Anion Requirements for Gastric Acid Secretion

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ABSTRACT The rate of hydrochloric acid production by isolated, bullfrog gastric mucosae depends critically on the supply of chloride ion to the serosal surface. Secretion of acid is negligible if chloride is completely replaced by glucuronate and gluconate ion. The experimental evidence indicates that the rate of acid secretion may be regarded as a reaction velocity, depending on chloride concentration in a manner closely resembling Michaelis-Menten kinetics. Bromide and iodide ions substitute, in varying degree, for chloride as substrate. A familiar inhibitor of gastric acid production, thiocyanate ion, appears to act by competition with chloride in a reaction leading to the formation of acid. This reaction is included in a hypothetical reaction cycle, generalized from the redox model for gastric acid production. Under certain conditions, the model predicts a dependence of secretion rate on chloride supply of the Michaelis-Menten type, as was observed.

INTRODUCTION

Both H and Cl ions are transported against an electrochemical gradient in the stomach actively secreting acid, and only Cl ion in the resting stomach (1). In the absence of external Cl ion, Heinz and Durbin showed that considerable secretion of H ion can still occur, presumably by way of an H ion "pump" (2). In their experiments, performed on isolated gastric mucosae of Rana pipiens (leopard frog), sulfate was substituted for Cl in the bathing solutions. An electrical potential difference (PD) was measured, positive on the lumen surface with respect to the serosal surface, and therefore opposite in sign to the normal PD of the stomach. An external circuit supplied the electrical charge necessary to maintain electroneutrality during secretion of H ions. Hogben, however, failed to observe a reversed PD in gastric mucosae of Rana catesbeiana (bullfrog) under similar conditions (3). Since he did not measure concomitant acid secretion, there is a possibility that such results represent the behavior of resting mucosae.

The experiments reported here were also performed on bullfrog gastric mucosae, and were designed to investigate the discrepancy in the above results, and the general relationship between rate of acid secretion and supply of Cl ion. A preliminary report of part of the results has been made (4).
METHODS

The bullfrog gastric mucosa was freed from its external smooth muscle and mounted as a membrane, 2.85 cm² in geometrical area, between two lucite chambers. These contained 12 ml each of solutions of varying composition. The solution bathing the serosal (submucosal) surface, referred to as the nutrient solution, was bubbled continuously with 95 per cent O₂ + 5 per cent CO₂. That bathing the lumen surface of the stomach, the secretory solution, was bubbled with 100 per cent O₂.

The normal nutrient solution contained in mM, 87 NaCl, 18 NaHCO₃, 3 KCl, 1.8 CaCl₂, 1.0 NaH₂PO₄, 0.8 MgSO₄, and 11 glucose. Cl-free nutrient solution was made up by replacing NaCl by Na glucuronate (Nutritional Biochemicals Corp.), CaCl₂ by Ca gluconate (Matheson, Coleman, and Bell Co.), and KCl by K₂SO₄. The concentration of sulfate in this solution was kept minimal, since there is evidence that this ion is actively transported by gastric mucosa (3). Analysis by means of a chloridometer (Aminco-Cotlove) showed that the Cl-free solution contained less than 0.05 mEq/liter Cl. Nutrient solutions of intermediate Cl concentration were obtained by combining Cl and Cl-free solutions.

Histamine diphosphate at a concentration of 0.1 mM was included in all nutrient solutions as a stimulant of acid secretion.

In early experiments, the secretory solution was similar to the nutrient in composition, except for the omission of buffers. Its use sometimes led to a significant "background" rate of acid secretion, presumably due to bacterial contamination. In later experiments, a simplified secretory solution, consisting of 0.12 M NaCl, 0.12 M Na glucuronate, or a combination of these two solutions, was made up on the day of the experiment.

