Progress report

Exocrine pancreatic function tests

Pancreatic disease may be classified as inflammatory, neoplastic, traumatic, or genetic. According to the definition of Marseilles, inflammatory diseases may be subdivided into (1) acute pancreatitis, (2) relapsing acute pancreatitis, (3) relapsing chronic pancreatitis, and (4) chronic pancreatitis (Table 1). The first classification is primarily based on aetiological, the second on clinical and functional and/or morphological criteria. For the diagnosis of pancreatic disease, pancreatic function tests are needed among other diagnostic procedures to assess the amount of functional damage. As pancreatic function returns to normal after attacks of acute or relapsing acute pancreatitis, unlike what is found in the chronic form of the disease, pancreatic function tests are necessary to differentiate between acute and chronic presentations of the disease.

Since the pioneer work of Chiray et al. and Lagerlöf on the evaluation of the secretin test in pancreatic diseases, a variety of exocrine pancreatic function tests have been developed over the last 50 years. They may be divided into the following categories (Table 2):

1. Direct tests, in which pancreatic flow, bicarbonate, and enzyme secretion are measured in duodenal or pure pancreatic juice after exogenous hormonal stimulation of the pancreas.
2. Indirect tests, which make use of nutrients for endogenous stimulation of pancreatic enzyme secretion.
3. Faecal tests, which include microscopic inspection of stools and estimation of faecal trypsin, chymotrypsin, fat and nitrogen content.
4. Serum enzyme or isoenzyme estimation, with or without previous hormonal stimulation.

The purpose of this progress report is (1) to discuss current exocrine pancreatic function tests with special reference to their practicability, (2) to state whether these tests are able to differentiate between impairment of pancreatic function of diverse aetiology, and (3) to suggest possible ways of refining tubeless tests. Three excellent reviews have been published

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<thead>
<tr>
<th>Classification of pancreatic diseases</th>
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<tbody>
<tr>
<td><strong>I Inflammatory</strong></td>
</tr>
<tr>
<td>Acute pancreatitis</td>
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<td>Relapsing acute pancreatitis</td>
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<td><strong>II Neoplastic</strong></td>
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<td>Ductal origin</td>
</tr>
<tr>
<td>Parenchymal origin (acinar cells)</td>
</tr>
<tr>
<td>Islet cell origin</td>
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<tr>
<td><strong>III Traumatic</strong></td>
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<td>Non-penetrating or blunt traumas</td>
</tr>
<tr>
<td>Penetrating injuries</td>
</tr>
<tr>
<td><strong>IV Genetic</strong></td>
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<td>Cystic fibrosis</td>
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<td>Hereditary and familial pancreatitis</td>
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recently, they deal mainly with direct tests and older indirect pancreatic function tests such as the Lundh test and faecal enzyme estimations. These will therefore be referred to only briefly and the role of more recently developed tests, such as tubeless pancreatic function tests and serum enzyme estimations, will be more extensively discussed.

**Direct tests**

**Determinations in duodenal juice**

The major technical problems of measuring pancreatic volume, bicarbonate and enzyme contents in duodenal juice after stimulation of the pancreas are to prevent contamination by gastric juice and to overcome the loss of duodenal contents into the jejunum or by reflux into the stomach. For intubation, double- or multiple-lumen tubes have been recommended; these offer the possibility of separate collection of gastric juice and balloons for occlusion of the pylorus and distal duodenum. In a comparative study the latter tube gave a higher percentage of recovery. Alternatively, non-absorbable markers such as polyethylene glycol (PEG) or $^{57}$Co-labelled vitamin $\text{B}_12$ (not allowed in several centres for legal reasons) have been recommended for calculating the loss of duodenal juice. The data for recovery of investigations using these markers, however, differ considerably (for review see Worning), and, unfortunately, are difficult or almost impossible to compare, as methods for intubation, position of patients, markers, their concentration, and infusion rate vary from study to study. Using PEG as a marker, recovery was very high or almost complete in some studies, but amounted to only up to 40% in others. This may be due to the position of the patient on his left side, or to the advanced distal position of the tube. As it is almost impossible to control adequate mixing of markers and duodenal juice, low recovery rates do not necessarily represent high losses of duodenal contents. Until new technical improvements appear, the following statement of Wormsley should be accepted: a recovery rate of above 85%
indicates that the collection technique is probably satisfactory and the magnitude of output of pancreatic juice can be satisfactorily inferred from experimental findings. Recovery of less than 85% means that the collection technique needs to be modified.

In comparison, loss of duodenal contents into the stomach and contamination of duodenal juice by gastric secretion present a minor problem. Duodenogastric reflux is only rarely significant and gastric secretion is low during pancreatic function tests. Errors in the interpretation of duodenal contents of bicarbonate and pancreatic enzymes can be avoided by the use of indicators and by estimating bilirubin in the gastric aspirate.

