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WATER EXCHANGE ACROSS FISH GILLS: THE SIGNIFICANCE OF TRITIATED-WATER FLUX MEASUREMENTS

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SUMMARY

1. Branchial turnover rates of tritiated water in the cyprinid teleost *Carassius auratus* are temperature dependent and show temperature acclimation with time.

2. Hypoxia produced abrupt increases in the rates of branchial water turnover.

3. The evidence suggests the importance of branchial blood flow parameters and ventilation on turnover rate. Membrane resistance is not a major barrier to water diffusion in these small fish.

4. Calculated apparent diffusional water permeabilities do not represent true branchial membrane permeabilities.

INTRODUCTION

The branchial epithelium of teleost fishes is admirably suited for respiratory gas exchange (namely large surface area, short diffusion distance and countercurrent flow). These same factors also enhance the diffusional exchange of water. Historically, estimates of branchial water permeabilities have been made using two different approaches. The first of these is based on a balance sheet of water fluxes. Water fluxes in the teleost fish may be summarized in the following manner:

$$\text{net balance} = \text{drinking} \pm \text{branchial and cutaneous net fluxes} - \text{urine flow} + \text{preformed water in food} + \text{metabolic water} - \text{faecal loss.} \quad (1)$$

Since feeding can be halted during experiments, contributions from faecal losses may be consequently reduced and those of preformed water excluded. In short-term experiments, especially those involving large water fluxes following salinity stress, contributions from metabolic water production would be small. Cutaneous fluxes may be ignored due to the relatively much larger surface area of the gill epithelium. Assuming the permeabilities, P, of the skin and branchial membranes are similar, the

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small relative area of the skin (ca. 2%, Parry, 1966) would tend to minimize the cutaneous contribution:

$$\text{Area}_{\text{gill}} . P_{\text{gill}} \gg \text{Area}_{\text{skin}} . P_{\text{skin}}. \tag{2}$$

The presence of scales and adjacent skin mucus would also act as obstructions to cutaneous fluxes. The resulting simplified equation contains those fluxes commonly measured in assessing net water fluxes (see, for example, Evans, 1969; Motais *et al.* 1969):

$$\text{net balance} = \text{drinking} \pm \text{branchial net flux} - \text{urine flow}. \tag{3}$$

In short-term experiments, weight loss due to use of metabolic stores can be neglected and any weight change equated with net water balance. Net branchial water flux, then, may be determined indirectly as:

$$\text{branchial net flux} = \text{weight change} - \text{drinking} + \text{urine flow}. \tag{4}$$

From the calculated branchial net flux, an estimate of the branchial surface area and the concentration gradient, an osmotic permeability coefficient (P_{os}) may be calculated:

$$P_{os} = \frac{\mathcal{J}_{\text{net}}(H_2O)}{A . \sigma \Delta C_s}, \tag{5}$$

where $\mathcal{J}_{\text{net}}(H_2O)$ is the branchial net water flux (mole.s^{-1}), A the branchial surface area (cm^2), σ the reflexion coefficient and ΔC_s the solute concentration gradient (mole.cm^{-3}).

Branchial water permeability may be estimated directly by measuring the flux rate of tritiated water across the branchial epithelium. The measured unidirectional flux may be determined as either the influx rate (appearance in fish) or efflux rate (appearance in external medium). The diffusional permeability coefficient (P_d) is calculated as:

$$P_d = \frac{\mathcal{J}_{\text{uni}}(H_2O)}{A . C_w}, \tag{6}$$

where $\mathcal{J}_{\text{uni}}(H_2O)$ is the unidirectional water flux (mole.s^{-1}), A the branchial surface area (cm^2) and C_w the water concentration in labelled solution (mole.cm^{-3}).

Comparisons of these two estimates (expressed as the ratio P_{os}/P_d) produce ratios typically close to 1 for teleosts in sea water and very much greater than 1 for teleosts in fresh water (Evans, 1969; Motais *et al.* 1969; Potts *et al.* 1967). Explanations of these 'isotope effects' in fresh water include the existence of unstirred layers in the fluid phases adjacent to the branchial membranes and the presence of pores allowing bulk inward flow retarding tracer efflux.

An unstated assumption made by some workers (Motais *et al.* 1969; Motais & Isaia, 1972; Isaia, 1972) is that changes in the apparent diffusional water permeability measured using tritiated water represent changes in the true branchial membrane permeability. This may not be a valid assumption for two reasons. First, the surface area of the branchial epithelium available for exchange may change under various circumstances. Although qualitative measurements may be made of the relative degree of perfusion of branchial secondary lamellae and central channels (vascular shunts not in close contact with the respiratory surfaces; Steen & Kruysse, 1964), no quant

tatively accurate measurements are available. Secondly, blood flow rates through the gills and/or water flow (ventilation) rates across the gills may change. This will have a small effect if the branchial membrane resistance to water diffusion is high and a substantial effect if membrane resistance is low. Typically, measured apparent water permeabilities are high and comparable with those of unstirred layer-limited systems (House, 1974). In such an unstirred layer-limited system, the membranes appear to offer negligibly more resistance to diffusion in comparison with the resistance of the bathing media. In light of these two factors, it may be more realistic to consider perfusion patterns or the existence of unstirred layers as the predominant factors limiting measured water fluxes.

