THE EFFECT OF ENVIRONMENTAL CALCIUM AND OVINE PROLACTIN ON SODIUM BALANCE IN *FUNDULUS KANSAE*

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INTRODUCTION

The ability of *Fundulus kansae* to live in both fresh water and 200% sea water, even when hypophysectomized, makes it a convenient experimental animal. In a previous paper it has been demonstrated that the permeability of the fish to water is affected both by ovine prolactin and by the concentration of calcium ions present in the medium (Potts & Fleming, 1970). Calcium ions have an effect on the permeability to water independent of prolactin and serve to reduce the permeability to water. Hypophysectomized fish had a lower permeability to water than intact fish, especially in fresh water, where prolactin production is highest, and prolactin restored the water permeability of hypophysectomized fish to normal levels. The higher permeability to water of fish adapted to fresh water (Potts, Foster, Rudy & Howells, 1967; Evans, 1969) is due to the combined effects of a high internal concentration of prolactin and a low external concentration of calcium ions.

Calcium ions are known to effect the permeability of biological membranes to other ions. The fluxes of sodium ions through fish adapted to sea water are much higher than those seen in fish adapted to fresh water, and transfer from one medium to another is usually associated with dramatic changes in flux, but the significance of such changes is not entirely understood (Motais, Garcia Romeu & Maetz, 1966; Potts, Foster & Stather, 1970). The effects of calcium ions on the flexus of sodium through *F. kansae* in various media have therefore been investigated.

MATERIALS AND METHODS

The animals were collected from Boones Lick, Missouri, and kept in the laboratory at 20 °C. They ranged in weight from 0·5 to 2·5 g.

Sodium efflux

Fish were injected intraperitoneally with 2 μC of ^{22}NaCl. The fish were then left for an hour for the sodium to equilibrate and were then counted in a thallium-activated sodium iodide well-crystal scintillation counter. The count was repeated either 2 h later or, when the rate of efflux was very rapid, 1 h later. If C_0 was the original count

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and C_T the count after time t the rate constant of the efflux K_e was calculated from the expression:

$$K_e = \frac{1}{t}\ln\left(\frac{C_0}{Ct}\right).$$

Fish were equilibrated for at least 1 week in the appropriate salinity, except in certain solutions containing little or no calcium where survival was poor. Fish in these solutions were previously equilibrated for 1 week to dilute or concentrated sea water of the same sodium content as the experimental solution and then equilibrated for a further 3 days to the experimental solution alone. Fish held in high salinities did not survive hypophysectomy if they were returned immediately to their medium. Fish held in 150 and 200% sea water were placed in 40% sea water for c. 12 h after the operation and then returned to their original salinity. All rate constants were converted to that of a fish of 1·5 g weight on the assumption that the rate constant varies as the (weight)[0·88] (Evans, 1969).

Short-term changes in the rate of efflux were monitored by placing the fish in a small volume of medium, usually 180 ml, continuously recirculated by a peristaltic pump at a rate of 630 ml/min through a T-shaped chamber constructed to fit over and into the crystal. The activity of the counter was monitored by a ratemeter which operated a pen recorder. The counter was heavily screened by lead from the activity in the fish. The bath could be emptied, flushed thoroughly, and the medium replaced in less than 2 min. At the end of an experiment the fish was killed, digested in concentrated nitric acid and diluted to the volume of the bath. From the activity of this solution and the activity lost to each volume of solution, values of K_e were calculated. Injections of radiosodium, hormones and drugs were made by a Kensington Scientific Corp. no. 1036 microsyringe with a 27-gauge needle. Injections did not exceed 5 μl in volume and were usually less. Ovine prolactin was supplied by N.I.A.M.D.

The effect and handling of injection on *F. kansae* was also investigated using the continuous flow technique. An injection was found to produce a small increase in the rate of efflux in both sea-water-adapted and in fresh-water-adapted fish. This increase was only temporary and the efflux had returned to normal after 15 min. The effects were similar in both intact and hypophysectomized fish.

Sodium concentrations were estimated by flame photometry. Fish were digested in concentrated nitric acid before dilution.

