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ACTIVE CHLORIDE TRANSPORT BY THE GILLS OF RAINBOW TROUT (*SALMO GAIRDNERI*)

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INTRODUCTION

Active sodium uptake from dilute media has been described extensively in a number of aquatic vertebrates, and we have some understanding of the cellular mechanisms involved (Koefoed-Johnsen & Ussing, 1958; Ussing & Windhager, 1964; Biber, Chez & Curran, 1966). While chloride transport has been neglected by comparison, chloride uptake systems are found in diverse groups of animals and deserve more attention. Krogh (1937) reported that several teleosts were able to absorb Cl^- from dilute media, and he also showed that this could occur without simultaneous transfer of an accompanying cation (Krogh, 1938). More recent work has provided values for Cl^- exchange in a number of fish (Mullins, 1950; Gordon, 1963; Motais, 1967; Potts & Evans, 1967).

But the mechanism of chloride transport in teleosts has received little attention. Because of its independence of simultaneous cation transport, Krogh (1938) suggested that exchange with another anion, perhaps bicarbonate, preserved electrostatic neutrality. Garcia-Romeu & Maetz (1964) confirmed the report that Cl^- uptake was independent of Na^+ transport in the goldfish. They also provided some evidence for a Cl^-/HCO_3^- exchange by showing that injections of $NaHCO_3$ stimulated Cl^- absorption in *Carassius*. Such an exchange would also explain the observation by Dejours (1969) thal CO_2 excretion by *Carassius* decreased temporarily when the animal was placed in a low-Cl^- medium and increased when the Cl^- concentration was raised. We have recently shown that ion transport across the teleost gill could be studied with a small volume system which, in effect, externally isolates the gill (Kerstetter, Kirschner & Rafuse, 1970). In the present work we have used the same system to evaluate the kinetics of Cl^- transfer and to study the characteristics of the transport system in rainbow trout.

METHODS

Rainbow trout (*Salmo gairdneri*), 150–350 g in weight, were obtained from a commercial hatchery in Soap Lake, Washington. They were stored unfed at 6–9 °C in continuously refiltered tap water ($[Na^+]$ = 0·9 mM; $[Cl^-]$ < 0·1 mM). For flux determination individual fish were anaesthetized in 0·1 % tricaine methane sulphate (TMS) and suspended dorsal side down in the holding device described earlier (Kerstetter *et al.* 1970). The gill was irrigated by recycling 100 ml of solution from a reservoir through the mouth and gill cavity. Anaesthesia was maintained by adding urethane

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to the reservoir to a final concentration of 0·1–0·3 %, depending on the requirements of each fish. The temperature of the irrigating medium was 12–13 °C.

Chloride influx was determined by measuring the disappearance of ^{36}Cl from the irrigating solution. Samples were taken for analysis at the start of the run and at 20 min intervals for 1 h. Fig. 1 shows the time course of isotope disappearance from the bathing solution and changes in total Cl^- in a typical experiment. Chloride influx

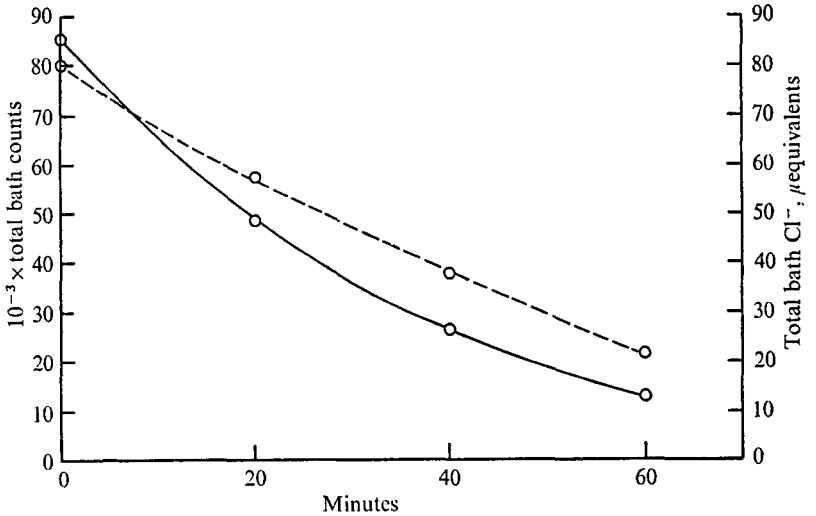

Fig. 1. Total counts per minute in the irrigating bath and total chloride *v.* time, Expt. 8–19–70. The irrigating solution was 1 mM-NaCl with ^{36}Cl. The solid line is total bath counts and the dashed line is total chloride.