The formation of gastric acid was followed by maintaining the pH of the secretory solution constant during an experiment, at (or near) pH 6.5. A radiometer combined electrode (GK 2024 C) and titrator (TTT 1c) controlled the admission of 8 mM NaOH from a graduated 2 ml buret. In measuring secretory rate, the output of acid was integrated over an interval at least a few minutes in duration to reduce the effect of small errors in timing and measurement of titrant.

Despite the appreciable buffer capacity of the glucuronate secretory solution, control titrations with known amounts of HCl showed that the pH-stat method was as accurate for this solution as for the normal Cl secretory solution.

In making a change in Cl ion concentration, the chambers were filled 2 to 4 times (depending on the direction and magnitude of the change) over an interval of 5 to 15 minutes. After an additional period of 5 to 10 minutes, the rate of acid secretion usually reached an appropriate, steady-state level. Control measurements of acid secretion were made at the beginning and end of each experiment; about one in five mucosae was rejected because either initial or final control rate fell markedly below normal.

The potential difference developed by the mucosa was measured between two saline agar bridges, each less than a millimeter from the respective mucosal surface. These were connected through calomel reference electrodes (Beckman 39270) to an electrometer (Keithley 610 A) and recorder (Varian G-10). Two other agar bridges,
terminating 2.5 cm from the mucosa, were used when needed to deliver direct current from an external source. The amount of current necessary to bring the measured PD to zero yields the short-circuit current (i sc).

Precautions were taken in measuring PD or i sc to eliminate external, asymmetric diffusion potentials and other stray effects. The glass electrode was removed completely, and nutrient solution (aerated with 95 per cent O₂ + 5 per cent CO₂) instilled in both secretory and nutrient chambers. For this reason it was necessary to measure rate of acid secretion and electrical activity alternately rather than simultaneously.

![Figure 1](image_url)

**Figure 1.** The effect of removing Cl ion from nutrient (N) and secretory (S) solutions on the rate of acid production.

**RESULTS**

*Effects of Cl Removal*

The rate of acid secretion, stimulated maximally by histamine, was found to depend critically on Cl concentration in the solutions bathing the mucosa. The requirement is primarily for Cl ion in the nutrient solution, as illustrated in the experiment of Fig. 1. Removal of all Cl from the secretory solution depressed the rate of acid secretion to about 60 per cent of control, while removal of nutrient Cl reduced the secretory rate to less than 10 per cent of control.

In many other experiments, the use of Cl-free nutrient solution had a similar, profound inhibitory effect on acid secretion. The effect of Cl-free secretory solution was variable, however, and often negligible; that illustrated in Fig. 1 was maximal. In most cases in which removal of secretory Cl had no effect, the rate of acid production was high, and it is possible that the Cl
concentration within the lumen of the gastric glands was not greatly affected
by the change in the secretory solution.

The results of removing Cl simultaneously from both bathing solutions
are summarized in Table I. Under these circumstances, acid production is
essentially abolished. Normal electrical activity was also eliminated with the
appearance of a small PD and short-circuit current in the direction opposite
to that usually found. Since the PD was measured just before and after
the short-circuit current, an effective mucosal resistance may be calculated (final
column of Table I). The resistance was more than tripled by the replace-
ment of Cl with the larger anions.

<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFFECTS OF REMOVING Cl FROM BOTH BATHING SOLUTIONS (11 MUCOSAE)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Solutions</th>
<th>Secretory rate</th>
<th>Potential difference</th>
<th>i_{sc}</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Cl)</td>
<td>7.8±1.2 μEq H⁺/hr.</td>
<td>25±6 mV</td>
<td>220±80 μA</td>
<td>110 ohms</td>
</tr>
<tr>
<td>Chloride-free</td>
<td>0.1±0.2 μEq H⁺/hr.</td>
<td>-4±2 mV</td>
<td>-11±6 μA</td>
<td>360 ohms</td>
</tr>
</tbody>
</table>

Standard deviations given throughout.

Kinetic Dependence of Secretory Rate on Cl

Intermediate concentrations of Cl ion led to steady-state rates of acid produc-
tion, as shown in Fig. 2A. The Cl concentration in the two bathing solutions
was kept the same, except in the control measurements.

