¹³C labelled cholesteryl octanoate breath test for assessing pancreatic exocrine insufficiency

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Abstract

Background—A non-invasive test for assessment of fat digestion has been developed based on the intraluminal hydrolysis of cholesteryl-[1-¹³C]octanoate by pancreatic esterase.

Aims—To determine the diagnostic performance of this breath test in the assessment of exocrine pancreatic function.

Methods—The test was performed in 20 healthy controls, 22 patients with chronic pancreatic disease (CPD), four with bilio-pancreatic diversion (BPD), and 32 with non-pancreatic digestive diseases (NPD); results were compared with those of other tubeless tests (faecal chymotrypsin and fluorescein dilaurate test).

Results—Hourly recoveries of ¹³CO₂ were significantly lower in CPD when compared with healthy controls or NPD. In patients with CPD with mild to moderate insufficiency, the curve of ¹³CO₂ recovery was similar to that of healthy controls, while in those with severe insufficiency it was flat. In three patients with CPD with severe steatorrhoea, a repeat test after pancreatic enzyme supplementation showed a significant rise in ¹³CO₂ recovery. The four BPD patients had low and delayed ¹³CO₂ recovery. Only eight of the 32 patients with NPD had abnormal breath test results. There was a significant correlation between the results of the breath test and those of faecal chymotrypsin, the fluorescein dilaurate test, and faecal fat measurements. For the diagnosis of pancreatic disease using the three hour cumulative ¹³CO₂ recovery test, the sensitivity was 68.2% and specificity 75.0%; values were similar to those of the other two tubeless pancreatic function tests. In seven healthy controls, nine patients with CPD, and nine with NPD a second breath test was performed using Na-[1-¹³C]octanoate and a pancreatic function index was calculated as the ratio of ¹³C recovery obtained in the two tests: at three hours this index was abnormal in eight patients with CPD and in three with NPD.

Conclusion—The cholesteryl-[1-¹³C]octanoate breath test can be useful for the diagnosis of fat malabsorption and exocrine pancreatic insufficiency.

(Gut 1998;42:81-87)

Keywords: cholesteryl octanoate breath test; exocrine pancreatic insufficiency; lipid malabsorption; stable isotopes

The ideal exocrine pancreatic function test would be sensitive, specific, easy to perform, non-invasive, and inexpensive. The secretin-cholecystokinin (CCK) or caerulein test, because of its high sensitivity and specificity, is considered the gold standard; unfortunately, this test is complex, time consuming, unpleasant for the patient and, in some conditions, not feasible. For these reasons, its use is limited to a few specialist centres. A large number of tubeless tests have been proposed in recent decades; however, only a few have been fully accepted in clinical practice. The *p*-amino-benzoic acid (PABA) and fluorescein dilaurate tests, and the determination of serum trypsin and faecal chymotrypsin concentrations are widely used, but all lack sensitivity in mild or moderate pancreatic insufficiency.¹ More recently, enzyme linked immunosorbent assay (ELISA) determination of elastase in faeces has been introduced in clinical practice, but this test also seems to have limited sensitivity in patients with mild chronic pancreatitis.² CO₂ breath tests for fat malabsorption using ¹⁴C labelled substrates appeared attractive because they are simple procedures that do not involve handling of faeces; however, they have not been widely adopted due to several drawbacks, including radiation exposure, interference by some metabolic and pulmonary disorders, and the high cost of equipment and markers.

Cholesteryl octanoate is very similar to cholesteryl esters naturally present in food³; it is specifically hydrolysed by pancreatic cholesterol esterase but not gastric lipase,⁴ and is aided in this process by the permissive action of pancreatic lipase, phospholipase, and bile salts.⁵ The octanoic acid produced by this hydrolysis is then rapidly and passively absorbed without micellar solubilisation, transported to the liver via the portal vein, and efficiently oxidised to CO₂ and water.^{6,7} A breath test with cholesteryl-[1-¹⁴C]octanoate has been developed for the evaluation of exocrine pancreatic insufficiency and fat malabsorption. Preliminary studies in rats and humans have shown that this test is able to detect severe pancreatic insufficiency⁸⁻¹⁰; the small number of patients enrolled in these studies, however, does not permit conclusive evaluation of its sensitivity and specificity. In addition, use of this test is limited by the need for a radioactive isotope. Encouraged by the results of these preliminary studies, and prompted by the greater accessibility of automated mass spectrometers capable of measuring ¹³CO₂, we carried out the test using cholesteryl-[1-¹³C]octanoate, in order to assess its value in the diagnosis of fat malabsorption and exocrine

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Accepted for publication
31 July 1997

pancreatic insufficiency. The test was performed in a large number of healthy subjects and patients with pancreatic or non-pancreatic disease. A control test with Na-[1-¹³C]octanoate was performed in some cases to ascertain whether this combination could offer improved diagnostic efficiency with respect to the single test.

