THE EFFECTS OF PROLACTIN AND DIVALENT IONS ON THE PERMEABILITY TO WATER OF *FUNDULUS KANSAE*

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INTRODUCTION

Fundulus kansae (Cyprinidae) is widely distributed in the western parts of the Mississipi basin. Although a continental form the fish is a powerful osmo-regulator. It can survive in the laboratory in solutions containing as little as 0·2 mM/l of sodium and 0·1 mM/l of calcium up to concentrations as high as 250% normal sea water, and some individuals can survive in 300% sea water. The fish can stand immediate transfer from a more concentrated to a more dilute medium, but not all individuals can survive an immediate transfer from fresh water up to full strength sea water or higher. The animals described below were obtained from an area containing saline springs and lived in water of variable salinity. The fish has often been the subject of physiological researches, particularly by Fleming and his co-workers (Fleming & Stanley, 1965; Fleming, Stanley & Meier, 1964; Stanley & Fleming, 1964*a, b*, 1966*a, b*, 1967).

The fish is easily hypophysectomized and differs from *F. heteroclitus* in that it survives in fresh water after such treatment (Pickford, Pang, Stanley & Fleming, 1966). Hypophysectomized *F. kansae* living in fresh water had a slightly lower blood concentration and a markedly reduced rate of urine production (Stanley & Fleming, 1966*a, b*). Injections of mammalian prolactin restored urine production approximately to the level in control animals. Stanley & Fleming (1967) suggested that prolactin increased the permeability of the body wall to water. Lahlou & Sawyer have recently reported (1969) that hypophysectomized goldfish, *Carassius auratus*, have lower rates of turnover of tritiated water than have intact fish. It has been noted that a number of fishes, including *Tilapia mossambica*, the yellow eel and the flounder, are more permeable to water when in fresh water than when in sea water (Potts, Foster, Rudy & Parry Howells, 1967) and in a systematic study of a variety of fishes from both sea water and fresh water it was noted that in general the fresh-water fishes are more permeable to water than are marine species (Evans, 1969). This evidence that teleosts in fresh water are more permeable to water than teleosts in sea water, together with the observation that prolactin is found in fresh-water teleosts, but is rare or absent in marine teleosts (Ensor & Ball, 1968; Dharmamba & Nishioka, 1968) and in euryhaline teleosts adapted to fresh water, strengthens the hypothesis that permeability to water in teleosts is increased by the prolactin hormone. The ability of *Fundulus kansae* to survive indefinitely in fresh water after hypophysectomy, in the absence of prolactin treatment, makes it a very convenient animal for the examination of this hypothesis.

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Measurements have therefore been made of the permeability of *F. kansae* to water in both the hypophysectomized and normal states and in a wide variety of different solutions.

MATERIALS AND METHODS

Animals were collected from Boones Lick, a small stream fed by a series of salt springs situated north of Petersburg, Missouri. They were held in the laboratory at 20 °C and all experiments were carried out at that temperature unless otherwise stated. The animals ranged in weight from 0·5 to 2·5 g.

Permeability to water. The animals were loaded with tritiated water by placing a dozen animals at a time in 650 c.c. of the appropriate solution containing 650 μC of tritiated water. Animals were removed in turn and washed in three changes of a similar inactive solution for a total period of 2 min and then placed in 150 c.c. of inactive solution. After a suitable period of time (t), usually 20 or 30 min, the approximate half-time for equilibration, a 1·5 c.c. water sample was removed from the beaker containing the fish and a second sample 10 h later. Water samples were distilled in vacuum and condensed using a mixture of dry ice and acetone; 1 ml aliquots were then counted in Aquafluor scintillation fluid (New England Nuclear Corp.). From the activity of the second sample and the volume of the bath the initial activity present in the fish was determined, A_0. From the activity in the first sample the activity lost from the fish during the first period of time was determined $(A_0 - A_t)$. The rate constant of the efflux, K, was then calculated from the relationship:

$$K = \frac{1}{t} \log_e \left(\frac{A_0}{A_t} \right).$$

Corrections for the volume of fluid removed at time t and for the volume of water in the fish were less than 1 % and of opposite signs and were neglected except in the case of the larger fish. At the end of the experiment the total weight of the fish was determined and the individual weights were calculated from the relative values of A_0. Each experiment was carried out, whenever possible, on a minimum of twelve fish. The fish were selectĕd so that they provided as wide as possible a range of weights. Evans, 1969, showed that the permeability to water of a wide variety of fishes varied as $(\text{weight})^{0.88}$. In these experiments all permeabilities were standardized to an animal of a weight of 1·5 g using Evans's formula.

