

THE EFFECTS OF CALCIUM ON WATER BALANCE OF THE BROWN TROUT *SALMO TRUTTA*

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SUMMARY

Water space, tritiated water flux, drinking and urine flow rates of the brown trout were decreased by increasing the environmental calcium concentration.

Diffusional and osmotic permeabilities were identical in fresh water. This suggests that water flow across the gill occurs entirely by diffusion. Previous suggestions of a discrepancy between the two permeabilities in freshwater teleosts could have been due to an overestimation of urine flow rate upon which the determination of osmotic permeability coefficient is based.

The wide-ranging effects of calcium on hydro-mineral regulation suggests that the ion interacts with the gills.

INTRODUCTION

Euryhaline teleosts maintain an internal environment which is different in composition from their external environment. The maintenance of a relatively stable body fluid composition by euryhaline teleosts in varying salinities involves complex physiological functions, which were first described by Smith (1930) and Krogh (1937). Seawater teleosts, because they are hypotonic with respect to the medium, face an obligatory loss of water and gain of electrolytes, especially sodium and chloride ions. In fresh water, however, the situation is reversed. The work of Smith (1930) demonstrated that marine teleosts solve their osmotic problem by drinking sea water and excreting salts extrarenally. Water lost in the urine is compensated by the absorption of some of the water drunk. Krogh (1937) postulated an active uptake of ions and the production of 'copious' urine in freshwater teleosts.

Considerable advance has been made in the understanding of electrolyte balance by euryhaline teleosts in varying salinities (see reviews by Potts, 1968; Motais & Garcia-Romeu, 1972; Maetz, 1972; Potts, 1972). The same cannot be said however for water balance. The regulation of water is as vital as that of electrolytes, although more attention has been given to the latter. Like the movement of ions, the net transfer of water in euryhaline teleosts in fresh water and sea water are opposite in direction. Permeability studies using tritiated water have shown that the gills of some teleosts are more permeable to water in fresh water than in sea water (Evans, 1969; Motais *et al.* 1969), while in others there is no apparent change in permeability with salinity

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(Evans, 1969). Thus, while euryhaline teleosts exhibit functional modification of branchial electrolyte permeability, the same is not true, in all teleosts, of water permeability.

The regulation of water and electrolyte permeability is under endocrine control (Lahlou, 1967; Lahlou & Giordan, 1970). Houston (1964) drew attention to the effect of passive factors, such as environmental calcium and pH, on the osmoregulation of teleosts. Environmental calcium was shown to promote the survival of hypophysectomized *Fundulus kansae* in de-ionized water (Pickford *et al.* 1966). More recently, there has been quantitative evidence for the influence of environmental calcium on water and electrolyte fluxes in teleosts. Increased calcium concentrations decreased both water (Potts & Fleming, 1970) and sodium (Potts & Fleming, 1971) fluxes in *Fundulus kansae*. The removal of calcium caused a reversible increase in sodium influx in the goldfish *Carassius auratus* (Cuthbert & Maetz, 1972). In the eel *Anguilla anguilla* in sea water, the removal of calcium caused a reversible fourfold increase in passive sodium outflux (Bornancin, Cuthbert & Maetz, 1972).

The importance of divalent cations, particularly calcium, in controlling many physiological functions has been recognized for a long time (see review by Brink, 1954). The calcium ion is known to affect the permeability of biological membranes to other ions and to water, and is responsible for the maintenance of intercellular cement.

This study was carried out to assess the relative roles of environmental calcium and the pituitary on water balance in the brown trout. This paper deals with the role of calcium.

MATERIALS AND METHODS

Brown trout *Salmo trutta* L. were purchased from commercial sources at Dunsop-Bridge and Pickering, Yorkshire, England. It was not possible to control the size of fish exactly as it depended on the season of the year. Experiments therefore had to be planned to suit the size of animals available. The bigger fish were often used for urine flow experiments and the smaller ones for tritiated water permeability studies. The fish were kept in large fibreglass tanks with a continuous flow of dechlorinated tap water. The aquarium was maintained at 15 ± 1 °C and this was the temperature at which all the experiments were carried out.

Ionic composition of media

Dechlorinated Lancaster tap water contained 0.25 m-equiv/l Na⁺; 0.2 m-equiv/l K⁺; 0.3 m-equiv/l Cl⁻ and 0.3 mM-Ca²⁺ (pH = 6.8). Calcium-rich fresh water was prepared by adding CaCl₂ to normal tap water to a concentration of 5 mM or 10 mM. Calcium-enriched fresh water was bubbled with air for 24 h to bring the pH to normal. Animals were kept in a medium for 2 weeks before being used for experiment.

