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ADAPTIVE CHANGES OF THE WATER PERMEABILITY OF THE TELEOSTEAN GILL EPITHELIUM IN RELATION TO EXTERNAL SALINITY

BY R. MOTAIS, J. ISAIA, J. C. RANKIN* AND J. MAETZ

Laboratoire de physiologie animale, Faculté des Sciences de Nice (06) *et Groupe de biologie marine du Département de Biologie du Commissariat à l'Energie Atomique. Station Zoologique. Villefranche-sur-Mer* (06) *France*

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INTRODUCTION

Fresh-water teleosts, whose blood is hypertonic to the external medium (about 330 m-osmoles/kg. *vs.* a few m-osmoles/kg.), face the problem of osmotic entry of water and loss of electrolytes, while sea-water teleosts live in an environment (1150 m-osmoles/kg.) which induces osmotic loss of water and a diffusional influx of electrolytes.

Many data are available on the electrolyte balance of fish (see reviews by Smith, 1932; Krogh, 1939; Parry, 1966; Motais, 1967; Maetz, 1968; Potts, 1968).

Much less work has been devoted to water equilibrium since Smith (1932) suggested the contrasting ways by which this equilibrium is achieved in fresh-water and sea-water teleosts. The fresh-water fish balances the net influx of water through the gill by excreting large quantities of hypotonic urine, the drinking rate being considered as negligible, while the sea-water fish balances the gill outflux by drinking sea water, absorbing monovalent ions together with water in the gut and simultaneously excreting excess ions through the gill.

While urine flow rates have been measured in numerous fishes including euryhaline fishes in low and high external salinity (see reviews by Potts, 1968 or Maetz, 1968), drinking rate studies have been resumed only recently (Motais & Maetz, 1965; Evans, 1967*a*, 1968, 1969; Potts & Evans, 1967; Potts *et al.* 1967; Hickman, 1968; Maetz & Skadhauge, 1968; Oide & Utida, 1968; Lahlou, Henderson & Sawyer, 1969; Lahlou & Sawyer, 1969*b*). The surprising observation is that the drinking habit occurs also in fresh-water fish.

The quantification of the components of the Smith model requires measurements of urine flow and drinking rate in the same species, and the assumption that the difference between them balances the osmotic water flow (positive in fresh water, negative in sea water) across the permeable boundary of the fish. Therefore the osmotic permeability of this boundary can only be determined indirectly. The present report is an attempt to evaluate this permeability for a few fresh-water, marine and euryhaline teleosts. The euryhaline fishes have been studied in both environments in order to measure the permeability of the same membranes when the water flow occurs in opposite directions. More recently a few investigators have also studied the diffusional

* Royal Society European Research fellow.

water permeability of the boundaries of fish with the help of labelled water (Vartapetjan, 1962; Evans, 1967*b*; Potts *et al.* 1967; Lahlou & Sawyer, 1969*a*, *b*). In the present study we shall also compare both diffusional and osmotic permeabilities in the light of the latest theories of membrane structure in relation to water transfer. Finally, one major problem recurring in these *in vivo* studies, the relative permeabilities of the gill and the skin, will be dealt with.

MATERIAL AND METHODS

In our investigation we have used the goldfish *Carassius auratus* (mean weight 96 g.), a typical fresh-water fish, the sea-water perch *Serranus cabrilla* or *S. scriba* (92 g.), a stenohaline marine fish, and two species of euryhaline teleosts, *Anguilla anguilla* (mean weight: 129 g. in fresh water or 116 g. in sea water) or the flounder *Platichthys flesus* (mean weight: 245 g. in fresh water and 220 g. in sea water).

Both euryhaline fishes were caught in fresh water, in the Rhône estuary for the eels and in the Loire estuary for the flounders. The eels were kept in running fresh water and the flounders in running sea water at various temperatures. The animals were considered to be adapted to their experimental external medium after a week. The measurements of diffusional and osmotic flow were made at the same temperature—$19 \pm 1°$ C. for the goldfish and the eel and at $16 \pm 1°$ C. for the flounder and *Serranus*—after at least 1 day's adaptation to this temperature.

(1) *Diffusional permeability to water*

The diffusional permeability was measured in a closed circuit (1 l. of external medium per 100 g. body weight) after intraperitoneal injection of 10 μl. of an isotonic solution labelled with tritium per 100 g. (THO). The specific activity was 50–66 mC. ml.$^{-1}$. The measurement began after a delay of 30 min. to allow the isotope to become uniformly distributed in the water space. The system was then closed and samples of the external medium (250 μl.) were withdrawn at 10 min. intervals for 4 hr. and then at 30 min. intervals for 3 hr. They were added to 10 ml. Bray's solution and counted on a Nuclear Chicago (mark I, model 6860) scintillation counter. The cumulative appearance of tritium is therefore followed in the external medium (Fig. 1). The curve may be described by an exponential function with the following equation:

$$Q = Q_{eq}\,(1 - e^{-\lambda t}),$$

where Q is the quantity (d.p.m.) of THO in the external compartment in time t, Q_{eq} the quantity of THO at equilibrium and λ the turnover rate between the water of the fish and the external water. λ can be deduced from the experimental curve by plotting on a semilogarithmic scale either log $\Delta Q/\Delta t$ for $\Delta t = 2$ hr. or log $(Q_{eq} - Q)$ against time (see Motais, 1967).

λ is the slope of the straight line (in % hr.$^{-1}$), given the equation:

$$\lambda = ([1/V_1] + [1/V_2])\, f_{out}.$$

V_1 and V_2 being respectively the internal and external water pools (in ml.). The unidirectional flux, given in ml. hr.$^{-1}$ (100 g.)$^{-1}$ can be obtained from:

$$f_{out} = \left(\frac{V_1 V_2}{V_1 + V_2}\right)\lambda,$$

V_1 and V_2 being expressed in ml. We have assumed that the water space was 70% of the body weight for all the species of fishes (Thorson, 1961). Lahlou & Sawyer (1969*a*) have shown that in the goldfish THO distributes within a space representing 70% of the body weight. The diffusional permeability coefficient, P_d, expressed in cm. sec.$^{-1}$, can be deduced from the unidirectional flux by the relation:

$$P_d = \frac{f'_{\text{out}}}{A \times C_{\text{in}}},$$

where f'_{out} is expressed in mM sec.$^{-1}$ (100 g.)$^{-1}$, A is the surface of the gills in cm^2 per 100 g. fish, C_{in} is the water concentration in the blood expressed in mM. cm.$^{-3}$.

