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BRANCHIAL SODIUM EXCHANGE AND AMMONIA EXCRETION IN THE GOLDFISH *CARASSIUS AURATUS*. EFFECTS OF AMMONIA-LOADING AND TEMPERATURE CHANGES

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Krogh (1939) first suggested that in freshwater animals branchial or cutaneous excretion of ammonia may be related to sodium absorption. In both freshwater Crustacea (*Astacus pallipes*) and teleosts (*Carassius auratus*) independent active transport of Na^+ and Cl^- from the outside medium have been definitely established (Shaw, 1960*a–c*; Garcia Romeu & Maetz, 1964). Sodium in particular may be absorbed at very high rates without an accompanying anion. The maintenance of the electroneutrality of the external and internal media necessitates an exchange with an endogenous ion during sodium movement. The possibility that ammonium ions may fulfil this role is suggested by the fact that the gill is the main site of ammonia excretion (Smith, 1929, 1953) and indirect experimental evidence has supported this hypothesis. Addition of ammonia to the outside medium, a procedure which inhibits ammonia excretion, interferes with the sodium uptake (Shaw, 1960*a*; Maetz & Garcia Romeu, 1964). Injection of ammonium ions, a procedure which increases ammonia excretion by the gill, produces an increase of sodium uptake in the goldfish (Maetz & Garcia Romeu, 1964) and in the eel (Garcia Romeu & Motais, 1966). No stoichiometric relationship between ammonia excretion rate and sodium net uptake in relation to experimental external salinity changes is, however, observed in the crayfish (Shaw, 1960*b*). Thus the Na^+/NH_4^+ exchange cannot be obligatory. In particular when ammonia excretion falls short of sodium uptake, a second exchange process, Na^+ against H^+, is thought to be operative, or when sodium uptake is blocked at low external sodium concentration, ammonium is thought to be accompanied by endogenous bicarbonate ions excreted by the gill (Shaw, 1960*b*).

The occurrence of a Na^+/H^+ exchange has recently been demonstrated by Garcia Romeu, Salibian & Pezzani-Hernandez (1969) working on the amphibian *Calyptocephallela gayi*. This type of exchange is thought to be related to the switch from ammonotelism to ureotelism accompanying amphibiosis.

In the teleosts, Maetz & Garcia Romeu (1964) considered that the Na^+/NH_4^+ exchange was obligatory on grounds of the evidence presented by Goldstein & Forster (1961) indicating that enzymic deamination and deamidation within the gill tissue accounts for most of the ammonia excreted by the gills. This point, however, was challenged by Pequin & Serfaty (1963) who demonstrated in the carp that most if not all of the excreted ammonia originates in the liver and that the gill is simply the

clearance site. Goldstein, Forster & Fanelli (1964) showed that in the marine *Myoxocephalus* 50% of the ammonia excreted results from blood clearance by the gill, the remaining being formed by the gill itself.

More recently de Vooys (1968) showed in the carp that transfer from tap water to de-ionized water, which blocks sodium uptake, is not accompanied by any reduction in ammonia excretion, rather the reverse. NH_4^+/Na^+ exchange cannot be obligatory in this fish. Finally, Kerstetter, Kirschner & Rafuse (1970) made similar observations on the rainbow trout. They demonstrated in addition that when sodium uptake greatly exceeds ammonia excretion a significant decrease of the pH of the external medium occurs, showing that a Na^+/H^+ exchange is operative. These authors propose that in the teleostean gill, as in amphibian skin and in the distal tubule of the mammalian kidney, an Na^+/H^+ exchange prevails, ammonia being excreted independently in the free base form by the teleostean gill and by the distal tubule.

This paper is a reconsideration of the evidence concerning Na^+/NH_4^+ exchanges in the goldfish. Observations will be presented demonstrating that the gill excretes ammonia in the ionized and not in the free-base form. Nevertheless, no correlation between ammonia excretion and sodium uptake is found in either control or ammonia-loaded fish. The effects of abrupt temperature changes on sodium exchange and ammonia excretion have also been studied. The results suggest that ammonia clearance by the gill is passive in nature at least in ammonia-loaded fish, the temperature coefficient being similar to that observed for the sodium efflux. Sodium influx, the active component of the sodium balance, is on the contrary highly temperature dependent. This paper is to be followed by a second dealing with Na^+/H^+ exchanges and the relative role of NH_4^+ and H^+ in sodium uptake.

MATERIALS AND TECHNIQUES

Carassius auratus purchased from a Paris dealer and weighing from 102 to 222 g (mean: 145 g) were used.

The fish were left unfed and kept at 16 ± 2 °C in a closed-circuit temperature-controlled freshwater aquarium (Na concentration about 100 μ-equiv.l^{-1}).

The animals were prepared at least 24 h before experiments, by being fitted with a urinary catheter and an indwelling intraperitoneal catheter (PE 20) and placed in a small individual tank (500 ml/100 g fish) in an open circuit with controlled temperature.

The urine was collected outside the bath in order to permit branchial sodium-exchange fluxes and ammonia excretion to be measured without interference from the urinary components of Na efflux and ammonia excretion. In about half of the experiments the urinary and branchial components were compared. In the remaining, the collected urine was discarded.

1. *Sodium fluxes and ammonia excretion experiments*

On the day of an experiment the circulating system was closed and ^{24}Na or ^{22}Na was added to the outside bath (about 20 μC per fish and 500 ml). The bathing medium was continually recirculated through a cryostat (16 ± 1 °C) and a flow-counter by means of a pump (200 ml min^{-1}). The aquarium was aerated continuously.

The concentrations of radio sodium and total sodium were followed by a printout every 5 min of the γ or β radiation flowing through the counter and by withdrawing 5 ml samples periodically for flame photometry. The volume of the fluid in the system was measured at the end of the experiment.

All fluxes were expressed in μ-equiv per 100 g and per h and calculated from the changes of the radio sodium and total sodium concentrations in the outside bath (Maetz, 1956). In most experiments on fish with high rates of sodium uptake the radioactive backflux from the fish has to be taken into account. The correction amounts up to 20% of the influx.

Sodium concentration in urine was measured by flame photometry. Urine flow was determined gravimetrically. At the end of some experiments a blood sample was taken from the caudal artery by means of a heparinized syringe. Plasma sodium was measured by flame photometry.

Total ammonia concentration in whole blood, urine and in the external bath samples was measured with a Technicon autoanalyser by a modification of the phenol-hypochlorite reaction followed by dialysis across a cuprophan membrane (Logsdon, 1960; Assous, Dreux & Girard, 1960). To compare the branchial permeability to unionized and ionized forms of ammonia, the concentrations of both forms were calculated for blood and external media taken at the end of the experiments. The following relation was used

$$pH = pK + \log \frac{NH_3}{NH_4^+}$$

(NH_3) being the concentration of the free-base form and (NH_4^+) that of the ionized form. The pK is taken as 9·3 (see Mossberg, 1967).

The pH of the blood and external medium were compared in a capillary glass pH microelectrode (Instrument Laboratory or Beckman).

In a few experiments, the chloride net flux of the fish was measured to comparison with the sodium net flux. Cl^- was measured in the external samples by amperometric titration (Aminco Cotlove).

2. *Experimental protocols*

Three types of experiment were performed.

The first consisted in following branchial sodium exchange and ammonia excretion in control fish at low and high external sodium concentrations. The consecutive low and high sodium periods each lasted about 3 h. An intermediate period of 0·3–0·5 h was allowed for homogenization of the NaCl molar solution added to the external bath to raise the external sodium from a 100 μ-equiv to a 1000 μ-equiv concentration. Radiosodium was also added (up to 50 μC per bath) to maintain a high external specific radioactivity.

