# ELECTROMOTIVE CHLORIDE TRANSPORT AND GASTRIC ACID SECRETION IN THE FROG*

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ABSTRACT

The total active transport of chloride ions across the gastric mucosa can be considered as the sum of two fractions; an acidic one which is equivalent to the acid secreted, and an electromotive one which accounts for the electric energy generated by the gastric mucosa. In the present studies, the relationship between this electromotive chloride transport and acid secretion has been investigated, using specific inhibitors. The rate of electromotive chloride transport was found to be essentially unaffected by changes in the rate of acid secretion, and also by inhibition of acid secretion by thiocyanate. On the other hand, diamox, in combination with histamine, was shown to depress or abolish the gastric electromotive force and to inhibit partially the total chloride transport, while acid was secreted at an almost normal rate. This kind of inhibition is undefined as to its mechanism but seems to be more specific for the gastric chloride transport than any other inhibitor known. It is concluded that acid secretion and electromotive chloride transport involve two different mechanisms, and are not absolutely essential for each other. The present results do not support the view that carbonic anhydrase is essential for acid secretion. They rather suggest an important function of this enzyme in the mechanism of active chloride transport.

## INTRODUCTION

The production of gastric acid is a complex phenomenon which may involve different processes, including the accumulation of H ions, the movement of Cl ions, and the generation of an electromotive force. The Cl movement has drawn special attention in recent years due to the identification of a special mechanism of active Cl transport (3, 4). This Cl "pump" has been shown to be linked to metabolism, and a model accounting for the phenomena concerned has been devised (5). It is of special interest that Hogben has found the rate of the active Cl transport to be electrochemically equivalent to the electrical current produced by the mucosa. This has been taken as evidence

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in favor of the assumption that Cl transport and E.M.F. are both manifestations of the same mechanism.

The question as to whether, and how, such an "electromotive" transport mechanism for Cl ions is connected with the gastric acid formation, is still unanswered. It has frequently been noticed that the onset of acid secretion has a comparatively small effect on the electrical properties of the mucosa, such as resistance and potential difference. It has been questioned, therefore, whether the electromotive Cl pump has any function in acid secretion at all (6). On the other hand, no appreciable acid secretion has been obtained hitherto in the absence of the electromotive force (E.M.F.); and increasing the gastric potential difference by an external E.M.F. was found to augment the acid secretion rate (7, 8). These observations have been considered as evidence in favor of an essential, or at least auxiliary, function of the chloride pump in acid secretion. The present investigations were intended to provide further information on the relationship between the secretory mechanism and the electromotive force of the gastric mucosa in the frog.

## *Methods*

Most of the procedures are described fully in a previous publication (5). The gastric mucosa of the frog was mounted between two lucite chambers, and bathed with oxygenated, physiological solutions. The solution in contact with the serosal surface contained bicarbonate and phosphate buffers, and is referred to as the *nutrient* solution. In contact with the mucosal surface was a similar solution, with buffers omitted, called the *secretory* solution.

In previous work, acid secretion was followed by the use of a glass electrode. In the present experiments, a procedure of semicontinuous titration was used, to maintain the pH at or near 5.5. The secretion is then calculated from the strength and volume of titrant added. This method has the advantage of essentially eliminating back-diffusion of H ions, and obviates the need to correct for the activity coefficient of the H ion.

Electrical potential difference measurements were made, using a recording millivoltmeter (varian, model G-10), either alone, or in conjunction with a radiometer, pH meter 22. For conductance measurements, a fixed, direct current of 75 microamperes was passed through the membrane, and the change in potential across the mucosal membrane recorded. This change occurred in two distinct phases, as shown in Fig. 1. In the initial phase, complete within a second, the potential difference changed from the spontaneous value $E_0$ to the value $E_1$. In the final phase, complete within 1 to 2 minutes (depending on the previous treatment of the mucosa), the potential difference changed to $E_2$. As indicated in Fig. 1, changing the direction of the current merely changes the direction of the potential changes.

If $I$ is the current per square centimeter of membrane, and $R_s$ the resistance of the bathing solution, 1 sq. cm. in area, between the two potential electrodes, we obtain $R_i = (E_1 - E_0)/I - R_s$, and $R_f = (E_2 - E_0)/I - R_s$. Here $R_i$ is defined as the *initial* resistance of the mucosa, and $R_f$ the *final* resistance, both in ohms·cm.[2]

The initial conductance ($\kappa_i$) and the final conductance ($\kappa_f$), in mhos/cm.$^2$, are obtained by taking the respective reciprocals of $R_i$ and $R_f$.