Patients and methods

SUBJECTS

After giving written, informed consent, the subjects were enrolled and assigned to one of the following four groups.

Healthy controls

Twenty healthy subjects (14 men and six women, median age 28 years, range 21–52) were recruited from medical staff or persons undergoing routine medical check up. For all subjects, reported alcohol consumption was less than 30 g per day; none had evidence of digestive disease or exocrine pancreatic dysfunction, the latter having been excluded by normal faecal chymotrypsin.

Chronic pancreatic disease

Twenty two patients (19 men and three women, median age 51 years, range 16–80) with chronic pancreatic disease (CPD) were studied; 17 had chronic pancreatitis, three cystic fibrosis, one pancreatic cancer, and one had undergone resection of the head of the pancreas for ampullary carcinoma six years prior to the study. The aetiology of chronic pancreatitis was alcoholic in 11 (alcohol consumption more than 100 g per day) and idiopathic in the remaining six. The diagnosis of chronic pancreatitis was based on typical clinical history (recurrent upper abdominal pain with hyperamylasaemia), and on positive findings at ultrasonography in 15 patients, computed tomography in nine, and endoscopic retrograde cholangiopancreatography (ERCP) in eight. The diagnosis was further confirmed by radiological evidence of pancreatic calcification in eight cases and by surgical and histological findings in six. Based on morphological findings at ultrasonography or ERCP,^{11 12} 10 patients were considered to have mild to moderate chronic pancreatitis, and seven, a severe form of the disease. All patients with severe pancreatitis had steatorrhoea and six also had diabetes. All patients were studied during a stable pain free period, and those with pancreatic insufficiency stopped their enzyme supplementation five days before the study. The diagnosis of cystic fibrosis was made on the basis of the clinical picture (chronic pulmonary disease, fat malabsorption) and was confirmed by the sweat test. In the patient with pancreatic cancer, malignancy was confirmed by surgery performed two months later.

Biliopancreatic diversion

Four patients (one man and three women, median age 41 years, range 34–48) who had undergone biliopancreatic diversion (BPD) according to Scopinaro *et al*¹³ for the treatment

of obesity were also studied. Surgery had been performed from a minimum of one to a maximum of 11 years prior to the study.

Non-pancreatic digestive disease (NPD)

Thirty two patients (17 men and 15 women, median age 46 years, range 19–73) suffering from various digestive diseases with symptoms or laboratory data also common to CPD (steatorrhoea and/or diarrhoea in 30, abdominal pain in 14, weight loss in 12, hyperamylasaemia in two) were studied. The final diagnoses were: inflammatory bowel disease (seven), coeliac disease (six), chronic gastritis (four), food allergy (three), chronic hepatic disease (three), gastrectomy (two), ischaemic colitis (two), intestinal giardiasis (one), carcinoid tumour of the ileum (one), scleroderma (one), irritable bowel (one), and colon diverticula (one).

TEST MEAL AND EXPERIMENTAL PROCEDURES

Cholesteryl-[1-¹³C]octanoate and [1-¹³C]octanoic acid (atom percentage excess ¹³C 99%) were purchased from Mass Trace and CIL, USA. Unlabelled cholesteryl octanoate and octanoic acid were obtained from Sigma Chemical Co., USA. [1-¹³C]octanoic acid was used as a sodium salt, prepared by a titrimetric method, in order to improve its taste. The substrates were given as an emulsion in an isotonic liquid meal. For the cholesteryl-[1-¹³C]octanoate breath test, labelled (500 mg) and unlabelled (800 mg) substrate was dissolved in 20 ml of olive oil heated to 90°C. For emulsification of the substrate, 5 ml of glycerol (Merck, Germany) and 5 g of lecithin (Gazzoni, Italy) were dissolved in 100 ml of water, to which 200 ml of normal saline solution and 60 ml of vegetable broth were added. The oil was poured gently into the aqueous phase and emulsified by stirring with a high speed mixer for 10 minutes. Prior to mixing, 5 g of D-xylose (Carlo Erba, Italy) was also added in order to assess gastric emptying of the meal. For the control test, an equimolar dose (163 mg) of Na-[1-¹³C]octanoate along with 260 mg of this substrate, unlabelled, was used instead of cholesteryl-[1-¹³C]octanoate. All subjects received the same amount of substrates; the quantities given were previously found to be the minimum necessary to achieve adequate discrimination of ¹³CO₂ outputs above baseline.

