Secretin pancreozymin stimulation test and confirmation of the diagnosis of cystic fibrosis

L T K WONG, S TURTLE, and A G F DAVIDSON*

From the Departments of Pediatrics and Pathology, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada

SUMMARY We have studied the secretin-pancreozymin stimulation test of pancreatic function with particular regard to its usefulness in the diagnosis of cystic fibrosis patients without gastrointestinal symptoms. We have compared the test results in 38 control individuals, 14 patients with cystic fibrosis and five other patients with problems affecting pancreatic function. Of the cystic fibrosis patients tested, six had no gastrointestinal symptoms and were shown to have normal or raised output of pancreatic enzymes measured (lipase, trypsin, chymotrypsin, and amylase). There was a positive correlation between both volume and bicarbonate measurement versus body weight in all subjects, but this relationship was clearly different in the cystic fibrosis patients compared with others regardless of the cystic fibrosis patients' ability to secrete pancreatic enzymes. Among the cystic fibrosis patients, bicarbonate concentration tended to be higher in those able to secrete a significant amount of pancreatic enzymes. However, the actual output of bicarbonate measured in all cystic fibrosis patients (range 0.001–0.037 mmol/kg/45 min post-stimulation) was below that found in all control patients (range 0.104–0.516 mmol/kg/45 min post-stimulation). Therefore the secretin-pancreozymin test of pancreatic function appears to be useful in the diagnosis of cystic fibrosis even in those patients with adequate enzyme production.

We have evaluated the secretin-pancreozymin stimulation test of pancreatic function with regard to its usefulness in the diagnosis of cystic fibrosis. Difficulty in performing the test plus the high proportion (approximately 15%) of cystic fibrosis patients with normal or high pancreatic enzyme output has in the past severely limited its value for this purpose. It has been suggested, however, that even those cystic fibrosis patients with 'normal' enzyme output may have significantly decreased pancreatic bicarbonate and fluid secretion.^{1 2} The pathophysiological implications of these findings have been carefully discussed,²⁻⁴ but the diagnostic implications have been given relatively little attention since then. If the decrease in pancreatic fluid and bicarbonate output in cystic fibrosis does reflect a basic disturbance in fluid and electrolyte transport, however, measurement of these parameters could prove of comparable reliability to the sweat test in the diagnosis of cystic fibrosis. This

suggests that the secretin-pancreozymin stimulation test of pancreatic function warrants further consideration with regard to its value in the confirmation of the diagnosis of cystic fibrosis.

A supportive diagnostic test for cystic fibrosis distinct from the sweat test would be very useful in many instances. Although the sweat test remains the mainstay for the laboratory confirmation of the diagnosis, recent reports emphasise that there are still problems in its use.⁵⁻⁹ Careful clinical evaluation plus performance of the sweat test by the standard Gibson and Cooke method¹⁰ at a cystic fibrosis reference centre may often resolve a doubtful situation, but uncertainty can remain in many instances – for instance, where there is a 'border-line' sweat result, where there is direct conflict between sweat test results from the reference centre and the local laboratory, where the diagnosis is considered in an adult,⁹ or where (rarely) the diagnosis may be considered despite an undeniably normal sweat test.^{11 12}

We quite frequently encounter such instances in patients referred to the Cystic Fibrosis Assessment Clinic in Vancouver.⁵ We have used the secretin-

* Address for correspondence: Dr A G F Davidson, Children's Hospital, 4480 Oak Street, Vancouver, BC, V6H 3N1, Canada.

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pancreozymin test as a complement to the sweat test and have reviewed our secretin-pancreozymin test results in order to assess how reliable it has been in separating patients with cystic fibrosis from those without the disease.

Methods

SUBJECTS

Patients undergoing the secretin-pancreozymin stimulation test are listed in Tables 1 and 2. Patients 1-14 had cystic fibrosis, while patients A-E showed other causes for pancreatic enzyme abnormalities. No patients had received pancreatic enzyme supplements for at least two days before the test.

