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Title: ACTIVE UPTAKE OF CHLORIDE ACROSS THE SKIN OF THE FROG, RANA PIPIENS (SCHREBER)

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Chloride is actively transported across the skin of the intact leopard frog, Rana pipiens. The influx and efflux in 0.2 mM NaCl are 0.67 and 0.72 μeq/10 g-hr, respectively. The renal efflux of chloride is 0.02 μeq/10 g-hr. The transepithelial potential differences (TEP) range from -1 to +32 mV (sign refers to the inside). The mean measured ratio of influx to efflux is 0.93. The flux ratio predicted from the flux ratio equation is 0.005. Clearly, chloride is actively transported.

With 0.2 mM KCl in the external bath, the influx and efflux of chloride (0.57 and 1.05 μeq/10 g-hr, respectively) are not significantly different from the values obtained in 0.2 mM NaCl. The measured and predicted flux ratios are 0.54 and 0.002, respectively. Chloride is actively transported, and this transport is not dependent on the movement of sodium.

The TEP is independent of the concentration of anion (chloride

or sulfate) in the external bath. With increasing sodium concentration (NaCl or Na_2SO_4) in the external medium, the body fluids become more electropositive relative to the bath (the slope of the line is 22 mV/log[mM]). At high sodium concentrations (50 mM), the magnitude of the TEP is reduced with increasing bath concentration probably as a result of increased permeability of the skin to chloride. The TEP is independent of the K^+ concentration in the external medium.

The influx of chloride increases with increasing chloride concentration in the external bath at lower concentrations. The transporting system shows Michaelis-type kinetics. For NaCl, V_{max} = 1.3 μeq/10 g-hr and K_m = 0.18 mM. For KCl, V_{max} = 1.2 μeq/10 g-hr and K_m = 0.38 mM.

Salt-depleted animals absorb net amounts of chloride (0.78 μeq/10 g-hr) from 1.0 mM KCl and lose net amounts of sodium and potassium (1.10 and 0.58 μeq/10 g-hr, respectively). When animals "pump" chloride from 1.0 mM KCl, the bath becomes more alkaline. The pH increases at the rate of 0.056 pH units/hr. The animals excrete net quantities of base (3.23 μeq/10 g-hr). The pK_b of the base excreted is 6.0. The pK_b of $KHCO_3$ in KCl is 6.1. The excreted base is probably bicarbonate ion which exchanges for the absorbed chloride.

Active Uptake of Chloride across the Skin of the Frog, Rana pipiens (Schreber)

by

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INTRODUCTION

The amphibians evolved from crossopterygian ancestors toward the close of the Devonian period. The earliest amphibian fossil remains are associated with fresh-water deposits which indicates that the primitive tetrapods, as well as their piscine ancestors, were fresh-water inhabitants (Romer, 1962; Noble, 1931).

Like all fresh-water animals, amphibians must continually combat the movement of water from a hypotonic environment (e.g., pond water) into the body tissues by producing a dilute urine equal in volume to the amount of water moving across the skin. A considerable amount of salt (principally sodium chloride) is lost in this process coupled with a diffusive loss of ions across the body surface. Fresh-water animals compensate by actively absorbing Na^+ and Cl^- from the external medium through specialized surface structures (gills in fishes and larval amphibians, skin in adult amphibians).

The active transport of sodium across amphibian skin (a subject already blessed with a wealth of literature) will receive limited treatment here. The primary focus of this paper will be the various aspects of in vivo active chloride transport across the skin of the common leopard frog, Rana pipiens.

A mechanism for the transport of chloride ions against an

electrochemical gradient is widely distributed in both the plant and animal kingdoms and has been documented for algal cells (MacRobbie, 1962), frog gastric epithelium (Hogben, 1955 a, b), turtle bladder (Finn et al., 1967), crayfish gills (Shaw, 1960), rat ileum (Curran and Solomon, 1957), rabbit gall bladder (Martin and Diamond, 1966), squid giant axons (Keynes, 1963) and anal papillae of mosquito larvae (Stobbart, 1967).

The first evidence for the existence of an active mechanism for chloride transfer was presented by August Krogh in 1937. His classical in vivo studies on Rana esculenta showed that salt-depleted animals absorb net amounts of chloride (0.1 μeq/cm^2-hr) from dilute (< 1.0 mM) NaCl solution. He also demonstrated that chloride does not of necessity passively follow the active absorption of sodium, because chloride is selectively absorbed from solutions of potassium, ammonium and calcium chlorides. Krogh postulated, but did not prove, that absorbed chloride is replaced by the extrusion of bicarbonate into the external medium (1937a, b, 1938, 1939). Krogh did not consider electrical gradients, and he was limited by the fact that he did not have radioactive isotopes which would have enabled him to measure unidirectional fluxes. Thus, his data are inadequate to unequivocally show the active transport of chloride as the criteria for active transport are presently understood (see below).

In 1954, Jørgensen, Levi and Zerhan noted an active transport

of chloride across the skin of Bufo bufo, R. temporaria and R. esculenta. The influx and efflux of chloride and the transepithelial skin potentials were measured with 3.0 mM NaCl or KCl in the external medium. However, recent statistical evaluation of their data by Kirschner (1970) did not support their original conclusion.

The existence of an active transfer of chloride has also been documented for the South American frog, Leptodactylus ocellatus (Salibián et al., 1968) and for the Chilean frog, Calyptocephalella gayi (García Romeu et al., 1969). The transport mechanism in these two frogs is independent of the active sodium system. When adult L. ocellatus are depleted of sodium and chloride they take up equal amounts of both ions from dilute NaCl; however, if pretreated with Na_2SO_4, they take up chloride three times more rapidly than sodium. In the case of C. gayi, the chloride net flux values are similar and a possible exchange of chloride for tissue bicarbonate has been proposed.

Amphibian skin has also been studied in vitro and, over the past 20 years, has provided invaluable information related to the mechanism of transepithelial ionic movements. Koefoed-Johnson, Levi and Ussing (1952) determined influx and efflux of chloride across isolated skins of R. temporaria and R. esculenta with Ringer's solution on the inside and 1/10 Ringer's on the outside. The flux ratio (influx/efflux) did not differ from the values predicted by Ussing's

flux ratio equation (Ussing, 1949b) indicating that chloride movement was passive. Koefoed-Johnson, Ussing and Zerahn (1952) measured chloride fluxes in short-circuited skins from R. temporaria which were exposed on both sides to Ringer's solution. No net flux of chloride was observed under these conditions, again indicating passive transfer. They did find, however, an active outward transport of chloride when adrenaline was added to the inside bathing medium. It was concluded that adrenaline triggers a secretory response in the mucous glands of the skin which involves active transport of chloride to the outside. Using skin bags from R. pipiens, Huf et al. (1955) also presented evidence to indicate that chloride is not actively transported in vitro.

In 1963, Zadunaisky et al. showed a net inward movement of chloride across the isolated skin of the South American frog Leptodactylus ocellatus. The skins were short-circuited and bathed on both sides with Ringer's solution. This active transfer of chloride could also be inhibited by 10^{-5} M ouabain. In view of this evidence, Zadunaisky et al. suggested that the mechanism for chloride transport does exist in vivo in all kinds of frog skin, but that it shows up in vitro only in some species in which it is more potent and lasts longer after removal of the skin from the animal.