For convenience, we may refer to the shift in potential difference from $E_1$ to $E_2$ as a "membrane polarization." Whether the resulting $R_i$ or $R_f$ represents the true resistance of the mucosa, depends on the interpretation of this polarization phenomenon (9, 10). If polarization is due to a change in any electromotive force present within the conducting parts of the mucosa, then $R_f$ has no meaning, and $R_i$ gives the real resistance. If, however, the polarization may be treated as a special kind of capacitance with a very high time constant, then $R_f$ has to be considered as the true, "non-polarizable" resistance of the mucosa. At the present time, it cannot be decided which interpretation of the polarization is the more appropriate. Both values of the mucosal resistance are therefore reported in the Results.

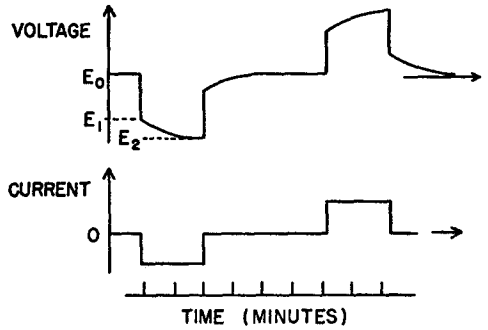


FIG. 1. Electrical response of the gastric mucosa to direct current.

## RESULTS

The short-circuit current, $i_{sc}$, has been taken in these studies to represent the most reliable index to the electromotive force developed by the mucosa. This is the current supplied externally, necessary to bring the measured potential difference across the mucosa to zero. Unlike the open-circuit potential difference, the parameter $i_{sc}$ is independent of changes in the shunt resistance of the membrane; *i.e.*, to changes in the permeability of the membrane for "passive" ions. Hogben has shown that, for the normal mucosa, the short-circuit current is equal to the net chloride flux in excess of the chloride in the secreted acid (4). In terms of the unidirectional fluxes, this becomes

$$\Phi_{ns}^{Cl^-} - \Phi_{sn}^{Cl^-} = \sigma_{H^+} + i_{sc} \quad (1)$$

in which $\Phi_{ns}^{Cl^-}$ and $\Phi_{sn}^{Cl^-}$ are the chloride fluxes from nutrient to secretory, and secretory to nutrient surfaces, respectively, and $\sigma_{H^+}$ is the rate of acid secretion. All terms are expressed in the unit, microequivalents per square centimeter per hour.

The net chloride flux may be divided into two fractions, using Equation 1. The first is equivalent to the acid secreted, and may be called the *acidic* chlo-

ride output. The second fraction, which is equivalent to the short-circuit current, probably accounts for the gastric E.M.F., and may be called the *non-acidic*, or electromotive chloride. The short-circuit current can accordingly be used as a partial index to the activity of the chloride pump, with the advantage that it is much easier to measure than are simultaneous unidirectional chloride fluxes.

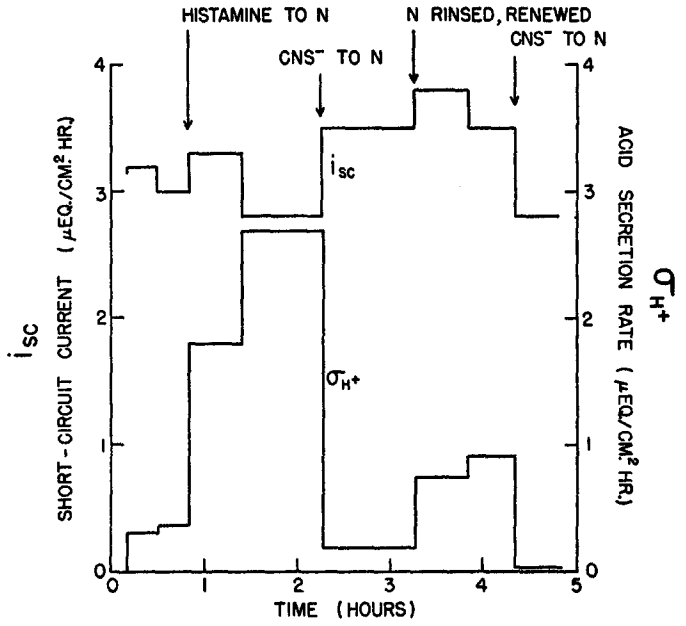


FIG. 2. Time course of a typical experiment with a normal mucosa, showing short-circuit current ($i_{sc}$) at varying rates of acid secretion ($\sigma_{H^+}$).