All cystic fibrosis patients had positive sweat tests (sodium and chloride concentrations over 70 mmol/l in a sweat collection of over 100 mg), performed at our reference centre by the Gibson and Cooke method,¹⁰ and had clinical and radiological changes suggestive of the diagnosis. Six of the cystic fibrosis patients had no gastrointestinal symptoms (CF, numbers 9-14).

Subject no. 9 is a 26 year old male who had a history of heat stroke, chronic sinusitis, and one episode of hemoptysis. Repeat sweat chloride

measurements showed 150 and 113 mmol/l. His sputum grew *Pseudomonas aeruginosa* and he was also shown to have oligospermia. A cousin has cystic fibrosis, diagnosed in infancy.

Subject no. 10 is a 33 year old male who is the brother of no. 9. He also has chronic sinusitis. His repeat sweat chloride tests were 129 and 112 mmol/l.

Subject no. 11 is a 3 year old girl who presented with recurrent chest infections. Radiographs of the chest showed peribronchial thickening and sweat chloride levels were 111 and 92 mmol/l.

Subject no. 12 is a 7 year old boy who had a history of recurrent chest infections and sputum on repeated occasions grew *Staph aureus* and *Haemophilus influenzae*. His sweat chloride levels were 115 and 84 mmol/l.

Subject no. 13 is a 14 year old male who also had a history of recurrent chest infections with sputum growing *Staph aureus* and *Haemophilus influenzae*. Sweat chloride levels were 78 and 90 mmol/l.

Subject no. 14 is a 5½ year old girl who is the sister of subject no. 6. She had had an episode of atelectasis which resolved after treatment, and her chest radiograph is now normal. Her sputum had grown *Staph aureus* and *Pseudomonas aeruginosa*. Her sweat chloride levels were 90 and 112 mmol/l.

Table 1 Maximum level during pancreozymin secretin stimulation test

		Age (yr)	Weight (kg)	pH	Bicarbonate (mmol/l)	Lipase (IU)	Trypsin (IU)	Chymotrypsin (IU)	Amylase (IU)	Remarks
1	LMc	0.5	2.85	—	5.4	26	2.9	9.5	10	CF+a
2	JC	10	28.80	6.2	5.6	10	0	0	0	CF+
3	MM	0.6	5.98	6.6	27.0	0	0	0	0	CF+
4	CP	7	15.90	6.8	17.0	0	0	0	0	CF+
5	DA	3	11.90	6.6	18.0	14	1.0	1.0	1	CF+
6	RS	7.5	23.30	6.8	18.0	16	1.7	3.0	5	CF+
7	MC	1.2	7.70	6.8	10.4	2	0.4	0.4	10	CF+
8	LW	22.0	43.00	6.6	16.0	183	9.3	74.4	84	CF+
9	GB	26.0	65.50	7.6	57.0	4852	62.6	422.0	971	CF-b
10	JB	33.0	68.00	6.8	48.0	1130	43.5	253.0	493	CF-
11	BW	3.0	15.30	6.8	15.0	3810	81.6	608.0	—	CF-
12	JW	7.0	18.80	6.8	33.0	964	35.7	254.0	213	CF-
13	WH	14.5	91.40	7.6	53.0	3238	53.5	451.5	426	CF-
14	BS	5.7	18.80	6.0	6.0	1506	19.1	117.0	219	CF-
A	JN	0.7	5.00	8.0	21.0	22	1.0	2.3	0	Shwachman
B	DE	17.0	50.00	6.6	45.0	116	1.5	21.0	4	Shwachman
C	GV	14.0	32.80	6.4	23.4	180	2.6	33.6	3	Shwachman
D	KM	0.2	2.60	7.4	31.2	37	4.0	12.2	1	c
E	SP	0.5	6.30	7.6	49.0	120	0	0	0	c
Controls (n=38)										
Mean		5.3	16.94	7.5	75.5	1637	29.5	181.0	262	
10th pc.		1.2	6.50	7.0	60.0	525	15.9	75.8	50	
90th pc.		13.7	37.50	8.5	99.3	2869	40.9	333.0	508	
Range		0.5-16.0	5.90-43.00	6.6-8.5	53.9-110.0	388-3490	10.7-95.0	66.4-646.0	2-1080	

a: CF+ cystic fibrosis with gastrointestinal symptoms.

b: CF- cystic fibrosis without gastrointestinal symptoms.

c: Intractable diarrhoea of infancy with severe intestinal mucosal damage.

