

STUDIES ON THE MECHANISM OF CHLORIDE ABSORPTION BY THE GOLDFISH GILL: RELATION WITH ACID-BASE REGULATION

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(Received 19 February 1973)

INTRODUCTION

In freshwater fish the gill absorbs sodium and chloride from the external medium by an active transport mechanism which compensates for the urinary salt loss and that resulting from passive diffusion along the electrochemical gradient (Maetz, 1971).

From preliminary observations Krogh (1939) suggested that the two ions are absorbed independently by exchange process with endogenous ions of the same charge. Garcia Romeu & Maetz (1964) have confirmed this independence in *Carassius auratus*. They showed that the absorption fluxes of sodium and chloride were influenced in different ways by different pre-adaptation conditions *i.e.* by de-ionized water or by dilute saline solutions containing sodium accompanied by either chloride or an impermeant ion (SO_4^{2-}) or chloride accompanied by the impermeant cation (choline). They also provided indirect experimental evidence supporting the hypothesis of $\text{Na}^+/\text{NH}_4^+$ and $\text{Cl}^-/\text{HCO}_3^-$ exchanges.

The linkage between sodium absorption and ammonium-ion excretion was subsequently questioned because de Vooy (1968) found that fish continue to excrete ammonium ions in the absence of sodium in the external medium. Recently Kerstetter, Kirschner & Rafuse (1970) have found evidence for a Na^+/H^+ exchange independent of ammonium excretion in *Salmo gairdneri*. Such an exchange has been demonstrated experimentally by titration of external acidity in relation to sodium absorption in *Calyptocephalella gayi*, an amphibian which excretes its nitrogenous waste as urea rather than ammonia (Garcia-Romeu, Salibian, Pezzani-Hernandez, 1969). Using the same technique, Maetz (1972) confirmed the presence of a Na^+/H^+ exchange in the goldfish and showed that ammonia can be exchanged in the ionized form (NH_4^+) against sodium or excreted in the non-ionized form (NH_3) depending on the prevailing external conditions.

Garcia-Romeu *et al.* (1969) also found a significant correlation between the amounts of base excreted and chloride absorbed, which is thus direct evidence of a $\text{Cl}^-/\text{HCO}_3^-$ exchange in parallel with the Na^+/H^+ exchange.

In the present work on the goldfish the same techniques were used in order to confirm experimentally $\text{Cl}^-/\text{HCO}_3^-$ exchange. In addition, chloride exchanges in the gill were measured in relation to the external chloride concentration. The effects of substituting the impermeant accompanying ion choline for the permeant sodium on the chloride pump were also studied.

The fish were pre-adapted in the various external media used by Garcia-Romeu & Maetz (1964), which theoretically result in selective depletions of the Na^+ ions in relation to Cl^- ions in the internal milieu. Whether such depletions were in fact obtained was investigated, and the possibility of other parameters relating to the acid-base equilibrium of the internal medium being modified by the various pre-treatments was studied.

MATERIAL AND METHODS

Preparation of animals

The *Carassius auratus* were obtained from a dealer in the Paris region. They weighed between 75 and 150 g were stocked, unfed, in aquaria in running water of uncontrolled temperature (9–18 °C).

Two adaptation series were made. The first, of long duration (3 weeks or more), prepared the fish for the study of chloride fluxes in relation to external chloride concentration, and for direct demonstration of the $\text{Cl}^-/\text{HCO}_3^-$ exchange. It was carried out between December and May. The second, of short duration (9 days), was made in October and served for confirming the effects of the different preadaptations on various internal parameters observed in the first series. Pretreatment media were maintained at 16–18 °C.

In the second series blood samples of 300 μl for measurement of pH and sodium (P_{Na}) and chloride (P_{Cl}) plasma concentrations were taken. The fish were weighed and then placed in one of three different media: de-ionized water, or 0.5 mM/l sodium sulphate solution, or 1 mM/l choline chloride solution. All media were buffered with an imidazole (2 mM/l)–sulphuric acid (0.07 mM/l) mixture to a pH of 7.4. The aquarium water was changed each day, and daily samples were taken to follow the changes in the external sodium and chloride concentrations. The fish were also weighed each day. On the 9th day a further blood sample of 600–750 μl was taken and P_{Na} , P_{Cl} , blood pH and the plasma concentrations of total CO_2 and ammonium were estimated. This procedure applies to all fish except those placed in the sodium sulphate solution, which never survived more than 5 days and which showed disorganized motor activity generally followed by death. No doubt the experimental procedure, involving daily handling for weighing, added further stress.

In the first series the fish were adapted for 3 or 4 weeks to de-ionized water or to the above sodium sulphate solution. In the latter solution only six of the original 12 fish survived to the end of the adaptation period and could be used for flux measurements. No study was made on fish adapted to chloride since it was already known that their sodium and chloride pumps are very similar to those of fish adapted to de-ionized water (Maetz & Garcia Romeu, 1964). Control fish were taken directly from the running water aquaria ($\text{Na}^+ = 105 \mu\text{-equiv./l}$, $\text{Cl}^- = 40 \mu\text{-equiv./l}$, $\text{Ca}^{2+} = 1340 \mu\text{-equiv./l}$).

The day before flux measurements were made, a blood sample was taken from the caudal artery and blood pH and plasma concentrations of sodium, chloride, total CO_2 and total ammonium were determined. A polyvinyl catheter was then inserted into the urinary papilla to discharge the urine outside the aquarium and thus assure that ionic changes in the external medium were due to branchial fluxes. Each fish was placed in an experimental aquarium of about 500 ml. capacity, abundantly aerated

And kept overnight in open circuit with the solution of the previous adaptation period. During the 2 h preceding experimentation the fish was rinsed with 30 l of buffered de-ionized water before being switched to closed-circuit conditions. The closed circuit comprised a pump (Ministaltic), a refrigerating unit maintaining a temperature of 16 °C and a β -radiation detector linked to an automatic counter and recorder (Tanguy, 1970).

Flux measurements

To obtain the low chloride concentrations of the external medium, 3 M solutions of Na³⁶Cl or choline ³⁶Cl (4.16 mCi/g Cl) prepared from H³⁶Cl obtained from the Centre d'Etudes Nucléaires de Saclay were added to the experimental aquaria. For higher chloride concentrations 'cold' solutions of NaCl or choline chloride were added.

The experimental procedure consisted of 3–5 flux measurement periods of 40–60 min duration at different external chloride concentrations. Each period involved taking 11 ml samples of the external medium every 20 min. Three or four such samples were taken and were used for measuring pH, alkalinity and sodium, chloride and total ammonium concentrations. Between each period the aquarium volume was readjusted to its initial value by adding rinsing solution, and the Cl⁻ and ³⁶Cl⁻ concentrations were adjusted to the desired levels. A 20–30 min homogenization interval separated each measurement period.

At the end of an experiment the aquarium volume was measured in a graduated cylinder and a further blood sample was taken for the various analyses. The radioactivity of the internal medium was measured on a suitable diluted plasma sample in the circulation counter.

Flux calculations

Since the experiments were of long duration (5–6 h) and certain fish absorbed a lot of chloride, it proved necessary to take the radioactive back flux into account when calculating influxes. The fluxes were calculated as described by Maetz (1956) for sodium. The internal specific radioactivity of chloride, measured in diluted plasma at the end of experimentation, was used in calculating the radioactive back-flux.

The chloride net flux for each period was calculated from the changes in the external chloride concentration during the period, and the outflux from the difference between the influx and net flux. The fluxes were calculated for each period of measurement and expressed in micro-equivalents per 100 g/h.

The net fluxes of sodium, total ammonium and base were also calculated from the concentration variations of sodium, total ammonium and titratable alkalinity in the aquarium water. The alkalinity titrations were restricted to the experiments using choline chloride, since in the presence of sodium in the external solution the H⁺ ion liberated in exchange for the Na⁺ ion combines with the HCO₃⁻ ion exchanged against the chloride to give CO₂ which escapes. As will be discussed below, it is necessary to take the excretory ammonium flux into account to obtain a correct estimation of the base excreted in exchange for the chloride absorbed because the ammonia remains in the external medium associated with respiratory CO₂, as ammonium bicarbonate.

