Progress report

The Lundh test

The idea of assessing pancreatic function by measuring the pancreatic response to a test meal is not new. Before 1940 several groups, using a variety of test meals, often of bizarre composition, had shown that in pancreatic carcinoma or chronic pancreatitis the exocrine secretions are frequently reduced¹⁻⁵. The enthusiasm, rightly engendered, for the use of secretin as a standard stimulus to pancreatic secretion in terms of total volume and bicarbonate output, caused a diminution in interest in test meals for about 20 years. But the introduction of specific substrates whereby hydrolytic enzymes could be assayed in the duodenal contents led to the reintroduction of the test meal as a measure of pancreatic function.

Technique

In the Lundh test, as currently performed, the fasting subject is intubated with a radioopaque tube of internal diameter about 2 mm (French gauge 12). Metaclopramide, 10 mg, is given by mouth shortly before intubation to minimize nausea and to speed the passage of the tube to the duodenum. The tube has a number of collecting holes in its distal few centimetres and is weighted at the end by a mercury bag. The tube is screened under *x*-ray control until it lies well within the duodenum. The whole procedure usually takes about 45 minutes. The exact position of the tube in the duodenum is not important since trypsin concentrations in different parts of the duodenum following the test meal are very similar⁶. The test meal is then swallowed and constant aspiration from the duodenum by syphonage or low-power suction is carried out for two hours. The aspirate is collected into ice-cooled containers and divided into four consecutive half-hour specimens. The samples are stored at -20°C until enzyme estimations are carried out.

The composition of test meals used by different workers has varied⁷⁻¹². The meal devised by Lundh, and used by the Central Middlesex Hospital and the Royal Free Hospital groups, London, comprises 18 g corn oil, 15 g Casilan, and 40 g glucose in 300 ml water. This consists of 6% fat, 5% protein, and 15% carbohydrates. Recently, Bergstrom and Lundh showed that 300 ml water alone produced mean trypsin values in duodenal contents only slightly lower than those obtained with a test meal, but with a greater coefficient of variability¹³.

Since Lundh's first work on the test meal most workers have agreed that total recovery of duodenal juice is unnecessary since it is the enzyme concentration rather than the total production which is being measured^{8,11,14,15}. Worning and Mullertz (1966) and Thaysen, Mullertz, Worning, and Bang (1964) reported that the volumes aspirated via the tube used for this type of test varied considerably and had no diagnostic value^{11,14}. Cook, Lennard-

Jones, Sherif, and Wiggins (1967) showed, by perfusion studies using ^{51}Cr -labelled marker, that the recovery of juice from the duodenum was 10-25%¹⁵. Similarly, the pH of the duodenal juice measured during the test is of no value because of many variables, including the buffering effect of the ingested meal, speed of gastric emptying, and gastric acid secretion. Worning, Mullertz, Thaysen, and Bang (1968), measured the pH in the duodenum of patients with abnormal tryptic activity and found it no different from a group of normal subjects¹⁶.

It is thought that the mean enzyme activity throughout the test produces a low coefficient of variation and has the best diagnostic value^{8,15,17,18}. A two-hour collection period appears to be optimal since studies using a shorter collection period have had higher coefficients of variation of mean enzyme activity¹⁹.

The enzyme most frequently measured is trypsin; comparison between results obtained on the same normal subjects measuring trypsin and amylase show fairly close correlation but Hartley, Gambill, Engstrom, and Summer-skill (1966), Worning *et al* (1968), and Goldberg and Wormsley (1970) have suggested that trypsin estimation is slightly more reliable in the diagnosis of pancreatic carcinoma^{16,19,20}.

Methods of Measuring Tryptic Activity in Duodenal Fluid

All methods measure the rate of hydrolysis of specific substrates. Most methods in current use arise from modifications to the method described by Schwert and Takenaka using N-benzoyl-arginine ethyl ester (BAEE) as substrate^{21,22,23}. These methods depend either upon the rate of H^+ liberated from the substrate by trypsin and are thus measurements of pH titre, or upon spectrophotometric changes in the products of the reaction^{24,25,26,27,28}. The method described by Wiggins involving measurements of pH titre is simple and needs only normal laboratory equipment; it is suitable when trypsin estimations are performed occasionally. It is essential that laboratories setting up trypsin estimations determine their own range of normal values²⁹. The precision of both groups of assay is very good, replicate determinations within a group of samples being within 3% of each other¹⁷. Results are expressed in international units (m-equiv H^+ released/min/ml aspirate) or as μmol substrate released/ml aspirate/min, or directly as μg trypsin/ml aspirate, depending on the method used.

