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The Influence of Swimming Activity on Sodium Balance in the Rainbow Trout (*Salmo gairdneri*)

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Summary. 1. Radiotracer and cannulation techniques have been used to measure branchial fluxes and internal distributions of sodium in freshwater adapted rainbow trout at rest, during one hour of swimming activity, and during one hour of post-exercise recovery.

2. Activity was imposed by manual chasing in a small chamber. Ventilatory and cardiovascular changes occurring during and after this procedure were similar to those associated with normal swimming.

3. Sodium efflux rate equalled influx at rest, increased 70% during exercise, and returned to slightly below resting levels during recovery; influx rate remained invariant under the three treatments. The switch from a negative to a positive branchial sodium balance at the end of exercise occurred extremely rapidly.

4. Despite the branchial deficit, plasma sodium levels tended to rise in active fish. This effect was associated with an apparent reduction in blood volume.

5. Terminal concentrations of sodium and water in "white" muscle did not differ significantly among treatment groups.

6. Expansion of the radiosodium space in active and recovering trout exceeded that in resting animals because of a faster rate of dispersal of influxed sodium out of the plasma compartment into tissues other than "white" muscle.

Introduction

Unfavourable passive fluxes of ions and water between internal and external milieux occur across the respiratory epithelia of water breathing vertebrates. The possession of a gas exchanger with a relatively small functional "size" or permeability may be viewed as an adaptation to this osmotic problem. Thus the respiratory surface per unit body weight in teleosts is only $\frac{1}{2}$–$\frac{1}{7}$ that in mammals (Hughes, 1966; Hildebrandt and Young, 1965), while diffusion distances between ventilatory medium and blood are up to six times greater in the gill than in the lung (Newstead, 1967; Hildebrandt and Young, 1965). However, this limitation of exchange capacity has not, by itself, been sufficient to fully control water and electrolyte movements. Tolerance of the osmotic deficit has been effected through the development of compensatory mechanisms which may utilize up to 30% of the organism's total oxygen consumption (Rao, 1968; Farmer and Beamish, 1969). Active transport apparatus on

the gills 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 environment have all been necessitated by the use of an aqueous respiratory medium.

These considerations indicate that the anatomical development of the teleost branchial surface is limited by the level of hydromineral exchange which the animal can tolerate. Yet it is known that fish are able to elevate their oxygen uptake ten times or more during exercise (Brett, 1964) through increases in ventilation, cardiac output, and the oxygen transfer factor of the gills (Randall *et al.*, 1967; Davis, 1968). The latter expression refers to units of gas transferred per unit gradient and is affected by the resistance to diffusion of the entire pathway between ventilation stream and red blood cell. Thus teleosts can apparently vary the effective "size" of the respiratory epithelium within structural limits; the concept that, at any one time, this branchial permeability must represent a dynamic compromise between respiratory and osmoregulatory demands has been advanced repeatedly (Steen and Kruysse, 1964; Randall *et al.*, 1967; Taylor *et al.*, 1968; Kirschner, 1969).

Such permeability changes are probably mediated by the double nature of the circulation in a typical branchial filament. Several workers (Steen and Kruysse, 1964; Richards and Fromm, 1969) have demonstrated *in vitro* that blood may pass from afferent to efferent filamental arteries through either the thin walled, high surface area respiratory lamellae, or through alternate shunts in the body of the filament overlain by the thick interlamellae filamental epithelium (Conte, 1969). Considerations based on known dimensions, surface areas, widths of blood/water barriers, and routes of water flow (Hughes and Grimstone, 1965; Hughes, 1966; Newstead, 1967) indicate that the lamellar blood pathway will be most effective in promoting gas exchange and least effective in curtailing water and electrolyte movements; conversely, the central filamental shunts will be virtually non-functional in respiration but highly efficient in preventing passive hydromineral fluxes between plasma and external medium. Catecholamines vasodilate the gills and stimulate lamellar perfusion, while acetylcholine increases branchial resistance and favours filamental flow (Krawkow, 1913; Keys and Batemen, 1932; Ostlund and Fange, 1962; Steen and Kruysse, 1964; Richards and Fromm, 1969). Thus the augmentation of circulatory levels of adrenaline and noradrenaline during swimming activity (Nakano and Tomlinson, 1967) may well facilitate the decrease in vascular resistance of the gills and elevation of transfer factors for respiratory gases observed during exercise in salmonids (Randall *et al.*, 1967; Randall and Stevens, 1967; Stevens, 1968; Davis, 1968).

An ionic and osmotic penalty should, by the above arguments, be concomitant with the increased rate of gas exchange accompanying exercise. The primary objective of the present investigation was to determine whether such a phenomenon does in fact occur. Preliminary studies by Randall *et al.* (1972) demonstrated that activity and catecholamine administration increased the rate of release of injected radiosodium from the gills of rainbow trout in freshwater. However, these experiments did not reveal whether any electrolyte imbalance resulted, for a redistribution of branchial blood flow might influence carrier-mediated sodium transport processes (Wood and Randall, 1971) as well as exerting simple permeability effects. Measurements of unidirectional fluxes alone cannot detect ionic deficits, while observed net fluxes may represent only a small fraction of total salt exchange. In these experiments Na^+ influx, efflux and net flux across the gills was measured in resting and swimming fish and in fish immediately after a period of exercise.

Methods

1. Preparation of Animals

Sexually mature rainbow trout *(Salmo gairdneri)* weighing between 180 and 350 g were obtained from a commercial supplier, Sun Valley Trout Farm, Port Coquitlam, B. C. Prior to experimentation, the fish were held in dechlorinated fresh water at $14.5 \pm 1.5°$ C for 1–4 weeks. During this time, the animals were not fed in order to prevent faecal contamination of the external water in subsequent experiments. Periodic determinations of sodium concentration in the acclimation water yielded values from 1.0–4.0 μg/ml.

Investigation of sodium balance during exercise demanded the use of radiotracer techniques (Maetz, 1956; Garcia-Romeu and Maetz, 1964) capable of distinguishing influx, efflux, and net flux components of electrolyte transfer. These methods necessitate the use of a relatively small water volume with attendant difficulties in supporting swimming activity. Fish were exercised by manual chasing in a small chamber. Branchial sodium flux rates measured during activity were compared with values determined during rest and post-exercise recovery. Pressure recordings from dorsal aortic and buccal cavity cannulae (Smith and Bell, 1964; Saunders, 1961) implanted in the animals permitted an evaluation of the physiological significance of the imposed treatments. Vascular catheterization also allowed serial blood sampling with minimal disturbance to the trout, through which internal levels of radiosodium and total sodium could be followed during the experiments.

Dorsal aortic, buccal, and urinary cannulae were implanted in all trout while perfused with a 1:20000 solution of MS 222 on an operating table (Smith and Bell, 1964). The urinary catheterization technique is reported in detail elsewhere (Wood and Randall, 1973a). Dorsal aortic and buccal cannulations were performed as described by Holeton and Randall (1967). All cannulae comprised 80 cm of PE 60 polyethylene tubing (Clay-Adams), and the aortic catheter was filled with heparinized (20 I.U./ml) Cortland saline (Wolf, 1963). Fish were allowed to recover from these procedures in individual temperature controlled ($14.5 \pm 1.5°$ C) aquaria for 10–48 hours.