Subjects were studied after a 12 hour overnight fast. Breath samples were obtained using a disposable plastic straw placed approximately 1 cm from the bottom of an open 10 ml vacutainer. Patients exhaled down the straw until condensation appeared. While the patient continued to exhale, the vacutainer was slowly moved away from the straw and quickly resealed. Samples were taken immediately before the meal and every 15 minutes thereafter for six hours; the subjects were asked to remain seated, and smoking and eating were forbidden for the duration of the test.

The ¹³C/¹²C isotopic ratio was measured using a fully automated continuous flow isotope ratio mass spectrometer (TracerMass, Europa Scientific, UK). Isotope ratios were

converted to $\delta^{13}\text{C}$ values using the PDB international standard¹⁴ and corrected for the oxygen isotope effect.¹⁵ The ^{13}C recovery per hour was calculated as a percentage of the administered dose from the enrichment of breath samples in ^{13}C using the Schoeller formula,¹⁶ assuming total CO_2 production to be 300 mmol per m^2 of body surface per hour.

To assess the reproducibility of this method, replicate tests were performed within one week in two healthy controls, in three patients with CPD, and in two with NPD. In order to determine the intra-assay variability of the measurements, 10 repeat breath samples were collected for each hour in three patients.

To assess the effect of pancreatic enzyme supplementation on the test results, in three patients (two with chronic pancreatitis and one with cystic fibrosis), the test was repeated after treatment for one week with pancreatic enzymes administered in acid resistant microgranules equivalent to 125 000 U of lipase per day, divided over three meals (Kreon 25000, Kali-Chemie, Germany). The repeat test was then performed with the addition of pancreatin (75 000 U of lipase) in the same form as for the test meal.

In seven healthy controls, nine patients with CPD, and nine with NPD, the control test with Na-[1- ^{13}C]octanoate was carried out within two weeks of the cholesteryl-[1- ^{13}C]octanoate breath test. The cholesteryl-[1- ^{13}C]octanoate/Na-[1- ^{13}C]octanoate $^{13}\text{CO}_2$ recovery ratio was used as a pancreatic function index. Blood samples were taken every 30 minutes for xylose measurement using the bromoaniline method.¹⁷

In all subjects, exocrine pancreatic function was evaluated by means of faecal chymotrypsin determination using a colorimetric method commercially available in kit form (Chymo, Boehringer Mannheim GmbH, Germany). To reduce the effect of day to day variations, we analysed three consecutive stool specimens and calculated the mean values of the single results. The lower normal limit, previously established

on a group of 32 healthy controls, was 7 U/g. In addition, 11 healthy controls, all patients with CPD or BPD, and 31 with NPD underwent the fluorescein dilaurate test, according to the method used in our laboratory and previously described.¹⁸ In particular, results were given as the percentage ratio (T/C) of fluorescein excretion on the test day (T) relative to that on the control day (C).

In 15 healthy controls, all patients with CPD and BPD, and 28 patients with NPD, fat excretion balance was carried out on faeces collected on the last three of five days during which a standard diet (70 g lipids/day) was followed; near infrared reflectance analysis was used for the faecal fat determination.¹⁹

STATISTICAL ANALYSIS

According to the D'Agostino-Pearson omnibus κ^2 normality test, the distribution of values in patients with CPD was not normal; hence, results are given as the median and 10–90th centiles. Non-parametric tests were performed for statistical significance: the Mann-Whitney U test for the differences between groups of patients, and the Spearman rank test for correlation between the breath test results and those of the exocrine pancreatic function studies.

Results

BREATH TEST VARIABILITY AND REPEATABILITY

Intra-assay variability was excellent: the coefficient of variation among the $^{13}\text{C}/^{12}\text{C}$ determinations carried out on multiple breath samples from three patients was less than 4%. Repeatability of the test was fairly good: variations in the cumulative three and six hour recovery were small and overall results remained in their respective normal or abnormal ranges (fig 1).

