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MOVEMENT OF CO_2 AND HCO_3^- ACROSS ISOLATED FROG SKIN*

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SUMMARY

1. Comparison of the electrical responses of isolated frog skin (*Rana pipiens*) to the sodium salts of HCO_3^-, Cl^-, SO_4^{2-} and NO_3^- (120 mequiv/l) suggested low conductance (permeability) for HCO_3^-.

2. This was confirmed by studies in which gain in total CO_2 on the corium side was measured across electrically open skins between $NaHCO_3$ and Na_2SO_4 solutions, pH 8.00. After 3 h the $NaHCO_3$ and Na_2SO_4 were found to be more and less alkaline, respectively, (average pH's 8.26 and 7.88). From this permeability coefficients (P) for CO_2 varying from 0.5 to 1.1 cm/h were calculated. This agrees with data from the literature for CO_2 gas diffusion in skin, in the absence of $NaHCO_3$.

3. For short circuited skins between $NaHCO_3$ solutions to which $NaH^{14}CO_3$ was added, ^{14}C influx equalled ^{14}C outflux. Hence, there was not an indication of active HCO_3^- transport. The carbon fluxes were approx. 6 times the short circuit current equivalent for Na^+, suggesting that ^{14}C moved as $^{14}CO_2$. From this a P for CO_2 of 3–4 cm/h was calculated. The conclusion is reached that P for HCO_3^- must be very low, comparable to P for SO_4^{2-} ($3 \cdot 10^{-9}$–$4 \cdot 10^{-9}$ cm/sec). From net influx experiments (NaCl outside, Na_2SO_4 inside) a P for Cl^- of $3 \cdot 10^{-6}$–$4 \cdot 10^{-6}$ cm/sec was calculated. In this situation the skin potential difference was near zero, or reversed.

4. For skins in mixtures of $NaHCO_3$ + $KHCO_3$ the potential difference changed by 24 mV per decade change in $[Na^+]$ on the outside over a range from 14 to 110 mequiv/l. Similar responses are most often seen for skins in SO_4^{2-} saline. It is unlikely that HCO_3^- shunting is the reason for not obtaining an ideal 58-mV response, assuming Na^+ permselectivity of the outside of the skin.

INTRODUCTION

The predominant inorganic anions present in the skin of the frog (*Rana pipiens*) are Cl^- and HCO_3^-. They are present in amounts of approx. 60 and 30 mequiv/g of wet skin, respectively[1–3]. Both anion species, therefore, could play a significant role in active Na^+ transport and electrogenesis in frog skin. Thus far only Cl^- movement across skin has been extensively investigated. Depending on experimental conditions

Abbreviation: PD, potential difference.

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Cl^- may move either passively or, under special conditions, actively in either direction across the skin[1-7]. Only few data on movement of HCO_3^- in this tissue have been reported. It has been claimed that in live frogs HCO_3^- can be exchanged for Cl^- (refs. 8, 9). In live normal frogs as well as in isolated skin, release of HCO_3^- has been found to occur when the solution at the epithelial side has a pH < 6 (refs. 1, 2). Injection of epinephrine into live frogs produces a voluminous secretion of fluid of high pH and $[HCO_3^-]$. Values of pH > 8 and $[HCO_3^-] > 30$ μequiv/ml are not uncommon[1,2,10]. From CO_2 gas diffussion experiments WRIGHT[11] has calculated diffusion coefficients of CO_2 in normal and acidified skins. Indirect evidence led him to suggest that the skin is highly impermeable to HCO_3^-.

The present investigations were undertaken to obtain additional information on the movement of CO_2 and HCO_3^- from HCO_3^- solutions across isolated skin of *R. pipiens* by studying its effects on skin potential and by measuring net as well as unidirectional fluxes of ^{14}C given as $NaH^{14}CO_3$.

METHODS

Animals: Mounting of the skin

The frogs (*R. pipiens*) were kept in a bin at 20°. They were not fed and were used within 3–4 weeks after arrival at the laboratory. After double pithing of the frogs the abdominal skin was removed, cleaned by washing it briefly in the appropriate salt solutions and then mounted in a plastic double chamber which permitted short circuiting when needed[7]. Mixing of solutions was accomplished by magnetically driven stirrers. The free skin area was 7.06 cm^2, and each half chamber had a volume of 26 ml. The 1–4 h experiments were conducted in an air conditioned laboratory at approximately 25°. During the experiments the chambers were kept closed so that no CO_2 from atmosphere could enter the solutions, except during short periods when samples were taken. To estimate CO_2 absorption from the air during these periods, control experiments were carried out with a cellophane membrane replacing the skin. Values for CO_2 absorption, and for blanks, are given in Table I.