Water content

After acclimation to the experimental medium, animals were killed, weighed and then dried to a constant weight at 100 °C. The water content was estimated from the difference in wet and dry weights of a fish.

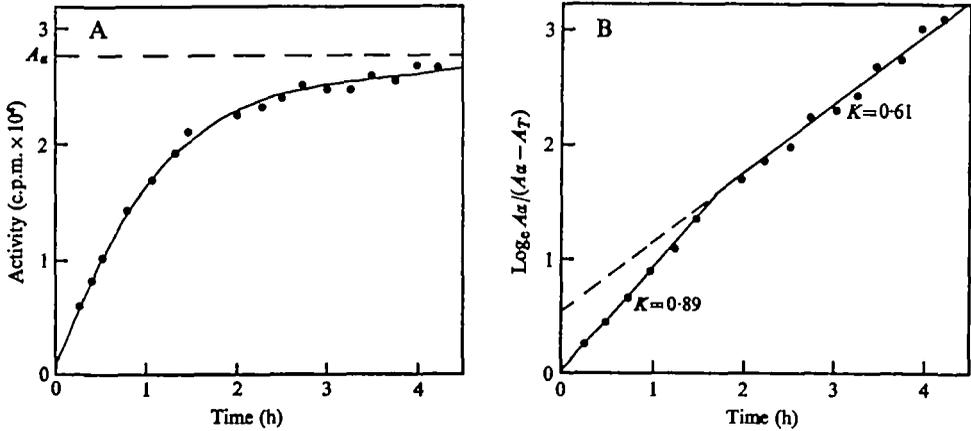


Fig. 1. Determination of water efflux rate. (A) The rate of appearance of THO in the efflux bath. (B) Analysis of the exponential appearance of THO.

Determination of tritiated water flux rate

The turnover rate of tritiated water was measured after injecting a fish with isotopically labelled water. About 3–5 mc of tritiated water contained in 20 μ l of isotonic saline was injected into the peritoneal cavity with a micrometer-driven syringe. After injection, the fish was put into a small tank containing the experimental medium of known volume. The system was first opened for a period of 15–30 min and the effluent run to waste. This was to allow the injection to become uniformly distributed in the water space of the fish. No more than half an hour was allowed for equilibration as the animal has a multiple water pool and the fast component, the blood, was largely emptied in the first 3 h after injection.

After the equilibration period, the system was closed and the medium circulated by means of a peristaltic pump. Samples of the system were removed every 10 min for one and a half hours, and a final sample was removed after 24 h, when equilibrium of the isotope in the whole system would have been established.

To avoid quenching during counting, pure water was extracted from the samples by means of a freeze-drying apparatus (Rudy, 1967). Samples were counted on the Packard Tri-carb Liquid Scintillation Spectrometer model 3375 after the addition of 5 ml of Insta-Gel (Packard).

The progressive increase in activity of the efflux bath is shown in Fig. 1 and can be expressed as a simple exponential function:

$$A_t = A_\alpha (1 - e^{-kt}),$$

where A_t is the activity in the bath at any time t , A_α is the activity in the system at equilibrium, and K is the exchange rate constant of the bath (h^{-1}). The exchange constant, K , was deduced by plotting $\log_e A_\alpha / (A_\alpha - A_t)$ against time (h). The slope of the graph gave the rate of exchange of water between the fish and the external medium (Fig. 1).

The rate of renewal of internal water, λ , (ml h^{-1}) was obtained from the expression:

$$\lambda = \frac{KV_2}{V_1 + V_2} \quad (\text{Motais \& Isaia, 1972}),$$

where V_1 and V_2 are the internal and external volumes of water (ml).