Columbia, Missouri, tap water contained 2·54 mM-Na/l and 1·0 mM-Ca/l. Standard sea water, prepared from Commercial Salts (Instant Ocean), contained 35 g salt/l or equivalent to about 550 mM-Cl/l or 470 m-equiv. Na/l. Animals were adapted to new media for at least 3 days before measurement, unless otherwise stated. The loss of sodium from the fish raised the concentration to about 0·05 mM-Na/l in the 'distilled water' tank after 3 days. Similarly, the 'calcium-and-magnesium-free' solution must have acquired low concentrations of these ions during adaptation of the fish.

Drinking rates

Drinking rates were determined by the use of ^{125}I-labelled polyvinylpyrrollidone (PVP). Fish were placed in an appropriate solution containing approximately 1 μC/ml of ^{125}I-PVP for 2 h. They were then transferred to a similar non-labelled solution for

a further hour to remove surface activity and to allow the active solution to move down the gut. M.S. 222 was added to the solution and the fish were left until dead. Each fish was opened up and the gut was removed with oesophagus and rectum clamped to prevent loss of fluid.

<div align="center">RESULTS</div>

The mean rate constant of the exchange of tritiated water in normal (non-hypophysectomized) fish in Missouri tap water was $1\cdot38$ h^{-1}. With increasing salinity the rate of exchange declined, as shown in Table 1. In 200 % sea water the rate of exchange was little more than half that in tap water. In distilled water, on the other hand, the rate increased to over $2\cdot0$ h^{-1}. In order to investigate whether the change in rate

Table 1. *Rate of exchange of tritiated water in* Fundulus kansae 20 °C *corrected to* $1\cdot5$ *g body weight*

	K h^{-1} ± s.e. (N)
Distilled water 3 days or more	$2\cdot02 \pm 0\cdot08$ (31)
FW/10 (0·1 mM-Ca/l)	$2\cdot03 \pm 0\cdot11$ (15)
FW (1·0 mM-Ca/l)	$1\cdot38 \pm 0\cdot04$ (42)
FW (5·0 mM-Ca/l)	$1\cdot36 \pm 0\cdot10$ (6)
FW (10 mM-Ca/l, 25 mM-Mg/l)	$0\cdot93 \pm 0\cdot05$ (11)
33 % SW	$1\cdot30 \pm 0\cdot10$ (12)
67 % SW	$1\cdot08 \pm 0\cdot04$ (18)
100 % SW	$0\cdot87 \pm 0\cdot03$ (19)
150 % SW	$0\cdot81 \pm 0\cdot04$ (10)
200 % SW	$0\cdot74 \pm 0\cdot05$ (23)
Mg free 'SW' (10 mM-Ca/l)	$0\cdot87 \pm 0\cdot15$ (8)
Mg free 'SW' (1 mM-Ca/l)	$1\cdot19 \pm 0\cdot06$ (17)
Ca free 'SW' (50 mM-Mg/l)	$1\cdot45 \pm 0\cdot10$ (11)
Ca free 'SW' (1 mM-Mg/l)	$1\cdot75 \pm 0\cdot09$ (12)
Ca rich 'SW' (50 mM-Ca/l, 50 mM-Mg/l)	$1\cdot00 \pm 0\cdot04$ (10)
FW ± 10 mM-Ca/l, 25 mM-Mg/l	$0\cdot93 \pm 0\cdot05$ (11)
'33 % SW' (10 mM-Ca/l, 50 mM-Mg/l)	$0\cdot80 \pm 0\cdot09$ (9)
'33 % SW' (20 mM-Ca/l, 100 mM-Mg/l)	$0\cdot70 \pm 0\cdot07$ (10)

constant was due to changes in the concentration of calcium and magnesium ions, or whether it was due to some intrinsic change resulting from adaptation to higher salinities, the fish were transferred to a variety of synthetic media. Sea water contains about 10 mM-Ca/l and 53 mM-Mg/l. *Fundulus kansae* did not survive for more than a few hours in solutions containing much magnesium and little sodium. The highest concentration to which fish were successfully adapted was Columbian tap water with calcium and magnesium chlorides added to concentrations of 10 mM-Ca/l and 25 mM-Mg/l. In this medium the rate constant for water exchange was $0\cdot93$ h^{-1} compared with $0\cdot87$ in normal sea water. *F. kansae* survived in a medium containing sodium, potassium and chloride equivalent to 33 % sea water but containing 20 mM-Ca/l and 100 mM-Mg/l, roughly equivalent to 200 % sea water in divalent ions. In this medium the rate constant was $0\cdot70$ h^{-1} similar to that in 200 % sea water. In a control group of twelve fish in 33 % sea water at the same time the rate constant was only $1\cdot33$ h^{-1} It is clear that the rate constant of exchange depends more on the concentration of calcium or magnesium ions than on the total salinity *per se*. Conversely, in a synthetic 'sea water' containing as much sodium and chloride as normal strength sea water but

