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

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Summary. 1. In the freshwater adapted rainbow trout, urine flow markedly increased during periods of exercise in a swimming respirometer.

2. Over a range of oxygen consumptions from "standard" to nearly "active metabolic rates", oxygen and water fluxes exhibited a highly significant positive correlation in individual fish. The results were indicative of covariation of the oxygen and water permeabilities of the gills.

3. A triphasic fluctuation in body weight, considered a measure of water balance, occurred during the course of exercise. On the basis of weight change and urine flow data, a tentative model of water regulation during swimming is presented.

4. Urinary levels of sodium and potassium tended to fall at high urine flows and rise at low flows. Calcium and magnesium concentrations underwent simultaneous fluctuations which did not always correspond to alterations in urine volume.

5. The renal excretion rates of all four cations increased significantly during swimming activity.

Introduction

Previous papers (Wood and Randall, 1973a, b) have dealt with the effects of exercise on general aspects of sodium balance of the freshwater rainbow trout, and, in particular, with sodium fluxes across the gills. The present study comprised a complementary investigation of water flux and regulation during swimming activity. If the concept of a respiratory/osmoregulatory compromise in branchial permeability (Steen and Kruysse, 1964; Randall *et al.*, 1967; Taylor *et al.*, 1968; Kirschner, 1969) is correct, then exercise should enhance water entry, as well as sodium efflux, across the gills.

Since it is extremely unlikely that any carrier mediated processes are directly involved in water exchange at the gills, determination of net water flux alone should provide adequate measure of the effective permeability of the organism to this substance. In freshwater teleosts, water enters across the gills and the intestine (Hickman and Trump, 1969), the skin being impermeable (Bentley, 1962; Fromm, 1968), and is eliminated through a single pathway, the kidney. The rainbow trout does not drink in fresh water (Shehadeh and Gordon, 1969), so urine flow should

approximate net branchial water entry in this species. Thus collection of urine provides a method for estimation of branchial water permeability, which, unlike radiotracer techniques, does not necessitate the use of a relatively small water volume. Such volume limitation had previously prevented the imposition of standard swimming conditions or any direct quantification of gas exchange during exercise in sodium flux studies (Wood and Randall, 1973a, b). Direct measurement of urine flow in the present investigation made it possible to use a swimming respirometer (Brett, 1964) in which the effects of controlled exercise on net branchial water entry and oxygen consumption could be studied simultaneously. Changes in body weight were ascertained as measures of water balance, while cation analyses of collected urine samples provided further information on kidney function during activity. Urine production and renal ion losses have not previously been measured in any freshwater teleost under controlled swimming conditions.

Methods

Sexually immature rainbow trout (*Salmo gairdneri*; 90–180 g) supplied by Sun Valley Trout Farm, Port Coquitlam, B. C., were held in dechlorinated fresh water for at least 3 weeks before experimentation. The fish were fed regularly with a commercial trout pellet. Water temperature throughout the study was $7.5 \pm 0.5°C$.

1. Urine Flow Versus Oxygen Consumption Experiments

Urinary catheters were implanted in trout anaesthetized in a 1:10000 MS 222 solution. The cannula was constructed by gluing, with epoxy resin, approximately 1.5 cm of Clay-Adams PE 190 polyethylene tubing around a 45 cm length of PE 60, 1–1.5 cm from the lightly heat-flared tip (proximal) of the latter. Both ends of the PE 190 jacket had also been flared, the distal to a much greater extent than the proximal. Numerous small holes were punched in the proximal 0.5 cm of PE 60 with a heated #22 needle. This tip was inserted into the urogenital aperture and pushed dorsad until the proximal flange of the PE 190 jacket lay just inside the papilla. The papilla was tied tightly around the jacket behind the proximal flange with 2 silk ligatures. A stitch was then sewn into the ventral body wall between the pelvic fins and the loose ends of thread led back around the distal flange of the catheter in a purse string ligature. Tension of the thread was adjusted such that a ventro-caudad pull on the cannula exerted force on the ventral body wall rather than on the delicate papilla itself. Finally, the catheter was anchored to the anal fin with a single stitch. This design ensured durability of the preparation under swimming conditions. During the cannulation procedure, the catheter was filled with distilled water. Immediately after insertion, 0.5 ml of water was infused into the urinary system and the cannula plugged with a pin; the plug was removed only when the fish was returned to water.

The animals were allowed to recover in darkened plexiglass chambers which permitted restricted movements but no change of position. Withdrawal of incurrent and excurrent water samples and measurement of flow through the metabolism

box (80–200 ml/min) allowed oxygen consumption determinations. PO_2's in water were ascertained with a thermostatted oxygen electrode system (Radiometer-Copenhagen). Rates of oxygen consumption, ventilation, and urine flow had stabilized by the third post-operative day. On the fifth day, the urine production of each fish was measured over a 5 hour period during which at least 5 determinations of metabolic rate were perfomed.

Between the fifth and eighth days, the trout was gently transferred, without anaesthesia, to the swimming respirometer and left overnight to accustom itself to the chamber. During this time, freshwater was circulated through the apparatus at 10.7 cm/sec, the minimum current into which the trout would continuously orient without swimming. The respirometer (volume = 23.75 L), originally used by Stevens and Randall (1967) and similar to that described by Brett (1964), could be either flushed with freshwater or sealed for measurement of oxygen consumption.