Table 2 Rate of secretion during pancreozymin-secretin stimulation test (per kg during 45 minutes)

		Volume (ml)	Bicarbonate (mmol/l)	Lipase (IU)	Trypsin (IU)	Chymotrypsin (IU)	Amylase (IU)
1	LMc	1.65	0.004	26	1.8	8	17
2	JC	0.86	0.001	8	0	0	0
3	MM	0.60	0.004	0	0	0	0
4	CP	0.73	0.009	0	0	1	19
5	DA	0.58	0.008	7	0.6	1	1
6	RS	0.60	0.009	7	0.6	1	3
7	MC	0.82	0.004	1	0.2	0	6
8	LW	0.62	0.009	86	5.5	40	51
9	GB	0.67	0.032	2838	28.8	237	545
10	JB	0.32	0.013	257	5.9	435	74
11	BW	0.50	0.005	1933	38.0	228	—
12	JW	1.16	0.037	7626	27.1	186	164
13	WH	1.20	0.034	1309	19.5	115	89
A	JN	4.60	0.068	54	2.0	5	0
B	DE	1.33	0.046	122	1.9	22	4
C	GV	3.72	0.061	330	5.5	58	7
D	KM	4.19	0.078	123	6.2	26	3
E	SP	4.27	0.272	359	0	0	0
<i>Controls (n=38)</i>							
Mean		3.95	0.256	4050	62.2	385	585
10th pc		2.58	0.147	1598	38.9	192	107
90th pc		5.52	0.412	7973	111.5	695	1465
Range		1.33–6.64	0.104–0.516	1064–8735	21.0–146	120–846	3–2139

Three patients (A–C) had Shwachman's syndrome, with neutropenia, recurrent infections, and growth failure. Patient A died at 3 months of age from fulminating infection. Only patient C had metaphyseal dysostosis. Patients B and C were mildly mentally retarded.

Two patients (D, E) had intractable diarrhoea of infancy. Examination of their small bowel mucosa showed extensive severe damage of unknown aetiology. In view of the nature and extent of the mucosal damage, it was felt likely that there might be secondary deficiency of small intestinal hormones and/or enzymes, including enterokinase.

The 38 'control' patients were tested in the course of investigation for a variety of complaints, such as chronic diarrhoea, recurrent abdominal pain, failure to thrive. These after a full evaluation were felt to be unrelated to pancreatic dysfunction.

Patients A–E and all controls had negative sweat tests (sodium and chloride less than 50 mmol/l). The age and weight of the three groups tested were comparable with the exception of the older cystic fibrosis patients (nos. 8, 9, 10, and 13).

PROCEDURES

The secretin-pancreozymin stimulation test was performed according to the method described by Delachaux-Salem and Sarles.¹³ After a six to eight hour fast, a double lumen Salem Sump tube was passed into the third part of the duodenum and

position confirmed by fluoroscopy. Duodenal secretions were aspirated for a 15 minute baseline period, and then for three consecutive 15 minute periods after intravenous secretin (Boots) 1 unit/kg and pancreozymin-cholecystokinin (Boots) 3 units/kg, given immediately one after the other.

Volume and pH were noted for each specimen, and concentrations of bicarbonate, lipase, trypsin, chymotrypsin were determined as previously described.¹³ Amylase was determined by the method of Dahlquist.¹⁴

Results

Maximal post-stimulation values for pH bicarbonate, lipase, trypsin, chymotrypsin and amylase concentration values are shown in Table 1. The total output in 45 minutes after secretin-pancreozymin stimulation test, expressed as secretion rate per kilogram body weight, is shown in Table 2. The results obtained from the 38 control patients are expressed in terms of mean, range, 10th and 90th percentiles.

Pancreatic enzyme results fell into two groups in the cystic fibrosis patients, demonstrating the expected divergence between those with gastrointestinal symptoms who have very low enzyme values (nos. 1–8) and those without whose enzyme results were well within the control range (nos. 9–12). All enzymes behaved similarly within each of

the groups of cystic fibrosis patients and controls. Amylase had the widest range of control values.