The purpose of the experiments reported here was to examine aspects of water exchange across fish gills and to consider the features limiting the diffusion of water. Tritiated-water effluxes were measured in goldfish at different temperatures and under conditions of hypoxia.

METHODS AND MATERIALS

Animals

Goldfish (*Carassius auratus*, 1–4 g, of both sexes) were obtained from tropical fish stores in Los Angeles County and were kept in freshwater aquaria at 19 ± 1 °C under natural photoperiods unless specified otherwise. Fish were fed ground trout chow daily. Experiments were performed in December 1975 to March 1976.

Flux measurements

Unidirectional tritiated-water effluxes were measured on weighed fish following intraperitoneal injections of approximately 30 μCi [^{3}H]water (ICN, Irvine, California) in 3 μl 0·9% NaCl solution. Injected fish were placed in a chamber containing 100 ml of continuously aerated water for 30 min to provide adequate time for the tracer to distribute throughout the body water. The fish were then removed from the water, gently rinsed and placed singly into test chambers consisting of 250 ml beakers, containing 100 ml water. The test chambers were supplied with air bubblers to aerate the water and provide mixing of the external medium. Aliquots (100 μl) of the external medium were taken from the test chambers at regular intervals and pipetted into 10 ml of liquid scintillation fluid containing 80% (v) toluene, 20% (v) Triton X-100, 3 g.l^{-1} PPO and 0·3 g.l^{-1} dimethyl POPOP. All samples were counted in a Beckman Liquid Scintillation Counter (LS-230). Since all samples were quenched to the same extent, no correction was made. Equilibration of tracer activities between the fish and the external medium occurred within 4–5 h after placement of fish into the test chambers.

A typical record of the washout of [^{3}H]water is shown in Fig 1. The kinetics of this two-compartment exchange have been described by Motais (1967), whose formulation will be followed here. The shape of the curve can be described by the following equation:

$$Q = Q_{eq}(1 - e^{-\lambda t}), \tag{7}$$

where Q is the counts per minute (cpm) in aliquot at time t, Q_{eq} the cpm in aliquot at equilibrium, λ the turnover rate (h^{-1}) and t time (h).

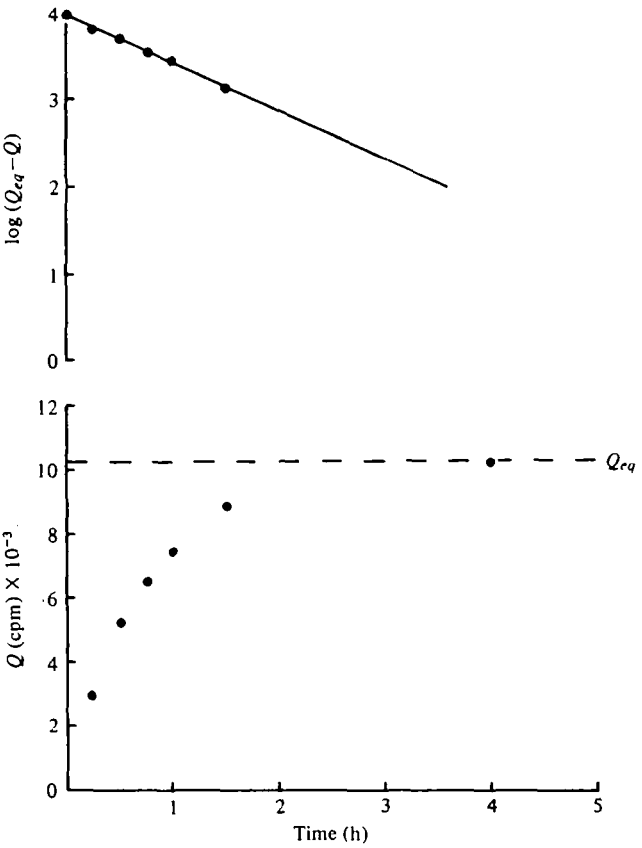

Fig. 1. Tritiated-water washout curve and log-transformed data for a 2·2 g goldfish acclimated to 19 °C and transferred acutely to an external bath of 100 ml water at 15 °C. Equation for least squares regression line of log-transformed data: log $(Q_{eq}-Q) = -0{\cdot}54\,t+3{\cdot}99$ ($r = -1{\cdot}00$).

The turnover rate, λ, may be calculated from the slope of the graph of the log-transformed data (Fig. 1):

$$\log (Q_{eq}-Q) = \log Q_{eq} - \lambda t \log e. \tag{8}$$

The slope, $\lambda \log e$, was obtained from standard least squares linear regression of the data. The unidirectional efflux of water was calculated as:

$$f_{out} = \frac{V_1 V_2 (100\text{ g})\lambda}{(V_1+V_2)\,\text{wt}}\,0{\cdot}01543, \tag{9}$$

where V_1 is the water space inside fish (assumed to be 70% body weight; ml), V_2 the volume of external bath (ml), wt the weight of fish (g) and 0·01543 the conversion factor from ml.h^{-1} (100 g fish)$^{-1}$ to mmol.s^{-1} (100 g fish)$^{-1}$.