In some patients duodenal intubation for aspiration of duodenal contents or pure pancreatic juice may be difficult. To make the procedure easier diazepam may be given. Hyoscine butylbromide, however, should not be used, as it reduces trypsin secretion into the duodenum and delays the appearance of both trypsin and bilirubin in duodenal aspirate.

**Secretin test**

Tests of secretory capacity such as the secretin test are based on the assumption that a decrease in the capacity to secrete fluid, bicarbonate, and enzymes indicates pancreatic damage, and that the dose of stimulant which provides submaximal or maximal stimulation to normal glands also exerts the same effect on the diseased pancreas.

After the evaluation of the secretin test by Lagerlöf, Dreiling and his colleagues, Sun and Shay, Burton et al., Petersen and Myren and other investigators (for review see) have provided more information on the usefulness of the test. Secretin has been applied by rapid intravenous injection, subcutaneously, and by continuous intravenous infusion. The results of these studies are difficult to compare because the type of secretin preparation is important and potent, dosage, and the test procedures used differed from study to study. When making comparisons it also has to be considered whether plastic material has been used for injections and especially for infusions, because plastic surfaces are able to bind secretin. This binding can be abolished by dissolving secretin in serum albumin.

Recently, the augmented secretin test (bolus intravenous test repeated with 4 clinical units/kg body weight intravenously) has been introduced and this is said to enhance the accuracy of the standard function test. The procedure is of interest but needs to be further investigated.

**Secretin-CCK (caerulein)-test**

After secretin administration, only a correct evaluation of the hydrokinetic function of the pancreas may be expected, whereas the enzyme output after the secretin wash-out varies considerably and may even be normal in many patients with chronic pancreatitis. CCK was therefore added to the test procedure in order to assess the ebolic pancreatic function.

Like secretin, CCK has also been given by different methods as a single intravenous injection before, with and after secretin, or by continuous intravenous infusion alone, or in combination with secretin. CCK may also be replaced by caerulein (Farmitalia, Research Laboratories, Milano, Italy) for testing the ebolic function of the
pancreas. It can be obtained in pure form and is cheaper than CCK. When it is combined with secretin infusion it provides a similar stimulation of pancreatic enzyme secretion.41–43

False abnormal test results of the secretin-CCK-test caused by an inadequate contraction of the gallbladder after administration of these substances are prevented by the intraduodenal application of dried ox bile (Kali-Chemie Pharma GmbH, Hannover, FRG) followed by another intravenous injection of secretin and CCK after the standard secretin-pancreozymin test.44 45

The most satisfactory stimulant or combination of stimulants for measuring pancreatic secretory capacity and thus for separating normal from impaired pancreatic function has not yet been defined.6 A pilot study has been set up by the European Pancreatic Club to investigate interlaboratory quality control of data in the secretin-pancreozymin test, but such great difficulties were initially found between the different centres involved in the study that rigorous and detailed standardisation of enzyme assays was introduced.46

The substances used in this study are two synthetic secretin preparations (Hoechst AG, Frankfurt/M, FRG, and Hoffmann-La Roche, Basel, Switzerland), and both preparations have been shown to have an effect on pancreatic secretion similar to that of natural secretin (GIH, Stockholm, Sweden) in man14 and dogs.47 Instead of CCK caerulein (Farmitalia, Milano, Italy) is applied. Markers are not used in this study.

Until the results of this pilot study are published, despite its limitations of lack of standardisation and technical difficulties related to the performance and requirement of analytical methods, the secretin-CCK-test remains the standard diagnostic method of detecting diffuse pancreatic disease.1

In a recent survey of 2003 patients who were studied to evaluate the secretin-CCK-test, false abnormal results were reported in 8% and false normal results in 6% of the patients.48 It is unlikely that these percentages will be improved on because of the wide variation in normal pancreatic function.

Secretin-bombesin test
An intravenous infusion of bombesin has been found to stimulate pancreatic secretion, amylase and trypsin concentrations but not bicarbonate.49 The role of this procedure as an exocrine pancreatic function test still remains to be established.

