J. exp. Biol. **118**, 277–286 (1985)
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UPTAKE AND LOSS OF POTASSIUM BY RAINBOW TROUT (*SALMO GAIRDNERI*) IN FRESH WATER AND DILUTE SEA WATER

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Accepted 12 March 1985

SUMMARY

Potassium turnover was studied in rainbow trout, *Salmo gairdneri* Richardson, adapted to fresh water or 22‰ sea water using ^{42}K and ^{86}Rb. Potassium space of the whole body increased with time and was about 5 mmol kg^{-1} after 20 h, while Rb^+ space under the same conditions was only about 0·5 mmol kg^{-1}, indicating slow penetration of body K^+ by Rb^+, especially in muscle and red blood cells. Potassium influx, measured by decrease in specific activity of the medium, was 0·07 mmol kg^{-1} h^{-1} in fresh water and 0·48 mmol kg^{-1} h^{-1} in 22‰ sea water; the values for efflux were comparable, indicating that unfed fish are able to maintain K^+ balance. In both fresh water and dilute sea water, K^+ fluxes are 5 % or less of the simultaneous Na^+ and Cl^- fluxes. The mechanism for K^+ fluxes is discussed in terms in K^+-ATPases.

INTRODUCTION

Although the regulation of sodium and calcium has been closely studied, the regulation of the major intracellular cation, potassium remains little investigated. This is particularly so in fish, where the use of radioisotopes has been helpful in revealing many aspects of Na^+ and Cl^- regulation, but the lack of a long-lived radioisotope for K^+ has not encouraged studies of this type, and even studies on the net uptake (or loss) of potassium from fish are uncommon.

The experiments described below attempted to answer a number of questions. These were (i) what is the rate of K^+ loss from the fish, (ii) are fish able to absorb K^+ from the water and (iii) if active absorption does occur, what is the mechanism?

Studying ionic regulation in freshwater fish, Krogh (1939) observed absorption of Na^+ and Cl^- but not K^+. One of the earliest studies to use ^{42}K was that of Mullins (1950), who showed an increase in the specific activity of *Gasterosteus aculeatus* relative to the medium both in fresh water and in dilute sea water,

Key words: Rainbow trout, potassium turnover.

indicating uptake of potassium by the fish. Other studies using ^{42}K are those of Maetz (1969) on seawater-adapted flounders and of Kerstetter & Kirschner (1972) on freshwater-adapted rainbow trout.

There have been many mammalian studies on whole body potassium using ^{42}K and these indicate some important points relating to the present work. Whole-body potassium space can be determined by comparing, at equilibrium, the total body activity with the specific activity of the blood plasma: it is about 42 mequiv kg^{-1} in man, nearly all of which is intracellular, labile and readily exchangeable with only about 2 % in the extracellular fluid. Thus a shift of a small fraction of intracellular K^+ could lead to large changes in the blood plasma K^+ concentration (Pitts, 1964). Such studies are lacking in fish, but Mayer & Nibelle (1969) used this technique to study sodium space in eels.

MATERIALS AND METHODS

Rainbow trout, weighing 15–30 g, were obtained from College Mill Trout Farm, Almondbank, Perthshire and were kept in 250 litre tanks at 10 °C supplied with flowing water. The fish were fed three times weekly with commercial trout pellets but not immediately prior to or during experiments. Fish selected for an experiment were placed in 2-litre glass conical flasks, covered over with black plastic sheet and containing a known volume (usually 600 ml) of constantly aerated aquarium water. For influx experiments, approximately 15 μCi ^{42}K (as $KHCO_3$) was added to each flask, an initial water sample of 2 ml was taken and further samples were taken at intervals over the next 24 h. For efflux experiments the same procedure was adopted except that the fish was first injected with ^{42}K, an operation which could be completed in a few seconds without stressing the fish to any major extent.