### *A. Normal Mocosae (without Diamox)*

For the representative experiment shown in Fig. 2, the short-circuit current is plotted as a function of time. Widely different rates of acid secretion were obtained by using histamine and thiocyanate as potentiator and inhibitor of secretion, respectively. It was found that changes in the rate of acid secretion had no consistent effect on the measured short-circuit current.

Results of 14 similar experiments are shown in Fig. 3. The average short-circuit current during a period (½ to 1 hour) is plotted against the simultaneous rate of acid secretion. Despite the fluctuation introduced by the plotting of data from many mucosae, it can be seen that the current is approximately constant over a considerable range of secretory activity.

### *B. Mucosae Treated with Diamox*

*1. Short-Circuit Current.*—The above results, though inconclusive, suggest that electrical activity is independent of acid secretion. This is consistent with the observation that general metabolic inhibitors, such as anoxia or DNP, affect first the rate of acid secretion, and then the electromotive force of the mucosa.[1] The use of inhibitors, specific for either secretory or electrical activity, would be expected to give a more reliable indication as to whether or not a single mechanism is responsible for both phenomena.

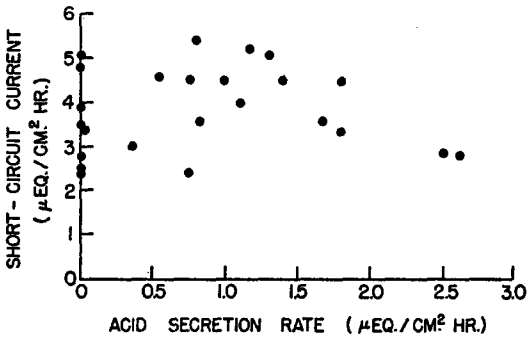


FIG. 3. Compilation of 24 experimental periods, showing short-circuit current and corresponding rate of acid secretion for normal mucosae.

Thiocyanate, a well known inhibitor of gastric secretion, has been found to restore the gastric potential difference to the resting level (11–13). In our experiments we also tested the short-circuit current and found it essentially unaffected by thiocyanate. Apparently this inhibitor specifically blocks acid secretion without appreciably interfering with the electromotive chloride transport. The question arises as to whether the opposite effect is also possible; namely, the inhibition of electrical activity while acid secretion is undisturbed. Experiments by Hogben (14), using the carbonic anhydrase inhibitor, diamox, showed a depression in the magnitudes of both phenomena; however, the effect on the short-circuit current was more pronounced than on the secretion rate.

We found that this discrepancy could be strikingly intensified by the addition of histamine, after diamox. The course of one such experiment is shown in Fig. 4. After diamox plus histamine, the mucosa maintained approximately its spontaneous rate of secretion for several hours. Meanwhile, a drastic reduction of electrical activity was observed. In this experiment, the short-circuit current fell to zero, as did the open-circuit potential difference. A number of

[1] Unpublished observation.

such experiments have shown similar results, although the exact magnitude of the effect is variable. In a few instances, the measured potential difference reversed in sign; in the most extreme case, to 8 mv., serosal surface negative with respect to mucosal surface.

The abolishment of the short-circuit current must be due either to a direct inhibition of the gastric E.M.F., or to a sharp drop of the internal conductance associated with the E.M.F. In order to distinguish between these possibilities the mucosal conductance was measured in the presence and absence of diamox.

