The Mechanism of Sodium and Chloride Uptake by the Gills of a Fresh-Water Fish, *Carassius auratus*

11. Evidence for NH_4^+ / NA^+ and HCO_3^- / Cl^- exchanges

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ABSTRACT   The addition of ammonium ions to the external medium results in an inhibition of the sodium influx and net uptake in *Carassius auratus*, while intraperitoneal injection of ammonium produces the opposite effect. The simultaneous chloride balance is not significantly affected by these treatments. The addition of bicarbonate ions to the external medium results in a reduction of the influx and net flux of chloride, while injection of bicarbonate produces the opposite effect. The simultaneous sodium balance is not significantly altered. The effects of the external additions are reversible after elimination of the excess ammonium or bicarbonate ions by rinsing. Inhibition of carbonic anhydrase in the gill by injection of acetazoleamide produces a simultaneous inhibition of both sodium and chloride exchanges. These results confirm the hypothesis of an exchange of sodium for ammonium, and of bicarbonate for chloride across the gill. A tentative schematic representation of the ionic absorption mechanisms in the branchial cell of the fresh-water teleosts is given. Similarities with other biological membranes and especially with the renal tubule are pointed out.

In the preceding publication (García Romeu and Maetz, 1964) substantial evidence was advanced to show that in *Carassius auratus* the absorption of sodium and chloride ions by the gill is independent. Such independence can only be explained by the assumption that these ions are independently exchanged with endogenous ions. Krogh (1937, 1939) has suggested that the NH_4^+ and HCO_3^- ions might play this role. The work presented here is an attempt to verify this hypothesis.

In support of the postulated sodium-ammonium exchange is the fact that the gill is the principal site for nitrogenous excretion in teleosts (Smith, 1929;

Delaunay, 1929), and that this excretion is mainly in the form of ammonia. More recent work by Wolbach, Heinemann, and Fishman (1959) on *Ameiurus*, Goldstein and Forster (1961) on *Myoxocephalus* and *Anguilla*, and Thornburn and Matty (1963) on *Carassius* and *Salmo* gives figures for branchial ammonia excretion which, at 15 to 100 (μEq/hr.)/100 gm, are of the same order of magnitude as those which we have found for the net flux of sodium. Goldstein and Forster (1961) have also shown that the major part of the endogenous ammonia is produced in the branchial cells by the activity of enzymes such as glutaminase and glutamic acid dehydrogenase, blood ammonia clearance by the gills accounting for only about 10 per cent of the total output.

In support of the postulated chloride-bicarbonate exchange is the fact that the gill is the site of the excretion of respiratory carbon dioxide [up to 500 (μEq/hr.)/100 gm] and the branchial cells contain carbonic anhydrase catalyzing the reaction:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \text{ (Leiner, 1938)}$$
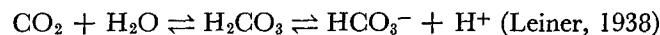
Carbonic anhydrase inhibition produces a significant change in the plasma chloride level, an increase in the marine fish, *Serranus* (Maetz, 1953), and a decrease in the fresh-water fish, *Perca* (Maetz, 1956 *a*). In *Carassius*, Maetz (1956 *b*) has observed that acetazolamide, the most specific carbonic anhydrase inhibitor, causes an important inhibition of the sodium uptake. In view of the suggested HCO_3^-/Cl^- exchange mechanism, it appeared necessary to verify whether this inhibition is accompanied by a simultaneous reduction of the chloride uptake by the gills. In point of fact, inhibition of carbonic anhydrase has been shown to produce a decrease of the bicarbonate excretion by the gills of the dogfish (Hodler, Heinemann, Fishman, and Smith, 1955).

There are two possible methods of approach to verifying the postulated sodium-ammonium and chloride-bicarbonate exchanges. The *direct* method, involving chemical and physicochemical analysis of the external medium during ion transport, would enable a complete balance sheet of Na^+/NH_4^+ and Cl^-/HCO_3^- exchanges to be drawn up. In practice, however, such a procedure would be difficult to carry out with precision. On the one hand, the relative concentrations of the ionized and unionized forms of ammonia and carbon dioxide [$HCO_3^-]/[CO_2]$ and $[NH_4^+]/[NH_3]$ are influenced by the pH of the aquarium water, and on the other hand, the aeration of the external medium would result in an escape of the volatile CO_2 and NH_3 and a shift of the pH. The *indirect* method was therefore chosen for this study. This consisted of an analysis of the effects on sodium and chloride absorption rates of experimental changes of ammonium or bicarbonate concentrations in the external or the internal media. In *Astacus*, the presence of a Na^+/NH_4^+ exchange is suggested by the fact that addition of ammonium ions to the external medium inhibits sodium uptake (Shaw 1960 *c*).

MATERIAL AND METHODS

The origin and maintenance of the goldfish and the techniques for measuring ion exchange are described in the preceding publication.

Sodium and chloride absorptions were measured in sodium chloride solutions (initial concentrations 300 to 1500 μEq/liter) using animals previously kept in running tap water or in sodium chloride solutions (400 μEq/liter). In many experiments designed to study the inhibition of ionic exchange, the initial absorption rate was stimulated by an internal osmotic shock by means of an injection of a hypotonic sodium chloride solution (20 to 30 mM) (Maetz, 1963; Bourguet, Lahlouh, and Maetz, in preparation).

