20,000 MS 222) or fresh water could be perfused through the mouth or backwards over the gills. The duration of the entire operation was about 30 minutes. During this time, water temperature was not controlled, but remained in the range of 11°C. to 17°C. The following cannulations were performed:

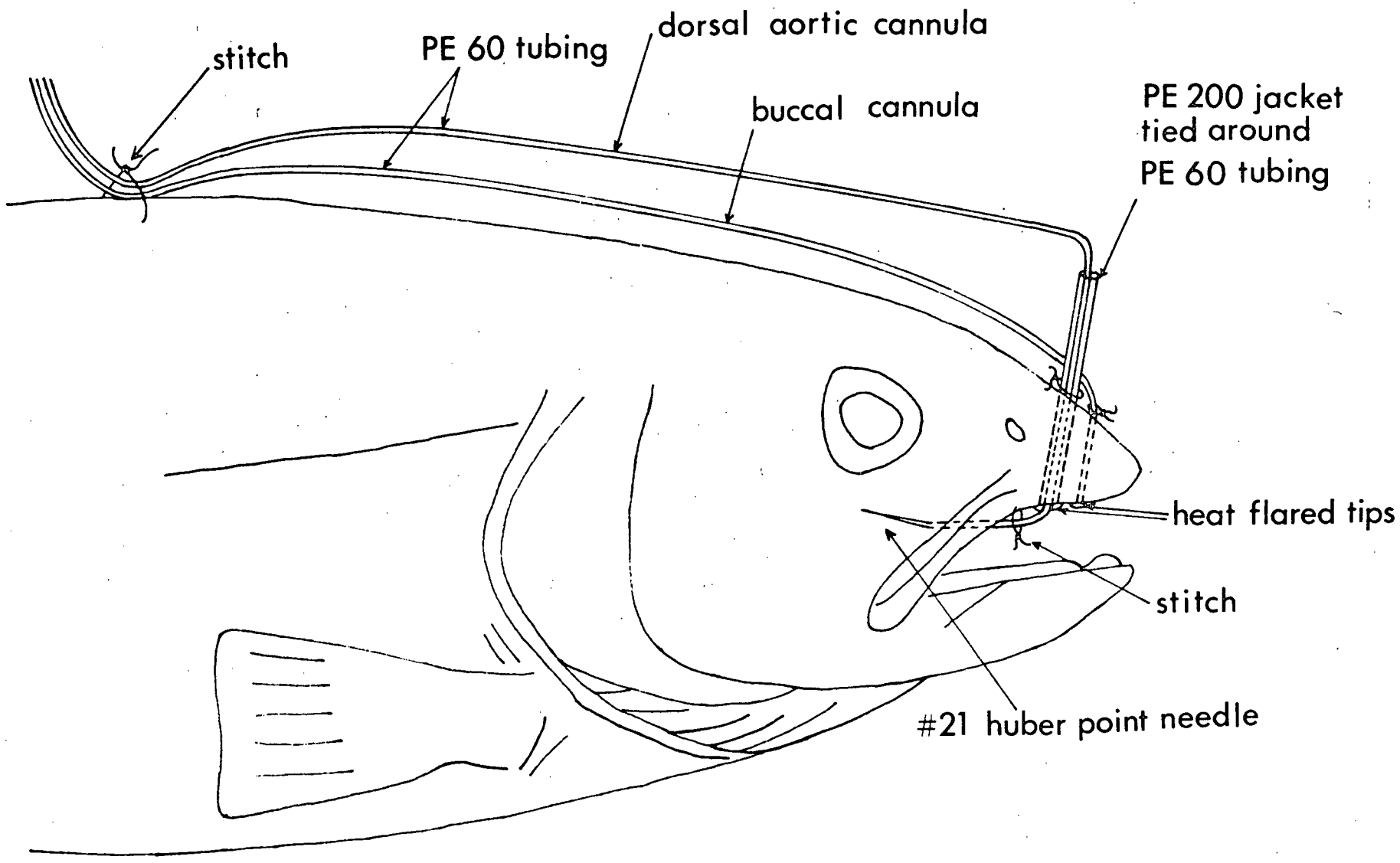
(a) Buccal Cavity Cannulation (Fig.1)

A small hole was punched through the cartilaginous portion of the snout slightly left of the midline using an iris scalpel. Care was taken to avoid damage to either the external naris or the buccal valves in both this and the dorsal aortic cannulation technique. Eighty cm of PE 60 polyethylene tubing (Clay-Adams), with a heat flared tip to act as an anchor, was passed out through this hole and tied on the dorsal side of the rostrum.

(b) Dorsal Aortic Cannulation (Fig.1)

The dorsal aorta was cannulated in the midline at the level of the first gill arch (Smith and Bell,1964) with a #21

Figure 1 A drawing of the anterior portion of a rainbow trout showing the location and size of the buccal and dorsal aortic cannulae used in the experiments of Section I.


stitch
PE 60 tubing
dorsal aortic cannula
buccal cannula
PE 200 jacket
tied around
PE 60 tubing
heat flared tips
stitch
#21 huber point needle

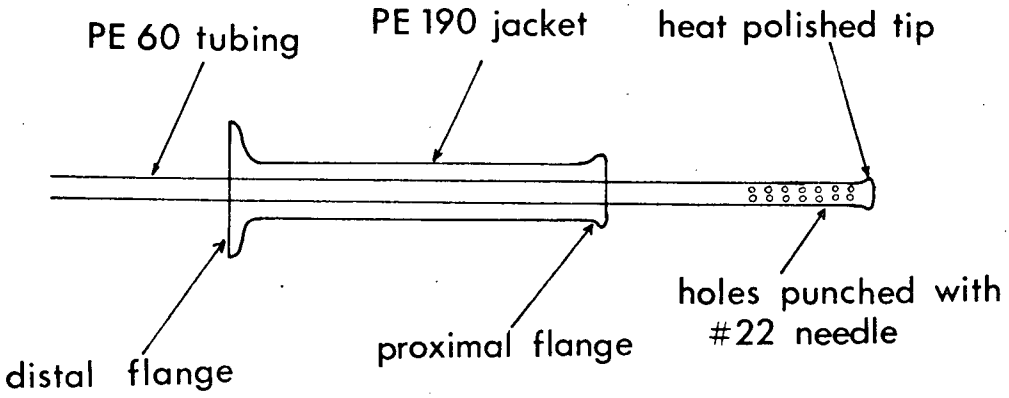
Huber point needle connected to 80 cm of PE 60 tubing. The catheter was passed out of the buccal cavity through a hole punched on the right hand side of the snout; the hole was lined with a short length of heat-flared PE 200 tubing. The PE 60 was loosely anchored to the roof of the mouth with one or two silk stitches, and the PE 200 jacket tied tightly around the inner tubing at its point of emergence from the snout to prevent inward movement of the cannula. Immediately after successful catheterization, 0.5 ml of heparinized (20 I.U./ml) Cortland saline (Wolf,1963) was infused into the dorsal aorta to prevent clotting, and the cannula plugged with a stainless steel pin. Both catheters were anchored with a single stitch to the dorsal side of the fish approximately 6 cm posterior to the snout.

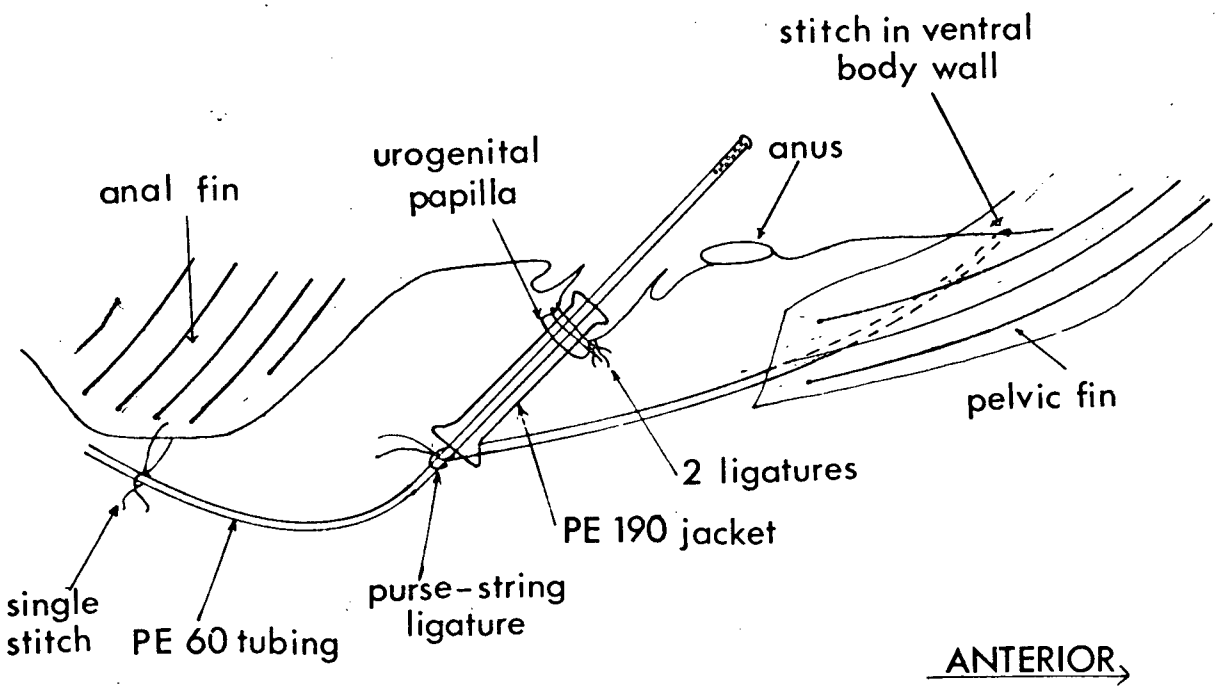
(c) Urinary Cannulation (Fig.2)

A urinary cannula was constructed by gluing, with epoxy resin, approximately 1.5 cm of PE 190 around an 80 cm length of PE 60 approximately 1.5 cm from the tip of the latter. This end of the smaller tubing had been very lightly heat polished. The distal end of the PE 190 jacket had been heavily heat flared, and the proximal end similarly flattened, but to a lesser degree. Numerous small holes were punched in the proximal 0.5 cm of PE 60 with a heated #22 needle. Successful cannulation was facilitated by having a variety of pre-constructed catheters on hand, representing a range of flare widths, jacket lengths, and proximal tip lengths. A cannula could then be fitted to the particular urogenital morphology of a specific trout; length and diameter of the uro-

Figure 2 Upper: A drawing of the construction of the proximal end of the urinary catheter.

Lower: A drawing of the placement and fixation of the urinary catheter used in this study.


PE 60 tubing
PE 190 jacket
heat polished tip
holes punched with
#22 needle
proximal flange
distal flange


stitch in ventral
body wall
anus
urogenital
papilla
anal fin
pelvic fin
2 ligatures
PE 190 jacket
purse-string
ligature
single
stitch
PE 60 tubing
ANTERIOR

genital papillae are features which vary widely among individual fish.

Cannulation was performed by opening the aperture of the urogenital papilla with a pair of fine forceps, and inserting the proximal tip of the catheter dorsad until the proximal flange of the PE 200 jacket was just inside the papilla. The papilla was then tied tightly around the jacket behind the proximal flange with two silk ligatures. During the cannulation procedure, the catheter was filled with Cortland saline. Immediately following insertion, 0.5 ml of saline was infused into the urinary system and the cannula plugged with a pin. A stitch was then tied into the ventral body wall between the pelvic fins and the loose ends of thread led back around the distal flange of the catheter in a purse string ligature. Tension of the threads was adjusted such that a ventro-caudad pull on the cannula exerted force on the ventral body wall rather than on the delicate papilla itself. Finally the catheter was loosely anchored to the anal fin with a single silk stitch. The plug was removed from the cannula only when the fish was returned to water, and was reinserted during any further transfer procedures.

This technique of urinary catheterization evolved from trials with many different procedures. From the point of view of the demands placed on the cannulae by the experimental protocol in this study, the method seemed superior to other published techniques (e.g. Enomoto,1967; Holmes and Stainer, 1966; Hammond,1969) for several different reasons:

(i) The purse string ligature ensured durability of the preparation during the experimental exercising

procedure.

(ii) The presence of a large number of holes in the collecting tip of the catheter reduced the incidence of mucous occlusion.

(iii) Ligation of the urogenital papilla behind a flange prevented leakage of urine around the outside of the catheter.

Following completion of the above procedures, the fish was revived by perfusion of the gills with fresh water and then transferred to a temperature controlled ($14.5 \pm 1.5^{\circ}C.$) 15 gallon aquarium for a recovery period of 10 to 48 hours.

3. Experimental System

Successful experiments were performed on 32 rainbow trout prepared as described above; results from a number of other fish were rejected because of cannulae failure and/or contamination of samples. The trout were divided into three treatment groups of approximately equal size: resting, active, and recovery. The experiments consisted of simultaneous measurements of sodium influx, efflux, and net flux rates, plasma sodium levels, radiosodium spaces, urine production rates, dorsal aortic blood pressures, buccal pressure amplitudes, ventilatory and cardiac rates, and hematocrits in each treatment group over a one or two hour period. In addition, samples for determination of plasma and tissue water, extracellular fluid volume, and tissue radiosodium and total sodium levels were taken at the end of the experiment for each set of animals.

(a) Experimental Chamber (Fig.3)

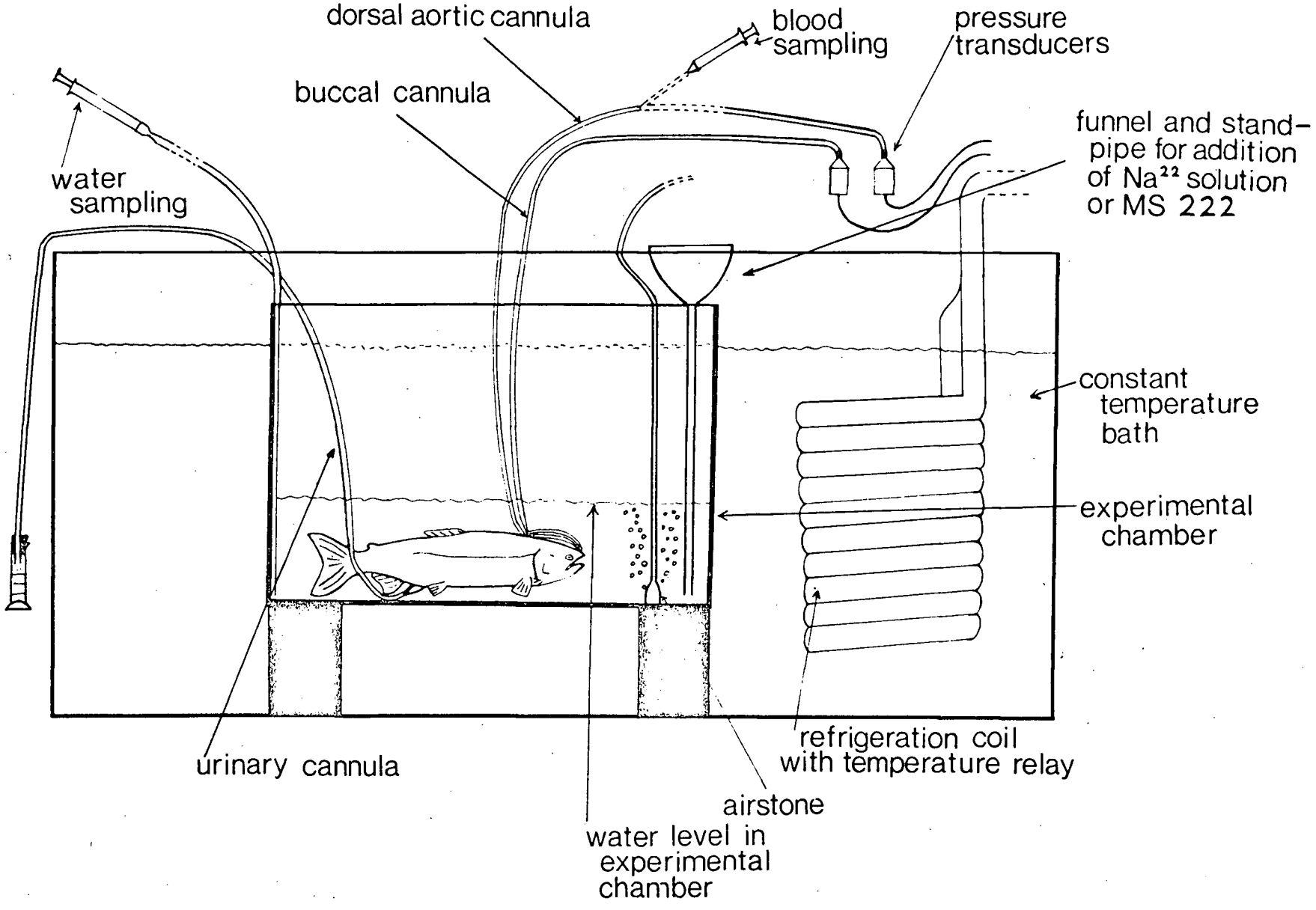
All experiments were performed in a small glass aquarium (length = 35 cm; width = 20 cm; depth = 24 cm) containing 5.5 or 6.0 L of dechlorinated fresh water (mean sodium concentration = 1.70 ug/ml) and covered on all sides with black plastic to reduce visual stimulation. The aquarium was equipped with a glass standpipe and funnel for the addition of Na^{22} solutions or anaesthetic without disturbance to the fish, polythylene tubing for the withdrawal of samples from beneath the water level, and airstones to provide adequate aeration and mixing of the water. The chamber was suspended inside a water filled 15 gallon aquarium containing a refrigeration unit with temperature relay. The apparatus maintained a stable temperature of $14.5 \pm 0.5^{\circ}C$. in the experimental tank. Adsorption of Na^{22} by the glass walls of the aquarium was negligible.

(b) Treatment Groups and Experimental Procedure

Cannulated trout were transferred to the small aquarium containing 5.5 L of water and left undisturbed for at least 2 hours prior to the start of an experiment. Dorsal aortic and buccal cannulae were attached to pressure transducers immediately after this transfer. The urinary catheter, however, was led out of the chamber only a few minutes before the beginning of an experiment to avoid excessive tangling of the 3 pieces of tubing.

(i) Resting Trout. At zero time, 500 ml of fresh water containing a known amount of $Na^{22}Cl$ (7 - 10uC.) were added to the aquarium. The changes in total sodium and Na^{22} concentrations of the external medium were monitored over the

Figure 3 A drawing of the experimental system used in Section I.


dorsal aortic cannula
blood sampling
pressure transducers
buccal cannula
funnel and stand-pipe for addition of Na^{22} solution or MS 222
water sampling
constant temperature bath
experimental chamber
urinary cannula
refrigeration coil with temperature relay
airstone
water level in experimental chamber

following 60 minutes; water radioactivity generally declined by 33 - 50% of the zero time level. With the exception of periodic blood sampling from the dorsal aortic catheter, the fish were left undisturbed during this time. At 60 minutes, a solution of MS 222 sufficient to provide a concentration of 1/10,000 in the external medium was added to the chamber. Immediately after loss of equilibrium, the fish was removed from the aquarium and stunned by a blow on the head in preparation for immediate tissue sampling and weighing procedures. Finally, the patency of the urinary catheter was checked by injection of a small amount of dyed saline into the cannula, followed by gentle pressure on the ventro-posterior body wall. All data from trout exhibiting leakage, and therefore the release of urine during the experiment, were discarded.

(ii) Active Trout. The fish was chased manually with a fire-polished glass rod during the experimental hour. Chasing was terminated 1 minute before each blood sampling and resumed 1 minute after its completion. As each of the 5 blood sampling procedures during the hour took approximately 2 minutes, the trout was kept active for about only 40 minutes out of a total of 60. In effect, merely an occasional movement of the rod was sufficient to keep the animal swimming during the first minutes of the experiment. However by the end of the hour, it became necessary to continually tap the fish in the caudal region to induce activity. With practice, it was possible to avoid excessive tangling of the three cannulae through periodic reversals of the direction of chasing. In other aspects, the experimental procedure was identical to that used for the resting trout.

(iii) Recovery Trout. As for the active experiments, the fish was chased over a one hour period. However, no radioisotope was added to the chamber at zero time; thus the volume of water in the aquarium remained at 5.5 L for the first 60 minutes. In addition, no blood samples were withdrawn, but chasing was terminated for 4 minute periods at times corresponding to the interruptions necessitated by blood sampling in the active fish. At 60 minutes, 500 ml of water containing 7 - 10uC of $Na^{22}Cl$ were added to the external medium, and the experiment proceeded for another hour with methods identical to those used for resting animals.

(c) Recording Techniques

Dorsal aortic blood pressures and buccal pressures were measured throughout the experiment in all three treatment groups in an attempt to quantify the physiological significance of the activity level imposed on the fish by chasing. Heart rate and breathing rate could also be ascertained from the recordings. Each cannula was connected to a Statham P-23 B.B. (venous) pressure transducer. The pressure changes were displayed together with a time signal on two channels of a Beckman Type R.S. Dynograph Recorder. The system was electrically balanced at zero against the water level in the tank before each experiment and calibrated at the end of each run. Fluctuations in water level due to movement of the fish, water sampling, and addition of the $Na^{22}Cl$ solution prevented computation of exact positive and negative pressures in the buccal cavity; however, the total pressure amplitude of the respiratory movements was still obtainable from the records. After the first few trials, it became apparent that both traces

became highly irregular during chasing (see Fig.4). Thus systematic pressure recordings were generally only taken during the 1 minute interval before and after each blood sampling period; recordings from active trout are, therefore, representative of an immediately post-exercise condition.

(d) Sampling Procedures

All apparatus used in the handling and subsequent processing of water, plasma, and tissue samples had been carefully washed with glass-distilled water and dried prior to use.

(i) Water Samples. Ten ml water samples were drawn from the aquarium through PE 60 tubing into plastic syringes; samples were subsequently transferred to polyethylene vials (Nalgene Corporation) and frozen at -12°C. For all groups, zero time represented the start of the experiment but coincided with the addition of isotope to the chamber in only the resting and active treatments. Samples were withdrawn at zero time, 1, 5, 15, 30, 45, and 60 minutes during the resting and active experiments, while in the recovery studies, water was removed at zero time, 5, 15, 30, 45, 58, 61, 65, 75, 90, 105, and 120 minutes. In addition, aliquots of the Na^{22} solution were taken in all experiments.

(ii) Blood Samples. Blood samples were withdrawn via the dorsal aortic catheter from resting and active trout at zero time (approximately 100 uL) and 5, 15, 30, 45, and 60 minutes (all approximately 200 uL); (total volume removed including "spillage" = 1.45 ml). From recovery fish, blood was taken at zero time and 60 minutes (both 100 uL) and at 65, 75, 90, 105, and 120 minutes (all 200 uL); (total volume removed including "spillage" = 1.60 ml). Blood was allowed

to flow from the catheter into a 1 ml plastic syringe under its own pressure or under slight suction. The syringe had been prerinsed with a 100 I.U./ml sodium heparin/distilled water solution and allowed to dry; this level of heparinization was adequate to prevent clotting or haemolysis but was not great enough to cause significant sodium contamination of plasma samples. As it was impossible for a single experimenter to take blood and water samples simultaneously, the following scheme was used. Aspiration of water commenced 15 seconds before the designated time and lasted approximately 30 seconds; only then was blood sampling (a 1 to 2 minute procedure) started. Thus while water samples were on time, blood samples were systematically 90 seconds late.

The blood sampling technique was designed as a compromise to reduce three undesirable effects:

1. Contamination of the blood aliquot with saline through "smearing" in the cannula.
2. Reduction of internal sodium levels and hematocrit through excessive bleeding.
3. Contamination of plasma still inside the trout through the reintroduction of blood mixed with saline.

Thus after disconnection of the dorsal aortic catheter from the manometer, blood was allowed to flow up the tubing under its own pressure, thereby forcing out the saline. The first 3 red drops of fluid emerging from the catheter were simply discarded ("spillage"), and the blood sample collected. Then the cannula was reconnected to the pressure transducer and saline slowly reinjected until the blood in the tubing had just disappeared into the trout.

Blood was transferred to Clay-Adams 0.6 ml glass

hematocrit tubes and spun at 13000 x G for 10 minutes; hematocrits were read directly from the centrifuge tubes. The supernatant plasma was aspirated into small polyethylene vials. A small portion of the plasma (20 uL) from terminal samples was analyzed for water content with a Goldberg Refractometer (American Optical TS meter). This device measured refractive index which could be converted to percent plasma water through tables supplied with the instrument. The samples were then frozen and stored at -12°C. until further analysis.

(iii) Urine Samples. Urine production was measured over the 1 or 2 hour experimental periods. In some experiments, the catheter, although patent, was apparently occluded; urine production data from these fish were rejected. None of these trout exhibited symptoms (urogenital distension, high tissue water levels) characteristic of chronic urinary occlusion (see Section II). It was therefore assumed that cannula failure was recent and probably associated with the suction of siphon drainage started at the beginning of the experiment. On this basis, data on all other parameters for these animals were accepted. Contamination of the urine sample with pre-experimental production from the dead space of the cannula (0.35 ml) rendered ionic analyses uninformative. However, assay of Na^{22} in urine from a few experiments demonstrated that renal excretion of the radioisotope was negligible and therefore had no effect on flux rate and sodium space determinations.

(iv) Tissue Samples. Anaesthetization of the trout with MS 222 prior to sacrifice was performed in an attempt to maintain any physiological difference in tissue samples

resulting from differences in experimental treatments. Extreme hyper-activity associated with handling in normal trout could well have eliminated characteristics distinctive of the resting state or recovery conditions. After removal from the water and stunning, the fish was quickly rinsed with fresh water and dried with paper towels. Three portions (approximately 1.5 g each) of dorsal epaxial muscle were excised from the right side of the trout below the dorsal fin. Any samples visibly contaminated with lateral line red muscle or skin were discarded.

In early experiments, each piece of tissue was immediately transferred to a tared aluminum dish and weighed to .1 mg accuracy. In later runs, all three tissue samples from an individual fish were immediately sealed in a small polyethylene vial and left for 20 - 30 minutes before separation and weighing. Tests showed that identical results were obtained with the two methods. Tissues were dried at 103°C. for 72 hours and water contents calculated by weight difference. The dishes of dried tissue were thereafter stored in a closed container for later ionic analysis.

(v) Physical Measurements of the Fish. Ionic flux rates of the gills should theoretically be proportional to the surface area implicated; over a limited range, gill area may be considered proportional to body weight. It is customary to express branchial flux rates on a per weight basis for a fairly homogeneous set of fish. Accurate and systematic determinations of body weight were therefore necessary. After tissue dissection, the carcass was carefully redried and weighed within 10 minutes of the end of the experiment.

A correction factor (2 x the weight of removed tissue) was added to give a final weight. In order to test whether the degree of body development (and therefore the body weight to gill area ratio) was similar among different treatment groups, records of length, fork length, and maximum depth, were also taken. The measurements permitted calculation of the coefficient of condition, an expression of "plumpness" (Toews, 1969) for each trout.

$$\text{Coefficient of Condition} = \frac{100 \times \text{Body Weight (g)}}{(\text{Fork Length})^3 (\text{cm})}$$

4. Analytical Procedures

(a) Na^{22} Concentration

(i) Water. Duplicate or triplicate 1.0 ml aliquots of each water sample were dried on planchets and counted for β emission in a Nuclear-Chicago Model 470 gas flow detector equipped with an automatic sample changer and decade scaler. All samples for radioactivity analysis were counted 3 times for 30 minutes/10,000 counts.

(ii) Plasma. Single 50 or 100 uL aliquots of each plasma sample were diluted with 1.0 ml of water on planchets, and then similarly counted and dried. Self absorption resulting from the small amounts of solid material in the plasma was insignificant.

(iii) Tissue. To avoid derivation of self-absorption correction factors for a variety of tissue weights, it was necessary to count the same amount of material (75 - 85 mg) in each planchet. Dried tissue samples were individually ground to a fine powder with a mortar and pestle. During the period of storage, the samples had rehydrated by 10 - 15% of their

true dry weight. Thus 85 - 95 mg amounts of powdered tissue were weighed into tared planchets and redried at 103°C. for 12 hours. The planchets were then reweighed; only those samples with a true dry weight within the range of 75 - 85 mg were accepted. Rejected samples were appropriately corrected and the dessication process repeated until acceptable values were obtained. One ml of water was then added to each planchet in order to obtain an even distribution of matter over the entire surface area; the resultant paste was mixed to a fine slurry with a needle. After slow drying under a heat lamp to avoid cracking and flaking of the powder surface, the planchets were counted as above. Triplicate aliquots were analysed for each experiment. A correction factor (x 1.4661) for self-absorption of emissions by the tissues was determined by assaying known amounts of Na^{22} which had been incubated and dried with 75 - 85 mg of powdered unlabelled dorsal epaxial muscle. In addition, it was necessary to apply appropriate corrections for radioactive decay and efficiency change of the counter to the results as tissue samples were counted 9 - 12 months after those of plasma and water from a particular experiment.

(b) Total Sodium Concentration

(i) Water. The sodium concentration of external water samples was assayed by flame emission photometry at 5890 Å against appropriate dilutions of a commercially prepared standard (Harleco). As the actual changes in sodium levels in a 6 L volume caused by a 200 g trout over 15 minute intervals were extremely small, dilution of all samples to a common concentration range was impractical. Two different methods

of analysis were applied, as determined by the properties of the two different photometers used in this study.

Samples from the resting state and active experiments were assayed on a Unicam Model Sp 900A Flame Emission/Atomic Absorption Spectrophotometer. Over the range 0 - 3.50 ug Na^+/ml, it was possible to calibrate the instrument over 0.50 ug/ml intervals (e.g. 1.75 - 2.25 ug Na^+/ml) bracketing the concentrations of experimental samples. A few samples with a concentration greater than 3.50 ug Na^+/ml were diluted 1/2 with distilled water and similarly read. Because of marked instability of the instrument in this mode of operation, multiple determinations on each unknown were performed at less than 50% scale deflection.

Samples from the recovery experiments were analysed on the emission mode of a Techtron Model AA 120 Atomic Absorption Spectrophotometer . Operation of the instrument at ranges exclusive of 0 ug Na^+/ml was not feasible. Thus the photometer was calibrated over intervals of 0 - 1.00, 0 - 2.00, and 0 - 3.00 ug Na^+/ml, with consequent loss of accuracy in the higher concentration ranges. However this fault was partially compensated for by the extreme stability and reproducibility of determinations even at nearly maximal scale deflections. Each unknown was read twice; those exceeding the highest calibration range were again diluted by two. Overall, the accuracy of the two techniques appeared to be comparable, but the latter was far more efficient.

(ii) Plasma. Triplicate dilutions (1 uL in 6.00 mls of distilled water) of each plasma sample were assayed in the 0 - 1.00 ug Na^+/ml calibration range on either flame photometer.

Each dilution was read 2 - 3 times. It should be noted that freezing of plasma may result in partial precipitation of proteins, producing a sample which is strictly neither plasma nor serum. However tests have shown that sodium concentrations of the fluid are not significantly changed by the freezing process (Toews, personal communication); for the sake of convenience, the term "plasma" concentration is used throughout this thesis for determinations on frozen samples.

(iii) Tissue. Single 115 mg aliquots of each powdered muscle sample were weighed into small tared polyethylene vials. The containers were heated at 70°C. for 24 hours and reweighed to obtain true dry weights. Exactly 1.0 ml of concentrated HNO_3 was then added, and the samples incubated for 48 hours at room temperature. Triplicate 50 uL aliquots were diluted to 10.0 mls and assayed on the Techtron flame photometer over a 0 - 50 uEq Na^+/L calibration range. Toews (1966) has shown that the high concentration of potassium in trout muscle enhances sodium flame emission by as much as 15%. Therefore final dilutions of both standards and unknowns contained a 200 ug K^+/ml swamp to eliminate this effect.

5. Calculations

(a) Sodium Flux Rates

Measurements of the decline in radioactivity and change in sodium concentration of the external medium over the one hour experimental period allowed calculation of sodium influx, efflux, and net flux across the external surface of the trout. Multiplication of the concentration of total sodium (ug/ml) and Na^{22}(cpm/ml) by the volume of water in the

chamber (after appropriate correction for sampling deficits) yielded absolute amounts of total sodium (Na ext) and Na^{22} (Q) in the external medium at any one time. Over any time period (Δt), the influx rate (Fi) of sodium(ug/min) into the fish could be calculated from the specific activity and loss in radioactivity (ΔQ) of the external medium:

$$Fi = \frac{-\frac{\Delta Q}{\Delta t}}{\frac{Q}{Na\ ext}} \qquad (1)$$

Similarly the net flux rate (Fn) of sodium over the same time interval could be computed from the change of total sodium (ΔNa ext) in the water:

$$Fn = -\frac{\Delta Na\ ext}{\Delta t} \qquad (2)$$

Thus the net flux was positive when external Na^+ concentration diminished. Finally the efflux rate (Fo) was calculated by difference:

$$Fo = Fi - Fn \qquad (3)$$

Flux rates have been expressed as ug Na^+/100 g body weight/min. It should be noted that at no time during the one hour experimental periods did the internal specific activity of sodium represent more than 3.5% of the external value, thereby rendering backflux of Na^{22} into the water insignificant.

(b) Apparent Volume of Distribution of Na^{22} or Na^{22} Space

This parameter is a theoretically derived measure of the distribution of the radioisotope within the organism. It is defined as the volume (ml/100g body weight) occupied by Na^{22} in the organism when uniformly distributed at the same concentration as that of the plasma. The radiosodium space (Vint)

at a particular time was calculated from the measured concentration of Na^{22} in the plasma (Na^{22}/ml pt) and the known amount of radioactivity in the trout (Qo - Q). Qo represents the amount of Na^{22}(cpm) added to the external medium at time zero.

$$\text{Vint} = \frac{Qo - Q}{Na^{22}/\text{ml pt}} \qquad (4)$$

(c) Fate of Sodium Taken up from the External Environment

The Na^{22} space provides a measure of dispersal of influxed sodium in the organism, but reveals no causes for the observed distribution. For example, a relatively high distribution volume could result from one or more of the following factors: a rapid exchange of plasma sodium with tissue sodium, a localized sodium "sink" within the system, or a large plasma volume. In order to investigate differences in radiosodium distribution volume among the treatment groups, the fate of sodium ions taken up from the external environment was traced by means of the radioisotope in both plasma and tissue.

(i) Plasma. At each blood sampling time, the concentration in plasma of sodium ions transported from the external medium (Na pt) was estimated from the measured concentration of Na^{22} in plasma and the average external specific activity over the experimental period up to that time. Na ext_o represents the total sodium content of the water at time zero.

$$\text{Na pt} = \frac{2 \times Na^{22}/\text{ml pt}}{\frac{Qo}{\text{Na ext}_o} + \frac{Q}{\text{Na ext}}} \qquad (5)$$

(ii) Tissue. Similarly the level of sodium ions in dorsal epaxial muscle which had originated from the external water during the experimental period (Na tt) could be approxi-

mated from the radioactivity per gram of the terminal tissue sample (Na^{22}/g tt)

$$\text{Na tt} = \frac{2 \times Na^{22}/g\ tt}{\frac{Qo}{Na\ ext_o} + \frac{Q}{Na\ ext}} \quad (6)$$

In this case, $\frac{Q}{Na\ ext}$ must represent the external specific activity at 60 minutes. It should be emphasized that Na tt merely estimates the concentration in muscle of sodium which had been influxed from the environment over the 60 minute period; the value is in no way an absolute measure of the incorporation of plasma sodium ions into tissue, nor even necessarily proportional to this parameter. Once external sodium has been taken up by the gills into the bloodstream, its specific activity (Na^{22}/Na total) will be drastically reduced by dilution with the large number of Na^{23}ions in plasma. However the amount of Na^{22} incorporated in tissue should still be proportional (in the ratio of the average external specific activity) to the total incorporation of sodium ions from the water.

(d) Extracellular Fluid Volume (E.C.F.V.)

Extracellular fluid volume may be estimated from the volume of distribution of total body sodium when appropriate corrections are applied for the heterogeneous distribution of the ion in the organism. E.C.F.V. calculated in this manner is known as sodium space, but should not be confused with radiosodium space. The following formula, as given by Manery (1954) was utilized for computation of E.C.F.V.:

$$\text{Na space} = \frac{\text{Na t} \times (H_2O)p}{\text{Na p} \times R} \quad (7)$$

Where:

Na t = tissue Na^+ concentration in mEq/kg wet tissue

$(H_2O)p$ = plasma water concentration in ml/kg wet plasma

Na p = plasma Na^+ concentration in mEq/L plasma

R = Gibbs Donnan ratio for Na^+

Unfortunately the Gibbs Donnan ratio for Na^+ has not yet been established for fresh water teleost tissue, so the value R = 0.942, obtained from mammalian studies, was used.

6. Presentation of Data

(a) Organization

In a study of this nature, involving simultaneous measurement of several parameters and the collection of multiple samples, it was inevitable that some data should be lost. Such losses occurred both during the experiment (e.g. cannula occlusion or transducer failure) and in the later processing of samples (e.g. contamination, dilution errors). Yet it seemed unreasonable to discard all data from a particular trout on the basis of an absence of one or two samples. Therefore the following scheme was used.

Within each treatment group, values for a particular parameter at each measurement time were presented as a mean accompanied by N = number of observations. Each piece of data acceptable in its own right was included in the average, with the exception of cases where failure to obtain a related sample would obviously bias the inclusion of the value in question. For example, plasma water values were used from an animal in which tissue sodium concentration was unavailable; however hematocrit and plasma sodium values were rejected for a fish from which only two blood samples were drawn, for

the normal serial sampling process was at least partially responsible for the observed changes in these factors with time in other trout.

(b) Statistical Analyses

The following techniques have been applied in all three sections of this study. Data have generally been expressed as a mean accompanied by the N number and the standard error of the mean. Possible relationships between parameters have been tested by the calculation of simple correlation coefficients. Straight lines have been fitted to data points by regression lines computed by the method of least squares. For some parameters, sets of data were subjected to a one way analysis of variance. The values of F obtained from the analyses and their levels of significance are presented. Means within each treatment group were then compared for significant differences at the 5% protection level by use of a modification of Duncan's New Multiple Range Test (Duncan,1955). Duncan's test is designed for the analysis of sets of data in which each measurement category contains an equal number of replicates, a condition which does not apply in the present study. The extension of the New Multiple Range Test for unequal numbers of replications, proposed by Kramer (1956), has been utilized. The technique is essentially conservative; "if the number of replicates differs greatly, there will be an increased probability of a significant difference within a subset of ranked means classed as homogeneous by this test".(Kramer,1956). In legends for figures, a single line under-scores subsets of means within which it has not been possible to demonstrate significant differences. Significance levels of difference between comp-

arable means of different treatment groups have been ascertained by application of the "Student's" t -test. Unless otherwise stated, significance has been assumed at the .05 level.

RESULTS AND DISCUSSION I

A. The Effect of Chasing on Ventilatory and Cardiovascular Parameters

Chasing of trout in a chamber only slightly longer than the animal's body initially appeared to be a far from satisfactory method for inducing activity, but was the only technique compatible with simultaneous sodium flux rate measurements. It was, therefore, critically important to the major part of this study, the investigation of sodium balance during exercise, to demonstrate that the procedure adopted did in fact cause real differences in cardio-respiratory function between experimental groups of fish, and that these changes were distinctly representative of resting state, swimming, and recovery conditions.

Two methods of presentation have been utilized for expression of cardiovascular and respiratory data. Figures 5, 6, 7, and 8 present the results as means of real values. In Table I, on the other hand, data are summarized at selected sample times in terms of "% routine" values (cf. Davis,1968). The term "routine" is here used in a sense homologous to Brett's (1962) description of the metabolic rate of fish undergoing continuous recording in the laboratory situation; determinations taken prior to zero time have been considered "routine". Expression of results in this manner is advantageous in considering changes in measured parameters for a specific fish relative to the value for that particular animal in a quiescent state; trends obscured by variability in the data may therefore become apparent. It will be noted that

Table I. Ventilatory and cardiovascular responses during the resting, active, and recovery experiments in terms of mean "% routine" values.

		5 or 65 minutes	30 or 90 minutes	60 or 120 minutes
Heart rate:	rest	104.25	103.00	107.44
	active	109.32	120.19	117.16
	recovery	108.14	103.00	105.97
Dorsal aortic blood pressure:	rest	98.06	93.86	92.08
	active	106.28	101.12	103.12
	recovery	87.92	94.41	88.93
Ventilation rate:	rest	99.86	99.73	99.35
	active	110.35	109.38	107.10
	recovery	101.85	98.41	96.21
Buccal pressure amplitude:	rest	108.40	102.52	92.52
	active	141.96	169.28	169.44
	recovery	132.26	102.45	104.11

Figure 4 Typical pressure recordings from the dorsal aorta and buccal cavity of a rainbow trout obtained during rest, chasing, and recovery in the protocol of Section I. Fish #U. 235.4 g. Recovery group.