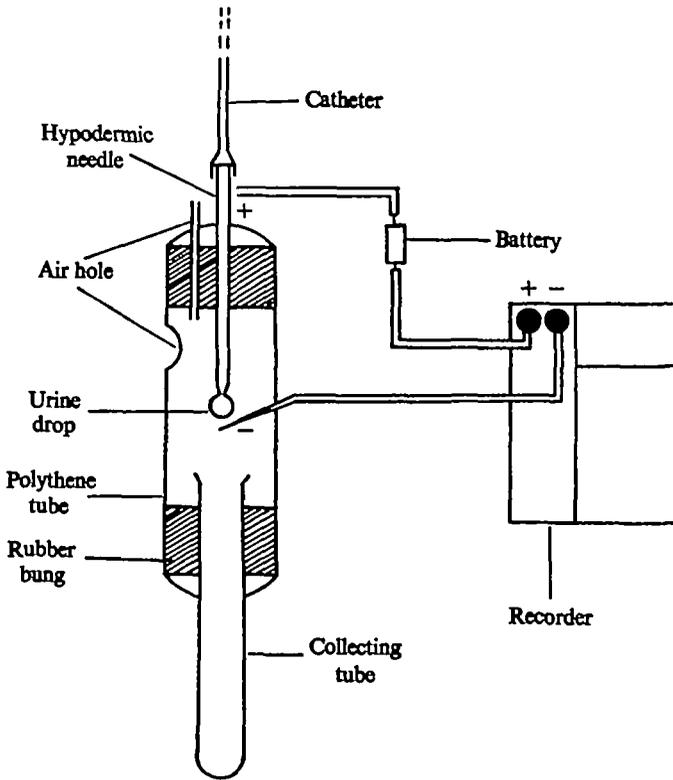


Fig. 2. Automatic urine drop recorder.

The diffusion permeability coefficient, P_d , may be calculated from the unidirectional water flux measurements and the gill surface area. P_d was calculated according to Motais & Isaia's (1972) equations. A gill surface area of $340 \text{ cm}^2 (100 \text{ g})^{-1}$ was assumed for the brown trout (Hughes, 1966). The net diffusion flux, F_{net} , was also calculated according to Motais & Isaia (1972) from the equation:

$$F_{\text{net}} = A \times P_d \times C,$$

where A is the gill surface area, P_d is the diffusion permeability coefficient and C is the difference in molar fraction of water in the plasma and the external medium.

Measurement of osmotic net flux

The osmotic permeability can be calculated from the difference between the rates of drinking and urine flow. In a freshwater-adapted teleost, the positive net water flux is equivalent to the urine flow rate minus the drinking rate.

Drinking rate was measured using ^{125}I -PVP by the method of Evans (1968) and Potts, Foster & Stather (1970) after adaptation to the experimental medium. Urine flow rate was measured by cannulation. Individual fish were cannulated and held in a small tank designed to hold the fish and allow limited movement without abrading the skin.

Two procedures were used for measuring urine volume; both were reliable. In the

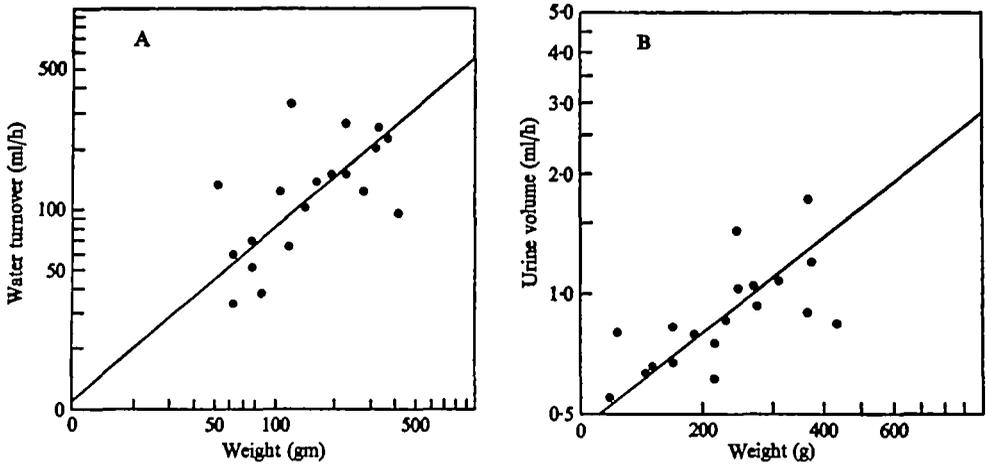


Fig. 3. Effect of weight on (A) THO flux; (B) urine flow rate.

first, the free end of the cannula was connected to a LKB UltraRac Fraction Collector. In the second, the cannula was connected to an electrode system (Fig. 2) designed to automatically record drops of urine. As the urine drop hanging from the upper electrode touched the lower electrode, an electrical circuit was completed. Since the lower electrode was inclined, however, the drop immediately broke from the upper electrode. The electrode circuit was linked to a Servoscribe pen recorder. The volume of a single drop of urine from the system was determined in trial experiments and thus the volume of urine produced could be recorded.

Urine was collected over a period of at least 48 h. The first 24 h was allowed for recovery from handling stress and anaesthetics. The flow rate during the second period was used in the calculation of osmotic water flux.

Osmotic net flux and osmotic permeability coefficient were calculated according to Motais & Isaia (1972). Water concentrations of 55.2 ml and 55.5 ml were assumed for the plasma and fresh water respectively.