It is assumed that all the water within the fish is exchanging as a single pool, i.e. internal water compartments are exchanging much more rapidly with the plasma than the plasma is with the external medium. The calculation of exclusively linear log-transformed data supports this notion. With an estimate of the surface area

available for diffusion and the water concentration inside the fish, an apparent diffusional water permeability was calculated:

$$\text{appar } P_d = \frac{f_{\text{out}}}{A.C_{\text{in}}}, \tag{10}$$

where A is the branchial surface area [cm^2 (100 g fish)$^{-1}$] and C_{in} the water concentration inside fish (mmol.cm^{-3}).

For all calculations, surface area was assumed constant at 400 cm^2 (100 g fish)$^{-1}$ (as per Isaia, 1972) and C_{in} was 55·3 mmol.cm^{-3}. Appar P_d is expressed as cm.s^{-1}.

Acute temperature change

Goldfish were acclimated to 19 ± 1 °C for at least 2 weeks prior to use. Fish were injected with tracer as described and at the end of the 30 min tracer distribution period at 19 °C were transferred to a test chamber at one of five different temperatures: 8·5, 15, 19, 25 or 30 °C, maintained by means of temperature-controlled water baths.

Temperature acclimation

Groups of goldfish were acclimated to one of three temperatures (8·5, 19 or 25 °C) for at least 2 weeks in small aquaria whose temperatures were regulated by temperature-controlled water baths. Injection of tracer was performed as described and both the tracer distribution and test periods were spent in temperature-controlled chambers.

E_a and Q_{10} values

Calculation of activation energies (E_a) for these temperature effects were made through the use of the Arrhenius Equation:

$$\ln(\text{appar } P_d) = \ln A - \frac{E_a}{RT}, \tag{11}$$

where A is the frequency factor (constant), E_a the apparent activation energy of the process (cal.mole^{-1}), R the gas constant (1·987 cal.mole^{-1} °K^{-1}) and T the temperature (°K).

E_a was determined from the slope of the graph of ln (appar P_d) versus T^{-1} where the slope equals E_a/R. The Q_{10} for the process was calculated from the activation energy:

$$Q_{10} = \exp(0{\cdot}056\, E_a). \tag{12}$$

Hypoxia

Prior to experiments on the effects of hypoxia, the extent to which the oxygen content of the water could be acutely lowered without affecting the activity patterns of the fish was determined. To maintain normoxic conditions, test chambers were bubbled with room air. Fish experiencing normoxic oxygen concentrations remained still in the test chambers and did not appear excited. Hypoxia was produced in the test chambers by altering the composition of the gas used to aerate and stir the chambers. A pair of flowmeters allowed for the mixing of room air and nitrogen gas in known proportions so as to lower the oxygen content of the water in the chambers.

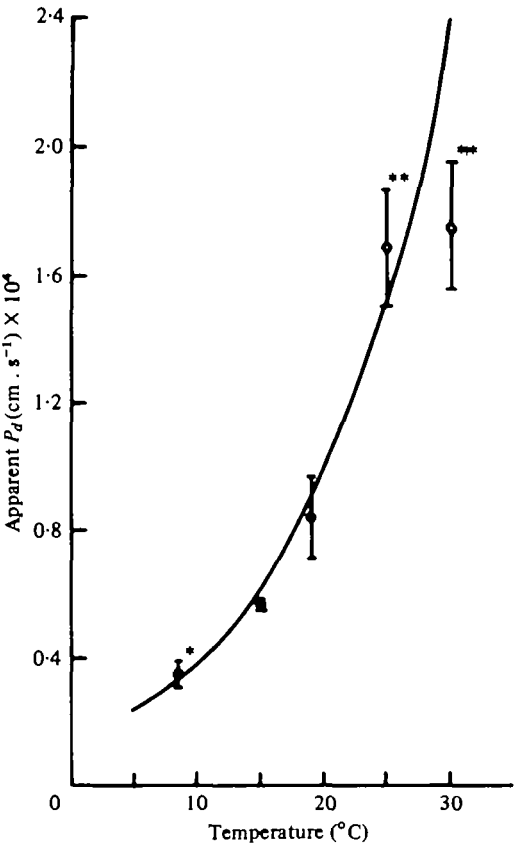

Fig. 2. Relationship of apparent diffusional water permeability coefficient (apparent P_d) of 19 °C acclimated goldfish to acute temperature change. Means ± one standard error of the means of measurements on four animals at each temperature are presented. Line is that derived from the Arrhenius plot in Fig. 3. * $P < 0.05$, ** $P < 0.01$ (compared to 19 °C controls, Dunnett's test).

