

Figure 2 shows one of four experiments similar to that of Figure 1, except that carbonic anhydrase was totally inhibited throughout the procedure. Plasma $p\text{CO}_2$ was the same in the two situations, since it was dependent on the level in the ambient water. Results in Figure 2 were qualitatively like those in the uninhibited fish (Figure 1) but quantitatively different in that CSF HCO_3^- rose more slowly.

Figure 3 shows the mean data for plasma and CSF HCO_3^- in all nine experiments. Inhibition of carbonic anhydrase cuts the rate of HCO_3^- formation in CSF to half the normal value. This agrees with the effect of inhibition on the rate constant for transfer of plasma $^{14}\text{CO}_2$ to CSF $\text{H}^{14}\text{CO}_3^-$ (Bull. MDIBL 9:33, 1969).

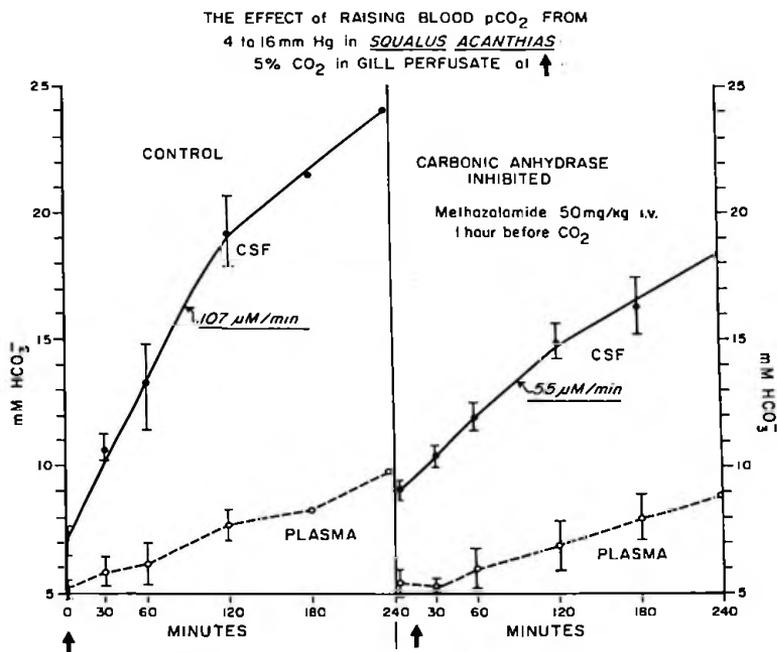


Figure 3. Showing mean data \pm S.E. from all experiments, for plasma and CSF HCO_3^- . $n = 3-6$ except where no S.E. is shown, $n = 2$.

The data strongly support the idea that the choroid plexus and other secretory cells in brain containing carbonic anhydrase have the function of polarizing water to H^+ and OH^- ions. CO_2 reacts with the latter to form HCO_3^- , which diffuses or is secreted into CSF. This provides a rapid means of regulating CSF pH in the face of alterations in blood $p\text{CO}_2$, and may be the chemical means by which a close feedback control of respiration is achieved. We thank Mr. Róbert Woodworth and Miss Marjorie Peirce for their excellent assistance.

The work was supported by NIH Grant GM AI 16934-02.

1970 #29

IN VIVO IONIC EXCHANGE THROUGH THE SKIN OF THE FROG *Rana clamitans*

René Motais and B. Schmidt-Nielsen, University of Nice, France and Case Western Reserve University, Cleveland, Ohio.

Fresh water animals compensate their renal loss of ions by absorbing these ions from the external medium through the gills (crustaceans, fishes) or the skin (frogs). There is evidence that in crustaceans and fishes the uptake of both chloride and sodium are independent, the sodium being exchanged for an endogenous cation and chloride for an endogenous anion. These mechanisms cannot be demonstrated in the short-circuited frog skin. Recently it was shown that in the South American frog the skin "in vivo" absorbs Na and Cl independently from dilute external solutions, H^+ and HCO_3^- being exchanged against respectively Na^+ and Cl^- (Garcia-Romeu et al, J. Gen. Physiol. 53:816-35, 1969).

