

Assessment of fat malabsorption

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SUMMARY For the assessment of fat malabsorption, the standard method of measuring faecal fat excretion using a 5 day stool collection has been compared with the alternative methods: stool microscopy, a lipid tolerance test and a continuous marker technique for the estimation of fat content on a single stool sample. The lipid test, using an emulsion of arachis oil (Prosparol), was less reliable than had been expected with a sensitivity of 33% and a specificity of 45.4%. Stool microscopy using Oil Red O to stain fat globules had a sensitivity of 72.2% and a specificity of 95.4%. Fat estimation of a single stool sample using copper (1) thiocyanate showed a high correlation with that determined on a 5 day stool collection ($p < 0.001$).

It is concluded that lipid tolerance tests have little place in the estimation of fat absorption. In laboratories where faecal fats are not measured, microscopic examination of stool for fat globules provides a specific and relatively sensitive method for detecting steatorrhoea. The use of a continuous marker provides a method for assessing the degree of steatorrhoea on a single stool sample without the disadvantages of the conventional method of faecal fat analysis.

Estimation of faecal fat excretion has been the traditional method for assessing steatorrhoea. However, it suffers from the difficulties of obtaining accurate collections and involves the handling of large quantities of faeces. To overcome these problems a number of indirect tests of fat absorption have been described including the measurement of serum carotene,¹ vitamin A absorption² and a ¹⁴C-labelled triolein breath test.³ These tests can only screen for steatorrhoea and are unable to indicate its degree. A further test, which has been used in Oxford, is the fat tolerance test which measures the rise in blood lipids following a 100 g fat breakfast.⁴ This test has been shown to correlate well with faecal fat estimations.⁴ Due to the unpalatability of the fatty breakfast, a modification has been introduced whereby the breakfast is substituted for by a fat emulsion (Prosparol).

The purpose of this study was to compare the results obtained from the Prosparol test with faecal fat determinations. In addition, a qualitative test for fat droplets in the stool was performed and faecal fat was also measured by a continuous marker technique using copper (1) thiocyanate.^{5,6}

Patients and methods

PATIENTS

Three groups of patients have been studied: 26 patients with suspected fat malabsorption from a variety of causes, 10 patients with diarrhoea without fat malabsorption, and four healthy volunteers. The Table lists the details of the patients' diagnoses. All patients were known to have normal renal function. The study was approved by the Ethics Committee and written consent was obtained from each patient after the purpose of the study had been explained and after they had read the explanatory notes.

EXPERIMENTAL PROTOCOL

All patients had fat absorption assessed by: (i) staining a faecal smear with Oil Red O; (ii) a Prosparol test, and (iii) the continuous marker technique. Twenty-five patients were in hospital for investigation and, during this time, they were placed on a 100 g fat diet. A 5 day stool collection was obtained for faecal fat estimation. The other 15 patients were studied as outpatients and, in these, 5 day faecal fat estimations were not obtained due to the difficulties and unreliability of outpatient collections. The outpatients were on their normal diet. However, patients were instructed to avoid a low fat diet and to stop pancreatic supplements, where appropriate. During the period of study for all patients, no laxatives were

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Diagnoses of patients studied

1	Patients with suspected fat malabsorption	
	<i>Intestinal</i>	
	Crohn's disease	5
	Radiation enteritis	1
	Coeliac disease	4
	Postgastrectomy	3
	Dermatitis herpetiformis	1
	<i>Pancreatic</i>	
	Chronic pancreatitis	7
	<i>Cholestasis</i>	
	Primary biliary cirrhosis	3
	Sclerosing cholangitis	1
	<i>Unknown</i>	1
Total		26
2	Patients not suspected to have fat malabsorption	
	Irritable bowel syndrome	7
	Hypolactasia	1
	Diverticulitis	1
	Crohn's colitis	1
Total		10

allowed and barium contrast studies were withheld for at least one week prior to the study. At the end of the study week, a serum sample was obtained from all patients for thiocyanate determination.

OIL RED O STAINING

This was performed by modifying the test described for Sudan III staining.⁷ Small pellets of stool were smeared on to two glass slides. For the detection of neutral fat, two drops of water were added to one slide, mixed, and then two drops of ethyl alcohol (95%) were added and mixed. Two drops of Oil Red O (British Drug House) were applied and mixed with the edge of a cover-slip. The smear was then examined under low-power magnification ($\times 100$) for the presence of fat globules. To detect split-fats, two drops of 36% (vol/vol) acetic acid were added to the other smear, mixed and then followed by drops of Oil Red O. After mixing, the preparation was covered with a cover-slip and gently heated until it began to boil. While still warm, the slide was examined under low-power magnification ($\times 100$).