Radioselenium test
Radioactive labelled amino acids given intravenously are rapidly taken up by the acinar cells of the pancreas. Youngs et al reported a good correlation between the radioactivity of radioselenium present in the duodenal aspirate after stimulation with the Lundh test meal followed by an intravenous injection of (75Se) selenomethionine.50 Duodenal 75Se-activity gave good separation between normal subjects and patients with diseases of the exocrine pancreas. The results of this study have been confirmed by others estimating 75Se in basal51 or secretin-CCK-stimulated state,52 53 whereas Brom et al54 reported that the Lundh test was superior to the radioselenium test.
By an extension of the procedure of Youngs et al\textsuperscript{50} Shichiri et al\textsuperscript{55} measured \(^{75}\text{Se}\)-radioactivity in the protein fraction of duodenal aspirates after stimulation with pancreozymin and secretin. They also found a satisfactory discrimination between normal control subjects and patients with pancreatic diseases. It has been suggested that this test may be combined with a standard secretin-pancreozymin test, serum enzyme response to pancreozymin, and secretin and pancreatic scanning. Direct measurements of enzyme activities after hormonal stimulation of the pancreas, however, is sufficiently simple and accurate and pancreatic isotope scanning is not worthwhile in the diagnosis of pancreatic disease.\textsuperscript{56} Thus the additional or single measurement of \(^{75}\text{Se}\)-radioactivity confers no advantage.

**Determinations in Pure Pancreatic Juice**

**Viscosity measurement**
The observation that duodenal secretion is more viscous in patients with chronic pancreatitis than in healthy controls led to the viscosity measurement of pure pancreatic juice obtained after stimulation with secretin (1 CU/kg body weight) via ERCP.\textsuperscript{57, 58} Viscosity was, indeed, significantly increased in the patients' group. In a similar study, viscosity of duodenal juice was also significantly higher in patients with pancreatic insufficiency after intravenous infusion of secretin and pancreozymin.\textsuperscript{59} Unfortunately, neither group supplied data on the extent to which exocrine pancreatic function was impaired. It is possible that the increase in viscosity is due to protein precipitation in pancreatic secretion\textsuperscript{60} and that it occurs only in advanced exocrine insufficiency. At present, this invasive test offers too little information and additional studies are necessary to determine whether viscosity measurement should become a supplementary procedure in the assessment of pancreatic function.

**Enzyme measurement**
Studies of human pure pancreatic juice are technically demanding and invasive. Endoscopic duct cannulation cannot guarantee complete recovery of pancreatic secretion and measurements of volume and output may be inaccurate.\textsuperscript{61} There is a considerable overlap in the values of bicarbonate concentration: in some preliminary studies abnormally low values were reported in patients with chronic pancreatitis,\textsuperscript{62, 63} whereas in other studies normal values were found.\textsuperscript{61, 64} In healthy control subjects bicarbonate concentration was found to be higher in pure pancreatic juice than in duodenal aspirates.\textsuperscript{65} In an extensive study by Denyer and Cotton\textsuperscript{61} volume, bicarbonate, and total protein concentrations were measured in pure pancreatic juice after bolus injection of 1, 4, and 70 CU secretin. There was a considerable overlap in all measured indices and no convincing differences were found between normal subjects and patients with chronic pancreatitis. It cannot be excluded, however, that these patients suffered only from mild to moderately severe exocrine insufficiency, as only a few showed calcification on plain radiographs of the abdomen and none had overt diabetes mellitus.

Although these studies are disappointing from the clinical point of view, they are of interest for studying the physiology of pancreatic secretion and
provide an ideal avenue for further detailed research into constituents of pancreatic juice that may have diagnostic significance (see below).

**Indirect tests**

**TESTS REQUIRING DUODENAL INTUBATION**

**Lundh test**

The indirect pancreatic function test that is most widely used at present has been described by Lundh and involves the endogenous stimulation of the pancreas by a test meal. The test procedure is simple: after intubation of the duodenum with a tube a test meal (300 ml) is given which contains 6% fat, 5% protein, and 15% carbohydrates. Duodenal contents are aspirated either in four consecutive 30-minute fractions or, more commonly, in a single two-hour collection. Trypsin is the most frequently measured enzyme, it is less sensitive to changes of pH and more discriminating than lipase and phospholipase. Because all enzymes may be reduced, however, it is recommended that at least two enzymes should be measured, preferably trypsin and lipase, to improve the reliability of the results.

Recently, Bergström and Lundh showed that 300 ml water alone provided mean trypsin values in duodenal contents that were only slightly lower than those obtained with a test meal. The latter procedure, however, is still used by all investigators of the test.

Several groups have shown that the Lundh test is a simple and helpful test in the diagnosis of chronic pancreatitis, particularly when associated with steatorrhoea – that is, severely impaired exocrine pancreatic function. James in 1973 found that the overall rate in the diagnosis of chronic pancreatitis was 90% and that of pancreatic carcinoma 79%.

Several groups have compared the Lundh test with a direct pancreatic function test, the secretin or the secretin-CCK test (for review see also ). The results showed considerable disagreement: in some studies there was a good correlation between both tests, especially in severe, but not in slight, exocrine insufficiency. Rolny and Jagenberg reported that the Lundh test was much less sensitive than the secretin-CCK test. Braganza and Rao found the opposite: that the indirect test was distinctly superior. This was partly because of the disproportionate reduction in trypsin response to endogenous stimulation in chronic pancreatitis; it was only a third of the response to exogenous stimulation.