containing only 1 mM-Ca/l the rate constant of exchange was 1·19 h^{-1}, that is, close to the value of the rate constant in fresh water containing the same concentration of calcium.

The increase in rate constant that occurs on transfer to low-calcium solutions takes place over several days, but the addition of external calcium produces an almost immediate decrease in the rate constant of exchange. Eleven fish which had been maintained in distilled water for 24 h had a rate constant of 1·66 h^{-1}. In the 20 min immediately following the addition of 1 mM-CaCl$_2$/l the permeability declined to 1·41 h^{-1}.

Table 2. *Rate constant of exchange of tritiated water in intact and hypophysectomized* Fundulus kansae *20 °C corrected to 1·5 g weight*

| Medium | K h^{-1}±s.e. (N) | |
	Intact	Hypophysectomized
FW	1·38±0·04 (42)	1·13±0·05 (17)
33% SW	1·30±0·10 (12)	1·29±0·09 (12)
100% SW	0·87±0·03 (19)	0·78±0·07 (12)
200% SW	0·74±0·05 (23)	0·68±0·05 (11)

Table 3. *Decline of permeability to water following hypophysectomy.* Fundulus kansae, *fresh water, 20° C*

	K h^{-1}±s.e. (N)
Before hypophysectomy	1·38±0·04 (42)
Two days after hypophysectomy	1·34±0·17 (6)
Five days after hypophysectomy	0·95±0·04 (6)
Six days after hypophysectomy	0·95±0·04 (6)
Two weeks or more after hypophysectomy	1·13±0·05 (17)

The effects of hypophysectomy

In fresh water the rate of exchange of tritiated water in hypophysectomized fish was approximately two-thirds that in normal fish, but in higher concentrations the difference was reduced (Table 2). In full strength and in concentrated sea water the rate of exchange in hypophysectomized fishes was lower than that in intact fish, but the difference may not be significant. The rate of exchange in hypophysectomized fish in 33% sea water was similar to that found in normal fish in 33% sea water and is anomalous. The mean rate constant of exchange in 33% sea water is biased by three very permeable fish (K *c.* 1·8 h^{-1}) but no valid reason can be suggested for rejecting them.

The decline in permeability following hypophysectomy takes place over several days (Table 3).

As shown in Table 4 low doses of ovine prolactin (30 mu) served to stimulate water turnover. The animals used in Expts 1 and 2 were the same animals and were tested 3 and 4 weeks after hypophysectomy respectively. In the first experiment the animals were given a 5 μl injection of saline or saline plus prolactin. Four hours later they were placed in the loading solution and treated as described above. At the end of the

test period the animals were returned to separate aquaria and left for a week. In the second experiment the fish which had previously acted as controls received the prolactin and the fish which had previously received prolactin acted as controls. The higher turnover rates reported in Expts 2 and 3 may be due to the higher temperature at which the experiments were performed. Evans (1969) found that the Q_{10} of the relationship between permeability of fish to tritiated water and temperature was 1·9. When corrected to 20 °C with this value of Q_{10} Expts 2 and 3 give average values as follows: controls 0·97 and 1·03 h⁻¹, prolactin-treated 1·14 and 1·17 h⁻¹ respectively.

Table 4. *Rate constant of exchange of tritiated water in hypophysectomized* Fundulus kansae *with and without prolactin treatment*

	K h⁻¹±s.e. (N)		
	Controls	Prolactin-treated	T °C
Expt 1	1·14±0·03 (12)	1·35±0·05 (10)	20
Expt 2	1·25±0·02 (8)	1·47±0·04 (8)	24
Expt 3	1·33±0·04 (6)	1·51±0·06 (6)	24

Table 5. *Drinking rates*

	Drinking rate % body weight/h±s.e. (N)
FW	0·94±0·15 (20)
33% SW	0·66±0·16 (9)
100% SW	0·95±0·12 (10)
150% SW	1·56±0·25 (10)
'100% SW' (1 mM-Ca/l, 0 mM-Mg/l)	1·77±0·27 (14)

Drinking rates

The water lost by exosmosis in hyperosmotic solutions must be replaced by drinking. The rate of drinking of *Fundulus kansae* was measured in fresh water, sea water, 150% sea water and finally in 'sea water' containing 1 mM-Ca/l in order to determine whether the higher rate of exchange of tritiated water in low-calcium solutions was correlated with a higher drinking rate. The results are shown in Table 5.