A sample of radioisotope – usually about 100 μCi – was made up to 350 ml with fish saline and each of six fish received a 50-μl intraperitoneal injection, with the remaining 50 μl being made up to 2 ml with water to determine the activity administered. Two to three minutes after introducing the fish to the flask, a 2-ml sample was removed to determine radioisotope leakage from the injection site. Similar experiments were carried out using about 1–2 μCi ^{86}Rb per fish.

A group of fish were kept in 2/3 sea water (22 ‰) for at least a week prior to determination of potassium and rubidium fluxes.

Experiments were terminated by killing the fish with a blow to the head, severance of the tail to collect blood in 70-μl heparinized capillary tubes and removal of a muscle sample (usually 0·5–1·0 g) from the area below the dorsal fin. The activities of blood plasma, red cells and muscle were determined immediately.

Ionic analysis

The K^+ contents of water, blood plasma and muscle (dissolved in a known volume of concentrated nitric acid) were determined using an EEL 100 flame photomotor or a Pye Unicam SP 900 atomic absorption spectrophotometer.

Determination of gamma activity

Because of the short half-life of ^{42}K, its activity was determined immediately using a manual Panax Reigate counter, while accumulated ^{86}Rb samples were counted in a Packard automatic 5250 counter.

Calculation of fluxes

Influx was calculated from the change in specific activity between successive samples of the medium. Because of the relatively short time periods between successive samples of the medium the error in treating disappearance of activity as a linear function instead of an exponential function was very small.

Efflux was calculated using the equations from Motais (1967).

$$K = \frac{1}{T} \ln \frac{Co - Ct}{Co},$$

where K is the turnover, T is the time, Co is the initial counts and Ct is counts at time T, or, alternatively, from the increase in specific activity of the medium; the two methods gave similar results over the short time periods involved.

Ionic content of water

A typical analysis of aquarium water gave (in mmol l^{-1}) Na^+, 0·2; Cl^-, 0·3; Ca^{2+}, 0·1; K^+, 0·05; pH = 7·5–8·0 and temperature 10 °C. All water samples were analysed for K^+ to determine the specific activity of this ion and a typical concentration in fresh water after addition of radioisotope was 0·1 mmol l^{-1}.

Calculation of potassium space

Potassium space at any time was calculated from the relationship:

$$K^+ \text{ space (mmol kg}^{-1}) = \frac{\text{activity administered} - \text{activity in water}}{\text{activity in 1 ml plasma/K}^+ \text{ in 1 ml plasma}}$$

Activity lost to the water will include branchial potassium efflux as well as urinary and body surface losses. No attempt was made to differentiate between the various losses but it is likely that the greater part of the K^+ efflux is branchial.

RESULTS

Table 1 shows the potassium space of whole body, muscle and blood at different times after injection with ^{42}K, and the K^+ content of the various tissues for both freshwater- and 2/3 seawater-adapted fish. Similar results are shown for rubidium; the most noticeable feature is that Rb^+ space is much smaller than K^+ space in all tissues examined.

The efflux of K^+ from fish injected with ^{42}K either in 2/3 sea water or fresh water is shown in Fig. 1A,B, which also shows the uptake of activity by fish from