The gill surface was estimated according to Gray (1954) for the eel (300 cm.^{2}/100 g.) and the flounder (200 cm.^{2}/100 g.) For the more active *Serranus* and goldfish we estimated the gill area to be somewhat larger (400 cm.^{2}/100 g.) (see Hughes, 1966). These calculations suppose that the major route of water exchange is the gill, a point that additional experiments will attempt to settle (see below).

(2) *Osmotic permeability to water*

The drinking rate minus the urine flow was taken as a measure of the rate of water loss through the gills and skin in the sea-water fish. The urine flow minus the drinking rate correspond to the osmotic inflow of water in the fresh-water fish.

The urine flow of the goldfish and of the sea-water-adapted and fresh-water-adapted eel was measured by catheterization of the urinary papilla with a Portex tubing no. NT 3 (internal diameter 1·4 mm.; external diameter 2 mm.). The urine flow was followed for about 24 hr., but the first 6 hr. only were taken into account in order to evaluate this parameter in similar experimental conditions as for THO turnovers.

Some values of urine flow were taken from Motais (1967) for the flounders and from Lahlou (1967) for *Serranus*.

The drinking rate was measured by either of the two methods making use of radioactive water markers ^{131}I phenol red and colloidal ^{198}Au. The phenol red technique has been described by Maetz & Skadhauge (1968). During the course of the present investigation the colloidal gold method was developed and was found to be preferable because no radioactivity appears in the body fluids of the fish and therefore no correction need be made. 0·5 mC. of colloidal gold from the Département des Radioéléments (Saclay), presented in plastic 1 ml. syringes, was used in about 10 l. of aquarium water. Two hours after addition of the water marker the fishes were removed and anaesthetized in 'cold' medium with MS 222. The gut was dissected and its radioactivity was measured against aliquots of the external medium in a well-type γ counter. In two series of experiments on flounder both techniques were used concurrently. The results agreed within the limits of variation. The colloidal gold technique yielded 212 ± 34 μl. ($n = 6$) and 49 ± 25 ($n = 4$) respectively for the sea-water-adapted and fresh-water-adapted fishes, while the phenol red technique gave 167 ± 68 ($n = 5$) and $27 \pm 3{\cdot}4$ ($n = 5$). The mean values are given in Table 3.

From the osmotic flow of water given in Table 3 (in μl. hr.$^{-1}$ (100 g.)$^{-1}$), the osmotic permeability coefficient P_{os} (in cm. sec.$^{-1}$) has been calculated by the relation:

$$P_{os} = \frac{F_{\text{net}}}{A \times \sigma \times \Delta C_s},$$

with F_{net} in mM sec.$^{-1}$ (100 g.)$^{-1}$, A the gill area in cm.^{2} (100 g.)$^{-1}$, ΔC_s in m-osmoles/kg. water as determined by cryoscopy (Lucarain, 1962), σ the Staverman or reflexion coefficient is a coefficient $\leqslant 1$ which measures the effective semipermeability of the membrane to the osmolyte. (See Discussion.)

(3) '*Dorso-ventral THO gill clearance*' *technique*

In order to evaluate the relative importance to the gills *versus* the skin in the THO permeability we have measured the cardiac output [C_0 in ml. hr.$^{-1}$ (100 g.)$^{-1}$] by two independent methods, with the help of a electromagnetic flow meter or by making use of the Fick principle by dividing f^*_{out}, the diffusional THO outflux (in d.p.m. h^{-1} (100 g.)$^{-1}$) by the difference of the THO concentration between the afferent (Q_V) and the efferent (Q_D) branchial blood (d.p.m. ml.$^{-1}$):

$$C_0 = \frac{f^*_{out}}{Q_V - Q_D}.$$

If the C_0 values given by the two methods are comparable, it means that the skin plays a minor role in the process.

$\frac{Q_V}{Q_V - Q_D}$ represents the dorso-ventral THO clearance fraction in %.

If this fraction were important, the cardiac output would be a limiting factor in the measurement of the diffusional water flux and would have to be taken into account in the calculation, because the specific activity of the gill blood would be much lower than that of the body fluids.

(*a*) *Operative procedures*

Polythene catheters were introduced into the ventral and dorsal aortae via the bulbus arteriosus of the heart and pneumogastric artery respectively and into the swimbladder vein of eels anaesthetized with MS 222. The operational technique was similar to that of Chester Jones, Chan & Rankin (1969) (see Fig. 1). After a 24 hr. recovery period the eels appeared unstressed (normal blood pressure and heart rate). For measurement of cardiac output the probe of a Statham M 4001 electromagnetic flowmeter was placed around the ventral aorta immediately anterior to the heart. Recordings could be made for several days in the free-swimming animal.

(*b*) *Experimental procedure*

All experiments were performed on fresh-water-adapted eels in fresh water. HTO (about 200 μC./100 g. body weight) was injected intravenously and 20–30 min. were allowed for equilibration within the animal. In experiments in which outflux was measured the animal was kept in a closed circuit and samples were taken throughout the experiment. Blood samples were taken at 5 or 10 min. intervals alternately from the dorsal and ventral aortae. Blood was first withdrawn into a heparinized syringe to clear the dead space in the cannula, and then allowed to flow into a centrifuge tube. After centrifugation 50 μl. of plasma was taken for measurement of radioactivity. The red-blood cells were then resuspended in the remaining plasma and re-injected, as was the blood in the syringe. It was found that up to 50 such blood samples could be taken from a large eel without significantly lowering the haematocrit or causing haemolysis.

The THO clearance fraction was calculated in the following way. Let the THO concentrations of the successive ventral (afferent) and dorsal (efferent) samples be Q_{V_n}, $Q_{D_{n+1}}$, $Q_{V_{n+2}}$, $Q_{D_{n+3}}$ ... taken at times n, $n+1$, $n+2$, $n+3$ (0–5–10–15 min., for example).