Weight correction

Since there were differences in the weights of fish used in this study, it was desirable to eliminate variations which were due solely to this. Potts *et al.* (1967) and Evans (1969) established that there is a relationship between weight and water permeability in fishes. It was suspected that urine flow rate might also vary with weight.

To obtain a relationship between weight, water flux and urine flow rate, animals were cannulated and injected with THO, and the cumulative appearance of the isotope and the urine flow rate were followed for at least 48 h. Assuming that the total body water of a fish is 75% of its body weight, the efflux constants were converted to ml water/fish/h. The water flux and urine flow (ml/h) were plotted against body weight (Fig. 3) in a manner similar to that of Potts *et al.* (1967) and Evans (1969). A line drawn through the resulting points could be expressed by the equation: $m = aw^x$, where m is the water flux or urine flow rate in ml water/fish/h, a is the Y-intercept, w is the body weight in g and x is the slope of the line. The slope was obtained by regression analysis.

Table 1. *Effect of calcium on water space*

| Medium | Body water (%) |
|--|----------------------|
| Fresh water (0.3 mM-CaCl ₂) | 75.6 ± 0.7 n = 5 |
| Fresh water (5 mM-CaCl ₂) | 71.6 ± 0.44 n = 6 |
| Fresh water (10 mM-CaCl ₂) | 68.5 ± 1.0 n = 6 |

Mean values are given ± standard error. n = number of determinations.

The x value is 0.85 for water flux and 0.79 for urine flow rate. Correction factors for both parameters were calculated from the following relationship:

$$\frac{100}{(100)^x} \times \frac{(wt)^x}{wt},$$

so that a 100 g fish had a correction factor of 1. All experimental values for THO flux and urine flow rates have been corrected for weight by this method.

RESULTS

Diffusion permeability

Water space. The effect of environmental calcium concentration on total body water in the brown trout is shown in Table 1. In normal fresh water (0.3 mM-Ca²⁺), total body water was about 76%. Animals adapted to fresh water containing 5 mM-Ca²⁺ maintained a water content of 72% of their total wet weight. In 10 mM-Ca²⁺ the water space decreased further to 69% of wet weight.

Evidence for a multiple water compartment. Analysis of the efflux bath (Fig. 1) in tritiated water efflux experiments showed that the rate of exchange was not constant over a long period and consequently water must be contained in more than one water pool in the fish (Rudy, 1967). An experiment was therefore carried out to confirm this hypothesis and to localize the water pools. Animals were loaded to equilibrium for 24 h in a medium containing 1 µc/ml THO. By this time the activity in the loading bath and the water in the fish were the same, A_0 . Individual fish were then unloaded for $\frac{1}{2}$ -1 h in a large bath containing inactive medium. The fish were then removed, rinsed quickly in three changes of the inactive medium and killed. 100 µl aliquots of water from tissue samples were counted separately along with a sample of the loading medium. The efflux rate, K_{eff} , of each tissue was derived from the relationship:

$$K_{\text{eff}} = \frac{1}{T} \log_e \frac{A_0}{A_T}$$

where A_0 is the initial activity in the tissue and A_T is the activity remaining after efflux time, T (in h). The result is shown in Table 2. The volume of water in the fish is negligible compared with the volume of the efflux bath and back-flux of THO into the animals was neglected. Allowance was however made for the rinsing time.

Because of the multiple water pool, efflux bath sampling in THO efflux experiments

Table 2. Tritiated water efflux rates of some tissues in the brown trout (*Salmo trutta* L).

| Tissue | K_{eff} |
|--------|------------------|
| Blood | 0.87 |
| Kidney | 0.80 |
| Liver | 0.66 |
| Eye | 0.65 |
| Muscle | 0.53 |

Table 3. Effect of calcium on water flux deduced from the efflux rate

| Medium | K (% h ⁻¹) | Diffusional flux ml h ⁻¹ (100 g) ⁻¹ | Net diffusion flux $\mu\text{l h}^{-1}$ (100 g) ⁻¹ |
|-------------------------------|------------------------|--|--|
| FW (0.3 mM-Ca ²⁺) | 89.6 ± 0.05 | 67.2 | 321.9 |
| FW (5 mM-Ca ²⁺) | 77.3 ± 0.03 | 58.0 | 277.9 |
| FW (10 mM-Ca ²⁺) | 69.1 ± 0.04 | 51.8 | 248.2 |

Mean values are given ± standard error. n = number of determinations.