The plateau rates of acid secretion from Fig. 2A rise sharply with in-
creasing Cl concentration and then level off, in a manner resembling the
familiar Michaelis-Menten kinetics. The adequacy of this description may
be tested by the use of the reciprocal plot (cf. reference 5):

\[ \frac{1}{V} = \frac{1}{V_a} \left( 1 + \frac{K_{Cl}}{[Cl]_a} \right) \]  

Here V, the velocity of the enzyme-substrate reaction, is identified with the
rate of acid production; the substrate is taken to be the concentration of Cl
ion in the nutrient solution, so that Vₐ is the limiting rate as [Cl]ₐ becomes
very large, and K_{Cl} is a constant. Fig. 2B gives the plot, 1/V against 1/[Cl]ₐ,
for the data of Fig. 2A. From an empirical viewpoint, their fit to the straight
line appears to be satisfactory. A theoretical justification for the use of Equation 1 is given in a later section.

In another group of experiments, only the nutrient Cl concentration was
changed and Cl-free secretory solution used throughout. One of these is plotted in Fig. 3. In this particular experiment, the concentration of nutrient Cl was reduced to 1.25 mM, and the reciprocal plot exhibits the non-linearity usually found for $[\text{Cl}]_n < 2.5 \text{ mM}$ ($[\text{Cl}]_n^{-1} > 0.4$). The source of the non-

![Figure 2A](image)

**Figure 2A.** Effects of varying Cl concentration, in milliequivalents per liter, from zero to normal values. The open spaces correspond to intervals during which solutions were changed.

![Figure 2B](image)

**Figure 2B.** Plot of the reciprocals of the plateau rates from Fig. 2A against reciprocals of the corresponding, nutrient Cl concentrations.

linearity may be related to the observation that gastric mucosae exposed to Cl-free solutions for several hours still retain 2 to 3 milliequivalents of chloride per kilogram tissue (unpublished results).

Analysis of seven experiments in which nutrient and secretory concentrations of Cl were maintained equal, and seven in which the secretory con-
concentration was zero, showed no significant difference between the mean values of the parameters, $V_o$ and $K_{Cl}$, calculated for the two groups. Within each group, these values varied widely. The extreme values did not seem to be associated, however, with larger deviations of the reciprocal plot from non-linearity. For all fourteen experiments, $V_o$ varied from 5.6 to 10.5 μEq H+/hour, with the mean $\overline{V_o} = 7.8$; $K_{Cl}$ ranged from 3.1 to 14 mM, with $\overline{K_{Cl}} = 6.4$.

![Figure 3](image)

**Figure 3.** Plot of the reciprocals of secretion rate against reciprocals of corresponding, nutrient Cl concentration, with secretory Cl maintained at zero level. Nutrient Cl has been lowered below minimum of Fig. 2 to show non-linearity at low Cl values. $V_o$ and $K_{Cl}$ have been calculated from linear portion of curve, at larger values of nutrient Cl.

![Figure 4](image)

**Figure 4.** Utilization of bromide ion for acid production, compared with that of Cl in reciprocal plot. Open circles, Br; filled circles, Cl.

**Comparison of Br, I, and Cl**

It is known that anions other than Cl are actively transported by the stomach (6–9), and it would appear that the above kinetic approach might be useful in assessing their effectiveness in substituting for Cl. Because of the variations reported in the preceding section, it was necessary to make the comparison on the same mucosa.