2. *Experimental System*

Successful experiments were performed on 32 fish divided into 3 treatment groups of approximately equal size: resting, active, and recovering. At least 2 hours prior to an experiment, a trout was gently transferred to a darkened aquarium containing 5500 ml of dechlorinated freshwater (mean sodium concentration = 1.90 μg/ml). Dorsal aortic and buccal cannulae were attached to pressure transducers at this time; the urinary catheter, however, was led out of the chamber only a few minutes before the start of an experiment to avoid tangling of the three pieces of tubing. The tank was equipped with a standpipe and funnel for addition of $Na^{22}Cl$ solutions or anaesthetic without disturbance to the animal, polyethylene tubing for withdrawal of samples from beneath the water level, and airstones to provide adequate mixing and aeration of the water. The chamber was suspended in a bath which maintained a stable temperature of $14.5 \pm 0.5°$ C.

(*i*) *Resting Trout.* At zero time, 500 ml of freshwater containing a known amount (7–10 μC) of $Na^{22}Cl$ were added to the aquarium. Changes in total sodium and Na^{22} concentrations of the internal and external media were monitored over the following 60 minutes. The fish was left undisturbed during this time. The specific activity of sodium in the water declined by approximately 33% of the zero time level; adsorption of Na^{22} by the glass walls of the chamber was negligible. Dorsal aortic blood pressures and buccal pressures were measured throughout the experiment from the respective cannulae. Each catheter was connected to a Statham P-23 B.B. (venous) pressure transducer, and the output, together with a time signal, displayed on a Beckman Type R.S. dynograph recorder. At 60 minutes, a solution of MS 222 sufficient to provide a concentration of 1:10000 in the external medium was added to the aquarium. Immediately after loss of equilibrium, the fish was removed, stunned by a blow on the head, washed quickly with freshwater, and then dried with paper towels in preparation for immediate tissue sampling and weighing procedures. The tightness of the urinary catheter was checked post-experimentally; all data from trout exhibiting leakage were discarded.

(*ii*) *Active Trout.* As before, 500 ml of water containing 7–10 μC of Na^{22} were added to the aquarium at the beginning of an experiment. The fish was chased manually with a 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 only about 40 minutes out of a total of 60. With practice, it was possible to prevent excessive tangling of the three cannulae through periodic reversals of the direction of chasing. In other aspects, the experimental protocol was identical to that used for resting trout.

(*iii*) *Recovering Trout.* No radioisotope was placed in the external medium at zero time, but the animal was subjected to an exercise regime identical to that for the active group during the first hour. At 60 minutes, chasing ceased, 500 ml of water containing 7–10 μC of Na^{22} were added to the chamber, and the experiment proceeded for a further hour with methods identical to those used for the resting fish.

3. *Sampling Procedures*

(*i*) *Water.* Ten ml samples of the external medium were withdrawn immediately prior to zero time, and at 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. Aliquots of the $Na^{22}Cl$ solution were also taken in all experiments. Samples were subsequently stored at $-12°$ C in polyethylene vials (Nalgene Corporation).

(*ii*) *Blood.* Blood was withdrawn into sodium heparinized syringes via the dorsal aortic catheter from resting and active animals at zero time (100 μl) and at 5, 15, 30, 45 and 60 minutes (all 200 μl: total volume removed, including "spillage" = 1.45 ml); from recovering fish, blood was taken at zero time and 60 minutes (both 100 μl), and at 65, 75, 90, 105, and 120 minutes (all 200 μl; total volume removed, including "spillage" = 1.60 ml). The level of heparinization was adequate to prevent clotting or haemolysis, but was not great enough to cause significant sodium contamination of the plasma. Blood samples were spun in a Clay-Adams microhematocrit centrifuge for 10 minutes and hematocrits read directly from the centrifuge tubes. A small portion of each terminal plasma sample was analyzed for water content with a Goldberg refractometer (American Optical TS meter). Plasma was then frozen at −12° C in small polyethylene vials until further analysis.

(*iii*) *Urine.* Urine flow was measured over the 1 or 2 hour experimental periods. In some experiments, the catheter, although patent, was apparently occluded; urine production data from such fish were rejected. However none of these trout exhibited symptoms characteristic of chronic urinary blockage (see Wood and Randall, 1973b). It was therefore assumed the cannula failure was recent, and probably associated with the suction of siphon drainage at the beginning of the experiment. On this basis, data for all other parameters from these animals were accepted.

(*iv*) *Tissue.* Anaesthetization of trout prior to sacrifice was performed in order to maintain any differences in tissue samples resulting from differences in experimental treatment; hyperactivity associated with handling could otherwise have eliminated characteristics distinctive of resting or recovery conditions. Three 1.5 g pieces 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. Tissues were dried at 103° C for 72 hours, a procedure known to cause complete dehydration, and water contents calculated by weight difference. The samples were thereafter stored in a closed container for later ionic analysis.

4. *Analytical Procedures*

(*i*) *Na^{22} Concentrations.* 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 automatic sample changer and decade scaler. Suitable dilutions of each plasma sample were similarly assayed. After being ground to a fine powder and redried to a constant weight, a known amount (75–85 mg) of each tissue sample was mixed to a smooth paste with 1 ml of water on a planchet. After slow drying to avoid cracking and flaking of the preparation, the planchets were counted as above. A correction factor (×1.4661) for self-absorption of emissions by the tissues was determined by assaying known quantities of Na^{22} which had been incubated and dried with 75–85 mg of powdered, unlabelled, dorsal epaxial muscle.

(*ii*) *Total Sodium Concentrations.* Sodium levels were assayed against appropriate dilutions of a commercially prepared standard (Harleco) by flame emission photometry at 5890 Å. Analyses were performed on either a Unicam Model Sp 900A Flame Emission/Atomic Absorption Spectrophotometer or a Techtron Model AA 120 Atomic Absorption Spectrophotometer. As the actual changes in sodium level in a 6 L volume caused by a 250 g trout over the experimental intervals were extremely small, dilution of all water samples to a common concentration range proved impractical. Instead, samples were measured directly. The Unicam instrument was calibrated over full scale ranges of 0.50 μg/ml bracketing the con-

centrations of unknowns (e.g. 1.75–2.25 μg/ml), and the Techtron spectrophotometer over intervals of 0–1.00, 0–2.00, and 0–3.00 μg Na^+/ml. In a typical experiment, the net flux of sodium by the fish might result in a concentration change of 0.05 μg/ml during a 15 minute sampling period. The difference measured in this example was thus 10% of the calibration scale on the Unicam instrument, and 5%, 2.5%, and 1.7% at ranges of 0–1.00, 0–2.00, and 0–3.00 μg/ml respectively on the Techtron instrument. Thus, with the latter, there occurred some loss of accuracy in samples of higher concentration, though this fault was partially compensated for by the extreme stability and reproducibility of determinations even at nearly maximal scale deflections. Triplicate dilutions of each plasma sample were assayed on either photometer. Known weights (100 mg) of redried powdered muscle samples were digested with 1.00 ml of concentrated HNO_3 for 48 hours; triplicate aliquots were appropriately diluted and analysed on the Techtron flame photometer. Both standards and unknowns were swamped with 200 μg/ml potassium to eliminate interference effects from the high concentrations of this ion in salmonid muscle (Toews, 1966).

