

# Micropuncture Analysis of the Cellular Mechanisms of Electrolyte Secretion by the In Vitro Rabbit Pancreas

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**ABSTRACT** Micropuncture techniques have been used to study electrolyte secretion by the spontaneously secreting in vitro rabbit pancreas over a wide range of environmental conditions. Pancreatic secretion does not have a strong requirement for  $\text{HCO}_3^-$  and secretion continues at nearly normal rates when exogenous  $\text{HCO}_3^-$  is replaced by acetate. Acetate concentration in the juice averages 70 meq/liter, nearly three times the environmental concentration. The similar characteristics exhibited by  $\text{HCO}_3^-$  and acetate secretion indicate that they are secreted by a common mechanism involving active H transport. In vitro acid-base alterations demonstrate that the secretion rate is controlled by the environmental  $\text{HCO}_3^-$  concentration and pH, while the  $\text{HCO}_3^-$  content of the primary secretion is controlled by the environmental  $\text{HCO}_3^-$  concentration and to a much lesser extent by the  $\text{pCO}_2$ . Secretion also requires active Na transport across the mucosal membrane. The effects of ouabain and a low Na environment strongly suggest coupling between the transport of Na and H and a cellular mechanism for electrolyte secretion is proposed involving Na-H exchange mechanisms at both the mucosal and serosal membranes.

In a previous paper we have shown that electrolyte secretion is the product of two distinct events (1). Primary secretion is produced in the intralobular ducts during spontaneous secretion and in both the intralobular and the smaller extralobular ducts in response to secretin stimulation. The anion composition of this primary secretion is dependent on the secretion rate and is modified further in the main collecting duct by flow-dependent Cl- $\text{HCO}_3^-$  exchange. Evidence has been presented that secretion results from the active transport of both Na (1-4) and  $\text{HCO}_3^-$  (1, 5) against their electrochemical potential differences.

Since most of the previous work on pancreatic electrolyte secretion has been based on the analysis of the externally collected juice, it offers little informa-

tion on the cellular events of secretion. In contrast, the micropuncture techniques, which we have used to study the composition of the primary secretion directly, have enabled us to investigate the cellular mechanisms. The spontaneously secreting *in vitro* rabbit pancreas preparation was chosen not only because of its suitability for micropuncture work but also because environmental conditions can be widely varied and closely controlled. Our objectives were threefold: to determine the nature of  $\text{HCO}_3^-$  transport, to examine the relations between the secretion of Na and  $\text{HCO}_3^-$ , and to determine the factors which control secretion at the cellular level. Although  $\text{HCO}_3^-$  is secreted against its electrochemical potential difference (1, 5), active transport of the  $\text{HCO}_3^-$  ion per se is not required.  $\text{HCO}_3^-$  secretion could result from the primary production of a transtubular pH gradient to which  $\text{HCO}_3^-$  accommodates itself passively by permeating as  $\text{CO}_2$ . This mechanism will be discussed in terms of the active transport of H from the duct lumen to the plasma or bathing solution. However, it is recognized that this mechanism is indistinguishable from either OH transport in the opposite direction or the intracellular splitting of water with H and OH being transported across the serosal and mucosal membranes, respectively.

Our data demonstrate that  $\text{HCO}_3^-$  secretion is mediated by active H transport. The transport of both Na and H are the rate-controlling steps in pancreatic secretion since secretion is sensitive to environmental factors affecting either ion. Finally our evidence suggests that the transport of Na and H are coupled and we propose a cellular mechanism for electrolyte secretion involving Na-H exchange at both the mucosal and serosal membranes which is consistent with our experimental data.

#### METHODS

The rabbit pancreas was removed and prepared for *in vitro* micropuncture study by the methods previously described (1). The tissue was mounted in 350 ml of a Krebs-Henseleit  $\text{HCO}_3^-$  buffer (6) (118.5 mM NaCl, 4.75 mM KCl, 2.54 mM  $\text{CaCl}_2$ , 1.18 mM  $\text{KH}_2\text{PO}_4$ , 1.18 mM  $\text{MgSO}_4$ , 24.9 mM  $\text{NaHCO}_3$ , and 5 mM glucose) which was maintained at  $30 \pm 0.5^\circ\text{C}$  and a pH of  $7.40 \pm 0.03$  by gassing with 95%  $\text{O}_2$ -5%  $\text{CO}_2$ .

To examine the effect of environmental alterations on secretion the following experimental procedure was adopted. After initial equilibration in the normal  $\text{HCO}_3^-$  buffer, samples of final juice and one or two micropuncture samples of juice from the small extralobular ducts (50-100  $\mu\text{m}$  in diameter) were obtained as a control. The composition of the juice in the small extralobular ducts reflects that of the primary secretion and the differences between the composition of this ductal juice and the final juice reflect the action of Cl- $\text{HCO}_3^-$  exchange in the main collecting duct (1). The desired change in environmental conditions was then made and after an equilibration period of 1 h, samples of ductal and final juice were collected again. Up to three different experimental conditions could be examined in this manner in each experiment.

### *Environmental Conditions*

The environmental  $\text{HCO}_3^-$  concentration was varied over a range of 10–75 meq/liter with appropriate changes in the Cl concentration to maintain a normal bathing solution osmolality of  $285 \pm 5$  mosmol/kg  $\text{H}_2\text{O}$ . When the  $\text{HCO}_3^-$  concentration was varied, the bathing solution pH was either maintained at the normal value of 7.4 by changing the  $\text{CO}_2$  content of the gas mixture correspondingly or allowed to vary with the  $\text{HCO}_3^-$  concentration by keeping the  $\text{CO}_2$  content fixed. Bath pH was also varied at constant  $\text{HCO}_3^-$  concentration by changing the  $\text{CO}_2$  content of the gas.  $\text{O}_2$ - $\text{CO}_2$  gas mixtures were used which contained 2, 5, and 10%  $\text{CO}_2$  by volume. In another series of experiments  $\text{HCO}_3^-$  was removed entirely from the bathing solution and replaced by 24.9 meq/liter acetate or by using a Krebs-Henseleit  $\text{PO}_4$  buffer (6). In both of these cases the bathing solution was gassed with pure  $\text{O}_2$ . When a low Na environment was used to examine the role of active Na transport, normal osmolality was maintained in the bathing solution by replacing NaCl with either sucrose or choline chloride.

### *Micropuncture and Analytical Techniques*

The in vitro micropuncture techniques have been described previously (1). However, we found it possible to discontinue the ATP procedure for estimating contamination of the micropuncture samples of ductal juice with the bathing solution. Our previous work had shown that contamination was generally 50% or more when it occurred and could be discerned readily by comparing the Cl concentration with other samples. Moreover, contaminated samples were generally characterized by abnormally high aspiration rates and the inability to control the position of the oil droplet within the duct through small variations in the aspiration rate. These observations indicate that contamination occurs during sample aspiration rather than in transferring the micropipette through the bathing solution to the overlying oil layer. With experience it was possible to estimate accurately the probability of contamination from the rate of sample aspiration and the behavior of the oil droplet within the duct. Samples were rejected which did not satisfy the criteria of appropriate Cl concentration and sample collection characteristics.

The analytical procedures for determining juice and cellular concentrations of Na, K, Cl,  $\text{HCO}_3^-$ , and pH have been described previously (1). The acetate concentration in the ductal and final juice was determined by adding sodium acetate -  $1\text{-}^{14}\text{C}$  (New England Nuclear, Boston, Mass.) to the bathing solution and determining the juice-to-bath activity ratio with a liquid scintillation counter (model 6801s, Nuclear-Chicago Corp., Des Plaines, Ill.). All labeled acetate was assumed to be in the ionic form since the concentration of un-ionized acetic acid is less than 1% of that of the acetate ion in the physiological pH range. The possibility of metabolism of acetate by the pancreas was assessed in each experiment by acidifying aliquots of the bathing solution and the juice with an equal volume of 1 N  $\text{H}_2\text{SO}_4$ . Acidified samples were either allowed to stand for 24 h or heated almost to boiling before analysis to drive off  $\text{CO}_2$ . The juice-to-bath activity ratio after acidification averaged  $97.0 \pm 0.8\%$  ( $n = 7$ ) of the unacidified value. Thus, only a small fraction of the

radioactivity in the juice can be [ $^{14}\text{C}$ ] $\text{HCO}_3$ . All acetate concentrations in the juice have been calculated using the acidified activity ratios.