An attempt is made in the present work to extend these observations concerning independence between Na and Cl uptake to the North American frog Rana clamitans, by promoting significant differences in the rates of Na and Cl uptake from NaCl solutions.

Before the experiments, and for the purpose of enhancing Cl or Na fluxes, the animals were kept at room temperature for seven days in Na_2SO_4 or choline chloride. The solutions were renewed once a day. After the preadaptation period, the animals were placed in individual flasks and urine was drained out of the external bath by cloacal cannulation in order to measure exclusively the epithelial electrolyte net fluxes. The external bath, during the two days prior to the flux measurement, remained the preadaptation solution. On the third day, the flux measurements were started after replacement of the preadaptation solution by a test solution: NaCl (1.7 mEq/L buffered at pH 7 by imidazole).

In animals preadapted in choline chloride solution (1.7 mEq/L), net Na^+ flux exceeds net Cl^- flux (Figure 1, Table 1), a result which is expected if the Na and Cl uptake mechanisms are

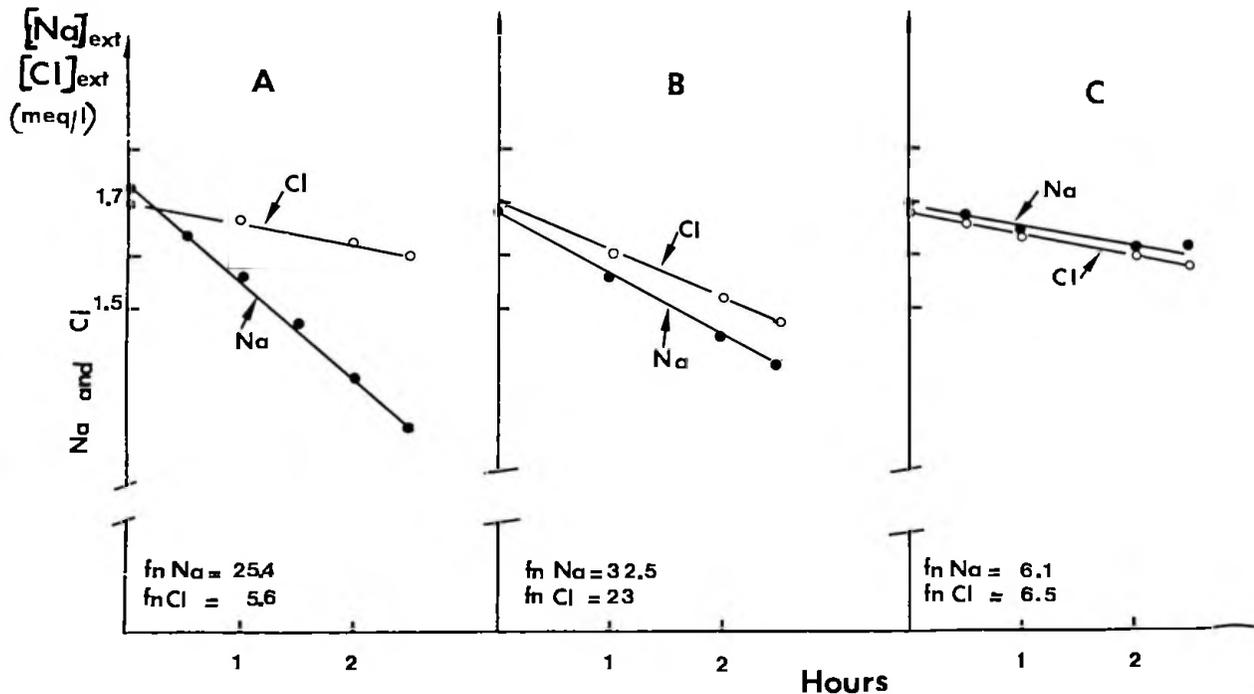


Figure 1. Net fluxes of Na^+ and Cl^- from NaCl solutions (1.7 mEq/L) from frogs preadapted in A, choline-Cl (1.7 mEq/L); B, Na_2SO_4 (1.7 mEq/L); C, Na_2SO_4 (10 mEq/L) solutions. The net fluxes (fn) are expressed in $\mu Eq/h \times 100 g$.