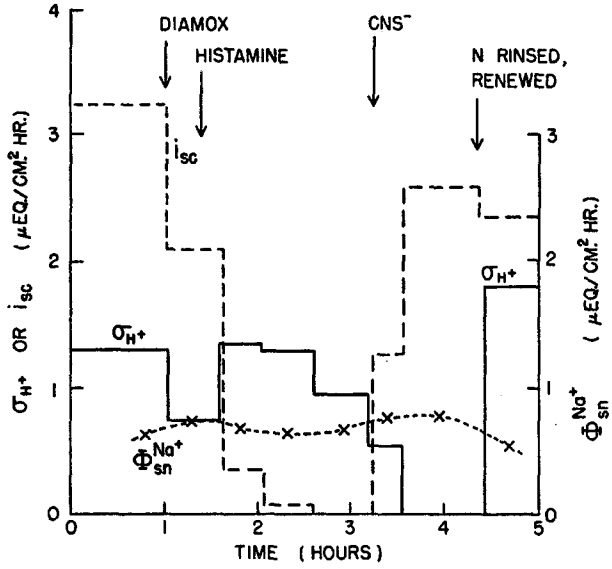


FIG. 4. Time course of a typical experiment with a mucosa, showing short-circuit current ($i_{sc}$), rate of acid secretion ($\sigma_{H^+}$), and sodium flux ($\Phi^{Na^+}_{sn}$) under the influence of diamox, histamine, and thiocyanate.

*2. Conductances.*—The mucosal conductance was measured as described in Methods, obtaining an initial conductance $\kappa_i$; and a final conductance $\kappa_f$. The difference between these conductances,

$$\Delta\kappa = \kappa_i - \kappa_f$$

for each measurement, is also of interest, and the three parameters are given in Table I for (*a*) normal, untreated mucosae, and for mucosae treated with (*b*) diamox, (*c*) thiocyanate, (*d*) general metabolic inhibitors, *e.g.*, anoxia. Data from our previous studies (5) in the last named category have been included for purposes of comparison.

Table I shows that diamox does not significantly change either the initial or the final conductance. These values, however, do not depend solely on the

internal conductance but also on the shunt conductance, associated with the diffusing ions. Measurements of sodium flux (Fig. 4) indicate that diamox does not appreciably change the partial conductance of that ion. If this behavior may be assumed typical of all passive ions, we may conclude that the shunt conductance is hardly disturbed by diamox. It follows that the internal conductance cannot be drastically affected under these conditions. The disappearance of the short-circuit current must be primarily due to a direct inhibition of the E.M.F.

A statistically significant increase in the mucosal polarization ($\Delta\kappa$) under diamox may be demonstrated from the data of Table I. Furthermore, this

TABLE I

*Electrical Conductances*

| | Control mucosae | With diamox | With thiocyanate | Under metabolic inhibition |
|---|---|---|---|---|
| No. of Measurements | 15 | 23 | 13 | 11 |
| $\kappa_i$ | 6.1 | 7.0 | 4.6 | 1.4 |
| S.D. | ±0.9 | ±1.4 | ±1.0 | ±0.4 |
| $\kappa_f$ | 3.6 | 3.0 | 3.4 | 1.3 |
| S.D. | ±0.9 | ±0.7 | ±0.8 | ±0.4 |
| $\Delta\kappa$ | 2.5 | 4.1 | 1.2 | 0.15 |
| S.D. | ±0.9 | ±1.2 | ±0.5 | ±0.18 |

parameter is greatly reduced under thiocyanate, and disappears under general metabolic inhibition. The mucosal polarization, therefore, seems to be associated with the acid-secreting mucosa.

*3. Effects of Secretion.*—The sharp decrease observed in short-circuit current under diamox would indicate that diamox acts as a specific inhibitor of the mucosal E.M.F. If this were the case, the further addition of thiocyanate should not disturb significantly the potential difference or short-circuit current. On the contrary, both indices to electrical activity show appreciable recovery after thiocyanate (Fig. 4). It must be concluded that the action of diamox depends on the presence of secretory activity. This conclusion is borne out by the results of the addition of diamox to a resting (non-secreting) mucosa (Fig. 5). In this case the effect of diamox on short-circuit current is small.

The dependence of the diamox effect on secretion is not merely a pH effect; the instillation of 0.12 N HCl on the secretory surface (while diamox was present in the nutrient solution) was observed to augment, rather than depress, the short-circuit current.

*4. Fluxes.*—In one series of experiments, $\Phi_{ns}^{Cl^-}$ was measured; in the second series, $\Phi_{sn}^{Cl^-}$. All the mucosae in these experiments were maintained under short-circuit conditions, and simultaneous values of $i_{sc}$ and $\sigma_{H^+}$ obtained.

As a preliminary check, a mean value of $\Phi_{sn}^{Cl^-}$ was calculated from the data in the first series by the use of Equation 1. This value is compared with the mean, measured $\Phi_{sn}^{Cl^-}$ from the second series of measurements, in Table II.