The presence of acetate in the juice does not affect the analysis of juice  $\text{HCO}_3$  by the equilibrium pH method (1). Since the pK of acetic acid, 4.75, is about 2 pH units below the pH at which the samples were analyzed, the buffer capacity of acetate in the juice can be neglected, as confirmed by pH measurements on test solutions with a similar composition containing as little as 2 meq/liter  $\text{HCO}_3$  in equilibrium with 2 or 5%  $\text{CO}_2$ .

## RESULTS

### *HCO<sub>3</sub> Secretion*

In view of the strong specificity generally exhibited by active transport mechanisms, pancreatic secretion should have a strong requirement for  $\text{HCO}_3$  if the  $\text{HCO}_3$  ion itself is actively transported. However, if secretion is mediated by an active H pump, it should be possible to replace exogenous  $\text{HCO}_3$  by other suitable weak acid anions without markedly affecting secretion. Table I shows the effects on pancreatic secretion of replacing environmental  $\text{HCO}_3$  by either  $\text{PO}_4$  or acetate. With  $\text{PO}_4$  replacement the secretion rate and  $\text{HCO}_3$  output are inhibited by 90% or more which is comparable with values reported previously for both the *in vitro* rabbit pancreas (2) and the perfused cat pancreas (7). While this inhibition is consistent with a primary  $\text{HCO}_3$  pump, it could also be due to impermeability of  $\text{PO}_4$  since  $\text{PO}_4$  concentration in the juice is normally about  $\frac{1}{10}$  of that in the plasma (8). Furthermore, Table I shows that in a  $\text{HCO}_3$ -free acetate environment juice is secreted at a much greater rate than in a  $\text{PO}_4$  buffer indicating that there is no unique requirement for exogenous  $\text{HCO}_3$ . The secretion rate in an acetate environment averages 60% of the control value and rates as high as 90% of the control have been observed. Acetate concentration in the juice averages 70 meq/liter or nearly three times the bathing solution concentration.  $\text{HCO}_3$  is still present in the "acetate" juice at low concentrations and the  $\text{HCO}_3$  output, about 6% of the control, is in agreement with estimates of the contribution of metabolic  $\text{CO}_2$  to the normal  $\text{HCO}_3$  output (2, 7). The total secreted output of base, acetate plus  $\text{HCO}_3$ , is about 50% of the control. Cl- $\text{HCO}_3$  exchange normally occurs in the main collecting duct and appears to be a passive process (1, 9). Similarly, the differences between the ductal and final juice acetate concentration indicate the presence of Cl-acetate exchange in the main duct. The difference in Table I is not statistically significant but this is due primarily to the large variability in both the secretion rate and juice composition between experimental animals. Significant differences between the ductal and final juice acetate content are observed in individual experiments.

The ability to maintain nearly normal secretion rates when exogenous

TABLE I  
EFFECT OF REPLACING EXOGENOUS  $\text{HCO}_3$  BY  $\text{PO}_4$  OR ACETATE ON  
PANCREATIC SECRETION\*

	Secretion rate	$\text{HCO}_3$	Acetate	$\text{HCO}_3$ output	Acetate output
	mg/h	meq/liter	meq/liter	$\mu\text{eq/h}$	$\mu\text{eq/h}$
Control ( $n = 3$ )	470 (401-538)				
Final juice		92 (82-103)	—	44 (33-55)	—
$\text{PO}_4$ buffer	45 (36-51)				
Final juice		32 (22-40)	—	1.4 (1.1-1.6)	—
Control ( $n = 5$ )	524 (437-704)				
Final juice		83 (73-95)	—	44 (32-56)	—
Ductal juice		90 (82-95)	—	48 (36-64)	—
Acetate buffer	321 (220-440)				
Final juice		8.7 (5.0-11.2)	63 (54-71)	2.6 (2.2-2.9)	20 (13-24)
Ductal juice		8.9 (5.2-13.2)	70 (58-73)	2.7 (2.3-2.9)	22 (16-31)

\*  $n$  represents the number of experiments. Values reported are the mean of all experiments with the range indicated in parentheses.

$\text{HCO}_3$  is replaced by acetate argues that  $\text{HCO}_3$  and acetate are secreted via a common mechanism mediated by an active H pump. If the juice-to-bath acetate concentration difference reflects an equilibrium distribution in response to a transtubular pH difference, the juice must be 0.3–0.5 pH units more alkaline than the bath. The pH of acetate juice must be determined by its  $\text{pCO}_2$  and  $\text{HCO}_3$  content, since acetate has virtually no buffering capacity in the physiological pH range. For technical reasons, juice pH could not be measured in situ. However, the juice  $\text{pCO}_2$  required to give an equilibrium distribution for acetate between the bath and the juice can be calculated and was computed to be  $5.7 \pm 0.7$  mm Hg ( $\pm$  SE,  $n = 12$ ). The consistency of this computed  $\text{pCO}_2$  tends to support the existence of an equilibrium distribution for acetate resulting from a primarily produced transtubular pH difference. Since the bathing solution was gassed with pure  $\text{O}_2$ , this calculated  $\text{pCO}_2$  may reflect the  $\text{pCO}_2$  of the actively metabolizing cells of the duct wall. Hubel (10) has reported that under normal in vitro secretory conditions the juice  $\text{pCO}_2$  is 7–9 mm Hg greater than that of the bath equilibrated

with 5% CO<sub>2</sub> which also may reflect the increased pCO<sub>2</sub> of metabolizing cells.

Since acetate juice pH is determined by its HCO<sub>3</sub> content, it is conceivable that the transtubular pH difference could be produced by the transport of endogenously produced HCO<sub>3</sub> across the mucosal membrane rather than by H transport. Such a mechanism would require the recycling of HCO<sub>3</sub> and hence be dependent on the carbonic anhydrase catalyzed hydration of CO<sub>2</sub>. The effect of carbonic anhydrase inhibition with Diamox (kindly provided by Lederle Laboratories, Pearl River, N. Y.) on secretion in a normal HCO<sub>3</sub> and HCO<sub>3</sub>-free acetate environment is shown in Table II. The individual

TABLE II  
EFFECT OF DIAMOX ON HCO<sub>3</sub> AND ACETATE SECRETION\*

	Experiment 1		Experiment 2		Experiment 3	
	Control	+300 mg/liter Diamox	Control	+300 mg/liter Diamox	Control	+300 mg/liter Diamox
<b>HCO<sub>3</sub> secretion</b>						
Secretion rate (mg/h)	450	276	412	290	298	135
Ductal HCO <sub>3</sub> (meq/ liter)	96±4	66±3	82±6	67±5	81±6	62±3
HCO <sub>3</sub> output (μeq/h)	43.2	18.2	33.8	19.7	24.1	8.4
<b>Acetate secretion †</b>						
Secretion rate (mg/h)	222	166	324	314		
Ductal acetate (meq/ liter)	73±2	63±2	72±2	70±2		
Acetate output (μeq/h)	16.3	10.4	23.3	22.0		

\* Control for the acetate experiment is secretion in a HCO<sub>3</sub>-free acetate environment without Diamox.

† Results of individual experiments. HCO<sub>3</sub> concentrations are reported ± SE in analyses. Estimated error in the determination of the secretion rate is ±5 mg/h.

results of three experiments with HCO<sub>3</sub> buffer and two experiments with acetate are presented to help alleviate the problem of variability between experimental animals. At 300 mg/liter Diamox inhibits the secretion rate and HCO<sub>3</sub> output on the average by 41 and 55%, respectively, in the normal HCO<sub>3</sub> buffer. However, in a HCO<sub>3</sub>-free acetate environment Diamox produces only moderate inhibition in one experiment and essentially none in the other. This marked difference in the effects of carbonic anhydrase inhibition on HCO<sub>3</sub> and acetate secretion argues against the recycling of endogenous HCO<sub>3</sub> through a HCO<sub>3</sub> pump.