The second type of experiment permitted the comparison of sodium exchange and ammonia excretion at low and high external sodium in ammonia-loaded fish. Ammonia-loading was obtained by an intraperitoneal injection of an equimolar ammonium sulphate solution (1 m-equiv per 100 g). The experiment was started about 45 min after the ammonia load was given. The same group of ten fishes were used on 2 consecutive days, first as controls and then after ammonia injection.

In the third type of experiment the effects of abrupt temperature change were

followed in fishes before and after ammonia-loading. In the control group the sodium fluxes and ammonia excretion were followed during three consecutive 1·5 h periods first at 16 °C, then at 6 °C and finally back at the adaptation temperature. To avoid shocking the fish, transfer from high to low temperature was arranged by cooling or re-heating the aquarium water during an intermediate period of 20–30 min, the maximum rate of change of temperature being 0·5 °C min^{-1}. During this intermediate period sodium and radiosodium were added in order to maintain as constant as possible the external concentration and specific radioactivity. In the ammonia-loaded fish the duration of each experimental period was reduced to 1 h.

RESULTS

1. *Branchial sodium exchange and ammonia excretion in control fish*

Table 1 and figure 1 summarize the observations concerning branchial sodium exchange in relation to external sodium concentration (Na_{ext}). Ten fishes were used successively at low (mean conc. 150 μ-equiv.l^{-1}, ranging from 80 to 350 μ-equiv.l^{-1}) and high (mean conc. 990 μ-equiv.l^{-1}; 900–1265 μ-equiv.l^{-1}) external Na. Eight

Table 1. *Branchial sodium exchange and ammonia excretion in control* Carassius *in relation to the external sodium concentration*

External Na concentration		Sodium				Ammonia excretion by gill
Mean ± S.E.	Range	Influx	Efflux	Net flux	$^{\bullet}f_{in}/[Na]_{ext}$	
109 ± 6·8 (8)	80–150	17·3 ± 1·15 (8)	12·4 ± 1·38 (8)	+4·8 ± 1·47 (8)	161 ± 12	14·0 ± 3·77 (8)
288 ± 30 (7)	150–400	31·7 ± 4·22 (7)	19·4 ± 3·71 (7)	+12·3 ± 3·60 (7)	118 ± 20	17·3 ± 1·60 (5)
501 ± 17 (9)	400–600	47·1 ± 5·98 (9)	30·0 ± 3·08 (9)	+17·2 ± 4·77 (9)	93 ± 10	30·7 ± 3·53 (9)
952 ± 34 (12)	800–1265	44·5 ± 3·03 (12)	20·6 ± 2·67 (12)	+23·9 ± 2·78 (12)	46 ± 2	16·0 ± 3·32 (12)

Fluxes in μ-equiv.h^{-1} (100 g)$^{-1}$; concentration in μ-equiv.l^{-1}. Ammonia excretion in μM.h^{-1} (100 g)$^{-1}$. In brackets, number of flux periods.
$^{\bullet}$ $f_{in}/[Na]_{ext} \times 10^3$.

additional control fish destined for temperature-change experiments (see below) were also first tested at 16 °C in the medium range of Na_{ext} (mean 470 μ-equiv.l^{-1}; range: 250–850 μ-equiv.l^{-1}). The flux periods (duration 1–3 h) were grouped according to arbitrarily chosen ranges of Na_{ext} as given in the table. It may be seen that in the lower range sodium influx (f_{in}) increases with Na_{ext} but the ratio f_{in}/Na_{ext} decreases with increasing Na_{ext}. Above 500 μ-equiv.l^{-1} a maximal flux of about 50 μ-equiv.h^{-1} 100 g^{-1} is attained. Sodium efflux (f_{out}) is more or less constant except for the lowest range of Na_{ext} where it is significantly lower ($P < 0{\cdot}01$).

In Fig. 1, the curve illustrating the variation of f_{in} in relation to Na_{ext} has been assumed to fit the well-known Michaelis-Menten equation

$$f_{in} = \frac{f_{max} \times Na_{ext}}{K_m + Na_{ext}}.$$

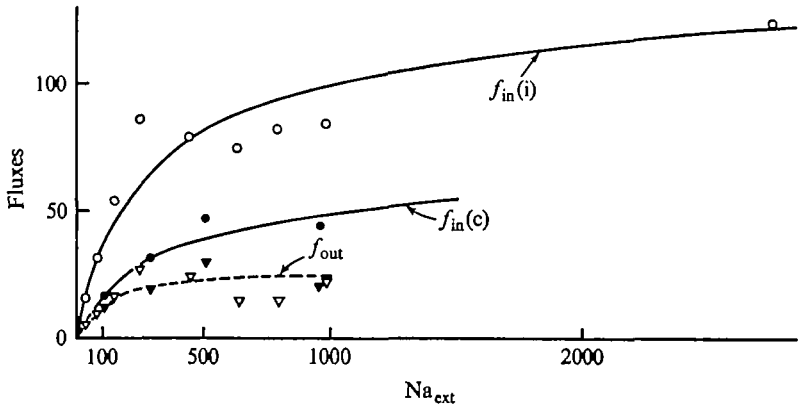

Fig. 1. Sodium influx and efflux as a function of external sodium concentration in ammonia-loaded and control *Carassius auratus*. Abscissa: Na_{ext} in μ-equiv.l^{-1}. Ordinate: f_{in}, influx of control (*c*) and injected (*i*) fish. f_{out}, efflux of control (black triangles) and ammonia-loaded fish (white triangles). Fluxes in μ-equiv.h^{-1}.100 g^{-1}.

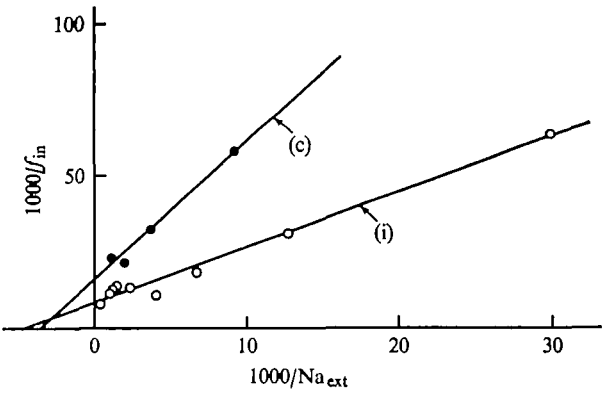

Fig. 2. Reciprocal plot of sodium influx *vs.* external sodium concentration (Na_{ext}) for calculation of K_m and f_{max}. In the equations given in the text $y = 1000/f_{in}$ and $x = 1000/Na_{ext}$.

The parameters $f_{max} = 65$ μ-equiv.h^{-1}.100 g^{-1} and $K_m = 300$ μ-equiv.l^{-1} were calculated from the reciprocal plot represented in Fig. 2.

The ammonia excretion rates by the gill are also given in Table 1. No correlation between ammonia excretion and sodium influx or sodium net uptake is observed. For the fish studied before and after raising Na_{ext} ($+842 \pm 35$ μ-equiv.l^{-1}), the resulting f_{in} and f_{net} increases are $20{\cdot}3 \pm 2{\cdot}9$ and $26{\cdot}2 \pm 5{\cdot}3$ μ-equiv.h^{-1} 100 g^{-1} while ammonia excretion remains constant ($-0{\cdot}5 \pm 1{\cdot}0$ μM.h^{-1}.100 g^{-1}).