The University water supply contained 2·82 mM-Na/l. and 1·0 mM-Ca/l. Standard sea water was prepared from 'Instant Sea' salt mixture and contained 35 g salt/l, equivalent to 460 m-equiv Na/l. Magnesium-free sea water was prepared by dissolving 26·7 g NaCl, 0·85 g K_2SO_4, 0·15 g $NaHCO_3$, 1·47 g $CaCl_2$ $2H_2O$ in one litre ($\equiv$ 458·5 mM-Na/l, 9·7 mM-K/l, 10 mM-Ca/l, 476·8 nM-Cl/l, 4·9 mM-SO_4/l and 1·7 mM-HCO_3/l). Sea water rich in calcium and magnesium was prepared by dissolving 27·6 g NaCl, 0·85 g K_2SO_4, 0·15 g $NaHCO_3$, 7·35 g $CaCl_2$ $2H_2O$, and 10·2 g $MgCl_2$ $6H_2O$ in one litre ($\equiv$ 458·5 mM-Na/l, 9·7 mM-K/l, 50 mM-Ca/l, 50 mM-Mg/l, 656 mM-Cl/l; 4·9 mM-SO_4/l and 1·7 mM-HCO_3/l).

RESULTS

Normal fish

The sodium content of the whole fish increased in more concentrated media but the content of potassium remained almost constant (Table 1). On the other hand the rate of turnover of sodium increased markedly from about 1%/h in fresh water to 40%/h in 200% sea water (Table 2). A five-fold increase in the calcium concentration

Table 1. *Water content and total body sodium and potassium of*
Fundulus kansae. 20 °C

	Medium	Water content (%)	Na m-equiv. kilo	K m-equiv. kilo
	F.W.	—	51·9±0·9 (8)	57·8±0·9 (8)
Intact	33% s.w.	—	48·3±1·7 (8)	59·8±2·1 (8)
	100% s.w.	—	52·6±2·9 (8)	58·0±1·0 (8)
	150% s.w.	79·3±0·6 (6)	57·7±3·3 (14)	63·4±1·3 (8)
Intact prolactin-treated	100% s.w.	—	108·3±7·0 (10)	55·2±1·5 (5)
	F.W.	—	48·3±3·9 (5)	—
Hypophysectomized	100% s.w.	—	73·7±2·7 (4)	—
	150% s.w.	79·3±0·6 (6)	76·0±3·0 (6)	59±2·4 (6)

Table 2. *Rate constant of exchange of sodium in normal and hypophysectomized*
Fundulus kansae. 20 °C. K h^{-1} ± S.E. (N)

Medium	Normal	Hypophysectomized
F.W./10	0·0194±0·0017 (15)	—
F.W.	0·0120±0·0014 (21)	0·0226±0·0030 (12)
33% s.w.	0·103±0·008 (18)	0·0257±0·0021 (12)
70%	0·119±0·0073 (24)	—
100% s.w.	0·236±0·016 (17)	0·124±0·014 (11)
150% s.w.	0·336±0·020 (28)	0·217±0·022 (11)
200% s.w.	0·397±0·027 (10)	0·273±0·030 (8)
Mg-free s.w. 10 mM-Ca/l	0·38±0·060 (8)	—
Ca-free s.w. 50 mM-Mg/l	0·53±0·034 (16)	—
Ca-rich s.w. (50 mM-Mg/l, 50 mM-Ca/l)	0·25±0·04 (10)	—
1 mM-Ca/l sw.	0·79±0·08 (14)	—
Prolactin-treated fish in 100% s.w.	0·084±0·02 (6)	—

in sea water produced no significant change in the rate of sodium turnover, but a reduction in the calcium or magnesium content produced a large increase in the rate of turnover. Fish adapted to calcium-free sea water, containing 50 mM-Mg/l, had a rate constant of sodium efflux of 0·53 h^{-1}. The efflux rose to 79%/h in a sea water containing no magnesium and only 1 mM-Ca/l. Thirty minutes after they had been returned to normal sea water the rate of efflux of the fish from 1 mM-Ca/l 'sea water' had fallen to 0·35 h^{-1} and it declined to only 0·18 h^{-1} after 1 h. A reduction of the calcium content of fresh water from 1·0 to 0·1 mM/l resulted in an increase of more than 50 % in the rate of turnover in fresh water (Table 2).

F. kansae survived for only a few days or even hours in de-ionized water containing 2 mM-NaHCO$_3$/l but the rate of efflux increased before death to 0·05 or 0·10 h^{-1}. The effect of the generally high acidity of de-ionized water may be partly responsible, but

fluxes as high as 0·128 h⁻¹ were observed in tris buffered de-ionized water, pH 7·5. Th[e] addition of 1 mM-Ca/l reduced the rate of efflux to 0·063 h⁻¹ within 5 min.