Electrical measurements

In experiments requiring only potential difference (PD) measurements, two matched pencil-type calomel electrodes (Radiometer Corporation, Copenhagen) were momentarily inserted through holes in the half chambers into the bathing solutions. The PD between electrodes when dipped into solutions of the type used was < 1 mV. PD's were measured with a high impedance-input Varian strip chart recorder, Model G-10. For measuring the short circuit current the same recorder was used. The external current was applied *via* Ringer–agar bridges and the PD was also measured *via* Ringer–agar bridges positioned with 1 mm of the skin surfaces. Small differences in diffusion potential (ΔV_j) between the agar bridges and the electrolyte solutions used undoubtedly existed. They were neglected, however, since with the solutions used in the present studies ΔV_j could not have been greater than 3 mV, using the Henderson equation for calculations.

Salt solutions

It was intended to compare effects and behavior of HCO_3^- in frog skin with effects and behavior of others anions, namely Cl^-, SO_4^{2-} and NO_3^-. Therefore, several

TABLE I

NET INCREASE IN TOTAL CO_2 IN THE CORIUM COMPARTMENT AND pH CHANGES IN OUTSIDE (o) AND INSIDE (i) SOLUTIONS

Type of experiments: S = SO_4^{2-} solution, pH = 8.00 ± 0.02; B = HCO_3^- solution, pH = 8.00 ± 0.02.***

Time	*Total CO_2 (μequiv in corium compartment (26 ml))*		*pH_o*	*pH_i*	*pH_o*	*pH_i*
	Control	*Experimental*	*Control*		*Experimental*	
	S_oS_i	S_oS_i	S_oS_i	S_oS_i	S_oS_i	S_oS_i
o	o*	o*	8.00 ± 0.02	8.00 ± 0.02	8.00 ± 0.02	8.00 ± 0.02
10 min	7.5 ± 1.5	—				
1 h	11.3 ± 1.2	5.5 ± 2.6				
	S_oS_i	B_oS_i**	S_oS_i	S_oS_i	B_oS_i**	B_oS_i**
2 h	14.5 ± 0.8	13.6 ± 1.1				
3 h	12.8 ± 0.9	17.9 ± 1.7	8.00 ± 0.03	8.02 ± 0.03		
4 h	—	24.5 ± 1.7			8.26 ± 0.04§	7.88 ± 0.02§§

* After subtracting blank value. All figures are given corrected for blank and CO_2 pick-up. Blank value = 0.2 μequiv CO_2 per ml. Pick-up values = 0.1–0.7 μequiv CO_2 per ml, increasing during the 1–4-h sampling period.

** S_o was replaced by B_o, leaving S_i.

*** P_{CO_2} = 41.3 mm Hg for B_o = 120 μequiv/ml.

§ P_{CO_2} = 23.1 ± 2.0 (S.E.) mm Hg for B_o = 120 μequiv/ml.

§§ P_{CO_2} = 0.42 ± 0.04 (S.E.) mm Hg for B_i = 0.94 μequiv/ml.

P_{CO_2} data were calculated for each of the five experiments and averages obtained. Calculations were by the Henderson–Hasselbalch equation. CO_2 solubility coefficient = 0.0460 μmole/ml per mm Hg[15]. pK' = 6.20.

types of saline solutions were prepared which contained (mequiv/l): 110 Na^+, 10 K^+, 10 Tris, and as anions equivalent amounts of one of the anions mentioned above. Tris is a weak monacidic base, $K_b = 1.202 \cdot 10^{-6}$ (25°) which, according to its manufacturer, does not absorb CO_2 (see ref. 12). After brief oxygenation the solutions were adjusted to pH 8, using the appropriate acid. CO_2 was used in the case of HCO_3^- saline. No non-electrolyte was added to the SO_4^{2-} saline to achieve isosmolarity with the other saline solutions.

In the studies on the electrical response of the epidermis to Na^+ in the presence of only HCO_3^-, the Na^+ concentration on the epidermal side was reduced from 110 to 13.8 mequiv/l in four steps (see under RESULTS). $NaHCO_3$ was replaced by equivalent amounts of $KHCO_3$. Solutions were removed by aspiration and a new solution was immediately poured into the empty chamber half. It is estimated that by this method, contamination of the bathing solution with the anion of the previous solution was less than 0.5 mequiv/l. All solutions were prepared using CO_2-free water.

Chemical and isotope methods

Cl^- estimations were carried out by the conductometric method of COTLOVE *et al.*[13], using the Aminco Cl^- titrator. Total CO_2 estimations in HCO_3^- saline were

carried out using the Natelson microgasometer. At pH 8.0 approx. 98 % of the total CO_2 is present as HCO_3^-. Reproducibility of data was within 1 % for both Cl^- and total CO_2.