The oxygen content of the water was simultaneously monitored with a Clark-type oxygen electrode and polarographic oxygen analyser (Instrumentation Laboratories Model 125A). The behaviour and activity of the fish were observed as the oxygen content of the water was lowered in a stepwise manner. The fish showed no behavioural response until the oxygen content reached 40% that of normoxic; the only adjustment observed in fish until this point was an increased ventilation rate. Below this critical oxygen content, fish appeared excited, moved to the surface of the water, and normal ventilation was replaced by 'gulping'. Beamish (1964) found goldfish to regulate oxygen consumption until the environmental oxygen content declined to between 50 and 25% of normal. It was decided to use an oxygen content 50% that of normoxic as the activity and behaviour of fish at this concentration appeared normal, although ventilation rate was increased.

In experiments to examine the effects of acute hypoxia, fish were acclimated to 19 °C and normoxic water. Injections and pretest preparation were as described earlier. The fish were placed in test chambers under normoxic conditions and samples taken as usual for 45 min or 1 h. After these periods, the gas mixture to the test chambers was

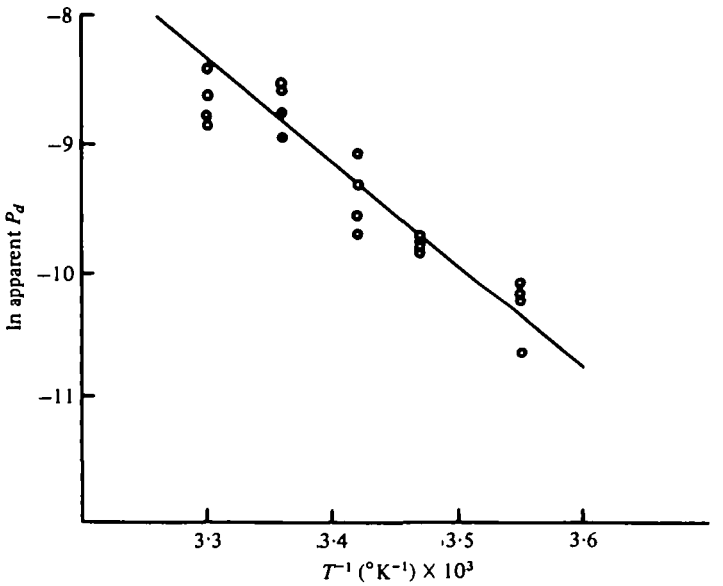

Fig. 3. Arrhenius plot of data in Fig. 2. Line is least squares regression line of data from 8·5 to 25 °C. Equation of line: ln (apparent P_d) = −8155 (°K^{-1}) + 18·59 (r = −0·94).

changed and samples collected during the ensuing periods of hypoxia. In this manner, rates of exchange under normoxic and hypoxic conditions were obtained for each fish.

Statistics

All data are presented as the mean ± one standard error of the mean (number of animals). Statistical comparisons were made by means of Dunnett's test (Winer, 1971), *t*-tests and *t*-tests for paired observations (Dixon & Massey, 1969).

RESULTS

The effect of acute temperature change on the apparent diffusional water permeability is shown in Fig. 2. There is an exponential increase in the apparent permeability coefficient from 8·5 to 25 °C and no change from 25 to 30 °C. The Q_{10} and E_a of the water exchange process were determined from the Arrhenius plot in Fig. 3. The observed acute temperature change effect shows a Q_{10} of 2·48 below 25 °C and 1·07 above 25 °C in animals acclimated to 19 °C. The apparent E_a for the exchange process below 25 °C is 16·2 kcal.mole^{-1}; the values at 30 °C were excluded from the calculation of E_a.

The apparent permeability coefficients for goldfish acclimated to three different temperatures are shown in Fig. 4. The Q_{10} derived from these data is 1·4 and the apparent E_a is 6·2 kcal.mole^{-1} as determined from the Arrhenius plot in Fig. 5. The apparent permeability coefficients for fish acclimated to 8·5 and 25 °C are significantly ($P < 0{\cdot}01$) higher and lower, respectively, than those measured in fish acclimated to 19 °C and acutely transferred to 8·5 and 25 °C.

The washout rate of tritiated water increased 55% during hypoxia (Table 1).