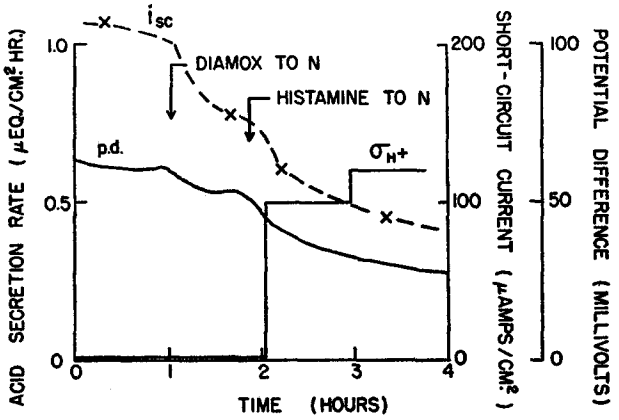


FIG. 5. Time course of a typical experiment, showing the effect of diamox on the gastric mucosa in the resting and secreting states. The symbols are those used in previous figures.

TABLE II

*Comparison of Measured and Calculated Chloride Fluxes, S → N*

| | Measured flux | Flux calculated from Equation 1 |
|---|---|---|
| Chloride flux, secretory to nutrient surface, μeq./cm.² hr. | 2.7 (37 periods) | 2.7 (12 periods) |
| S.D. | ±0.9 | ±1.0 |

From the good agreement of the measured and calculated fluxes, it is clear that Equation 1 is valid in the presence and absence of diamox.

The effect of diamox is thus shown to be an effect on the net transport of chloride ion. How this reduction is accomplished may be seen from the plot of secretory-to-nutrient chloride flux $\Phi_{sn}^{Cl^-}$ as a function of short-circuit current (Fig. 6). As pointed out above, the abscissa $i_{sc}$ is the net, non-acidic chloride transport. It may be seen that the flux $\Phi_{sn}^{Cl^-}$ is constant over a wide range of electrical activity, for normal mucosae. Furthermore, the addition of diamox, which lowers the short-circuit current, does not change (on the average) the flux, $\Phi_{sn}^{Cl^-}$. Thus, a change in short-circuit current is reflected in a corresponding change in $\Phi_{ns}^{Cl^-}$, while $\Phi_{sn}^{Cl^-}$ remains constant (Equation 1).

This result yields information concerning the status of the carrier *exchange diffusion* of chloride, as defined by Ussing (15). There is evidence that most of the chloride exchanged across the mucosal membrane is in combined form (3–5). Since normally the net movement of chloride is always in the same direction, nutrient to secretory, the exchange diffusion is equal to the chloride flux in the opposite direction, less the contribution of free diffusion to this flux. The rate of free diffusion of chloride across the normal mucosa is not exactly known, but is presumably rather small and constant at a given electrical P.D. Changes in the secretory-to-nutrient flux of chloride may therefore

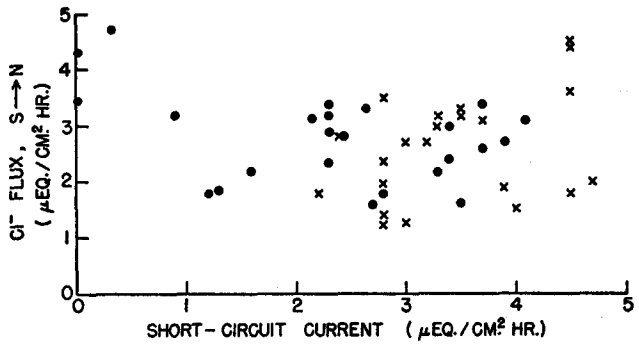


FIG. 6. Compilation (46 experimental periods) of secretory-to-nutrient chloride flux and corresponding short-circuit current. Crosses denote periods with normal mucosae, and solid circles, periods with mucosae treated with diamox. The data include both secreting and non-secreting mucosae.

be taken as indicating changes in the carrier exchange diffusion rate. Consequently, the constancy of the flux $\Phi_{sn}^{Cl^-}$, with and without diamox, shows a corresponding stability in the magnitude of the exchange diffusion of chloride across the gastric mucosa.