Two types of $H^{14}CO_3^-$ flux experiments were done on short circuited skins, giving the same results. First, a more complicated glass double chamber was used which had attached to it two reservoirs containing 200 ml of $NaHCO_3$ (+ $KHCO_3$) solution. The solutions in the two half chambers were kept constant at pH 8 throughout the experiments by using two Radiometer pH stats. The solutions were pumped through the half chambers by the use of two peristaltic pumps. The pH adjustment was done with CO_2 before the experiments, and with 0.1 M HCl during the flux studies. Three influx experiments and four outflux experiments were carried out in this manner. In addition, four experiments of each type were carried out with the simple plastic double chamber used in all of the other studies. In these eight experiments the pH of the $NaHCO_3$ (+ $KHCO_3$) solutions was adjusted with CO_2 only at the start of the experiments. 1 h was allowed for equilibration of the skin in $NaHCO_3$ solution. Then 0.01 mC $NaH^{14}CO_3$ (New England Nuclear Corporation) was added to either the inside or outside bathing solution, for measuring outflux or influx, respectively. 2-ml samples of the bathing solutions were taken immediately before and after a 1-h flux period. Aliquots of these samples were mixed with scintillation fluid (10 ml toluene, 5 ml ethylene glycol monomethyl ether, 6 g/l 2,5-diphenyloxazole) and counted in a Packard liquid scintillation counter. The probable per cent error of the counting procedure was $\pm$ 3.8 %.

RESULTS

Potentiometric studies

The change in skin potential upon replacement of one anion by another was followed, allowing 15 min between solution changes for establishment of a reasonably stable potential difference across the skin. Under conditions leading to relatively large changes in PD, the time-course was such that the PD decreased or increased rapidly during the first few minutes. This followed by a more gradual fall or rise of PD and during the last 10–12 min following fluid replacement very little PD change was seen. "Average potentials" were calculated from the data collected during the 10–12 min period. Had one chosen 3 min the PD changes could have been different from those reported by only about 5 %, except when the transitions from SO_4^{2-} to Cl^- on the outside were made. Here the drop in PD would have been 10 % smaller than the steady state value.

Fig. 1 gives the average data obtained on ten skins. The left side shows PD changes which were found when anion replacement was carried out on the epidermal side of the skin. It shows the expected result that replacement of SO_4^{2-} by Cl^- greatly reduced the total skin PD. The reversal of skin PD seen with Cl^- saline was unexpected and is of interest (see DISCUSSION). Replacement of Cl^- by HCO_3^- raised the skin PD, but not to the level seen in SO_4^{2-} saline. Subsequent replacement of HCO_3^- by NO_3^- and then back to SO_4^{2-} reestablished the original relatively high PD. In these, as in all other similar studies, experiments in which the final PD was less than 90 % of the initial PD (in the same solution environment) were discarbed. The histogram on the right side of Fig. 1 shows that the skin did not respond significantly to sequential anion

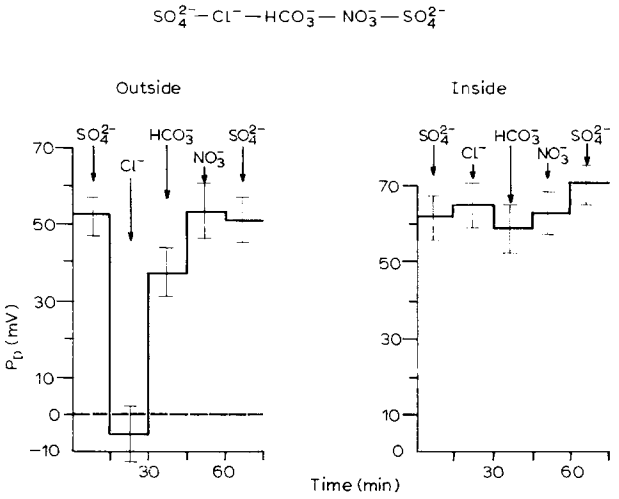

Fig. 1. Histogram representing frog skin potential changes when solutions of different anion contents were added to the two sides of the skin. The left side of the figure represents changes in the outside bathing solution (SO_4^{2-} saline inside), and the right side represents changes in the inside bathing solution (SO_4^{2-} saline outside). The values are averages ± S.E. from studies on skins from ten frogs. The value of PD for Cl^- on the outside was significantly different from those for the other anions ($P < 0.0005$). No other differences were significant. PD = $\phi_i - \phi_0$, where ϕ_i is the potential at the corium side; and ϕ_0 is the potential at the epidermal side.

