Evidence for a Primary Defect of Pancreatic HCO_3^- Secretion in Cystic Fibrosis

KEVIN J. GASKIN,⁽²²⁾ PETER R. DURIE, MARY COREY, PATRICK WEI, AND
GORDON G. FORSTNER

*Division of Gastroenterology and the Kinsmen Cystic Fibrosis Research Centre, The Hospital for Sick Children
Toronto and the Department of Paediatrics, University of Toronto, Toronto, Ontario Canada*

Summary

The specificity of impairment of pancreatic water and HCO_3^- secretion in cystic fibrosis (CF) and the role of this impairment in diagnosing CF were studied in 62 CF patients and 66 non-CF controls. A quantitative pancreatic stimulation test using a duodenal bromsulphthalein marker perfusion technique was performed on each subject. The hourly secretion of trypsin, HCO_3^- , and water was measured. Over three ranges of tryptic activity (<50, 50-1000, and >1000 units per kg body weight per h, selected to represent low, intermediate, and normal acinar function respectively), mean water secretion was significantly lower in CF patients than in controls only when acinar function was low or intermediate. Mean water secretion per kg body weight per h did not vary among controls. In the CF patients, water secretion per kg body weight per h was significantly greater in those with trypsin secretion >1000 (8.28 ± 3.52 ml) than in those with trypsin secretions <50 (4.22 ± 5.8 ml, $P < 0.02$) or 50-1000 (3.07 ± 2.46 ml, $P < 0.001$). Mean HCO_3^- secretion in the CF patients was significantly impaired over all three ranges of tryptic activity when compared with controls. In both CF patients and controls there was a significant positive correlation between HCO_3^- and trypsin secretion. Analysis of covariance showed that HCO_3^- secretion was significantly impaired in the CF patients as compared with the controls over the total range of trypsin secretion.

These data demonstrate that CF patients have impairment of pancreatic water secretion in association with impaired acinar function but in those with normal acinar function, water secretion appears intact. The impairment of water secretion is thus probably the result of destruction of pancreatic tissue. Pancreatic HCO_3^- secretion, however, is generally impaired in CF patients even in those with normal pancreatic enzyme and water secretion. Because impairment of HCO_3^- secretion appears to occur independently of the extent of pancreatic damage, it may represent a primary secretory defect which is related to the defective CF gene. Inasmuch as a few CF patients had HCO_3^- secretory rates within the control range, the diagnosis cannot be excluded by the demonstration of HCO_3^- values within this range.

Speculation

Patients with cystic fibrosis have evidence of exocrine gland dysfunction specifically in regard to electrolyte secretion. In the pancreas, impaired HCO_3^- secretion may be the result of a primary genetic defect. Further investigation of pancreatic HCO_3^- secretion is warranted to elucidate the nature and controlling factors of secretion as they may provide evidence for the underlying abnormality in cystic fibrosis.

Hadorn *et al.* (8) reported that the secretin-pancreozymin stimulation test was useful in assessing pancreatic function in patients with cystic fibrosis (CF). They studied 10 CF patients without steatorrhea to determine whether any anomaly of pancreatic

function could be detected and found a wide range of pancreatic enzyme secretion with some values in the control range. Of considerable importance was their demonstration that pancreatic water and bicarbonate HCO_3^- secretion was markedly impaired; this finding was similar to their later observation in steatorrheic CF patients (9). They suggested that CF patients have a primary defect in pancreatic water and HCO_3^- secretion.

Later, Smalley *et al.* (19) emphasized the importance of the work by Hadorn *et al.* and recommended that the diagnosis of CF be confirmed in non-steatorrheic patients by demonstrating impaired pancreatic enzyme and/or water and HCO_3^- secretion. They indicated that this approach was most applicable to young non-steatorrheic patients in whom chronic suppurative lung disease had not yet developed and in whom the sole evidence for diagnosis was the finding of high sweat chlorides.

Our initial experience was not always consistent with these observations. We found that both steatorrheic and non-steatorrheic patients with CF occasionally secreted normal amounts of HCO_3^- and water. Therefore, we reinvestigated these aspects of pancreatic function using a larger number of patients and a more quantitative pancreatic stimulation technique to determine the specificity of the defect in pancreatic water and HCO_3^- secretion and its use in diagnosing CF.