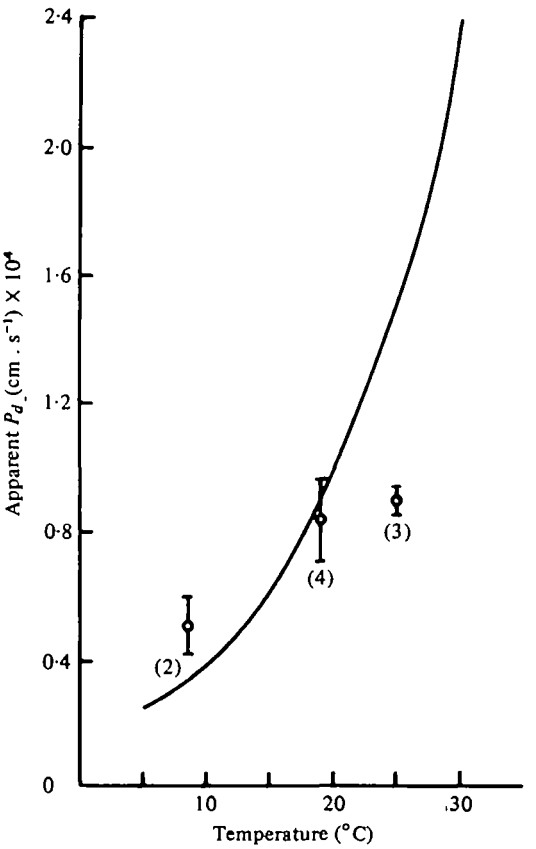

Fig. 4. Relationship of apparent diffusional water permeability coefficient (apparent P_d) of goldfish to temperature of acclimation (no significant differences compared to 19 °C acclimated fish, Dunnett's test). Means ± one standard error of the means (number of animals) are presented. Line is same as that in Fig. 2 for comparison.

DISCUSSION

The utilization of tritiated water as a tracer offers the advantage of being a direct measure of branchial water flux and is not dependent on accurate estimation of drinking rate, urine flow or other water balance components. In addition, the contributions of other water balance components to the measured tracer flux are negligible. The theoretical basis for this can be seen from the relationship between the net and unidirectional tracer fluxes. The net flux across the branchial epithelium is the difference between the two unidirectional fluxes (influx and efflux):

$$J_{net} = P_d A(C_{in} - C_{out}), \tag{13}$$

where C_{in} and C_{out} are the water concentrations in the fish and the external medium, respectively. The branchial net flux for fish in water balance equals predominantly urine flow in freshwater fish and drinking in sea-water fish.

The contributions of either urine flow or drinking on the measured unidirectional tracer fluxes must be small (*ca.* 1%). Three experimental lines of evidence support this contention. Using a Fick analysis of the difference in the specific activities of

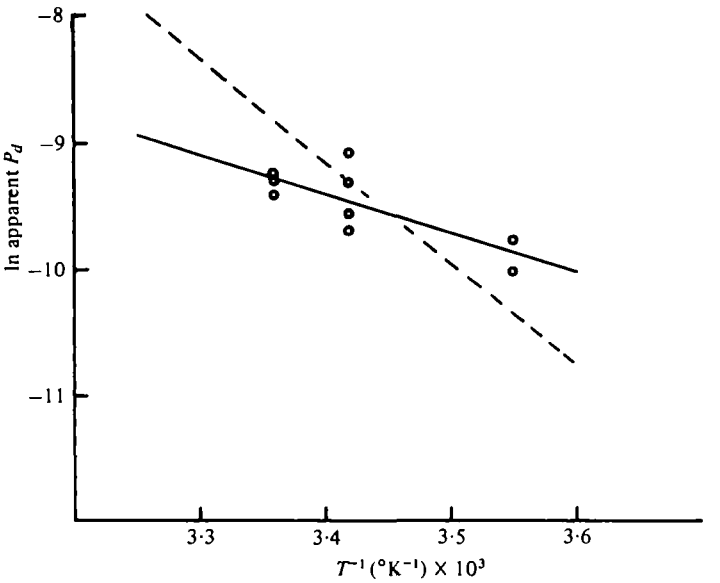

Fig. 5. Arrhenius plot of data in Fig. 4. Solid line is least squares regression line of data. Equation of line: ln (apparent P_d) = -3126 (°K^{-1}) + 1·23 ($r = -0{\cdot}78$). Dashed line is same as that in Fig. 3. The slopes of these two lines are significantly different ($P < 0{\cdot}01$, t-test).

Table 1. *Apparent diffusional water permeability coefficients* (appar P_d) *for goldfish during an initial period of normoxia and a subsequent period of hypoxia*

[Data are expressed as the mean ± one standard error of the mean (number of animals).]

Treatment	appar P_d (cm.s^{-1})
Normoxia	0·90 ± 0·03 (3)
Hypoxia	1·41 ± 0·23 (3)*

* $P < 0{\cdot}05$ (one-tailed t-test for paired observations) compared to normoxic period.

tritiated water in the ventral and dorsal aortae of *Anguilla anguilla* in fresh water, Motais *et al.* (1969) found the loss through the branchial circulation sufficient to account for the appearance rate in the external medium. Data on urine flow rates of *Carassius auratus* (MacKay, 1974) were used to estimate that the contribution of urine flow to the overall appearance rate of tritiated water would be less than 1%. In experiments using [^{14}C]inulin as an extracellular space marker, appearance rate of inulin in the external medium can be used as a gross measure of urine flow. Compared with total body water turnover rates, urine flow contributions in the freshwater-acclimated teleosts *Sarotherodon mossambicus* and *Gillichthys mirabilis* were calculated to be less than 3 and 1%, respectively (Loretz, unpublished data). Since inulin clearance is a commonly used indicator of glomerular filtration rate and the effect of any water reabsorption has been ignored in these calculations, these figures may be slight overestimates.