Most investigators who favour the Lundh test stress that it is a simple, physiological, and inexpensive test that is possible to perform in most hospitals. There are, however, certain disadvantages which limit the usefulness of the test. (1) The test is based on the endogenous release of secretin and CCK. Thus, where there is mucosal damage – for example, in coeliac disease – the test may not be valid because of impaired hormonal release. Therefore the test cannot differentiate between malabsorption of pancreatic or non-pancreatic origin. (2) Hormonal release depends on the integrity of the gastroduodenal anatomy and the results may be difficult to interpret after operations such as Billroth II gastrectomy. (3) Volume and bicarbonate secretory capacity cannot be assessed.

**Small intestinal infusions**

Hydrochloric acid had been perfused into the jejunum to release secretin and amino acids to release CCK. The response to this indirect
stimulation was as high as to direct stimulants and was impaired in patients with pancreatic disease. Both tests are at the moment primarily research procedures, however, and are still not standardised for clinical use.

**TUBELESS TESTS**

**General principle**

In tubeless pancreatic function tests, usually with a test meal for stimulation of pancreatic secretion, a compound is given which is split up into two or more parts by pancreatic enzymes in the duodenum. One of these split products is readily absorbed in the small intestine, conjugated in the liver, and excreted in the urine. The recovery rate in the urine within a given time is taken as an index for pancreatic function. As the test result may be influenced by an impairment of absorption, liver function, and renal excretion the test may be repeated on another day, this time applying the split product only.

**NBT-PABA test**

This test is based on the specific hydrolysis of a synthetic tripeptide, N-benzoyl-L-tyrosyl-p-aminobenzoic acid (NBT-PABA) by chymotrypsin in the duodenum. The recovery rate of split PABA reflects intraluminal chymotrypsin activity. Thus, this oral pancreatic function test is essentially an indirect test of chymotrypsin secretion.

The test procedure is not yet standardised but this may easily be achieved when it becomes commercially available. Usually the peptide is applied together with a test meal at a dosage between 150 mg and 2 g. To increase the sensitivity of the test it would seem to be logical to increase the applied dose of NBT-PABA. A comparative investigation using 150 mg and 1 g NBT-PABA indeed showed an improvement in sensitivity. The dosage cannot, however, be increased too much because after 4 g a decrease in PABA excretion has been observed, and probably the appropriate dosage is between 500 and 2000 mg NBT-PABA. The content of the test meal does not seem to be of importance judging by the studies carried out in man. It seems to be necessary, however, to give some sort of stimulant to the pancreas, as the lowest sensitivity rate of the NBT-PABA test was obtained in a study in which no stimulation of pancreatic secretion was performed. Recent studies showed that the liquid test meal may be replaced by a standard breakfast and additional meals may even be taken during the period of urine collection without interfering with the test.

In animal experiments the application of chymotrypsin-specific inhibitors (raw egg white) led to a better separation between normal controls and animals with pancreatic insufficiency.

To obtain an adequate diuresis, tea or mineral water is given at certain intervals and PABA is measured in the urine collected for six, eight, or nine hours. A collection period of six hours is probably sufficient, because prolonging the test for up to nine hours did not improve its sensitivity. Drugs such as sulphonamides, sulphonyl ureas, etc., or food ingredients such as prunes and cranberries interfere with the chemical determination of aromatic amines and should be discontinued for two to three days and pancreatic enzyme substitution for five days before the test.

In animal experiments Imondi *et al* reported that the NBT-PABA test reflected the impairment of exocrine pancreatic function induced by pancreatic duct ligation. It was subsequently found to be a useful test for
diagnosing pancreatic insufficiency in protein-deficient patas monkeys.\textsuperscript{95}

Since the first promising studies in man,\textsuperscript{96-100} the NBT-PABA test has been subjected to a considerable number of studies by different groups.\textsuperscript{89-91 93 101-108} With one exception,\textsuperscript{93} the sensitivity rate (true abnormal results in patients with pancreatic insufficiency) was between 80 and 100\% compared with the secretin-pancreozymin test\textsuperscript{89 91 93 98 103-106} or the Lundh test meal.\textsuperscript{90 99 101 102} In a recent review based on five years' experience with the test a sensitivity rate of 86-1\% was given for chronic pancreatitis.\textsuperscript{90} While the sensitivity of the test seems to be good in severe and moderately severe exocrine insufficiency, it is not fully established in cases with only slight impairment of pancreatic function, and seems to be inferior.