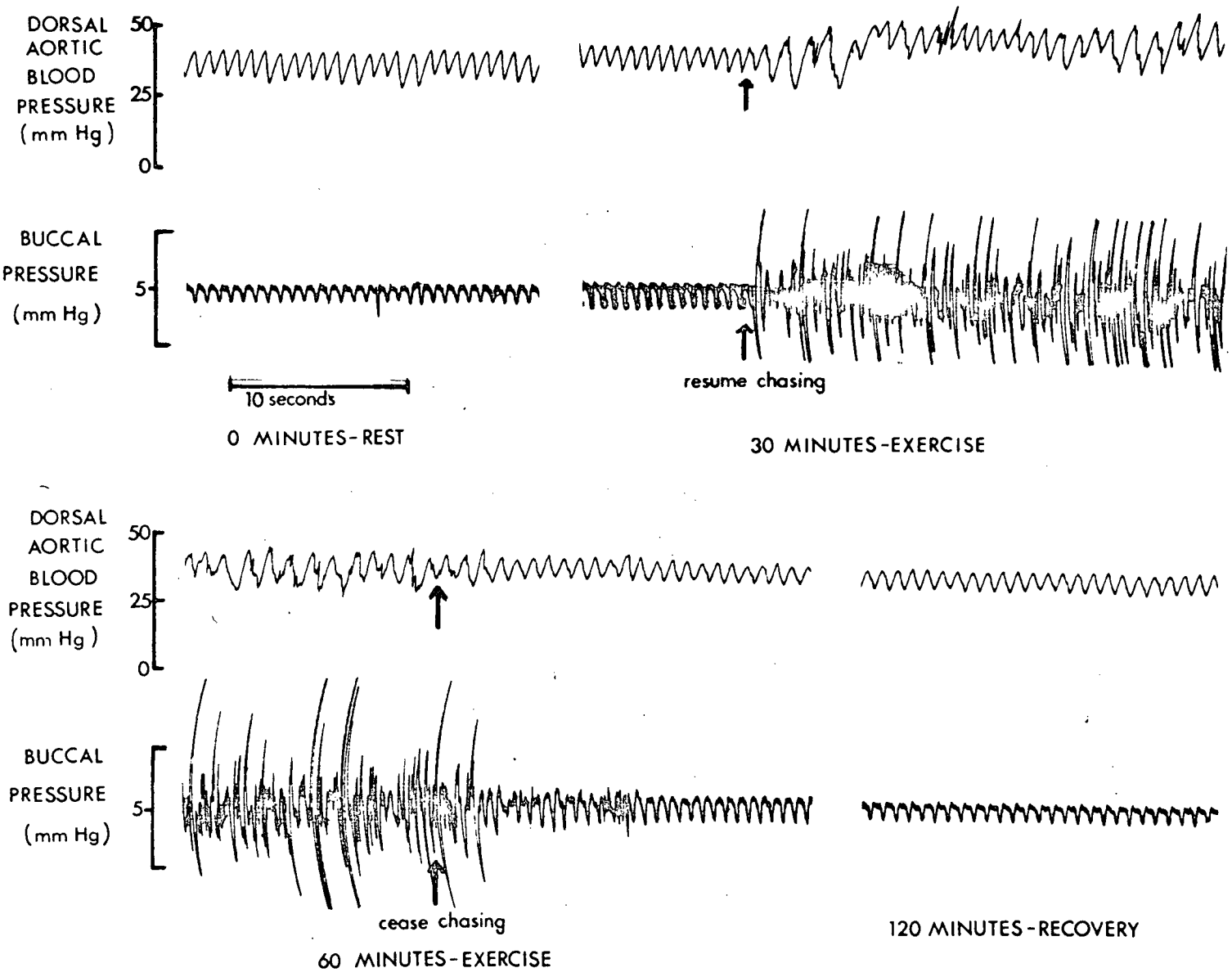
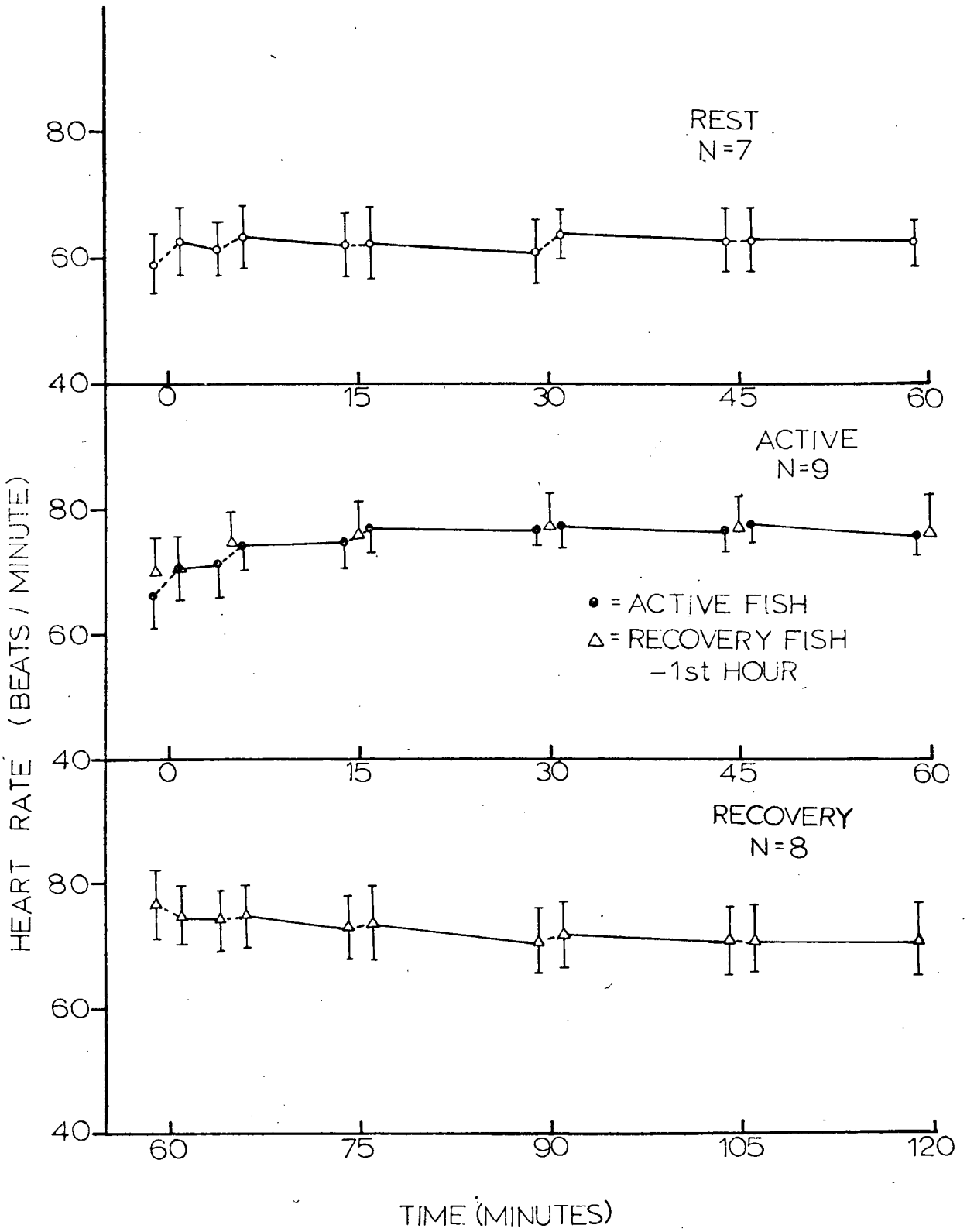

DORSAL AORTIC BLOOD PRESSURE (mm Hg)
50
25
0
BUCCAL PRESSURE (mm Hg)
5
10 seconds
0 MINUTES-REST
resume chasing
30 MINUTES-EXERCISE
DORSAL AORTIC BLOOD PRESSURE (mm Hg)
50
25
0
BUCCAL PRESSURE (mm Hg)
5
cease chasing
60 MINUTES-EXERCISE
120 MINUTES-RECOVERY

Figure 5 Variations in heart rate over the experimental periods in resting, active, and recovery groups of trout. First hour values for recovery fish were obtained during their initial chasing treatment. Means ± 1 standard error.


REST
N=7
ACTIVE
N=9
RECOVERY
N=8
● = ACTIVE FISH
△ = RECOVERY FISH
–1st HOUR
HEART RATE (BEATS / MINUTE)
TIME (MINUTES)
80
60
40
0
15
30
45
60
75
90
105
120

"%routine" values presented in Table I cannot be computed from points in Figs. 5 - 8; the former are means of percentages while the latter would be percentages of means.

The onset of chasing was associated with immediate increases in ventilatory amplitude and rate, dorsal aortic blood pressure, and, in many animals, a noticeable short-term bradycardia changing to a tachycardia after 15 - 30 seconds (Fig. 4). However both traces remained highly erratic throughout chasing, thereby precluding systematic determinations of rates and pressures during actual exercise periods. Consequently, only values extracted from recordings taken during the one minute rest intervals immediately before and after each blood sampling time have been reported. These values are probably somewhat lower than the mean rates and pressures attained by exercised fish during the experimental periods.

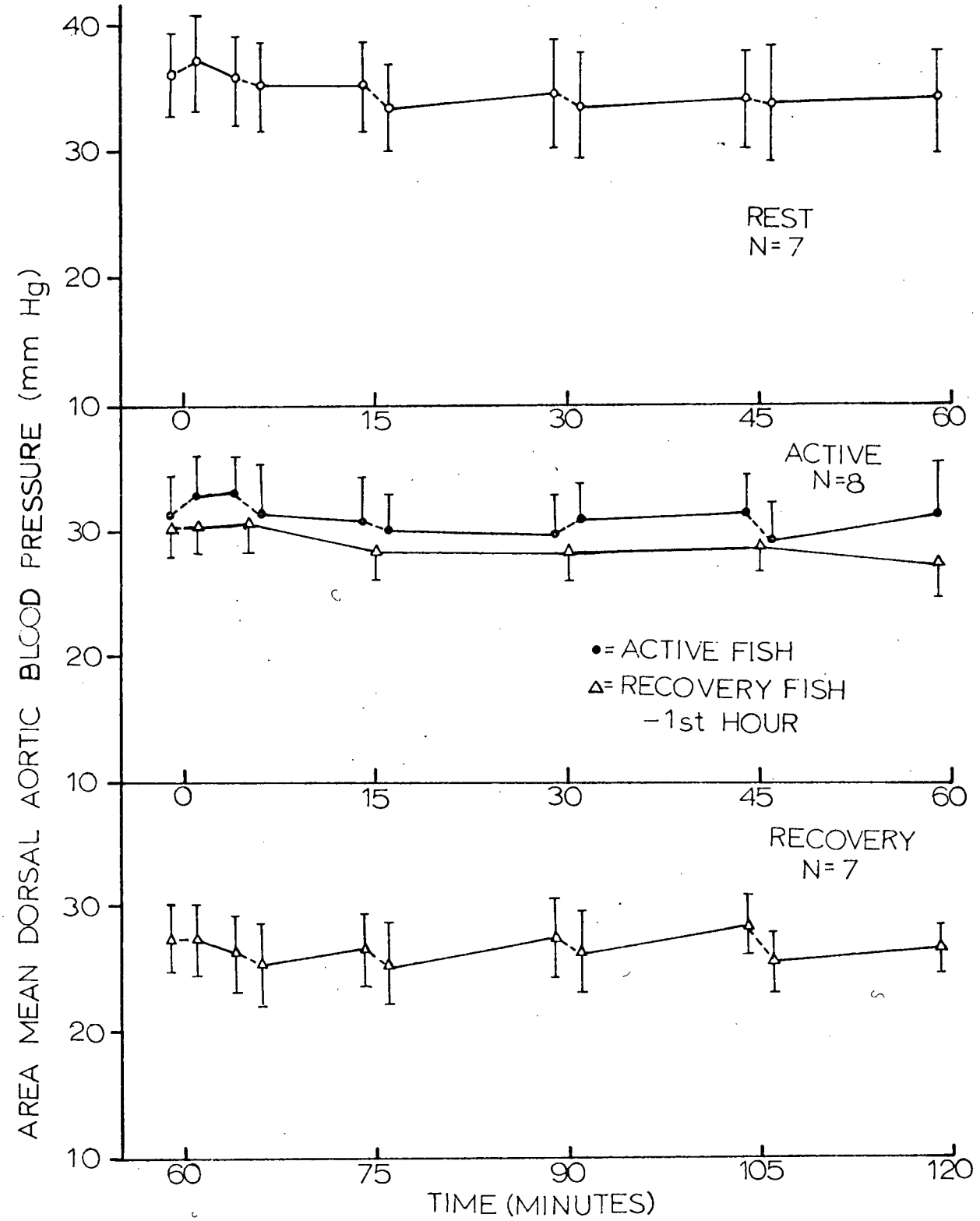
Heart rate (Fig. 5) remained stable during rest, increased a maximum 20% during chasing, and declined to pre-exercise levels during recovery. This cardioacceleration is comparable to that (15%) recorded in Salmo gairdneri during ten minutes of moderate swimming (up to 52 cm/sec.) in a water tunnel (Stevens and Randall,1967 a). The increase is far smaller than the 40 - 80% tachycardia observed at fatigue speeds in mature sockeye salmon, Onchorynchus nerka, (Smith et al.,1967; Davis,1968). However, Stevens (1968 b) found that imposition of severe exercise on rainbow trout could elicit an increase in cardiac frequency of no more than 15%. The difference in the cardiac rate response to swimming between trout and salmon is probably related to the absence in the

former (Stevens and Randall,1967 a), but presence in the latter (Randall and Stevens,1967) of vagal tone at rest. Stevens and Randall (1967 a, b,) calculated that the 15% cardioacceleration during moderate activity in rainbows was associated with a 4.5 fold increase in cardiac output. It seems probable that similar changes occurred in chased fish in the present study resulting in augmentation of blood flow through the gills. The cardiac output of the isolated heart of Salmo gairdneri can be related to a series of Starling curves at different filling pressures and catecholamine concentrations of the perfusion fluid; an elevation of either factor causes an increase in output (Bennion,1968). Venous return probably increases during swimming, but there are as yet no measures of venous filling pressures in vivo in teleosts. Ample stores of adrenaline and noradrenaline plus an as yet unidentified catecholamine ("catechol - 4") have been reported in teleost tissue, (Ostlund,1954; von Euler and Fange,1961; Nakano and Tomlinson, 1967; Gannon and Burnstock,1969).

Significant elevations of plasma catecholamine concentrations have been found in correspondence with diurnal activity rhythms in the channel catfish, Ictalurus punctatus, (Boehlke et al.,1967) and in response to induced exercise in the rainbow trout (Nakano and Tomlinson,1967). It seems probable that mobilization of these hormones is at least partially responsible for the maintenance of high cardiac delivery during swimming. Arguments were presented in the general introduction that adrenaline and noradrenaline are also responsible for vasodilation of the branchial capillary network and stimulation of lamellar blood flow. Increased passage of

Figure 6 Variations in dorsal aortic blood pressure over the experimental periods in resting, active, and recovery groups of trout. First hour values for recovery fish were obtained during their initial chasing treatment. Means $\pm$ 1 standard error.

$$\text{Area mean blood pressure} = \frac{2(\text{diastolic}) + 1\ \text{systolic}}{3}$$


AREA MEAN DORSAL AORTIC BLOOD PRESSURE (mm Hg)
40
30
20
10
REST
N= 7
0
15
30
45
60
ACTIVE
N=8
●= ACTIVE FISH
△= RECOVERY FISH
-1st HOUR
RECOVERY
N= 7
60
75
90
105
120
TIME (MINUTES)

blood through these respiratory pathways should in turn facilitate oxygen and carbon dioxide exchange. It would therefore appear that catecholamines are instrumental in effecting circulatory adaptation to the increased metabolic demands of exercise.

Dorsal aortic blood pressure varied greatly between individual animals but the mean generally remained stable or fell slightly over the experimental period (Fig. 6). Expression of the data in relation to routine values (Table I) revealed that exercised trout maintained pressures slightly above resting levels despite the serial removal of blood demanded by the experimental protocol. This reduction in blood volume explains the progressive fall in pressure observed in resting animals (Table I). Similar effects have been noted in salmon (Smith et al.,1967; Randall and Smith,1967), the drop in blood pressure being proportioned to the volume withdrawn. During recovery, "%routine" pressures were least immediately after termination of chasing and did not decrease in response to further depletion of circulatory fluid. When appropriate corrections for the effects of blood sampling are applied to the pressure changes measured in the present study, the data follow trends similar to those reported during moderate exercise and recovery under more controlled conditions (Stevens and Randall,1967 a; Davis,1968); in these studies, pressure changes could be correlated with variations in cardiac output.

Both breathing rate (Fig. 7) and buccal pressure (Fig. 8) remained constant in the resting group of trout. Chased fish demonstrated a slight polypnea (10%) and very large increases in buccal pressure (70%) (Table I); these trends were

Figure 7 Variations in ventilation rate over the experimental periods in resting, active, and recovery groups of trout. First hour values for recovery fish were obtained during their initial chasing treatment. Means $\pm$ 1 standard error.

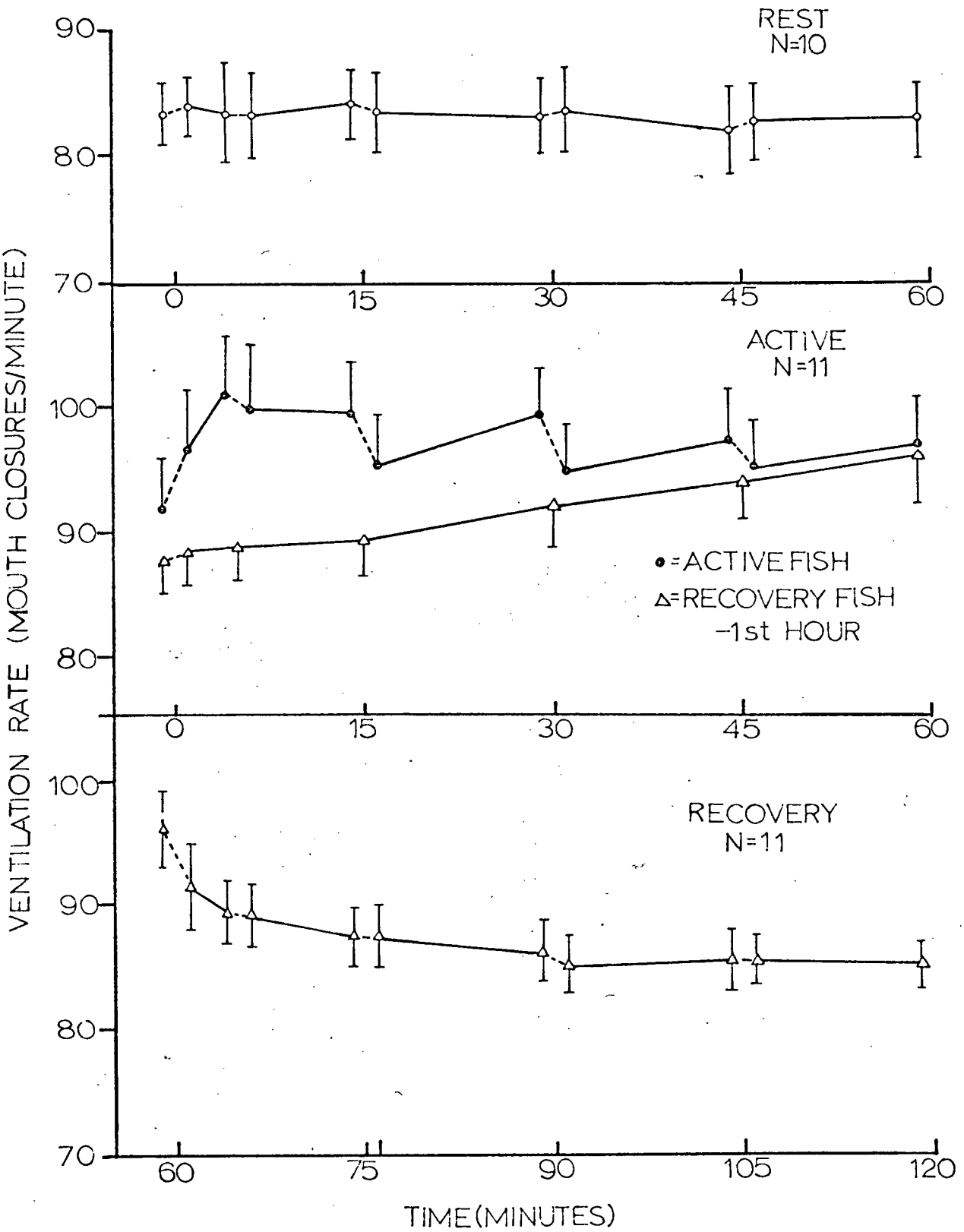
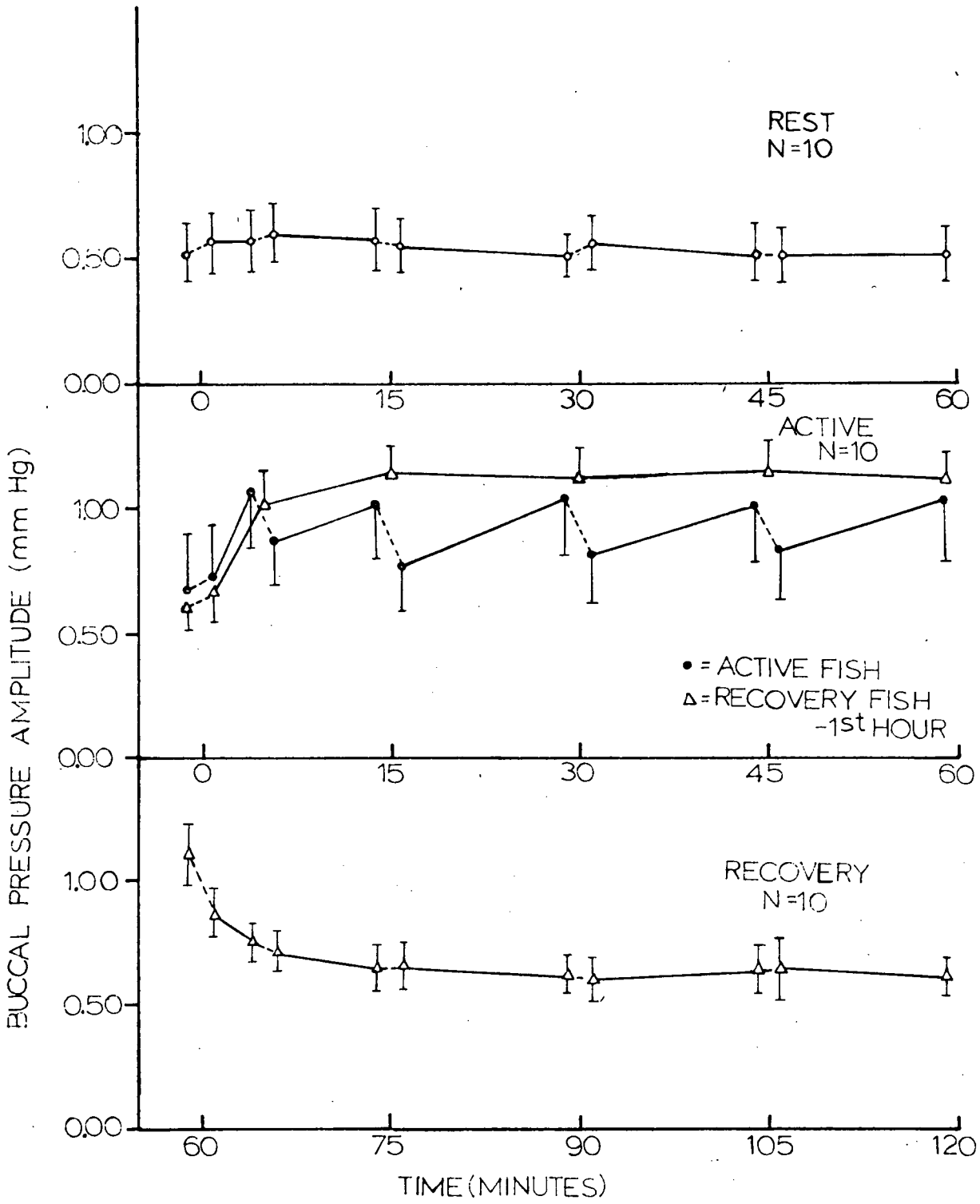

REST
N=10
ACTIVE
N=11
RECOVERY
N=11
●=ACTIVE FISH
△=RECOVERY FISH
-1st HOUR
VENTILATION RATE (MOUTH CLOSURES/MINUTE)
TIME (MINUTES)
90
80
70
100
90
80
100
90
80
70
0
15
30
45
60
0
15
30
45
60
60
75
90
105
120

Figure 8 Variations in buccal pressure amplitude over the experimental periods in resting, active, and recovery groups of trout. First hour values for recovery fish were obtained during their initial chasing treatment. Means $\pm$ 1 standard error.


REST
N=10
ACTIVE
N=10
• = ACTIVE FISH
Δ = RECOVERY FISH
-1st HOUR
RECOVERY
N=10
BUCCAL PRESSURE AMPLITUDE (mm Hg)
TIME (MINUTES)
1.00
0.50
0.00
0
15
30
45
60
75
90
105
120

quickly reversed during recovery. Even the short intervals of rest at each blood sampling time produced marked reductions in both components. Increases in ventilation are commonly associated with exercise in teleosts (Saunders,1962; Stevens and Randall,1967 a, b; Davis,1968) and may be effected by either a mechanism similar to the Harrison joint-tendon reflex of mammals (Stevens and Randall,1967 a) or a direct stimulatory action of circulatory catecholamines on central respiratory centres (Waitzenegger,1967).

The rate increases reported here (10%) are lower than those (30%) observed by Stevens and Randall (1967 a), but inspection of recordings taken during actual chasing indicate that the immediately post-exercise values reported are significant underestimations of mean ventilatory frequencies attained by the exercised treatment group. Elevations of buccal pressure, however, are similar to those measured at moderate to fatigue swimming speeds in salmon (Davis,1968). Extrapolation from the data presented in a recent study of ventilation in the rainbow trout (Hughes and Saunders,1970) suggests that chased fish elevated their breathing volumes 2.5 - 3.0 fold in the present study. A 4 fold increment in ventilatory flow was calculated for moderately swimming trout by Stevens and Randall (1967 a, b). However, it is likely that ventilation volume would be enhanced by "ram" ventilation (Muir and Kendall,1968) during the mouth opening phase of the breathing cycle when swimming against a constant current in a water tunnel.

The data reported above adds nothing new to our knowledge of teleost physiology by themselves, but do demonstrate

that the desired effects were produced. With the exception of a slight fall in dorsal aortic pressure attributable to serial blood sampling, the resting treatment group remained in the "routine" condition throughout the experimental period. The ventilatory and cardiovascular correlates of chasing were very similar to those previously documented in rainbow trout during controlled swimming at moderate speeds (Stevens and Randall,1967 a, b). The latter workers measured a 5 fold rise in metabolic rate at this exercise level; it does not seem unreasonable to estimate a similar increase in oxygen consumption in the chased animals. Recovery was characterized by a return to resting levels in the parameters measured.

B. The Effect of Exercise on Branchial Sodium Fluxes

Before presentation and evaluation of these results, use of the term "branchial" in reference to the measured fluxes needs justification, for ions may move between internal and external media across four main regions of a teleost: the gill epithelium, the urinary system, the intestinal system, and the skin of the general body surface. In these experiments, urinary release of electrolytes was prevented by catheterization. Intestinal absorption of sodium was negated by the fact that rainbow trout do not drink in fresh water (Shehadeh and Gordon,1969). Pre-experimental starvation eliminated faecal contamination of the external medium, but during some trials, a gelatinous tube of mucoid material was vented from the anus. However Shehadeh and Gordon (1969) have demonstrated that this material, which normally encloses faeces in fed fish, is devoid of sodium. The impermeability of Salmo gairdneri skin to

sodium has been proven repeatedly (Holmes,1959; Fromm,1968). The assumption of an exclusively branchial origin for the observed fluxes would, therefore, seem sound.

The physical dimensions of trout in each experimental group are tabulated in Table II; the mean size of resting fish was obviously slightly larger than that of the other two sets. However coefficients of condition for the groups did not differ significantly, indicating a common body weight to gill surface area relationship for the narrow weight range used, and therefore permitting comparison of flux rates between groups on a per 100 g basis. Data has been expressed in ug/100 g/minute for convenience in discussing the movements of very small amounts of sodium over short time intervals.

Branchial sodium flux rates are presented for the resting, active, and recovery treatment groups in Fig. 9 and are summarized in Table III. Average external sodium concentration (Table III) did not differ significantly among the three experimental groups and thus was not a factor causing the observed differences in flux rates. Resting animals maintained a state of sodium equilibrium at the gills, influx (17.62 ug/100 g/minute) and efflux (16.88 ug/100 g/minute) being approximately equal so that there was only a very slight mean net uptake of the ion. During activity, however, branchial efflux increased markedly (70%, $p < 0.01$) while influx remained identical to resting levels. Consequently, there was an extremely significant ($p < .001$) net loss of sodium across the gills. Over the recovery period, after an hour of exercise, the fish returned to a state of positive balance through a reduction ($p < 0.001$) in the efflux parameter to

Table II. Physical dimensions of trout in the three experimental groups of Section I. Means ± standard error.

	Resting N = 10	Active N = 11	Recovery N = 11
Body weight (g)	259.66 ± 12.79	233.95 ± 11.19 p_1 = n.s.	228.17 ± 8.05 $p_1 < .05$ p_2 = n.s.
Length (cm)	29.95 ± 0.50	29.65 ± 0.49 p_1 = n.s.	28.81 ± 0.45 p_1 = n.s. p_2 = n.s.
Fork length (cm)	28.63 ± 0.47	28.01 ± 0.51 p_1 = n.s.	27.62 ± 0.40 p_1 = n.s. p_2 = n.s.
Max.depth (cm)	6.18 ± 0.14	5.91 ± 0.14 p_1 = n.s.	5.87 ± 0.09 p_1 = n.s. p_2 = n.s.
Coefficient of condition $\frac{\text{weight} \times 100}{(\text{fork length})^3}$	1.101 ± 0.022	1.062 ± 0.028 p_1 = n.s.	1.084 ± 0.029 p_1 = n.s. p_2 = n.s.

p_1 = significance with respect to corresponding resting value.

p_2 = significance with respect to corresponding active value.

Figure 9 Branchial sodium flux rates over consecutive intervals of the experimental periods in resting, active, and recovery groups of trout. Black bars = mean branchial sodium influx rates; dotted bars = mean branchial sodium efflux rates; clear bars = mean branchial sodium net flux rates. The vertical lines represent 1 standard error of each mean.

Statistical Comparisons: (Numbers refer to means of intervals as labelled at the head of the graph.)

Resting: Influx Rate. $F = 1.20$, n.s.

4 5 2 3 1

Efflux Rate. $F = 0.53$, n.s.

3 5 4 2 1

Net Flux Rate. $F = 0.34$, n.s.

4 2 5 1 3

Active: Influx Rate. $F = 1.37$, n.s.

4 3 2 5 1

Efflux Rate. $F = 0.48$, n.s.

2 3 1 5 4

Net Flux Rate. $F = 1.25$, n.s.

1 2 3 5 4

Recovery: Influx Rate. $F = 2.44$, n.s.

3 5 4 2 1

Efflux Rate. $F = 1.48$, n.s.

3 4 5 2 1

Net Flux Rate. $F = 0.80$, n.s.

3 5 4 1 2

A = significantly different from corresponding resting value ($p < .05$)

B = significantly different from corresponding active value ($p < .05$)

—= not significantly different from other corresponding values ($p > .05$)

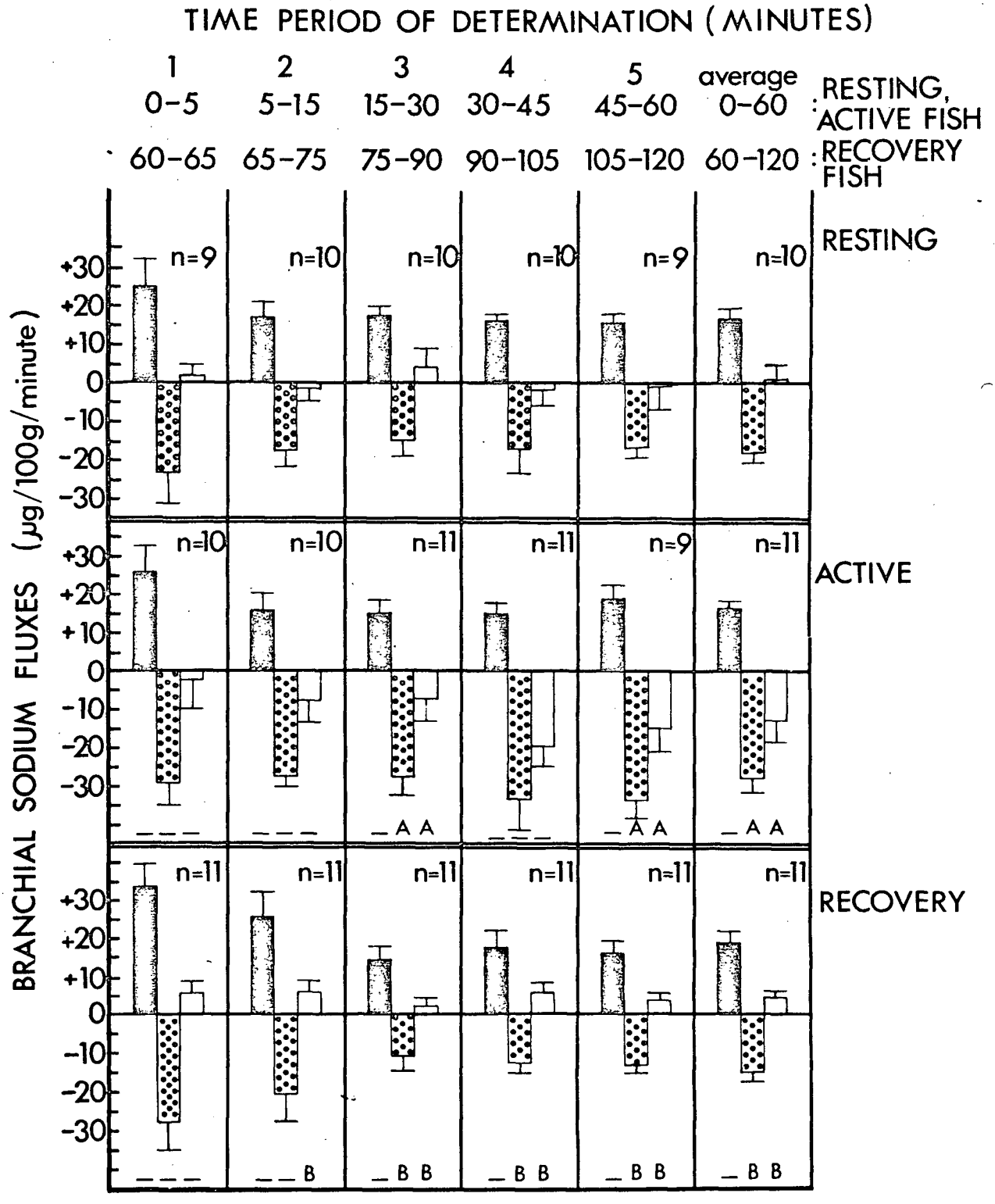

TIME PERIOD OF DETERMINATION (MINUTES)
1
0-5
2
5-15
3
15-30
4
30-45
5
45-60
average
0-60
RESTING, ACTIVE FISH
60-65
65-75
75-90
90-105
105-120
60-120
RECOVERY FISH
BRANCHIAL SODIUM FLUXES (µg/100g/minute)
RESTING
ACTIVE
RECOVERY
n=9
n=10
n=11
+30
+20
+10
0
-10
-20
-30
A
B

Table III. Average branchial sodium flux rates over the experimental periods. Means ± 1 standard error.

	Resting	Active	Recovery
External Sodium Concentration (ug/ml)	N = 10 1.550 ± 0.151	N = 11 1.612 ± 0.151 p_1 = n.s.	N = 11 1.851 ± 0.254 p_1 = n.s. p_2 = n.s.
Sodium Influx Rate (ug/100 g/min)	N = 10 + 17.62 ± 1.15	N = 11 + 16.41 ± 1.49 p_1 = n.s.	N = 11 + 19.24 ± 2.86 p_1 = n.s. p_2 = n.s.
Sodium Efflux Rate (ug/100 g/min)	N = 10 - 16.88 ± 1.57	N = 11 - 28.50 ± 3.55 $p_1 < .01$	N = 11 - 14.70 ± 2.36 p_1 = n.s. $p_2 < .001$
Sodium Net Flux Rate (ug/100 g/min)	N = 10 + 0.74 ± 1.43	N = 11 - 12.14 ± 2.94 $p_1 < .01$	N = 11 +4.54 ± 1.99 p_1 = n.s. $p_2 < .001$

p_1 = significance with respect to corresponding resting value.

p_2 = significance with respect to corresponding active value

resting values, again without significant change of the influx parameter. It would appear, therefore, that branchial sodium influx is insensitive, but efflux extremely labile, to some factor or factors associated with exercise. The present results clarify the observations of Randall et al.(1969) who noted that activity augmented the release of injected Na^{22} from the gills of fresh water adapted trout but were unable to decide whether the effect represented a stimulation of sodium turnover rate or of the efflux component alone. Data presented here indicates the latter to be true.

In each treatment group, fluxes were measured over the first 5, the next 10, and the following three 15 minute time periods of the experimental hour (Fig. 9). The error in estimating exchange rates over the initial 5 minute interval was high and is manifested by the large standard errors on all three flux components for this period in each set of fish. This inaccuracy can be traced to a number of causes: the changes in total sodium and radiosodium concentration of the water were minimal and subject to limitations of analytical precision; the measurements assumed instantaneous and complete mixing of the labelled solution with the originally unlabelled external medium, which was probably untrue; most importantly, there could have occurred a slight exchange of Na^{22} ions with the small number of Na^{23} ions adsorbed to the mucus of the animal's epithelium (Fromm,1968). This latter factor would only have been effective until the specific activity of adsorbed skin sodium equilibrated with that of free sodium in the water, but over this short interval would have been detected as an influx due to the disappearance of radiotracer from the external

compartment. As efflux was not directly measured, but computed as the difference between influx and net flux, artificial enhancement of influx would have automatically elevated this component. There was in fact a tendency for higher unidirectional fluxes during the first 5 minutes than during subsequent periods for all three groups, but these elevations were not statistically significant in the resting and active trout. In the recovery group, the initial high influx attained borderline significance (Duncan's Test, 5% protection level, although not reflected in $F_{.05}$) with respect to values for the final three intervals of measurement. However, in light of the above discussion, no biological importance is attached to this difference.

Figure 9, and the accompanying significance tests, therefore indicate that, within the detection limits of the methods used, there occurred no variation of influx, efflux, and net flux components of gill sodium exchange within each treatment group over the experimental hour. This in turn implies that the lag phases in the changeover from a branchial flux rate pattern distinctive of rest to that characteristic of activity, or from the latter to the typical recovery arrangement, were extremely short.

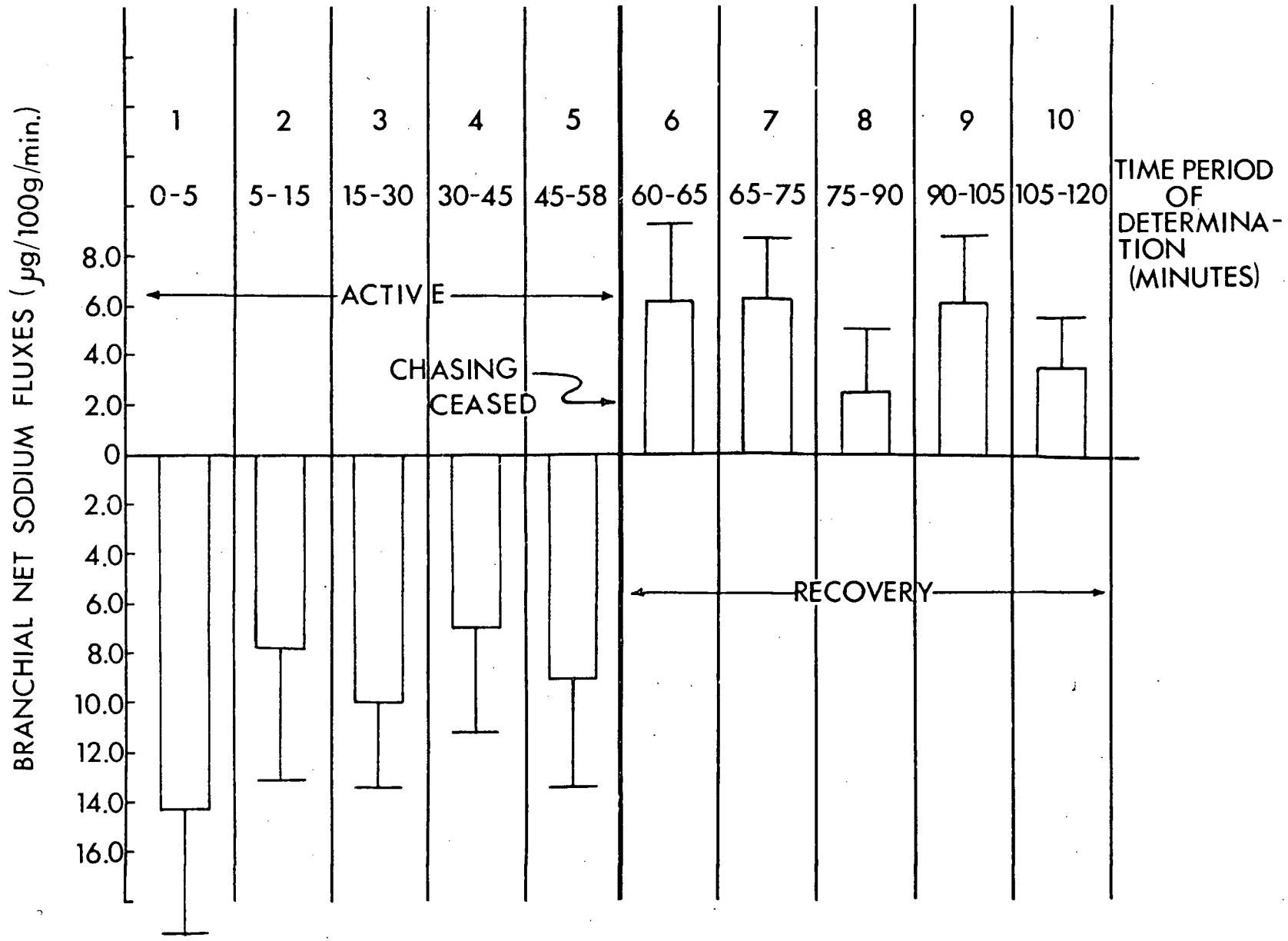
An examination of net flux rates in the recovery group (Fig. 10) is especially persuasive of the point, for measurements of this parameter were based solely on flame photometric analyses which, during the first 5 minute period, were far more accurate than isotope uptake determinations (and therefore, unidirectional fluxes). These data demonstrate that the switch from a negative to a positive sodium balance with the onset of

Figure 10 Branchial sodium net flux rates over consecutive intervals of the experimental period for the recovery treatment group. The vertical lines represent 1 standard error of each mean. Chasing ceased at 60 minutes.

Statistical Comparisons: (Numbers refer to means of intervals as labelled at the head of the graph.)

