Secretion of Exocrine Pancreatic Enzymes in Patients with Tropical Sprue, Following Stimulation by a Test Meal

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Pancreatic function in response to a test meal was studied in 24 control subjects, 24 patients with tropical sprue, and 7 patients with chronic pancreatitis. A non-absorbable marker was used to estimate recovery of the meal from the duodenum. Trypsin, amylase and esterase were measured and the results expressed both as concentration and total enzyme output per h. Patients with tropical sprue had a significant reduction in pancreatic function compared with control subjects, as shown by a decreased output and decreased concentration of all three enzymes. The output of pancreatic enzymes in the sprue patients showed a significant correlation with the serum albumin concentration but not with any parameter of intestinal structure or function. The different parameters of pancreatic function showed general overall agreement. In detection of severe pancreatic dysfunction any one enzyme measurement would suffice, but in studying minor degrees of pancreatic hypofunction it is advantageous to measure several enzymes.

Introduction

Steatorrhoea has long been recognized as one of the hall-marks of tropical sprue. This defect of fat absorption has most often been assumed to be due to the damage to the mucosal cells which can be demonstrated morphologically. Recently, however, it has been shown that bile salt abnormalities may occur in some patients with tropical sprue and these may contribute to the pathogenesis of steatorrhoea (Kapadia et al., 1971; Kapadia and Baker, 1973). A search of the literature failed to reveal any detailed investigation of pancreatic function, which has usually either been studied only by detecting the presence or absence of enzymes in duodenal juice, or has been assumed to be normal. This investigation was therefore undertaken to study the pancreatic response of patients with tropical sprue to a test meal and to compare this with the responses of normal subjects and patients with chronic pancreatitis.

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Material and Methods

Twenty-four patients with tropical sprue, 24 apparently healthy asymptomatic volunteers of similar socio-economic status and 7 patients with chronic pancreatitis were studied.

All the subjects were admitted to a metabolic ward, a detailed clinical and dietary history was obtained and a physical examination performed. All were given a diet containing 50 g. of fat per day and the daily fat excretion measured throughout their stay in hospital. Xylose absorption was studied using a 5 g. dose of d-xylose and measuring the 5 h. urinary xylose excretion. Vitamin B₉₂₆ absorption was measured by the serum method following 1 μg dose of Co³⁺ labelled vitamin B₂₆. All subjects also had a jejunal biopsy and a barium meal examination with particular emphasis on the small intestine.

The results of the intestinal studies in the control subjects were similar to those recorded previously from this Unit (Baker and Mathan, 1972). None of the subjects had steatorrhoea, six had decreased xylose excretion and none had vitamin B₂₆ malabsorption. The biopsy and X-ray findings were in the normal range for the local population.

The patients with tropical sprue all had steatorrhoea, xylose malabsorption, radiological changes in the small intestine, and jejunal biopsy abnormalities and 18 had vitamin B₂₆ malabsorption. In all cases other known causes of malabsorption were excluded. These patients were typical cases of tropical sprue as described in detail previously (Baker and Mathan, 1971).

The seven patients with chronic pancreatitis all had gross steatorrhoea but a normal xylose and vitamin B₂₆ absorption, and their jejunal biopsies were normal for this population. Three had pancreatic calcification seen in a plain X-ray of the abdomen.

Exocrine pancreatic function was studied following stimulation with a test meal similar to that described by Lundh (1957). After an overnight fast, a radio-opaque polyvinyl tube with an internal diameter of 2 mm. and a tip of 3 mm. internal diameter, 5 cm. long, with multiple lateral holes, was passed per os and the tip positioned at the end of the fourth part of the duodenum under fluoroscopic control. A second tube was passed and positioned so that the distal end lay at the most dependent part of the stomach. Using small mechanical pumps, suction was applied to both the gastric and intestinal tubes. Periodically small amounts of air were injected down both tubes to ensure that the distal ends were not blocked. Aspiration was carried out for a period of 20 min. and the fasting collection discarded.