Comparative studies have been performed together with the pancrconauryl test (see below) and the estimation of chymotrypsin in stool. The results are inconsistent: faecal enzyme estimation was of higher diagnostic value than the NBT-PABA test in one study\textsuperscript{91} but not in another investigation.\textsuperscript{93} A third one reported that both function tests were of the same sensitivity in severe exocrine insufficiency, whereas, in slightly impaired cases, the oral function test was superior.\textsuperscript{109}

In addition to the studies in man, the NBT-PABA test has also been successfully used in children to diagnose pancreatic insufficiency due to cystic fibrosis.\textsuperscript{110 111} Similar results were obtained by using 4-(N-acetyl-L-tyrosyl)amino benzoic acid as substrate in adults\textsuperscript{112} and children.\textsuperscript{113}

In a study of 353 patients the specificity (true normal results in patients with non-pancreatic diseases) of the NBT-PABA test was found to be 92-93\%.\textsuperscript{90} This study did not include patients with renal insufficiency in whom PABA excretion is reduced.\textsuperscript{102} As false abnormal test results have been reported, however, in patients with impaired liver function,\textsuperscript{104 108} inflammatory bowel diseases,\textsuperscript{108} Billroth II gastrectomy\textsuperscript{108} (not found in other studies\textsuperscript{90 107}), and diabetes mellitus\textsuperscript{104 105} the specificity may still be improved by repeating the test using free PABA alone.\textsuperscript{114} Further studies of the test's specificity are necessary, as PABA excretion has also been found after total pancreatectomy.\textsuperscript{93 99 103} This may be due to a too-recent discontinuation of pancreatic enzyme therapy but also to an intact NBT-PABA absorption which has been reported in \textit{in vivo}\textsuperscript{115} and \textit{in vitro}\textsuperscript{116} animal experiments. It is therefore necessary to determine whether such an intact absorption is also possible in man to establish whether the NBT-PABA test offers quantitative or only qualitative assessment of pancreatic function.

At the moment the test seems to be a simple, non-invasive exocrine pancreatic function test which is feasible in most hospitals and may even be used in outpatient departments provided that it is possible to guarantee adequate urine collection. It is not intended to replace more sensitive and specific, but also more invasive function tests such as the secretin-pancreozymin test but could be used as a screening procedure in places where tests requiring more nursing or laboratory facilities are not possible. In addition to its diagnostic possibilities, the test may possibly be used to control pancreatic enzyme therapy.\textsuperscript{117}

\textit{Pancreolauryl test}

The test compound, fluorescein dilaurate, a poorly water-soluble synthetic
Exocrine pancreatic function tests

ester, is hydrolysed by the pancreas by specific arylesterases from pancreatic juice into lauric acid and free, water-soluble fluorescein. This is readily absorbed in the small intestine, partly conjugated in the liver, and excreted in the urine. The test procedure is standardised: on the first day the test capsules are given orally in the middle of a standard breakfast (50 g white bread, 20 g butter, a cup of tea) which is followed by 1 l of weak tea between the third and fifth hour of the test. The urine is collected for 10 hours. To evaluate individual absorption, conjugation, and excretion the test is repeated on the third day using a control capsule which contains free fluorescein only. The recovery rate of both days is expressed as a ratio and taken as an index of pancreatic function. Treatment with pancreatic enzymes or vitamin B₂, which interfere with the fluorescein measurement, has to be discontinued five days before the test.

Although this oral pancreatic function test was developed 11 years ago it has only recently become commercially available (Temmler, Marburg, FRG) and there is little information on its sensitivity and specificity in pancreatic disease.

Kaffarnik et al found a confirmation of the final diagnosis in 93% of 255 healthy controls and patients with different pancreatic diseases. In two preliminary studies the sensitivity of the pancreolauryl test was distinctly higher in severe exocrine pancreatic insufficiency (estimated with the secretin-pancreozymin test) compared with cases with only slightly impaired pancreatic function. A comparative study with the NBT-PABA test showed that both oral pancreatic function tests led to similar results.

By modifying test doses and fluid intake the pancreolauryl test has also been adapted to children, in whom it was clearly able to differentiate between healthy controls and patients with pancreatic insufficiency due to cystic fibrosis.

Like the NBT-PABA test the pancreolauryl test is a simple and non-invasive tubeless function test. Its specificity should theoretically be higher because individual absorption, conjugation, and excretion are considered. Further experience, however, is obviously needed in a larger number of patients with different pancreatic and other gastrointestinal diseases to assess the sensitivity and specificity of the test. Like the NBT-PABA test, it remains to be determined whether there is a non-pancreatic hydrolysis of the ester, as some fluorescein excretion has been measured even after total pancreatetectomy.

FAECAL TESTS

Microscopic examination

Microscopic examination of stools may reveal meat fibres, neutral or split fats as an index of malabsorption but cannot distinguish its aetiology. According to a recent report, qualitative faecal fat estimation by means of Sudan III staining and light microscopy is a highly sensitive test for detecting steatorrhoea.