2. *Branchial sodium exchange and ammonia excretion in ammonia-loaded fish*

The same ten fish which had served as control were re-studied at low (40–245; mean 110) and high (425–1050; mean 870) Na_{ext} after ammonia-loading, and 6 additional fish were studied in the medium range (200–500; mean 265 μ-equiv) during temperature-effect experiments. As the rate of sodium net uptake was very high, the range of sodium concentrations encountered by the same fish during the course of an experiment was wider and thus the flux periods (usually of 1 h duration and for a narrow range of Na_{ext}) were more numerous.

Table 2. *Branchial sodium exchange and ammonia excretion in* Carassius *injected with ammonia, in relation to the external sodium concentration*

External Na concentration		Sodium				Ammonia excretion by gill
Mean ± S.E.	Range	Influx	Efflux	Net flux	$^{*}f_{in}/[Na]_{ext}$	
33 ± 5 (7)	10–50	14·9 ± 1·86 (7)	5·7 ± 1·36 (7)	+9·2 ± 2·27 (7)	505 ± 72	97·6 ± 9·3 (7)
78 ± 4 (8)	60–90	31·5 ± 4·98 (8)	9·8 ± 2·18 (8)	+21·7 ± 3·00 (8)	396 ± 52	99·5 ± 6·0 (8)
146 ± 8 (11)	105–180	54·4 ± 8·30 (11)	16·9 ± 2·89 (11)	+37·5 ± 8·50 (11)	357 ± 39	110·6 ± 15·8 (11)
247 ± 8 (8)	215–280	86·8 ± 8·74 (8)	30·3 ± 6·24 (8)	+56·5 ± 11·56 (8)	356 ± 41	134·1 ± 20·4 (8)
443 ± 15 (4)	410–480	79·1 ± 10·80 (4)	24·7 ± 11·31 (4)	+54·4 ± 9·96 (4)	178 ± 22	67·5 ± 18·4 (4)
635 ± 20·9 (8)	545–680	74·2 ± 4·04 (8)	14·7 ± 3·52 (8)	+59·5 ± 6·69 (8)	117 ± 6	67·5 ± 8·3 (8)
794 ± 24·0 (6)	725–850	82·1 ± 8·57 (6)	15·1 ± 3·72 (6)	+67·0 ± 5·71 (6)	103 ± 9	86·0 ± 10·5 (6)
986 ± 19·7 (6)	930–1050	84·9 ± 3·54 (6)	22·3 ± 4·51 (6)	+62·6 ± 3·91 (6)	86 ± 4	82·8 ± 7·1 (6)

Same legend as preceding table.

Table 2 and Fig. 1 illustrate the results. Ammonia-loading produces at all external sodium concentrations a significant increase of f_{in} and f_{net}, while sodium efflux remains unchanged. This confirms our previous observations obtained on a smaller number of fish (Maetz & Garcia Romeu, 1964). As in the controls, sodium influx increases with Na_{ext}, but above 400 μ-equiv.l^{-1}. the transport system appears saturated. The highest fluxes measured attained 124·1 ± 18·8 μ-equiv.h^{-1}(100 g)$^{-1}$ for Na_{ext} = 2754 ± 313 μ-equiv.l^{-1} (n = 5). This mean value represented in Fig. 1 is not given in table 2 as for most of these fish branchial ammonia excretion and sodium efflux were not measured simultaneously. The f_{in}/Na_{ext} ratio remains more or less constant up to 300 μ-equiv.l^{-1} Na_{ext}, then declines at higher Na_{ext}. This ratio is 2–3 times higher than that calculated for control fish, at the lowest range of Na_{ext}.

As in control fish, the sodium efflux is significantly less at the lower ranges of Na_{ext} ($P < 0{\cdot}01$, for 33–78 μ-equiv.l^{-1} compared with 146–986 μ-equiv.l^{-1}). In Fig. 1 it is assumed that the curve illustrating the variation of f_{in} as a function of Na_{ext} fits the Michaelis–Menten equation in ammonia-loaded as in control fish. Fig. 2 compares the reciprocal plots of f_{in} in relation to Na_{ext}. The calculated parameters are f_{max} = 123 μ-equiv (twice the value characterizing control fish) while K_m = 225 μ-equiv.l^{-1} which is not significantly different from the control value. The equations of the linear functions represented in Fig. 2 are:

$$\text{for control fish } y = 4{\cdot}63x + 15{\cdot}11,$$

$$\text{for injected fish } y = 1{\cdot}84x + 8{\cdot}15.$$

As expected, branchial ammonia excretion is considerably increased (about five- to seven-fold) after ammonia-loading. As in control fish, there is no correlation between the ammonia excretion rate and the sodium influx or net uptake. For example, in the ten fish studied before and after raising Na_{ext} (+755 ± 120 μ-equiv.l^{-1}) the increases

Table 3. *Ammonia excretion rate as a function of time*

	h_1	h_2	h_3	h_4	h_5
Intact fish (8)					
Excretion rate in μM (100 g)$^{-1}$ h^{-1}	14·0 ± 3·75	14·0 ± 3·75	14·0 ± 3·75	14·6 ± 3·87	14·7 ± 3·86
Relative to h_1	[100]	100 ± 0·0	100 ± 0·0	100·4 ± 0·04	100·5 ± 0·05
Injected fish (10)					
Excretion rate in μM (100 g)$^{-1}$ h^{-1}	114·3 ± 13·94	94·7 ± 9·96	91·7 ± 6·72	71·6 ± 7·85	—
Relative to h_1	[100]	85·8 ± 6·09	85·7 ± 6·67	70·7 ± 9·38	—

h_1, h_2, ... are successive hourly periods.
The intraperitoneal injection 1 mM per 100 g was given about 1 h before starting the excretion rate measurements. After 5 h (i.e. h_4) about one-half of the injected dose is excreted.

of f_{in} and f_{net} are 48·2 ± 5·0 and 49·4 ± 5·2 μ-equiv.h^{-1} 100 g^{-1} respectively while ammonia excretion declines significantly (−34·2 ± 14·0 μM). This decrease, however, results from the design of the experiments. Ammonia excretion diminishes steadily in the hours following the initial injection while it is constant in the control fish (see Table 3). The external sodium concentration was raised experimentally in the second part of the experiment, when about one-third of the ammonia load had already been excreted.

When sodium influx and uptake and ammonia excretion are compared in the ten fish before and after ammonia-loading, at low and high Na_{ext}, there is again no correlation between the extra ammonia excretion and the extra sodium uptake. At low Na_{ext}, the increase in ammonia excretion is 83·9 ± 13·9 μM. h^{-1}. 100 g^{-1} while the increases in f_{in} and f_{net} are 13·2 ± 5·3 or 16·6 ± 7·2 μ-equiv.h^{-1}. 100 g^{-1} respectively (correlation between ammonia excretion and sodium f_{net}: $r = 0{\cdot}467$; $P < 0{\cdot}1$). At high Na_{ext} the increase in ammonia excretion is 50·3 ± 5·4 while the increases in f_{in} and f_{net} are in the same range, being 40·8 ± 5·4 and 39·7 ± 4·3 μ-equiv.h^{-1} 100 g^{-1} respectively, but these two variables are not correlated (correlation between ammonia excretion and sodium f_{net}: $r = 0{\cdot}272$).

3. *Comparison between gill and kidney as sites of sodium loss and ammonia excretion*

Table 4 gives the data concerning urine flow and urinary concentration of sodium and ammonia for control and ammonia-injected fishes.

Table 4. *Urine flow, sodium and ammonia concentrations in urine of* Carassius

	$\dot{V}$	U_{Na}	U_{NH_3}
Controls (12)	730 ± 15·4	7·0 ± 2·93	0·57 ± 0·18
Injected (11)	741 ± 95·4	19·0 ± 3·83	11·90 ± 2·76

$\dot{V}$ in μl h^{-1} (100 g)$^{-1}$; concentrations in μ-equiv or μM per litre.

