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SOME FEATURES OF HYDROGEN (ION) SECRETION BY THE FROG SKIN

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SUMMARY

We have studied the movements of H^+ from the in vitro frog skin into the outside solution because it has been suggested that the movement of sodium from the outside solution into the skin may result from the forced exchange of Na^+ by H^+.

Our main observations can be summarized as follows: (a) Hydrogen moves from the skin into the outside solution at a rate of 0.04 μequiv $\cdot$ $cm^{-2} \cdot h^{-1}$ while Na^+ influx had a value of 0.49 μequiv $\cdot$ $cm^{-2} \cdot h^{-1}$. (b) The rate of H^+ secretion is not significantly affected by substituting the Na^+ in the outside solution by K^+ nor by inhibiting Na^+ influx with amiloride ($5 \cdot 10^{-5}$ M). (c) Acetazolamide ($5 \cdot 10^{-3}$ M) blocked H^+ secretion without altering the potential difference across the skin. (d) The rate of H^+ production is not underestimated because it may have been neutralized by HCO_3^- secreted into the outside solution in exchange for Cl^-. Substituting all the Cl^- by SO_4^{2-} in the outside solutions does not result in an increase in the rate of H^+ production. (e) The steady-state rate of H^+ secretion is not affected by large changes in electrochemical potential gradients for H^+. Neither abolishing the potential difference across the skin nor a 10-fold change in H^+ concentration in the outside solution affected significantly the steady-state rate of H^+ secretion. (f) The H^+ secretion was abolished by the metabolic inhibitors dinitrophenol ($1 \cdot 10^{-4}$ M) and Antimycin A ($1.5 \cdot 10^{-6}$ M) which also markedly reduced the potential difference across the skin.

Observations (a), (b), and (c) suggest that H^+ and Na^+ movements across the outer border of the isolated frog skin are not coupled. The ratio of Na^+ to H^+ movements is very different from unity and Na^+ movements can be abolished without any effects on H^+ secretion and conversely H^+ movements can be abolished without interruption of Na^+ uptake.

A second conclusion suggested by these results is that the H^+ secretion does not result from movement of H^+ following its electrochemical potential gradient since

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that rate of secretion is not affected by marked changes in either potential or $[H^+]$. Furthermore, the effects of metabolic inhibitors suggest that H^+ secretion requires the expenditure of energy by the cell.

INTRODUCTION

One of the most interesting features of salt-absorbing epithelia is the process by which sodium penetrates the cells. In some epithelia there is evidence that may imply that Na^+ absorption occurs in exchange for H^+ (gall bladder [1], jejunum [2], kidney tubule [3], and gills [4]; a comprehensive review of the problem has been recently published by Steinmetz [5]). In our investigation we have examined whether the process by which Na^+ enters the frog skin epithelium is coupled with an excretion of hydrogen. Such a possibility was raised in 1938 by Krogh [6] and in 1949 by Ussing [7]. A few years later, Huf, et al. [9] and Fleming [8] found that frog skins indeed acidify the outside solution.

Since then a number of investigators have confirmed this observation [10–12] and have concluded that the acidification is not related to Na^+ absorption. However, the interest in the problem has been recently revived by two groups of investigators [13, 14] who, working in whole frogs with low salt concentrations in the bathing solutions, have concluded that Na^+ is absorbed in exchange for H^+ across the outer border of the frog skin. This discrepancy led us to examine further the problem of a Na/H^+ coupled exchange at the outer border of the skin in vitro with 115 mM NaCl as the outside bathing solution.

The results described here do not support the hypothesis that there is an obligatory link between Na^+ absorption and H^+ secretion at the outer border of the frog skin. Further experiments suggest that H^+ secretion by the frog skin is an active process. The results of in vivo experiments [13, 14] are not necessarily contradictory, for these investigators conducted experiments with 1–2 mM NaCl as the outside solution. The ion transport characteristics of the skin may differ with high, contrasted to low salinites on the outside [29, 30]. A brief description of some of our results was published elsewhere [15].

METHODS

For most experiments frogs (*Rana temporaria*) kept in a container partially filled with running tap water were used. In a few experiments, specifically mentioned in the text, we used frogs kept at room temperature (18–25 °C) in separate chambers filled with running, double-distilled water for at least 1 week.