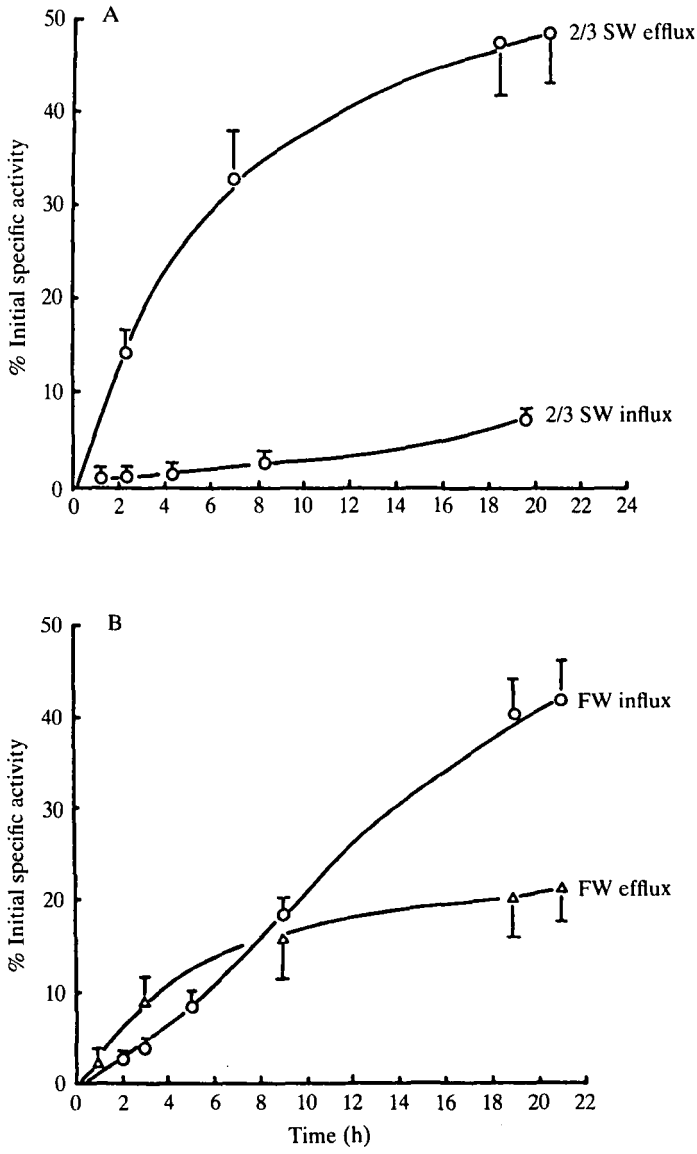

Fig. 1. Change in specific activity of K^+ in the water during flux experiments, (A) in dilute sea water and (B) in fresh water. Results are expressed as percentage of the initial specific activity of ^{42}K in the medium, influx as a decrease and efflux as an increase in specific activity. Mean and standard error of six determinations are indicated. SW, sea water, FW, fresh water.

these media. The results are expressed as the percentage of the total activity appearing or disappearing from the medium and indicate that fish lose K^+ to the outside medium but are also able to absorb K^+ from the water, certainly against a concentration gradient in fresh water.

Fig. 2 shows influx and efflux rates of K^+ from fish in fresh water and 2/3 sea water. The results for influx are inherently more accurate since they are calculated

Table 1. *Tissue K^+ concentration and K^+ space in rainbow trout*

Time (h)	Experimental conditons	K^+ (mmol l^{-1})			Ion space (mmol kg^{-1})		
		Plasma	RBC	Muscle	Body	RBC	Muscle
1·3	Fresh water efflux K^+	4·62 ± 0·75	130·0 ± 7·2	97·4 ± 6·9	1·4 ± 0·43	33·0 ± 21	2·8 ± 1·5
4	Fresh water efflux K^+	4·81 ± 0·64	133·8 ± 3·2		2·74 ± 1·5	46·6 ± 9·5	
21	Fresh water efflux K^+	3·2 ± 0·33	117·7 ± 5·2	110 ± 5·0	4·93 ± 1·48	69·4 ± 13·9	22 ± 10
1	Fresh water efflux Rb^+	5·1 ± 2·2			0·31 ± 0·09	0·098 ± 0·004	
18	Fresh water efflux Rb^+	3·8 ± 0·83			0·53 ± 0·17		
18	Fresh water efflux Rb^+	3·1 ± 0·7			0·5 ± 0·15	0·057 ± 0·008	
19·5	Sea water influx K^+	4·89 ± 0·46	157·9 ± 11·3		5·9 ± 2·1	9·0 ± 2·3	
21	Sea water efflux Rb^+	6·9 ± 0·84		130·7 ± 11·8	0·9 ± 0·33		0·0023 ± 0·0007
28	Sea water influx Rb^+	4·06 ± 0·13		107·5 ± 6·8	0·153 ± 0·07		$8·5 \times 10^{-5} \pm 6·5 \times 10^{-5}$

K^+ and Rb^+ spaces were calculated from the relationship: $\text{Ion space} = \dfrac{\text{counts kg}^{-1}\text{ tissue}}{\text{specific activity of } K^+ \text{ in counts mmol}^{-1}}$.