Table 4. Osmotic water flux deduced from drinking and urine flow rates

| Medium | Drinking rate | Urine flow | Osmotic net flux |
|-------------------------------|------------------|---------------|---------------------|
| FW (0.3 mM-Ca ²⁺) | 46.5 ± 7.6 | 382.3 | 335.8 |
| FW (5 mM-Ca ²⁺) | 25.5 ± 9.0 | 300.7 | 275.2 |
| FW (10 mM-Ca ²⁺) | 21.0 ± 7.4 | 254.4 | 233.4 |

Mean values are given ± standard error. Drinking rate, urine flow and osmotic net flux in $\mu\text{l h}^{-1}$ (100 g)⁻¹. Urine flow values were obtained after giving the fish a 24 h post-operation recovery period.

had to be completed within 2 h of the injection of the isotope. Up to this time the effect of the slower compartments on the rate of efflux was minimal.

Tritiated water flux. Table 3 shows the effect of calcium on tritiated water turnover. In normal fresh water, the fish had an exchange constant of about 90 % h⁻¹. When the calcium content of the medium was raised to 5 mM, the exchange rate was reduced by 14 %. There was a reduction of 23 % when the calcium content of the medium was raised to 10 mM.

Osmotic permeability

Drinking rate. The effect of calcium on drinking rate in the brown trout is shown in Table 4. In normal fresh water, the fish ingested 46.5 $\mu\text{l h}^{-1}$ (100 g)⁻¹ of the medium. In 5 mM-Ca²⁺ drinking decreased to 25.5 $\mu\text{l h}^{-1}$ (100 g)⁻¹. This was further decreased to 21.0 $\mu\text{l h}^{-1}$ (100 g)⁻¹ in 10 mM-Ca²⁺.

Urine flow rate. The effect of calcium on urine flow pattern is shown in Fig. 4 and summarized in Table 4. Maximum urine flow rates were obtained in the three groups in the first 4 h following cannulation. This dropped sharply for the next 6 h. There then followed a gradual drop, with occasional irregularities until 16–18 h post-operation. Even 24 h after operation, urine flow was not really steady. Fish with low urine flow rate had a more consistent urine output pattern 18 h after operation compared with those having high flow rates.

On the basis of the result taken 24 h after operation (Table 4), an increase in the