Trypsin and chymotrypsin estimation

The development of synthetic low molecular substrates has made possible the specific titrimetric estimation of trypsin and chymotrypsin in stools. Both random samples and 24-hour collection of faeces
have been used. The test is obviously not invasive but is not popular with the laboratory technicians and requires a titrimeter, which is not available in all hospitals. Stool samples may be sent by post, however, to specially equipped centres without affecting test results. Pancreatic enzyme therapy has to be discontinued five days before the test, while antibiotics may lead to false high faecal trypsin values.\textsuperscript{129}

Several studies have shown that trypsin estimation has only a low sensitivity.\textsuperscript{93 127 130} The sensitivity rate of chymotrypsin estimation is between 72 and 95% in severe and between 41 to 64% in slight exocrine insufficiency demonstrated with the secretin-pancreozymin test.\textsuperscript{93 127 131 132} Studies of the specificity of the test are contradictory: whereas in some of them the rate was in the range of 90 to 100%,\textsuperscript{130 133 134} false abnormal results were found in 29% of non-pancreatic diseases.\textsuperscript{135} They are reported in non-pancreatic diarrhoea/steatorrhoea (low enzyme concentration due to dilution), after Billroth II gastrectomy, and sprue (decreased liberation of hormones stimulating pancreatic secretion), after reduced oral feeding, and in cachectic state (decreased pancreatic enzyme synthesis due to protein malnutrition) and in obstructive jaundice (lacking stimulation of bile). The test has also been successfully applied in children with cystic fibrosis for the detection of pancreatic insufficiency.\textsuperscript{136 137}

From the present data chymotrypsin estimations in stools may be used as a screening test for pancreatic insufficiency. An abnormal test result should be taken as an index of pancreatic insufficiency when other causes of malabsorption can be ruled out. A normal result does not exclude pancreatic diseases.

Of special interest is the faecal enzyme estimation after stimulation of the pancreas with secretin and CCK; the patient should first be purged to ensure rapid intestinal transit of the enzymes. There was a good correlation between enzymes in duodenal aspirate and faeces.\textsuperscript{138}

**Faecal fat and nitrogen measurement**
Steatorrhoea and azotorrhoea occur when stimulated lipase output falls below 10% of normal.\textsuperscript{139} The estimation of fat in stools collected for 72 hours\textsuperscript{140} is a safe procedure for the diagnosis of steatorrhoea. It may be used as an index of pancreatic function when performed before and after enzyme replacement therapy or as a control of the effect of the latter therapy. It is also obviously not invasive but again is not popular with laboratory technicians and requires some laboratory equipment. Attempts have been made to replace this method by breath tests but the results are not promising (for review see \textsuperscript{141}).

**Enzyme estimation in saliva, serum, and urine**

**Parotid saliva test**
In acute experimental pancreatitis in rats, atrophy and reduction of amylase content of parotid glands were found.\textsuperscript{142} In dogs the same experimental condition induced degenerative changes of the parotid gland associated with a marked decrease of maximal bicarbonate concentration of parotid saliva.\textsuperscript{143} In man, a decrease of basal but not of stimulated salivary secretion was reported in chronic pancreatitis.\textsuperscript{144} In contrast, Kakizaki et al found that pilocarpine-stimulated volume, maximal bicarbonate concentration, and amylase content of the saliva of patients
Exocrine pancreatic function tests

with pancreatic disorders were significantly lower than in patients with non-pancreatic diseases.\textsuperscript{145,146} Therefore the parotid saliva test was introduced as allegedly superior to the secretin-pancreozymin test.\textsuperscript{146} The test procedure is simple and non-invasive. Parotid saliva is collected after stimulation with pilocarpine hydrochloride (intramuscular injection) or by application of citric acid to the tongue. For evaluation volume, bicarbonate, amylase, and protein concentration and output are measured.

The results of different studies are at variance. Some groups have confirmed the results of Kakizaki et al.,\textsuperscript{147-149} whereas in two studies the sensitivity was very low\textsuperscript{150} or practically zero.\textsuperscript{151} In both studies pancreatic insufficiency had been demonstrated by the secretin-pancreozymin test. These data do not support the role of the parotid saliva test as a screening test for pancreatic function.