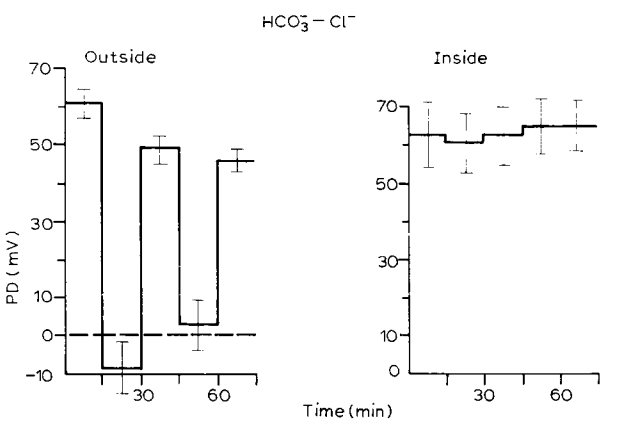

Fig. 2. Histogram representing frog skin potential changes when HCO_3^- saline was replaced by Cl^- saline. Two tests: the left side of the figure represents changes in the outside bathing solution (HCO_3^- saline inside), and the right side of the figure represents changes in the inside solution (HCO_3^- saline outside). Solutions were used in the sequence: HCO_3^-, Cl^-, HCO_3^-, Cl^-, HCO_3^-. The difference in PD found when Cl^- saline replaced HCO_3^- saline on the epidermal side was significant ($P > 0.0005$). PD found when the solutions were changed on the corium side were not significant. The values given are averages ± S.E. for ten experiments.

replacement on the corium side during the 15-min test period. Testing of the behavior of the epidermal side and the corium side of the skin was done on skins obtained from different frogs. Many experiments of the same type were carried out altering the order of anion replacement. Results of some of these additional experiments are shown in Figs. 2 and 3. The findings were in agreement with those shown in Fig. 1.

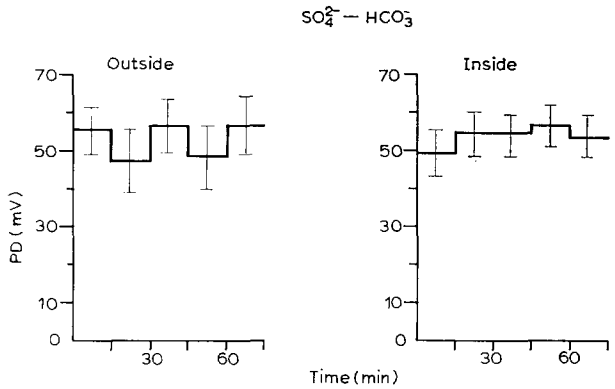

Fig. 3. Histogram representing frog skin potential changes when SO_4^{2-} saline was replaced by HCO_3^- saline. Two tests; the left side of the figure represents changes in the outside bathing solution (SO_4^{2-} saline inside), and the right side represents changes in the inside solution (SO_4^{2-} saline outside). Solutions were used in the sequence: SO_4^{2-}, HCO_3^-, SO_4^{2-}, HCO_3^-, SO_4^{2-}. The differences in PD found when HCO_3^- saline replaced SO_4^{2-} saline at either the epidermal or corium side were not significant. The values given are averages ± S.E. for ten experiments.