Experience with the Lundh Test in Diagnosis of Pancreatic Disease

In the diagnosis of pancreatic carcinoma, published series show 111 markedly abnormal tests out of a total 141 patients (79%)^{6,14,15,16,18,19,30,31,32,33}. The accuracy of diagnosis ranges from nine abnormal out of 16 to 39 abnormal out of 44 (Lundh, 1965) and six abnormal out of six^{6,14,30}. In few series is there any differentiation as to the situation of the carcinoma within the pancreas.

In the diagnosis of chronic pancreatitis, published series show 151 markedly abnormal results in 168 patients (90%)^{6,14,15,16,17,18,19,30,31,33,34}. The abnormality in the test ranges from 15 out of 22 to 17 out of 17, 11 out of 11, and 27 out of 28^{6,16,30,33,34}. A high proportion of these patients had sufficiently severe pancreatic insufficiency to cause steatorrhoea. To these series must be

added that of Waller, Mottaleb, Wiggins, Kellock, and Kapp who found 85% abnormal tests in 45 patients with proven pancreatic carcinoma or chronic pancreatitis.³⁵ It should be stressed that the Lundh test assesses pancreatic exocrine function in relation to the production of one or more enzymes and does not distinguish between carcinoma and chronic inflammation: this must be a clinical decision based upon history, examination, and if necessary, other tests.

It seems appropriate to include here studies on patients with obstructive jaundice due to bile duct obstruction, either by a gallstone or a bile duct carcinoma, since this is an important group to differentiate clinically from pancreatic carcinoma. Out of 65 such patients, 50 (77%) had normal tests and a further eight were borderline (total 86%),^{6,14,15,16,17,30}. Thus it appears that in obstructive jaundice due to presumed extrahepatic obstruction a normal test suggests a non-pancreatic cause for the obstruction.

Comparison with Tests Involving Direct Pancreatic Stimulation

Since the classic studies of Chiray and Lagerlof the most generally accepted test of exocrine pancreatic function has been aspiration of duodenal contents following injection of secretin or, more recently, of secretin and pancreozymin^{36,37}.

Despite the variations in the methods of stimulation and the statement by Wormsley that 'comparison of different studies and methods of administration is quite impossible', it is necessary to compare the diagnostic accuracy of the test meal with direct hormonal pancreatic stimulation³⁸.

Dreiling and Janowitz, in a series which they and others regard as the standard by which others should be judged, have reported on over 5000 'standard' secretin tests using 1.0 clinical unit/kg body weight^{39,40,41,42}. They found extremely good agreement between results obtained using different types of secretin, despite evidence which suggests that of the two types of secretin now most commonly available, one, the Vitrum preparation, is eight or nine times stronger than the other, Boots, per clinical unit⁴³. In their impressive reports Dreiling and Janowitz, using the criteria of quantitative secretory deficiency as implying duct obstruction, found only 4.5% errors in tests on 242 patients with carcinoma of the head of the pancreas. Percentage errors for neoplasms of the body and tail were much higher (41% errors in 76 cases of carcinoma of the body, 97% errors in 29 cases of carcinoma of the tail) in their 1962 series, although by 1970 they claimed higher diagnostic accuracy. In chronic pancreatitis, Dreiling recorded only 4% errors among 296 cases, using the criterion of qualitative bicarbonate secretory deficiency.

These figures are thus slightly more impressive than the totals produced in Lundh tests on patients with pancreatic carcinoma or chronic pancreatitis.

Hansky⁴⁴, however, found that a 'standard' secretin test, using the same dose schedule as Dreiling, was associated with 50% error in the diagnosis of pancreatic carcinoma and 23% in chronic pancreatitis. Prolla, Settles, and Kirsner agree that in the 'standard' secretin test, the clinical significance of the test was greatly decreased by the large overlap of values among carcinoma, pancreatitis, and normals.