The measurement of true branchial membrane permeability to water is dependent on two factors. First, the branchial surface area available for exchange must be known.

Since actual branchial surface areas have been estimated for only a limited number of species (Gray, 1954; Hughes, 1966) estimates must be based on existing measurements for similar species or types. Even if reliable anatomical measurements of total branchial surface area are available, the actual surface area participating in water exchange is probably smaller since not all branchial secondary lamellae are perfused simultaneously or equally (Steen & Kruysse, 1964; Richards & Fromm, 1969; Booth, 1978). No direct measurements of total or effective branchial surface areas have been made by other workers investigating water exchange but reliance has been placed instead on estimates based on similarity of species types and activities (Motais *et al.* 1969; Motais & Isaia, 1972; Isaia, 1972). Inasmuch as the measured surface areas of Gray (1954) and Hughes (1966) are total anatomical surface areas, permeability coefficients reported in the literature are probably underestimates. In this study, the branchial surface area of the goldfish was assumed to be 400 $cm^2.(100\ g\ fish)^{-1}$ as this was the value used in Isaia's (1972) study on the goldfish.

The determination of true membrane permeability is also dependent on boundary conditions (i.e. the degree of mixing on either or both sides of the membrane). This is important since the apparent water permeability in goldfish is so high. It is a well-known phenomenon that transepithelial permeabilities are very high for small molecules like water and urea (Bindslev & Wright, 1976). The permeability to water is sufficiently high that in some systems unstirred layers may limit the measurement of true permeability. The presence of unstirred layers increases the diffusion distance of tracer molecules such that the gradient across the epithelium itself is very much less than that for the bulk solutions on either side. An unstirred layer-limited system of this kind would lead to underestimates of true membrane permeability. An estimate of unstirred layer effects can be made using the data of Hughes (1966) on the dimensions of fish gills from a variety of species. Average values for gill dimensions from measurements on a number of species are:

h = height of secondary lamellae	0·46 mm	(1·10),
d = distance between secondary lamellae	0·034 mm	(0·070),
l = length of secondary lamellae	0·75 mm	(1·60).

The maximum dimensions recorded by Hughes are shown in parentheses. Fig. 6 shows the structure of the branchial arch and primary and secondary lamellae and illustrates the dimensions considered. The most important surfaces for water exchange are the secondary lamellae, which are arranged as closely apposed broad plates; this arrangement provides a short diffusion pathway from blood to the external medium.

The possible effects of an unstirred layer can be calculated from the following equation where the membrane and adjacent unstirred layers are treated as resistances in series (House, 1974):

$$\frac{1}{P_{obs}} = \frac{1}{P_{memb}} + \frac{\delta}{D_w}, \tag{14}$$

where P_{obs} is the observed permeability coefficient ($cm.s^{-1}$), P_{memb} the true membrane permeability coefficient ($cm.s^{-1}$), δ the total unstirred layer thickness (cm), D_w the self-diffusion coefficient of [^{3}H]water ($2{\cdot}12 \times 10^{-5}\ cm^2\ s^{-1}$ at 20 °C from Kohn, 1965)

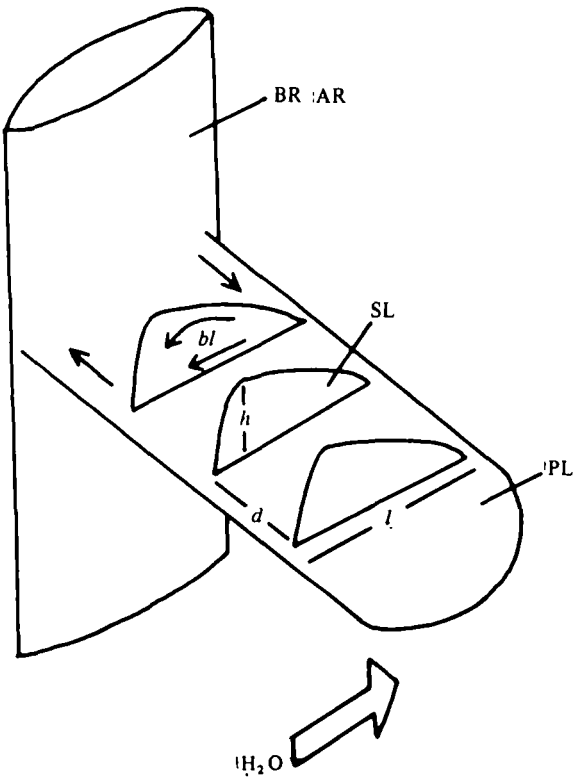

Fig. 6. Simplified diagram of branchial arch (BR AR), primary lamella (PL) and secondary lamellae (SL) of teleost gill. Directions of water (H_2O) and blood (*bl*) flows are indicated. h = height of secondary lamella, l = length of secondary lamella, d = distance between secondary lamellae.