A test meal of the following composition—spray dried milk powder 48 g., dextrose 15 g., groundnut oil 6 g., polyethylene glycol (PEG) 1 per cent solution, 50 ml., with water to make 250 ml. was given by mouth. This meal contained 4:2 per cent protein, 15-6 per cent carbohydrate and 5-9 per cent fat. Suction was applied to the duodenal
tube for the next h. and the aspirate collected on ice. At the end of the hour the stomach was aspirated as completely as possible.

PEG was estimated by the method of Hyden (1955) and the recovery of the PEG marker in the aspirates calculated. The amount recovered from the stomach (S) was subtracted from the total given (T). It was assumed that the remainder (T-S) entered the duodenum and the amount recovered from the duodenum (D) was expressed as a percentage of this.

\[
\text{Per cent recovery from duodenum} = \frac{D}{T-S} \times 100
\]

Those tests in which this per cent recovery was less than 50 were considered unsatisfactory and repeated. With some experience in carrying out the procedure this happened only occasionally.

Trypsin was estimated by the method of Lundh (1957) using n-benzoyl arginine ethyl ester substrate; amylase by the method of Bernfield (1955) and esterase by the method of Bier (1955) using p-nitrophenyl acetate substrate. Enzyme output was expressed both as total/h. (mg/h. in the case of trypsin and kilounits/h in the case of amylase and esterase) corrected to a recovery of 100 per cent (Zieve et al., 1966) and as average concentration per ml. of aspirate over the whole hour.

Results

The mean per cent recovery of PEG from the duodenum for the whole group was 80 per cent. The output of the enzymes in the three groups of subjects is shown in the figure and the means and standard error of the means in Table I. The mean output of each enzyme, whether expressed as total per hour or as enzyme concentration per ml. was highest in the control subjects, was significantly lower in the patients with sprue and lowest of all in the subjects with chronic pancreatitis.

In one patient with tropical sprue whose initial response resembled that of a patient with chronic pancreatitis, pancreatic function was re-evaluated eight months later, when the patient was in partial remission, when the output of all enzymes had improved considerably although trypsin and amylase still remained subnormal (Table II).

In the patients with sprue there was no correlation between enzyme output and the severity of the malabsorption as judged by the stool fat excretion, vitamin B₁₂ absorption, xylose excretion or jejunal biopsy appearances. However there was a significant correlation between the serum albumin concentration in the sprue patients and the total output of trypsin, amylase and esterase (Table III). No such relationship was found among the control subjects or those with chronic pancreatitis.

Discussion

The test meal as a provocative test in the evaluation of exocrine pancreatic function was introduced by Lundh (1962) and has subsequently been used by a number of
Table I. Mean enzyme output and the standard error of the mean, expressed both as total per h. and mean enzyme concentration, for trypsin, amylase and esterase and the significance of the differences between the means in the three groups of subjects.

<table>
<thead>
<tr>
<th></th>
<th>Total output per h.</th>
<th>Mean concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard error of</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>Standard error of</td>
</tr>
<tr>
<td></td>
<td></td>
<td>µg./ml.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mean</td>
</tr>
<tr>
<td>Trypsin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>65.1</td>
<td>3.47</td>
</tr>
<tr>
<td>Sprue</td>
<td>26.6</td>
<td>3.66</td>
</tr>
<tr>
<td>Chronic pancreatitis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amylase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>27.9</td>
<td>2.42</td>
</tr>
<tr>
<td>Sprue</td>
<td>9.8</td>
<td>1.47</td>
</tr>
<tr>
<td>Chronic pancreatitis</td>
<td>4.0</td>
<td>1.44</td>
</tr>
<tr>
<td>Esterase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>11.1</td>
<td>0.59</td>
</tr>
<tr>
<td>Sprue</td>
<td>5.7</td>
<td>0.84</td>
</tr>
<tr>
<td>Chronic pancreatitis</td>
<td>1.8</td>
<td>0.28</td>
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</table>

Table II. Patient G: results of Lundh test meal initially and after eight months therapy.