was estimated by taking a tangent to the curve at 20 min and dividing by the specific activity of the medium at that time. External specific activity was always more than 10 times that of the body fluids, so no correction for back flux of isotope was necessary (cf. Kirschner, 1970). Net chloride movement was determined from changes in total amount of chloride in the external solution (cf. Fig. 1). Chloride efflux was the difference between influx and net flux. In a few experiments unidirectional fluxes of sodium and chloride were determined simultaneously. In these runs ^{22}Na and ^{36}Cl were both added to the external irrigating solution, aliquots of which were counted for both isotopes.

In single-labelled experiments aliquots of the external medium were plated on aluminum planchets and counted on a gas-flow GM tube with an ultra-thin window. In the double-labelled experiments one aliquot of the irrigating medium was pipetted into tubes and the ^{22}Na was counted in a well scintillation counter. A second aliquot of the medium was plated and counted as for chloride alone. The total counts were then corrected for ^{22}Na to obtain the ^{36}Cl in the sample. Chloride concentration in the external medium was measured by electrometric titration, sodium concentration by atomic absorption spectrophotometry. Blood pH was measured with a glass capillary pH electrode maintained at 13 °C. Intracellular potentials were measured with micropipette bridges filled with 2·5 M-KCl and connected by Ag/AgCl electrodes to an amplifier of input impedance. A potentiometric recorder was used for read-out. The micropipettes used had tip resistances of 10 MΩ or greater.

RESULTS

Kinetics of chloride movement

Influx of chloride ions was dependent on the external Cl^- as shown in Fig. 2. Influx appears to show saturation kinetics, with a half-saturation value (K_m) between 0·2 and 0·3 mM. If the values in Fig. 2 are converted to reciprocals, the three highest fluxes fall on a straight line, but the lowest does not. It is possible that the flux/concentration relationship is not really hyperbolic, but it is more likely that the lack of fit at the lowest concentration is due to analytical difficulties. The electrometric

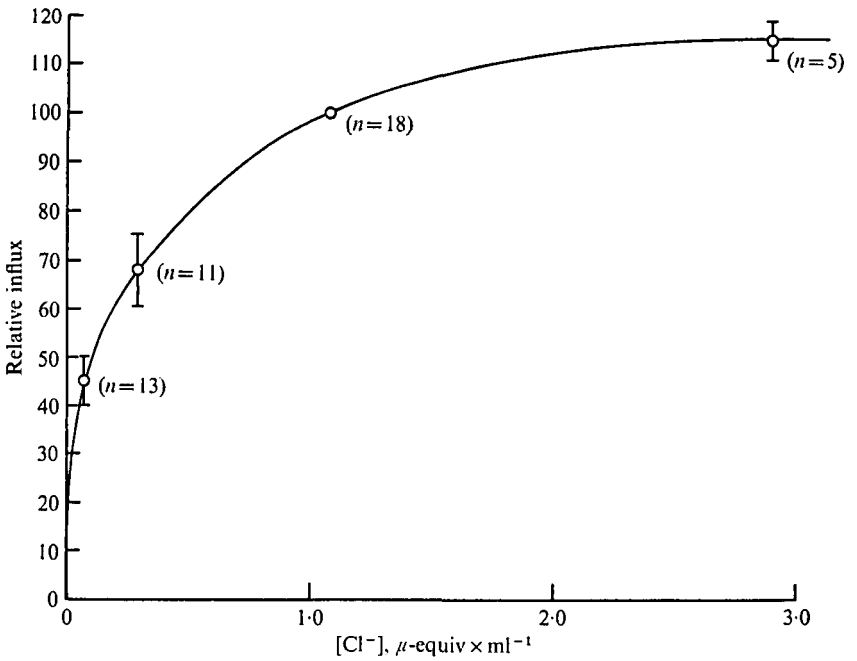

Fig. 2. Chloride influx *v.* bath [Cl]. Vertical lines are standard errors of the mean.

