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

1. *Evidence for an independent uptake of sodium and chloride ions*

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**Abstract** *Carassius auratus* placed in a dilute sodium chloride solution (400 μM) is able to absorb sodium and chloride ions at very different rates, or to absorb one ion and to lose the other. This is the case not only for fish which have been previously kept in choline chloride or sodium sulfate solutions or deionized water, in order to stimulate their absorption processes, but also in control fish which have not been deprived of sodium or chloride. The absorption of sodium or chloride appears to be unaffected by the presence of a non-permeant co-ion such as choline or sulfate. Conductivity measurements of the external medium show that during ion uptake the conductivity is constant or increases slowly. This suggests the existence of exchange processes between the ions absorbed and endogenous ions excreted. It is unlikely that potassium or calcium is exchanged for sodium, because of the low permeability of the gills to these ions. Finally, the flux ratios observed for both sodium and chloride ions in the present investigation can only be explained, in relation to their electrochemical gradients across the gills, in terms of active transport.

Fresh water organisms maintain a concentration gradient between the electrolytes of their internal medium and those of their very dilute external medium. Invertebrates, fish, and amphibians have been the objects of numerous investigations designed to elucidate the processes by which they maintain this gradient. Special attention has been paid to sodium- and chloride-concentrating mechanisms. In this field diverse experimental techniques on various taxonomic groups yield results which may be classified into two categories: (a) those which indicate the presence of a special transport mechanism for the sodium (active transport) while the chloride follows passively (Ussing, 1954)
and (b) those which suggest independent concentration mechanisms for both sodium and chloride: active transport for both ions according to Jørgensen, Levi, and Zerah (1954) and Zadunaisky, Candia, and Chiarandini (1963), transport associated with ionic interchange processes which account for the different rates of absorption of the cation and anion, according to Krogh (1937 a, b, 1939) and Shaw (1960 a, b, c).

It is of particular interest that the skins of the amphibians Rana esculenta and R. temporaria in vivo (Jørgensen et al., 1954) gave results contradictory to those obtained in vitro (Ussing, 1954). The reasons for these contradictory results remain to be elucidated. Shaw (1960 a) without underestimating the importance of such research for an understanding of the processes, has stressed the necessity for extreme caution when interpreting results obtained in vitro in terms of processes occurring in the intact animal. It is therefore of importance to determine whether the active transport of sodium involving passive chloride absorption, as has been observed in vitro, does in fact, also occur in vivo.

Although sodium transport mechanisms and their regulation have been studied in fresh-water fish (see Maetz, 1963), no research correlating sodium and chloride absorptions in these organisms has been carried out since the classical work of Krogh (1937 b). Krogh concludes that in Carassius “it can be stated on the basis of experiments so far made, that the power of absorbing cations is independent, at least in principle, of the anion absorption” and that the chloride ions “are as a rule accompanied by cations, but may to a certain extent be exchanged against bicarbonate ions.” Furthermore, according to the same author (1939), the sodium may be absorbed “in exchange of ammonia” when the sodium absorption is not accompanied by an equivalent quantity of chloride.

As Krogh published very few experimental details, and as radioactive tracers furnish a new approach to the study of electrolyte exchange in vivo, we undertook a systematic reinvestigation of this subject in Carassius auratus. The results will be published in two parts, the first dealing with the independence of the mechanisms of sodium and chloride absorption, and the second with the evidence for exchanges between sodium and ammonium ions, and bicarbonate and chloride ions.

MATERIAL AND METHODS

Preparation of Animals

The Carassius auratus, of 80 to 330 gm body weight, were obtained from a dealer in Paris. They were kept in aquaria with running tap water, at an uncontrolled temperature, and fed with minced meat. Before experimentation fish were placed under special conditions first to adapt them to the temperature (18–23°C) at which the subsequent sodium and chloride flux measurements were to be made, and second to deplete their internal sodium or chloride concentration in order to stimulate their
absorption processes. This technique has been used by Krogh (1937 a, b) and by Jørgensen et al. (1954) on various fish and amphibians, and by Shaw (1960 a, b, c) on the crustacean Astacus.