The effects of acid-base alterations on pancreatic secretion have been studied extensively in vivo to gain insight into secretory mechanisms (11-13). However, the interpretation of these results is complicated by the inability to control plasma pH, pCO<sub>2</sub>, and HCO<sub>3</sub> concentration independently and,

therefore, we have reexamined this problem using the *in vitro* preparation. The Henderson-Hasselbalch equation indicates that two of the three variables can be independently controlled.

$$\text{pH} = \text{pK} + \log \frac{\text{HCO}_3}{\alpha \text{pCO}_2}$$

Environmental pH and  $\text{pCO}_2$  can be varied inversely at constant  $\text{HCO}_3$  concentration;  $\text{pCO}_2$  and  $\text{HCO}_3$  can be varied proportionately at constant pH; and pH and  $\text{HCO}_3$  can be varied directly at constant  $\text{pCO}_2$ . Thus by systematic variation of the environmental conditions it should be possible to determine the individual effects of pH,  $\text{pCO}_2$ , and  $\text{HCO}_3$  concentration.

The effect of varying pH and  $\text{pCO}_2$  at constant  $\text{HCO}_3$  concentration is shown in Fig. 1 *a*. Increasing pH (or decreasing  $\text{pCO}_2$ ) stimulates secretion and the data further suggest that this stimulatory effect is enhanced at higher  $\text{HCO}_3$  concentrations. Fig. 1 *b* presents the pH dependence of secretion in a  $\text{HCO}_3$ -free acetate environment. Since the acetate environment was buffered only by the small amount of  $\text{PO}_4$  present in the normal  $\text{HCO}_3$  buffer, its pH was altered by the addition of a small amount of acid or base. The environmental acetate concentration was not significantly altered by this

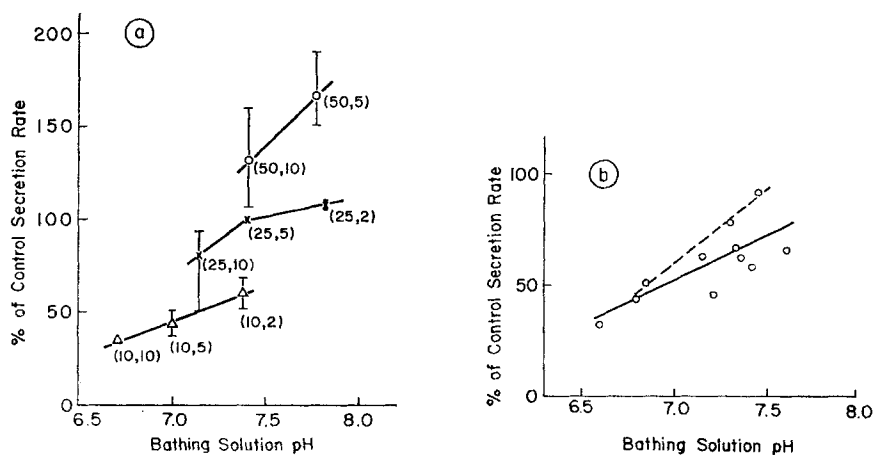


FIGURE 1. The effect of bathing solution pH on pancreatic secretion (*a*) in  $\text{HCO}_3$  buffers with 10, 25, and 50 meq/liter  $\text{HCO}_3$  and (*b*) in  $\text{HCO}_3$ -free acetate buffer with 25 meq/liter acetate. The secretion rate is expressed as the percent of the control value in a normal  $\text{HCO}_3$  buffer (25 meq/liter  $\text{HCO}_3$ , 5%  $\text{CO}_2$  in the gas mixture). The numbers in parentheses for each point for  $\text{HCO}_3$  secretion indicate the  $\text{HCO}_3$  concentration and  $\text{pCO}_2$  (as percent  $\text{CO}_2$  in the gas mixture) in the bathing solution, respectively. Bars indicate the range of observation in two to five experiments. The data for acetate secretion are the results of eight experiments. The solid line is a regression line through the data with a slope of  $39 \pm 11$  while the dashed line connects three points from a single experiment.

procedure and the environmental pH remained constant for test periods of an hour or more. The slope of the regression line through the data is significantly different from zero ( $P < 0.01$ ) indicating that acetate secretion is markedly stimulated by an increased environmental pH. The scatter in the data primarily reflects animal variability as indicated by the dashed line which connects three points from a single experiment. Both the  $\text{HCO}_3^-$  and acetate data extrapolate linearly to zero secretion at an environmental pH of about 6. The similar effects of pH on  $\text{HCO}_3^-$  and acetate secretion support their secretion by a common mechanism.

The effects of environmental  $\text{HCO}_3^-$  alterations at constant pH or  $\text{pCO}_2$  on secretion are shown in Fig. 2. Both the secretion rate and the ductal  $\text{HCO}_3^-$  concentration are strongly dependent on environmental  $\text{HCO}_3^-$ . Close examination of Fig. 2 *a* reveals that the secretory stimulation in Fig. 1 *a* can be attributed to the increased pH rather than the decreased  $\text{pCO}_2$  since the secretion rate can be enhanced in the presence of an increase in  $\text{pCO}_2$  (constant pH), or a decrease in  $\text{pCO}_2$  (constant  $\text{HCO}_3^-$  concentration), or at constant  $\text{pCO}_2$ . Moreover, the greatest secretory stimulation occurs at constant  $\text{pCO}_2$ , indicating that the effects of pH and  $\text{HCO}_3^-$  are additive.

The ductal  $\text{HCO}_3^-$  concentration in Fig. 2 *b* exhibits a Michaelis dependence on environmental  $\text{HCO}_3^-$ . The data also suggest that increasing the environmental  $\text{pCO}_2$  (or decreasing pH) at constant  $\text{HCO}_3^-$  increases the ductal  $\text{HCO}_3^-$  concentration. These small differences at 10 and 50 meq/liter in Fig. 2 *b* are not statistically significant again due primarily to the variability in juice composition between experimental animals. The significance of these effects, however, can be seen in individual experiments and the results of four typical experiments are presented in Table III. When the environmental

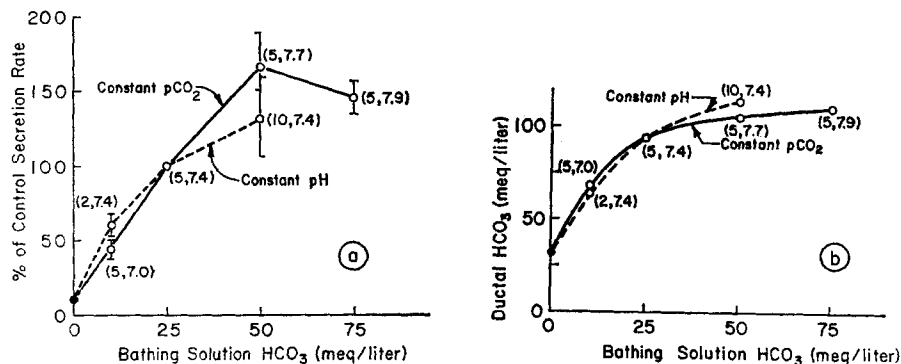


FIGURE 2. The effect of altering the environmental  $\text{HCO}_3^-$  concentration at constant  $\text{pCO}_2$  (solid line) and at constant pH (dashed line) on (a) the secretion rate and (b) the ductal  $\text{HCO}_3^-$  concentration. Values in parentheses indicate the  $\text{pCO}_2$  (as percent  $\text{CO}_2$  in the gas mixtures) and pH of the bathing solution, respectively. Points on the ordinate represent secretion in a  $\text{HCO}_3^-$ -free  $\text{PO}_4$  buffer, taken from Table I.



