A Micropuncture Investigation of the Whole Tissue Mechanism of Electrolyte Secretion by the In Vitro Rabbit Pancreas

C. H. SWANSON and A. K. SOLOMON

From the Biophysical Laboratory, Harvard Medical School, Boston, Massachusetts 02115. Dr. Swanson's present address is the Department of Chemical Engineering and Materials Science, University of Minnesota, Minneapolis, Minnesota 55455.

ABSTRACT Micropuncture techniques have been used to examine electrolyte secretion by the in vitro rabbit pancreas. The concentration profiles of the major secreted ions and digestive protein and the electrical potential profile within the pancreatic ductal system have been determined during spontaneous and secretin-stimulated secretion. The active transport of both Na and HCO₃ are the rate-controlling steps in primary secretion. Spontaneous secretion is produced primarily within the intralobular ducts. The anion composition of this primary secretion depends on the secretion rate with HCO₃ ranging from about 70 meq/liter at low rates to about 110 meq/liter at high rates. With secretin stimulation the smaller extralobular ducts also secrete and this extralobular secretion has a higher HCO₃ content than that of the intralobular secretion. In the main collecting duct the anion composition of the juice is modified further by Cl-HCO₃ exchange which appears to be a passive process depending on the average residence time of the juice in the main duct.

Little is known definitively about the mechanisms responsible for pancreatic electrolyte secretion either at the whole tissue or the cellular level. The pancreas secretes a fluid rich in HCO₃ into a highly branched ductal system through which it is transported to the duodenum to aid in the neutralization of gastric secretion. Due to the complex geometry of the ductal system, electrolyte secretion has been studied primarily by collecting and analyzing the juice leaving the gland. The characteristic feature of the externally collected juice is a hyperbolic dependence of the juice HCO₃ concentration on the secretion rate (1). Over the years three major whole tissue mechanisms, admixture (2, 3), Cl-HCO₃ exchange (4), and unicellular (5-7) have been proposed to explain this dependency. These mechanisms differ only in
the number and function of the individual processes assumed to be involved, however, and are indistinguishable by this black box approach.

Clearly, further insight into the mechanisms of pancreatic secretion requires information from within the ductal system. Recent investigators (8, 9) have adapted the micropuncture techniques of renal physiology for use with the in vivo rabbit pancreas and have obtained the first direct information on the concentrations of Cl and HCO₃ within the ductal system. These investigations, however, have not fully clarified the whole tissue aspects of secretion in part because analysis has been restricted only to the major secreted anions, Cl and HCO₃.

In this and a subsequent paper¹ we present the results of a quantitative investigation of the secretion of the four major ions, Na, K, Cl, and HCO₃, present in the juice of the in vitro rabbit pancreas. The diffuse structure of the rabbit pancreas not only makes it highly suitable for micropuncture studies but also allows the oxygen requirements of the tissue to be satisfied from a controlled saline environment gassed with 95% O₂ - 5% CO₂ without vascular perfusion (10). The in vitro preparation was used since secretion can be studied over a much wider range of experimental conditions than is possible in vivo (7, 10). In this paper the whole tissue aspects of electrolyte secretion are considered; the actively transported species are determined and the individual processes contributing to electrolyte secretion are identified and characterized. The cellular mechanisms of pancreatic electrolyte secretion are considered in a subsequent paper.¹

METHODS

After fasting for 12–24 h, New Zealand white rabbits of either sex weighing 1.5–2.5 kg were anesthetized by intravenous injection of 0.7 ml/kg Dial-Urethane (Ciba Pharmaceutical Co., Summit, N. J.). The pancreas is located in the mesomentum of the first loop of duodenum distal to the pylorus. The main duct was cannulated through the intestinal papilla and the pancreas containing intestinal loop was removed following the procedure of Rothman and Brooks (10). The loop was mounted horizontally with the attached loop of rectum facing up in a Lucite chamber containing 350 ml of a Krebs-Henseleit HCO₃ buffer (118.5 mM NaCl, 4.75 mM KCl, 2.54 mM CaCl₂, 1.18 mM KH₂PO₄, 1.18 mM MgSO₄, 24.9 mM NaHCO₃, and 5 mM glucose) (11). The bathing solution was oxygenated and maintained at a pH of 7.40 ± 0.03 by bubbling 95% O₂-5% CO₂ through sintered glass frits in each corner of the chamber, maintained at 30° ± 0.5°C with an immersion heater, and mixed with a small motorized impellor. The chamber was covered to prevent the loss of CO₂ from the large gas-liquid interface and only the center third exposed during micropuncture.

As suggested by Reber and Wolf (8), the most suitable portion of the pancreas for micropuncture lies beneath the attached loop of rectum which was carefully dissected

away after the tissue had been mounted. After this procedure, the tissue was allowed to equilibrate for an hour before micropuncture was begun.

**Micropuncture**

A schematic drawing of the pancreatic ductal system is presented in Fig. 1. Small lobules of acinar parenchyma, scattered throughout the connective tissue of the duodenal loop, are connected through a network of ducts, the extralobular ducts, consisting of a single main collecting duct 200–500 μm in diameter which empties into the duodenum and a highly branched network of smaller ducts 50–200 μm in diameter. The lobules are ramified by a further network of ducts, the intralobular ducts, 5–50 μm in diameter which terminate in the blind-ending acini lined by the protein-secreting acinar cells. For technical reasons outlined below, in vitro micropuncture has been restricted to the extralobular ducts.

Because of the irregular geometry of the ductal system no quantitative measure of the position within the ductal system exists. However, the caliber of the ducts increases continuously in the direction of fluid movement, from the acini to the main collecting
duct, and duct diameter has been used as a qualitative index of position in the ductal system.

