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Sodium Balance in the Rainbow Trout (*Salmo gairdneri*) during Extended Exercise

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Summary. 1. Sodium flux rates in the freshwater adapted rainbow trout were measured by radiotracer techniques during longterm swimming (up to 8 hours) and during recovery from extended exercise.

2. Branchial sodium influx rate remained constant under different activity conditions, while whole animal efflux rate was highest during the first hour of swimming (negative sodium balance), declined during the second hour, and reached levels lower than influx rate during the third and subsequent hours of exercise (positive sodium balance). A minimum efflux value occured during the second hour of recovery accompanied by a maximum positive net flux. These changes in efflux rate appeared to be mainly branchial in origin.

3. Branchial sodium influx rate was dependent on external sodium concentration in a manner well described by the Kirschner (1955) equation with K_s= 0.02 mEq/L and $M_{i\,max}$=61.68 μEq/100 g/hr.

4. Branchial sodium efflux rate was also dependent on external sodium levels in a manner loosely paralleling that of influx rate and suggestive of an exchange diffusion mechanism.

Introduction

A net sodium loss across the gills of rainbow trout during exercise has been demonstrated in the preceding publication (Wood and Randall, 1973a). Over a 1 hour period this deficit amounted to only 31.68 μEq Na^+, 100 g, or about $^1/_{20}$th of the total plasma sodium content. The effect of this small loss was apparently counteracted by a decrease in blood volume which produced a slight elevation of plasma sodium concentration. However, if such a net branchial efflux rate were maintained during prolonged swimming, the trout would soon encounter osmoregulatory difficulties; after only 6–7 hours, approximately $^1/_3$ 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 excretion, which was suspected at the time these experiments were performed (Hammond, 1969; Hickman and Trump, 1969), and later directly confirmed (Wood and Randall, 1973b). Thus the sodium deficit, if unchecked, would clearly limit the duration of exercise to a matter of hours. Yet freshwater salmonids can swim almost indefinitely at sub-fatigue speeds (Bainbridge, 1962; Brett, 1964). The induction of some

compensatory mechanisms to reduce sodium loss during extended activity was therefore indicated; the present study was designed to test the validity of this assumption.

The development of a small volume revolving chamber in which trout would swim continuously for long periods facilitated the application of radiotracer techniques to the problem. As direct collection of urine from an animal in the apparatus was not feasible, an indirect method was employed in an attempt to distinguish between branchial and renal contributions to the total sodium efflux.

Flux rates were recorded during long term exercise from both normal unencumbered trout and individuals in which occlusion of the urogenital papilla prevented the release of urine. Urinary blockage has been used previously to eliminate the renal discharge of electrolytes in several studies of osmoregulation in rainbow trout (Holmes, 1959; Gordon, 1963; Randall *et al.*, 1972), and in fact has been recently endorsed (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 set of fish (shams) were subjected to urinary intervention (without blockage) to evaluate the possible influence of this procedure alone on sodium exchanges. Weight changes over the experimental period were determined on the urinary blocked and sham trout, and terminal samples for the determination of hematocrit, plasma sodium, and plasma and tissue water concentrations were taken from all fish. 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.

Examination of three treatment groups produced a large number of flux rate measurements. Because of the extensive duration of these experiments, trout were often able to effect marked alterations of external sodium concentrations. Consequently this body of flux rate data was distributed over a range of environmental sodium levels. Analysis of the concentration dependence of branchial flux rates, which comprises the second part of this paper, casts some light on the mechanisms of sodium exchange in the rainbow trout gill.

Methods

1. Preparation of Animals

Animals used in this study included both immature and sexually mature rainbow trout (*Salmo gairdneri*; 160–280 g) which were obtained, held, and acclimated to $14.5 \pm 1.5°$ C as described previously (Wood and Randall, 1973a). Two groups of trout (urinary blocked and shams) were subjected to urinary catheterization (Wood

and Randall, 1973b) under MS 222 (1:20000) anaesthesia, while a third group (normals) were not handled prior to the experiment. The cannula was cut off approximately 2 cm posterior to the anal fin and firmly tied to this fin with several silk stitches. A pin could later be inserted into the open end of the catheter to cause urinary blockage. Operated fish were allowed to recover for 24 to 72 hours in individual aquaria at 14.5±0.5° C.

2. *Experimental System*

The exercise apparatus comprised a circular polyethylene chamber (diameter= 40 cm; depth=13.5 cm) mounted on a variable speed kymograph motor. The plexiglass lid contained a small central hole to accommodate tubing for water sampling and aeration, and a large hinged port for addition of the trout. In all experiments the chamber was rotated at 20.5 revolutions/minute for the entire 8 hour 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/sec. Behaviour of the animal during each one 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. Average experimental temperature was 15.2° C with extremes of 13° C and 16.5° C; maximum change observed during an 8 hour run was 1.8° C.

Prior to an experiment, the swimming chamber was filled with 6000 ml of dechlorinated fresh water (mean sodium concentration=1.60 μg/ml) containing 15 to 25 μC of $Na^{22}Cl$ and then sealed with the plexiglass lid. After thorough mixing, an aliquot of the medium was taken for later analyses of total sodium and Na^{22} concentrations.