The results indicate that diamox changes the normal relationship between secretory and electrical activity. Without diamox, the short-circuit current is independent of the rate of acid secretion; with diamox, the short-circuit current varies in the direction opposite to changes in the rate of acid secretion. The latter effect might be explained as follows. Assume an increase in the active, net transport of H ions, while chloride ion output remains constant. The requirement for an equivalent amount of $Cl^-$ in HCl would result in an equivalent reduction of the short-circuit current. If this were the case, a one-to-one ratio between increase in acid secretion and decrease in short-circuit current (and *vice versa*) would be found after diamox.

The experimental relationships may be seen in Table III. For A, in which histamine was added in the presence of diamox, the decrease in current is four times the increase in secretion rate. If diamox and histamine, together,

are treated as the effective agent (B), no change in rate of acid secretion was observed on the average, with an accompanying large reduction in short-circuit current. In C, the effects of the addition of thiocyanate, in the presence of diamox and histamine, are listed. In this case, the rate of acid production was decreased, and short-circuit current increased; but as in A and B, the observed changes are far from equivalent.

TABLE III

*Stoichiometric Relationship between Changes in Short-Circuit Current and Acid Secretion*

| | A<br>Effect of histamine, in presence of diamox | B<br>Effect of diamox + histamine in normal mucosae | C<br>Effect of thiocyanate in presence of diamox |
|---|---|---|---|
| No. of measurements | 13 | 15 | 13 |
| Change in acid secretion rate ($\Delta\sigma_{H^+}$) | 0.3 | −0.2 | −0.8 |
| S.D. | ±0.3 | ±0.8 | ±0.4 |
| Change in short-circuit current ($\Delta i_{sc}$) | −1.35 | −3.1 | 1.5 |
| S.D. | ±0.51 | ±1.1 | ±0.54 |

## DISCUSSION

It is known that normally some chloride transport, accompanied by the output of electrical energy, takes place in the absence of any detectable acid secretion. This is true for the resting state, and also after inhibition of acid secretion by thiocyanate. We shall call the mechanism responsible for this chloride transport, at rest, the *basal* chloride pump. The question arises as to whether, and how, this basal Cl transport mechanism participates in the formation of acid. It might do so chemically, by providing the Cl ions for the acid secretion, or physically, by maintaining the electrical P.D. which seems favorable to the movement of H ions. In the first case the acidic chloride would be secreted at the expense of the non-acidic chloride. If the basal chloride output remained constant, any increase in acid secretion would be accompanied by an equivalent drop in short-circuit current. This, however, has not been found for the normal mucosa, as shown schematically in Fig. 7 A. The short-circuit current changes very little, if acid secretion is initiated or increased. The same is true if the acid secretion is inhibited by thiocyanate. Hence, acid secretion at any rate, entails an equivalent and genuine increment in active chloride transport. This increment does not seem to be a direct effect of histamine. Histamine does not change the Cl output if acid secretion is inhibited by thiocyanate, which by itself does not affect the basal Cl transport.

The incremental or acidic chloride output is either due to an increased activity of the basal Cl pump, or derives from a mechanism other than the basal Cl pump. In the latter case, the basal Cl pump need not have any chemical function in acid secretion at all, whereas the accessory pump for acidic chloride might be linked with, or even identical to, the mechanism of H ion secretion.

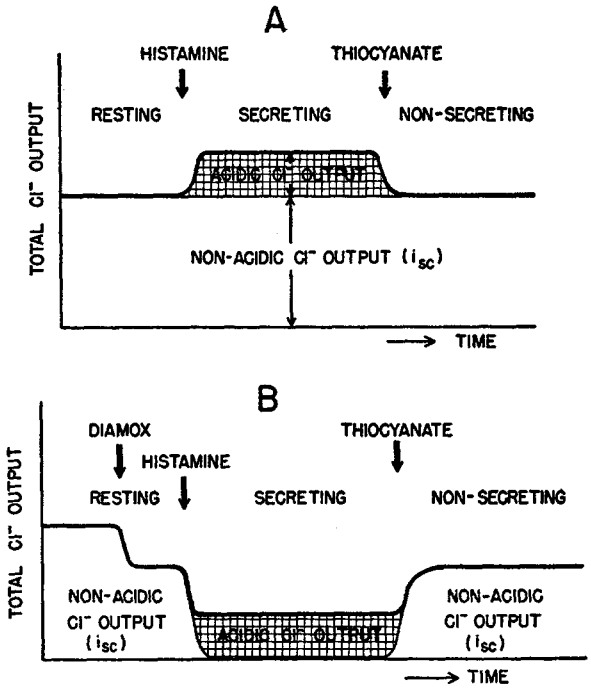


FIG. 7. Schematic diagram, showing the rates of acidic and non-acidic chloride outputs for the secreting and non-secreting mucosa. A, normal mucosae; B, mucosae under the influence of diamox.

