

Mechanisms of disposal of acid and alkali in rabbit duodenum

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FIDDIAN-GREEN, R. G., AND W. SILEN. *Mechanisms of disposal of acid and alkali in rabbit duodenum*. Am. J. Physiol. 229(6): 1641–1648. 1975.—Stripped duodenal mucosa of rabbits was mounted in Ussing chambers containing a Ringer solution gassed with 100% O₂. The disappearance of acid or alkali from the mucosal solution of short-circuited tissue was measured with a pH stat while the serosal pH was kept at 7.4. The duodenum rapidly disposed of both acid and alkali; neither property was altered by gassing with N₂ while iodoacetate was in the perfusing solutions. Prevention of release of CO₂ from the mucosal chamber obliterated the early rapid phase of acid disposal by the mucosa while a similar maneuver in the serosal chamber increased the appearance of serosal acid without altering the rate of acid disposal. Gut sacs of rabbit duodenum in vitro and in vivo showed a positive correlation between acid disposal and the rate of luminal CO₂ production. While acid disposal progressively decreased with time for the in vitro gut sacs, the in vivo gut sac showed no fatigue in this respect. Luminal acidification in the Ussing chamber was associated with a profound reduction in short-circuit current (*I*_{sc}), partially reversible by elevation of the mucosal pH but not by luminal glucose. Our data suggest that acid disposal occurs in part by intraluminal neutralization and in part by diffusion into the mucosa.

intestinal mucosa; bicarbonate secretion; hydrogen backdiffusion

DUODENAL MUCOSA can dispose of acid to which it is exposed in the absence of alkaline pancreatic and biliary secretions (3, 14, 15, 17, 21, 23, 26, 27, 30, 36, 40, 45, 46). Most of the acid appears to be neutralized by sodium bicarbonate secreted by the duodenal mucosa (3, 15, 26, 27), but some of the acid may diffuse across the mucosa in exchange for diffusible sodium (15, 23, 27). Jejunal mucosa can also dispose of acid, but in addition, disposes of alkali by absorbing sodium bicarbonate (40).

The purpose of this study was to examine further the mechanisms by which the duodenal mucosa can dispose of acid and alkali.

METHODS

All of these studies have been performed on duodenal mucosa obtained from white New Zealand rabbits weighing between 2 and 4 kg.

Ussing chamber experiments. The rabbit was sacrificed by a blow on the neck. The proximal 3 cm of duodenum were immediately excised, stripped of adherent muscularis, and mounted as a mucosal sheet between the two halves of a

Lucite chamber. The Lucite chamber was connected to an Ussing chamber so that each side of the mucosal sheet was perfused with identical separate solutions of isotonic, bicarbonate-free Ringer solution oxygenated and circulated by a humidified stream of 100% O₂. The composition of this solution was: Na⁺ 136, K⁺ 5, Ca⁺⁺ 3.6, Mg⁺⁺ 1.2, Cl⁻ 145.8. No nutrients were added to either the mucosal or the serosal solutions, and both the solutions were maintained at 37°C by a water jacket which encased the Ussing chamber. The pH of the mucosal and serosal solutions was adjusted by the addition of 10 or 50 mM HCl or NaOH and then maintained by automatic titration at values described below with two separate pH stats (Radiometer, Copenhagen). The rates of titration were recorded on the Titrigraphs attached to each of the pH-stats (Radiometer, Copenhagen), and these provided a continuous record of the rates of acid loss or acid gain from each side of the mucosal sheet.

Preliminary studies were first conducted in which an inert plastic sheet was substituted for the duodenal mucosal sheet in the Lucite chamber. These experiments revealed that 1 h was required before a steady state was established in which only small, negligible constant rates of titration were necessary to maintain a constant pH. For that reason, in all subsequent experiments both the mucosal and serosal solutions were gassed and circulated with 100% O₂ in the absence of the tissue for 1 h prior to beginning the experiment by connecting the inlet and outlet tubes with each other on each side of the Ussing chamber.

In each experiment, the potential difference (PD) across the tissue was measured on a digital voltmeter (Keithley Instruments, Inc.) using salt agar bridges and calomel electrodes. The tissue resistance (*R*) was calculated in ohms times centimeter squared from the magnitude of deflection of the PD which occurred when a standard current of 100 μA in excess of the short-circuit current (*I*_{sc}) was passed across the tissue. The standard current was passed as a pulse through a second set of silver-silver chloride electrodes so that the tissue was exposed to the current for 2–3 s while the measurements were recorded. The measurement of tissue resistance was corrected for artifact produced by the fluid resistance of the system (16). The measurements of PD and *R* were used to calculate the *I*_{sc}, since the anameter was insufficiently sensitive to measure an *I*_{sc} of less than 50 μA. All measurements of acid disposal were conducted while the tissue was in the short-circuited state (43). The alkali experiments were not conducted in the

short-circuited state because the PD always fell to zero. The alkali disposal was not changed by altering the transmucosal PD.