Drinking occurs in fresh water at a high but very irregular rate. Drinking is minimal, but not insignificant in approximately iso-osmotic sea water (33%) and increases steadily with increasing salinity. In low-calcium sea water the drinking rate was nearly double that in normal sea water.

DISCUSSION

A difference between the diffusional permeabilities to water of fish in fresh water and in sea water was first observed in *Tilapia mossambica* (Potts *et al.* 1967) and was confirmed in a variety of species by Evans (1969). A detailed study of *Platichthys*, *Serranus* and *Anguilla* by Motais, Isaia, Rankin & Maetz (1969) showed that in sea water the osmotic and diffusional permeability coefficients were almost identical, while in fresh water the osmotic permeability coefficient was several times that of the diffusional coefficient. An even larger discrepancy between the osmotic permeability coefficients and the diffusional coefficients has been apparent in amphibia for many

years. Koefoed-Johnsen & Ussing (1953) suggested that the discrepancy was due to the existence in the rate-limiting membrane of water-filled pores through which bulk flow occurred. Calculations have been made of the diameter of these hypothetical pores from the discrepancies between the two coefficients, on the assumption that Poiseuille's law can be applied to flow through the pores under the action of the osmotic concentration differences. Dainty & House (1966a, b) suggested that some of the discrepancies, between the two coefficients in anuran skin, arose from the presence of unstirred layers which reduced the flow of tritiated water across the membrane proportionally more than the osmotic flow. Since the tritiated water flow is so large, a concentration gradient of tritiated water would exist across the unstirred layers, reducing the tritiated water gradient across the membrane itself. As the net flow (osmotic flow) is much smaller than total flow, the solute gradient, which determines the osmotic flow, would not be significantly effected. Very vigorous stirring was found to reduce the discrepancy between the two permeability constants significantly. However, it is evident from the concurrence of the two permeability coefficients in the gills of fishes in sea water that the rapid circulation both inside and outside the gill eliminates any effect of this nature. A variation of this hypothesis by Hays (1968) suggested that the discrepancy is due to the presence of two rate-limiting membranes: a thin, non-porous membrane which limits osmotic flow and a thicker porous membrane which limits diffusion in the same way as an unstirred layer. Motais et al. (1969) suggested that a mucous coat present on the gill filaments of Fundulus heteroclitus in fresh water but absent in sea water (Philpott & Copeland, 1963) might constitute the thick porous layer. On the other hand the presence of a substantial cell layer between the blood and external medium in all fish must constitute a much thicker 'unstirred layer' yet the two rate constants are concordant in sea water. Motais et al. also considered briefly and inconclusively the hypothesis that the osmotic permeability exceeds the diffusional permeability in marine fish as well as in fresh water fish but that the difference is not apparent because the water flow outwards is reduced as a result of interaction with the inflow of ions down the concentration gradient. Under this hypothesis the similarities of the coefficients in fresh water and in sea water in Platichthys, Anguilla, Serranus and Tilapia would all be coincidences. It is worth noting also that this hypothesis requires an improbably large solute/solvent interaction. The change from a high permeability in fresh water to a low permeability in sea water is necessarily associated with a reversal of the osmotic gradient, and Motais et al. draw attention to the studies of Patlak, Goldstein & Hoffman (1963) on asymmetrical membranes and to the observation of Bentley (1964) that the permeability of the toad bladder is dependent on the direction of the osmotic gradient. While the data reported here do not demonstrate that permeability to water is independent of the orientation of the osmotic gradient they do show that in the case of Fundulus kansae at least both high and low permeability can occur with the osmotic gradients running in either direction. Further, it seems that permeability is controlled in part by exogenous factors (ions) and in part by endogenous factors (hormones).

