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THE EFFECT OF INHIBITORS OF HCl SECRETION ON THE UNIDIRECTIONAL FLUXES OF CHLORIDE ACROSS BULLFROG GASTRIC MUCOSA*

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SUMMARY

1. The unidirectional fluxes of Cl^- across isolated bullfrog gastric mucosa were measured under normal conditions and after inhibition of acid secretion. Inhibition was accomplished by deprivation of oxidative metabolism or by addition of SCN^-.

2. Removal of O_2 from the solutions bathing the gastric mucosa reduced the net Cl^- flux and short-circuit current, reversibly. Unidirectional Cl^- flux from nutrient to secretory solution ($J^{Cl^-}_{ns}$) was decreased, while the backflux, from secretory to nutrient solution ($J^{Cl^-}_{sn}$) was increased. A generalized increase in tissue permeability does not account for the observed rise of $J^{Cl^-}_{sn}$ since the total mucosal conductance was decreased during anoxia. Other explanations based on the accompanying decrease of bulk flow from nutrient to secretory solution as well as an alteration of carrier-site availability might explain the increased $J^{Cl^-}_{sn}$ which occurred with anoxia.

3. Addition of SCN^- to gastric mucosa depressed $J^{Cl^-}_{ns}$ and this agent appears to be relatively specific for the component of Cl^- flux directly associated with H^+ secretion. The exchange diffusion component of Cl^- flux is only slightly decreased by SCN^-.

4. The action of SCN^- in the reduction of HCl secretion is interpreted on the basis of a competition with Cl^- for enzymic sites which normally operate for the translocation of H^+ by an ATP-driven mechanism.

INTRODUCTION

The secretion of HCl by gastric mucosa occurs against a large electro-chemical gradient and is well-known to be an active transport process. HOGBEN[1] has shown that net Cl^- transport in the short-circuited gastric mucosa was equal to the short-circuit current of Cl^- *plus* the HCl secreted. Gastric secretion of HCl is inhibited by anoxia, 2,4-dinitrophenol, iodoacetate *etc.*, most likely as a result of interference with the metabolic energy supply[2–6]. Other agents, such as SCN^-, also reduce gastric HCl production and appear to be more specific for the secretory mechanism[7,8]. LEFEVRE, GOHMANN AND REHM[9] have demonstrated that SCN^-, CNO^-, NO_2^- or NH_4^+ decreased H^+ secretion and produced characteristically similar alterations in the electrical properties of gastric mucosa. They pointed out that these compounds

shared the common feature of a nitrogen atom with a pair of unshared electrons and proposed that this common feature was the basis for the inhibition of H^+ secretion (they assumed that NH_3 was the intracellularly active form of NH_4^+). In seeking to explain the mechanism of SCN^- inhibition of gastric secretion, DURBIN[10] showed that SCN^- appears to act by competing with Cl^- in a reaction leading to the formation of acid by frog gastric mucosa.

In the present experiments the unidirectional fluxes of Cl^- were measured in the *in vitro* bullfrog gastric mucosa before and after inhibition of the metabolic machinery, and after the addition of SCN^-. The observed changes in Cl^- flux, as effected by these inhibitors, are interpreted in terms of a metabolically dependent, enzyme-mediated transport of HCl.

METHODS AND MATERIALS

Bullfrog (*Rana catesbeiana*) gastric mucosae were isolated from the underlying muscle coat and suspended between two Lucite chambers previously described[11]. In most experiments both the nutrient and secretory surfaces of the mucosa were bathed by identical Ringer's solution having the following composition: 85.3 mM NaCl; 3.4 mM KCl; 1.8 mM $CaCl_2$; 0.9 mM KH_2PO_4; 0.9 mM $MgSO_4$; 17.6 mM $NaHCO_3$; and 0.2 % (w/v) glucose. In experiments where H^+ secretion was measured directly, the secretory solutions contained no added HCO_3^-. The Cl^--containing secretory solution was composed of 95 mM NaCl, 21 mM sodium isethionate and 2.5 mM K_2SO_4 whereas the Cl^--free secretory solution contained 116 mM sodium isethionate and 2.5 mM K_2SO_4. All solutions were continually bubbled with a gas phase of 5 % CO_2 with the gas balance made up of either O_2 or N_2.