A positive test⁸ was the presence of 10 or more fat globules per field of greater than $10 \mu\text{m}$ in diameter for neutral fat or $20 \mu\text{m}$ for split fats. The diameter was measured using an eyepiece graticule.

PROSPAROL TEST

After an overnight fast, patients were given a 10 mg tablet of metoclopramide 30 min before taking 100 ml of Prosparol (British Drug House: emulsion of 50% arachis oil in water) mixed with 30 ml of double cream (Express Dairy). It was flavoured with butterscotch to improve palatability. This provided an intake of approximately 65 g of fat.

Serum was obtained fasting at two and four hour intervals after ingestion. Total esterified fatty acids

were measured by a modification of the method of Stern and Shapiro.⁹

A positive test indicating fat malabsorption was defined as a rise of total esterified fatty acid of equal or less than 0.5 mmol/l measured at either two or four hours. Normal absorption was indicated by a rise of 1.0 mmol/l or more. These arbitrary limits are in current use in the Biochemistry Department.

CONTINUOUS MARKER TEST

This was performed as described by Lee *et al.*⁶ Briefly, copper (I) thiocyanate (Koch-Light Laboratories) was administered orally for six days as a non-absorbable marker. It was given in capsule form in a dose of 240 mg (80 mg tds). At the end of this period, a single stool sample was obtained and analysed for copper and fat. The copper content ($x \text{ mmol}$) was measured by atomic absorption spectrometry and the fat ($y \text{ grams}$) by the method of van de Kamer.¹⁰ Given a daily dose of 240 mg of marker (containing 1.17 mmol copper) the faecal fat excretion (G) is calculated by $\frac{1.17}{x} \times y$.

FAECAL FAT ESTIMATION

For five day collections, patients were instructed to collect their own stools in special plastic bags (Seward Laboratories) and this was carefully monitored by the ward staff and by Dr T L-B. The stools were homogenised within the bags after adding an appropriate volume of deionised water, using a Laboratory Blender ("Stomacher", Seward Laboratories). The fat content was measured by the method of van de Kamer *et al.*¹⁰ using an aliquot of the homogenate. The upper limit of normal was taken as 7% of dietary fat,¹⁹—that is $< 7 \text{ g/24 h}$ (equivalent to 25 mmol fatty acids/24 h).

THIOCYANATE ESTIMATION

Thiocyanate concentrations of serum were measured by the method of Bowler.¹¹

Results

Eighteen patients were found to have fat malabsorption as assessed by the 5 day faecal fat estimation ($> 7 \text{ g/day}$) or, in the case of outpatients, by the continuous marker technique.

PROSPAROL TEST

Fig. 1 shows the correlation between the faecal fat excretion and the results of the Prosparol test for all the 40 patients studied. No meaningful correlation exists. The sensitivity of the Prosparol test was 33%, calculated as the proportion of patients with steator-

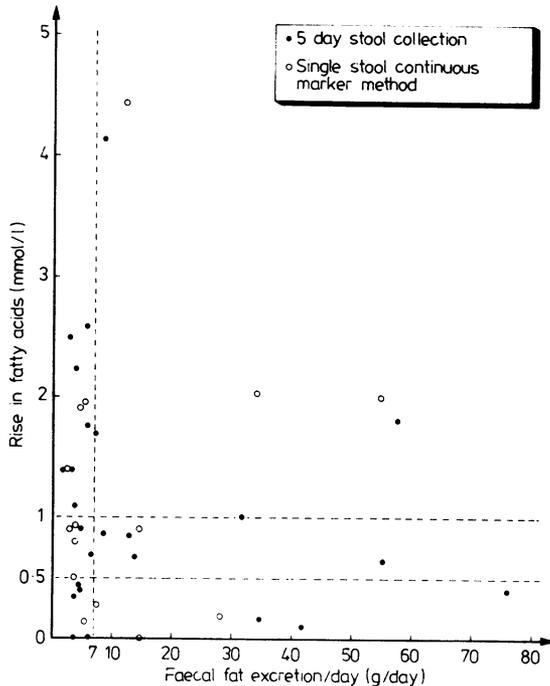


Fig. 1 Comparison of the Prosparol test and faecal fat excretion. The results of the Prosparol test are expressed as the rise in serum fatty acids. Points above the upper horizontal dotted line are considered normal, those below the lower horizontal line are considered definitely abnormal. The vertical dotted line represents the upper limit of normal fat excretion.