Pieces of belly skin were mounted between two Ussing-type half chambers [16] with an area of 3.14 cm^2. The inside half chamber (see Fig. 1) had a capacity of 7 ml and was filled with buffered Ringer's solution while the outside half chamber contained 15 ml of non-buffered Ringer's solution and was stirred with a magnetic bar.

Solution changes were made by withdrawing and replacing the solutions with polyethylene syringes through small ports in the tops of each half chamber.

H^+ secretion determinations. H^+ secretion was determined by the pH-stat tech-

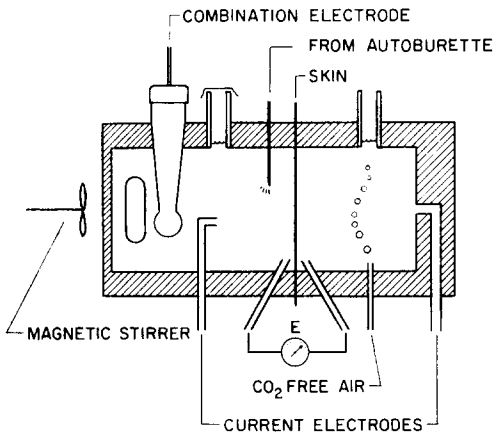

Fig. 1. Diagram of the experimental chamber. The skin was placed with the outside surface facing the combination electrode. E indicates the PD-measuring circuit.

nique. The outside half chamber had a hole which allowed access to a combination pH electrode (Radiometer 6K 202 G C). The unbuffered outside solution was titrated (Radiometer autoburette, pH meter and titrator) to a constant pH value of 7.8 by adding 0.01 M KOH.

Special care was taken to reduce the amount of CO_2 in the solutions. These solutions were prepared with boiled distilled water and bubbled overnight with CO_2-free O_2 up to the moment of being used. The CO_2-free O_2 was obtained by pumping 100 % O_2 successively through a flask containing 2.5 or 5 M KOH, then a soda-lime filter, and finally a flask of distilled water. The pH of these solutions did not change more than 0.05 pH units when placed in the experimental chamber without skin, bubbled with CO_2-free O_2 and stirred magnetically.

To find whether the skins were secreting into the outside solution any buffers which could markedly influence the H^+ secretion measurements, the bathing solutions were collected at the end of an experiment or at the end of an extended period of equilibration with the outside of the skin and then titrated with HCl. These titration curves were compared with titration curves of freshly prepared solutions.

We found that the isolated skin secreted a buffering substance with pK 5.9 into the outside Ringer's solution. The buffering capacity of this substance did not affect our measurement at pH > 7.7. Since most of our experiments were performed with the outside bathing solution pH 7.7–7.9, no correction of measured H^+ secretion rates was made. However, at pH 7.0 the buffer secreted throughout the experiment could cause a maximum underestimation of H^+ secretion rates by about 10 %.

Electrical measurements. Four 3 M KCl/4 % agar polyethylene bridges were provided to measure potential differences (PD) and short circuit current I_{sc}. The bridges used for measuring the PD were situated approx. 1.5 mm from the skin and led to calomel electrodes which were connected to a Vibron high impedance electrometer. The other two bridges also led to calomel electrodes which were used to pass current from a battery to short circuit the tissue [16].

Na^+ fluxes. $^{24}Na^+$ was used to measure outflux and influx on paired halves of the same skin following the method described by Ussing [7].

In four experiments we measured outside-to-inside $^{24}Na^+$ fluxes in skins bathed with a 1 mM Na_2SO_4 solution on the outside. The radioactive Na_2SO_4 solution was prepared by neutralizing $^{24}Na_2CO_3$ solution with H_2SO_4.

Solutions. The basic NaCl Ringer's solution contained 115 mM NaCl, 2.5 mM KCl and 2.0 mM $CaCl_2$. Na^+-free Ringer was prepared by replacing all Na^+ with K^+. Sulfate Ringer had the following composition: 59.5 mM Na_2SO_4, 1.25 mM K_2SO_4, and 2.0 mM $CaSO_4$. The outside solution was always unbuffered while the inside solution was usually buffered to pH 7.7–7.9 with 5 mM Tris.