Measurement of disposal of acid from mucosal solution in vitro. The rate of acid disposal by the mucosa represented that amount of HCl added to the mucosal solution to maintain the pH at 2.5 in four successive 30-min periods during which the serosal pH was kept at pH 7.4 by the addition of NaOH with a pH stat. The quantity of NaOH added to the serosal solution provided a measure of the appearance of acid in this chamber. Measurements of I_{sc} and R were recorded at the midpoint of each of these four successive periods. The pH of 2.5 was selected because the duodenal bulb in man is frequently exposed to this pH (32, 33).

This basic experimental pattern was modified in three ways: 1) the effects of simultaneous inhibition of both aerobic and anaerobic metabolism were tested in the system described above by gassing both sides of the mucosa with 100% nitrogen while 5 mM iodoacetate was present in both the mucosal and serosal solutions. 2) To ascertain whether disappearance of luminal acid is influenced by exogenous bicarbonate or by CO_2 , measurements of acid disposal under control conditions (see above) were compared with those obtained when a bicarbonate serosal solution (Na^+ 136, K^+ 5, Ca^{++} 3.6, Mg^{++} 1.2, HCO_3^- 25, Cl^- 120.8) was gassed with 5% CO_2 ·95% O_2 while the mucosal solution and gas remained the same as control. 3) The possibility that there might be an obligatory exchange diffusion between H^+ and Na^+ (15) was examined by comparing the rate of acid disposal from oxygenated tissue with the rate of acid disposal from oxygenated tissue in which the sodium of the serosal solution was substituted with choline (choline 136, K^+ 5, Ca^{++} 3.6, Mg^{++} 1.2, Cl^- 145.8).

The relationship between the rate of acid disposal and the hydrogen ion concentration within the mucosal solution was determined by measuring the rate of acid disposal when the mucosal pH was maintained successively at pH 7.4, 6.0, 5.0, 4.0, 3.5, 3.0, and pH 2.5 while the serosal pH was kept at pH 7.4.

Gut-sac experiments—relationship between rate of acid disposal and rate of intraluminal CO_2 production. The dependence of acid disposal on the secretion of bicarbonate was next examined by comparing the rate of intraluminal CO_2 production with the rate of acid disposal. In order to measure the intraluminal CO_2 production, a different experimental model was designed.

Immediately after sacrifice of the rabbit, the proximal 10 cm of the duodenum were dissected free from adherent pancreas and excised. A cannula, with attached stopcock, was tied into one end of this isolated duodenal loop. After rinsing the loop with the control Ringer solution, the other end of the loop was ligated so as to form a gut sac. A test solution identical to the control solution except that the pH was adjusted to pH 2.5 was then instilled in a volume sufficient to fill but not to distend the gut sac. The gut sac was immersed into a flask containing the bicarbonate-free control Ringer solution adjusted to pH 7.2 by the addition of 5 mM Tris buffer. This solution was maintained at 37°C in a water bath and gassed with 100% O_2 . Thirty minutes later the contents of this sac were aspirated into an air-

tight syringe which was immediately sealed. The gut sac was then reimmersed into the flask for a further incubation. The pH and P_{CO_2} of the luminal contents were measured (Radiometer, Copenhagen). Six or seven successive 30-min incubations were performed in each of four in vitro gut-sac experiments.

A single experiment was performed on a live rabbit. After sedation with 40 mg diazepam intravenously, the abdominal wall was infiltrated with 1% lignocaine and the abdomen opened. The 10-cm portion of duodenum immediately distal to the entrance of the pancreatic duct was isolated and a gut sac made in the same manner as described above, but taking care to preserve the blood supply to that segment of duodenum. The experiment was performed in the same way as the in vitro experiments and using the same solutions. The loop of the duodenum with intact blood supply was returned to the peritoneal cavity of the anesthetized animal between manipulations. After three successive 30-min incubations, the duration of the incubation was reduced from 30 to 10, 5, 2 min and finally to 35 s. Thereafter, one further 30-min incubation was performed. Finally, a standardized pH electrode was secured into the lumen opposite the infusion site. An identical volume of the test solution was then injected rapidly into the gut sac and the pH changes were recorded at 2-s intervals. Throughout this procedure, which was repeated several times, the pH electrode was continually agitated to insure adequate exposure to the luminal contents.

In vitro studies of relationship between acid disposal and CO_2 . The following set of experiments was performed to ascertain whether neutralization contributed to the disposal of acid from the lumen of the duodenum. The contribution which neutralization might have upon the capacity to dispose of acid was inhibited by preventing the release of CO_2 from the mucosal solution. After preliminary oxygenation of both the mucosal and serosal solutions with 100% O_2 , the gassing of the mucosal solution was discontinued to discourage escape of CO_2 while circulation through this chamber was maintained with a Holter roller pump. The serosal solution was continually circulated by bubbling with 100% O_2 , a procedure adequate to maintain viability of the tissue (25). The serosal solution was maintained at pH 7.4 and the mucosal solution at pH 6.0, a value much closer than pH 2.5 to the pK_a of HCO_3^- (6.1) and consequently less conducive to the spontaneous release of CO_2 . As an additional precaution against loss of CO_2 from the chamber, the surface of the mucosal solution was covered with liquid paraffin after the pH electrode and pipette had been placed in situ. The latter maneuver caused such problems with cleaning of the apparatus at the conclusion of the experiment that the experiment was not repeated. The results of this single study were compared with those of a separate set of experiments in which the rate of acid disposal was measured from a gassed mucosal solution which was maintained at pH 6.