Table 1. *Flux-ratio analysis of chloride fluxes*

Cl	J_i*	J_o*	n	J_i/J_o	TEP expected†
0·29±0·02	11·0±1·4	12·9±1·8	11	0·85	+148 mV
1·08±0·04	19·3±2·3	11·4±1·4	18	1·69	+134·5 mV

* Influx and efflux, μ-equiv h^{-1} (100 g)$^{-1}$.
† Transepithelial potential expected if fluxes are diffusive only.

titration for Cl^- is reliable down to about 0·2 mM, but determinations at lower concentrations approach the 'noise' level of the method and hence have a large uncertainty. In any case, the transport system is saturable, and the parameter K_m is approximately correct. In a previous paper (Kerstetter *et al.* 1970) we reported that the K_m for sodium influx was about 0·45 mM, about double that for chloride. It is interesting that Shaw (1960) reported that the K_m for chloride influx in the crayfish *Astacus* was also lower than that for sodium transport. Chloride fluxes in two different NaCl concentrations

were analysed by the flux ratio equation (Ussing, 1949). The results, summarized in Table 1, show the transepithelial potentials (TEP's) expected if chloride fluxes were passive. But TEP's previously measured in Ca^{2+}-free NaCl solutions are 10–15 mV, body fluids negative (Kerstetter *et al.* 1970), values which are clearly incompatible with passive Cl^- influx.

Data from which the curve in Fig. 2 was constructed were obtained by changing the concentration of the irrigating solution at hourly intervals and measuring chloride influx at each concentration. In practice, each fish was usually used for three different flux determinations. For each animal chloride influx at 1·0 mM-NaCl was determined first, then influxes from higher or lower concentrations were measured and expressed as a percentage of the initial rate. The points in Fig. 2 are the means of these percentages, with the influx at 1·0 mM assigned a value of 100%. This procedure was necessary because of wide variations in chloride fluxes between individual fish.

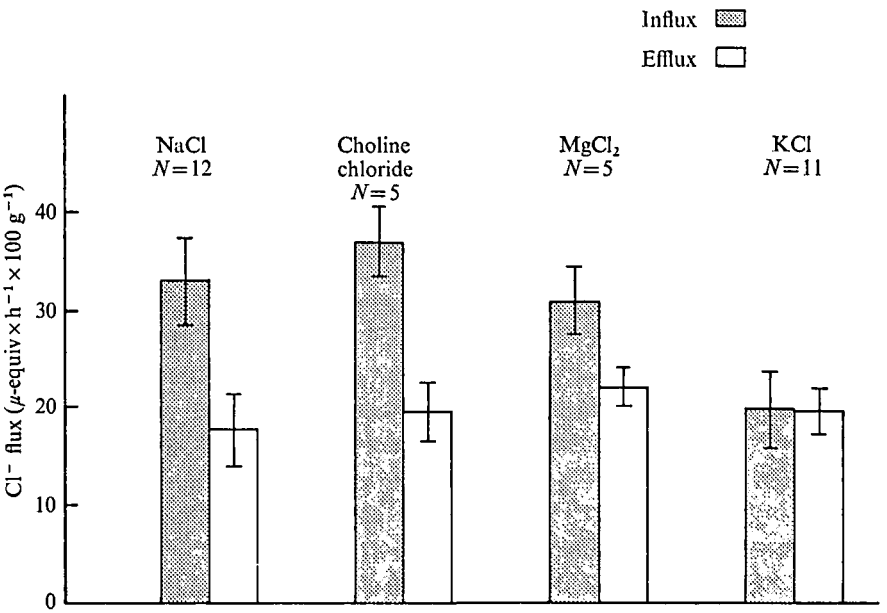

Fig. 3. Chloride fluxes in four different cation-chloride solutions. In all cases [Cl] was about 0·8 μ-equiv/ml. Vertical lines are standard errors.