Nutrient solutions containing Br or I were made up in the same way as normal Cl nutrient, and combined with Cl-free, glucuronate solution to obtain intermediate concentrations. The rate of acid production was measured...
for three different values of \([\text{Cl}]_n\), not in order. Just before or after a determination with Cl, the secretory rate was measured for the same value of \([\text{Br}]_n\) or \([\text{I}]_n\). Figs. 4 and 5 illustrate the results. For three mucosae, the ratio of \(K_{\text{Br}}/K_{\text{Cl}}\) was 0.77, 0.80, and 0.71; in three other mucosae, \(K_{\text{I}}/K_{\text{Cl}}\) was 2.4, 3.3, and 2.4. The results indicate that Br is best utilized by the acid-producing mechanism, followed by Cl and I in order. The reciprocal plot of data obtained with Br and I, however, exhibited considerably more deviation from linearity than those for experiments in which only Cl was used. In addition, the use of iodide ion occasionally led to some irreversible damage to the mucosa.

**Mode of Thiocyanate Action**

The halide ions, Br, Cl, and I may be classified as potentiating anions, i.e. as the concentration of the ion is increased, the rate of acid secretion is increased. Glucuronate and gluconate may be considered indifferent anions.
A well known example of an inhibiting anion is thiocyanate, which reduces acid secretion in increasing concentration (10).

Assuming the present (and previous) results demonstrate that Cl is an essential substrate in a chain of reactions leading to acid production, the possibility arises that SCN competes with Cl for a place in this sequence. Thus, considerable evidence indicates that SCN acts to block I uptake in the thyroid by competitive inhibition (11).

Normally, large concentrations of SCN have been used to inhibit gastric acid secretion. For example, Hogben found that even 25 mEq SCN/liter did not completely stop acid production by bullfrog mucosae in vitro (12). Experience in the present studies indicated that much smaller concentrations (0.25 to 1 mEq/liter) were effective at reduced Cl concentrations, as expected if SCN were to compete with Cl.

A given concentration of SCN in the nutrient solution was used with three different values of [Cl]. The rate of acid secretion was measured before and after, as well as in the presence of SCN, so that a sequence at a single Cl concentration required several hours and the experiment 6 to 8 hours. Cl-free secretory solution was used throughout.

As before, it is convenient to plot the results in reciprocal form, as illustrated in Fig. 6. The data with SCN fall on a straight line, with approximately the same intercept, \(1/V_0\), as the line corresponding to control measurements, but with an increased slope. This is formally the behavior expected for competitive inhibition in Michaelis-Menten kinetics (5). The equation for the reciprocal of reaction velocity, \(1/V\), in terms of reciprocal of substrate concentration, \(1/[Cl]\), becomes:

\[
\frac{1}{V} = \frac{1}{V_0} + \frac{K_{CL}}{V_0} \left(1 + \frac{[i]}{K_i} \right) \frac{1}{[Cl]_n}
\]

where \([i]\) is the concentration of inhibitor, SCN, and \(K_i\) a constant which can be obtained from the experimental plot of Equation 2.

In nine experiments, the values obtained for \(K_i\) ranged from 0.15 to 0.83 mm, with a mean of 0.45 mm. The large scatter observed is probably due, in part, to the dependence of this parameter on both control (Cl) and thiocyanate (Cl + SCN) data. Moreover, the rate of acid secretion in the presence of SCN tended to drift downward with time, suggesting that more than one site of action is ultimately involved.

A Kinetic Model

It is well known that the formal properties of Michaelis-Menten kinetics may apply to a system of reactions in which a catalyst enters (13). This must be the case in the present studies, since the actively transporting gastric mucosa is clearly much more complex than the single, well stirred compartment as-
assumed for the classical enzyme-substrate reaction. In what follows, a hypothetical reaction sequence is presented and its possible relationship to the gastric mucosa discussed.

The idealized system, or cycle of reactions, is shown in Fig. 7. The reactions take place at the two boundaries of an otherwise homogeneous phase representing a critical membrane of the actively transporting tissue. At the nutrient surface, Cl and a mobile constituent ("carrier") of the membrane, X, combine to form the complex, XCl. This diffuses through the membrane to the secretory boundary, where it reacts with the substance A to form the products, B, Y, and HCl. Y represents the inactive form of the carrier, having negligible affinity for Cl; like other forms of the carrier, it is assumed to be confined to the critical phase. The HCl formed diffuses preferentially through the surface S and thence into intracellular canaliculi. The inactive carrier Y diffuses from S to N and is restored to X by reaction with substance C at the nutrient boundary, and the cycle is ready to repeat.