A complementary set of experiments was designed to ascertain whether any of the acid which left the lumen might be neutralized by the bicarbonate contained within the tissue. In these experiments, the relationship between the rate of acid disposal and the release of CO_2 into the "serosal" solution was examined. The mucosal pH was maintained

at pH 2.5 and the mucosal solution gassed with 100% O₂. The serosal chamber was preoxygenated and circulated by means of a Holter roller pump. The pH of the serosal solution was kept at 7.4, and thus much less likely to release of CO₂ than at pH 6.0 or 2.5. For this reason, the need to take additional precautions to prevent the spontaneous release of CO₂ from the solution was reduced, and the serosal chamber was merely sealed with parafilm. At the conclusion of these experiments, the serosal chamber was gassed with 100% O₂ after connecting the inlet and outlet tubes of the Lucite chamber to each other, thus excluding the tissue from the system. HCl was titrated into the serosal chamber to maintain the pH at 7.4 as CO₂ was released into the atmosphere when the gassing with 100% O₂ was begun after removal of the parafilm. The magnitude of this titration provided an indication of the contribution by a gaseous acid to the appearance of acid within the serosal solution.

Measurement of disposal of alkali from mucosal solution in vitro. The ability of the duodenal mucosa to dispose of alkali was examined by maintaining the mucosal pH at 9 while the serosal pH was kept at 7.4. The metabolic dependence of this property was tested by measuring the rate of alkali disposal while both aerobic and anaerobic metabolism was inhibited with the combination of 100% nitrogen and 5 mM iodoacetate. The possibility of an obligatory exchange of H⁺ and Na⁺ was again examined by comparing the rate of alkali disposal from oxygenated tissue with the rate of alkali disposal from oxygenated tissue in which the sodium in the mucosal solution was replaced with choline.

Influence of pH and Na⁺ gradient on I_{sc} in vitro. The I_{sc} was determined at the middle of each of the four successive 30-min periods during which the mucosal solution was maintained at pH 2.5. The results of these experiments were compared with the results of another set of experiments in which the hydrogen concentration gradient was reversed by maintaining the mucosal pH at 7.4 instead of at pH 2.5, and the serosal pH at 2.5 instead of pH 7.4.

The influence of a sodium gradient on the I_{sc} was examined by mounting adjacent pieces of duodenal mucosa from the same rabbit in two separate Ussing chambers. In these experiments, both sides of the mucosa were maintained at pH 7.4, and the I_{sc} was recorded at intervals of 10–15 min. After a basal period, the serosal solution in one chamber was replaced with a solution in which all the sodium had been substituted with choline. At the same time, the mucosal solution of the other Ussing chamber was replaced with the same sodium-free choline-substituted solution. One hour after the substitution with sodium-free solution, both tissues were rendered anoxic by gassing with 100% nitrogen.

In several experiments the changes in PD were recorded when the mucosal pH was raised to 7.4 after the tissue had been exposed to pH 2.5 for a long period. In one experiment the influence of sequential lowering of mucosal pH on I_{sc} was examined after replacement of the serosal solution with the sodium-free choline-substituted solution.

RESULTS

Disposal of acid in vitro. The rabbit duodenum disposed of acid in the mucosal solution at an initial rapid rate which

decreased exponentially as the experiment progressed (Figs. 1 and 5). After 1 h in the chamber, however, the rate of acid loss was related to the [H⁺] within the mucosal solution (Fig. 2). The capacity to dispose of acid was not diminished by inhibiting tissue metabolism and was not enhanced by supplying bicarbonate and CO₂ to the serosal solution, nor was it changed when sodium in the serosal solution was replaced with choline (Table 1).

Only a very small proportion of the acid which left the mucosal solution at pH 2.5 appeared as H⁺ within the serosal solution (Fig. 1). There was no correlation between the rate at which acid disappeared from the mucosal solution and the rate at which it entered the serosal solution, even during the third and fourth 30-min periods (mucosal acid loss during 3rd and 4th periods = 0.13 serosal acid gain + 6.89; $n = 23$, $r = 0.04$).