(i) Normal Trout. A fish was removed directly from the acclimation tank and placed in the chamber. The apparatus was then rotated slowly and the desired velocity attained by gradual increments in speed; the details of this procedure varied with the behaviour of the individual animal. Exactly 10 minutes after addition of the fish, a water sample (10 ml) was withdrawn; further samples were taken at 60 minute intervals for 8 hours to measure the changes in total sodium and Na^{22} concentrations of the external medium. Observations of the animal's behaviour were made twice hourly 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. The animal was weighed, a blood sample (0.5 ml) drawn by cardiac puncture into a heparinized syringe, and dorsal epaxial muscle samples excised (Wood and Randall, 1973a).

(ii) Urinary Blocked Trout. The weight changes of animals in both this and the sham groups were recorded as gross measures of net water fluxes. Before the start of an experiment, a urinary catheterized trout was removed from its aquarium and weighed twice by the method of Wood and Randall (1973b). Towel drying of the animal was omitted as it could have produced undesirable stress effects on sodium flux rates; the accuracy of the procedure remained adequate for the purpose of the measurements. The urinary cannula was then sealed with a stainless steel pin and the fish returned to its 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 8 hour run, the fish was again weighed twice before blood and tissue sampling. Urinary blockage was checked post-experimentally by injection of dyed saline into the cannula.

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

3. Analytical Procedures

Determinations of terminal hematocrit, plasma and tissue water, plasma and water sodium, and plasma and water radioactivity were performed as described by Wood and Randall (1973a). All flame emission analyses were carried out on the Techtron Model AA 120 Atomic Absorption Spectrophotometer.

4. Calculations

The method used previously (Wood and Randall, 1973a) for calculation of sodium 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. Consequently, in computation of influx rates, it was necessary to use the equation of Maetz (1956) to correct for the backflux of the radioisotope. Net flux rates were derived from changes in the total sodium content of the external medium, and efflux rates obtained by difference. The terminal radiosodium space was calculated from the terminal plasma radioactivity and the known amount of Na^{22} in the animal after 8 hours 10 minutes (see Mayer and Nibelle, 1969).

Results and Discussion

1. Sodium Flux Rates during Extended Exercise

Table 1 demonstrates that the trout used in this study were generally smaller and thinner (as manifested by the low coefficients of condition) than those of the previous work (cf. Table 1 of Wood and Randall, 1973a), and many were in breeding condition. These batch differences probably accounted for the slightly higher plasma sodium concentrations and flux rates observed in the present investigation. However body weights and coefficients of condition did not vary significantly among the three experimental groups, thereby permitting comparison of flux rates between treatments on a body weight basis (Wood and Randall, 1973a).

Data derived from the weighing procedure and analysis of terminal blood and tissue samples has been summarized in Table 2. The radiosodium space of the normal 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 freshwater teleosts (Maetz, 1956; Maetz *et al.*, 1967b; Motais, 1967; Lahlou and Sawyer, 1969; Lahlou *et al.*, 1969). Both Na^{22} distribution volumes and plasma sodium levels were lower in the shams than the normals, although neither difference was significant by itself. The effective product of these two parameters, total exchangeable internal sodium, did

Table 1. Physical dimensions of trout. Means ± 1 standard error

	Normal $N=10$	Sham $N=10$	Urinary blocked $N=9$
Body weight (g)	186.92 $\pm$ 5.42	190.60 $\pm$ 10.19	180.09 $\pm$ 6.95
Coefficient of condition $\frac{\text{weight} \times 100}{(\text{fork length})^3}$	0.882 $\pm$ 0.026	0.928 $\pm$ 0.036	0.889 $\pm$ 0.036

No significant differences between corresponding means.

however show a statistically confirmed decrease. Disturbances in electrolyte balance after MS 222 anaesthesia and operative procedures may be associated with both an elevated renal ion loss (Hunn and Willford, 1970) and an increased branchial permeability accompanying greater respiratory exchange during the recovery period (Houston *et al.*, 1969, 1971). The sham animals did in fact exhibit slightly higher sodium efflux rates than the normals under most exercise conditions (Fig. 3).

An extremely significant weight gain amounting to a net water entry of about 1% body volume per hour occurred in the urinary occluded animals (Table 2). [Shams underwent a 2.5 g weight reduction over the experimental period; a smaller but significant weight loss associated with extended exercise has been observed under more carefully controlled conditions by Wood and Randall (1973b)]. Plasma sodium levels were drastically depressed despite the stability of total exchangeable internal sodium content. A very small elevation of plasma water concentration and depression of hematocrit in the ligated trout relative to the shams suggested a slight hemodilution. However, the large augmentation of water levels in tissue ("white" muscle) indicated that most of the superfluous water accumulated in the extravascular space. This oedema was accompanied by an apparent redistribution of body sodium reflected in both a significant expansion of the radiosodium space and reduction of the plasma sodium concentration. Urinary blockage was thus extremely disruptive of internal hydromineral homeostasis and hence would appear to be an unsatisfactory technique for partitioning branchial and renal efflux rates in freshwater trout. In particular, it has been shown in both the goldfish, *Carassius auratus* (Bourguet *et al.*, 1964) and the ammocoete, *Lampetra planeri* (Morris and Bull, 1970) that unidirectional branchial sodium movements are sensitive to internal sodium concentrations. Indeed, Richards and Fromm (1970) have recently demonstrated that the net sodium flux across isolated gill arches of *Salmo gairdneri* is inversely proportional to the sodium concentration of the internal perfusion fluid. Some decrement in passive sodium efflux from the urinary blocked trout would be expected

Table 2. Terminal measurements of internal sodium and water levels, radiosodium spaces, hematocrits, and weight changes over the experimental period. Means ± 1 standard error