A basically similar sequence has been an essential part of many models for gastric acid production, especially those of the redox type (14-16). In the latter, XCl is reduced at the secretory surface, with the concomitant oxidation of a substance containing an H atom (A in Fig. 7) and formation of free HCl. In the reaction, an electron is transferred from the H atom to the carrier, yielding the inactive (e.g., neutral) form Y. The electron may be accepted by oxygen (C in Fig. 7), regenerating active carrier. The OH or HCO₃ ion thus formed must be presumed to have little affinity, in comparison with Cl, for the carrier and therefore exchanges with Cl across the nutrient surface of the critical phase.

To define the system in more detail, some explicit assumptions need to be made: (a) The interior of the critical phase is homogeneous and nearly impermeable to ions of either sign. This assumption is consistent with the observed separation of H and OH ions by the secreting gastric mucosa. It does not exclude channels, permeable to ions in varying degree, adjacent to the critical phase. (b) All chemical reactions in the critical phase are assumed to take place at the two boundaries. These are taken to be of finite thicknesses, t₁ and t₂ (Fig. 7). If the kinetic equations are written for unit area of membrane, then t₁ and t₂ are also the respective reaction volumes. (c) The concentration of Cl ion at the nutrient (or secretory) surface of the critical phase is proportional to its concentration in the nutrient (or secretory) bathing solution. The concentrations at the internal surfaces are denoted by [Cl]ᵢ and [Cl]ᵢ, respectively. (d) The reactions involving substances A and C are irreversible. This assumption is made for mathematical simplicity. (e) The effect of electrical potential gradients can be neglected. This allows the same permeability coefficient to be used for diffusion in either direction across the critical phase. It may be noted that the transmucosal PD is nearly zero for
Figure 7. A kinetic model of HCl formation. The distance between vertical lines does not represent the thickness of the mucosa, but may instead be of the order of Angstroms. It is assumed to be divided into three homogeneous regions, the two boundaries of the thickness shown, and the intervening space. N denotes the nutrient surface of the critical phase; S, the secretory surface.

[C1]n between 0 and 10 mEq/liter in the secreting stomach, a range which encompasses most of the observed change in rate of acid secretion (unpublished results).

Kinetic equations may now be written for the steady-state formation and disappearance of the three forms of carrier, X, XCl, and Y, at the two boundaries of the critical phase.

\[
k_{1} t_{1} [C1]_{n} [X]_{n} = k_{-1} t_{1} [XCl]_{n} + k_{XCl} ([XCl]_{n} - [XCl]_{o})
\]

\[
k_{XCl} ([XCl]_{n} - [XCl]_{o}) + k_{2} t_{2} [C1]_{n} [X]_{n} = k_{-1} t_{2} [XCl]_{o} + k_{2} t_{2} [A]_{o} [XCl]_{o}
\]

\[
k_{-1} t_{2} [XCl]_{o} = k_{1} t_{2} [X]_{o} [C1]_{o} + k_{X} ([X]_{o} - [X]_{n})
\]

\[
k_{2} t_{2} [A]_{o} [XCl]_{o} = k_{Y} ([Y]_{o} - [Y]_{n})
\]

\[
k_{Y} ([Y]_{o} - [Y]_{n}) = k_{3} t_{3} [C]_{o} [Y]_{o}
\]

In the equations, \(k_{1}, k_{-1}, k_{2}, \) and \(k_{3}\) are rate coefficients with appropriate dimensions; \(k_{X}, k_{XCl}, \) and \(k_{Y}\) are permeability coefficients, with the usual dimension of length/time. The final equation in the sequence, for \([X]_{n}\), has been omitted, since it is the sum of the preceding five. Instead, the equation for the total concentration of carrier, \([X]_{n}\), may be written in terms of the average concentration of the various forms:

\[
[X]_{n} = \frac{1}{3} ([X]_{n} + [X]_{o}) + \frac{1}{3} ([XCl]_{n} + [XCl]_{o}) + \frac{1}{3} ([Y]_{n} + [Y]_{o})
\]
Equation (8) is strictly valid only if $t_1$ and $t_2$ are negligible compared to the thickness of the critical membrane; if this is not the case, the numerical factors will differ somewhat from one-half.