Gut-sac experiments—relationship between acid disposal and CO₂ production. These experiments were done to study the dependence of acid disposal on the secretion of bicarbonate. There was a positive and significant correlation between the rate of acid disposal and the rate of CO₂ production in gut sacs both in vitro and in vivo (Table 2). The highest rate of acid disposal was associated with the highest rate of CO₂ production in both the in vitro and the in vivo experiments. In vitro the rate of acid disposal decreased with each successive 30-min exposure, whereas in vivo the ability to dispose of acid was not only greater but was also not

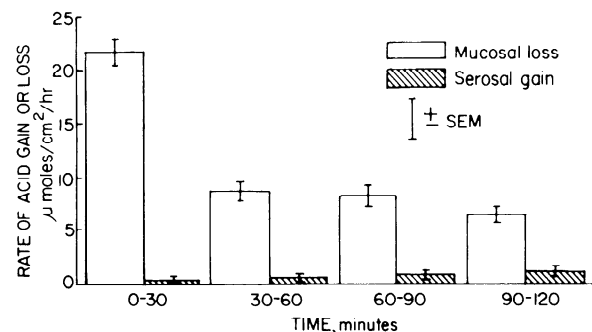


FIG. 1. Disposal of acid from mucosal solution and appearance in serosal solution (Ussing chambers). Rate of acid loss from mucosal solution maintained at pH 2.5, and rate of acid gain within serosal solution maintained at pH 7.4 while both sides were gassed with 100% O₂.

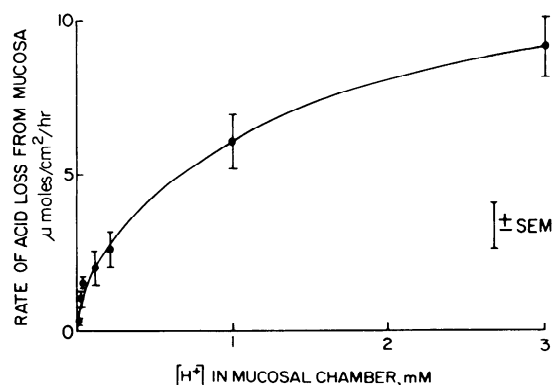


FIG. 2. Relationship between [H⁺] in mucosal chamber (mM calculated from pH) and rate of acid disposal from mucosal chamber. All readings depicted in this figure were obtained beginning 1 h after tissue had been introduced into system.

TABLE 1. Rate of acid loss from mucosal solution maintained at pH 2.5 while serosal solution was maintained at pH 7.4

	No. of Tissues	Acid Loss, $\mu\text{mol}/\text{cm}^2$ per h			
		0-30 min	30-60 min	60-90 min	90-120 min
Standard solutions and 100% O_2 on both sides	16	21.7 ± 1.2	8.7 $\pm .9$	8.2 $\pm .8$	5.7 $\pm .5$
N_2 and 5 mM iodoacetate on both sides	6	25.0 ± 1.5	5.5 $\pm .7$	6.7 ± 1.2	5.2 $\pm .6$
100% O_2 and standard Ringer in mucosal solution; 5% CO_2 -95% O_2 and 25 mM HCO_3^- in serosal solution	2	19.6	7.4	6.6	5.2
Na^+ -free choline-substituted serosal solution and standard mucosal solution; 100% O_2 on both sides	4	17.7 ± 2.0	7.2 ± 1.3	5.0 ± 1.6	5.3 ± 1.3

The rates were measured in four successive 30-min periods and are expressed as the mean acid loss \pm SE. The rates of acid loss during a given time period did not differ significantly from each other, regardless of the experimental circumstances.

TABLE 2. Correlation between rate of CO_2 production and rate of acid loss

Experimental Situation	Regression Equation	n	r	P
In vitro	$\text{PCO}_2 = 24.8 \text{ pH} - 25$	6	.99	<.001
	$\text{PCO}_2 = 32.6 \text{ pH} - 24$	6	.87	<.05
	$\text{PCO}_2 = 15.1 \text{ pH} + 29$	7	.95	<.001
	$\text{PCO}_2 = 23.1 \text{ pH} + 14$	7	.96	<.001
In vivo	$\text{CO}_2 = 18.3 \text{ pH} - 119$	8	.998	<.001

Rate of CO_2 production is expressed as the rate of rise of PCO_2 in 30 min, and the rate of acid loss is expressed as the rate of rise of pH from pH 2.5 in pH U/30 min. The results from each of the four in vitro and the single in vivo experiments are depicted.

fatigued despite eight successive incubations with acid (Fig. 3). After each 30-min incubation in vivo, the intraluminal pH was always pH 8. The disposal of acid invariably occurred so rapidly in vivo (Fig. 4) that a pH change could only be observed when the pH electrode was placed within 2 cm of the acid infusion site. Whenever acid was instilled into a gut sac, there was a profuse discharge of opalescent fluid into the lumen.

In vitro studies of relationship between acid disposal and CO_2 production. When CO_2 was prevented from leaving the "mucosal" solution, the early rapid rate of acid disposal was abolished, and the rate of acid loss approximated that seen when the gassed tissue had been allowed to equilibrate with its new environment (Fig. 5). On the other hand, when CO_2 was prevented from leaving the serosal solution, the rate at which acid left the mucosal solution was not inhibited or different from normal, but the rate at which the acid appeared within the serosal solution was significantly increased (Fig. 6). The enhanced rate of acid appearance within the serosal solution accounted for almost 70% of the acid lost from the mucosal solution during the third and fourth 30-min periods. Moreover, there was a significant positive correlation between the rate at which the acid