TABLE III  
EFFECT OF ENVIRONMENTAL ACID-BASE ALTERATION ON PANCREATIC SECRETION\*

	Environmental condition		Secretion rate	Ductal HCO <sub>3</sub>	Ductal Cl	
	HCO <sub>3</sub>	pH				
	meq/liter		mg/h	meq/liter	meq/liter	
HCO <sub>3</sub> secretion	25	7.45	427	118±6	47.0±0.4	
	10	7.01	214	86±4	73.5±0.6	<i>P</i> <0.002‡
	10	7.42	294	77±4	83.8±0.6	<i>P</i> <0.01§
	25	7.15	198	95±4	66.1±0.5	
	25	7.44	396	89±4	69.3±0.5	<i>P</i> <0.05§
	25	7.88	433	83±4	74.7±0.5	<i>P</i> <0.01§
	25	7.42	219	92±6	65.8±0.5	
	50	7.46	287	109±6	48.8±0.3	<i>P</i> <0.002‡
	50	7.73	343	109±6	49.6±0.3	<i>P</i> >0.1§
		Acetate meq/liter		Final juice acetate meq/liter		
Acetate secretion	25	7.42	277	71.1±0.3		
	25	7.61	312	63.4±0.3		<i>P</i> <0.005§

\* The results of four individual experiments. Ductal HCO<sub>3</sub> and Cl concentrations are reported ±SE in analysis. Estimated error in the determination of the secretion rate is ±5 mg/h.

‡ The significance of the change in ductal Cl concentration produced by altering environmental HCO<sub>3</sub> at either constant pH or pCO<sub>2</sub>.

§ The significance of the change in ductal Cl concentration produced by altering environmental pH at constant HCO<sub>3</sub> (or acetate) concentration.

pH is increased by reducing the pCO<sub>2</sub> at constant HCO<sub>3</sub> concentrations of 10 and 25 meq/liter ductal HCO<sub>3</sub> falls and Cl increases. While HCO<sub>3</sub> analysis is not sufficiently accurate to show statistically significant changes, the sum of Cl and HCO<sub>3</sub> is constant (1) and statistically significant differences are observed in Cl concentration which can be determined with much greater precision. With environmental HCO<sub>3</sub> of 50 meq/liter the Cl change is small and not statistically significant. pH alterations in a HCO<sub>3</sub>-free acetate

environment also produce a significant change in juice acetate concentration further indicating that  $\text{HCO}_3^-$  and acetate are secreted by a common mechanism. These changes in ductal Cl and  $\text{HCO}_3^-$  composition produced by pH alterations at a constant environmental  $\text{HCO}_3^-$  concentration are a direct result of the environmental alterations and not attributable to the secretion rate changes produced since the present ductal  $\text{HCO}_3^-$ -secretion rate behavior is the opposite of that observed under normal conditions (1). Since pH-induced changes in the secretion rate will also alter the extent to which flow-dependent Cl- $\text{HCO}_3^-$  exchange in the main duct can modify the composition of the final juice, these small effects of environmental pH can be seen best in the ductal juice. If the secretion rate change is large, the resultant change in Cl- $\text{HCO}_3^-$  exchange may be sufficient to obscure or even reverse the variation of the anion composition of the final juice with pH. In the acetate experiment, however, the secretion rate change was small and the effect of environmental pH on the primary juice composition was still evident in the final juice. Comparison of secretion with environmental  $\text{HCO}_3^-$  concentrations of 10 and 50 meq/liter with their controls at 25 meq/liter also demonstrate the more marked effects of environmental  $\text{HCO}_3^-$  alterations as well as the additive effects of simultaneously increasing or decreasing environmental pH and  $\text{HCO}_3^-$  concentration.

The secreted output of  $\text{HCO}_3^-$  is strongly dependent on the environmental  $\text{HCO}_3^-$  concentration. Increasing environmental pH at constant  $\text{HCO}_3^-$  concentration also stimulates  $\text{HCO}_3^-$  output since the stimulatory effect of pH on the secretion rate is much greater than its apparent inhibitory effect on the ductal  $\text{HCO}_3^-$  concentration. The Na content of the juice was not affected by the *in vitro* acid-base alterations (final juice Na averages  $151.5 \pm 0.5$  meq/liter ( $\pm$  SE,  $n = 23$ ) for all the abnormal acid-base conditions versus  $151.2 \pm 0.5$  meq/liter for their corresponding controls). The juice Na concentration is determined primarily by the environmental Na concentration and osmolality (3) both of which were held constant in these experiments. Thus the secreted output of Na varies directly with the secretion rate and depends on both the environmental pH and  $\text{HCO}_3^-$  concentration.

Electrochemical potential difference measurements indicate that  $\text{HCO}_3^-$  secretion requires active transport of either  $\text{HCO}_3^-$  or H across the serosal membrane (1). We have also shown that the cellular pH exhibits the same linear dependence on environmental pH when the latter is altered by varying either the  $\text{HCO}_3^-$  concentration or the  $\text{pCO}_2$  (14). Fig. 3 indicates that a similar dependence is observed in both  $\text{HCO}_3^-$  and  $\text{HCO}_3^-$ -free acetate environments. Cellular pH is markedly increased in the acetate environment, however, which may be a result of the decreased buffer capacity of the cell. Since acetate has no buffering capacity in this pH range this cellular pH behavior strongly argues for active H transport across the serosal membrane.

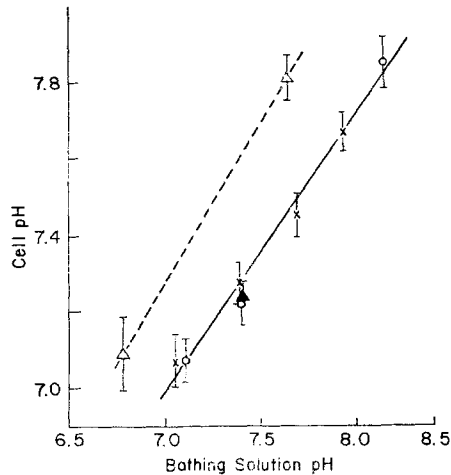


FIGURE 3

FIGURE 3. Effect of environmental pH on the cellular pH in  $\text{HCO}_3^-$  and  $\text{HCO}_3^-$ -free acetate buffers. The solid line represents behavior in a  $\text{HCO}_3^-$  buffer when the bathing solution pH is altered by changing the  $\text{HCO}_3^-$  concentration (X) or the  $\text{pCO}_2$  (O). The dashed line indicates behavior in a  $\text{HCO}_3^-$ -free acetate environment with constant acetate concentration. The solid diamond is the control for the acetate experiment, the cell pH in a normal  $\text{HCO}_3^-$  buffer. The bars represent the estimated error in analysis of cellular pH.

FIGURE 4. Schematic representation of the proposed cellular mechanism for pancreatic electrolyte secretion. Serosal Na-H exchange is linked indirectly to metabolism through the Na-K pump. The mucosal Na-H pump requires direct metabolic coupling although not necessarily in the form of ATP.

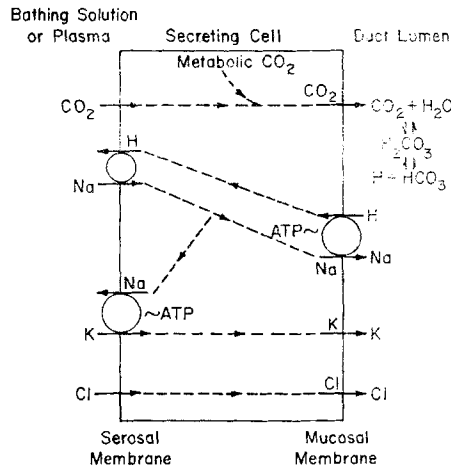


FIGURE 4

#### Active Na Transport and Secretion

The requirement for active Na transport across the mucosal membrane for pancreatic secretion has been demonstrated (1) and secretion is sensitive to factors affecting Na transport (2-4). The effects of  $10^{-6}$  and  $10^{-4}$  M ouabain (Sigma Chemical Company, St. Louis, Mo.) on secretion rate and juice composition are shown in Table IV. The degree of secretion rate inhibition is comparable to that reported by Ridderstap and Bonting (4). The cation composition of the final juice is not strongly affected by ouabain with Na slightly reduced and K elevated only at  $10^{-4}$  M ouabain. The cation composition of the ductal juice was not determined but it is unlikely to be different from that of the final juice. Under normal conditions the ductal and final juice cation compositions are identical (1) and juice Na concentration appears to be set primarily by the isosmotic nature of pancreatic secretion (3).