The fish were kept for 2 to 6 weeks in one of the following solutions: (a) tap water or a sodium chloride solution of approximately 400 μM for the control animals; (b) deionized water, renewed frequently to ensure that the concentrations of the sodium and chloride ions remained below 5 μeq/liter; (c) a sodium sulfate solution renewed frequently, Na concentration approximately 400 μeq/liter, Cl concentration below 5 μeq/liter; (d) a choline chloride solution renewed frequently: Cl concentration approximately 400 μeq/liter, Na concentration below 5 μeq/liter.

The fish were not fed during the week preceding the flux measurements. These experiments were carried out over a period of 1 year, irrespective of season.

Measurement of Electrolyte Exchange

A method for measuring simultaneously the ionic fluxes in the intact fresh-water animal has been described previously by one of us (Maetz, 1956, 1958, 1963). Essentially it is as follows, the animal and the surrounding water form a closed “two compartment system,” the external medium and the internal medium; i.e., the extracellular fluid or the so called sodium or chloride space of the animal. Either the sodium or the chloride space, measured by isotopic dilution techniques, represents about 30 per cent of the body volume. In order to measure the rate of exchange of the ions between these two compartments, a certain amount of tracer is added to the outer medium. The net flux, influx, and outflux can then be calculated from the changes of the concentration of the stable isotope and of the tracer in the external medium without perturbing the animal. In some cases the uptake of the tracer is so rapid that it is necessary to take the radioactive outflux into account (see Maetz, 1956, 1958) in the calculations. In all experiments the urine of the fish is collected outside the aquarium; thus it can be assumed that it is the ionic exchanges across the gills that are measured by the technique.

Each fish was placed in a narrow experimental aquarium sufficiently constrictive to limit its movements. The urine was collected by means of a polyvinyl catheter inserted into the urinary papilla and tied to the anal fin.

Each aquarium contained a volume of water equal to three or five times the volume of the fish. The water was well aerated and kept in circulation by means of a small motor pump (200 ml/min. flow) in a closed circuit consisting of aquarium, thermostat, and a flow type Geiger counter.

The flux measurements were carried out in solutions of sodium chloride, choline chloride, or sodium sulfate, prepared with deionized water and containing at the start of any one experiment the same concentrations of ions as in one of the solutions, described above, used to adapt the fish. In the majority of experiments the anions and cations were marked simultaneously in order that the flux measurements could be carried out at the same time for both ions on the same individual. Cl36 Na (specific radioactivity 200 to 500 μc/gm) and choline Cl34 chloride (40 μc/mg) supplied by the Radiochemical Center, Amersham, Na36 Cl (50 to 250 mc/gm) and S35O3Na2 (1 mc/mg) supplied by the Department of Radioelements, Saclay, were used. The initial iso-
tope concentration in the aquarium was of the order of 0.1 μc/ml in the case of Na\textsuperscript{24} and of 0.01 μc/ml for the other isotopes.

**Simultaneous Measurements of Cl\textsuperscript{18} and Na\textsuperscript{24} or \textsuperscript{35}S\textsuperscript{4} and Na\textsuperscript{24}**

The flow type Geiger counter was of the type 3 B 17 (Laboratoire Central des Télécommunications, Paris) with glass walls practically insensitive to the relatively soft β-radiations of Cl\textsuperscript{18} (714 kev) and of S\textsuperscript{35}O\textsubscript{4} (167 kev) but sensitive to the higher energy β-radiations of Na\textsuperscript{24} (1390 kev). The radiosodium concentration in the external medium was recorded by means of a ratemeter connected to a millivolt meter. The radiochloride concentration was measured after Na\textsuperscript{24} decay by means of a Geiger counter with a thin mica end-window from 2 ml samples taken every half-hour and dried on planchets. The S\textsuperscript{35}O\textsubscript{4} concentration was measured on 0.2 ml samples in a liquid scintillation counter (tri-carb, Packard Instrument Company, La Grange, Illinois) after allowing for the Na\textsuperscript{24} decay. Choline C\textsuperscript{14} concentrations were also measured by liquid scintillation. No simultaneous tagging of chloride was carried out in these experiments with Choline C\textsuperscript{14}.