The urinary catheter was extended with a further 35 cm of PE 60 and led out through a rubber stopper at the top rear of the respirometer at least 2 hours before the start of an experiment; the cannula drained by siphon. The fish was then subjected to the following regime of water velocities:

10.7 cm/sec for 3 hours
21.4 cm/sec for 3 hours
10.7 cm/sec for 3 hours
21.4 cm/sec for 15 min
32.1 cm/sec for 3 hours
10.7 cm/sec for 3 hours
21.4 cm/sec for 15 min
32.1 cm/sec for 15 min
42.8 cm/sec for 3 hours
10.7 cm/sec for 3 hours

The animals swam at all velocities greater than 10.7 cm/sec. The anterior two-thirds of the swimming tube were covered with black plastic; trout generally oriented under the posterior end of the plastic, using it as a visual cue to maintain position during exercise. Total urine production was collected and measured for each 60 min section of the 3 hour periods and for the 15 min intermediate intervals. Samples were immediately frozen at −12°C in polyethylene vials for later ionic analysis. Oxygen consumption was also determined during each of the one hour periods by sealing the respirometer for a length of time (30–55 min) sufficient for the animal to reduce the ambient PO_2 by 10–15%. At no time was the PO_2 allowed to fall below 120 mm Hg. Oxygen uptakes during the 15 min velocity increment intervals were not large enough to quantify accurately. Opercular rates and tail beat frequencies were counted regularly throughout the experiments with the aid of mirrors placed under the swimming chamber.

All ionic analyses were performed in duplicate on a Techtron Model AA 120 Atomic Absorption Spectrophotometer against commercially prepared standards (Harleco). Sodium, potassium, and calcium concentrations were assayed in appropriate dilutions of urine on the emission mode of the instrument at 5890 Å, 7664 Å, and 4227 Å respectively. To overcome known interference effects, both standards and unknowns were swamped with 200 μg/ml sodium in the potassium analyses, and with 200 μg/ml sodium and 100 μg/ml potassium in the calcium analyses. No correction was made for the reported depressant action of phosphate ion on the spectral emission of calcium (Teloh, 1958); however addition/recovery

tests demonstrated that remaining interference effects were negligible. Magnesium assays were carried out on the atomic absorption mode of the instrument at 2852 Å; interference from inorganic ions is insignificant in this analysis (Dawson and Heaton, 1961).

As the primary purpose of this study was to correlate urine volume with oxygen uptake, urine was collected in hourly samples corresponding to the swimming regime periods. The volume of the collecting ducts and ureter was negligible, and as the catheter drained by siphon, urine could not have accumulated in the bladder. However the deposition into the collecting vial of urine formed at any one moment was delayed by a factor corresponding to the volume of the catheter and the rate of urine flow; at low flows, collection delay approached 3 hours. Consequently it was necessary to refer the measured ionic composition of collected urine to its time of formation by the method of Hickman (1968). Unfortunately the production times of samples often spanned two water velocities; variations in electrolyte concentration associated with different activity levels would have been somewhat dampened by this mixing process.

2. *Weight Change Versus Swimming Duration Experiments*

Experiments were performed on a batch of approximately 70 trout held in a 250 gallon concrete tank supplied with running freshwater. As 151 determinations were made in this study, each fish was used 2–3 times. The animals were starved for 6 days prior to experimentation to prevent faecal release contributing to weight changes. Because of the known sensitivity of branchial permeability (Evans, 1969) and renal function (R. M. Holmes, 1961; Hickman, 1965; Hammond, 1969; Hunn and Willford, 1970) to handling effects, the experimental procedure was designed to impose as little stress as possible on the trout. As a further precaution, the alterations in weight of an identically handled set of control animals were also measured.

For weighing, an unanaesthetized trout was removed from the water, placed on a bed of paper towels, and gently but throughly dried for 15 sec. The animal was then added to a tared styrofoam container fitting the shape of the fish and lined with a plastic bag. The bag contained 500 ml water and was sealed immediately after entry of the fish. Trout rarely struggled in the apparatus. After weighing, the animal was returned to its aquarium and gently released underwater. Total duration of the procedure was about 60 sec. Tests with dead fish showed that measurements were accurate to 0.1 g.

For weight change determinations, rainbows were transferred to individual darkened and covered aquaria (25 or 50 L) and left undisturbed for at least 8 hours to recover from this initial handling. Then individual fish were netted from their tanks, weighed, and returned for a further 8 hours recuperation. After this interval, experimental animals were separately transferred by dip-net to the darkened respirometer (5 sec). Water velocity was set at 10.7 cm/sec (15 sec), then 21.4 cm/sec (15 sec), and finally 32.1 cm/sec for the desired swimming period of 1, 5, 15, 30, 60 or 240–480 min (long term). Inactive fish were gently tapped on the tail to induce swimming; chronic non-swimmers were discarded. At the end of the exercise period, trout were removed from the respirometer and weighed as before. Control fish were netted from their aquaria and held in the air for 5 sec as for the experimental fish. However they were then returned to their individual tanks and left undisturbed until the appropriate sample time (1, 5, 15, 30, 60 or 240–480 min) when they were removed and weighed in the same manner.

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

Results

Four trout (#55, #59, #63 and #65) were subjected to the exercise regime in the swimming respirometer. For fish #59, the protocol was interrupted by a lengthy interval (24 hours), during which the trout was kept at 10.7 cm/sec, before imposition of the highest swimming speed. This break had no obvious effects on the animal's respiratory responses to exercise, but apparently depressed its rate of urine flow. Consequently, individual sections of the swimming regime were imposed consecutively on each of the other three fish during continuous experimental periods of 21.75 hours.