The clearance fraction was taken as

$$\frac{Q_V - Q_D}{Q_V} \times 100 = \frac{\frac{Q_{V_n} + Q_{V_{n+2}}}{2} - Q_{D_{n+1}}}{\frac{Q_{V_n} + Q_{V_{n+2}}}{2}} \times 100.$$

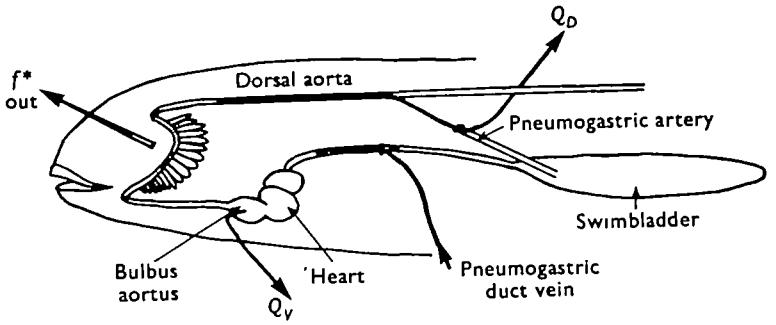

Fig. 1. Schematic arrangement of the afferent (ventral) and efferent (dorsal) cannulation of the branchial vessels. The pneumatic duct vein permitted the injection of the labelled water. Compare with Fig. 1 in Holeton & Randall (1967*a*) and Chester Jones *et al.* (1969).

The diffusional water flow was also measured in a few experiments and calculated as the quotient f^*_{out} (external THO appearance rate) divided by the mean afferent and efferent plasma specific activity for the period.

$$f_{out} = \frac{Q_{ext}/\Delta t}{\frac{Q_V + Q_D}{2}}.$$

In these measurements the different quenching of plasma and external water was taken into account by the use of an external standard, the channel ratio method and a standard quenching curve. The radioactive backflux arising from the accumulation of THO in the external water and the progressive labelling of the water influx was also taken into account in the calculations. The rate of appearance of THO has to be divided by the difference between the internal and external specific radioactivities.

RESULTS

(1) *Dorso-ventral clearance experiments. Relative diffusional water permeability of the gills and the skin in the eel*

Table 1 summarizes the results of seven dorso-ventral gill clearance experiments. The clearance fraction was found to be higher in the smaller animal. This is probably related, at least in part, to the observation that the unidirectional outflux (expressed in terms of body weight) is relatively higher in smaller animals (see Evans, 1969). The comparison of the unidirectional outfluxes calculated for the eels of more than 1 kilo body weight (Table 1) and for the eels of 100 g. body weight (Table 2) emphasizes this

point. The fact that the clearance fraction does not exceed 30% and is more often in the range of 5% suggests that the cardiac output is not a limiting factor in the THO flux measurements. The cardiac output calculated by the application of the Fick principle and measured by the electromagnetic flowmeter is of the order of 100–165 ml hr.$^{-1}$ (100 g.)$^{-1}$, a value which incidently agrees rather well with the previously known range given for other species of fish (see Holeton & Randall, 1967*b*) and is at least ten times higher than the unidirectional water outflux measured in the same animals.

Table 1. *Branchial THO clearance fraction*

(1) Results of the dorso-ventral gill clearance studies

Date	Body weight	Percentage clearance fraction (mean ± s.e.)	No. of periods
17. iv. 68	400 g.	29·1 ± 2·5	8
18. iv. 68	Same fish	29·3 ± 2·9	3
30. v. 68	1500 g.	6·7 ± 3·7	3
31. v. 68	Same fish	6·1 ± 0·7	10
3. vi. 68	1220 g.	4·8 ± 0·7	6
6. ix. 68	1500 g.	7·0 ± 2·7	5
11. ix. 68	1225 g.	7·3 ± 0·8	12

(2) Cardiac output (C_{out})

Date	Body weight	C_{out} ml. hr.$^{-1}$ (100 g.)$^{-1}$	Method
3. vi. 68	1220 g.	165	Fick
6. ix. 68	1500 g.	125	Fick
11. ix. 68	1225 g.	125	Fick
3. vii. 68	350 g.	130	magnetic
21. ix. 68	1100 g.	100	magnetic

(3) Unidirectional outflux (f_{out})

Date	Body weight	f_{out} ml. hr.$^{-1}$ (100 g.)$^{-1}$
3. vi. 68	1200 g.	10·0
6. ix. 68	1500 g.	7·2
11. ix. 68	1225 g.	8·7

Table 2. *Diffusional water flux deduced from the THO turnover experiments*

External medium	Genus	*n*	λ (%) hr.$^{-1}$	Unidirectional flux in ml. hr.$^{-1}$ (100 g.)$^{-1}$	Unidirectional flux in *μl.* hr.$^{-1}$ cm.$^{-2}$
F.W.	*Carassius*	8	74·0 ± 7·0	48·6 ± 4·7	121
F.W.	*Anguilla*	8	42·4 ± 4·7	27·3 ± 3·0	91
F.W.	*Platichthys*	4	31·1 ± 2·0	19·5 ± 1·3	97
S.W.	*Platichthys*	6	19·8 ± 1·6**	12·3 ± 1·0**	61
S.W.	*Anguilla*	8	9·3 ± 2·4*	19·1 ± 1·5*	64
S.W.	*Serranus*	6	20·7 ± 1·6	13·3 ± 1·0	33

The means ± s.e. are given; *n*, the number of measurements; λ is the sum of the external and internal fraction renewed per hr. (see techniques). The outflux is given relative to the body weight or the gill surface area. Statistical analysis of the comparison: F.W. and S.W. for the euryhaline fishes ** $P < 0.01$ and * $P < 0.05$. External medium: F.W. freshwater; S.W. sea water.

Finally the two cardiac output values given by the electromagnetic flowmeter techniques are similar to those calculated by the dorso-ventral clearance technique assuming that all the THO outflux occurs through the gill. The conclusion that the

skin plays a minor role in the diffusional flow of water seems justified. A better quantitative evaluation of this role is expected from experiments in which both methods are used simultaneously on the same fish.