Urine flow is unaltered by the intraperitoneal injection of ammonia. The urinary sodium is increased almost three-fold ($P < 0{\cdot}02$) while the urinary ammonia is increased 20-fold ($P < 0{\cdot}001$).

Table 5 gives the rates of branchial sodium efflux, urinary sodium excretion and ammonia excretion by the gill and the kidney. All the values are given irrespective of

Table 5. *Urinary excretion and branchial efflux or excretion of sodium and ammonia in* Carassius auratus

1. Control fish	
(*a*) Sodium	
Efflux across gill	20·7 ± 1·67 (37)
Excretion via urine	5·1 ± 2·16 (12)
(*b*) Ammonia	
Excretion by gill	19·7 ± 2·13 (31)
Excretion via urine	0·4 ± 0·15 (11)
2. Ammonia injected fish	
(*a*) Sodium	
Efflux across gill	17·2 ± 1·70 (59)
Excretion via urine	13·7 ± 2·96 (10)
(*b*) Ammonia	
Excretion by gill	97·3 ± 7·29 (32)
Excretion via urine	8·3 ± 2·10 (10)

μM or μ-equiv.h^{-1} (100 g)$^{-1}$

the external sodium concentration which varied experimentally during the collection period. In control fish the urinary sodium excretion rate amounts to about 25 % of the branchial sodium efflux, while the excretion rate of ammonia by the kidney is only about 2 % of the branchial excretion rate. After injection of ammonia the sodium efflux across the gill remains unchanged while the excretion rate by the kidney is increased, attaining 80 % of the sodium efflux by the gill. Ammonia excretion via the urine is also increased but it still represents only 8·5 % of the excretion via the gill, which shows a five-fold increase.

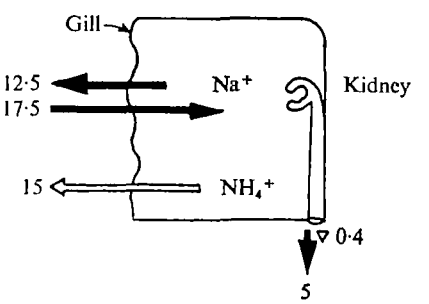

Fig. 3. Schematic representation of the relative role of gill and kidney in the handling of sodium and ammonia. The values given are in μ-equiv or μM.h^{-1}.100 g^{-1} for Na_{ext} = 100 μ-equiv.l^{-1}.

In conclusion, the importance of the gill as the major site of nitrogenous waste product clearance is confirmed for *Carassius*. Fig. 3 illustrates schematically the relative roles of the various sites of excretion and the relative roles of kidney and gill in handling sodium and ammonia in a fish kept in a 100 μ-equiv Na solution.

4. *Comparison between pH, (NH_3) and $(NH_4)^+$ concentrations in blood and external medium*

Table 6 summarizes observations concerning the relative concentrations of unionized and ionized forms of ammonia in the blood and in the closed-circuit aquarium water at the end of experiments. For both control and ammonia-injected fish, the pH of the external medium is higher than that of the blood. It should be noted that Villefranche tap water is rich in HCO_3^- and Ca^{2+} (1·5–2 m-equiv). This explains its relative alkalinity. The blood becomes slightly more acid after injection of ammonium sulphate.

Table 6: *pH*, $[NH_3]$, $[NH_4^+]$ *gradients across the gill of* Carassius

	External	Internal	Ext − Int	Ratio Ext/Int	Total ammonia excretion rate (end of exp.)
1. Control fish (n = 7)*					
pH	7·88 ± 0·04	7·55 ± 0·04	+0·33 ± 0·08	—	
$[NH_3]$	11·7 ± 2·42	4·1 ± 0·66	+ 7·6 ± 2·00 $P < 0·01$	2·75 ± 0·47 $P < 0·02$	17·7 ± 3·33
$[NH_4^+]$	271 ± 45·5	223 ± 26·4	+48 ± 40·6 N.S.	1·29 ± 0·19 N.S.	
2. Injected fish (n = 12)					
pH	7·81 ± 0·06	7·41 ± 0·09	+0·40 ± 0·11	—	
$[NH_3]$	43·8 ± 6·64	26·2 ± 4·60	+17·6 ± 5·67 $P = 0·01$	1·90 ± 0·24 $P < 0·01$	55·1 ± 8·90
$[NH_4^+]$	1310 ± 171	2386 ± 563	−1076 ± 586 N.S.	1·01 ± 0·24† N.S.	

Concentrations in $\mu M.l^{-1}$, rates in $\mu M.h^{-1}$ (100 g)$^{-1}$.

* These experiments include four fishes submitted to abrupt temperature changes; the comparison between plasma and external media was made at the end of the 'return period' in 16 °C.

† Mean ± S.E. of the individual Ext/Int ratios. Most of the ratios higher than 1 were in the small concentration range. This explains why the ratio of the means (0·55) is much less than 1.

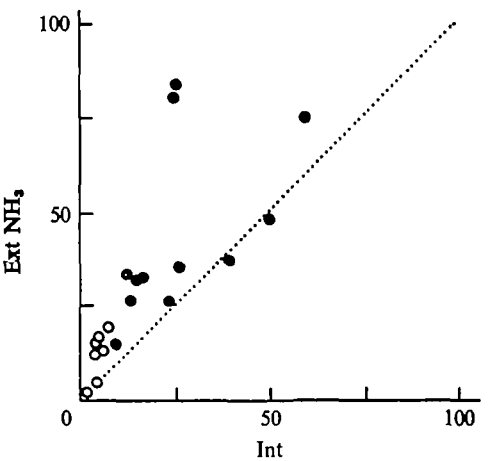

Fig. 4. External ammonia concentration as a function of internal ammonia concentration in the free base form (NH_3) at the end of the closed-circuit experiments. Open circles: control fish. Filled circles: ammonia-loaded fish. Concentration in $\mu M.l^{-1}$. The dotted line stands for $NH_{3\,ext} = NH_{3\,int}$.

As a result of this pH difference the concentration of ammonia in free base form appears to be about two to three times higher in the external bath than in the blood. As seen in Table 6, the concentration ratio is significantly higher than 1 and the concentration difference is significantly different from 0 in both control and ammonia-loaded fish. In fig. 4 the individual values of external (NH_3) concentration have been plotted against the internal values to illustrate this point.

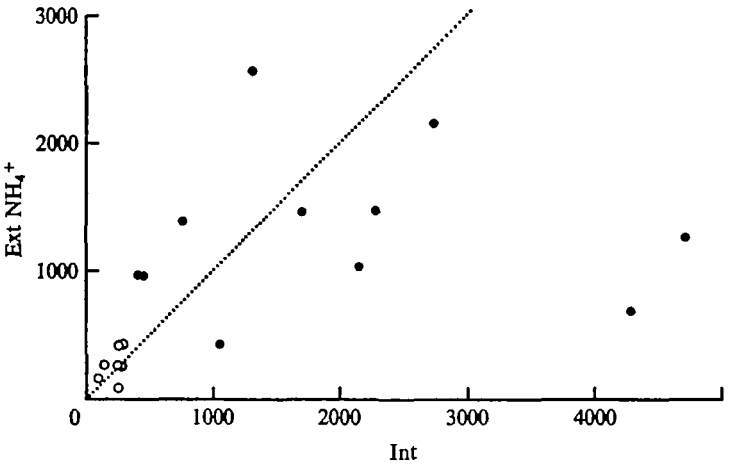

Fig. 5. External ammonia concentration as a function of internal ammonia concentration in the ionized form (NH_4^+). Same legend as Fig. 4.