Experimental design. Because H^+ secretion sometimes decayed slowly during the course of an experiment, a "bracketed" design was most often used for testing the effects of different experimental procedures. Control values for H^+ secretions were first determined; then we measured the effects on H^+ secretion of a desired experimental procedure; finally H^+ secretion was determined again under control conditions.

In some types of experiments, for example when testing the effects of some inhibitors, the "bracketed" design was not feasible. Then the electrical measurements and H^+ secretion rates were expressed as the steady-state values immediately before the experimental procedure was carried out and at sequential times after the experimental modification was introduced.

RESULTS

Control levels of H^+ secretion

In agreement with previous findings [8–12] we found that the in vitro abdominal skin of *R. temporaria* secretes H^+ into the outside bathing solution. In 42 experiments in which pH 7.80 NaCl Ringer's solution initially bathed both sides of the skin (outside unbuffered; inside Tris-buffered), the average rate of H^+ secretion into the outside solution was 41.1 ± 4.2 nequiv $\cdot$ cm$^{-2} \cdot$ h^{-1}. The transepithelial PD in these same skins ranged from 22 to 111 mV.

In three skins bathed in NaCl Ringer's solution acid secretion proceeded at a stable level for about 1 h and then slowly decreased until it finally stopped while the PD did not change throughout this period. Such spontaneous decrease in H^+ secretion has already been observed [17]. Furthermore in four other skins whose PD values ranged between 30 and 88 mV no acid secretion could be detected between 1.5 and 3 h.

Sodium fluxes

The H^+ secretion rates measured in the experiments described in the previous sections are very low when compared with Na^+ influx values previously found for the skin [7]. We thought that it would be necessary to confirm, at least in a few experiments, that in our observations Na^+ influx had values similar to those reported previously. We measured transepithelial Na^+ influx in two halves of the same skin bathed with NaCl Ringer's solution. Sodium influx had an average of 487 ± 39 nequiv $\cdot$ cm$^{-2} \cdot$ h^{-1}, very similar to values found previously. This value is eight times larger than the largest H^+ secretion we measured in any of our experiments. In another set of experiments we measured Na^+ influx from an outside bathing solution of 2 mM Na_2SO_4 Ringer's to compare it with rates with H^+ secretion measured in the same ex-

periments. The average H^+ secretion rate was 9.5±2.1 nequiv · cm^{-2} · h^{-1} while the average Na^+ influx was 27.7±5.2 nequiv · cm^{-2} · h^{-1}. The average PD was 27.4± 3.1 mV ($n = 4$). Since we were interested in comparing the Na^+ influx to H^+ secretion, the outflux of Na^+ was not measured in these experiments.

The effect of Cl^--free solutions

Garcia-Romeu et al. [13] observed that when frogs are immersed in Cl^--containing solutions, there is a secretion of HCO_3^-. They found a ratio of 2 : 3 between the Cl^- absorbed and the HCO_3^- secreted. Since in the experiments summarized in the previous section the skins were immersed in Cl^- solutions, it was possible that an exchange of HCO_3^- for Cl^- was taking place. If in these experiments HCO^-_3 were secreted in exchange for Cl^-, it could neutralize the H^+ secreted by the skin. This process would result in an underestimation of the rate of acidification. We therefore decided to measure the effects of solutions in which all the Cl^- had been replaced by SO_4^{2-} since Garcia-Romeu et al. [13] found that there is no HCO_3^- secretion into SO_4^{2-} solutions. In ten experiments the substitution of Cl^- with SO_4^{2-} resulted in a large increase in PD without any change in H^+ secretion rate (Table I). In two additional experiments we measured the effect of replacing Cl^- with SO_4^{2-} in skins obtained from frogs that had been kept, as in the experiments of Garcia-Romeu et al. [13], for at least 1 week in distilled water. As in the previous experiments, Cl^--free solutions had no effect on the rate of H^+ secretion.

Effects of reducing Na^+ transport on H^+ secretion

One of the simplest ways of exploring whether H^+ secretion and Na^+ uptake are directly coupled is to measure the effects of reducing or abolishing transport on the rate of H^+ secretion by the skin. We selected two procedures to reduce Na^+ transport. In one group of experiments we abolished Na^+ uptake by substituting all outside Na^+ with K^+. In the second group of experiments we measured the effects of amiloride on H^+ secretion.

