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Effects of *p*-Chloromercuribenzene Sulfonic Acid on Na and Cl Transport by Isolated Gastric Mucosa[1] (40466)

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There is general agreement that binding of sulfhydryl groups in the gastric mucosa is followed by inhibition of acid secretion, but it is unclear if this is a specific, or isolated, ion transport effect of chloromercuribenzene compounds. The effects of thiol group binding on other active, or net, ion transport processes (for example, Na and Cl) are poorly understood. It has been assumed, primarily on the basis of red blood cell studies (1), that one of the more frequently used sulfhydryl binding agents, *p*-chloromercuribenzene sulfonic acid (*p*CMBS), penetrates cells slowly. Thus, the effects of this agent are believed to be related to the binding of superficially located thiol groups. However, as a result of an *in vivo* study showing that systemic absorption of *p*CMBS occurs from the fundus of canine stomach (2), it cannot be assumed that *p*CMBS reacts primarily with superficially located thiol groups of isolated gastric mucosa. The purpose of this study is to examine the effects of a free and an impermeable form of *p*-chloromercuribenzene on active ion transport processes across isolated gastric mucosa.

Methods. New Zealand white rabbits (2.5–3.0 kg) were sacrificed by giving pentobarbital intravenously. Either a segment of fundus or antrum was excised, stripped of serosa and muscularis and mounted in an Ussing type chamber. The mucosal and serosal sides of the tissue were bathed in Ringer's solution (3) bubbled with 100% O_2 and circulated by individual gas-life reservoirs kept at 38°. The mucosal and serosal bathing solutions were kept at pH 6.5 by the addition of phosphate buffer prior to the flux measurements.

Unidirectional fluxes of ^{24}Na, ^{22}Na, and ^{36}Cl were measured simultaneously across short-circuited mucosa as described previously (3). Unidirectional Cl fluxes were measured across adjacent segments of mucosa, whereas bidirectional Na fluxes were measured across each tissue exposed to *p*CMBS. The unidirectional Na fluxes and short-circuit current were averaged for each tissue pair and counted as one measurement. In additional experiments with dextran bound *p*-chloromercuribenzoate (see below), only one unidirectional Na flux (^{22}Na) was measured simultaneously with the same unidirectional Cl flux. The isotopic fluxes were measured before and after the addition of free or bound chloromercuribenzene compound, 0.5 m*M*, to the mucosal side of the tissue. Preliminary studies indicated that steady state isotopic fluxes were achieved within 10 min in the absence of *p*CMBS and within 30 min after the addition of *p*CMBS. The techniques for isotopic assay and measurement of short-circuit current, tissue electrical resistance, and acid secretion are those described previously (3).

Aminoethyl–dextran (AED) was prepared from dextran (molecular weight approximately 10,000) with 2-aminoethyl–hydrogen sulphate according to the method of Eldjarn and Jellum (4). The AED, however, was not washed after drying. Washing with water to free the AED of alkali was done in an ultrafiltration cell with UM-2 membrane (Amicon Corp.) as described by Simon and associates whose method was used to bind *p*-chloromercuribenzoate (*p*CMB) to AED (5). After the *p*CMB-AED mixture was further purified on a Sephadex G 25 column (50 × 2.5 cm, elution fluid = NaCl, 0.01 *M*) and subsequently concentrated to approximately 10 ml by ultrafiltration, the concentration of *p*CMB bound to AED was determined by UV spectrometry. The O.D. at 243 nm has been shown to be directly proportional to the concentration of *p*CMB bound to aminoethyl–

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dextran as determined by direct mercury analysis (5). The electrolyte content of the preparative solution containing *p*-CMB-AED was adjusted to match the Ringer's solution bathing the mucosa. The effect of *p*-CMB-AED on acid secretion was measured at pH 6.5 using a pH stat system (3). The *p*CMB-AED was used when freshly prepared in order to avoid loss of activity (5).

Results. pCMBS - Na and Cl Fluxes. Unidirectional and net Na and Cl flux measurements for control and *p*CMBS - treated fundic and antral tissues are shown in Table I. In the absence of *p*CMBS, there is, as observed previously (3, 6), net secretion of Na and Cl by both fundic and antral mucosa. There is a significant unmeasured ion flux (J^R net) for fundic mucosa, and this has been shown previously to be due to acid secretion (3). In contrast to fundic mucosa, the residual ion flux for antrum is negligible, since there is no active H or HCO_3 transport in the absence of exogenous HCO_3 (6). The unidirectional and net fluxes, short-circuit, current tissue electrical resistance and unmeasured ion flux of fundus and antrum do not change significantly over two 30 min intervals of measurement.