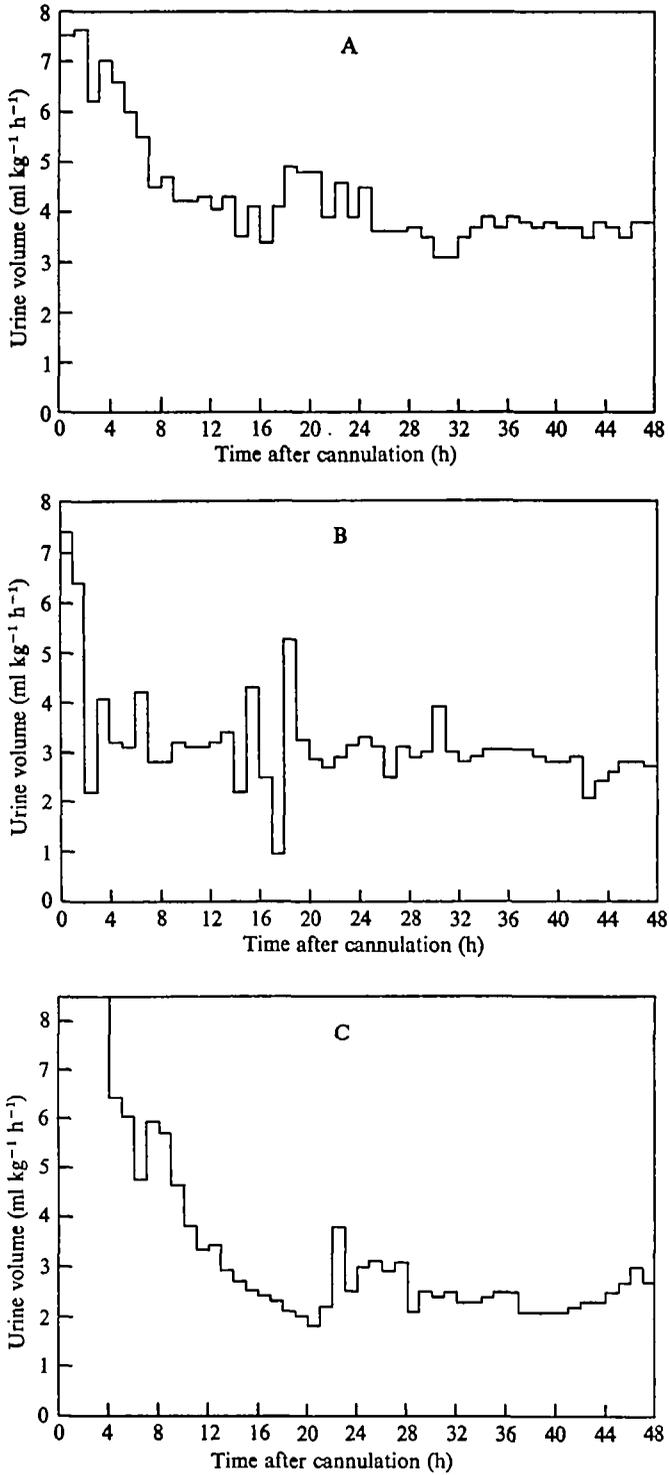


Fig. 4. Effect of calcium on urine flow. (A) Normal tap water (0.3 mM-Ca²⁺). (B) Tap water containing 5 mM-Ca²⁺. (C) Tap water containing 10 mM-Ca²⁺.

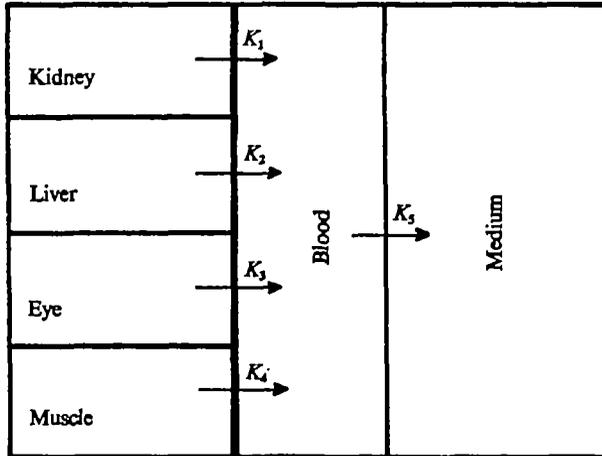


Fig. 5. Diagrammatic representation of the mode of exchange of water pools in the brown trout. K_1 – K_5 represent the exchange rates of the different tissues.

calcium content of the medium from 0.3 to 5 mM produced a 22 % decrease in urine flow rate, while 10 mM- Ca^{2+} produced a 32 % decrease.

DISCUSSION

Water space

The water content of the brown trout in fresh water (Table 1) was about 76 %. In general, water space in teleosts is fairly uniform and varies only slightly between fresh water and sea water (Thorson, 1961). The water content of the European eel *Anguilla anguilla* is 70 % in fresh water at 25 °C. (Motais & Isaia, 1972).

A large proportion of the body water in teleosts is contained in the muscles. In winter, adult *Salmo salar* have a muscle water content of 80 % in fresh water, which is reduced to 68 % when they migrate into sea in summer (Parry, 1961). The muscle water content of the American variety of the brown trout is 79 % in fresh water and decreases to 75 % in sea water (Gordon, 1959). Thorson (1961) is of the opinion that water and electrolyte spaces are of ecological significance in aquatic vertebrates.

Increasing the concentration of external calcium caused a decrease in water space. The decrease in water content at high calcium levels may be due partly to the decrease in permeability and partly to the fact that the fish had not been fed during the 3 weeks they were maintained in the experimental media. The water contents of the fish in the different media were used in the appropriate calculations of the diffusion water permeability.

Multicompartment system

Table 2 demonstrates that the brown trout has a multiple water pool, with various organs exchanging with the blood at different rates. The probable mode of this exchange is shown in Fig. 5. As expected, the blood has the highest efflux rate, since it exchanges directly with the medium through the gills. The more vascularized an organ the higher will be its efflux rate. The dorsal musculature, which has a very poor blood supply, has the lowest efflux constant.

Tritiated water flux

Comparison of water exchange rates in different species is made difficult by the fact that weight and temperature markedly affect water permeability. The Q_{10} for *Carassius auratus*, *Phoxinus phoxinus* and *Platichthys platessa* are 2.12, 1.77 and 1.81 respectively (Evans, 1969). The temperature range also affects the magnitude of the Q_{10} . Between 5–15 °C the Q_{10} for *Anguilla anguilla* is 2.1; whereas it is 1.5 between 15–25 °C (Motais & Isaia, 1972). The rate of water exchange in freshwater-adapted euryhaline teleosts ranges from 30 % h⁻¹ for *Platichthys platessa* at 10 °C (Evans, 1969) to about 140 % h⁻¹ in *Fundulus kansae* at 20 °C (Potts & Fleming, 1970). The value of 90 % for brown trout in normal fresh water (Table 3) is intermediate between these two extremes. Generally, inactive species have low water permeability compared with active ones. Gill surface area (Hughes, 1966) and respiratory rate (Marshall, 1965) are directly related to the level of activity in animals. Active freshwater teleosts are doubtless more permeable than inactive ones because of their greater gill surface area and higher respiratory rate. Oxygen consumption is correlated with water permeability in the rainbow trout (Wood & Randall, 1973).