5. *Calculations*

Rates of sodium influx, efflux, and net flux across the external surface of the trout were computed from the decline in radioactivity and the change in total sodium content of the external medium after appropriate adjustments for sampling deficits (see Maetz, 1956). At no time did the internal specific activity of sodium represent more than 3.5% of the external value, thereby rendering correction for backflux of Na^{22} into the water unnecessary. The radiosodium space at each blood sampling time was calculated from the plasma radioactivity and the known amount of Na^{22} in the animal (see Mayer and Nibelle, 1969). The concentration in plasma of sodium ions transported from the external medium ("plasma sodium incorporation") was estimated from the plasma Na^{22} concentration and the average external specific activity over the experimental interval:

$$\text{plasma sodium incorporation} = \frac{2 \times Na^{22}/\text{ml}\ p}{\frac{Q_0}{\text{Na ext}_0} + \frac{Q}{\text{Na ext}}}$$

where: $Na^{22}/\text{ml}\ p$ = plasma Na^{22} concentration at time t (cpm/ml), Q_0 = amount of Na^{22} originally added to external medium (cpm), Q = amount of Na^{22} in external medium at t (cpm), Na ext_0 = total amount of sodium originally in external medium (μg), Na ext = total amount of sodium in external medium at t (μg).

Similarly, the level of sodium in dorsal epaxial muscle which had originated from the external medium over the experimental period ("tissue sodium incorporation") could be approximated from the radioactivity per gram of the terminal tissue sample.

$$\text{tissue sodium incorporation} = \frac{2 \times Na^{22}/\text{g}\ t}{\frac{Q_0}{\text{Na ext}_0} + \frac{Q}{\text{Na ext}}}$$

In this case, $\frac{Q}{\text{Na ext}}$ must represent the external specific activity at 60 minutes after addition of the radioisotope. Estimates of the size of the effective blood pool from which samples were removed over a particular interval were obtained from the resulting change in hematocrit.

$$\text{blood pool/100 g} = \frac{\sum_{k=1}^{f-1} H_k V_k}{H_i - H_f}$$

where: H_i = hematocrit (%) of initial sample, H_f = hematocrit (%) of final sample, H_k = hematocrit (%) of each individual sample between H_i and H_{f-1} inclusive, V_k = volume (ml/100 g) of blood removed in each individual sample between H_i and H_{f-1} inclusive.

6. Treatment of Data

In this and the following studies (Wood and Randall, 1973a, b), values have generally been expressed as a mean accompanied by the N number and standard error. Each piece of data acceptable in its own right was included in the average except where failure to obtain a related sample would have obviously prejudiced inclusion of the value in question. The "Student's" t-test (two-tailed) was applied between the means of different treatment groups. For some parameters, sets of data within a treatment group were subjected to a one way analysis of variance; the values of F obtained and their significance levels are presented. Means were then compared at the 5% protection level by a modification (Kramer, 1956) of Duncan's Multiple Range Test (Duncan, 1955). In legends for figures, a single line underscores subsets of means within which it has not been possible to demonstrate significant differences (see Duncan, 1955). Possible relationships between parameters have been evaluated by the calculation of simple correlation coefficients, and straight lines have been fitted to data points by the method of least squares regression.

Results

1. Ventilatory and Cardiovascular Parameters

Immediate increases in ventilatory frequency and amplitude, dorsal aortic blood pressure, and, in many animals, a noticeable short term bradycardia, later changing to a tachycardia, accompanied the start of chasing (Fig. 1). However, both traces became highly erratic, thereby precluding systematic determinations of rates and pressures during actual exercise. Consequently, only data extracted from readings taken during the 1 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 the active trout.

Heart rate remained approximately stable during rest, increased about 15% during chasing, and declined to pre-exercise levels during recovery (Fig. 2A). Dorsal aortic blood pressures tended to fall slightly in most animals, probably as a result of the serial removal of blood demanded by the experimental protocol (Smith *et al.*, 1967; Randall and Smith, 1967), but there did not occur any definite differences between treatment groups (Fig. 2B). It must be emphasized, however, that since these measurements were taken immediately after the termination of chasing, they are not reflective of the blood pressure elevations consistently observed at the onset of activity (Fig. 1). Both breathing rate and buccal pressure amplitude remained constant in the resting trout. Chased fish demonstrated a slight polypnea (10%; Fig. 2C) and large increases in buccal pressure (50–90%; Fig. 2D). These trends