After the addition of *p*CMBS, net secretion of Na and Cl by fundic mucosa is no longer significant. Like its effect on fundus, *p*CMBS also inhibits net Na and Cl transport across antrum. However, the inhibition of Cl secretion across antral mucosa is less profound than that observed for fundic mucosa ($p < 0.001$). The changes in net Na and Cl fluxes across fundic and antral mucosa were due to decreases in the unidirectional serosal-to-mucosal fluxes; the opposite unidirectional fluxes did not change significantly. Associ-

TABLE I. EFFECTS OF *p*CMBS ON NA AND CL FLUXES ACROSS FUNDUS AND ANTRUM—LUMINAL pH 6.5.[a]

N	Agent Added	J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{net}	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{net}	I_{sc}	J^{R}_{net}	R
Fundus										
10	—	3.0	3.6	−0.6	4.4	8.0	−3.6	1.3	0.17	109.9
				±0.2			±0.4	±0.2	±0.5	±10.1
	—	3.3	3.8	−0.5	4.6	7.9	−3.3	1.1	−1.7	105.1
				±0.2			±0.5	±0.2	±0.4	±11.0
Δ		0.3	0.2	0.1	0.2	0.1	0.3	0.2	0.0	4.8
		±0.3	±0.1	±0.1	±0.3	±0.2	±0.2	±0.1	±0.2	±5.2
p		NS	NS	NS	NS	NS	NS	NS	NS	NS
20	—	3.9	4.6	−0.7	4.9	7.9	−3.0	0.7	−2.0	99.6
				±0.2			±0.5	±0.1	±0.4	±8.6
	*p*CMBS	3.6	3.7	0.1	4.5	4.7	−0.2	0.3	0.0	114.6
				±0.2			±0.1	±0.0	±0.1	±9.1
Δ		−0.3	−0.9	0.8	−0.4	3.2	2.8	0.4	−2.0	15.0
		±0.2	±0.3	±0.2	±0.4	±0.6	±0.4	±0.1	±0.3	±5.6
p		NS	<0.01	<0.001	NS	<0.001	<0.001	<0.001	<0.001	<0.025
Antrum										
10	—	2.7	4.0	−1.3	3.6	6.6	−3.0	1.8	0.1	148.5
				±0.1			±0.4	±0.1	±0.2	±15.2
	—	2.9	3.9	−1.0	3.8	6.5	−2.7	1.7	0.0	157.6
				±0.2			±0.3	±0.1	±0.3	±16.5
Δ		0.2	−0.1	0.3	0.2	−0.1	0.3	−0.1	−0.1	−9.1
		±0.1	±0.1	±0.2	±0.2	±0.2	±0.3	±0.1	±0.3	±10.2
p		NS	NS	NS	NS	NS	NS	NS	NS	NS
20	—	2.4	3.8	−1.4	3.2	5.6	−2.4	1.7	0.7	166.2
				±0.1			±0.3	±0.1	±0.3	±13.6
	*p*CMBS	2.1	2.5	−0.4	3.0	4.3	−1.3	0.9	0.0	208.7
				±0.2			±0.5	±0.1	±0.3	±18.7
Δ		−0.3	−1.3	1.0	−0.2	−1.3	1.1	−0.8	−0.7	42.5
		±0.1	±0.2	±0.2	±0.1	±0.2	±0.2	±0.1	±0.3	±7.9
p		<0.01	<0.001	<0.01	NS	<0.001	<0.01	<0.001	NS	<0.001

[a] *p*CMBS = 0.5 m*M* added to mucosal (m) but not serosal (s) sides. J_{ms} = unidirectional m-to-s flux. J_{sm} = unidirectional s-to-m flux. J_{net} = net flux = $J_{ms} - J_{sm}$. I_{sc} = short-circuit current. J^R_{net} = unmeasured ion flux = $I_{sc} - ({}^{Z}J^{Na}_{net} + {}^{Z}J^{Cl}_{net})$. Fluxes (J) and I_{sc} are given as mean or ± 1 SEM μEq/hr cm^2. R = tissue electrical resistance given as mean ± 1 SEM ohms cm^2. Δ = mean difference ± 1 SEM between the two flux periods. *p* determined from Student's *t* test for paired variates. NS = $p > 0.05$. Negative J_{net} = secretion.