**Total Ionic Concentration Determinations and Water Conductivity Measurements**

The half-hourly water samples were used to determine the total sodium concentration by flame photometry (eppendorf photometer) and the total chloride concentration by potentiometric titration (Sanderson, 1952). The concentrations are expressed in milliequivalents per liter. Stable choline and sulfate concentrations were not measured and therefore only the radioactive influxes for these ions were calculated in order to verify the degree of impermeability of the gills. In some experiments the changes of the total electrolyte concentration of the aquarium water were followed by means of conductivity measurements carried out with a Philips PR 9500 conductivity bridge and a PR 9513/00 dip-type cell. The results are expressed in mho cm\textsuperscript{-1}.

**Calculation of Fluxes**

The sodium influx was calculated from recorded changes in radiosodium concentration after correction for the decay, taking into account the volume of the external medium, the specific radioactivity, and in some cases, the radioactive outflux. For the chloride influx, the variations in radiochloride concentration were calculated from the slope of the regression line obtained from successive concentration values over a period of 2 to 5 hours. When the exchange rate was very high (as in Fig. 2), the slopes were calculated between each two successive measurements, and the radioactive outflux was taken into account for correction. The net fluxes were calculated in a similar manner from the volume of the external medium and from the rate of change of the total concentration of a particular ion as measured from the slope of the regression line or the successive individual slopes in cases of rapid absorption. The outflux values were obtained from the influx and net flux values. All fluxes are expressed in (milliequivalents per hour)/100 gm. The potassium net flux was likewise determined by following the external potassium concentration by flame photometry on the successive samples.

In an independent series of experiments the permeability of the gills to Ca ions was
measured by a technique similar to that used for the sodium or chloride ions. Measurement of Ca\(^{45}\) concentration was made on 0.2 ml samples in a liquid scintillation counter. Total calcium concentration was determined by flame photometry. The initial concentration of calcium was of the order of 200 \(\mu\)M.

**TABLE I**

<table>
<thead>
<tr>
<th>Pretreatment solution</th>
<th>Date of experiment</th>
<th>(\text{Na}^+) exchange</th>
<th>(\text{Cl}^-) exchange</th>
<th>(\text{K}^+) exchange</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(f_{\text{in}})</td>
<td>(f_{\text{net}})</td>
<td>(f_{\text{out}})</td>
</tr>
<tr>
<td>(\text{NaSO}_4)</td>
<td>July 11</td>
<td>5</td>
<td>-8</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>July 18</td>
<td>18</td>
<td>+4</td>
<td>14</td>
</tr>
<tr>
<td>(\text{Choline chloride})</td>
<td>July 16</td>
<td>57</td>
<td>+17</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>July 17</td>
<td>40</td>
<td>+23</td>
<td>17</td>
</tr>
<tr>
<td>(\text{Deionized water})</td>
<td>July 10</td>
<td>79</td>
<td>+34</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Oct. 31</td>
<td>66</td>
<td>+48</td>
<td>18</td>
</tr>
<tr>
<td>(\text{NaCl})</td>
<td>Feb. 27</td>
<td>25</td>
<td>+2</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Mar. 12</td>
<td>40</td>
<td>+1</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Oct. 22</td>
<td>1</td>
<td>-5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Nov. 21</td>
<td>8</td>
<td>-2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Dec. 3</td>
<td>27</td>
<td>+6</td>
<td>21</td>
</tr>
</tbody>
</table>

* \(f_{\text{in}},\) influx; \(f_{\text{net}},\) net flux; \(f_{\text{out}},\) outflux; in milliequivalents per hour/100 gm body weight, to the nearest integer.
‡ The \(\text{K}^+\) net flux is given for some of the fishes.