Stress has been shown by Evans (1969) to affect water flux in large trout but not in small ones. The degree of stress will also have an effect on water flux. When rainbow trout are exercised for 30 min, water flux measured by the change in weight did not significantly exceed the control means; but prolonged exercise produced an increase in water flux (Wood & Randall, 1973). Unavoidably, the stress factor inherent in the determination of water flux by isotope injection could not be completely controlled in this work. Attempts were made to catheterize the caudal vein and the dorsal aorta by the method of Houston (1971) so that injection of isotope could be done after a fish had recovered from the shock of handling. This failed, however, as the brown trout will not stand such manipulation. Measurements of water permeability using tritiated water may be slightly affected by shock even when every effort has been made to limit stress.

The effect of calcium in reducing the water permeability in the brown trout is similar to that obtained by Potts & Fleming (1970) for *Fundulus kansae* in both fresh and sea water.

Drinking rate

As shown by the magnitude of the SEM (Table 4) drinking rates were highly variable. Evans (1968) also found irregularities in the drinking rate of teleosts. Shehadeh & Gordon (1969) found that the related salmonid *Salmo gairdneri* does not drink in fresh water. Oide & Utida (1968) also could not find any evidence of drinking in the Japanese eel *Anguilla japonica*. In the brown trout, there is no direct evidence that the water drunk is actually absorbed through the gut, but this is most likely, although about 20–40 % of the water ingested by seawater-adapted *Paralichthys lethostigma* is lost through the anus (Hickman, 1968). In fresh water there are no large ions to retain water in the gut and most of the water drunk will be absorbed by osmosis.

Although they may have been slightly affected by handling, it has been definitely established that a variety of teleosts drink when in fresh water. A drinking rate of about 0.05 % body wt/h by the brown trout is low compared with other teleosts. *Tilapia mossambica* drinks about 0.3 % of its body weight/h (Potts *et al.* 1967), while

The eel *Anguilla anguilla* drinks about 0.1 % of its body weight/h (Motais *et al.* 1969). The drinking rate of the brown trout is similar to that of the goldfish but slightly higher than that of the flounder in fresh water (Motais *et al.* 1969).

Urine flow rate

Urine output shortly after operation was high in the brown trout and may be attributed to anaesthetics and the stress of handling during cannulation (Hunn & Willford, 1970). Urine flow rates on the second day after operation were used in the determination of osmotic permeability so as to allow renal function to return to normal. Even after 24 h urine output was still variable. The slight hourly variations by this time, however, could be due to intermittent vigorous activity by the fish in the 'cannulation' tank. Wood & Randall (1973) observed that vigorous exercise by the rainbow trout causes a diuretic response similar but less severe than that caused by handling stress.

It is general practice to use an anaesthetic prior to cannulation. Diuresis has been reported on several occasions following such treatment (Holmes & Stainer, 1966; Hunn, Schoettger & Willford, 1968; Hunn & Willford, 1970). In the brown trout, urine output on the first day following operation was almost double that on the second day.

This diuretic response is not due to anaesthesia alone. Urine volume and chloride concentration may both rise ten times their normal values shortly after handling and mechanical restraint (Holmes, 1961). When rainbow trout are anaesthetized with MS₂₂₂ independently of the catheterization stress, diuresis occurs in the first 2-4 h post-anaesthesia (Hunn & Willford, 1970). Such prolonged diuresis as shown by the brown trout and other salmonids is also common in marine teleosts (Grafflin, 1931; Forster, 1953) where the phenomenon will have a greater physiological disadvantage.

In marine teleosts, increased urine flow rate is associated with an increase in filtration rate (Grafflin, 1931; Forster, 1953). Holmes & Stainer (1966) found a similar increase in filtration rate with increase in urine flow rate in a study of renal function in smolting and non-smolting rainbow trout. It is most probable that all aspects of renal function, including tubular absorption and secretion, are disturbed during diuresis. Obviously, an antidiuretic factor which is normally functional in both freshwater and seawater teleosts is disturbed or broken down as a result of handling, anaesthetics and stress.

'Normal' urine flow rate in the brown trout in fresh water (Table 4) is less than that of the rainbow trout (Hunn & Willford, 1970). Urine flow rate in freshwater-adapted *Salmo irideus* (Holmes, 1961) is similar to that obtained for the brown trout.