Table 1

DIFFERENTIAL RATE OF Cl^- AND Na^+ UPTAKE IN FROGS PREADAPTED IN
DIFFERENT SALT SOLUTIONS

Mean of net fluxes \pm SEM in $\mu\text{Eq/h} \times 100\text{g}$ measured in the experimental
solutions (NaCl 1.7 mEq/1 - pH7 buffered by imidazole).

Preadaptation solutions	No. of experiments	Na^+ net flux	Cl^- net flux
Choline chloride (1.17 mEq/1)	11	+ 18.10 \pm 2.14	+ 4.08 \pm 1.19
Na_2SO_4 (1.7 mEq/1)	10	+ 20.83 \pm 3.60	+ 18.99 \pm 2.19
Na_2SO_4 (10 mEq/1)	5	+ 7.28 \pm 1.26	+ 4.18 \pm 1.53

Experiments made in July - August.

Temperature = 22° C.

independent. But, surprisingly, in frogs preadapted in Na_2SO_4 (1.7 mEq/L, net Na^+ flux equals or even exceeds net Cl^- flux (Figure 1, Table 1). This unexpected observation may be explained if, during the preadaptative period in Na_2SO_4 , the frogs were as depleted in Na as they were depleted in Cl. It has been shown (Garcia-Romeu et al, op. cit.) that the uptake of Na^+ is higher in NaCl than in Na_2SO_4 , at equal Na concentrations, an observation which suggests that the accompanying anion affects the Na permeability. To test this hypothesis, frogs were adapted in a more concentrated Na_2SO_4 solution (10 mEq/L). But, as can be seen in Figure 1 and Table 1, the relative rates of Cl^- and Na^+ uptake were not modified by this experimental procedure. It can be observed, however, that both Na and Cl uptakes are lower if the animals are preadapted in 10 mEq/L rather than in 3 mEq/L sodium sulfate solutions.

It must be noted that the pH of preadaptation solutions being acid (5.7 - 5.9), a sudden change of pH occurs when frogs are transferred in experimental solution, which is buffered at pH 7.0. Then, it is possible that the unexpected pattern of Na_2SO_4 preadapted frogs could be explained by the effect of the shift of pH. To test this possibility, two groups of frogs were adapted in the same preadaptation solutions as before, either Na_2SO_4 (1.7 mEq/L) or choline (1.7 mEq/L).

The net fluxes of sodium or chloride were measured successively in a non buffered NaCl solution and in an identical NaCl solution buffered to pH 7 by imidazole (1 mM/L). Two experiments of this type are illustrated in Figure 2 and the results are reported in Table 2.

Several remarks may be made:

1) In animals adapted to Na_2SO_4 , during the first testing period in non buffered NaCl solution (pH : 5.6), the Cl net flux is significantly higher than the sodium net flux. In the second testing period in neutral NaCl solution, an important augmentation of Na^+ net flux is observed, such that the net Na^+ and Cl^- fluxes become equal. No significant effect of pH on the net Cl^- flux is observed. Preliminary observations indicate furthermore that imidazole per se has no effect on the fluxes and that it is the pH changes which are of importance.

2) For animals adapted to choline Cl, in the non buffered NaCl solution, the net Na^+ flux is somewhat higher than the net Cl^- flux although the small number of observations and individ-