Martin and Curran, in 1966, noted an active transfer of chloride across isolated skins of R. pipiens and R. esculenta only when

the skins were bathed with 2 mM KCl on the outside and Ringer's solution containing only 2 mM chloride on the inside. Values for chloride net flux with 56 mM Na_2SO_4 + 2 mM KCl on both sides of the skin were 33.2 mμeq/cm^2-hr for R. pipiens and 53.4 mμeq/cm^2-hr for R. esculenta. With 2 mM KCl on the outside, transepithelial skin potentials were negative and ranged from -29 to -60 mV. These results could indicate that at higher concentrations of chloride, the transport phenomenon appears to be masked by a relatively large passive transfer of chloride. However, a 2 mM chloride solution bathing the inside surface of the skin is highly unphysiological.

Most of the studies on ion transport in amphibia have been made within the framework of the isolated skin. By comparison, the amount of literature on in vivo studies is noticeably smaller. Salibián et al. (1968) suggest that sometimes the advantage of a model (the in vitro skin) in which the variables are reduced obscures the fact that the model is a system in itself and that it may behave differently from the system it represents.

A review of the literature suggests a mechanism for active chloride transport across the skin of intact adult frogs, but the data are not conclusive for Rana pipiens, the most commonly used frog in this country for in vitro skin studies. The evidence to date indicates that there is no active transport of chloride across the isolated skin of this species. This study presents conclusive evidence that

1) there is an active chloride transport system in the skin of intact R. pipiens; 2) the transport system displays saturation kinetics typical of most active transport systems; 3) this transport system is not dependent on the activity of a cation transporting system; 4) the chloride transport system does not appreciably affect the trans-epithelial skin potential; and 5) the chloride transport system may operate by exchanging for an internal anion, probably bicarbonate.

CRITERIA FOR ACTIVE TRANSPORT

Passive diffusion is a process which can be explained by ordinary physical forces--a concentration gradient, an electrical gradient, a solvent drag, or any combination of these. Active transport, on the other hand, cannot be explained by any of these forces. Active transport is usually defined as the movement of a substance against an electrochemical gradient.

Ussing (1949b) has shown that if an ion moves independently and passively in response to its electrochemical gradient, the flux ratio (influx/efflux) is given by the following equation:

$$\log \frac{M_i}{M_o} = \log \frac{C_o}{C_i} - \frac{ZFE}{2.3RT} \qquad (1)$$

where M_i = influx,

M_o = efflux,

C_i = concentration of ion in inside in mM/l,

C_o = concentration of ion in outside in mM/l,

Z = charge on the ion,

F = 96,500 coulombs/mole,

E = potential difference in volts,

R = 8.3 joules/degree-mole, and

T = temperature in °K.

This equation indicates that passive diffusion is proportional to the electrochemical gradient. However, if the observed flux ratio (M_i/M_o) is not in agreement with the calculated flux ratio (equation (1)) or if the measured and the calculated transepithelial potential differences are not equal, then it may be concluded that the ion in question is actively transported.

MATERIALS AND METHODS

Animals

The experimental animals were adult Rana pipiens obtained from Steinhilber and Company, Oshkosh, Wisconsin. They were maintained in tap water at room temperature. The average size of the animals used was 30 g. When salt-depleted animals were needed, they were kept in distilled water which was changed once daily for a period of 10 to 12 days.

Analyses

All chloride determinations were made with an Aminco-Cotlove chloridometer (precision ±1%). Urine and bath samples were titrated directly. Serum samples were diluted 100 times and then analyzed. Sodium and potassium were determined by flame photometry (precision ±1%). Total acidity was estimated by titration with 0.001 N NaOH and total base with 0.001 N HCl.

Flux Determination

Chloride influx was measured with ^{36}Cl. The isotope, obtained as HCl, was neutralized with NaOH or KOH and then added to the bathing medium. In some instances, the animals were allowed to

equilibrate in the bath for a period of 12 hours before the medium was replaced and isotope added. One ml samples were removed from the bath at specific time intervals. Each sample was transferred to a planchet, air dried, and then counted on a gas-flow Geiger counter. Separate bath samples were analyzed for chloride using electrometric titration and the net flux (M_{net}) was calculated from the following expressions:

$$A_o = C_o \cdot V_o \tag{2}$$

$$A_t = C_t \cdot V_t \tag{3}$$

$$M_{net} = \frac{A_o - A_t}{t} \tag{4}$$

where A_o = total ion in bath at time zero,

A_t = total ion in bath at time t,

C_o = concentration of ion in bath at time zero,

C_t = concentration of ion in bath at time t,

V_o = volume of bath at time zero,

V_t = volume of bath at time t.

The influx (M_i) was calculated from the rate of disappearance of the isotope from the bath using the equations derived by Jørgensen et al. (1946). In a steady-state condition, when the concentration of the ion in the bath does not change in time, the equation is

$$M_i = -\frac{2.3A}{t} \log \frac{y_o}{y_t} \tag{5}$$

However, when steady-state conditions do not prevail and the concentration of the ion in the bath changes with time, the equation is

$$M_i = B\frac{\log\frac{y_o}{y_t}}{\log\frac{A_o}{A_t}} \qquad (6)$$

where M_i = influx,

B = rate of net uptake or excretion,

y_o = radioactivity/unit volume at time zero,

y_t = radioactivity/unit volume at time t,

A_o = total ion in bath at time zero, and

A_t = total ion in bath at time t.

The total efflux (M_o) can then be calculated from the following expression:

$$M_{net} = M_i - M_o \qquad (7)$$

where

$$M_o = M_{o(renal)} + M_{o(extrarenal)}. \qquad (8)$$

The renal efflux ($M_{o(renal)}$) is obtained by measuring the rate of urine production and the concentration of chloride ion in the urine.

$$M_{o(renal)} = u \cdot V \qquad (9)$$

where u = the concentration of chloride in urine (μeq/ml)

V = the volume of urine produced in ml/10 g-hr (measured by the osmotic uptake of water).

Transepithelial Potential Difference (TEP)

Animals were anesthetized in 2% urethane, rinsed and then transferred to the experimental solution. The skin and body wall were punctured and a fine polyethylene tube filled with 3% agar-Ringer's solution was inserted into the body cavity. A second salt bridge containing 1/10 Ringer's solution was placed into the bathing medium. Both bridges were connected through calomel electrodes to a recording potentiometer. Corrections for asymmetry were made after each potential measurement (Dietz et al., 1967).

Serum and Urine Samples

Blood samples were taken by direct heart puncture, centrifuged for two minutes at 3000 x g and a sample of the serum removed.

Urine was collected at random from laboratory animals by blotting the animals thoroughly and then pressing gently on the lower abdominal region to empty the bladder.

RESULTS

Flux Data

The influx and efflux (both renal and extrarenal) of chloride were determined for animals maintained in 300 ml of 0.2 mM NaCl. The experiment lasted 48 hours and samples were taken at eight-hour intervals. The measured flux ratio (M_i/M_o) was compared with the predicted flux ratio based upon Ussing's equation. The known values of C_i, C_o and TEP were used in the calculation. The results are given in Table 1.

Table 1. Active transport of chloride from 0.2 mM NaCl.