MATERIALS AND METHODS

The study involved 62 patients with CF (age range 0.1-33 years), a control group of 66 non-CF subjects (age range 0.1-28 years) that included 12 patients with Shwachman's syndrome (exocrine pancreatic hypoplasia and neutropenia), 11 normal individuals and 43 patients with various gastrointestinal problems. The diagnosis of CF was based on clinical symptoms and signs, chest roentgenograms and pulmonary function abnormalities, with sweat chloride levels consistently greater than 60 mEq/litre. The diagnosis of Shwachman's syndrome was established by evidence of exocrine pancreatic hypoplasia, persistent or cyclic neutropenia and, in four patients, associated metaphyseal dysostosis. The 43 patients with gastrointestinal diseases had a variety of disorders, including congenital and surgical short gut syndromes, celiac disease, malnutrition, chronic intractable diarrhea and failure to thrive. Seven subjects who had no gastrointestinal disease on investigation and four volunteers comprised the group of 11 normal subjects. Their investigation was approved by the Experimentation Committee of the Hospital for Sick Children, Toronto.

Pancreatic stimulation tests were done as part of the assessment of pancreatic function in each individual. Pancreatic enzyme supplements were withheld for at least 48 h before subjects entered the study. Five patients with Shwachman's syndrome had multiple stimulation tests: three of the five patients had a total of two tests each and the remaining two patients had three tests each.

The pancreatic stimulation test, which was done after an overnight fast, was a modification of a previously published method (6). Under fluoroscopic control, a double lumen tube was inserted

into the duodenum. It was constructed so that one lumen opened near the ampulla of Vater and the other had several ports at least 5 cm distal to this, with the most distal being positioned close to the ligament of Treitz. Through the proximal opening a nonabsorbable bromsulphthalein (BSP) marker solution, 0.5 ml of 5% BSP in 250 ml of 5% mannitol, was infused into the duodenum at a constant rate of 1.6 ml/min throughout the test period. The pancreatic juice mixed with marker solution was aspirated through the distal ports and was collected for four 20-min periods. The first, or baseline period, was allowed for equilibrium of marker perfusion fluid with pancreatic secretions. In the three remaining periods the duodenal aspirate was collected during a continuous intravenous infusion of secretin and pancreozymin (Boots, U.K.), both at a dose of 0.125 units per kg body weight per min. All duodenal juice was collected on ice. Throughout the test, gastric juice was aspirated through a separate nasogastric tube by low Gomco suction to prevent acid contamination of the duodenal contents.

Tryptic activity was assessed by a titrimetric assay (3) and HCO_3^- by an autoanalyser colorimetric method (18). BSP concentration was determined by a colorimetric technique (17) so that the volume correction factor

$$\frac{\text{BSP infused}}{\text{BSP aspirated}}$$

could be calculated. The correction factor times the measured volume of duodenal aspirate gave the total volume of duodenal aspirate, thus correcting for the incomplete collection. The actual volume of pancreatic secretion was calculated by subtracting the volume of marker solution infused from the total volume of duodenal aspirate.

RESULTS

The relationship between pancreatic trypsin secretion and pancreatic water secretion is shown for CF patients and controls in Figure 1. Three ranges of trypsin secretion were selected for comparison, to represent low, intermediate and normal acinar function: <50 units, 50–1000 units and >1000 units per kg body weight per h respectively. BSP marker recovery rates did not differ significantly between the control and CF patients in the three ranges of trypsin secretion (<50 units: recovery rate in controls, $59.4 \pm 31.2\%$ versus CF, $63.29 \pm 27.38\%$, mean ± 1 S.D. NS; 50–1000 units: controls, $70.74 \pm 19.64\%$ versus CF, $72.87 \pm 21.0\%$, NS; >1000 units: controls, $69.79 \pm 22.13\%$ versus CF $67.02 \pm 18.86\%$ NS). Compared with controls, water secretion (Fig. 1) was significantly lower in the CF patients except when trypsin secre-

Fig. 1. Range of H_2O secretion in ml/kg body weight/h for controls (● Shwachman's syndrome, ○ other controls) and CF patients (■) in three ranges of trypsin secretion measured in units/kg body weight/h. [] = mean ± 1 S.D. Comparison of CF patients versus controls: <50, $P < 0.001$; 50–1000, $P < 0.001$; >1000, not significant.