The transmucosal electrical potential difference (P.D.) was measured with a recording potentiometer (Sargent MR recorder) *via* calomel electrodes. Agar-saline bridges placed in close juxtaposition to the mucosa (about 2 to 3 mm) made contact with the calomel half-cells by a saturated KCl liquid junction. Short-circuit current was manually applied and measured using a circuit similar to that of USSING AND ZERAHN[12]. Ag–AgCl electrodes were imbedded in agar–saline and inserted into the the ends of both chambers. The electrodes were connected in series with a battery for delivering current and a microameter for measuring the short-circuit current. Mucosal conductance was measured by passing a square wave of current (50 μA) and recording the steady state change in P.D. A correction for the resistance of the bathing solution between Agar bridges measuring P.D. was applied in the following way:

$$G = \frac{I}{\Delta \text{P.D.} - IR_s}$$

where G is the mucosal conductance in $m\Omega^{-1} \cdot cm^{-2}$, I is the applied current in $\mu A/cm^2$, ΔP.D. is the mV change in electrical potential and R_s the resistance of the solution between the agar bridges in $\Omega \cdot cm^2$.

In the more recent experiments an automatic voltage clamp device was constructed for application and measurement of the short-circuit current. The voltage clamp greatly facilitated the operations of the experiments. Details for the circuitry of this device are shown in Fig. 1.

The unidirectional fluxes of Cl^- were measured using radioactive ^{36}Cl. The

isotope (2–3 μC of a solution with specific activity of 11.9 mC/g Cl^-) was added to one of the chambers and the unidirectional flux measurements commenced after a 45-min-equilibration period. The amount of ^{36}Cl appearing on the opposite side was measured by completely removing the solution and counting an aliquot. That side was then quickly washed and replaced with fresh Ringer's solution for the flux measurement of the subsequent period. Small amounts of the solution to which the isotope was initially added were periodically removed and counted. A similar procedure was used for the ^{35}SCN flux experiments. The amounts of ^{36}Cl and/or ^{35}SCN in the various samples were measured using a Packard liquid scintillation spectrometer. There was sufficient separation in the energy levels of these two isotopic components to permit double labelling experiments.

A Radiometer pH-stat was used to measure the rate of H^+ secretion. The secretory side was maintained at pH 5.0–5.5 by the addition of 0.025 M NaOH to the

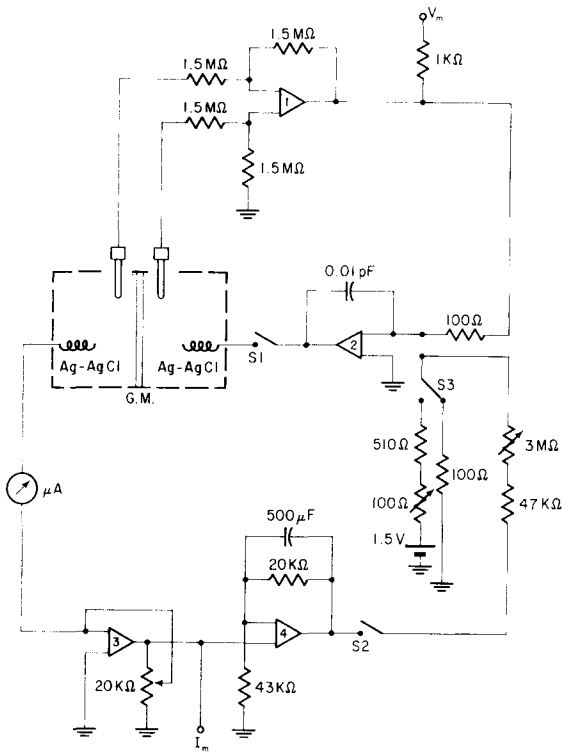

Fig. 1. Circuit diagram for automatic voltage clamp. Four Burr–Brown operational amplifiers are used: (1) a differential amplifier input; (2) an amplifier to provide current through a feed-back loop so that the input is maintained at the desired potential; (3) an amplification stage to provide a suitable signal for recording current at I_m; and (4) an additional feedback loop that is adjusted so that the input to amplifier No. 2 is corrected to account for the resistance of the Ringer's solution between P.D. measuring electrodes. This is done by adding to the input signal at amplifier No. 2 an E equal to I_m times the empirically measured resistance of the Ringer's solution. Switches are provided for the application of the current (S1), the inclusion of the correction stage for the solution resistance (S2), and for switching to a circuit containing a battery (S3) so that the P.D. across gastric mucosa may be maintained at any value. The Lucite chambers for mounting the gastric mucosa are indicated by dotted lines.

