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Assessment of pancreatic exocrine function

Impairment of pancreatic exocrine function in childhood is most commonly seen in association with cystic fibrosis but occurs in a range of different disorders.¹ Function may be assessed by aspiration of duodenal juice and measurement of enzymes secreted by the gland in response to stimulation by hormones or nutrients. Such stimulatory tests are intended to define whether or not the pancreas is diseased, abnormal secretions implying abnormal pancreas. There is, however, no clear agreement on what constitutes abnormal results.

Among the indications for pancreatic function testing are clinical suspicion of pancreatic insufficiency such as in the child with steatorrhoea and failure to thrive, the assessment of enzyme replacement therapy in a child with known pancreatic disease such as cystic fibrosis, and serial monitoring of function for example after pancreatitis or partial pancreatectomy. Alternatives to duodenal intubation include a number of indirect function tests. These are often easier to perform and rely upon analysis of enzymes in faeces or serum or investigation of how completely an administered test substance is digested. Indirect tests are usually based on the assessment of a single specific pancreatic enzyme; it is not always appropriate to make assumptions about other enzymes. For example, isolated enzyme deficiencies are well recognised,^{2,3} and in newborns and young infants there appears to be variation in the rates of development of individual pancreatic enzymes.⁴ In general, however, such tests when combined with clinical assessment are highly suitable for identifying those patients who require more detailed study. As pancreatic insufficiency is relatively uncommon in childhood it is important that an indirect test should reliably give a negative result in the absence of disease.

Intraduodenal tests

PANCREOZYMIN-SECRETIN TEST

The standard intraduodenal test involves stimulation of the pancreas with intravenous cholecystokinin (pancreozymin) followed by secretin.⁵ These specific intestinal peptides are used because they provide a powerful stimulus and appear to be important in normal control of pancreatic function. A

simplified representation of their physiological role would be that secretin, released by acid in the duodenum, provides a bicarbonate rich secretion, while cholecystokinin released by the products of protein and fat digestion stimulates the production of a fluid rich in enzymes including trypsin, chymotrypsin, carboxypeptidase, pancreatic amylase, and lipases. Trypsin, released as inactive trypsinogen, changes spontaneously into its active form in solution, converting the other proteases into their active forms. This change is accelerated both by enterokinase secreted by enterocytes of the proximal small intestine, and the presence of activated trypsin.⁶ Pancreatic lipase is prevented from being denatured as a result of adsorption to the oil:water interface through being anchored by a colipase, a small protein also secreted by the pancreas.

During the test, secretin and cholecystokinin are given at an unphysiological dose of 2 units/kg body weight with the aim of exciting the maximal possible response in the pancreas. Children in the first few months of life seem refractory to stimulation, probably because there are insufficient numbers of secretagogue receptors or receptor affinity is low. The total volume of secretions, bicarbonate output, lipase and amylase activities are measured together with one or more proteases⁷ and expressed in terms of output/kg body weight/50 minutes of test. A correction for incomplete juice recovery can be made if a non-absorbable marker is perfused into the proximal duodenum and aspirated distally.⁸ The validity of this test refinement which adds greatly to an already technically complex procedure has not been clearly established. Enzyme analysis should be performed without delay, but addition of glycerol (50% v/v) and storage at -20°C will stabilise lipase and trypsin for at least a month. Although reference ranges for stimulated duodenal juice have been published for children,⁷ problems in standardising analysis of enzymes make comparisons between laboratories extremely difficult. Ideally, stimulation tests should only be performed in a centre with its own reference ranges. Normal amylase and lipase activities with low protease activity should suggest congenital enterokinase deficiency.⁹ Addition of enterokinase to a sample of duodenal fluid causes a marked increase in tryptic activity.