Ventilation rates, tail beat frequencies, and oxygen uptakes associated with different periods of the protocol are summarized in Table 1. Despite the fact that the trout had already spent 12 hours in the respirometer when an experiment commenced, the initial oxygen uptakes (at the non-swimming velocity of 10.7 cm/sec) were 2–3 times greater than those determined in the same animals after 5 days in the darkened plexiglass chambers. Similarly elevated metabolic rates at sub-swimming speeds in a water tunnel have been observed in salmon (Brett, 1964), and are associated with "restlessness" (spontaneous activity). Such effects are extremely difficult to eliminate, but tend to disappear with exercise. Thus oxygen uptake increased only slightly at the lowest swimming speed (21.4 cm/sec), probably reflecting a decrease in the consumption associated with "restless" behaviour. Metabolic rates at the highest velocity (42.8 cm/sec at 7.5°C, were greater than those observed at maximum swimming speeds in rainbows of similar size at 5°C (Rao, 1968). It would therefore appear that trout in the present study approached "active metabolic rates" (Brett, 1962) at relatively low speeds. This effect probably reflected the drag created by a trailing urinary catheter which would greatly elevate the cost of maintaining a particular swimming velocity. However the precipitous drops in oxygen consumption to original levels at the end of the exercise periods indicated that little, if any, oxygen debt was incurred.

Although urine production rates varied considerably, flows consistently increased during periods of exercise and decreased after their termination in all fish (Table 2). Alterations in net water flux (urine flow) corresponded with changes in oxygen uptake. Fig. 1 presents plots of oxygen consumption versus urine volume for each simultaneous deter-

Table 1. Tail beat frequencies (T.B.F.), ventilation rates, and oxygen consumptions during each 60 min period of the imposed swimming regime. Means ± 1 standard error for 4 fish

Velocity (cm/sec)	10.7	10.7	10.7	21.4	21.4	21.4	10.7	10.7	10.7	32.1	32.1
T.B.F. (tail beats/min)	—	—	—	77.8 ±12.7	78.3 ±13.1	58.0 ±16.0	—	—	—	124.6 ±4.1	124.2 ±2.0
Ventilation rate (closures/min)	63.0 ±2.4	63.5 ±2.7	61.6 ±3.0	79.0 ±3.2	75.3 ±3.0	71.6 ±3.5	62.8 ±2.0	63.2 ±2.4	62.2 ±2.3	89.8 ±1.8	86.7 ±2.4
Oxygen consumption (ml/kg/hour)	128.35 ±24.74	116.14 ±22.67	101.55 ±19.21	150.62 ±26.27	119.20 ±13.78	109.74 ±9.14	98.28 ±9.30	112.97 ±7.41	90.97 ±12.35	222.06 ±14.29	232.24 ±27.29
Continuation of regime:											
Velocity (cm/sec)	32.1	10.7	10.7	10.7	42.8	42.8	42.8	10.7	10.7	10.7	
T.B.F. (tail beats/min)	124.3 ±3.7	—	—	—	144.6 ±4.5	133.0 ±4.4	137.8 ±3.5	—	—	—	
Ventilation rate (closures/min)	92.0 ±3.2	66.8 ±1.1	64.1 ±1.4	60.8 ±1.8	102.2 ±2.7	96.7 ±8.2	84.0 ±8.9	66.6 ±1.5	61.4 ±1.4	61.5 ±2.1	
Oxygen consumption (ml/kg/hour)	248.38 ±19.69	139.63 ±19.81	113.72 ±12.60	98.14 ±9.17	299.81 ±6.05	272.35 ±9.11	296.56 ±5.03	92.61 ±6.93	81.50 ±12.97	94.46 ±14.70	

Table 2. Urine flows (ml/kg/hour) of individual trout during each period of the imposed swimming regime

Velocity (cm/sec)	10.7	10.7	10.7	21.4	21.4	21.4	10.7	10.7	10.7	21.4[a]	32.1	32.1
55 166.8 g	3.87	2.04	3.12	4.20	4.02	5.94	3.31	3.53	3.57	5.52	5.22	5.94
59 152.1 g	8.62	8.35	6.82	11.90	8.35	10.06	5.33	9.28	10.07	6.45	24.86	23.09
63 120.1 g	7.07	5.41	4.75	5.66	5.91	5.66	4.29	4.29	4.16	5.66	6.70	7.79
65 138.9 g	6.16	5.94	5.72	21.45	6.80	5.40	4.44	4.32	3.27	4.17	6.77	6.62
Mean	6.43	5.44	5.10	10.80	6.27	6.77	4.34	5.36	5.27	5.45	10.89	10.86
Continuation of regime:												
Velocity (cm/sec)	32.1	10.7	10.7	10.7	21.4[a]	32.1[a]	42.8	42.8	42.8	10.7	10.7	10.7
55 166.8 g	5.07	4.26	3.90	2.97	3.36	5.00	5.40	5.52	5.28	2.19	2.49	3.16
59 152.1 g	7.23	6.25	6.25	3.95	4.74[b]	5.26[b]	7.30[b]	8.22[b]	7.37[b]	3.09[b]	2.27[b]	2.24[b]
63 120.1 g	6.04	2.62	2.79	3.16	4.66	5.34	6.95	6.50	7.74	2.91	2.96	3.62
65 138.9 g	7.52	4.82	3.92	3.51	3.74	6.48	11.08	11.51	7.05	1.55	1.02	2.01
Mean	6.47	4.49	4.22	3.40	4.13	5.52	7.68	7.94	6.86	2.44	2.19	2.76

[a] Denotes 15 min periods. All other periods of the regime were 60 min.
[b] Denotes values taken after 24 hour interruption of experimental protocol.