	Normal $N=10$	Sham $N=10$	Urinary blocked $N=9$
Plasma sodium concentration (mEq/L)	144.75 ± 5.23	132.62 ± 10.05 p_1 = n.s.	106.69 ± 3.87 $p_1 < 0.001$ $p_2 < 0.05$
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. $p_2 < 0.02$
Total exchangeable internal sodium (μEq/100 g)	4846.51 ± 255.19	3912.73 ± 288.36 $p_1 < 0.05$	3671.00 ± 165.65 $p_1 < 0.01$ p_2 = n.s.
Plasma water concentration (ml/100 g 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/100 g initial weight)	—	−2.57 ± 1.05 p_1 = —	+8.57 ± 0.93 p_1 = — $p_2 = < 0.001$
Tissue water concentration (ml/100 g tissue)	81.047 ± 0.530	81.851 ± 0.570 p_1 = n.s.	83.899 ± 0.257 $p_1 < 0.001$ $p_2 < 0.01$

p_1 = significance with respect to corresponding normal value.
p_2 = significance with respect to corresponding sham value.

simply from the decrease in the plasma to water sodium gradient, and could be reinforced by any hormonally induced decrease of branchial permeability elicited by hyponatremia.

Interpretation of the flux rate results was complicated by several factors. Individual trout swam for varying periods of time; classification of a particularly hourly value was dependent not only on whether the animal was swimming or not, but also on the length of time prior to the measurement period during which the fish had been active or resting. Inspection of data from experiments in which the trout had swum for most or all of the 8 hours revealed no marked variation in sodium movements after the first 3 hours of exercise. Similarly, flux rates of fish which had spent most of their time at rest had stabilized by 3 hours after the cessation of swimming. Fig. 1 presents characteristic examples of these cases. Consequently, hourly determinations in the swimming category were broken down

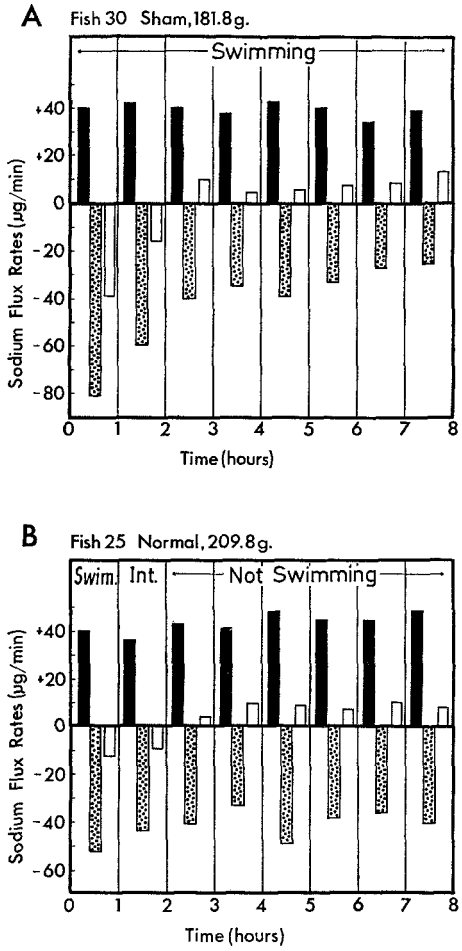

Fig. 1 A and B. Sodium flux rates of rainbow trout in two experiments. A. Example of an animal which swam continuously for 8 hours. B. Example of an animal which was inactive for most of the experimental period. Black=influx; dotted=efflux; clear=net flux

into first, second, and subsequent hours swimming, and measurements in the not swimming category into first, second, and subsequent hours recovery. The intermittent category was not further subdivided. The classification procedure is illustrated by the experiment outlined in Fig. 2. All values for a particular category from every animal in the treatment group were then averaged. Each hourly measurement was thus weighted equally in the mean although some fish obviously contributed more values to a category than did others.

A further problem evolved from the large reductions in external sodium concentrations caused by the highly positive net flux rates of some trout (e.g. Fig. 2), an effect especially prevalent in the urinary blocked group. Investigation of the interaction between sodium concentration and influx rate revealed that at average environmental sodium levels greater than 0.8 μg/ml, the slope of the regression line of influx on concentration did not differ significantly from zero, whereas below this figure, there existed a definite positive correlation. It will be shown later that the data agree well with a Michaelis-Menten relationship. Comparison of influx rates within and between groups was therefore valid only for determinations taken over that part of the curve where "substrate" concentration had little effect on "velocity". A similar argument must apply to net flux rates and efflux rates if the latter are either directly (exchange diffusion) or indirectly (back-transport) linked to influx rates. Consequently all flux measurements taken at average external sodium concentrations less than 0.8 μg/ml were discarded from the means (e.g. Fig. 2).

Fig. 3 displays the results of the above analysis for the normal and sham trout. These two groups demonstrated extremely similar trends of flux rate variation in response to the state of activity. Sodium efflux rates were slightly higher in the shams and thus net movements marginally less positive, but the differences were generally not large enough for statistical confirmation. In both treatments, branchial influx rate exhibited remarkable constancy under different activity conditions. The insensitivity of sodium influx across the gills to factors associated with short term exercise (one hour) and recovery has been established previously (Wood and Randall, 1973a). This conclusion may now be extended to prolonged swimming and the recovery period following such activity.