rhoea by faecal fat estimation who had an abnormal Prosparol test. The specificity of the test was 45.4%, calculated as the proportion of patients without steatorrhoea who had a normal Prosparol test. A further problem in interpreting the data of the Prosparol test is that 10 patients had a rise in blood lipids between the 0.5 mmol/l and 1.0 mmol/l limits. Only five of these had increased fat excretion. However, as can be appreciated from Fig. 1, altering the limits for an abnormal Prosparol test does not increase the sensitivity and specificity of the test, nor were these improved by excluding patients with cholestatic or pancreatic disease.

STOOL MICROSCOPY

Fig. 2 shows the data for stool microscopy following staining with Oil Red O. Of the 18 patients with steatorrhoea by faecal fat estimation, 13 had positive stool microscopy for fat (sensitivity 72.2%). Of the 22 patients who did not have steatorrhoea, 21 of them were negative on stool microscopy (specificity 95.4%).

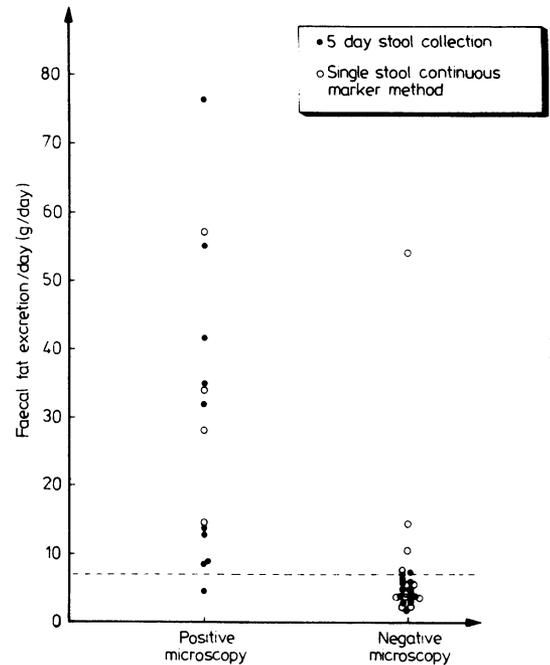


Fig. 2 Faecal fat excretion related to the results of stool microscopy.

CONTINUOUS MARKER METHOD

Twenty-four patients had their fat excretion measured by the two methods—5 day stool collection and the continuous marker technique using a single stool sample. The data are shown in Fig. 3 and demonstrate a high correlation between the two methods ($r = 0.94$, $p < 0.001$). However, especially at low values of fat excretion, the single stool sample technique tends to overestimate the fat content. Thus, the equivalent of 7 g/day on the 5 day stool collection method was 10.5 g/day on the single stool method. One possible source of error is that there was incomplete recovery of copper in the stool. Two experiments were performed in which a known quantity of copper (1) thiocyanate was added to a normal stool and then analysed for copper content. Recovery of copper was 83% and 93% in each of the experiments.

Fig. 4 shows the serum thiocyanate concentrations in all patients at the end of the study period. In no case did the concentration exceed more than 4 mg/dl which is well below the accepted toxic level of 10 mg/dl.¹¹ Furthermore, patients with small intestinal disease were not at particular risk of developing higher concentrations.

VARIABILITY OF FAECAL FAT ESTIMATION

Faecal fat estimations by the van de Kamer titrimetric

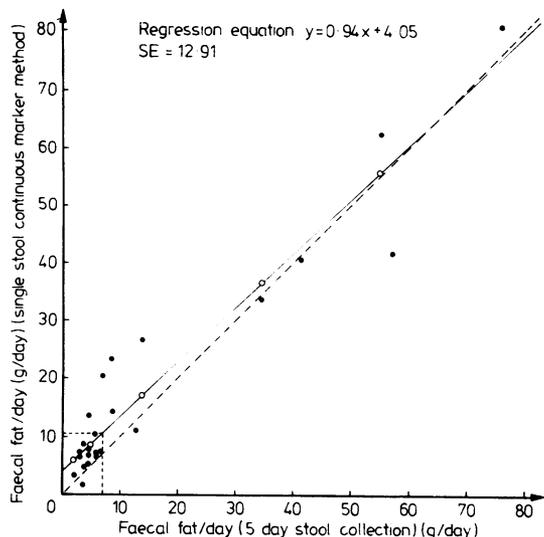


Fig. 3 Correlation between faecal fat excretion measured using a 5 day collection and a single stool sample with a continuous marker.