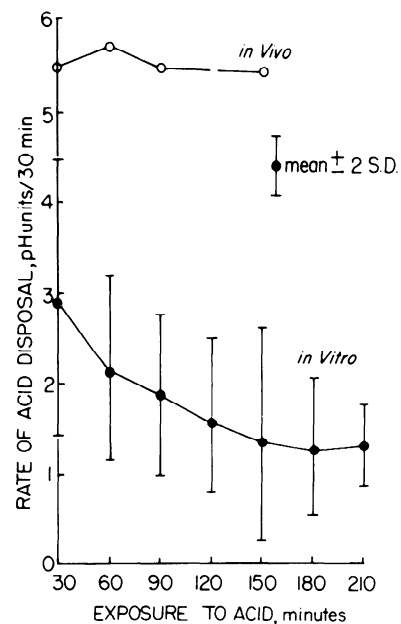


FIG. 3. Rate of acid disposal in gut sacs expressed as rise in pH from pH 2.5. Means \pm 2 SD for in vitro experiments are compared with values obtained from first, second, third, and eighth successive incubations in in vivo experiments. Equilibrium pH was 8 in in vivo experiments after each 30-min incubation.

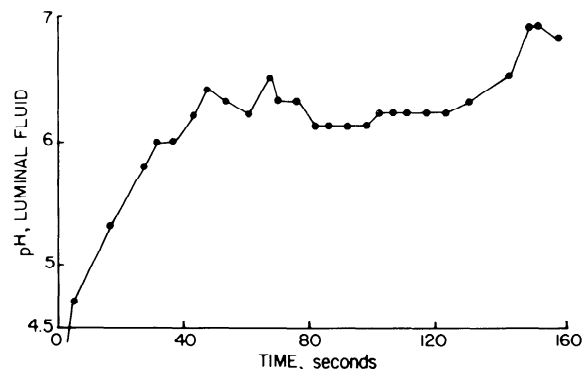


FIG. 4. Gut-sac experiment in vivo recording intraluminal pH 2 cm from site at which solution of pH 2.5 was infused rapidly into lumen. pH readings were made at 2-s intervals. Lowest pH to be recorded was pH 4.7, 2 s after infusing acid.

left the mucosal solution and entered the serosal solution during these periods, the slope of the regression line being 0.95 (mucosal acid loss = 0.95 serosal acid gain + 2.2, $n = 16$, $r = 0.59$, $P < 0.05$). The magnitude of this enhanced gain of serosal acid was approximately the same in each of the four successive 30-min periods (Fig. 6). The total amount of acid which appeared within the serosal solution during 2 h, $11.4 \pm 1.6 \mu\text{mol}$, was significantly reduced to $3.4 \pm .5 \mu\text{mol}$ ($t = 4.73$, $P < .01$) when gassing with 100% oxygen was resumed.

Disposal of alkali from mucosal solution in vitro. The rabbit duodenum was also found to dispose of luminal alkali (Table 3) with a pattern resembling that of acid disposal (Fig. 1). The ability to dispose of alkali was not inhibited by N_2 and iodoacetate and was not influenced by the removal of sodium from the mucosal solution (Table 3). Throughout these experiments, the pH of the serosal solu-

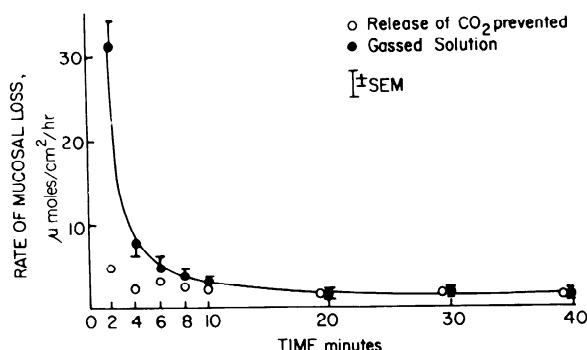


FIG. 5. Rate of acid loss from gassed mucosal solution compared with that from mucosal solution in which release of CO_2 was prevented. Mucosal solutions were maintained at pH 6.0 throughout. No H^+ appeared in serosal chamber during these experiments.

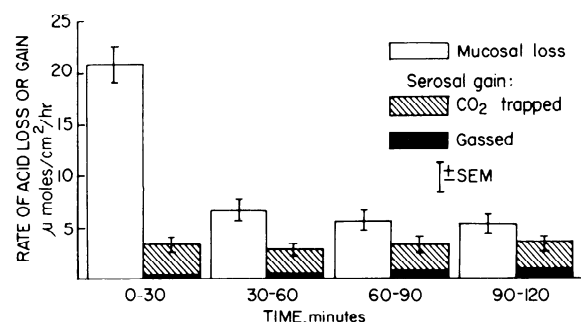


FIG. 6. Rate of mucosal acid loss from gassed mucosal solution maintained at pH 2.5 compared with rate of acid gain within serosal solution maintained at pH 7.4 when CO_2 was trapped in serosal solution. Rate of serosal acid gain in gassed serosal solution (Fig. 1) is included for comparison. There was no significant difference between corresponding rates of mucosal acid loss illustrated in Fig. 1 and rate of acid loss in these experiments.

tion remained at pH 7.4 without the need for addition of either acid or alkali.