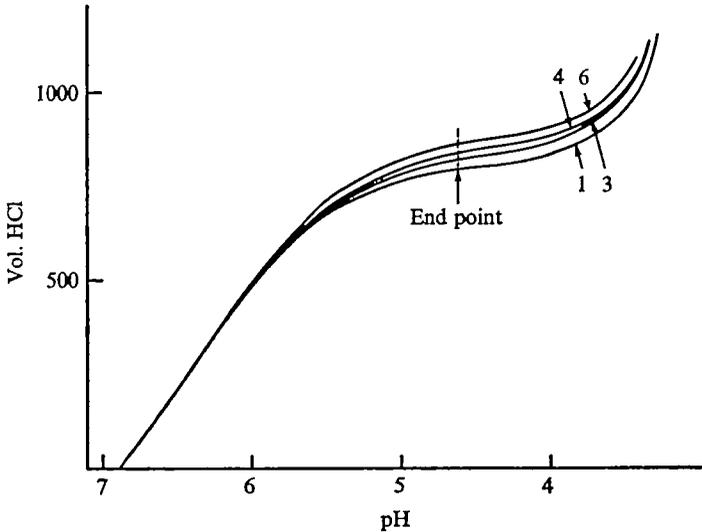


Fig. 1. Titration of alkalinity of aquarium water. Ordinate: volume of titrant in μ l added in 2 ml sample. Abscissa: pH. Note: despite identical initial pH of sample, a progressive augmentation of the total titrable alkalinity indicating an increase of the buffering capacity of the external medium. 1, 3, 4, 6: successive sample number. Samples 2 and 5 were omitted for the sake of clarity.

Measurements of the various parameters

Chloride was measured by amperometric titration (Aminco-Cotlove), sodium with an Eppendorf flame photometer, total ammonium with a Technicon auto-analyser (Maetz, 1972) and total CO_2 with a Natelson gas micro-analyser. The pH of the external medium was determined by means of a Tacussel macroelectrode and that of the internal medium by an I.L. microelectrode. Total alkalinity was determined by titration of a 2 ml sample with 5 mM/l HCl in a Tacussel automatic titrator (Fig. 1). The samples were left in contact with air for 24 h before titration to remove respiratory CO_2 (Maetz, 1972). No correction was made to take into account the HCO_3^- content resulting from solution of atmospheric CO_2 as the pH of the successive samples remained more or less constant at 7.02, the shift for the experimental periods being 0.05 ± 0.005 ($n = 98$). All the results given below correspond to means \pm standard error. Statistical comparison of the data are made using Student's t test.

RESULTS

(1) Effects of adaptation to different media on the internal milieu

The data concerning the various parameters of the internal medium in animals kept for 3 or more weeks in either de-ionized water or sodium sulphate solution are given in Table 1 together with values obtained from control individuals kept in tap water.

It can be seen that pretreatment in de-ionized water results in a significant decrease of P_{Na} and P_{Cl} ($P < 0.001$). Unexpectedly, a very significant decrease ($P < 0.001$) of both these parameters was also recorded in the surviving individuals kept in the sodium sulphate solution. There was no selective reduction of internal chloride as

Table 1. *Data concerning the internal medium of goldfish: comparison of fish from tap water and fish kept for 3-4 weeks in artificial media*

| | External medium | | |
|--------------------------------------|------------------|--------------------------|---------------------|
| | Tap water | Sodium sulphate solution | De-ionized water |
| Plasma sodium (m-equiv./l) | 158.5 ± 4.2 (32) | 128.0 ± 1.9**** (6) | 120.1 ± 2.0**** (8) |
| Plasma chloride (m-equiv./l) | 128.8 ± 2.9 (32) | 105.5 ± 3.6**** (6) | 100.0 ± 4.6**** (8) |
| Blood pH | 7.64 ± 0.05 (9) | 7.83 ± 0.02*** (6) | 7.53 ± 0.04 (8) |
| Total plasma CO ₂ (mm/l.) | 9.3 ± 0.30 (9) | 12.0 ± 1.80 (6) | 7.9 ± 0.50 (8) |
| Total plasma ammonium (μM/l) | 191 ± 30 (9) | 992 ± 263*** (6) | 1085 ± 294*** (8) |

Comparison with tap water: *** $P < 0.01$; **** $P < 0.001$.

Number of flux periods in parentheses.

Table 2. *Data concerning the internal medium of goldfish: fish kept for 5-9 days in various artificial media*

| | External medium | | |
|--------------------------------------|---------------------------------|-------------------------|----------------------------------|
| | A. Sodium sulphate solution (6) | B. De-ionized water (5) | C. Choline chloride solution (6) |
| Plasma sodium: initial | 169.7 ± 9.7*** | 167.3 ± 8.8* | 155.1 ± 9.8** |
| final | 121.8 ± 4.6 | 116.0 ± 10.6 | 110.7 ± 5.8 |
| Plasma chloride: initial | 135.6 ± 6.6*** | 141.2 ± 3.7** | 131.3 ± 8.1*** |
| final | 79.8 ± 9.2 | 88.3 ± 10.1 | 90.2 ± 3.7 |
| Blood pH: final | 7.91 ± 0.068 | 7.61 ± 0.022 | 7.50 ± 0.089 |
| Total plasma CO ₂ : final | 18.3 ± 3.3 | 4.2 ± 0.35 | 5.3 ± 0.93 |
| Total plasma ammonium: final | 270 ± 58 | 315 ± 26 | 304 ± 28 |

Same units as in Table 1.

P_{Na} , P_{Cl} . Comparison between initial and final concentration: * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$.

Statistical comparison. pH: A, B, $P < 0.001$; A, C, $P < 0.001$. Total CO₂: A, B, $P < 0.001$; A, C, $P < 0.001$.

Comparison with tap-water fish (values Table 1). pH: A, $P < 0.001$.

Total CO₂: A, $P < 0.001$; B, $P < 0.001$; C, $P < 0.001$.

Total Am.: A, NS.; B, $P < 0.05$; C, $P < 0.05$.

might have been expected; it would seem that a diminution of chloride level brings about a reduction of internal sodium.

This phenomenon was verified in the second series of fish kept either in de-ionized water or in sodium sulphate or in choline chloride solutions (Table 2).

Plasma sodium and chloride values were determined before and after adaptation. In all cases there was a simultaneous reduction of the two concentrations even in fish in sodium sulphate which only survived 5 days. This confirmed that it is not possible to cause selective depletion of sodium or chloride in the internal medium by removing the ion from the external medium.

The losses of sodium and chloride in any adaptation medium were checked in this

Table 3. *Body weight and internal sodium and chloride changes in goldfish in relation to pretreatment*

| Total loss | Conservation medium | | |
|--|------------------------------|--------------------------------------|---------------------------------------|
| | De-ionized water (9 days) | Sodium sulphate solution (5 days) | Choline chloride solution (9 days) |
| Body weight (% initial weight) | -10.1 | +1.4 | -8.2 |
| Na _{int} (μ -equiv/100 g initial weight) | -1655 | -955 | -1820 |
| Cl _{int} (μ -equiv/100 g initial weight) | -945 | -1155 | -1250 |

second experiment by recording daily the total quantities of the ions appearing in the aquarium. Table 3 shows the total losses of weight, sodium and chloride per 100 g initial weight.

Although the plasma concentrations of these two ions do not show any differences which can be correlated to the different compositions of the adaptation media, this is not the case for the acid-base equilibrium of the blood; it can be seen from Tables 1 and 2 that fish kept in sodium sulphate solution have a well-marked alkalosis ($P < 0.01$) compared with controls kept in tap water. This condition is accompanied by an increase of the total CO_2 concentration of the plasma, especially well marked in fish after a short stay in sodium sulphate solution. Fish kept in de-ionized water, on the other hand, have a plasma tending towards acidosis (difference of pH from control fish not significant, however) and a reduced total CO_2 level (difference highly significant in Table 2). Fish kept in choline chloride resemble fish kept in de-ionized water in showing a tendency towards acidosis and reduced total CO_2 level (see Table 2).

Table 1 shows that there is a considerable increase of plasma ammonia concentration in fish kept in de-ionized water or sodium sulphate solution. This increase, however, is probably a result of the experimental procedure: the fish were confined in relatively small volumes and at the end of the adaptation period the water was only changed every 2 or 3 days. It was changed every day, however, for the fish yielding the data recorded in Table 2. Comparison of these data with those from animals kept in running water is therefore more legitimate. In animals kept in de-ionized water or choline chloride solution there is a significant ($P < 0.05$) increase of about 60% of the plasma ammonia concentration. The increase recorded from the fish treated with sodium sulphate is not significant.