However, the prevailing exercise condition had great influence on the sodium efflux rate in normal and sham animals (Fig. 3). Unidirectional outward movements were greatest during the first hour of swimming but decreased significantly to post-exercise levels by the third and subsequent hours of activity, while intermittent exercise had an intermediate effect on this parameter. A minimum efflux value occurred during the second hour of recovery accompanied by a maximum positive net flux. Subsequent hours recovery were characterized by a return to a slightly greater efflux associated with a somewhat lower net sodium uptake. These results confirmed that the rainbow trout can overcome the net sodium deficit associated with short periods of exercise through the implementation of compensatory mechanisms both during and after more prolonged exercise. This adaptation apparently occurs entirely through reduction of the efflux rate, the same factor whose original elevation had been responsible for the situation of negative balance.

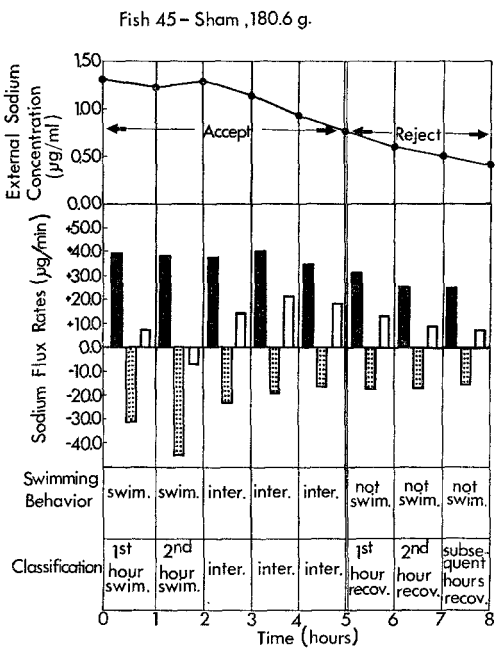

Fig. 2. Changes in external sodium concentration and sodium flux rates from a typical experiment illustrating the method of data classification applied (see text). Black=influx; dotted=efflux; clear=net flux. Below approximately 0.8 μg Na/ml, influx rate became dependent on external sodium concentration. Inter.=intermittent swimming

It must be emphasized that the sodium efflux rates of the normals and shams (Fig. 3) were whole animal determinations representing the sum of branchial and renal contributions. The experiments with urinary occluded rainbows were performed in order to ascertain the role of each component in the observed response. Rejection of many flux rate determinations from the ligated fish was unfortunately necessitated by the 0.8 μg/ml external sodium level criterion. The remaining data are summarized in Table 3. Branchial influx rates showed no significant variation with respect to either different activity 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 derives from the fact that three of the four values in the mean came from a single animal which exhibited low influx rates throughout the experiment. Thus sodium influx across the gills of urinary blocked animals showed similar magnitude and stability to that of the unligated fish.

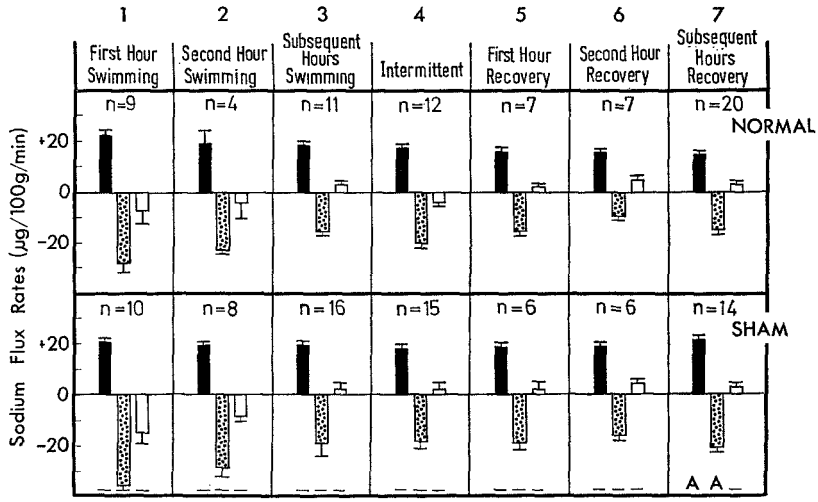

Fig. 3. Mean sodium flux rates, measured at external concentrations greater than 0.8 μg Na/ml, in normal and sham trout. Black=influx; dotted=efflux; clear=net flux. Vertical bar=1 standard error. A = significantly different ($p<0.05$) from corresponding normal value. Statistical comparisons: numbers refer to means of exercise conditions as at head of figure; see Wood and Randall (1973a).

	Normal	Sham
Influx rate:	$F=1.59$; n.s. 6 5 7 4 3 2 1	$F=1.61$; n.s. 4 5 6 2 3 1 7
Efflux rate:	$F=13.90$; $p<0.005$ 6 7 5 3 4 2 1	$F=5.63$; $p<0.005$ 6 4 5 3 7 2 1
Net flux rate:	$F=4.59$; $p<0.005$ 1 2 4 5 3 7 6	$F=6.62$; $p<0.005$ 1 2 4 3 5 7 6

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, and remained extremely constant under different activity conditions (Table 3). This result implies that both the initial elevation of whole body efflux rate and its compensatory reduction during prolonged swimming in unligated fish may be ascribed to wholly renal events. There exist a number of reasons to believe this conclusion false.