The six equations, (3) through (8), may be solved by the use of determinants for any of the unknowns on the right of Equation 8. In particular, the rate of acid production, $k_2 t_2 [A]_o [XCl]_o$, requires the solution for $[XCl]_o$. The calculation is tedious but straightforward. The final expression is not given here because of its length. It may be vastly simplified, however, by making the further condition that the critical phase be essentially impermeable to the free carrier, $X$. For example, if the complex $XCl$ is neutral, $X$ is charged and the condition represents an extension of assumption (a).

Terms containing $k_x$ as a factor in the complete solution can then be neglected, and the following equation is obtained:

$$\text{Secretory rate} = k_2 t_2 [A]_o [XCl]_o$$  \hspace{1cm} (9)

$$= \frac{a [Cl]_o^i}{\left(\beta + \frac{\gamma}{[Cl]_o^i}\right) [Cl]_o^i + \delta}$$  \hspace{1cm} (10)

where $\alpha$, $\beta$, $\gamma$, and $\delta$ are relatively complicated functions of the rate coefficients, permeability coefficients, substrate concentrations $[A]_o$ and $[C]_o$, and total carrier $[X]_o$. If none of these vary with external Cl concentration, $\alpha$, $\beta$, $\gamma$, and $\delta$ may be treated as constants. If the experimental conditions are such that $[Cl]_o^i$ is either maintained constant or equal to $[Cl]_o^i$, Equation 10 may be further simplified:

$$\text{Secretory rate} = \frac{a [Cl]_o^i}{[Cl]_o^i + b}$$  \hspace{1cm} (11)

Here $a$ and $b$ are constants obtained from the preceding constants. Equation 11 has the form of the Michaelis-Menten equation in which $V_o$ is replaced by $a$ and $K_{Cl}$ by $b$.

If $[Cl]_o^i$ can be maintained at a value of zero, Equation 11 predicts that the rate of acid production will also be zero. Alternatively, $[Cl]_o^i$ may be kept at a constant, non-zero value (as was presumably the case in the experiment illustrated in Fig. 1). Equation 10 then reduces to the form:

$$\text{Secretory rate} = \frac{c [Cl]_o^i}{[Cl]_o^i + d}$$  \hspace{1cm} (12)

Here $c$ and $d$ are constants obtained from $\alpha$, $\beta$, $\gamma$, $\delta$, and $[Cl]_o^i$. The form of Equation 12 is again that of the Michaelis-Menten equation.
DISCUSSION

The profound depression in both acid and current production by the stomach caused by the removal of Cl ion is not surprising, in view of the essential role of Cl transport in both phenomena. The effects of changes in Cl concentration in the present studies were rapidly and completely reversible. The observation that the rate of acid secretion is near maximal, with 10 to 20 mEq Cl/liter and 85 to 75 mEq glucuronate and gluconate/liter in the nutrient solution, insures that the inhibitory action is not due to the presence of the large anions, but rather to the absence of chloride.

Chloride ion, to be utilized for acid production, must be supplied to the gastric mucosa in the solution bathing the nutrient surface. The dependence of this rate, or reaction velocity, on the nutrient Cl concentration closely resembles Michaelis-Menten kinetics. An attempt to account for these two properties of the mucosa has led to the reaction cycle considered here.