Non-dependence on penetrating cations

To test whether chloride transport was independent of simultaneous sodium uptake, chloride fluxes, measured in $MgCl_2$ and choline chloride, were compared with those from NaCl solutions of equal chloride concentration. Fig. 3 shows that influxes were about the same in all three solutions. The passive efflux also seemed to be independent of which cation was used. When $MgCl_2$ was the irrigating solution the concentration of Mg^{2+} (determined by atomic absorption spectrophotometry) did not change in any of the experiments. The ability of choline to enter was not determined for the trout, but Garcia-Romeu & Maetz (1964) reported that it does not pass across the gill of *Carassius*. The data show that Cl^- uptake does not require simultaneous transfer

of Na^+ and is probably independent of any cation influx. An anion exchange system is therefore suggested.

A curious finding, also shown in Fig. 3, was that Cl^- influx was significantly lower from KCl than from the other salts of the same concentration ($P < 0{\cdot}025$). Flux measurements (^{42}K) on four fish showed that the gill epithelium was permeable to K (influx was $4{\cdot}25 \pm 1{\cdot}3$ μ-equiv h^{-1} (100 g)$^{-1}$; efflux was $5{\cdot}8 \pm 1{\cdot}6$ μ-equiv h^{-1} (100 g)$^{-1}$ with 0·5 mM-KCl in the external irrigating solution). The potential difference across the gill epithelium (TEP) was not significantly different with KCl as the irrigating solution from the value of -10 mV found in Ca^{2+}-free NaCl (Kerstetter *et al.* 1970). Flux-ratio analysis (Ussing, 1949) suggests that K^+ influx is active, but none of these observations indicates why Cl^- uptake is depressed.

The possibility of chloride-bicarbonate exchange

Maetz & Garcia-Romeu (1964) showed that chloride uptake was stimulated in goldfish when bicarbonate was injected. We measured chloride influx before and after intraperitoneal injection of HCO_3^- and also followed blood pH during the experiment. A detailed protocol for these experiments is described in Table 2. Control animals

Table 2. *Sequence of procedures in bicarbonate loading and ringer-injected control experiments*

Event	Sequence in experiment
1. Control influx measurement	First hour
2. Blood sample 1	End of first hour
3. First injection	Immediately following (2)
4. Blood sample 2	Thirty min after (3)
5. Second injection	Immediately following (4)
6. Second influx measurement	One-hour period immediately following (5)
7. Third blood sample	Immediately after (6)
8. Third influx measurement	One-hour period immediately following (7)
9. Final blood sample	Immediately after (8)

were injected intraperitoneally with Ringer's solution. The results, shown in Table 3 and Fig. 4, are the same in the trout as were reported for the goldfish; Cl^--influx was stimulated by the bicarbonate injection. When compared with the Ringer's-injected controls, influx changes in the experimental animals were significant at the 2·5% level for the first hour after loading and at the 1% level for the second hour. Fig. 4 also shows that blood pH increased in the experimental animals as expected. Chloride effluxes, not shown in Fig. 4, did not change significantly in the experiment.

In these experiments, as well as in those of Maetz & Garcia-Romeu, the bicarbonate load was administered as $NaHCO_3$ which also changes the Na^+/Cl^- ratio of the body fluid. To eliminate the possibility that a shift in this ratio could be responsible for stimulating Cl^- influx, $(NH_4)HCO_3$ was injected in two experiments. The results, shown in Table 3, were similar to those from the first group and indicated that bicarbonate ion was responsible for the stimulation of chloride influx.

Inhibition of both sodium and chloride uptake by the carbonic anhydrase inhibitor acetazolamide (Diamox^R) was reported in *Carassius* by Maetz (1956). We have shown that Diamox inhibits sodium uptake in the trout, but have found that it is almost without effect on chloride uptake. The data in Table 4 summarize the results from