The anion composition of the juice is strongly affected by ouabain. Even with the moderate secretory inhibition produced by  $10^{-6}$  M ouabain, there is a sharp drop in ductal  $\text{HCO}_3^-$  and a corresponding increase in ductal Cl.

TABLE IV  
EFFECT OF OUABAIN ON PANCREATIC SECRETION\*

	Experiment 1		Experiment 2		Experiment 3	
	Control	+Ouabain	Control	+Ouabain	Control	+Ouabain
$10^{-6}$ M Ouabain						
Secretion rate (mg/h)	181	117	309	200	352	244
Final juice composition (meq/liter)						
Na	152 ± 1	146 ± 1	154 ± 1	151 ± 1	151 ± 1	153 ± 1
K	6.71 ± 0.05	6.96 ± 0.06	7.35 ± 0.05	7.10 ± 0.04	6.31 ± 0.08	7.50 ± 0.10
Cl	105.0 ± 0.8	117.1 ± 0.8	82.8 ± 0.4	100.5 ± 0.5	87.3 ± 0.4	106.4 ± 0.6
HCO <sub>3</sub>	57 ± 3	41 ± 4	84 ± 4	58 ± 3	70 ± 5	54 ± 5
Ductal juice composition (meq/liter)						
Cl	72.3 ± 0.7	102.7 ± 0.6	73.4 ± 0.4	90.0 ± 0.4	73.8 ± 0.5	103.0 ± 0.7
HCO <sub>3</sub>	87 ± 6	51 ± 3	92 ± 4	68 ± 3	86 ± 5	58 ± 5
$10^{-4}$ M Ouabain						
Secretion rate (mg/h)	352	24	365	31	368	39
Final juice composition (meq/liter)						
Na	151 ± 1	147 ± 1	156 ± 1	154 ± 2	154 ± 2	145 ± 1
K	6.31 ± 0.08	7.78 ± 0.10	7.95 ± 0.10	7.31 ± 0.12	7.31 ± 0.12	8.41 ± 0.08
Cl	87.3 ± 0.4	118.1 ± 0.5	73.0 ± 0.7	116.7 ± 0.5	79.6 ± 0.4	112 ± 1
HCO <sub>3</sub>	70 ± 5	37 ± 4	83 ± 5	40 ± 5	82 ± 5	32 ± 5
Ductal juice composition (meq/liter)						
Cl	73.8 ± 0.5	112.4 ± 0.7	—	—	—	—
HCO <sub>3</sub>	86 ± 5	44 ± 4	—	—	—	—

\* The results of individual experiments with juice composition reported ± SE in analysis. Estimated error in the determination of the secretion rate is ± 5 mg/h.

This marked decrease in the HCO<sub>3</sub> content of the primary secretion is greater than would be expected from the secretion rate changes alone (1) suggesting that ouabain inhibits the secretion of HCO<sub>3</sub> more strongly than that of Na.

Secretion also can be inhibited by reducing the environmental Na concentration (2, 3). Table V presents the results of four representative experiments where environmental Na was reduced from its normal value of 143 meq/liter to 100, 75, and 50 meq/liter while maintaining normal osmolality by replacing environmental NaCl with either sucrose or choline chloride. With a bath Na of 100 meq/liter using sucrose replacement the Na concentration in the smallest extralobular ducts (<100 μm in diameter), which should approximate the composition of the primary secretion, is not significantly different from that under control conditions. However, a marked reduction in ductal Na is observed with a bath Na of 75 and 50 meq/liter using both sucrose and choline chloride replacement. Juice K is markedly increased above control values at all environmental Na concentrations. The use of sucrose versus choline chloride replacement at a bath Na of 75 meq/liter does not significantly affect the cation content of the juice although sucrose replacement appears to inhibit secretion to a greater extent which may reflect the simultaneous reduction in environmental Cl. With low Na, the Na

TABLE V  
EFFECT OF A LOW Na ENVIRONMENT ON PANCREATIC SECRETION\*

	Secretion rate	Na	K	Cl	HCO <sub>3</sub> ‡
	mg/h	meq/liter	meq/liter	meq/liter	meq/liter
Control	402 (382-436)				
Final juice		150.8 (149.6-152.0)	6.55 (6.44-6.78)	77.4 (72.0-81.6)	80 (74-86)
Ductal juice				67.2 (64.0-72.4)	90 (87-92)
<i>Sucrose replacement</i>					
Na = 100 meq/ liter ( <i>m</i> = 4)	219				
Final juice		143.2	8.99	65.1	87
Ductal juice		148 (145-150)	8.9 (8.8-9.1)	59.2 (57.7-61.4)	97 (92-100)
Na = 75 meq/liter ( <i>m</i> = 4)	94				
Final juice		118.8	9.76	56.1	73
Ductal juice		128 (125-133)	10.9 (10.6-11.2)	47.4 (45.2-48.6)	92 (90-96)
<i>Choline chloride replacement</i>					
Na = 75 meq/liter ( <i>m</i> = 4)	182				
Final juice		118.2	9.81	71.6	83
Ductal juice		124 (121-127)	10.2 (9.9-10.4)	65.4 (62.0-69.2)	90 (86-93)
Na = 50 meq/liter ( <i>m</i> = 5)	149				
Final juice		94.3	12.7	69.5	85
Ductal juice		96 (95-98)	12.9 (11.2-14.0)	60.7 (58.0-66.6)	94 (88-97)

\* The results of four individual experiments. Control values represent mean of all experiments with the range indicated in parentheses. For low environmental Na conditions *m* represents the number of micropuncture samples obtained in each experiment and the ductal juice composition presented is the mean of the micropuncture samples with the range indicated in parentheses.

‡ Juice HCO<sub>3</sub> concentrations under both control and low Na conditions have been estimated from the requirement of electroneutrality as described in the text.

concentration in the final juice is less than that in the smallest extralobular ducts. This is seen most strikingly with sucrose replacement at an environmental Na of 75 meq/liter and could be due to the diffusion of Na out of the duct down its concentration difference or to the dilution of the juice by the diffusion of sucrose and water into the duct. The presence of sucrose in the juice is indicated by the fact that juice osmolality remains essentially constant, in equilibrium with its environment even though the total ion content

of the juice is markedly reduced. The mean juice osmolality is 289 mosmol/kg H<sub>2</sub>O (range: 285–292,  $n = 4$ ) at an environmental Na of 75 meq/liter using sucrose replacement versus  $284 \pm 1$  mosmol/kg H<sub>2</sub>O ( $\pm$  SE,  $n = 24$ ) under normal conditions. Mean juice osmolalities of 280 (range: 276–285,  $n = 4$ ) and 279 mosmol/kg H<sub>2</sub>O (range: 274–282,  $n = 5$ ) for environmental Na concentrations of 75 and 50 meq/liter, respectively, using choline chloride replacement also require the presence of choline in the juice.

Juice HCO<sub>3</sub> in these experiments has been estimated from the requirement of electroneutrality between the major secreted ions. Juice HCO<sub>3</sub> can be estimated accurately from the concentrations of Na, K, and Cl under normal secretory conditions (1) and using sucrose to replace environmental NaCl cannot affect the requirement of electroneutrality between these four major secreted ions. With choline chloride replacement juice HCO<sub>3</sub> has been estimated from the requirement of electroneutrality between the five major secreted ions (Na, K, Cl, HCO<sub>3</sub>, and choline). The total juice cation concentration (Na + K + choline) was assumed to be 155 meq/liter based on a comparison of juice osmolality with control conditions when juice osmolality is  $284 \pm 1$  mosmol/kg H<sub>2</sub>O and the total juice cation content (Na + K) is  $158.8 \pm 0.4$  meq/liter ( $n = 24$ ). In contrast to the sharp reduction in ductal HCO<sub>3</sub> produced by secretory inhibition with ouabain, ductal HCO<sub>3</sub> remains high in a low Na environment with both sucrose and choline chloride replacement. The maintenance of a high ductal HCO<sub>3</sub> concentration here cannot be attributed to alteration in the environmental Cl concentration since the Cl concentration is unchanged using choline chloride replacement.