<table>
<thead>
<tr>
<th></th>
<th>Trypsin</th>
<th>Amylase</th>
<th>Esterase</th>
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<tr>
<td></td>
<td>Total mg.</td>
<td>Concentration</td>
<td>Total kilounits</td>
</tr>
<tr>
<td></td>
<td>µg./ml.</td>
<td></td>
<td>units/ml.</td>
</tr>
<tr>
<td>Initial</td>
<td>0</td>
<td>0</td>
<td>5.2</td>
</tr>
<tr>
<td>After eight months</td>
<td>16.2</td>
<td>50</td>
<td>16.0</td>
</tr>
</tbody>
</table>

Table III. Correlation, in sprue patients, between serum albumin concentration and total enzyme output.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Correlation coefficient</th>
<th>t</th>
<th>Degrees of freedom</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypsin</td>
<td>0.72</td>
<td>4.822</td>
<td>22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Amylase</td>
<td>0.59</td>
<td>3.454</td>
<td>22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Esterase</td>
<td>0.64</td>
<td>3.919</td>
<td>22</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
other workers (Hartley et al., 1966; Worning and Müllertz, 1966; Zieve et al., 1966; Worning, et al., 1961). It was decided to employ this test, rather than one using secretin-pancreozymin, since the meal is a more physiological stimulus and tests the integrity of both the pancreas and endogenous hormone production.

Any test dependent on gastrointestinal aspiration is beset with problems. The composition of the fluid aspirated from the duodenum is modified by variables such as sporadic gastric emptying, biliary secretion, pancreatic secretion, variable absorption from the duodenum and loss of fluid from the duodenum into the jejunum or reflux into the stomach. Further, aspiration of luminal contents is seldom, if ever, complete.

In an attempt to minimise some of these difficulties Lundh (1962) incorporated a non-absorbable marker in the test meal but only calculated the results in terms of enzyme concentrations. Zieve et al. (1966) also used a marker and calculated total enzyme output as in this study. The use of a marker in this way assumes that the meal entering the duodenum and the pancreatic secretion mix thoroughly and that the aspirate
represents a true aliquot of the whole. These assumptions may not be fully valid, but Worming (1967) has shown that the marker technique is more valid when used with a test meal than when used with a secretin-pancreozymin test.

In this study the results were expressed both as total output corrected for recovery of the meal and as mean enzyme concentration per ml. of aspirate per h. Both methods appear to give similar information. The correlation coefficients for the two sets of values for the three enzymes were trypsin $r=0.69$; amylase $r=0.82$ and esterase $r=0.72$ ($P<0.0001$ in each case). It appears that for routine use little is gained by attempting to estimate total enzyme output.

The estimation of esterase as a parameter of pancreatic function needs some explanation. The lipolytic activity of the pancreatic secretion can be broadly divided into the true lipase fraction and the non-specific carboxylic ester hydrolyase (esterase). The true physiological significance of esterases are not known (Erlansen and Borgstrom, 1970). However, it has been demonstrated that, in the presence of bile salts, esterases also cleave long chain triglycerides in emulsion form, a situation obtained under normal physiological circumstances in the duodenum (Barrowman and Borgstrom, 1968)
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Methods for estimating lipase such as those described by Marchis-Mouren et al. (1959) and Cherry and Crandall (1922) are not suitable for a system where the pancreatic juice is mixed with a test meal in an emulsion form. Barrowman and Borgstrom (1968) showed that in a system containing bile salts, p-nitrophenyl laurate was hydrolyzed both by lipase and esterase, whereas p-nitrophenyl acetate was hydrolysed only by esterase. It was therefore decided to use the latter to measure esterase activity as a further estimate of pancreatic function in addition to trypsin and amylase.