$F = 4.68$, $p < .005$

1 3 5 2 4 8 10 9 6 7


BRANCHIAL NET SODIUM FLUXES (µg/100g/min.)
8.0
6.0
4.0
2.0
0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
1
2
3
4
5
6
7
8
9
10
0-5
5-15
15-30
30-45
45-58
60-65
65-75
75-90
90-105
105-120
TIME PERIOD OF DETERMINATION (MINUTES)
ACTIVE
CHASING CEASED
RECOVERY

recovery occurred almost instantaneously. The net fluxes of the recovery trout changed significantly between the last 15 minute period of chasing and the first 5 minutes of recovery. Figure 10 also confirms that recovery fish, during their initial hour of exercise, suffered a net branchial sodium deficit similar to that of the active group (Fig. 9).

These extremely rapid effects, which apparently implicated only branchial sodium efflux, were probably associated with a readjustment of the respiratory/osmoregulatory compromise at the gills. Reference to Figs. 5 - 8 and Table I indicates that the ventilatory and cardiovascular correlates of these changes had occurred by 5 minutes after the inception of exercise or recovery. As outlined earlier (pp. 8 - 10) an osmotic deficit should be concomitant with elevation of gas exchange because of increased blood perfusion of the high surface area, thin walled respiratory lamellae. A principal manifestation of such a deficit would be an increased simple diffusion of sodium, the major plasma cation, from internal to external milieux. The 70% rise in branchial sodium efflux from 16.88 to 28.50 ug/100 g/minute during exercise is interpreted in these terms.

There exist, as yet, no measurements of the diffusion coefficient for sodium in teleost gill tissue so it has not been possible to determine whether the observed increase in sodium efflux was attributable to simple diffusion on a theoretical basis. However, a very tentative calculation, based on a number of unproven suppositions, was undertaken to see if this explanation of the enhanced efflux was in any way reasonable. These suppositions included that of a branchial

sodium diffusion coefficient identical to that of mammalian capillaries (Pappenheimer,1953) because of the marked similarities between lamellar and capillary structure (Newstead, 1967). Other assumptions comprised the use of the lamellar area of Hughes (1966) for Salmo trutta, the diffusion distance of Newstead (1967) for Onchorhynchus kisutch, and the transepithelial potential of Kerstetter et al. (1970) for anaesthetized rainbow trout at comparable external sodium concentrations. This computation yielded a maximum diffusional efflux of approximately 3 ug/100 g/minute; Kerstetter et al.(1970), by methods unstated, estimated a very similar diffusional loss (2 ug/100 g/minute). Active trout in the present study increased branchial efflux by 12 ug/100 g/minute. In light of the uncertainties in the assumptions taken, agreement between calculated and observed values within an order of magnitude was encouraging.

Resting animals maintained only a negligible net branchial uptake of sodium (0.74 ug/100 g/minute) yet presumably suffered greater renal loss (Section III); on a whole body basis, the trout were in negative sodium balance, a situation which obviously could not be indefinitely endured. This condition has been encountered in many other studies (e.g. Maetz,1956; Maetz et al.,1964 a; Lahlou and Sawyer,1969) and has generally been attributed to shock, stress, or handling effects without further explanation. It is probable that increases in respiratory exchange were associated with recovery from anaesthetization and cannulation (see Section III, Fig. 25) and caused disturbance of the branchial respiratory/osmoregulatory adjustment with concomitant increases in diffusional sodium

efflux. Houston et al., (1969) have detected very marked disturbances in water and electrolyte concentrations in plasma and tissue of the brook trout, Salvelinus fontinalis, for up to 72 hours after MS 222 anaesthesia and vascular catheterization, the same procedures to which rainbows in the present study had been subjected 10 - 48 hours prior to experimentation. For individual resting fish, net flux rate bore no relationship to influx rate but was positively correlated ($r = 0.713$, $p < .05$) with efflux rate, indicating that the state of balance for a particular trout was determined largely by the magnitude of efflux. This correlation was accentuated during exercise ($r = 0.915$, $p < .01$). Despite the fact that resting animals were not in a truly "normal" state, differences demonstrated between groups of similarly cannulated trout should still represent effects independent of those caused by the common pre-experimental treatment.

Arguments have been presented previously that the mobilization of catecholamines during exercise is responsible for both the branchial vasodilation increasing the effective permeability of the respiratory exchanger and for the elevation of cardiac output. Therefore it is of interest to ask whether the true course of variation in plasma levels of these substances can be correlated with the almost instantaneous changes in branchial sodium efflux and heart function. The work of Nakano and Tomlinson (1967) on rainbow trout demonstrated a rapid increase (less than 10 minutes) in plasma levels at the onset of exercise, but this elevation was maintained for at least 3 hours into recovery. Sodium efflux (Fig. 9), heart rate (Fig. 5), and cardiac output (Stevens and Randall,1967 b)

drop rapidly after termination of swimming. Thus catecholamines may well be involved in the initial branchial and cardiac adaptation to the respiratory demands of activity, but other factors may over-ride these adrenergic effects as soon as they are no longer required. The persistence of high circulatory concentrations of adrenaline and noradrenaline after exercise may reflect a low activity of breakdown mechanisms in teleost tissue. One could also argue that catecholamines exert only partial control over cardiac activity and branchial permeability, and act as potentiators for the observed effects. Thus an increased cardiac output could be caused by greater venous return at the onset of activity, but facilitated by the action of adrenaline on the myocardium to favourably change the relationship between stroke volume and filling pressure (Bennion,1968). At the same time, catecholamines would stimulate the dilation of respiratory shunts at the gills, but the filling of a particular lamella would still be dependent upon a specific opening pressure. At recovery, venous return, cardiac output, and ventral aortic pressure would tend to fall in causative sequence, and thus the number of respiratory lamellae perfused (i.e. branchial permeability) would be reduced despite the continuance of high catecholamine levels in plasma. Clearly further research on the nature of action of catecholamines and the integration of cardiovascular adjustment to exercise in teleosts is necessary.

Ion exchange mechanisms of the fish gill are poorly understood at present and the little knowledge available is confounded by obvious species dependent differences; exchange diffusion, active transport, active coupled transport, back-

transport, drinking, and simple diffusion have all been implicated in sodium fluxes between internal and external milieux in different radiotracer studies (Maetz,1956; House,1963; Maetz and Garcia-Romeu,1964; Garcia-Romeu and Maetz,1964; Garcia-Romeu and Motais,1966; Motais et al.,1966; Evans,1967; Maetz,1969; Potts et al,1970; Morris and Bull,1970; Kerstetter et al,1970). The only general conclusion pertinent to the present study which can be drawn from these investigations is that branchial sodium influx in fresh water occurs by an active transport mechanism which may in some way be linked to the extrusion of another cation (NH_4^+ : Maetz and Garcia-Romeu,1964; Garcia-Romeu and Motais,1966; H^+: Kerstetter et al,1970) and which is independent of chloride transport (Garcia-Romeu and Maetz,1964). Exchange diffusion, in the sense of Motais et al.(1966) (an "organized leakiness" in which unidirectional sodium fluxes are linked on a one for one basis by a hypothetical carrier) is characteristic of some euryhaline forms but has not been observed when the animals were adapted to fresh water (Motais et al.,1966; Evans,1967).

In light of this paucity of information, analysis of unidirectional branchial sodium flux rates, which have not previously been measured in intact Salmo gairdneri, must proceed from very basic considerations. Recent measurements of transepithelial potentials in fresh water adapted rainbows (Kerstetter et al.,1970; Randall and Conte, personal communication) have revealed that internal fluids are slightly positive with respect to the external medium. Therefore the inward movement of sodium occurs against both an electrical and a chemical gradient. Application of the Ussing equation (Ussing,1949a)to this

situation ($[Na]_i$ = 117.97 uEq/ml; $[Na]_o$ = .067 uEq/ml; T.E.P. = +6.0 mv.) suggests that the efflux/influx ratio should approximate 2244 if only passive mechanisms are involved. On the contrary, the ratio for resting trout is almost unity, and not greatly different during exercise or recovery; clearly the Ussing criterion for the contribution of active transport mechanisms to the inward flux of sodium is satisfied. Richards and Fromm (1970) have recently demonstrated *in vitro* that the sodium uptake of the perfused trout gill is an A.T.P. dependent process. Thus at least part of the measured sodium influx rate must be attributable to active transport; the remainder, if any, could consist of simple diffusion (negligible) and exchange diffusion. This latter mechanism is essentially passive and would simply exchange internal and external sodium ions (no net transport of the electrolyte), but would be detected in both influx and efflux parameters by the radiotracer technique. As such, it would contribute equally to the two unidirectional fluxes. Exchange diffusion could hypothetically utilize either the same carrier as active transport or a completely separate agent (Motais *et al.*,1966; Maetz, 1969). *A priori*, an active transport contribution to efflux seems most unlikely, and has never been demonstrated in fresh water teleosts; efflux should, therefore, consist of simple diffusion and exchange diffusion if present. The 70% rise in efflux during exercise was not associated with any change in influx rate and thus would not seem to involve exchange diffusion. Consequently the increment in efflux probably consisted entirely of simple diffusion.

Effective branchial permeability to either ions or gases will be determined by the factor $\frac{AD}{X}$ (area x diffusion coefficient/distance, p. 4). As concentration gradients for both oxygen (Stevens and Randall,1967 b) and sodium will remain essentially unchanged during exercise, simple diffusion fluxes may be taken as representative of effective permeabilities and vice versa. Changes in A and X should have the same effect on the diffusion of both sodium and oxygen. If it is assumed that D either remains stable or changes equally for the two substances during augmented lamellar blood flow, then the following arguments may be advanced.

(1) A particular increase in the simple diffusional efflux of sodium will imply a similar rise in the oxygen transfer factor (which is directly proportional to oxygen uptake as the gradient is invariant). Thus if all branchial sodium efflux occurs by simple diffusion, then a 70% rise during exercise should be associated with only a 70% increase in metabolic rate. Yet this seems impossible as trout more than double their oxygen uptake in response to only minor disturbance. Ventilatory and cardiovascular data presented in the first part of this section was indicative of a 5-fold rise (Stevens and Randall,1967 a, b) in oxygen uptake during chasing. Thus the 70% elevation of efflux must represent an approximately 5-fold increase in the simple diffusion component (γ) of efflux. This statement in turn implies the existence of an exchange diffusion component (β) to efflux; the following simple calculation may be performed:

At rest $\gamma + \beta =$ 17 ug/100 g/min.

During activity $5\gamma + \beta =$ 29 ug/100 g/min.

γ = 3 ug/100 g/min.

β = 14 ug/100 g/min.

This very elementary analysis would indicate that, if the above assumptions are correct, only a small portion of the efflux at rest was caused by simple diffusion, the majority being due to exchange diffusion. Consequently influx would also be largely exchange diffusion (14 ug/100 g/min.) with only about 3 ug/100 g/min. of active uptake. The fact that exchange diffusion has not previously been demonstrated in fresh water teleosts must weigh against this hypothesis.

(2) Again presuming an interlocking of D for oxygen and sodium in the gills, it could alternately be hypothesized that the only efflux process present is simple diffusion. Thus an approximately 5-fold elevation of sodium efflux did in fact occur, but the techniques used were incapable of detecting most of this increase. Such a situation can occur if a back-transport pump is operative, as has been postulated in the ammocoete, Lampetra planeri (Morris and Bull,1970). Under this system, escaping internal sodium ions diffusing through the branchial tissue are back-transported by the unsaturated active sodium pump before they can effectively enter the external medium. Adrenaline stimulates the net sodium uptake of the isolated perfused trout gill (Richards and Fromm,1970). In the present study, no increase in influx was observed during exercise when plasma adrenaline levels are known to rise (Nakano and Tomlinson,1967). This result could well be explained by back-transport in the following manner. Active inward transport of sodium was in fact augmented by the stimulatory action of catecholamines during activity, but this

additional activity was occupied in back-transporting ions moving out through increased simple diffusion. Consequently no increase in influx and only a relatively small elevation (70%) of efflux were observed. Against this argument, however, must be stated the fact that the sites of maximum diffusional loss (respiratory lamellae) and of active transport (inter-lamellae filamental epithelium) are spatially separated in the teleost gill (Conte,1969).

If the assumptions on which the above arguments are based are valid, then the real situation should be representative of either Case 1, Case 2, or some combination of the two schemes. If the assumptions are invalid, then some completely different mechanism could be involved. The most tenuous supposition used in these considerations is that of co-variation in oxygen and sodium diffusional coefficients, on which there appears to be no relevant evidence. In a system where gases can move directly through the plasma membrane but ions must pass through restrictive "pores", it is possible to imagine that recruitment of new exchange area during activity could change one coefficient more than the other. Until more is known about the physical properties of the gills, consideration of this subject will remain supposition.

Simultaneous unidirectional fluxes of sodium have not previously been determined across the gills of intact Salmo gairdneri, so comparison of the present data with reported measurements for the few other teleost species which have been examined in fresh water seems worthwhile (Table IV). For the sake of convenience, flux rates have been expressed in both ug/100 g/min. and uEq/100 g/hr., the unit popularized by Maetz

Table IV. Summary of branchial unidirectional sodium flux rates in fresh water teleosts.

Species	Weight (g)	Influx rate uEq/100g/hr	Efflux rate uEq/100g/hr	Influx rate ug/100g/min	Efflux rate ug/100g/min	Ext.Na^+ mEq/L	Reference
Salmo gairdneri	180-350	46.0	44.1	17.6	16.9	0.07	present study
Salvelinus fontinalis	0.91-7.72	59.5	* -	22.8	-	0.10	Packer & Dunson(1970)
Salmo salar(smolts)	20-45	68.0	66.0	26.0	25.3	0.20	Potts et al. (1970)
Fundulus heteroclitus	2.0-5.0	58.0	-	22.2	-	1.00	Potts & Evans(1967)
Fundulus heteroclitus	9 - 20	23.0	-	8.8	-	1.00	Maetz et al. (1967b)
Platichthys flesus	85-115	12.4	-	4.7	-	0.43	Motais & Maetz(1964)
Platichthys flesus	60-220	14.0	-	5.4	-	0.45	Motais et al.(1966)
Anguilla anguilla	107-378	21.3	-	8.2	-	0.55	Garcia-Romeu, Motais(1966)
Anguilla anguilla	60-120	4.0	-	1.5	-	0.12	Maetz et al. (1967a)
Carassius auratus	300-670	21.4	12.3	8.2	4.7	0.70	Maetz(1956)
Carassius auratus	80-330	20.2	19.4	7.7	7.4	0.90	Garcia-Romeu & Maetz(1964)

Table IV. (Continued)

Species	Weight (g)	Influx rate uEq/100g/hr	Efflux rate uEq/100g/hr	Influx rate ug/100g/min	Efflux rate ug/100g/min	Ext.Na^+ mEq/L	Reference
Carassius auratus	80-330	34.1	20.6	13.1	7.9	0.90	Maetz & Garcia-Romeu (1964)
Carassius auratus	80-225	10.8	14.6	4.1	5.6	0.65	Maetz et al. (1964a)
Carassius auratus	15-80	16.0	-	6.1	-	0.12	Lahlou et al. (1969)
Carassius auratus	40-120	16.8	25.0	6.4	9.6	0.50	Lahlou & Sawyer(1969)

* Efflux rates are not tabulated for many reports as workers failed to distinguish between branchial and whole organism effluxes.

and his co-workers. All values fall within the same order of magnitude, but there is wide variability, much of which may be ascribed to a size effect. Gill area per unit body weight, and presumably the fluxes for which the surface is responsible, decrease with increasing body weight (Muir,1968). Thus on a per 100 g basis, very small fish will have apparently greater turnovers than those of larger animals. Branchial sodium fluxes of 260 g Salmo gairdneri from the present study were relatively large and comparable to rates observed in very small fish in other investigations. Even more surprising is the fact that these high rates for rainbow trout were ascertained at external sodium levels 1/2 - 1/20 of those used by other workers; over the concentration range normally encountered in fresh water, there is a definite positive relationship between influx rate and external sodium levels (Maetz,1956; Chester Jones et al.,1969). The magnitude of both unidirectional fluxes in the rainbow trout relative to other euryhaline forms (e.g. Anguilla anguilla, Platichthys flesus) lends support to hypothesis (1) stated previously (i.e. presence of exchange diffusion). Further consideration of the mechanisms of sodium exchange in Salmo gairdneri will be applied in Section II when data relating to the concentration dependence of the fluxes are presented.

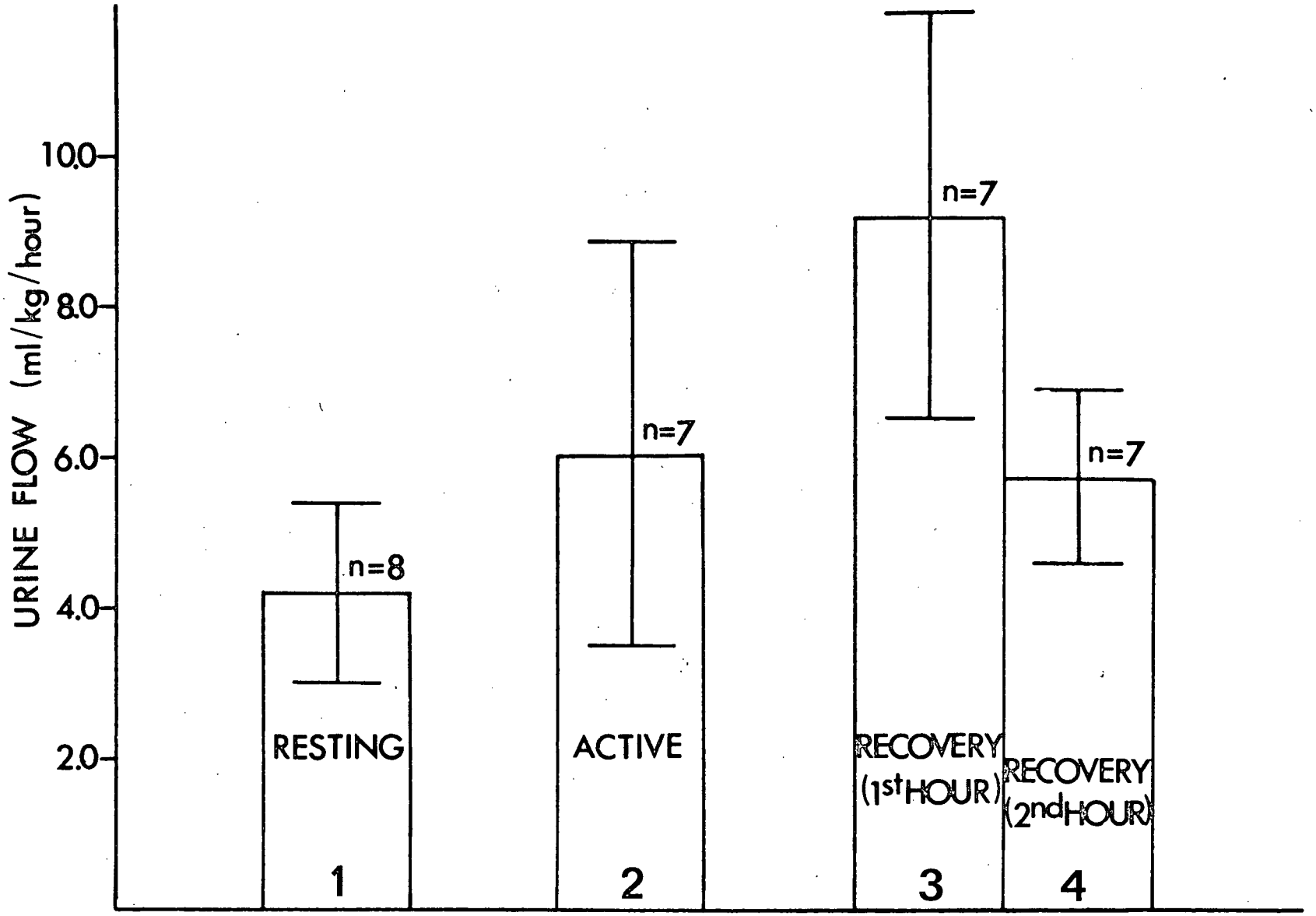
Augmentation of branchial permeability to gases and sodium should also enhance the net osmotic entry of water. Urine production may be considered equivalent to the total influx of water as long as the fish does not drink (Hickman and Trump,1969). The work of Shehadeh and Gordon (1969) renders this a relatively safe assumption for rainbow trout. Urine

Figure 11 Mean urine flow rates over the experimental periods in resting, active, and recovery groups of trout. First hour values for recovery fish were obtained during their initial chasing treatment. The vertical lines represent $\pm$ 1 standard error of each mean.

Statistical Comparisons: (Numbers refer to means as labelled on the graph.)

$F = 1.01$, n.s.

1 4 2 3

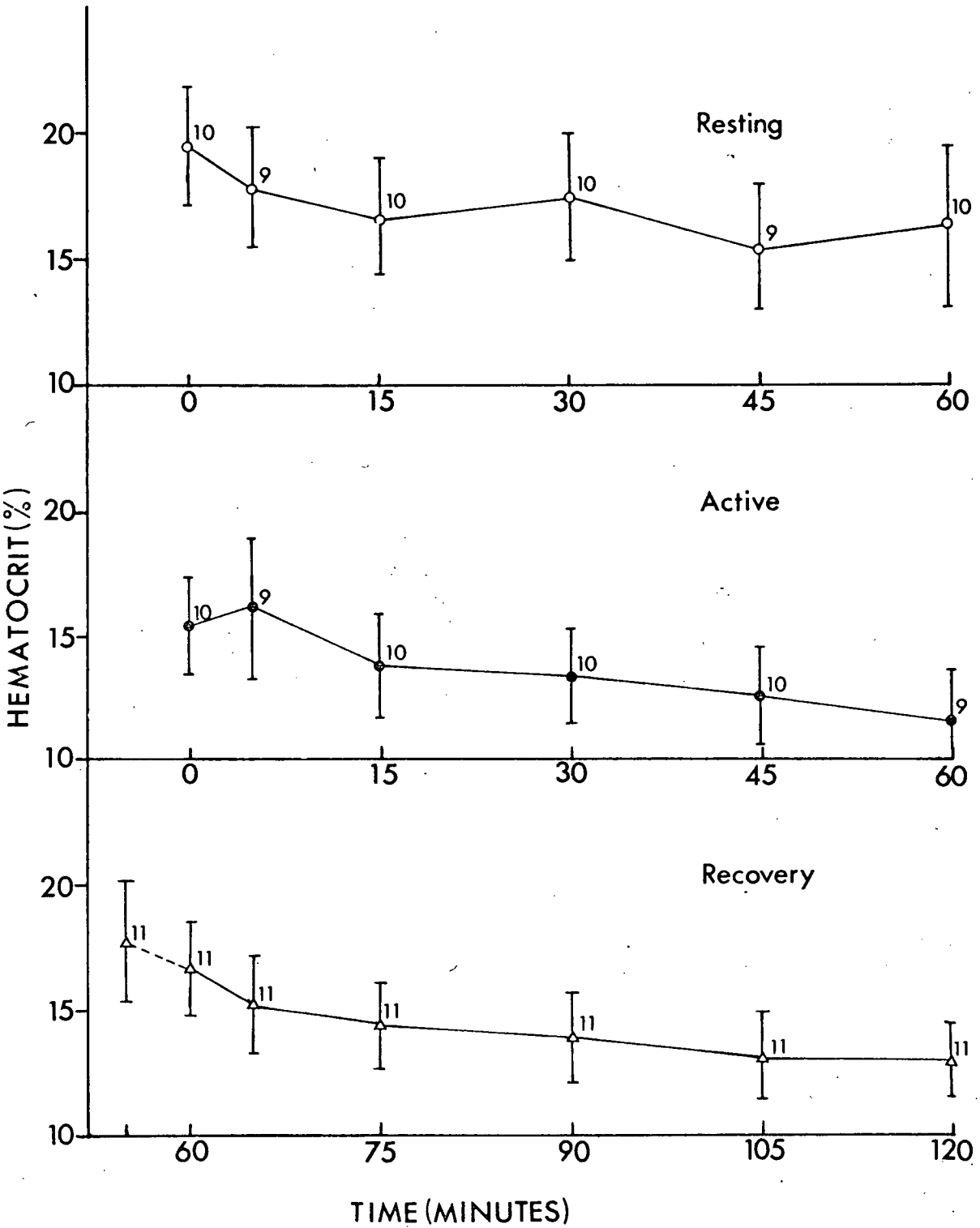

URINE FLOW (ml/kg/hour)
10.0
8.0
6.0
4.0
2.0
n=8
n=7
n=7
n=7
RESTING
ACTIVE
RECOVERY (1st HOUR)
RECOVERY (2nd HOUR)
1
2
3
4

flow (Fig. 11) tended to rise during exercise and decrease during recovery, although large variability in the data precluded significance. The higher production rates by recovery trout during their hour of exercise than by active fish may be attributed to the continual removal of fluid in serial blood sampling throughout the experimental period in the latter group. The effect of exercise on urine flow and water fluxes has been examined in much greater detail in Section III so the topic will not be pursued at present.

C. The Effect of Exercise on Sodium and Water Distribution

Many of the results presented in this section were obtained through the serial removal of small blood samples from the fish via the dorsal aortic catheter. Interpretation of these values is complicated by the effect of the repetitive blood removal necessary to ascertain them. The slight fall in blood pressure over the experimental hour in resting fish (Table I) implies that there resulted some decrease in blood volume. Hematocrit data (Fig.12)were informative in this respect for it was improbable that the trout could mobilize erythrocytes to replace sampling losses over the short experimental time period (Stevens 1968 b). If the animal's blood volume simply decreased as a function of the amount removed, then there would have occurred no net change in red cell concentration. On the contrary, packed red cell volumes declined progressively over the sampling period in all three groups (Fig. 12); the fish must have obviously effected compensatory changes to help maintain the circulatory volume. The difference in urine flows between the recovery group during

Figure 12 Changes in hematocrit over the experimental periods in resting, active, and recovery groups of trout. Means $\pm$ 1 standard error.


Resting
Active
Recovery
HEMATOCRIT (%)
TIME (MINUTES)
20
15
10
0
15
30
45
60
75
90
105
120
10
9
10
10
9
10
9
10
10
10
9
11
11
11
11
11
11
11

their hour of exercise (only 0.16 ml/blood/fish removed) and the active group (1.20 ml/blood/fish removed) indicated that this occurred at least partially through water retention. Fluid shifts from the intracellular to extracellular phases (Houston,1964) could also have been important in this respect. Thus in all three groups, plasma constituents were expected to have undergone some progressive dilution during the experiment. However, as sampling regimes were similar in all experiments, then the relative differences observed should still be valid.

Hematocrit changes also provided a basis for estimation of the effective blood volume or mixing pool from which samples were removed over a particular interval. If, for the purposes of comparison, the assumption was taken that volume did not decline in response to sampling, then the size of the blood pool could be calculated:

$$100\ \frac{\left(\frac{Hi}{100}\ Y - \frac{H\bar{x}}{100}\ \Sigma V\right)}{Hf} = Y$$

Y = blood pool/100 g

Hi = initial hematocrit

$H\bar{x}$ = average hematocrit of samples between Hi and Hf (including Hi)

Hf = final hematocrit

ΣV = total volume of blood removed/100 g between Hi and Hf (including the Hi sample)

Because the assumption was not entirely true, the compartments calculated may represent over-estimations. Nevertheless, they allow legitimate comparisons of blood pool sizes between different treatment groups and experimental intervals on a relative basis.

The blood pools calculated from the hematocrit decline over 0 - 30 minutes (or 60 - 90 minutes) and over 15 - 60 minutes (or 75 - 120 minutes) are presented in Table V. These values,

Table V. Effective blood pools calculated from hematocrit changes over the first 30 and final 45 minute periods of the experiments.

	Effective blood pools (ml/100 g)	
	First measurement	Second measurement
	or $^{0\ -\ 30}_{60\ -\ 90}$ minutes	or $^{15\ -\ 60}_{75\ -120}$ minutes
Resting (N = 9)	1.98 ± 0.19	2.78 ± 0.38 $p < 0.10$
Active (N = 9)	1.81 ± 0.22	2.01 ± 0.34 p = n.s.
Recovery (N = 11)	1.82 ± 0.35	2.83 ± 0.39 $p < 0.10$

p = significance with respect to first measurement.

despite the probable exaggeration inherent in the computation, are inferior to total blood volume figures obtained by direct measurement in salmonids (Smith and Bell,1964; Smith,1966; Houston and Dewilde,1969). It is possible that the distribution volume of erythrocytes is lower than that of the whole blood due to "skimming" in capillary beds; Conte (1963) in fact measured lower blood volumes with labelled red blood cells than with labelled plasma proteins. However, a more likely explanation may be found in the work of Smith (1966) who demonstrated that the blood volume obtained was dependent on the time allowed for mixing of the label in the compartment. His dye dilution curves exhibited one, and occasionally two, changes in slope before stabilization 2 - 3 hours after injection of the marker. These shifts were probably reflective of pools with different turnover rates comprising the total circulation, a fast mixing pool in the larger vessels, and at least one slow mixing compartment in the peripheral capillaries.

The present values (Table V) were taken over only 30 and 45 minute "mixing" intervals, and therefore probably dealt largely with the fast equilibrating pool of the major arteries and veins. As there could be no distinct division between the component pools, the volumes determined over a short interval would be dependent on the time allowed for interchange. Thus the greater volumes ($p < 0.1$) for the second measured period (45 minutes) in resting and active trout (Table V) were attributable to this time dependent mixing effect. The fact that this increase did not occur in the active fish was indicative of a volume reduction of the pool.

Stevens (1968 a) in fact observed a haemoconcentration associated with exercise in Salmo gairdneri which he attributed to a decrease in the water content of the vascular fluids. The data of Section III indicates that this blood volume reduction during swimming was caused by an imbalance between net water influx and efflux, urinary output exceeding branchial influx.

Variations in plasma sodium levels over the experimental period are presented in Fig. 13. Pre-experimental concentrations (zero time) were identical to those reported by Toews (1969) for rainbows after long term acclimation to 14^{o} - $16^{o}C$. Data variability precluded significance, but there was a progressive fall in plasma sodium concentration in resting trout; this trend was probably effected by the dilution of serial sampling. During activity, however, this diminution failed to occur, despite the existence of increased branchial (Table III) and renal (Section III) sodium loss in addition to the systematic withdrawal of blood. In recovery trout, which were not sampled during the first hour, there was in fact an increase in plasma sodium levels during exercise followed by a significant decline over the post-exercise hour. Analyses of tissue samples provide no evidence for the movement of intracellular sodium into the vascular compartment during exercise, so the plasma sodium rise associated with activity may be attributed to a reduction, computed previously, in blood volume through inequality of net water fluxes. This concept is supported by the work of Rao (1969) who found a significant increase in plasma osmolarity after exercise in rainbow trout. Rao postulated that this osmoconcentration was related to the increased release of metabolites during

Figure 13 Changes in plasma sodium concentration over the experimental periods in resting, active, and recovery groups of trout. Means $\pm$ 1 standard error.

Statistical Comparisons: (Numbers refer to the sample times of the respective means.)

Resting: $F = 0.52$, n.s.

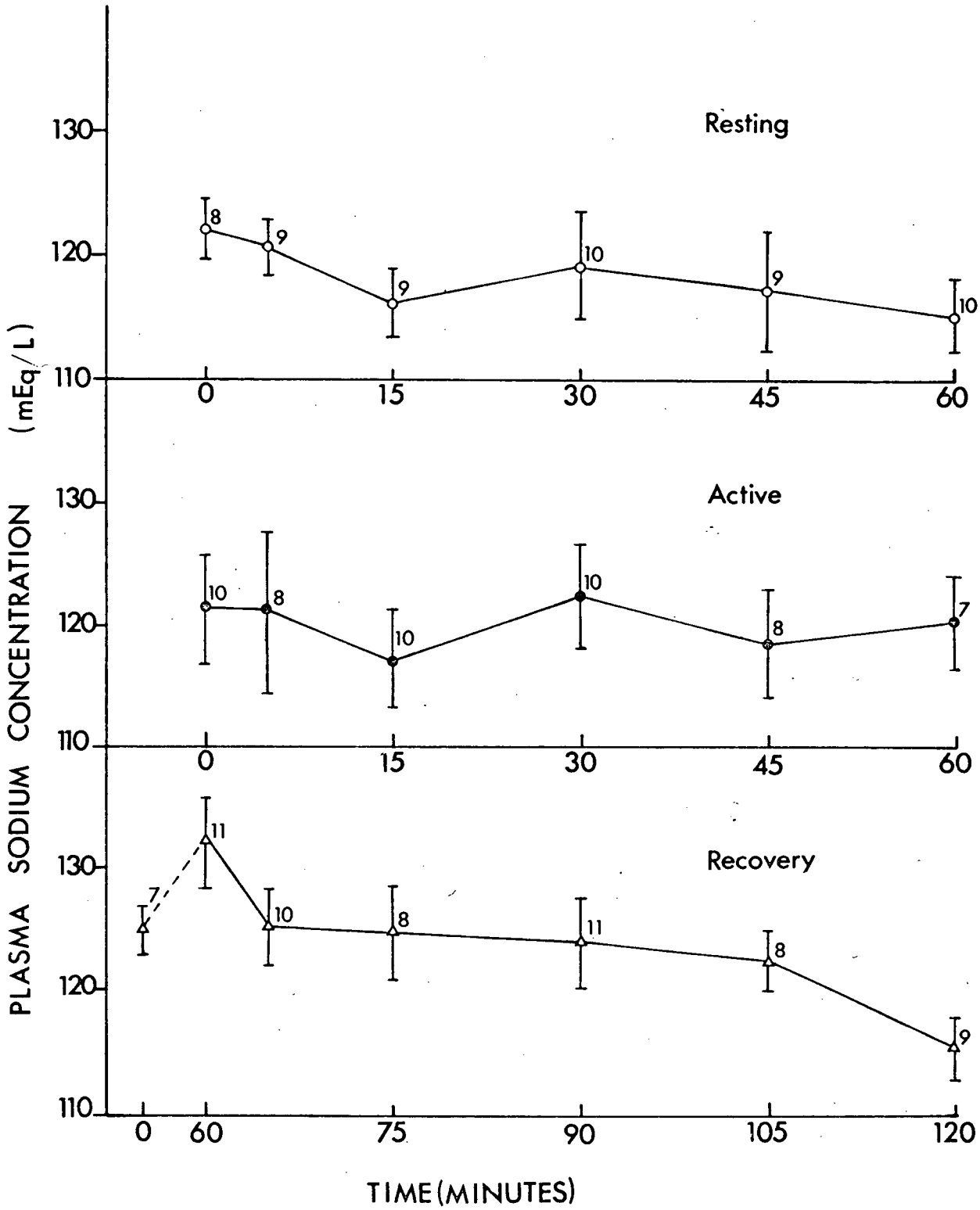
60 15 45 30 5 0

Active: $F = 0.15$, n.s.

15 45 5 60 0 30

Recovery: $F = 2.31$, $p < .05$

120 105 90 75 0 65 60


Resting
Active
Recovery
PLASMA SODIUM CONCENTRATION (mEq/L)
TIME (MINUTES)
130
120
110
0
15
30
45
60
75
90
105
120
8
9
9
10
9
10
10
8
10
10
8
7
7
11
10
8
11
8
9

activity, but did measure a slight elevation of plasma chloride levels; his results can well be explained by a reduction of plasma water. Farmer and Beamish (1969), however, observed a non-significant drop in osmolarity after exercise in fresh water adapted Tilapia nilotica; in this species, there may be a greater loss of electrolytes across the branchial epithelium and/or lesser ability of the kidney to handle water loads.

Terminal concentrations of sodium and water in plasma and tissue are summarized in Table VI. There occurred no significant difference between the three groups in any of the parameters measured, despite the prediction of lower plasma water levels in exercised trout by the preceding statements. However plasma water values were higher than previous measurements in salmonids (Houston,1959; Stevens,1968 a; Miles and Smith,1968; Toews,1969) probably due both to the dilution of serial sampling and the protein catabolism associated with pre-experimental starvation. Differences in water concentration sufficient to explain the slightly higher terminal plasma sodium levels in active fish would have fallen well below the analytical accuracy of the refractometer. In an attempt to detect changes in plasma water levels during exercise without the confounding effects of repetitive sampling, the zero time and 60 minute samples from recovery fish were analyzed in a number of experiments. The results were encouraging, but not conclusive: water concentration decreased in four trout, increased in two, and remained stable in one. The difference in the means (96.48 ml/100 g plasma vs. 96.30 ml/100 g plasma) was sufficient to account for the observed elevation of plasma

Table VI. Terminal concentrations of sodium and water in plasma and tissue. Means ± 1 standard error.

	Resting	Active	Recovery
Terminal plasma sodium conc'n. (mEq/L)	N = 10 115.05 ± 2.71	N = 7 120.64 ± 3.97 p_1 = n.s.	N = 9 115.19 ± 2.87 p_1 = n.s. p_2 = n.s.
Tissue sodium concentration (mEq/kg wet tissue)	N = 9 8.13 ± 0.51	N = 11 8.93 ± 0.51 p_1 = n.s.	N = 11 8.03 ± 0.50 p_1 = n.s. p_2 = n.s.
Plasma water concentration (ml/100g plasma)	N = 10 96.90 ± 0.29	N = 11 97.15 ± 0.14 p_1 = n.s.	N = 11 96.85 ± 0.20 p_1 = n.s. p_2 = n.s.
Tissue water concentration (ml/100g tissue)	N = 10 81.420 ± 0.329	N = 11 81.569 ± 0.452 p_1 = n.s.	N = 11 82.107±0.399 p_1 = n.s. p_2 = n.s.
Sodium space E.C.F.V. (g water/kg/wet weight)	N = 9 72.38 ± 3.99	N = 11 76.57 ± 4.80 p_1 = n.s.	N = 11 71.14 ± 3.71 p_1 = n.s. p_2 = n.s.

p_1 = significance with respect to corresponding resting value.

p_2 = significance with respect to corresponding active value.

sodium levels. Stevens (1968 a) witnessed a triphasic fluctuation in plasma water levels at the onset of exercise in trout, culminating in a final gradual decrease as swimming time was prolonged.

Tissue water levels were about 2% higher than commonly quoted values for salmonids (Houston,1959; Houston et al,1968; Toews,1969); again the hydration was a probable consequence of starvation (Brett, personal communication), the apparently higher water contents being due to a reduction in the fat content of the tissues. Muscle sodium levels, however, agreed well with previously reported figures (Toews,1969) for rainbows acclimated at 14^{o} - $16^{o}C$. As there is little intracellular sodium (Manery,1954), muscle sodium should be largely reflective of concentrations in plasma. In both resting and recovery groups, there occurred significant positive correlations ($r = 0.754$, $p < .01$; $r = 0.678$, $p < .05$ respectively) between plasma and tissue levels of the electrolyte in individual animals. This relationship, however, did not exist for active trout ($r = .033$, n.s.) indicating a possible buffering during exercise of the tissues from changes in electrolyte levels in the fast-mixing plasma pool. An ischemia of "white" muscle during swimming (Stevens,1968 b) would be a probable cause for the phenomenon; additional evidence for this phenomenon was provided by Na^{22} distribution data which will be discussed presently. As the parameters from which the sodium space estimate of extracellular fluid volume were calculated did not show significant variation, E.C.F.V's were similar in all three treatment groups. Toews (1969) has demonstrated that in fresh water teleosts, unlike mammals, sodium space

provides a more accurate measure of E.C.F.V. than chloride or chloride-potassium space because of the significant chloride content of the intracellular phase.

The evolution of the Na^{22} space with time during rest, activity, and recovery is illustrated in Fig. 14, while the temporal sequence of the accumulation in plasma of sodium transported from the external water ("plasma sodium incorporation") is presented in Fig. 15. Plasma sodium incorporation is a factor which permits the comparison of plasma Na^{22} levels between individual fish, which would not be valid through use of just activity values due to differences in external specific activity between experiments. As such, this factor is the ratio between plasma Na^{22} levels and the average external specific activity prior to the measurement time. Thus values are expressed in terms of the actual concentration of sodium from the external medium resident in the internal medium at the particular sample time. The analogous values for tissue ("tissue sodium incorporation") are summarized in Table VII together with the radiosodium space and plasma sodium incorporation values at 60 (or 120) minutes for each experimental group.

The time course of the radiosodium space expansion in teleosts has been followed in only one other study (Mayer and Nibelle,1969) while the relative incorporation of external sodium into plasma and tissue represents a completely new approach. Interpretation of these data must therefore proceed from a basic level. The form of the evolution curve (Fig. 14) is similar in the three treatments although the magnitudes of the component points differ between experimental groups.

Figure 14 The evolution of the radiosodium space with time in resting, active, and recovery groups of trout. Means $\pm$ 1 standard error.