In experiments in which all the outside Na^+ was substituted by K^+, the PD

TABLE I

EFFECTS OF SEVERAL TREATMENTS ON H^+ SECRETION AND POTENTIAL DIFFERENCE (PD) OF ISOLATED FROG SKIN

n denotes number of experiments. All values are means ±S.E.

Treatment	*n*	H^+ secretion (nequiv · cm^{-2} · h^{-1})		PD (mV)	
		Control	Experimental	Control	Experimental
Cl^--free solution	10	16.5±2.1	15.6±1.9	43.2±5.2	87.8±7.1
Na^+-free solution	5	43.0±6.9	37.6±7.2	39.8±4.7	9.3±1.9
Amiloride	5	23.0±2.1	21.4±1.9	42.1±3.8	4.8±1.2
Acetazolamide*	6	41.8±5.7	0	42.8±7.1	35.2±8.1
pH change**	5	29.8±4.1	36.5±4.0	58.1±5.7	61.8±6.9
Short circuit	8	36.2±5.6	30.2±4.8	59.1±7.1	0

* Inside solution was pH 9.

** H^+ concentration of outside solution was changed from pH 7 (during the control) to pH 8.

was reduced nearly to zero without marked alteration in the rate of H^+ secretion (Table I). The effects of amiloride on PD and H^+ secretion were compared in five experiments. As with Na^+ substitution the PD was nearly abolished while H^+ secretion rate was not altered. After washing the amiloride-containing solution from the outside solution, no H^+ secretion could be detected for 70 min, then H^+ secretion was resumed at its original rate. Such an interruption of H^+ secretion by long exposure (more than 60 min) to amiloride was observed in three out of the five experiments, in one of them even when amiloride was present in the outside solution. However, the temporal dissociation between the inhibition of Na^+ influx, which occurs within seconds of adding the drug, and of H^+ secretion, which occurs after 1 h or more and is not observed constantly, is beyond doubt. Table I summarizes the effects of amiloride on H^+ secretion.

The effects of acetazolamide

It is well known that acetazolamide inhibits H^+ secretion by several epithelia. Furthermore Emilio et al. [11] found that in the isolated frog skin acetazolamide blocked H^+ secretion without changing the short circuit current and potential difference. In preliminary experiments we tested the effects of 0.5 mM acetazolamide added to the inside solution of skins bathed with Cl^- at pH 7.8. With these concentrations we could not measure any effects on either the PD or H^+ secretion. When we raised acetazolamide concentration to 5 mM we found that it slowly blocked H^+ secretion. However, it was necessary to wait for 70–80 min to observe maximum inhibitory effects. In a total of six experiments where the inside solution was pH 7.8, the PD and H^+ secretion rates were 42.8 ± 7.1 mV and 41.8 ± 5.7 nequiv $\cdot$ cm^{-2} $\cdot$ h^{-1}, respectively, before acetazolamide treatment. 1 h after adding 5 mM acetazolamide the PD decreased to only 35.2 ± 8.1 mV ($n = 6$) while H^+ secretion was always completely inhibited after 90 min with no further effect on the PD.

In contrast to these slow effects observed with pH 7.8 inside solutions, when the inside solution had a pH of 9.0, acetazolamide (5 mM) produced a more rapid (within 40–50 min) inhibition of H^+ secretion. This decrease in H^+ secretion with inside solutions of pH 9.0 was not due to the increased pH, since H^+ secretion and PD did not change significantly at inside pH values ranging between 7.8 and 9. At pH 7.8, H^+ secretion averaged 53.1 ± 5.7 nequiv. $\cdot$ cm^{-2} $\cdot$ h^{-1}; when the pH was increased to 9.0 the H^+ secretion rate was 41.8 ± 5.7 nequiv $\cdot$ cm^{-2} $\cdot$ h^{-1}. In four experiments in which the effects of 5 mM acetazolamide (pH 9 on the inside solution) were tested on the I_{sc} we found no inhibitory effects of the drug. The control values of I_{sc} were 24 ± 3.2 μA/cm^2; 100 min after the addition of acetazolamide it was 22 ± 2.8 μA/cm^2. The results of the effects of acetazolamide on H^+ secretion and PD are given in Table I.