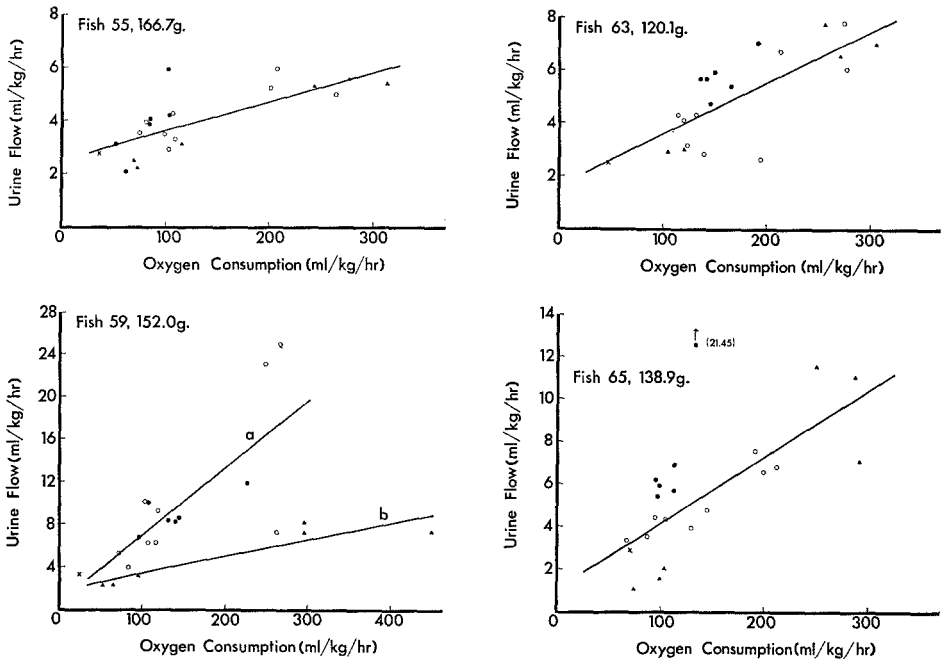

Fig. 1. Relationships between oxygen consumption (X) and urine flow (Y) in individual trout for each simultaneous determination of the swimming regime.
#55: $Y = 2.55 + 0.011\ X$; $r = 0.744$, $p < 0.001$.
#59a: (before interruption of regime) $Y = 0.71 + 0.063\ X$; $r = 0.707$, $p < 0.02$.
#59b: (after interruption of regime) $Y = 1.76 + 0.016\ X$; $r = 0.913$, $p < 0.02$.
#63: $Y = 1.72 + 0.019\ X$; $r = 0.742$, $p < 0.001$.
#65: $Y = 1.12 + 0.031\ X$; $r = 0.783$, $p < 0.001$. • data from first 6 hours of regime; ∘ from middle 9 hours; ▲ = from final 6 hours; × from same animal at rest in metabolism box (mean of 5 determinations for each parameter). All ×'s and labelled point (21.45 ml/kg/hour) of #65 (an extremely diuretic value; see Fig. 2) were excluded from regression and correlation calculations

mination of the swimming regime. For animals #55, #63 and #65, there existed highly significant ($p < 0.001$) positive correlations between the two parameters, although the slopes and intercepts of the regression lines differed between individuals. In fish #59, the slope of the relationship between urine production and metabolic rate declined after interruption of the swimming protocol, so separate correlations ($p < 0.02$) have been calculated for the two sets of data. Oxygen consumption and urine flow values taken in the metabolism boxes were in good agreement with the lines fitted to the points from the swimming experiments (Fig. 1).

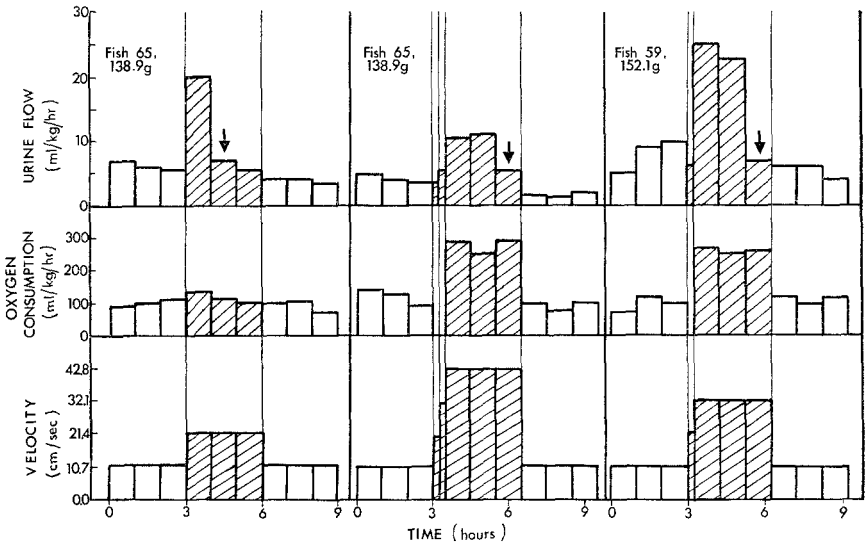

Fig. 2. Three examples of the phenomenon in which extremely high urine flows at the start of exercise were markedly reduced (as indicated by the arrows) during continued swimming without alteration of metabolic rate. Cross-hatching indicates periods of exercise

Thus over a range of oxygen uptakes from "standard" to almost "active metabolic rates" (Brett, 1962), net water and oxygen fluxes were in approximately linear positive proportion. This correspondence is interpreted in terms of gill water permeability being largely defined by the pattern of branchial blood perfusion necessary to effect a certain oxygen uptake.