CHOLESTERYL-[1- ^{13}C]OCTANOATE BREATH TEST

Healthy controls

$^{13}\text{CO}_2$ excretion in breath generally began to rise within 30 minutes, but the time at which peak values were reached varied widely among individuals (median 225 minutes, 10–90th centile range 105–315 minutes). After reaching peak values there was a progressive decline, but none returned to baseline within the six hour observation period (fig 2). The median $^{13}\text{CO}_2$ cumulative recoveries at three and six hours were 12.0% (7.4–26.3%) and 30.4% (18.3–47.2%), respectively (fig 1).

Patients with pancreatic disease

The $^{13}\text{CO}_2$ output was significantly reduced ($p < 0.01$) for each hour with respect to the values of healthy controls or patients with NPD (fig 2). The median cumulative recoveries at three and six hours were 2.5% (0.1–14.6%) and 6% (0.4–30.2%), respectively, values which were significantly lower ($p < 0.001$) than those of healthy controls or patients with NPD (fig 1). The time at which peak output was achieved (210 minutes, 120–255) did not differ from that observed in healthy controls. The behaviour of expired $^{13}\text{CO}_2$ in single patients varied greatly: in those with mild chronic pancreatitis the curve of $^{13}\text{CO}_2$ output was very similar to that of controls; among the patients

Figure 1 Individual values of the results of the cholesteryl-[1- ^{13}C]octanoate breath test expressed as cumulative $^{13}\text{CO}_2$ recovery after three and six hours. Horizontal bars indicate median values. Arrows show the variation of a repeat test in two healthy controls and five patients. Inverted triangle, mild to moderate CPD; upright triangle, severe CPD.

Figure 2 Hourly time course $^{13}\text{CO}_2$ recovery shown as median values in healthy controls and in patients. The 10-90th centile ranges are shown in the table below the graph.
*Significance of the differences between chronic pancreatic disease and healthy controls or non-pancreatic digestive disease: $p < 0.01$.

with severe chronic pancreatitis, pancreatic cancer, or cystic fibrosis, it was flat, or nearly so. The $^{13}\text{CO}_2$ cumulative six hour recovery was significantly lower ($p < 0.01$) in patients with severe chronic pancreatitis (1.2%, 0.1-22.5%) than in those with mild to moderate disease (20.2%, 9.7-34.2%).

The $^{13}\text{CO}_2$ cumulative output in the six hour period was found to be directly correlated with faecal chymotrypsin concentration ($n=78$, $r=0.46$, $p < 0.001$), and fluorescein dilaurate test results ($n=68$, $r=0.56$, $p < 0.001$); moreover, it was inversely correlated with faecal fat excretion ($n=69$, $r=-0.56$, $p < 0.001$) (fig 3).

In the three patients with severe exocrine insufficiency, pancreatic enzyme supplementation significantly improved the recovery of $^{13}\text{CO}_2$ and reduced the steatorrhoea (table 1).

Biliopancreatic diversion

In these four patients the $^{13}\text{CO}_2$ output was both reduced and delayed compared with healthy controls (fig 4).

Patients with non-pancreatic disorders

The median $^{13}\text{CO}_2$ cumulative outputs at three and six hours were 8.7% (2.3-18.0%) and 21.6% (4.7-40.1%), respectively. The differences with respect to CPD were significant ($p < 0.01$) (fig 1). The median peak time (180 minutes, 105-315) did not differ significantly from those of the other two groups.

DIAGNOSTIC SENSITIVITY AND SPECIFICITY

Because the distribution of values of cumulative outputs was not normal, we arbitrarily chose the lowest values found in healthy subjects as cut off levels. Patients with CPD were best discriminated from healthy controls and patients with NPD using the three hour

$^{13}\text{CO}_2$ cumulative output value with a cut off of 5.7%; this resulted in abnormal values for 15/22 patients with CPD (diagnostic sensitivity for CPD: 68.2%), and for 8/32 patients with NPD (specificity: 75.0%). The diagnoses of these latter eight patients were as follows: gastritis in two, gastrectomy in two, and coeliac disease, irritable bowel disease, hepatic cirrhosis, and scleroderma in one each. The six hour $^{13}\text{CO}_2$ cumulative recovery with a cut off of 15.2% indicated abnormal results for the same number of patients with CPD as the three hour test (15/22), but gave abnormal results for a higher number (12/32) of those with NPD (specificity: 62.5%). Faecal chymotrypsin was abnormally low in 17/22 patients with CPD (sensitivity: 77.3%) and in 10/32 with NPD (specificity: 68.8%); the fluorescein dilaurate test gave abnormal results in 15/22 of the former group (sensitivity: 68.2%) and in 10/31 of the latter (specificity: 67.7%). Table 2 summarises the diagnostic sensitivity and specificity of the cholesteryl-[1- ^{13}C]octanoate breath