Fluxes (μeq/10 g-hr)[a]		M_i/M_o		C_o/C_i	E(mV)[b]	
M_i	M_o	exp.	calc.		mean	range
0.67 ±0.07	0.72 ±0.03	0.93	0.005	0.003	+16	-1 to +32

[a] Mean values ± SEM for eight animals. Samples taken at eight hour intervals over a period of 48 hours.

[b] TEP for eight animals with 0.2 mM NaCl in the external bath. Sign is that of the body tissues.

The total efflux (0.72 μeq/10 g-hr) is larger than the influx (0.67μeq/10 g-hr); so that, M_{net} is negative (-0.05 ± 0.07 μeq/10 g-hr). The rate of urine production as measured by the osmotic uptake of water was 0.14 ± 0.007(6) ml/10 g-hr and the concentration of chloride in the urine was 0.13 ± 0.009 (6) mM. The renal efflux

($u \cdot V$, see equation (9)) is 0.02 μeq/10 g-hr which represents less than 3% of the total efflux. The concentration of chloride in plasma is 72 ± 2.0 (8) mM. The ratio of influx to efflux is 0.93. For chloride to be passively distributed, the flux ratio equation predicts a value of 0.005 which differs by a factor of 100 from the observed flux ratio.

Independence of Active Chloride Transport

To show that the mechanism for active chloride transport is independent from the active absorption of sodium ions, animals were kept in 300 ml of 0.2 mM KCl and the flux ratio and TEP differences were measured as before. The data are given in Table 2.

The influx and efflux of chloride from 0.2 mM KCl are not significantly different from the values obtained for 0.2 mM NaCl. These animals also show a negative salt balance. The measured and predicted flux ratios are 0.54 and 0.002, respectively, which indicates that chloride is actively transported.

Table 2. Active transport of chloride from 0.2 mM KCl.

Fluxes (μ eq/10 g-hr)[a]		M_i/M_o		C_o/C_i	E(mV)[b]	
M_i	M_o	exp.	calc.		mean	range
0.57 ±0.06	1.05 ±0.19	0.54	0.002	0.003	-14	+1 to -32

[a] Mean values ±SEM for eight animals. Samples taken at eight hour intervals over a period of 48 hours.

[b] TEP for eight animals with 0.2 mM NaCl in the external bath. Sign is that of the body tissues.

TEP Differences, In Vivo

Figure 1 shows the effect of various concentrations of NaCl, Na_2SO_4, KCl, K_2SO_4 and Ringer's solution on the TEP measured in vivo. Each point on the graph represents a mean value for four animals. The whole series of measurements was carried out on each frog by aspirating the external medium and replacing it with the appropriate solution.

The TEP is not affected by potassium, chloride or sulfate ions. However, the potential increases (slope = 22 mV/log [mM]) with increasing sodium concentration. There is no difference between the effect of NaCl and Na_2SO_4 on the in vivo TEP. At higher sodium concentrations (50% Ringer's solution is approximately 55 mM sodium), the magnitude of the TEP is decreased.

Kinetics

Figures 2 and 3 show the influx of chloride as influenced by the concentration of chloride ion in the external medium (either NaCl or KCl). Animals were maintained in a particular solution for 32 hours, and samples were taken at eight hour intervals. M_i values for each animal in the experiment are indicated on the graphs. Clearly, saturation kinetics apply. For the NaCl series, $V_{max} = 1.3$ and $K_m = 0.18$. With KCl in the external

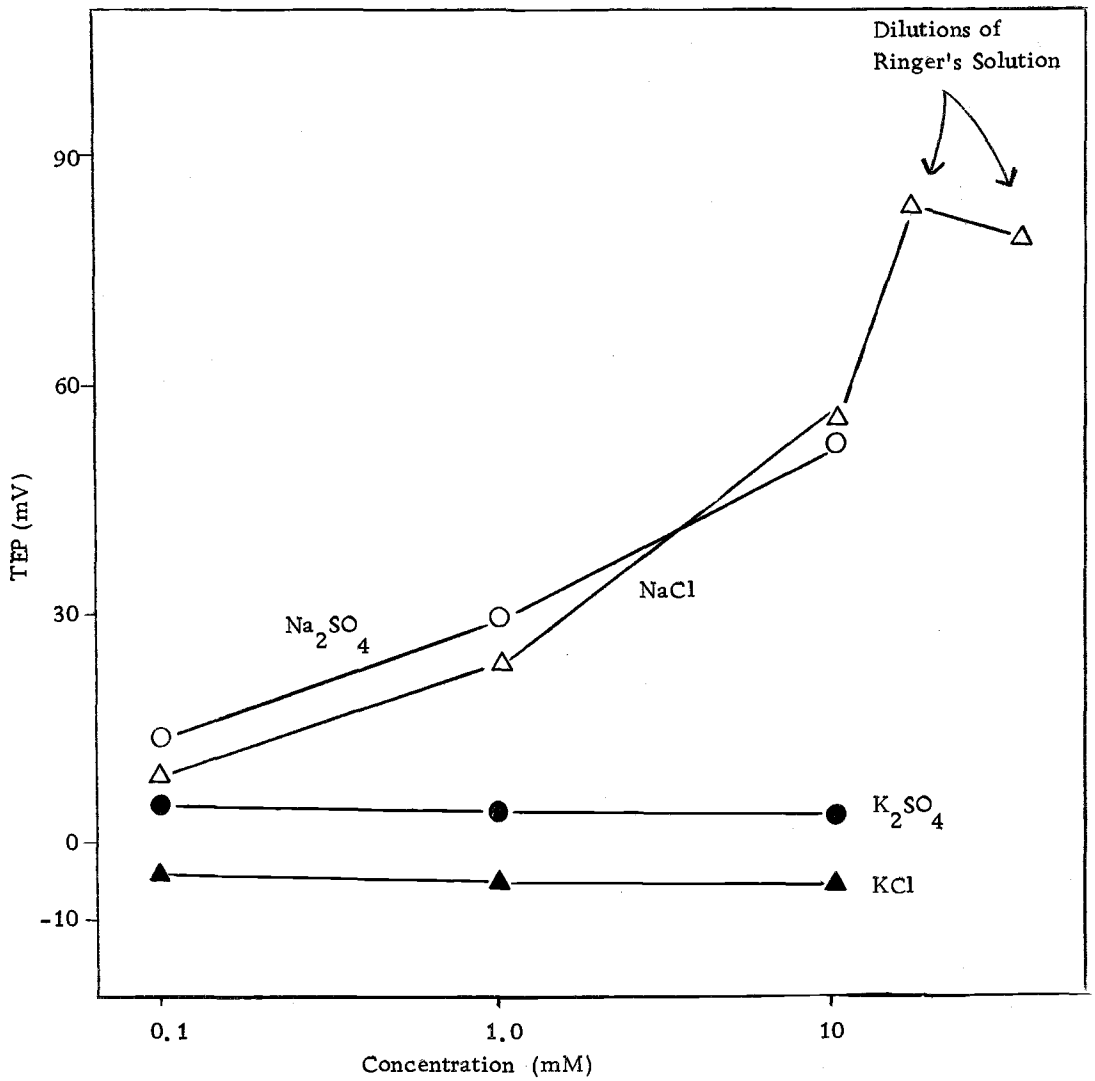

Figure 1. Transepithelial potential differences vs. concentration for various salts. Each point represents a mean of 4 animals. Standard error of the means ranged from ±0.7 to ±9.0 mV