Pancreatic fluid and electrolyte outputs were assessed by measurement of duodenal aspirate pH, volume, and bicarbonate content. The maximum pH of duodenal aspirate showed considerable overlap between patients and controls (Fig. 1). There were differences in duodenal aspirate volume between control and cystic fibrosis patients. Comparison of volume output per kilogram body weight (Fig. 1B), showed that all cystic fibrosis patients were below the control range except for patient no. 1 whose volume output was in range but below the 10th percentile. The relationship between volume output and body weight (Fig. 2) appeared to be different in cystic fibrosis patients ($y = 0.8x - 1.3$, $p < 0.001$, $r = 0.84$) compared with the controls ($y = 3.6x + 6.5$, $p < 0.001$, $r = 0.88$). The non-cystic fibrosis patients (A-E) with abnormal pancreatic function results seemed to follow the same volume/weight relationship as the control group. For any given weight, cystic fibrosis patients, including those with higher enzyme output, had a lower volume of output than controls.

Bicarbonate measurement also showed significant differences between cystic fibrosis and controls (Fig. 3). The maximal bicarbonate concentration in cystic fibrosis patients was below the control range in all but two patients whose results were below the 10th percentile (Fig. 3). Low bicarbonate concentration was also seen in patients with abnormal pancreatic function, not due to cystic fibrosis. As can be seen in

Fig. 2 Correlation between total aspirated volume of duodenal juice and subjects' weight in cystic fibrosis and control patients. $\square$ CF+. $\blacksquare$ CF-. $\bullet$ Others. $\triangle$ Control.

Fig. 4, cystic fibrosis patients who secreted a higher concentration of bicarbonate, also tended to secrete higher concentrations of trypsin ($0.01 < p < 0.025$), but the control patients did not seem to demonstrate this trend ($p < 0.25$).

The rate of bicarbonate output per kilogram of body weight showed a clear separation between cystic fibrosis and non-cystic fibrosis individuals (Fig. 3). All cystic fibrosis results were lower than

Fig. 1 Duodenal aspirate pH (left) and volume (right) results after secretin-pancreozymin stimulation. Solid bar indicates control range, plus mean, 10th, and 90th percentiles. Cystic fibrosis patients with (+) and without (-) gastrointestinal symptoms. A-E, see text.

Fig. 3 Duodenal aspirate maximum bicarbonate concentration (left) and bicarbonate output (right) after secretin-pancreozymin stimulation. For explanation of symbols see Fig. 1.

Fig. 4 Correlation between maximum duodenal aspirate bicarbonate and trypsin levels in controls ($y = 0.03x + 72.8$, $r = 0.03$, $p > 0.25$) and cystic fibrosis patients ($y = 0.4x + 145$, $p = 0.62$, $0.025 < p < 0.01$). ■ All CF patients. ● Others. △ Control.

the control range. In all subjects there appeared to be a positive linear correlation between bicarbonate output and trypsin output (Fig. 5) but the relationship appeared different in cystic fibrosis ($y = 0.001x + 0.007$, $r = 0.70$, $0.01 < p < 0.025$) as compared with controls or those with abnormalities of pancreatic function due to other causes ($y = 0.002x + 0.105$, $r = 0.68$, $p < 0.0005$).

Fig. 5 Correlation between rate of bicarbonate and trypsin output in controls ($y = 0.002x + 0.105$, $r = 0.68$, $p < 0.0005$), and cystic fibrosis patients ($y = 0.001x + 0.007$, $r = 0.70$, $0.01 < p < 0.025$). Key: see Fig. 4.

Discussion

We performed the secretin-pancreozymin test using a relatively simple technique in the hope of minimizing discomfort to the patient. The tube used was readily passed and easy to aspirate with little tendency to block. The test procedure was generally well tolerated by our patients, though some discomfort remained during passage of the tube. Gastric juice contamination was usually readily apparent by change in pH and turbidity of the aspirate, and could usually be avoided by positioning and by aspiration of the stomach.