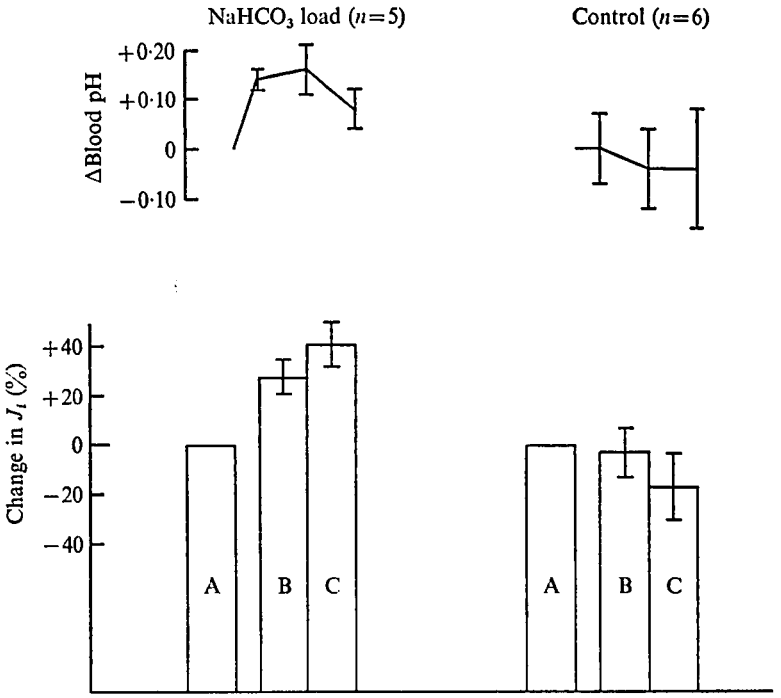

Fig. 4. The response of chloride influx to a bicarbonate load. The bars in each group represent 1 h flux measurements. Period A in each group is the control, and fluxes in periods B and C are expressed as means of the individual percentage changes following the experimental treatment. The changes in blood pH for each group are plotted to correspond in time to the actual measurements. Vertical lines are standard errors. See Table 1 for the detailed protocol.

Table 3. *Changes in chloride influx in response to bicarbonate loading*

Experimental treatment*	*n*	A†	B†	% Change‡	C†	% Change‡
$NaHCO_3$ load	5	14·0±1·9	17·5±2·0	27·7±6·7	20·0±3·4	40·0±9·0
$(NH_4)HCO_3$ load	2	15·8	20·5	25·3	26·5	68·2
Control (Ringer's injection)	6	26·1±4·9	23·9±2·8	−3·2±9·0	19·9±3·7	−17·4±13·5

* See Table 1 for procedures.

† A, B and C are successive 1 h flux determinations. The data are μ-equiv h^{-1} (100 g)$^{-1}$. The experimental manipulation followed 'A' in all cases.

‡ Mean of the percentage change of influx for each fish, based on the influx in period 'A'.

one set of experiments. As in the loading experiment a control influx was determined first for each experimental animal. Diamox (33 mg/kg) was then injected intraperitoneally, following which a second flux determination was made. A second Diamox injection was given 1 h after the first and was followed by a third flux measurement. The data show that chloride influx was unchanged by the two injections. In another experiment fluxes of both Na^+ and Cl^- were measured before and after Diamox. The procedure was similar to that of the previous experiment, except that both ^{36}Cl and ^{22}Na were added to the irrigating solution, and only one injection of the inhibitor was given. The results in Table 5 show that Diamox markedly depressed sodium influx without

causing much change in that of chloride. The small decrease in mean chloride influx was caused by a large negative change in only one fish. Inhibition of sodium uptake shows that the inhibitor was acting in the gill, and the indifference of chloride transport to its presence shows that the transport system does not require carbonic anhydrase.

Intracellular potentials

The electrical potential difference (TEP) across the gill is strongly calcium dependent. In the absence of Ca^{2+} the body fluids are 10–15 mV negative to the external irrigating solution. When Ca^{2+} is present the polarity reverses, and potentials up to + 10 mV are seen. In either case the electrochemical gradient is against inward movement of chloride, and active transport must be involved. In order to learn more

Table 4. *Effects of acetazolamide on chloride fluxes*

	A*	B*	C*	n
J_i*	15·4 ± 1·7	17·5 ± 2·9	17·5 ± 4·5	6
J_o†	14·1 ± 2·0	15·1 ± 3·3	9·9 ± 1·8	6

* A, B, C are successive 1 h time periods. Acetazolamide (33 mg/100 g) was injected after A and again after B.
† μ-equiv h^{-1} (100 g)$^{-1}$ ± S.E.