Influence of pH and sodium gradients on I_{sc} in vitro. Under control conditions with mucosal and serosal solutions at pH 7.4, the I_{sc} remained between 30 and 40 $\mu\text{A}/\text{cm}^2$ for long periods. When the sodium in the serosal solution was substituted with choline, there was a dramatic increase in I_{sc} which persisted for at least 1 h (Fig. 7). This increase in I_{sc} could be abolished by anoxia. Conversely, when the Na^+ in the mucosal solution was substituted with choline, there was an abrupt reversal of I_{sc} which was also maintained for 1 h and was abolished by anoxia (Fig. 7). In four pairs of experiments, the effects of the Na^+ -free solutions upon the I_{sc} were similar and were not altered by the addition of mannitol to the mucosal solution. The addition of D-glucose to the mucosal solution enhanced the rise in I_{sc} induced by a Na^+ -free serosal solution and impaired the reversal of I_{sc} induced by a Na^+ -free mucosal solution.

When the mucosal surface was exposed to pH 2.5 (serosal pH 7.4), the I_{sc} was abolished (Fig. 8). In 10 experiments at mucosal pH of 2.5 and serosal pH of 7.4, 5 mM glucose in the mucosal solution had no effect on the PD and I_{sc} , whereas at mucosal pH of 6.0 or 7.0 a definite rise in PD and I_{sc} was always observed when 5 mM glucose were added. Exposure of the serosal surface to pH 2.5 while the mucosal surface was maintained at pH 7.4 caused a gradual decrease in I_{sc} , but it was never abolished (Fig. 8).

TABLE 3. Rate of alkali lost from mucosal solution maintained at pH 9.0 while serosal solution was maintained at pH 7.4

	No. of Tissues	Alkali Loss, mol/cm ² per h			
		0-30 min	30-60 min	60-90 min	90-120 min
Standard solutions; 100% O_2 on both sides	10	9.0 ±.8	4.8 .7	4.7 ±.7	3.9 ±.7
Standard mucosal solution and Na^+ -free serosal solution; 100% O_2 on both sides	3	8.4 ±.8	5.0 ±1.1	5.4 ±2.6	6.8 ±2.6
Standard solutions; 100% N_2 and iodoacetate on both sides	6	9.8 1.0	4.4 ±.7	3.0 ±.7	2.5 ±.7

Each value represents the mean \pm SE. None of the experimental values was significantly different from controls under standard conditions. These rates compare with similar rates measured in experiments in which Na^+ in the serosal solution was replaced with choline and to those obtained in tissues treated with N_2 and iodoacetate.

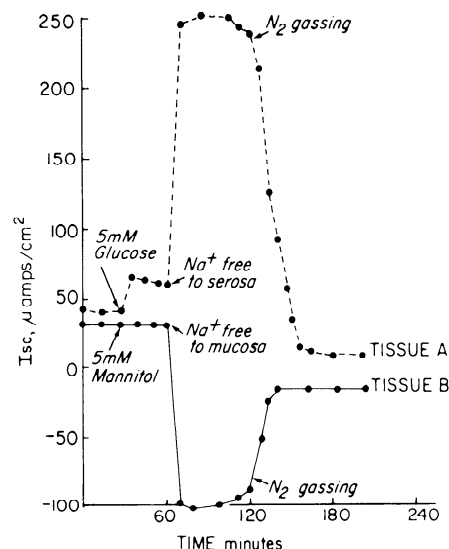


FIG. 7. Effect of Na^+ -free solutions in mucosal and serosal solutions on I_{sc} in paired tissues A and B from same rabbit mounted simultaneously in separate chambers. Note increase in I_{sc} in response to 5 mM glucose in mucosal solution but not to 5 mM mannitol.

When the mucosal pH was raised in increments toward pH 7.4, the rise in pH was accompanied by a partial recovery of PD. When the serosal sodium was replaced with choline so that the I_{sc} was enhanced, lowering of the mucosal pH was associated with a decrease in the I_{sc} . The effect of acid on I_{sc} was first evident at pH 4.5 and most marked below pH 2.5 (Fig. 9).

DISCUSSION

Mechanisms of disposal of acid. These experiments demonstrate that the duodenum of a rabbit can dispose of either acid or alkali to which it is exposed in the absence of pancreatic and biliary secretion. The disposal of acid occurs with great rapidity in vivo, and the luminal pH always returns to pH 8. In these respects, the duodenum of rabbit

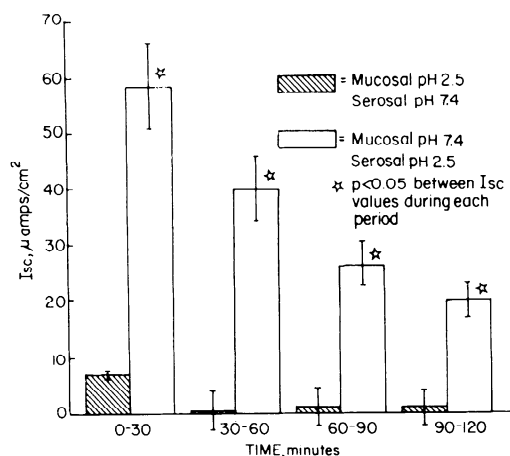


FIG. 8. I_{sc} at mucosal pH of 7.4 and serosal pH of 2.5 compared with I_{sc} at mucosal pH of 2.5 and serosal pH of 7.4.