Micropuncture was carried out in the field of a 40 X stereoptic microscope using transillumination. Micropipettes with tips of 5-15 μm in diameter prepared from 1 mm OD glass capillaries were placed in a pipette holder filled with Sudan black-stained mineral oil mounted on a micromanipulator (Laboratory Associates Inc., Belmont, Mass.). Samples of ductal juice were obtained by the following free-flow procedure. The bathing solution over the puncture site was covered with mineral oil. The duct was punctured, and a small column of oil injected into the duct. Samples of 50-500 nl (requiring 1-10 min) were collected by aspirating at a rate sufficient to keep the position of the oil column steady. The micropipette was then rapidly withdrawn into the overlying oil layer and the tip occluded with oil. The sample was immediately transferred to a piece of siliconized glass under oil and its volume determined by measuring the drop diameter with a calibrated eyepiece micrometer.

During the experimental period which lasted 60-90 min, four to eight micropuncture samples were generally obtained and the final juice was also collected in tared vessels under oil. Complete analyses required 12-24 h, and between analyses the micropuncture samples were stored in thin siliconized glass capillaries (50-200 μm in diameter) between columns of oil. Experiments with test solutions indicated that samples as small as 25 nl could be stored in this manner for 48 h without detectable loss of water.

Since micropuncture was carried out in a saline environment, contamination of the sample of ductal juice with the bathing solution was possible, and in order to determine possible contamination it was necessary to have a marker in the bathing solution which did not affect pancreatic secretion, was not secreted, and could be assayed in aliquots of the micropuncture sample. ATP was chosen as such a marker and assayed by a modified version of the method of Addanki et al. (12). ATP (di-Na salt, Sigma Chemical Co., St. Louis, Mo.) was added to the bathing solution at concentrations of 0.5 mg/ml and had a half-life as ATP of 30-60 min both in the bathing solution and when added separately to the juice. Since ATP binds Ca and has been shown to affect the properties of red cell membranes (13), Ca was added with the ATP in a 2:1 molar ratio and neither the secretion rate nor the final juice composition were affected. Contamination of 2-10 % could be detected by analyzing 25- to 50- nl aliquots of the micropuncture sample and the bathing solution. All contaminated samples were discarded. Contamination of less than 5 % did not markedly affect estimates of ductal juice composition. At the maximum HCO₃ concentration of about 110 meq/liter in the pancreatic juice of the rabbit, 5 % contamination would result in an underestimate of ductal juice HCO₃ by about 4 meq/liter which corresponds to the experimental error in HCO₃ analysis.

The minimum requirement of a 25 nl aliquot for ATP analysis effectively ruled out micropuncture of the intralobular ducts. The higher probability of contamination in puncturing the small intralobular ductules clearly necessitated the use of the ATP analysis. However, the time necessary to aspirate 50 nl, the minimum volume necessary for all analyses, from most of the intralobular system is prohibitive.
Analysis

With the exception of Na and K analysis, the same ultramicroanalytical procedures were used on samples of both ductal and final juice. Na and K in the final juice were determined by flame photometry (model 143, Instrumentation Laboratory, Inc., Lexington, Mass.) while Na and K in the ductal juice were determined with a helium plasma picomole spectrophotometer (Clifton Technical Physics, Hartford, N.Y.) following the procedure of Vurek and Bowman (14). Cl was analyzed by the electro-

metric titration method of Ramsay et al. (15).

Juice pH was determined on 25-50- nl samples in equilibrium with known partial pressures of CO 2 using an antimony electrode developed by Vieira and Malnic (16) and a Keithley model 603 electrometer amplifier (Keithley Instruments Inc., Cleveland, Ohio). The antimony electrode was found to have a slope of 40-45 mV/pH in the normal juice pH range of 7.5-8.0 in agreement with the values reported by Vieira and Malnic (16). Juice HCO 3 concentration was calculated from the equilibrium pH using the Henderson-Hasselbalch equation

$$pH = pK + \log \frac{HCO_3}{\alpha pCO_2}$$

with appropriate values for $\alpha$ and $pK$ obtained from Severinghaus et al. (17, 18).

Juice protein concentration was determined by the Lowry method (19) using bovine serum albumin as a standard. The protein concentration in ductal juice (normal range 1-10 mg/ml) could be determined accurately using the microcolorimeter developed by Solomon and Caton (20) which increased the sensitivity of the Lowry method to 0.1 $\mu$g protein by reducing the volume of the total reaction mixture to 0.150 ml.

Cell and transtubular electrical potential differences (PD's) were measured using standard 3 M KCl Ling-Gerard microelectrodes and a Keithley model 610A electrometer (Keithley Instruments Inc.). Microelectrodes having a resistance of 2-5 M$\Omega$ and a tip potential of less than 5 mV were connected to a calomel electrode. The reference calomel electrode in 3 M KC1 was connected to the bathing solution by an agar bridge containing HCO 3 buffer. All PD's are measured and reported relative to a zero potential in the bathing solution.