In all experiments described below, the net fluxes of both sodium and chloride ions were measured. In a few experiments, both ions were tagged by Cl^{36} and Na^{24} and therefore simultaneous exchanges of both ions were measured. In certain experiments in which sodium was not tagged, the concentration of external Cl^{36} was registered by a flow type scintillation counter sensitive to the relatively low β-energy of the isotope. The counter was connected to a rate meter and a millivolt meter. This counter (Istin, 1964) consists of a photomultiplier 53 AVP (La Radiotechnique) of which the window is mounted against a cylinder of polystyrene containing a dissolved scintillation substance, para-terphenyl and tetraphenyl butadiene (Scintillateur Plastique Fluorescent, La Radiotechnique). The aquarium water circulates in a spiral cavity (volume, 1 ml) bored in the plastic. Special care has to be taken to shield this counting equipment from daylight.

Addition of Ammonium or Bicarbonate Ions to Internal or External Media

In these experiments the unidirectional fluxes of sodium and chloride were compared before and after the addition of ammonium or bicarbonate ions to the external water or their injection into the intraperitoneal cavity. This injection was carried out by means of a previously inserted catheter and a slow injection apparatus (maximum flow, 500 μl/ min.). A volume corresponding to between 2 and 5 per cent of the body volume was injected. The volume added externally amounted to about 0.5 to 1 per cent of the volume of the aquarium, the addition being made rapidly behind the fish by means of a syringe. The circulating pump and aerator assured a rapid mixing of the fluid added to the aquarium water.

The salts added to the external medium were molar solutions of ammonium sulfate and potassium bicarbonate. They did not modify the concentration of specific radioactivity of the sodium and chloride. The final concentration of ammonium ions varied between 16 and 40 μEq/liter giving a concentration ratio of $[NH_4^+]/[Na^+]$ of between 25 and 115. For bicarbonate ions the actual figures were 11 to 32 μEq/liter and 25 to 65 for $[HCO_3^-]/[Cl^-]$.

Molar solutions of ammonium sulfate (doses between 800 and 1400 μEq/100 gm) and sodium bicarbonate (doses between 300 and 1700 μEq/100 gm) were given in intraperitoneal injections. Control injections of sodium sulfate were also made to check any possible effects of the sodium and sulfate ions accompanying the ammonium and bicarbonate ions under study.

Reversibility of the Effects of External Addition, "Rinsing"

In certain experiments the reversibility of the effects of adding ammonium or bicarbonate ions externally was studied after removal of these ions by flushing the aquarium with deionized water. Tagged sodium chloride was then added after about one-half hour and the flux measurements restarted after a further one-half hour allowing for equilibration of the counting equipment. The unidirectional fluxes were thus compared 1 hour before, and 1 hour after the completion of the rinsing process.

Inhibition of Carbonic Anhydrase

Carbonic anhydrase inhibition was produced by intraperitoneal injection of acetazoleamide (diamox, Lederle Laboratories) at doses of 2 mg/100 gm of the sodium salt in a 2 per cent aqueous solution.

RESULTS

Control Injections

Intraperitoneal injection of isotonic saline solution has been shown to have no perturbing effects on the osmoregulatory processes of the goldfish (Maetz, Bourguet, and Lahlouh, 1964) providing precautions are taken to avoid handling the fish during experimentation. Injections of a hypertonic sodium chloride solution (a molar solution at a dose of 1 mmole/100 gm) however, result in a progressive inhibition of the sodium uptake (Maetz, 1963; Bourguet, Lahlouh, and Maetz, in preparation). As simultaneous effects of injection on the chloride exchange were not measured in the above experiments a further set of control injections was made. Hypertonic solutions of sodium sulfate at the same dose as above were injected into 5 fish. The sodium and chloride exchanges 1 hour before and 1 hour after injection are given in Table I. It can be seen that although injection is without significant effect on the chloride exchanges, it causes a decrease of the influx and of the net flux of sodium, the latter being just significant at the 5 per cent level. The sodium outflux remains unchanged.

The Action of Ammonium Ions on the Sodium Exchange

Fig. 1 shows the effect of the addition of ammonium sulfate to the external medium on the sodium exchange. It can be seen from the change of slope of the curve representing the radiosodium disappearance (left-hand graph) that at an external concentration ratio $[NH_4^+]/[Na^+]$ of approximately 40, the addition of ammonium ions causes a marked inhibition of the sodium influx. This inhibition is reversible, for after rinsing, the slope of the radiosodium concentration curve returns to its initial value. The changes in total external sodium concentrations show that the net flux of sodium, at first positive, becomes negative after addition of ammonium ions, and returns to

TABLE I

EFFECT OF SODIUM SULFATE INJECTIONS ON THE SODIUM AND CHLORIDE EXCHANGES OF CARASSIUS AURATUS

	Na exchange									Cl exchange								
	f_{in} (5)			f_{net} (5)			f_{out} (5)			f_{in} (4)			f_{net} (4)			f_{out} (4)		
	Before	After	Dif.*	Before	After	Dif.	Before	After	Dif.	Before	After	Dif.	Before	After	Dif.	Before	After	Dif.
	51.4	40.0	−11.4	+24.0	+9.0	−15.0‡	27.4	31.0	+3.6	19.0	19.5	+0.5	−20.8	−10.0	+10.8	39.8	29.5	−10.3
	±10.0	±7.2	±4.6	±3.1	±8.8	±6.6	±7.2	±7.5	±10.0	±3.6	±3.5	±0.6	±5.6	±9.8	±11.7	±10.9	±6.2	±11.9

Number of flux determinations are given in parentheses.

f_{in}, influx, f_{net}, net flux, f_{out}, outflux, are given in (µEq/hr.)/100 gm body weight ± standard error of the mean.

* Dif., mean differences of paired data before and after treatment ± standard error of the mean.

‡ $P \leqq 0.05$, probability level that difference is significantly different from 0.