Observations with the urine drop recorder (Fig. 2) showed that a change of behaviour occurred about 20 h after cannulation. For the first 18-20 h urine flow was continuous, proceeding at about a drop per minute. In several fish, urine flow then became discontinuous, with an outflow of 18-25 drops every 20 min or so. It is not known whether a similar behaviour is shown in normal conditions. It is possible that this was an artifact due to the deposition of mucus or other material in the catheter opening in the urinary papilla. This would allow urine to accumulate in the bladder and force its way out periodically under pressure.

Table 5. Comparison of osmotic and diffusion permeability coefficients of the gill, assuming $\sigma = 1$

| Medium | P_{os} ($\mu\text{m} \cdot \text{sec}^{-1}$) | P_d ($\mu\text{m} \cdot \text{sec}^{-1}$) | P_{os}/P_d |
|-------------------------------|---|--|--------------|
| FW (0.3 mM-Ca ²⁺) | 0.58 | 0.55 | 1.05 |
| FW (5 mM-Ca ²⁺) | 0.47 | 0.48 | 0.98 |
| FW (10 mM-Ca ²⁺) | 0.40 | 0.43 | 0.93 |

σ is the reflexion coefficient which expresses the effective semi-permeability of the gill to water. The coefficient has a maximum value of 1 for a semi-permeable membrane and decreases with increasing porosity of a membrane.

Comparison of osmotic and diffusion permeabilities

A comparison of the osmotic (P_{os}) and diffusion (P_d) permeability coefficients of the trout gill in three freshwater media containing different calcium concentration is shown in Table 5. To a first approximation, both coefficients are identical in the three media. The concordance of the two coefficients is contrary to the results obtained by Motais *et al.* (1969) for the goldfish, eel and flounder, and to the results for anuran skin, where P_{os}/P_d ratio is several times higher than unity (Maetz, 1968; Dainty & House, 1966*b*). It is however in agreement with that of the crustacean *Gammarus duebeni* in 2% sea water (Lockwood & Inman, 1973). Discrepancy between the two coefficients may be due to the presence of aqueous channels in biological membranes (Koefoed-Johnsen & Ussing, 1953; Ussing, 1954). The agreement of the two coefficients in fresh water suggests that no channels or pores large enough to allow bulk flow of water exist in the brown trout gill and that water passes entirely by diffusion.

The main lines of evidence for the existence of pores in biological membranes are first, a discrepancy between P_{os} and P_d , second, the molecular sieve behaviour of certain membranes in discriminately allowing the passage of molecules through them on the basis of size, and third, the apparent interaction between water and solutes crossing a membrane. Assumptions of a solute-solvent interaction are based on irreversible thermodynamics (Kedem & Katchalsky, 1958; Dainty & Ginzburg, 1963).

Osmotic permeability determinations, as deduced from the difference between drinking and urine flow rates, can be grossly affected especially in the first 12 h following anaesthetics and stress. However, there is at the most a 50% increase in tritiated water flux in the period following anaesthetics and cannulation (Evans, 1969). The only quantitative comparisons of osmotic and diffusional permeability coefficients of teleost gill are those of Motais *et al.* (1969) and Motais & Isaia (1972), who based the comparison on urine flow rates established within 6 h of anaesthetization and urinary cannulation. Isolated gill preparations of the eel *Anguilla dieffenbachi* incubated in fresh water and sea water do not show any significant difference in osmotic permeability coefficients (Shuttleworth & Freeman, 1974). Over-estimation of urine flow rate can thus account for the apparent discrepancy between P_{os} and P_d of some teleosts in fresh water. It is possible for a fresh water teleost to run down its water reserve in the form of urine during diuresis without a change in branchial water permeability or any discernible physiological consequences.

The agreement of P_{os} and P_d in the brown trout shows that water flow across the fish occurs entirely by diffusion. In the absence of bulk flow of water, alteration of the branchial water permeability by calcium could be due to a modification of the diffusion barrier. The mucous coat present in the gill of teleosts (Philpott & Copeland, 1963) was suggested by Motais *et al.* (1969) and Potts & Fleming (1970) to constitute an unstirred layer and consequently a diffusion barrier (Dainty & House, 1966*a, b*). The action of calcium in the stabilization of the mucous coat was also suggested by Potts & Fleming (1970) as the reason for the modification of water permeability in *Fundulus kansae*. The action of calcium in increasing the diffusion barrier may be extended to the reduction of the effective size of membrane pores when they do exist (Whittembury, Sugino & Solomon, 1960).

Variations in water permeability have been produced in the brown trout without a change in the magnitude or direction of the osmotic gradient. Changes in gill permeability to water with changes in salinity cannot therefore be due entirely to salinity difference or osmotic gradient. Changes in structural properties of the diffusion barrier may partly account for the modification of water and electrolyte permeabilities in teleosts.

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