Our control results showed a distribution skewed toward the lower values, much as was observed in their series by Hadorn *et al.*² Because of this skewed distribution, it was more appropriate to express our control values in terms of percentiles rather than mean and standard deviations as was done by Delachaux-Salem and Sarles.¹³ Our range of control values was very similar to theirs, however, and the conclusions for our cystic fibrosis patients remain constant whether the cystic fibrosis results are interpreted with respect to our control range or that of Delachaux-Salem and Sarles.¹³

Our pancreatic enzyme results separate clearly two groups of cystic fibrosis patients with and without adequate pancreatic enzyme function. It was interesting to note that the ability to secrete a significant amount of enzyme was not strictly familial. In one pair of siblings tested (patients nos. 9 and 10) both secreted significant amounts of enzymes, but, in the other pair, one had low enzymes (patient 6) and the other had normal enzymes (patient 14). It is not clear why some patients with cystic fibrosis should remain capable of secreting normal amounts of pancreatic enzymes into the duodenum, while others are not. Recent studies of blood immunoreactive trypsin levels in infancy¹⁵⁻¹⁸ suggest that all patients with cystic fibrosis are initially able to produce pancreatic enzymes, but there is some degree of obstruction to their secretion. The cystic fibrosis patients who have significant amounts of pancreatic enzymes in duodenal aspirate are presumably those in which this obstruction or its effect is less severe.

The ability of the pancreas to put out fluid and electrolytes was assessed by measuring duodenal aspirate pH, volume, and bicarbonate content. As pH is also affected by various other factors, it is perhaps not surprising that the maximal pH of duodenal aspirate showed considerable overlap between patients and controls. The duodenal aspirate volume and bicarbonate measurements probably reflect pancreatic fluid and electrolyte output more directly. These showed significant

differences between cystic fibrosis and non-cystic fibrosis subjects regardless of whether the cystic fibrosis subject was able to secrete measurable amounts of pancreatic enzymes or not. Cystic fibrosis patients appeared to have an altered relationship between volume output and body weight, and between the two main types of pancreatic secretions – that is, between enzyme and bicarbonate output. These differences, which suggest abnormal fluid and electrolyte transport in the cystic fibrosis pancreas, become most apparent for the individual patient when the rate of output of bicarbonate after pancreatic stimulation is considered. This measurement reflects both the bicarbonate concentration and fluid volume output together, and is probably a more accurate indicator of the functional capacity for fluid and electrolyte secretion of the pancreas than either measurement alone. In our series, this measurement clearly separated cystic fibrosis from non-cystic fibrosis subjects in all cases.

These results seem very promising with regard to the usefulness of the secretin-pancreozymin test in confirming the diagnosis of cystic fibrosis, but a number of factors have to be considered when interpreting them. The problem of completeness of sample collection arises when considering results based on volume measurements. It may be that markers such as polyethylene glycol and bromsulphalein should be used to assess and correct for incomplete collections or gastric juice contamination. Use of bromsulphalein as marker by one group seemed to suggest that a higher recovery of secretions occurred in cystic fibrosis compared with control patients.¹⁹ If this was the case in our series, our data would relatively underestimate control values, and the true difference between cystic fibrosis and non-cystic fibrosis would be even more marked than we have demonstrated.

The method used to stimulate pancreatic secretion seems also to be important. Our results and those of Hadorn *et al*² were obtained using 'standard' single dose stimulation with secretin and pancreozymin, but repeated high dosage or continuous secretin infusions may be capable of increasing bicarbonate secretion by cystic fibrosis patients to levels overlapping those of controls.^{3 18} It is possible that these differences in results reflect an analogous situation to that of the sweat test, where valid results depend upon use of a carefully selected and standardised method of sweat stimulation.

Conclusions

Our results support the premise that all cystic

fibrosis patients, regardless of their ability to produce pancreatic enzymes, have an abnormality in pancreatic fluid and bicarbonate secretion. This abnormality is most apparent when the rate of bicarbonate output after pancreatic stimulation is compared for cystic fibrosis and non-cystic fibrosis individuals. This measurement appears to be capable of differentiating between these two types of patient.