A = significantly different from corresponding resting value ($p < .05$)

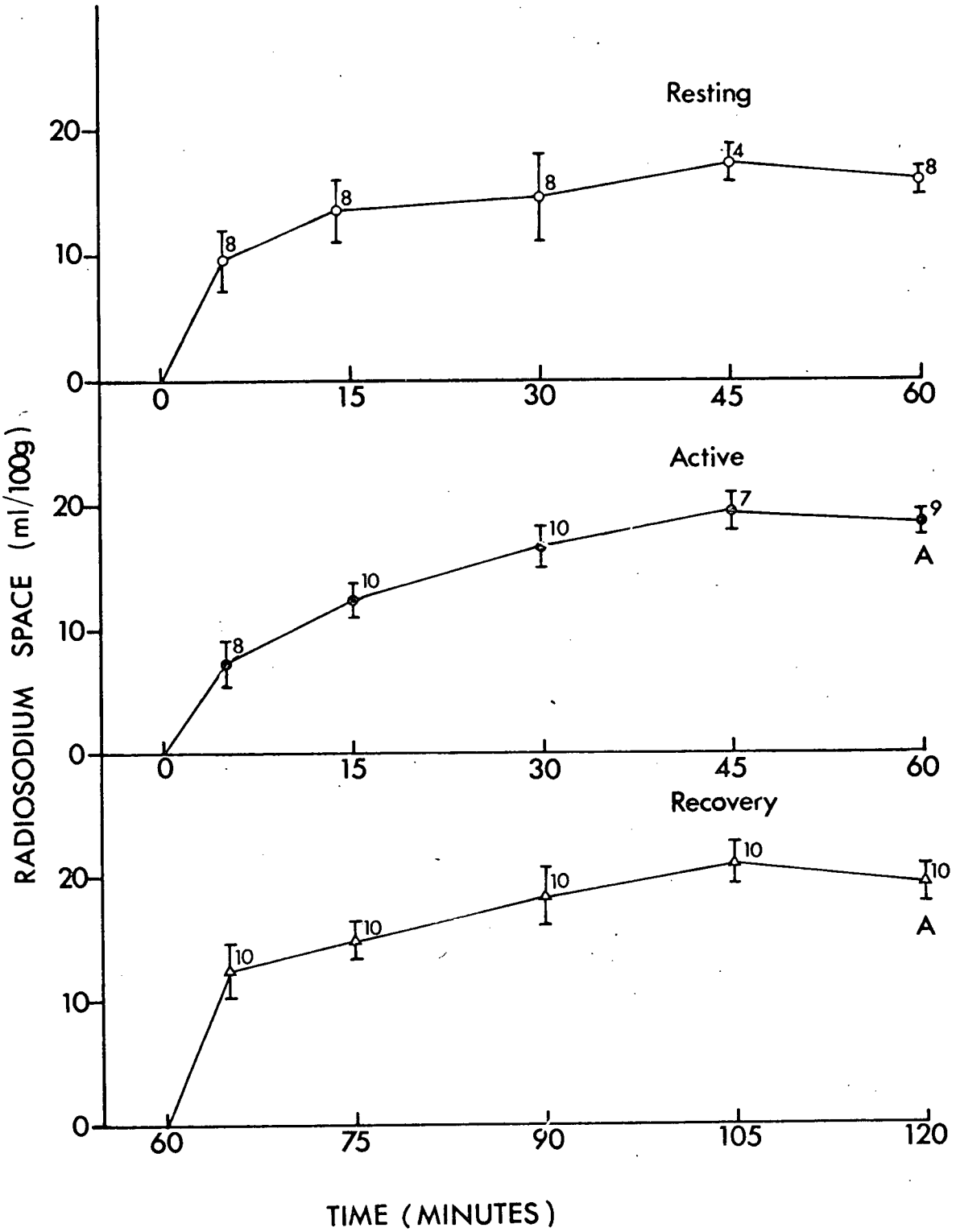
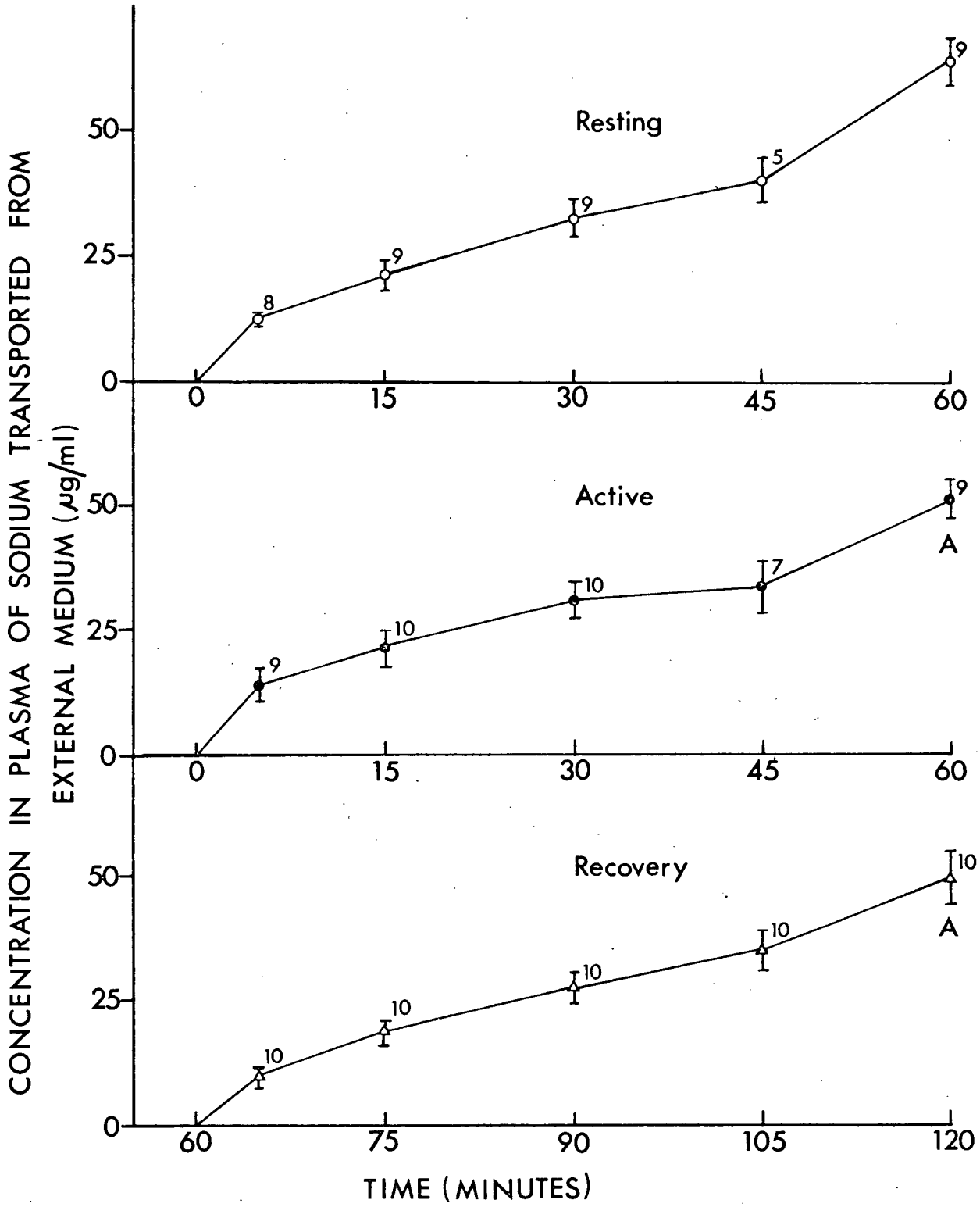

Resting
Active
Recovery
RADIOSODIUM SPACE (ml/100g)
TIME (MINUTES)
0
10
20
0
15
30
45
60
75
90
105
120
8
8
8
4
8
10
10
7
9
A
10
10
10
10
10
A

Figure 15 The evolution with time of the concentration in plasma of sodium transported from the external environment ("plasma sodium incorporation") in resting, active, and recovery groups of trout. Means $\pm$ 1 standard error.

A = significantly different from corresponding resting value ($p < .05$)


CONCENTRATION IN PLASMA OF SODIUM TRANSPORTED FROM EXTERNAL MEDIUM (μg/ml)
Resting
Active
Recovery
A
A
TIME (MINUTES)
0
25
50
0
15
30
45
60
75
90
105
120
8
9
9
5
9
9
10
10
7
9
10
10
10
10
10

The general configuration of the observed changes will be dealt with first.

By definition, the radiosodium space is the ratio between the amount of radioisotope in the whole animal and the amount per ml of plasma. The precipitous increase in Na^{22} distribution volume over the first 5 minute interval was probably related to the initial exchange of Na^{22} ions with Na^{23} ions adsorbed to the skin of the animal. This apparent influx would tend to elevate the value of the term ($Q_o - Q$) (see Methods p. 38)disproportionately to the radioactivity of the plasma (Na^{22}/ml pt), thereby exaggerating the calculated distribution space. Arrival of the expansion curve at a zenith, or at least a plateau, 45 minutes after introduction of radiotracer, is however, more difficult to explain, for the volume of Na^{22} distribution at equilibrium is about 34 ml/100 g (Section II), considerably greater than this apparent peak at 17 - 21 mls/100 g. Reference to the plasma sodium incorporation data (Fig. 15) provides the obvious mathematical explanation for the phenomenon. The evolution curve of plasma sodium incorporation (representative of plasma Na^{22} concentration) underwent an abrupt upward deflection at the same point (45 minutes) as the levelling of the radiosodium space curve. Consequently, there probably occurred a large increase in the parameter Na^{22}/ml pt relative to the rise in ($Q_o - Q$), resulting in a lower terminal estimate of Na^{22} distribution volume. The biological reason for this event, however, remains unclear. Up to 45 minutes, the slopes of the plasma incorporation curves tended to slowly decline as radiosodium became distributed over an increasing volume, presumably through the

natural enlargement with time of the effective vascular mixing pool and the concomitant diffusional dispersal to a greater tissue mass. The acclivity of the curve after 45 minutes could be due to either an increase in the rate of influx of external sodium across the gills into the plasma pool, or a decrease in the rate of movement out of the pool. It seems unlikely that there would be any significant backflux of influxed sodium from the tissues into the mixing compartment). It has already been shown, however, that branchial influx rate remained constant (Fig. 9) over the interval in question, so the second interpretation must apply. The most probable reason for a decreased rate of movement out of the mixing pool is that the size of this compartment approached or attained constancy such that dispersal of influxed sodium became limited by diffusion into the extravascular E.C.F.V. spaces and perhaps the intracellular phase. Smith (1966), in using the dilution of Evan's Blue dye to measure blood volumes in salmonids found that the intersection of the initial "mixing plus excretion" slope with the stable "excretion" slope of the dye dilution curve took place "about one hour after injection". Although Smith's experiments differed from those reported here in the incidence of only a single injection of marker rather than constant entry of it across the gills, it is tempting to speculate that the plateau of the radiosodium space curve was attributable to a similar stabilization of the mixing compartment.

The only previous examination of radiosodium space evolution in teleosts is that of Mayer and Nibelle (1969) in the eel, Anguilla anguilla. These workers demonstrated that

the rate of completion of the radiosodium space, but not its equilibrium value, depended upon the site of introduction of the label. They argued that capillary barriers were negligible in the evolution of the Na^{22} distribution volume, and that the "filling up" of the compartment (radiosodium space) could be likened to the diffusion of electrolytes in an ionized solution. The presence of a plateau effect in the present data casts some doubt on this interpretation. However, Mayer and Nibelle (1969) took only widely spaced samples and thus would have been unable to detect an intermediate levelling of the curve. In light of the widespread use of radiosodium space values as the "internal compartment" in the calculation of branchial flux rates and exchangeable sodium pools (e.g. Maetz,1956; Motais, 1967), more detailed studies on the kinetics of radiosodium space evolution are urgently required. Until the work of Mayer and Nibelle (1969), there had apparently been no systematic attempt to find even the equilibrium values of this parameter in a particular species, despite its ubiquitous application to the study of teleost ionic regulation for over twelve years. For example, the data of Wood and Randall (1971) indicate that the commonly used radiosodium space value of the flounder (Motais et al.,1966; Motais,1967), on which perhaps the majority of ion exchange work has been performed (Maetz, 1969), may well be a significant underestimation of the equilibrium volume.

Differences in the evolution of the Na^{22} distribution volume between treatment groups may now be examined. The curves (Fig. 14) for active and recovery trout followed a similar course; however the radiosodium space expansion lagged

noticeably behind this pattern during rest, the difference becoming significant by the terminal sample. It is not possible, however, to say whether the equilibrium values would also have differed; in any case, the point is somewhat hypothetical as an active fish would stop swimming at some time, and a recovery fish would eventually return to the resting condition. The higher Na^{22} distribution volumes during exercise and post-exercise cannot be related directly to the plasma volume changes discussed earlier for at least two reasons. Firstly the larger spaces were characteristic of both active and recovery states, while the reduction of blood volume through dehydration occurred only during exercise (Table V). Secondly, a decrease in the size of the plasma volume alone would increase the concentration of Na^{22} on a simple dilution basis, and, in addition, would tend to depress the diffusion of the isotope out of the vascular pool into the tissues. The net effect of hemoconcentration would be an inhibition, rather than a stimulation, of the radiosodium space evolution. Plasma sodium incorporation values (Fig. 15) indicate that the reverse was in fact true; by the end of the experiment, plasma "Na^{22} concentrations" were significantly lower than the resting value in both active and recovery trout, thereby generating the higher Na^{22} distribution volume. It must be noted that this effect cannot be related to slower entry rates of sodium, for influxes were identical in all three groups (Table IV). Thus there must have occurred a greater emigration rate of previously influxed sodium out of the sampled plasma pool during activity and recovery than at rest, despite the probable decrease in size of this compartment

during exercise. The terminal epaxial muscle samples were analyzed for Na^{22} as a possible reception site for this lost sodium; the results, expressed as tissue sodium incorporation on a wet weight basis, are presented in Table VII. "White" muscle obviously did not receive this extra sodium, the incorporation being somewhat lower in the active trout than in the resting animal, and significantly smaller in the recovery fish. ("White" muscle is a misnomer in salmonids, as Webb (personal communication) has found that this tissue may contain up to 17% red muscle fibres by volume). The tissue incorporation data is thus reflective of plasma incorporation values. However, correction of tissue incorporation measurements for hypothetical enclosed plasma volumes of 1 - 4% did not change the fact that the extravascular region of "white" muscle accumulated less influxed sodium during exercise and recovery than during rest. It must therefore be concluded that the higher radiosodium spaces resulted from a greater accumulation of influxed sodium, and thus the radioisotope, in other regions of the body with an accompanying decrease in "white" muscle incorporation.

Due to the time lag in moving from the gills to the tissues, the majority of radiosodium found in muscle after 60 minutes probably entered the animal during the earlier portion of the experimental hour when external specific activities were greatest. Use of the average external specific activity figures over the whole hour to calculate tissue sodium incorporation may therefore have been responsible for a systematic overestimation of this parameter. However, such an error would have been common to all three groups, and would in fact tend to

Table VII. Measures of internal distribution of influxed sodium at 60 minutes after introduction of Na^{22}. Means ± 1 standard error.

	Resting	Active	Recovery
Na^{22} space (ml/100 g) (60 minutes)	N = 8 15.73 ± 1.05	N = 9 18.45 ± 0.70 $p_1 < 0.05$	N = 10 19.56 ± 0.90 $p_1 < 0.05$ p_2 = n.s.
Plasma sodium incorporation (ug/ml) (60 minutes)	N = 9 64.86 ± 3.83	N = 9 51.99 ± 3.81 $p_1 < 0.05$	N = 10 49.17 ± 4.09 $p_1 < 0.02$ p_2 = n.s.
Tissue sodium incorporation (ug/kg/wet tissue) (60 minutes)	N = 10 3.077 ± 0.243	N = 10 2.579 ± 0.351 p_1 = n.s.	N = 10 2.218 ± 0.203 $p_1 < 0.02$ p_2 = n.s.

p_1 = significance with respect to corresponding resting value.
p_2 = significance with respect to corresponding active value.

Table VIII. Balance sheet of fate of influxed sodium at 60 minutes after introduction of Na^{22}.

	Resting	Active	Recovery
Sodium influxed/100g over 60 minutes	1057.2 ug	984.6 ug	1154.4 ug
Sodium incorporated in plasma (3 ml/100 g)	194.6 ug	156.0 ug	147.5 ug
Sodium incorporated in tissue (97 g/100 g)	298.5 ug	250.2 ug	215.2 ug
Total in tissues and plasma	493.1 ug	406.2 ug	362.7 ug
% of total sodium influx represented	46.64%	41.25%	31.42%

It has been assumed in the above calculation that all tissue sodium incorporation was equal to "white" muscle sodium incorporation and that plasma volume in the major vessels was 3 ml/100 g.

enforce the substance of the following argument.

If a generous estimate of 3 ml/100 g body weight for plasma in the major vessels and the assumption of tissue incorporation values equal to that of epaxial muscle for the remainder of the body mass are taken, then the balance sheets of Table VIII may be constructed for the trout in each group at 60 minutes after introduction of the isotope. Even at rest, such a scheme accounts for the fate of less than 47% of the influxed sodium; this figure decreases to 31.5% in the post-exercise condition. It is apparent that under all conditions some tissues must exchange sodium faster than the "white" muscle, and that this effect is accentuated during activity and recovery by a further reduction of sodium turnover in "white" muscle and an increase in the turnover rates of other unknown tissues.

The sodium turnover rate of a particular tissue might well be proportional to its blood supply. If this is true, then the discrete red muscle and cardiac muscle, which, on a unit weight basis, contain 2½ times as much blood as "white" muscle (Stevens,1968 a), may preferentially accumulate sodium. The increased blood flow to these tissues during exercise could therefore account for the lower concentrations of influxed sodium in the plasma, and higher Na^{22} distribution volumes measured during exercise. This effect could be further augmented by an ischemia of the "white" muscle, as indicated by the reduced sodium incorporation of this tissue, and the lack of correlation between plasma and tissue levels of total sodium discussed previously. It is difficult, however, to see how this explanation could also apply to the similar sodium

space elevation and decreased plasma and "white" muscle sodium incorporation determined during recovery, when blood flow patterns should return to a more resting configuration. Thus blood flow variation may contribute to the observed Na^{22} space differences, but is probably not the major factor involved. A property common to both active and recovery conditions is the presence of high circulating levels of catecholamines (Nakano and Tomlinson,1967), substances which are known to stimulate the active transport of sodium across trout gills in vitro (Richards and Fromm,1970). It is possible that these hormones may also promote the uptake or turnover of sodium by specific sinks in the system, thereby contributing to the elevation of radiosodium space. Again, such sites could well be those tissues containing high blood volumes, such as the liver, spleen, red muscle, and cardiac muscle (Stevens,1968 a).

SUMMARY I

1. The ventilatory and cardiovascular responses of trout to chasing in the present study were similar to those reported to occur during normal swimming activity in other studies.
2. Unidirectional branchial sodium fluxes of *Salmo gairdneri* were higher than those recorded for other fresh water teleosts of comparable size despite the extremely low external sodium concentrations used in this study.
3. The uptake of sodium by the gills was apparently an active transport process.
4. Resting trout maintained a state of sodium equilibrium at the gills. During one hour of exercise there occurred a net branchial sodium loss which was reversed during recovery.
5. The alterations in net flux rate at the onset of exercise and the onset of recovery occurred extremely rapidly (within 5 minutes).
6. Branchial sodium influx rate remained constant during rest, exercise, and recovery.
7. Branchial sodium efflux rate increased 70% with swimming activity, causing a negative balance situation. This unidirectional outward movement decreased to slightly below resting levels during one hour recovery.
8. The elevation of efflux apparently occurred through increased simple diffusion resulting from an augmented branchial permeability.
9. Calculations based on hematocrit changes suggested that blood volume decreased during exercise.

10. Plasma sodium levels tended to increase in exercised fish even though there was a net loss of sodium. This effect was apparently caused by the blood volume decrease.

11. Terminal tissue sodium and water levels, and sodium space (E.C.F.V.), were similar in resting, active, and recovery animals.

12. In all groups, the evolution of the Na^{22} distribution volume reached a temporary plateau 45 - 60 minutes after introduction of the radioisotope. This levelling effect corresponded to an acclivity in the plasma sodium incorporation curve.

13. Sodium gained from the external environment over the experimental period was preferentially accumulated (on a per unit weight basis) in tissues other than "white" muscle in all treatments.

14. Radiosodium space expansion during rest lagged behind that occurring under active and recovery conditions, the difference becoming significant at 60 minutes after addition of the tracer. The opposite course was seen in plasma sodium incorporation values, this factor in active and recovery fish becoming significantly inferior to resting values by 60 minutes. A parallel trend occurred in tissue sodium incorporation values for the three groups. Thus greater amounts of influxed sodium were taken up by "sites" other than"white"muscle during activity and recovery than during rest.

SECTION II

THE EFFECT OF EXTENDED EXERCISE ON SODIUM BALANCE

INTRODUCTION II

The experiments of Section I demonstrated the occurrence of a net branchial sodium loss during exercise. The results strongly indicated that this negative balance was caused by an increased simple diffusional efflux due to a readjustment of effective gill permeability necessitated by the increased metabolic demands of activity. Over a one hour period the branchial sodium deficit (12.14 ug/100 g/min.) amounted to only 31.68 uEq/100 g or about 1/20th of the total plasma sodium content (600 uEq/100 g). The effect of this small loss was apparently negated by a decrease in plasma volume through a reduction of water content, thereby producing a slight augmentation of plasma sodium concentration. However, if such a net branchial efflux rate were maintained during prolonged swimming, the trout would soon suffer osmoregulatory embarassment. After only 6 - 7 hours, about 1/3 of the total plasma sodium would be lost, a situation obviously disadvantageous to the fish. In addition, the swimming animal's problems would be compounded by an increased urinary sodium loss, which was suspected at the time these experiments were performed (Hammond, 1969; Hickman and Trump,1969) and confirmed by the data of Section III. Thus the sodium deficit, if unchecked, would

clearly limit the duration of exercise to a matter of hours. Yet there exists a great deal of evidence (see Brett,1964) that fresh water salmonids can swim almost indefinitely at sub-fatigue speeds. The induction of some compensatory mechanism to reduce sodium loss during extended exercise was therefore indicated; the experiments of this section were designed to test the validity of this assumption.

As a correction of the negative sodium balance could occur through a modification of influx, efflux or both parameters, it was again desirable to separate the unidirectional movements of the ion by radiotracer techniques. The employment of these methods once more demanded the use of a relatively small water volume with its attendant difficulties in supporting swimming activity. Manual chasing was precluded by the extended length of the exercise periods. These problems were eventually overcome through the development of a small revolving chamber in which trout would swim continuously, albeit at low speeds, for many hours.

A means for distinguishing between branchial and renal contribution to the whole body efflux was also necessary. Collection of urine outside the experimental chamber through an implanted catheter as in Section I would have been the most accurate solution, but was prevented by the use of a constantly revolving system in which the fish repeatedly changed its position. Therefore a more indirect approach was taken. Sodium flux rates were measured during long term exercise in both normal unencumbered individuals and in animals in which the urogenital papillae had been occluded, thereby eliminating the release of urine into the external compartment. The difference

in mean efflux rates between normal and ligated fish would thus represent the renal component of sodium loss under a particular condition. Urinary blockage has been used previously to eliminate renal discharge of electrolytes in several studies of osmoregulation in rainbow trout (Holmes,1959; Randall et al.,1969) and in fact has been recently recommended (Kirschner,1970) as an acceptable technique for partitioning unidirectional fluxes in hypertonic regulators. However none of these workers have considered the possible traumatic effects of this treatment on the animal, effects which could disturb normal ionic balance. Thus a third group of trout (shams) were subjected to urinary intervention to evaluate the influence of this procedure alone on sodium exchanges. Weight changes over the experimental period were determined on the urinary blockage and sham fish, and terminal samples for determination of hematocrit, plasma sodium, and plasma and tissue water concentrations were taken from the experimental trout. These measurements were designed to test whether the urinary intervention associated with ligation, and/or the occlusion itself, produced disruptions of internal homeostasis likely to disturb normal sodium flux rates.

Because of the extended duration of the experiments, the net sodium fluxes of the trout in many cases brought about large alterations of external sodium concentrations; a marked concentration dependence of branchial flux rates was noted as a result of these changes. The information provided by these observations held considerable pertinence to arguments concerning the mechanisms of branchial sodium exchange presented in Section I. In addition, the data WERE not completely conson-

ant with the results of a recent examination of the concentration dependence of sodium fluxes across the gills of anaesthetized perfused Salmo gairdneri (Kerstetter et al.,1970). In light of these facts, the results seemed worthy of analysis in some detail; the topic has been dealt with in Part B of Results and Discussion in this Section.

METHODS II

1. Experimental Animals

Animals used in this study included both immature and sexually mature rainbow trout (Salmo gairdneri) weighing between 160 and 280 g. Some of the fish were in breeding condition. The trout were obtained, held, and acclimated as described in Section I. During the acclimation period, the fish were maintained under almost constant illumination to sustain the desired water temperature ($14.5 \pm 1.5^{\circ}C.$). Experiments were performed during December, 1969, and January and February, 1970.

2. Operating Procedures and Cannulations

Two groups of trout (urinary blockage and shams) studied in this section were subjected to urinary catheterization, while a third group (normals) were not handled prior to the experiment. Urinary cannulae were implanted as in Section I, but were cut off approximately 2 cm posterior to the anal fin. The cannula stump was then firmly tied to the anal fin with several silk stitches. Operated fish were allowed to recover for 24 to 72 hours.

3. Experimental System

Sodium influx, efflux, and net flux rates were determined hourly for the 3 treatment groups on trout placed individually in a revolving swimming chamber for 8 hours. Simultaneous records of the fish's behaviour were made, and terminal blood and tissue samples taken.

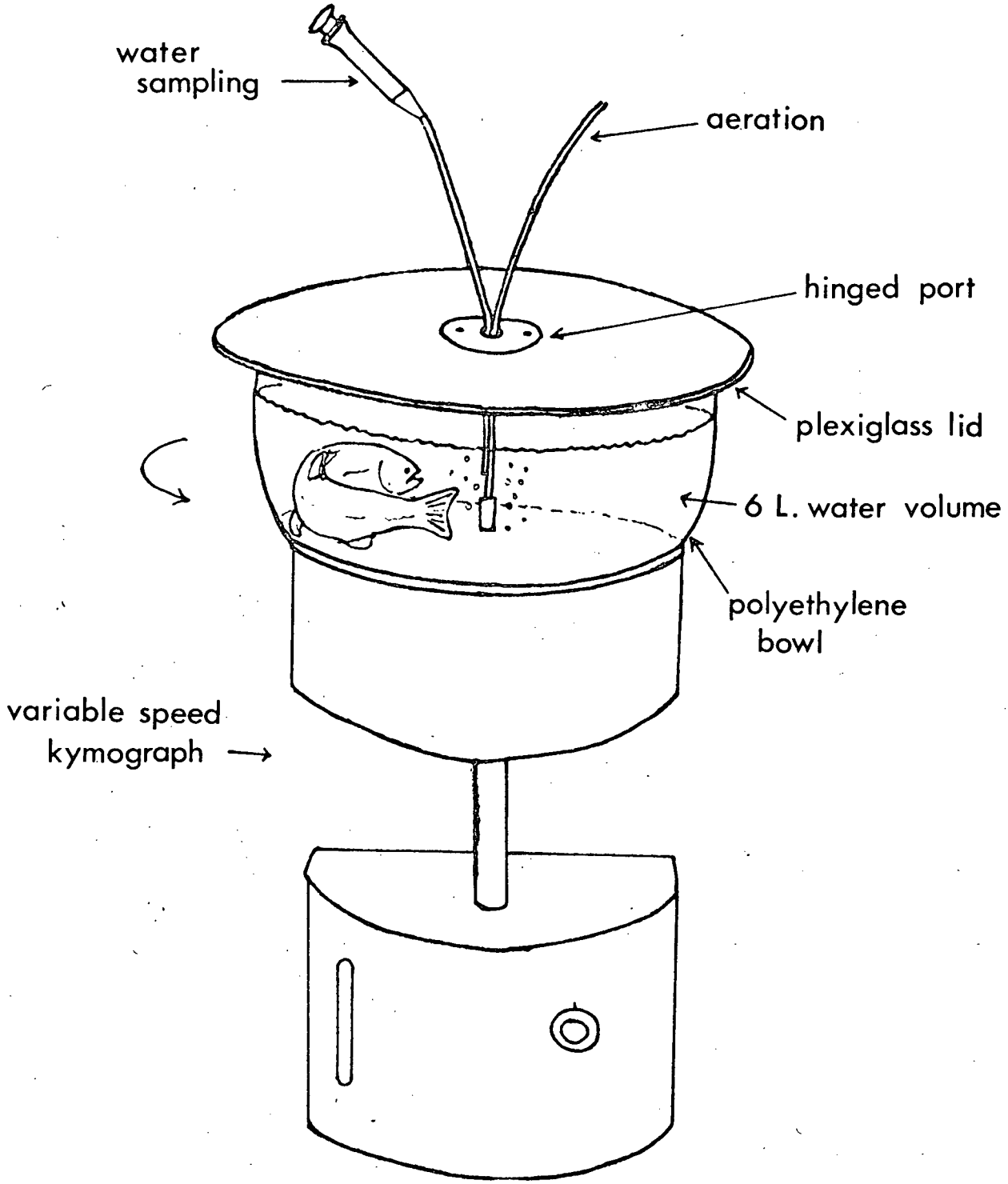
(a) Swimming Chamber

The exercise chamber comprised a circular polyethylene bowl (diameter = 40 cm; depth = 13.5 cm) mounted on a variable speed motor (kymograph) which permitted rotation at different velocities (Fig.16). A hinged port (diameter = 14 cm) through the plexiglass lid allowed addition of the fish to the bowl. Continuous aeration was effected by an airstone suspended through the centre of the lid; a length of PE 60 tubing adjacent to the airstone permitted periodic water sampling. Water temperature was dependent on the relatively constant ambient air temperature. Average experimental temperature was 15.2°C. with extremes of 13.0°C. and 16.5°C; maximum change observed during an 8 hour run was 1.8°C.

(b) Exercise Conditions

In all experiments, the chamber was rotated at 20.5 revolutions/minute for the entire run. Individual trout swam in opposition to the current for periods ranging from less than 60 minutes to the full 8 hours. The most rapidly swimming fish maintained their position in the chamber relative to external cues, and thus a maximum velocity of about 32 cm/second. Behaviour of the animal during each hour period was recorded as one of three well defined categories: swimming, not swimming, or intermittent. The first two categories are self-explanatory; the third refers both to periods during which the fish demonstrated interrupted bouts of activity and to periods in which the trout was swimming at the start, but had stopped by the end, of the hour. In a few cases, a "not swimming" fish would become active again during water sampling, probably in response to overhead visual stimulation. As these

Figure 16 A drawing of the small volume revolving chamber used for long term exercise of trout in the experiments of Section II.


water
sampling
aeration
hinged port
plexiglass lid
6 L. water volume
polyethylene
bowl
variable speed
kymograph

swimming bouts were extremely short (1 - 2 minutes), assignment to the "not swimming" category remained unchanged.

(c) Weighing Procedure

The weight changes of the shams and urinary blockage treatment groups over the experimental period were recorded as gross measures of net water fluxes. A trout was removed without anaesthesia from its recovery tank and placed in a bucket of water one hour before the start of an experiment. The animal was then picked up by hand, shaken briefly to remove excess water, and added to a tared plastic sack containing 500 mls of water. The bag was stapled closed and weighed. The trout was then returned to the bucket and the process repeated. After completion of the weight determinations, the fish was placed back in its aquarium. An identical weighing procedure was used on the stunned animal at the end of the experiment. Duplicate weight measurements were averaged, and the change over 9 hours expressed in g/100 g initial weight. It must be emphasized that the procedure, although adequate for the purposes of this section, was relatively inaccurate due to variable amounts of water adhering to the surface of the fish; 5 successive determinations on a dead trout ranged from 271.5 g to 273.4 g. However thorough drying of the animal (as in Section III) could well have produced undesirable stress effects on ion flux rates.

(d) Experimental Procedures and Treatment Groups

Prior to an experiment, the swimming chamber was filled with exactly 6 L of dechlorinated fresh water (mean sodium concentration = 1.61 ug/ml) containing 15 - 25 uC of Na^{22}, and then sealed with the plexiglass lid.

(i) Normal Trout. A fish was removed directly from the acclimation tank and allowed to slip through wet hands into the chamber through the port. It was then generally necessary to wait for the trout to position itself near the outside of the bowl in opposition to the proposed direction of revolution. The chamber was then rotated slowly and the desired velocity attained by gradual increments in speed. The details of the procedure varied with the behaviour of the individual animal. Exactly 10 minutes after addition of the fish to the bowl, a water sample (10 ml) was drawn; further water samples were taken at 60 minute intervals for 8 hours to measure the decline of Na^{22} and the change in total sodium concentration of the external medium. Water radioactivity decreased approximately 85% during the experimental period. Observations of the fish's behaviour were made twice an hour as described above. After 8 hours, the trout was quickly removed from the chamber, stunned by a blow on the head, rinsed with fresh water, and dried with paper towels. A blood sample (500 ul) was drawn by cardiac puncture into a heparinized syringe and centrifuged immediately; tissue sampling, weighing, and measuring procedures were performed as in Section I.

(ii) Urinary Blockage Trout. After the weight determinations, a stainless steel pin was inserted into the stump of the urinary cannula, and the fish returned to a recovery aquarium. Exactly one hour later, the trout was transferred to the swimming chamber and the experimental protocol followed as for the normal group. At the end of the experiment, the fish was again weighed twice before blood and tissue sampling.

Patency of the urinary cannula was checked by injection of dyed saline. Of the 17 fish subjected to urinary blockage, hydrostatic pressure resulting from the build-up of urine had caused rupture of the urogenital papilla in 8. All data from these trout were discarded.

(iii) Sham Trout. These animals were subjected to the same experimental regime as the urinary blockage group. However, the procedure of plugging the urinary cannula was merely mimicked through several firm tugs on the tubing; the catheter was checked post-experimentally to ensure that it had remained free-draining.

4. Analytical Procedures

Determinations of terminal hematocrit, plasma and tissue water, plasma and water sodium, and water radioactivity were performed as in Section I. All flame emission analyses were done on the Techtron Model AA 120 Atomic Absorption Spectrophotometer (see Section I). Na^{22} in terminal plasma was assayed on duplicate 25 uL aliquots which had been diluted to 1 ml with water and dried under a heat lamp.

5. Calculations

(a) Sodium Flux Rates

The method described in Section I for calculation of flux rates is complicated in long term experiments by the return of the labelled isotope Na^{22} from the fish to the water. In the present study, the internal specific activity of sodium approached 35% of the external specific activity after 8 hours. Computation of influx rate without compensation for backflux of the radioisotope would yield a value inferior to the true

influx rate. Thus it was necessary to utilize the equation of Maetz (1956) to determine ion influx rates in fresh water during long term experiments. The following description of the theoretical derivation of this equation has been modified from Maetz (1956). The analysis is based on the assumption that the fish (internal) and the water (external) represent a two compartment system between which all ion movements occur.

Symbols:

Na ext : Total quantity of sodium(ug) contained in the external water at a particular time t.

Na int : Quantity of exchangeable sodium(ug) contained in the fish at t.

Qo : Total quantity of Na^{22} (c.p.m.) added to the external water at time zero.

Q : Total quantity of Na^{22} (c.p.m.) remaining in the external water at t.

Qf : Total quantity of Na^{22} (c.p.m.) remaining in the external water at the end of the experiment (8 hrs).

Δt : Time period over which the influx rate is measured (60 minutes).

Na^{22}/ml pf: Concentration of Na^{22} in a terminal plasma sample (c.p.m./ml).

Na/ml pf : Concentration of sodium in a terminal plasma sample (ug/ml).

Fi : Na influx rate (ug/minute).

Fo : Na efflux rate (ug/minute).

Fn : Na net flux rate (ug/minute).

At time t the specific activity of sodium in the external compartment is $\frac{Q}{Na\ ext}$, while that of the internal compartment is $\frac{Qo - Q}{Na\ int}$. Thus over Δt, the amount of Na^{22} entering the internal compartment may be expressed:

$$Fi \times \frac{Q}{Na\ ext} \times \Delta t \qquad (1)$$

Conversely, the amount of Na^{22} leaving the internal compartment is:

$$Fo \times \frac{(Qo - Q)}{Na\ int} \times \Delta t \qquad (2)$$

Thus the change in total external Na^{22} (ΔQ) over Δt is given by the difference between expressions (1) and (2):

$$\Delta Q = \left[\frac{-Q}{Na\ ext} \times Fi \times \Delta t \right] + \left[\frac{(Qo - Q)}{Na\ int} \times Fo \times \Delta t \right] \qquad (3)$$

In fresh water teleosts, Fi rarely equals Fo, and thus Fn does not usually equal zero over the time interval of an experiment. Consequently, Na ext varies with time in a non-linear fashion, preventing integration of equation (3). It is therefore necessary to calculate fluxes by numerical resolution over each time interval. The net flux is considered positive when the amount of sodium in the external compartment diminishes:

$$Fn = -\frac{\Delta Na\ ext}{\Delta t} \qquad (4)$$

and represents the difference between Fi and Fo

$$Fn = Fi - Fo \qquad (5)$$

Solution of equation (4) and (5) for Fo and substitution of this term in (3) produces the following equation:

$$\Delta Q = \Delta t \left[\frac{-Q}{Na\ ext} \times Fi + \frac{Qo - Q}{Na\ int} \times \left(Fi + \Delta \frac{Na\ ext}{\Delta t}\right) \right] \qquad (6)$$

and by solution for Fi:

$$Fi = \frac{\frac{\Delta Q}{\Delta t} - \left[\frac{Qo - Q}{Na\ int}\right]\left[\frac{\Delta Na\ ext}{\Delta t}\right]}{\frac{Qo - Q}{Na\ int} - \frac{Q}{Na\ ext}} \qquad (7)$$

Fo may then be deduced from equation (5). It is interesting to note that during a short term experiment, the quantity (Qo - Q) is negligible, and equation simplifies to:

$$Fi = - \frac{\frac{\Delta Q}{\Delta t}}{\frac{Q}{Na\ ext}} \qquad (8)$$

which is the relationship used to calculate Fi in Section I. All terms in equation (7) with the exception of Na int, are readily extractable from data on changes in sodium and Na^{22} concentrations in the water. As Fn varies during the experiment, Na int must vary to mirror changes in Na ext. However, since Na int is large relative to Na ext, these slight deviations may be ignored, and Na int may be approximated from the terminal plasma sample:

$$Na\ int = \frac{Qo - Qf}{\frac{Na^{22}/ml\ pf}{Na/ml\ pf}} \qquad (9)$$

(b) $\underline{Na^{22}\ Space}$

The distribution volume of radiosodium (Vint) was calculated as in Section I from the total mount of Na^{22} in the fish after 8 hours and the radioactivity of the terminal plasma sample:

$$Vint = \frac{Qo - Qf}{Na^{22}/ml\ pf} \qquad (10)$$

RESULTS AND DISCUSSION II

A. Sodium Flux Rates during Extended Exercise

The results of this section were obtained from a completely different set of rainbows from those used in Section I; their physical dimensions are presented in Table IX. The animals were generally smaller and thinner (as manifested by the low coefficients of condition) than the first group (cf. Table II) and many were in prime breeding condition. These batch differences probably accounted for the slightly higher sodium influx rates and plasma concentrations observed in the present study. However Table IX demonstrates that body weights and coefficients of condition did not vary significantly among the three experimental groups of this section, thereby again allowing comparison of flux rates between treatments on a per 100 g body weight basis.

It was the object of this investigation to measure on a temporal basis any progressive changes which occurred in flux components in response to long term swimming. Interpretation of the results was however complicated by several factors. Flux rate values from a typical experiment have been graphically presented in Fig. 17 to illustrate the difficulties encountered in evaluation of data and the method of analysis applied. Individual trout exhibited variable behaviour in the exercise chamber, swimming for periods ranging from less than one hour to the entire eight hours of the experiment. Classification of a particular hourly flux rate value was therefore dependent not only on whether the animal was

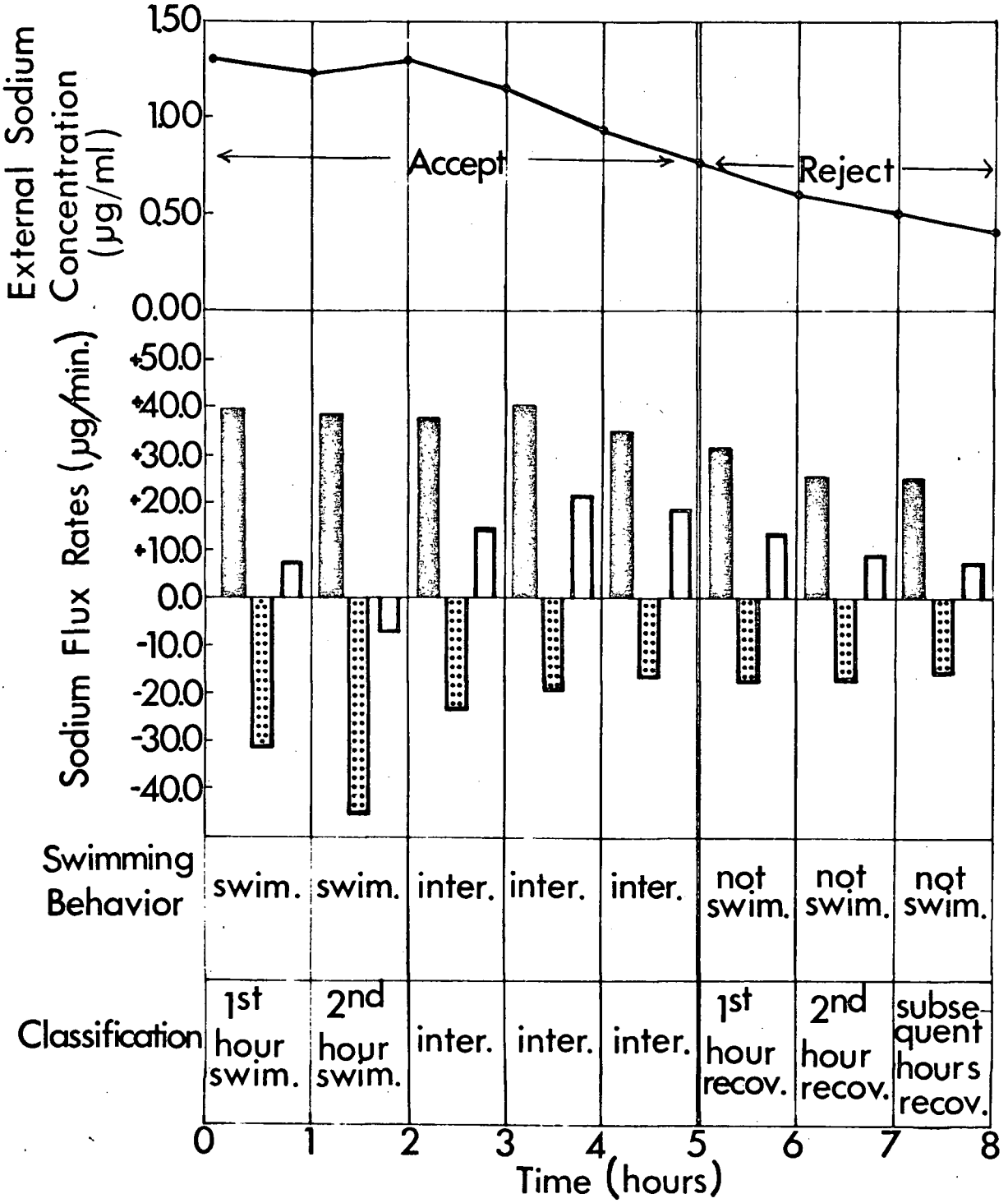
Table IX. Physical dimensions of trout in the three treatment groups of Section II. Means ± 1 standard error

	Normal N = 10	Sham N = 10	Urinary Blockage N = 9
Body weight (g)	186.92 ± 5.42	190.60 ± 10.19	180.09 ± 6.95
Length (cm)	28.84 ± 0.40	28.73 ± 0.57	28.42 ± 0.35
Fork length (cm)	27.70 ± 0.39	27.38 ± 0.49	27.26 ± 0.33
Max.depth (cm)	5.50 ± .08	5.52 ± .08	5.65 ± .11
Coefficient of condition $\frac{\text{weight} \times 100}{(\text{fork length})^3}$	0.882 ± .026	0.928 ± .036	0.889 ± .036

No significant differences between corresponding values in different groups

Figure 17 Results of a typical experiment of Section II illustrating the method of data analysis applied. Black bars = sodium influx rates; dotted bars = sodium efflux rates; clear bars = sodium net flux rates. Below approximately 0.8 ug Na^+/ml, influx rate became dependent on external sodium concentration.