Figure 3 Relations between the six hour $^{13}\text{CO}_2$ recovery of the cholesteryl-[1- ^{13}C]octanoate breath test and faecal chymotrypsin concentration (A), fluorescein dilaurate test results (B), and faecal fat (C). In graph C, the curve was drawn manually and represents the best fit for the points. Inverted triangle, mild to moderate CPD; upright triangle, severe CPD.

Table 1 Results of cholesteryl-[1-¹³C] octanoate breath test before and during pancreatic enzyme supplementation in three patients with severe pancreatic exocrine insufficiency

Patient	Diagnosis	Before pancreatin supplementation		During pancreatin supplementation	
		Faecal fat (g/24h)	Cumulative 6h ¹³ CO ₂ recovery (%)	Faecal fat* (g/24h)	Cumulative 6h ¹³ CO ₂ recovery† (%)
SS	Cystic fibrosis	30.1	0.6	16.5	6.5
IG	Chronic pancreatitis	27.0	0.5	14.4	27.8
PF	Chronic pancreatitis	22.6	0.1	11.3	21.5

*Faecal fat balance of the last three of seven days of treatment with 125 000 U of lipase/day (Kreon, five capsules). Upper normal limit=6 g/24 h.

†¹³CO₂ excretion after the test meal with 75 000 U of lipase (Kreon, one capsule at the beginning, during, and at the end of the meal). Lower normal limit=15.2%.

test (three hour ¹³CO₂ cumulative recovery), faecal chymotrypsin, and fluorescein dilaurate test.

Twenty three of the 36 patients (63.9%) with steatorrhoea showed abnormal three hour ¹³CO₂ cumulative recovery (14/16 CPD; 9/20 healthy controls + NPD + BPD); 31/33 (93.9%) patients without steatorrhoea had normal cholesteryl-[1-¹³C]octanoate breath test results (5/6 CPD; 26/27 healthy controls + NPD + BPD).

PANCREATIC FUNCTION INDEX

The pancreatic function index (the ratio between the ¹³CO₂ recovery after cholesteryl-[1-¹³C]octanoate and after Na-[1-¹³C]octanoate) was calculated for values obtained at three and six hours in seven healthy controls, in nine patients with CPD, and in nine with NPD (fig 5). Eight of nine with CPD had abnormally low

index values at both three and at six hours, while among those with NPD, three and four had an abnormal index at three and six hours, respectively. In comparison, ¹³CO₂ recoveries after cholesteryl-[1-¹³C]octanoate were low at both three and six hours in 7/9 patients with CPD and 3/9 patients with NPD.

BLOOD XYLOSE

The xylose appeared rapidly (within 30 minutes) in the serum in all subjects. There were no differences in median peak concentration times among healthy controls (90 minutes, 60–120), CPD (60 minutes, 60–120), and NPD (90 minutes, 60–120).

Discussion

Few studies have thoroughly investigated the utility of breath tests that include non-radioactive labelled substrates for the evaluation of lipid digestion. In 1982 Watkins *et al* compared the effectiveness of trioctanoin, triolein, or palmitic acid breath tests in children with malabsorption and reported that pancreatic insufficiency could be readily differentiated from mucosal disease or bile salt deficiency by abnormal triolein or trioctanoin breath test but normal palmitic acid breath test results.²⁰ A mixed triglyceride breath test using 1,3-distearyl, 2[¹³C]octanoyl glycerol was described by Vantrappen *et al* in 1989 as a sensitive and specific means of diagnosing pancreatic insufficiency.²¹ In 1993 Kato *et al* performed a breath test using trioctanoin in patients after pancreatoduodenectomy and found it to be as sensitive as the secretin test as well as more reliable than conventional tubeless tests.²² More recently, a new ¹³C breath test using hiolein (a mixture of different uniformly labelled triglycerides) has proved to be highly sensitive and specific for detecting steatorrhoea.²³

Cholesteryl octanoate has been proposed as a substrate for the study of lipid maldigestion.⁸ The test, performed using ¹⁴C as a marker, has seemed to be very useful for assessing exocrine pancreatic insufficiency and monitoring the efficacy of pancreatic replacement therapy.^{9 10 24} The recent availability of automated mass spectrometry for ¹³C has given us the possibility of performing the test without exposing patients to radioactivity.