In Fig. 5 the external (NH_4^+) concentration has been plotted against the internal concentration. In about half of the fish the concentration ratio Ext/Int is higher than 1. It can be seen from Table 4, however, that the mean ratio is not different from 1, nor is the difference significantly different from 0 in either control or in ammonia-loaded fish.

In conclusion, at the end of the closed-circuit experiments the fish excretes ammonia against a concentration gradient of free ammonia. In about half of the individuals, mostly controls, ammonia is also excreted against the concentration gradient of ammonia in the ionized form. In the remaining fish, ammonia excretion proceeds along the concentration gradient of the ionized form.

These data support the hypothesis that the gill of *Carassius* is only permeable to the ionized form of ammonia.

5. *Effect of abrupt temperature changes on sodium exchange and ammonia excretion by the gill*

The temperature sequence studied to gain an insight into the temperature dependence of the various parameters was 16–6–16 °C.

Table 7 summarizes the relevant data for the control fish (n = 8) and Fig. 6 illustrates a typical experiment. The Na influx was measured at an Na_{ext} of 500 μ-equiv.l^{-1} which assures saturation of the carrier mechanism at 16 °C. As this concentration was kept more or less constant during the experiment, the comparison of either the mean f_{in} or mean f_{in}/Na_{ext} values obtained during the 'warm' and 'cold' periods was taken

Table 7. *Effects of temperature change on ammonium excretion and sodium exchange by the gill of* Carassius (*control fishes*; $n = 8$)

	16 °C	6 °C	16 °C	Q_{10} *m*
Am. excr. μM h^{-1} (100 g)$^{-1}$	25·0 ± 4·3	8·2 ± 1·7	27·1 ± 4·1	3·9 ± 0·52
Mean Na_{ext} (μ-equiv.l^{-1})	462 ± 36	574 ± 86	563 ± 109	—
f_{in} μ-equiv (100 g)$^{-1}$ h^{-1}	41·3 ± 5·8	17·1 ± 2·9	44·6 ± 7·3	2·6 ± 0·09
f_{out}	27·2 ± 4·0	23·4 ± 8·5	30·5 ± 3·1	2·0 ± 0·40
f_{net}	+14·1 ± 3·2	−6·3 ± 7·5	+14·1 ± 5·5	—
f_{in}/Na_{ext}	88·9 ± 9·8	31·6 ± 5·0	88·3 ± 14·2	2·9 ± 0·16

Q_{10} *m*: mean ± S.E. of the individual ratios $\frac{\text{mean flux or excretion rate at 16 °C}}{\text{flux or excretion rate at 6 °C}}$.

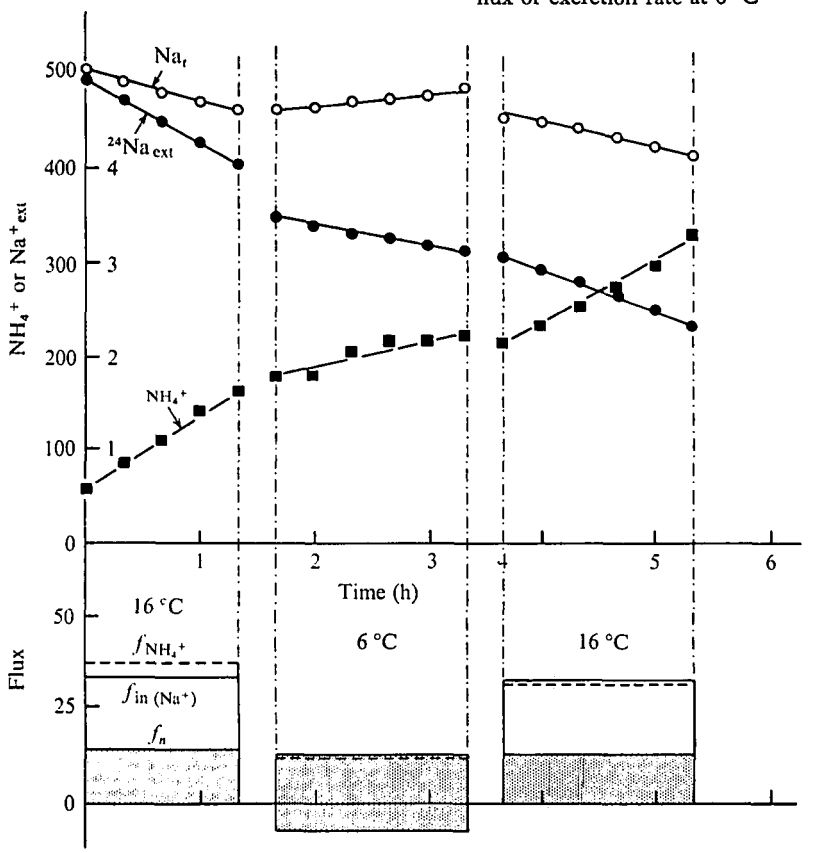

Fig. 6. Effect of abrupt temperature changes on branchial sodium exchange and ammonia excretion in control goldfish *Carassius*. Upper graphs: Ordinates, external total sodium or ammonia concentration in μ-equiv or μM.l^{-1} (left-hand side of axis). External ^{24}Na conc. in cpm ml^{-1} ($\times 10^{-3}$) (right-hand side of axis). Abscissae, time in hr. Lower graphs: Ordinates, sodium fluxes or ammonia excretion rates in μ-equiv or μM.h^{-1}.100 g^{-1}. Abscissae, time in h. f_{in}, Na influx; f_{net}, Na net flux in shaded column. f_{NH_4}, ammonia excretion rate. Note: Intermediate 20 min. periods between flux-periods allowed for temperature change.

as representative of the effect of the temperature change on the sodium influx. The Q_{10} obtained was 2·6 or 2·9 which corresponds to an activation energy, A, of about 16 kcal.mole^{-1}, as calculated from the Arrhenius relation

$$f_{in} = f_0 e^{-A/RT},$$

R and T being the usual thermodynamic constants and f_0 the theoretical flux at 0 °K.

The sodium efflux is much less temperature-dependent. The Q_{10} ratio obtained by dividing the mean fluxes is 1·2 while the mean Q_{10} taking into account the individual Q_{10} values is 2. The corresponding activation energy is about 8 kcal.mole^{-1}.

The obvious consequence of the large difference in temperature dependence between sodium influx and efflux is that the sodium balance, positive during the 'warm' period, becomes negative in the 'cold' period (see Table 7).

When the fluxes obtained during the first and second 'warm' period are compared, it is apparent that the effects of cold shock are reversible. Thus the fish regains a positive sodium balance during the second 'warm' period.

The Q_{10} calculated for the ammonia excretion rate is very high, being nearly 4. The corresponding activation energy is 22 kcal.mole^{-1}, even larger than that of the sodium influx. The effect of cold shock is reversible.