MEAL TEST

A test meal of glucose, protein, and fat may be used to provide a more physiological stimulus to pancreatic secretion. A two hour postprandial collection of duodenal fluid is made and bile salt concentration as well as enzyme activity per millilitre measured.¹⁰ The test can therefore be used not only to detect pancreatic pathology but also to investigate other factors in the pathophysiology of steatorrhoea. Disadvantages include an appreciable technical failure rate, the need for a two hour collection, and the fact that bicarbonate analysis is not possible. It is, however, simpler to perform than hormonal stimulation and could be carried out in units experienced in taking jejunal biopsies, with appropriate analytical facilities.

Non-invasive tests

'Tubeless' tests of pancreatic exocrine function involve ingestion of a non-absorbed compound that is cleaved by a specific pancreatic enzyme, producing a fraction which is absorbed in the small intestine and excreted in the urine. The proportion recovered in a timed urine specimen, or the peak plasma concentration, give a measure of pancreatic enzyme activity. At best they appear to be semiquantitative. Evaluation has largely been in adult patients where they have a clear role in identifying those in need of further investigation.

BENTIROMIDE TEST

The synthetic peptide *N*-benzoyl-L-tryosyl-p-aminobenzoic acid is used in the bentiromide (paba-peptide, NBT-PABA test), 15 mg/kg body weight being given together with a test meal. The composition of the test meal does not seem to be too important as long as some nutrient stimulation is used. NBT-PABA is hydrolysed by chymotrypsin and the p-aminobenzoic acid (PABA) fraction released is absorbed, conjugated in the liver, and excreted in the urine. The proportion of PABA recovered in a six hour urine collection is expressed as the percentage of PABA given as NBT-PABA. Measurement of serum PABA at 120 minutes obviates the need for urine collection and, in one study using a higher dose of peptide (30 mg/kg) together with a liquid meal, distinguished between 34 controls with normal fat absorption and 35 patients with fat maldigestion due to pancreatic insufficiency.¹¹ Interference with the spectrophotometric assay of PABA by drugs including sulphonamides, diuretics, paracetamol, chloramphenicol, and food such as prunes and cranberries causes a high test failure rate.¹²

False positive tests may occur in the newborn, when there is immature renal function or when urine collection is incomplete. False negative results have been described in Shwachman's syndrome,¹³ possibly due to hydrolysis of NBT-PABA by mucosal paba-peptide hydrolase.¹⁴ False positive results may also occur when there is malabsorption due to enteropathy. In this case, recovery of free PABA given on a second day test will also be low and the ratio of the two percentage results can be expressed as an excretion index. In malabsorption for example, PABA recovery will be low on both days giving a ratio approaching unity, as one would find in normal controls. While this approach improves test specificity, the two stages can be combined in a single investigation using the structural and pharmacokinetic relation to PABA, p-amino salicylic acid (PAS), as a marker for PABA handling, and expressing the result as urine PABA:PAS ratio.¹⁵ This test was reliable in separating 28 children with pancreatic insufficiency from 20 controls.¹⁴ Separation of PABA and PAS in urine requires high performance liquid chromatography; separation has proved technic-

ally more difficult to achieve in plasma, so that an alternative to urine collection is not yet available.

FLUORESCEIN DILAURATE TEST

Fluorescein dilaurate (pancreolauryl) is an alternative substrate to NBT-PABA, hydrolysed by pancreatic cholesterol ester hydrolase to produce lauric acid and fluorescein. The latter is excreted in a 10 hour urine collection and can be assayed using spectrophotometry or fluorimetry. A second day test with unesterified fluorescein improves test specificity, as in the bentiromide/PABA test, at the cost of complicating the procedure. Serum fluorescein can be measured as an alternative to urine collection. Using a dose of 0.25 mmol, results in normal controls appear widespread and overlap with those found in children with cystic fibrosis.¹⁶ However, another study in which half this dose was given demonstrated complete separation between 20 patients with cystic fibrosis and 12 controls.¹⁷ Bile salt abnormalities may influence the test result by an effect on esterase activity.

¹³CO₂ BREATH TEST

A further non-invasive test relies upon ingestion of fat containing the non-radioactive isotope of carbon, ¹³C, and the measurement of the ¹³C labelled carbon dioxide in the breath.¹⁸ This investigation provides a measure of the overall functional level of lipase activity in the duodenum. A mass spectrometer is required for analysis, and accurate end expiratory breath sampling is difficult in infants and young children.