Fish 45 – Sham ,180.6 g.

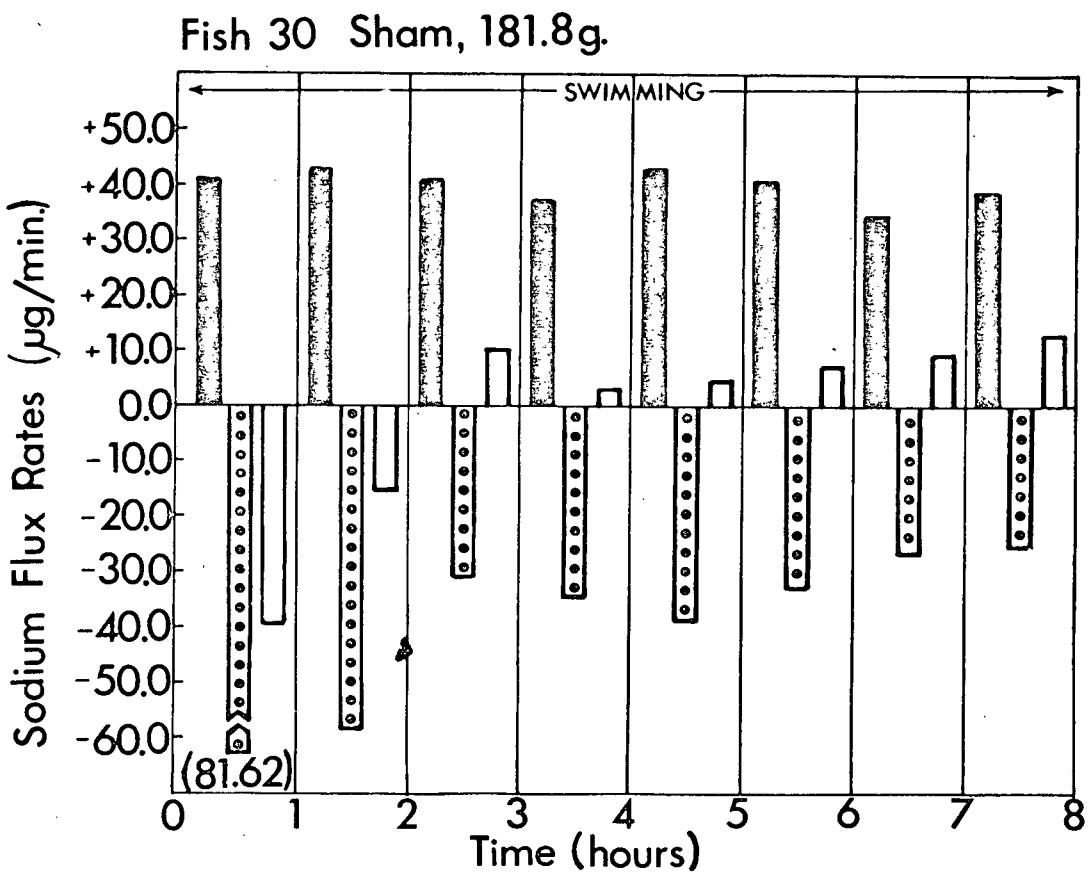


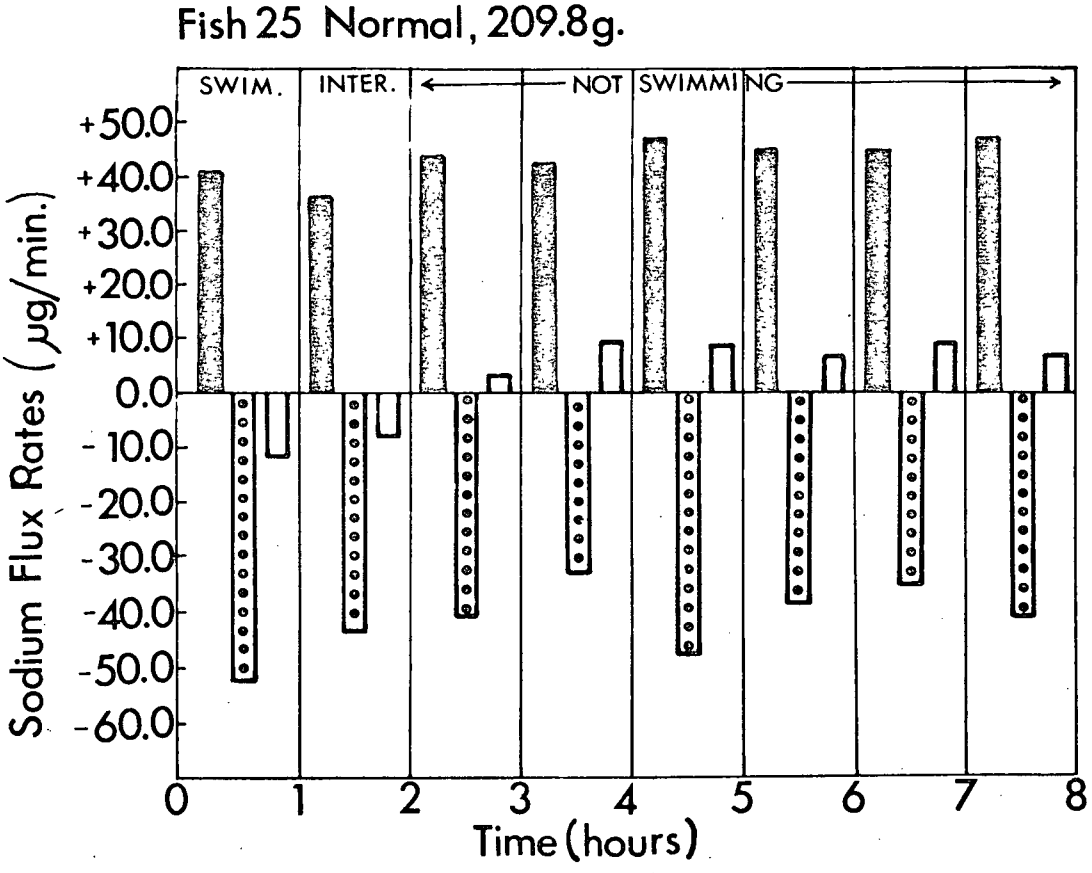
swimming or not, but on the length of time prior to the measurement period during which the animal had been active or resting. Inspection of data from experiments in which the trout had swum for most or all of the eight hours revealed no marked variation in sodium movements after the first three hours of exercise. Similarly, flux rates of fish which spent most of their time at rest had stabilized by three hours after the cessation of swimming. Fig. 18 presents characteristic examples of these cases. Consequently, hourly determinations were divided into the following self-explanatory categories: first hour swimming, second hour swimming, subsequent hours swimming, intermittent, first hour recovery, second hour recovery, and subsequent hours recovery. The classification procedure is illustrated by the experiment outlined in Fig. 17. All the values of a flux component for a particular category from every animal in the treatment group were then averaged. Each hourly measurement was therefore weighted equally in the mean although some fish obviously contributed more values to a particular category than did others. A further problem in data analysis evolved from the large diminutions in external sodium concentration caused by the highly positive net flux rates of some trout; the effect was especially prevalent in the urinary blockage group which suffered no renal efflux. In all groups, there occurred a marked depression of branchial influx rate at low sodium levels of the outside water. Investigation of the interaction between sodium concentration and influx rate revealed that at average external sodium levels greater that 0.8 ug/ml, the slope of the regression line of influx on concentration did not differ significantly from

Figure 18 Results of two experiments of Section II. Black bars = sodium influx rates; dotted bars = sodium efflux rates; clear bars = sodium net flux rates.

Upper: Example of an animal which swam continuously for 8 hours. Sodium flux rates had stabilized by the third hour of exercise.

Lower: Example of an animal which was inactive for most of the experimental period. Sodium flux rates had stabilized by the third hour after termination of exercise.


Fish 30 Sham, 181.8g.
SWIMMING
+50.0
+40.0
+30.0
+20.0
+10.0
0.0
-10.0
-20.0
-30.0
-40.0
-50.0
-60.0
(81.62)
Sodium Flux Rates (µg/min.)
0
1
2
3
4
5
6
7
8
Time (hours)


Fish 25 Normal, 209.8g.
SWIM.
INTER.
NOT SWIMMING
+50.0
+40.0
+30.0
+20.0
+10.0
0.0
-10.0
-20.0
-30.0
-40.0
-50.0
-60.0
Sodium Flux Rates (µg/min.)
0
1
2
3
4
5
6
7
8
Time (hours)

zero, whereas below this figure, there existed a definite positive correlation. In Part B of this section, it has been shown that the data agrees well with a typical Michaelis-Menten curve (cf. Fig. 20) which tends to approach the zero-order relationship after 0.8 ug/ml. Comparison of influx rate means between and within groups was therefore valid only if all the components of the averages had been determined over that part of the curve where "substrate" concentration had little effect on "velocity". A similar argument must apply to net flux rates, and to efflux rates if the latter are directly (exchange diffusion) or indirectly (back-transport) linked to influx rates. Thus it was necessary to discard from the means all flux measurements taken at average external sodium concentrations less than 0.8 ug/ml. (Fig. 17). Fig. 19 displays the results of the above analysis for the three treatment groups.

Sodium efflux rates (Fig. 19) were slightly higher in the shams and thus net movements marginally less positive relative to the normals, but the differences were generally not large enough to obtain statistical validation. These two sets of trout demonstrated extremely similar trends of flux rate variation in response to the state of exercise. Disregarding for the present the data from the urinary blockage fish, a number of conclusions may be drawn from the sodium exchanges of these two groups. Firstly, branchial sodium influx rate exhibited remarkable stability over the experimental period. The only statistically significant divergence in this parameter occurred between 1st hour swimming and 2nd hour recovery values in the normals, and between the

Figure 19 Sodium flux rates, measured at external concentrations greater than 0.8 ug/ml, under different exercise conditions in normal (10), sham (10), and urinary blockage (9) trout. Black bars = sodium influx rates; dotted bars = sodium efflux rates; clear bars = sodium net flux rates. Vertical lines represent 1 standard error of each mean. Each average includes all acceptable data in the category; thus it was possible for a single fish to contribute more than one value to the mean in classifications 3, 4, and 7.

Statistical Comparisons: (Numbers refer to means of exercise conditions as labelled at the head of the graph.)

Normal: Influx Rate. $F = 1.59$, n.s.

6 5 7 4 3 2 1

Efflux Rate. $F = 13.90$, $p < .005$

6 7 5 3 4 2 1

Net Flux Rate. $F = 4.59$, $p < .005$

1 2 4 5 3 7 6

Sham: Influx Rate. $F = 1.61$, n.s.

4 5 6 2 3 1 7

Efflux Rate. $F = 5.63$, $p < .005$

6 4 5 3 7 2 1

Net Flux Rate. $F = 6.62$, $p < .005$

1 2 4 3 5 7 6

Urinary Blockage: Influx Rate. $F = 1.94$, n.s.

7 6 5 4 2 1

Efflux Rate. $F = 0.20$, n.s.

5 2 7 1 6 4

Net Flux Rate. $F = 1.52$, n.s.

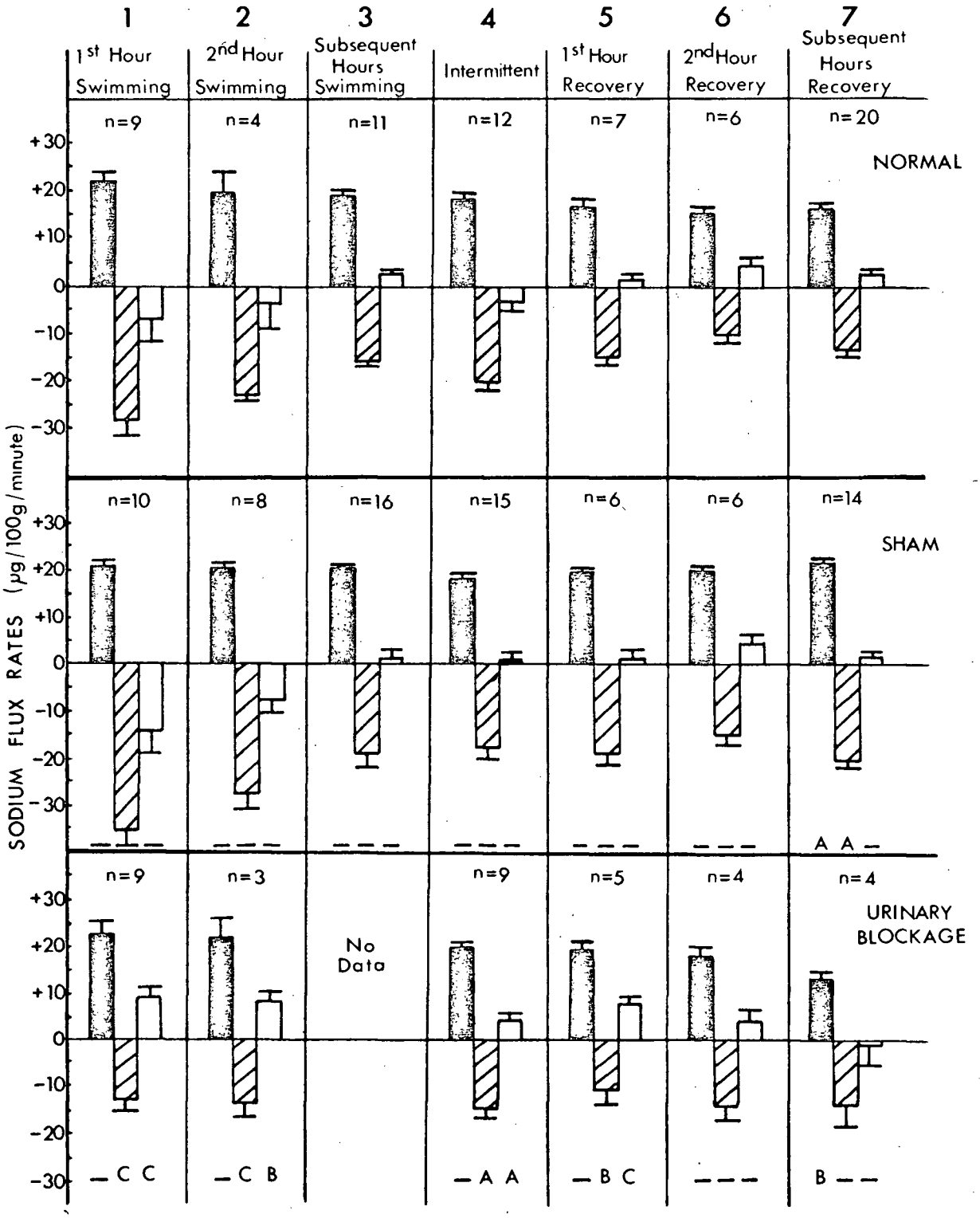
7 6 4 5 2 1

A = significantly different from corresponding normal value ($p < .05$)

B = significantly different from corresponding sham value ($p < .05$)

C = significantly different from both normal and sham values ($p < .05$)

— = not significantly different from other corresponding values ($p > .05$)


1
1st Hour Swimming
2
2nd Hour Swimming
3
Subsequent Hours Swimming
4
Intermittent
5
1st Hour Recovery
6
2nd Hour Recovery
7
Subsequent Hours Recovery
n=9
n=4
n=11
n=12
n=7
n=6
n=20
NORMAL
n=10
n=8
n=16
n=15
n=6
n=6
n=14
SHAM
A A
n=9
n=3
No Data
n=9
n=5
n=4
n=4
URINARY BLOCKAGE
C C
C B
A A
B C
B
+30
+20
+10
0
-10
-20
-30
SODIUM FLUX RATES (µg/100g/minute)

intermittent and subsequent hours recovery figures of the shams. As these differences attained only borderline statistical confirmation, were not consistent between the two treatments, and were not concordant with any general trends in the data, it seems doubtful that they represented any meaningful variation. An augmentation of spurious statistical effects is unfortunately concomitant with the unequal contribution of data from single sources to the mean. The insensitivity of branchial sodium influx rate to factors associated with short term exercise and recovery (one hour) was established in Section I. This conclusion may now be extended to prolonged swimming, at least at low velocity, and the subsequent recovery period following such exercise.

Sodium efflux rate (Fig. 19) was, however, vulnerable to the influence of the prevailing exercise condition. Unidirectional outward movements were greatest during the first hour of swimming but declined significantly to post-exercise levels by the third and subsequent hours of activity. Variations in net flux rates followed a similar pattern. The hypothesis which these experiments were designed to test, namely that the trout is able to overcome the net sodium deficit associated with short periods of swimming through the implementation of compensatory mechanisms during prolonged exercise, was therefore confirmed. This adaptation apparently occurred entirely through reduction of the efflux rate, the same factor whose elevation was originally responsible for the negative balance situation over the initial exercise interval. The sodium exchange data of trout #30 (Fig. 18) which swam continuously throughout the 8 hours, provide excellent illustration

of this phenomenon; sodium efflux was extremely high during the first hour of measurement producing a pronounced net loss of the electrolyte to environment. This deficit persisted over the second hour despite a large reduction in the outward moving component. By the third hour, fish #30 had returned to a positive balance situation which was maintained throughout the following five hours of exercise.

It may be noted in Fig. 19 that at least for the normal group, the efflux rate of the intermittent category was greater than that of subsequent hours swimming, a difference which was reflected by the return of the next flux to a negative value. This effect was probably explainable by the behaviour of trout in the intermittent category, which consisted of short rapid bursts of swimming into the current followed by intervals of rest. Such activity produces large increases in oxygen uptake (Brett,1964), which, combined with possible disruption of the compensatory phenomenon through the irregular nature of the activity, could have been responsible for an increase in the efflux element. Both groups exhibited a minimum efflux value during the second hour of recovery (Fig. 19) accompanied by a maximum positive net flux. Subsequent hours of recovery were characterized by a return to a slightly greater efflux associated with a somewhat lower net sodium uptake. A similar increase in net flux through reduction of the branchial efflux parameter during recovery was observed in the experiments of Section I.

It must be emphasized that the sodium efflux rates discussed above were whole organism measurements for the normals and shams and thus represented the sum of branchial and renal

contributions. The experiments with urinary occluded rainbows were performed in order to ascertain the role of each constituent in the observed responses. Rejection of many flux rate determinations from the ligated fish was unfortunately necessitated by the 0.8 ug/ml external sodium level criterion. The acceptable data, with one exception, however, was well distributed between different experiments and exhibited only moderate variability. On this basis, one may have some confidence in the representative nature of the means calculated from the remaining measurements (Fig. 19).

Branchial influx rates of ligated trout showed no significant variation with respect to either different exercise conditions within the treatment group, or comparable means of the other two sets of fish, with the exception of the subsequent hours recovery value. This lone anomaly, which was also true of net flux rate, can be ascribed to the fact that three of the four values in the average came from a single animal which demonstrated characteristically low influx rates throughout the experiment. Thus the inward movement of sodium across the gill epithelium of urinary blockage animals showed similar magnitude and stability to that of the unligated fish.

Sodium efflux rates of the occluded trout were in most cases significantly lower than the corresponding values for either or both of the other two treatments, but remained extremely constant under different activity conditions. If the assumption is taken that branchial sodium efflux, like influx, was unaffected by urinary occlusion, then the observed changes in whole body unidirectional losses during prolonged

swimming must be attributed to variation in the renal efflux component alone. On this basis, the urinary excretion of sodium under different conditions could be approximated by subtraction of urinary blockage sodium efflux means from sham efflux means. Table X presents the results of this calculation. These estimates indicate that the renal discharge of sodium was high during the first hour of exercise but had decreased to recovery levels by the third and subsequent hours of swimming.

These conclusions seriously disagree with the results of Section I which demonstrated a very significant augmentation of the rate of sodium efflux across the gills during exercise. This discrepancy could be explained if the branchial sodium effluxes of urinary occluded rainbows were not representative of rates in the normals and shams; i.e. if the efflux variations of these two groups did in fact include a significant branchial component which was not evident in the ligated individuals. An assessment of the validity of the calculated renal efflux rates (Table X) provides cogent support for this hypothesis. In Section III, urinary sodium outputs were measured directly through collection and analysis of the urine of rainbows during rest, controlled swimming at various speeds, and recovery. There actually did occur an increased sodium loss through the kidney associated with diuresis during swimming, but the magnitude of this discharge was extremely low relative to the estimate of renal efflux calculated in the present section. Table XI compares the maximum renal discharge of the electrolyte observed in Section III with the urinary release calculated by subtraction of the sodium efflux rate of the

Table X. Urinary sodium efflux rates of shams under different exercise conditions estimated by subtraction of the mean branchial efflux rate of the urinary blockage group from the mean whole animal efflux rate of the shams.

Exercise condition	Calculated urinary sodium efflux rate (ug/100 g/minute)
1st hour swimming	21.72
2nd hour swimming	14.88
subsequent hours swimming*	5.82
intermittent	3.39
1st hour recovery	7.53
2nd hour recovery	1.25
subsequent hours recovery	6.86

* Computed assuming an efflux rate for subsequent hours swimming in urinary blockage fish equal to 13.81 ug/100 g/minute, the average of 2nd hour swimming and intermittent values.

Table XI. Comparison of calculated renal sodium efflux rates during swimming in shams with reported direct measurements of maximum urinary sodium discharge in Salmo gairdneri.

Urinary sodium efflux rate. (ug/100 g/minute)	Conditions	Method	Reference
6.86	control (subsequent hours swimming)	calculation	present study Section II
21.72	*1st hour swimming up to 32 cm/sec.	"	"
14.88	*2nd hour swimming up to 32 cm/sec.	"	"
1.95	control	direct measurement	present study Section III
3.51	*1st hour swimming - 21.4 cm/sec.	"	"
2.61	*1st hour swimming - 32.1 cm/sec.	"	"
2.83	*1st hour swimming - 42.8 cm/sec.	"	"
1.43	control	direct measurement	Hunn (1969)
5.35	*1 hour after acute hypoxia	"	"
0.30	control	direct measurement	Hunn and Willford (1970)
3.38	*6 hours after MS 222 anaesthesia	"	"
4.71	*2 hours after methyl pentynol anaesthesia	"	"

* Maximum urinary sodium loss under different experimental treatments.

urinary blockage fish from the corresponding sham value. A number of other direct measurements of the maximum renal sodium loss of Salmo gairdneri in response to various stresses has been included from the work of Hunn (Hunn,1969; Hunn and Willford,1970). These data indicate that rather than comprising over half the whole organism efflux rate during the first and second hours of exercise, as the calculations suggest, urinary sodium loss can probably account for no more than about 15% of the unidirectional outward movement. This evidence, although not conclusive, strongly suggests that branchial sodium efflux rates of urinary blockage fish were abnormally low; a possible cause for this irregularity will be advanced presently. Consequently, in non-occluded animals, most of the variations in efflux rate associated with swimming probably occurred at the gills. The branchial efflux rate of the shams would therefore have been approximately 30 ug/100 g/min. during the first hour of swimming, a figure nearly identical to that observed during one hour of chasing in Section I. In addition, the unknown compensatory mechanism responsible for the reduction of efflux during prolonged activity would have been largely active on the branchial component of sodium loss.

Data derived from the weighing procedure and analysis of terminal blood and tissue samples has been summarized in Table XII. The radiosodium space of the normal rainbow trout, which at 8 hours after external administration of the radioisotope probably represented an equilibrium value (Mayer and Nibelle,1969) was very similar to the few other measurements reported for "intact" fresh water teleosts (Maetz,1956; Motais, 1967; Lahlou et al.,1969). Both Na^{22} distribution volumes

Table XII. Terminal measurements of internal sodium and water levels, radiosodium spaces, hematocrits, and weight changes over the experimental period. Means ± 1 standard error.

	Normal	Sham	Urinary blockage
	N = 10	N = 10	N = 9
Plasma sodium concentration (mEq/L)	144.75 ± 5.23	132.62 ± 10.05 p_1 = n.s.	106.69 ± 3.87 $p_1 < .001$ $p_2 < .05$
Na^{22} space (ml/100 g)	33.82 ± 1.83	29.86 ± 1.43 p_1 = n.s.	34.39 ± 0.78 p_1 = n.s. $p_2 < .02$
Total exchangeable internal sodium (ug/100 g)	111,426.00 ± 5,867.11	89,957.50 ± 6,629.68 $p_1 < .05$	84,399.90 ± 3,808.42 $p_1 < .01$ p_2 = n.s.
Plasma water concentration (ml/100g plasma)	96.19 ± 0.26	95.90 ± 0.27 p_1 = n.s.	96.38 ± 0.31 p_1 = n.s. p_2 = n.s.
Hematocrit (%)	28.05 ± 3.17	33.75 ± 2.56 p_1 = n.s.	28.49 ± 3.86 p_1 = n.s. p_2 = n.s.
Weight change (g/100g initial weight)	-	- 2.57 ± 1.05 p_1 = -	+8.57 ± 0.93 p_1 = - $p_2 < .001$
Tissue water concentration (ml/100g/tissue)	81.047 ± 0.530	81.851 ± 0.570 p_1 = n.s.	83.899 ± 0.257 $p_1 < .001$ $p_2 < .01$

p_1 equals significance with respect to corresponding normal value

p_2 equals significance with respect to corresponding sham value

and plasma sodium levels were lower in the shams than the normals, although neither difference was significant by itself. The effective products of these parameters, total exchangeable internal sodium, did however show a statistically confirmed decrease. Disturbances in electrolyte balance for up to 72 hours after MS 222 anaesthesia and operative procedures may be associated with an increase in effective branchial permeability accompanying elevation of respiratory exchange during the recovery period (Houston et al.,1969). As discussed in Section I, a greater leakage of sodium out across the gill could result from this permeability increase. The trout bladder is contractile in nature (Lederis,1970) and probably releases urine intermittently. Recent unpublished studies (Hirano, personal communication to D.J.Randall) have demonstrated a sodium transport capacity in the isolated bladder wall of Salmo gairdneri, although its function in the intact animal is not yet understood. It is therefore possible that a secondary sodium conservation mechanism in series with the tubular reabsorptive activity normally increases the efficiency of sodium retention. The urinary catheter would drain the bladder of urine as soon as it was produced and thus negate this reabsorptive function, thereby further augmenting the sodium deficit of the cannulated trout. The slightly higher whole body efflux rates recorded under most exercise conditions in the shams (Fig. 19) may therefore have reflected elevation of both branchial and renal losses. However as noted earlier, the presence of the urinary cannula did not interfere with the pattern of efflux changes exhibited during extended exercise.

A very significant weight gain amounting to a net water

entry of about 1% body volume per hour occurred in the urinary blockage animals (Table XII). (Shams underwent a 2.5 g/100 g weight reduction over the experimental period; a smaller but significant weight loss associated with extended swimming was observed under more carefully controlled conditions in Section III). On autopsy, the ligated fish exhibited some distension of the urogenital papillae although the volume contained in the bladder itself did not appear to be large. It seems probable that the accumulation of urine in this organ continued only until its hydrostatic pressure, referred back to the kidney tubules, opposed the glomerular filtration pressure. This effect may have been accelerated by the known contractility of the bladder (Lederis,1970). Thus most of the influxed water was probably not removed from the blood by the kidney. Plasma water concentration was however only marginally (and non-significantly) elevated in the ligated trout and hematocrit remained similar to that of the other two groups. The implementation of some mechanism to prevent excessive expansion of the intravascular volume was therefore indicated. The extremely significant augmentation of tissue water levels suggested that this compensation involved accumulation of the superfluous water in either or both the intracellular and extracellular phases of the extravascular space. Plasma sodium levels were drastically depressed despite the apparent stability of plasma volume and total exchangeable internal sodium. It would therefore appear that the removal of excess water from the vascular compartment was accompanied by a redistribution of body sodium which was reflected in a significant expansion of the radiosodium space relative to the shams. As sodium appears to

be effectively excluded from the intracellular space in salmonids (Houston,1964; Toews,1969) a net movement of this ion into the interstitial fluids to help maintain the constancy of the immediately extracellular milieu may well have occurred at the expense of plasma homeostasis.

This profound drop in plasma sodium levels could have been responsible for the depression of branchial efflux rate earlier postulated to have occurred in the urinary blockage trout. Infusion of hypotonic saline stimulated the net uptake of sodium across the gills of the goldfish, Carassius auratus, (Bourguet et al.,1964) and the eel, Anguilla anguilla, (Mayer and Nibelle,1970), while administration of a hypertonic solution reversed this effect. The former response occurred through the elevation of the influx component alone, while the latter involved both inhibition of influx and augmentation of efflux. At least in the goldfish, the responses were directly mediated by the changes in plasma sodium levels resulting from the injections. The ammocoete, Lampetra planeri, increased net uptake in response to experimental sodium depletion through both elevation of influx and depression of efflux parameters (Morris and Bull,1970). The net sodium flux across isolated hemibranchs of Salmo gairdneri was inversely proportional to the sodium concentration of the perfusion fluid (Richards and Fromm,1970). At internal sodium concentrations inferior to normal plasma sodium levels there occurred a net inward movement of the electrolyte, while the opposite occurred at high sodium concentrations of the perfusion solution. This investigation did not, however, separate the relative contributions of the two unidirectional flux components to the observed

effects. The data of the present study indicate that branchial sodium influx of rainbow trout is remarkably stable *in vivo*; the same is not, however, true of the efflux parameter. Influx was apparently unaffected by the plasma sodium depletion associated with renal blockage (Fig. 19). Thus in this species, modification of net sodium transport across the gills may normally occur through alterations in the outward moving component alone. Indeed some decrement in passive efflux would be expected simply from the decline in the plasma to water sodium concentration gradient caused by reductions in plasma sodium levels, and could be reinforced by an induced decrease of branchial permeability. The existence of such a mechanism would explain the depression of branchial efflux proposed to accompany urinary ligation.

Hence urinary blockage would appear to be an extremely unsatisfactory technique in light of the massive disturbance in internal hydroelectrolyte homeostasis associated with the procedure and its likely consequences with respect to sodium exchanges. Conclusions about sodium flux behaviour based on its use (e.g. Holmes, 1959) must be gravely suspect. The occlusion experiments were therefore uninformative in discriminating between branchial and renal contributions to the sodium efflux changes measured during prolonged exercise. Evidence from other sources, however, indicated that the majority of efflux rate variation, including the compensatory reduction in sodium loss during extended swimming, involved branchial function. The possible nature and mechanism of this adjustive phenomenon will be considered in the General Discussion.

B. The Concentration Dependence of Branchial Sodium Fluxes

As each experiment in this section entailed eight hourly flux rate measurements, over 230 individual values for each unidirectional sodium movement were obtained from 29 trout. The large external concentration changes effected over the experimental period by the net fluxes of the animals produced measurements at a wide variety of water sodium levels. The results presented in Part A of Section II indicated that branchial sodium influx was insensitive to the exercise condition or experimental treatment of the trout (Fig. 19). It therefore seemed valid to analyze the interaction between external sodium level and branchial influx rate using all available paired measurements (231) of these parameters. Consequently all hourly values of sodium influx rate were assigned to categories on the basis of the average ambient water sodium level over the particular period. The mean influx value for each 0.2 ug/ml range, plotted at the midpoints of the intervals, are presented in Fig. 20. As noted earlier, means at sodium levels below 0.8 ug/ml demonstrated an obvious positive correlation with external concentrations; this was not true at sodium levels greater than this figure. The form of the relationship over the whole concentration range was similar to that observed for a variety of sodium-transporting systems - e.g. isolated frog skin (Ussing,1949b;Kirschner,1955); isolated toad bladder (Frazier et al.,1962); whole crayfish (Shaw,1959); whole gammarid crustaceans (Sutcliffe and Shaw,1968); whole ammocoetes (Morris and Bull,1970); and the perfused gills of anaesthetized rainbow trout (Kerstetter et al.,1970).

Figure 20 The relationship between branchial sodium influx rate and external sodium concentration. All data from normal, sham, and urinary blockage treatment groups has been averaged over 0.2 ug/ml sodium concentration intervals. Means $\pm$ 1 standard error are plotted at the midpoints of each interval. The line fitted to the points was generated by the Kirschner (1955) equation:

$$Fi = \frac{Fi(max) \cdot [Na]o}{Ks + [Na]o}$$

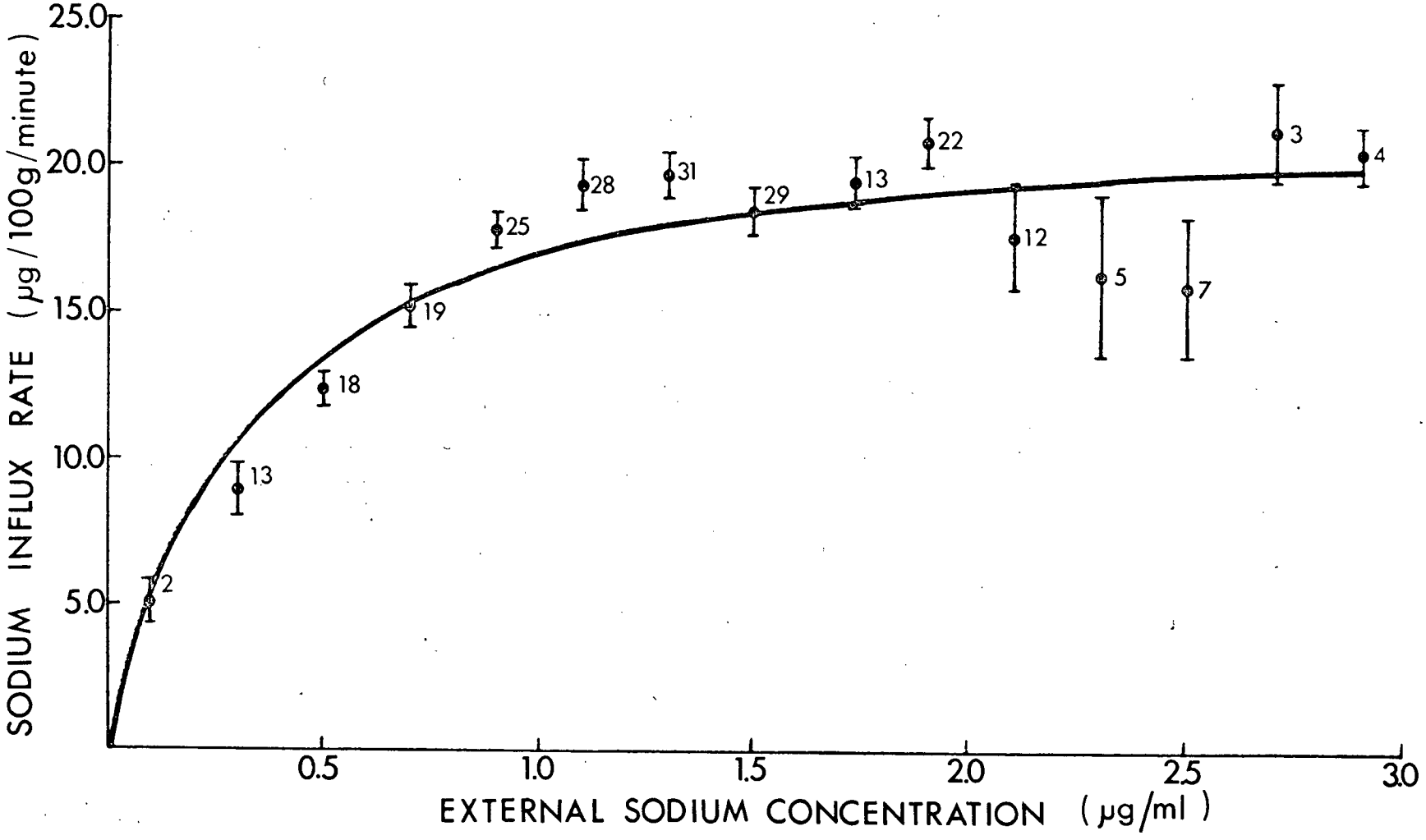
where $[Na]o$ = external sodium concentration

Fi = sodium influx rate

$Fi(max)$ = maximum sodium influx rate

Ks = half-saturation constant

Values for Fi(max) and Ks were obtained from a Lineweaver-Burke plot of the data.


SODIUM INFLUX RATE (µg/100g/minute)
25.0
20.0
15.0
10.0
5.0
0.5
1.0
1.5
2.0
2.5
3.0
EXTERNAL SODIUM CONCENTRATION (µg/ml)
2
13
18
19
25
28
31
29
13
22
12
5
7
3
4

Kirschner (1955), assuming the implication of a specific carrier with which sodium complexed in the transport process, developed a theoretical relationship describing the rate limited nature of influx through the frog skin. When the concentration gradient between internal and external media is large, the simple diffusion constituent of the equation may be ignored and the relationship becomes

$$Fi = \frac{Fi(max) \cdot [Na]o}{Ks + [Na]o}$$

where $[Na]o$ = external sodium concentration
Fi = sodium influx rate
$Fi(max)$ = maximum sodium influx rate
Ks = half-saturation constant

which has a form identical to the familiar Michaelis-Menten equation relating enzymatic reaction rate to substrate concentration. A double reciprocal plot (Lineweaver and Burke, 1934) of the present data yielded values for Fi(max) and Ks which were used to generate a curve describing Fi at different $[Na]o$'s. This theoretical curve is the line fitted to the values in Fig.20. The concentration dependence of sodium influx across the gills of intact Salmo gairdneri is well described by the Kirschner equation. Similar accord has been demonstrated in the crayfish, Astacus pallipes, the ammocoete Lampetra planeri, and the gills of anaesthetized rainbow trout (Kerstetter et al.,1970; Kirschner,1970). As Shaw (1959) has pointed out, there is nothing singular about coincidence of the data with the Kirschner relationship, and other expressions may exist which describe the process more accurately (e.g. through also considering potential difference). However, the

correspondence does suggest a saturable rate limited system based on a complexing carrier (Kirschner,1955), and a general similarity to other reported active sodium transport mechanisms.

The Ks and Fi(max) value for Salmo gairdneri derived from the present data (Fig. 20) were 0.32 ug/ml (0.014 mEq/L) and 22.38 ug/100 g/minute (58.40 uEq/100 g/hour) respectively. Table XIII compares these figures with values from an assortment of other systems. The transport system of rainbow trout in this study obviously demonstrated an extremely high sodium affinity relative to that of other animals. However, it must be noted that the Ks value of the present study is 30 times lower than that obtained by Kerstetter et al. (1970) for the same species, with lesser dissimilarities in Fi(max). These discrepancies may have arisen from the extremely different experimental conditions of the respective investigations. The present trout were in a relatively normal state, while the the rainbows of Kerstetter et al. (1970) were in a distinctly unphysiological condition. These workers used MS 222 anaesthetized fish held upside down and perfused at 100 ml/minute through a #15 needle sewn into the buccal cavity. Such a system would have involved only a small portion of the normal branchial area and was probably inadequate to satisfy the gas exchange requirements of the animal (Davis and Cameron,1970). The latter effect is sufficient to account for the difference in maximum influx rate on a body weight basis, while the general metabolic depression resulting from anaesthesia could well have elevated the observed Ks. If the concentration dependence curve of sodium influx in trout gill is determined in some way by the permeability characteristics of the membranes

Table XIII. Summary of maximum sodium influx rates (Fi(max)) and half saturation concentrations (Ks) for sodium uptake systems in a variety of animals.

Ks (mEq/L	Fi(max) (uEq/100g/hour	Preparation	Reference
.014	58.40	whole intact rainbow trout, *Salmo gairdneri*	present study
0.46	33.30	gills of perfused, anaesthetized rainbow trout, *Salmo gairdneri*	Kerstetter *et al.* (1970)
0.26	36.00*	whole ammocoete, *Lampetra planeri*	Morris and Bull (1970)
0.13	45.00*	sodium depleted whole ammocoete, *Lampetra planeri*	Morris and Bull (1970)
0.25	97	whole crayfish, *Astacus pallipes*	Shaw (1959)
4.3	-	isolated frog skin, *Rana* sp.	Kirschner (1955)
20	-	isolated toad bladder, *Bufo marinus*	Frazier *et al.* (1962)
0.15	~200	amphipod crustacean, *Gammarus pulex*	Sutcliffe and Shaw (1968)
0.5	~1000	amphipod crustacean, *Gammarus duebeni*	Sutcliffe and Shaw (1968)

* corrected from an apparent typographical error in the paper.

through which the ion must pass before binding with the carrier, as apparently occurs in the toad bladder (Frazier et al.,1962), then an increase in diffusion resistance resulting from inefficient gill perfusion could also have raised the Ks.

The water sodium level to which rainbows were acclimated and in which flux rates were measured was determined by the nature of Vancouver tap water, which contains very low levels of this ion (generally less than 2.0 ug/ml or .09 mEq/L; see Holmes and Stainer,1966). The trout of Kerstetter et al. were preadapted to a sodium concentration about 10 times higher than that in the present study. Morris and Bull (1968, 1970) have shown that the Ks of sodium influx in the ammocoete could be markedly reduced by pretreatment with dilute solutions; Fi(max), on the other hand, was elevated by this procedure. Indeed Sutcliffe and Shaw (1968) have demonstrated that races of the amphipod Gammarus duebeni living in streams with low sodium concentrations exhibit Ks values considerably smaller than those of other races of the species perennially inhabiting environments in which the ion is more abundant. Thus the quantitative difference in the results of the present study and that of Kerstetter et al.(1970) on the same species may also reflect adaptational or perhaps even genetic influences on the experimental stocks.