Experiments concerning temperature effects in 6 ammonia-loaded fish are illustrated in Fig. 7 and summarized in Table 8. The comparison of the sodium influx

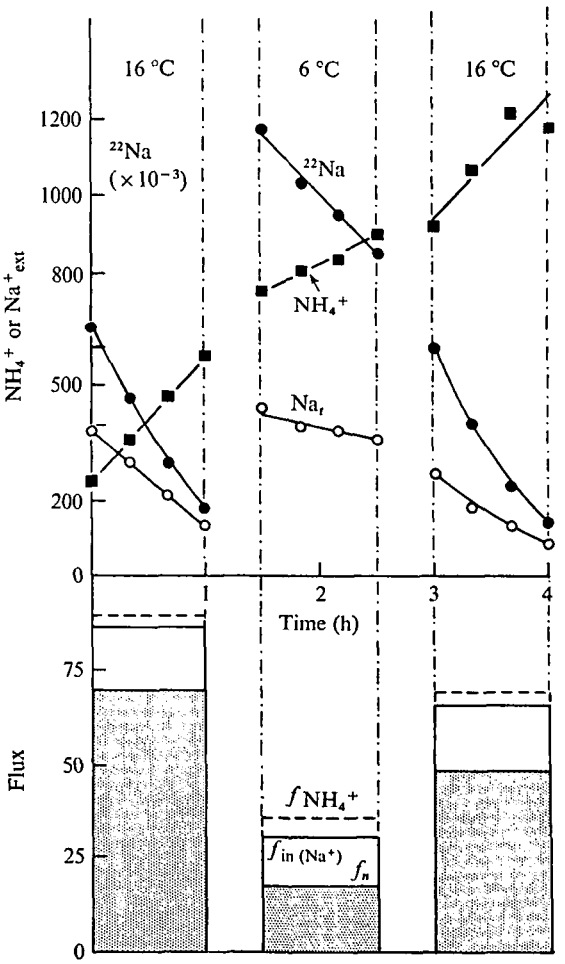

Fig. 7. Effect of abrupt temperature changes on branchial sodium exchange and ammonia excretion in ammonia-loaded *Carassius*. Same legend as in Fig. 6. Note that during the first 30 min intermediate period allowed for cooling of the bath, sodium and ^{24}Na were added to the external bath.

Table 8. *Effects of temperature change on ammonia excretion and sodium exchange by the gill in ammonia-loaded* Carassius ($n = 6$)

	16 °C	6 °C	16 °C	Q_{10}, m
Am. excr. μM h^{-1} (100 g)$^{-1}$	147·3 ± 16·4	54·7 ± 10·2	86·5 ± 15·0	2·3 ± 0·25
Mean Na_{ext} μ-equiv.l^{-1}	264 ± 37	373 ± 102	214 ± 95	—
f_{in} μ-equiv (100 g)$^{-1}$ h^{-1}	107·2 ± 5·4	44·1 ± 9·2	58·5 ± 8·5	2·2 ± 0·32
f_{out}	30·4 ± 7·8	20·0 ± 2·9	17·2 ± 2·9	1·2 ± 0·26
f_{net}	+76·8 ± 10·8	+24·1 ± 10·2	+41·3 ± 7·8	—
f_{in}/Na_{ext}	443·8 ± 63·1	167·7 ± 43·6	454·0 ± 90·7	3·3 ± 0·50

during 'warm' and 'cold' periods is complicated by the fact that attempts to maintain the same Na_{ext} during all three successive periods by addition of NaCl solution during the intermediate cooling and warming periods were not very successful. The mean Na_{ext} during the 6 °C period is in fact higher than during the two 16 °C periods. The Q_{10} for f_{in} is thus underestimated. As f_{in}/Na_{ext} is more or less constant up to 300 μ-equiv.l^{-1}, the Q_{10} for this ratio, 3, which is relatively high and which corresponds to an activation energy of 19·5 kcal.mole^{-1}, is thought to be a better estimate of the temperature sensitivity. This value is similar to that calculated for control fish. The Q_{10} for the sodium efflux is very low giving an activation energy of about 4 kcal.mole^{-1}, comparable to that usually given for free diffusion of Na^+ in an aqueous solution.

Despite the differential effects of temperature on sodium influx and efflux, the sodium balance remains positive in the 6 °C fish. The net uptake decreases considerably, however.

The study of the temperature effects on branchial ammonia excretion is complicated by the fact that in ammonia-loaded fish but not in control fish the excretion rate declines spontaneously as shown in table 3. Thus the excretion rate observed during the 'return' period at 16 °C is barely 60% of that measured during the initial period at 16 °C. To take this decline into account, the excretion rate observed at 6 °C was compared to the mean of the rates measured during the two 'warm' periods. The temperature coefficient ($Q_{10} = 2·3$) thus obtained corresponds to an activation energy of 13·5 kcal.mole^{-1}, which is considerably less than that calculated for the control fish.

DISCUSSION

1. *Concentration dependence of branchial sodium fluxes*

Sodium influx in *Carassius* varies with external sodium concentration in a manner suggesting a saturable process. This is the third freshwater fish whose sodium transport system has been characterized by a maximal rate of influx and an affinity constant (see Table 9).

After injection of ammonium sulphate, the sodium influx, which is increased for several hours, remains dependent upon the external sodium concentration, but f_{max} is increased two-fold while K_m diminishes though not significantly.

The effects of sodium depletion (obtained by keeping goldfishes in renewed deionized water) upon the parameters of sodium influx are being studied (Maetz, in preparation). Table 9 gives the relevant data showing that maximal sodium transport is increased without modification of the K_m. Sodium-depleted *Carassius* resembles ammonia-loaded *Carassius* in this respect. These results may be interpreted as

Table 9. *Comparison of the parameters characterizing sodium influx as a function of Na_{ext} in various freshwater teleosts*

Species	Temperature of test (°C)	Na_{ext} adaptation medium (μ-equiv.l^{-1})	f_{max} (μ-equiv.h)	K_m	Reference
Platichthys flesus	16	100	35	800	Maetz (1971)
Salmo gairdneri	13	700	33	460	Kerstetter *et al.* (1970)
Carassius auratus	16	100	65	300	This paper
C. auratus	16	0–25*	140	260	Maetz in preparation

* Kept in renewed de-ionized water for 3 weeks.

indicating that salt-depletion or ammonia-loading allows more sites to be accessible for Na transport but the sites have more or less the same affinity for sodium ions.

Branchial sodium efflux remains unchanged after ammonia-loading. In both control and ammonia-loaded fish sodium efflux was found to be independent of Na_{ext} except at the lower range of Na_{ext} (100 μ-equiv.l^{-1}). Similar observations were made on the rainbow trout by Kerstetter *et al.* (1970). The possibility of an operative

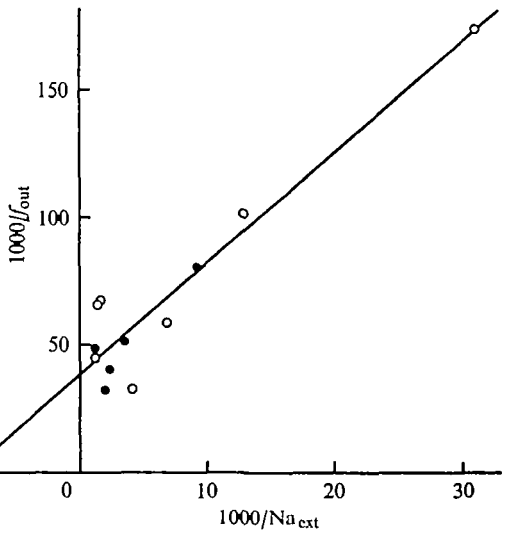

Fig. 8. Reciprocal plot of sodium efflux as a function of external sodium. Open circles: ammonia-loaded fish. Filled circles: control fish. The equation given in text holds for $y = 1000/f_{out}$ and $x = 1000/Na_{ext}$. f_{out} in μ-equiv.h^{-1}.100 g, Na_{ext} in μ-equiv.l^{-1}.

exchange-diffusion system has been put forward by these authors. In Fig. 8 the reciprocals of Na_{ext} for both control and injected fish have been plotted. A good correlation is observed ($r = 0{\cdot}944$). The equation of the regression line thus obtained is

$$y = 4{\cdot}43x + 38{\cdot}92.$$

The calculated parameters of the exchange-diffusion system are f_{out}, max = 25·5 and K_m = 115 μ-equiv.l^{-1}. This value indicates that the affinity of the transport carrier is rather high. In Fig. 1 f_{out} as a function of Na_{ext} (dotted line) has been represented according to this equation.

