

Diagnosis of exocrine pancreatic insufficiency in cystic fibrosis by use of fluorescein dilaurate test

J G R CUMMING, J S FORSYTH, E J S BOYD, G J FROST, AND A CUSCHIERI

Departments of Surgery, Child Health, Clinical Pharmacology and Surgery, Ninewells Hospital and Medical School, Dundee

SUMMARY In the fluorescein dilaurate test fluorescein dilaurate is cleaved by the pancreas specific cholesterol ester hydrolase activity and the liberated fluorescein is absorbed and excreted in the urine. Fluorescein recovery is a reflection of exocrine pancreatic function. The test was evaluated in 14 patients with cystic fibrosis and 16 healthy volunteers. The test was well tolerated by patients, was easy to perform, and gave significantly lower values in the patients suffering from cystic fibrosis. The result of the pancreolauryl test was also correlated with the result of the faecal chymotrypsin test in 11 of the patients suffering from cystic fibrosis. A positive correlation was found between the two test results. The test is a practical and reliable index of pancreatic exocrine function and may have a useful role as a screening procedure.

The most accurate test of pancreatic exocrine state is the analysis of duodenal aspirate after maximal stimulation of the gland with secretin and cholecystokinin,¹ but the test is unpleasant and time consuming and requires inpatient facilities. Duodenal tubes have to be positioned by skilled operators using *x* ray control, and the assay of bicarbonate and enzymes requires skilled technical services. There is therefore a need for a single non-invasive test of pancreatic exocrine function, especially for children. The fluorescein dilaurate test was recently reported to be useful in screening for pancreatic disease in adults,^{2,3} and we have now evaluated its use in patients with cystic fibrosis, the most common cause of pancreatic insufficiency in childhood. The results of a new test of exocrine pancreatic function must correlate with the more widely used variables of disease detection. For this reason, the results of the pancreolauryl test were compared with activities of faecal chymotrypsin estimation in 11 patients suffering from cystic fibrosis.

Patients and methods

Fourteen patients aged 4–19 years, suffering from cystic fibrosis (confirmed by sweat chloride concentrations, which exceeded 70 mEq/l as determined by using the Orion electrode), and 15 healthy volunteers aged 3–16 years all performed a fluorescein dilaurate test. The experimental nature of the study

was explained to the parents and the children and informed consent was obtained.

Fluorescein dilaurate test. Fluorescein dilaurate is hydrolysed by the pancreas specific cholesterol ester hydrolase activity in the upper small intestine. Fluorescein is released, absorbed, and excreted in the urine. The urinary output of fluorescein is, therefore, an indication of pancreatic exocrine function.

The test was carried out on two consecutive days. On the first day the patient swallowed two capsules each containing 0.25 mmol of fluorescein dilaurate together with a light breakfast to act as a physiological stimulus to exocrine pancreatic function. Diuresis was encouraged by a liberal fluid intake, and urine was collected for 10 hours after the ingestion of the capsules.

On the following day the patient swallowed a capsule containing 0.5 mmol of unesterified fluorescein under similar conditions. Urine was again collected for 10 hours. Pancreatic enzyme supplements and vitamin B preparations (which absorb light at 492 nm) were stopped two days before the test. The urine was collected in five litre plastic containers and the volume noted. A 0.5 ml aliquot of the sample was mixed with 4.5 ml of 0.1M sodium hydroxide solution and heated to 70° for 10 minutes in a water bath. The optical extinction *E* at 492 nm (Lambda maximum of fluorescein) was compared

with water in a spectrophotometer (Pye Unicam SP 600), and the excretion of fluorescein was calculated as

$$\frac{E \ 492 \times \text{urine volume.}}{35}$$

The denominator 35 was derived from the dose, dilution, and molar extinction coefficient of fluorescein. Excretion on the test day was expressed as a percentage of excretion on the control day. The fluorescein dilaurate test as 'Pancreolauryl Test' was supplied by International Laboratories Ltd (Alton, Hampshire, England).

Faecal chymotrypsin estimation. Chymotrypsin was measured titrimetrically according to the method of Haverback *et al.*⁴ Faecal samples were obtained during the performance of the pancreolauryl test. They were homogenised, buffered to pH 7.8, and mixed with acetyl-tyrosine-ethyl ester as chymotrypsin substrate.

Statistical methods. The results in the two groups

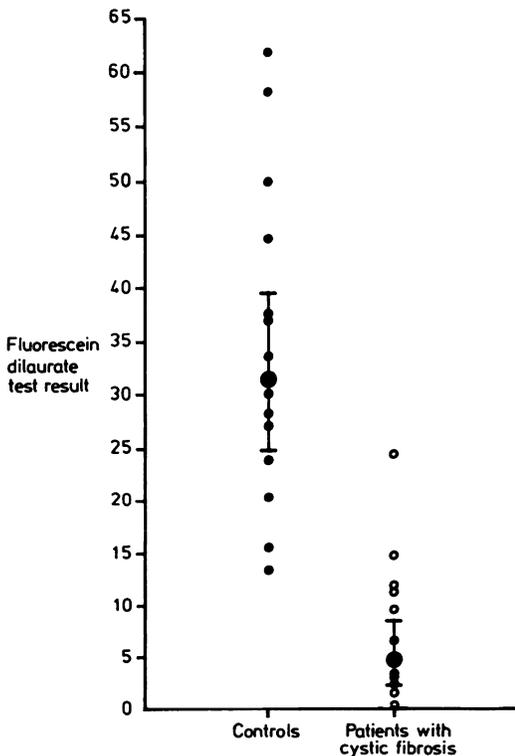


Fig. 1 Results of fluorescein dilaurate test in patients suffering from cystic fibrosis and controls. Values are geometric means with 95% confidence limits.