Using the average values from Hughes (1966) for gill dimensions and the apparent permeability coefficient measured at 19 °C in these experiments, an estimate can be made of the unstirred layer effect if there was no stirring of the interlamellar (secondary) spaces and the unstirred layer thickness was one-half of the interlamellar distance. Under these conditions, the measured coefficient of $0{\cdot}84 \times 10^{-4}$ cm.s^{-1} would be an underestimate by 1% of $0{\cdot}85 \times 10^{-4}$ cm.s^{-1}, the true coefficient. If the same calculation is made for the highest observed coefficient and the largest interlamellar distance, the measured coefficient would be a 4% underestimate. A 10% error would require an interlamellar distance of 0·48 mm, ten times the observed average. The effects of unstirred layers within the cytoplasm of the branchial epithelial cells are negligible as the thickness of the respiratory epithelium is but 3–5 μm (Maetz, 1971). It seems clear that unstirred layers would not significantly impair mixing of the interlamellar spaces.

The presence of mucus secretion from branchial mucus cells is apparent in some fish but in no case will it be as thick as the unstirred layers discussed above. In any case it would be maladaptive for the mucous coat to be so large as to impede the diffusion of respiratory gases. The secretion of mucus is greater in some freshwater fishes (Mattheij & Sprangers, 1969) but at the same time water exchange can be much

greater in freshwater-acclimated teleosts than in sea-water-acclimated teleosts (Evans, 1969; Motais *et al.* 1969; Motais & Isaia, 1972; Isaia, 1972; Loretz, 1978). Water will diffuse through the mucus at the same rate as through water or at a lower rate due to obstructional effects by the protein components of the mucus. Since mucus may potentially limit water exchange, the increased exchange in fresh water must result from other factors. The primary function of branchial mucous secretion is probably not osmoregulatory.

There are two possible explanations for the temperature dependence observed in the acute temperature change experiment. First, the system might be membrane-limited below 25 °C and unstirred layer-limited above 25 °C. Second, the branchial membranes might not limit water diffusion and as a result the system would be unstirred layer- or perfusion-limited at all temperatures.

The first explanation is supported by the magnitudes of the calculated activation energies over the temperature ranges 8–25 °C and 25–30 °C. The effects of temperature on water diffusion through phospholipid-cholesterol bilayers have been compared with those expected on theoretical grounds. Price & Thompson (1969) found activation energies between 12·7 and 13·3 kcal.mole^{-1} for lecithin-cholesterol-decane membranes over the range 7–49 °C. Redwood & Haydon (1969) measured an activation energy of 14·6 kcal.mole^{-1} for lecithin-cholesterol-hydrocarbon membranes over the range 20–45 °C. These authors concluded that the high values for the activation energies were consistent with a solution-diffusion mechanism for passage through the hydrocarbon interior of the membrane rather than passage through aqueous (water-filled) pores. The activation energy of 16·2 kcal.mole^{-1} measured in this study would support a solution-diffusion mechanism and is inconsistent with the activation energy expected for an unstirred layer-limited system (4·6 kcal.mole^{-1}). A molecular mechanism and statistical model for this process of diffusion through membranes have been described (Trauble, 1971; Wright & Bindslev, 1976). Temperature dependences (expressed as Q_{10} and E_a) of tritiated-water exchange for other species are listed in Table 2. It should be noted that some of these fish did not experience acute temperature transfers; tritiated-water exchange was measured in some at least several days after transfer. The activation energies are all larger than the activation energy for the self-diffusion of water.

The apparent permeabilities I measured were the same for fish transferred to 25 and 30 °C. This would be expected if the true membrane permeability had increased sufficiently that unstirred layers became the major resistance to diffusion. Since morphological considerations would not support the existence of large unstirred layers between secondary lamellae, the limiting factor is probably perfusion. In a sense, the system is unstirred layer-limited since the interlamellar spaces are not sufficiently irrigated to provide mixing with the remainder of the ventilatory water volume.

The second explanation, perfusion limitation at all temperatures, is supported by the similarity of the measured Q_{10} for water exchange and that typical for metabolic functions in ectotherms, *ca.* 2·5 (Prosser, 1973). Data from Beamish & Mookherjii (1964) indicated a Q_{10} for metabolic rate in the goldfish of *ca.* 2; this may be an underestimate of Q_{10} for acute temperature change since these fish were allowed 2 weeks for acclimation at each test temperature. From this explanation, the observed change

Table 2. *Summary of activation energies (E_a) and temperature coefficients (Q_{10}) for tritiated-water exchange in a number of teleost species*

(Specific information regarding experimental temperature regimes is presented in parentheses.)