In conclusion, therefore, total absence of either Na^+ or Cl^- , or the presence of one accompanied by an impermeant co-ion, in an adaptation medium does not induce the selective depletion of either ion, but does upset the acid-base equilibrium.

This study of the effects of different pretreatments on the internal medium was followed by chloride flux measurements on the two groups of fish (adapted to de-ionized water and to sodium sulphate solution) which yielded the data given in Table 1.

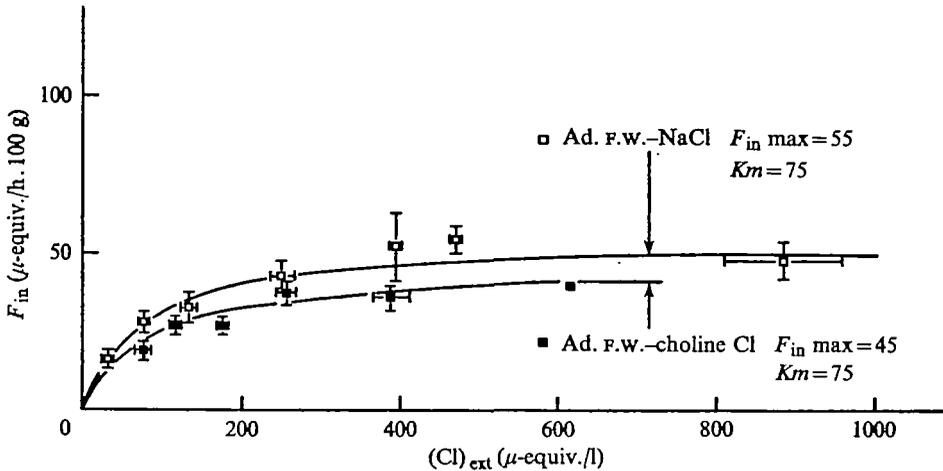


Fig. 2. Chloride influx from sodium chloride and choline chloride solutions as a function of external chloride concentration in *C. auratus* previously kept in tap water. Ad. F.w.-NaCl: fish adapted in fresh water, fluxes measured in NaCl solution; Ad. F.w.-choline Cl: fish adapted in fresh water, fluxes measured in choline Cl solution.

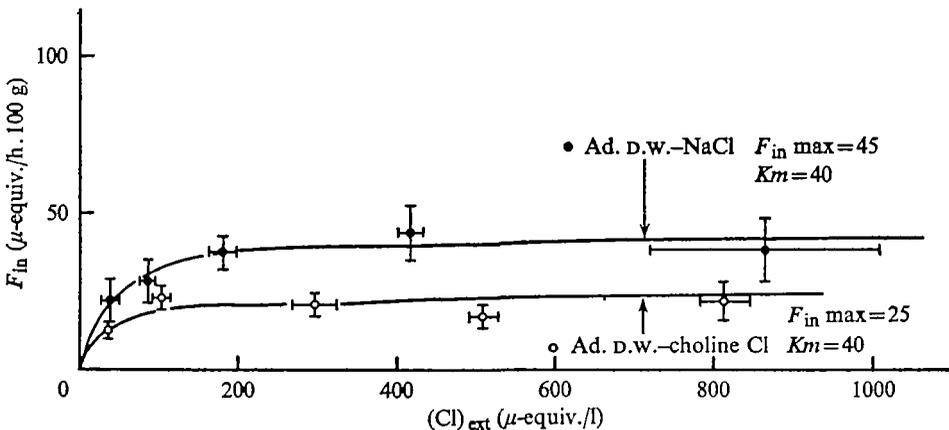


Fig. 3. Chloride influx from sodium chloride and choline chloride solutions as a function of external chloride concentration in *C. auratus* previously kept in de-ionized water (Ad. D.w.).

(2) Effect of external chloride concentration on chloride influx

In order to judge the effect on the chloride fluxes of the presence or absence of a permeant ion, experiments were carried out with or without external sodium (choline in the latter case replacing sodium).

The chloride influx values obtained are illustrated in Figs. 2-4; chloride influx, efflux and net flux data are given in Tables 4-9.

A general survey of all the results obtained shows a pattern common to all types of fish studied, i.e. the form of the curve of change in the fluxes as a function of the external chloride concentration, and the effect of exchanging choline for sodium; but there are also differences, concerning the kinetics of the pump mechanism, related to the different adaptation media.

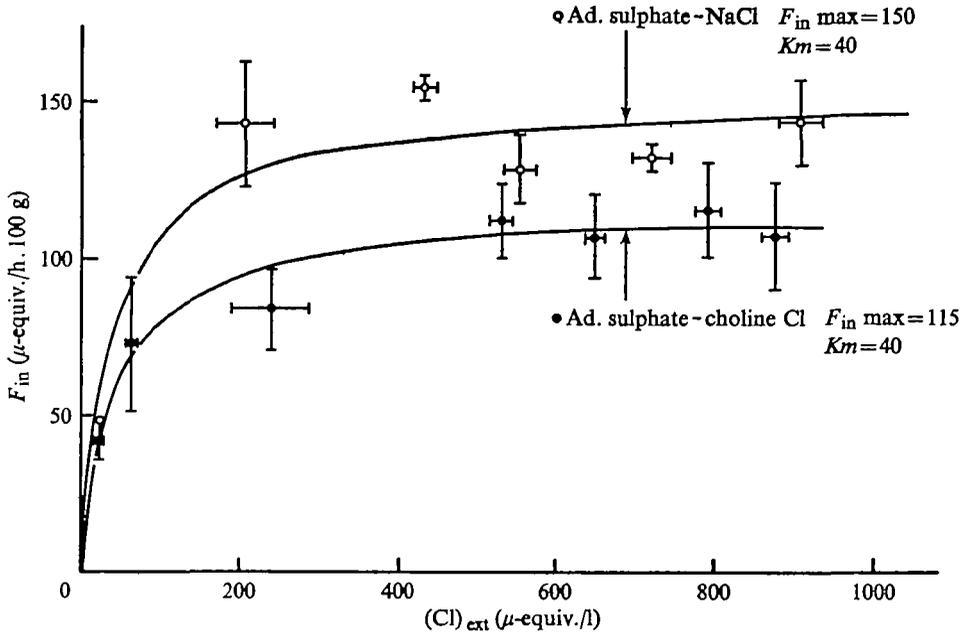


Fig. 4. Chloride influx from sodium and choline chloride solutions as a function of external chloride concentration in *C. auratus* previously kept in a sodium sulphate solution (Ad. sulphate).

Table 4. Branchial chloride exchange measured in a choline chloride solution, in relation to external chloride concentration

(Fish were previously kept in tap water.)

| External Cl concentration (mean \pm S.E.) | Chloride | | |
|--|--------------------|--------------------|---------------------|
| | Influx | Efflux | Net flux |
| 77.1 \pm 7.6 (7) | 19.0 \pm 3.2 (7) | 8.7 \pm 3.4 (7) | +10.3 \pm 3.4 (7) |
| 116.6 \pm 5.3 (8) | 26.6 \pm 2.6 (8) | 15.6 \pm 1.9 (8) | +11.0 \pm 3.1 (8) |
| 176.4 \pm 6.2 (7) | 25.6 \pm 2.7 (7) | 14.1 \pm 2.7 (7) | +11.5 \pm 1.7 (7) |
| 255.7 \pm 12.1 (4) | 37.6 \pm 3.5 (4) | 25.0 \pm 3.6 (4) | +12.6 \pm 3.6 (4) |
| 389.0 \pm 23.4 (4) | 35.7 \pm 3.1 (4) | 17.3 \pm 2.9 (4) | +18.4 \pm 1.8 (4) |
| 616.0 (2) | 40.1 (2) | 24.1 (2) | +16.0 (2) |

Fluxes in μ -equiv. h^{-1} (100 g) $^{-1}$; concentration in μ -equiv. l^{-1} . Number of flux periods in parentheses

General form of the influx curves

A consideration of Figs. 2-4 shows that the flux curves obtained here are analogous to sodium and chloride curves already published by various authors. They probably represent a state of saturation of the pump mechanism at high external chloride concentrations, corresponding to hyperbolic functions with an equation similar to that of Michaelis-Menten.

$$F_{in} = F_{in \max} \times C / (K_m + C),$$

where F_{in} is the influx, $F_{in \max}$ the maximum influx, C the external Cl concentration and K_m the external Cl concentration at which $F_{in} = \frac{1}{2}F_{in \max}$.