Cellular electrolyte content was determined by incubating 30- to 60- mg pieces of pancreatic tissue (about 1 g wet tissue per animal) for an hour or more in the normal HCO 3 buffer containing RISA ($[^{14}I]$ human serum albumin, Abbott Laboratories, Chemical Marketing Div., Chicago, Illinois) as an extracellular marker. One portion of the tissue was dried at 100°C for 48 h to determine total tissue water and the other extracted in distilled water for 48 h to determine electrolyte content and extracellular space. Cell pH was determined following the same procedure after incubating tissue in the presence of RISA and $[^{14}C]$DMO (5, 5-dimethyl oxazolidene-2, 4-dione-[2,14C], New England Nuclear, Boston, Mass.). RISA was assayed with a gamma scintillation counter (model 4222, Nuclear-Chicago Corp., Des Plaines, Ill.) and $[^{14}C]$DMO with a liquid scintillation counter (model 6801 s, Nuclear-Chicago Corp.).
ACTIVELY TRANSPORTED SPECIES

Results

The identification of the actively transported species is based on the construction of the electrochemical potential difference profiles from the source of the secreted ions to the duct lumen at the site of active transport. These differences are computed from chemical concentration and PD measurements. The contribution of metabolic CO$_2$ to HCO$_3$ secretion has been estimated to be 5–10% of the total secreted HCO$_3$ (7, 21) so that the primary source of all secreted ions is the plasma in vivo and the bathing solution in vitro (7, 21–24). The basic assumption also is made that all ions reach the duct by transport through the cell. Thus cellular composition and PD data are required so that electrochemical potential differences across both the bath facing or serosal and duct facing or mucosal membranes can be computed.

| TABLE I |
| SATISFACTION OF THE REQUIREMENT OF ELECTRONEUTRALITY IN PANCREATIC JUICE |
| Final juice (meq/liter ± SD) | Ductal juice (meq/liter ± SD) |
| Na | 151 ± 2 (11)* |
| K | 7.3 ± 0.8 (11) |
| Na + K | 158 ± 2 (11) |
| Cl | 77 ± 15 (11) |
| HCO$_3$ | 81 ± 17 (11) |
| Cl + HCO$_3$ | 159 ± 5 (11) |
| Ductal juice (meq/liter ± SD) | |
| Na | 151 ± 2 (11)* |
| K | 7.3 ± 0.8 (11) |
| Na + K | 158 ± 2 (11) |
| Cl | 77 ± 15 (11) |
| HCO$_3$ | 81 ± 17 (11) |
| Cl + HCO$_3$ | 159 ± 5 (11) |

* The number of individual samples from six experiments.

The evidence for active HCO$_3$ transport is already compelling. The HCO$_3$ concentration in the final juice increases with the secretion rate approaching four times plasma concentrations in the rabbit (10) and six times that in the dog (1) and cat (25). For this reason we have focused attention primarily on the other secreted ions. However, as indicated in Table I, HCO$_3$ concentrations in the juice can be accurately estimated from the concentrations of Na, K, and Cl and the requirement of electroneutrality. In a series of experiments Na and K were determined in the final juice and Cl and HCO$_3$ determined in both the ductal and final juice. The sum of Na and K was not significantly different from that of Cl and HCO$_3$ in either the ductal or final juice. In spite of wide variation in the juice anion composition (HCO$_3$ range 40–111 meq/2

Active HCO$_3$ transport, as used in this paper, signifies an energy-requiring process and not necessarily active transport of HCO$_3$ per se. HCO$_3$ secretion could be coupled to the active transport of H or OH. No attempt is made in this paper to discriminate between these two mechanisms, which are considered in detail in a subsequent paper of this series (footnote 1).
there is little variation in the sum of Cl and HCO₃ which reflects the isosmotic nature of pancreatic secretion (26, 27). Thus, information on HCO₃ secretion can be obtained from data on the other secreted ions when the HCO₃ concentration is not computed from pH determinations.

Table II presents the average concentration profiles of Na, K, and Cl in the extralobular ducts obtained during spontaneous secretion. The cation concentrations are constant throughout the extralobular ductal system. Both juice Na (range 145–160 meq/liter) and K (range 6–8 meq/liter) exceed their concentrations in the bathing solution (143 and 5.9 meq/liter, respectively). The final juice cation data are in excellent agreement with those reported by Rothman and Brooks (10) for the final juice of the in vitro rabbit pancreas.

From animal to animal the anion composition of both the ductal and final juice is highly variable, reflecting both a dependence on the rate of secretion and animal variability. However, the average Cl concentration profile is typical of that seen in any individual experiment in which the ductal Cl concentration is essentially constant over the range of duct sizes examined but is generally significantly less than the final juice concentration. Since the largest ducts punctured were generally more proximal portions of the main collect-
ing duct, the region where the increase in Cl concentration occurs is the main collecting duct. As will be shown below, this change is the result of Cl-HCO₃ exchange. Cl concentration range is 47–100 meq/liter in ductal juice and 52–113 meq/liter in the final juice, in all cases less than the 128 meq/liter in the bathing solution. These data agree with the values reported by Rothman and Brooks (10) for the final juice in vitro and by Reber and Wolf (8) and Schulz et al. (9) for ductal juice in vivo.

The average HCO₃ concentration profile can be constructed from the data in Table II and the requirement of electroneutrality; the HCO₃ concentration is constant in the small extralobular ducts and decreases in the main collecting duct to its final juice value. The data in Table I also indicate that the HCO₃ concentration in the ductal juice is greater than that in the final juice (P < 0.05 for paired samples of ductal and final juice). Juice HCO₃ varies from 50 to 110 meq/liter, which is greater than the 25 meq/liter in the bathing solution.

Fig. 2 presents the results of a typical experiment when pancreatic secretion is stimulated with secretin (GIH Research Unit, Karolinska Institut, Stockholm). Only the anion profiles are significantly affected by the hormone. Following the direction of fluid movement in the smallest extralobular ducts, Cl decreases and, by electroneutrality, HCO₃ increases. Schulz et al. (9) also have reported a progressive decline in Cl concentration from the acini to the final juice with secretin stimulation in vivo. Cl-HCO₃ exchange is evident in the main collecting duct in the present data but is not apparent in the data of Schulz et al. (9). This may be due to the differences in secretion rate produced by secretin in vivo and in vitro as indicated in the next section.