The different effects of ouabain and low Na on ductal HCO<sub>3</sub> concentration as well as the ability of acid-base alterations to affect Na secretion suggest that the Na and H transport mechanisms are not independent. We have previously reported that the effects of ouabain and low Na on cellular pH and Na concentration are consistent with a Na-H exchange mechanism at the serosal membrane (14). This exchange mechanism utilizes the potential energy of the Na concentration difference from bath to cell, produced by the Na-K pump, to achieve net uphill H transport from cell to bath associated with HCO<sub>3</sub> secretion. The data in Table VI indicate that the Na-H exchange mechanism is involved in pancreatic electrolyte secretion. Increasing the environmental pH at constant HCO<sub>3</sub> concentration continues to stimulate secretion and the secreted Na output even after inhibition of active cation transport with  $10^{-6}$  M ouabain. If the Na and H transport systems were independent and if secretion is made Na limited, factors affecting H transport, such as acid-base alterations should have no effect on the secretion rate and, in particular, on the secreted Na output. The effects of bath pH and pCO<sub>2</sub> on the secretion rate and ductal HCO<sub>3</sub> concentration in the presence of ouabain are qualitatively similar to those observed without ouabain (cf. Table III).

TABLE VI  
EFFECT OF ENVIRONMENTAL pH ON OUABAIN INHIBITED SECRETION\*

	Control pH <sub>B</sub> = 7.42	+ 10 <sup>-6</sup> M Ouabain		
		pH <sub>B</sub> = 7.12	pH <sub>B</sub> = 7.42	pH <sub>B</sub> = 7.78
<b>Experiment 1</b>				
Secretion rate (mg/h)	181	78	117	106
Na output (μeq/h)	27.5	11.4	17.0	15.7
Na (meq/liter)	152±1	146±1	146±1	148±1
HCO <sub>3</sub> (meq/liter)				
Final juice	57±3	41±2	41±4	41±4
Ductal juice	87±6	57±3	51±3	51±3
<b>Experiment 2</b>				
Secretion rate (mg/h)	309	139	200	232
Na output (μeq/h)	47.7	21.3	30.2	35.2
Na (meq/liter)	154±1	154±1	151±1	152±1
HCO <sub>3</sub> (meq/liter)				
Final juice	84±4	47±2	58±3	45±2
Ductal juice	92±4	65±3	68±3	56±3

\* Bathing solution pH was varied by changing the CO<sub>2</sub> content of the gas mixture at constant HCO<sub>3</sub> concentration. Juice composition is reported ±SE in analysis. Estimated error in determination of the secretion rate is ±5 mg/h.

#### DISCUSSION

The evidence presented above strongly argues that HCO<sub>3</sub> secretion by the pancreas results from the primary production of a transtubular pH difference to which HCO<sub>3</sub> accommodates itself passively by permeating as CO<sub>2</sub> rather than active transport of the HCO<sub>3</sub> ion per se. The ability of the pancreas to secrete acetate in the absence of exogenous HCO<sub>3</sub> at nearly the same rate that HCO<sub>3</sub> is secreted under normal conditions indicates that secretion does not have a strong requirement for HCO<sub>3</sub> and is more consistent with the secretion via active H transport. In addition to acetate we have tested other weak organic acid anions: formate, propionate, and butyrate (15). All promote pancreatic secretion at greater rates than those obtained in PO<sub>4</sub> buffers and all anions are found in the juice at higher concentrations than those in the bathing solution. However, acetate is the most effective in attaining secretion rates and juice anion concentrations that are comparable to those obtained during normal HCO<sub>3</sub> secretion. Schulz et al. (16) also have found that the perfused cat pancreas secretes at greater rates when exogenous HCO<sub>3</sub> is replaced by sulfamerazine (2-sulfanilamido-4-methyl-pyrimidine) than when replaced by PO<sub>4</sub>. Secretion rates and juice anion concentrations attained with sulfamerazine, however, are much less than those observed with acetate, which may, in part, be explained by differences in the size and lipid solubility of their corresponding un-ionized acids. The secretion of sulfamerazine, a noncarboxylic acid, by the perfused cat pancreas further argues against HCO<sub>3</sub> secretion via a relatively nonspecific anion pump.

Comparison of the effects of environmental factors on  $\text{HCO}_3^-$  and acetate secretion also support their secretion by a common mechanism involving active H transport. For example, the increase in the  $\text{HCO}_3^-$  or acetate content of the primary secretion as the environmental pH is reduced is not explicable in terms of the normal ductal juice  $\text{HCO}_3^-$ -secretion rate behavior (1) but is consistent with the H transport mechanism. At a lower pH, the concentration of the un-ionized acid is increased increasing the flux of the un-ionized acid in response to the primarily produced transtubular pH difference. This would facilitate the attainment of an equilibrium distribution of the anion between bath and juice and reduce the passive back diffusion of H into the duct lumen. Schulz et al. (16) have reported a similar relationship between the concentration of ionized sulfamerazine in the juice and the concentration of an un-ionized sulfamerazine in the vascular perfusate.

The marked difference in the effect of Diamox on  $\text{HCO}_3^-$  and acetate secretion supports the H transport mechanism. If Diamox specifically inhibited a  $\text{HCO}_3^-$  pump which is also capable of transporting acetate, comparable degrees of secretory inhibition would be expected. With the H transport mechanism, however, inhibition of carbonic anhydrase activity would have a stronger effect on  $\text{HCO}_3^-$  secretion. During  $\text{HCO}_3^-$  secretion Diamox would alter the transtubular pH difference by disrupting the pH- $\text{HCO}_3^-$ - $\text{CO}_2$  equilibria and inhibit the response of  $\text{HCO}_3^-$  to the transtubular pH difference by suppressing the rate of hydration of  $\text{CO}_2$  to  $\text{HCO}_3^-$ . In contrast, Diamox would have a smaller effect on the transtubular pH difference during acetate secretion because the juice  $\text{pCO}_2$  and  $\text{HCO}_3^-$  concentration, which determine juice pH, are much smaller and Diamox would not affect the ability of acetate to respond to this pH difference. Schulz et al. (16) have also reported that Diamox inhibits secretion by more than 50% even at the low rates produced when the vascular system of the cat pancreas is perfused with  $\text{HCO}_3^-$ -free  $\text{PO}_4$  buffers, indicating that Diamox markedly affects the secretion of endogenously produced  $\text{HCO}_3^-$ . The difference between the effect of Diamox on secretion in  $\text{HCO}_3^-$ -free  $\text{PO}_4$  and acetate environments probably reflects differences in juice  $\text{HCO}_3^-$  concentration (cf. Table I) and the H-donating capacity of acetic acid.

The cellular pH behavior in Fig. 3 provides the strongest argument for the H transport mechanism.  $\text{HCO}_3^-$  secretion and the maintenance of cellular pH require the active transport of either  $\text{HCO}_3^-$  from bath to cell or H from cell to bath across the serosal membrane (1). Since cellular pH in an acetate environment exhibits a similar dependence on environmental pH to that in a  $\text{HCO}_3^-$  environment, H must be the actively transported species. Acetate has no buffering capacity in the physiological pH range and thus the cellular pH could not be affected by the transport of acetate ions across the serosal membrane by a  $\text{HCO}_3^-$  pump.