TABLE II

EFFECTS OF EXTERNAL ADDITION OR INJECTION OF AMMONIUM SULFATE ON THE SODIUM EXCHANGE OF CARASSIUS AURATUS

	External addition									injection								
	f_{in} (7)			f_{net} (7)			f_{out} (7)			f_{in} (7)			f_{net} (7)			f_{out} (7)		
	Before	After	Dif.*	Before	After	Dif.	Before	After	Dif.	Before	After	Dif.	Before	After	Dif.	Before	After	Dif.
	34.1	16.0	−18.1‡	+13.5	−18.5	−32.0‡	20.6	34.5	+13.9§	25.4	52.1	+26.7‡	−14.3	+19.4	+33.1§	39.7	32.7	−7.0
	±7.4	±5.0	±3.8	±12.5	±15.8	±6.6	±7.6	±12.7	±5.6	±5.5	±7.1	±7.2	±10.3	±13.3	±10.7	±12.4	±9.0	±8.2

Number of flux determinations on 7 animals are given in parentheses.

* Dif., see Table I.

‡ $P \leqq 0.01$.

§ $P \leqq 0.05$.

positive after rinsing. The addition of ammonium ions results also in a well marked increase in the outflux of sodium, calculated from the influx and net flux data (right-hand graph). The outflux returns to its initial value after rinsing.

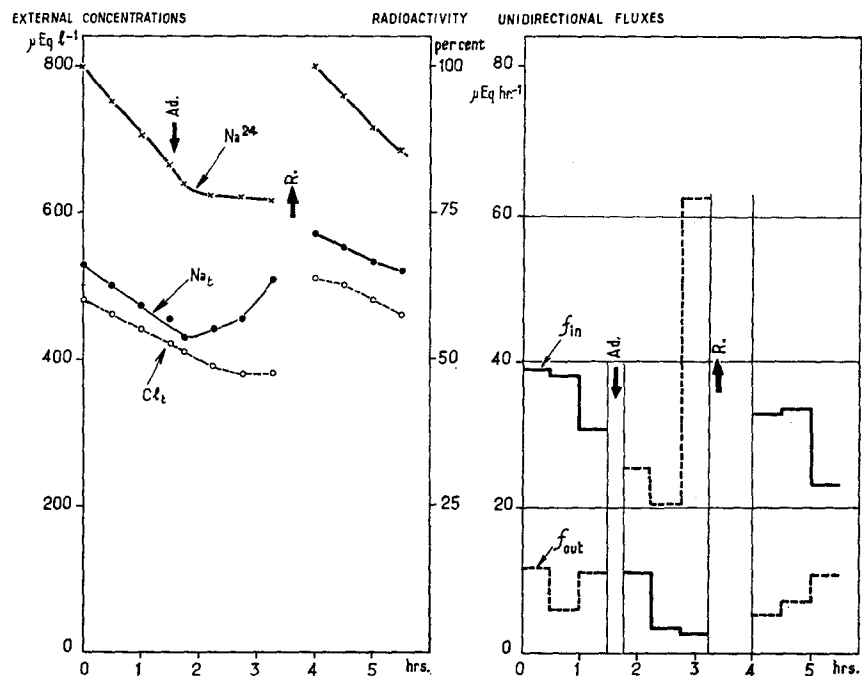Fig. 2 illustrates the effects of an ammonium sulfate injection on the sodium

FIGURE 1.   Effect of addition of ammonium ions to the external medium on the sodium exchange of *Carassius auratus*. (Experiment of Oct. 31, 1963), 85 gm. Initial volume of aquarium water, 570 ml. On left, ordinates, concentrations in external medium of radiosodium in per cent of initial radioactivity and of total sodium and chloride in microequivalents per liter. Abscissa, time in hours. On right, ordinates, influx and outflux of sodium, calculated for $\frac{1}{2}$ hr. periods from data given on left (in microequivalents per hour). Note that outflux is shown by dashed line. Abscissa, time in hours. Arrow *Ad.* indicates external addition of 5 ml of a molar sodium sulfate solution giving a 9 mM concentration in the aquarium water. Arrow *R* marks the start of "rinsing."

exchange. Approximately 20 minutes after the injection there is a considerable increase in the influx and net flux of sodium (left-hand graph), whereas the outflux is not significantly changed (right-hand graph).

Table II summarizes all data from experiments on the effects of internal and external addition of ammonium sulfate, of which Figs. 1 and 2 are individual examples. Seven animals were studied in each type of experiment and as in Table I, the values of influx, net flux, and outflux of sodium have been compared 1 hour before and 1 hour after the experimental treatment.

It can be seen that injection of ammonium ions increases the sodium influx to twice its initial value, while the sodium outflux remains unchanged, resulting in a significant increase of the net flux. This is in sharp contrast to the results of the control injections.