method were performed in duplicate. The intra-assay variability had a median of 1.4% (range 0–7.5%) for the five day stool assays and 3.4% (range 0–12.1%) for the single stool assays. Stool homogenates from the same 5 day collection from five patients were

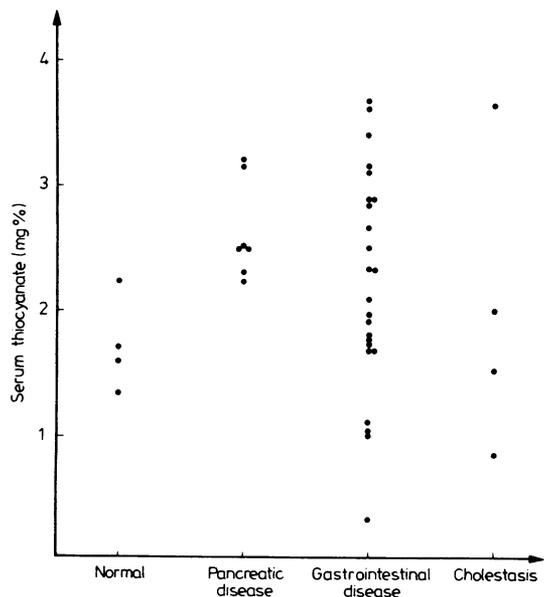


Fig. 4 Serum thiocyanate concentrations in patients receiving copper thiocyanate as a continuous marker.

tested on two separate occasions with an interval ranging from 6–37 days. The mean inter-assay variability was 4.6%.

Discussion

The standard method for assessing fat absorption is the 5 day stool collection with measurement of fat content. It has many disadvantages, of which its dependence on reliable collections and the social problems of handling large quantities of faeces are the major ones. Indirect tests have therefore been explored to avoid faecal fat determinations, but none of them is able to measure the degree of steatorrhea. Serum carotene concentrations correlate well with the presence or absence of steatorrhea^{1 2} and vitamin A tolerance tests may also be a useful screening test.² More recently, breath tests using ¹⁴C-labelled triglycerides have been re-evaluated.³ ¹⁴C-labelled triolein was found to be the most reliable but this is a relatively expensive test and has the disadvantage of using an isotope with a very long half-life making it inappropriate for use in children. The same disadvantages apply to the use of ¹⁴C-phenylacetic acid.¹³ The use of ¹³C, a non-radioactive isotope, to label triolein overcomes this difficulty but is extremely expensive and requires mass spectrometry to detect the isotope.³

Fat tolerance tests have proved to be both sensitive and specific when compared with faecal fat estimations^{4 14 15} but, as many patients are unable to take the fatty breakfast, it is limited in its use. A more palatable substitute was a mixture of arachis oil (Prosparol) mixed with double cream and flavoured with butterscotch. This was readily taken by all patients and delay in gastric emptying was minimised by using metoclopramide. However, this study shows that, when compared with faecal fat estimations, it has poor sensitivity and low specificity. Therefore, it cannot be recommended as a screening test for steatorrhea.

The other two methods which have been studied appear more promising. Stool microscopy using Oil Red O to stain fat globules was highly specific and was reasonably sensitive (72%). It may therefore provide a rapid and easy screening test. Similar results have been previously reported using Sudan III to detect neutral and split fats in faecal smears.^{7 8}

A variety of markers have been used to allow fat excretion to be calculated on the basis of the fat content of a single stool. These include chromic oxide,¹⁶ barium sulphate,¹⁷ cuprous thiocyanate⁶ and polyethylene glycol.¹⁸ Polyethylene glycol suffers from the disadvantage that an abnormal jejunal mucosa may be permeable to it and therefore its use as a non-absorbable marker may be suspect. The

present results using copper (1) thiocyanate confirm the excellent correlation with the 5 day stool collection method previously found by Lee *et al.*⁶ It tends to overestimate fat excretion which appears, at least in part, to be due to incomplete recovery of copper in the stool but may also be compounded by the well-recognised errors of the 5 day collection. Further development of this test to improve the recovery of the marker seems worthwhile in order to achieve a test which is practicable, easy to perform, and quantifies fat excretion.

Diets were organised and supervised by Miss Ruth Rolph.

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