There exist only a few other measurements of the concentration dependence of sodium influx in "intact" fresh water fish, and all were obtained at sodium concentrations of the acclimation and experimental media considerably greater than those used here. However comparison of the trout curve with this limited information (Fig. 21) does emphasize the

Figure 21 Comparison of the concentration dependence of branchial sodium influx in the rainbow trout (1) with that reported in the goldfish (2), the lamprey ammocoete (3), the eel (4), and the killifish (5). Lines have been redrawn from data presented in:

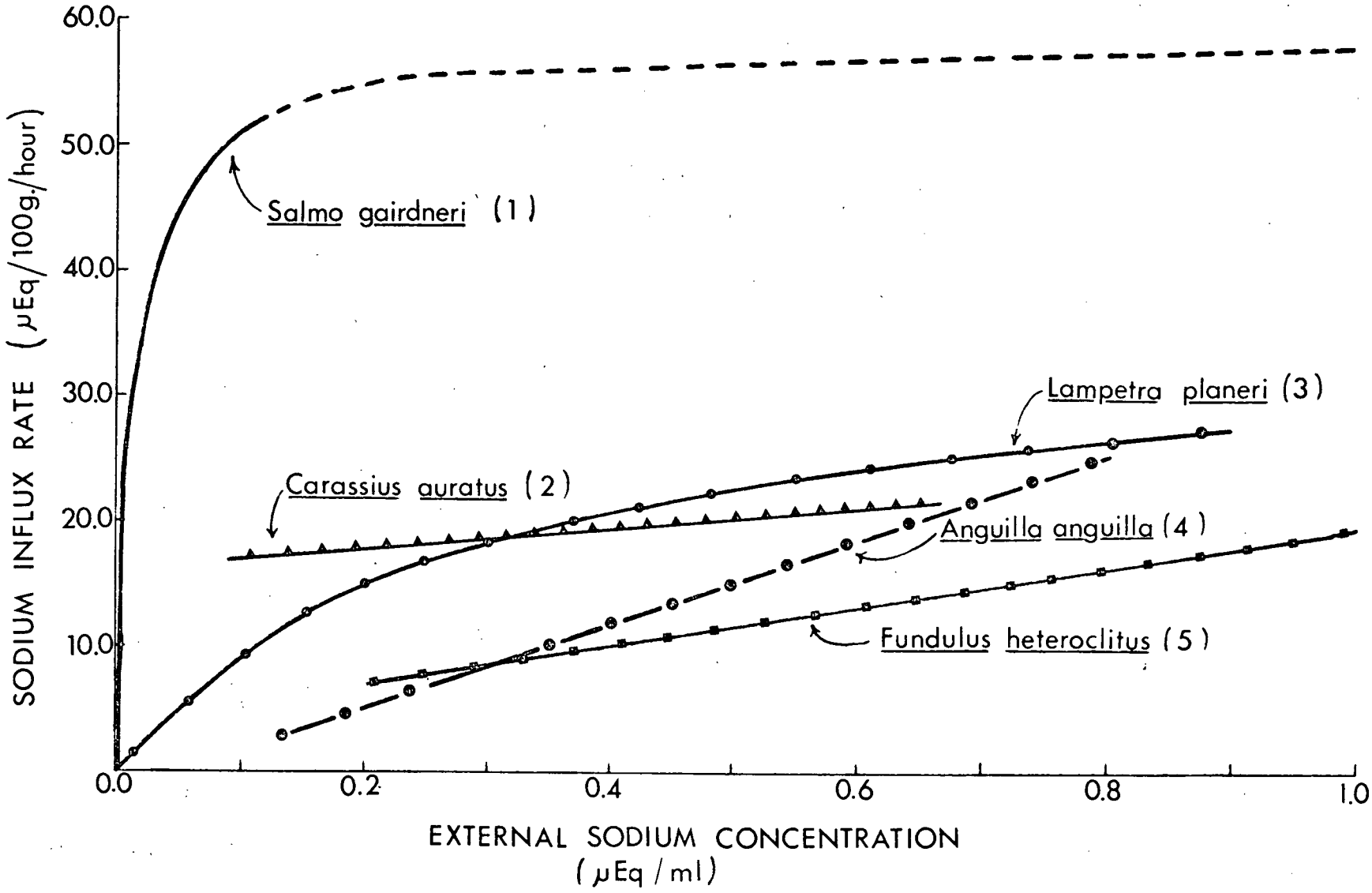
(1) the present study (Fig. 20). This curve has been extrapolated (a broken line) beyond the region of direct determination through use of the Kirschner (1955) equation.

(2) Maetz (1956).

(3) Morris and Bull (1970).

(4) Chester Jones et al. (1969).

(5) Maetz et al. (1967 b).


60.0
50.0
40.0
30.0
20.0
10.0
0.0
0.2
0.4
0.6
0.8
1.0
SODIUM INFLUX RATE (μEq/100g./hour)
EXTERNAL SODIUM CONCENTRATION (μEq / ml)
Salmo gairdneri (1)
Carassius auratus (2)
Lampetra planeri (3)
Anguilla anguilla (4)
Fundulus heteroclitus (5)

extremely high efficiency of this animal's system for sodium uptake from dilute solutions relative to the eel, Anguilla anguilla, the killifish, Fundulus heteroclitus, and the lamprey ammocoete, Lampetra planeri. The data of Maetz (1956) on the goldfish, Carassius auratus, however, show a rate limited trend parallel to that of Salmo gairdneri down to 0.1 uEq/ml, which may be indicative of a similar high affinity transport mechanism.

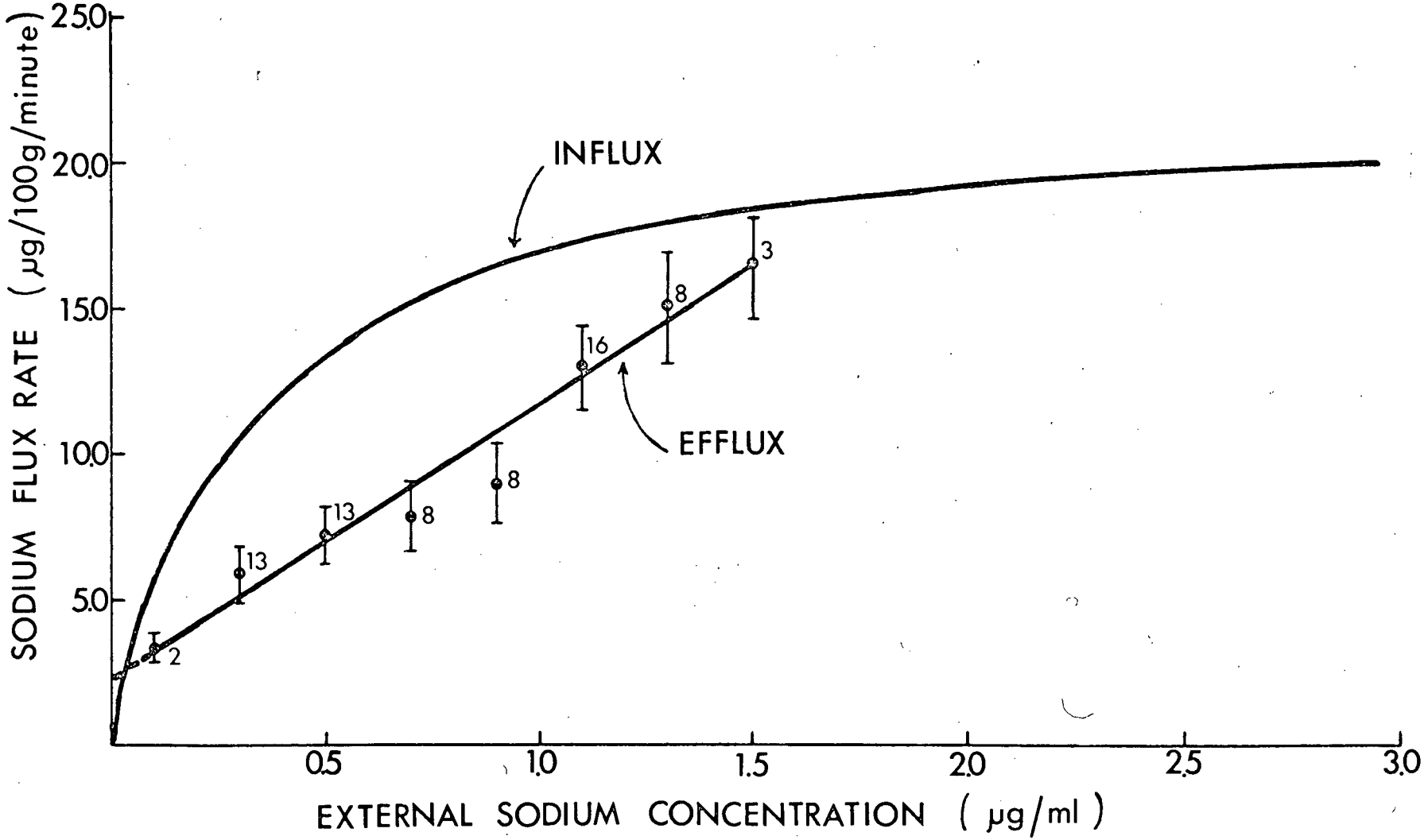
The possible occurrence of a sodium exchange diffusion effect in the gills of rainbow trout was proposed in Section I. Arguments for this phenomenon were based on the extremely high branchial flux rates of trout relative to other fresh water teleosts and the fact that the efflux of sodium across the gills did not apparently increase in proportion to their oxygen permeability. Exchange diffusion of sodium has not previously been demonstrated in the branchial regions of fresh water teleosts, but, a priori, there seems no definite reason why it could not exist. Such a mechanism evidently accounts for 20 - 40% of the normal sodium exchange of the fresh water crayfish, Astacus pallipes, (Shaw, 1959) and of various gammarid amphipods (Sutcliffe and Shaw, 1968). A general criterion for the presence of this process is covariation of unidirectional flux rates with changes in external sodium concentration, or a marked reduction in efflux when influx is abolished.

To further pursue the problem, the efflux rates (70) of the urinary blockage group of trout alone were averaged over 0.2 ug/ml sodium concentration ranges as previously described for influx rates. These efflux values were generated by admittedly abnormal animals but were produced by the

only treatment group in which measured sodium movements were exclusively branchial in origin. As the influx rates of these fish were identical to those of normal rainbows (Fig. 19), it seems unlikely that the exchange diffusion component of efflux, if present, would have been affected by trauma. In addition, the use of these data was advantageous inasmuch as the unidirectional loss of sodium across the gills, perhaps due to the effect of plasma sodium depletion discussed earlier, was apparently insensitive to the exercise condition of the animal. The results are presented in Fig. 22. Over the concentration interval (0.10 - 1.50 ug/ml) for which adequate data were available, there prevailed an approximately linear positive relationship between external concentration and branchial sodium efflux rate which loosely paralleled the concentration dependence of the influx parameters over the same range. This pattern is extremely suggestive of exchange diffusion. Kerstetter et al. (1970) have obtained similar correlation of unidirectional branchial flux rates in their perfused trout preparations. Thus, at least on the qualitative nature of the influx curve and influx-efflux linkage, the results of the present study and that of Kerstetter et al. (1970) are in good agreement.

Assuming for the present that exchange diffusion does contribute to branchial sodium transfer, then the extension of the efflux concentration dependence line (Fig. 22) to zero external sodium should approximate the non-carrier mediated sodium leak across the gills of ligated fish. The extrapolation yielded a value of 2.3 ug/100 g/minute for the simple diffusion component of efflux, which should be stable over the

Figure 22 The relationship between branchial sodium efflux rate and external sodium concentration. All values for urinary blockage animals only have been averaged over 0.2 ug/ml sodium concentration intervals. Means $\pm$ 1 standard error are plotted at the midpoints of each interval. The fitted line has been extrapolated to 0 external sodium level for the purposes of the calculation presented in Table XIV. The concentration dependence curve of sodium influx rate from Fig. 20 is included for comparison.


SODIUM FLUX RATE (µg/100g/minute)
25.0
20.0
15.0
10.0
5.0
INFLUX
EFFLUX
1
2
13
13
8
8
16
8
3
0.5
1.0
1.5
2.0
2.5
3.0
EXTERNAL SODIUM CONCENTRATION (µg/ml)

relatively narrow concentration range of external sodium encountered in the study. Consequently, the contribution of different mechanisms to branchial sodium exchange in the ligated trout could be computed for a water sodium level of 1.5 ug/ml from the data of Figs. 20 and 22. (This concentration has been arbitrarily chosen as it corresponds to the average level used in Section I and represents a point where both influx (Fig. 20) and efflux (Fig. 22) means lie on the fitted lines). The results are presented in Table XIV. It is encouraging to note that these figures are in excellent agreement with those calculated in Section I (pp.68,69) for resting rainbows through a number of assumptions about changing branchial permeability during exercise. The present data therefore lends credence to the idea presented in Section I that the effective permeability of the branchial epithelium to sodium increased about 5 fold in parallel with that for oxygen during the one hour of chasing. However both the original idea and the support presented here are presumptive of exchange diffusion in the sodium transfer system of the rainbow trout.

Kirschner (1970), presumably referring to the work of Kerstetter et al.(1970), has recently claimed that exchange diffusion of sodium does exist in Salmo gairdneri gills. Such a conclusion seems very probable, but premature until the problem of back-transport is solved. The conceivable operation of this mechanism in the branchial epithelium was also discussed in Section I. Morris and Bull (1968, 1970) for the ammocoete, Lampetra planeri, and in a somewhat circuitous fashion, Bryan (1960) for the crayfish, Astacus fluviatilis, have argued that flux linkages similar to those of the

Table XIV. Calculated contribution of different mechanisms to total branchial sodium exchange in urinary blockage trout assuming all influx-efflux linkage to be caused by exchange diffusion. External sodium concentration equals 1.50 ug/ml.

Influx	Exchange diffusion	=	+	14.3	ug/100 g/minute
	Active transport	=	+	4.2	"
	Total influx	=	+	18.5	"
Efflux	Exchange diffusion	=	-	14.3	"
	Simple diffusion	=	-	2.3	"
	Total efflux	=	-	16.6	"
	Net flux	=	+	1.9	"

present study were caused by an unsaturated carrier back-transporting sodium ions leaving by simple diffusion. At high external sodium levels, more of the carrier would be bound up actively transporting external ions, and the measured efflux (by simple diffusion) would be augmented. Such a system demands that outward diffusion occur in series with the active sodium pump (Kirschner,1955). In the teleost, this is probably not true, for most of the passive leakage is thought to take place through the thin walled respiratory lamellae while active uptake apparatus is situated in the interlamellae filamental epithelium (Conte,1969). Thus it seems likely that back-transport could offer only a minimal contribution to the observed concentration dependence of efflux. However, definitive statements about the relative roles of exchange diffusion and back-transport in sodium flux linkages across Salmo gairdneri gills cannot be made from the result of either the present study or that of Kerstetter et al.(1970). Such assessments must await the results of experiments in which sodium efflux to a sodium free solution is measured during inhibition of active transport.

The utility of a back-transport mechanism for an organism inhabiting an environment where sodium is extremely scarce is clear; however the advantage of an exchange diffusion system in such a situation is not immediately obvious. It could in fact merely represent the remnant in fresh water of the exchange diffusion process characteristic of the sea water adapted forms of many euryhaline teleosts (Motais et al., 1966; Motais,1967) which is important in adaptation to lower salinities. Such a mechanism, as proposed by Motais et al.

(1966) organizes the inherent "leakiness" of the gills so that efflux may be instantaneously lowered by reduction of the external sodium concentration, thereby preventing excessive salt loss on entry into a more dilute medium. This system could presumably continue to be beneficial during adaptation to fresh water environments in which sodium is extremely scarce. However one may also speculate that if the active uptake of external sodium ions across the trout gill occurs through a coupled exchange to hydrogen ions, for which Kerstetter et al. (1970) have provided some evidence, then the presence of exchange diffusion may play some role in regulation of acid-base balance. Imagining a mechanism similar to that originally proposed for the frog skin (Ussing, 1949b), where external sodium ions can exchange, through the mediation of a carrier, with either internal sodium ions (no net transport) or hydrogen ions (net transport), then the existence of exchange diffusion would allow the trout to keep a large number of carrier sites available for excretion of protons during acutely acidotic conditions. Under normal circumstances the system would effect mostly sodium - sodium exchanges which would entail only a minimal maintenance cost of the carrier and offer no osmoregulatory disadvantage to the animal.

SUMMARY II

1. Branchial sodium uptake remained constant during swimming periods of up to 8 hours and during recovery from extended exercise.
2. Whole organism sodium efflux rate was highest during the first hour of exercise in trout which were not urinary ligated. Unidirectional outward movements declined during the second hour of swimming, and reached levels lower than influx rate during the third and subsequent hours of swimming. This evidence was indicative of a compensatory mechanism implemented during exercise to reduce the osmotic penalty of activity.
3. Efflux rate demonstrated a further reduction during the second hour of recovery after which it increased slightly to a level slightly below influx rate.
4. Alterations in whole body sodium net flux rates of the non-occluded sets of fish paralleled changes in efflux rate under different exercise conditions.
5. The efflux rate (branchial) of the urinary blockage treatment group was unaffected by the exercise condition of the animal. However, subtraction of the mean efflux rates of this group from that of the shams yielded unrealistically high values for renal sodium efflux rates during activity. Further evidence was presented which suggested that the branchial sodium losses of the ligated trout were abnormally depressed due to the effect of renal occlusion.
6. Consequently, most of the variation in whole animal efflux rates with different exercise conditions of the unligated fish

has been attributed to a branchial source.

7. Branchial sodium influx rate was unaffected by urinary catheterization or blockage.

8. Whole organism efflux rate was slightly elevated in trout bearing open urinary catheters (shams) relative to that of normal fish. However the two groups exhibited very similar responses in efflux rate under various exercise situations.

9. The higher efflux rates of the shams was reflected in significantly lower total internal exchangeable sodium contents. This effect may be ascribed to elevation of both branchial and renal losses following cannulation.

10. Urinary ligated animals suffered a pronounced net gain of water over the experimental period accompanied by significant increases in radiosodium space and tissue water levels, and a decrease in plasma sodium concentrations. Plasma water levels and hematocrit, however, did not change markedly. These effects were interpreted in terms of the relegation of excess water to the extravascular space which prevented a large increase of plasma volume; this water shift was apparently accompanied by a net movement of sodium from the plasma into the extra-cellular but extra-vascular compartment. The reduction of plasma sodium levels may have been responsible for the postulated depression of branchial sodium efflux rates in renal blockage fish.

11. Branchial sodium influx rate was affected by external sodium levels. This concentration dependence was well described by the Kirschner (1955) equation (analogous to the Michaelis-Menten equation) with Ks = .014 mEq/L and Fi max = 58.40 uEq/100 g/hour. These values were indicative of an

extremely efficient system for sodium uptake from dilute solutions in the rainbow trout.

12. Branchial sodium efflux rates, as determined from the urinary occlusion group alone, were also dependent on external sodium levels. This phenomenon was considered indicative, although not conclusive, of the presence of an exchange diffusion mechanism for sodium in the gills of fresh water adapted Salmo gairdneri.

SECTION III

THE EFFECT OF EXERCISE ON WATER BALANCE

INTRODUCTION III

The experiments of the first two sections were designed to assess the effect on sodium balance in general, and sodium fluxes across the gills in particular, of shifts in the branchial respiratory/osmoregulatory compromise caused by the particular metabolic demands of exercise. The present study was designed as a complementary investigation of water flux and regulation during activity. Because the necessary experimental facilities were available for only a short period of time, the work was somewhat preliminary in nature and involved only a small number of animals, but yielded a good deal of useful information.

As the effects on sodium balance of alterations in branchial blood flow and distribution could be manifested in both carrier mediated and simple diffusional fluxes, it had previously been desirable to separate the unidirectional components of net flux. The small volume system necessitated by this purpose had not permitted the imposition of standard swimming conditions or any direct measurement of the respiratory changes occurring during exercise. However, it is extremely unlikely that any carrier mediated processes are

directly involved in water exchange at the gills. Consequently determination of the net flux of water alone should provide adequate measure of the effective branchial permeability to this substance at a particular time.

In fresh water teleosts, water gained from the environment enters across the gills and the intestine (Evans,1967; Potts and Evans,1967), the skin being impermeable, (Bentley, 1962; Fromm,1968), and is eliminated through a single pathway, the kidney. Thus if the assumption is taken that the animal maintains a steady state, the urine flow should be equivalent to net water flux. The rainbow trout does not drink in fresh water (Shehadeh and Gordon,1969) further simplifying the situation so that urine flow equals net branchial water entry in this species. Consequently, collection of urine affords estimates of branchial permeability without accompanying limitation of the volume of the experimental system. Use of this technique in the present study allowed simultaneous determinations of oxygen consumption and net branchial water entry under controlled exercise conditions in a swimming respirometer (Brett, 1964; Stevens,1968 b). It was hoped that such data would provide a quantitative relationship between gas and water exchange and further information about the compensatory limitations in branchial hydromineral permeability which in Section II were postulated to occur during prolonged exercise. In order to further pursue the question of a compensatory reduction in gill permeability, weight changes at different swimming durations were determined as measures of water regulation. The data obtained also provided explanation for the decrease in blood volume and increase in plasma sodium levels

measured during activity in Section I.

In addition to relating water and gas fluxes, the collection of urine allowed direct mensuration of urinary sodium efflux rate. The results aided interpretation of the data of Section II and have already been noted in brief summary (Table XI). A fairly extensive cation analysis (Na^+, K^+, Mg^{++}, Ca^{++}) of the collected samples provided further information on kidney function during exercise. Urine flows and renal ion losses have not previously been measured in any fresh water teleosts under controlled swimming conditions.

Thus determination of water fluxes through collection of urine provided an opportunity for examining some characteristics of the respiratory/hydroelectrolytic adjustment which the use of sodium exchange as the the osmoregulatory variable had previously precluded. Furthermore the investigation served to solve several problems raised by the results of the first two sections, and provided information on renal physiology during swimming.

METHODS III

Experimental Animals

Experiments were performed on sexually immature rainbow trout (Salmo gairdneri) with weights ranging from 90 to 180 g. The animals were kept indoors for at least 3 weeks prior to experimentation in a 250 gallon concrete holding tank supplied with fully oxygenated fresh water at 7 - 8°C; during this time, they were fed regularly with a commercial trout pellet. All experiments were carried out at the Vancouver Public Aquarium during February and March, 1970.

I. Urine Flow versus Oxygen Consumption Experiments

1. Operating Procedure and Cannulations

Implantation of urinary catheters was performed as described in Section I on trout anaesthetized in a 1/10,000 MS 222 solution at 7 - 10°C. The higher MS 222 concentration was necessitated by the colder water temperature. Cannulae (length = 45 cm) were constructed from PE 60 and PE 190 tubing as in Section I. However, because of the low body weights and sexual immaturity of these fish, the urogenital papillae were extremely small. This fact necessitated a reduction in length of the proximal tip and in diameter of the flanges of the catheter. Despite these modifications, many cannulation attempts failed; of the 18 animals originally catheterized, only 5 exhibited both patent and free-draining cannulae. Following completion of the operation, the fish was sealed into a metabolism box while still anaesthetized.

Recovery was aided by directing a water flow of 300 ml/min. through the chamber.

2. Experimental System

Urine production and oxygen consumption were simultaneously determined during rest in metabolism boxes (5 fish) and then during controlled levels of swimming activity (4 fish). Urine samples were collected for later ionic analysis. Terminal weighing and measuring was carried out as in Section I.

(a) Metabolism Boxes

Resting state measurements were taken from trout sealed in blackened plexiglass metabolism chambers (length = 43.1 cm; width = 8.6 cm; depth = 8.2 cm) continually flushed with fresh water at 7 - 8°C. A moveable partition in the boxes permitted restricted movements, but did not allow the fish to change position. Opercular rates could be visually counted with the aid of mirrors placed beneath the chambers. The urinary catheter was led out of the box via attachment to a #21 needle piercing a rubber stopper in the rear of the chamber. The catheter drained by gravity. The withdrawal of incurrent and excurrent water samples and measurement of water flow rate (80 - 200 ml/min) through the chamber permitted oxygen consumption determinations. Representative effluent samples were ensured by mixing chambers at the rear of each box. Partial pressure of oxygen (PO_2's) in water were determined with a thermostatted oxygen micro-electrode system (Radiometer - Copenhagen); conversion of differential PO_2's to differential oxygen contents by means of temperature dependent

solubility factors allowed calculation of oxygen consumption rates.

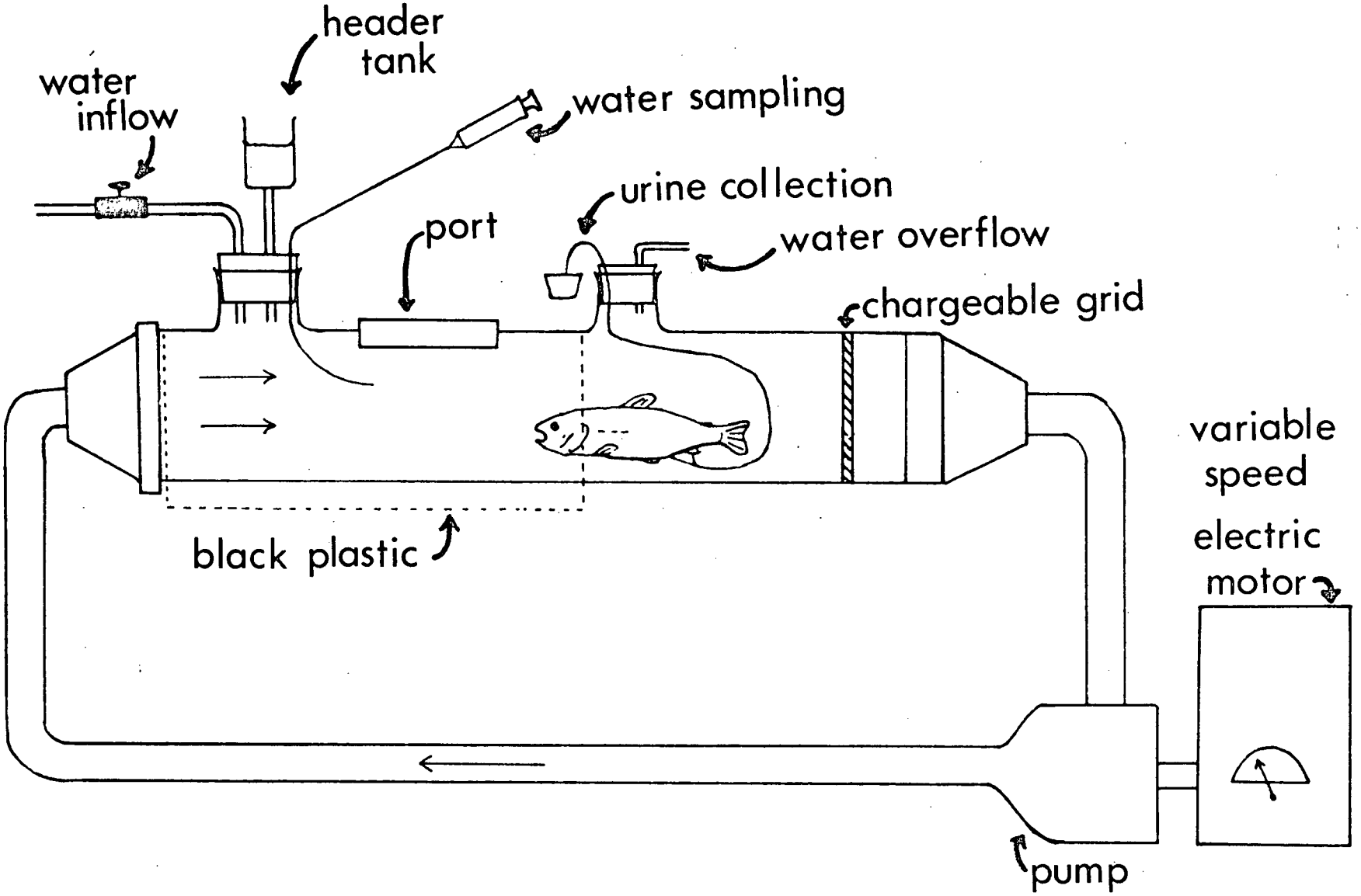
(b) Respirometer

Data at various levels of swimming activity were taken from trout exercised at 7 - 9°C. in the swimming respirometer originally used by Stevens (1968 a). The design was similar to that described by Brett (1964) (Fig.23). In brief, the respirometer consisted of a plexiglass tube (length = 70 cm; inside diameter = 13 cm) through which water could be recirculated at a range of velocities. A variable speed electric motor propelled the pump, allowing continuous variation of speed from 0 to 45.7 cm/sec. The volume of the system was 23.75 L. The respirometer could be either continually flushed with fresh water or sealed for measurement of oxygen consumptions. In the latter case, a small header tank connecting to the chamber prevented bubble formation when water samples were withdrawn. The anterior two thirds of the swimming tube were covered with black plastic; trout generally oriented under the posterior end of the plastic, using it as a visual cue to maintain position during exercise. A metal grid at the rear of the respirometer could be electrified at a low voltage to encourage swimming performance. The urinary catheter was extended with a further 20 cm of PE 60 and brought out through the top of the chamber via a #21 needle through a rubber stopper; in this system, the cannula drained by siphon.

(c) Experimental Procedures

(i) Resting Trout. Catheterized fish were maintained in metabolism chambers for up to 8 days after operation. During the first 4 days, urine was collected from 5 fish as 24

Figure 23 A diagram of the swimming respirometer used in the experiments of Section III.


header
tank
water
inflow
water sampling
urine collection
port
water overflow
chargeable grid
variable
speed
electric
motor
black plastic
pump

hour samples. Oxygen consumption and opercular rates were ascertained periodically on a further 3 trout during the first 48 - 60 hours after cannulation. Unfortunately, the urinary catheters were not patent on the latter animals and urine production could not be quantified. On the fifth post-operative day, the urine production of each fish was measured over a 5 hour period during which at least 5 determinations of metabolic rate were carried out.

(ii) Swimming Trout. Between the 5th and 8th post-operative days, a fish was gently transferred, without anaesthesia, to the swimming respirometer and left overnight to accustom itself to the chamber. During this time, the respirometer was flushed with fresh water at a velocity of 10.7 cm/sec., the minimum current into which the trout would continuously orient without swimming. The urinary catheter was led out of the chamber and allowed to drain for at least 2 hours before the start of an experiment. Trout were then subjected to the following regime:

10.7 cm/sec.	-	3 hours
21.4 cm/sec.	-	3 hours
10.7 cm/sec.	-	3 hours
21.4 cm/sec.	-	15 minutes
32.1 cm/sec.	-	3 hours
10.7 cm/sec.	-	3 hours
21.4 cm/sec.	-	15 minutes
32.1 cm/sec.	-	15 minutes
42.8 cm/sec.	-	3 hours
10.7 cm/sec.	-	3 hours

For one fish (#59), the swimming regime was interrupted for lengthy breaks near the beginning and end of the experiment. However, as possible shifts in the base line rate of urine production occurred during these intervals, periods of the swimming regime were imposed consecutively on each of the other

3 animals tested (#55, #63, #65) during a continuous experimental period of 21.75 hours.

Total urine production was collected and measured for each 60 minute section of the 3 hour treatment periods, and during the 15 minute intermediate periods. Oxygen consumption was also determined over each of the one hour intervals by sealing the respirometer for a length of time sufficient for the organism to reduce the PO_2 in the chamber by 10 - 15% (30 - 55 minutes). At no time was the PO_2 in the respirometer permitted to fall below 120 mm Hg. PO_2 analyses on duplicate water samples drawn at the beginning and end of each closure period allowed calculation of metabolic rates. Oxygen uptakes during the 15 minutes velocity increment periods were not great enough to quantify accurately. Opercular rates and tail beat frequencies were counted regularly throughout the experiments with the aid of mirrors placed under the respirometer.

3. Analytical Procedures

Urine samples were collected in small polyethylene vials, measured with a Hamilton syringe, and then frozen in the vials at -12°C. for later ionic analyses. All assays were performed in duplicate against calibration curves constructed with appropriate dilutions of commercially prepared standards (Harleco); the Techtron Model AA 120 Atomic Absorption Spectrophotometer was used for all determinations.

(i) Sodium in Urine. Aliquots of each sample were diluted 1/600 with distilled water and read at 5890 Å on the flame emission mode of the spectrophotometer (calibration

range = 0 - 1.0 ug Na^+/ml.

(ii) Potassium in Urine. Potassium concentrations were ascertained on the flame emission mode of the instrument at 7664 Å. Samples were diluted 1/250 and assayed against calibration curves in the range 0 - 20 uEq K^+/L. Because of the known interference of high sodium levels on potassium flame emission, both standards and unknowns were swamped with 200 ug Na^+/ml.

(iii) Calcium and Magnesium in Urine. The same dilution (1/60) of each sample was utilized for determination of both ions. Sodium and potassium have been shown to potentiate calcium flame emission (Teloh,1958); thus unknowns and standards were diluted with both a 200 ug Na^+/ml swamp and a 100 ug K^+/ml swamp. No correction was made for the reported depressant effect of phosphate ion on the spectral emission of calcium (Teloh,1958). However, tests revealed that additions of constant known amounts of calcium (approximately 30% of total concentration) to a range of different, but identically diluted,urine samples could be recovered analytically 93 - 101%. This evidence indicates that, after Na^+ and K^+ swamping, only minimal interference effects remained. Calcium analyses were performed at 4227 Å over a calibration range of 0 - 100 uEq Ca^{++}/L.

The remainder of each dilution was analyzed for magnesium at 2852 Å on the atomic absorption mode of the spectrophotometer over a calibration range of 0 - 80 uEq Mg^{++}/L. As the unknowns had already been swamped (200 ug Na^+/ml; 100 ug K^+/ml)for calcium measurements, the magnesium standards were similarly fortified as a safety precaution. However, sodium,

potassium, and phosphate ion apparently do not interfere with the spectral absorption of magnesium in urine (Dawson and Heaton,1961).

As the primary purpose of this study was to correlate urine volume with oxygen uptake, urine was collected in hourly samples. The volume of the collecting ducts and ureter was negligible, and as the catheter drained by siphon, urine would not have accumulated in the bladder. However the deposition into the collecting vial of urine formed at any one moment was delayed by a factor corresponding to the volume of the catheter (0.35 ml) and the rate of urine flow. Thus an hourly sample would represent the exact volume excreted during that 60 minute period, but, at low urine flow rates, may have been produced 2 - 3 hours previously. Thus it was necessary to refer the measured ionic composition of collected urine to its production time in the following manner. Cumulative urine volume was plotted against elapsed time. The cannula volume was graphically subtracted from the cumulative volume at the end of each sample period, and a perpendicular dropped to the time axis at these points, thereby defining a new set of time intervals over which each urine sample was formed. The measured ionic composition of each sample was then referred to the midpoint of these periods. Unfortunately, the production times of collected samples often spanned two swimming speeds, creating composition values representative of neither condition. Thus any variations in ionic content associated with different activity levels would have been somewhat dampened by this mixing process.

II. Weight Change vs. Swimming Duration Experiments

Weight changes were measured as an indication of water fluxes in trout swum for various lengths of time in the respirometer at 32.1 cm/sec. and in identically handled sets of control animals. Experiments were performed on a batch of approximately 70 trout held in the 250 gallon concrete tank; as 151 determinations were made in this study, each fish was used 2 - 3 times. The animals were starved for 6 days prior to experimentation to prevent faecal evacuation contributing to weight changes. Water temperature throughout the study was 7 - 8°C.

For weighing, an unaesthetized trout was removed from the water, placed on a bed of paper towels, and gently but thoroughly dried for 15 seconds. The animal was then added to a tared container and weighed to the nearest .05 g. The weighing container consisted of a light styrofoam chamber (length = 30 cm; width = 7 cm; depth = 12 cm) fitting the shape of the fish and lined with a plastic sack. The bag contained 500 ml of water and was sealed with staples immediately after entry of the fish. Animals rarely struggled in the apparatus. The trout was finally returned to its aquarium and gently released under water. Total weighing time was about 60 seconds. Tests with dead fish showed that the weighing procedure was accurate to 0.1 g.

For weight change measurements, fish were transferred from the holding tank to individual blackened and covered aquaria (5 or 10 gallon) served with a constant supply of fully oxygenated fresh water. The animals were left undisturbed for at least 8 hours to recover from this initial handling.

Then individual trout were netted from their aquaria, weighed, and returned to the tanks for a further 8 hours recuperation. After this interval, experimental animals were removed from their chambers and quickly airdipped to the respirometer (5 seconds). Water velocity was set at 10.7 cm/sec. (15 seconds), then 21.4 cm/sec. (15 seconds), and finally 32.1 cm/sec. for the desired swimming period of 1, 5, 15, 30, 60 minutes or 4 - 8 hours (long term). To avoid stress, the grid at the rear of the water tunnel was not electrified; inactive fish were gently tapped on the tail with an aluminum rod to induce swimming. Chronic non-swimmers were discarded. At the end of the experimental period, the trout was removed from the respirometer and weighed as before.

Control fish were netted from their aquaria and held in the air for 5 seconds as for the experimentals. However, they were then returned to their individual tanks and left undisturbed until the appropriate sample time (1, 5, 15, 30, 60 minutes or 4 - 8 hours), when they were removed and weighed as before.

After an experiment, an animal was returned to the general holding tank for at least 24 hours before the next trial. During the course of the study, a few fish became noticeably de-scaled and were sacrificed; results from these trout were rejected. Weight changes were expressed as g/100 g original weight.

RESULTS AND DISCUSSION III

Before presentation and discussion of the results, several points of data interpretation must first be clarified. A major portion of this section has been devoted to consideration of the relationship between branchial permeability to oxygen and permeability to water. As concentration gradients for oxygen (Stevens and Randall,1967 b) and water between internal and external milieux will remain essentially invariant under different exercise conditions, it has again been assumed that the measured fluxes of both substances are representative of permeabilities. However oxygen transfer across the respiratory epithelium occurs by simple diffusion (Randall,1970 a); the same is not necessarily true of water movement, the nature of which is dependent on the properties of the membranes through which transfer occurs. Under an osmotic gradient, water flux through a porous membrane proceeds by both quasi-laminar flow (described by the Poiseuille equation) and simple diffusional flow (Davson,1964). As pore size increases, diffusional transport becomes increasingly less important. The bulk flow of water through such pores (Davson,1964; Evans, 1969b) and the influence of an unstirred layer bounding the membrane in question (Dainty and House,1966) have been postulated to account for discrepancies in many biological systems between directly measured net water transfer (osmotic permeability) and that calculated from the gross flux of labelled water (diffusional permeability). At present, it is not clear whether in teleosts, the osmotic permeability (Pos), as

measured by urine production in fresh water or drinking rate in sea water, is representative of simple diffusional permeability (Pd) or a more complex phenomenon affected by flow through pores or unstirred layers. In the intertidal fish Xiphister atropurpureus (Evans,1967) and Pholis gunnelis (Evans,1969a) Pos and Pd were in fair agreement, but from a survey of a wide range of both fresh water and marine species, Evans (1969b) has concluded that it is not yet possible to make any definite statements about Pos - Pd relationships, and thus about the membrane structure of the permeable surfaces of teleosts. Consequently the present study, in comparing simultaneous oxygen consumptions and urine productions may or may not be relating two simple diffusional permeabilities. The fact remains, however, that the data must represent effective branchial permeabilities to the two substances, which are the parameters of importance to the animal itself in any alteration of the respiratory/osmoregulatory adjustment at the gills.

It is possible that the sodium reabsorptive mechanism (Hirano, personal communication to D.J.Randall) observed in the isolated trout bladder wall may serve to alter the composition or reduce the volume of the fluid finally released through the urogenital papilla in vivo. Such functions would be largely negated by the presence of the cannula, thus introducing some inaccuracy in the results with respect to the normal condition. All previous data on urine flow and composition in trout have been obtained through similar techniques of bladder intervention (Fromm,1963; Holmes and Stainer,1966; Enomoto,1967; Hammond,1969; Hunn et al.,1968; Hunn,1969; Hunn and Willford,1970), so there exists no comparative information

on this point. Until methods are developed for the collection of urine after it leaves the animal, the normal in vivo activity of the bladder will remain unknown. It seems likely, however, that reabsorption of water and ions by this organ, if indeed occurring at all, would be minimal due to its poor vascularization and high volume to surface area ratio. Consequently errors introduced by direct collection from the bladder are probably small and relatively systematic in nature; for the purposes of this study, they have been disregarded.

A marked diuresis subsequent to urinary catheterization and handling has been repeatedly demonstrated in fresh water salmonids (R.M.Holmes,1961; Hammond,1969; Hunn and Willford,1970). Evans (1969 b) has correlated this phenomenon with an immediate increase in water permeability, presumably branchial, after stress. Hickman and Trump (1969) have suggested that this augmented permeability is associated with an elevation of oxygen uptake during the post-operative period. Measurements of urine flow (Fig. 24) and respiratory functions (Fig. 25) after MS 222 anaesthesia and cannulation in the present study supported the hypothesis of Hickman and Trump (1969). "Laboratory diuresis" therefore seems to result from a disturbance of the respiratory/osmoregulatory adjustment in the gills. Oxygen uptake and ventilation rate had declined to a constant level by about 45 hours after operation (Fig. 25) corresponding to a similar stabilization of urine production (Fig. 24).

Determinations of urine flow and metabolic rate for resting state values (Table XVI) were performed on the fifth post-operative day. The mean oxygen consumption was extremely

Figure 24 The decline in urine flow with time after MS 222 anaesthesia and catheterization in 5 trout. Each point represents the flow calculated from a 24 hour sample plotted at the midpoint of the collection interval. Different symbols have been used for values from different animals. One 72 - 96 hour sample was lost.