The external addition of ammonium ions on the other hand, causes a significant inhibition of the sodium influx to about 50 per cent of its initial
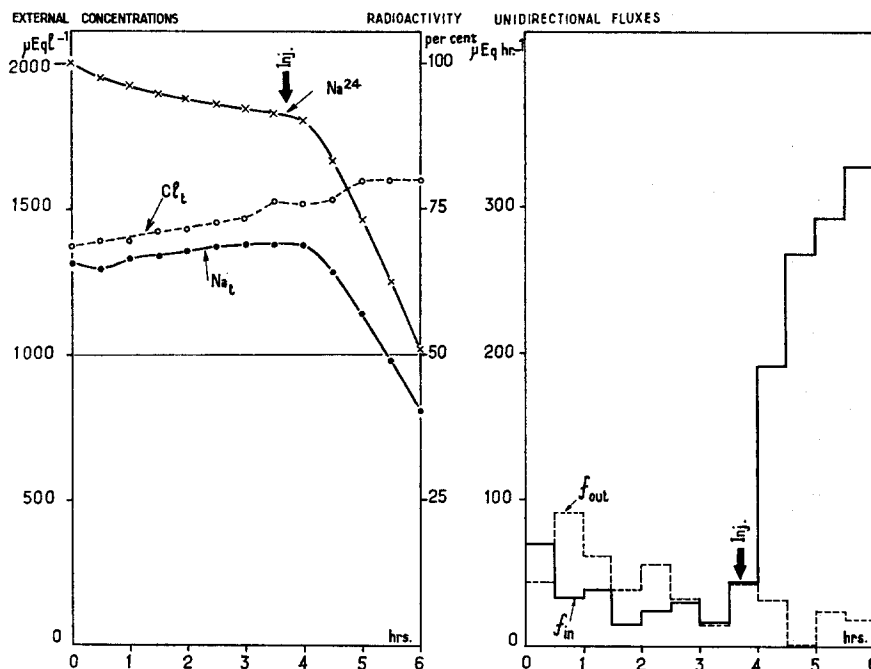

Figure 2. Effect of intraperitoneal injection of ammonium ions on the sodium exchange of *C. auratus.* (Experiment of Dec. 4, 1963), 312 gm. Volume, 960 ml. Coordinates as in Fig. 1. The arrow *Inj.* indicates the injection of 2 ml of molar solution of ammonium sulfate equivalent to 1250 μEq/100 gm.

value and also a decrease of the net flux, whereas the outflux increases by about 70 per cent.

Table III summarizes 4 experiments in which the reversibility of the inhibitory effect of external ammonium ions was studied. The sodium influxes and net fluxes before and after addition of ammonium sulfate and after rinsing are given. It can be seen that while in 3 out of the 4 individuals studied the inhibitory effect of ammonium ions on the influx was reversible, in all 4 cases the effect on the net flux was found to be reversible.

The Action of Bicarbonate Ions on the Chloride Exchange

The effect on the chloride exchange of adding bicarbonate ions to the external medium is shown in Fig. 3. The addition of potassium bicarbonate to

TABLE III

REVERSIBILITY OF THE EFFECTS OF AMMONIUM SULFATE EXTERNAL ADDITION ON THE SODIUM UPTAKE OF CARASSIUS AURATUS

Date of experiment	Influx			Net flux		
	Before addition	After addition	After rinsing	Before addition	After addition	After rinsing
1963						
Oct. 31	43	9	41	+32	−20	+32
Nov. 5	20	12	29	+4	−13	+13
Dec. 4	13	6	11	0	−12	−2
Dec. 4	27	13	12	+6	−24	−4

TABLE IV

EFFECTS OF EXTERNAL POTASSIUM BICARBONATE ADDITION OR SODIUM BICARBONATE INJECTION ON THE CHLORIDE EXCHANGE OF CARASSIUS AURATUS

	External addition									Injection								
	f_{in} (4)			f_{out} (4)			f_{net} (4)			f_{in} (5)			f_{out} (5)			f_{net} (5)		
	Before	After	Dif.*	Before	After	Dif.	Before	After	Dif.	Before	After	Dif.	Before	After	Dif.	Before	After	Dif.
	21.0	5.3	−15.7‡	8.0	5.8	−2.2	+13.0	−0.5	−13.5‡	13.6	33.2	+19.6§	25.2	17.0	−8.2	−11.6	+16.2	+27.8§
	±4.5	±3.9	±2.9	±2.7	±1.9	±0.9	±6.5	±6.2	±2.4	±7.1	±8.3	±3.5	±8.5	±5.6	±5.3	±10.4	±9.9	±5.9

Number of flux determinations are given in parentheses.
* Dif., see Table I.
‡ $P \leqq 0.05$.
§ $P \leqq 0.01$.

the aquarium water to give a concentration ratio $[HCO_3^-]/[Cl^-]$ of approximately 25, causes within a quarter of an hour a marked inhibition of the influx of chloride ions. Furthermore, the fish which previous to the inhibition had a positive chloride net flux began to lose chloride ions. No significant change of the outflux was observed. After removal of the excess bicarbonate by rinsing, the fish again began to absorb chloride ions. The inhibitory effect thus reversible.
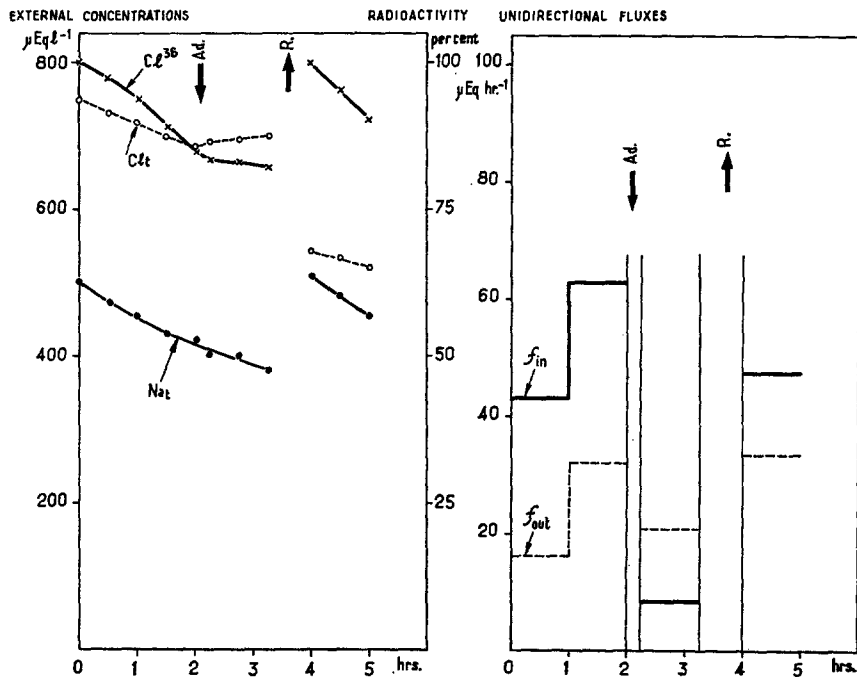