Table 3. Influx (M_i), efflux (M_0), and net flux (M_n) rates of sodium (μg/100 g/min) in urinary occluded trout under different exercise conditions. Means ± 1 standard error. External sodium concentration >0.8 μg/ml

First hour swimming $N=9$			Second hour swimming $N=3$			Intermittent $N=9$		
M_i	M_0	M_n	M_i	M_0	M_n	M_i	M_0	M_n
+22.42	−13.40	+9.02	+21.41	−13.10	+8.31	+19.48	−14.51	+4.97
± 2.29	± 2.37	±2.45	± 3.88	± 3.21	±2.04	± 1.33	± 1.70	±1.60
—	c	c	—	c	b	—	a	a

First hour recovery $N=5$			Second hour recovery $N=4$			Subsequent hours recovery $N=4$		
M_i	M_0	M_n	M_i	M_0	M_n	M_i	M_0	M_n
+19.01	−11.02	+7.99	+18.23	−13.77	+4.46	+12.95	−13.39	−0.44
± 2.23	± 2.78	±1.78	± 1.82	± 2.16	±2.67	± 1.73	± 4.70	±5.25
—	b	c	—	—	—	b	—	—

No significant differences between corresponding means within the treatment group.
a = significantly different ($p<0.05$) from corresponding normal value; b = from corresponding sham value; c = from both normal and sham values.
No data for "Subsequent hours swimming".

Firstly, as discussed previously, the traumatic disturbance in internal sodium regulation found to accompany urinary blockage (Table 2) would be expected to abnormally depress branchial sodium efflux. Conclusions about sodium flux behaviour based on the use of this technique must be gravely suspect.

Secondly, an increase in unidirectional sodium loss during one hour of exercise and a decrease in this parameter during one hour of recovery were observed as totally branchial phenomena in the previous study (Wood and Randall, 1973a). These changes were of similar magnitude to those seen during first hour swimming and subsequent hours swimming respectively in unligated fish of the present work (Fig. 3).

Finally, calculation of urinary sodium efflux rates by subtraction of the urinary blockage values (Table 3) from comparable means of the sham treatment group (Fig. 3) yielded values of 21.72 and 14.88 μg/100 g/min for the first and second hours of swimming respectively. However, direct measurements of maximum renal sodium loss in similarly cannulated rainbows subjected to a variety of stresses, including exercise itself (3.64 μg/100 g/min; Wood and Randall, 1973b), acute hypoxia (5.35 μg/

100 g/min; Hunn, 1969), MS 222 anaesthesia (3.38 μg/100 g/min; Hunn and Willford, 1970) and methyl pentynol anaesthesia (4.71 μg/100 g/min; Hunn and Willford, 1970) yield figures of considerably smaller magnitude. These data again suggest that the branchial sodium efflux rates of urinary blocked fish in the present study were abnormally low. The evidence further indicates that rather than comprising over half the whole animal efflux rate during the first and second hours of exercise (as the calculations suggest), urinary sodium loss can probably account for no more than 15% of this parameter. The branchial efflux rate of the shams would therefore have been approximately 30 μg/100 g/min during the first hour of swimming, a figure nearly identical to that measured during one hour of chasing by Wood and Randall (1973a).

The above points suggest that both the initial sodium deficit and its reversal during long term swimming occur largely at the gills. The original elevation of efflux has already been explained in terms of an increased blood perfusion of the highly permeable respiratory lamellae accompanying greater respiratory gas exchange during exercise (Wood and Randall, 1973a). It is highly unlikely that the reduction in branchial sodium efflux during prolonged activity represents a return of blood flow through the gills to a resting configuration because an elevated rate of oxygen uptake must be maintained. The nature and time course of the compensation are far more indicative of a progressive hormonal action on the sodium permeability of the branchial epithelia. Paralactin (or mammalian prolactin which mimics its action) has been shown to limit the passive efflux of sodium, mainly at the gills, in a variety of teleosts (e.g. Potts and Evans, 1966; Maetz *et al.*, 1967a, b; Ensor and Ball, 1968; Ball, 1969). It is now evident that paralactin 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). As such, this hormone could be of prime importance in reducing the augmented branchial sodium efflux associated with swimming in the rainbow trout.

2. The External Concentration Dependence of Branchial Sodium Fluxes

The preceding results have shown that branchial sodium influx was insensitive to the exercise condition or experimental treatment of the trout. It therefore seemed valid to analyse the interaction between external sodium level and branchial influx rate using all available paired values for these parameters from the same batch of rainbows. Each simultaneous hourly measurement of the two variables from the normal, sham, and urinary blocked treatment groups, and from ligated fish in which rupture of the urogenital papillae had previously necessitated rejection of flux

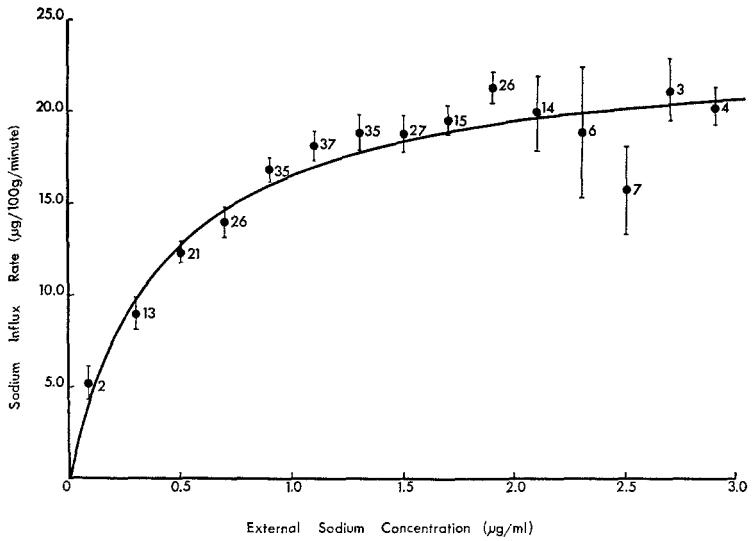

Fig. 4. The relationship between branchial sodium influx rate and external sodium concentration. Means ± 1 standard error. The curve fitted to the points represents the equation:

$$\text{influx rate} = \frac{23.64\ [\text{Na}]_0}{0.43 + [\text{Na}]_0}$$

where $[\text{Na}]_0$=external sodium concentration

rate data, were included in the analysis. These 271 determinations of sodium influx rate were averaged over successive 0.2 µg/ml ranges of external sodium concentration; the resulting means were plotted at the midpoints of the concentration intervals (Fig. 4).