tion was >1000 units per kg body weight per h. At all levels of function there was a considerable overlap of results, with many CF patients (even those with reduced enzyme secretion) having water secretion in the control range. The mean water secretion per kg body weight per h did not vary among the three control groups (<50, 11.47 ± 7.09 ml; 50–1000, 8.63 ± 4.36 ml; and >1000, 11.00 ± 5.07 ml) indicating that the ability of the subjects without CF to maintain their water secretion did not depend on preservation of acinar function. Mean water secretion was significantly greater in CF patients with trypsin secretion >1000 units per kg body weight per h (8.28 ± 3.52 ml) than in those with trypsin secretion of 50–1000 units per kg body weight per h (3.07 ± 2.46 ml, $P < 0.001$) or those with secretion of <50 units per kg body weight per h (4.22 ± 5.18 ml, $P < 0.02$).

In contrast to water secretion, the mean HCO_3^- secretion (Fig. 2) was significantly lower in the CF patients than in controls in all three ranges of tryptic activity but again some CF patients had values in the control range. The mean HCO_3^- secretion also rose with tryptic activity in both the control and CF groups and therefore, HCO_3^- secretion appeared to correlate with the degree of acinar preservation.

Figure 3 shows HCO_3^- output plotted against trypsin in con-

Fig. 2. Range of HCO_3^- secretion in mEq/kg body weight/h for controls (● Shwachman's, ○ others) and CF patients (■) in three ranges of trypsin secretion measured in units/kg body weight/h. [] = mean ± 1 S.D. Comparison of CF patients versus controls: <50, $P < 0.001$; 50–1000, $P < 0.001$; >1000, $P < 0.001$.

Fig. 3. Secretion of HCO_3^- (mEq/kg body weight/h) versus trypsin (units/kg body weight/h) in Shwachman's syndrome patients (●) and other controls (○). A significant direct linear relationship is demonstrated between HCO_3^- and trypsin secretion ($y = 0.25 + 1.29 \times 10^{-4}X$, $r = 0.67$, $P < 0.001$).

Fig. 4. Secretion of HCO_3^- (mEq/kg body weight/h) versus trypsin (units/kg body weight/hr) in CF patients (■). A significant direct linear relationship is demonstrated between HCO_3^- and trypsin secretion ($y = 0.04 + 1.19 \times 10^{-4}X$, $r = 0.73$, $P < 0.001$). Control regression line (---) as in Fig. 3.

trols. There was a direct linear relationship between HCO_3^- and trypsin secretion ($y = 0.25 + 1.29 \times 10^{-4}X$, $r = 0.67$, $P < 0.001$). When HCO_3^- output was plotted against trypsin in CF patients (Fig. 4) there was a significant direct linear relationship ($y = 0.04 + 1.19 \times 10^{-4}X$, $r = 0.73$, $P < 0.001$). Plotted on the same graph is the regression line (dotted) for the control subjects from Figure 3 which is almost parallel to the CF regression line. The regression line for the 26 CF patients with trypsin secretions greater than 50 units per kg body weight per (not plotted) was almost identical to the total regression line. The direct linear relationship of HCO_3^- to trypsin output remained highly significant ($y = 0.04 + 1.18 \times 10^{-4}X$, $r = 0.64$, $P < 0.001$) indicating that no negative bias to the results was introduced by including the 36 patients with very low acinar function.

Regression lines for the total cystic fibrosis and control groups were compared by analysis of covariance and no significant difference was found between the two slopes. There was a significant difference in the adjusted means when the lines were fitted to a common slope ($t = 6.48$, $P < 0.001$). One can conclude, therefore, that HCO_3^- secretion was significantly less per unit of trypsin output in the CF patients at all levels of acinar function.