Table 5. Branchial chloride exchange measured in a sodium chloride solution, in relation to external chloride concentration

(Fish were kept previously in tap water.)

| External Cl concentration (mean \pm S.E.) | Chloride | | |
|--|---------------------|---------------------|---------------------|
| | Influx | Efflux | Net flux |
| 32.8 \pm 4.6 (5) | 15.8 \pm 2.4 (5) | 2.0 \pm 3.0 (5) | +13.8 \pm 3.9 (5) |
| 79.3 \pm 6.0 (6) | 27.7 \pm 3.2 (6) | 16.8 \pm 2.7 (6) | +10.9 \pm 5.1 (6) |
| 133.4 \pm 9.0 (5) | 32.5 \pm 4.4 (5) | 12.3 \pm 7.4 (5) | +20.2 \pm 9.0 (5) |
| 253.1 \pm 15.4 (7) | 42.4 \pm 5.2 (7) | 23.0 \pm 3.3 (7) | +19.4 \pm 4.4 (7) |
| 394.0 \pm 3.7 (4) | 51.9 \pm 10.6 (4) | 21.6 \pm 6.0 (4) | +30.3 \pm 5.8 (4) |
| 470.1 \pm 5.5 (8) | 53.2 \pm 4.3 (8) | 45.4 \pm 27.9 (8) | +7.8 \pm 8.2 (8) |
| 883.0 \pm 75.5 (4) | 47.3 \pm 6.1 (4) | 27.9 \pm 6.9 (4) | +19.4 \pm 4.0 (4) |

Same legend as in Table 4.

Table 6. Branchial chloride exchange measured in a choline chloride solution, in relation to external chloride concentration

(Fish were kept previously in de-ionized water.)

| External Cl concentration (mean \pm S.E.) | Chloride | | |
|--|--------------------|---------------------|----------------------|
| | Influx | Efflux | Net flux |
| 35.9 \pm 5.9 (9) | 13.4 \pm 3.4 (9) | 13.0 \pm 5.9 (9) | +0.4 \pm 3.0 (9) |
| 102.9 \pm 10.9 (7) | 22.8 \pm 3.8 (7) | 30.9 \pm 6.5 (7) | -8.1 \pm 8.5 (7) |
| 296.1 \pm 29.3 (7) | 20.9 \pm 3.4 (7) | 40.4 \pm 13.7 (7) | -19.5 \pm 12.1 (7) |
| 509.7 \pm 20.7 (7) | 17.5 \pm 3.7 (7) | 18.6 \pm 5.2 (7) | -1.1 \pm 3.0 (7) |
| 811.5 \pm 29.8 (8) | 22.5 \pm 5.9 (8) | 15.5 \pm 7.2 (8) | +7.0 \pm 4.5 (8) |

Same legend as in Table 4.

Table 7. Branchial chloride exchange measured in a sodium chloride solution, in relation to external chloride concentration.

(Fish were kept previously in de-ionized water.)

| External Cl concentration (mean \pm S.E.) | Chloride | | |
|--|--------------------|---------------------|---------------------|
| | Influx | Efflux | Net flux |
| 37.8 \pm 10.1 (4) | 21.9 \pm 7.0 (4) | 13.7 \pm 7.4 (4) | +8.2 \pm 8.9 (4) |
| 86.2 \pm 8.0 (4) | 27.9 \pm 6.9 (4) | 24.8 \pm 6.5 (4) | +3.1 \pm 11.9 (4) |
| 181.7 \pm 17.2 (6) | 36.8 \pm 4.8 (6) | 22.9 \pm 6.5 (6) | +13.9 \pm 7.2 (6) |
| 417.0 \pm 15.8 (5) | 43.4 \pm 7.6 (5) | 39.3 \pm 12.3 (5) | +4.1 \pm 14.9 (5) |
| 864.0 \pm 145.5 (6) | 38.5 \pm 9.6 (6) | 22.6 \pm 6.1 (6) | +15.9 \pm 5.9 (6) |

Same legend as in Table 4.

The values of K_m and of F_{in} max are recorded on the curves obtained for each adaptation medium and for the two groups of measurements made with or without sodium.

It should be noted that the influx more or less attains its maximum at external chloride concentrations above 3 K_m .

Table 8. *Branchial chloride exchange measured in a choline chloride solution, in relation to external concentration*

(Fish were kept previously in a sodium sulphate solution.)

| External Cl Concentration (mean \pm S.E.) | Chloride | | |
|---|----------------------|---------------------|----------------------|
| | Influx | Efflux | Net flux |
| 23.4 \pm 2.6 (5) | 41.5 \pm 5.4 (5) | 30.7 \pm 6.8 (5) | +10.8 \pm 2.5 (5) |
| 63.3 \pm 6.6 (6) | 73.0 \pm 21.0 (6) | 48.8 \pm 18.7 (6) | +24.2 \pm 4.6 (6) |
| 240.3 \pm 48.2 (3) | 84.4 \pm 15.1 (3) | 59.5 \pm 11.3 (3) | +24.9 \pm 26.2 (3) |
| 536.5 \pm 19.7 (4) | 113.2 \pm 12.5 (4) | 70.2 \pm 16.3 (4) | +43.0 \pm 13.9 (4) |
| 655.0 \pm 13.3 (5) | 99.4 \pm 13.0 (5) | 40.3 \pm 8.3 (5) | +59.1 \pm 14.4 (5) |
| 792.6 \pm 14.8 (5) | 115.7 \pm 15.2 (5) | 69.3 \pm 16.4 (5) | +46.4 \pm 16.6 (5) |
| 876.5 \pm 16.2 (6) | 107.2 \pm 16.8 (6) | 69.0 \pm 8.2 (6) | +38.2 \pm 10.3 (6) |

Same legend as in Table 4.

Table 9. *Branchial chloride exchange measured in a sodium chloride solution, in relation to external chloride concentration*

(Fish were kept previously in a sodium sulphate solution.)

| External Cl concentration (mean \pm S.E.) | Chloride | | |
|---|----------------------|---------------------|----------------------|
| | Influx | Efflux | Net flux |
| 25.0 (2) | 48.0 (2) | 23.3 (2) | +24.7 (2) |
| 206.4 \pm 33.7 (8) | 142.4 \pm 19.8 (8) | 75.7 \pm 15.6 (8) | +66.7 \pm 6.9 (8) |
| 434.0 \pm 17.5 (3) | 153.8 \pm 3.9 (3) | 92.3 \pm 9.1 (3) | +61.5 \pm 12.8 (3) |
| 556.0 \pm 18.2 (3) | 128.4 \pm 10.3 (3) | 32.3 \pm 17.8 (3) | +96.1 \pm 26.3 (3) |
| 721.5 \pm 24.5 (4) | 131.9 \pm 5.1 (4) | 59.0 \pm 23.1 (4) | +82.9 \pm 17.1 (4) |
| 907.5 \pm 25.8 (4) | 143.7 \pm 13.4 (4) | 67.9 \pm 12.2 (4) | +75.8 \pm 7.5 (4) |

Same legend as in table 4.