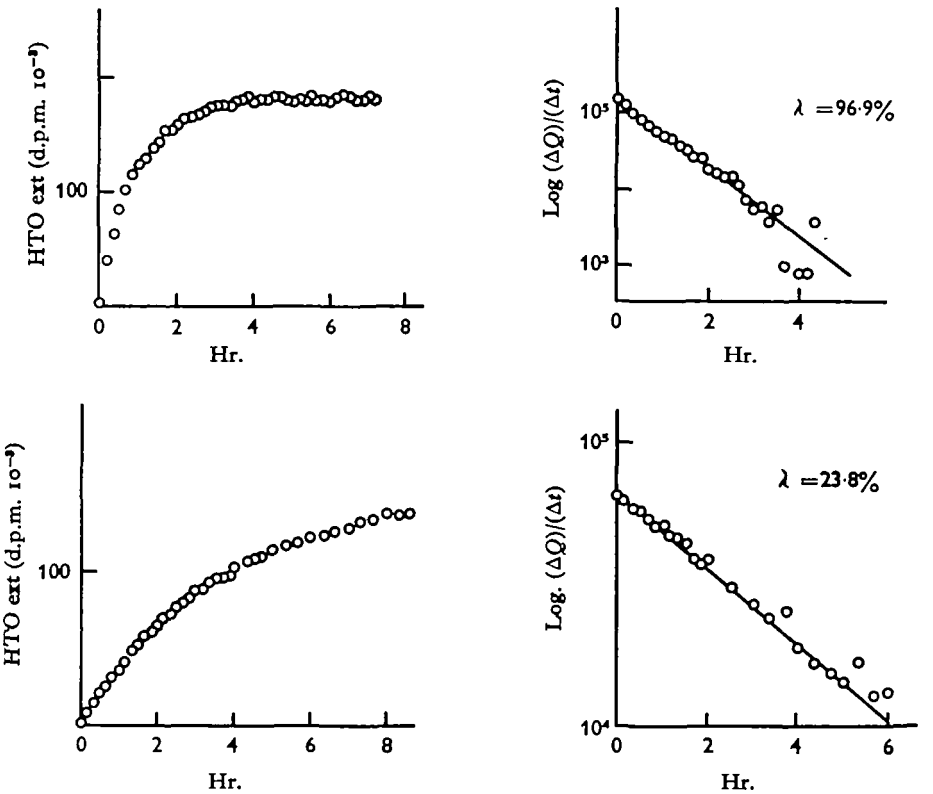

Fig. 2. Comparison of the THO appearance curve in the external medium for the goldfish (top) and the seawater perch (bottom). On the right-hand side the mathematical analysis of the exponential function giving λ (the slope) proportional to the turnover rate. Note high turnover rate for *Carassius* and low rate for *Serranus* (different logarithmic scales).

(2) *Diffusional water permeabilities in fresh-water, sea-water and euryhaline fishes*

Figures 2, 3 and 4 illustrate the results of several turnover experiments with, on the left-hand side, the actual THO appearance curve as a function of time. The right-hand side shows the mathematical analysis used to determine λ, the fraction renewed per hr. Figure 2 emphasizes clearly the rapid turnover observed for the goldfish, in contrast to the slow turnover found for the marine perch. Figure 3 illustrates one experiment in which the THO turnover of the fresh-water eel was calculated simultaneously by two different methods, either by the external cumulative THO appearance curve (see below) or by the disappearance curve of the internal THO as a function of time. The successive THO values measured in the urine samples were taken to represent the progress of the THO concentration of the internal medium. Figure 4 illustrates the differences in THO turnover found when the fresh-water and sea-water flounders are compared, being much faster in the former than the latter. Table 2 summarizes the

data collected in this study. The difference between the goldfish and *Serranus* is highly significant ($P < 0{\cdot}001$). The THO turnover and unidirectional outflux are also definitely faster in fresh-water-adapted than in sea-water-adapted euryhaline fish, especially in the flounder.

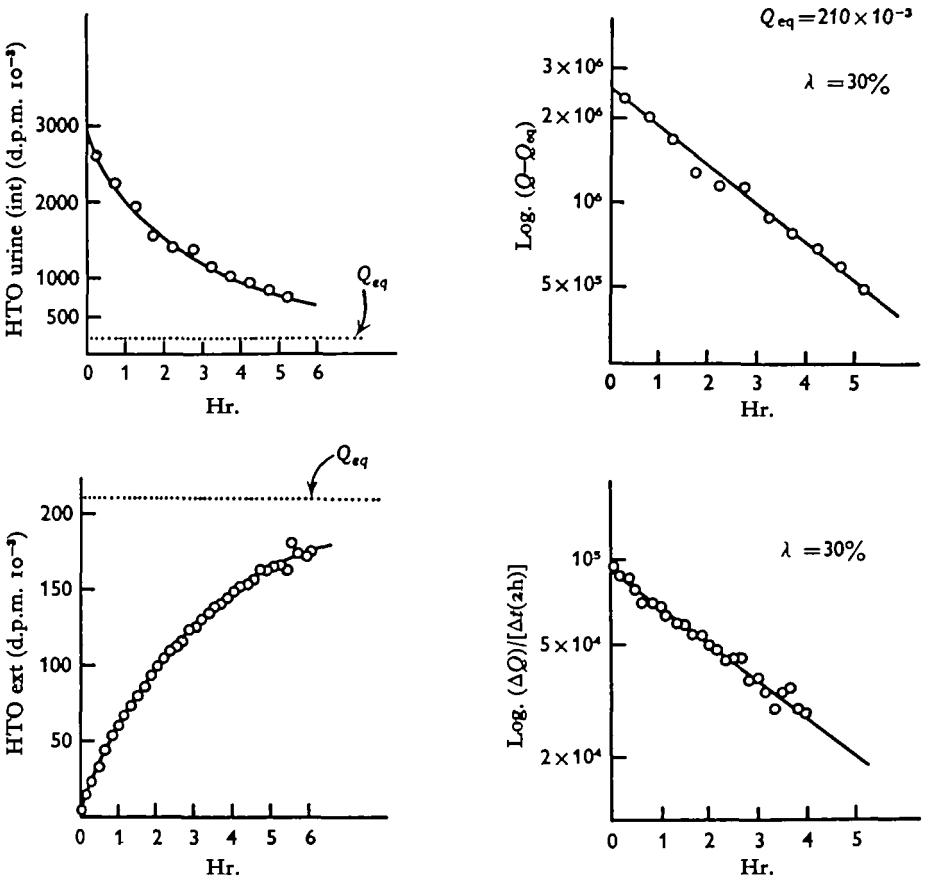

Fig. 3. Comparison of the THO appearance curve in the external medium (bottom) and of the THO disappearance curve in the internal medium (urine; top). The analysis of the curves yield identical turnover rates. Freshwater eel.