FIGURE 3. Effect of addition of bicarbonate ions to the external medium on the chloride exchange of *C. auratus*. (Experiment of Dec. 4, 1963), 273 gm. Volume 953 ml. Addition of 16 ml of a molar solution of potassium bicarbonate, giving a 18 mM concentration in the aquarium water. Coordinates as in Fig. 1, substituting "chloride" for "sodium."

Fig. 4 shows that the injection of sodium bicarbonate produces within half an hour a considerable increase in the rate of total chloride and radiochloride uptake. In this particular experiment the increase was of short duration. In others it lasted for several hours.

Table IV summarizes all the results obtained from the external addition (data from 4 fish) and the injection (data from 5 fish) of bicarbonate ions. It can be seen that bicarbonate ions in the external medium inhibit by about 75 per cent the chloride influx and also reduce significantly the net flux, while the outflux remains unchanged. The injection of bicarbonate ions, on the other hand, has the opposite effects, namely, a significant increase of

influx of approximately 150 per cent, and of net flux. No significant change of the outflux was observed.

Three experiments showing the reversibility of the inhibitory effects of bicarbonate ions after their removal from the external medium by rinsing, are given in Table V. In all 3 individuals, both influx and net flux returned
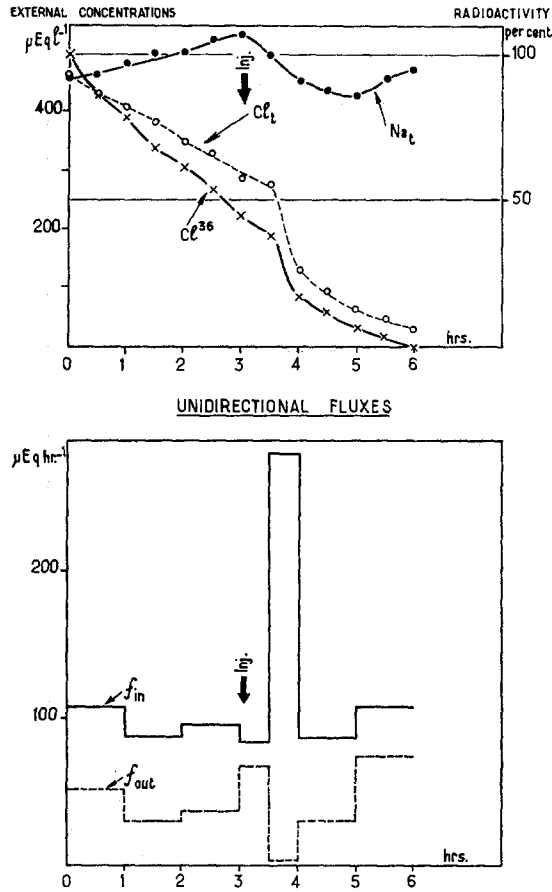

FIGURE 4. Effect of intra-peritoneal injection of bicarbonate ions on the chloride exchange of *C. auratus*. (Experiment of Nov. 15, 1963), 305 gm. Volume, 970 ml. Upper graph, variations of external concentrations of radiochloride, total chloride, and sodium. Coordinates as in Fig. 3, on left. Lower graph, unidirectional fluxes at $\frac{1}{2}$ hr. or hourly periods. Coordinates, as in Fig. 3, on right. At arrow, injection of 1 ml of a molar solution bicarbonate.

to their previous levels after the elimination of the external bicarbonate ions.

It may be concluded from the above series of experiments that the external addition of ammonium or bicarbonate ions produces parallel effects on the ionic absorption processes of the goldfish, the former inhibiting the absorption of sodium ions, the latter that of chloride ions. Such inhibitory effects are reversed when the ions in question are removed by rinsing.

Conversely, the injection of ammonium ions into the fish stimulates the absorption of sodium and the injection of excess bicarbonate stimulates the uptake of chloride.

TABLE V

REVERSIBILITY OF THE EFFECTS OF POTASSIUM
BICARBONATE EXTERNAL ADDITION ON THE CHLORIDE
UPTAKE OF *CARASSIUS AURATUS*

	Influx			Net flux		
Date of experiment	Before addition	After addition	After rinsing	Before addition	After addition	After rinsing
1963						
Nov. 8	31	18	62	+31	+18	+38
Dec. 4	23	3	17	+11	−5	+5
Dec. 5	21	0	12	+10	−8	+7

Specificity of the Actions of Ammonium and Bicarbonate Ions

The postulated ionic exchange relationship between sodium and ammonium ions and between chloride and bicarbonate ions has thus been confirmed. The extent to which each relationship is specific remains to be considered. In other words, does the internal or external addition of ammonium ions influence exclusively the sodium exchange or does it also affect chloride exchange? Similarly do bicarbonate ions act solely on the chloride uptake or also influence the sodium balance? To study these questions the net fluxes of chloride were recorded during the experiments designed to show the influence of ammonium ions on the sodium absorption (see in Figs. 1 and 2 the total chloride external concentration) and similarly the net fluxes of sodium were studied in the experiments using bicarbonate (see Figs. 3 and 4, the total external sodium concentrations).