The pancreatic secretion rate can be altered by small changes in environ-



mental factors affecting the transport of either Na or H which indicates that the active transport of both ions are the rate-determining steps in secretion. Furthermore, the ability of factors affecting H transport, such as environmental pH or  $\text{HCO}_3$  concentration, to affect Na transport also and vice versa suggests that their transport mechanisms are not independent. In the gall bladder, increasing the environmental pH has been found to increase cation permeability presumably by the titration of fixed charges in the membrane making it more electronegative (17). Such a mechanism might explain the stimulation of secretion rate and secreted Na output by environmental pH under normal conditions by increasing the passive permeability of the serosal membrane to Na making it more available to the mucosal Na pump. However, this mechanism is not consistent with the continued ability of environmental pH to stimulate the secretion rate and Na output after secretion has been partially inhibited with ouabain. Ouabain increases cellular Na concentration (14) so that secretion should not be limited by the availability of Na to the mucosal pump. Thus the stimulatory effect of pH on secretion must occur through the H transport mechanism and strongly argues for coupling between the transport of Na and H.

Calculation of the electrochemical potential differences across the mucosal and serosal membranes indicates that pancreatic electrolyte secretion requires active Na transport across the mucosal membrane and active H transport across the serosal and possibly the mucosal membrane as well (1). Coupled with the demonstrated sensitivity of secretion to factors affecting both Na and H transport this suggests that transport across both cell membranes is important in determining the secretion rate and juice composition. Based on the evidence we have presented above and elsewhere (1, 14) we propose the mechanism for pancreatic secretion depicted schematically in Fig. 4. This mechanism satisfies the energetic requirements of secretion and also explains the action of environmental factors which control the juice secretion rate and composition as well as the cellular composition. At the serosal membrane transport occurs via a Na-H exchange mechanism which is linked indirectly to metabolism through the serosal Na-K pump. While maintaining the normal high K, low Na cellular environment, the Na-K pump provides the energy in the form of the Na concentration difference across the serosal membrane to pump H out of the cell against its electrochemical potential difference via the Na-H exchange mechanism. At the mucosal membrane transport also occurs via a Na-H exchange mechanism. The electrochemical potential differences for Na and H across the mucosal membrane, however, require that this exchange mechanism be directly linked to metabolism (1). The secretion of K and Cl into the juice is not rate limiting under normal secretory conditions although their transport may not be purely passive (1). For example, net K transport across the serosal membrane likely occurs via the

Na-K pump but the amount of K in the juice is too small for this to be the rate-limiting event in secretion.

We originally proposed serosal Na-H exchange on the basis of the effects of ouabain or a low Na environment on the cellular composition and the electrochemical potential difference facing net H transport out of the cell across the serosal membrane (14). When secretion and net H transport from duct lumen to bath are inhibited by 90% or more with ouabain, the cellular pH and the serosal pH difference are unaffected. However, at a comparable degree of secretory inhibition in a low Na environment the cellular pH and the serosal pH difference are markedly increased (cellular pH increases from a normal value of  $7.28 \pm 0.04$  to  $7.48 \pm 0.04$  in an environmental Na concentration of 54 meq/liter [14]). Furthermore, ouabain or low Na do not significantly affect the cellular PD:  $-40 \pm 2$  mV ( $\pm$  SE,  $n = 15$ ) cell negative under control conditions versus  $-41 \pm 4$  mV ( $n = 2$ ) with an environmental Na of 30 meq/liter and  $-45 \pm 3$  mV ( $n = 5$ ) with  $10^{-4}$  M ouabain (15). Thus the serosal electrochemical potential difference for H is not significantly altered by ouabain but is increased by a low Na environment. This behavior suggests that in the presence of ouabain net H transport across the mucosal and serosal membranes associated with electrolyte secretion is inhibited to similar extents whereas in the presence of low Na net H transport is more strongly inhibited at the mucosal membrane than at the serosal membrane. These observations are difficult to reconcile with an independent H transport system but can be readily explained in terms of the secretory mechanism presented above. Ouabain inhibits the serosal Na-K pump which is actively transporting Na in the opposite direction to that required for secretion and as a result electrolyte secretion is inhibited directly by the reduction in the energy source for serosal H transport, the serosal Na concentration difference. The corresponding inhibition of mucosal H transport suggested by the cellular pH behavior likely is due to the effect of disruption of the normal cellular ionic environment on the mucosal Na-H pump and this would result in the inhibition of mucosal Na transport as well. For example, in vitro pancreatic Na-K-dependent ATPase activity is inhibited by high Na concentrations (4). Direct inhibition of the active Na transport component of secretion at the mucosal membrane by ouabain seems unlikely. Direct action on a mucosal Na-K pump would require the presence of ouabain in the duct lumen (18-20), but evidence suggests that ouabain does not rapidly permeate cell membranes (21). A low Na environment inhibits secretion directly by reducing the cellular Na concentration (14) thereby increasing the concentration difference against which Na is actively transported across the mucosal membrane. The requisite inhibition of mucosal H transport is produced through the mucosal Na-H pump. At the same time serosal H transport is inhibited by the reduction of the serosal Na concentration difference in a low

Na environment. However, for a comparable degree of secretory inhibition, serosal H transport is more strongly inhibited by ouabain than by a low Na environment, as suggested by the cellular pH behavior. The potential energy for serosal H transport is proportional to the logarithm of the bath to cellular Na concentration ratio,  $Na_B/Na_C$  and the ratio is reduced from a control value of 2.8 (143 meq/liter/51 meq/kg cell  $H_2O$ ) to 1.1 (143/129) for 90% secretory inhibition with ouabain versus a value of 1.5 (54/35) for 90% inhibition with low Na (14). With a one-for-one exchange mechanism, where the effect of cellular PD is eliminated this would represent a 90% reduction in the potential energy for serosal H transport with ouabain and a 60% reduction with low Na.

The differences in the  $HCO_3$  content of the juice in the presence of ouabain or low Na also can be reasonably explained in terms of the mechanism of secretory inhibition indicated above. In a low Na environment the major inhibitory effect is at the mucosal Na-H pump where both ions are actively transported. As a result secretion of Na and  $HCO_3$  are inhibited to comparable extents and juice  $HCO_3$  concentration remains high. With ouabain the activity of the serosal Na-H pump is more strongly affected. Since Na can still diffuse passively into the cell across the serosal membrane serosal H transport would be more strongly affected than Na transport. As a result  $HCO_3$  secretion is more strongly inhibited than that of Na and the  $HCO_3$  content of the juice falls.

The results of our acid-base studies also can be explained in terms of the secretory mechanism in Fig. 4. The changes in secretion rate produced by variations in the environmental pH,  $pCO_2$ , and  $HCO_3$  concentration can be correlated with the pH differences facing net H transport from duct lumen to bathing solution as indicated in Fig. 5. The pH differences have been calculated from the experiments reported in Figs. 1-3. The ductal juice pH was computed from the ductal  $HCO_3$  concentrations in Fig. 2 *b* assuming that the  $pCO_2$  of the bathing solution and ductal juice are identical. An elevated juice  $pCO_2$  suggested by Hubel's data (10) would reduce juice pH somewhat, but is unlikely to have any effect on the changes in the pH differences produced by environmental alterations. The cellular pH was estimated from the dependence on environmental pH in Fig. 3. The pH differences are shown in the sense that a positive value indicates net H transport against its concentration difference. Electrochemical potential differences for H, expressed as an equivalent pH difference, are also presented and have been calculated assuming that the cellular and transtubular PD's are unaffected by acid-base alterations. While PD measurements were not carried out in these experiments, this assumption appears reasonable. More drastic environmental alterations such as low Na or  $HCO_3$ -free  $PO_4$  buffers do not significantly alter the cellular PD and produce only small changes, of the order