The form of the relationship appeared qualitatively similar to that observed for a wide variety of sodium transporting systems, i.e. a non-linear pattern in which influx was approximately proportional to concentration at low external sodium levels but levelled off to approach a maximum at higher concentrations. 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 influx equation may be ignored and the relationship becomes.

$$M_i = \frac{M_{i\max} \cdot [\text{Na}]_0}{K_s + [\text{Na}]_0}$$

where: M_i=sodium influx rate;
$M_{i\max}$=maximum sodium influx rate;
K_s=half-saturation concentration;
$[Na]_0$=external sodium concentration.

which has a form analogous to the Michaelis-Menten equation relating enzymatic reaction rate to substrate concentration.

A double reciprocal plot of the present data (Lineweaver and Burke, 1934) yielded values for $M_{i\max}$(23.64 μg Na/100 g/min=61.68 μEq Na/100 g/hr) and K_s(0.43 μg Na/ml=0.02 μEq Na/ml) which were used to generate a curve describing M_i at different $[Na]_0$'s. This theoretical curve is the line fitted to the points in Fig. 4; the concentration dependence of sodium influx across the trout gill was therefore well described by the Kirschner equation. Similar accord has been demonstrated in many other intact animals—e.g. the fresh water crayfish, *Astacus pallipes* (Shaw, 1959); various gammarid crustaceans (Shaw and Sutcliffe, 1961; Sutcliffe, 1967a, b; Sutcliffe and Shaw, 1967); the amphibious crab, *Eriocheir sinensis* (Shaw, 1961); the mosquito larva, *Aëdes aegypti* (Stobbart, 1965, 1967); the freshwater shrimp, *Triops longicaudatus* (Horne, 1967); the freshwater gastropod, *Limnaea stagnalis* (Greenaway, 1970); and the lamprey ammocoete, *Lampetra planeri* (Morris and Bull, 1970)—and in the externally perfused gills of the anaesthetised rainbow trout (Kerstetter *et al.*, 1970; Kirschner, 1970). As Shaw (1959) has pointed out, there is nothing singular about coincidence of the data with a Michaelis-Menten type relationship, and other expressions may exist which describe the process more accurately. However, the correspondence does suggest a saturable rate-limited system, and a general similarity to other reported active sodium transport mechanisms.

The K_s and $M_{i\max}$ figures for *Salmo gairdneri* derived from the present data (Fig. 4) have been compared with values from several other fresh water organisms (Table 4). The transport system of rainbow trout in this investigation obviously demonstrated an extremely high sodium affinity (i.e. low K_s) relative to that of other animals. However, it must be noted that the K_s value of the present study is about 20 times lower than that obtained by Kerstetter *et al.* (1970) for the same species with lesser dissimilarities in $M_{i\max}$ (Table 4).

There exist several possible causes for these discrepancies. The present trout were in a relatively normal state, while Kerstetter *et al.* (1970) used anaesthetised fish held upside down and perfused at 100 ml/minute through a #15 needle sewn into the buccal cavity. Such a system probably involved only a small portion of the normal branchial area and may have been inadequate to satisfy the gas exchange requirements of the animal (Davis and Cameron, 1971). This effect would be sufficient to

Table 4. A comparison of K_s (half-saturation concentration) and $M_{i\,max}$ (maximum influx rate) values for sodium influx in several freshwater animals

Animal	K_s (mEq/L)	$M_{i\,max}$ (μEq/100 g/hr)	Reference
Limnaea stagnalis	0.25	22.50	Greenaway (1970)
Aëdes aegypti	0.55	1200	Stobbart (1965, 1967)
Astacus pallipes	0.25	~100	Shaw (1959)
Gammarus pulex	0.10–0.15	~500	Shaw and Sutcliffe (1961) Sutcliffe (1967a)
Gammarus lacustris	0.14	270	Sutcliffe and Shaw (1967)
Gammarus duebeni	0.4–2.5	~1500	Shaw and Sutcliffe (1961) Sutcliffe and Shaw (1968) Sutcliffe (1967b, 1971)
Lampetra planeri	0.26	36.00	Morris and Bull (1970)
Salmo gairdneri (perfused, anaesthetised)	0.46	33.30	Kerstetter *et al.* (1970)
Salmo gairdneri (intact)	0.02	61.68	Present study

account for the differences in flux rate magnitudes on a body weight basis, while the metabolic depression resulting from anaesthesia and/or hypoxia could well have elevated the observed K_s. Furthermore, if the concentration dependence curve of sodium influx across the trout gill is determined in some way by the permeability characteristics of the membranes 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 K_s. The nature of Vancouver tap water determined the water sodium levels to which rainbows were acclimated and in which flux rates were measured in the present investigation. Vancouver tap water contains very low concentrations of sodium [generally less than 2.0 μg/ml or 0.09 mEq/L; see also Holmes and Stainer (1966)]. The trout of Kerstetter *et al.* (1970) were preadapted to a sodium concentration about 10 times higher than this value. There exists considerable evidence from both invertebrates (e.g. Shaw, 1959, 1961; Bryan, 1960; Shaw and Sutcliffe, 1961; Stobbart, 1965; Horne, 1967; Sutcliffe and Shaw, 1968; Greenaway, 1970) and vertebrates (Garcia-Romeu and Maetz, 1964; Morris and Bull, 1968, 1970) that pretreatment with dilute solutions can stimulate sodium uptake through decreases in K_s and/or increases in $M_{i\,max}$. Indeed, genotypic differences may even be involved; it has been