TABLE VI

SPECIFICITY OF THE EFFECTS—ACTION
OF BICARBONATE ON Na UPTAKE—ACTION OF
AMMONIUM ON Cl UPTAKE

	Treatment	No. of flux determinations	Ion	New flux		
				Before	After	Dif.*
$KHCO_3$	Addition to external medium	4	Na^+	+10.7±6.8	+5.0±8.6	−5.7±5.9
$NaHCO_3$	Injected intraperitoneally	5		−14.0±6.7	−1.6±6.8	+12.4±8.2
$(NH_4)_2SO_4$	Addition to external medium	6	Cl^-	−10.0±10.0	−18.0±11.1	−8.0±8.6
$(NH_4)_2SO_4$	Injected intraperitoneally	5		−33.4±13.2	−23.2±5.8	+10.2±11.0

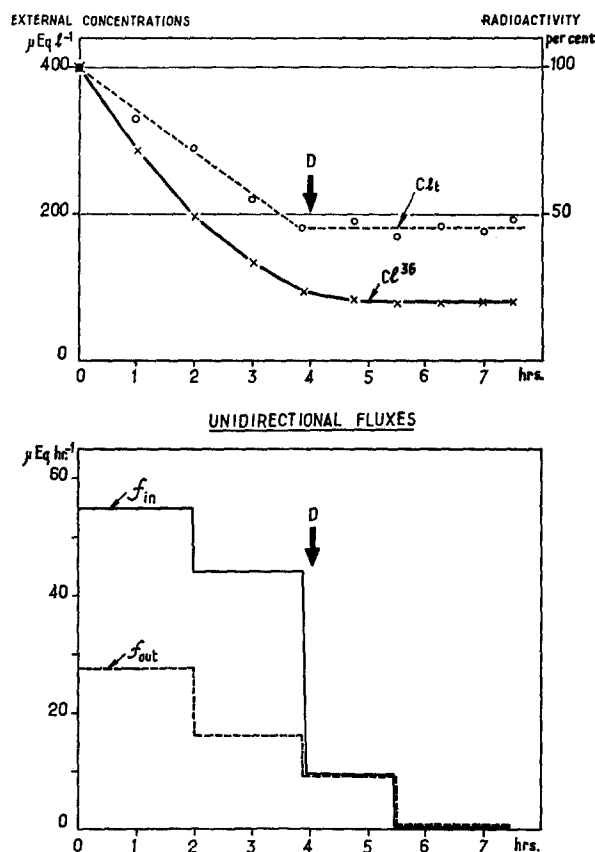Data recorded in part in Figs 1 to 4.
* See Table I.

FIGURE 5. Effect of acetazoleamide injection on the chloride exchange of *C. auratus*. (Experiment of October 22, 1963), 160 gm. Volume, 510 ml. Upper graph, variations in external concentration of total chloride and radiochloride. Coordinates as in Fig. 3, on left. Lower graph, unidirectional chloride fluxes calculated from the data in upper graph, in 1.5 to 2 hr. periods. Coordinates as in Fig. 3, on right. Arrow D indicates the injection of a 2 per cent solution of diamox at a dose of 2 mg/100 gm.

TABLE VII

EFFECT OF CARBONIC ANHYDRASE INHIBITION
BY ACETAZOLEAMIDE INJECTION ON THE CHLORIDE
EXCHANGE OF *CARASSIUS AURATUS*

	f_{in} (5)			f_{net} (5)			f_{out} (5)	
Before	After	Dif.*	Before	After	Dif.	Before	After	Dif.
23.0	6.2	−16.8‡	−11.2	−6.4	−4.8	34.2	12.6	−21.6‡
±5.9	±6.2	±2.8	±6.8	±2.7	±5.8	±7.5	±4.4	±4.6

Number of flux determinations on 5 animals given in parentheses.
* See Table I.
‡ $P \leq 0.01$.

Table VI summarizing these results shows that there is no clear-cut influence of bicarbonate ions on sodium absorption or of ammonium ions on chloride net uptake. Differences in net fluxes before and after treatment have been observed in several cases, but since the effect is very variable and is sometimes in one direction and sometimes in the other (as can be observed from the large standard errors), no definite conclusion can be drawn from these observations.
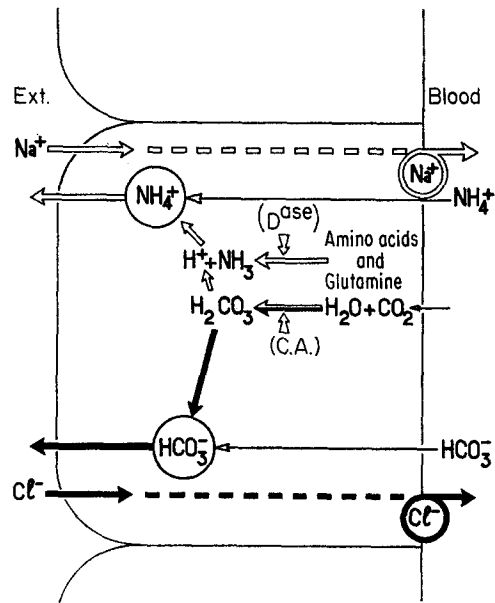

FIGURE 6. Schematic representation of ionic exchanges in the branchial cell of *Carassius*. (D^{ase}), deamidation and deamination enzymes. (C.A.), carbonic anhydrase.

The Effect of Carbonic Anhydrase Inhibition on the Chloride Exchange

The effect of acetazoleamide injection on the chloride exchange has been studied in 5 experiments, one of which is represented graphically in Fig. 5. It can be seen that approximately 2 hours after injection the chloride uptake is completely inhibited. The results of all experiments of this type are given in Table VII, which shows clearly that acetazoleamide injection produces a very significant inhibition of both influx (by approximately 75 per cent) and outflux (by about 60 per cent) of chloride ions. In two experiments given above the sodium exchange was studied simultaneously and was observed to be reduced to the same extent as the chloride exchange.