Osmotic permeability studies

Table 3 collects the values for the drinking rates, urine flows and net osmotic water flow. The net flow is positive in the fresh-water fishes and negative in the sea-water fishes. The mean osmotic gradients are also given. It may be seen that the absolute value of the osmotic flow is much higher in the fresh-water-adapted fishes even though the osmotic gradient is much smaller. This suggests a higher osmotic permeability for the fresh-water fish gill.

The drinking rate found for the goldfish is very similar to that reported by Lahlou *et al.* (1969). That measured for the flounder is much less than originally given by Motais & Maetz (1965) or by Hickman (1968). It is somewhat higher than the value reported by Evans (1969) for *Platichthys platessa* at 10° C. The urine flows given for

Carassius are very high but comparable to those reported by Maetz *et al.* (1964) or Lahlou & Sawyer (1969*c*). The values reported here for *Anguilla* are higher than those given by Sharratt, Chester Jones & Bellamy (1964). It should be noted that the urine flows are given for animals placed in an experimental tank for 6 hr. without previous adaptation. During the course of this investigation it was observed for the fresh-water eel that the urine flow measured over a 24 hr. period is only 353 μl. hr.$^{-1}$(100 g.)$^{-1}$ $\pm$ 43 (n = 5), a value identical to that given by Sharratt *et al.* (1964) and significantly ($P < 0{\cdot}01$) less than the mean flow reported here. The 'shock effect' has been reported to induce diuresis (see Fleming & Stanley, 1965).

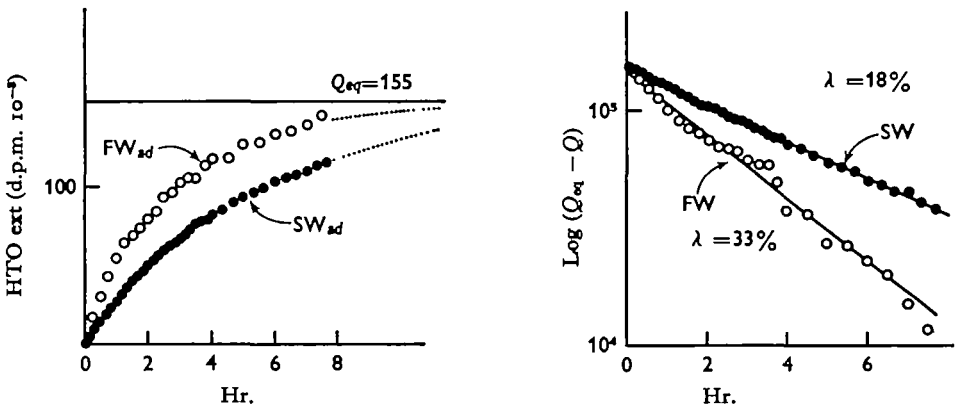

Fig. 4. Comparison of the external THO appearance curves of flounders adapted either to fresh water (FW_{ad}) or to sea water (SW_{ad}). The body weights (and internal water space), external volume and injected radioactivity were identical. The equilibrium value Q_{eq} for the external THO concentration is therefore identical for both fishes. This equilibrium is reached much faster for the fresh-water-adapted fish.

Table 3. *Osmotic water flux as deduced from drinking rate and urine flow*

External medium	Genus	Drinking rate	Urine flow	Net flux	Osmotic gradient
F.W.	*Carassius*	51 $\pm$ 28 (5)$^{\beta}$	1445 $\pm$ 132 (8)	+1394	+250
F.W.	*Anguilla*	135 $\pm$ 28 (17)$^{\alpha\bullet\bullet\bullet}$	538 $\pm$ 32 (6)	+403	+255
F.W.	*Platichthys*	37 $\pm$ 11 (9)$^{\alpha\beta}$	287 $\pm$ 27 (27)$^{\bullet\bullet\bullet}$	+250	+265
S.W.	*Platichthys*	192 $\pm$ 35 (11)$^{\alpha\beta}$	47 $\pm$ 9 (5)$^{\bullet\bullet\bullet}$	−145	−825
S.W.	*Anguilla*	325 $\pm$ 34 (12)$^{\bullet\bullet\alpha}$	31 $\pm$ 9 (5)	−294	−815
S.W.	*Serranus*	277 $\pm$ 35 (6)$^{\alpha}$	70$^{\bullet}$ (2)	−207	−820

The means $\pm$s.e. are given; in parentheses, the number of measurements. Drinking rate and urine flow in μl. hr.$^{-1}$(100 g.)$^{-1}$. Osmotic gradient in m-osmoles.kg.$^{-1}$. External medium: 10 m-osmoles and 1150 m-osmoles: $^{\alpha}$ measured with ^{131}I phenol red; $^{\beta}$ measured with colloidal ^{198}Au; $^{\bullet}$ according to Lahlou (1967); $^{\bullet\bullet}$ according to Maetz & Skadhauge (1968); $^{\bullet\bullet\bullet}$ according to Motais (1967).

(4) *Comparison of diffusional and osmotic water permeabilities*

It is interesting to note that the net (osmotic) water flow across the gills represents 1–3% of the unidirectional fluxes of water. Accordingly, the urine flow in the fresh-water fish and the drinking rate in the sea-water fish represent a negligible fraction of the diffusional water outflux or influx respectively. To compare both permeabilities it is, however, necessary to take into account on the one hand the huge water concentration involved in the diffusion flux (more than 55 moles), and on the other hand, the

small osmotic pressure difference related to the water concentration gradient across the membrane (a fraction of a mole), which is involved in the osmotic net flux of water. The corresponding permeability coefficients P_{os} and P_d will be discussed below.

DISCUSSION

(1) *Validity of the diffusional and osmotic flow values obtained in the present investigation*

Before considering diffusional and osmotic permeability properties of teleostean gills in relation to life in fresh water and sea water, we must discuss the validity of our data obtained *in vivo*.