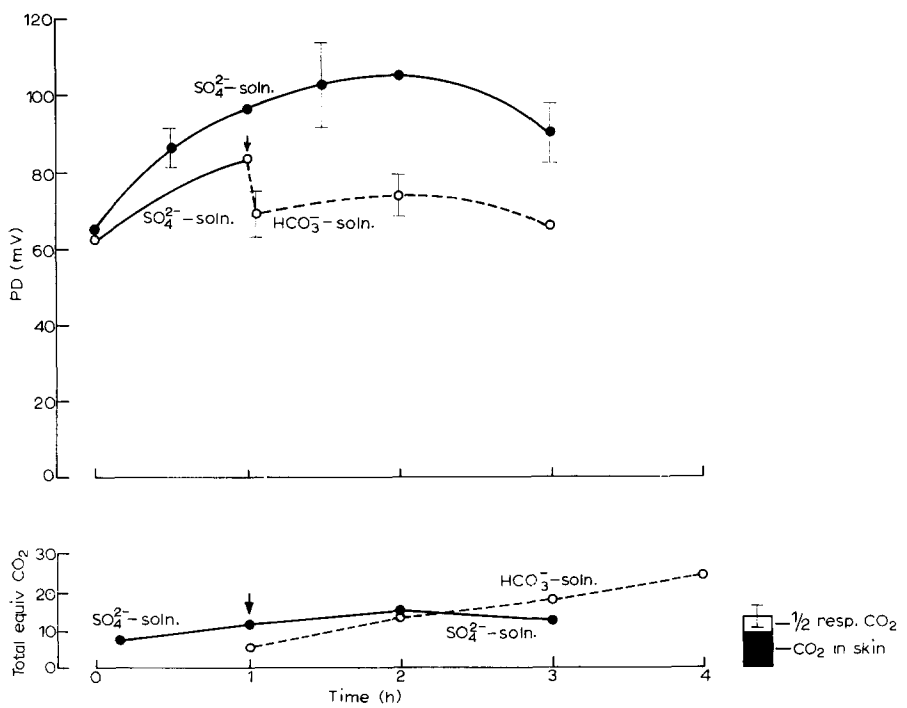

Fig. 4. Results of experiments in which net inward HCO_3^- flux was measured chemically. The solid line in the lower part of the figure represents the accumulation of total CO_2 in the solution bathing the inside of the skin when the bathing solutions on both sides of the skin were SO_4^{2-} saline. The solid bar to the right represents the total CO_2 of the skin; the open bar represents one-half of the respiratory CO_2 produced during the experiment. The broken line represents the accumulation of total CO_2 in the inside bathing solution when the outside SO_4^{2-} solution was changed to HCO_3^- saline (arrow). The solid line in the upper part of the figure represents the skin PD measured when the skin was bathed by SO_4^{2-} saline on both sides. The broken line represents the skin PD after the outside bathing solution was changed to HCO_3^- saline. The S.E. value is shown for some points.

Net total CO_2 and net Cl^- transport

A control study in which SO_4^{2-} saline remained on both sides of the skin throughout the 3- or 4-h experimental period is contrasted to experiments in which the solution on the outside was changed from SO_4^{2-} to HCO_3^- saline (Fig. 4), or to Cl^- saline (Fig. 5) after a 1-h equilibration period. For each series five skins were utilized. The points plotted in the figures are average values. For some points standard errors of the mean are also given. The upper halves of the graphs give potential readings, the lower halves give data on net change of total CO_2 and Cl^-, respectively, in the inside compartment. At the times indicated 3-ml samples were taken from the the inside compartment for chemical analysis. The same volume of the appropriate solutions were added to the chamber half, and the amounts of CO_2 or Cl^- removed with the sample were taken into account in the calculations of net influx.

As has been mentioned in the introduction, frog skin contains significant amounts of total CO_2 and Cl^-. It must be expected that they are at least partially washed out during the experiment. Furthermore, the amount of metabolic CO_2 produced during the experiments must be taken into account in the calculations of net carbon fluxes. The bars shown at the right hand side of the lower graphs in Figs. 4 and 5 give the amounts of total CO_2 and Cl^- that can be expected to be present in the HCO_3^-- and CO_2-free SO_4^{2-} saline solution at the corium side of the skin at the end of the experiments because of washout from the piece of skin used (7.06 cm^2; 0.31 g). The arbitrary assumption was made that one-half of the metabolic CO_2 was present on each side of the skin. Fresh skin contains 27.8 $\pm$ 2.0 μequiv total CO_2 per g of wet skin[2]. The rate of respiratory CO_2 production[1] is 0.36 $\pm$ 0.05 μequiv/h per cm^2. From this an approximate calculated washout of 12–14 μequiv total CO_2 is obtained for a 3–4-h experiment. Cl^- estimation in abdominal skins from fifteen frogs were carried

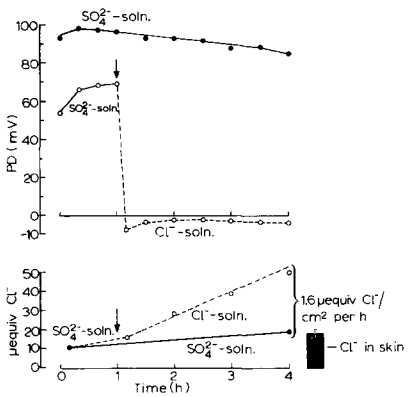

Fig. 5. Results of studies in which net inward Cl^- flux was determined chemically. The solid line in the lower part of the figure represents Cl^- accumulation in the solution bathing the corium side of the skin when both bathing solutions were SO_4^{2-} saline. The solid bar represents the Cl^- content of the skin. Evidently in this case the Cl^- appearing in the inside bathing solution is due to washout from the skin. The broken line in the lower part of the figure shows Cl^- accumulation in the inside bathing solution when the outside of the skin was bathed in Cl^- saline. The net Cl^- influx corrected for washout is 1.6 μequiv/cm^2 per h. The upper part of the graph represents the skin PD measured during the experiments. The solid line is the skin PD when both bathing solutions were SO_4^{2-} saline. The broken line shows the skin PD after the outside bathing solution had been changed to Cl^- saline (arrow). A potential reversal is seen which persists for the duration of the experiments.

out by the method of VAN SLYKE AND SENDROY[14]. The result was 58 ± 4 μequiv Cl^- per g wet skin, in good agreement with earlier measurements[3].

It can be seen that the potential responses of the skin are quite in agreement with the data shown in Figs. 1–3. Attention is called to the long-lasting, slightly reversed skin PD's in the case when NaCl saline is on the epidermal and Na_2SO_4 saline is on the corium side of the skin. In these skins, NaCl moved from the outside to the inside at near zero potential, with indication that Cl^- was leading Na^+. Calculations showed (Fig. 5) that Cl^- moved from the epidermal to the corium side of the skin (1.6 ± 0.4 μequiv/h per cm^2).