The effects of modifying the electrochemical gradient for H^+ on secretion

Two methods were selected to modify the electrochemical gradient for H^+ across the frog skin. In one set of experiments the H^+ secretion rates into outside solutions of different pH were compared. Table I shows that a 10-fold change of outside (H^+) from pH 7.0 to 8.0, had no significant effects on the H^+ secretion rate.

In another group of experiments the potential across the skin was shortcircuited following the method of Ussing and Zerhan [16]. Under short circuit current

conditions the PD across the outer border of the skin is modified markedly. In open circuit conditions the cell interior of the outermost layer of cells is positive with respect to the outside solution while during I_{sc} the cytoplasm is about 17 mV negative with respect to the outside solution [18, 19].

Reduction of the PD produced no changes in H^+ secretion, however. The results of eight experiments are summarized in Table I. (It was noted in these experiments that the average I_{sc} was 1 200±142 nequiv · cm^{-2} · h^{-1} ($n = 8$) while the H^+ secretion was only 36±5.6 nequiv · cm^{-2} · h^{-1}). In one experiment performed in SO_4^{2-} Ringer's solution and in two further experiments where 2 mM NaCl replaced the outside NaCl Ringer's solution, similar dissociation between Na^+ and H^+ fluxes was obtained.

Metabolic inhibitors

We compared the effects on PD and H^+ secretion of two metabolic inhibitors Antimycin A and dinitrophenol.

In four experiments antimycin A was added to the inside solution at $3 \cdot 10^{-6}$ M. After 30 min the inhibitor reduced H^+ secretion to very low levels in all experiments. Secretion was completely abolished after 45 min. The PD was also reduced by the inhibitor from 82.8±1.1 to 31.9±5.9 mV after 45 min. Reduction of the PD and I_{sc} caused by antimycin followed a similar time course. Inhibition to 10 % of the control levels takes place in about 2 h.

As shown in Fig. 2 the addition of dinitrophenol (to give a concentration of $1 \cdot 10^{-4}$ M) to the inside solution caused a rapid and transient increase in H^+ secretion followed by a decline until no more H^+ was secreted. The PD was slowly reduced by dinitrophenol. In three other experiments we found that, in agreement with Schoffeniels [20], the reduction of PD is followed very closely by a reduction of I_{sc}. In eight experiments in which the effects of dinitrophenol were tested, the control H^+ secretion rate was 12.6±2.1 nequiv · cm^{-2} · h^{-1} and the PD was 73.5±8.2 mV. When dinitrophenol was added, secretion increased to a peak of 111.3±12.7 nequiv ·

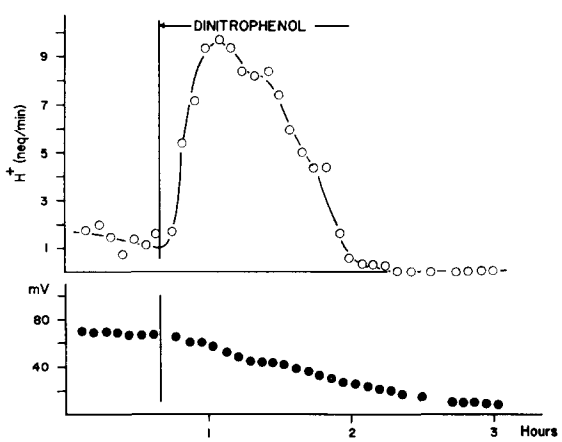

Fig. 2. Effects of dinitrophenol ($1 \cdot 10^{-4}$ M) on H^+ secretion (empty circles) and PD (filled circles) of the isolated frog skin. All solutions had pH 7.8. The inhibitor was added only to the inside solution.

$cm^{-2} \cdot h^{-1}$ in 16 ± 2 min and then decreased and reached zero level after 84 ± 21 min.

The addition of dinitrophenol to two skins that were not secreting spontaneously also produced an increase in H^+ secretion with characteristics similar to those observed in skins that were secreting spontaneously. However, when dinitrophenol was added to two skins whose H^+ secretion had been blocked with acetazolamide, the uncoupler did not stimulate H^+ secretion.