Our 'dorso-ventral clearance' studies show that the branchial epithelium is the major if not the sole site for tritiated water efflux. The diffusional flux as deduced from our THO turnover experiments for the eel corresponds more or less to the product of the gill blood flow and the fraction of the water cleared through the branchial epithelium. We made the same assumption for the other species. Furthermore, we have admitted that the net osmotic flow calculated indirectly from drinking rates and urine flows is also to be ascribed to the gills. If the skin were of importance the values reported here for the osmotic permeability of the gill would be an over-estimate.

As discussed by many authors, notably by Koefoed-Johnsen & Ussing (1953) and Kedem & Katchalsky (1958), tritiated water fluxes should be measured in the absence of an osmotic gradient because of the interaction of bulk flow of water with the diffusion flux of labelled water. This condition is not met in our experiments. It follows that our tritiated water outfluxes are probably underestimates in the fresh-water fishes and overestimates in the sea-water fishes. The observation that the diffusional permeabilities are found to be higher in the fresh-water living species in accordance with the observations of Potts *et al.* (1967) and Evans (1969) is therefore strengthened by this consideration.

We have measured the osmotic flow across the gills by taking into account urine flow and drinking rate. We have seen that the drinking rate cannot be neglected in the fresh-water animals. Our present data concerning the goldfish and the flounder, together with those reported for the eel by Maetz & Skadhauge (1968), confirm earlier reports by Evans (1967*a*), Potts & Evans (1967), Potts *et al.* (1967) and Evans (1969) for other fresh-water-living stenohaline and euryhaline species. Our values for the goldfish are also very similar to that given by Lahlou & Sawyer (1969*a*). On the other hand, Oide & Utida (1968) did not find any drinking in the Japanese fresh-water-adapted eel. Because of the important osmotic gradient between fresh water and the internal milieu, we admitted that all the water swallowed by the fresh-water fish is absorbed by the gut. A similar assumption has been made in our calculation of the net rate of water loss of the sea-water-adapted species, which is admitted as being the difference between drinking rate and urine flow. Only part of the water swallowed by the fish is absorbed according to Smith (1930), and this has been confirmed for the eel, the trout and two other marine fishes by Oide & Utida (1968). About 20–40% of the water swallowed is lost by the anal route. This has been directly confirmed by Hickman (1968) for the flounder by the use of an anal indwelling catheter. The opening of the anal sphincter may however prevent complete absorption by the hind gut and

overestimates of the rectal water loss may be expected. Nevertheless, to admit complete fluid absorption as we have done in the present calculations probably leads to an overestimation of the water loss through the gills.

Finally, fishes with differing body weights have been compared in the present study. The maximal weight ratios is about 2·5 for the turnover experiments and 10 when the dorsoventral clearance experiments are included. According to Potts *et al.* (1967) and Evans (1969)

$$f_{out} = aw^x,$$

where f_{out} is the unidirectional flux in ml. (fish)$^{-1}$ hr.$^{-1}$, a is a constant, w the body weight and x varies from 0·81 to 0·94 for different species.

Similarly, the tritiated water flux has been found to be temperature-dependent by Evans (1969), the temperature coefficient (Q_{10}) being about 1·90. Our studies were made at 19° C. for the goldfish and eel against 16° C. for the flounder and *Serranus*.

Table 4. (1) *Comparison of the permeability coefficients of the gills of sea-water and fresh-water fishes assuming* $\sigma = 1$

External medium	Genus	P_{os}	P_{dif}	P_{os}/P_{dif}
		(in cm. sec.$^{-1} \times 10^4$)		
F.W.	*Carassius*	2·08	0·34	6·12
F.W.	*Anguilla*	0·79	0·25	3·16
F.W.	*Platichthys*	0·70	0·27	2·59
S.W.	*Platichthys*	0·14	0·17	0·83
S.W.	*Anguilla*	0·19	0·18	1·05
S.W.	*Serranus*	0·10	0·10	1·0

(2) *Comparison with the permeability coefficients of amphibian epithelia*

				References
Xenopus laevis skin	2·80	0·30	9·3	Maetz (1968)
Rana esculenta skin	5·60	0·55	10·1	Maetz (1968)
Rana esculenta bladder	7·50	1·0	7·5	Maetz (1968)
Rana temporaria skin	3·30	0·65	5·1	Dainty & House (1966*b*)
Bufo regularis skin	15·5	0·57	27·2	Maetz (1968)
Bufo bufo skin	23·6	1·48	16·0	Koefoed-Johnsen & Ussing (1953)
Bufo marinus bladder	6·90	0·95	7·3	Hays & Leaf (1962)

(2) *Diffusional and osmotic permeabilities of the freshwater teleosts. Comparison with Amphibia*

(*a*) *Diffusional flow*

Using the gill area evaluation, it is possible to compare the values of the diffusional permeabilities per unit surface for the various teleosts studied here and for other aquatic vertebrates. The average fluxes are summarized in Table 2. All three fresh-water-adapted fishes have a tritiated water efflux of the order of 100 μl hr.$^{-1}$ cm.$^{-2}$. These values compare well with that reported by Maetz (1968) for the aquatic living *Xenopus laevis*, which has been found to possess the lowest diffusional permeability of the anurans studied so far (see Table 4). Some fresh-water fishes have gills even less permeable than reported here, e.g. *Xiphister atropurpureus* (Evans, 1967*b*) and the aglomerular toadfish *Opsanus tau* (Lahlou & Sawyer, 1969*b*), which exhibits the

lowest recorded permeability (50 μl. hr.$^{-1}$ cm.$^{-2}$)* in a fresh-water animal. As Lahlou and Sawyer point out, this extremely low permeability is probably an adaptive feature permitting the survival of this aglomerular fish in a hypotonic medium.