The cellular composition of the rabbit pancreas determined by whole tissue analysis is presented in Table III and is in good agreement with data reported by Solomon (6) for the pancreas of the dog. The HCO₃ concentration has been calculated from the cellular pH, assuming that the cellular pCO₂ is the same as that of the bathing solution and that the pK of H₂CO₃ is not altered by the intracellular environment. In an inhomogeneous tissue such as the pancreas the cellular pH calculated using DMO, a weak acid, may not be the true mean cellular pH but has been shown to be directly related to the true mean cellular pOH (28). However, DMO can be used to determine the true mean cellular concentration of weak acid anions such as HCO₃. We have presented our results in terms of pH since it is still meaningful and more widely used than pOH.

The transtubular PD exhibits considerable variability between animals (range −4 to −10 mV, lumen negative) but this variability does not correlate with either the secretion rate (60–425 mg/h) or the final juice anion composition (Cl range 70–112 meq/liter). The transtubular PD also does not vary with position in the extralobular ducts averaging −7.4 ± 0.3 mV.
FIGURE 2. Concentration profiles of Na, K, and Cl in the extralobular ducts for a typical experiment with secretin stimulation (215 U/liter). Secretin increased the secretion rate from 135 to 342 mg/h. Values at the far right are those of the externally collected juice. Bars represent the standard error in analysis.

FIGURE 3. The electrochemical potential difference profiles of the four major ionic constituents of pancreatic juice. The electrochemical potentials have been calculated from concentration and PD data as outlined in the text and expressed as an equivalent electrical potential. The shaded area for Cl and HCO$_3$ represent the range in ductal electrochemical potential due to variations in juice anion composition.

TABLE III
INTRACELLULAR COMPOSITION OF THE RABBIT PANCREAS*

<table>
<thead>
<tr>
<th></th>
<th>Bathing solution (meq/liter)</th>
<th>Rabbit pancreas (meq/kg cell H$_2$O ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>143</td>
<td>45 ± 3 (5)</td>
</tr>
<tr>
<td>K</td>
<td>5.9</td>
<td>126 ± 2 (5)</td>
</tr>
<tr>
<td>Cl</td>
<td>128</td>
<td>60 ± 4 (5)</td>
</tr>
<tr>
<td>HCO$_3$</td>
<td>25</td>
<td>18 ± 2 (6)</td>
</tr>
<tr>
<td>pH</td>
<td>7.40</td>
<td>7.25 ± 0.03 (6)</td>
</tr>
</tbody>
</table>

* The dry to wet tissue weight ratio was 0.191 ± 0.010 (± SE) and the magnitude of the extracellular space was 0.160 ± 0.013 ml/g wet tissue (± SE). The number of experiments are indicated in parentheses. Intracellular HCO$_3$ concentration is based on cell pH measurement.
(SE, \( n = 54 \)). However, the transtubular PD in the intralobular ducts, 
\(-9.1 \pm 0.4 \text{ mV (SE, } n = 30\) \), is significantly higher \((P < 0.05)\) than that 
in the extralobular ducts which could reflect either the site of electrolyte 
secretion or a Donnan effect due to increased protein concentration near the 
site of protein secretion. These in vitro PD data are in general agreement 
with the \(-5 \text{ mV observed in vivo in the pancreas of the rabbit (9) and the} \)
cat (29).

The PD between the ductal cells of the extralobular ducts and the bathing 
solution averages \(-40 \pm 2 \text{ mV (SE, } n = 15\) \) cell negative. Intralobular 
cell PD (acinar or ductal cells) averages \(-34 \pm 2 \text{ mV (SE, } n = 6 \) \). The 
difference may reflect greater cell injury in the intralobular cells. The in 
vitro preparation is not highly suited for cell PD measurement since tissue 
movement cannot be eliminated completely. However, fluid pressure in the 
extralobular ducts provides greater stabilization for the extralobular cells 
than can be provided for the intralobular cells.

**Discussion**

Using the data presented thus far, the electrochemical potential difference 
profiles from bath to cell to duct of the four major secreted ions can be 
constructed using the equation

\[
\Delta \mu_{ij} = zF\psi_{ij} + RT \ln \left( \frac{C_i}{C_j} \right)
\]

where \(\Delta \mu_{ij}\) is the electrochemical potential difference between the \(i^{th}\) 
and \(j^{th}\) phase (e.g. cell and duct), \(\psi_{ij}\) is the electrical potential difference, 
\(C_i\) and \(C_j\) are the chemical concentrations in the two phases, \(R\) is the gas 
constant, \(T\) is the absolute temperature, \(z\) is the valence of the ion, and \(F\) 
is Faraday's constant. No attempt has been made to account for possible 
differences in the activity coefficients between phases. Although the cellular 
site of electrolyte secretion has not yet been identified, three reasons can be 
advanced to support the use of concentration data from the extralobular 
ducts in computing the ionic electrochemical potential differences between 
the juice and the bathing solution which will be representative of that found 
throughout the entire ductal system. First, both the juice in the extralobular 
ducts and the final juice are in osmotic equilibrium with the bathing solu-
tion (27). The constant total cation concentration in the extralobular ducts 
can be extended to include the juice in the intralobular ducts if it is as-
sumed that the intralobular juice is also isosmotic with the bathing solution. 
If it is further assumed that the transport mechanisms are the same in the 
intralobular and extralobular ducts, the individual Na and K concentra-
tions in the extralobular juice can also be extended to include the intralobular 
juice. The recent observation of Mangos and McSherry (30) that the Na and
K concentrations in the acinar fluid in vivo are identical with that of the final juice indicates that these assumptions are probably valid. Second, the Cl concentrations range that has been found in the acinar fluid in vivo (9, 30) falls within the range of our data for the extralobular ducts in vitro so that the present data on both Cl and HCO₃ probably apply to the juice in the intralobular ducts as well. Third, although secretin stimulation changes the local shape of the anion concentration profiles in the extralobular ducts, the total range is not altered. Thus our computed differences between the juice and the bathing solution will, on the average, apply to both spontaneous and secretin-stimulated secretion.