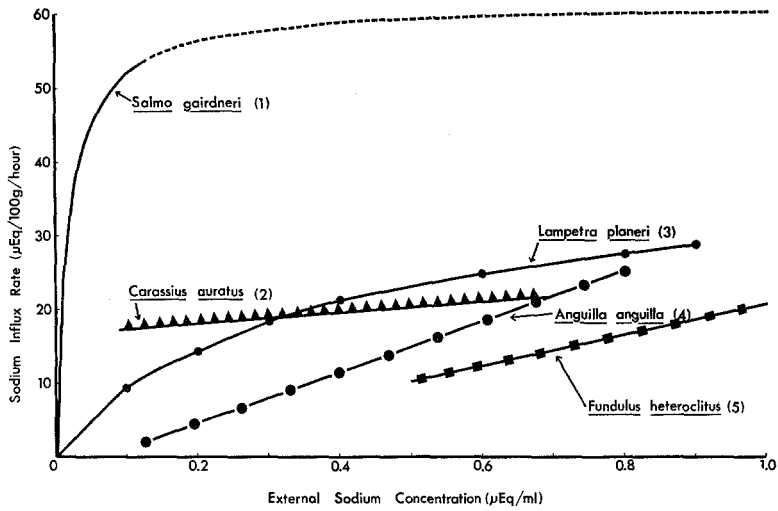

Fig. 5. Comparison of the external concentration dependence of branchial sodium influx rate in the rainbow trout (*1*) with that reported in the goldfish (*2*), the lamprey ammocoete (*3*), the eel (*4*), and the killifish (*5*). Lines have been redrawn from data presented in: (*1*) the present study. The curve has been extrapolated (broken line) beyond the region of direct determination by the equation:

$$\text{influx rate} = \frac{61.68\ [\text{Na}]_0}{0.02 + [\text{Na}]_0}$$

where $[\text{Na}]_0$=external sodium concentration, (*2*) Maetz (1956), (*3*) Morris and Bull (1970), (*4*) Chester Jones *et al.* (1969), and (*5*) Maetz *et al.* (1967b)

demonstrated that populations of both the amphipod, *Gammarus duebeni* (Sutcliffe and Shaw, 1968; Sutcliffe, 1971), and the isopod, *Mesidotea entomon* (Croghan and Lockwood, 1968) occurring in natural waters of low sodium concentration exhibit K_s values considerably lower (i.e. higher sodium affinities) than those of the same species inhabiting environments in which the ion is more abundant. Thus the quantitative differences (Table 4) between the results of the present study and those of Kerstetter *et al.* (1970) on *Salmo gairdneri* may reflect experimental, adaptational or perhaps even genetic influences.

There exist only a few other measurements of the concentration dependence of sodium influx in intact freshwater fish, and all were obtained at sodium concentrations of the acclimation and experimental media greater than those used here. However, comparison of the trout curve with this limited information (Fig. 5) does emphasize the 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 mEq Na/L, which may be indicative of a similar high affinity transport mechanism.

Comparison of branchial unidirectional sodium movements in the rainbow trout with those reported from a variety of other fresh water teleosts has revealed the considerably greater magnitude of the former (Wood, 1971); Fig. 5 also illustrates this difference. The presence of an exchange diffusion mechanism (Ussing, 1947) contributing equally to and enhancing influx and efflux rates in *Salmo gairdneri* could explain this effect; the contribution of an exchange component to branchial sodium fluxes has previously been suggested on other grounds (Wood and Randall, 1973a). Exchange diffusion of sodium has not been demonstrated in the gills of other freshwater adapted teleosts, but, *a priori*, there seems no definite reason why it could not occur. Such a system evidently accounts for a significant fraction of the normal sodium exchange of many freshwater invertebrates (Shaw, 1959; Stobbart, 1959, 1967; Bryan, 1960; Sutcliffe and Shaw, 1968; Greenaway, 1970). A general criterion for the presence of this mechanism is covariation of unidirectional flux rates with changes in external sodium concentration, or a marked reduction in efflux when influx is abolished.