**RESULTS**

The Independence of Sodium and Chloride Absorption from a Sodium Chloride Solution

When a goldfish is placed in a sodium chloride solution, the sodium and chloride exchanges are frequently of very different intensities. These exchanges result in net transfers which may be of the same sign, but of different values or they may be of opposite signs, indicating a net absorption of one ion and a loss of the other. This independence always occurs when the internal sodium or chloride has been depleted by keeping the fish in a solution lacking one or the other or both of these ions. It may even be recorded from control fish previously kept in a sodium chloride solution.

Table I summarizes 11 experiments selected from a total of 65 which show most clearly the independence of sodium and chloride absorptions in fish previously kept in various solutions. The selection of certain experiments is justi-
fied because the object of these experiments was to demonstrate that such independence exists and not to make a statistical analysis of its occurrence. It can be seen that fish previously kept in a sodium sulfate solution show a high rate of chloride exchange and a positive net flux for this ion. The opposite is generally the case for the sodium ion. Fig. 1 (left) illustrates such a case, in which, after sodium sulfate treatment, the two net fluxes, although both of the same sign, are of markedly different values. Animals previously kept in choline chloride, and also those from deionized water, have a high rate of exchange of sodium resulting in a positive net flux, whereas the chloride influx is noticeably smaller than the outflux. An example of this difference in sign of net fluxes is given in Fig. 1 (right) by a fish previously kept in choline chloride. The fish in Fig. 2 (right), also previously treated with choline chloride, shows an extremely high rate of sodium absorption with a feeble uptake of chloride. The net flux of sodium was so high (+38 to +86 (µEq/hr.)/100 gm depending upon the external sodium concentration) that the external sodium ions became rapidly depleted, necessitating renewal (by the addition of sodium...
sulfate to the aquarium water without changing the external chloride concentration) at 2 and 4 hours after the start of the experiment. The addition of sodium is indicated by arrows on the graph. In this experiment, the sodium influx was equal to the net flux, that is, there was no measurable outflux of sodium.

The five series of data at the bottom of Table I are those from control animals previously kept in sodium chloride solution. It can be seen that here also there is considerable variation in the net flux of sodium in relation to chloride flux. Out of 50 control fish studied, only 5 had similar sodium and chloride positive net fluxes (not differing by more than 5 (\(\mu\)Eq/hr.)/100 gm). The curves in Fig. 3 are from such a fish. It can be seen that the diminutions of external sodium and chloride concentration have parallel time courses. In Fig. 2 on the other hand a control fish shows an independence of sodium

![Graph](https://example.com/graph.png)
and chloride fluxes, the sodium net flux being zero, and the chloride net flux positive (+13(μEq/hr.)/100 gm).

The Absorption of Chloride from a Choline Chloride Solution-Impermeability of the Gill to Choline Ions

The preliminary treatment of fish in solutions of sodium sulfate and choline chloride raises two questions. First, what is the degree of impermeability of the gill to the choline and sulfate ions, and second if choline and sulfate are impermeant ions, what is the rate of absorption of the sodium and chloride ions in the presence of these co-ions?

To study these problems concerning choline chloride, fish were prepared in a sodium sulfate solution in order to stimulate their subsequent absorption
of chloride. Two experiments, one of which is given in Fig. 4 on the right, demonstrated that such fish, placed in a choline C\textsuperscript{14} chloride solution, showed a high rate of chloride absorption and at the same time a total impermeability to choline, the radioactive influx of this ion as indicated by the regression line, being zero. These control experiments were necessary in view of the doubts raised about the impermeability of frog skin to choline (Kirschner, 1960; Macey and Koblick, 1963).