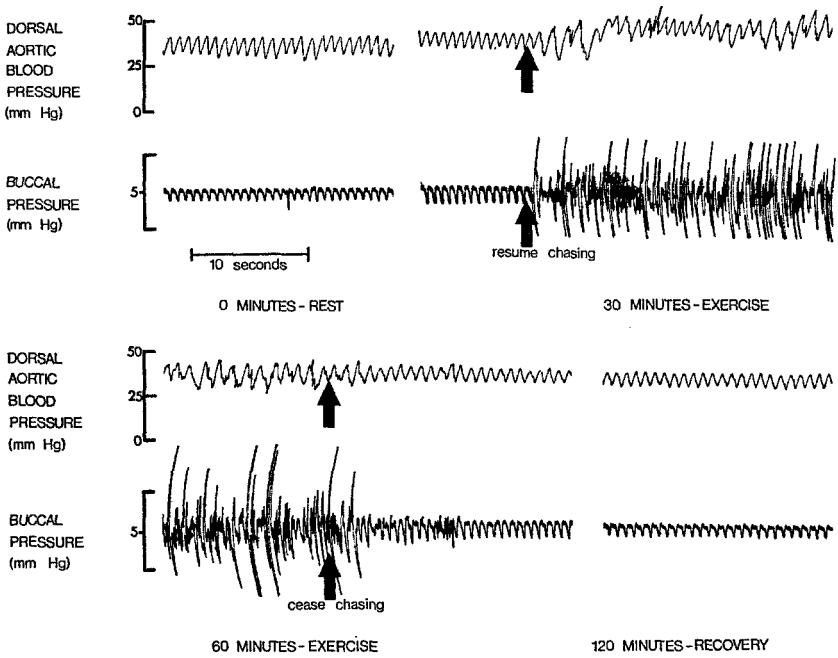


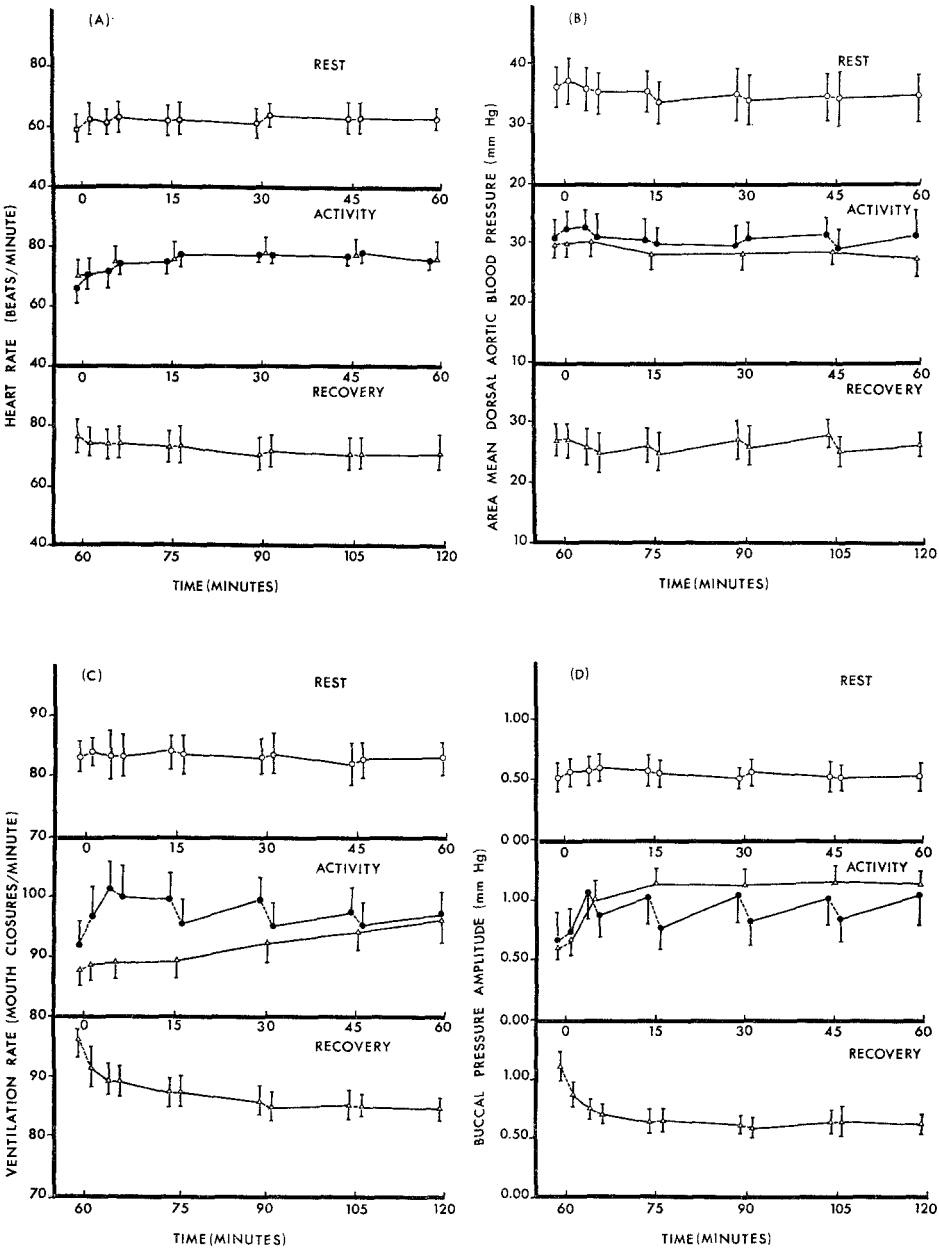
Fig. 1. Typical pressure recordings from the dorsal aorta and buccal cavity of a rainbow trout during rest, chasing, and recovery

were quickly reversed during recovery, even the short intervals of rest at each blood sampling time producing marked reductions in both components.

2. Branchial Fluxes

Ionic flux rates of the gills should be related to the surface area implicated; over a limited weight range, branchial area may be considered proportional to body weight as long as the degree of body development (and thus the gill area to weight ratio) remains constant. This latter parameter was estimated by calculation of the coefficient of condition, an expression of "plumpness" (Toews, 1966), for each

Fig. 2. Variations in (A) heart rate; (B) area mean dorsal aortic blood pressure = 2 diastolic + 1 systolic/3; (C) ventilation rate; (D) buccal pressure amplitude over the experimental periods. Each point represents the mean value from 7–11 trout, ± 1 standard error. ○ resting treatment group, ● active treatment group, △ recovering treatment group; the values for this group under activity were obtained



during their initial chasing treatment. Unlike the active treatment group, recovering trout were not subject to blood sampling during this hour of exercise. Dotted lines join determinations taken immediately before and after each blood sampling

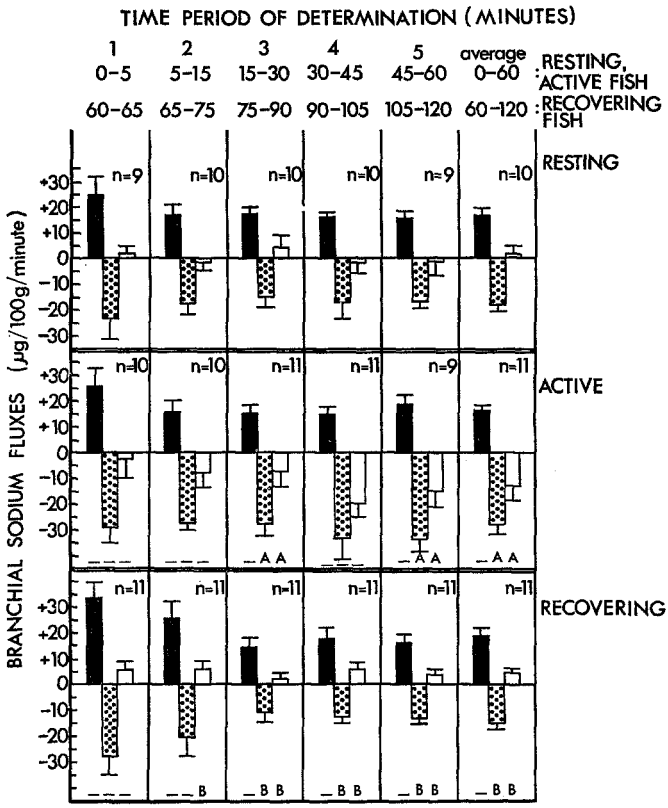


Fig. 3. Mean branchial sodium flux rates over consecutive intervals of the experimental periods in resting, active, and recovering groups. Black = influx; dotted = efflux; clear = net flux. Vertical bar = 1 standard error. A = significantly different ($p < 0.05$) from corresponding resting value; B = from corresponding active value. There were no significant differences in corresponding means between different measurement intervals within any of the three groups

trout. The mean size of resting fish was slightly greater than that of the other two sets, but coefficients of condition were similar in the three groups (Table 1), thereby justifying comparison of flux rates between treatments on a weight basis.