Table 5. *Comparison of the effects of acetazolamide on sodium and chloride fluxes*

	A*	B*	*n*
J_i (Na^+)†	21·1 ± 6·8	3·5 ± 1·7	4
J_i (Cl^-)†	18·0 ± 12·2	14·9 ± 8·1	4
J_o (Na^+)†	16·1 ± 4·5	15·0 ± 3·4	4
J_o (Cl^-)†	15·1 ± 7·6	16·5 ± 4·9	4

* A and B are successive 1 h time periods. Acetazolamide (33 mg/kg) was injected after A.
† μ-equiv h^{-1} (100 g)$^{-1}$ ± S.E.

precisely where the TEP originates we undertook to measure the intracellular potentials in the gill epithelium. For these measurements a single gill filament was sucked into the end of a vacuum tube whose bore was just slightly larger than the filament tip. The microelectrode was then driven by a vertical micromanipulator into the filament near the point of entry into the vacuum tube. With the free end of the filament immobilized in this way potential differences between the internal microelectrode and the reference bridge in the external solution were stable for at least 10–15 min. Five successful penetrations in two different fish gave a mean potential difference of $-32 \pm 2{\cdot}5$ mV. In these experiments a potential measurement was considered acceptable only when (1) it was stable for several minutes after inserting the microelectrode and (2) the potential difference between microelectrode and reference returned to within 2–3 mV of its original value when the electrode was withdrawn. The potentials reported above are corrected for electrode asymmetry.

DISCUSSION

The data presented make it clear that chloride uptake by the perfused trout gill requires an active transport system. It is independent of that for sodium and shows saturation kinetics which generated the usual hyperbolic curve for concentration

dependence. Chloride influx averaged 19·7 μ-equiv h^{-1} (100 g)$^{-1}$ in a 1·0 mM-NaCl solution, which is not significantly lower than the value of 21·7 μ-equiv h^{-1} (100 g)$^{-1}$ reported for sodium uptake from the same salt solution (Kerstetter *et al.* 1970). But chloride uptake was much more variable from individual to individual than sodium influx, and in addition, the behaviour of the former was sometimes erratic. For example, the rate of isotope uptake occasionally slowed markedly and sometimes accelerated during a single flux measurement. In an occasional individual, tracer uptake simply stopped for a period of time. Neither phenomenon was common in studying sodium influx, and the observations suggest that chloride influxes may be highly sensitive to changes in the internal milieu. The cause is unknown and deserves further study. A further peculiarity was that, at concentrations above 5·0 mM-NaCl, chloride influx often slowed abruptly or even stopped, suggesting a substrate inhibition. A similar observation has been reported by Bielawski (1964) in his study of Cl^- transport across crayfish gills.

Few measurements have been made of unidirectional chloride fluxes in intact animals. Gordon (1963) measured chloride effluxes of freshwater-adapted rainbow trout housed in aquaria. His value, about 20 μ-equiv h^{-1} (100 g)$^{-1}$, is remarkably similar to our influx values. Values from *Carassius* are also similar to those recorded here (Maetz & Garcia-Romeu, 1964), while those in the flounder (Motais, 1967) and in the European eel (Kirschner, 1969) are an order of magnitude lower. In bullfrog (*R. catesbeiana*) tadpoles adapted to pond water chloride influx from 1·0 mM-NaCl was about 10 μ-equiv h^{-1} (100 g)$^{-1}$, while the influx in salt-depleted animals was 2–3 times higher (Alvarado & Moody, 1970). In the frogs *R. esculenta* and *R. temporaria* influx was about the same as in our fish, did not require the simultaneous uptake of sodium, and was stimulated about threefold in salt-depleted animals (Jørgensen, Levi & Zerahn, 1954).

Where chloride uptake has been systematically investigated in intact animals it has been assumed that electrical neutrality is maintained by simultaneous efflux of bicarbonate ions. The best evidence that the exchanging anion is bicarbonate has come from the work of Garcia-Romeu, Salibián & Pezzani-Hernandez (1969) with the Chilean frog, *Calyptocephalella*. They reported that when an intact frog was placed in choline chloride solution for a period of time uptake of chloride was exactly balanced by an output of base. The titration curve at the end of the experiment was identical to that of a choline bicarbonate solution. Additional evidence was provided by Maetz & Garcia-Romeu (1964) showing that chloride influx in the goldfish was stimulated by injecting bicarbonate. Similar results with the trout were described above. But there is one important difference between the two fish. Chloride transport in the goldfish was markedly inhibited by Diamox while this compound had no effect in the trout. The model for chloride uptake proposed by Maetz & Garcia-Romeu (1964) demands a continuous supply of bicarbonate generated by the enzyme carbonic anhydrase in the gill epithelium. Clearly such a model needs to be modified for the trout gill. The fact that chloride uptake is independent of cation transport and is stimulated by injected bicarbonate in both fish suggests that a common anion exchange system is involved. The Diamox data suggest that in the goldfish exchangeable HCO_3^- is produced by catalytic hydration of CO_2 entering gill epithelium from the blood; movement of HCO_3^- across the nutrient membrane must be relatively slow and be-