Table 10. *Maximal chloride exchange fluxes.*

(Effect of various pretreatments and of substitution of sodium for an impermeant cation during flux measurements.)

| Adaptation medium | Fluxes measured in | Fluxes measured at $Cl_{ext} \geq 3 K_m$ | | |
|----------------------|-----------------------|--|-----------------------|--------------------------|
| | | $F_{in} \max$ | $F_{out} \max$ | $F_{net} \max$ |
| Tap water | Na Cl | 50.13 \pm 2.99** (20) | 33.24 \pm 4.29 (20) | +16.89 \pm 3.97 (20) |
| | Choline chloride | 37.41 \pm 1.90 (10) | 21.81 \pm 2.35 (10) | +15.60 \pm 1.78 (10) |
| Sodium sulphate | Na Cl | 133.82 \pm 5.65** (19) | 61.57 \pm 6.25 (19) | +72.25 \pm 6.00** (19) |
| | Choline chloride | 105.46 \pm 6.61 (23) | 61.81 \pm 5.62 (23) | +43.65 \pm 6.56 (23) |
| De-ionized water | Na Cl | 39.22 \pm 4.17*** (17) | 27.48 \pm 4.84 (17) | +11.74 \pm 5.23* (17) |
| | Choline chloride | 20.19 \pm 2.59 (23) | 23.61 \pm 5.37 (23) | -3.42 \pm 4.66 (23) |

For units, see preceding tables. Comparison between fluxes measured in sodium chloride solution and in choline chloride solution: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

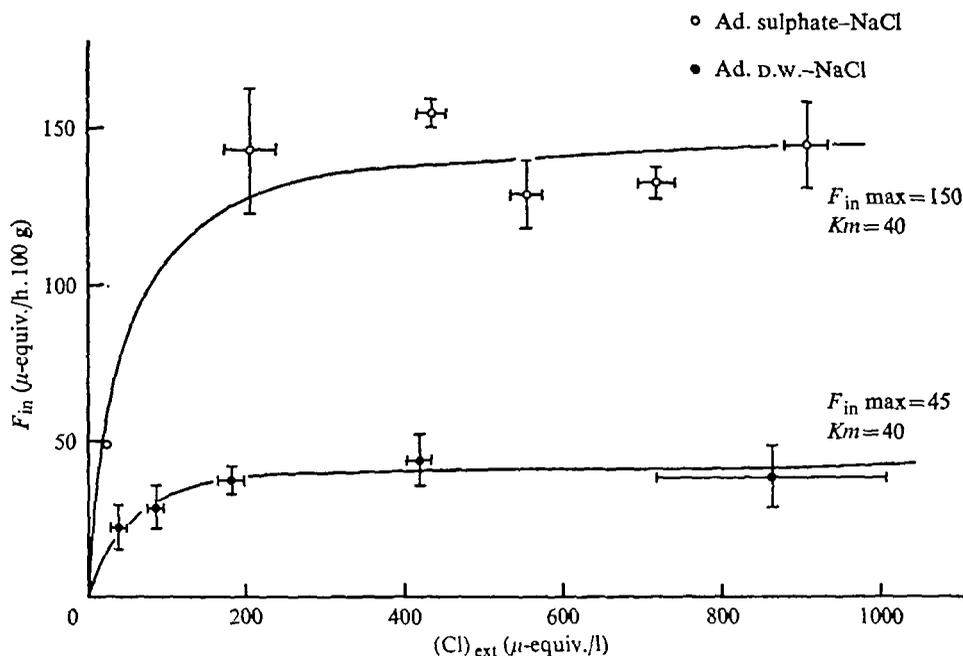


Fig. 5. Chloride influx from sodium chloride solution as a function of external chloride concentration in goldfish pretreated in deionized water or in a sodium sulphate solution.

Effect of replacement of choline by sodium

The curves of fluxes measured in the presence of sodium have a higher F_{in} max than those measured in its absence. This difference is small in freshwater-adapted fish but well-marked in fish adapted to de-ionized water.

Only the F_{in} max is affected by the presence or absence of sodium. The value of Km remains the same.

The averages of the maximum fluxes observed for external chloride concentrations more than 3 times the value of Km , $[Cl]_{ext} > 3 Km$, clearly show the significant increase of F_{in} max in the presence of sodium (see Table 10). This increase would suggest that chloride absorption is not totally independent of sodium absorption.

Effects of different pretreatments on the kinetic characteristics of chloride absorption

The changes in the internal medium brought about by adapting fish to different external media (de-ionized water, sodium sulphate solution), are reflected, when compared with control animals, not only in modifications of the maximum absorption rate but also in changes of the affinity constant (Km).

(1) *Freshwater/de-ionized water.* Adaptation to de-ionized water results in an increase of the apparent affinity of the hypothetical carrier for its substrate – as shown by the diminution of the value of Km (Figs. 2, 3). The F_{in} max values in sodium chloride or choline chloride solutions (Table 10) are significantly lower in fish adapted to de-ionized water ($P < 0.05$ and $P < 0.001$ respectively).

(2) *De-ionized water/sodium sulphate solution.* The apparent affinity of the transport

mechanism is unchanged by adaptation to either of these media, but the maximum influx is very significantly increased in fish pre-adapted in sodium sulphate. (Figs. 3, 4; Table 10). This increase is significant whether fluxes are measured in NaCl or choline chloride solutions ($P < 0.001$).

(3) *Freshwater/sodium sulphate solution.* The fish adapted to sodium sulphate show an increased F_{in} max (Table 10, $P < 0.001$) and an increased apparent affinity of the hypothetical carrier (Figs. 2, 4) in both of the measuring media, when compared with control animals.

Fig. 5 illustrates the two extremes of chloride transport observed in this series of experiments. The maximal influx from a NaCl solution is three times higher in fish pretreated with sodium sulphate than in fish adapted to de-ionized water, the Km values being identical.

(3) *Changes in efflux and net flux as a function of the experimental and adaptation media*

Effect of external chloride concentration. Tables 4-9 show that the efflux increases in relation to the external concentration of chloride irrespective of the type of adaptation or the experimental solution. Plotted graphically these results would show that the change of the efflux is a hyperbolic function, as is the change of the influx.

Effect of sodium in the experimental water. It can be seen from Table 10 that the maximum effluxes (F_{out} max), calculated by averaging all the efflux values for $[Cl]_{ext} > 3 Km$ are not significantly modified by the presence or absence of sodium in the external medium.

The maximum net fluxes (F_{net} max) which in control animals are not influenced by the nature of the accompanying ion in the experimental water, are considerably higher in fish adapted to sodium sulphate or to de-ionized water in the presence of a permeant cation ($P < 0.01$ and $P < 0.05$ respectively).

Modifications resulting from the various adaptations. Statistical comparison of the F_{out} max and F_{net} max from control fish and from fish adapted to de-ionized water shows that there is no difference between the effluxes but that the net flux is lower in fish adapted to de-ionized water especially when the measurements are made without sodium in the external medium ($P < 0.02$) (Table 10).

Adaptation to sodium sulphate solution causes significant rises of efflux and net flux ($P < 0.001$ in all experimental media).

(4) *Direct demonstration of the Cl^-/HCO_3^- exchange*

In addition to studying the various aspects of chloride absorption, an attempt was made to demonstrate directly the existence of the Cl^-/HCO_3^- exchange mechanism. This verification can only be made when the external sodium concentration is around zero. It was essential, as discussed above, to subtract the ammonium net flux from the total base net flux, thereby taking into account the diffusion of NH_3 and its reaction with the respiratory CO_2 in the aquarium water to give NH_4HCO_3 which is titrated (Fig. 6).

Figs. 7 and 8 gives base net fluxes as function of chloride net fluxes. The correlation is highly significant ($P < 0.001$). Such data were obtained from the three types of fish, but to simplify the figures only values obtained at $[Cl]_{ext}$ above $3Km$ have been shown; but a good correlation ($P < 0.01$) exists when all the data are considered.

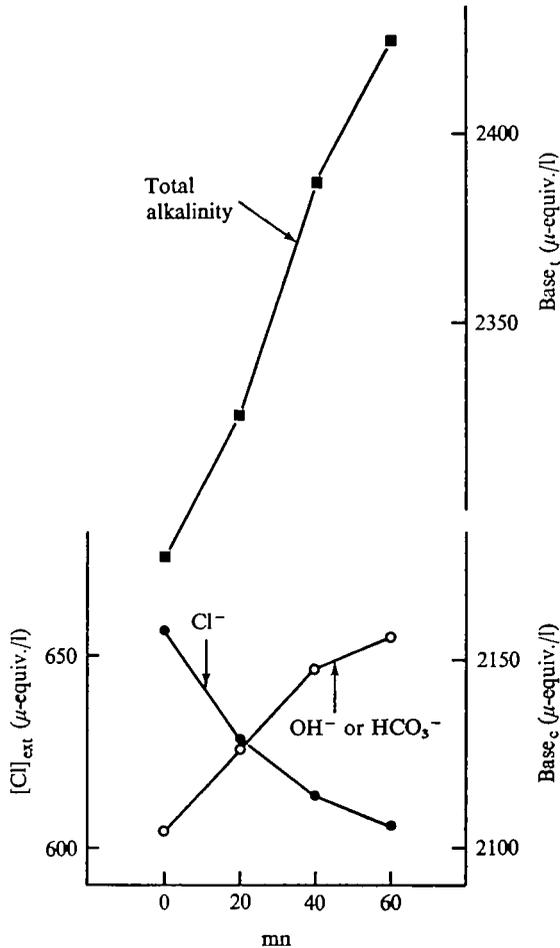


Fig. 6. Changes as a function of time (minutes) in external concentrations of Cl^- and total base (base_e) or base corrected for ammonia present in the bath (base_c), in a typical experiment.