The results obtained in this study show that the esterase, amylase and trypsin values correlate well with each other, whether expressed as total output or as enzyme concentration (Table IV). If only the measurements of enzyme concentration are considered, and if the lower limit of normal is defined as the mean in the control group minus twice the standard deviation, then the results of the individual tests in the sprue and chronic pancreatitis patients are given in Table V. All seven patients with chronic pancreatitis had abnormal results in all tests. Eighteen out of 24 patients with sprue had concordant results, whereas in six the results of the measurement of the three different enzymes were discordant. For detecting grossly abnormal pancreatic function, such as in chronic pancreatitis, measurement of any one enzyme would probably be adequate, but when it is desired to employ minor degrees of pancreatic hypofunction then a battery of tests seems preferable. This may particularly be so when hormonal stimulation alone is employed (Goldberg and Wormsley, 1970).

Table IV. Correlation between the different measurements of pancreatic response, data from all 55 subjects pooled.

<table>
<thead>
<tr>
<th>Enzyme 1</th>
<th>Enzyme 2</th>
<th>Correlation coefficient r</th>
<th>t</th>
<th>Degrees of freedom</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypsin total</td>
<td>Esterase total</td>
<td>0.87</td>
<td>12.709</td>
<td>53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trypsin conc.</td>
<td>Trypsin conc.</td>
<td>0.85</td>
<td>11.909</td>
<td>53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Amylase total</td>
<td>Esterase total</td>
<td>0.90</td>
<td>9.861</td>
<td>53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trypsin conc.</td>
<td>Amylase conc.</td>
<td>0.75</td>
<td>8.371</td>
<td>53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trypsin total</td>
<td>Amylase total</td>
<td>0.72</td>
<td>7.582</td>
<td>53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trypsin conc.</td>
<td>Amylase conc.</td>
<td>0.67</td>
<td>6.394</td>
<td>53</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The results for the concentration of trypsin in the control subjects in this study are of the same order, though slightly lower, than those obtained by Lundh (1962), who found a range from 161-612 µg./ml and Hartley et al. (1966) who found a mean concentration of 333 µg./ml. Unfortunately Lundh did not measure other enzymes, and in the studies of Zieve et al. (1966) and Worning and Möllerz (1966) different methods of analysis and data presentation were used, making direct comparisons with the present series impossible.

The seven subjects with chronic pancreatitis showed considerable reduction in all enzymes, the most affected being trypsin which was absent in all. These findings
are in conformity with those of other workers (Hartley et al., 1966; Worning et al., 1968).

In an earlier report from this laboratory (Balagopal, 1971) studies on a small number of patients with tropical sprue were presented. Though the enzyme output in these patients was decreased compared with controls, the difference in the means did not attain statistical significance. However, the present larger study indicates that in tropical sprue there is in fact a significant reduction in the mean enzyme output and that many patients with tropical sprue have enzyme values lower than those found in control subjects. In some of the patients the enzyme output is so low as to be in the same range as subjects with chronic pancreatitis. At times this may produce problems in differential diagnosis. The distinguishing features are the absence of other absorptive defects, the absence of the classical bariurn meal changes of sprue and a normal jejunal biopsy in patients with chronic pancreatitis. Although we have not found vitamin B₁₂ malabsorption in patients with chronic pancreatitis, this has been reported from other centers (McIntyre et al., 1956), and if present, would further confuse the picture. Patients with pancreatitis may have calcification of the pancreas which does not occur in sprue. Patients with sprue tend to improve either spontaneously or following therapy (Baker and Mathan, 1971) whereas patients with chronic pancreatitis have their disease for life. It may perhaps also be possible to distinguish patients with sprue, with a very low enzyme output in response to a test meal, from patients with chronic pancreatitis by a secretin-pancreozymin test. This is currently under study.