comes rate limiting when carbonic anhydrase is inhibited. In the trout diffusion of that ion from the blood appears to be commensurate with the rate of its extrusion from the cell into the environment. Bicarbonate concentration in fish blood is low. Based on the mean blood pH of 7·47 in our fish, and mean blood CO_2 tensions of about 4·0 mmHg through the gill (Stevens & Randall, 1967), blood bicarbonate concentration would be about 3·5 mM. Intracellular potentials measured with reference to the outside bathing solution averaged −32 mV, and the total TEP is between −10 and +10 mV, with reference to the outside solution, depending on the presence or absence of Ca^{2+}. Thus the blood border membranes would have a potential difference of between 20 and 50 mV with the cell interior negative. Passive distribution of HCO_3^-, calculated from the Nernst equation, would give intracellular levels between 0·4 and 1·0 mM. Although this concentration is low, it is certainly not incompatible with the operation of a high-affinity mechanism for extruding the ion into the external bathing solution. The only requirement is that the membrane permeability on the nutrient side be sufficiently high to permit bicarbonate to diffuse in from the blood as rapidly as it is extruded. Thus, the difference between goldfish and trout would be explained if the passive bicarbonate permeability of the nutrient membrane were higher than in the goldfish.

An alternative to this argument is that in the trout a coupled chloride/bicarbonate exchange system is located on the nutrient membrane of the transporting cells. This possibility cannot be ruled out, but in any case a passive step at the outer membrane is unlikely. The apparent potential difference at that membrane and the very low concentrations from which net chloride uptake occurs both suggest an active entry step at the outer border. Finally, even though the above discussion is based partly on our intracellular potential measurements, it should be noted that these values are only suggestive. There are several morphologically distinct cell types making up the gill epithelium (see review by Conte, 1969), not all of which may be involved in chloride transport. Until marking techniques can be used to identify cell types from which electrical measurements are made, definite conclusions about the electrical barriers to chloride movements are unwarranted.

SUMMARY

1. The kinetics of chloride transport by the irrigated trout gill have been studied. The transport system is saturable, and the half-saturation value is about 0·25 mM.

2. Chloride uptake occurs equally well from solutions of non-penetrating cations and from NaCl solutions. The presence of potassium in the irrigating solution, however, significantly inhibits chloride uptake.

3. The trout gill is permeable to potassium, and there appears to be an active component to potassium influx.

4. Chloride transport is stimulated by injections of $NaHCO_3$ and $(NH_4)HCO_3$. The carbonic anhydrase inhibitor, acetazolamide, has no apparent effect on chloride influx, and it is suggested that if a Cl^-/HCO_3^- exchange exists in the trout gill, sufficient HCO_3^- can be supplied by the blood.

5. The mean intracellular potential (relative to the irrigating solution) of five gill filament cells was −32 mV. This indicates the presence of an energy barrier to chloride

uptake at the outer membrane of the epithelium, and therefore it is postulated that an active step for chloride transport is located on this membrane.

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REFERENCES

ALVARADO, R. H. & MOODY, A. (1970). Sodium and chloride transport in tadpoles of the bullfrog *Rana catesbeiana*. *Am. J. Physiol.* **218**, 1510–16.

BIBER, T. U. L., CHEZ, R. A. & CURRAN, P. F. (1966). Na^+ transport across frog skin at low external Na^+ concentration. *J. gen. Physiol.* **49**, 1161–76.

BIELAWSKI, J. (1964). Chloride transport and water intake into isolated gills of crayfish. *Comp. Biochem. Physiol.* **13**, 423–32.