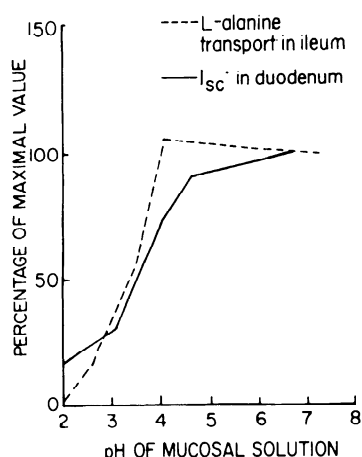


FIG. 9. Effect of changes in luminal pH on I_{sc} when serosal solution was replaced with a sodium-free choline substituted solution. Results were expressed as a percentage change of I_{sc} observed at pH 7.4. These findings are compared with percentage changes of sodium-coupled L-alanine absorption induced by luminal acidification (ref. 18).

behaves in a manner similar to the duodenum and the jejunum of a dog (14, 15, 30, 40).

Because the ability to dispose of acid in vitro was not impaired by N_2 and iodoacetate and was not enhanced by supplying HCO_3^- and CO_2 to the serosal solution, the disposal of acid appears to be achieved by passive physicochemical forces. In the third and fourth 30-min periods after mucosal exposure to acid in the absence of significant hydrostatic, osmotic, and electrochemical gradients, the only constant physical force that could induce the observed disposal of acid was the hydrogen ion concentration gradient which would cause diffusion of H^+ into mucosa. Since the rate of acid disposal was also unchanged when the sodium within the serosal solution was replaced with choline (Table 1) and the disposal of alkali was not impaired by eliminating Na^+ from the mucosal solution (Table 3), it is unlikely that H^+ leaves the mucosal solution by means of an obligatory exchange diffusion between mucosal hydrogen and serosal sodium, as has been previously implied (15). The processes of simple diffusion and exchange diffusion are not saturable (11) and therefore would not explain the

findings observed in the current experiments. Rather, the relationship between the rate of acid disposal and luminal $[H^+]$, which resembles a hyperbolic enzyme saturation curve, suggests but does not prove that H^+ like Na^+ leaves the mucosal solution by means of a passive, carrier-mediated facilitated transfer.

Since only a small proportion of the acid which left the mucosal solution maintained at pH 2.5 could be recovered from the gassed serosal solution (Fig. 1) and because H^+ did not appear in the serosal solution when the mucosal surface of the tissue was exposed to pH 6 or 9, it is unlikely that the appearance of H^+ in the serosal solution at a mucosal pH of 2.5 was the result of metabolic acid. The relation between $[H^+]$ in the mucosal solution and the rate of acid loss in the 2nd h (Fig. 2) is consistent with diffusion of H^+ down a transmucosal gradient. However, the lack of correlation between the rate at which the acid leaves the mucosal solution and the rate at which it enters the serosal solution during this 2nd h (3rd and 4th 30-min periods) implies that the H^+ which enters the tissue does not traverse the mucosa as such to enter the serosal solution. The positive correlation between mucosal acid loss and serosal acid gain when CO_2 was trapped in the serosal solution (slope 0.95) suggests that the acid which leaves the mucosal solution appears as CO_2 on a 1:1 basis in the serosal solution, possibly after neutralization of the H^+ by tissue HCO_3^- .

In addition to the possible disposal of H^+ by facilitated diffusion, our gut-sac experiments support the suggestion of others (3, 14, 15, 17, 21, 26, 27, 30, 36, 40, 45, 46) that intraluminal neutralization by HCO_3^- also occurs. The correlation we observed between the rate of disappearance of luminal H^+ and the luminal CO_2 production during the in vitro and in vivo gut-sac studies (Table 2) is consistent with the process of intraluminal neutralization and is similar to the rise in intraluminal P_{CO_2} noted in man when duodenal acid is neutralized by HCO_3^- (46). In agreement also is our finding in vitro that prevention of release of CO_2 from the mucosal solution abolishes the early rapid phase of acid disposal which indicates that this phase is dependent on neutralization by HCO_3^- . The subsequent steady phase of acid loss is more likely the result of intramucosal diffusion of H^+ . While the failure of N_2 and iodoacetate to inhibit the early rapid phase of acid disposal suggests that secretion of HCO_3^- during this phase is not an "active" process, the inability to fatigue the process of acid disposal during the in vivo gut-sac experiments is consistent with the presence of an active process of HCO_3^- secretion as appears to occur in canine duodenum in vivo (15, 27). Our findings do not permit further analysis of the contribution of these various processes to acid disposal.