In Fig. 7 the ordinate is the total base net flux. The slope of the regression line (0.68 ± 0.02) is significantly different from 1. The line meets the ordinate at -33.41 ± 4.96 which is significantly different from zero ($P < 0.001$). This difference probably represents the average rate of ammonium excretion occurring during the experiments since direct measurements of this excretion rate give a mean of $-29.4 \pm 3.1 \mu\text{M}/\text{h} \cdot 100 \text{ g}$ ($n = 50$).

In Fig. 8 the ordinate represents the base net flux after correction for the ammonium net flux. The correction does not significantly change the regression-line slope (0.71 ± 0.02) but shifts the line to intersect the ordinate at (-3.43 ± 5.69) which is not significantly different from zero.

Table 11 compares the ammonia-excretion values in the three groups of fish during ionic flux measurements in choline chloride with that obtained in fish during adaptation to this medium. It may be seen in Table 11 that 'de-ionized' fish excrete significantly more ammonia than 'tap-water' fish and 'sodium sulphate fish'. Thus the correction

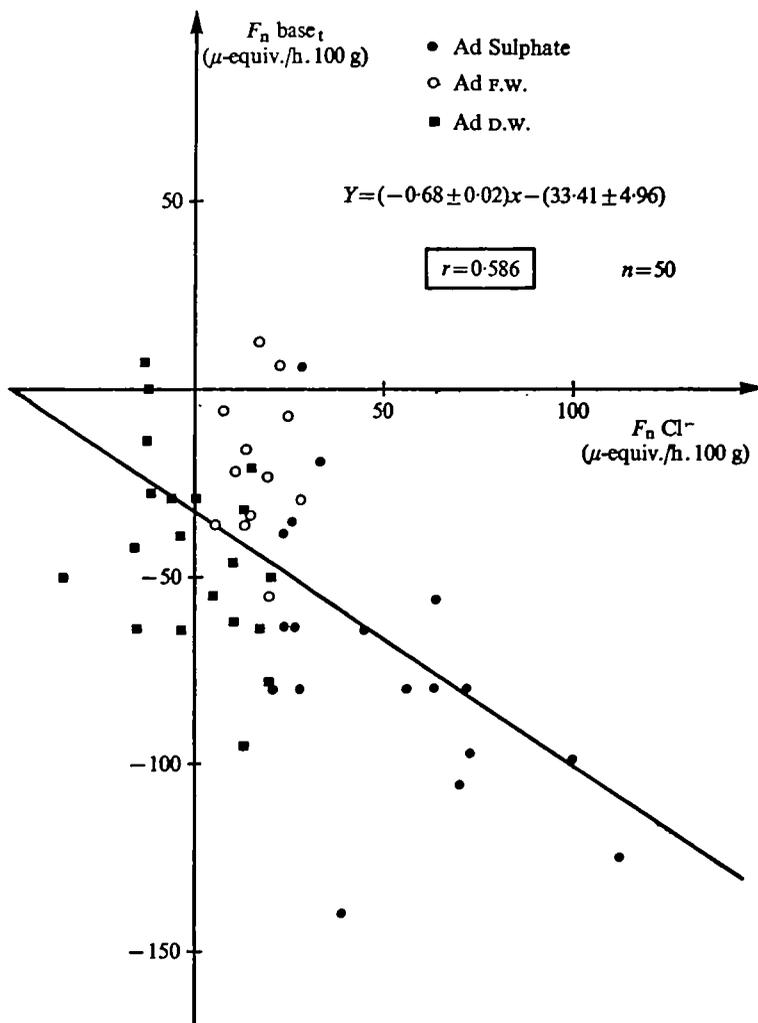


Fig. 7. Correlation between total base excretion and chloride uptake from a choline-Cl solution in *C. auratus*.

factor for ammonia excretion was different for the various groups of fish. The highest rate of ammonia excretion is observed in fish during pre-adaptation in choline chloride. This confirms and extends previous observations by Maetz (1973).

DISCUSSION

(1) *Kinetics of sodium and chloride absorption*

Present data about chloride uptake will be discussed in the light of previous observations concerning sodium uptake. The results given above in relation to Figs. 2-4 show that a relationship between chloride influx and external chloride concentration exists and that this relationship is a hyperbolic function indicating that at higher external chloride concentrations a saturation phenomenon appears. This

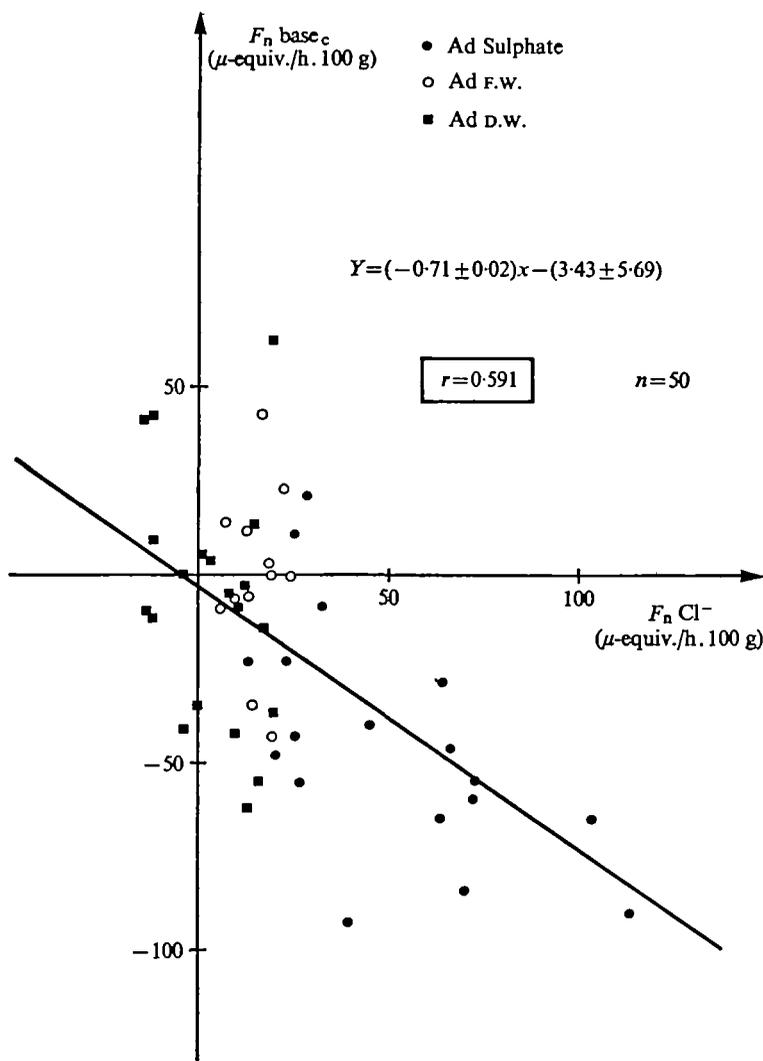


Fig. 8. Correlation between the net flux of base corrected for the net flux of ammonia and chloride uptake from a choline chloride solution in *C. auratus*.

characteristic of the chloride absorption mechanism has already been described by Shaw (1960a) in the crayfish and by Kerstetter & Kirschner (1972) in the trout. A similar relationship is also found in the goldfish for sodium as shown by Maetz (1972, 1973). Important differences in the F_{in} max or Km appear, however, when these sodium or chloride relationships are considered with reference to the different pretreatments. Although the maximum influx values for chloride in freshwater-adapted animals (45–55 $\mu\text{-equiv./h. 100 g}$) are similar to the values for sodium influx published previously (F_{in} max = 65 $\mu\text{equiv./h. 100 g}$), the Km values for the two ions differ appreciably. The affinity constant (Km) for chloride (40–75 $\mu\text{M/l}$) was found in *Carassius* to be lower than the sodium value (300 $\mu\text{M/l}$ according to Maetz, 1972, 1973). A similar difference in the Km values was also reported for other freshwater animals,

Table 11. *Branchial ammonia excretion in goldfish in relation to pretreatment*

| Pretreatment solution | Ammonium excretion, $\mu\text{M}\cdot\text{h}^{-1} (100\text{ g})^{-1}$ |
|-----------------------|--|
| Tap water (a) | 18.4 ± 1.9 (32) |
| De-ionized water (b) | $44.6 \pm 4.6^{**}$ (35) |
| Sodium sulphate (c) | $28.0 \pm 2.3^*$ (27) |
| Choline chloride (d) | $98.7 \pm 6.5^{**}$ (9) |

abc, excretion measured after transfer to choline chloride; *d*, excretion measured during pretreatment in choline chloride.