To pursue the problem, two sets of efflux rate data were averaged over 0.2 μg/ml sodium concentration ranges as previously described for influx rates. The first set, distributed over generally low external sodium levels, comprised the efflux rates (71) of the urinary blocked group. Although generated by admittedly abnormal animals, these efflux values were exclusively branchial in origin and apparently unaffected by the activity condition of the fish (Table 3). Furthermore, as the influx rates of these ligated trout were identical to those of normal rainbows (Table 3; Fig. 3), it seems unlikely that the exchange diffusion component of efflux, if present, would have been altered by trauma. The second set of data, ranging over slightly higher environmental sodium concentrations, consisted of efflux values (49) recorded from trout of the normal treatment group only during periods of subsequent hours swimming, and first, second, and subsequent hours recovery. Under these conditions, the high efflux rates associated with the early hours of exercise did not occur (Fig. 3), and renal sodium loss was probably minimal (Wood and Randall, 1973b). The results of this analysis, presented in Fig. 6, indicated that branchial sodium efflux rate loosely paralleled the concentration dependence of the influx parameter over the same range. This pattern is extremely suggestive of exchange diffusion. Kerstetter *et al.* (1970) have obtained similar covariation of unidirectional branchial flux rates in their externally perfused trout preparation. Thus, at least on the qualitative nature of the influx

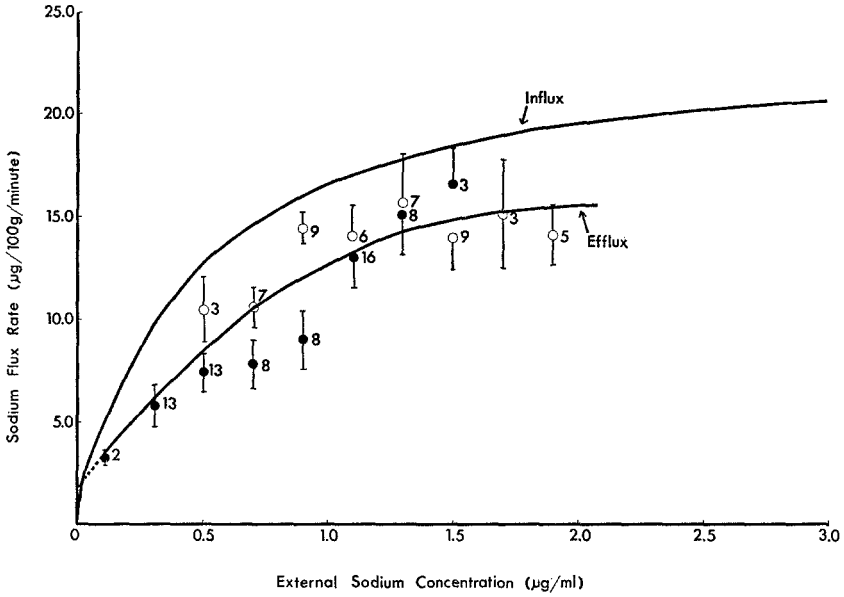

Fig. 6. The relationship between branchial sodium efflux rate and external sodium concentration. • =urinary blocked trout; ∘ =normal trout during subsequent hours swimming, and first, second, and subsequent hours recovery. Means ±1 standard error. The curve has been fitted by eye and extrapolated (broken line) to zero external sodium concentration for the purposes of the calculation in Table 5. The sodium influx curve from Fig. 4 has been included for comparison

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 extension of the efflux concentration dependence line to zero external sodium (Fig. 6) should approximate the non-carrier-mediated sodium leak across the gills. The procedure yielded a value of 2.0 μg Na/100 g/min for the simple diffusion component of efflux although there was not enough data to provide an exact basis for the extrapolation. Kerstetter *et al.*, by methods unstated, estimated an identical figure for this parameter. The value in any case must be somewhat greater than 0 μg/100 g/min, and is obviously less than 5 μg/100 g/min, so 2.0 μg/100 g/min would seem a reasonable estimate. The magnitude of this passive loss should have been stable over the relatively narrow external sodium concentration range encountered in the study. Consequently, from Figs. 4 and 6, the contribution of different mechanisms to branchial sodium exchange could be computed for a water sodium level of 1.6 μg/ml (Table 5), a concentration which was close to the acclimation

Table 5. Calculated contribution of different mechanisms to total branchial sodium exchange in the rainbow trout at an external concentration of 1.6 μg Na/ml

		μg/100 g/min
Influx	Exchange diffusion	+13.1
	Active transport	+ 5.7
	Total influx	+18.8
Efflux	Exchange diffusion	−13.1
	Simple diffusion	− 2.0
	Total efflux	−15.1
	Net flux	+ 3.7

It has been assumed that all influx-efflux linkage is caused by exchange diffusion.

value and at which influx rate approached maximum. This calculation indicates that a very large proportion (70%) of normal branchial sodium influx in *Salmo gairdneri* may be due to exchange diffusion, a situation similar to that observed in the freshwater mosquito larva, *Aëdes aegypti* (Stobbart, 1959, 1967). It should also be noted that the intersection of influx and efflux lines (Fig. 6), which gives an approximate estimate of the external concentration at which sodium balance can be maintained (Stobbart, 1967), occurred at the extremely low value of 0.06 μg Na/ml (2.6 μEq/L). This low balance point derives primarily from the low K_s of sodium influx; Shaw (1961) has demonstrated how the acquisition of a high affinity uptake mechanism obviates excessive permeability reductions in adaptation to dilute environments.

Kirschner (1970), presumably referring to the work of Kerstetter *et al.* (1970), has recently claimed that exchange diffusion of sodium does exist in freshwater rainbow trout gills. Such a conclusion seems very probable, but premature until the problem of back-transport is solved. Morris and Bull (1970) have argued that sodium flux linkages in the lamprey ammocoete, *Lampetra planeri*, similar to those observed in the present study, were caused by an unsaturated carrier back-transporting sodium ions leaving by simple diffusion. At high environmental sodium levels, more of the carrier would be bound up actively transporting external ions, and the measured efflux (by simple diffusion) would increase. 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 external concentration dependence of efflux. However, definitive state-

ments about the relative roles of exchange diffusion and back-transport in sodium flux linkages across the gills of *Salmo gairdneri* cannot be made from the results 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.

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