SERUM ENZYMES

Serum enzyme measurement is sometimes helpful in assessment of exocrine pancreatic function. Normal serum amylase concentrations tend to rise with age and may be low in patients with cystic fibrosis. Raised serum cationic trypsinogen has been used in the newborn period as a screening test for cystic fibrosis¹⁹ but initial false positive tests are relatively common. As disease of the pancreas progresses, serum concentration falls and by 5–7 years of age is subnormal, reflecting the level of exocrine insufficiency.²⁰ The finding of low or undetectable serum immunoreactive trypsinogen suggests severe impairment of pancreatic function as, for example, in Shwachman's syndrome.

FAECAL FATS

Faecal analysis is unpopular in most biochemistry laboratories and while no longer considered useful in the investigation of pancreatic disease²¹ still has a role in comparison of enzyme replacement therapy. Although in theory increased stool triglyceride might distinguish between maldigestion and malabsorption, this does not seem to be the case in practice.²² The 'steatocrit' test is a relatively simple measure of fat malabsorption/maldigestion but lacks both sensitivity and specificity for investigating pancreatic disease. Serial measurements may be useful for determining adequacy of enzyme replacement.

FAECAL ENZYMES

Although possessing the attraction of ease of sampling, faecal enzyme analysis in the assessment of pancreatic exocrine function enjoys a poor reputation, which is undeserved. This negative view²³ probably derives from experience in adult patients that stool enzyme measurements are not sensitive enough to be used as a diagnostic test for a range of conditions that produce very variable effects on exocrine function.

There are fewer problems in children in whom a more rapid bowel transit means less degradation of enzyme, and who more often present with severe pancreatic insufficiency.

Chymotrypsin can be measured using a kinetic, potentiometric method, in which the rate of hydrolysis of enzyme specific synthetic substrate (acetyl tyrosine ethyl ester) is recorded with an automatic titrator and recorder.²⁴ A proprietary test kit, the Boehringer-Mannheim C-system, is also available. This is an automated colorimetric assay for chymotrypsin in stools based on the method of Kaspar *et al.*²⁵ Chymotrypsin activity remains stable for several days at ambient temperature so that samples can be sent to the laboratory by post without difficulty. Low levels may occur when there is constipation.

Faecal chymotrypsin concentration showed a significant positive correlation with duodenal chymotrypsin secretion after hormonal stimulation in 25 children who responded to intravenous cholecystokinin.²⁶ Correlation was appreciably poorer using a single stool estimation compared with the mean of three taken within 72 hours of the stimulation test. In 46 children with cystic fibrosis and clinical evidence of malabsorption, chymotrypsin measured in a single stool was unequivocally subnormal in each case, and in five children with undetectable or only traces of chymotrypsin in the duodenum after stimulation, the mean faecal chymotrypsin concentrations were only 3–10% of the lower reference range.²⁷ In infants with meconium ileus, faecal chymotrypsin was below the reference range in all 22 who were subsequently shown to have cystic fibrosis and within the normal range in all eight who later had negative sweat tests.²⁷ This test is therefore useful for identifying pancreatic insufficiency in the surgical newborn too young for sweat testing, and in this clinical situation is both sensitive and specific. Concentrations in preterm infants are similar to those at term, and lower in growth retarded compared with appropriately grown infants,²⁸ a finding consistent with the effects on the pancreas of antenatal malnutrition in animal models and starvation in childhood.

When clinical symptoms suggest the possibility of pancreatic insufficiency a reliable non-invasive test should be performed. From the point of view of simplicity, cost, and repeatability there seems little doubt that faecal chymotrypsin is the best first line test for the district general hospital paediatrician. If faecal chymotrypsin or another tubeless test is abnormal further investigation is necessary. Duodenal intubation with test meal may be the most appropriate next step in units with the necessary laboratory facilities, while pancreozymin-secretin testing should be confined to regional paediatric gastroenterology units.

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