Statistical comparisons: comparison with tap water, * $P < 0.01$; ** $P < 0.001$. Comparison: *b-c*, $P < 0.01$; *b-d*, $P < 0.001$; *c-d*, $P < 0.001$.

Astacus pallipes (250 $\mu\text{M/l}$ against 100 $\mu\text{M/l}$; Shaw, 1959, 1960*a, b*), *Salmo gairdneri* (450 $\mu\text{M/l}$ against 200 $\mu\text{M/l}$; Kerstetter *et al.*, 1970; Kerstetter & Kirschner, 1972). This difference indicates that the chloride transport mechanisms has a much higher affinity than the sodium mechanism. Perhaps this affinity difference should be considered in relation to the concentration differences of these two ions in this adaptation medium (100 $\mu\text{M/l}$ sodium and 40 $\mu\text{M/l}$ chloride). The affinity differences would allow for a similar absorption of the two ions in spite of the concentration differences.

Pretreatment in artificial media results in important differences in the maximum absorption intensities of chloride and sodium. Thus animals adapted to de-ionized water have a chloride F_{in} max of 25 $\mu\text{-equiv./h}\cdot 100\text{ g}$ in choline chloride, while Maetz (1973) found the sodium F_{in} max to be 120 $\mu\text{-equiv./h}\cdot 100\text{ g}$ in sodium sulphate. This would seem to show that when the internal concentration of the two ions becomes reduced by pretreatment, readjustment of the internal sodium level takes priority over that of the chloride level.

Pre-adaptation in sodium sulphate solution also brings an important difference in the maximum rates of influx of chloride and sodium. This treatment results in a characteristic reduction of the sodium influx (Garcia-Romeu & Maetz, 1964) and a large increase of the chloride influx maximum (Fig. 4, Table 10).

Thus, for a given Km value, the maximum fluxes differ very considerably between fish treated with de-ionized water and fish treated with sodium sulphate (Table 10). The change implies either an augmentation of the chloride turnover or an increase of the number of transport sites available.

The difference of the Km values of pretreated fish as compared with control fish remains to be explained. A reduction of Km , indicating an increase of the hypothetical carrier affinity, may be the result of the effect of prolonged calcium lack on the structure of the carrier. Cuthbert & Maetz (1972) found that the addition of calcium to the external medium slowed up sodium exchange across the goldfish gill. Whether the calcium acts by modifying the Km or the F_{in} max has still to be ascertained.

(2) Confirmation of a $\text{Cl}^-/\text{HCO}_3^-$ exchange

The significant correlation ($P < 0.001$) between quantity of base excreted and quantity of chloride absorbed found in the present work, directly confirms the $\text{Cl}^-/\text{HCO}_3^-$ exchange postulated by Krogh (1939) and indirectly demonstrated by Maetz & Garcia Romeu (1964) in the goldfish.

There remains the problem of the stoichiometry of this exchange (4Cl^- for 3HCO_3^-) for theoretically a 1 to 1 exchange should be expected on the grounds of the equilibration of electrical charges. Garcia Romeu *et al.* (1969) found a 2Cl^- for 3HCO_3^- exchange in *Calyptocephalella gayi*, while F. Garcia Romeu & J. Ehrenfeld (personal communication) found a 1 to 1 exchange in *Rana esculenta*.

The chloride/endogenous ion exchange in the goldfish parallels the Na^+/H^+ or $\text{Na}^+/\text{NH}_4^+$ exchange (Maetz, 1973). The stoichiometry of this latter exchange is also 4Na^+ for 3 endogenous ions (H^+ or NH_4^+). The fact that the two exchange mechanisms involve different endogenous ions may explain the independence of the sodium and chloride absorptions, in so far as the counter-ions NH_4^+ , H^+ or HCO_3^- are available directly from the internal medium. In the functional scheme of the branchial cell advanced by one of us (see fig. 15 in Maetz, 1971) it was suggested, however, that the H^+ and HCO_3^- ions are produced *de novo* by hydration of respiratory CO_2 catalysed by carbonic anhydrase. The sodium and chloride pumps would in this case be closely linked. The degree of independence of the two pumps would thus depend on the source of the endogenous counter-ions.

(3) *The problem of the independence of the sodium and chloride absorption mechanisms*

The present study confirms that the goldfish can, to a certain extent, absorb chloride independently of sodium from the external medium. This is clearly the case for fish put into choline chloride after being kept in freshwater and is even more striking in fish previously kept in sodium sulphate solution. The independence of the sodium and chloride absorption mechanisms is relative, however, as can be seen from two types of experiment.

The first type reveals a short-term interdependence of the two mechanisms. The data summarized in Table 10 suggest that the efficiency of the chloride absorption mechanism is increased when sodium replaces choline. The effect is that of a significant rise of $F_{\text{in}} \text{max}$ while the $F_{\text{out}} \text{max}$ remains the same. In the trout, on the other hand, this change of ions does not modify either the influx or efflux of chloride (Kerstetter & Kirschner, 1972). Shaw (1964), however, also found an interdependence of the sodium and chloride absorption mechanisms, but manifesting itself in a different way when an impermeant ion (K^+) was replaced by Na^+ , the chloride influx remained the same but the efflux decreased. According to Shaw it was as if a Cl^-/Cl^- exchange was replaced by a $\text{Cl}^-/\text{other endogenous ion}$ exchange, the substitute ion being probably HCO_3^- . An interdependence of the sodium and chloride absorption mechanisms has also been shown in other biological membranes, such as the intestinal mucosa of the flounder *Pseudopleuronectes americanus* (Huang & Chen, 1971), the cornea of *Rana catesbiana* (Zadunaisky, 1972) and the skin of *Calyptocephalella gayi* (Garcia-Romeu *et al.* 1969).

The second type of observation indicates a long-term interdependence of the two absorption mechanisms. Thus, as has been discussed above, fish coming from freshwater into a saline solution with an impermeant ion such as choline chloride have a positive ionic balance for the permeant ion (the Cl^-) for the 5 or 6 h that the flux measurements last (Table 4). Considered over a long period, however, the chloride balance becomes markedly negative (Table 3). It would seem that the diminution of internal sodium caused by the lack of this ion in the external medium results, over a

long period, in a parallel drop of the internal chloride concentration. After transfer from fresh water to sodium sulphate solution there is also a drop of the internal sodium and chloride concentrations. It was because of this that it was impossible to produce long-term selective depletions of either ion from the internal medium.

This long-term interdependence of the sodium and chloride absorption mechanisms is related to the regulatory mechanisms controlling the internal concentrations of these ions.

(4) *Regulatory mechanisms of the internal sodium and chloride concentrations: importance of the internal acid-base equilibrium*

In his review 'The control of the salt balance in the Crustacea' Shaw (1964) quotes experimental data showing that variations of the mass of the sodium or chloride trigger off feed-backs controlling the absorption intensities of these ions. In *Carassius* also, Bourget, Lahlou & Maetz (1964) showed that intraperitoneal injections of hypertonic or hypotonic Ringer solutions, changing the internal sodium or chloride concentrations, resulted in changes in the absorption rates of these ions towards a re-establishment of the initial equilibrium.

The stimulus afforded by the variation of the internal masses or concentrations of sodium and chloride is not, however, the only factor concerned. Thus, all the experimental pre-treatments result in a reduction of internal sodium and chloride, but transfer to a NaCl solution does not stimulate the absorption mechanisms of these two ions simultaneously. Fish kept in de-ionized water or in choline solution, for example, subsequently show an accelerated absorption of Na^+ but not of Cl^- (see Table 10, Garcia-Romeu & Maetz (1964) and Maetz (1973)). Animals pretreated in sodium sulphate on the other hand subsequently accelerate their Cl^- absorption but not of Na^+ (Table 10 and Garcia-Romeu & Maetz (1964)). Some other stimulus must determine the relative pumping rates of these ions. Shaw (1964) also, by putting *Astacus* in solutions of either Na_2SO_4 or KCl, showed that the gill could vary the relative intensities of its Na^+ and Cl^- absorptions. He suggested that the stimulus was related to the difference between the internal concentrations of sodium and chloride, perhaps in fact, the pH of the internal medium. The results given in Tables 1 and 2 would suggest that the intervening factor is internal pH variation rather than a concentration difference between Na^+ and Cl^- .