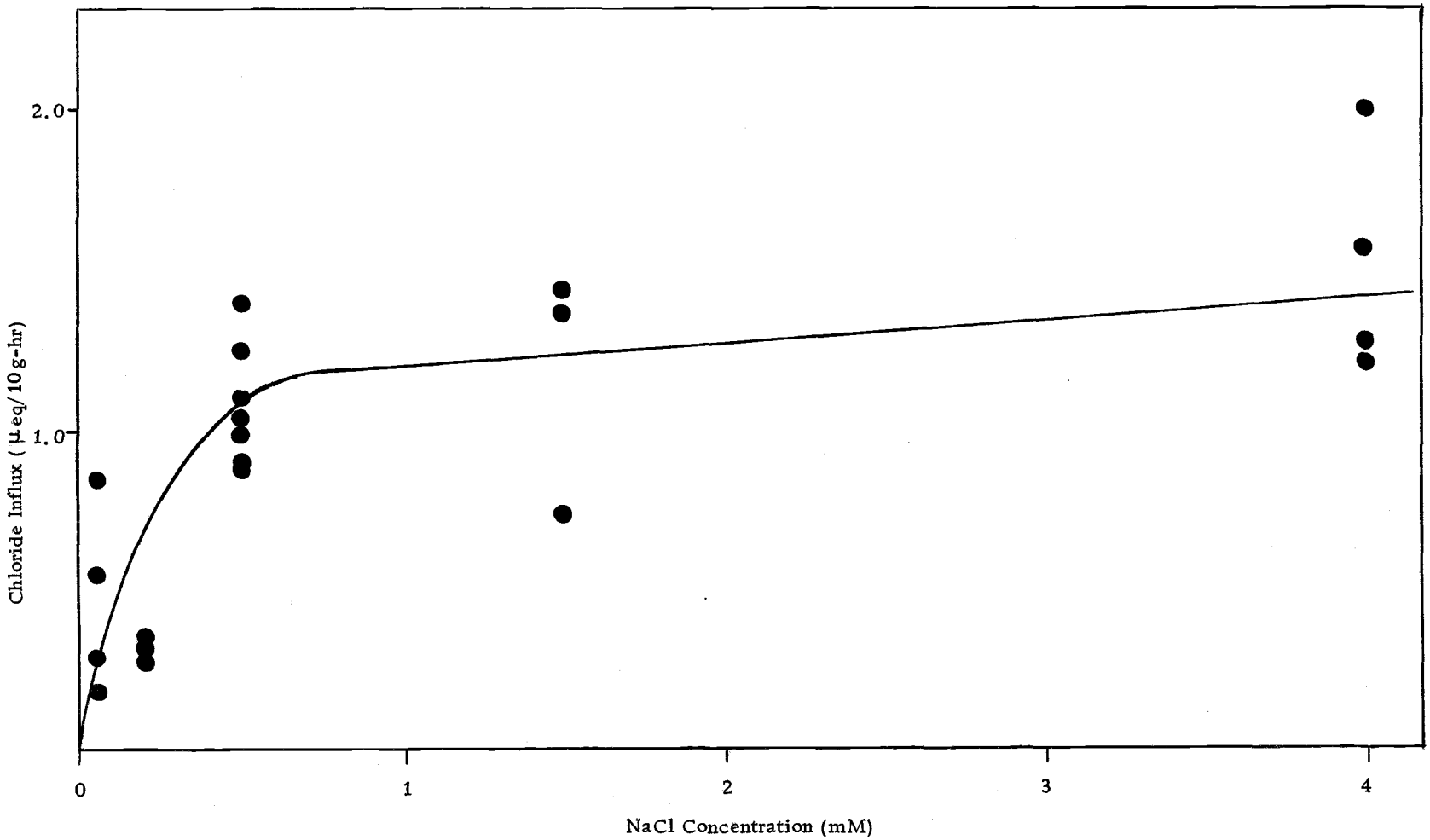

Figure 2. Influx of chloride from NaCl vs. external chloride concentration. Each point on the graph represents one animal.

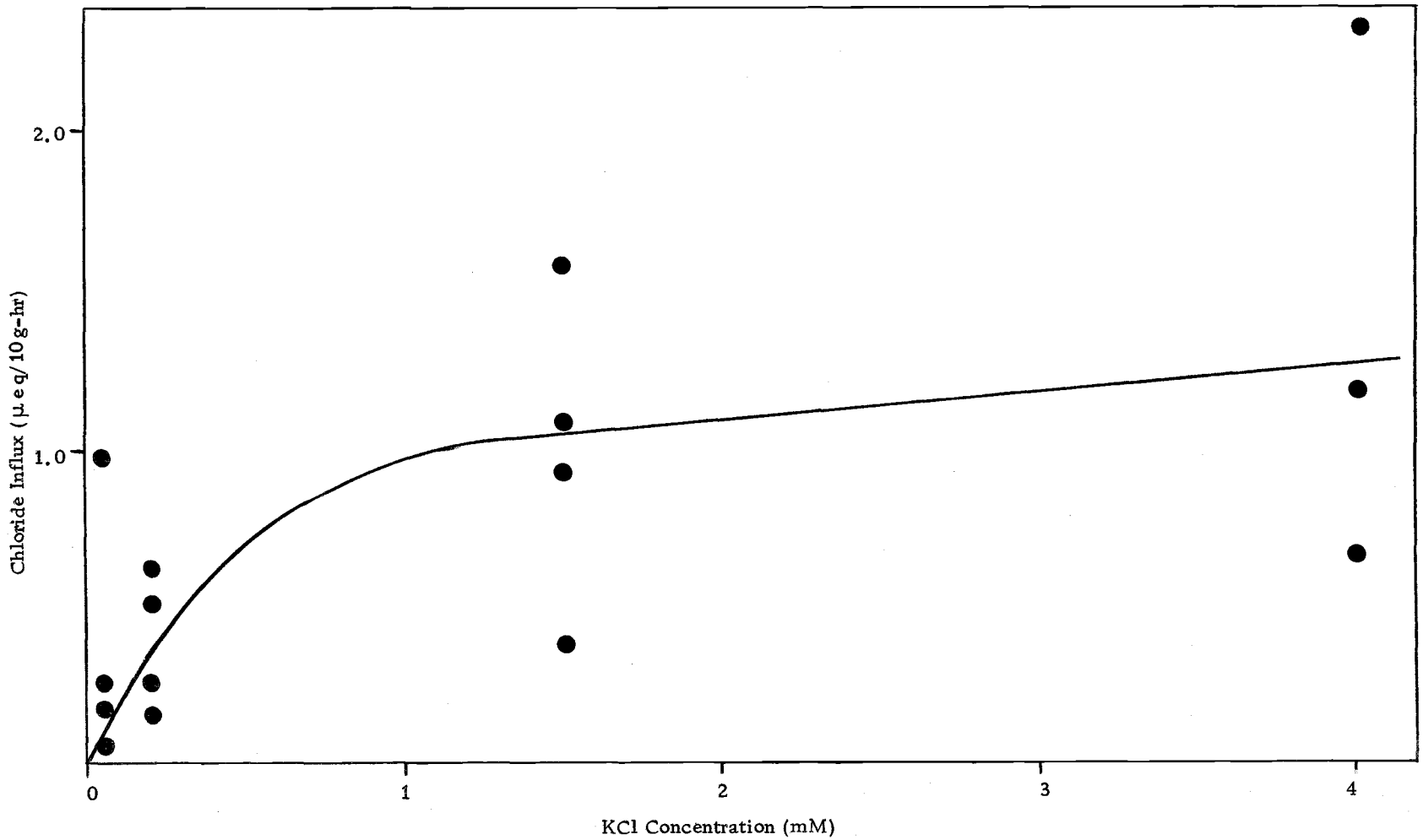

Figure 3. Influx of chloride from KCl vs. external chloride concentration. Each point on the graph represents one animal.