The data also provided some evidence of compensations to reduce the osmotic penalty of exercise. Such phenomena were occasionally seen within a swimming period, and in a more general trend over the whole experimental regime. In the former, extremely high urine discharges at the beginning of an exercise interval were drastically reduced by the end of the period without any marked alterations of metabolic rate (Fig. 2). The more general tendency is illustrated by the plots of Fig. 1. For trout #55, #63, and #65, points taken from the first 6 hours of the regime generally lie above the common regression line, those from the middle 9 hours straddle it, while the values from the final 6 hours tend to fall below it. In fish #59, the conspicuous change in the urine flow/oxygen consumption relationship after interruption of the regime

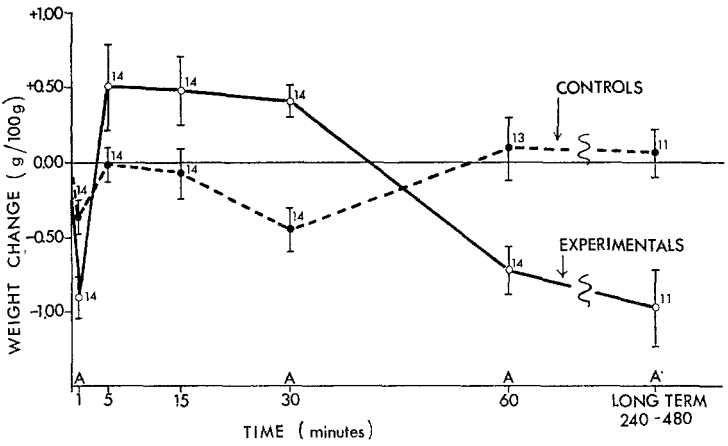

Fig. 3. Changes in body weight in response to various exercise durations at 32.1 cm/sec (experimentals) and in response to the handling necessary for the determinations alone (controls). Means ± 1 standard error. A = sample times at which control and experimental values are significantly different ($p<0.05$). Statistical comparisons: numbers refer to the sample times of respective means; see Wood and Randall (1973a).

Controls: $F=2.29$; $p<0.05$
30 1 15 5 240 60

Experimentals: $F=12.54$; $p<0.005$
240 1 60 30 15 5

may represent an extension of this effect. Water flux per unit oxygen uptake thus decreased as swimming experience increased.

The results of the weight change studies from experimentals (swimming at 32.1 cm/sec) and controls (resting in darkened aquaria) are presented in Fig. 3. Elevations of weight could only be caused by the gain of water from the environment; decreases could represent net water efflux, excretion of electrolytes (negligible), extusion of intestinal mucus, and "metabolic" losses. Estimates of the latter two factors based on the data of Shehadeh and Gordon (1969) for mucous discharge and the measured oxygen uptake of comparable trout (Table 1) for "metabolic" weight loss amounted to less than 5% of the mean decline of fish swum for 60 min. Consequently, it seems legitimate to interpret all weight shifts in terms of water movements. The net water balance of the exercised fish exhibited a triphasic response as swimming duration increased. The large decrease observed after 1 min was followed by a rapid rise to positive balance; however because of variability in values contributing to the 5 and 15 min experimental means, the increase did not significantly exceed control means until 30 min. Between 30 and

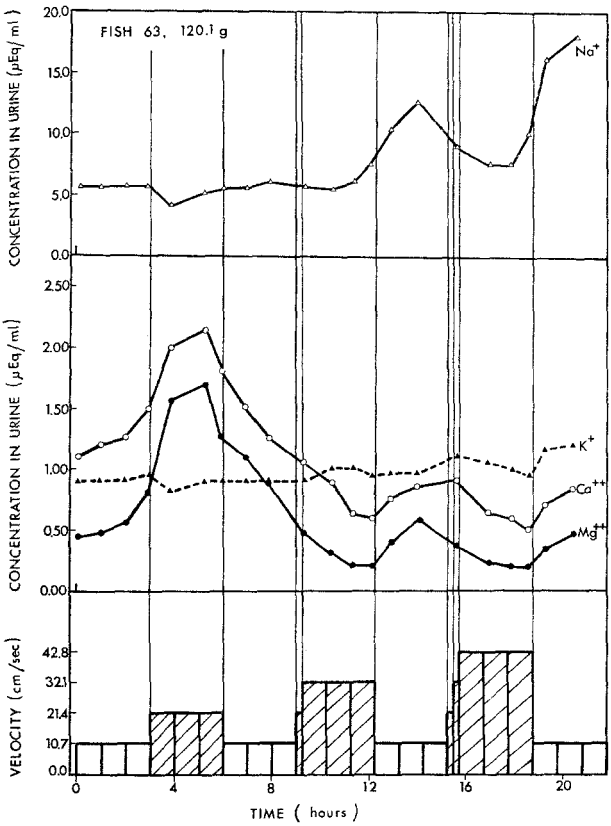

Fig. 4. Changes of cation concentration in urine from a single trout during the imposed swimming regime. Cross-hatching indicates periods of exercise

60 min exercise, the animals returned to a negative balance. There occurred only a very slight further decrement over the following 3–7 hours of activity. Fluctuations in control values were of much smaller magnitude but mimicked the oscillations of the experimentals until 30 min posthandling. It seems probable that the effects of handling were similar to but less severe than those of swimming. By 60 min, controls had returned to their initial weight which was maintained during long term activity.