solution bathing that surface. Isotopic flux values were corrected to account for the amount of base added during titration.

RESULTS

When the gas phase of the Ringer's solution bathing the gastric mucosa was changed from O_2–CO_2 (95:5, v/v) to N_2–CO_2 (95:5, v/v) a decrease in the nutrient to secretory flux of the Cl^- ($J^{Cl^-}_{ns}$) and an increase in the secretory to nutrient Cl^- flux ($J^{Cl^-}_{sn}$) occurred. Short-circuit current fell to zero during anoxia and was followed

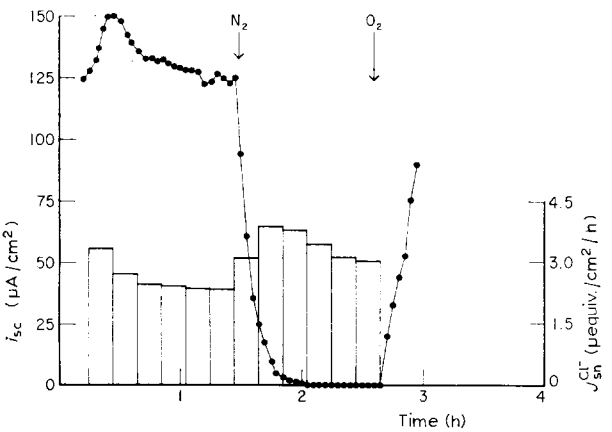

Fig. 2. Changes in short-circuit current, i_{sc}, and $J^{Cl^-}_{sn}$ after oxygen was removed from solutions bathing gastric mucosa.

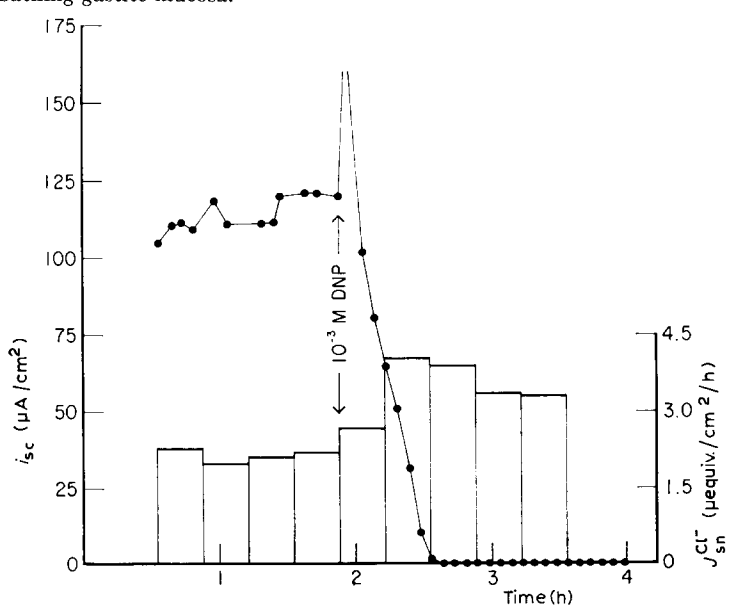

Fig. 3. Changes in short-circuit current, i_{sc}, and $J^{Cl^-}_{sn}$ after the addition of 2,4-dinitrophenol (DNP) to solutions bathing gastric mucosa. Shortly after the addition of dinitrophenol the short-circuit current abruptly increased to a value of about 175 $\mu A/cm^2$ and then decreased as shown in the figure.

rather closely by the net Cl^- flux. Reintroduction of O_2 into the system produced a recovery of the short-circuit current, and the unidirectional fluxes of Cl^- also returned to about their control levels. These experiments clearly show the influence of oxidative metabolism on active Cl^- transport.