Branchial sodium flux rates are presented for each measurement period of the experimental hour in Fig. 3 and are summarized in Table 1. Average external sodium concentrations did not differ significantly among the three treatment groups (Table 1) and thus were not a factor causing the observed differences in sodium movements. Resting animals maintained a state of sodium equilibrium at the gills, influx and efflux

Table 1. Physical dimensions of trout, external sodium levels, and average branchial sodium flux rates over the experimental periods. Means $\pm$ 1 standard error

	Resting $N=10$	Active $N=11$	Recovering $N=11$
Body weight (g)	259.66 ± 12.79	233.95 ± 11.19 p_1 = n.s.	228.17 ± 8.05 $p_1 < 0.05$ 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.
External sodium concentration (μg/ml)	1.550 ± 0.151	1.612 ± 0.151 p_1 = n.s.	1.851 ± 0.254 p_1 = n.s. p_2 = n.s.
Sodium influx rate (μg/100 g/min)	$+17.62 \pm 1.15$	$+16.41 \pm 1.49$ p_1 = n.s.	$+19.24 \pm 2.86$ p_1 = n.s. p_2 = n.s.
Sodium efflux rate (μg/100 g/min)	-16.88 ± 1.57	-28.50 ± 3.55 $p_1 < 0.01$	-14.70 ± 2.36 p_1 = n.s. $p_2 < 0.001$
Sodium net flux rate (μg/100 g/min)	$+0.74 \pm 1.43$	-12.14 ± 2.94 $p_1 < 0.01$	$+4.54 \pm 1.99$ p_1 = n.s. $p_2 < 0.001$

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

being approximately equal so that there occurred only a very slight mean net uptake of the ion. During activity, sodium efflux increased markedly (70%, $p<0.01$) while influx remained identical to resting levels. Consequently, there resulted a highly significant ($p<0.001$) net loss of sodium across the gills. Over the recovery period, after one hour of exercise, the fish returned to a state of positive balance through a reduction ($p<0.001$) in the efflux parameter to slightly below resting levels, again without change of the influx element.

Unidirectional flux rates during the first 5-minute interval were greater than determinations over the subsequent three 15-minute intervals in all groups; however, the inaccuracies inherent in measurements over such a short time period precluded significance. These elevations probably reflected an initial exchange of Na^{22} ions with Na^{23} ions adsorbed to the mucus of the animals' epithelia (Fromm, 1968), resulting in artificial enhancement of both influx and efflux rates. Consequently no biological importance is attached to these differences. No significant

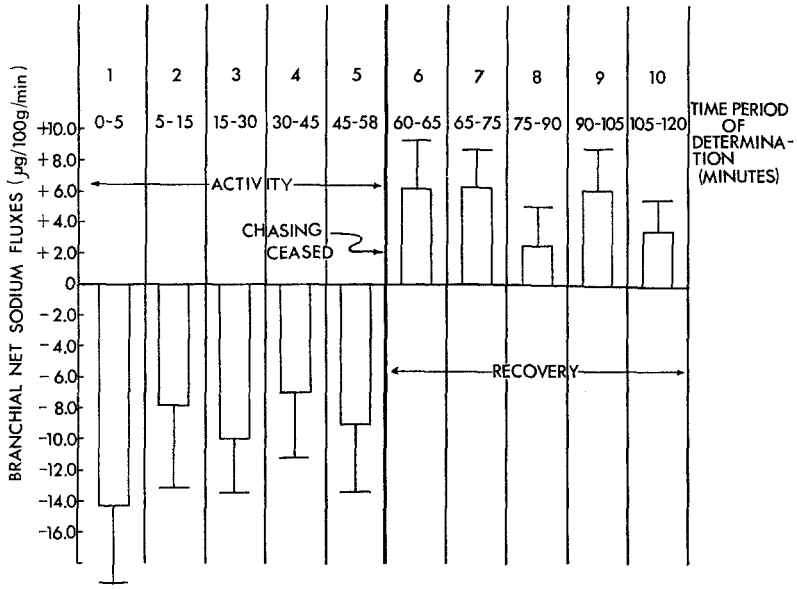


Fig. 4. Mean branchial sodium net flux rates over consecutive intervals of the experimental period in the recovering group. $N = 11$. Vertical bar = 1 standard error. $F = 4.68$, $p < 0.005$. 1 3 5 2 4 8 10 9 6 7 (numbers refer to means of intervals as at head of graph)

variations in flux elements occurred over the experimental hour within a treatment group. Thus it seems probable that the lag phases in the changeover from a branchial flux rate pattern distinctive of rest to that characteristic of activity, and from the latter to the typical recovery arrangement, were extremely short. Sodium net flux rates, recorded from the recovery group during both their initial hour of exercise and their following hour of recovery, are especially persuasive of this point (Fig. 4). These measurements were based solely on flame photometric analyses, which, especially during the first 5-minute period, were more accurate than radioisotope uptake determinations (and therefore, unidirectional fluxes). Fig. 4 demonstrates that the switch from a negative to a positive sodium balance at the end of activity occurred almost immediately, the net flux rates of the fish changing significantly between the final 15-minute period of chasing and the first 5 minutes of recovery. These data also confirm that the recovering trout, during their initial hour of exercise, suffered a net branchial sodium deficit similar to that of the active group.

Table 2. Urine flows (ml/kg/hour) over the experimental periods. Mean ± 1 standard error

Resting ($N=8$)	4.19±1.21
Active ($N=7$)	6.04±2.56
Recovering ($N=7$)	
activity (1st hour)	9.18±2.70
recovery (2nd hour)	5.73±1.19

Urine flow was extremely variable, but tended to rise during exercise and decrease during recovery (Table 2). These trends may have reflected changing branchial permeabilities to water (Wood and Randall, 1973a) similar to those occurring for sodium. Contamination of urine samples with pre-experimental production from the dead space of the cannula rendered ionic analyses uninformative, but assay of Na^{22} in urine from a few trials demonstrated that renal excretion of the radioisotope was negligible, and therefore had no effect on flux rate or radiosodium space determinations.

3. Sodium and Water Distribution

Hematocrits declined progressively over the blood sampling period in all three groups, indicating that the animals effected compensatory changes to help maintain the circulatory volume. These reductions in erythrocyte concentration provided a basis for estimation of the effective blood space from which aliquots were removed over a particular interval (see Methods). The calculation assumed that there occurred no mobilization of erythrocytes to replace sampling losses, and that blood volume did not decline. Because these assumptions may not have been entirely true, the compartments calculated may represent over-estimations. Nevertheless, as sampling regimes were similar in the three groups, the technique permits comparison of blood pool sizes between different treatments and experimental intervals on a relative basis.

The values obtained by this method increased in approximate proportion to the duration of the measurement interval in resting and recovering fish. This relationship reflects the fact that the volume ascertained over a short time period is dependent upon the interval permitted for equilibration within the compartment; Smith (1966) found that dye dilution curves did not stabilize until 2–3 hours after injection of the marker. Thus the present values (Table 3), despite the probable inaccuracies inherent in the estimation, were lower than total blood volume figures obtained by direct measurements in salmonids (Smith and Bell, 1964; Smith, 1966; Houston and De Wilde, 1969). The

Table 3. Effective blood pools (ml/100 g) calculated from hematocrit changes over the first 30 and final 45 minute intervals of the blood sampling periods. Means ±1 standard error

	First measurement	Second measurement
	0–30 or 60–90 minutes	15–60 or 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.
Recovering ($N=11$)	1.82±0.35	2.83±0.39 $p<0.10$

$p=$ significance with respect to first measurement.

fact that blood volumes were greater ($p<0.1$) for the second measurement period (45 minutes) than for the first (30 minutes) in resting and recovering trout was explained by the time dependent mixing effect. That this increase did not occur in the active fish was indicative of a reduction of the pool during the latter part of the exercise period. The data presented in Wood and Randall (1973a) suggest that this blood volume decrease resulted from an imbalance between net water influx and efflux, renal output exceeding branchial entry.