Hypophysectomized fish had a higher rate constant of turnover of sodium than intact fish, in fresh water, but a lower rate of turnover in sea water (Table 2). These differences were compensated to some extent by a higher sodium content in sea-water-adapted fish and a lower sodium content in fresh-water-adapted fish (Table 1), but the total fluxes are clearly reduced in sea water after hypophysectomy.

Prolactin

Normal fish adapted to sea water, treated with 5 μg/g of ovine prolactin 24 h before measurement, had a much lower rate of sodium turnover (Table 1). Prolactin is essential for the survival of hypophysectomized *F. heteroclitus* in fresh water and raises the level of sodium in the blood of hypophysectomized *F. kansae* in fresh water
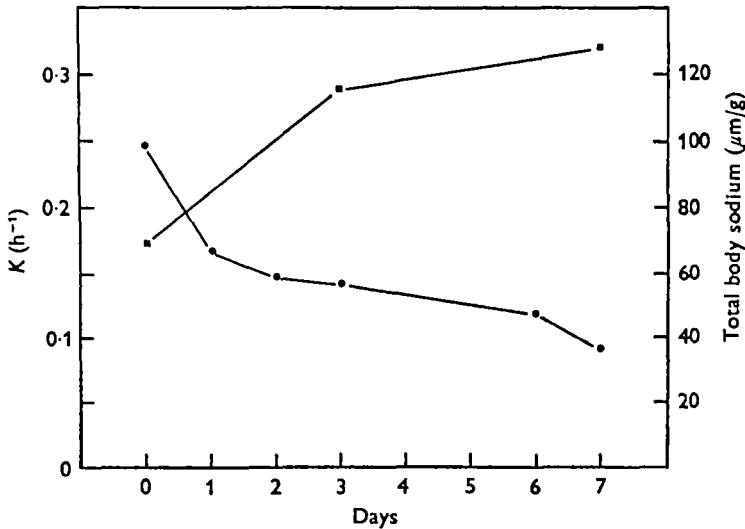

Fig. 1. The effect of injections of 5 μg prolactin/day on the rate of efflux of sodium (K) and on the total body sodium of *F. kansae*. 20 °C, ● K h⁻¹ ■ Total body sodium. Each point mean of six fish.

(Stanley & Fleming, 1965). Both intact and hypophysectomized *F. kansae* died after a few hours in sea water when treated with large doses, c. 30 μg/g, of prolactin. Smaller doses, 5 μg/g, produced a reduction in the rate of sodium efflux. Successive treatment with 5 μg/g doses for several days resulted in a high mortality and a steady decline in the rate of turnover of sodium down to an average of 8·4 %/h amongst the survivors after 1 week (Table 2). The total efflux was also reduced but not in proportion as the total body sodium rose during treatment from an average of 52·6 μM/g to 108·3 μM/g (Table 1). After 3 days the sodium content of the blood rose to 193 ± 12(5) mM/l compared with 148 ± 1·3(5) mM-Na/l in the controls. The results of a second experiment, showing the progressive changes in rate of efflux and total body sodium, following regular injection of 5 μg/g prolactin, are shown in Fig. 1.

The effect of single doses of 20 μg/g was investigated with the continuous flow

echnique. In intact fish adapted to 125% sea water the rate constant of the efflux declined from a mean of 0·30 h⁻¹ to 0·21 h⁻¹ during the 2 h following injection, and some of the fish died during the following 24 h. Similar results were obtained with hypophysectomized fish, but here the rate constant declined from a mean of 0·20 h⁻¹ to 0·10 h⁻¹ during the 2 h following injection.