In the cycle, generalized from the familiar redox scheme, Cl ion forms a neutral complex with an unknown substance in the transporting membrane. This part of the hypothesis is supported by other lines of evidence. Hogben found that the measured electrical conductance of the mucosa was considerably less than the value predicted from the unidirectional flux of tracer chloride, and concluded that the major part of this flux represented movement of a non-conducting, combined form of chloride (1). Heinz and Durbin observed potentiation of nutrient to secretory Cl flux by increased secretory Cl concentration ("transconcentration" effect), and interpreted this on the basis of a carrier model for Cl transport (17).

The reaction cycle considered in the present studies is a direct extension of the latter model. The principal changes are the explicit introduction of the reaction yielding HCl, and the reactions involving the inactive form of the carrier, Y. In their model, Heinz and Durbin also found that it was necessary to assume negligible permeability of free carrier, k_x, to fit the experimental results. A calculation of the nutrient to secretory Cl flux, k_x[Cl]_x, from Equation 3 through Equation 8 yields their expression for the transconcentration effect, provided k_x is set equal to zero.

The present results indicate that a second transconcentration effect, on rate of acid production, is present in the isolated gastric mucosa (Fig. 1). Calculations from the model indicate that both phenomena, acid production and nutrient to secretory Cl flux, should be affected in the same way by changes in [Cl]_. An appropriate test would have to be performed simultaneously on the secreting mucosa, and this has not yet been done.

The inhibition of PD and acid production due to Cl removal obtained in the present studies is in apparent conflict with other results previously reported. In the experiments of Heinz and Durbin (2) and Rehm et al. (18),
sulfate ion was used to replace Cl in isolated gastric mucosae of *Rana pipiens* and *Rana esculenta*. Under Cl-free conditions, the mean rate of acid secretion was 0.6 to 0.7 μEq/cm² hr., compared to an average control rate (with optimal oxygenation) of 3 to 5 μEq/cm² hr. Forte, Adams, and Davies found, using isethionate to substitute for Cl in *Rana catesbeiana*, average secretion rates of 1.0 μEq/cm² hr. in the absence of Cl, and 4.8 μEq/cm² hr. in control measurements (19). Thus, all three groups found appreciable secretion in Cl-free solutions and observed a reversed potential characteristic of active transport of H ions. All agree, however, that most of the H ion output is suppressed by substituting large anions for Cl. Their results, to this extent, are in qualitative agreement with the present findings. It seems likely that the variation in amount of depression reflects a species difference in frogs.

In terms of the model reaction cycle, the species difference may be interpreted as a difference in relative permeability of the critical phase to free carrier, X, and bound carrier, XCl. If free carrier can enter into the reaction with substrate A to form H ion, the total acid production will be given by the sum of the net fluxes of free and combined carrier. Under short-circuited conditions in the absence of Cl, only the net flux of free carrier remains, giving rise to a current equal to the rate of acid secretion (2, 18). The results would indicate that permeability to free carrier is somewhat larger in *Rana pipiens* than in *Rana catesbeiana*, although remaining in both cases much less than the permeability to bound carrier.

The net flux of free carrier must also depend on the orientation and size of the electrical PD across the gastric mucosa. In confirmation, Rehm et al. observed that clamping the secretory solution at an optimal negative potential with respect to the nutrient, increased acid production in Cl-free media by more than threefold (18).

While the interpretation of the present studies by the use of the reaction cycle is undeniably complex, it would seem difficult to simplify without sacrificing the asymmetrical properties of the postulated, secreting membrane. It is, unfortunately, impossible to deduce specific parameters for the model (e.g., k₋₁) from the measured values of Vₑ and KCl. Thus the present data are not sufficient to compare the affinity (k₁/k₋₁) of the carrier for Br, I, and Cl.

These studies suggest that the inhibitory action of SCN is by competition with Cl for free carrier. It is assumed that the complex thus formed, XSCN, does not participate in the reaction with A to yield acid. However, the introduction into the cycle of the further set of reactions of X with SCN leads to formidable difficulties in calculation. A more direct approach, preferably with an appropriate cell constituent, might better serve to test the hypothesized competition of Cl and SCN.
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