Urinary cation levels differed widely between animals; such diversity of values seems characteristic of urinary electrolyte data in freshwater teleosts (Hickman, 1965; Hammond, 1969; Hunn and Willford,

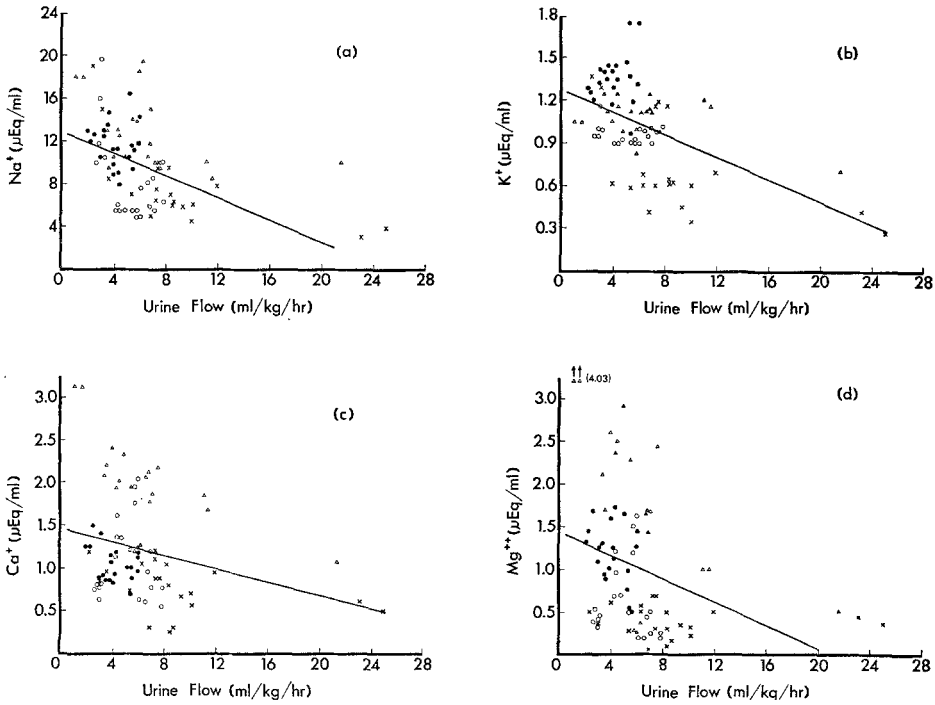

Fig. 5a—d. Relationships between urine flow (X) and urinary cation concentrations (Y) for each simultaneous determinations of the swimming regimes. a) Sodium: $Y = 12.70 - 0.426\ X$; $r = -0.462$, $p < 0.001$. b) Potassium: $Y = 1.28 - 0.040\ X$; $r = -0.543$, $p < 0.001$. c) Calcium: $Y = 1.46 - 0.039\ X$; $r = -0.264$, $p < 0.02$. d) Magnesium: $Y = 1.43 - 0.068\ X$; $r = -0.335$, $p < 0.01$. • data from fish #55; × from fish #59; ∘ from fish #63; ▵ from fish #65

1970; Miles, 1971). Nevertheless, several trends were common to all four trout and are illustrated by the data for fish #63 (Fig. 4). Sodium and potassium levels varied only moderately, tending to fall during exercise and rise during recovery in inverse relationship to urine volume. Calcium and magnesium concentrations, however, underwent large simultaneous fluctuations which did not always correspond to alterations in urine production.

Average electrolyte levels for each urine flow determination were estimated from curves of concentration versus time (e.g., Fig. 4). Overall, there existed highly significant negative correlations between urine flow and concentrations of sodium and potassium in the product ($r = -0.462$;

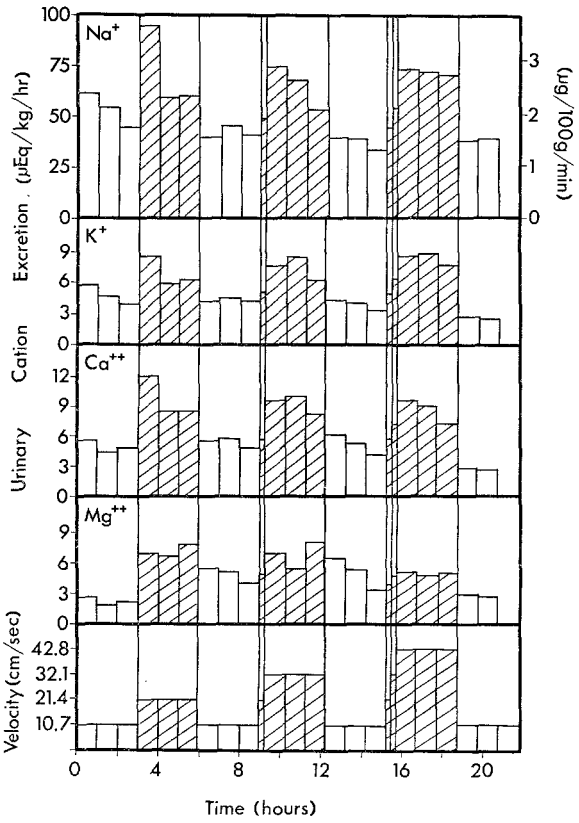

Fig. 6. Renal excretion rates of four cations during each period of the imposed swimming regime. Means for 4 fish. Cross-hatching indicates periods of exercise

Table 3. Renal excretion rate (μEq/kg/hour) of four cations. Means ± 1 standard error of all hourly determinations during "rest" (10.7 cm/sec) and swimming activity (21.4, 32.1 and 42.8 cm/sec)

	Rest $n=44$	Swimming activity $n=36$	p
Sodium	43.15 ± 2.99	69.78 ± 5.36	<0.001
Potassium	4.05 ± 0.18	7.70 ± 0.40	<0.001
Calcium	4.86 ± 0.36	9.28 ± 0.84	<0.001
Magnesium	3.84 ± 0.45	6.36 ± 0.70	<0.01

p = significance level of difference between means.