Adherents of an 'augmented' secretin test claim that a higher dosage of secretin producing 'maximal' stimulation improves diagnostic accuracy or

at least reduces the coefficient of variation for maximal volume and bicarbonate values^{44,46,47}.

The combined secretin/pancreozymin test has been advocated as a method of stimulating not only fluid and bicarbonate output but also enzyme production from the pancreas; it is therefore thought to have possibly more potential diagnostic accuracy⁴⁸⁻⁵³. Several groups have felt that the assay of an enzyme (usually amylase) secreted in response to pancreozymin added accuracy to the diagnosis of pancreatic carcinoma. Wormsley⁵² stated that 'patients with pancreatic insufficiency due to chronic pancreatitis show greater loss of capacity to secrete bicarbonate than enzymes while carcinoma of the pancreas shows the reverse of the pattern'. Accuracy in diagnosis of pancreatic disease was between 75 and 90% in these series.

Since some importance is attached to the completeness and accuracy of the collection of duodenal contents in direct stimulating tests, and to the question of maximal stimulation of the pancreas, the work of Go, Hofmann, and Summerskill is of interest^{54,55}. These workers showed that using standard secretin/pancreozymin testing there was considerable duodenal reflux into the stomach and that the mean recovery of duodenal marker from the duodenum was only 36.7%. By perfusing the duodenum with amino acids while simultaneously giving an intravenous infusion of pancreozymin they demonstrated that there was a significant increase in pancreatic enzyme productivity above 'maximal' values obtained when pancreozymin alone is administered.

Direct comparisons between the Lundh test and secretin or secretin/pancreozymin tests are few and rather unsatisfactory^{9,19,34,56,57,58}. The first comparison was on only six subjects: Schon, Rico-Irles, and Henning⁹ gave secretin, pancreozymin, and a test meal in sequence and found the rise in six enzymes after the test meal to be very similar to that after pancreozymin. Hartley *et al* performed a similar study in 81 subjects¹⁹. They found that in patients with pancreatic disease the results of both tests showed significant differences when compared with those in a control group, except in respect of duodenal amylase measured after the test meal in subjects with pancreatic carcinoma; trypsin estimation gave a comparable accuracy in the diagnosis of pancreatic disease. These authors stated, however, that the information derived from maximal stimulation of volume and bicarbonate secretion with an augmented dose of secretin was the only test that allowed significant discrimination between pancreatic carcinoma and chronic pancreatitis. A valid criticism which has been levelled against this work is that the collection period following the test meal was only 80 minutes, not two hours, and this resulted in a high coefficient of variation in the expression of the results^{15,32}.

Zieve, Mulford, and McHale compared tests in 17 normal subjects and concluded: 'The test meal as a physiologic, reproducible, non-changing and cheap agent for stimulating pancreatic enzyme secretion may, in most instances, be a better test substance than exogenous secretin/pancreozymin for evaluating pancreatic function and detecting pancreatic disease'^{11,57}. Fiore, Dal Monte, and Sasdelli reached similar conclusions in a group of 10 normal subjects⁵⁸.

Worning compared the total secretion of amylase after an intravenous infusion of maximal secretin/pancreozymin using a marker technique to correct for loss of complete aspiration with the amylase in duodenal contents after a test meal⁵⁶. There was a linear correlation between the two measure-

ments, and he concluded that the concentration of pancreatic enzymes in the small intestine was a reflection of the secreting capacity of the pancreas. Thus the meal test for pancreatic function was suitable for assessing the secretory function of the pancreas.

Moeller, Dunn, and Klotz performed secretin/pancreozymin and Lundh tests on nine control subjects and seven patients with pancreatic disease⁶⁴; their conclusion that 'statistically significant differences in six parameters indicate that the pancreozymin-secretin test is more sensitive in detecting mild acute or chronic pancreatic disease ($P < 0.01$)' is based on evidence from one patient only and is thus rather thinly supported.

The Lundh Test in Conditions Not Primarily of Pancreatic Origin

The test meal has two functions: as a test of exocrine pancreatic function in pancreatic disease and to assess effective pancreatic function in conditions not primarily related to the pancreas.