Patients with Shwachman's syndrome had moderate to severe impairment of acinar function and are designated by black circles in Figures 1 and 2. In both ranges of tryptic activity, their water and HCO_3^- secretion did not differ significantly from that of other non-CF controls according to the unpaired t test. They maintained significantly higher water and HCO_3^- than the CF patients in both ranges of tryptic activity: <50 units of trypsin secretion; (1) HCO_3^- secretion (mEq/kg/h), Shwachman's 0.2 ± 0.1 versus CF 0.04 ± 0.05 , mean ± 1 S.D. $P > 0.001$ and (2) water secretion (ml/kg/hour), Shwachman's 10.82 ± 5.99 versus CF 4.22 ± 5.18 , $P < 0.01$. 50–100 units of trypsin secretion; (1) HCO_3^- secretion (mEq/kg/hour), Shwachman's 0.27 ± 0.21 versus CF 0.1 ± 0.09 , $P < 0.01$ and (2) water secretion (ml/kg/hour), Shwachman's 9.21 ± 5.04 versus CF 3.07 ± 2.46 , $P < 0.01$).

DISCUSSION

Our results show that CF patients have defective pancreatic HCO_3^- secretion at all levels of pancreatic enzyme secretion, whereas only those patients with decreased enzyme secretion have a defect in water secretion. Because the HCO_3^- secretory defect is not dependent upon the degree of pancreatic preservation, it may, as suggested by Hadorn *et al.* (8), occur as the result of a primary genetic abnormality in CF patients. In contrast, our evidence does not support the presence of a coexisting genetic defect in pan-

creatic water secretion since our CF patients with normal enzyme secretion had normal water secretion. Furthermore, over the three ranges of trypsin secretion, some CF patients had water and HCO_3^- secretion values within the control range. Thus we cannot support the contention that values in the control range exclude a diagnosis of CF (19).

In man the origin of pancreatic enzyme, water and HCO_3^- secretion has to be extrapolated from animal data. There is compelling evidence both histochemical (1, 20) and through induction of pancreatic acinar cell atrophy in experimental animals (7) that the acinar cell is the source of pancreatic enzymes. Scratcherd and Case (16) have indicated that the major locus of pancreatic HCO_3^- secretion is the centroacinar and ductular epithelium. Although, admittedly the evidence for this is only indirect, it is very supportive of this hypothesis. Several points are worthy of mention. Grossman and Ivy (7) have shown that intravenous injection of alloxan in rats induces vacuolization in pancreatic ductular epithelium and reduces the response of the gland to secretin stimulation, without effecting enzyme secretion in response to pancreozymin. Fölsch and Creutzfeldt (5) have recently extended this observation. They induced pancreatic acinar atrophy in rats fed a copper deficient diet and oral penicillamine and showed the gland continues to produce water and HCO_3^- in response to secretin but no enzyme in response to pancreozymin. Furthermore, the pancreatic micropuncture and microperfusion studies of Lightwood and Reber (13) performed in the cat have shown that the major HCO_3^- and water secretion occurs between the intralobular duct and the main pancreatic duct *i.e.*, in the interlobular system. Analysis of intralobular duct secretion revealed a reasonably high HCO_3^- secretion of 52 mEq/litre. Although its exact site of release remained unidentified, it most probably originated from small intralobular duct epithelium and centroacinar cells, as it has been demonstrated that secretin does not alter the electrical properties of acinar cells (15).

In the present study, water secretion was well maintained in the control subjects even when trypsin output was low, indicating that ductal function was relatively unaffected. This was not the case in patients with CF because water secretion was significantly reduced when trypsin output fell below the normal range. Thus, a sufficient degree of ductal impairment must have developed in the CF patients to affect fluid secretion. However, normal water output was seen in CF patients whose acinar function appeared normal as judged by trypsin secretion. Thus our data failed to provide any evidence that impaired water secretion is a primary gene-related abnormality in CF but instead suggested that fluid secretion falls as a result of greater functional destruction of ductular tissue in this disease.

In contrast to water secretion, HCO_3^- output fell when acinar impairment increased in both controls and CF patients. This agrees with the above suggestion that considerable HCO_3^- secretion must originate in a site that is closely associated with acini. This site could include centroacinar cells and small intralobular duct cells, which in CF are probably affected by the same disease process that impairs acinar function. Because a similar explanation would apply to the impairment of HCO_3^- secretion in the controls with poor acinar function (mainly patients with Shwachman's syndrome), the consistently lower HCO_3^- secretion in the CF patients over all ranges of acinar function must have an alternate explanation. It would seem likely that this defect occurs throughout the pancreatic centroacinar and ductular system and also occurs in addition to the disease process effecting the intralobular secretory unit. Evidence to support this proposal is provided by the group of CF patients with normal acinar function who presumably have a preservation of acinar cells and adjacent structures and yet still demonstrate a HCO_3^- secretory defect. Inasmuch as pancreatic HCO_3^- secretion is a centroacinar and ductular process, CF patients would appear to have a defect in this process. Considering that it was generally present in the CF group, the defect may be a primary gene-related abnormality.