Time course of adaptation from sea water to fresh water

Although there was some individual variation between fishes, the pattern of adaptation to fresh water was similar in all experiments. In every case the reduction of efflux took place in a series of sharp steps at intervals of 15 or 30 min (Fig. 2). No convincing evidence of exchange diffusion in sea water was observed either on transfer to fresh
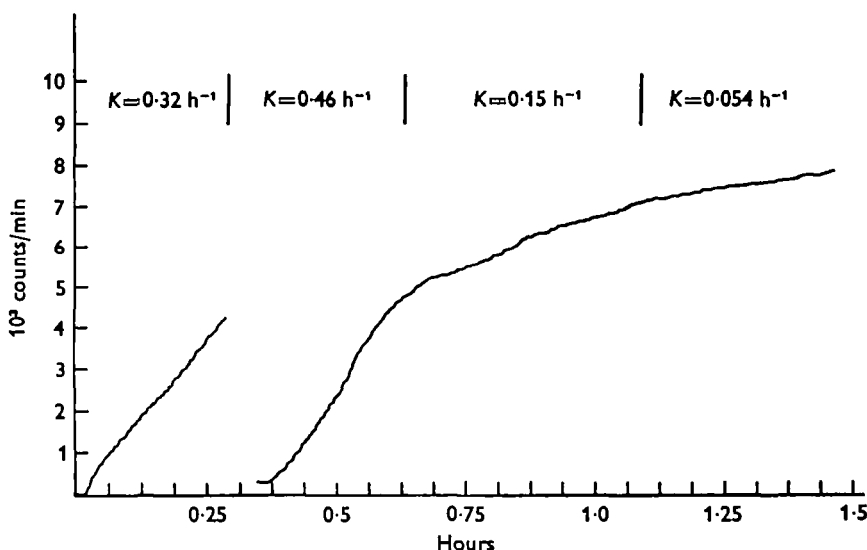

Fig. 2. The rate of efflux of sodium from *F. kansae* in sea water and during the first hour after transfer from sea water to fresh water. 20 °C.

water or on transfer back to sea water. In three experiments the rate of efflux declined immediately on transfer to fresh water but in two experiments the rates after transfer were unchanged and in three they were increased for a while, as in Fig. 2. The average rates of efflux at intervals following transfer from 150% sea water to fresh water are recorded in Table 3. The rates of efflux found both in sea water and in fresh water in the continuous flow experiments are higher than in the experiments recorded in Table 2. This is probably due to the greater stress inevitably associated with the continuous flow experiments.

Fish transferred from fresh water to sea water increased their rate of efflux more slowly (Table 4) but fish which had spent only a short term in fresh water re-adapted to sea water more rapidly.

Hypophysectomized fish adapted from sea water to fresh water in a similar manner to intact fish. Once again the reduction of the efflux took place in a series of steps (Fig. 3). The rates of efflux both in sea water and immediately after transfer to fresh

water were lower than in intact fish and the final rate of efflux in fresh water wa[s]
higher but the time courses of adaptation in intact and hypophysectomized fish were
similar (Table 3). Adaptation from fresh water to sea water also follows a similar course

Table 3. *Rate constant of loss of sodium at intervals following transfer from*
150% sea water to fresh water. 20 °C. K h⁻¹. Mean of eight fish

Medium	150% s.w.	F.W. 0–15 min	15–30 min	30–45 min	45–60 min	1–1·5 h	1·5–3 h
				Intact fish			
	0·43	0·40	0·17	0·14	0·14	0·10	0·04
			Hypophysectomized fish				
	0·22	0·185	0·133	0·095	0·092	0·086	0·075

Table 4. *Rate constant of loss of sodium at intervals following transfer of* Fundulus
kansae *from fresh water to 150% sea water. 20 °C. K h⁻¹. Mean of 6 fish*

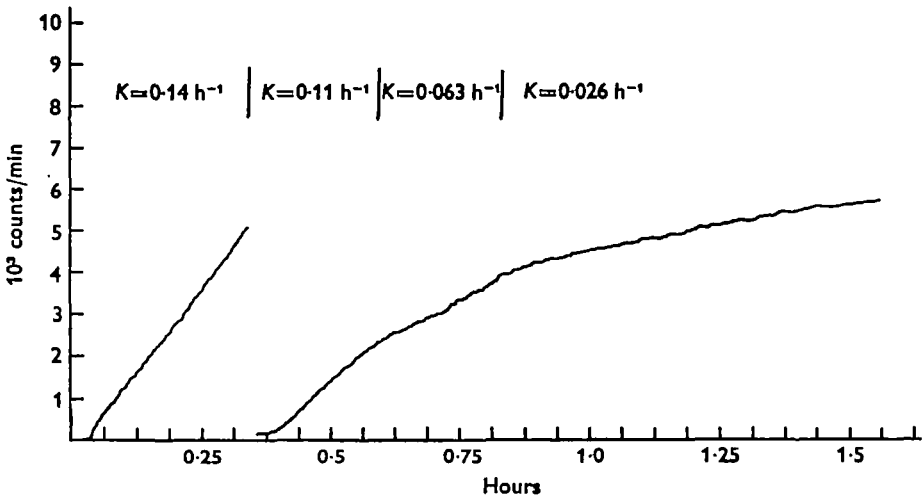| F.W. 24 h or more	s.w.	1 h	2 h	3 h	4 h	6 h	8 h	15 h
0·02		0·065	0·065	0·075	0·08	0·158	—	—
			Hypophysectomized fish					
0·10		0·08	0·095	—	0·1	0·138	0·173	0·22