DISCUSSION

The results given above are consistent with the hypothesis that the branchial cells of *Carassius auratus* exchange sodium for ammonium ions and chloride for bicarbonate ions.

A Tentative Schematic Representation of Ion Absorption by the Gill

For the purposes of this discussion a theoretical diagram of functional phenomena related to ion exchange in the branchial cell of the goldfish is given in Fig. 6, and the various hypotheses concerned will first be considered below.

1. It is reasonable to assume that in *Carassius*, as in *Myoxocephalus* (Goldstein and Forster, 1961), the branchial cells contain deamidation and oxidative deamination enzymes such as glutaminase and glutamic acid dehydrogenase catalyzing the production of ammonia. These enzymes are represented by (D^{ase}) in the figure. The results of a preliminary experiment carried out in connection with the present work lend support to this assumption; an intraperitoneal injection of glutamine (700 μmole/100 gm) was found to produce a 300 per cent increase in the rate of sodium absorption. However, the possibility remains that this increase was in point of fact due to a rise in the blood ammonia level, for Pequin and Serfaty (1963) have shown that in the perfused liver of the carp adding glutamine to the afferent blood causes a rapid increase of the ammonium concentration of the efferent blood as a result of ammonia-producing enzymes in the liver.

2. It has been assumed that these enzymes would produce ammonia in its molecular form within the cells of the gills. These molecules would then become transformed into ammonium ions by the addition of protons liberated by the dissociation of carbonic acid. A certain proportion of ammonium ions could actually be formed by the capture of protons released from α-ketoglutaric acid produced by deamidation of glutamine. The pK_2 of this acid (4.3) is in fact lower than the pH of the cell. But Goldstein and Forster (1961) state that only about 25 per cent of the ammonia produced by the gill of *Myoxocephalus* is derived from glutaminase activity, the remaining 75 per cent being accounted for by oxidative deaminations which do not produce hydrogen ions available for capture by ammonia. The fact that carbonic anhydrase inhibition reduces sodium uptake by about 75 per cent (Maetz, 1956 *b*) supports the hypothesis that ammonium ions involved in sodium exchange owe their formation largely to protons originating from carbonic acid dissociation.

3. With regard to the ammonium exchanged for sodium across the external surface of the branchial cells, the simplest hypothesis is to assume that ammonium ions as such pass out of the cell, for they are in the ionic form on both sides of the membrane, in view of the prevailing pH conditions. An alternative hypothesis would be that H^+ ions originating from carbonic acid dissociation and NH_3 molecules diffuse independently through the cell membrane, the capture of the proton occurring in the outer medium. The fact that an experimental increase of ammonium ion concentration in the external water causes an inhibition of the sodium uptake would seem to support the

hypothesis of a passive diffusion of ammonium ions out of the cell. This flux would then be stopped or even reversed as the concentration of ammonium ions in the external medium equalled or exceeded that in the cell. The increased outflux of sodium recorded under these conditions (see Table II) can thus be explained by a penetration of ammonium ions into the branchial cell reversing the normal direction of sodium-ammonium exchange. Further evidence in favor of ammonium diffusion is given by Wolbach, Heinemann, and Fishman (1959) who found that experimental increase of the concentration of this ion in the external medium brought about an inhibition of the branchial excretion of ammonia in *Ameiurus*.

Furthermore, these authors found that the greater part of the excess ammonium injected intraperitoneally into *Ameiurus* is rapidly eliminated, chiefly by the gills. Our results show that a similar injection in *Carassius* increases the rate of absorption of sodium ions, these ions being presumably exchanged for the excess ammonium.

Finally, in relation to ammonium excretion, it should be pointed out that a small fraction of the cellular ammonium pool available for exchange with sodium is probably derived from the blood. Goldstein and Forster (1961) have shown that in *Myoxocephalus* about 10 per cent of the ammonium excreted by the gills is the result of blood clearance.

4. The postulated exchange between chloride and bicarbonate ions is borne out by three series of experiments. First, the inhibition of carbonic anhydrase causes a considerable reduction of chloride exchange, suggesting that the rate of formation of carbonic acid in the cell is a limiting factor for the chloride absorption mechanism. Second, experimentally raising the external concentration of bicarbonate ions inhibits the uptake of chloride presumably by reducing or even reversing the outflux of bicarbonate ions from the branchial cells. Finally, the converse experiment of injecting an excess of bicarbonate into the fish augments the chloride uptake, which by the same hypothesis, would be accounted for by the increased rate of elimination of bicarbonate ions by the gills.

5. The principal route of excretion of excess bicarbonate is a question necessitating further study. In a preliminary report, Heinemann, Blum, Wolbach, and Fishman (1959) suggest that in *Ameiurus* excess bicarbonate is eliminated by the kidney, whereas in *Squalus* the excess is eliminated by the gills (Hodler, Heinemann, Fishman, and Smith, 1955). In our experiments on *Carassius* an increased alkalinity of the urine after bicarbonate injection has not been observed. As in *Squalus*, the gills are probably the principal route of bicarbonate elimination. A further question concerns the form in which this excess bicarbonate being excreted crosses the gill epithelium. A preliminary experiment lends support to the hypothesis that it is directly excreted in ionic form and that the reaction catalyzed by carbonic anhydrase

is not involved in the elimination of this excess. It was found that acetazoleamide injection had no effect on the chloride exchanges of 2 fish which had previously received an excess of bicarbonate ions by intraperitoneal injection. The possibility remains that the increased alkalinity resulting from these injections causes a compensatory rise in the blood pCO_2 to maintain the acid-base equilibrium. This rise would then increase the rate of formation of carbonic acid in the cell by shifting the equilibrium of the reaction $CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$ independently of carbonic anhydrase catalysis.