The collection system used in the present study did not exclude biliary secretions. It is possible therefore that defective bile cana-

lular secretion, gallbladder emptying or bile duct secretion contributed to the decreased HCO_3^- output in the duodenal fluid of CF patients. Abnormalities of both liver (11, 14) and gallbladder (4) function have been recognized in the CF group. To date however the non-steatorrheic CF patients have not been sufficiently investigated to exclude gallbladder dysfunction but they have been shown to have normal liver function biochemically (2). Thus liver or gallbladder dysfunction may not explain the HCO_3^- secretory deficit in the nonsteatorrheic group. Perhaps more pertinent is that in experimental dogs (21), secretin increases bile duct HCO_3^- secretion to as high as 70 mEq/litre. One could postulate that impairment of this mechanism in CF patients could substantially reduce HCO_3^- output from the biliary tree. This aspect has not been studied previously. In the dog experiments above, bile duct volume output increased only to a mean of 6 ml per 30 min during secretion stimulation. Thus the absolute HCO_3^- secretion during stimulation was extremely small (42 μEq in 30 min) compared with absolute pancreatic HCO_3^- secretion in the same species (approximately 1500 μEq in 10 min), as reported by Henriksen and Worning (10). In our study, even if secretin-induced, bile duct HCO_3^- secretion was completely suppressed in the CF patients, it would therefore account for only a small fraction of the HCO_3^- deficit observed in the duodenal aspirate.

The values obtained in our study differed markedly from those reported by Hadorn *et al.* (8). In our control subjects mean water, HCO_3^- and trypsin secretion rates were two to three times higher and in our CF patients two to ten times higher than those of Hadorn *et al.* The longer collection period in our system (60 min as opposed to 50 min) can account for only a minor part of these differences. The discrepancy could be explained by the volume of duodenal juice aspirated since this depends on the completeness of the collection and would affect all other results. Incomplete collections occur through loss of juice beyond the distal aspiration site as a result of a number of problems including blockage of the tube, displacement of the tube, and simple failure of the system to aspirate the volume of juice secreted. Previous reports (6, 12) demonstrated that these problems could be avoided by incorporating a non-absorbable marker perfusion technique into the pancreatic stimulation test. We used this technique in preference to the balloon catheter technique of Hadorn *et al.* (8) on the theory that it would produce a more precise estimation of pancreatic secretion, particularly in CF patients in whom the high viscosity and low volume of secretion interferes greatly with the collection. The marker perfusion technique not only permits a more complete collection but also dilutes the thick viscous secretions, thereby facilitating aspiration and preventing pooling of secretions and subsequent contamination by gastric acid which may be a problem with the balloon catheter technique. In the CF patient who has lower pancreatic HCO_3^- secretion, minor degrees of contamination with gastric acid could also lead to a significantly reduced HCO_3^- estimation. This factor may account for some of the difference between the pancreatic HCO_3^- secretion reported here and that reported by Hadorn *et al.* (8).

We have emphasized the importance of using a quantitative pancreatic stimulation test that employs a non-absorbable marker perfusion technique to ensure a precise evaluation of pancreatic function in CF patients. In addition, we have defined a primary HCO_3^- secretory defect in CF patients. In patients with high sweat chlorides, depressed pancreatic water and HCO_3^- secretion in association with low or even normal enzyme association strongly suggests a diagnosis of CF. But water and/or HCO_3^- secretion

within the control range does not exclude the diagnosis. CF patients demonstrate anomalies of both pancreatic acinar and ductal function, whereas patients with Shwachman's syndrome show evidence only of severe acinar dysfunction.

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22. Requests for reprints should be addressed to: Dr. K. J. Gaskin, Division of Gastroenterology, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8.
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