Fig. 3. The rate of efflux of sodium from hypophysectomized *F. kansae* in sea water and during
the first hour after transfer to fresh water. 20 °C.

in both intact and hypophysectomized fishes. Considerable individual variation
occurred between fishes. For example, after transfer from sea water to fresh water
one hypophysectomized fish had only reduced its rate of efflux to 15%/h after
1½ h in fresh water while another had cut down to 3%/h after 45 min.

Effects of solutions lacking sodium, potassium and chloride on efflux of sodium ions

In each experiment the rate of efflux was first measured in the normal medium,
generally 150% sea water, in the continuous circulation bath, then in the ion-

eficient medium and finally in the normal medium again. On occasions the fish was followed for two successive changes of the ion-deficient medium in order to ensure that the external concentration of the ion was as low as possible. The time in each medium was usually 15 min.

Potassium. Removal of potassium ions had no consistent effect on the rate of efflux of sodium ions (Table 5).

Table 5. *Effect of potassium-free* 150% *sea water on the rate of efflux of sodium ions from* Fundulus kansae. 20 °C. *K h^{-1}*

	150% s.w.	K-free 150% s.w.	150% s.w.
Fish no. 24	0·52	0·54	0·46
Fish no. 20	0·57	0·50	0·53
	0·53	0·54	0·53

Table 6. *Effect of sodium-free* 150% *sea water on the rate of efflux of sodium ions from* Fundulus kansae. 20 °C. *K h^{-1}*

Fish no.	150% s.w.	Na-free 150% s.w.	150% s.w.
21	0·24	0·15	0·20
22	0·46	0·20	0·33
23	0·33	0·21	0·23
24	0·52	0·24	0·46

Table 7. *Effect of chloride-free* 150% *sea water on the rate of efflux of sodium ions from* Fundulus kansae. 20 °C. *K h^{-1}*

Fish no.	150%	Cl-free 150% s.w.	150% s.w.
21	0·33	0·53	0·24
22	0·33	0·59	0·33
23	0·48	0·63, 0·56	0·42
24	0·28	0·41	0·36

Sodium. Removal of sodium and its replacement by choline always resulted in a marked reduction of efflux (Table 6). After return to normal 150% the rate of efflux rose again but usually remained below the original level (Table 6). The longer the period in choline sea water the greater the reduction in efflux on return to normal sea water. The effect of choline sea water therefore resembles that of fresh water to some extent.

Chloride. Removal of chloride ions always stimulated sodium efflux (Table 7). On return to complete 150% sea water the efflux was usually lower than before.

DISCUSSION

Sodium fluxes in normal animals. In 100% sea water the rate of turnover of sodium (total body sodium × rate constant) was 12·4 μM/g/h. This rose to about 19·4 μM/g/h in 150% sea water. The drinking rate rose from 0·95%/h in 100% sea water (4·4 μM-Na/g/h) to 1·56%/h in 150% sea water or 10·8 μM-Na/g/h (Potts and Fleming, 1970). The direct influx through the body surface therefore increased from 8·0 μM/g/h in normal sea water to 9·6 μM/g/h in 150% sea water. In sea water containing only

1 mM-Ca/l the total influx was 41·6 μM/g/h, assuming that the total body sodium wa̧
the same as in normal sea water, while drinking had risen to 1·77% body wt/h
equivalent to 8·1 μM-Na/h. Clearly the influx through the body wall has greatly in-
creased, from 9·6 μM/g/h to 33·5 μM/g/h. Similarly in diluted fresh water, containing
0·1 mM-Ca/l, the rate of sodium efflux was increased by more than half compared with
the efflux in the normal fresh water containing 1 mM-Cal (Table 2).