(*b*) *Osmotic flow*

Most of the studies of the osmotic permeability of amphibian skin have been made *in vitro* with the use of impermeant solutes such as sucrose or mannitol (see Dainty & House, 1966*b*). For frogs and toads *in vivo* placed in fresh water, the net osmotic flow of water observed for the osmotic gradient existing between the internal and external media (about 240 m-osmoles) corresponds well with that measured in experiments *in vitro*, if the urine flow is taken as a measure of the rate of water entry through the skin and if the skin surface is taken into account (see Deyrup, 1964; Maetz & Morel, 1965; Maetz, 1968). This shows that salt, which is the major osmolyte which intervenes to set up the osmotic gradient in the natural conditions, may be considered as being an impermeant solute for which the Staverman coffiecient σ is nearly equal to 1. The Staverman coefficient for sodium and chloride ions has been measured by House (1964) on isolated frog skin and was found to be nearly equal to 1. A similar value has been calculated for the toad bladder (Leaf & Hays, 1962).

In all the fresh-water fishes studied so far, the ionic exchange rate of Na and Cl determined with isotopes (Maetz, 1963; Motais & Maetz, 1964; Maetz *et al.* 1964; Motais 1967); is of the same order of magnitude as that found for amphibians studied *in vivo* (see Maetz, 1963), although the mechanisms implicated may be different (Maetz & Garcia Romeu, 1964). The assumption that the Staverman coefficient is also nearly equal to 1 may be justified from the similarity of the ionic exchange rates. In that case, the osmotic gradient given in Table 3 for the fresh-water fishes has to be used together with the gill area to calculate the permeability coefficient P_{os}. This calculation permits the comparison with the values found for amphibian skins (see Dainty & House, 1966*b*; Maetz, 1968). It appears that the osmotic permeability of the gill is definitely smaller than that of the amphibian skin. Table 4 summarizes the relevant data. Therefore not only the diffusional permeability but also the osmotic permeability is indicative of a fundamental impermeability of the teleostean gill to water.

(*c*) *Comparison of diffusional and osmotic permeability coefficients*

If these two coefficients are compared for the same fish, the P_{os}/P_d ratio is found to be greater than one (see Table 4). The discrepancy is however less than that reported for amphibian skin. Several suggestions have been made to account for the fact that P_{os} is always higher than P_d for all the epithelial membranes studied so far. Some authors, i.e. Koefoed-Johnsen & Ussing (1953), suggest the existence of water-filled channels or pores inside the membrane. This hypothesis stems from thermodynamical considerations assuming different laws governing the diffusional flow of water (Fick's law) and the bulk flow of water (Poiseuille's law) across a membrane. In the light of this well-known pore theory, the gill of the fresh-water fish appears to be less porous. Other authors suggest that the discrepancy between the two permeability coefficients

* This value has been recalculated from the turnover rate given by the authors and the gill surface given by Gray (1954).

arises from the possibility that the tritiated water flux is underestimated because of the presence of unstirred layers (Dainty & House, 1966*a*, *b*). It is quite possible that the anatomical arrangement of a gill which is ideally suited for maximal efficiency of gaseous exchange (Hughes, 1966) makes it also one of the best-stirred membrane systems. A third hypothesis has recently been put forward by Hays (1968). His model suggests that the hydraulic flow may be limited by one dense and thin barrier, while the diffusional flow of water may be reduced by a second, thick and porous barrier. The mucous coating of the epithelial membranes may be the barrier in which unstirred layers reduce the tritiated water flux. Such a mucous coating has been observed in electronmicroscopic studies of the gill epithelium, especially in fresh-water-adapted fishes (Philpott & Copeland, 1963; Newstead, 1967).

(3) *Comparison of the diffusional and osmotic permeabilities in the sea-water teleosts*

(*a*) *Diffusional flow*

The diffusional permeability of the gills of the three sea-water-adapted teleosts is even smaller than that reported for the fresh-water animals, being in the range of 30 to 60 μl. (Table 2). Lahlou & Sawyer (1969*b*) report 25 μl. for the sea-water-adapted toadfish. Potts *et al.* (1967) also reported for *Tilapia mossambica* a significant difference between the fresh-water-adapted and sea-water-adapted forms. In this fish the turnover values are much higher than those found for the species studied here but this difference is entirely accounted for by the high (25° C.) temperature at which the experiments were carried out and by the small size of the fish, almost two orders of magnitude lower than those reported here. In some species such as *Xiphister* (Evans, 1967*b*) no difference is found in relation to adaptation to varying external salinities. This is also the case for the few species studied by Vartapetjan (1962), but new experiments are necessary to duplicate his observations with more modern techniques and better precision. In his recent report, Evans (1969) also concluded that fresh-water fishes exhibit a higher diffusional permeability than the marine forms.

(*b*) *Osmotic flow*

The difficulty in taking into account the osmotic gradient as measured by osmometry for calculating the osmotic permeability coefficient P_{os} of the gill epithelium is even greater in the sea-water-adapted fishes. As shown by Mullins (1950), Motais (1961, 1967), Motais, Garcia Romeu & Maetz (1966), Potts & Evans (1967) and Evans (1967*b*), the gill of the sea-water living teleosts is the site of a very high exchange rate of sodium and chloride ions, the unidirectional fluxes being about 10 times higher than the net excretory flux of salt through the gill. About 25–60% of the total body content of salt is exchanged per hr. Obviously if the gill is freely permeable to salt, the osmotic gradient produced by this major osmolyte across the gill may have to be corrected for a reflexion coefficient which is unlikely to be equal to 1. It is, however, impossible to ascertain to what extent this high salt flow is interfering with the water flow. For instance, the carrier-mediated active transport and exchange diffusion components together representing about 90% of the total salt flux across the gill, may not interact with the passive fluxes of solute and solvent. The passive flux component, which in the eel and the flounder represents the remaining 10% of the ionic exchange, on the contrary, may interfere with the water flow, causing the reflexion coefficient to be less

than 1. It is quite possible for instance that the carrier-mediated fluxes may be located in the so-called chloride cells or mitochondria-rich cells suspected by Keys & Willmer (1932) to be the site of the excretion pump of the gill, while the passive flow of solutes may take place through the flat epithelial cells of the gill leaflets which are the site of the gaseous exchange. The passive component may even be more important in the gill of the stenohaline *Serranus*, for which no exchange-diffusion component can be demonstrated (Motais *et al.* 1966). Furthermore, in both *Serranus* and *Anguilla* the possibility of a 'solvent-drag' effect, which is indicative of solute-solvent interaction, has been suggested by Motais *et al.* (1966) and confirmed by Motais (1967). The addition to fresh water of varying amounts of mannitol permitting an experimental change of the osmotic gradient in the absence of external sodium and chloride results in a significant, and in the case of *Serranus* an important, change in the ionic outflux. It is unlikely therefore that $\sigma = 1$ in the gill of the eel or of *Serranus*. The question remains open for the flounder, for which no drag-effect is observed.