In our study this test proved to have good reproducibility and low variability, which may be partly accounted for by the easy absorption and the simple and rapid metabolism of octanoic acid.

Figure 4 Individual hourly time course recovery of ¹³CO₂ after cholesteryl-[1-¹³C]octanoate breath test in the four patients with biliopancreatic diversion compared with median values of healthy controls (dotted line).

Table 2 Diagnostic sensitivity and specificity of three hour ¹³CO₂ cumulative recovery of cholesteryl-[1-¹³C]octanoate breath test (COBT), and faecal chymotrypsin (CHT) and fluorescein dilaurate test (FDT) results

	Sensitivity (%)			Specificity (%)
	Mild-moderate (n=10)	Severe (n=12)*	Total	
COBT	40.0	91.7	68.2	75.0
CHT	50.0	100	77.3	68.8
FDT	30.0	100	68.2	67.7
COBT+CHT	60.0	100	81.8	50.0
COBT+FDT	50.0	100	77.3	54.8
COBT+CHT+FDT	60.0	100	81.8	35.5

*Three patients with cystic fibrosis, one with pancreatic resection, and one with pancreatic cancer were considered to have severe pancreatic exocrine insufficiency on the basis of notable steatorrhoea (>15 g/24 h).

Figure 5 Individual values of the pancreatic function index (cholesteryl-octanoate/Na-octanoate $^{13}\text{CO}_2$ recovery ratio). Inverted triangle, mild to moderate CPD; upright triangle, severe CPD.

To assess the sensitivity of the cholesteryl octanoate breath test in the diagnosis of exocrine pancreatic insufficiency, we compared its results with those of simple tubeless tests (faecal chymotrypsin, fluorescein dilaurate test, and faecal fat); these techniques measure different digestive activities and, considered together, represent a valid determination of pancreatic exocrine function. Use of the CCK-secreatin test would have been ideal, but this procedure was avoided because it is invasive and unpleasant for the patient. For greater certainty, the severity of chronic pancreatitis was also assessed on the basis of morphological features (ultrasound, computed tomography, and ERCP).

The majority of patients (14/18, 77.8%) with exocrine pancreatic insufficiency determined by faecal chymotrypsin and the fluorescein dilaurate test showed decreased excretion of $^{13}\text{CO}_2$ after cholesteryl-[1- ^{13}C]octanoate ingestion. These patients could be best discriminated from healthy controls by the three hour cumulative $^{13}\text{CO}_2$ values. This may reflect the fact that most of the digestive processes involving the substrate used in the test take place within this time period. As indicated both by ^{13}C recovery and xylose curves, patients with CPD did not show different gastric emptying with respect to healthy controls. The earlier peak of plasma xylose with respect to the peak of $^{13}\text{CO}_2$ suggests that the oil phase containing the substrate may have separated in the stomach and emptied later.

Recovery of $^{13}\text{CO}_2$ was found to be related to the severity of the exocrine pancreatic insufficiency; in fact, all but one of the seven patients with severe chronic pancreatitis as assessed by morphological findings and functional tests (steatorrhoea and abnormal faecal chymotrypsin and fluorescein dilaurate test) showed very low recovery, and in all three patients with cystic fibrosis it was virtually absent. Moreover, the $^{13}\text{CO}_2$ excretion proved to be closely correlated with the degree of pancreatic insufficiency as determined by faecal chymotrypsin

and the fluorescein dilaurate test. The breath test results were also compared with faecal fat output and found to be inversely correlated. Figure 3C shows the disposition of the values of $^{13}\text{CO}_2$ output plotted against faecal fat: steatorrhoea does not occur until there is about a 90% reduction in $^{13}\text{CO}_2$ recovery; this is an indirect demonstration of the great functional reserve of the exocrine pancreas. In fact, in the pioneering study of DiMaggio *et al* it was clearly shown that steatorrhoea does not occur until there is at least a 90% reduction in pancreatic lipase output.²⁵