DISCUSSION

Two types of experimental findings suggest the conclusion that there is coupling between two different ions that are exchanged across a membrane: (1) A more or less fixed stoichiometry exists between the movements of each of the exchanged species. (2) Interference with the movement of one of the exchanged species, either by eliminating the ion from the solutions or by adding a specific inhibitor, blocks the movement of the other species.

The simplest mechanism postulated for such a coupling is what has been designated sometimes as "chemical" coupling. At one side of the membrane one species of ions combines selectively with a membrane component. The complex moves across the membrane and the ion is discharged at the other side of the membrane where the membrane component is free to bind selectively the second ionic species. This second complex moves back across the membrane and the cycle is initiated again. One of the most distinctive features of the salt transport system of the frog skin and other salt-absorbing epithelia is the presence at the apical border of an amiloride-sensitive Na^+ absorption system (see ref. 21). It is therefore of interest to establish whether this Na^+-absorbing mechanism involves a direct exchange of Na^+ for H^+.

Several of the observations described here are not in agreement with the notion that Na^+ uptake at the outside border of the frog skin occurs through an obligatory one-for-one exchange for H^+. These observations are:

(1) The ratio of Na^+ influx to H^+ secretion is very large. Average hydrogen secretion was 0.04 μequiv $\cdot$ $cm^{-2} \cdot h^{-1}$ while Na^+ influx had a value of 0.49 μequiv $\cdot$ $cm^{-2} \cdot h^{-1}$. Although these measurements were carried out in Cl^- solutions where an exchange of this anion for HCO_3^- could result in neutralization of some of the secreted H^+, the experiments with SO_4^{2-} solutions obviate such a conclusion. Thus, the lack of effects on H^+ secretion of substituting Cl^- by SO_4^{2-} suggests that in our Cl^- experiments there was no important exchange of HCO_3^- for Cl^- that would offset the H^+ secretion determinations. We have not excluded the possibility that there is a non-coupled HCO_3^- secretion which occurs in both Cl^- and SO_4^{2-} solutions. It could be argued that when Cl^- is substituted with SO_4^{2-} there is a reduction in Na^+ penetration. This reduction of Na^+ penetration is in itself against the Na^+/H^+ exchange model since one of the most important features of the model is that Na^+ should be absorbed independently of anion movements. Indeed, substituting Cl^- with impermeable anions or reducing Cl^- conductance depresses net transepithelial salt transport, indicating that the anion accompanying the cation is of central importance during the salt absorption process [22]. Therefore, if there is any role for an H^+ exchange, it is secondary.

(2) Sodium influx can be suppressed either by eliminating Na^+ from the outside solution or by the addition of amiloride without interfering with H^+ secretion.

Conversely H^+ secretion can be blocked with acetazolamide without eliminating Na^+ absorption.

(3) The lack of effects of changing the skin PD from open circuit levels to zero transepithelial PD on H^+ secretion also suggests that H^+ secretion is not coupled with Na^+ uptake. It is well known that reducing the PD across the skin markedly increases the movement of Na^+ across the tissue [16]. Some recent measurements have shown that the increase is associated with increased Na^+ movement across the outer border of the epithelium [23, 24].

Although all these findings are not in agreement with a Na^+/H^+ exchange, the observations in whole frogs point in a different direction and require some comment. Thus in the whole animal a one-to-one ratio is observed between H^+ secreted and Na^+ absorbed [13, 14], and acetazolamide and amiloride block both Na^+ absorption and H^+ secretion [14]. Several arguments can be invoked to explain the discrepancy between results obtained with whole animals and those with isolated skins. First, since a large number of variables is involved in experiments with whole animals, the results are harder to interpret. For example, it has been shown that the skin glands of the frog secrete continuously into the outside solution, even when the animals remain unmolested [13]. The composition of this secretion may have marked effects on the results obtained on whole animals. There is the additional possibility that different parts of the skin may have different capacities for Na^+/H^+ exchange. In whole frogs other portions of the skin (and not only the abdomen as in the present experiments) are in contact with the fluid, and the skin of the back might exhibit some Na^+/H^+ exchange. In addition, it is well known that the skin is an important accessory respiratory organ for amphibians (for references see Garcia-Romeu et al. [13]). Since even the uncatalyzed hydration of CO_2 to form carbonic acid proceeds at a considerable rate [26], CO_2 exchange across the skin from blood to the bathing solution must certainly affect H^+ secretion rates.