In making such calculations it was assumed that K^+ and Rb^+ were identical. In the Table 'Sea water' refers to dilute sea water whose concentration was 22‰. Mean and standard deviation for six determinations are indicated. RBC refers to red blood cells.

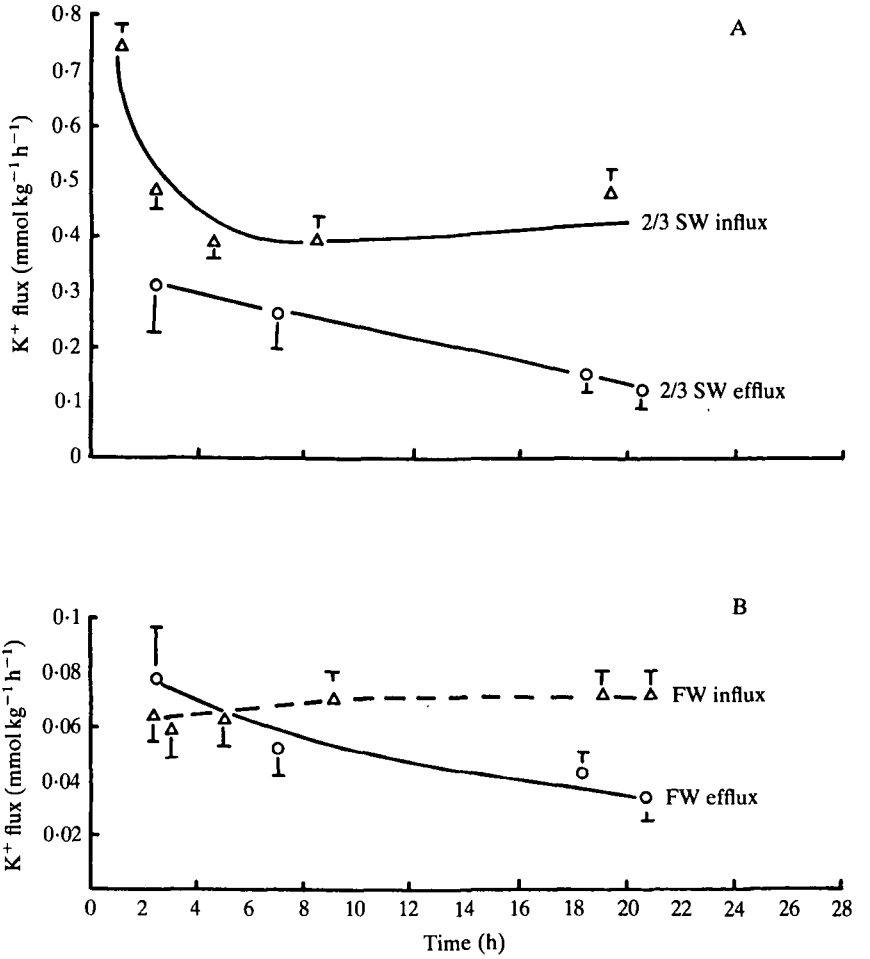

Fig. 2. K^+ fluxes for rainbow trout (A) in dilute sea water and (B) in fresh water. Values are in mmol kg^{-1} h^{-1}. Influx was calculated from the decrease in specific activity of the medium and efflux from the increase in specific activity together with an estimate of exchangeable K^+ in the fish at any time. See Table 1 and text for details. Mean and standard error of six determinations are indicated.

from differences in specific activity of the medium for any given time interval. However results for efflux are less accurate since they depend upon a value for K^+ space, or exchangeable K^+, which is difficult to determine precisely and also tends to increase with time (Table 1). There is reasonable agreement between influx and efflux rates in the two media and the results indicate that the fish has adequate mechanisms for maintaining K^+ balance and achieving a net K^+ uptake which is independent of diet.