CONTE, F. P. (1969). Salt secretion. In *Fish Physiology*, ed. W. S. Hoar and D. J. Randall. New York: Academic Press, Inc.

DEJOURS, P. (1969). Variations of CO_2 output of a fresh-water teleost upon change of the ionic composition of the water. *J. Physiol., Lond.* **202**, 113*P*.

GARCIA-ROMEU, F. & MAETZ, J. (1964). The mechanism of sodium and chloride uptake by the gills of a fresh water fish, *Carassius auratus*. I. Evidence for an independent uptake of sodium and chloride ions. *J. gen. Physiol.* **47**, 1195–207.

GARCIA-ROMEU, F., SALIBIÁN, A. & PEZZANI-HERNANDEZ, S. (1969). The nature of the *in vivo* sodium and chloride uptake mechanisms through the epithelium of the Chilean frog, *Calyptocephallela gayi* (Dum. et Bibr., 1841). Exchanges of hydrogen against sodium and of bicarbonate against chloride. *J. gen. Physiol.* **53**, 816–835.

GORDON, M. S. (1963). Chloride exchanges in rainbow trout (*Salmo gairdneri*) adapted to different salinities. *Biol. Bull. mar. biol. Lab., Woods Hole* **124**, 45–54.

JØRGENSEN, C. B., LEVI, H. & ZERAHN, K. (1954). On active uptake of sodium and chloride in anurans. *Acta physiol. scand.* **30**, 178–190.

KERSTETTER, T. H., KIRSCHNER, L. B. & RAFUSE, D. (1970). On the mechanisms of sodium ion transport by the irrigated gills of rainbow trout (*Salmo gairdneri*). *J. gen. Physiol.* **56**, 342–59.

KIRSCHNER, L. B. (1969). Ventral aortic pressure and sodium fluxes in perfused eel gills. *Am. J. Physiol.* **217**, 596–604.

KIRSCHNER, L. B. (1970). The study of NaCl transport in aquatic animals. *Am. Zool.* **10**, 365–76.

KOEFOED-JOHNSEN, V. & USSING, H. H. (1958). The nature of the frog skin potential. *Acta physiol. scand.* **42**, 298–308.

KROGH, A. (1937). Osmotic regulation in fresh water fishes by active absorption of chloride ions. *Z. Vergl. Physiol.* **24**, 656–66.

KROGH, A. (1938). The active absorption of ions in some fresh water animals. *Z. Vergl. Physiol.* **25**, 335–50.

MAETZ, J. (1956). Les échanges de sodium chez le poisson *Carassius auratus* L. Action d'un inhibiteur de l'anhydrase carbonique. *J. Physiol. (Paris)* **48**, 1085–99.

MAETZ, J. & GARCIA-ROMEU, F. (1964). The mechanism of sodium and chloride uptake by the gills of a fresh-water fish, *Carassius auratus*. II. Evidence for NH_4^+/Na^+ and HCO_3^-/Cl^- exchanges. *J. gen. Physiol.* **47**, 1209–27.

MOTAIS, R. (1967). Les mécanismes d'échanges ioniques branchiaux chez les téléostéens. *Ann. Inst. océanogr., Monaco* **45**, 1–84.

MULLINS, L. (1950). Osmotic regulation in fish as studied with radioisotopes. *Acta physiol. scand.* **21**, 303–14.

POTTS, W. T. W. & EVANS, D. (1967). Sodium and chloride balance in the killifish *Fundulus heteroclitus*. *Biol. Bull. mar. biol. Lab., Woods Hole* **133**, 411–24.

SHAW, J. (1960). The absorption of chloride ions by the crayfish, *Astacus pallipes* Lereboullet. *J. exp. Biol.* **37**, 557–72.

STEVENS, E. D. & RANDALL, D. J. (1967). Changes of gas concentrations in blood and water during moderate swimming activity in rainbow trout. *J. exp. Biol.* **46**, 329–37.

USSING, H. H. (1949). The distinction by means of tracers between active transport and diffusion. *Acta Physiol. Scand.* **19**, 43–56.

USSING, H. H. & WINDHAGER, E. E. (1964). Nature of shunt path and active sodium transport path through frog skin epithelium. *Acta physiol. scand.* **63**, 141–55.