More work is needed to confirm these results, to assess their diagnostic usefulness, and to establish the optimal conditions of pancreatic stimulation and collection of secretions for this purpose. In the meantime, we feel that careful use of the secretin-pancreozymin test, as described here, is warranted whenever there is doubt about the diagnosis of cystic fibrosis, despite a careful clinical assessment and adequately performed sweat testing.

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References

- 1 Rick W. Untersuchung zur exokrinen funktion des pankreas bei zystischer Pankreasfibrose. *Med Welt* 1963; **42**: 2158.
- 2 Hadorn B, Zoppi G, Shmerling DH, Prader A, McIntyre I, Anderson CM. Quantitative assessment of exocrine pancreatic function in infants and children. *J Pediatr* 1968; **73**: 39–50.
- 3 Hadorn B, Johansen PG, Anderson CM. Pancreozymin secretin test on exocrine pancreatic function in cystic fibrosis and the significance of the result for the pathogenesis of the disease. *Can Med Assoc J* 1968; **98**: 377.
- 4 Johansen PG, Anderson CM, Hadorn B. Cystic Fibrosis of the pancreas: a generalized disturbance of water and electrolyte movement in exocrine tissues. *Lancet* 1968; **1**: 455.
- 5 Davidson AGF, Wong LTK, Applegarth DA. Problems in the diagnosis of cystic fibrosis. *Clin Biochem* 1978; **II**(2): 2.

- 6 Smalley CA Addy DP, Anderson CM. Does that child really have cystic fibrosis? *Lancet* 1978; **2**: 415.
- 7 Rosenstein BJ, Langbaum TS, Gordes E, Brusilow SW. Cystic fibrosis: problems encountered with sweat testing. *JAMA* 1978; **240**: 1987.
- 8 Shwachman H, Mohmoodian A. Quality of sweat test performance in the diagnosis of cystic fibrosis. *Clin Chem* 1979; **25**: 158.
- 9 Davidson AGF, Applegarth DA, Wong LTK. Diagnosis of cystic fibrosis in adults. *N Engl J Med* 1979; **300**: 1164.
- 10 Gibson LE, Cooke RE. A test for concentration of electrolytes in sweat in cystic fibrosis of pancreas utilizing pilocarpine by iontophoresis. *Pediatrics* 1959; **23**: 545.
- 11 Sarsfield JK, Davies JM. Negative sweat tests and cystic fibrosis. *Arch Dis Child* 1975; **50**: 463.
- 12 Huff DS, Huang NN, Arey JB. Atypical cystic fibrosis of pancreas with normal levels of sweat chloride and minimal pancreatic lesions. *J Pediatr* 1979; **94**: 237.
- 13 Delachaux-Salem E, Sarles H. Evolution en fonction de l'age de la secretion pancreatique humaine normale. *Biol Gastroenterol (Paris)* 1970; **2**: 135.
- 14 Dahlquist A. A method for the determination of amylase in intestinal content. *Scand J Clin Lab Invest* 1962; **14**: 145.
- 15 Crossly JP, Elliott RP, Smith PA. Dried blood-spot screening for cystic fibrosis in the newborn. *Lancet* 1979; **1**: 472.
- 16 Applegarth DA, Davidson AGF, Kirby L, Bridges M, Sorensen P, Wong LTK. Dried blood spot screening for cystic fibrosis. *Lancet* 1979; **2**: 1236.
- 17 King DN, Heeley AF, Walsh MP, Kuzemko JA. Sensitive trypsin assay for dried blood specimens as a screening procedure for early detection of cystic fibrosis. *Lancet* 1979; **2**: 1217.
- 18 Kirby LT, Applegarth DA, Davidson AGF, Wong LTK, Hardwick DF. Use of a dried blood spot in immunoreactive-trypsin assay for detection of cystic fibrosis in infants. *Clin Chem* 1981; **27**: 678-80.
- 19 Gaskin K, Forstner G. Volume and HCO₃-secretin in CF patients. In: Proceedings of the 8th International Cystic Fibrosis Congress, Toronto: *Perspectives in cystic fibrosis*. 1980: 15a.