Table II gives the chloride fluxes of 3 fish similarly treated. It can be seen

<table>
<thead>
<tr>
<th>Date</th>
<th>Flux of chloride (fm)</th>
<th>Flux of sodium (f\textsubscript{Na})</th>
<th>Flux of choline (f\textsubscript{Ch})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apr. 10</td>
<td>167</td>
<td>+90</td>
<td>77</td>
</tr>
<tr>
<td>June 20</td>
<td>33</td>
<td>+5</td>
<td>28</td>
</tr>
<tr>
<td>June 20</td>
<td>79</td>
<td>+22</td>
<td>57</td>
</tr>
</tbody>
</table>

These fish were kept in a sodium sulfate solution, previous to the flux measurements.
that the unidirectional fluxes and positive net flux are comparable in intensity to those observed in similarly prepared fish studied in sodium chloride (see Table I for comparison). Fig. 5 illustrates an experiment of this kind. It can be seen that chloride is completely absorbed at the end of about 3 hours. Thus the chloride ion absorption appears to be unaffected by the presence of a non-permeant cation.

![Figure 5](image_url)

**Figure 5.** Absorption of sodium from a sodium sulfate solution and of chloride from a choline chloride solution. Coordinates as in Fig. 4. On left, fish previously kept in a sodium sulfate solution (experiment of June 10, 1963), 170 gm. Volume, 430 ml. Fluxes measured in choline chloride solution. On right, fish previously kept in deionized water. Fluxes measured in a sodium sulfate solution.

The Absorption of Sodium from a Sodium Sulfate Solution—Impermeability of the Gill to Sulfate

Two control experiments demonstrated that the gill is impermeable, at any rate over an experimental period of 5 hours, to sulfate ions (see Fig. 4, on the left). The regression line of the variation of external radioactive sulfate concentration has a slope not significantly different from zero, whereas the negative slopes of the total sodium and radiosodium regression lines indicate uptake of this ion.

Sodium absorption from sodium sulfate solutions is also stimulated in fish previously kept in deionized water. The results from 3 such fish are given in Table III. A comparison with the data from similarly treated fish studied in sodium chloride (see Table I) shows that the sodium unidirectional fluxes and the positive net flux are comparable in intensity even in the presence of a non-permeant co-ion. The sodium absorption curve of one such animal is
taken in Fig. 5 (on the right). Sodium is completely absorbed from the external medium within 4 to 5 hours.

**Experiments on Conductivity**

In a certain number of the above experiments, the conductivity of the external medium was also recorded as a measure of the total electrolyte concentration (Figs. 2 and 3). In all cases, the conductivity remained constant or increased slightly. In no experiment did it fall, even though sodium or chloride or both ions were being absorbed rapidly. In Fig. 2 (right), the conductivity appears to increase in a series of steps, but this was due to the addition of sodium sulfate at intervals as already explained.

**TABLE III**

SODIUM UPTAKE AND EXCHANGE BY CARASSIUS AURATUS FROM SODIUM SULFATE SOLUTION

<table>
<thead>
<tr>
<th>Date</th>
<th>Na_/ia</th>
<th>Na_/out</th>
<th>Na_/if</th>
<th>K_/net</th>
</tr>
</thead>
<tbody>
<tr>
<td>1963</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 8</td>
<td>111</td>
<td>+91</td>
<td>20</td>
<td>-7</td>
</tr>
<tr>
<td>June 26</td>
<td>62</td>
<td>+40</td>
<td>22</td>
<td>-3</td>
</tr>
<tr>
<td>June 26</td>
<td>70</td>
<td>+21</td>
<td>49</td>
<td>-10</td>
</tr>
</tbody>
</table>

These fish were kept in deionized water previous to the flux measurements. The K net flux is also given (see Discussion).

**Measurement of Potassium and Calcium Fluxes**

In most of the experiments summarized in Tables I and III, the potassium net flux was measured. This flux was observed to be always negative and of relatively low intensity varying from virtually 0 to $-10$ (μEq/hr.)/100 gm.

Calcium fluxes across the gills were studied in a series of 10 experiments. The average value for the influx was $3.2 \pm 1.6$ (standard error of the mean) (μEq/hr.)/100 gm with values ranging from 0 to 12; for the outflux, $3.6 \pm 1.0$ (extremes, 0 and 8); and for the net flux, $-0.4 \pm 5.5$ (extremes, $-8$ and $+4$).