Table 8. *Influx through body surface of* Fundulus kansae *in various conditions.* 20 °C

Medium	Total body sodium (μM Na/g)	Rate constant of of influx (K h^{-1})	Total influx (μM/g/h)	Drinking (μM/g/h)	Influx through surface (μM/g/h)
Intact					
100% s.w.	52·6	0·236	12·4	4·4	8·0
150% s.w.	57·7	0·336	19·4	10·8	9·6
1 mM-Ca/l s.w.	52·6	0·79	41·6	8·1	33·5
Hypophysectomized					
100% s.w.	73·7	0·124	9·1	4·0	5·1
150% s.w.	76	0·217	16·5	9·7	6·8

No measurements of drinking rates were made on hypophysectomized animals,
but the osmotic permeability and the diffusional permeability are similar in marine
teleosts (Potts *et al.* 1967; Motais *et al.* 1969) so that the rate of drinking can be
estimated from the water permeability (Potts & Fleming, 1970). The water permeability
of hypophysectomized animals in both 100 and 200% sea water was 0·9 of that of
normal animals in the same media. In 150% sea water the drinking rate of hypo-
physectomized animals would therefore be 1·4%/h equivalent to 9·7 μM/g/h, while
the total flux was 16·5 μM/g/h, in hypophysectomized fish in 150% sea water. Influx
through the body wall would therefore be 6·8 μM/g/h compared with 9·6 μM/g/h in
intact fish in the same medium. These calculations are summarized in Table 8. It is
clear that the influx of ions across the body surface is partly dependent on the external
calcium concentration but, like the water permeability, depends on a number of
extrinsic and intrinsic factors.

The nature of the large part of the influx into marine teleosts which does not take
place through the gut is currently the subject of investigation and speculation. Maetz
(1969) found that in the flounder, *Platichthys flesus*, the gastric influx was balanced
by an active output of sodium ions in exchange for potassium ions while the remainder
of the influx was due to a one-for-one, sodium-for-sodium, exchange by the same
carrier system, as demonstrated by Motais *et al.* (1966). The sensitivity of the non-
gastric influx in *Fundulus kansae* to calcium implies either that the influx takes place
by simple diffusion or that the carrier system is affected by calcium ions. The former
hypothesis involves the difficulty that the fish is more permeable to sodium ions when
in sea water, in spite of the high concentration of calcium ions in sea water, although it
must be noted that the fish is in a very different physiological state when in sea water
than when in fresh water. The latter hypothesis may be extended by suggesting that
the calcium ions may be competing for sites on the carrier, though evidently if this is
the case the calcium ions compete more effectively than magnesium ions. In either case,
in fresh water, calcium ions have a direct effect on the permeability of the body wall
to sodium ions.

The direct effect of calcium on the permeability to ions has previously been neglected in discussion of the problem of the adaptation of fish to sea water and fresh water. The effects of cortisol, prolactin and other hormones might be elucidated better by experiments in which the fish were transferred between sea water and fresh water containing the same activity of calcium ions, although the calculation or measurement of activity in solutions of widely different ionic strengths is difficult (Potts & Fleming, 1970). It is likely that in experiments of this kind the changes in the sodium and chloride fluxes would be greater than they are on transfer between normal sea water and normal fresh water.

The effect of calcium ions in reducing the permeability to sodium ions is similar to its effect on the permeability to water previously discussed (Potts & Fleming, 1970), but the intrinsic factors responsible for the changes in permeability to sodium and chloride ions are more difficult to identify. Prolactin serves to maintain a low permeability to sodium ions in hypophysectomized *F. heteroclitus* held in fresh water (Potts & Evans, 1966; Maetz, Sawyer, Pickford & Mayer, 1967) and causes a long-term reduction of sodium turnover of intact animals held in sea water (Maetz, Motais & Mayer, 1969). In *F. heteroclitus* treatment with prolactin is essential for the survival of the hypophysectomized fish in fresh water, while in *F. kansae* prolactin, although not essential, enables the fish to maintain a higher sodium concentration in the blood when in fresh water (Stanley & Fleming, 1965).