$p<0.001$; $r=0.543$; $p<0.001$ respectively) (Fig. 5A, B). Similar relationships applied to calcium and magnesium levels ($r=-0.264$; $\#p<0.02$; $r=-0.335$; $p<0.01$ respectively), but were less well defined due to the aforementioned synchronous fluctuations of these two cations (Fig. 5C, D). However increases in flow generally overshadowed the associated decreases in ionic concentration, resulting in elevated electrolyte losses from the kidney during exercise periods of the imposed regime (Fig. 6). At least for sodium and potassium, changes in renal excretion rate thus closely paralleled alterations in urine volume. Separate averaging of all determinations taken during periods of "rest" (10.7 cm/sec) and exercise (21.4, 32.1, and 42.8 cm/sec) confirmed that swimming activity significantly elevated the renal efflux rates of all four cations (Table 3). Urinary sodium discharge (2.0–4.0 μg/100 g/min, Fig. 6), however, remained small relative to either the unidirectional (28.50 μg/100/min) or net efflux (12.14 μg/100 g/min) of this ion across the branchial surface during exercise (Wood and Randall, 1973a).

Discussion

Shehadeh and Gordon (1969) have shown that the rainbow trout in fresh water, unlike some other teleosts (Potts *et al.*, 1967; Potts and Evans, 1967; Foster, 1969), does not drink under resting conditions, but the possibility of intestinal water absorption during activity cannot be completely dismissed. However, as drinking rates of salmonids even in full strength sea water are relatively low (Shehadeh and Gordon, 1969; Potts *et al.*, 1970), it seems unlikely that this element was of importance in the present study. Inequality of branchial influx and renal efflux rate, as detected in the weight change experiments, was probably a more significant source of error in the estimation of water entry across the gills. This factor could have contributed to much of the scatter in the relationships between urine production and metabolic rate (Fig. 1). Nevertheless, the highly significant correlations between oxygen uptake and the urine flow estimate of net water influx over a wide range of values seems indicative of a respiratory/osmoregulatory adjustment in gill water permeability. A branchial sodium deficit associated with activity has been demonstrated previously (Wood and Randall, 1973a). The same hypothesis, that an increased passive flux is caused by greater blood perfusion of the highly permeable respiratory lamellae, i.e. by an increase in the functional "size" of the branchial exchange area, can be used to account for both the net sodium efflux and increased water influx during activity. Mackay and Beatty (1968) suggested a similar explanation for the temperature dependence of urine production in the white sucker, *Cato-*

stomus commersonii. Evans' (1969) demonstration, through radiotracer techniques, of increased water permeability caused by "stress" in trout is also in agreement with the present results.

The weight change data (Fig. 3) must be interpreted in terms of differences between net water uptake across the gills and net water excretion through the urogenital papilla. The large magnitude of the weight loss after 1 minute indicated that it was probably caused by a bladder emptying effect, although an increase in glomerular filtration rate (G.F.R.) resulting from an initial "overshoot" n systemic blood pressure at the onset of exercise could have been involved. Hammond (1969) has demonstrated in the lake trout, *Salvelinus namaycush*, that pressor effects can markedly elevate the rate of urine formation.

The following water gain and final water loss must have been produced by inequalities of influx and efflux rates, but it is not clear whether the former, the latter, or both processes were changing. However the urine production data may be informative in this respect. After 60 min of swimming, the net weight loss relative to the gain at 30 min was 1.13 g/100 g. Even if no water influx occurred after 30 min, a highly unlikely situation, urine flow over the initial hour of exercise must have exceeded 11 ml/kg/hour. It is probable that the actual rate was much higher. Thus the weight change animals were apparently behaving as the examples of Fig. 2 which underwent such a pronounced diuresis during the first 1 or 2 hours of swimming acitivity; this diuresis was drastically reduced during subsequent exercise. Furthermore, there occurred little, if any, augmentation of urine production during the first 15 min of activity in such cases. That this type of response was observed only occasionally in the catheterized animals may be attributed to their spontaneous activity and experience in the respirometer, which could have "adapted" them to exercise prior to the actual swimming runs. The weight change group had, on the other hand, been quietly resting in darkened aquaria prior to obligatory swimming.

Thus by collating the weight change information (Fig. 3) with the type of urinary response illustrated in Fig. 2, the following tentative scheme describing water regulation during swimming (in a trout initially "unadapted" to exercise) may be developed. A graphical illustration of the proposed model is presented in Fig. 7. The net weight increment appearing between 5 and 30 min is caused by an extremely high influx rate at the onset of exercise without compensatory elevation of renal function. As swimming progresses, this high branchial water permeability tends to decrease; a similar reduction in branchial sodium permeability during exercise has been previously noted (Wood and Randall, 1973b). Efflux, however, increases as water uptake declines. This phenomenon finally