\textbf{Evocative tests}

To avoid duodenal intubation, the rise of serum amylase and lipase after stimulation with secretin and/or pancreozymin has been used as an index of pancreatic function. When the pancreas is normal, a negligible rise of both enzymes is to be expected, whereas in pancreatic insufficiency, especially at an early stage, the rise has been reported to be significant. The validity of the test has been debated for many years. In his review on this subject Wormsley\textsuperscript{5} concluded that, in view of the many factors other than pancreatic diseases which can modify the values of both fasting and stimulated levels of pancreatic enzymes in the blood, the test is of limited value. Two large studies published since then correlating the evocative test with the secretin-pancreozymin test in patients with pancreatic and non-pancreatic disease have shown that this test is neither sensitive nor specific and is not to be recommended.\textsuperscript{48,152}

\textbf{Immunoreactive serum trypsin}

Recently a radioimmunoassay for serum trypsin has been developed and recommended for the diagnosis of chronic pancreatitis.\textsuperscript{153} Further investigations showed that radioimmunoreactive trypsin is not detectable after total pancreatectomy.\textsuperscript{154} It increases in acute pancreatitis,\textsuperscript{155} acute exacerbation of chronic pancreatitis,\textsuperscript{154} renal failure,\textsuperscript{156,157} and may also be raised in the presence of pancreatic cysts with hyperamylasaemia.\textsuperscript{154}

Several studies have shown that the sensitivity of immunoreactive serum trypsin is limited: chronic pancreatitis was confirmed in 33\%,\textsuperscript{158} 52\%,\textsuperscript{155} 61\%,\textsuperscript{157} and 65%\textsuperscript{154} of the cases by low serum trypsin values. A review of the present experience leads to the conclusion that in approximately 60\% of uncomplicated cases of chronic pancreatitis an abnormal serum trypsin can be expected.\textsuperscript{159} Therefore pancreatic insufficiency is not excluded by normal values. The relatively high incidence of false normal test results may be explained by the fact that, unlike amylase, serum trypsin tends to be raised for a longer time after an acute attack of pancreatitis.

Low serum trypsin values have been reported in some patients with diabetes mellitus. Pancreatic function had, however, not been tested in these cases.\textsuperscript{160,161}

Recently a raised ratio of trypsin to creatinine clearance ($C_T/C_C$) has been found in pancreatic carcinoma.\textsuperscript{162} The ratio was normal in chronic pancreatitis and normal or raised in acute pancreatitis. Before the role of
the trypsin radioimmunoassay can be finally evaluated more data on its sensitivity and specificity in chronic pancreatitis and pancreatic carcinoma are needed.

Isoamylase determination
Determination of total amylase in serum and urine reveals normal values in chronic pancreatitis except during acute exacerbation of the disease. Aw et al\(^{163}\) could show by means of electrophoretic separation that pancreatic isoamylase is lowered in chronic pancreatitis. Since then, serum and urinary amylase have been fractionated by polyacrylamide gel electrophoresis, Sephadex chromatography, electrophoresis, and isoelectric focusing (for review see \(^{164}\)). As with any other serum enzyme estimation, isoamylase determinations are not invasive but need laboratory facilities.

Some of these methods have been applied in the diagnosis of chronic pancreatitis. Using agarose gel electrophoresis Skude and Eriksson\(^{165}\) detected low pancreatic isoamylase values in 15 patients with advanced chronic pancreatitis. All had steatorrhoea. Using the same method Magid et al\(^{166}\) could show that the incidence of abnormal pancreatic isoamylase increased with the impairment of pancreatic function. Berk et al (chromatographic method)\(^{167}\) and Johnson and Levitt (isoelectric focusing)\(^{168}\) reported that low isoamylase values support the diagnosis of chronic pancreatitis, whereas normal values do not exclude the diagnosis.

Though isoamylase determinations are helpful in the diagnosis of unexplained hyperamylasaemia,\(^{169}\) the above-mentioned methods are more or less restricted to specialised centres. Details of a new rapid method requiring simple photometric measurement have recently been published\(^{170,171}\) and it has already become commercially available (Phadebas, Uppsala, Sweden). An amylase inhibitor had been isolated from wheat (\textit{Triticum aestivum}) with a 100-fold higher specificity against salivary amylase than pancreatic isoamylase. Thus the test requires the photometric measurement of total serum amylase with and without the inhibitor.

This is a simple method but further studies are needed to assess its validity. A preliminary study showed a confirmation of pancreatic insufficiency (secretin-pancreozymin test) in 70\% of cases with uncomplicated pancreatitis.\(^{172}\) The test results of more demanding methods, such as agarose gel electrophoresis\(^{165}\) and radioimmunoassay of trypsin, were of slightly lower sensitivity.\(^{172}\) As with the trypsin-RIA, pancreatic isoamylase estimations cannot be used for the diagnosis of chronic pancreatitis during acute exacerbation of the disease.

DIFERENTIATION BETWEEN DIFFERENT TYPES OF PANCREATIC DISEASES
Unfortunately, none of the above-mentioned pancreatic function tests is able to solve the main diagnostic problem: how to differentiate between pancreatic insufficiency due to chronic pancreatitis or to pancreatic carcinoma. For this purpose, additional determinations in duodenal or, preferably, in pure pancreatic juice are necessary. These include, with different success rates, lactoferrin,\(^{173-175}\) carcinoembryonic antigen,\(^{63,176,177}\) \(\gamma\)-glutamyl transpeptidase,\(^{178}\) and cytology,\(^{177-180}\) the latter may be also obtained by endoscopic retrograde brushing.\(^{181}\)
Exocrine pancreatic function tests

Sensitive function tests, however, will be able to decide whether pancreatic function returns to normal and thus differentiate between acute or acute relapsing and relapsing chronic pancreatitis.