= 1.2 and K_m = 0.38.

Chloride for Bicarbonate

ove data show clearly that chloride can be actively transported inward with the simultaneous uptake of sodium. Under appropriate experimental conditions, it can be shown that chloride can be transported inward in the absence of a corresponding uptake of a cation. Thus, when frogs are salt-depleted for 10 days and then placed in 1.0 mM KCl, they experience a net uptake of chloride and a net loss of potassium and sodium (Figure 4 and Table 3). In the interest of maintaining electroneutrality, chloride must exchange with an endogenous anion.

Table 3. The net flux of Cl^-, base, K^+ and Na^+ in salt-depleted animals kept in 1 mM KCl (mean of net flux ±SEM in μeq/10 g-hr).

Number of Animals	M_{net} Cl^-	M_{net} Base	M_{net} K^+	M_{net} Na^+
6	+0.78 ±0.08	-3.23 ±0.94	-0.58 ± 0.16	-1.10 ±0.08

When animals "pump" chloride from 1.0 mM KCl, the bath becomes more alkaline. The pH increases at the rate of 0.056 ± 0.002 pH units/hr (mean of 6 experiments ±SEM). Titration showed that the animals excrete net quantities of base (see Figure 4 and Table 3). The pK_b of the system is 6.0 ± 0.02 (mean of 6 experiments

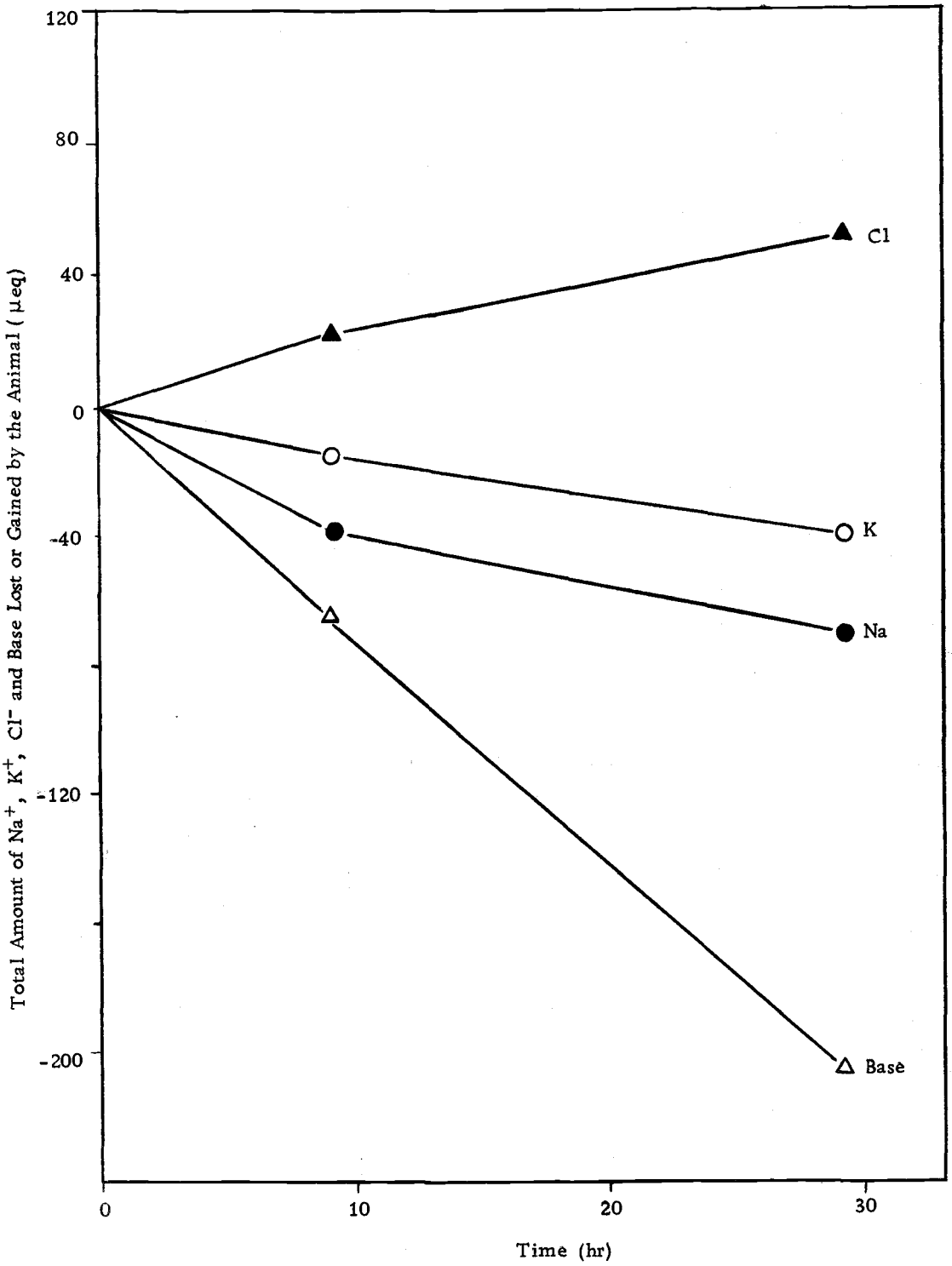

Figure 4. Comparison between the excretion of base, K^+ and Na^+ and the uptake of chloride from 1 mM KCl. Each point represents a mean for six animals. Standard error of the means ranged from ±3.0 to ±40.0 μeq.

± SEM) which agrees with the pK_b of $KHCO_3$ (6.13 ± 0.03) in mixture with KCl at concentrations equivalent to those of the experimental solutions.

In all cases, the base excreted exceeds the chloride incorporated. However, in order to maintain electrostatic neutrality, the net fluxes of sodium and potassium (-1.10 μeq/10 g-hr and -0.58 μeq/10 g-hr, respectively) may be assumed to represent the loss of these ions as bicarbonate salts. The resulting net flux of base is -1.55 μeq/10 g-hr which more closely approximates the net uptake of chloride (+0.78 μeq/10 g-hr, see Table 3) and strongly indicates an *in vivo* chloride/bicarbonate exchange mechanism for *Rana pipiens*.

DISCUSSION

Evidence for Independent Active Chloride Transport

The existence of a mechanism for the active transport of chloride has been demonstrated for Rana pipiens. The measured flux ratio in 0.2 mM NaCl is 100 times the value predicted by Ussing's flux ratio equation. Also, the transepithelial potential differences measured in vivo do not exceed +32 mV. The potential difference would have to be +145 mV (inside positive) in order for chloride to be passively distributed with the concentration gradient that existed in these experiments.