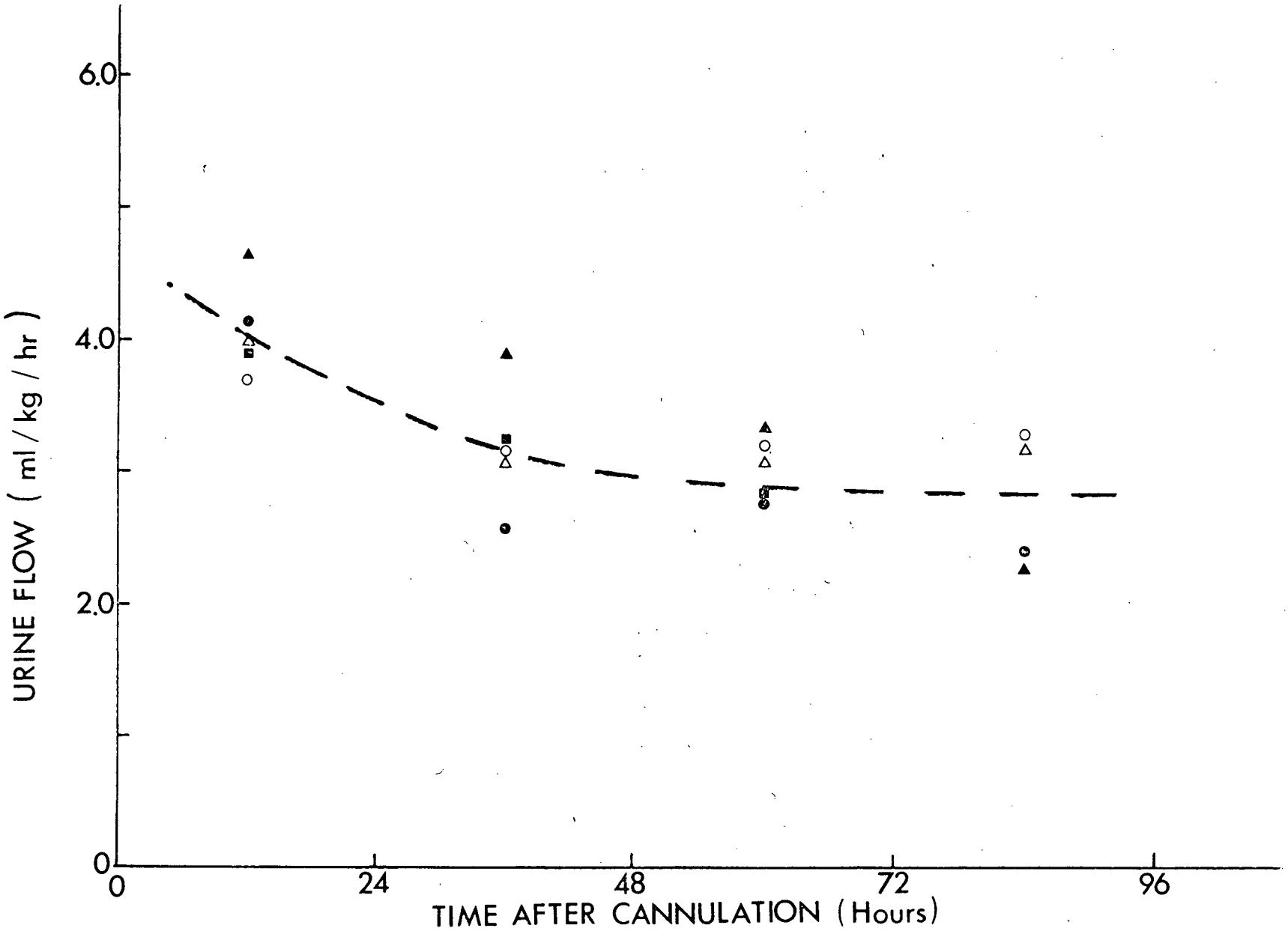

6.0
4.0
2.0
0
URINE FLOW (ml / kg / hr)
0
24
48
72
96
TIME AFTER CANNULATION (Hours)

Figure 25. The decline in ventilation rate (upper figure) and oxygen consumption (lower figure) with time after MS 222 anaesthesia and catheterization in 3 trout. The 5 points represented by black triangles at 96 - 120 hours in each figure are the resting rates of Table XVI.

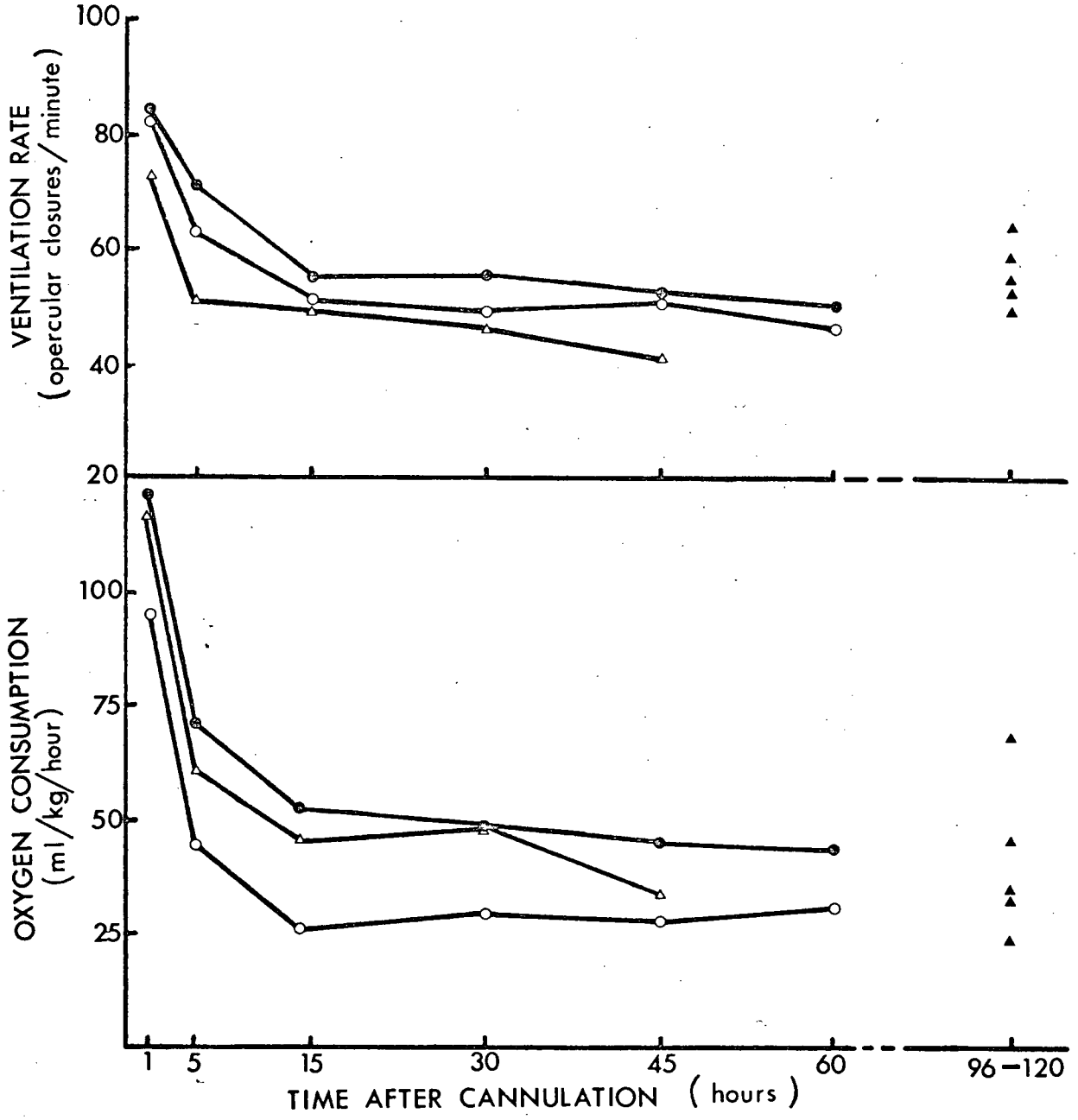

VENTILATION RATE
(opercular closures / minute)
100
80
60
40
20
OXYGEN CONSUMPTION
(ml/kg/hour)
100
75
50
25
1
5
15
30
45
60
96–120
TIME AFTER CANNULATION (hours)

Table XV. Physical dimensions of the trout used in Section III. Means ± 1 standard error.

	N = 8
Body weight (g)	144.50 ± 11.82
Length (cm)	24.80 ± 0.54
Fork length (cm)	23.80 ± 0.53
Maximum depth (cm)	4.93 ± 0.10
Coefficient of condition $\frac{\text{weight} \times 100}{(\text{fork length})^3}$	1.056 ± 0.036

Table XVI. Resting state values of urine flow, oxygen uptake, and ventilation rate for 5 trout in metabolism boxes. Determinations were made on the fifth day after cannulation.

Fish	Weight	Ventilation rate* (operc.closures/min.)	Oxygen consumption* (ml/kg/hour)	Urine flow^{x} (ml/kg/hr)
#55	166.7	52.9	34.95	2.76
#59	152.0	55.8	23.47	3.26
#63	120.1	58.6	46.39	2.48
#64	105.7	53.1	32.41	3.15
#65	138.9	63.6	69.57	2.78
Mean ± 1 standard error		56.8 ± 1.99	41.36 ± 7.94	2.89 ± 0.14

* mean of at least 5 determinations over 5 hours for each fish

^{x} over 5 hours

similar to the standard metabolic rate measured for Salmo gairdneri of this weight by Rao (1968) at 5°C. Furthermore the urine flow means were lower than those reported for resting state trout of similar size (Fromm,1963; Holmes and Stainer,1966) and comparable to those determined in much larger salmonids (Hammond,1969; Hunn,1969). Thus the values presented in Table XVI may be considered basal to standard rates (cf.Brett,1962). These data will be given further consideration when observations relating urine production to oxygen uptake are presented.

Four animals (#55, #59, #63, and #65) were subjected to a standardized exercise regime in the swimming respirometer as described previously. Lengthy interruptions in the regime of fish #59 may have caused alterations in urine flow rate but had no obvious effect on this trout's ventilatory and metabolic responses to exercise. The changes in respiration rates, tail beat frequency, and oxygen uptake associated with different sections of the protocol are tabulated in Table XVII. Several points are worthy of note. Firstly, despite the fact that the fish had already spent about 12 hours in the chamber when the experiment commenced, their initial "resting" oxygen uptake was 3 times greater than that determined in the same animals after 5 days in the metabolism boxes (Table XVI). Similarly high oxygen uptakes at sub-swimming speeds in a water tunnel have been observed in salmon (Brett, 1964) and are associated with "restlessness" (spontaneous activity). Such effects are extremely difficult to eliminate but tend to disappear with exercise. Consequently average metabolic rate increased only slightly at the lowest swimming

Table XVII. Tail beat frequencies (T.B.F.), ventilation rates, and oxygen consumptions during each hour of the imposed swimming regime. Means ± 1 standard error for 4 fish.

Velocity (cm/sec)	10.7	10.7	10.7	21.4	21.4	21.4	10.7	10.7	10.7
T.B.F. (tail beats/min) N = 12	-	-	-	± 77.8 ± 12.7	± 78.3 ± 13.1	± 58.0 ± 16.0	-	-	-
Ventilation rate (closures/min) N = 12	± 63.0 ± 2.4	± 63.5 ± 2.7	± 61.6 ± 3.0	± 79.0 ± 3.2	± 75.3 ± 3.0	± 71.6 ± 3.5	± 62.8 ± 2.0	± 63.2 ± 2.4	± 62.2 ± 2.3
Oxygen Consumption (ml/kg/hr) N = 4	± 128.35 ± 24.74	± 116.14 ± 22.67	± 101.55 ± 19.21	± 150.62 ± 26.27	± 119.20 ± 13.78	± 109.74 ± 9.14	± 98.28 ± 9.30	± 112.97 ± 7.41	± 90.97 ± 12.35
Continuation of regime:									
Velocity (cm/sec)	32.1	32.1	32.1	10.7	10.7	10.7	42.8	42.8	42.8
T.B.F. (tail beats/min) N = 12	± 124.6 ± 4.1	± 124.2 ± 2.0	± 124.3 ± 3.7	-	-	-	± 144.6 ± 4.5	± 133.0 ± 4.4	± 137.8 ± 3.5
Ventilation rate (closures/min) N = 12	± 89.8 ± 1.8	± 86.7 ± 2.4	± 92.0 ± 3.2	± 66.8 ± 1.1	± 64.1 ± 1.4	± 60.8 ± 1.8	± 102.2 ± 2.7	± 96.7 ± 8.2	± 84.0 ± 8.9
Oxygen consumption (ml/kg/hr) N = 4	± 222.06 ± 14.29	± 232.24 ± 27.29	± 248.38 ± 19.69	± 139.63 ± 19.81	± 113.72 ± 12.60	± 98.14 ± 9.17	± 299.81 ± 6.05	± 272.35 ± 9.11	± 296.56 ± 5.03

Table XVII. (Continued)

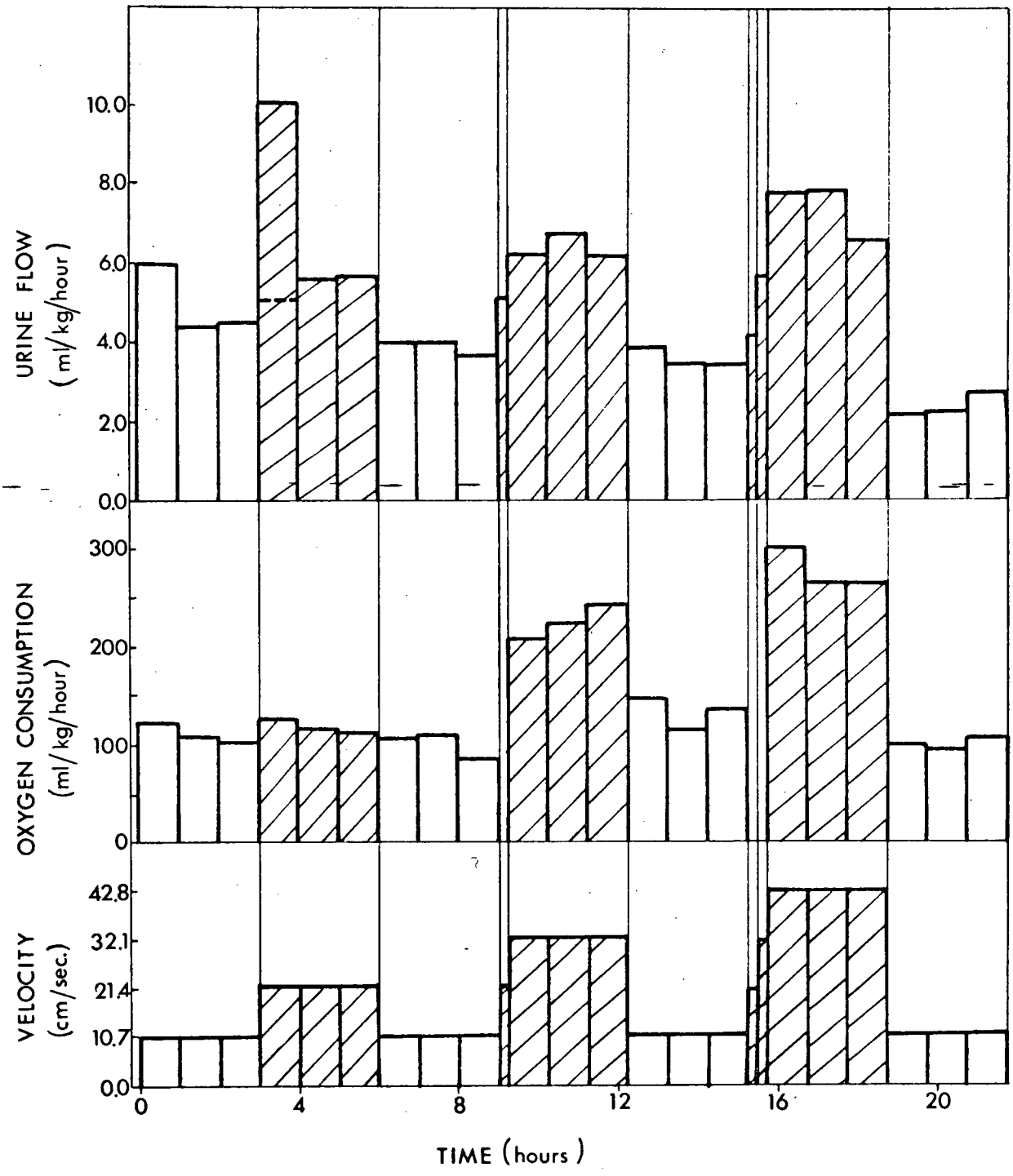
Continuation of regime:

Velocity (cm/sec)	10.7	10.7	10.7
T.B.F. (tail beats/min) N = 12	-	-	-
Ventilation rate (closures/min) N = 12	66.6 ± 1.5	61.4 ± 1.4	61.5 ± 2.1
Oxygen consumption (ml/kg/hr) N = 4	92.61 ± 6.93	81.50 ± 12.97	94.46 ±14.70

speed (21.4 cm/second), probably reflecting a decrease in the consumption associated with restless behaviour. The oxygen uptakes at the two highest velocities (32.1 cm/second and 42.8 cm/second at 7° - 9°C.) were comparable to those observed by Rao (1968) at these speeds for rainbows of the same size but at 15°C. The metabolic rates measured here at 42.8 cm/second were in fact greater than the maximum consumptions ascertained by Rao (1968) at 5°C. It would therefore appear that trout in the present study approached active metabolic rates (Brett, 1962) at relatively low swimming speeds. This effect was probably attributable to the drag created by the trailing urinary catheter which would greatly elevate the cost of maintaining a particular swimming velocity. The precipitous drop in metabolic rate to original levels at the end of the exercise periods indicated, however, that little, if any, oxygen debt was incurred. By the end of the regime, oxygen consumptions were actually slightly below initial "resting" values, again probably due to a diminution of spontaneous activity.

The averaged urine flows and oxygen consumptions of trout #55, #63, and #65 throughout the experiment are illustrated in Fig. 26. Urine production markedly increased during periods of exercise, the augmentation being approximately proportional to the velocity increment. A branchial sodium deficit associated with activity has been demonstrated in the first two sections; a similar conclusion may now be extended to branchial water balance. In addition, the data reveals that, in general, alterations in water flux (urine flow) corresponded with changes in oxygen consumption. Consideration of the data for individual animals is particularly convincing

Figure 26 Simultaneous changes in urine flows and oxygen uptakes during the continuous imposed swimming regime of Section III. Each bar represents the averaged responses of trout #55, #63, and #65. Crosshatching indicates periods of exercise. The broken line on the urine flow bar for the initial hour of the first exercise interval represents the mean without inclusion of the extremely diuretic value of fish #65.


URINE FLOW
(ml/kg/hour)
10.0
8.0
6.0
4.0
2.0
0.0
OXYGEN CONSUMPTION
(ml/kg/hour)
300
200
100
0
VELOCITY
(cm/sec.)
42.8
32.1
21.4
10.7
0.0
0
4
8
12
16
20
TIME (hours)

of this point. Fig. 27 presents plots of oxygen consumption versus urine production (water flux) for each simultaneous determination during the swimming regime. For each trout there existed a highly significant positive correlation between the two parameters, although the slopes and intercepts of the regression line varied between individuals. It may be noted that standard oxygen consumption and urine flow values taken in the metabolism boxes (Table XVI) were in good agreement with the lines fitted to the data from the swimming experiments. Thus over a range of oxygen uptakes from sub-standard to almost active metabolic rates, oxygen and water fluxes were in an approximately linear positive relationship. The results are interpreted in terms of gill permeability to water being largely defined by the pattern of branchial blood perfusion necessary to effect a certain oxygen uptake. Mackay and Beatty (1968) have suggested a similar explanation for the temperature dependence of urine production in the white sucker, Catostomus commersonii. This correspondence between gas and water net movements is perhaps indicative that the contribution of non-diffusional flow to total water entry across the gills is relatively unimportant.

However, close inspection of the data from individual fish reveals that more complex interactions, associated with reductions of the osmotic penalty of exercise, may be superimposed on the general relationship. Such compensations were occasionally seen in drastic modification of urine flow within a swimming period, and in a more general trend over the whole swimming regime. The former short term effect may be dealt with first; Fig. 28 presents three of the more pronounced

Figure 27 The relationship between oxygen consumption and urine flow, considered a measure of water influx, in 3 rainbows subjected to a continuous swimming regime. Each point,(except x's) represents a simultaneous determination of the two factors over a 1 hour period.

● = data from first 6 hours of the regime. The labelled point (21.45 ml/kg/hr) represents an extremely diuretic value noted in Fig. 28, and was not included in the calculation of the regression line or correlation coefficient.

O = data from middle 9 hours of the regime.

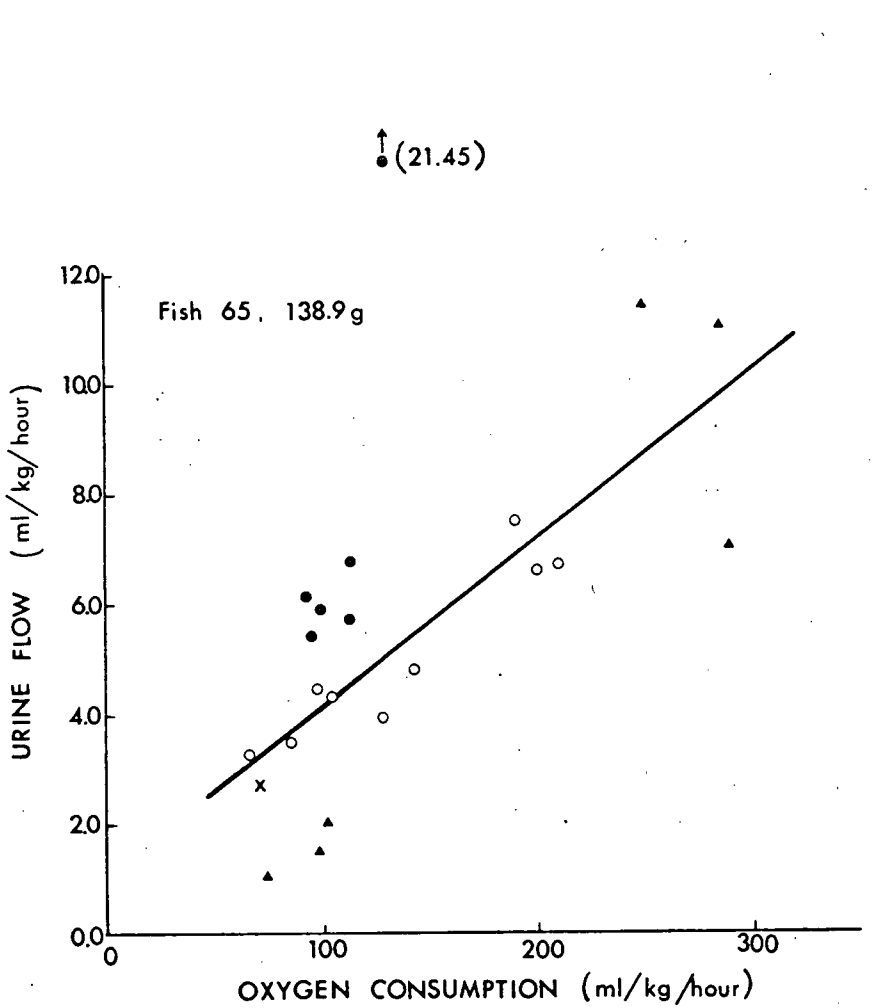
▲ = data from final 6 hours of the regime.

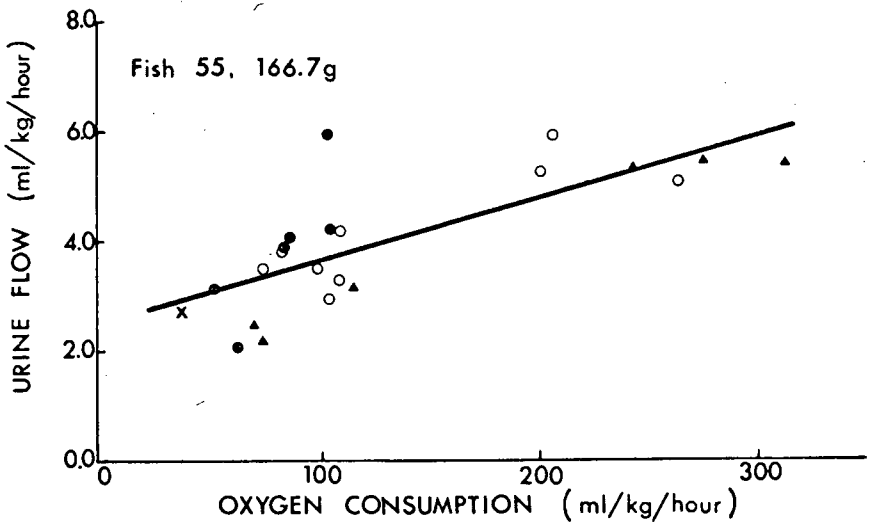
x = value obtained from the same trout at rest in a metabolism box (Table XVI); not included in the calculation of the regression line or correlation coefficient.

Fish #65: water influx = 1.12 + 0.031(oxygen uptake)
$r = 0.783$, $p < .001$

Fish #55: water influx = 2.55 + 0.011(oxygen uptake)
$r = 0.744$, $p < .001$

Fish #63: water influx = 1.72 + 0.019(oxygen uptake)
$r = 0.742$, $p < .001$


(21.45)
Fish 65, 138.9g
URINE FLOW (ml/kg/hour)
OXYGEN CONSUMPTION (ml/kg/hour)
0.0
2.0
4.0
6.0
8.0
10.0
12.0
0
100
200
300


Fish 55, 166.7g
URINE FLOW (ml/kg/hour)
OXYGEN CONSUMPTION (ml/kg/hour)
0.0
2.0
4.0
6.0
8.0
0
100
200
300

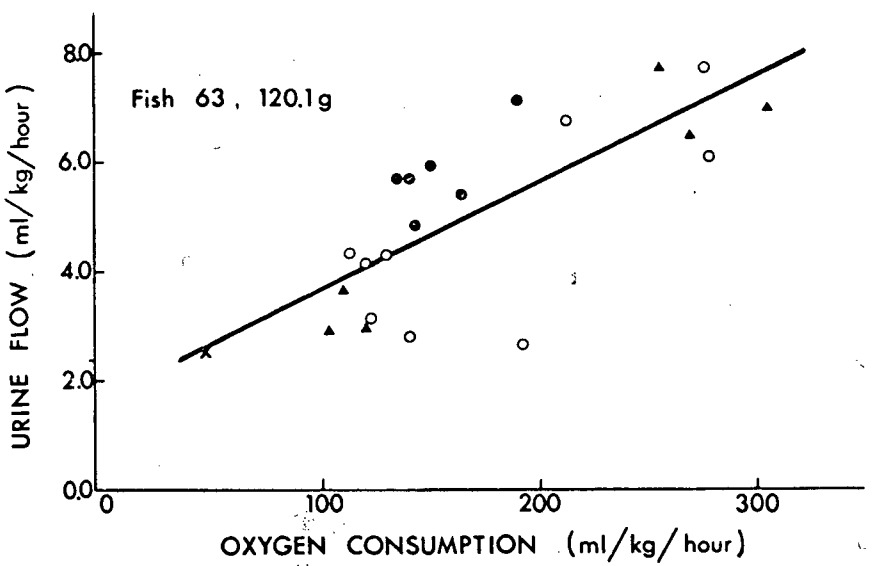
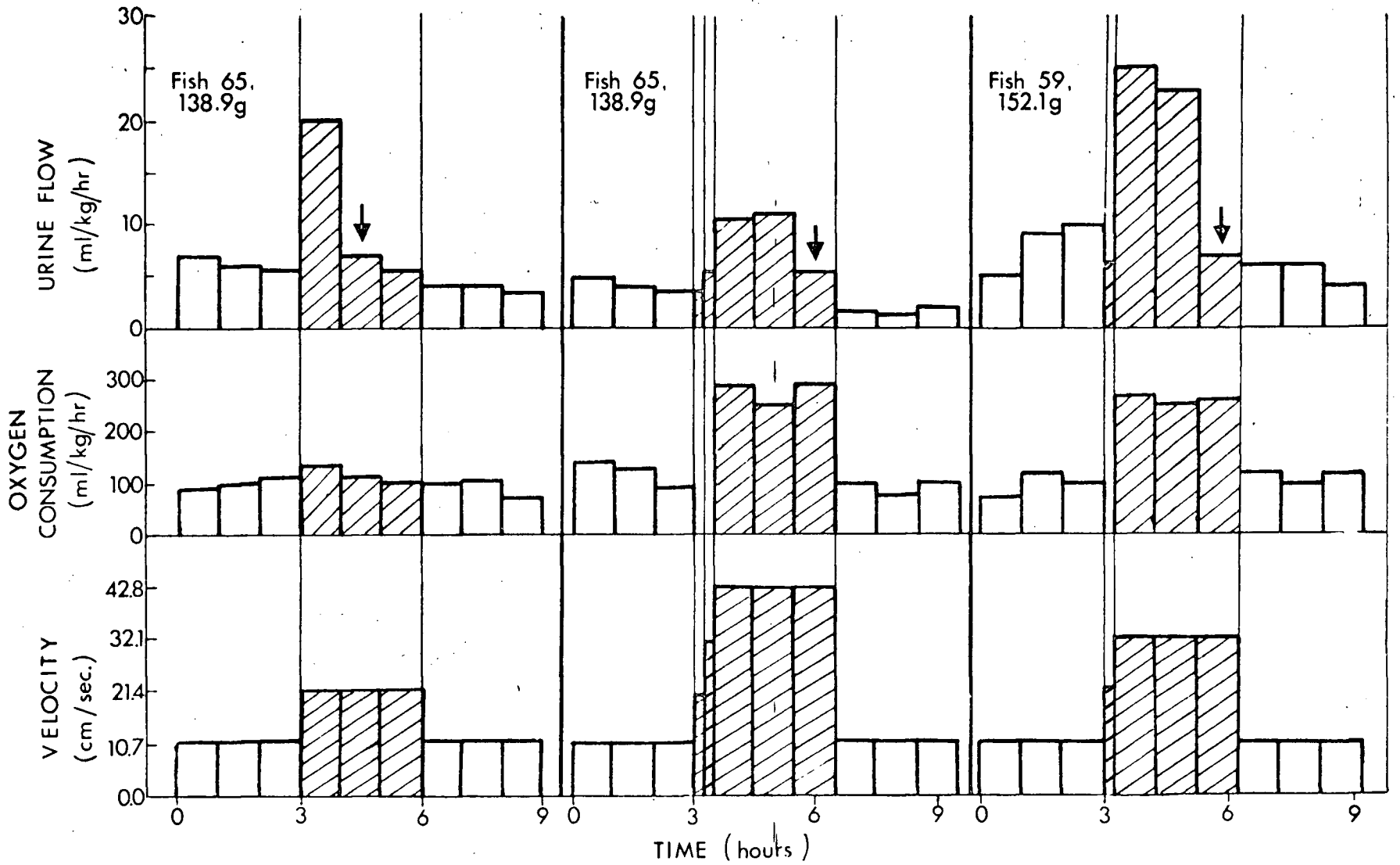

Fish 63, 120.1g
URINE FLOW (ml/kg/hour)
OXYGEN CONSUMPTION (ml/kg/hour)
0.0
2.0
4.0
6.0
8.0
0
100
200
300

Figure 28 Three examples of the phenomenon in which extremely high urine flows at the start of exercise were drastically reduced (as indicated by the arrows) during continued swimming. These changes were not associated with variations in oxygen consumption. This pattern of renal water efflux at the onset of activity is thought to be the typical response of trout which are not already "adapted" to swimming. Crosshatching indicates periods of exercise.


Fish 65,
138.9g
Fish 65,
138.9g
Fish 59,
152.1g
URINE FLOW
(ml/kg/hr)
30
20
10
0
OXYGEN
CONSUMPTION
(ml/kg/hr)
300
200
100
0
VELOCITY
(cm/sec.)
42.8
32.1
21.4
10.7
0.0
0
3
6
9
TIME (hours)

examples of this phenomenon. In such cases, extremely high urine discharges at the beginning of an exercise interval were dramatically reduced by the end of the period. The changes were not associated with any marked alterations of metabolic rate. As will be shown presently, this effect probably occurred because branchial water influx was initially very high but subsequently declined. This compensation appears similar in magnitude and timing to that observed for sodium efflux in Section II; the two effects may have been caused by a common mechanism. That this exceptional diuresis was observed only occasionally in the present study may be related to the spontaneous activity exhibited by the animals at the "resting" velocity prior to swimming. This behaviour may have been sufficient to induce the postulated lowering of branchial permeability before the imposition of the experimental exercise situation.

A more general tendency for curtailment of water influx during prolonged activity was suggested by the response of individual fish over the entire protocol. This effect is best illustrated by the plots of Fig. 27. For each trout, it can be seen that the points taken from the first 6 hours of the swimming regime generally lie above the common regression line; those from the middle 9 hours straddle it; while the values of the final 6 hours tend to fall below it. One may speculate that if enough data were available, it would be possible to fit three different regression lines of similar slope but progressively lower elevation to the values recorded at the beginning, middle, and end of the swimming protocol for each animal. The water entry per unit oxygen uptake obviously

decreased as swimming experience increased. Further consideration will be devoted to the possible mechanism of this phenomenon in the General Discussion.

The weight change experiments were performed to further elucidate the time course of water flux changes associated with exercise. Due to the known sensitivity of branchial permeability to handling effects (Evans,1969b), the experimental procedure was designed to impose as little stress as possible on the trout. As a further precaution, the weight alterations of an identically handled set of control animals was also ascertained. The results from experimentals (swimming at 32.1 cm/second) and controls (resting in separate aquaria) are presented in Fig. 29. Increase in weight could only be caused by the gain of water from the environment; decrease in weight could represent net water efflux, excretion of electrolytes, and "metabolic" losses. However very generous estimates of the latter two factors amounted to less than 3% of the mean decline of fish swum for 60 minutes. Consequently, it seems legitimate to interpret all weight shifts in terms of water movements. The net water balance of the exercised trout exhibited a triphasic response as swimming time increased. The large decrease observed after 1 minute was followed by a rapid rise to positive balance; however because of variability in the 5 and 15 minute experimental values, the increase did not become significantly greater than control weights until 30 minutes. Between 30 and 60 minutes exercise, the animals returned to a negative state; there occurred only a very slight further decrement over the following 3 - 7 hours of activity. Fluctuations in control values were of much smaller magnitude

Figure 29 Changes in body weight of rainbow trout (relative to original values determined at least 8 hours previously) in response to various exercise durations at 32.1 cm/sec. in the swimming respirometer (experimentals) and in response to the handling necessary for the determinations alone (controls). Means ± 1 standard error.

Statistical Comparisons: (Numbers refer to the times in minutes of the respective means on the graph.)

Control: $F = 2.29$, $p < .05$

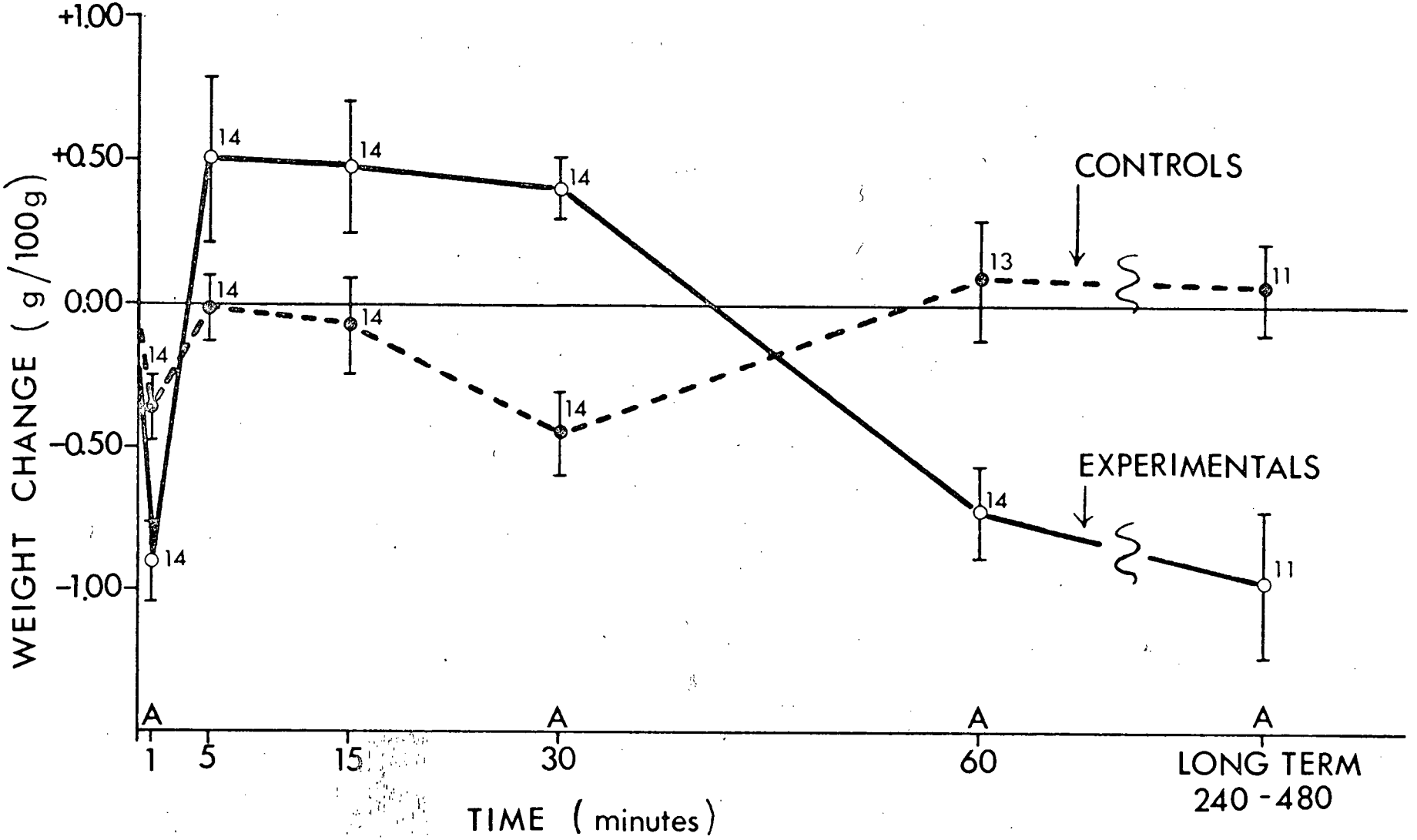
30 1 15 5 240 60

Experimentals:

$F = 12.54$, $p < .005$

240 1 60 30 15 5

A = sample times at which control and experimental means are significantly different ($p < .05$)


+1.00
+0.50
0.00
-0.50
-1.00
WEIGHT CHANGE (g /100g)
CONTROLS
EXPERIMENTALS
14
13
11
A
1
5
15
30
60
LONG TERM
240 - 480
TIME (minutes)

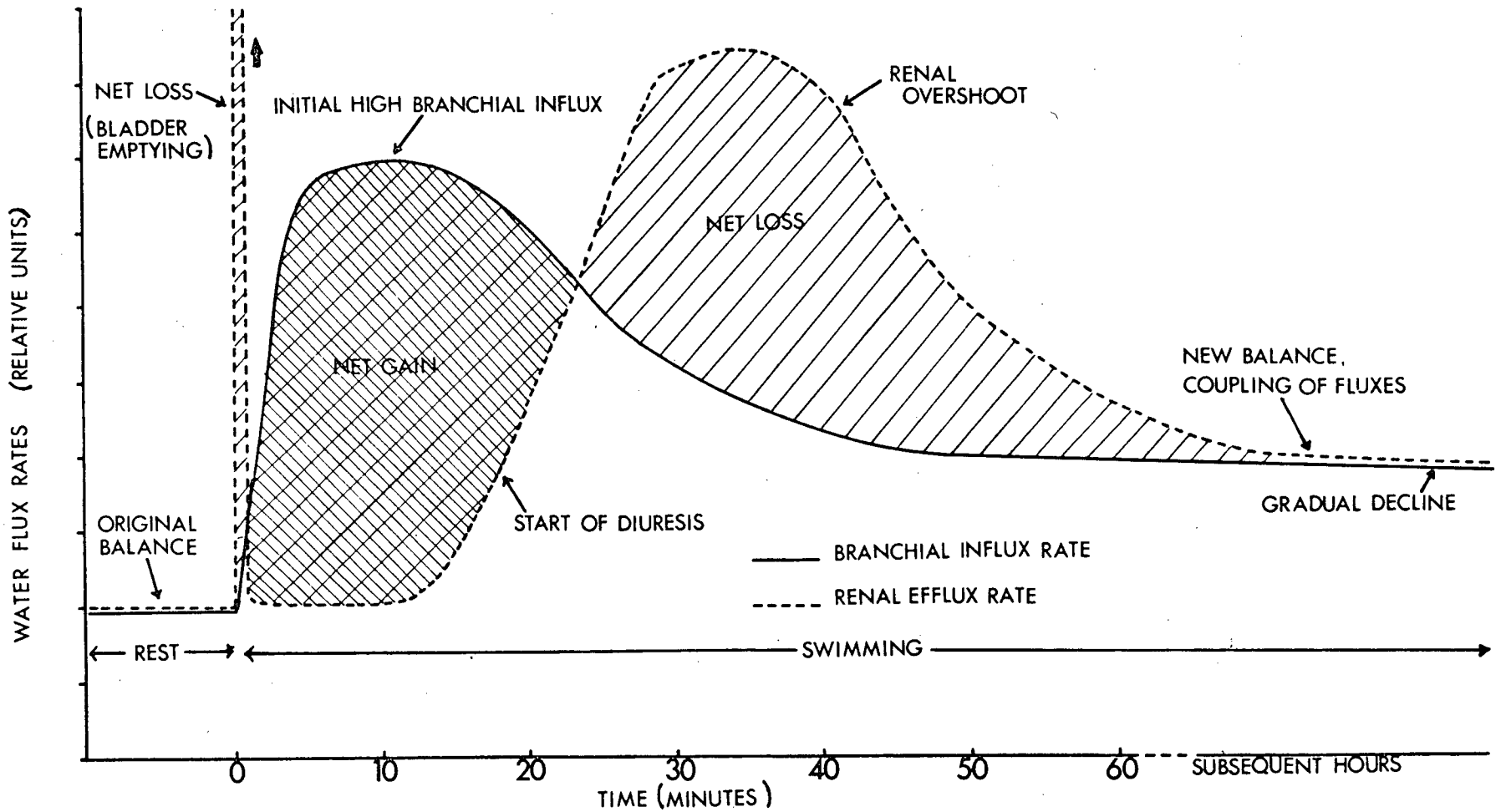
but mimicked the oscillations of experimentals until 30 minutes post-handling. It seems probable that the effects of handling were very similar but less severe than those of swimming. After 30 minutes, controls returned to their initial weight which was maintained through further activity (3 - 7 hours).