Secondly, the interpretation of the effects of inhibitory agents on whole animals may not be straightforward. For example, it has been shown that the blocking effects of acetazolamide on fluid secretion by the choroid plexus are not the result of a direct effect on the epithelium but of vasoconstriction caused by the drug [27]. In the particular case of the frog skin Garcia-Romeu and Ehrenfeld [28] found that the injection of acetazolamide caused an increase in the efflux of both Na^+ and Cl^- through the skin. Findings of this type raise the question of how much of the blockade of Na^+ absorption caused by acetazolamide in the whole frog, which is not observed in the isolated skins, is due to a direct effect on the epithelium and not to systematic effects of the drug. Similar considerations could be valid in the case of other inhibitors.

Finally, there is a fundamental difference in design between the in vitro and in vivo conditions. In vivo experiments have been conducted with low salt concentrations (1–5 mM) of the bathing solutions; the present in vitro results were obtained with high salt concentrations (115 mM). From the work of Kirschner [29] in vivo and Mandel and Curran [24] in vitro it seems that ion transport characteristics of the skin may be different in high, contrasted to low salinities of the outside of the skin. Specifically, when low salt concentrations of a sodium salt with an impermeant anion are used [13, 14] experimental conditions favor the unmasking of a Na^+/H^+ exchange. However, it should be stressed that this exchange does not necessarily occur via an obligatory coupling on some membrane carrier. When high salt concentrations

are used, Na^+/H^+ exchange does not occur to any significant extent.

The fact that H^+ secretion is not coupled directly to the transport of other electrolytes in vitro does not rule out the possibility of indirect interactions with the transport of Na^+ and other ions. For example, if a fraction of the H^+ secreted by the skin is derived from the hydration of CO_2 produced by cell metabolism, conditions of stimulated transport that increase the rate of respiration and of CO_2 production could lead to increased H^+ secretion. Another possible interaction could occur under conditions in which H^+ would be one of the more permeable ions available, then changes in the electrical forces like an increased outside negativity, caused by adding Na^+ to the solution, could increase outward H^+ movements.

Since Na^+ uptake does not seem to occur via an obligatory one-for-one exchange for H^+, what is the mechanism of H^+ secretion? Some of our results suggest that it may be an active process. For example the findings that show that changes of electrochemical potential gradient for H^+ across the outer border of the skin produced by either changing the PD by short-circuiting or altering 10-fold the H^+ concentration of the bathing solutions did not affect the rates of acidification are not compatible with a simple diffusion process for H^+ excretion. The results of the experiments with dinitrophenol and antimycin A described here and the inhibition of H^+ secretion by O_2 exclusion seen by Emilio et al. [11] are compatible with the suggestion that H^+ secretion by frog skin is an active process dependent on energy-producing reactions in the cell.

Finally, our experiments suggest that respiratory CO_2 serves as at least a partial source of the H^+ secreted and that this CO_2 is hydrated intracellularly via a carbonic anhydrase-catalyzed reaction. This conclusion arises from the experiments in which acetazolamide was added and H^+ secretion stopped. The initial stimulation of H^+ secretion caused by dinitrophenol could be interpreted along these lines since the uncoupler causes a large stimulation of the respiratory rate that could be connected with a fall in ATP levels.

In conclusion our results and arguments indicate that the H^+ secretion in frog skin does not occur via an obligatory one-for-one exchange for Na^+. These movements occur independently at the outer-resistive membrane. H^+ secretion seems to be an active process dependent on intracellular energy-producing reactions. Respiratory CO_2 which is hydrated intracellularly via a carbonic anhydrase-catalyzed reaction serves at least as a partial source of the secreted H^+

These conclusions are in agreement with findings in the toad urinary bladder [31, 32], an epithelium in which almost all Na^+ is absorbed by an amiloride-sensitive process and which shares many features with the amphibian skin, and where the available evidence is against a Na^+/H^+ exchange process. Since the Na^+/H^+ exchange does not play a central role in acidification by frog skin and toad urinary bladder, focus ought to be shifted to the H^+ transport system and undoubtedly the more profitable systems to study the process would be those tissues which secrete H^+ at high rates.

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