It is possible that amphibians are only capable of actively transporting sodium ions and that chloride must, of necessity, follow passively, or that the chloride pump is tightly linked to the sodium pump (as in the gall bladder, Martin and Diamond, 1966). This study shows that chloride can, in fact, be actively absorbed from solutions which contain virtually no sodium.

The influx and efflux of chloride from 0.2 mM KCl are not statistically different ($P > 0.05$) from the values obtained for 0.2 mM NaCl. The absence of sodium, therefore, does not affect the movement of chloride. The measured flux ratio in 0.2 mM KCl is 100 times the value predicted by the flux ratio equation. Also, the TEP would have to be +164 mV for chloride to move passively.

The actual TEP ranged between +1 and -32 mV.

The system for active chloride transport in Rana pipiens exhibits Michaelis-type kinetics. V_{max} with NaCl in the external bath is not significantly different from the V_{max} obtained when sodium is replaced by potassium. Clearly, the chloride transporting system is independent of the presence of sodium. The K_m value for the KCl series, on the other hand, is twice as large as the K_m for the NaCl series indicating that, perhaps, the absence of sodium reduces the affinity of the transport system for chloride. However, this conclusion is subject to question because data for animals maintained in 0.5 mM KCl is lacking. This information would serve to more precisely delineate the curve drawn in Figure 3.

Chloride Influence on TEP

Dietz et al. (1967) have shown that the TEP in larval salamanders in dilute NaCl is generated by active sodium transport and that external anions do not affect the potential difference. The data presented here indicate that this is also the case in Rana pipiens. The magnitude of the TEP increases with increasing NaCl or Na_2SO_4 concentration. The slope of the line is 22 mV/log[mM] which approximates the values calculated by Dietz et al. for larval salamanders (16-20 mV/log[mM]). At higher sodium concentrations, the magnitude of the TEP decreases appreciably. The same phenomenon is

seen in isolated frog skins (Linderholm, 1952) and larval salamanders (Dietz et al., 1967). The decrease in TEP reflects an increase in permeability of the skin to chloride which would effectively reduce the observed potential difference (isolated frog skin, Linderholm, 1953; intact frogs, Kirschner, 1970).

A Possible Ion Exchange Mechanism

It has been suggested that amphibian skin is capable of actively exchanging exogenous chloride ions for endogenous bicarbonate (Krogh, 1937a; García Romeu et al., 1969). The data presented here also support this hypothesis. A theoretical diagram of the processes which may be involved in this type of ion exchange system is given in Figures 5 and 6 with either NaCl or KCl in the external bath.

With NaCl in the external bath, sodium could be exchanged for H^+ produced from the dissociation of H_2CO_3. An alternate possibility is the exchange of sodium for NH_4^+. Due to the prevailing pH conditions (~pH 6 on the outside of the skin and ~pH 8 on the inside, Friedman et al., 1967b), ammonium ions are in the ionic form on both sides of the skin and, as such, could be moved to the outside.

Bicarbonate is present in frog blood (32 mM, Friedman et al., 1967b) from the dissociation of H_2CO_3 which is formed from $H_2O + CO_2$ via the action of carbonic anhydrase. Frog skin, according to the

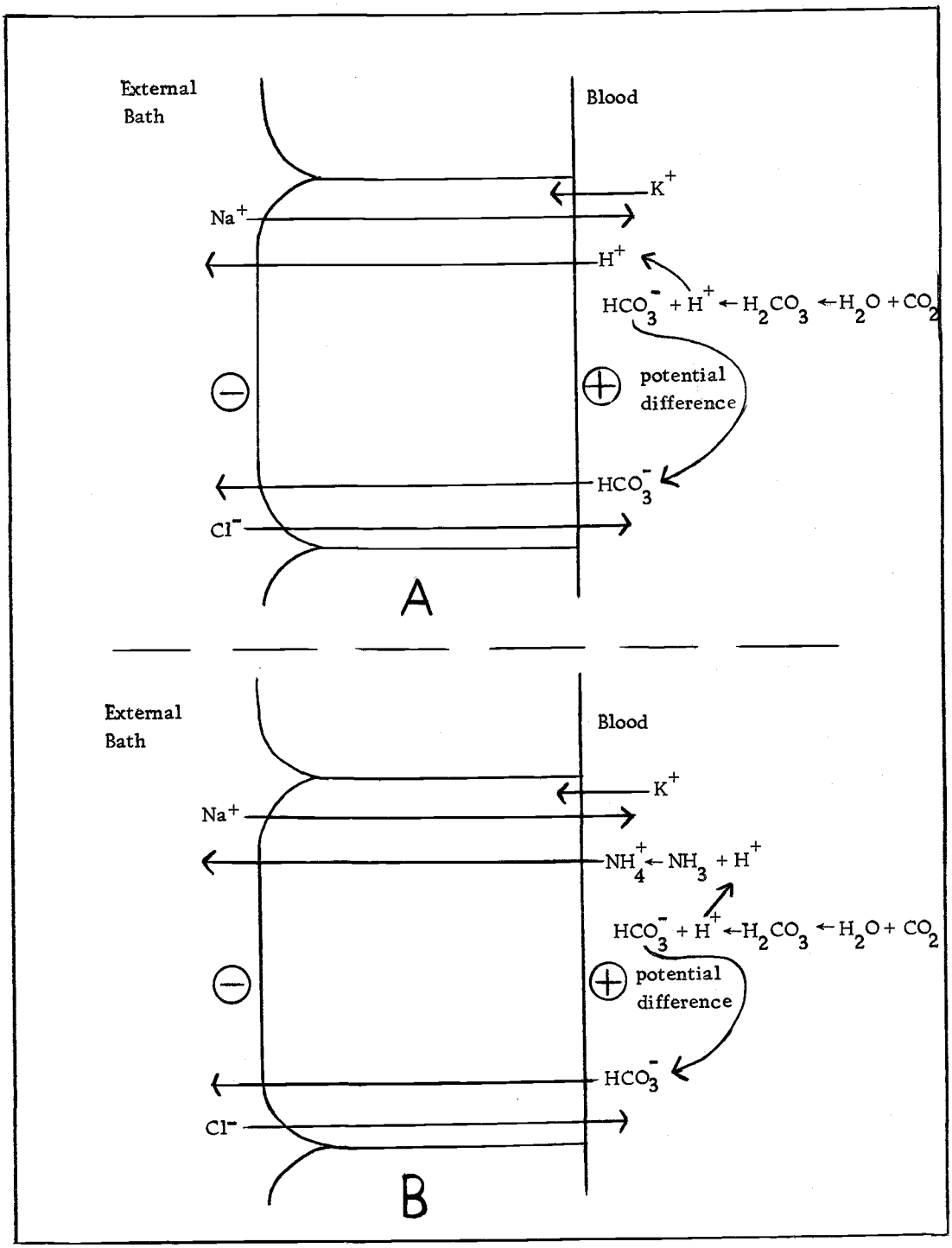

Figure 5. Schematic representation of ionic exchange across frog skin with NaCl in the external bath. Alternative A: Na^+ is exchanged for H^+. Alternative B: Na^+ is exchanged for NH_4^+.