The data must be interpreted in terms of differences between net water influx across the gills and net water efflux through the urogenital papilla. The large magnitude of the weight loss after 1 minute indicated that it was probably caused mainly by a bladder emptying effect, although an increase in glomerular filtration rate resulting from an initial "overshoot" in systemic blood pressure at the onset of exercise may have contributed to the enhanced water efflux. Hammond (1969) has demonstrated in the lake trout, Salvelinus namaycush, that pressor effects can markedly elevate urine formation. The following weight gain and final weight loss must have been produced by inequalities of influx and efflux, but it is not clear whether the former, the latter, or both processes were changing. The urine production data is informative in this respect. After 60 minutes of swimming, the net weight loss relative to the gain at 30 minutes was 1.13 g/100 g. Even if no influx occurred after 30 minutes, a highly unlikely situation, the urine production over the first hour of swimming must have exceeded 11 ml/kg. It is probable that the actual rate was much higher. Therefore the weight change animals were obviously behaving as the examples of Fig. 28 which underwent a much greater diuresis during the first one or two hours of swimming than during subsequent hours. In such cases there was little, if any, augmentation of urine production

during the first 15 minutes of exercise. The fact that this type of response did not commonly occur at the onset of exercise in the urinary catheterized trout may again be attributed to their spontaneous activity and experience in the respirometer which could have "adapted" them to exercise prior to the swimming runs. The experimentals of the weight change group had, on the other hand, been quietly resting in darkened aquaria prior to obligatory swimming.

Thus by integrating the weight change data (Fig. 29) with the type of urinary response illustrated in Fig. 28, the following explanation may be developed. The net weight increment appearing between 5 and 30 minutes was caused by an extremely high branchial water influx without compensatory elevation of renal function (except for the initial bladder emptying effect). As swimming progressed, influx tended to decrease while efflux eventually rose, thereby producing the pronounced diuresis. This phenomenon finally overcompensated for the original water gain resulting in the weight loss at 60 minutes. As weights measured at 1 and 4 - 8 hours after the start of swimming were similar, a balance situation between branchial entry and renal output was reached soon after 60 minutes. This new balance, however, was set at a net body water content lower, and a water turnover rate higher, than those originally maintained. A tentative scheme graphically describing the above interpretation of water balance during exercise in an initially "naive" trout is presented in Fig. 30. The main features of the model are an initial high water influx, a delay in renal adaptation, a subsequent overshoot of the efflux parameter as influx falls, and an eventual equilibrium

Figure 30 A tentative model of the temporal changes occurring in water influx and efflux of the rainbow trout during the onset and continuation of exercise. It has been assumed that the animal is not already "adapted" to swimming.


NET LOSS
(BLADDER EMPTYING)
INITIAL HIGH BRANCHIAL INFLUX
RENAL OVERSHOOT
NET LOSS
NET GAIN
NEW BALANCE, COUPLING OF FLUXES
GRADUAL DECLINE
START OF DIURESIS
ORIGINAL BALANCE
BRANCHIAL INFLUX RATE
RENAL EFFLUX RATE
REST
SWIMMING
WATER FLUX RATES (RELATIVE UNITS)
0
10
20
30
40
50
60
SUBSEQUENT HOURS
TIME (MINUTES)

between influx and efflux rates. It may be hypothesized that as the animal reaches this new balance, it becomes "adapted" to exercise so that branchial and renal components of water movement are now linked in some manner and change simultaneously with further alterations of metabolic demand. A final gradual decline in the coupled fluxes has been incorporated into the model to represent the progressive decrease in water flux per unit oxygen uptake discussed previously (Fig. 27).

The triphasic weight fluctuation (Fig. 29) may be correlated with a reciprocal triphasic response in haemoglobin and plasma protein concentrations ascertained at the beginning of exercise in Salmo gairdneri by Stevens (1968 a). Stevens concluded that since the ratio of haemoglobin concentration to plasma protein concentration did not change during their individual fluctuations, the effects were caused by differential rates of water movement into the blood across the gills and out of the blood into the tissues. On the basis of the present work, this explanation may be modified to inequalities of branchial water influx and renal efflux rates. The decline in blood volume and rise in plasma sodium levels over one hour of exercise reported in Section I can also now be explained by the significant water loss observed by 60 minutes (Fig. 29). The maintenance of a reduced plasma volume during extended exercise through a decrease in water content offers several obvious benefits to the trout. On an osmoregulatory basis, the resulting concentration of plasma electrolytes would tend to compensate for the augmented branchial and renal losses of ions suffered during swimming

and thus help to preserve the constancy of the internal medium Haemoconcentration would elevate the blood oxygen capacity and therefore favourably alter the relationship between total oxygen transport and cardiac work. In addition, this effect would increase the buffering capacity of the blood, and therefore serve to reduce acidosis associated with greater muscular activity. The accompanying rise in blood viscosity would be unfavourable in itself, but would probably be offset by the overall decrease in systemic resistance to blood flow calculated to accompany exercise in trout (Stevens, 1968 a). The volume reduction of the circulatory compartment during activity could be facilitated by an ischemia of "white" muscle postulated by Stevens (1968 b), and for which some limited evidence was presented in Section I. This tissue is in any case largely anaerobic; if in fact it continued to receive a blood supply during swimming, there would occur a disadvantageous release of lactate into the circulation, which would tend to inhibit oxygen uptake by haemoglobin at the gills. A decrease in plasma volume during prolonged exercise would therefore seem to play an integral role in the physiological adjustment of the fish to the increased metabolic demands of swimming.

Cation levels were measured in all hourly urine samples from the four exercised trout. As noted previously (p. 156) the method of sample collection probably dampened concentration change associated with different exercise conditions; the data presented in Table XVIII therefore represents average ion levels for each hour estimated from the curves of concentration versus time (e.g. Fig. 31). Because of large magnitude differences between the samples from different animals, ranges rather than

Table XVIII. Urine cation levels and flows during each hour of the imposed swimming regime. Means and ranges for 4 fish.

Velocity (cm/sec)	10.7	10.7	10.7	21.4	21.4	21.4	10.7
Urine flow (ml/kg/hr)	7.04 3.83-10.17	5.45 2.01-8.62	5.43 3.20-8.35	10.49 4.20-20.21	6.26 4.00-8.35	6.75 5.40-10.06	4.33 3.30-5.33
Urine Na^+ (uEq/ml)	10.0 5.5-19.5	10.8 5.5-18.5	9.3 4.0-13.8	8.5 4.8-11.3	9.5 4.8-15.0	9.5 5.5-14.0	9.5 5.5-13.0
Urine K^+ (uEq/ml)	0.95 0.62-1.17	0.95 0.62-1.29	0.89 042-1.40	0.94 0.70-1.44	1.00 0.65-1.31	1.00 0.60-1.32	1.01 0.58-1.40
Urine Ca^{++} (uEq/ml)	0.96 0.30-1.27	0.99 0.30-1.24	1.06 0.30-1.40	1.18 0.95-1.75	1.37 0.80-2.05	1.39 0.70-1.95	1.33 0.72-2.03
Urine Mg^{++} (uEq/ml)	0.51 0.15-1.01	0.56 0.15-1.30	0.57 0.05-1.26	0.83 0.50-1.20	1.15 0.30-1.62	1.36 0.21-2.28	1.31 0.28-2.47

Continuation of regime:

Velocity (cm/sec)	10.7	10.7	32.1	32.1	32.1	10.7	10.7
Urine flow (ml/kg/hr)	5.32 3.50-9.27	5.27 3.55-10.06	10.89 5.20-24.86	10.84 5.90-23.09	6.48 5.07-7.50	4.48 2.60-6.25	4.22 2.80-6.25
Urine Na^+ (uEq/ml)	9.6 5.8-13.0	9.4 4.5-14.7	9.5 13.8-16.5	8.5 3.0-14.3	8.5 6.5-10.5	9.3 7.5-10.5	9.9 7.5-11.8
Urine K^+ (uEq/ml)	0.99 0.45-1.36	0.98 0.35-1.44	1.05 0.26-1.74	1.07 0.42-1.74	1.01 0.60-1.47	0.99 0.68-1.36	1.01 0.60-1.43
Urine Ca^{++} (uEq/ml)	1.20 0.66-1.93	1.15 0.56-2.07	1.15 0.50-2.11	1.15 0.60-2.07	1.26 0.62-2.17	1.32 0.73-2.32	1.31 0.80-2.40
Urine Mg^{++} (uEq/ml)	1.15 0.35-2.34	1.00 0.32-2.10	0.86 0.35-1.70	0.97 0.25-1.90	1.25 0.20-2.45	1.39 0.38-2.90	1.33 0.54-2.60

Table XVIII. (Continued)

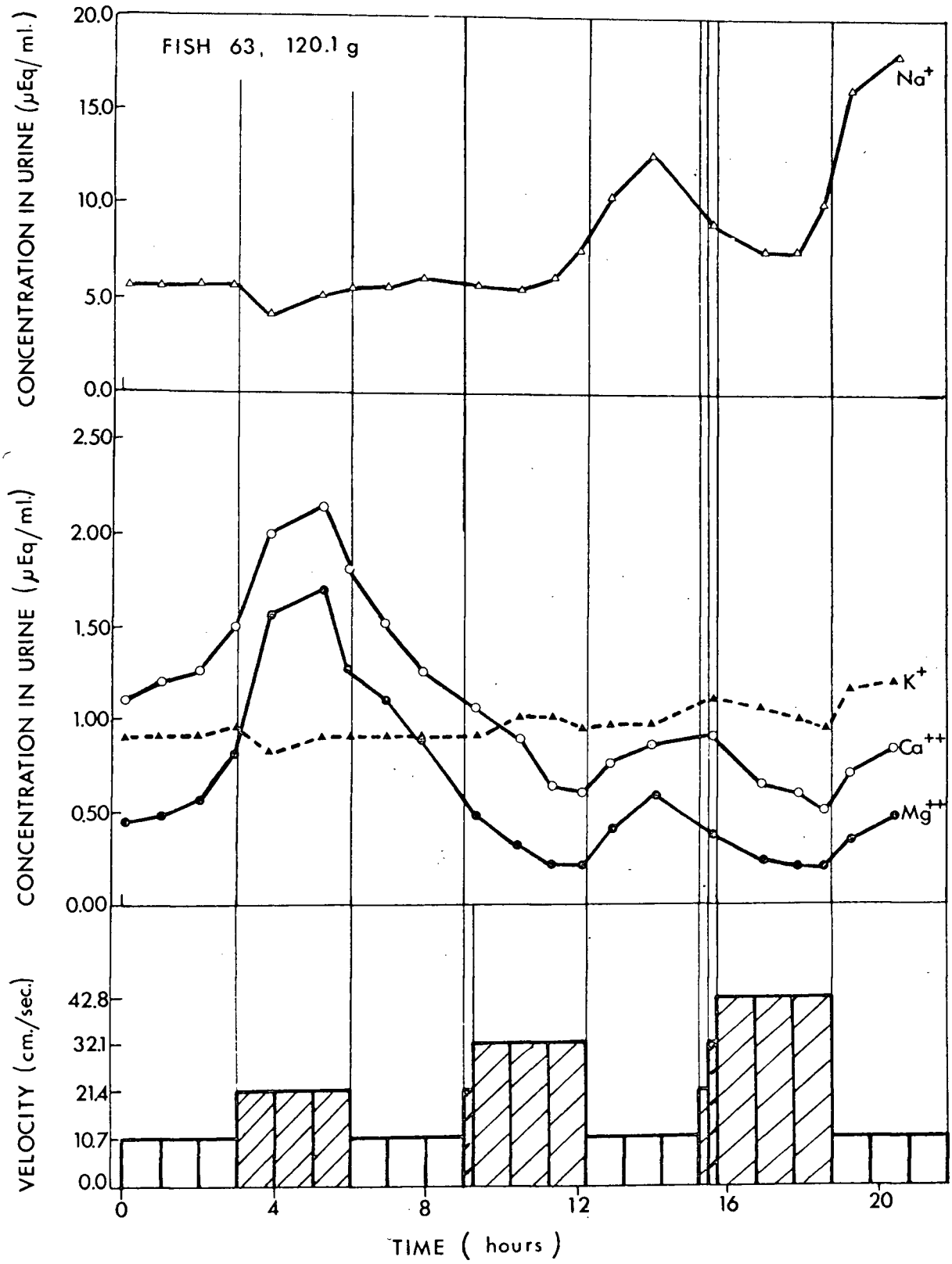
Continuation of regime:

Velocity (cm/sec)	10.7	42.8	42.8	42.8	10.7	10.7	10.7
Urine flow (ml/kg/hr)	3.48 2.97-4.50	7.68 5.40-11.10	7.91 5.50-11.50	6.84 5.30-7.70	2.40 1.40-3.09	2.23 1.20-2.95	2.62 2.00-3.60
Urine Na^+ (uEq/ml)	10.5 9.5-11.5	9.4 8.6-10.00	9.0 7.5-11.2	8.3 7.0-10.0	15.3 12.0-18.0	17.4 12.7-19.0	-
Urine K^+ (uEq/ml)	1.17 1.00-1.32	1.11 0.97-1.22	1.13 1.05-1.15	1.16 0.98-1.38	1.17 1.06-1.30	1.20 1.05-1.38	- -
Urine Ca^{++} (uEq/ml)	1.38 0.83-2.22	1.13 0.66-1.85	1.04 0.60-1.56	1.09 0.55-1.90	1.46 0.63-3.11	1.65 0.77-3.11	- -
Urine Mg^{++} (uEq/ml)	1.14 0.46-1.70	0.62 0.25-1.00	0.54 0.19-1.00	0.73 0.20-1.67	1.54 0.33-4.03	1.66 0.50-4.03	- -

standard errors have been presented as estimates of variability. Such diversity of values between individuals is characteristic of teleost urinary ion data (Hammond, 1969; Hunn and Willford, 1970).

Sodium, potassium, calcium, and magnesium concentrations in "resting" samples (10.7 cm/second) fell well within the ranges reported by other workers for this species (Fromm, 1963; Holmes and Stainer,1966; Hunn,1969; Hunn and Willford, 1970). During periods of exercise there was a tendency for the concentrations of all four minerals to fall as urine volume increased. The reverse occurred upon termination of swimming; these effects become more pronounced during the latter part of the exercise regime. In individual animals, sodium and potassium levels varied only moderately and generally in relation to alterations in urine flow. Calcium and magnesium concentrations,however, demonstrated large simultaneous fluctuations which did not always correspond to volume changes (Fig. 31). Similar oscillations in the concentrations of these two divalent cations have been observed in the urine of the lake trout, Salvelinus namaycush (Hammond,1969). In that study, these variations were associated with fluctuations in the tubular reabsorption of the ions. The data are suggestive of some coupling in the active transport of calcium and magnesium across the proximal tubule; Hickman and Trump (1969) have in fact proposed that the transport of the two electrolytes through the tubular epithelial cell is effected by closely related, if not identical, ATP requiring systems. However, the reason for apparent temporal fluctuations in the activity of this mechanism is not clear.

Figure 31 Changes of cation concentrations in urine from a single trout over the continuous swimming regime of Section III. Variations in sodium, and to a lesser extent, potassium levels were associated with urine volume alterations under different exercise conditions. Calcium and magnesium levels, however, fluctuated in synchrony, but independently of urine flows or exercise conditions.


FISH 63, 120.1 g
CONCENTRATION IN URINE (μEq/ml.)
20.0
15.0
10.0
5.0
0.0
Na+
CONCENTRATION IN URINE (μEq/ml.)
2.50
2.00
1.50
1.00
0.50
0.00
K+
Ca++
Mg++
VELOCITY (cm./sec.)
42.8
32.1
21.4
10.7
0.0
0
4
8
12
16
20
TIME (hours)

The electrolyte concentration changes observed in the present study indicate that the diuresis associated with exercise was somewhat different from that occurring after handling (R.M.Holmes,1961), severe hypoxia (Hunn,1969), or anaesthesia (Hunn and Willford,1970). In all these latter situations, high flow rates were associated with marked increases in electrolyte concentrations; the opposite occurred during swimming. Diuretic states involving increased urine ion levels probably reflect profound disturbances in tubular mineral reabsorptive functions as well as in glomerular filtration rate. The exercise diuresis,however,appears to represent merely an exaggeration of variations which normally can be observed in resting fish (Hammond,1969; Hickman and Trump,1969). Such alterations are mediated by changes in filtration with only minimal alterations in tubular salt conservation functions. The efficiency of water reabsorption, however, tends to decrease at high glomerular clearance rates, thereby creating lower electrolyte concentrations at high urine flows (Hammond,1969). Increased urinary discharges during swimming therefore seem to be an example of simple "water" diuresis (Hunn,1969) uncomplicated by perturbations of tubular ion transport mechanisms. It is interesting to note that non-smolting rainbows may be considered as demonstrating a similar type of diuresis relative to smolting individuals (Holmes and Stainer,1966). Smolting fish, like resting fish, are thought to have a lower urine production because of a reduced branchial water permeability.

As previously noted (Table XI) there occurred an increased net urinary efflux of sodium during swimming.The magnitude of the renal loss,however (2.0 - 4.0 ug/100 g/minute)

was small relative to either the simultaneous unidirectional (28.50ug/100 g/minute) or net efflux (12.14 ug/100 g/minute) across the branchial epithelium (Table III). The elevated renal loss was in fact common to all four cations measured; the averaged responses of animals #55, #63, #65 have been summarized in Fig. 32. In a diuresis of this nature, urinary salt excretion should be dependent largely on urine volume as percentage tubular reabsorption of ions will remain relatively stable (Hammond,1969). Comparison of Fig. 32 with Fig. 26 indicates that, at least for sodium and potassium, this relationship was confirmed in the present data. Increases in calcium and magnesium excretion with exercise were less pronounced due to the variations that were independent of exercise which apparently occurred in the activity of the reabsorptive mechanism for these minerals. It may be noted that there transpired gradual declines in urinary sodium and potassium loss rates under "resting" conditions (10.7 cm/second) over the duration of the experimental protocol (Fig. 32) which were entirely dependent on the concomitant decreases in urine flow. It has previously been argued that the latter effect resulted from the implementation of compensatory mechanisms during extended exercise to reduce the water permeability of the gills. Thus renal adaptation to reduce the urinary component of mineral deficit during exercise may be simply a secondary consequence of branchial permeability adjustment.

Figure 32 The net renal excretion of four cations during the continuous swimming regime of Section III. Each bar represents the averaged responses of trout #55, #63, and #65. Crosshatching indicates periods of exercise.

VELOCITY (cm/sec)
URINARY ELECTROLYTE LOSS (μEq/kg/hour)
0.0
10.7
21.4
32.1
42.8
0.0
3.0
6.0
9.0
Mg++
0.0
3.0
6.0
9.0
12.0
Ca++
0.0
3.0
6.0
9.0
K+
0.0
5.0
50.0
75.0
100.0
Na+
0
4
8
12
16
20
0.0
1.0
2.0
3.0
(μg/100g./min.)

SUMMARY III

1. Post-catheterization diuresis was associated with elevations of oxygen consumption and ventilation rate in rainbow trout. All three parameters had stabilized by 45 hours after operation.
2. Urine flow, considered a measure of branchial water entry, markedly increased during periods of exercise in a swimming respirometer. Alterations in water flux generally corresponded with changes in oxygen uptake.
3. In individual trout, over a range of oxygen consumptions from sub-standard to nearly active metabolic rates, oxygen and water influxes exhibited a highly significant positive correlation. The results were indicative of covariation of the water and oxygen permeabilities of the branchial exchanger.
4. Compensations apparently occurred to reduce branchial water permeability during exercise. In a few cases, high urine flows at the beginning of a swimming period were greatly reduced after 1 or 2 hours. Weight change experiments indicated that this response was characteristic of fish which were not "adapted" to exercise, and resulted from an extremely high branchial water entry which subsequently declined. In all animals, water entry per unit oxygen uptake tended to decrease over the duration of the imposed exercise regime as swimming experience increased.
5. Weight changes were determined during exercise as measures of water balance in trout which were "naive" to swimming. A triphasic fluctuation in body weight was observed as swimming time was prolonged; an initial loss (1 minute) was followed with a net gain by 30 minutes, and a final decrement below resting

level by 60 minutes. There occurred only a very slight further reduction over the following 3 - 7 hours of exercise.

6. Integration of weight change data with urine flow information permitted construction of a tentative model of water regulation by the previously unexercised trout during swimming. The main features of this scheme are: an immediate bladder emptying at the onset of activity (net loss); an initial high branchial water influx without appreciable modification of renal output (net gain); a subsequent decline in influx rate and rise in urinary efflux rate which eventually overcompensates for the water accumulation (net loss); and an eventual equilibrium between water entry and exit set at a higher turnover rate and lower body water content than during rest.

7. This net water deficit can account for the decrease in blood volume and increase in plasma sodium levels noted during exercise in Section I.

8. Sodium and potassium levels in urine collected during swimming were generally stable but tended to decrease at high urine flows and increase at low flows. However calcium and magnesium concentrations underwent simultaneous fluctuations which did not always correspond to urine volume alterations.

9. The augmented urine output of exercise thus appeared to represent a simple "water" diuresis resulting from elevated branchial permeability, and uncomplicated by disturbances in tubular electrolyte reabsorptive functions.

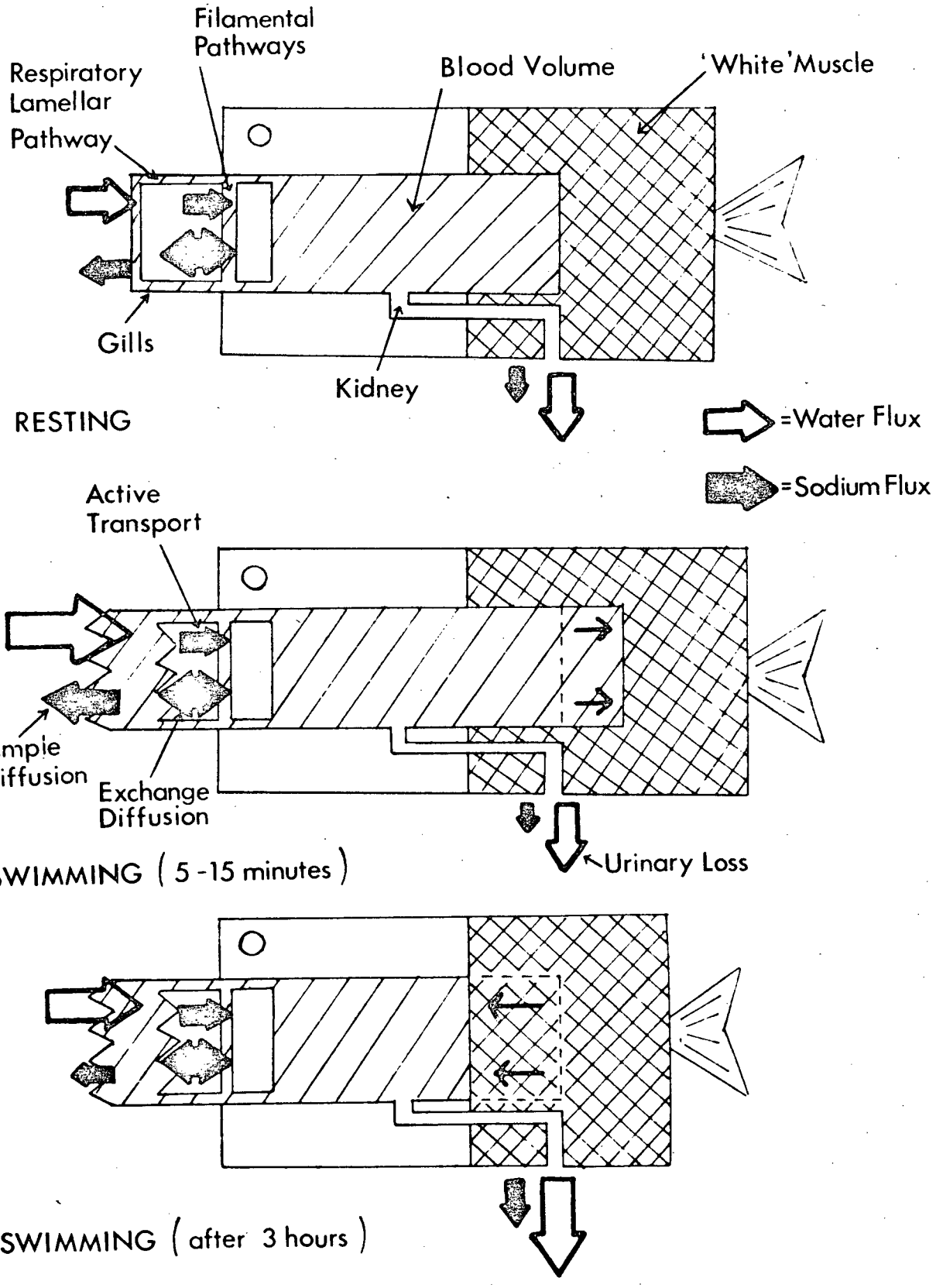
10. The urinary excretion of all four cations which were measured increased during swimming, although this trend was only well defined for sodium and potassium. Renal sodium efflux (2.0 - 4.0 ug/100 g/minute) associated with activity was small compared to branchial sodium net loss rate (12.14 ug/100 g/minute).

GENERAL DISCUSSION

The present study has demonstrated that increases in oxygen uptake across the gills of the fresh water adapted rainbow trout were associated with similar elevations of sodium and water movements through the branchial surface in the direction of their activity gradients. During extended exercise, mechanisms were apparently invoked to reduce these hydromineral fluxes and thus alleviate the osmotic penalty of swimming. The most important inferences and conclusions from the data have been summarized in Fig. 33 for previously unexercised animals at rest, during initial activity (5 - 15 minutes), and after "adaptation" to exercise (3 hours).

In these models, it has been assumed that back-transport of sodium is negligible in the trout gill. Thus at rest, branchial sodium transfer comprises a large exchange diffusion component and a small active influx element, both presumably generated in the interlamellae epithelium served by the filamental blood pathways; a small simple diffusional efflux occurs across the thin walled respiratory lamellae. This area is also the site of a constant influx of water which is eliminated through the kidney together with a small urinary ion loss. During initial swimming, the enhanced cardiac output causes increased circulatory perfusion of the respiratory lamellae and thus elevates the total gas exchange capacity of the gills. This redistribution of blood has no apparent effect on the carrier mediated sodium transport mechanisms

Figure 33 Models summarizing information on sodium and water balance in the rainbow trout presented in this study. Animals are illustrated at rest, shortly after the onset of activity (5 - 15 minutes), and after prolonged exercise. Blood volume changes are indicated by thin arrows and flux rates by thick arrows; the size of the latter represents the magnitude of the flux rate. It has been assumed that the animal is not already "adapted" to swimming.


Filamental Pathways
Respiratory Lamellar Pathway
Blood Volume
'White' Muscle
Gills
Kidney
RESTING
=Water Flux
=Sodium Flux
Active Transport
Simple Diffusion
Exchange Diffusion
Urinary Loss
SWIMMING (5 -15 minutes)
SWIMMING (after 3 hours)

supplied by the filamental pathways, but markedly augments the diffusional loss of the ion across the lamellar surface, thereby creating a sodium deficit. Water entry is also greatly elevated without appreciable change in renal output. An increased blood volume results. After prolonged exercise, the pattern of gill blood flow probably remains similar to that at the start of swimming. However compensatory reductions in branchial hydromineral permeability have returned the diffusional outward movement of sodium to resting levels. Active transport and exchange diffusion are again invariant. Urine flow now equals the rate of water entry; an increased renal sodium excretion accompanies the diuresis. Before this state of water equilibrium was attained however, urinary discharge had exceeded branchial influx, thereby reducing the blood space below resting volume. This effect was apparently accompanied by an increase in plasma sodium levels (through the net water loss) and an ischemia of "white" muscle. In summary, the results indicated that the effective permeability of the branchial exchanger was an accommodation between the demands of respiration and osmoregulation, and that both the dynamics of this compromise and the functioning of other systems could be influenced by compensations necessary to preserve sodium and water balance.

The discussions presented with the individual sections of this thesis have dealt largely with evaluation of the data obtained in terms of the salt and water balance situation of the animal under different exercise conditions, and with development of the concept of a respiratory/osmoregulatory compromise at the gills. The intent of this general discussion is to

provide a more speculative treatment of certain problems raised by the results. Firstly, the study has provided ample evidence that elevations of oxygen uptake hamper water and ion regulation; it is therefore pertinent to ask whether osmoregulatory necessities could in turn have a similar limiting effect on gas exchange. Secondly, the enactment of a compensatory mechanism to restrict the osmotic deficit of activity implies both the presence of a system for detection of internal misalignment and the activity of a mediator to change gill permeability; the possible natures of both these factors will be considered. Finally, some suggestions will be advanced on the actual way in which branchial permeability could be modified.

In discussing the interaction of gas exchange on the one hand, and ion and water exchange on the other hand, it is convenient to consider changes in branchial permeability in terms of primary (occurring at the gills) and secondary (occurring as a consequence of branchial phenomena) effects on respiration and osmoregulation. Thus the greater efflux of sodium and influx of water across the gills during exercise were primary penalties on hydromineral balance, while the increased urinary loss of ions was apparently a secondary problem resulting from the elevated permeability of the exchange surface. The present investigation has utilized gas exchange as the essentially independent variable and ion and water exchange as the dependent variables, and thus provides no evidence for parallel penalties on respiratory balance. Two recent studies have indicated, however, that the obligations of osmoregulation can exert at least severe

secondary effects on oxygen metabolism. Rao (1968) with Salmo gairdneri, and Farmer and Beamish (1969) with Tilapia nilotica, performed almost idential experiments in which the oxygen consumption of animals acclimated to and exercised in a variety of salinities were measured at several different swimming speeds. Both studies found that the metabolic rate at all velocities was lowest in an isosmotic salinity. Through the assumption that the cost of osmoregulation equalled zero in this medium, the proportion of total oxygen consumption devoted to hydroelectrolyte regulation in other salinities could be calculated. For fresh water, this figure was approximately 20% at rest, and did not change with increasing swimming speeds. Thus the cost of osmoregulation rose in proportion to the oxygen flux across the gills. This greater metabolic demand of osmoregulatory functions is an obvious secondary penalty on respiration caused by elevations in branchial permeability.

The present study provides some indication, at least for the rainbow trout, of the cause of this increased osmoregulatory cost. Branchial sodium influx did not change during exercise. As the role of back-transport in Salmo gairdneri gills is probably not important, it would appear that active sodium uptake remained constant. Indeed, the diversion of a greater blood flow through the respiratory lamellae with concomitant decreased perfusion of the central sinus filamental pathways serving the ion transporting cells would not favour an augmentation of active influx. Wood and Randall (1971) have in fact demonstrated in the southern flounder, Paralichthys lethostigma, that induced anaemia,

a treatment thought to produce changes in branchial blood distribution similar to those occurring during exercise, caused a decreased rate of carrier mediated sodium transport across the gills. The trout apparently overcomes the initial branchial sodium deficit of exercise through a reduction of passive permeability, a mechanism which may well be far less expensive yet achieve the same effect as an elevation of active influx. Branchial water entry, however, increased in approximately linear fashion with oxygen consumption, and was only partially reduced by compensatory changes. The expense of renal water elimination would, therefore, seem a more important factor in the increase of osmoregulatory cost with rising oxygen uptake, for both glomerular filtration rate and the active tubular reabsorption of some electrolytes probably increases in proportion to water influx.

There exists no direct evidence that hydromineral regulation can have a primary limiting effect on oxygen uptake at the gills. The existence of such a phenomenon would mean that the oxygen consumption for a given swimming speed was less than that optimal to support that exercise condition because osmoregulatory considerations prevented the necessary increase in effective gill permeability. A somewhat oblique indication that there is no primary inhibition of oxygen exchange is provided by the reports of Rao (1968) and Farmer and Beamish (1969) that the cost of swimming is the same in an isosmotic solution as in fresh water. However it must be noted that these workers calculated the oxygen consumption devoted to exercise through subtraction of the cost of osmoregulation from the difference between standard and swimming

metabolic rates; the cost of osmoregulation had previously been derived as stated above. One could object that the cost of osmoregulation was in fact greater than that computed, but was not manifested because the animals assumed an oxygen debt rather than further increasing their gill permeability and thus compounding their osmoregulatory disequilibria. This criticism is largely negated by the fact that Rao (1968) allowed his animals to come into a steady state at a particular activity level. However experiments in which both the oxygen uptake during the swimming and the amount devoted to repayment of any anaerobically produced debt after exercise are compared between fish in fresh water and isosmotic environments should demonstrate more clearly whether or not a primary depressant effect of osmoregulation on oxygen uptake is built into the physiology of the trout. That this phenomenon may not exist, i.e. that the branchial compromise is weighted in favour of gas exchange, seems reasonable inasmuch as hydromineral imbalance may be at least partially corrected by permeability and blood volume changes, while there apparently exists no parallel mechanism for reducing the metabolic cost of a certain exercise level.

The misalignment detection system which must be responsible for the initiation of compensatory osmoregulatory effects during swimming activity may act in one of two ways. Firstly, a direct sensing of disturbances in the hydromineral characteristics of the internal environment may occur. Such an arrangement, with osmoreceptors in the hypothalamus responsive to changes in plasma osmotic pressure, and volume receptors in renal afferent arterioles and the left atrial

wall sensitive to blood pressure variations, has been described in mammals (D.M.Woodbury,1965). Moreover, there exists limited evidence that an analogous, although qualitatively different, system may exist in teleosts. Infusion of hypotonic or hypertonic sodium chloride solutions into the goldfish, Carassius auratus, produced alterations of branchial unidirectional flux rates in directions tending to restore the ionic equilibrium of the plasma; mannitol injections of the same tonicity demonstrated that the responses were mediated by neither changes in extracellular space (volume) or osmotic pressure, but by the disturbance of internal electrolyte concentrations (Bourguet et al.,1964). Mayer and Nibelle (1970) have observed similar effects in the eel, Anguilla anguilla., The administration of the same dilute and concentrated saline loads produced a diuresis and a antidiuresis respectively in the goldfish; the osmotic diuresis resulting from mannitol injections however prevented elucidation of the specific stimuli involved in the renal responses (Bourguet et al.,1964). These workers postulated that the observed overall effects could well be caused by the direct action of circulatory ion levels on the activity of endocrine organs whose secretions were responsible for water and electrolyte economy. This concept is supported by the work of Sage (1968) which revealed a greater secretory rate by Xiphophorus sp. pituitary paralactin cells cultured on a dilute medium (112 mEq Na^+/L) than on a concentrated medium (160 mEq Na^+/L); paralactin is an important factor controlling sodium outflux in fresh water (Ensor and Ball,1968). However the recent study of Richards and Fromm (1970) on the isolated perfused hemibranchs of Salmo

gairdneri suggested a direct action of plasma concentrations on branchial sodium fluxes. Whatever the actual causes, there does seem to exist some detection of plasma salt levels intrinsic to the function of certain tissues and not necessarily dependent on discrete receptor sites.

The second possible monitoring system would be sensitive to some internal change(s) attendant to exercise other than hydroelectrolytic disarrangement. The present study indicates that changes in plasma composition occurring during swimming were slight relative to the experimental disturbances induced in the above investigations. In addition, plasma sodium levels tended to rise above resting values due to the blood volume reduction, a deviation which would elevate sodium efflux if the mechanisms shown by these workers were in operation. Thus the second type of detection mechanism may well be functional during swimming, the electrolyte sensitive system having perhaps been designed for the more drastic changes which occur upon entry into different salinities. It is possible that catecholamines, the factors largely responsible for cardiovascular adaptation to exercise, are also instrumental in the osmoregulatory adjustment. In such a scheme, the increased circulatory levels of adrenaline and noradrenaline during swimming (Nakano and Tomlinson, 1967) would be the internal "disturbance" detected, and would be responsible for the branchial permeability alterations. The plan offers an automatic adaptation to exercise without the necessity of large prior reductions of internal electrolyte levels. The stimulatory effect of catecholamines on the secretory activity of the anterior hypophysis in mammals provides tentative support for this hypothesis (Russel, 1965);

pituitary hormones are of great importance in teleost osmoregulation.

In recent years, it has become clear that osmoregulation in teleosts is an exceedingly intricate function dependent on an array of endocrine mechanisms. Perhaps because of the severe osmotic stress imposed by the aqueous environment, water and ion regulation in fish appears to be a much more complex process than in mammals. Three main endocrine systems, secretions of the anterior pituitary-adrenocortical axis, adenohypophysial paralactin ("fish prolactin"), and neurohypophysial octapeptides, are now known to exert control over hydromineral metabolism. Other agents, such as thyroid hormone, growth hormone, renin, and secretions of the ultimobranchials and urophysis may embroider the pattern laid down by these demonstrated influences, but their exact roles in hydroelectrolyte economy are not well understood at present. Consequently, it would be naive to suggest that the adjustments which reduce the osmoregulatory problems of swimming in trout are attributable to a single definite source. The following discussion will consider the possible roles, in the observed compensatory effects, of each of the three systems presently known to be of importance in water and salt balance; this treatment in no way precludes the intervention of other hormones whose actions have not yet been defined.

Adrenocorticosteroids, of which cortisol seems to be of major osmoregulatory importance, are released from the interrenal gland, probably under the stimulation of adrenocorticotropin (A.C.T.H.) from the anterior lobe of the pituitary; the two endocrine organs are thought to be linked

in a negative feedback relationship (Chester Jones et al.,1969). Plasma cortisol levels increase during exercise in Salmo gairdneri (Donaldson and McBride,1967), and thus this hormone would seem a prime candidate for involvement in the adaptation to swimming; however none of its demonstrated effects are in accord with the compensations (reductions in branchial sodium efflux rate and water permeability) noted in the present investigation. Early studies on the trout in fact indicated that adrenal steroids were generally deleterious to osmoregulatory homeostasis (Holmes,1959; Holmes and Butler,1963), although this work is now suspect because of the unphysiologically high doses of hormones administered. More recent investigations in the eel, Anguilla anguilla, using surgical adrenalectomy and cortisol therapy, have revealed that cortisol maintains the normal function of the active branchial sodium pump, promoting efflux in sea water and influx in fresh water; however it has no effect on efflux in fresh water. Furthermore, A.C.T.H. and cortisol augment branchial water permeability in the goldfish, Carassius auratus, without accompanying diuresis (Lahlou and Giordan,1970). This latter action is reminiscent of the observed weight gain during the first few minutes of swimming in the trout (Figs. 29 and 30). Thus, on present evidence, it would appear that the anterior pituitary-interrenal axis is not involved in the osmoregulatory adjustment to exercise, and may in fact contribute to water balance problems at the onset of activity. However the mobilization of cortisol during swimming is perhaps of overriding importance in carbohydrate metabolism.

Paralactin, originating from the teleost adenohypophysis, or mammalian prolactin which mimics its action, have been shown to limit the passive efflux of sodium, presumably largely at the gills, in a variety of teleosts (e.g. Maetz et al.,1967 a, b; Ensor and Ball,1968; Ball,1969). It is now evident that fish prolactin is not essential for life in fresh water in all species,and that its function, rather than as an "all or nothing survival mechanism", lies in the modulation, through simple permeability effects, of sodium efflux across the gills to maintain ionic homeostasis (Ball, 1969). Consequently this hormone could be of major importance in reducing the augmented branchial sodium loss which accompanies swimming in trout. However mammalian prolactin (and presumably paralactin), like A.C.T.H. and cortisol, tends to elevate branchial water permeability, at least in the goldfish (Lahlou and Giordan,1970) and the plains killifish Fundulus kansae (Potts and Fleming,1970).

Neurohypophysial octopeptides are presently of disputed osmoregulatory function in fresh water teleosts. They may produce an elevation of branchial sodium influx (Maetz and Julien,1961) or of both unidirectional fluxes (e.g. Motais and Maetz,1964; Maetz et al.,1964 b); and either a diuresis (e.g. Maetz et al.,1964 b;Lahlou and Giordan,1970) or an antidiuresis (Holmes,1961; Hammond,1969). It is probable that much of this disagreement arises from the failure of many investigators to adequately control stress or use physiological doses of purely teleostean principles (arginine vasotocin and isotocin). Neither of the reported salt balance effects are in agreement with the compensations observed in the

present study, but the whole problem of neurohypophysial influence on branchial sodium movements seems worthy of further investigation with more prudent techniques. Perks (1969) has in fact suggested that arginine vasotocin may act to control sodium fluxes across the gills during exercise. The most careful water balance study appears to be that of Hammond (1969) on the lake trout, Salvelinus namaycush, who demonstrated a marked antidiuresis, mediated almost entirely by a reduction in glomerular filtration rate, following intravascular infusion of only nanogram amounts of arginine vasotocin. Unfortunately Hammond did not measure branchial water entry or weight variation so could not conclude whether the decreased urine flow was a direct renal effect of the hormone or a secondary phenomenon resulting from a decrease in branchial water permeability. Lahlou and Giordan (1970), however, have recently demonstrated that at least pharmacological doses of the same hormone greatly depress the branchial water exchange of Carassius auratus. The implication of neurohypophysial principles in the correction of hydromineral disequilibrium during exercise therefore remains quite possible. One may conjecture that arginine vasotocin serves primarily to limit water influx while adenohypophysial prolactin reduces passive sodium efflux across the gills. The former agent can act almost immediately (less than 15 minutes after injection (Hammond,1969)), but the time course of the paralactin influence is ill-defined; however on the basis of the work of Ensor and Ball (1968), it would seem to exert permeability effects within 4 hours. These latencies do not offer serious disagreement to the time courses of compensatory phenomena

observed in the present study.

The mechanism by which endocrine agents act on the gill tissue raises fundamental questions about the manner of hormone action on membranes which are beyond the scope of this discussion. However one may speculate on the nature of the effective permeability reduction to sodium and water during extended exercise. Changes which are not dependent on a synthetic modification of cell structure and which are easily reversible would seem least costly and most practical. Three possibilities may be advanced. Firstly, divalent cations such as calcium and magnesium have long been known to depress cell permeability by reducing the repulsion of fixed anions in the membrane structure and therefore allowing closer apposition of organic molecules (Potts and Fleming,1970). Furthermore, the external administration of calcium ions reduced sodium loss in the ammocoete, Lampetra planeri,(Morris and Bull,1968) and branchial water exchange in the plains killifish, Fundulus kansae (Potts and Fleming,1970). A hormonally stimulated alteration in divalent cation metabolism could be involved in the permeability changes during swimming. Secondly, the secretion of mucus by branchial epidermal cells may change the effective diffusion distance and resistance of the exchange surface. Moreover, there exists some evidence that paralactin stimulates mucus production at the gills (see Ball,1969); this action may explain its effect on passive sodium efflux but not on branchial water permeability (Lahlou and Giordan, 1970). Finally an involvement of the cytoplasmic filaments, which are similar to the myofilaments of smooth muscle (Rhodin, 1964; Newstead,1967), found in the pillar cells of the respir-

atory lamellae may be suggested. It is possible that contraction of these elements could in some way alter the tension on the overlying epithelium and basal lamina to reduce their permeability; the known stimulatory action of neurohypophysial peptides on smooth muscle offers a possible mediation of this effect.

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