DISCUSSION

There have been few measurements of K^+ fluxes in fish and some of the results are reviewed in Table 2. Potassium fluxes in the flounder, with an efflux of

Table 2. *Potassium fluxes in various fish species*

Species	Medium	Influx	Efflux	Author
Flounder (*Platichthys flesus*)	SW	1·2		Maetz (1969)
Sculpin (*Leptocottus armatus*)	SW	0·58	1·56	Sanders (1979)
Rainbow trout (*Salmo gairdneri*)	SW	0·241	0·349	Sanders (1979)
Rainbow trout	FW	0·042	0·058	Kerstetter & Kirschner (1972)
Rainbow trout	FW	0·07	0·035	This study
Rainbow trout	SW	0·35–0·48	0·2–0·3	This study

Values in mmol kg^{-1} h^{-1}.

In the present experiments on rainbow trout, 2/3 sea water was used and the values for fluxes are derived from Fig. 2 and Table 1.

Values for K^+ efflux are strongly dependent upon the value for exchangeable body K^+. See text for details. SW, sea water; FW, fresh water.

1·45 μmol kg^{-1} h^{-1}, are much greater than in other species studied so far, and K^+ influx values in rainbow trout, whether in full strength or dilute sea water, are less than half the value for the flounder (Table 2). Fluxes for freshwater-adapted rainbow trout in this study are significantly lower than the values obtained by Kerstetter & Kirschner (1972), who used anaesthetized animals in an external medium of 0·5 mmol l^{-1}, K^+. K^+ fluxes are much smaller than Na^+ and Cl^- fluxes: e.g. in flounder, K^+ influx is about 5 % of the Na^+ influx (Maetz, 1969), for rainbow trout in 2/3 sea water K^+ efflux is about 2 % of Na^+ efflux, and rainbow trout in fresh water have a K^+ efflux about 5 % of the Na^+ efflux (Eddy & Bath, 1979).

Potassium and rubidium space

Rb^+ penetrates the body tissues to a much smaller extent than K^+ (Table 1). Thus, after about 20 h, whole-body Rb^+ space is some 10–20 % of the K^+ space, about 0·1 % of the red cell space, whilst the muscle compartment is barely penetrated by Rb^+. This condition could be clearly observed in all Rb^+ experiments because of the very high activity in blood plasma compared to that in other tissues. This observation was amplified when 0·5 ml trout whole blood was equilibrated with 0·1 μCi ^{86}Rb, centrifuged after 15 min, and the cells and plasma counted. About 45 % of the activity was in the plasma even though it contained about 4 % of the blood potassium, which is in contrast to the extensive penetration of red blood cell potassium by ^{42}K.

Rubidium has often been used as an analogue for K^+ in mammalian erythrocytes (Mills & Tupper, 1975; Beauge & Adragna, 1971) and in fish erythrocytes (Bourne & Cossins, 1982), where similar influx rates for ^{42}K and ^{86}Rb were noted (Bourne & Cossins, 1984), but the present results suggest that caution should be

exercised in applying this assumption to all fish tissues. In studies involving simultaneous use of Rb^+ and K^+ on insect midgut (*Hyalophora cecropia* larvae), Zerahn (1980) concluded that although Rb^+ is transported in a similar manner it could not be used quantitatively as a tracer for K^+. However in frog skin, K^+ and Rb^+ competed for transport in the ratio 1:0·9 (Zerahn, 1983).