The relative changes in the unidirectional flux components are of basic importance in the development of a molecular model of Cl^- transport. The increase in the backflux of Cl^- during anoxia and after the addition of 2,4-dinitrophenol are shown for individual mucosae in Figs. 2 and 3. The increase in passive flux was consistently observed and several possible explanations will be discussed later. Here it should be pointed out that the increase in passive Cl^- flux is not likely to be the result of a generalized increase in tissue permeability pursuant to the deprivation of oxidative metabolism. Several groups have shown that the d.c. electrical conductance of gastric mucosa was increased during hypoxia or complete anaerobiosis[13,14]. Such a change in conductance is shown in Fig. 4 where the voltage–current characteristics of a mucosal preparation were measured at varying times after O_2 was removed from the system. An increase in ionic permeability of the tissue would also have increased the electrical conductance. It is also significant that the partial ionic conductance of Cl^- during anoxia, calculated from the unidirectional Cl^- flux on the basis of the formulation described by HODGKIN[15], is always larger (by about 2-fold) than the

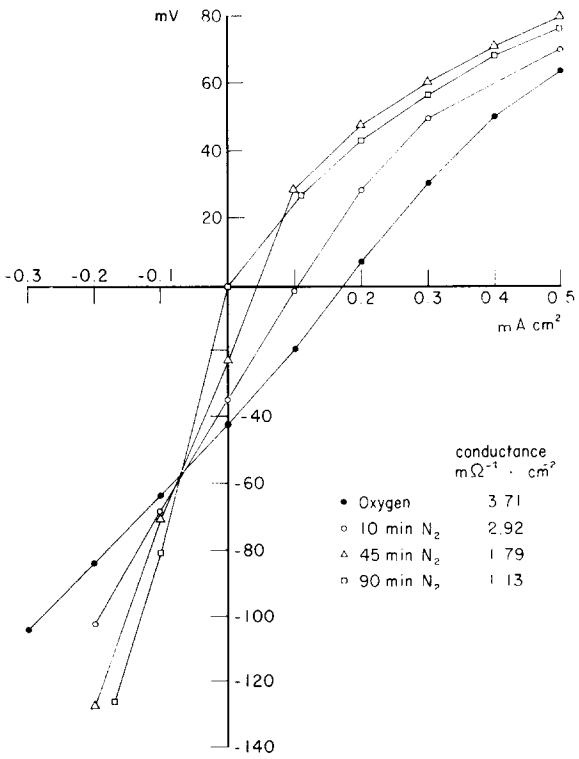

Fig. 4. Voltage–current characteristics for oxygenated gastric mucosa and for those maintained under anaerobic conditions for varying periods of time. The conductance values shown in the figure were obtained from the $\Delta I/\Delta V$ measured over the linear range of the curves at low current densities.

total measured conductance. This suggests, as HOGBEN[1] has pointed out for the oxygenated mucosa, that there is considerable carrier-mediated, or exchange, diffusion remaining during anaerobiosis. Thus the hypothetical Cl^- carrier is not grossly destroyed during anoxia.

The effects of 10 mM SCN^- on short-circuited mucosa are shown in Table II. Net Cl^- flux decreased while the short-circuit current usually increased. Even though the number of observations is limited, there appears to be very little net flux of SCN^- and thus this anion does not contribute significantly to the short-circuit current. H^+ secretion is ordinarily reduced to about 5–10 % of its orginal rate by 10 mM SCN^- and therefore net Cl^- flux under these conditions should more closely approximate the short-circuit current[8], as is demonstrated by the results in Table II. The subsequent removal of O_2 from the preparations produced a further decrease in the $J^{Cl^-}_{ns}$ as well as an increase in $J^{Cl^-}_{sn}$. The introduction of O_2 did not restore the unidirectional Cl^-

TABLE I

EFFECT OF NITROGEN ON Cl^- FLUX AND SHORT-CIRCUIT CURRENT IN BULLFROG GASTRIC MUCOSA

Unidirectional flux values are the mean ± S.E. of 3 individual experiments. All 6 of the values have been averaged for the short-circuit current, i_{sc}. Net Cl^- flux, $J^{Cl^-}_{net}$, was obtained by the difference of $J^{Cl^-}_{ns} - J^{Cl^-}_{sn}$. All flux periods are 20 min. Values are expressed as $\mu equiv \cdot cm^{-2} \cdot h^{-1}$.