When the respective role of kidney and gill as sites of sodium loss are compared

(see Fig. 3 and Table 5) it can be seen that the mean branchial efflux is 4 times higher than the urinary excretion rate. In the lower range of Na_{ext} the branchial f_{out} is probably only twice the urinary loss. In these short-term experiments the urinary sodium loss was assumed to be independent of Na_{ext} changes, but this point remains to be studied. In any case, as illustrated in Fig. 3, the overall sodium balance of the goldfish adapted to 100 μ-equiv.l^{-1} and studied in this medium is in equilibrium, as the net sodium uptake by the gill exactly compensates for the urinary sodium loss. Above 100 μ-equiv.l^{-1}, the sodium balance is positive.

2. *Ammonia excretion in* Carassius

The present results confirm that the gill is the main site of nitrogenous waste excretion. In *Carassius* the urine is responsible for at most 0·3 % of the total excretion rate. This dominant role of the gill was first demonstrated by Smith (1929). The branchial excretion rate reported by Fromm (1963), Fromm and Gillette (1968) and by Kerstetter *et al.* (1970) for the rainbow trout kept at 13 °C are in the same range (12–30 μM h^{-1}.100 g^{-1}) as those given here for *Carassius*.

After ammonia-loading (1 mM/100 g) the excretion rate is increased up to 6 times. Most of the load is excreted by the gill but the relative role of the kidney is increased, attaining 8 % of the total excretion rate. About 50 % of the injected load is eliminated in 4 h. In a recent set of experiments the internal space distribution of the injected ammonia was measured in four fish about 1–1·75 h after injection and found to be of the order of 150 ml 100 g^{-1}, indicating that most ammonia is located intracellularly.

In ammonia-loaded fish most of the ammonia excreted by the gill must be the result of branchial clearance of exogenous ammonia. In control fish we do not know which fraction of the excreted ammonia is the result of endogenous ammonia clearance. According to Pequin & Serfaty (1963) 100 % of the ammonia excreted by the carp originates from the liver. This observation contradicted the claim expressed by Smith (1953) that most of the ammonia is produced by the gill. *In vitro* studies of enzymic production of ammonia by gill extracts appeared at first to confirm this claim (Goldstein & Forster, 1961). More recently, by simultaneous measurement of branchial blood flow and comparison of the ammonia blood levels in dorsal and ventral aorta, Goldstein *et al.* (1964) observe in the marine *Myoxocephalus* that 50 % only of the excreted ammonia results from branchial clearance, the remainder being produced by the gill itself.

As to the form (ionized or unionized) in which ammonia is excreted by *Carassius*, the observations (see Figs. 4 and 5 and Table 6) concerning the concentration gradients for both forms and the pH gradient across the gill clearly demonstrate that transfer in the free base form is unlikely, at least under our experimental conditions. The free base, which is generally considered to be highly lipid-soluble and diffusible across biological membranes, would have to have a partial pressure (pNH_3) at most equal if not higher in the internal medium than in the external medium. Unless NH_3 is actively transported, the most likely explanation is that it is NH_4^+ as an ion which crosses the gill.

The possibility that as a result of 'local' epithelial ammonia production, the pNH_3 in the branchial cell itself could be higher than in both external and internal media has to be considered. The pNH_3 gradient to be overcome at the internal boundary of

the branchial eipthelium would be even higher, and branchial clearance of ammonia would be impossible unless the membrane were permeable to the ionized form of ammonia.

Whether NH_4^+ transfer is active or passive in nature remains in doubt. In about half of the fish NH_4^+ is excreted against its concentration gradient but the potential difference across the gill epithelium which is also of importance, remains unknown. Maetz & Campanini (1966) found that in the freshwater eel the inside was negative (16 mV) while Kerstetter *et al.* (1970) found the inside to be positive in the trout.

Other epithelial membranes have been found to be the site of ammonia transfer in the ionized form. In the case of the hamster ileum, active transport of NH_4^+ is strongly suggested by the observations of Mossberg (1967).

Concerning the gill, the data reported here are in line with preliminary experiments on the catfish published by Wolbach, Heinemann & Fishman (1959). According to these authors ammonia-loaded fishes kept in a closed-circuit aquarium are able to excrete ammonia at two-thirds of the normal rate against a 2 m-equiv.l^{-1} concentration gradient. Furthermore, the final concentration of total ammonia in the bath may be 2 to 3 times that found in the blood even when the blood is more acid than the bath.

My results are in complete disagreement with those obtained in the trout by Fromm & Gillette (1968) who found that NH_3 concentration in blood was always higher than in the outside medium.

In conclusion, in the catfish as well as in the goldfish, ammonia excreted in the ionized form is thus readily available as an endogenous ion to be exchanged against Na^+. In the trout more work is needed to verify whether the gill is the site of non-ionic diffusion of NH_3 exclusively and whether endogenous H^+ ions are to be exchanged against Na^+ ions as suggested by Kerstetter *et al.* (1970).

3. *The absence of correlation between ammonia excretion and sodium uptake*

Tables 1 and 2 show that ammonia excretion is independent of sodium uptake in both control and ammonia-loaded fish.

In some experiments, in those concerning control and ammonia-loaded fish at low Na_{ext}, for example, ammonia excretion greatly exceeds sodium net uptake. In a few experiments, especially in control fish at high Na_{ext}, sodium net uptake exceeds ammonia excretion.

No correlation was observed between the extra sodium uptake resulting from the experimental increase of Na_{ext} and the simultaneous variations of ammonia excretion. Nor was any correlation found between the extra ammonia output consequent upon ammonia-loading and the extra sodium uptake induced by this treatment.

As the experiments were made on fish kept in NaCl solutions, the chloride net movements may have intervened to maintain the electro-neutrality of the external and internal media either by accompanying the excreted NH_4^+ ions (Cl^- net flux negative) or by accompanying the Na^+ ions taken up (Cl^- net flux positive). That chloride movement does not intervene was verified by measuring the Cl^- net flux in six control and eight ammonia-loading experiments. At low Na_{ext}, for example, the chloride balance was found to be in equilibrium in control fish ($-3{\cdot}0 \pm 8{\cdot}3$ μ-equiv.h^{-1} 100 g^{-1}) and slightly negative in injected fish ($-12{\cdot}5 \pm 5{\cdot}2$), a net flux insufficient to account for the NH_4^+ excretion *minus* the Na^+ net uptake rate (about 80 μ-equiv.h^{-1}).

Furthermore, the extra sodium uptake induced by ammonia-loading is not accompanied by any significant variation in the chloride balance. At high Na_{ext}, for example, injection of ammonia produces a variation of $-9{\cdot}2 \pm 6{\cdot}8$ μ-equiv.h^{-1}.100 g^{-1} ($n = 6$). This confirms our previous observations (Maetz and Garcia Romeu, 1964).

Garcia Romeu & Maetz (1964) suggest that Na^+ and Cl^- movements are also independent when these ions are taken up simultaneously from an NaCl solution. In such a situation the conductivity of the external medium remains constant or increases slightly which indicates that Na^+ and Cl^- are exchanged independently with endogenous ions.