The electrochemical potential differences across the serosal and mucosal membranes are computed using the cellular composition determined by whole tissue analysis. The exocrine pancreas is an inhomogeneous tissue containing two major cell types: acinar and ductal. As is shown in the next section, the ductal cells are involved in electrolyte secretion but the role of the acinar cells in electrolyte secretion is not known. Since it is operationally impossible to separate the two cell types, we must assume that the composition determined on the whole tissue corresponds to that of the secreting cell. Also in calculating intracellular concentrations no correction has been made for the possibility that a portion of the extracellular space determined with RISA represents ductal fluid whose composition is different from that of the bathing solution. Only the anion concentrations would be affected since Na and K concentrations in the bathing solution and the juice are nearly equal. An upper limit of the error introduced in the calculation of the intracellular Cl and HCO₃ concentrations can be computed if we assume: (a) that the composition of the ductal fluid is 110 meq/liter HCO₃ and 50 meq/liter Cl, the maximum deviation from the bathing solution composition; and (b) that the ductal fluid represents half of the total extracellular space, a reasonable estimate of the upper limit of the ductal volume. These calculations indicate that the intracellular Cl concentration has been underestimated by no more than 10 meq/kg cell H₂O and HCO₃ overestimated by no more than 5 meq/kg cell H₂O. Since these represent the maximum errors, ignoring the possibility of an inhomogeneous ion distribution in the extracellular space will not significantly affect the computed electrochemical potential differences.

Fig. 3 presents the computed electrochemical potential difference profiles for the four major secreted ions expressed as an equivalent electrical potential relative to a zero potential in the bathing solution. The requirement of active HCO₃ transport is confirmed conclusively by the maximum electrochemical...

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3 Electron micrographs (32) indicate that the centroacinar and ductal cells are structurally very similar; we have considered them as a homogeneous class of cells.
potential difference of 45 mV between the bathing solution and the ductal juice. Active transport is necessary across the serosal membrane against a 30 mV difference which is due primarily to cellular electronegativity. The maximum electrochemical potential difference of 15 mV across the mucosal membrane suggests that HCO₃ may be actively transported at this cell face as well. This requirement is not fully convincing, however, because of the variable magnitude of the mucosal difference which is due to the variable juice HCO₃ concentration. The electrochemical potential difference profiles of HCO₃ and OH are nearly identical since HCO₃ is the primary buffer in all phases. Hence, these differences alone cannot provide information as to whether HCO₃ secretion is the result of active transport of the HCO₃ ion or is a secondary process coupled to the active transport of H or OH. This topic will be considered in a subsequent paper.

Although the electrochemical potential difference for Na from the bathing solution to ductal juice is downhill by about 5 mV, the uphill difference of over 60 mV across the mucosal membrane requires active Na transport. This difference is composed of nearly equal contributions due to cellular electronegativity and a low cellular Na concentration, and it is highly unlikely that cellular variations due to tissue inhomogeneity or the inclusion of activity coefficients in the computations can alter this basic conclusion. Whether active Na transport at the mucosal membrane is an essential requirement for pancreatic secretion, however, depends upon our initial assumption that ions are transported through the cells. If a significant intercellular pathway for Na is present, Na could reach the duct lumen passively. However, several pieces of evidence can be advanced to support our initial assumption that the major transport pathway for all ions, and Na in particular, is transcellular rather than intercellular. First, ouabain, which inhibits active cation transport, inhibits both pancreatic Na-K-dependent ATPase activity and electrolyte secretion to nearly identical extents over an ouabain concentration range of 10⁻⁷ to 10⁻³ M (footnote 1 and reference 32). Furthermore at low concentrations (10⁻⁹ to 10⁻⁸ M) ouabain stimulates both Na-K-dependent ATPase activity and secretion (27, 32). Second, electrolyte secretion is sensitive to the environmental Na concentration. Replacing Na in the bathing solution with Li abolishes electrolyte secretion by the in vitro rabbit pancreas which is consistent with the inability of the Na pump to handle Li (7). Similarly, replacing environmental NaCl by sucrose strongly inhibits secretion with normal Na concentrations of 150–160 meq/liter maintained in the juice when the environmental Na concentration is as low as 80 meq/liter in the cat (26) and 100 meq/liter in the rabbit. The sensitivity of secretion to ouabain and environmental Na concentration is readily explicable in terms of the transcellular Na pathway with active Na transport at the mucosal membrane being an integral part of the secretory mechanism but is difficult
to rationalize in terms of Na transport through an intercellular pathway, particularly the ability of ouabain at low concentrations to stimulate secretion. Third, we have presented evidence for a Na-H exchange mechanism at the serosal membrane based on the effects of ouabain and a low environmental Na concentration on intracellular pH and Na concentration that is consistent with the secretion of both HCO₃ and Na through transcellular pathways (33). Finally, in the next section it is shown that spontaneous electrolyte secretion occurs in the intralobular ducts, but that secretion also occurs in the small extralobular ducts during secretin stimulation. During spontaneous secretion no significant changes in juice Cl and HCO₃ concentrations are observed in the small extralobular ducts even at the lowest secretion rates (100–200 mg/h) in spite of large electrochemical potential differences between the bathing solution and the ductal juice which suggests that the intercellular pathway is impermeant to anions. Therefore, while ion transport through an intercellular pathway cannot be ruled out entirely, the evidence argues strongly that the major transport pathway is transcellular and that active Na transport across the mucosal membrane is an essential requirement for electrolyte secretion.