Flux period	*Gas phase*	$J^{Cl^-}_{sn}$	$J^{Cl^-}_{sn}$	$J^{Cl^-}_{net}$	i_{sc}
1	O_2	10.6 ± 0.7	3.1 ± 0.3	7.5	2.9 ± 0.5
2	O_2	10.0 ± 0.5	2.7 ± 0.3	7.3	2.8 ± 0.5
3	O_2	9.2 ± 0.5	2.6 ± 0.3	6.6	2.6 ± 0.4
4	N_2	6.8 ± 0.4	3.3 ± 0.2	3.5	1.4 ± 0.2
5	N_2	4.3 ± 0.2	4.2 ± 0.4	0.1	0.2 ± 0.05
6	N_2	3.3 ± 0.1	3.8 ± 0.4	−0.5	0
7	O_2	3.4 ± 0.1	3.1 ± 0.5	0.3	0.6 ± 0.15
8	O_2	7.4 ± 0.6	3.3 ± 0.4	4.1	2.2 ± 0.15
9	O_2	10.1 ± 0.8	3.5 ± 0.3	6.6	2.6 ± 0.5

TABLE II

EFFECT OF SCN^- AND NITROGEN ON THE UNIDIRECTIONAL FLUXES OF Cl^- ACROSS BULLFROG GASTRIC MUCOSA

Cl^- and SCN^- flux values are the mean ± S.E. of 3 individual experiments. All 6 experiments have been averaged for the short-curcuit current, i_{sc}. All flux periods are 20 min. Values are expressed as $\mu equiv \cdot cm^{-2} \cdot h^{-1}$.

Flux period	*Gas phase*	*10 mM NaSCN*	$J^{Cl^-}_{ns}$	$J^{Cl^-}_{sn}$	$J^{Cl^-}_{net}$	i_{sc}	$J^{SCN^-}_{ns}$	$J^{SCN^-}_{sn}$
1	O_2		9.9 ± 1.1	2.9 ± 0.6	7.0	2.4 ± 0.3	—	—
2	O_2		9.7 ± 1.3	2.5 ± 0.6	7.2	2.3 ± 0.3	—	—
3	O_2	+	6.5 ± 0.8	2.7 ± 0.6	3.8	3.1 ± 0.4	—	—
4	O_2	+	6.6 ± 0.8	3.0 ± 0.6	3.6	3.7 ± 0.6	0.23	0.19
5	O_2	+	6.9 ± 1.1	3.0 ± 0.6	3.9	3.8 ± 0.5	0.28	0.19
6	N_2	+	6.4 ± 1.1	2.6 ± 0.9	3.8	2.3 ± 0.1	0.27	0.19
7	N_2	+	4.3 ± 0.8	3.5 ± 0.5	0.8	0.3 ± 0.03	0.20	0.21
8	N_2	+	3.1 ± 0.3	3.3 ± 0.5	−0.2	0	0.14	0.20
9	O_2	+	2.7 ± 0.2	2.8 ± 0.1	−0.1	0.3 ± 0.1	0.12	0.18
10	O_2	+	2.8 ± 0.5	2.7 ± 0.3	0.1	1.0 ± 0.1	0.12	0.18
11	O_2	+	3.5 ± 0.4	3.1 ± 0.2	0.4	1.2 ± 0.2	0.16	0.19

fluxes to their pre-anoxic level, even after 1 h incubation. Similarly, the short-circuit current developed to only a fraction of that observed prior to anoxia, although the current was considerably larger than net Cl^- flux. The source of this short-circuit is unknown. It is clear, however, that the presence of SCN^- prevented the rapid reversibility and recovery from anoxia that was observed above (*cf.* Table I).