6. An active transport of both sodium and chloride has been assumed to exist in the inner membrane of the branchial cell, whereas the ammonium-sodium and bicarbonate-chloride exchanges occurring at the outer membrane would be passive phenomena.

The verification of these hypotheses must await precise measurements both of the cellular concentrations of sodium and chloride and of the differences of potential across the internal and external membranes of the branchial cells. In a preliminary study, Tosteson (1962) has measured potentials at the gill surface of *Anguilla* by means of microelectrodes and found the cellular potential always negative in relation to that of the external medium (whether fresh water, sea water or isotonic Ringer solution), and to that of the blood. One must thus assume that the sodium ions are transported across the inner surface of the cell against an electrochemical gradient. In the case of chloride, it will be necessary to verify whether the electric potential compensates for the chemical gradient.

Absorption of Chloride or Sodium Ions in the Presence of a Non-Permeant Ion

The absorption at the external surface of the branchial cell of sodium or chloride ions in the absence of accompanying ions of opposite charge, may satisfactorily be explained by admitting their exchange with ammonium or bicarbonate ions. Their passage across the inner cellular membrane must, in view of the maintenance of the electroneutrality of the cell, be accompanied by ions of opposite charges or, as at the external surface, result in exchanges of ions of the same charge.

Concerning transfer of chloride across the inner membrane, the simplest hypothesis is to suppose that this ion is accompanied by the proton originating from the dissociation of carbonic acid, the HCO_3^- ion produced simultaneously being exchanged at the outer surface for the Cl^- ions. It remains possible that the proton captured first an ammonia molecule and the resulting ammonium ion would then accompany the chloride. Similarly, the sodium would pass into the internal medium together with bicarbonate ions produced from carbonic acid when sodium is absorbed from the external medium by exchange with ammonium ions.

Under natural conditions, with both sodium and chloride present in the external water, the processes of internal ion transfer explained above may still be assumed to take place with varying degrees of interaction according to the different rates of absorption of the two ions.

Comparison of the Ion Exchange in the Branchial Cell with That in the Oxyntic Cells of the Stomach

An active secretion of H^+ ions together with an active transport of chloride into the lumen of the crypts is known to occur in the oxyntic cells of the stomach (Davies and Ogston, 1950; Hogben, 1955). The H^+ ions originate from the dissociation of carbonic acid and the liberated bicarbonate ions are exchanged for chloride ions across the internal surface of the cell. The rate of formation of carbonic acid from CO_2 and H_2O, catalyzed by the carbonic anhydrase present within the oxyntic cells, is a limiting factor in this process and its inhibition stops the hydrochloric acid secretion.

As in the gills, one finds therefore a chloride-bicarbonate exchange mechanism linked with carbonic anhydrase activity.

Comparison with the Distal Tubule of the Kidney

The presence of carbonic anhydrase and of glutaminase in both the gill and the distal tubule of the mammalian kidney would *a priori* suggest functional similarities between the two structures. The classical theories on the role of the kidney in the maintenance of the acid-base equilibrium (Smith, 1956; Berliner, 1960; Pitts, 1959) cite the cells of the distal tubules as responsible for the acidification of the urine by effecting an H^+/Na^+ exchange. Some of the protons secreted are captured by ammonia molecules excreted into the lumen of the tubules. The protons originate from the dissociation of carbonic acid within the tubular cell. The absorbed sodium ions, together with bicarbonate ions liberated by this dissociation, pass together into the blood stream. This classical scheme has been questioned by certain authors (Ullrich, Hilger, and Klümper, 1958; Rector, Seldin, Roberts, and Copenhaver, 1954; Seldin, Rector, and Teng, 1957) who consider that the reabsorption of the sodium ions is chiefly dependent on an exchange with ammonium ions. Furthermore, the classical theory does not take into consideration the movements of chloride ions which are reabsorbed in the same segment. More recent work on kidney function has led Morel and Boudiak (1962) and Rector and Clapp (1962) to postulate active transport for the chloride ions. Pursuing the analogy with the branchial cells, it would be of interest to study the reabsorption of chloride in relation to a possible bicarbonate ion exchange in the mammalian kidney tubule. Such a phenomenon is known to occur in Crocodilia. Hernandez and Coulson (1954) found that the cells of the distal tubule of the alligator excrete simultaneously ammonium and bicarbonate ions in

exchange with sodium and chloride ions which are reabsorbed, the resulting urine being alkaline.

Recent research provides evidence that the proximal tubule is also concerned with ammonia and H^+ ion secretions (Clapp, Watson, and Berliner, 9163; Glabman, Klose, and Giebisch, 1963) and these processes may thus be characteristic of the renal tubule as a whole.

In several aspects concerning absorption and transfer of ions, the branchial cells of *Carassius* show therefore great similarity to the renal tubules of the mammalian kidney.

The ionic exchange processes present in the gills of *Carassius* may possibly be of widespread occurrence in fresh-water animals, for similar mechanisms have been postulated by Krogh (1937, 1939) for the skins of amphibians, and by Shaw (1960 *a*, *b*, *c*) for the gills of the fresh-water crustacean *Astacus*. Marine teleosts are known also to possess in their gills enzymes similar to those of their fresh-water relatives. In these fish, however, sodium and chloride ions must be excreted along with ammonium and bicarbonate. They cannot therefore possess ionic exchange processes of the type described for *Carassius*.

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