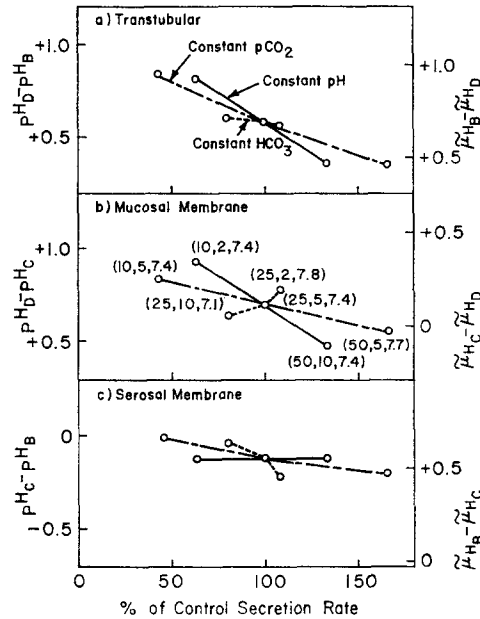


FIGURE 5. Effect of environmental acid-base alterations on the (a) transtubular, (b) mucosal, and (c) serosal pH and electrochemical potential differences faced by active H transport. Subscripts B, C, and D indicate bathing solution, cell, and duct lumen, respectively. The electrochemical potential differences are expressed as an equivalent pH as explained in the text. The three curves represent acid-base alterations at constant  $\text{HCO}_3^-$  concentration (---), constant pH (—), and constant  $\text{pCO}_2$  (— · —). The values in parentheses indicate the environmental  $\text{HCO}_3^-$  concentration,  $\text{pCO}_2$  (as percent  $\text{CO}_2$  in the gas mixture), and pH, respectively.

of 2–3 mV, in the transtubular PD, which averages  $-7.4 \pm 0.3$  mV ( $\pm$  SE,  $n = 54$ ) lumen negative under normal conditions (1, 15). Thus any change in the electrical portion of the electrochemical potential difference here is likely to be small in comparison with the changes in the concentration portion.

The pancreas is a heterogeneous tissue and only the ductal cells have been clearly implicated in electrolyte secretion (1, 5). Thus in attributing changes in secretion rate to changes in the pH differences across the mucosal and serosal membrane we must assume that the cellular pH, determined on whole tissue, reflects that of the secreting cell. The effects of ouabain or low environmental Na on cellular pH support this assumption. As noted above, the electrochemical potential difference against which H is transported across the serosal membrane is not significantly altered by ouabain and is increased by low Na. In addition to supporting the Na-H exchange mechanism, these results are more consistent with the cellular pH behavior of an actively secreting cell where H transport across both cell membranes is important in de-

termining cellular pH. With Na-H exchange in a nonsecreting cell where H transport across the serosal membrane alone is likely to determine cellular pH, ouabain or low Na would be expected to decrease the serosal electrochemical potential difference for H.

In Fig. 5 *a* a general correlation is observed between the secretion rate and the overall transtubular pH difference; decreasing the transtubular pH differences by acid-base alterations at constant pH, constant pCO<sub>2</sub> or constant HCO<sub>3</sub> concentration stimulate secretion. The secretion rate is controlled by the environmental pH and HCO<sub>3</sub> concentration and the individual effects of pH and HCO<sub>3</sub> can be seen by examining the differences across the mucosal and serosal membranes, Figs. 5 *b* and *c*, respectively. Increasing environmental HCO<sub>3</sub> at constant pH reduces the mucosal pH difference but has no effect on the serosal pH difference. Since HCO<sub>3</sub> is the major buffer, the overall transtubular pH difference is determined by the distribution of HCO<sub>3</sub> between the bath and the juice.

$$\text{pH}_D - \text{pH}_B = \log \frac{(\text{HCO}_3)_D}{(\text{HCO}_3)_B}$$

As bath HCO<sub>3</sub> is increased, ductal HCO<sub>3</sub> increases (cf. Fig. 2 *b*) but the HCO<sub>3</sub> distribution ratio, (HCO<sub>3</sub>)<sub>D</sub>/(HCO<sub>3</sub>)<sub>B</sub> and hence the transtubular pH difference decrease. Thus increased environmental HCO<sub>3</sub> stimulates secretion by reducing the overall difference against which H is actively transported. This HCO<sub>3</sub> effect is mediated at the mucosal membrane since the cellular pH and hence the serosal pH difference appear to depend only on the environmental pH (14).

Increasing environmental pH at constant HCO<sub>3</sub> concentration reduces the serosal pH difference but has the opposite effect on the mucosal membrane with a resulting small reduction in the transtubular pH difference. This suggests that environmental pH stimulates secretion primarily by its action at the serosal membrane which is consistent with its control over cellular pH. The antagonistic effect on the two cell membranes may explain why secretory stimulation produced by pH alterations is much less than that produced by HCO<sub>3</sub>. The data in Fig. 1 *a* tend to support this view. The stimulatory effect of pH is enhanced as the environmental HCO<sub>3</sub> concentration is increased. At a higher HCO<sub>3</sub> concentration the mucosal pH difference is reduced making secretion more dependent on H transport across the serosal membrane and hence more susceptible to pH stimulation.

The greatest secretory changes are observed when environmental pH and HCO<sub>3</sub> concentration are altered simultaneously at constant pCO<sub>2</sub>. The overall transtubular pH difference is not markedly different from that produced by HCO<sub>3</sub> alterations at constant pH. However, the additive effects of pH and HCO<sub>3</sub> are indicated by the decrease in the pH difference across both cell membranes.

This interpretation of the effects of acid-base alterations on pancreatic electrolyte secretion lead to the conclusion that H transport across both cell membranes plays an important role in determining the secretion rate. The secretory mechanism in Fig. 4 not only is consistent with the interpretation but also explains the ability of these environmental factors to affect simultaneously the secreted output of Na which varies directly with the secretion rate. Environmental pH, with its stimulatory action on H transport at the serosal membrane, stimulates the secretion of Na into the cell via the exchange mechanism. The ability of environmental pH to stimulate secretion after partial inhibition with ouabain is also consistent with the primary action of both ouabain and environmental pH on serosal Na-H exchange. Decreasing the serosal pH difference tends to counteract the reduction in the Na concentration difference stimulating net transport of both Na and H through the serosal exchange mechanism. Similarly environmental  $\text{HCO}_3^-$  which stimulates H transport by reducing the mucosal pH difference also stimulates Na transport through the mucosal Na-H pump.

The degree of coupling between Na and H in the exchange mechanisms cannot be determined from present data. The simplest mechanism would be one-for-one exchange which would eliminate the effects of PD. The potential energy of the serosal Na concentration difference is sufficient to pump H out of the cell against a maximum pH difference of 0.5 units on a one-for-one basis (14). As noted previously the secretion rate versus environmental pH data in Fig. 1 linearly extrapolate to zero secretion at an environmental pH of about 6 for both  $\text{HCO}_3^-$  and acetate secretion. Interestingly, the cellular pH dependence in Fig. 3 predicts that the cellular pH would be 6.3 and 6.5 at an environmental pH of 6.0 for  $\text{HCO}_3^-$  and acetate environments which is in reasonable agreement with the maximum predicted for one-for-one exchange but is not proof of its existence. The difference in the Na and  $\text{HCO}_3^-$  content of the juice (150 meq/liter for Na versus a maximum of 110 meq/liter for  $\text{HCO}_3^-$ ) and the partial dissociation between the secreted outputs of Na and  $\text{HCO}_3^-$  as evidenced by the fact that juice  $\text{HCO}_3^-$  varies with the secretion rate while Na is independent of the secretion rate (1) need not reflect the order of coupling in the exchange mechanisms. They could be produced by differences in the passive permeabilities of Na and H. The possibility of an additional pathway for Na also cannot be eliminated. Na can diffuse passively into the cell across the serosal membrane and a separate Na pump could be present at the mucosal membrane.

In summary, the mechanism we propose for pancreatic electrolyte secretion seems entirely consistent with the observed dependence of secretion on environmental factors. Secretion rate and juice composition are determined primarily by the environmental Na and  $\text{HCO}_3^-$  concentrations and pH through their effects on the Na and H concentration differences across the mucosal and serosal membranes. The effects of ouabain and environmental pH on

secretion rate as well as juice and cellular composition provide strong support for serosal Na-H exchange linked indirectly to metabolism through the Na-K pump. The effects of low Na and environmental HCO<sub>3</sub> also support the mucosal Na-H exchange pump although the evidence here is not as strong. However, a coupled mucosal pump would facilitate the cellular control of secretion since direct input of metabolic energy occurs only at this cell face. Thus our mechanism is not only consistent with the data presented above but also provides a simple effective means for the cellular control of secretion.

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