The numerical values for total CO_2 plotted in Fig. 4 are given in Table I for further analysis presented in DISCUSSION.

In the HCO_3^-(outside)–SO_4^{2-}(inside) experiments the total CO_2 in the corium compartment rose continuously to a value above the washout value after 3–4 h, when the experiment was terminated. This indicates that either CO_2, or HCO_3^-, or both have diffused into the corium compartment. Associated with this movement was a pH change in outside and inside solutions in all five HCO_3^-(outside)–SO_4^{2-}(inside) experiments which was not seen in the SO_4^{2-}(inside)–SO_4^{2-}(outside) controls (also not seen in the Cl^-(outside)–SO_4^{2-}(inside) experiments). The HCO_3^- solution at the epithelial side became more alkaline (pH 8.00–8.26), while the solution at the corium side turned more acid (pH 8.00–7.88). This suggests that CO_2, and not HCO_3^-, has diffused across the skin. The argument presented in DISCUSSION shows that this is indeed the most reasonable explanation.

Carbon fluxes from solutions containing $NaH^{14}CO_3$

A total of seven influx and eight outflux experiments were carried out on short circuited skins, using different pieces of skin. The skins were suspended in identical HCO_3^- saline solutions. They were kept in these solutions for 1 h before $NaH^{14}CO_3$ was added either to the epidermal or corium side of the skin. Results are given in Table II. The figures in Column 4 suggest that the skins actively transported Na^+ at no lower a rate than that found in our laboratory for skins bathed in Cl^- saline. FUNDER *et al.*[16] have found that in the skin of *Rana temporaria* the average values of short circuit current when using 115 mM HCO_3^- medium on both sides was 1.4–1.5 times higher than those found in skins bathed in 2.4 mM HCO_3^- saline solution.

Carbon influx and outflux were found to be equal and about 6 times the short circuit current equivalent, assumed to be net Na^+ flux. Calculations showed that the

TABLE II

1-h UNIDIRECTIONAL CARBON FLUXES ACROSS SHORT CIRCUITED SKIN

$NaH^{14}CO_3$ was added to either the inside or outside $NaHCO_3$ saline solution.

	Number of experiments	*Range of av. open skin PD (mV)*	*Carbon flux ± S.E (μequiv/cm² per h)*	*S.C.C. equivalent* ± S.E (μequiv/cm² per h)*
Influx	7	18–71	6.72 ± 1.07	1.06 ± 0.18
Outflux	8	32–70	6.70 ± 0.76	1.23 ± 0.19

* Short circuit current equivalent, taken as a measure of net inward Na^+ flux.

carbon fluxes amounted to about 0.4 % of the total $NaHCO_3$ present in the compartment, and that at pH 8 close to 1.5 % of the carbon was present as free CO_2. The data do not suggest active HCO_3^- transport in frog skin.

Electrical response of the epidermis to Na^+ in HCO_3^- saline

KOEFOED-JOHNSEN AND USSING[17] have shown that the skin of *R. temporaria* responds to a 10-fold change in outside Na^+ concentration ($[Na^+]_0$) with a PD change of nearly 58 mV, if the skins are kept in a solution containing an impermeant anion, such as SO_4^{2-}, and Na^+ is replaced by equivalent amounts of K^+ to vary $[Na^+]_0$. Since the studies described above suggested a rather low HCO_3^- permeability of the skin of *R. pipiens*, the response of the epidermis to Na^+ in the presence of this anion was tested. Mixtures of $NaHCO_3$ and $KHCO_3$ were prepared to give $[Na^+]_0$ values of 110, 55, 27.5, and 13.8 mequiv/l. The solution at the corium side was kept constant: 110 mM $NaHCO_3$ + 10 mM $KHCO_3$. Average PD values and the standard errors of the mean were plotted against log $[Na^+]_0$ (Fig. 6). The PD changed by 24 mV per decade change in $[Na^+]_0$. The deviation from the 58-mV response expected if the outside of the skin behaves as a Na^+-permselective membrane remains to be explained. Shunting of the PD by HCO_3^- is unlikely since HCO_3^- appears to be a rather impermeant ion as will be brought out in DISCUSSION.

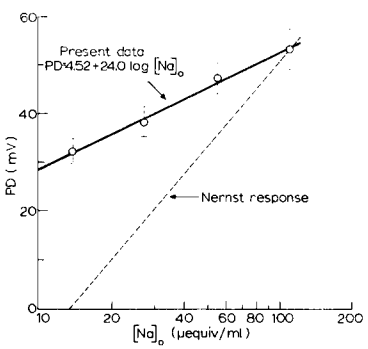

Fig. 6. Potentiometric response of frog skin in HCO_3^- saline to changes in the Na^+ concentration of the outside bathing solution. In all cases HCO_3^- saline was used on the corium side. The values are the average for ten skins ($PD = \phi_i - \phi_0$) ± S.E. The slope of the line is 24 mV per decade change in outside Na^+ concentration. The theoretical Nernst response is also indicated.