The assumption, that acidic and non-acidic Cl output originate from different mechanisms, would be supported if these mechanisms could be separated from each other. It has already been mentioned that the basal Cl pump may be active without acid secretion, *e.g.* in the resting state, or under thiocyanate. On the other hand, diamox in combination with histamine has been found to reduce, or even abolish, the short-circuit current, while acid is secreted at an almost normal rate (Fig. 7 B). These findings show that acid secretion is possible in the absence of any measurable electromotive force. One may conclude that non-acidic chloride transport is not essential for acid secretion.

Absence of the electromotive force, however, does not necessarily indicate complete inhibition of the basal chloride pump. The actual extent of this inhibition is difficult to evaluate, in view of the fact that complete disappearance of the short-circuit current under diamox requires the presence of strong acid secretion. Since the acidic chloride under these conditions may be provided by the basal chloride pump, it cannot be decided whether this pump is essential for acid secretion or not.

While under diamox, in the presence of acid secretion, the inhibition of the basal chloride may not be complete, a partial inhibition certainly takes place. This follows from the finding that under the influence of diamox the acid secretion is always much smaller than the concomitant drop in short-circuit current. Likewise thiocyanate makes the short-circuit current rise much higher than would be equivalent to the corresponding drop in acid secretion. Fig. 7 B illustrates schematically these relationships, which indicate a real depression of the total, and hence of the basal, chloride output.

It seems that diamox bares a peculiar dependence of the basal chloride pump on the acid-producing mechanism, which does not appear under normal conditions. The nature of this interdependence cannot be defined at present. It suggests some kind of linkage between the two mechanisms involved by a common factor. To the extent that the effects of diamox result from an inhibition of the enzyme carbonic anhydrase, this common factor might be $CO_2$. In this connection, it is of interest to note the results of Teorell (6). Under conditions in which the bicarbonate ion concentration was negligible in the nutrient solution, he found appreciable acid secretion with an unusually small, spontaneous potential difference across the mucosa.

The effect of diamox, in combination with histamine, on the basic Cl pump is more specific and of a different nature, than is the corresponding effect of metabolic inhibition. Unlike metabolic inhibition, diamox and histamine leave acid secretion and also the Cl exchange diffusion largely intact. In view of this dissimilarity, the common factor for which basal Cl pump and acid-producing mechanism might compete is not likely to be metabolic energy.

In summary it may be stated that the described observations are consistent with the hypothesis that the E. M. F. is a manifestation of an active chloride pump. The question as to whether the chloride required for acid secretion is supplied by this pump or by a separate mechanism, *e.g.* directly by the mechanism for acid secretion, cannot be answered at present. The experiments, however, strongly support the view that the gastric E.M.F. and acid secretion involve different mechanisms. The acid secretion does not seem essential for the E.M.F., nor does the E.M.F. seem essential for the acid secretion. The failure of diamox to block acid secretion in our experiments speaks against an essential function of the enzyme carbonic anhydrase in acid secretion. On the other hand, if the effect of diamox on the electromo-

tive force is due to an inhibition of carbonic anhydrase, we may conclude that this enzyme has an essential function in active chloride transport.

*Note Added in Proof.*—More recent observations seem to verify that the effect of diamox on the E.M.F. is due to the inhibition of carbonic anhydrase. CL 8490, an $N^5$-methyl derivative of diamox, without appreciable activity as an inhibitor of carbonic anhydrase, was found to have no effect on the gastric E.M.F. On the other hand, two inhibitors of carbonic anhydrase, dichlorphenamide and chlorothiazide, depressed the E.M.F. of the secreting stomach, the first more strongly, the second less strongly, than diamox. The potency of the three inhibitors, dichlorphenamide, diamox, and chlorothiazide in depressing gastric E.M.F. seems to be proportional to their relative activities as inhibitors of carbonic anhydrase, as measured *in vitro*.

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