Variations in plasma sodium levels over the experimental periods are presented in Fig. 5. Plasma concentrations tended to decline in resting fish, a trend reflecting the dilution of serial blood sampling. During activity, however, this diminution failed to occur, despite the additional loss of sodium through the gills (Table 1) and the kidney (Wood and Randall, 1973a). In recovering trout, which were not sampled during chasing, an elevation of plasma sodium levels was in fact associated with activity, followed by a significant decline over the post-exercise hour. As tissue sodium levels were the same in all three groups (Table 4), the plasma sodium rise during exercise may be attributed to the decline in blood volume through net water loss.

Terminal concentrations of sodium and water in plasma and tissue, and sodium space estimates (Manery, 1954) of the extracellular fluid volume of epaxial muscle, are summarized in Table 4. There occurred no significant differences among the three groups in any of the parameters measured, despite the prediction of lower plasma water values in the active fish. However, both plasma and tissue water concentrations were higher than previously reported values in salmonids (Houston, 1959; Stevens, 1968; Miles and Smith, 1968; Toews and Hickman, 1969),

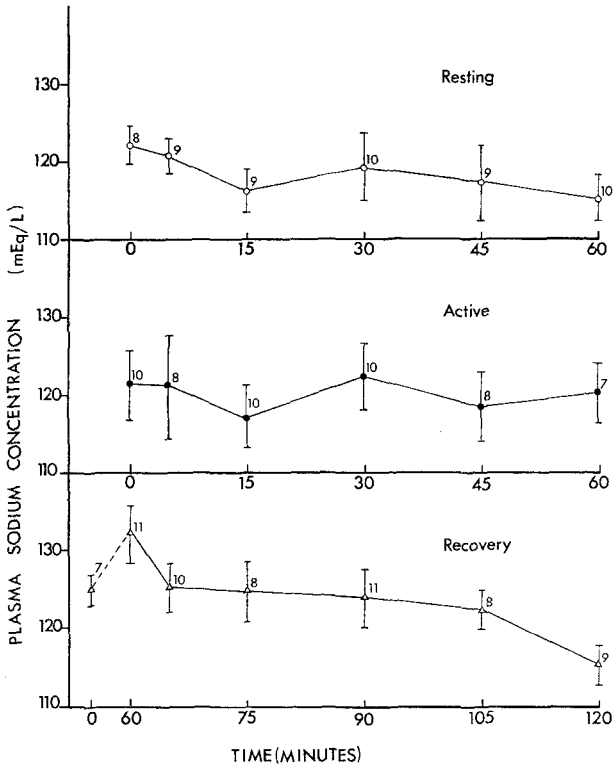


Fig. 5. Variations in plasma sodium concentration over the experimental periods in resting, active, and recovering groups of trout. Means ± 1 standard error. Resting: $F = 0.52$ n. s. Active: $F = 0.15$ n. s. Recovering: $F = 2.31$, $p < 0.05$
60 15 45 30 5 0 15 45 5 60 0 30 120 105 90 75 0 65 60

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

probably due to the effects of both pre-experimental starvation and serial sampling. Differences in plasma water levels sufficient to explain the slightly higher terminal sodium concentrations of the active fish would have fallen well below the analytical accuracy of the refractometer.

As there is little intracellular sodium (Manery, 1954), muscle sodium levels should have been largely reflective of concentrations in plasma. In both resting and recovering groups, there occurred significant positive correlations between terminal plasma and tissue levels of the electrolyte ($r = 0.754$, $p < 0.02$; $r = 0.678$, $p < 0.05$ respectively) in individual trout. This relationship, however, did not exist for active animals ($r = 0.033$,

Table 4. Terminal concentrations of sodium and water in plasma and dorsal epaxial muscle. Means $\pm$ 1 standard error

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

No significant differences between corresponding means.

n. s.), indicating a possible buffering during exercise of the tissue from changes in electrolyte levels in the fast mixing plasma pool; an ischemia of "white" muscle during swimming, suggested by Stevens (1968), would offer explanation for this phenomenon.

By definition, the radiosodium space is the ratio between the quantity of radioisotope in the whole animal and the amount per ml of plasma; plasma sodium incorporation is an expression which permits the comparison of influxed sodium levels between individual fish. Such comparison would not be valid through use of just plasma radioactivity values due to differences in external specific activity between experiments. In resting fish, expansion of the radiosodium space (Fig. 6) lagged behind that in active and recovering trout, the difference becoming significant by the final sample (Table 5). The higher Na^{22} distribution volumes during exercise and post-exercise could not be related directly to the calculated plasma volume change for at least two reasons. Firstly, the larger spaces were characteristic of both active and recovering states, while the apparent reduction in blood volume occurred only during the former (Table 3). Secondly, a decrease in plasma water alone would have increased the concentration of Na^{22} in plasma and the net effect would have been an inhibition, rather than a stimulation, of the radiosodium space evolution. Terminal plasma sodium incorporations (representative of plasma Na^{22} levels) were

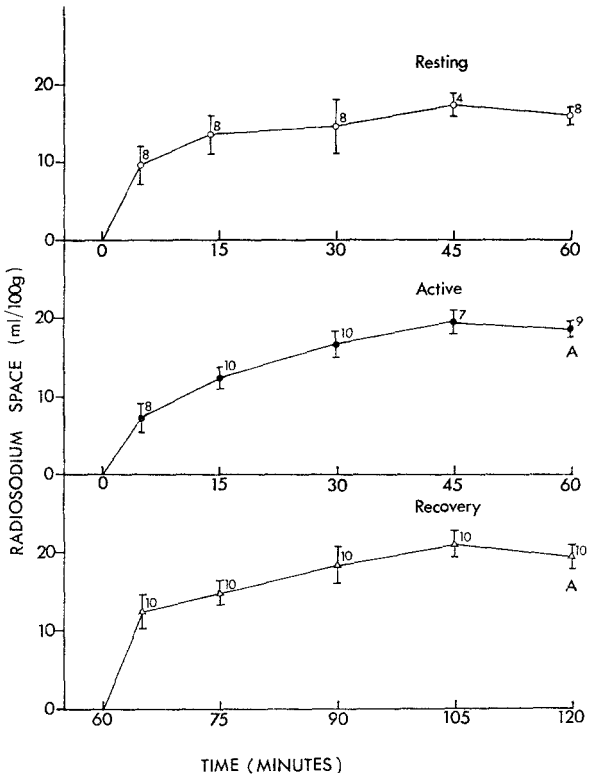


Fig. 6. The evolution of the radiosodium space with time in resting, active, and recovering groups of trout. Means ± 1 standard error. A = significantly different ($p < 0.05$) from corresponding resting value

significantly lower than the resting value in active and recovering groups (Fig. 7; Table 5), but sodium influx rates the same under all three treatments (Table 2). Thus the higher distribution volumes of the active and recovering animals must have resulted from a greater emigration rate of previously influxed sodium out of the sampled plasma compartment.