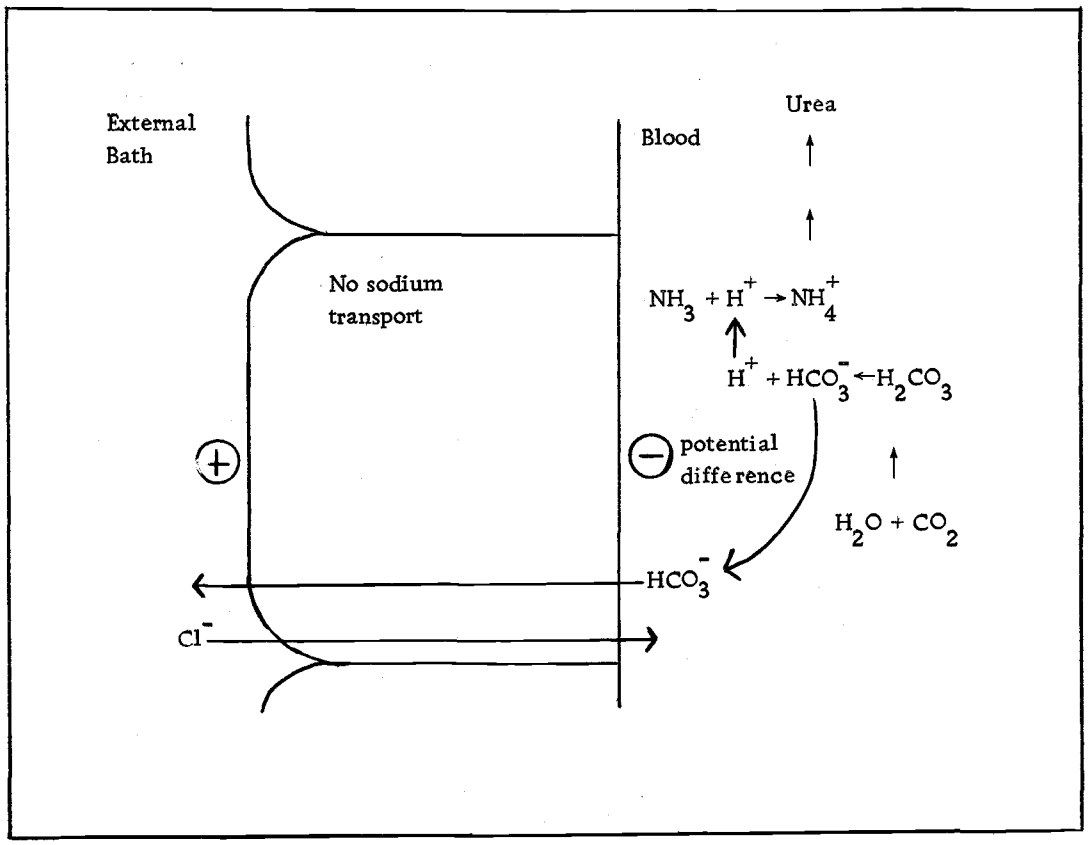

Figure 6. Schematic representation of ionic exchange across frog skin with KCl in the external bath.

literature (Friedman et al., 1967b; Maren, 1967), does not exhibit carbonic anhydrase activity; so that, the blood is the source of bicarbonate which exchanges for chloride in the external bath.

The potential difference across the skin in the presence of sodium (inside positive) results from an active exchange of sodium for potassium at the inner surface together with a high permeability of the cells on the inside surface of the skin to potassium.

On the other hand with KCl in the external bath (see Figure 6), there is no active transport of sodium. Ammonium ions, formed from $NH_3 + H^+$, could then be converted to urea and excreted. The TEP is reversed (inside negative) because sodium is not actively exchanged for potassium at the inside surface of the skin. Chloride is exchanged for blood bicarbonate as before.

In nature the external environment invariably contains some sodium, as well as, chloride. Conceivably, then, various combinations of cationic or anionic exchange systems or counter ion "drag" systems could operate. The significance of the evolution of an exchange system is probably more related to the maintenance of internal acid-base balance than to ionic balance. The question of whether the animal can control the relative intensity of each type of transport system remains unanswered.

A mechanism for the active uptake of chloride in vivo has been shown for Rana pipiens and, also for various related animals, e.g.,

larval salamanders, a fresh-water goldfish, and bullfrog tadpoles (Alvarado and Kirschner, 1963; Dietz et al., 1967; Alvarado and Dietz, 1970; Maetz and García Romeu, 1964; Alvarado and Moody, 1970). The ionic exchange mechanisms postulated here may also be of widespread occurrence in fresh-water animals. Similar mechanisms have been proposed for the gills of a fresh-water crustacean Astacus (Shaw, 1960), gills of a goldfish Carassius (Maetz and García Romeu, 1964) and skin of the Chilean frog Calyptocephalella gayi (García Romeu et al., 1969). Marine teleosts are also known to possess an ionic mechanism in the gills which extrudes sodium and chloride. However, ammonium and bicarbonate are also excreted at the same time. These fish cannot, therefore, possess an ionic exchange system of the type postulated for Rana pipiens.

It appears that in the process of evolution a variety of transport systems have evolved, perhaps independently, in a wide variety of organisms. Thus, care should be taken to avoid extrapolating what is observed in one species to include other species. More importantly, what is found in an isolated "model" system should not be interpreted as absolutely valid for that same system as it exists in nature.

BIBLIOGRAPHY

Alvarado, R. H. and T. H. Dietz. 1970a. Effect of salt depletion on hydromineral balance in larval Ambystoma gracile. I. Ionic composition. Comparative Biochemistry and Physiology 33: 85-92.

_____ 1970b. Effect of salt depletion on hydromineral balance in larval Ambystoma gracile. II. Kinetics of ion exchange. Comparative Biochemistry and Physiology 33:93-110.

Alvarado, R. H. and L. B. Kirschner. 1963. Osmotic and ionic regulation in Ambystoma tigrinum. Comparative Biochemistry and Physiology 10:55-67.

Alvarado, R. H. and A. Moody. 1970. Sodium and chloride transport in tadpoles of the bullfrog Rana catesbeiana. American Journal of Physiology 218:1510-1516.

Alvarado, R. H. and D. F. Stiffler. 1970. The transepithelial difference in intact and adult salamanders. Comparative Biochemistry and Physiology 33:209-212.

Baker, P. S. 1969. Basic principles and application techniques of isotope tracers. Transactions of the American Society of Agricultural Engineers 10:705-710.

Biber, T. U. L., R. A. Chez and P. F. Curran. 1966. Na transport across frog skin at low external Na concentration. Journal of General Physiology 49:1161-1176.

Brown, A. C. 1962. Current and potential of frog skin in vivo and in vitro. Journal of Cellular and Comparative Physiology 60: 263-270.

Cereijido, M. et al. 1964. The influence of Na concentration on Na transport across frog skin. Journal of General Physiology 47: 879-893.

Comar, C. L. and F. Bronner (eds.). 1960. Mineral metabolism: an advanced treatise. Vol. 1. New York, Academic Press. 386 p.

Curran, P. F. and A. K. Solomon. 1957. Ion and water fluxes in the ileum of rats. Journal of General Physiology 41:143-168.

Dietz, T. H., L. B. Kirschner and D. Porter. 1967. The roles of sodium transport and anion permeability in generating trans-epithelial potential differences in larval salamanders. Journal of Experimental Biology 46:85-96.

Finn, A. L., J. S. Handler and J. Orloff. 1967. Active chloride transport in the isolated toad bladder. American Journal of Physiology 213:179-184.