ated with the fundic response to *p*CMBS is a decrease in the unmeasured ion flux to a negligible value, which is consistent with the inhibitory effect of *p*CMBS on acid secretion (7).

pCMB-AED - Na, Cl, and H Fluxes. The effects of *p*CMB bound to aminoethyl–dextran (*p*CMB-AED) on unidirectional Na and Cl fluxes are shown in Table II. These fluxes were measured in one direction across two tissues from the same rabbit with one tissue exposed to AED and the other exposed to *p*CMB-AED. Thus, the net fluxes for tissue pairs are not shown in Table II. Addition of AED alone does not affect significantly either unidirectional flux of Na and Cl or short-circuit current. *p*CMB bound to AED also does not alter significantly the unidirectional mucosal-to-serosal fluxes of Na or Cl. However, *p*CMB-AED causes significant decreases in the unidirectional serosal-to-mucosal fluxes of Na and Cl, which would result in a decrease in net secretion of both ions.

The effect of *p*CMB-AED on acid secretion by fundic mucosa is shown in Fig. 1. Aminoethyl–dextran by itself, does not significantly alter the rate of acid secretion, short-circuit current or tissue electrical resistance. The bound organomercurial agent, however, causes a significant decrease in the rate of basal acid secretion ($p < 0.001$), but this decrease appears to be less than that previously observed with the unbound agent (7).

The mucosal bathing solution of 3 of the tissues exposed to *p*CMB-AED shown in Fig. 1 was collected at the end of the experiment and subjected to ultrafiltration through an Amicon UM-2 membrane. The ultrafiltrate was then analyzed for activity of *p*CMB (5), and none was detectable. Analysis of the unfiltered medium, however, showed activity of *p*CMB, indicating the presence of *p*CMB bound to AED.

*p*CMB bound to AED also caused a statistically significant decrease in short-circuit current (<0.01) and an increase ($p < 0.01$) in tissue electrical resistance of antral mucosa, Fig. 2. These changes are similar to those observed for *p*CMBS (Table I).

The effect of *p*CMB-AED on both fundic (N = 6) and antral ($N = 4$) mucosa were reversed by the addition of cysteine (4 m*M*) or diothiothreitol (2 m*M*).

Discussion. The present data support the concept that oxidation of thiol groups accessible to *p*CMBS are primarily associated with active ion transport processes. Chloromercu-

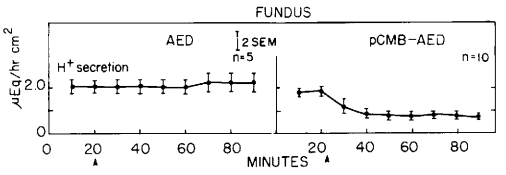

FIG. 1. Effects of aminoethyl–dextran (AED) and *p*-chloromercuribenzoate bound to aminoethyl–dextran (*p*CMB-AED) on acid secretion. AED and *p*CMB-AED were added after 20 min (arrows).

TABLE II. EFFECTS OF AED AND *p*CMB-AED ON Na AND Cl FLUXES ACROSS FUNDIC MUCOSA.[a]

Agent Added	N	J_{ms}^{Na}	J_{ms}^{Cl}	I_{sc}	R	N	J_{sm}^{Na}	J_{sm}^{Cl}	I_{sc}	R
—	7	2.1	4.6	1.1	116.7	8	2.4	8.5	1.3	120.1
		±0.3	±0.2	±0.2	±3.5		±0.2	±0.5	±0.2	±6.4
AED		2.1	4.7	0.9	120.8		2.7	8.3	1.2	123.1
		±0.2	±0.2	±0.2	±3.4		±0.3	±0.6	±0.2	±8.0
Δ		0.0	0.1	−0.2	4.1		0.3	−0.2	−0.1	3.0
		±0.3	±0.3	±0.1	±4.8		±0.3	±0.4	±0.1	±7.4
p		NS	NS	NS	NS		NS	NS	NS	NS
—	9	2.0	5.4	1.3	117.8	9	2.3	8.5	1.7	125.8
		±0.1	±0.4	±0.2	±4.6		±0.2	±0.7	±0.4	±10.5
*p*CMB-AED		2.1	5.1	0.8	138.2		1.8	6.8	1.2	146.6
		±0.2	±0.5	±0.1	±8.8		±0.3	±0.7	±0.2	±8.1
Δ		0.1	−0.3	−0.5	20.4		−0.5	−1.7	−0.5	20.8
		±0.2	±0.3	±0.1	±5.1		±0.2	±0.6	±0.1	±5.9
p		NS	NS	<0.01	<0.005		<0.05	<0.025	<0.01	<0.01