In any case in the present experiments Na^+ uptake and NH_4^+ excretion are certainly not tightly coupled. Such a conclusion has already been drawn by Shaw (1960*b*, *c*) from his study of the mechanisms of Na^+ uptake in the crayfish. He observed that in de-ionized water ammonia excretion continues unimpaired despite the absence of Na uptake. After addition of sodium to the external bath resumption of sodium uptake is not accompanied by an increased ammonia uptake. De Vooys (1968) made similar observations on the carp transferred from tap water to de-ionized water and back to tap water. Finally, Kerstetter *et al.* (1970) observed that ammonia excretion in the trout is more or less constant despite considerable changes of sodium uptake due to experimental changes of external sodium concentration. As discussed above, Kerstetter *et al.* (1970) propose that all ammonia is excreted in the molecular form, while Na^+ is exchanged against another endogenous cation, H^+. The significant pH decrease in the external bath accompanying Na^+ uptake demonstrates such an exchange, at least in cases in which sodium uptake greatly exceeds ammonia excretion. Earlier Shaw (1960) had proposed that the cationic exchange does not necessarily involve a single ion species. An alternative H^+/Na^+ exchange in addition to NH_4^+/Na^+ exchange was suggested on the grounds of the competition between protons and Na^+ ions for sodium uptake (Shaw, 1960*b*).

Recent experiments on the goldfish (Maetz, in preparation) in which H^+ and NH_4^+ excretion were measured simultaneously confirm Shaw's suggestion of alternative H^+/Na^+ and NH_4^+/Na^+ exchanges.

4. *Temperature effects on sodium balance and ammonia excretion*

The data demonstrate clearly how branchial sodium uptake is drastically affected by external temperature change. The sodium influx, which is undoubtedly active in nature in view of the huge concentration gradient between external and internal media (see Garcia Romeu & Maetz, 1964), is much more affected (mean Q_{10} for all experiments $= 3{\cdot}07 \pm 0{\cdot}23$ for f_{in}/Na_{ext}) than the branchial sodium efflux (mean Q_{10}: $1{\cdot}67 \pm 0{\cdot}26$). It follows that in control fish the sodium balance switches from positive to negative. The decrease in plasma sodium observed after transfer to a cold environment in various freshwater fishes including the goldfish is thus explained (Prosser, Mackay & Kato, 1970, for *Carassius auratus*; Umminger, 1970, for *Fundulus heteroclitus*; Houston & Madden, 1968, for *Cyprinus carpio*; Hickman, McNabb, Nelson, Van Breeman & Comfort, 1964, and Reaves, Houston & Madden, 1968, for the trout). The effects of abrupt cold exposure on osmoregulation are in many fishes followed by compensatory mechanisms (see Reaves *et al.* 1968; Houston, Reaves, Madden & de Wilde, 1968; Houston, Madden & de Wilde, 1970). These remain to be studied in

terms of branchial electrolyte exchange mechanisms. My observations confirm those made by Morris & Bull (1968) of the effects of temperature on the sodium balance of the ammocoete larvae of *Lampetra planeri*, although their study concerns uncatheterized animals. These authors concluded that 'low temperature lowers the permeability of the external surfaces to ions', but 'that this effect is, however, small compared with the inhibition of ion uptake so that the combined result is to increase the net loss of sodium from the animal'.

Almost two decades ago Wikgren (1953) observed that in various freshwater fishes low temperature decreases passive branchial chloride loss into de-ionized water, while in the presence of chloride in the external bath the ion net uptake is even more sensitive to the temperature effect. Unfortunately no isotopes were available at that time.

The effect of temperature on ammonia excretion in fish had not previously been studied. In control fish ammonia excretion is very temperature-sensitive (Q_{10} = 3·9 corresponding to the very high activation energy of 22). It is doubtful whether this high temperature-sensitivity reflects effects on gill permeability to ammonia. It is more probable that the limiting factor which is temperature-sensitive is ammonia production via metabolic pathways either in the gill or in the liver. In fact, ammonia production by the liver is probably even more temperature-sensitive than suggested by our observations, as it is doubtful whether the temperature change in the liver reflects that of the external medium.

To study temperature effects on the branchial permeability of the gill, it is necessary to work with fish in which the ammonia excretion rate is much higher than the metabolic ammonia production. Q_{10} studies were thus extended to ammonia-loaded fish in which about 6 times more ammonia is cleared by the gill than when the ammonia production is metabolic. It is significant that in such fish (compare tables 7 and 8) the Q_{10} of ammonia excretion is significantly less. The Q_{10} (2·3) corresponding to an activation energy of 13·5 is still relatively high. If the rate of ammonia production in control fish is subtracted from the rate of total ammonia excretion in ammonia-loaded fish in order to obtain an approximate rate of extra ammonia cleared by the gill for each period, a Q_{10} of 1·95 may be calculated corresponding to an activation energy of 10·5 which is only slightly higher than that calculated for the passive sodium efflux in control fish (A = 8·5). The data thus suggest that ammonia transfer, like sodium efflux across the gill, is passive in nature, but owing to the slightly bigger diameter of the charged ion, the temperature coefficient for membrane transfer is higher. Ammonia transfer in the molecular form which is highly lipid-soluble would be expected to have a much lower temperature coefficient.

When the present results concerning temperature effects on the branchial sodium exchange of *Carassius* are compared with those obtained by Maetz & Evans (1972) on the seawater-adapted flounder, several points emerge.

1. In fresh water the active component of sodium transport f_{in}, with a Q_{10} of 3, is definitely less sensitive to abrupt temperature changes than the Na/K exchange which according to Maetz (1969, 1971) represents the active sodium excretion component in sea water. This difference in sensitivity is surprising as the electrochemical gradient to be overcome during sodium uptake from fresh water seems definitely higher than that for sodium extrusion in sea water. The rates of net transfer are about 5 times higher for the flounder (100 μ-equiv.h^{-1}.100 g^{-1}) than for control *Carassius* in

0·1 m-equiv.l^{-1} sodium concentration. At 1 m-equiv.l^{-1} after ammonia-loading the rates are, however, very similar.

2. The passive component of sodium exchange in *Carassius* f_{out}, with a Q_{10} of 1·2 to 2 (mean 1·67), has exactly the same temperature coefficient as the f_{out} leak observed in *Platichthys* after transfer from sea water to fresh water. The Na/Na exchange component observed in the flounder is characterized by a slightly higher temperature-sensitivity (Q_{10} of 2·2).

SUMMARY

1. Sodium influx and efflux and ammonia excretion by the gill have been studied as a function of external sodium chloride concentration in *Carassius auratus* before and after loading the fish with ammonia.

2. No correlation between net sodium uptake and ammonia excretion is observed, either when the net uptake changes with an external sodium change or when net uptake increases with ammonia-loading. Branchial handling of chloride ions cannot explain this absence of correlation.

3. Comparison of the concentrations of free base ammonia (NH_3) and of ammonium ions (NH_4^+) in both blood (dorsal aorta) and external medium at the end of the closed-circuit experiments on control or ammonia-loaded fish demonstrates that the gill is permeable to the ionized form of ammonia.

4. An abrupt temperature decrease (16 → 6 °C) affects the sodium influx (Q_{10} = 3) much more than the sodium efflux (Q_{10} = 1·7). Sodium balance becomes negative unless the fish is ammonia-loaded. The observed effects of temperature are reversible when the fish is returned to 16 °C. Branchial ammonia excretion is highly temperature-sensitive (Q_{10} = 4) in control fish when metabolic production limits ammonia excretion. After ammonia-loading, when most of the ammonia cleared by the gill is exogenous, the effect of temperature on branchial permeability to ammonia (Q_{10} = 1·9) suggests a passive transfer of ammonium ions.

5. The contributions of the kidney and the gill in sodium loss and ammonia excretion are compared in intact and ammonia-loaded fish.

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