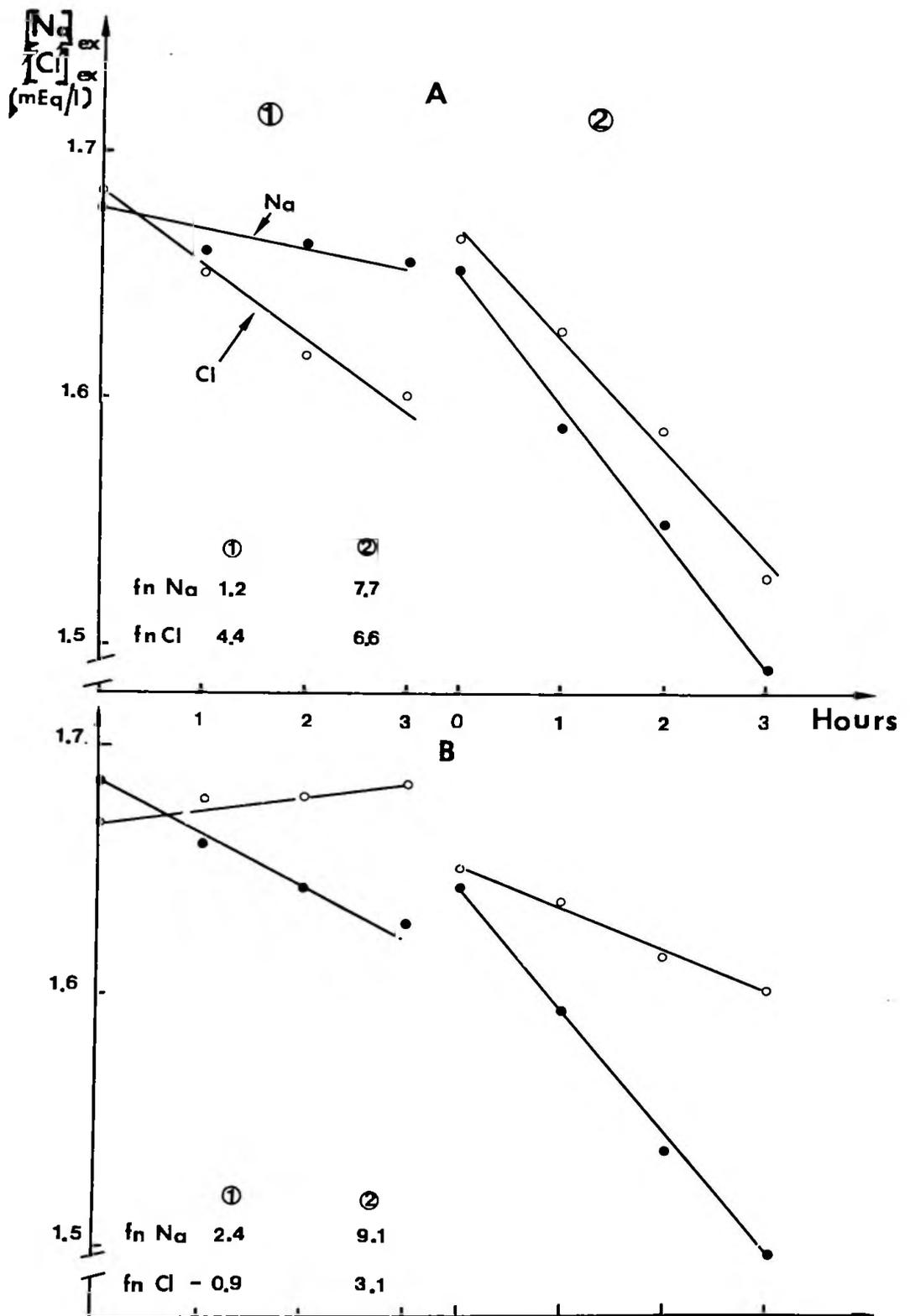


Figure 2. Net fluxes of Na^+ and Cl^- from not buffered (1) and buffered (2) NaCl solutions from frogs preadapted in A, Na_2SO_4 and in B, choline- Cl solutions. fn expressed in $\mu\text{Eq}/\text{h} \times 100 \text{ g}$.