DISCUSSION

Potentiometric data

The results presented in Figs. 1–3 are based on PD readings 15 min after solution changes were made. The PD transients were nearly over after 3 min since the readings differed by 10 % or less from those thaken after 15 min. When the skin was exposed on both sides to identical 110 mequiv/l Na^+ solutions, differing in anion species (Cl^-, SO_4^{2-}, NO_3^-, HCO_3^-), a considerable change in skin PD was seen only if Cl^- replaced any of the other anions used on the epidermal (outside) of the skin. From these figures and also from Fig. 4, the HCO_3^- behaved much more like SO_4^{2-} and NO_3^- which are generally considered as rather impermeant ions in whole skin[18]. Therefore, assuming

that the effect of Cl^- indicates high conductance of the skin for this ion, by these tests it seems that the skin has a low conductance for HCO_3^-.

Reversal of the skin PD was seen when the outside Na_2SO_4 or $NaHCO_3$ solution was replaced by NaCl solution (Figs. 1, 2 and 4). To conclude from this great leakiness of the skin for Cl^-, diffussing in the inward direction, does not explain, however, why no comparable rise in PD occurred when NaCl was placed on the inside of the skin (Figs. 1 and 2). It is possible that in this situation the conductance for Cl^- has greatly decreased because of secondary events such as swelling of the epithelium[19]. Or, with NaCl on the outside and Na_2SO_4 on the inside, active inward Cl^- transport occurs[5,6]. These observations warrant further investigations on the movement of Cl^- in skin which is not the particular concern of this study.

Flux data

From the results presented in Fig. 4 and Tables I and II it is clear that CO_2, or HCO_3^-, or both, moved from the outside to the inside of the skin. In the net flux studies (Table I) with $NaHCO_3$ solution (pH 8.00) at the outside and Na_2SO_4 solution (pH 8.00) at the inside it has been noted that after 3 h the $NaHCO_3$ solution was more alkaline (pH 8.26) and the Na_2SO_4 solution more acidic (pH 7.88). Such an acidifying effect of $NaHCO_3$ has first been described by WEHRLI-HEGNER AND WYSS[20] who used the wall of the frog *vena abdominalis* as a membrane. JACOBS[21] quotes this study in analogy to the alkalizing effect that NH_4Cl solutions can have, as first observed in the starfish egg[22]. The acidifying effect of $NaHCO_3$ solution seen here in frog skin is the more remarkable since the Na_2SO_4 solution was buffered with 10 mM Tris, which itself does not combine with CO_2 (see ref. 12). Following the reasoning of JACOBS[21], the present data suggest that CO_2, and not HCO_3^-, diffused from the outside $NaHCO_3$ solution into the inside Na_2SO_4 solution.

To further test this hypothesis, P_{CO_2} values were calculated for the original $NaHCO_3$ solution (pH 8.00; $P^{I}_{CO_2}$) and the $NaHCO_3$ solution at the end of experiment (pH 8.26; $P^{II}_{CO_2}$). The P_{CO_2} values obtained are given in Table I. If it is CO_2 that has diffused across the skin, one can then calculate a CO_2 permeability coefficient (P) by applying an equation derived by JACOBS[21] for the situation which applies here. Neglecting the very low P_{CO_2} in the SO_4^{2-} solution (Table I), one has:

$$P = \frac{V_1}{A \cdot t} \ln \frac{P^{II}{}_{CO_2}}{P^{I}{}_{CO_2}} \qquad (1)$$

$V_1 = 26$ ml; $A = 7.06$ cm^2; $t = 3$ h. The average P thus calculated is 0.73 cm/h. The lowest and highest values in the series are 0.51 and 1.10 cm/h. WRIGHT[11] has measured the CO_2 diffusion coefficient in frog skin (probably *R. pipiens*; 0.175–0.325 mm thickness). From his results one can calculate P values ranging from 0.78 to 1.44 cm/h. His experiments were done by studying CO_2 gas diffusion in the absence of $NaHCO_3$. He inferred that HCO_3^- contributes little to the total diffusion. This conclusion is now amplified by the present experiments in which CO_2 diffused from a 120 mM $NaHCO_3$ solution.