  Calcium ions, and indeed all cations, tend to reduce the hydration of polar organic molecules by reducing the repulsive forces between fixed anions thus allowing closer packing of the organic molecules. This ionic action is especially important in membrane structures. Divalent ions, such as calcium, are particularly effective in this respect as

they may link with two molecules at once thus further stabilizing the structure. The effect of calcium ions on cell permeability has been known for many years (references in Robertson, 1941; Bayliss, 1958, p. 45) and calcium ions are believed to play an important part in stabilizing cell membranes (Wolman & Wiener, 1965; Benedetti & Emmelat, 1968). Calcium ions also help to stabilize intercellular cements and in the absence of such ions certain tissues, including the embryos of some marine sponges and fresh-water *Tubifex*, disintegrate into separate cells (Gasic & Galanti, 1966). Low-calcium artificial 'sea water' containing fish very rapidly becomes opalescent, suggesting an unusual dispersal of mucus from the fish, although it may be that the artificial sea water 'irritates' the fish or alternatively that the rate of mucus production is normal, but that the mucins are usually precipitated by the divalent ions present in sea water. Although calcium is clearly the most important ion in determining the rate of exchange of tritiated water, nevertheless, magnesium is not without effect. Fish maintained in magnesium-free synthetic 'sea water' had the same rate of exchange as fish maintained in normal sea water; but, although fish could not survive in sea water containing neither calcium nor magnesium, fish could survive in synthetic 'sea water' containing normal amounts of sodium, magnesium and other ions but no calcium. The fish maintained in this medium had a high rate of exchange, but it was still lower than that of fish maintained in distilled water. It should be noted that the value for the rate constant in distilled water is probably not maximal as the fish do not survive indefinitely in this medium. The data are not sufficiently precise to enable the effect of calcium to be quantified exactly, but it is clear that the effect of calcium varies approximately as the logarithm of its concentration. Tap water diluted ten times contains about 0·1 mM-Ca/l. An increase in the calcium concentration from 0·1 mM/l to 1·0 mM/l reduced the rate constant of exchange from about 2·0 to 1·4 h^{-1}. A further increase from 1 to 10 mM/l reduced the rate constant of exchange from about 1·4 to 0·9 h^{-1}.

Motais *et al.* (1969) found that in fresh water the osmotic permeability increased proportionally more than the diffusional permeability. It is uncertain whether this is true also for *Fundulus kansae* and whether this differential effect is also due to the lower concentration of calcium ions in fresh water; but both hypotheses are possible. Calcium ions may simultaneously reduce the diffusional permeabilities of the cell membrane and of the intercellular cement and at the same time make them less porous.

The time taken to adapt to changes of external calcium requires further investigation. Preliminary experiments suggest that permeabilities continue to increase during the second day following transfer from high to low calcium concentrations. Adaptation to high calcium may be more rapid but is not instantaneous.

It is not possible to make an accurate allowance for the effect of different concentrations of sodium and chloride and other ions on the activity coefficients of calcium and magnesium ions. To a first approximation, in dilute solutions, the activity coefficient of an ion is a function of the ionic strength of its solution; but the theory is complex and not sufficiently exact at higher concentrations to allow accurate comparisons. Direct determination of the activity coefficient of calcium and magnesium chlorides show that they are constant (*c.* 0·4–0·6) over the range 0·1 to 1·0 molal ionic strength and both increase at higher concentrations (Robinson & Stokes, 1943). On the other hand the activity coefficient of magnesium sulphate solutions declines from 0·15 to

0·05 over the same range. As sea water contains both chloride and sulphate ions interpolation is difficult but it is unlikely that the activity coefficients change by more than a factor of two between fresh water and 200% sea water.

Although in general marine fish have a lower permeability to water than do fresh water fishes, and the permeability of a variety of euryhaline fishes declines on adaptation to sea water, this is not a universal characteristic of fish. The stickleback *Gasterosteus aciculatus* and the silver form of the eel *Anguilla anguilla* show no decrease in permeability on adaptation to sea water (Evans, 1969). The difference between these fishes and those that do change may well be quantitative. Sufficient exposure to a calcium-free medium might eventually induce an increase in permeability in these fishes. Whether the difference between the eel and *Fundulus* depends on peculiarities of the composition and structure of the gill or whether it depends on differences in calcium uptake and mobilization is uncertain.