Sanders (1979) measured K^+ and Rb^+ fluxes across the gills of rainbow trout and concluded that the gill epithelium was more permeable to K^+ than to Rb^+: e.g. $^{42}K/^{86}Rb$ efflux $= 1{\cdot}20 \pm 0{\cdot}1$. These experiments had the added refinement that the presence of a cannula in the dorsal aorta enabled determination of fluxes by comparing the specific activity of the radioisotope between blood and water, i.e. unidirectional fluxes across the gill epithelium could be measured directly. The influx values obtained are a little lower but in reasonable agreement with values from the present study (Table 2), where changes in specific activity of the medium were measured to calculate fluxes. The efflux values obtained by Sanders (1979) exceed the influx values, which suggests a significant net loss of K^+ by the fish which is not the case in the present experiments (Table 2). However, the present results indicate that Rb^+, compared to K^+, exchanges relatively slowly between the blood plasma and the body mass of the fish. Thus, Rb^+ and K^+ fluxes may be similar initially but over a period of hours or days the specific activity of Rb^+ in the blood plasma will tend to be higher than that for K^+, since it exchanges more slowly with the body tissues. Under these conditions, using Rb^+ as a substitute for K^+, calculation of efflux may give unrealistically high results, but this is an area worthy of further investigation.

Transport of K^+

In the majority of freshwater fishes the branchial surface rather than the body surface is the principal site for exchanges of Na^+ and Cl^- (Maetz, 1971; Haswell, Randall & Perry, 1980) and all the available evidence suggests that the same is true for K^+ (Sanders, 1979; Table 2). Trout in 2/3 sea water drink about 5 ml kg^{-1} h^{-1} of the medium giving a K^+ intake of about 0·015 mmol kg^{-1} h^{-1} or approximately 3 % of the branchial influx. The diet probably accounts for a significant intake of K^+, but the present work, carried out on fish which had not been fed for 2–3 days prior to the experiment, shows that trout have the ability to regulate K^+ independently of the diet. Although there are some theories to explain the movements of Na^+ and Cl^- (Maetz, 1971; Haswell *et al.* 1980), there are apparently as yet no theories to explain K^+ transport.

If K^+ was transported directly to the blood of a freshwater fish and if the potential was close to zero then the concentration gradient would be about $4/0{\cdot}1 = 40$ and the electrical gradient would be about 90 mV. It seems more likely that K^+ may be transported directly into the gill epithelium cells which would tend to have a relatively high K^+ content and may be strongly negative within. In this case the large concentration gradient may be offset to some extent by the possibility of a negative potential inside the cell, but as before energy would be required to transport K^+.

In seawater fish the concentration gradient would favour entry of K^+ directly into the blood in fish such as the trout where the potential is close to zero (Eddy & Bath, 1979), but in fish such as the flounder where the potential is positive by 20–30 mV (Potts & Eddy, 1973), K^+ would be more or less in equilibrium across paracellular routes. If the interior of the gill epithelial cells was strongly negative, this would tend to oppose loss of K^+ from the potassium-rich cell interior and passive movement of K^+ across the apical boundary of the cell may be very small. However, the K^+ content and potential of branchial and chloride cell are not precisely known.

There is good evidence for active secretion of K^+ by marine fish. In seawater-adapted trout at least part of the efflux appears to be active (Sanders, 1979), while in *Gillichthys* skin there is a net efflux of Rb^+ in the absence of electrochemical gradients (Marshall, 1981). It was also shown in this preparation that some Rb^+ may move passively by paracellular routes but the active component was dependent upon the presence of Cl^-, as has been shown in a variety of preparations, e.g. *Necturus* gallbladder (Reuss, Weinman & Grady, 1980) and in many insect preparations (Phillips & Lewis, 1983), while many other insect preparations transport K^+ in the absence of Cl^- (Harvey, Cioffi, Dow & Wolfersberger, 1983). A number of tissues, such as erythrocytes, show Na/K/Cl co-transport (Palfrey & Rao, 1983). At this point, it is impossible to say which, if any, of such systems operate in fish guts or skin, but it is possible to say that K^+ fluxes both in fresh water and sea water are at least an order of magnitude smaller than Na^+ and Cl^- fluxes. Therefore a system linked to another ion, unless extremely unbalanced, seems unlikely. Thus, it is suggested that future work may be directed to looking for a K^+-transporting system in fish gills.

I thank the Scottish Universities Research and Reactor Centre at East Kilbride for supplies of radioactive potassium.

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