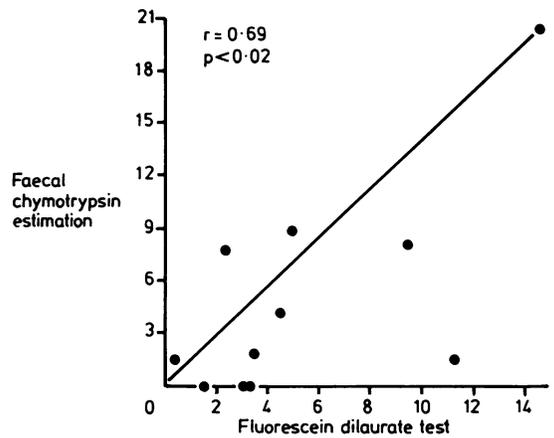


Fig. 2 Scatterplot of fluorescein dilaurate test result and faecal chymotrypsin estimation.

were compared by the Wilcoxon rank sum test. The results were abnormally distributed, especially in the patients with cystic fibrosis, and consequently 95% confidence limits were calculated for both groups. The correlation coefficient between the result of the pancreolauryl test and the faecal chymotrypsin estimation was assessed by linear regression analysis.

Results

Results of the fluorescein dilaurate test in the control group ranged from 13.3 to 62.2 with a geometric mean of 31.4 (25 to 39.5 upper and lower 95% confidence limits). Results of the tests performed in the patients with cystic fibrosis ranged from 0.3 to 24.6 with a geometric mean of 4.7 (2.6 to 8.6 upper and lower 95% confidence limits) (Fig. 1). These values were significantly reduced in the patients with cystic fibrosis compared with the healthy volunteers ($p < 0.01$).

The results of the faecal chymotrypsin estimation were markedly reduced and ranged from 0 to 20.5 $\mu\text{g/ml}$. The scatterplot of the results of the fluorescein dilaurate test against the faecal chymotrypsin estimation is illustrated in Fig. 2. The correlation coefficient was 0.69 ($p < 0.02$).

Discussion

Previous reports of peroral tests of pancreatic insufficiency using p-aminobenzoic acid,⁵ or its acetyl derivatives,⁶ have been performed on in-patients and required severe dietary restrictions during the test. The assay of the liberated p-

aminobenzoic acid is difficult and requires expert laboratory help. The pancreatic specificity of the test has recently been questioned.^{7 8} The fluorescein dilaurate test proved very acceptable to patients and was performed as an outpatient procedure. The investigation was completed during the course of one weekend and there was no interference with schooling. Patients were able to eat a normal lunch during the test procedure.

Our results show that the fluorescein dilaurate test is a useful screening test for detecting children with pancreatic insufficiency due to cystic fibrosis. We suggest that a test result of 20 or over indicates that there is no gross pancreatic insufficiency. A test result below 20 would be an indication to perform further studies to determine pancreatic exocrine state (faecal fat, direct stimulation intubation test).

Previous reports of the use of this test in the diagnosis of pancreatic insufficiency in patients with cystic fibrosis have failed to compare the results with other tests of pancreatic disease.^{9 10} We believe that the positive correlation found between our test results and the commonly used faecal chymotrypsin estimation provides confirmation that the fluorescein dilaurate test will be useful in aiding the diagnosis of cystic fibrosis. A more valid comparison might have been obtained if the pancreolauryl test result had been compared with faecal fat estimation. This study was, however, carried out on an outpatient basis and it proved impossible for the parents of the children involved to collect and store the faecal samples required to perform faecal fat studies. Attention has recently been drawn to the difficulty in obtaining an accurate sweat test result in centres in which the procedure is not frequently performed, and overdiagnosis of cystic fibrosis in children with chronic productive cough has been reported.^{11 12} It is important that the one patient in the cystic fibrosis group whose fluorescein dilaurate test result was above 17 indicated that there had been no change in bowel habits during the period of the test when no pancreatic supplement was being taken, and she may therefore not require supplementation. A more extensive assessment of her pancreatic function is indicated. A wide variation in pancreatic function in cystic fibrosis is recognised

and the fluorescein dilaurate test may be useful in detecting those patients not requiring supplementation.

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Correspondence to Professor A Cuschieri, Department of Surgery, Ninewells Hospital and Medical School, Dundee DD1 9SY, Scotland.

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