(*c*) *Comparison of diffusional and osmotic permeability coefficients*

Assuming nevertheless that $\sigma = 1$ for all three sea-water fishes studied here, a very curious point emerges from our calculations of P_{os} and P_d as seen in Table 4. For all three species the ratio P_{os}/P_d is equal to 1 or nearly so. It is surprising to find no discrepancy between the two permeability coefficients in all three fishes although some show a greater or smaller osmotic component in the ionic fluxes. Lahlou & Sawyer (1969*b*) also give values for the water permeabilities which may be recalculated, and for the condition that the reflexion coefficient is 1 for sodium and chloride, the ratio of P_{os} and P_d is also 1. Potts *et al.* (1967) also suggest concordance of the two permeability coefficients for *Tilapia*. Such an identity has so far been rigorously demonstrated only for the cellular membrane of a marine algae *Valonia* (Gutnecht, 1967, 1968). For this organism both osmotic and diffusional flows occur by the same mechanism, that is by diffusion. This may be due to the absence of water-filled pores in the cellular membrane, which would be rate-limiting for both types of water transfer. Such an assumption seems difficult to accept for the complicated gill epithelium as visualized by electronmicroscopic observation (Philpott, 1965; Newstead, 1967). Such a simple membrane would, however, explain the extremely low osmotic and diffusional permeabilities encountered in this membrane, which is 10 times less permeable than the *Valonia* cell wall, 100 times less permeable than the amphibian skin and 1000 times less permeable than the human erythrocyte (Sidel & Solomon, 1957).

Let us now reconsider briefly the hypothesis of $\sigma < 1$, which would mean that $P_{os} > P_d$. This would suggest that indeed the gill epithelium of the sea-water-adapted teleost, like that of the fresh-water teleost, is porous and that the net flow of water is reduced because of the leakiness of the gill to sodium and chloride.

In conclusion, experiments using techniques for the perfusion of the isolated gill are badly needed in order to permit the choice between these two opposite hypotheses.

(4) *Evolution of the permeability of the gill during transfer and adaptation from fresh water to sea*

Comparison of the reversed osmotic flows when gills of euryhaline fishes are compared in fresh water and in sea water suggest that the mucosal to serosal permeability

is higher than that in the opposite direction. Such a difference in permeability with reversed flows was first described by Bentley (1964) in the toad bladder and called rectification of flow. In the same paper the author suggests that this phenomenon may be of importance for the gill of the euryhaline fishes.

Theoretical studies by Patlak, Goldstein & Hoffman (1963), utilizing the methods of irreversible thermodynamics for the steady-state flow of solvent and solute across simple non-homogenous and structurally non-symmetrical membrane system, show that a solvent flow non-linearly related to the osmotic gradient should be expected in such a system. Diamond (1966) proposes an alternative explanation, suggesting that the cell membrane behaves as an osmometer, shrinking in concentrated solutions of impermeant solute and therefore increasing membrane resistance to water flow.

It must be emphasized, however, that in our experiments the measurements of the osmotic water flow across the gill were made in long-term adapted fishes and it is possible that the observed differences of the permeabilities found in sea water (serosal to mucosal) and in fresh water (mucosal to serosal) are the result of slow adaptive mechanisms modifying the epithelial permeability. A recent kinetic study concerning changes in intestinal absorption and renal excretion of water together with changes of body weight and water content in relation to the transfer of the Japanese eel from fresh water to sea water has shown that, during the first day after transfer, the rate of water loss through the gills is two to three times higher than after 3 days of adaptation (Oide & Utida, 1968). This strongly suggests adaptive changes in gill permeability. Additional experiments concerning weight changes of excised gills in sea water also show that the gills taken from a fresh-water-adapted trout are much more permeable than those taken from a sea-water-adapted animal. Adaptive changes of the osmotic permeability of the gut in the eel have been described by Skadhauge & Maetz (1967). One of the striking adaptive features observed in relation to salinity changes in the gill is its progressive modification of the permeability to Na and Cl ions discovered by Motais *et al.* (1966). It is quite possible that the resulting increase in the leakiness of the gill and the consequent reduction of the reflexion coefficient is the *primum movens* of the simultaneous reduction of water permeability.

An alternative explanation, if the reflexion coefficient remains close to 1 for the gills of both sea-water-adapted and fresh-water-adapted fishes, would be that the adaptive mechanism underlying the reduction of permeability to water during adaptation to higher salinity corresponds to the disappearance of the water-filled channels which are replaced by molecular pores. If the complex membrane hypothesis as suggested by Hays (1968) is to be taken into consideration, then we have to envisage the replacement of a dual membrane system by a unique barrier which is rate-limiting for both types of water transfer and across which water moves by diffusion only.

SUMMARY

1. Cannulation of afferent and efferent branchial vessels in the eel permitted studies of tritiated water clearance. It was observed that most of the diffusional water flow occurs through the gills.

2. Diffusional and osmotic water flows have been measured in a fresh-water (*Carassius*), a marine (*Serranus*) stenohaline fish and in two euryhaline species

(*Platichthys* and *Anguilla*) adapted to either fresh water or sea water, and are found to be lower than in any comparable epithelia so far studied.

3. The diffusional water flow deduced from THO turnover is significantly smaller in the sea-water fish.

4. The osmotic water flow, determined indirectly by measuring drinking rate and urine flow, is smaller in the sea-water fishes despite a greater osmotic gradient across the gills.

5. Attempts to compare diffusional and osmotic permeabilities for the gill are hindered by our ignorance of the extent of solute (salt)-solvent interaction in the epithelium. It is suggested that the gill of the fresh-water-adapted fishes is semi-permeable, while that of the sea-water teleosts may not be, because of the very high ionic exchange across the gill.

6. The surprisingly low diffusional and osmotic permeabilities of the gill epithelium in sea-water fish may be possibly related to the absence of water-filled pores.

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