[a] AED-aminoethyl–dextran. *p*CMB-AED = *p*CMB bound to aminoethyl–dextran with concentration of bound pCMBS being 0.5 *mM*. *p*CMB-AED and AED were added to the muscoal (m) but not serosal (s) sides. J_{ms} = unidirectional m-to-s flux. J_{sm} = unidirectional s-to-m flux. Other abbreviations are the same as those in Table I. (Two of J_{ms} and 1 of the J_{sm} fluxes of AED treated tissues were technically unsatisfactory and account for the differences in number of experiments (N) between AED and *p*CMB-AED treated tissues).

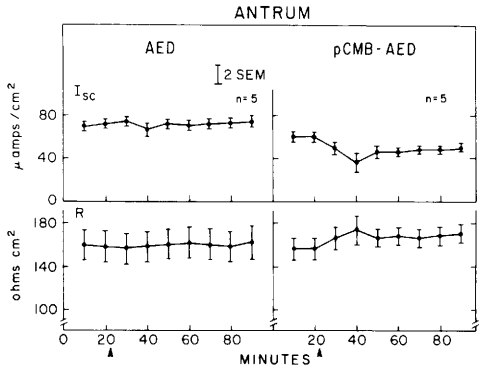

FIG. 2. Effects of aminoethyl-dextran (AED) and *p*-chloromercuribenzoate bound to aminoethyl-dextran (*p*CMB-AED) on short-circuit current (I_{sc}) and electrical resistance (R) of antral mucosa. AED and *p*CMB-AED were added after 20 min (arrows).

ribenzene compounds have been shown to inhibit basal as well as stimulated acid secretion (7–10). In addition to these effects on acid secretion, *p*CMBS also inhibits active Na and Cl transport by fundic mucosa. This action, however, is not restricted to parietal cell bearing mucosa, since inhibition of active Na and Cl transport by antral mucosa occurs as well. Although *p*CMBS inhibits active ion transport, this does not imply death of the tissue, since the effects of *p*CMBS have been shown to be reversible (7).

*p*CMBS is believed to penetrate cells slowly and thus react primarily with superficially located sulfhydryl groups. Even though the effects of *p*CMBS on the gastric mucosa have been shown to be readily reversible (7), this is not strong evidence that the agent reacts only with superficial thiol groups. Autoradiographic studies of canine stomach exposed to a concentration of *p*CMBS 20 times greater than that used in the present study show more or less diffuse penetration of the tissue and systemic absorption of the agent (2). In the present study, however, a presumably impermeable chloromercuribenzene compound (*p*CMB bound to aminoethyl-dextran) was found to have qualitatively similar effects to the free chloromercuribenzene compound. This suggests that in spite of the reported diffuse penetrability of a high concentration of *p*CMBS into canine gastric mucosa *in vivo*, active ion transport processes of isolated gastric mucosa are related to superficially located thiol groups. The quantitative differences between the bound and free agent may be related to penetrability of these compounds. *p*CMB bound to aminoethyl-dextran may not easily diffuse deeply into the lumina of the fundic or antral glands and thus only affect those cells associated with active transport that are closer to the necks of the glands.

Summary. The effects of the sulfhydryl reactive agent *p*-chloromercuribenzene sulfonic acid (*p*CMBS) were measured on isotopic Na and Cl fluxes across isolated fundic and antral mucosa. *p*CMBS inhibits active Na and Cl transport by both fundic and antral mucosa. Addition of *p*-chloromercuribenzoate bound to aminoethyl-dextran (with a mol wt of approximately 10,000) (*p*CMB-AED) decreases H secretion as well as net Na and Cl transport. Since the effects of a presumably impermeable chloromercuribenzene compound (*p*CMB-AED) are qualitatively similar to those of a free chloromercuribenzene compound (*p*CMBS), the active transport effects of *p*CMBS appear to be due primarily to the oxidation of superficially located thiol groups.

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