The electrochemical potential difference profiles for K and Cl display similar characteristics: an increase from bath to cell and a decrease of an equal or greater magnitude from cell to duct. K transport across the serosal membrane can be attributed to the Na-K pump which actively transports K into the cell in exchange for Na in order to maintain the normal high K and low Na concentrations in the cell. However, it is unlikely that the active transport of K from bath to cell is a controlling factor for normal secretion since K is a minor constituent of the juice (the secreted output of K is \( \frac{1}{10} \) that of HCO₃ and \( \frac{1}{50} \) that of Na).

The similarity of the K and Cl profiles suggests that Cl also is actively transported across the serosal membrane. However, the relatively small magnitude of the serosal difference for Cl (20 mV) is not a compelling argument for active Cl transport, especially since differences in the activity coefficients between the bathing solution and the cell have not been considered. However, even if active Cl transport is present, three arguments indicate that it cannot be a controlling factor in electrolyte secretion. First, the final juice Cl concentration varies inversely with the secretion rate and is always less than the plasma or bathing solution concentration (1, 10, 25). Second, replacing Cl in the bathing solution by HCO₃ enhances secretion. Both of these characteristics are consonant with HCO₃ rather than Cl limitation. Third, although Cl is present in significant concentrations in the pancreatic juice of the rabbit, in other species such as the dog (1) or the cat (25) which exhibit the same characteristic dependence of the juice anion composition on the secretion rate, Cl, like K, is a relatively minor constituent...
of the juice being as low as 20 meq/liter at high secretion rates. The only
evidence suggestive of Cl limitation is that secretion is inhibited when Cl in
the bathing solution is replaced by the more impermeant SO\textsubscript{4} ion (7), but
this could also reflect the inability of the rabbit pancreas to secrete HCO\textsubscript{3} at
concentrations much above 110 meq/liter. The sensitivity of secretion to
environmental factors affecting Na and HCO\textsubscript{3} transport coupled with the
fact that these two ions are the major juice constituents therefore strongly
argues that active transport of Na and HCO\textsubscript{3} are the rate-controlling steps
in pancreatic electrolyte secretion.

SITE OF ELECTROLYTE SECRETION

Results

The site of electrolyte secretion cannot be determined from the concentration
profiles of the secreted ions alone. For example, the constant composition of
the juice in the smaller extralobular ducts during spontaneous secretion
could be the result of a secretion of a uniform composition or of the ducts
acting as a passive conduit. The sites of secretion can be localized by examining
the concentration profile of the secreted proteins. Since the digestive proteins
are secreted by the acinar cells which line the most proximal portions of the
ductal system, any change in protein concentration distal to the acini must
reflect a site of electrolyte and water secretion. From the changes in protein
concentration it also should be possible to estimate qualitatively the relative
contribution of different portions of the gland to the total secretion.

The protein concentration profile obtained from a series of experiments
during spontaneous secretion is presented in Fig. 4a. The final juice protein
concentration exhibits considerable variability between animals (normal
range 1–10 mg/ml) and to facilitate comparison results are presented as the
ratio of the protein concentration in the ductal juice, \( P_D \), to that in a simul-
taneously collected sample of final juice, \( P_F \). Hence, a \( P_D/P_F \) of unity indi-
cates that there is no secretion distal to the puncture site; values greater
than unity, distal secretion, and less than unity, distal reabsorption. In the
larger extralobular ducts, \( P_D/P_F \) does not differ significantly from unity.
However, in the smaller ducts large variability is seen which cannot be at-
tributed to experimental error or animal variability. It seems highly unlikely
that this variability is the result of the secretion of electrolytes and water into
the small extralobular ducts in one region and their reabsorption in another.
Such a mechanism would be energetically wasteful and would serve no
useful function since we have found no variations in the electrolyte composi-
tion of the juice in the small extralobular ducts in individual experiments.
A more plausible explanation is that this variability represents differences
in the relative rates of protein and electrolyte secretion by different regions
of the pancreas. The variability disappears in the larger extralobular ducts
as the juice from different parts of the gland is mixed. If this is the case, the average protein concentration ratio in the small extralobular ducts should give an indication of the relative contribution of this region to secretion. The average ratios for the large and small ducts are also presented in Fig. 4a. The distinction between large and small ducts is made at 150 \( \mu \text{m} \) in diameter to isolate the variability in the small duct samples. The average protein concentration ratio is unity in both the large and small extralobular ducts which strongly argues that the intralobular ducts are the major source of spontaneous electrolyte secretion. While the variability makes it impossible to rule out secretion in the small extralobular ducts, a contribution in excess of 10% by volume is unlikely \( (P < 0.01) \).