Relationship between I_{sc} , luminal pH, and Na^+ transport. Both the I_{sc} and the PD, their direction, and their magnitude are reasonable reflections of the net Na^+ transport in the intestine (1, 2, 5, 7, 8, 37, 42). Absorption of sodium is stimulated by adding D-glucose or L-alanine to the mucosal solution, and this stimulation is manifest as an increase in the transmucosal I_{sc} and PD (28, 31, 35, 37-39).

In our experiments in which the mucosal pH was maintained at 6 or 7, the addition of D-glucose to the mucosal solution also stimulated an increase in PD and I_{sc} , sug-

gesting that there also might be a solute-coupled Na^+ absorptive mechanism in the rabbit duodenum. This stimulation by D-glucose was not evident when the mucosal pH was maintained at pH 2.5. Na^+ -coupled L-alanine absorption from the terminal ileum has also been shown to be inhibited at pH 2.5 (18). Indeed, the similarity between the effect of luminal pH on the I_{sc} in rabbit duodenum and on Na^+ -coupled L-alanine absorption in rabbit ileum is striking (Fig. 9). Moreover, the partial return of the PD toward control values and the recovery of L-alanine absorption (18) when the luminal pH was raised toward 7.4 are consistent with persistent tissue viability, and the observed decrease in PD with luminal acidification was unlikely to be attributable to tissue death.

In the case of Na^+ -coupled L-alanine absorption, it has been suggested that this inhibition by luminal acidification is the result of the competitive inhibition between H^+ and Na^+ for the cationic binding site on the carrier that mediates solute-coupled Na^+ transfer across the mucosal brush border (18). Our data are consistent with the possible existence of a similar mechanism in duodenal mucosa. It is unlikely that inhibition by H^+ was the result of an interference with the sodium pump, for the inhibitory effect of acid in our experiments was confined to the mucosal surface and not to the serosal surface where the sodium pump is located (37) (Fig. 9).

Proximal jejunum of the rabbit differs from ileum in possessing an active transport mechanism for Na^+ from serosa to mucosa (S-M) in addition to the active M-S absorptive flux (19). Our findings that the I_{sc} was reversed when the Na^+ of the mucosal solution was replaced with choline and that this I_{sc} was abolished by anoxia suggest that a similar active S-M flux is present in the duodenum of the rabbit. If luminal acidification interferes with the movement of Na^+ from M-S by competitive inhibition, then a net Na^+ flux from S-M could be unmasked by this luminal acidification. The direction of the change of the I_{sc} and PD induced by luminal acidification is the reverse of that anticipated from a passive diffusion potential. If this unmasked secretion of Na^+ carried with it fluid similar in composition to interstitial fluid, then not only will luminal acidification stimulate the secretion of bicarbonate from the pancreas (24), but also it will induce a net active secretion of sodium bicarbonate from the duodenal mucosa. That such a fluid movement can be inferred from the direction and magnitude of the net Na^+ flux has been demonstrated before in the rabbit intestine (4, 10, 29).

Comparison of studies of acid disposal in vitro and in vivo.

Sodium is probably absorbed by similar mechanisms in canine and rabbit intestine, since the rate of absorption in both animals depends on the luminal $[\text{H}^+]$ and is enhanced by D-glucose (31). The rates of net Na^+ and H_2O transport in rats and dogs are the same in vivo and in vitro, suggesting that extrapolation from the in vitro to the in vivo situation is justifiable (9, 13, 20, 22, 40). Our previous studies in canine duodenal pouches indicated that 67% of the acid disposal occurred by intraluminal neutralization and 33% by backdiffusion of H^+ (15). In those in vivo studies, the relationship between the luminal (H^+) and the rate of acid disposal was similar to that observed in the current in vitro experiments on rabbits and also resembled a hyperbolic saturation curve. It is possible that H^+ enters the canine duodenal mucosa by means of a carrier-mediated facilitated transfer, thereby inhibiting the absorption of sodium and water. A hypothetical replacement of Na^+ by H^+ on the carrier could account for the one-to-one exchange between H^+ and Na^+ which occurred independently of volume change in the canine duodenal pouches (15). Such a mechanism is also consistent with our observation in rabbits in vitro that acid disposal is not affected by Na^+ -free solutions, indicating there is not an obligatory exchange diffusion between Na^+ and H^+ as previously implied (15). If the canine duodenum possesses a bidirectional Na^+ transport system as exists in rabbits, then the intramucosal diffusion of H^+ will induce a volume of bicarbonate secretion proportional to the magnitude of the H^+ backdiffusion by selectively inhibiting the M-S absorptive flux of Na^+ . This would account for the linear relationship which was observed between H^+ backdiffusion and the volume of sodium bicarbonate secretion in those dogs with newly constructed duodenal pouches (15).

Disposal of alkali. As in dogs, the duodenum in rabbits can dispose of alkali (40). It is implied by our experiments in vitro, showing that N_2 and iodoacetate did not affect the disappearance of alkali, that an active process is not involved, whereas an active secretion of acid has been suggested in dogs and in man (40, 41). Species differences could explain these discrepancies, since the equilibrium pH of the duodenum in the rabbit is 8 and in dogs and man is 6 (32, 33, 40). It is also possible that the rabbit under different circumstances might have demonstrated an active secretion of acid.

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