Tissue sodium incorporation figures (Table 5) reveal that "white" muscle, at least in the dorsal epaxial region examined, did not receive this elevated sodium dispersal, the incorporation being somewhat lower in active than in resting trout, and significantly smaller in the recovering animals. Correction of the tissue figures for enclosed plasma volumes of 1–3.5% did not alter the fact that the extravascular region of "white" muscle accumulated less influxed sodium during exercise and recovery

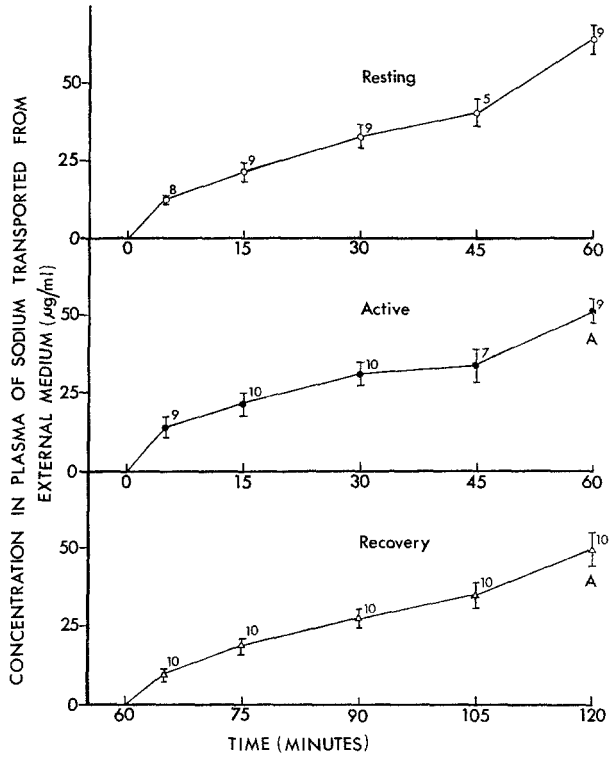


Fig. 7. The evolution with time of the concentration in plasma of sodium transported from the external environment ("plasma sodium incorporation") in resting, active, and recovering groups. Means $\pm$ 1 standard error. A = significantly different ($p < 0.05$) from corresponding resting value

than during rest. Thus the higher radiosodium spaces were apparently a consequence of a greater accumulation of influxed sodium in other regions of the body with an accompanying decrease in "white" muscle incorporation.

Movement of influxed sodium from the gills to the tissues must involve a time lag. Thus more of the radiosodium found in "white" muscle after 60 minutes probably entered the fish during the first half than during the second half of the experimental hour. Use of average external specific activities over the whole hour to calculate tissue sodium incorporation may therefore have been responsible for a systematic over-estimation of this parameter. However, such an error would have been common to all three groups, and would in fact tend to enforce the substance of the following argument. The balance sheets of Table 6

Table 5. Measures of internal distribution of influxed sodium at 60 minutes after the introduction of Na^{22}. Means $\pm$ 1 standard error

	Resting	Active	Recovering
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 (μg/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 (μg/g 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 6. Balance sheet of fate of influxed sodium at 60 minutes after introduction of Na^{22}

	Resting	Active	Recovering
Sodium influxed/100 g over 60 minutes	1057.2 μg	984.6 μg	1154.4 μg
Sodium incorporated in plasma (3 ml/100 g)	194.6 μg	156.0 μg	147.5 μg
Sodium incorporated in tissue (97 g/100 g)	298.5 μg	250.2 μg	215.2 μg
Total in tissues and plasma	493.1 μg	406.2 μg	362.7 μg
% 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 the plasma volume in the major vessels was 3 ml/100 g.

were constructed from a generous estimate of the amount of plasma in the major vessels (3 ml/100 g) and the assumption of a tissue incorporation value for the remainder of the body mass equal to that of the sampled epaxial muscle. Even in the resting group, this scheme accounted for less than $\frac{1}{2}$ of the influxed sodium, and less than $\frac{1}{3}$ in the recovering animals. Thus under all three conditions, some tissues must have received more influxed sodium on a per unit weight basis than did

"white" muscle, this effect being accentuated during activity and recovery.

Discussion

The elevations of dorsal aortic blood pressure at the start of chasing and of heart rate (15%) throughout the treatment were similar to those recorded for *Salmo gairdneri* during moderate swimming in a water tunnel (Stevens and Randall, 1967a, b). These workers calculated that the tachycardia was associated with a 4.5-fold increase in cardiac output. It seems probable that a similar effect occurred in chased fish in the present study, resulting in augmentation of blood flow through the gills. The mobilization of catecholamines during swimming (Nakano and Tomlinson, 1967), appears to play a major role in this response, as well as causing branchial vasodilation (Randall and Stevens, 1967; Bennion, 1968). Measured breathing rate increases (10%) were smaller than those observed by Stevens and Randall (1967a) (30%), but inspection of recordings taken during actual chasing indicated that the immediately post-exercise values reported here were significant underestimations of mean ventilatory frequencies attained by the active treatment groups. Buccal pressure elevations (50–90%) were, however, comparable to those measured at moderate to fatigue speeds in salmon (Davis, 1968). The return of cardiac and ventilatory rates to resting levels followed a similar time course to that seen after moderate swimming in rainbow trout (Stevens and Randall, 1967a, b). In the latter study, the enforced swimming activity produced a 5-fold augmentation of metabolic rate; a similar increase in oxygen consumption of the chased animals in the present investigation probably occurred.

Chasing in a chamber only slightly longer than the trout's body initially appeared to be a far from satisfactory method for inducing swimming, but, at the time, was the only procedure compatible with simultaneous sodium flux determinations. It was therefore imperative to the major point of this study, the investigation of sodium balance during exercise, to demonstrate that the adopted procedures caused real differences in cardiorespiratory function distinctive of resting state, swimming, and recovering conditions. The present rate and pressure data alone add nothing new to our knowledge of teleost physiology, but do demonstrate that the desired effects were produced.

Use of the term "branchial" in reference to measured fluxes needs justification, for ions may move between internal and external media across four main regions of the teleost: the gills, the urinary system, the intestinal system, and the skin of the general body surface. In these experiments, renal contribution to measured ionic exchange was avoided

through urinary catheterization. Rainbow trout at rest do not drink in freshwater (Shehadeh and Gordan, 1969), thereby negating intestinal absorption of sodium. It is not known whether active trout ingest the external medium; however, even if swimming fish drink as much water as trout in full strength sea water (Shehadeh and Gordan, 1969), which is highly unlikely, the sodium available for transport by the gut would represent only about $^1/_{1000}$th of the sodium influx rate recorded in the present experiments. Pre-experimental starvation prevented faecal contamination of the external medium. During some trials, a gelatinous tube of mucoid material was eliminated from the anus, but this material is devoid of sodium (Shehadeh and Gordon, 1969). The impermeability of *Salmo gairdneri* skin to the ion has been proven repeatedly (Holmes, 1959; Fromm, 1968). The assumption of an exclusively branchial origin for the observed sodium movements, would, therefore, seem sound.

Randall *et al.* (1972) noted that activity elevated the release of injected Na^{22} from the gills of freshwater adapted rainbow trout, but were unable to determine whether the effect represented a stimulation of sodium turnover rate or of the efflux component alone. The present study has demonstrated that branchial efflux increases during exercise without modification of influx, resulting in a net sodium deficit at the gills. The concept of a respiratory/osmoregulatory compromise in branchial permeability (Steen and Kruysse, 1964; Randall *et al.*, 1972; Taylor *et al.*, 1968; Kirschner, 1969) therefore appears substantiated.