In small intestinal malabsorption states, notably coeliac disease, an abnormal pancreatic trypsin response to a test meal has been found by several groups. Thus Worning *et al* found 11 abnormal results out of 40 patients^{14,15,59}. A criticism levelled against the Lundh meal as a test of pancreatic function has been that an abnormal result may be found in a subject who has essentially a disorder of intestinal absorption, thus giving erroneous information. Cook replied when he pointed out that in patients with steatorrhoea due to primary pancreatic insufficiency the trypsin levels in duodenal aspirate are very low indeed whereas in intestinal malabsorption states they are only mildly abnormal if at all¹⁵. It should also be pointed out that the result of any test for malabsorption should not be taken in isolation, unrelated to other investigations. The secretin test usually confirms normal pancreatic ability to secrete bicarbonate and fluid in malabsorption states^{60,61}. Dreiling, however, found reduced pancreatic secretion in three of 36 patients with malabsorption syndromes and Newsome in two of seven^{62,63}. The secretin/pancreozymin test also revealed 'false positive' results in patients with intestinal malabsorption⁵⁷.

If we accept that the Lundh test does give an indication of reduced effective pancreatic exocrine function in about 25% of patients with small intestinal malabsorption, this may be due either to impaired production of pancreozymin by the damaged small intestine⁶⁴, or, in severe malabsorption at any rate, to a diminution in the amount of amino acids available to the pancreas with which to synthesize enzymes. This could be reflected in the smaller number of abnormal tests found with direct stimulation as well as with stimulation by a test meal⁶⁵.

The use of the Lundh test has thrown some light on digestion in peptic ulcer and after gastric surgery. In patients with duodenal ulcer, Worning *et al* found abnormal trypsin and amylase concentrations in the duodenum following a test meal: 10% had markedly decreased trypsin levels and 17% lowering of duodenal amylase: these results are in agreement with those of others using the secretin test^{59,62}. Holmquist and Colleen noted no abnormal duodenal enzyme levels in 12 patients with duodenal ulcer following a test meal, and also reported normal results following vagotomy in five of these subjects^{66,67}. Wormsley agrees with their findings⁵².

Lundh gave his test meal originally to patients who had undergone gastric

surgery with the object of assessing effective pancreatic function during digestion of food rather than to register total possible pancreatic response after an artificial stimulus⁷. He demonstrated low concentrations of trypsin in intestinal contents in patients following a Billroth II gastrectomy; by including a non-absorbable marker it was possible to calculate the ratio of food concentration to trypsin concentration at different stages of digestion. A marked lack of coordination between gastric emptying and pancreatic secretion was observed which resulted in poor mixing of food with enzymes. Patients with Billroth I gastrectomies had normal duodenal trypsin levels. Worning *et al*⁷⁰ confirmed that 21-50% (depending on enzyme measured) of patients with Billroth II gastrectomy had abnormally low concentrations of duodenal enzymes in all collections, and suggested that there might be two factors operating in Billroth II patients. There may be a genuine decrease in pancreatic secretion, and secondly, there may be delayed emptying of the afferent loop. The mean concentration of lipase in the efferent loop was found to correlate closely with fat absorption. They confirmed that neither Billroth I gastrectomy nor gastric ulcer were associated with reduced pancreatic output of enzymes⁵⁹.

Pancreatic function in patients with liver disease has been the subject of studies using both test meals and secretin and secretin/pancreozymin tests^{32,68,69,70}. The latter have shown that pancreatic output of fluid and bicarbonate is variable and often increased, and that enzyme output may be low in liver disease. This finding may well be due to dilution since Worning *et al*⁷⁰ and Youngs⁷⁷ have found normal duodenal enzyme concentrations in response to a test meal in patients with liver disease, including alcoholic cirrhosis^{32,70}. Although abnormalities in the structure of the pancreas are found in association with liver disease they do not explain satisfactorily the malabsorption which has been found. Forrell (1967) suggested that, since secretory volume and bicarbonate output may be normal or even raised in liver disease, there may be poor hepatic inactivation of secretin⁷¹.