**DISCUSSION**

The above results clearly show the independence of sodium and chloride absorptions by the gills of Carassius. In certain cases, either of these ions may be absorbed unaccompanied by an ion of opposite charge, if the gills happen to be impermeable to the latter. In cases in which both sodium and chloride ions are available in the external medium, they may both be absorbed but at different rates, or one may be absorbed and the other lost. These experiments thus confirm the conclusions of Krogh (1937 a, b, 1939) for Rana and Carassius, and of Shaw (1960 a, b, c) for Astacus.
These differences in the absorption of ions of different charges can only be explained in conformity with the law of electroneutrality of solutions, if one assumes they exchange with endogenous ions of the same charge. The existence of such exchanges is confirmed by the results of the conductivity measurements of the external medium during ion uptake. The relative stability of the conductivity level clearly points to a process of ionic exchange even when sodium and chloride ions are being absorbed at equivalent rates (see Fig. 3).

What then are the ions exchanged during sodium and chloride uptake? In the case of sodium, possible exchange ions are potassium or calcium. The very low flux values found for both these ions however, eliminate the possibility that either plays an important role in branchial exchange. Krogh (1939) and Shaw (1960 a) have both suggested that ammonium is the ion exchanged for sodium.

With regard to the ion exchanged for chloride, Krogh (1937 a, b) and Shaw (1960 c) postulated the bicarbonate ion for this role. Further evidence in support of sodium-ammonium and chloride-bicarbonate exchanges will be given in the publication to follow (Maetz and García Romeu, 1964).

When ionic exchanges linked with uptake of ions across the gill from a medium of low sodium and chloride concentrations are considered, the question arises whether the mechanisms involve endothermic processes, either of the type "facilitated diffusion" as in the Cl--HCO₃⁻ exchange through the red blood cell (Jacobs and Stewart, 1942), or of the type "active transport" (Ussing, 1954). The experimental results presented here strongly suggest the occurrence of active transport for both sodium and chloride. Thus the data presented in Fig. 5 give during the first 2 hours of the experiment a ratio of influx to outflux \( \frac{f_{in}}{f_{out}} \) of about 2.5 for chloride and 5.5 for sodium, for a ratio of internal to external concentration \( \frac{C_{in}}{C_{ext}} \) averaging 300 for the sodium and 650 for the chloride. One can therefore calculate the difference of potential across the gill that would be necessary to explain these ratios in terms of passive transport by the use of Ussing's equation (Ussing, 1954)

\[
f_{in}/f_{out} = \frac{C_{in}/C_{ext}}{e^{zFe/R}}
\]

where \( z \) is the charge of the ion (negative or positive), \( E \) is the potential difference, and \( F, R, \) and \( T \) the usual constants. Applying this equation to our data, one calculates a potential difference of approximately 180 to 190 mV, positive inside for the chloride and negative inside for the sodium. Such high potentials have never been observed in any biological membrane. We made some rough preliminary measurements (between the ventral aorta at the base of the branchial arches and the external medium) of the gill potential in Carassius and obtained very low values, of the order of a few millivolts. That the ions are transported passively would thus appear to be extremely unlikely. Not only are sodium and chloride transported independently therefore, but
also by an active transport mechanism. Active sodium and chloride transport has also been suggested for Blennius (House, 1963) and for Anguilla (Tosteson, Spivack, and Nelson, 1962), though in these fish the transport is in the opposite direction permitting excretion of these ions when the external medium is isotonic Ringer's or hypertonic sea water. House (1963) also postulates active absorption of sodium and chloride ions when Blennius is studied in hypotonic brackish water. Since Jørgensen et al. (1954) and Zadunaisky et al. (1963) have also demonstrated active transport of sodium and chloride across amphibian skins, it would therefore appear that such simultaneous active transports are widespread phenomena in aquatic animals.

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