In order to show that SCN^- is primarily effective in reducing the component of Cl^- flux associated with H^+ secretion, 2 groups of experiments were designed. In

TABLE III

EFFECT OF SCN^- ON THE FLUXES OF Cl^- ACROSS BULLFROG GASTRIC MUCOSA AFTER PREPARATIONS WERE MADE ANOXIC

Cl^- and SCN^- fluxes are the mean ± S.E. of 3 individual experiments. All 6 experiments have been averaged for the short-circuit current, i_{sc}. All flux periods are 20 min. Values are expressed as μequiv·cm^{-2}·h^{-1}.

Flux period	*Gas phase*	*10 mM NaSCN*	$J^{Cl^-}_{ns}$	$J^{Cl^-}_{sn}$	$J^{Cl^-}_{net}$	i_{sc}	$J^{SCN^-}_{ns}$	$J^{SCN^-}_{sn}$
1	O_2		8.8 ± 0.8	3.1 ± 0.2	5.7	2.5 ± 0.4		
2	O_2		8.4 ± 0.6	2.6 ± 0.4	5.8	2.6 ± 0.5		
3	O_2		8.2 ± 0.2	2.5 ± 0.3	5.7	2.6 ± 0.4		
4	N_2		6.2 ± 0.8	3.2 ± 0.1	3.0	1.5 ± 0.3		
5	N_2		4.1 ± 0.5	3.2 ± 0.1	0.9	0.4 ± 0.1		
6	N_2		2.9 ± 0.2	2.9 ± 0.2	0	0.1 ± 0.04		
7	N_2	+	2.3 ± 0.1	2.6 ± 0.2	−0.3	0*		
8	N_2	+	2.2 ± 0.2	2.5 ± 0.2	−0.3	0*	0.12	0.14
9	N_2	+	2.1 ± 0.2	2.4 ± 0.2	−0.3	0*	0.14	0.14

* One of the 6 mucosal preparations maintained a residual short-circuit current (2–3 μA/cm^2) even after 2 h in N_2.

TABLE IV

$J^{Cl^-}_{ns}$ AFTER REMOVING Cl^- FROM THE SECRETORY SOLUTION AND UPON THE ADDITION OF INCREASING CONCENTRATIONS OF SCN^- TO A BULLFROG GASTRIC MUCOSA

$J^{Cl^-}_{ns}$, qH^+ (rate of H^+ secretion), and $J^{SCN^-}_{ns}$ are given in μequiv·cm^{-2}·h^{-1}. Each flux period was 20 min.

Flux period	*Secretory solution*	*NaSCN in nutrient solution (mM)*	$J^{Cl^-}_{ns}$	qH^+	$J^{SCN^-}_{ns}$	*P.D. (mV)*
1	Cl^-		11.1	4.5		33
2	Cl^-		10.6	4.1		31
3	Cl^--free		6.7	3.9		36
4	Cl^--free		5.9	3.4		37
5	Cl^--free		5.8	3.3		38
6	Cl^--free	0.1	5.8	3.3	2.4×10^{-3}	38
7	Cl^--free	0.1	5.6	2.9	2.4×10^{-3}	40
8	Cl^--free	1.0	4.8	2.2	2.5×10^{-2}	48
9	Cl^--free	1.0	5.2	2.2	2.7×10^{-2}	48
10	Cl^--free	2.0	4.6	1.7	5.2×10^{-2}	52
11	Cl^--free	2.0	4.7	1.7	5.3×10^{-2}	53
12	Cl^--free	10.0	3.4	0.4	0.21	60
13	Cl^--free	10.0	3.0	0.3	0.19	59
14	Cl^-	10.0	6.0	0.2	0.32	42
15	Cl^-	10.0	7.0	0.2	0.33	41

the first group 10 mM SCN^- was added to gastric mucosae previously made anoxic and maintained in N_2–CO_2 (95:5, v/v) during the test period (Table III). There was a small and comparable reduction in both $J^{Cl^-}_{ns}$ and $J^{Cl^-}_{sn}$. Such an effect may have been predicted on the basis of a limited competition between SCN^- and Cl^- for an exchange carrier. The results shown in Table III may be contrasted with the changes induced by SCN^- in the oxygenated mucosa shown above.