In three patients with severe pancreatic steatorrhoea, the total $^{13}\text{CO}_2$ recovery over the six hour test period was significantly improved during administration of pancreatin; however, the recovery normalised only in the two with chronic pancreatitis, while it remained abnormal in the patient with cystic fibrosis. This latter finding was probably due to alterations of the intestinal contents typically associated with cystic fibrosis, such as precipitation of bile acids and impaired micellar formation, at reduced intraluminal pH.²⁶ Mundlos *et al*²⁴ also reported incomplete correction of maldigestion by pancreatin formulated in enteric coated microspheres, as shown by this test, in patients with pancreatic insufficiency, but attributed this finding to retention of spheres in the stomach.

The cholesteryl octanoate breath test was also found to be abnormal in some patients with NPD. It is well known that hydrolysis of the substrate requires both pancreatic enzymes and bile acids. When bile acids are deficient, as occurs in liver disease, irritable bowel disease, or coeliac disease (because of impaired CCK release) the test will be positive despite normal pancreatic function. In such cases a control test with bile acid administration might be useful for excluding bile acid deficiency. Furthermore, other factors, such as alteration of intestinal mixing of the lipid phase (in gastrectomy and scleroderma) and/or impaired octanoic acid oxidation (in hepatic cirrhosis), may also account for the pathological results.

$^{13}\text{CO}_2$ recovery was abnormally low in all four patients with BPD performed for obesity. These patients also showed moderate to severe steatorrhoea (range 8.9–71.9 g lipid/24 hours), and had had considerable weight loss. The curves of ^{13}C appearance in the breath were flat or shifted to the right, showing a significant impairment or delay in fat digestion. This may be dependent on anatomical diversion of pancreatic secretion and/or impaired CCK release.¹³

According to the results of this study, the breath test with cholesteryl-[1- ^{13}C]octanoate as a test of fat malabsorption appears to have a good diagnostic specificity, but limited sensitivity in steatorrhoea of non-pancreatic origin. Likewise, the sensitivity of the breath test in the diagnosis of exocrine pancreatic insufficiency was not optimal; indeed, abnormal results were found in only 15/22 patients with CPD (68.2%). However, the presence in our series of cases with mild chronic pancreatitis (with normal faecal chymotrypsin or fluorescein

dilaurate test and slight morphological changes) may account for the less than excellent diagnostic performance of the test. Nevertheless, the sensitivity and specificity of this breath test for chronic pancreatic disease were similar to those of the other tubeless tests performed for comparison. The combination of the cholesteryl octanoate breath test with the faecal chymotrypsin and fluorescein dilaurate test did not increase diagnostic capacity for exocrine pancreatic insufficiency, since the gain in sensitivity was counterbalanced by a loss of specificity.

In a limited number of cases we performed a combined test, first with cholesteryl-[1-¹³C]octanoate and subsequently with Na-[1-¹³C]octanoate. The combination of the two tests should have (theoretically) improved the diagnostic performance of the cholesteryl-[1-¹³C]octanoate breath test, because the ratio of ¹³CO₂ recovery from the first and second test should correct the abnormality of octanoic acid absorption and metabolism in patients with NPD. In our study, however, we saw no advantage in diagnostic specificity with the combined test. One reason for this finding may be the presence of associated pancreatic insufficiency in the NPD group, as shown by low faecal chymotrypsin in one patient with coeliac disease.

In conclusion, the present study has shown that the cholesteryl-[1-¹³C]octanoate breath test has some validity in the assessment of lipid malabsorption. It can also be used for the diagnosis of exocrine pancreatic insufficiency, for which its sensitivity and specificity are comparable to those of two commonly used tubeless pancreatic function tests. The test is easy to carry out and can be performed in three hours. Unfortunately, at present the cost of the labelled substrate (US\$50–75 for each test) and apparatus, greatly exceeds that of the currently used pancreatic function tests. However, the potential uses of isotope ratio mass spectrometry in gastroenterology (such as for urea and liver function breath tests), and the possibility of performing the test at the patient's home and mailing the specimens to a test centre may eventually make the test more cost effective. In addition, the use of non-radioactive substrate means that this procedure is suitable for infants, children, and pregnant women.

The preliminary results of this study were presented in Oslo (Norway) at the 3rd United European Gastroenterology Week, 25–29 June 1994. This study was partially supported by the University and Technological and Scientific Research Ministry of Italy in 1994.

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