A priori, an active transport contribution to branchial sodium efflux seems most unlikely, and has never been demonstrated in freshwater teleosts; efflux should, therefore, consist of simple diffusion and exchange diffusion if present. The 70% rise in efflux during exercise was not accompanied by any change in influx rate, and thus would not seem to involve exchange diffusion. Consequently, this elevation of the unidirectional outward sodium movement has been attributed to simple diffusion caused by enhanced circulatory perfusion of the thin walled, high surface area respiratory lamellae.

As concentration gradients for both oxygen (Stevens and Randall, 1967b), and sodium remain essentially unchanged during exercise, simple diffusional fluxes may be considered representative of effective permeabilities. Ventilatory and cardiovascular data were consistent with a 500% rise in oxygen uptake during chasing, while branchial sodium efflux increased by only 70%. This discrepancy may, of course, be explained by a change in the relationship between the diffusion coefficients for oxygen and sodium associated with the recruitment of new exchange area in the gills. However, if this is not the case, two alternate explanations derived from possible branchial sodium transport mechanisms may be offered.

Firstly, it could be argued that the release of adrenaline during exercise (Nakano and Tomlinson, 1967), a substance known to elevate the sodium uptake of the isolated perfused trout hemibranch (Richards and Fromm, 1970), would stimulate the active influx pump. However, this additional activity would be occupied in back-transporting ions moving out through increased simple diffusion (Kirschner, 1955; Morris and Bull, 1970). If this were so, no increase in influx and only a small portion of a 5-fold rise in passive efflux would be observed. The spatial separation of sites for maximum diffusional loss (respiratory lamellae) and carrier-mediated transport (interlamellae filamental epithelium) in the teleost gill (Conte, 1969) must weigh heavily against this hypothesis. In addition, the diversion of blood flow away from 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 (Cameron and Davis, 1970), causes a decreased rate of carrier-mediated sodium transport across the gills.

Alternatively, the 70% rise in efflux could represent a 5-fold elevation of sodium loss by simple diffusion. This statement implies that simple diffusion (X) comprises only a small portion of the total efflux in resting fish, the remainder being exchange diffusion (Y), a mechanism which, by definition, will contribute equally to both unidirectional fluxes. The following simple calculation may be performed on the basis of Table 1:

At rest: $X + Y = 17$ μg/100 g/minute
During activity: $5X + Y = 29$ μg/100 g/minute

This assumption yields values of approximately 14 μg/100 g/minute for the exchange element and 3 μg/100 g/minute for simple diffusional loss during rest, increasing by a factor of five to 15 μg/100 g/minute during activity. These figures are in close agreement with those resulting from an analysis of the concentration dependence of branchial sodium fluxes in the rainbow trout (Wood and Randall, 1973b).

As the release of catecholamines during exercise may be responsible for both the branchial vasodilation increasing the effective permeability of the respiratory exchanger and for the elevation of cardiac output, it is of interest to ask whether the time course of variation in plasma levels of these hormones can be correlated with the rapid changes in branchial sodium efflux and heart function observed. Nakano and Tomlinson (1967), in rainbow trout, found an immediate rise (less than 10 minutes) of plasma levels at the onset of exercise, but this elevation was maintained for at least 3 hours into recovery. Sodium efflux rate (Fig. 4),

heart rate (Fig. 2A), and oxygen consumption and cardiac output (Stevens and Randall, 1967b) drop rapidly after the termination of activity. Thus catecholamines may well be involved in the initial branchial and cardiac adaptations to the respiratory demands of swimming, but other factors may over-ride these adrenergic effects as soon as they are no longer required; their persistence after exercise may simply reflect a low activity of breakdown mechanisms in teleost tissue. One could also hypothesize that adrenaline and noradrenaline exert only partial control over cardiac activity and branchial permeability, acting as potentiators for the observed effects. Thus an increased cardiac output would be caused by a greater venous return (Labat *et al.*, 1961) at the onset of activity, though facilitated by the action of adrenaline on the myocardium to favourably alter the relationship between stroke volume and filling pressure (Bennion, 1968). At the same time catecholamines would stimulate the dilation of respiratory shunts in the gills, but the filling of a particular lamella would still remain 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 lamellae perfused (i.e. branchial permeability) would be reduced despite the continuance of high catecholamine levels in plasma. Clearly, further research on the action of catecholamines and the integration of cardiovascular adjustment to exercise in teleosts is required.

Elevation of plasma sodium concentrations during activity has been attributed to a reduction in plasma volume through net water loss. This concept is supported by the work of Stevens (1968) who offered a similar explanation for the increase of both haemoglobin and plasma protein concentrations in rainbow trout during exercise. Furthermore Rao (1969) demonstrated a significant rise in plasma osmolarity during swimming in the same species. Rao postulated that this osmoconcentration was related to an increased release of metabolites associated with activity, but did measure a slight rise in plasma chloride levels; his results can well be explained by a reduction of plasma water. Further consideration is applied to the topic of water balance during activity in Wood and Randall (1973a).

The arrival, consistent to all three treatment groups, of the radiosodium space expansion curve at a plateau of 15–21 ml/100 g only 45 minutes after introduction of the radiotracer (Fig. 6) deserves comment, for the volume at equilibrium is about 34 ml/100 g (Wood and Randall, 1973b). A parallel phenomenon is seen in the upward deflection of the plasma sodium incorporation curve (Fig. 7) at this time without change in branchial influx rate (Fig. 3). However, the biological reason for this apparent retardation of the dispersal of influxed sodium is

unknown; stabilization of the mixing compartment and limitation by diffusion into the extravascular phase may be involved. Mayer and Nibelle (1969), from studies on the eel, *Anguilla anguilla*, argued that capillary barriers were negligible in the evolution of the Na^{22} distribution volume, and that the filling up of the compartment 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 such an intermediate levelling of the curve.

If the relatively low sodium incorporation of "white" muscle (Table 6) is related to its poor blood supply, then red muscle fibres and cardiac muscle, which contain $2^1/_2$ times as much blood on a wet weight basis (Stevens, 1968) may preferentially accumulate influxed sodium. Increased blood flow to these tissues and an ischemia of "white" muscle (Stevens and Black, 1966) could account for the higher Na^{22} distribution volumes and lower plasma and tissue sodium incorporations measured during exercise (Table 5). It is difficult, however, to see how this explanation could apply to the similar effects observed during recovery, when circulatory patterns should return to a more resting configuration. Thus blood flow variation may contribute to the observed differences, but is probably not the major factor involved. A property common to both active and recovering animals is the presence of high circulatory levels of catecholamines (Nakano and Tomlinson, 1967). It is possible that these hormones may promote uptake or turnover of sodium by specific "sinks" within the system, thereby contributing to the elevation of the radiosodium space expansion under both exercise and post-exercise conditions. The cerebral spinal fluid (CSF) is a possible sink. Maren (1972) has suggested that acidosis in the dogfish is associated with increased Na^+ levels in the CSF. In these experiments the exercise and post-exercise periods were undoubtedly associated with a fall in plasma pH and may have resulted in increased levels of Na^+ in the CSF of the trout.

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