Table 2
 COMPARISON OF THE EFFECT OF pH ON Na⁺ AND Cl⁻ NET FLUXES IN FROGS PREADAPTED
 IN Na₂SO₄ (1.7 mEq/l) OR CHOLINE CHLORIDE (1.7 mEq/l)

Preadaptation solutions	No. of experiments	Na ⁺ net flux			Cl ⁻ net flux		
		1st solution	2nd solution	Difference*	1st solution	2nd solution	Difference*
Na ₂ SO ₄	4	+ 2.82 ± 1.36	+ 8.60 ± 1.81	+ 5.99 ± 1.26	+ 6.14 ± 1.81	+ 8.18 ± 1.71	+ 0.97 ± 0.81
Choline	3	+ 3.88 ± 1.44	+ 13.17 ± 1.44	+ 9.22 ± 1.16	+ 1.31 ± 1.82	+ 5.78 ± 1.06	+ 3.74 ± 0.94

Net flux expressed in $\mu\text{Eq/h} \times 100\text{g} \pm \text{SEM}$.

1st experimental solution: NaCl solution (1.7 mEq/l) without buffer.

2nd experimental solution: NaCl solution (1.7 mEq/l) with imidazole 1mM/1 pH = 7.

* Mean differences of paired data during first and second period \pm SEM.

Experiments made in October.

Temperature = 15°C.

ual variations did not permit statistical analysis. In neutral NaCl solution an important increase of net Na^+ flux is observed while the net Cl^- flux increases less although still significantly.

To summarize, Rana clamitans placed in dilute NaCl solutions may pump Na^+ and Cl^- at very different rates. The difference in pumping rates depends on the electrolyte composition of external solutions in which the animals were preadapted. This observation is considered as evidence that the mechanisms for sodium and chloride absorption are different. It can be observed however that both Na and Cl uptakes are lower if the animals were preadapted in 10 mEq/L rather than 3 mEq/L sodium sulfate solutions. Furthermore a shift from acid to neutral pH by buffering the experimental NaCl test solution may produce a simultaneous increase in Na and Cl net uptake. It appears, therefore, that the transepithelial transports of Na and Cl are not completely independent.

1970 #30

RESPONSE OF THE KIDNEY OF Squalus acanthias TO DIURETICS

H. V. Murdaugh and Mark Epstein, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

The kidney of the dogfish, Squalus acanthias, has in several aspects been characterized as an organ with a relatively fixed pattern of function. Urine pH remains unchanged in the face of acid loading (Sharks, Skates and Rays, 1967, p. 249), and there is no demonstrable change in titratable acid excretion save for the increase that occurs with phosphate loading (W. W. Smith, *J. Cell. & Comp. Physiol.* 14:95, 1939). Although mercurials in the past have not been demonstrated to induce a renal response in the dogfish, Forster and coworkers reported that a diuresis and chloruresis was induced in the dogfish by epinephrine (*Bull. MDIBL* 9:14, 1969).

As part of a study of trace metal excretion in the dogfish, response to a series of diuretics was investigated. Inulin clearances were conducted 10 or more hours after the intramuscular administration of 3-4 ml 3.3% inulin in dogfish Ringers in divided doses. Following 2 control clearance collections, epinephrine (0.5-1.1 mgm/Kg), furosimide (8-17 mgm/Kg), ethacrynic acid (11-12 mgm/Kg), or chlorthiazide (80-125 mgm/Kg) were administered by intravascular route, and clearance collections were continued. Some fish received no drug and served as controls. Each drug studied, with the exception of chlorthiazide, produced diuresis and natriuresis. There was no measurable difference in the response with the higher doses of drugs used, indicating that the smallest doses used were effective. The higher dose of ethacrynic acid was associated with sluggish behavior of the fish. The large doses of chlorthiazide used were a result of previous failure to demonstrate response with lesser amounts of the drug. Following administration of chlorthiazide there appeared a white precipitate in the urine, presumably the chlorthiazide.

Change in sodium excretion was from a mean of 124 ± 15 (SE) to 310 ± 32 $\mu\text{Eq/Kg/Hr}$ following epinephrine, 174 ± 26 to 438 ± 35 $\mu\text{Eq/Kg/Hr}$ after ethacrynic acid and 136 ± 40 to 485 ± 118 $\mu\text{Eq/Kg/Hr}$ in response to furosimide. There was no measurable change in sodium excretion in the fish that received chlorthiazide or no drug.

Inulin clearance increased in most fish studied, but the increase in sodium excretion was greater in magnitude and occurred in studies when no increase in inulin clearance was found.