The results for secretin-stimulated secretion are shown in Fig. 4b. In the large ducts the average protein ratio again is not significantly different from unity, suggesting that this region lacks secretory capability. However, the average protein concentration ratio increases significantly in the small extralobular ducts, indicating that the hormone stimulates electrolyte secretion in this region. The fall in ductal Cl concentration in the small extralobular ducts during secretin stimulation (cf. Fig. 2) further indicates that
this extralobular secretion has a lower Cl and a higher HCO₃ concentration than that of the intralobular secretion. The magnitude of the average protein concentration ratio suggests that the small extralobular ducts produce about 30% of the total juice which corresponds roughly to the degree of secretory stimulation produced in vitro by secretin in these experiments. As previously reported (10), however, the in vitro preparation does not respond well to the hormone. At 215 U/liter in the bathing solution, secretin increases the secretion rate 30–50% on the average as opposed to the 3- to 10-fold increases reported in vivo (30). Therefore, secretin may also stimulate secretion by the intralobular ducts in vivo either by further stimulating cells which also secrete spontaneously or by turning on cells which secrete only in the presence of the hormone. The reason for the poor response of the in vitro preparations to secretin is not known, but if hormonal stimulation is mediated through specific receptor sites, it could be related to diffusional problems encountered when secretin is placed in the bathing solution rather than presented vascu-
larly.4

Discussion

Our general conclusions as to the sites of secretion are supported by the split-drop microperfusion studies of Schulz et al. (9). They perfused the extralobular ducts with plasma solutions containing radioactive inulin and after 3–6 min found little or no change in the volume of the perfusion fluid during spontaneous secretion but large increases in volume during secretin stimulation. Because of the irregular geometry of the ductal system, however, their data cannot be used to estimate the relative contribution of the intralobular and extralobular ducts to secretion. Schulz et al. (9) concluded that the entire ductal system is involved in electrolyte secretion, but our data indicate that the largest extralobular ducts lack secretory capability. The present distinction between the large and small extralobular ducts at 150 μm in diameter has been made to isolate protein concentration variability in the small duct samples and should not be taken as an absolute demarcation between regions which secrete in the presence of the hormone and those which do not. More likely, the secretion occurs in all of the extralobular system except the main collecting duct where Cl-HCO₃ exchange is localized.

The composition of the juice secreted by the extralobular ducts in vitro is qualitatively similar to that found in vivo in spite of the marked difference in the volumetric response of the two preparations to secretin. From changes in the volume and Cl concentration of their microperfusion fluid, Schulz et al. (9) estimated that the Cl content of the extralobular secretion in vivo was 52 meq/liter (and by electroneutrality about 110 meq/liter HCO₃). For

4 Secretin is a sizable polypeptide containing 27 amino acids (34).
the secretin experiments in Fig. 4 where about 30% of the secretion was of extralobular origin, the average Cl concentration of the small and large duct samples were 78 ± 2 and 72 ± 3 meq/liter (± SE), respectively. Taking the former as representative of the composition of the intralobular secretion and the latter that of the final mixture gives a qualitative estimate of a composition of 58 meq/liter (and by electroneutrality about 100 meq/liter HCO₃⁻) for the extralobular secretion in vitro.

Secretion by the extralobular ducts demonstrates that the ductal cells, the only cell type present in this region, are involved in electrolyte secretion. While it seems reasonable to assume that the ductal cells are also involved in secretion within the intralobular ducts, secretion by the acinar cells cannot be ruled out especially in view of the differences in the composition of the intralobular and the extralobular secretions. It is not known whether this difference in HCO₃⁻ secreting capability is due to differences between regions of the pancreas, between cell types, or between cells which secrete spontaneously and those which secrete only in the presence of the hormone. However, it is not an artifact due to the poor secretin response in vitro since this difference in HCO₃⁻ secreting capability also has been found in vivo (9).

### CI⁻—HCO₃⁻ EXCHANGE

**Results**

The absence of changes in protein concentration in the large extralobular ducts argues that the HCO₃⁻ decrease and Cl increase in the main collecting duct is the result of Cl-HCO₃⁻ exchange without net transport of salt and water. Conclusive proof that this is indeed the case is presented in Table IV.

**Table IV**

<table>
<thead>
<tr>
<th></th>
<th>Clᵢ(meq/liter)</th>
<th>Clₒ(meq/liter)</th>
<th>P&lt;ᵢ/Pₒ</th>
<th>P&lt;ᵢ/Pₒ for secretion of isosmotic NaCl⁻</th>
<th>P&lt;ᵢ/Pₒ for reabsorption of isosmotic NaHCO₃⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>88 ± 2</td>
<td>74 ± 2</td>
<td>1.02 ± 0.02</td>
<td>1.19</td>
<td>0.84</td>
</tr>
</tbody>
</table>

* Experimental data are those of the nine samples from the large extralobular ducts reported in Fig. 4a for spontaneous secretion. Values are ± SE and difference between Clᵢ and Clₒ is significant (P < 0.001).
† Protein concentration ratios which would be observed if the Cl change between the ductal and final juice were the result of secretion of NaCl or reabsorption of NaHCO₃ in the main duct. Both are significantly different from the observed value.
which compares the differences in Cl and protein concentration between the ductal and final juice for samples from the large extralobular ducts reported in Fig. 4 a. If the anion changes are the result of a secretion of NaCl into the ducts or reabsorption of NaHCO₃ out of the ducts, the total salt concentration in the secretion or reabsorbate cannot differ from the isosmotic concentration of about 160 meq/liter since juice Na concentration and osmolality do not change in the main duct. The last two rows of Table IV present the protein concentration ratios that should be observed if the anion changes were the result of an isosmotic secretion of NaCl or an isosmotic reabsorption of NaHCO₃. Both values are significantly different from the observed average protein concentration ratio; hence, the anion changes in the main duct must be the result of Cl-HCO₃ exchange.