It was a clinical impression that the patients with the greater degrees of pancreatic hypofunction were, in general, those who were most ill. However, with one exception, there was no correlation between enzyme production and any other parameter such as steatorrhoea, vitamin B₁₂ absorption, xylose excretion or jejunal biopsy. The one exception was a correlation with the concentration of serum albumin. A similar correlation between the concentration of serum albumin and pancreatic enzyme output following stimulation with secretin-pancreozymin has been observed in children with
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kwashierkor (Barbezat and Hansen, 1968) and in patients with a variety of malabsorptive states (Novis et al., 1972). Pancreatic function has long been shown to be reduced in conditions of protein deprivation both in man and animals (Grossman et al., 1943; Veghelyi et al., 1950; Thompson and Trowell, 1952; Gomez et al., 1954; Lemire and Iber, 1967; Tandon et al., 1969; Klotz et al., 1972). The precise pathogenesis of the pancreatic hypofunction has not been fully elucidated, but in all cases it has tended towards normal on protein re-feeding. The lower serum albumin concentration in our patients is probably an indication of a generalised disorder of protein and aminoacid metabolism secondary to the malabsorptive state (Vaish et al., 1965). The synthesis of enzymes by the pancreatic acinar cells presumably requires the availability of appropriate aminoacids and in the case of trypsin and lipase, sulphur containing aminoacids appear to be essential (Veghelyi and Kemeny, 1962). However the amino acid composition of the plasma in our four subjects with the greatest diminution of pancreatic function did not reveal any consistent pattern of hypoaminoacidemia except for a uniform depression of valine. The significance of this finding is not clear and further studies on the pathogenesis of decreased pancreatic function in hypoalbuminemic states are needed.

There are several reports of pancreatic function in subjects with non-tropical sprue. Comfort et al. (1949) studied 13 patients using hormonal stimulation and found normal lipase activity. However other workers using hormonal stimulation (Newsome, 1949; Drelling, 1957; Working, 1971; Novis et al., 1972) have found abnormal pancreatic function in a proportion of the patients studied. The findings of the latter authors are perhaps compatible with post-mortem studies in patients with idiopathic sprue by Himes and Adlesberg (1957) who found some degree of microscopic pancreatic fibrosis in five out of eight subjects and evidence of acinar atrophy in one. Using a test meal Worning et al. (1967) found reduced concentrations of amylase, lipase and trypsin in subjects with gluten induced enteropathy. DiMagno et al. (1972) extended these observations when they infused a solution of essential aminoacids into the duodenum and found a depression of output of trypsin, amylase and lipase in patients with non-tropical sprue as compared with control subjects, whereas after maximal stimulation with exogenous hormones, the outputs in the two groups were similar. These authors logically conclude that there was impaired secretion of cholecystokinin-pancreozymin in their patients but that the pancreas itself was normal. In summary it would appear that in non-tropical sprue pancreatic response to a test meal stimulus is not infrequently abnormal, whereas response to hormonal stimulation is only abnormal in a smaller proportion of cases. Recently Polak et al. (1973) have shown that 60 per cent of children with coeliac disease have an increase in secretin containing cells in their jejunal biopsies and suggest that there may be a failure of secretin release. Such failure could explain a poor response to a test meal.

We have only been able to locate two reports on pancreatic function in tropical sprue. Brown (1921) studied 5 cases of tropical sprue, 2 from Puerto Rico and 3 from the Philippines. "All had been diagnosed as sprue by experts in this disease". He found extremely low values of pancreatic enzymes both in stools and duodenal
contents. Four out of the 5 cases improved markedly on administration of pancreatin or some form of pancreatic extract. However, in the absence of present day diagnostic methods, the differentiation of tropical sprue from chronic pancreatitis must have been very difficult, if not impossible, and the history of response to pancreatic extracts suggests that these may have been cases of chronic pancreatitis. Ashford (1932) reported that in tropical sprue "there is an intermittent reduction in pancreatic output" and "the duodenal contents showed reduction in amylase and lipase" but we have been unable to locate details of this work.

Although this study has demonstrated defective pancreatic enzyme secretion in response to a test meal, it does not permit of differentiation of the relative roles of abnormalities in the production of endogenous hormones in the intestine and abnormalities in the pancreas itself. Further, from this study it is not possible to determine to what extent pancreatic hypofunction contributes to the malabsorptive state of tropical sprue. It seems probable that in those with very low outputs the decreased enzyme secretion may make a considerable contribution to the fat malabsorption. However, the extent of this contribution can only be precisely determined by a quantitative study of intraluminal hydrolysis. Further studies to elucidate these various points are in progress.

Acknowledgment

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