Tables 1 and 2 demonstrate that considerable disturbance of the acid-base equilibrium occurs when goldfish are kept in media containing a single permeant ion; a tendency towards acidosis results from choline chloride treatment and a tendency towards alkalosis from sodium sulphate treatment. The acidosis shown by fish adapted to de-ionized water indicates that the absence of sodium from the external medium plays a more important role than the absence of chloride. This dominance of the Na^+ ion over the Cl^- ion in the regulatory processes remains unexplained. It can also be seen when fish adapted to de-ionized water are put into a sodium chloride solution, for they react by adjusting first the internal sodium level by absorbing Na^+ more rapidly than Cl^- . In any case, the direction of the shift of the acid-base balance, *i.e.* towards acidosis or alkalosis, determines the relative intensities of the sodium and chloride absorptions from NaCl solutions. Alkalosis, characterized by an increase of the plasma bicarbonate concentration, results in an acceleration of chloride uptake by

The $\text{HCO}_3^-/\text{Cl}^-$ exchange. Acidosis, characterized by raised H^+ and plasma ammonia concentrations, brings about a stimulation of sodium absorption by H^+/Na^+ or $\text{NH}_4^+/\text{Na}^+$ exchanges.

As to why the acid-base balance becomes perturbed by treatments in the various solutions one can only theorize. According to Maetz (1973) when sodium is absent from the external medium the ammonium ion is excreted in the non-ionic form of NH_3 . The H^+ ions of metabolic origin, which would normally be excreted as such or as part of the NH_4^+ exchanged against Na^+ , accumulate in the internal medium partly as NH_4^+ and partly neutralized by the buffering system of the blood. Animals kept in choline chloride or de-ionized water do in fact show a reduction of bicarbonate level and an increase of the plasmatic concentration of the ammonium ion (Table 2).

In the absence of chloride in sodium sulphate medium, on the other hand, the observed alkalosis would be the result of a blockage of the $\text{Cl}^-/\text{HCO}_3^-$ exchange. The alkalosis is probably partly alleviated by a CO_2 retention, such as Dejours (1969) observed.

A comparison of Tables 1 and 2 suggests that the perturbations of the acid-base balance show an initial acute phase after the transfer of the fish to an artificial medium, followed after several weeks by a tendency to return to the original equilibrium. The mechanism of this re-equilibration is unknown.

Under the present experimental conditions it would certainly seem that the stimulus *pH-shift* takes precedence over the stimulus *internal Na^+ and Cl^- concentration drop* in the feedback modulating the relative intensities of the branchial absorptions of sodium and chloride and thus regulating their internal concentrations.

SUMMARY

1. Pretreatment of *Carassius auratus* in artificial media (de-ionized water, sodium sulphate or choline chloride solutions), resulted in simultaneous loss of internal Na and Cl, variations in the total CO_2 and NH_3 plasma content; blood pH shifts towards alkalosis in fish treated with sodium sulphate and towards acidosis in fish treated with de-ionized water or choline chloride.

2. Fish kept in de-ionized water and in sodium sulphate solution were compared with control (tap water) fish. Branchial chloride fluxes were studied as a function of external chloride concentration, the accompanying cation being impermeant (choline) or permeant (sodium). For chloride influx saturation kinetics prevails, the maximal rate of uptake being about 3 times faster in sodium sulphate than in fish kept in de-ionized water, the apparent affinity of the uptake mechanisms (*K_m*) remaining the same.

3. Substitution of choline by sodium increases the maximal rate of influx without changing the *K_m* or efflux in all types of fish.

4. The quantity of base excreted is correlated to the quantity of Cl^- absorbed confirming the $\text{Cl}^-/\text{HCO}_3^-$ exchange process.

5. The degree of linkage between Na and Cl absorption is discussed in relation to the maintenance of acid-base balance by the gill.

The authors wish to thank Dr B. M. Walshe-Maetz for translating the manuscript and Mr R. Tanguy for technical help with the electronics and computer programs.

REFERENCES

- BOURGUET, J., LAHLOU, B. & MAETZ, J. (1964). Modifications expérimentales de l'équilibre hydro-minéral et osmorégulation chez *Carassius auratus*. *Gen. comp. Endocr.* **4**, 563-76.
- CUTHBERT, A. W. & MAETZ, J. (1972). The effect of calcium and magnesium on sodium fluxes through gills of *Carassius auratus* L. *J. Physiol., Lond.* **221**, 633-43.
- DEJOURS, P. (1969). Variations of CO₂ output of a fresh-water teleost upon change of the ionic composition of water. *J. Physiol. Lond.* **202**, 113P-14P.
- GARCIA-ROMEU, F. & MAETZ, J. (1964). The mechanism of sodium and chloride uptake by the gills of a freshwater fish, *Carassius auratus*. I. Evidence for an independent uptake of sodium and chloride ions. *J. gen. Physiol.* **47**, 1195-207.
- GARCIA-ROMEU, F., SALIBIAN, A. & PEZZANI-HERNANDEZ, S. (1969). The nature of the *in vivo* sodium and chloride uptake mechanisms through the epithelium of the chilean frog, *Calyptocephalella gayi* (Dum. et Bibr., 1841). *J. gen. Physiol.* **53**, 816-35.
- HUANG, K. C. & CHEN, T. S. T. (1971). Ion transport across intestinal mucosa of winter flounder, *Pseudopleuronectes americanus*. *Am. J. Physiol.* **220**, 1734-8.
- KERSTETTER, T. H. & KIRSCHNER, L. B. (1972). Active chloride transport by the gills of rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **56**, 263-72.
- KERSTETTER, T. H., KIRSCHNER, L. B. & RAFUSE, D. D. (1970). On the mechanism of sodium ion transport by the irrigated gills of rainbow trout (*Salmo gairdneri*). *J. gen. Physiol.* **56**, 342-59.
- KROGH, A. (1939). *Osmotic Regulation in Aquatic Animals*. Cambridge University Press.
- 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. (1971). Fish gills: mechanism of salt transfer in fresh water and sea water. *Phil. Trans. Roy. Soc. Lond. B* **262**, 209-51.
- MAETZ, J. (1972). Branchial sodium exchange and ammonia excretion in the goldfish *Carassius auratus*. Effects of ammonia-loading and temperature changes. *J. exp. Biol.* **56**, 601-20.
- MAETZ, J. (1973). Na⁺/NH₄⁺, Na⁺/H⁺ exchanges and NH₃ movement across the gill of *Carassius auratus*. *J. exp. Biol.* **58**, 255-75.
- 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 NH₄⁺/Na⁺ and HCO₃⁻/Cl⁻ exchanges. *J. gen. Physiol.* **47**, 1209-27.
- SHAW, J. (1959). The absorption of sodium ions by the crayfish, *Astacus pallipes* Lereboullet. I. The effect of external and internal sodium concentrations. *J. exp. Biol.* **36**, 126-44.
- SHAW, J. (1960a). The absorption of chloride ions by the crayfish *Astacus pallipes* Lereboullet. *J. exp. Biol.* **37**, 557-72.
- SHAW, J. (1960b). The absorption of sodium ions by the crayfish, *Astacus pallipes* Lereboullet. II. The effect of external anion. *J. exp. Biol.* **37**, 534-47.
- SHAW, J. (1964). The control of the salt balance in the crustacea. *Symposia Exp. Biol.* **18**, 237-54.
- TANGUY, R. (1970). Ensembles électroniques destinés à l'étude de l'osmorégulation d'animaux aquatiques. *Bull. Inf. Sci. Techn. C.E.A.* **144**, 11-15.
- DE VOOYS, G. G. N. (1968). Formation and excretion of ammonia in teleostei. I. Excretion of ammonia through the gills. *Arch. int. Physiol. Biochim.* **76**, 268-73.
- ZADUNAISKY, J. A. (1972). Sodium activation of chloride transport in the frog cornea. *Biochim. biophys. Acta* **282**, 255-7.