During spontaneous secretion, the externally collected juice is therefore the product of two distinct processes: primary electrolyte secretion in the intralobular ducts and Cl-HCO₃ exchange in the main collecting duct. The relative contribution of these two processes to the dependence of the anion composition of the final juice on the secretion rate can be assessed from the plots of the Cl concentration in the ductal and final juice (Figs. 5 a and 5 b) and their difference (Clₚ - Clₜ) (Fig. 5 c) as a function of secretion rate. The Cl concentration in the smaller extralobular ducts, Clₚ, is relatively constant in each experiment and is an index of the composition of the primary secretion, while (Clₚ - Clₜ) is a measure of Cl-HCO₃ exchange. In spite of the large animal variability, both Clₚ and (Clₚ - Clₜ) exhibit significant negative correlations with the secretion rate (P < 0.001). Comparison of the slopes of linear regression lines through the data suggests that changes in the primary secretion composition and Cl-HCO₃ exchange contribute equally to the secretion rate dependence of the final juice anion composition. At low secretion rates Cl-HCO₃ exchange may alter juice Cl and HCO₃ by 20 meq/liter or more while at high secretion rates final juice composition approaches that of the primary secretion. The present data also suggest that Cl-HCO₃ exchange ceases to alter juice composition significantly in the range of 500-1,000 mg/h which is well below the maximum secretion rates obtained in vivo in response to secretin.

Discussion

The data of Case et al. (25) support the present conclusion that Cl-HCO₃ exchange occurs in the main collecting duct. They perfused the main duct of the nonsecreting cat pancreas with a high HCO₃ fluid characteristic of the final juice at high secretion rates and found variations in the perfusate anion composition with the rate of perfusion that were similar to the normally observed dependence of the final juice anion composition on the secretion rate. They attributed the differences between the two dependencies to Cl-
HCO₃ exchange in the smaller ducts. The present evidence, however, suggests that this difference is due to variations in the composition of the primary secretion rather than Cl-HCO₃ exchange in the smaller ducts. Even at the lowest secretion rates studied (100–200 mg/h) when main duct Cl-HCO₃ exchange produced changes of 20 meq/liter or more, no significant changes in Cl concentration were observed in the smaller extralobular ducts. Since the surface area per unit volume of ductal fluid varies inversely with the duct diameter, this means that the smaller ducts must have a lower anion permeability than that of the main duct. In vivo micropuncture studies also support this conclusion. Reber and Wolf (8) found no measurable change in the Cl concentration of juice obtained from the same small extralobular duct puncture site before and after blocking flow for 30–60 s. Schulz et al. (9) also have found that the Cl concentration is constant throughout the intralobular ducts. In contrast, Mangos and McSherry (30) have recently reported...
large increases in juice Cl between the acini and the extralobular ducts but sufficient information is not available from their data to determine either the site or the cause of this Cl change. Thus, most of the evidence to date suggest that variations in the anion composition of the juice from the small extralobular ducts with the secretion rate in Fig. 5 b can be attributed to changes in the composition of the primary secretion and not to Cl-HCO₃ exchange.

The transtubular electrochemical potential differences for Cl and HCO₃ in Fig. 3 indicate that Cl-HCO₃ exchange can be achieved passively. The inability of diamox (25) or metabolic inhibitors (35) to alter the anion changes observed in the main duct perfusion studies also supports a passive exchange process. The localization of Cl-HCO₃ exchange in the main collecting duct in our experiments coupled with the demonstrated absence of secretory capability indicate that the main collecting duct has a markedly increased

Figure 6. Schematic representation of pancreatic electrolyte secretion at the whole tissue level. The separation of spontaneous electrolyte and protein secretion in the intralobular ducts indicates functionally distinct and not necessarily spatially distinct processes.
anion permeability and is functionally very different from the rest of the ductal system.

The data presented above are consistent with the whole tissue mechanism for pancreatic electrolyte secretion shown schematically in Fig. 6. Primary secretion, driven by the active transport of Na and HCO₃⁻, is produced spontaneously in the intralobular ducts and in the extralobular ducts as well during secretin stimulation. During spontaneous secretion, the anion composition of the primary secretion varies from about 90 meq/liter Cl and 70 meq/liter HCO₃⁻ at low secretion rates to about 50 meq/liter Cl and 110 meq/liter HCO₃⁻ at high rates. Thus spontaneous secretion appears to follow the unicellular hypothesis (5-7) in that it contains all the ions normally found in the final juice and its composition varies with the secretion rate. Since the HCO₃⁻ concentration of the extralobular secretion during secretin stimulation is greater than that of the intralobular secretion both in vitro and in vivo (9), secretin-stimulated secretion appears to follow the admixture hypothesis (2, 3) in the sense that it is a mixture of two primary secretions (extralobular and intralobular) of different composition. The compositional differences, however, are small and do not represent a separate secretion of Cl and HCO₃⁻ as originally proposed by the admixture hypothesis. Finally, Cl-HCO₃⁻ exchange as originally proposed by Dreiling and Janowitz (4) modifies the composition of the primary secretion in the main collecting duct. The evidence suggests that this is a passive process depending on the average residence time of the juice in the main duct. At low secretion rates, Cl-HCO₃⁻ exchange plays an important role in the conservation of secreted base, but at the high secretion rates normally produced by secretin stimulation in vivo it does not significantly alter juice composition. Thus we have now shown that pancreatic electrolyte secretion is the product of spatially distinct processes which can be separated through the use of micropuncture techniques.

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