In another experimental design $J^{Cl^-}_{ns}$, $J^{Cl^-}_{sn}$, and the rate of H^+ secretion were measured in mucosae left on open circuit as shown in Table IV. When Cl^- was completely replaced in the secretory solution by the non-permeating isethionate anion ($HOCH_2CH_2SO_3^-$) there was always observed a decrease in $J^{Cl^-}_{ns}$. This dependence of $J^{Cl^-}_{ns}$ on the trans-concentration of Cl^- has previously been noted[16] and it has been described as the exchange diffusion component of Cl^- flux[17]. Subsequent additions of SCN^- to the nutrient bathing solution up to 10 mM produced a decrease in both $J^{Cl^-}_{ns}$ and the H^+ secretory rate. Upon the introduction of the normal Cl^--containing secretory solution there occurred an increase in $J^{Cl^-}_{ns}$ and an increase in J^{SCN}_{ns} (but the latter was proportionately less). The magnitude of the exchange diffusion Cl^- flux component in the presence of 10 mM SCN^- was only partially reduced from that observed when the inhibitory anion was not present. In 6 similar experiments the exchange component of Cl^- flux, as measured by the decrease in $J^{Cl^-}_{ns}$ upon removing Cl^- from the secretory solution, was 4.2 $\pm$ 0.4 (S.E.) $\mu equiv \cdot cm^{-2} \cdot h^{-1}$. This is to be compared to the increment in $J^{Cl^-}_{ns}$ of 3.5 $\pm$ 0.3 $\mu equiv \cdot cm^{-2} \cdot h^{-1}$ which occurred in these tissues upon the replacement of the normal Cl^--containing secretory solution after 10 mM SCN^- had been added to the nutrient bathing solution.

These results suggest that SCN^- is only partially effective in the reduction of Cl^--exchange diffusion, or passive Cl^- flux, whereas it is a considerably more potent inhibitor of H^+ secretion and that component of $J^{Cl^-}_{ns}$ associated with H^+ secretion.

DISCUSSION

It is clear that the active transport of Cl^- by gastric mucosa is dependent upon oxidative metabolism. Net Cl^- flux and short-circuit current are reversibly abolished in the absence of oxygen.

HEINZ AND DURBIN[16] reported that for *Rana pipiens* gastric mucosa both $J^{Cl^-}_{ns}$ and $J^{Cl^-}_{sn}$ were reduced by 0.1 mM 2,4-dinitrophenol. In the present experiments an increase in $J^{Cl^-}_{sn}$ was always observed when oxidative metabolism was abolished or after the addition of dinitrophenol to the gastric mucosa. This apparent discrepancy may be resolved on the basis of the large differences in the secretory rates, since the preparations used by HEINZ AND DURBIN were transporting H^+ and Cl^- at very much lower rates than the bullfrog mucosae used in the present studies. COOPERSTEIN[18] noted an increase in $J^{Cl^-}_{sn}$ in gastric mucosa treated with 10 μM strophanthidin in order to reduce HCl secretion.

It was pointed out earlier that the increase in $J^{Cl^-}_{sn}$ which occurred upon deprivation of oxidative metabolism was not likely to be the result of a generalized increase in tissue permeability. Explanations have been offered to account for an increase in the backflux of a transported species involving the redistribution of carrier molecules which might occur as a result of metabolic inhibition. These characteristics are shown by the carrier model developed by ROSENBERG AND WILBRANDT[19]. The

experiments presented here do not rule out such a possibility to explain the observed changes in Cl^- flux.