The loss of CO_2 from the outside compartment can be calculated as $V_1 \alpha (P^{I}_{CO_2} - P^{II}_{CO_2}) = 26 \cdot 0.046 \cdot 18.1 = 21.6$ μequiv. The experimentally measured gain of total CO_2 in the inside compartment was $24.5 - 12.9 = 11.6$ μequiv. The main reason for the underestimation may be found in the two relatively large corrections applied to

the results. No attempts were made, therefore, to calculate a P value for CO_2 based on the measured gain of total CO_2 at the corium side. The good agreement of our results with those of WRIGHT[11] leads one to conclude that, whereas CO_2 diffuses readily across the skin, the conductance permeability of the skin for HCO_3^- must be very low and was not measureable by the method used, although there existed a favorable electrical PD of 70 mV across the skin. From the potentiometric studies it seems that P for HCO_3^- must be close to that of SO_4^{2-}. From data in the literature[23,24] one can calculate a P value for SO_4^{2-} of approx. $4 \cdot 10^{-9}$–$5 \cdot 10^{-9}$ cm/sec for skins of *R. pipiens*. By comparison, from the Cl^- flux of 1.6 μequiv/cm^2 per h (Fig. 5) one obtains a P for Cl^- of $3.7 \cdot 10^{-6}$ cm/sec, at nearly zero skin PD. This checks well with the value of $1.5 \cdot 10^{-6}$ cm/sec given by GARBY AND LINDERHOLM[25].

For unidirectional "C" flux a value $J = 6.7$ μequiv/cm^2 per h was found (Table II). Neglecting back flux in the isotope studies, which is justified because only 0.4 % of the total ^{14}C activity had crossed the skin, a P value for CO_2 can be calculated from $P = 6.7/(\alpha \cdot P^{I}_{CO_2})$, or $P = 6.7/(\alpha \cdot P^{II}_{CO_2})$; $\alpha = 0.046$ μmole/ml per mm Mg; $P^{I}_{CO_2} = 41.3$ mm Hg. The pH remained constant. One obtains $P = 3.5$ cm/h. This P value is somewhat higher than the one obtained by WRIGHT[11] and us. It is, however, the same order of magnitude. The discrepancy may be in methodology. In the net flux studies the skin was between HCO_3^- solution on the epidermis and SO_4^{2-} solution on the corium side of the skin. In the ^{14}C-flux experiments, identical HCO_3^- solutions were placed on both sides of the skin which was kept short circuited. In this situation it is also possible that isotope exchange diffusion of CO_2 cannot be ruled out.

MACEY AND MEYERS[26] give a rather high value of 0.7 μequiv/cm^2 per h for Cl^- influx in short circuited skins (*R. pipiens*) between NaCl Ringers' solution. If the unidirectional "C" fluxes of 6.7 μequiv/cm^2 per h skins of *R. pipiens* between $NaHCO_3$ solutions were in fact flux of HCO_3^-, it would mean that the skin has a 10 times higher conductance for HCO_3^- than for Cl^-. This is incompatible with the potentiometric data presented. As has been mentioned, (Fig. 3) a small decrease in PD occurred when SO_4^{2-} solution was replaced by HCO_3^- solution suggesting low conductance of the skin for HCO_3^-. The potentiometric method, however, is an indirect method only for studying ion permeabilities. Distribution of ions, including H^+, across the outer cell membranes could have been slightly altered by the presence of HCO_3^-. Intracellular acidosis in the face of diffusion of CO_2 from the $NaHCO_3$ solutions appears to be a distinct possibility.

The results presented in Table II show that the "C" flux was about 6 times greater than the net Na^+ flux calculated from the short circuit current. This is also best explained by assuming that carbon moved in the form of CO_2, and not as HCO_3^-. Influx and outflux of carbon were equal, hence there is no indication for active HCO_3^- transport, at least not in the unstimulated skin with the glands at rest.

The conclusion drawn from this study that the skin has a very low conductance (permeability) for HCO_3^- appears to be at variance with the work of GARCIA-ROMEU *et al.*[9] who have concluded that in frog skin (*Calyptocephalella gayi*) Cl^- is exchanged for HCO_3^-. Whereas such a mechanism is quite possible, it seems doubtful to us that their results give proof of this. From their Fig. 11 one notes that the diluted, acidified Na_2SO_4 solution in which the frogs were kept steadily rose in $[Na^+]$ and $[Cl^-]$ above the originally established levels. This indicates that the frogs leaked electrolyte into the bath. For the same reason, and because of titrations of "total acidity' from

unspecified pH starting and end points under uncontrolled conditions (open vessels), it remains an unanswered question whether H^+ (from CO_2) is exchanged for Na^+ of the bath. These authors[9] do not consider the need to include K^+ in the ionic balance. The study of such exchange reactions at the epidermal level, at rates of 0.05 μequiv/cm^2 per h, or less, (calculated by the use of Meek's formula) in the presence of Na^+- and $KHCO_3$-secreting glands[2,10], which can be activated by epidermal acidification, is a difficult problem. It should also be mentioned that, contrary to their statement[9], in our earlier work on the function of the HCO_3^- – secreting skin glands[2], a clear distinction was made between results with and without glandular stimulation resulting from handling of the frogs.

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