Species	E_a (kcal.mole^{-1})	Q_{10}	Temperature range	Reference
Anguilla anguilla				
(SW)	17·7	2·70	5–25 °C	Motais & Isaia (1972)
(FW)	21·5	3·32	5–25 °C	
(acute temperature change)				
Serranus scriba, *Serranus cabrilla* (acute temperature change)	11·3	1·88	10–20 °C	Isaia (1972)
Carassius auratus (acute temperature change)	15·6	2·40	5–25 °C	Isaia (1972)
Carassius auratus (2 weeks at 19 °C, acute temperature change)	16·2	2·48	8·5–30 °C	Present study
Carassius auratus (3 days at each temperature)	13·5	2·14	10–20 °C	Evans (1969)
Phoxinus phoxinus (3 days at each temperature)	10·2	1·77	10–20 °C	Evans (1969)
Platichthys platessa (3 days at each temperature)	10·6	1·81	10–21 °C	Evans (1969)

in exchange rate represent changes in branchial perfusion patterns of fish as a response to abruptly increased metabolic rates at higher temperatures and decreased rates at lower temperatures. Gill blood flow is directly related to oxygen consumption in the carp, *Cyprinus carpio* (Garey, 1970). In the present sense, perfusion includes both the velocity of blood flow through the secondary lamellae and the total surface area available for exchange (i.e. number of perfused lamellae) and the ventilation rate. The calculated activation energy of the process, therefore, is not that of the actual diffusion process. It is merely coincidental that the calculated E_a is similar to that for diffusion through lipid bilayers. The unchanged apparent permeability above 25 °C could here represent the achievement of maximal branchial perfusion.

The apparent permeability is a function of the thermal history of the fish. The observed apparent permeabilities show temperature acclimation with time. The Q_{10} calculated from the data for temperature-acclimated goldfish over the range 8·5–25 °C is 1·40. This pattern of temperature acclimation is similar to the temperature acclimation seen in oxygen consumption in teleost fishes (Bullock, 1955; Prosser, 1973). This phenomenon was also observed by Motais & Isaia (1972) for the European eel, *Anguilla anguilla*. Temperature acclimation is consistent with both explanations. In long-term-acclimated fish, the lipid composition of the branchial membranes may be altered in response to temperature and this would subsequently affect exchange rates if diffusion is membrane limited. At the higher temperatures (25 °C and above), decreased lipid saturation could bring the system into the membrane-limited domain. Roots & Johnston (1968) and Knipprath & Mead (1968) have shown for the goldfish that saturated fatty acids are replaced by more-unsaturated fatty acids at lower temperatures and that unsaturated fatty acid synthesis increases after exposure to low temperature. The relationship between fluidity and saturation of fatty acids might in part be

responsible for the temperature acclimation of water exchange. Any surface area adjustments related to alterations in oxygen demand would also affect water exchange rate in a membrane-limited system.

If membranes do not limit the exchange of water, temperature acclimation of exchange rate may result from alterations in either surface area, blood flow rate or ventilation.

Abrupt increases in branchial exchange at constant temperature occurred during hypoxia. Since a change in membrane composition (fluidity) over such a short time is unlikely, a more probable alternative is that the surface area was increased. Such a change in branchial perfusion pattern would be consistent with the observed short time course. Increased branchial surface area during hypoxia would produce increased exchange rates in both models while increased blood flow rate through a fixed number of lamellae or increased ventilation would only have an effect on exchange described by the perfusion-limited model. Since not all secondary lamellae are perfused simultaneously, lamellar recruitment is a possible and reasonable response to hypoxia (Steen & Kruysse, 1964; Richards & Fromm, 1969; Booth, 1978). Increased lamellar perfusion occurred in the trout, *Salmo gairdneri*, during hypoxia (Holeton & Randall, 1967).

Motais *et al.* (1969) observed tritiated-water clearance fractions up to 30% during passage of the blood through the branchial circulation of *Anguilla anguilla*; larger fish exhibited smaller clearance fractions. If less than the total anatomical surface area was perfused then clearance fractions would be even larger than the calculated values. For small goldfish such as those used in my study, calculations using an estimated cardiac output of 120 $ml.h^{-1}$ (100 g fish)$^{-1}$ (Motais *et al.* 1969; Garey, 1970; Itazawa, 1970; Prosser, 1973) and the measured exchange rates predict a clearance fraction near 100%. Branchial water exchange is size-dependent in a number of species; larger fish exhibit slower exchanges (Evans, 1969). This relates well to the lower clearance fractions in large fish. Whether branchial tritiated-water exchange is operating within the membrane-limited or perfusion-limited domain may depend on the size of the fish. The high clearance fractions of small fish are inconsistent with a membrane-limited exchange. In either case, the potential limitation of tracer exchange by perfusion must be considered.

In conclusion, careful consideration must be taken of the potential limitations before equating the apparent permeability coefficient derived from tracer flux measurements with the true branchial membrane permeability coefficient. Perfusion appears to limit tracer exchange in smaller fish and may be important in larger fish as well. Inaccurate estimation of functional branchial surface area is another limitation to the technique. Although Motais & Isaia (1972) acknowledge that changing surface area is a factor which must be considered, the present model emphasizes that changing surface area and blood and water perfusion patterns can be major factors influencing the observed fluxes. For this reason, apparent diffusional permeability coefficients (P_d) may not be equivalent to permeability coefficients derived from osmotic measurements (P_{os}).

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