However, a very simple explanation for the observed increase in $J^{Cl^-}_{ns}$ during anoxia is available on the basis of the concomitant changes in net solvent flow. The osmotic flow from nutrient to secretory side which normally accompanies HCl secretion may limit the diffusion of Cl^- from the bulk secretory solution, where stirring is vigorous, to the apical surface of the acid-secreting cells deep within the gastric glands. As secretion is reduced by anoxia, and net solvent flow is reduced, the effective concentration of $^{36}Cl^-$ at the ion-exchange site would be increased and the apparent $J^{Cl^-}_{sn}$ would similarly be increased. A formal treatment of this problem for gastric mucosa as well as for other tissues where bulk solvent flow occurs has been presented in preliminary form[20]. Where the initial secretory rates are very low, as in the experiments of HEINZ AND DURBIN[16] mentioned above, it would be expected that metabolic inhibitors would not produce an increase in observed $J^{Cl^-}_{sn}$. In accord with this explanation, a consistent increase in $J^{Cl^-}_{sn}$ was produced by a totally different type of inhibition, SCN^-, although the change was not so large as that observed with anoxia (Table II). Perhaps the competition by SCN^- for anionic carrier sites[10] is responsible for a general diminution of Cl^- flux.

There has been considerable interest to seek an explanation for the mechanism of SCN^- inhibition in the HCl secretory process. DURBIN[10] measured the rate of H^+ secretion as a function of Cl^- concentration in the nutrient solution and found that SCN^- was more effective in reducing H^+ secretion at lower concentrations of Cl^-. The kinetics for these reactions suggested a competition between SCN^- and Cl^- for a reaction leading to secretion of H^+.

From the present experiments it is clear that SCN^- is very effective in reducing the component of $J^{Cl^-}_{ns}$ that is associated with H^+ secretion, whereas the exchange diffusion of Cl^- is only slightly decreased by addition of SCN^- to the gastric mucosa. These results may be interpreted in terms of a competition between SCN^- and Cl^- for a primary carrier or ion-exchange site. The site would serve for both the metabolically linked Cl^- transport and the anionic exchange diffusion process. The following initial reactions are proposed:

$$[Cl^-] + [X]_m \rightleftharpoons [XCl^-]_m \quad (1)$$

$$[SCN^-] + [X]_m \rightleftharpoons [XSCN^-]_m \quad (2)$$

where the reactions involve the physicochemical association of the anions with a binding site, or carrier substance, within the membrane ($[X]_m$). A closely associated enzymic site, similar in principle to the asymmetric ATPase proposed by MITCHELL[21,22], could operate either by the specific release of protons to the secretory lumen or by the specific uptake of OH^- from that surface.

An ATPase has been shown in microsomes isolated from gastric mucosa by KASBEKAR AND DURBIN[23]. They found that the gastric microsomal ATPase was sensitive to SCN^- and that kinetics for the inhibition by this anion were not of the competitive type.

These results with isolated microsomes are still compatible with the proposed competition between Cl^- and SCN^- in the intact gastric tissue. It may well be that the $[XSCN^-]_m$ complex does not interfere with the binding of the ATPase substrate

to the enzyme involved with proton deposition but only with the velocity constant. However, from the reactions for a common binding site given above, the concentration of $[XSCN^-]_m$ is an inverse function of the concentration of Cl^- and the relative dissociation constants as shown in Eqn. 3.

$$[XSCN^-]_m = \frac{K_d{}^{XCl}}{K_d{}^{XSCN}} \cdot \frac{[SCN^-]\,[XCl^-]_m}{[Cl^-]} \qquad (3)$$

$K_d{}^{XCl}$ and $K_d{}^{XSCN}$ are the dissociation constants for the membrane-anionic complexes given above in Eqns. 1 and 2. Such a relationship indicates that in the intact gastric mucosa Cl^- and SCN^- might appear as competitive substrates in the production of H^+, and thus would be consistent both with DURBIN's results showing a competition between Cl^- and SCN^- in intact mucosal preparations[10] and the SCN^- inhibition kinetics observed using the gastric microsomal ATPase[23–25]. Further support for this interpretation is provided by the results of KASBEKAR AND DURBIN showing that Cl^- (100 mM) or HCO_3^- (20 mM) reduced the effective inhibition of SCN^- on the ATPase of frog gastric microsomes[23]. The protective effect of HCO_3^- has special significance in view of the recent work of IMAMURA[26] who has shown for intact gastric mucosa that the inhibitory effect of SCN^- on the rate of H^+ secretion and $J^{Cl^-}{}_{ns}$ was diminished at high partial pressures of CO_2. Further experiments investigating the interaction of Cl^-, HCO_3^- and SCN^- for a common site would be of interest.

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