The rate constant of the sodium efflux in sea water declined dramatically after the injection of prolactin and it is probable that the rate of sodium efflux also declined initially. As there was a net gain of about 1 μM-Na/g/h after injection the rate of influx does not decline to the same extent and might even remain unchanged. After a few days, probably as a consequence of the reduction of the concentration gradient across which the pump operates, the rate of efflux increased again, although this may be due in part to the fact that the survivors were a selected group. The rate of efflux from normal fish in sea water is 12·4 μM/g/h. In the prolactin-treated fish shown in Fig. 1 the rate of efflux was apparently 16·1 μM/g/h after 3 days and 10·4 μM/g/h after 7 days, but as both the rate of efflux and the total body sodium were changing simultaneously and the values at any time were very variable, the exact values are not too reliable. The increased blood concentration should have reduced the need to drink, which would imply an even higher non-gastric influx, but the prolactin, by increasing the permeability to water, might have counteracted this effect, although we have no direct evidence that prolactin increases water permeability in sea-water-adapted fish (Potts & Fleming, 1970). The decline on sodium output is probably associated with a decline in the Na^+K^+ ATPase which is reduced in hypophysectomized *F. heteroclitus* in fresh water following prolactin injection (Pickford, Griffiths *et al.* 1970).

The low rate of turnover of sodium in hypophysectomized animals when in concentrated solutions cannot be influenced by prolactin, which is a product of the pituitary. It might be due in part to a reduction in cortisol levels as a result of the absence of ACTH. Cortisol has been shown to facilitate sodium turnover in sea-water-adapted eels. Chan, Chester Jones, Henderson & Rankin (1967) reported that interrenalectomy of sea-water-adaptive eels caused a rise in tissue plasma sodium, while cortisol reversed these effects. A series of papers from Maetz's laboratory have shown that either ACTH or cortisol will increase that rate of sodium turnover in eels adapted

to sea water, and that hypophysectomy of such animals will reduce the turnover rate. Both ACTH and cortisol served to restore sodium turnover to near normal values (Mayer & Maetz, 1967; Maetz, Motais & Mayer, 1969; Mayer, Maetz, Chan, Forester & Chester Jones, 1967). On the other hand the cortisol content of the blood of sea-water-adapted *F. kansae* is less than half that of fish adapted to fresh water. Hypophysectomy caused cortisol to drop almost to zero in *F. kansae*, no doubt because of the absence of ACTH (Hawkins, Ball & Fleming, unpublished data). The low permeability to sodium found in hypophysectomized fish adapted to sea water and more concentrated solutions might therefore be related to their very low cortisol level. However, if cortisol increases the permeability to sodium in marine fish it is anomalous that the permeability to sodium of fresh water *Fundulus* should be much lower although the blood cortisol is higher. Possibly part of the low permeability in fresh water is due to the presence of prolactin in intact fish in fresh water and some of the slow increase in permeability and sodium turnover that takes place on adaptation to sea water is due to the disappearance of prolactin. If this were the case then a cortisol-treated hypophysectomized fish in sea water should be superficially equivalent to a normal sea-water-adapted fish (Pickford, Parry *et al.* (1970)). However, the rate of adaptation of hypophysectomized fish from fresh water to sea water is very similar to that of intact fish, and the characteristics of the adaptation, a sequence of discreet changes of rate of influx, are identical, which implies that the gradual disappearance of prolactin is not a controlling factor and indeed that the great difference in effluxes and permeability in the two media are not directly related either to a pituitary hormone or to cortisol. Studies on the nervous control of gill function and on the fine structure of the gill epithelium would seem to be indicated. Transfer back to sea water after a few minutes only in fresh watet resulted in the restoration of approximately the initial rate of efflux, but after an inflexion had occurred the rate of efflux, on return to sea water, was much lower than before. The rapidity and reversibility of these changes suggests that the concentration of the Na^+K^+ ATPase is not immediately changing but that the immediate regulation involves some change in the fine structure or blood flow through the gill.

There is a close similarity between the effects of calcium ions on water permeability and on sodium permeability. In both cases calcium ions decrease permeability, probably by stabilizing cell membrane and intracellular cements (Potts & Fleming, 1970). However, prolactin has reciprocal effects on the permeabilities of the fish to sodium ions and to water, decreasing the former and increasing the latter. It is difficult to conceive of a simple model to explain these diverse effects.

The results of the immediate transfer experiments from sea water to fresh water and from sea water to sodium-free and chloride-free sea water suggest that exchange diffusion is not an important component of the sodium fluxes in sea water but indicate that changes in electrical potential are significant. An immediate change of medium is probably associated with a change of potential which in turn would produce an immediate change in the fluxes. In some cases the transfer to fresh water was associated with a small increase rather than a decrease in efflux, which could be due to a temporary increase in electro-positivity by the fish.