While the incidence of diabetes mellitus among patients with chronic pancreatitis or carcinoma is well documented, the reverse is not true. In the largest survey of pancreatic function in randomly selected diabetics Chey, Shay, and Shuman found 18 out of 50 patients with abnormal secretin/pancreozymin tests⁷². Joslin, Rost, White, and Marble and Peters, Dick, Hales, Orrell, and Sames, however, found no abnormality of exocrine pancreatic function in diabetics^{73,74}. Using the Lundh test, Youngs found four out of five diabetics with a normal test and concluded that an abnormal Lundh test was only found in longstanding insulin-dependent diabetics³². At present it seems reasonable to suppose that the presence of mild diabetes is not a sufficient reason to explain abnormal tryptic activity in the duodenal aspirate following a test meal.

Pancreatic exocrine function in haemochromatosis has not been extensively investigated. Worning *et al* (1967) found enzyme levels to be in the lower part of the normal range in three patients with haemochromatosis⁷⁸. Wormsley has found large secretory volume and bicarbonate output in patients with haemochromatosis following a secretin/pancreozymin test⁵². James *et al* have found pancreatic function following a test meal and pancreatic scan to be abnormal in three out of eight patients⁷⁵.

The Place of the Lundh Test in Clinical Practice

Unfortunately, the Lundh test like other tests of pancreatic function, does not bring us nearer to solving two of the most important problems in pancreatic disease, namely, the early diagnosis of pancreatic carcinoma, and the assessment of the importance of minor degrees of pancreatic malfunction in patients with otherwise unexplained abdominal pain.

The Lundh test does have certain advantages over the secretin test and its variants. It is simpler to perform, it is cheaper, it is less unpleasant for the patient, and the test meal does not have the unpleasant side effects sometimes associated with secretin/pancreozymin injection. Against these advantages must be set the fact that the stimulus is not exactly measured and cannot be guaranteed as precisely as can the secretin or secretin/pancreozymin tests; more important these tests do claim to differentiate between pancreatic carcinoma and chronic pancreatitis.

The following may be regarded as being the circumstances in which the Lundh test may be of value: 1, in the differential diagnosis of obstructive jaundice; 2, in the diagnosis of pancreatic steatorrhoea; 3, in the diagnosis of chronic pancreatitis even without steatorrhoea; 4, in the follow up of pancreatic function following one or more episodes of acute pancreatitis; 5, in the investigation of effective function of the pancreas in a variety of circumstances, ie, in the investigation of malabsorption following Billroth II gastrectomy; 6, in the diagnosis of pancreatic carcinoma without obstructive jaundice (a very abnormal test being helpful, a normal test non-contributory).

Recently a new test using a Lundh meal has been described, in which instead of trypsin estimation the radioactivity of radioselenium is measured following an intravenous injection of ^{75}Se selenomethionine given 10 minutes after a test meal. Youngs *et al* reported very good correlation between the radioselenium activity in duodenal aspirate at 90-120 minutes after the Lundh meal and mean tryptic activity of the two-hour test period in 96 subjects, including 20 patients with pancreatic carcinoma and 15 with chronic pancreatitis^{32,76}. The test may be combined with a ^{75}Se selenomethionine pancreatic scan. The same group has shown that labelled amino acids given intravenously to patients with pancreatic fistula appear in the pancreatic juice about 50 minutes after injection⁷⁷. Bron *et al* have been less enthusiastic about the use of duodenal radioselenium measurements following a test meal but at present their comments are only in abstract and not supported by concrete results³³.

Work by Kalser *et al* has been of great interest both as throwing light on pancreatic function and suggesting a further practical use for the Lundh test¹⁰. This group showed that following partial gastrectomy steatorrhoea due to ensuing pancreatic insufficiency was only present when about 95% of functioning pancreatic tissue had been removed. Their elegant studies indicated that duodenal trypsin and lipase levels were improved considerably in pancreatectomized patients who were taking pancreatic supplements with their food. Thus in patients with pancreatic steatorrhoea, it seems reasonable to test the efficacy of treatment by oral pancreatic supplements by repeat test meals.

In this review the Lundh test has been considered alone, or in relation to other tests involving duodenal intubation. However, it should be stated strongly that the use of the Lundh test, as with other pancreatic function

tests, should not be taken in isolation but in conjunction with clinical information and other pancreatic tests. Experience with a group of such tests, including either a test meal or a secretin or secretin/pancreozymin test, will yield the best diagnostic result.

OLIVER JAMES

Department of Medicine,
The Royal Free Hospital, London

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