Friedman, R. T. et al. 1967a. Effects of NH_4^+-ions on acid-base properties and ion movements in isolated frog skin. Comparative Biochemistry and Physiology 23:847-869.

Friedman, R. T. et al. 1967b. Chemical basis for the $[H^+]$ gradient across frog skin. American Journal of Physiology 212:962-972.

García Romeu, F. and J. Maetz. 1964. The mechanism of sodium and chloride uptake by the gills of a fresh-water fish, Carassius auratus. I. Evidence for an independent uptake of sodium and chloride ions. Journal of General Physiology 47:1195-1207.

García Romeu, F., A. Salibián and S. Pezzani-Hernández. 1969. The nature of the in vivo Na^+ and Cl^- uptake mechanisms through the epithelium of the Chilean frog Calyptocephalella gayi (Dum. et Bibr., 1841). Journal of General Physiology 53:816-835.

Green, H. H., P. R. Steinmetz and H. S. Frazier. 1970. Evidence for proton transport by turtle bladder in presence of ambient bicarbonate. American Journal of Physiology 218:845-850.

Hogben, C. A. M. 1955a. Active transport of chloride by isolated frog gastric epithelium: origin of the gastric mucosal potential. American Journal of Physiology 180:641-649.

_____ 1955b. Biological aspects of active chloride transport. In: Electrolytes in biological systems, ed. by A. M. Shanes. Washington, D.C., American Physiological Society. p. 176-204.

House, C. R. 1963. Osmotic regulation in the brackish water teleost, Blennius pholis. Journal of Experimental Biology 40:87-104.

Huf, E. G., J. P. Wills and M. F. Arrighi. 1955. Electrolyte distribution and active salt uptake in frog skin. Journal of General Physiology 38:867-888.

Jørgensen, C. B. 1954. On excretion of chloride in sodium chloride loaded frogs and toads. Acta Physiologica Scandinavica 30:171-177.

Jørgensen, C. B., H. Levi and H. H. Ussing. 1946. On the influence of the neurohypophyseal principles on the sodium metabolism in the axolotl (Ambystoma mexicanum). Acta Physiologica Scandinavica 12:350-370.

Jørgensen, C. B., H. Levi and K. Zerahn. 1954. On active uptake of sodium and chloride ions in anurans. Acta Physiologica Scandinavica 30:178-190.

Keynes, R. D. 1963. Chloride in the squid giant axon. Journal of Physiology, London 169:690-705.

_____ 1969. From frog skin to sheep rumen: a survey of transport of salts and H_2O across multicellular structures. Quarterly Reviews of Biophysics 2:177-281.

Kirschner, L. B. 1970. The study of NaCl transport in aquatic animals. American Zoologist 10:365-376.

Kirschner, L. B., R. Maxwell and D. Fleming. 1960. Non-osmotic water movement across the isolated frog skin. Journal of Cellular and Comparative Physiology 55:267-273.

Koch, A. R. 1970. Transport equations and criteria for active transport. American Zoologist 10:331-346.

Koefoed-Johnson, V., H. Levi and H. H. Ussing. 1952. The mode of passage of chloride ions through the isolated frog skin. Acta Physiologica Scandinavica 25:150-163.

Koefoed-Johnson, V. and H. H. Ussing. 1958. The nature of the frog skin potential. Acta Physiologica Scandinavica 42:298-308.

Koefoed-Johnson, V., H. H. Ussing and K. Zerahn. 1952. The origin of the short-circuit current in the adrenaline stimulated frog skin. Acta Physiologica Scandinavica 27:38-48.

Krogh, A. 1937a. Osmotic regulation in the frog (R. esculenta) by active absorption of chloride ions. Skandinavisches Archiv für Physiologie 76:60-74.

_____ 1937b. Osmotic regulation in fresh water fishes by active absorption of chloride ions. Zeitschrift für vergleichende Physiologie 24:656-666.

_____ 1938. The active absorption of ions in some fresh-water animals. Zeitschrift für vergleichende Physiologie 25: 335-350.

_____ 1939. Osmotic regulation in aquatic animals. New York, Dover Publications. 242 p.

LeFevre, P. G. 1955. Active transport through animal cell membranes. Protoplasmatologia 8:1-123.

Linderholm, H. 1952. Active transport of ions through frog skin with special reference to the action of certain diuretics. Acta Physiologica Scandinavica 27 (Suppl. 97):1-144.

_____ 1953. The electrical potential across isolated frog skin and its dependence on the permeability of the skin to Cl^-. Acta Physiologica Scandinavica 28:211-217.

MacRobbie, E. A. C. 1962. Ionic relations of Nitella translucens. Journal of General Physiology 45:861-878.

Maetz, J. and F. García Romeu. 1964. The mechanism of sodium and chloride uptake by the gills of a fresh-water fish, Carassius auratus. II. Evidence for NH_4^+/Na^+ and HCO_3^-/Cl^- exchanges. Journal of General Physiology 47:1209-1227.

Maren, T. H. 1967. Carbonic anhydrase: chemistry, physiology and inhibition. Physiological Reviews 47:595-781.

Martin, D. W. and P. F. Curran. 1966. Reversed potentials in isolated frog skin. Journal of Cellular Physiology 67:367-374.

Martin, D. W. and J. M. Diamond. 1966. Energetics of coupled active transport of Na and Cl. Journal of General Physiology 50: 295-315.

Meyer, D. K. 1948. Physiological adjustments in chloride balance of the goldfish. Science 108:305-307.

Moore, J. A. 1944. Geographic variation in Rana pipiens Schreber of eastern North America. Bulletin of the American Museum of Natural History 82:345-369.

Moore, John A. 1964. Physiology of the amphibia. New York, Academic Press. 654 p.

Noble, G. K. 1931. The biology of the amphibia. New York, McGraw-Hill. 577 p.

Romer, A. S. 1962. The vertebrate body. Philadelphia, W. B. Saunders. 627 p.

Salibián, A., S. Pezzani-Hernández and F. Garciá Romeu. 1968. In vivo ionic exchange through the skin of the South American frog, Leptodactylus ocellatus. Comparative Biochemistry and Physiology 25:311-317.

Shaw, J. 1960. The absorption of chloride ions by the crayfish, Astacus pallipes Lereboullet. Journal of Experimental Biology 37:557-572.

Stobbart, R. H. 1967. The effect of some anions and cations upon the fluxes and net uptake of chloride in the larva of Aedes aegypti (L.), and the nature of the uptake mechanisms for Na and Cl. Journal of Experimental Biology 47:35-57.

Ussing, H. H. 1949a. Active ion transport through isolated frog skin in the light of tracer studies. Acta Physiologica Scandinavica 17:1-37.

_____ 1949b. The distinction by means of tracers between active transport and diffusion. Acta Physiologica Scandinavica 19:43-56.

_____ 1960. The frog skin potential. Journal of General Physiology 43:135-148.

Wright, C. I. 1934. The diffusion of carbon dioxide in tissues. Journal of General Physiology 17:657-676.

Zadunaisky, J. A., O. A. Candia and D. J. Charandini. 1963. The origin of the short-circuit current in the isolated skin of the South American frog Leptodactylus ocellatus. Journal of General Physiology 47:393-402.

Zadunaisky, J. A. and F. W. deFisch. 1964. Active and passive chloride movements across isolated amphibian skin. American Journal of Physiology 207:1010-1014.