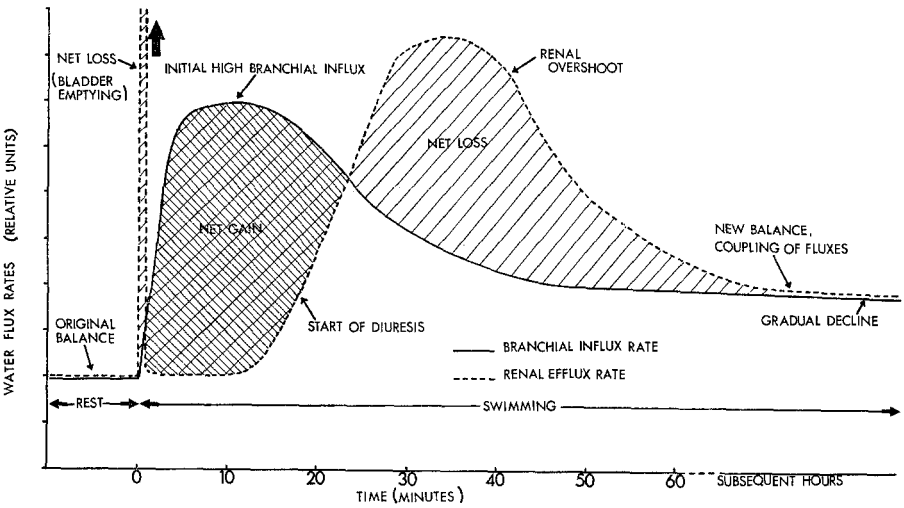

Fig. 7. Proposed model of the temporal changes occurring in branchial water influx and renal water efflux rates of the rainbow trout during the course of swimming activity

overcompensates for the original water gain resulting in a weight loss at 60 min. As weights measured 1 and 4–8 hours after the start of swimming are similar, a balance between branchial entry and renal output is reached soon after 60 min. The new balance, however, is set at a net body water content lower, and a water turnover rate higher, than those originally maintained. It may be hypothesized that as the trout reaches this new balance, it becomes "adapted" to exercise so that branchial and renal water movements are now linked in some manner, changing simultaneously with further alterations of metabolic demand. A final gradual decline in the coupled fluxes has been incorporated into the model to represent the decrease in water flux per unit oxygen uptake observed with increased swimming experience. Such a scheme satisfies the present information, but contains considerable speculation from limited data. More extensive research, employing if possible a direct measure of branchial permeability, will be required to determine its validity.

The triphasic weight fluctuations (Fig. 3) may be correlated with a reciprocal triphasic response in haemoglobin and plasma protein levels observed at the beginning of exercise in *Salmo gairdneri* by Stevens (1968). Since the ratio of haemoglobin concentration to plasma protein concentration did not change during their individual fluctuations, Stevens concluded that the effects were caused by differential rates of water

movement into the blood across the gills and out of the blood into the tissues. On the basis of the present work, this explanation may be modified to include inequalities of branchial water influx and renal efflux rates. The rise in plasma sodium levels and apparent decline in blood volume over one hour of exercise in the same species (Wood and Randall, 1973a) can also now be related to the significant water loss by 60 min. An increase in concentration of plasma electrolytes resulting from a fall in plasma water content would tend to compensate for augmented branchial and renal ion losses, while haemoconcentration would elevate both the oxygen and buffering capacities of the blood. Any accompanying rise in blood viscosity would be offset by the overall decrease in systemic resistance associated with swimming acitivity (Stevens, 1968). Thus maintenance of a reduced blood volume through net water loss appears distinctly advantageous to a trout undergoing prolonged exercise.

Urinary sodium levels were similar to those reported by other workers for this species; potassium, calcium and magnesium concentrations fell within the ranges but were generally lower than the mean values of other studies (Fromm, 1963; Holmes and Stainer, 1966; Hunn, 1969; Hunn and Willford, 1970). An inverse relationship between sodium and potassium levels in urine, as seen in the white sucker, *Catostomus commersonii* (Hickman, 1965), did not occur in *Salmo gairdneri*. The large simultaneous oscillations in calcium and magnesium levels, observed in all animals of the present study, have also recently been noted in one freshwater specimen of the coho salmon, *Onchorhyncus kisutch* (Miles, 1971). It seems likely that these variations were associated with fluctuations in the tubular reabsorption of the ions (Hammond, 1969). Hickman and Trump (1969) have proposed that the transport of calcium and magnesium through the tubular epithelial cell is effected by closely related, if not identical, ATP requiring systems. However the reason for apparent temporal fluctuations in the activity of this mechanism is unknown.

The urinary cation concentration changes observed in the present study indicate that the diuresis associated with exercise is somewhat different from that occurring after handling (R. M. Holmes, 1961), severe hypoxia (Hunn, 1969), or anaesthesia (Hunn and Willford, 1970). In all these latter situations, high flow rates were associated with marked increases in electrolyte concentrations; the opposite occurred during swimming. Diuretic states involving increased urinary ion levels probably reflect profound disturbances in tubular mineral reabsorptive functions as well as in G.F.R. The exercise diuresis, however, appears to represent merely an exaggeration of variations which can normally be observed in resting fish (Hammond, 1969; Hickman and Trump, 1969). Such altera-

tions are mediated by changes in filtration rate with only minimal variation in tubular salt conservation functions. The efficiency of water reabsorption, however, may decrease at high G.F.R.'s, resulting in lower electrolyte concentrations at high urine flows (Hammond, 1969). Increased urine discharge during swimming therefore seems to be a simple "water" diuresis (Hunn, 1969), uncomplicated by perturbations of tubular ion transport mechanisms. In a phenomenon of this nature, salt excretion will be largely dependent on urine flow. Thus renal adaptation to reduce the urinary component of mineral deficit during or after exercise may be simply a secondary consequence of branchial permeability adjustment. It is interesting to note that non-smolting rainbows may be considered to demonstrate a similar type of diuresis relative to smolting individuals (Holmes and Stainer, 1966). Smolting fish, like resting fish, are thought to have a lower urine production because of a reduced branchial water permeability.

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