The interactions between calcium and prolactin and the osmotic and diffusional permeabilities are too complex to be completely elucidated at this time, but it is clear that calcium ions and prolactin are to some extent antagonistic. In marine fish both the high calcium and low prolactin combine to produce a low permeability to water and in this condition both the diffusional and osmotic permeabilities are similar. In fresh water hypophysectomized fish have a higher diffusional permeability than in sea water, which may be attributed to the lower concentration of calcium ions. Normal fish in fresh water or prolactin-treated hypophysectomized fishes have still higher diffusional permeabilities and even greater osmotic permeabilities. It would be interesting to know whether hypophysectomized fish in fresh water have a low or high osmotic permeability. It is possible that prolactin, by opening pores, acts more on the osmotic than on the diffusional permeability, but this seems unlikely in view of the fact that prolactin reduces the passive loss of ions in fresh water (Potts & Evans, 1966). The interpretation of the results is complicated by the finding that injections of 200 mu doses of prolactin have an ACTH-like action in *F. kansae* in that such injections markedly stimulate cortisol production (Hawkins, Ball & Fleming, unpublished data). Whether the changes in water turnover observed are due to prolactin *per se* or to the effect of prolactin on the interrenals or some other target organ remains to be determined.

The effects of salinity on permeability to water have been investigated in a number of invertebrates. It is noteworthy that several investigators have begun with the notion that permeability is likely to be lowest in fresh water for reasons of economy. Smith (1964) found no significant change of permeability of *Nereis limnicola* to D_2O in different salinites. Similarly neither *Carcinus maenas* nor *Palaemonetes varians* showed any significant change of permeability to tritiated water over a considerable range of salinities (Rudy, 1967). However, neither *Carcinus* nor *Palaemonetes varians* survived in fresh water. As the effect of calcium varies approximately with the logarithm of the concentration any increase of permeability is not apparent unless the animal can survive in a very wide range of salinities. Smith (1967) found that the permeability to water of the euryhaline crab *Erythropanopeus* declined in low salinities and has found similar effect with *Nereis diversicolor* (personal communication). The concomitant changes in calcium concentrations would act to reduce these effects.

The protective effects of calcium ions on animals in fresh water have been known

for many years. Pantin (1931 *a*, *b*) found that *Procerodes (Gunda) ulvae* can only survive in hard fresh water. In soft fresh water both the rate of swelling and the rate of loss of salt are higher than in hard water. Similarly *Nereis diversicolor* cannot osmoregulate in calcium-free brackish water (Ellis, 1933, 1937). Breder (1934) found a variety of marine fish living in a fresh-water lake on the island of Andros in the Bahamas. The water was hard, but not particularly calcium-rich, containing 1 mM-Ca/l. Breder reported that he was able to adapt a variety of marine fish to calcium-enriched fresh water but not to fresh water containing little calcium. Although *Fundulus kansae* can survive in fresh water even after hypophysectomy the addition of calcium enables the hypophysectomized fish to maintain a higher blood concentration, *c*. 300 m-osmole compared with 200–250 m-osmole in low-calcium fresh water. It is notable that the sham-operated fish also had slightly higher blood concentrations in calcium-rich water (Pickford *et al.* 1966).

The special effects of calcium on permeability to water constitutes an additional barrier to the penetration of marine and brackish species into fresh water but it will facilitate the return of fresh-water animals to the sea or the adaptation of originally fresh-water animals to saline lakes. The high calcium content of sea water reduces the permeability of *Fundulus kansae* by 35 % compared with its value in fresh water, and 200 % sea water reduces the permeability by 43 %. The need to drink sea water and to excrete the surplus salt will be reduced in proportion.

The presence of a high concentration of divalent ions in the gut of sea-water fishes might be expected to reduce the permeability of the gut wall as compared to that of fresh-water fish, but Utida, Isono & Hirano (1967) have demonstrated that the rate of water uptake is greater in sea-water-adapted fishes, and Skadhauge & Maetz (1967) found that the permeability to water of the gut of the eel (*Anguilla anguilla*) was higher in sea water than in fresh water, 7·3 μl/100 g/m-osmole and 4·1 μl/100 g/m-osmole respectively.

SUMMARY

1. Measurements have been made of the rate of exchange of tritiated water in both intact and hypophysectomized *Fundulus kansae* in a variety of media.

2. Hypophysectomy reduces the rate of exchange in fresh water.

3. Low doses (30 mu) of ovine prolactin stimulate water turnover in hypophysectomized fish in fresh water.

4. The rate of exchange declines in both intact and hypophysectomized animals with increasing salinity.

5. Experiments with synthetic solutions show that the decline in the rate of exchange in sea water and in higher salinities is due mainly to the effects of calcium ions.

6. Fishes maintained in synthetic sea water containing a low concentration of calcium have both a higher rate of exchange of tritiated water and a higher drinking rate than fish in normal sea water.

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