The crustacean *Artemia salina* osmoregulates in sea water in a fashion very similar to a teleost. However, in *Artemia* the permeability to chloride ions is much lower than

þo sodium ions. When transferred to fresh water or to sodium-free solutions the animal behaved almost as a sodium electrode. Smith (1970) has shown that the negative potential then set up reduced the efflux of sodium ions. It is interesting to note that this reduction, when first observed, was interpreted as an example of exchange diffusion (Croghan, 1958; Thuet *et al.* 1968). The high chloride flux found in *F. heteroclitus* (Potts & Evans, 1967) may indicate that *Fundulus* is relatively permeable to chloride. If so, it will not act in quite the same manner as *Artemia*, i.e. the potential change on transfer to a sodium-free solution will be less and the sodium efflux correspondingly greater, but the effect will be similar.

The spectacular increase in sodium efflux which occurred in chloride-free water may be attributed to the increased positivity of the fish consequent on the cessation of chloride influx. Similarly, the absence of sodium influx in choline sea water should have rendered the fish more electronegative. The sodium pump would then be working against an increased electrochemical gradient and the rate of efflux should have declined (Table 6).

The absence of potassium in the external medium had no detectable effect on the sodium fluxes, which renders a sodium-potassium exchange as described by Maetz in the flounder *Platichthys flesus* (Maetz, 1969), unlikely in *Fundulus kansae*. In the flounder the influx through the gut amounts to only 3% of the total influx and is balanced by a net sodium outflux in the gills. In potassium-free sea water the net outflux ceased so the total flux dropped by only 3%. In the much smaller *F. kansae* the influx through the gut amounts to about 30% of the total efflux which must be balanced on an equivalent efflux. Total efflux should therefore have dropped by about 30% in potassium-free sea water, even if there were no passive influx through the body surface.

F. kansae differs from *F. heteroclitus* in surviving in fresh water after hypophysectomy. On the second day after transfer from sea water to fresh water intact *F. heteroclitus* lost 0·23 μM-Na/g/h while hypophysectomized fish lost 0·50 μM/g/h (Maetz *et al.* 1967). In contrast intact *F. kansae* lost 0·6 μM/g/h while a hypophysectomized one lost 1·15 μM/g/h (from Tables 1 and 2). Exact comparison is difficult because *F. heteroclitus* weighed ten times as much (9–20 g) as *F. kansae*, which would reduce fluxes by about one-third, and the temperature was lower, 16–17 °C (Maetz *et al.* 1967 and *pers. com.*) compared with 20 °C of these experiments. However, hypophysectomy doubled the rate of efflux in both fishes. The ability of *F. kansae* to survive hypophysectomy must be due to the presence of a more efficient uptake system. In hypophysectomized *F. heteroclitus* uptake never exceeded 0·24 μM/Na/g/h even when the fish was suffering a net loss of sodium and had a declining blood concentration. This level of uptake would have maintained a normal *F. heteroclitus*.

SUMMARY

1. The sodium contents and rates of turnover of sodium have been determined in both intact and hypophysectomized *Fundulus kansae* in a variety of media.

2. A reduction of the calcium content of fresh water from 1·0 to 0·1 mM/l increased the rate of sodium efflux by half while transfer to calcium-free water increased the rate several fold.

3. The rate of sodium efflux increased fourfold in sea water containing no magnesium and only 1 mM/l calcium.

4. Hypophysectomy increased the rate of efflux in fresh water but reduced it in sea water.

5. Intact fish adapted to sea water and treated with prolactin showed a net gain of sodium probably due to a reduction in the activity of the sodium efflux pump.

6. Both intact and hypophysectomized fish on transfer from sea water to fresh water reduced the rate of efflux of sodium in a series of stages.

7. Studies of the rate of efflux of sodium before and after transfer between various media showed no evidence of exchange diffusion or of a linked sodium-potassium efflux pump, but did show evidence or interaction between sodium and chloride fluxes probably as a consequence of potential changes.

8. The rate of efflux of sodium from *F. kansae* doubled following hypophysectomy. The ability of hypophysectomized *F. kansae* to survive in fresh water after hypophysectomy, unlike *F. heteroclitus*, is due to a more effective uptake system.

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