There is some debate on whether, for the diagnosis of exclusion of pancreatic diseases, a particular sequence of tests or, alternatively, a battery of more or less simultaneous diagnostic procedures should be applied. We prefer the latter, as clinical experience has taught us that single function tests or endoscopic/radiological examinations cannot exclude chronic pancreatitis or pancreatic carcinoma. Our diagnostic procedure includes exocrine (secretin-pancreozymin test or, if this is not possible, tubeless tests such as NBT-PABA test and PLT) and endocrine (oral glucose tolerance) function tests, plain radiographs of the abdomen, sonography (in case of enlargement of the pancreas with sonographically guided fine-needle puncture), and ERCP (in selected cases also PTC).

OUTLOOK FOR FURTHER PROGRESS

Direct tests
At the moment, the secretin-pancreozymin or the secretin-caerulein test seems to be the safest procedure for the assessment of pancreatic function. To prove this, one must await the completion of the multicentre study of the European Pancreatic Club. It is hoped that results of this study will be available in the next two years and will decide whether the chosen combination is really the best and preferable to the single secretin test and also which parameter(s) are the best for diagnosing pancreatic diseases. Furthermore, it will settle the long-lasting debate as to whether or not an impaired secretory rate and normal content of bicarbonate and enzymes mean the presence of pancreatic carcinoma, or whether a reduction of bicarbonate and/or enzyme output and normal secretory rate should be taken as an indication of chronic pancreatitis. Finally, the study will present information upon whether bicarbonate or enzymes are reduced first in early chronic pancreatitis.

This study could also be used as a reference for large comparative studies for the evaluation of morphological methods such as sonography and computed tomography and especially ERCP. Several studies have shown that pancreatic function is always impaired when ERCP shows abnormal findings but others found in some cases abnormal ERCP results combined with normal pancreatic function. The study may show whether these changes really are relevant or whether they are due to scarring as a result of acute attacks of relapsing acute pancreatitis and may enable the interesting finding of the relationship between ductal obstruction and pancreatic secretion to be re-evaluated.

Finally, these comparative studies could help decide whether those morphological findings not available at the time of the definition of Marseilles should be included in a new definition of chronic pancreatitis.

‘POOR MAN’S’ PANCREATIC FUNCTION TESTS
It is common experience that the incidence of pancreatic cancer and chronic pancreatitis (due to alcoholism) is increasing. There is, therefore, a need for many hospitals and physicians in practice, and not just for specialised centres, to be able to diagnose or exclude pancreatic disease, but highly sensitive pancreatic function tests are often beyond their
resources. The role of faecal tests, especially of chymotrypsin determinations in stools and fat estimation, has been fully established in recent years. They are not often used, however, because they are either unpopular or time consuming or require special laboratory equipment.

Further progress may be made by proceeding with the evaluation of serum enzyme estimation and tubeless pancreatic function tests. At the moment, serum enzyme estimations (trypsin-RIA, isoamylase) seem to supply information when abnormal. It has yet to be demonstrated in large series whether they are specific, and to what extent pancreatic function has to be impaired before these tests become abnormal.

Tubeless pancreatic function tests such as the NBT-PABA test and the pancreolauryl test have also to be studied further. It must first of all be established whether an intact absorption of the NBT-PABA-peptide or a non-specific hydrolysis of fluorescein dilaurate can occur in order to decide whether these tests offer quantitative or only qualitative assessment of pancreatic function. The pancreolauryl test then needs to be applied in more cases with pancreatic and non-pancreatic diseases before it can finally be evaluated. If both tests offer quantitative information, it should be established to what extent pancreatic function has to be impaired before tubeless test results become abnormal. It would still be of interest, however, if the tests provide only qualitative assessment of pancreatic function for centres in which direct function tests are not available. As both tests are based on different principles, it may be useful to combine them to increase their sensitivity.109 121

Further studies on tubeless tests should be directed to discovering whether the time of the urine collection may be shortened without impairing the test result. This applies especially to the pancreolauryl test. In order to adapt the test to outpatient demands, attempts have been made to estimate PABA in serum and to take the PABA concentration after one hour as an index of pancreatic function.190 If this finding could be confirmed in larger studies and if the serum test has the same diagnostic value as the urine test, this would be of interest for outpatient departments, avoid the problem of renal excretion, and could possibly be used in combination with other tests such as xylose absorption or the oral glucose tolerance test.

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