Clinical evaluation of the ^{14}C fat absorption test

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SUMMARY The absorption of ^{14}C -labelled glyceryl tripalmitate was tested in 62 patients by determining the maximum specific activity of CO_2 in the breath after an oral dose. A significant correlation with faecal fat excretion was found. The absorption of ^{14}C labelled palmitic acid was tested subsequently in 54 patients. In cases of pancreatic steatorrhoea, normal absorption of palmitic acid with impaired absorption of glyceryl tripalmitate was found, whereas in most patients with coeliac disease the absorption of both substances was depressed to the same extent.

Malabsorption of fat is usually diagnosed by the demonstration of excess fat in the stools. Various techniques have been devised to measure absorption of fat directly. These have either taken the form of lipid tolerance tests or have used radioactive tracer-labelled fats. Stanley and Thannhauser (1949) gave ^{131}I -labelled glyceryl trioleate orally and measured radioactivity in blood. Subsequently others modified this technique, but there remained difficulty in purifying the iodinated fat and the problem of loss of the label before absorption (Kennedy and Kinloch, 1964). These problems were overcome by intrinsic labelling of the fat with ^{14}C but large doses were required in order to detect and measure this label in the blood.

Abt and Von Schuching (1966) investigated utilization of ^{14}C fat using long-chain fatty acids. They showed that it was unnecessary to collect the total output of $^{14}\text{CO}_2$ to measure fat absorption; the expired air could be sampled at intervals, and the highest concentration of the label in the CO_2 (maximum specific activity) was proportional to the absorption of the test dose. Kaihara and Wagner (1968) developed a test of fat absorption using this principle with ^{14}C glyceryl tripalmitate. They were able to correlate their results with measurements of faecal fat excretion.

Malm, Reemtsma, and Barker (1956), using radioiodine, tested the absorption of fats and their related fatty acids. This enabled them to distinguish coeliac disease, where the fatty acid is poorly absorbed, from pancreatic steatorrhoea, where its absorption is

normal. Lubran and Corsini (1960) devised a double isotope test using ^{82}Br oleic acid in ^{131}I triolein to differentiate pancreatic from other forms of steatorrhoea.

We have combined the principle of triglyceride compared with fatty acid absorption with the test of Kaihara and Wagner, using ^{14}C -labelled glyceryl tripalmitate (GTP) and palmitic acid (PA). This paper reports our experience in 62 patients.

Method

The method of Kaihara and Wagner (1968) is followed. Five microcuries of glyceryl tripalmitate (Radiochemical Centre, Amersham) in arachis oil was taken orally. At hourly intervals from three to eight hours after the dose, expired carbon dioxide was sampled by the patient exhaling into alkaline hyamine solution standardized to trap a known quantity of carbon dioxide. Radioactivity in the solution was then measured by liquid scintillation counting. All results were expressed as a percentage of the administered dose per millimole of carbon dioxide $\times 10^{-4}$. The maximum value at any of the hourly estimations was taken as the result of the test. The test was repeated on a subsequent day using palmitic acid in an identical procedure. The total body radiation dose is stated by Kaihara and Wagner (1968) to be 3×10^{-4} rad/test.

Faecal fats were estimated by Cammidge's dry weight method using ether extraction (Varley, 1962). The patients were taking a standard ward diet containing 70-100g fat/24 hours, and stools were collected for not less than four days.

	Mean ($\pm$ SD) of Test Results (% dose/m mole $\text{CO}_2 \times 10^{-4}$)		Faecal Fats (g/day)	
	Glyceryl Tripalmitate	Palmitic Acid	Mean	Range
Normals (14)	76 ($\pm$ 13)	86 ($\pm$ 17)	4.3	(2.3-6.0)
Coeliacs (8)	32 ($\pm$ 18)	44 ($\pm$ 13)	15	(7.0-37)
Pancreatics (9)	16 ($\pm$ 8)	92 ($\pm$ 39)	35	(9.9-120)
Blind loops (5)	47 ($\pm$ 11)	73 ($\pm$ 16)	31	(10-62)

Table 1 Results grouped by diagnosis

Results

NORMAL RESULTS

Normal values were established for each test in a group of eight healthy volunteers together with six patients in whom there was no suggestion of malabsorption (faecal fat <6g/day). These are shown in fig 1 and the mean results with standard deviation are given in table I. There is no significant difference between maximum specific activity after glyceryl tripalmitate and after palmitic acid. The mean minus 2 standard deviations is 51.3% administered dose/m mole $\text{CO}_2 \times 10^{-4}$. However, the data presented, both with regard to the separation of normal and malabsorbing subjects, and to the correlation with faecal fat excretion, suggest that 60 is a more discriminating lower reference limit.

Fig 1 Linked results grouped by diagnosis for absorption of glyceryl tripalmitate (GTP) and palmitic acid (PA). The results are expressed as a percentage of the administered dose/m mole $\text{CO}_2 \times 10^{-4}$.

RESULTS GROUPED BY DIAGNOSIS

In three groups of patients the tests proved useful. These are shown as linked results for individual cases in fig 1, and as mean results ($\pm$ 1 standard deviation) in table I.

Fig 2 Ratio of maximum breath CO_2 specific activity after ingestion of ^{14}C -labelled glyceryl tripalmitate (GTP) and ^{14}C -labelled palmitic acid (PA) in the four groups of patients.

COELIAC DISEASE

Eight patients with malabsorption and a flat jejunal mucosa on biopsy were tested before starting on a gluten-free diet. One patient unresponsive to gluten withdrawal was also studied. Mean absorption of both glyceryl tripalmitate and palmitic acid were impaired similarly ($P < 0.001$). Only one patient had a glyceryl tripalmitate:palmitic acid ratio of less than 0.4 (fig 2).

PANCREATIC INSUFFICIENCY

Studies were carried out on nine patients with abnormal faecal fat excretion due to pancreatic exocrine insufficiency which was supported either by a Lundh test meal, pancreatic calcification, or a marked clinical response to pancreatic supplements (two cases). Results (fig 1) showed impaired glyceryl tripalmitate absorption ($P < 0.001$) but normal palmitic acid absorption. All the patients showed a glyceryl-tripalmitate:palmitic acid ratio of less than 0.4 (fig 2).

BACTERIAL COLONIZATION OF THE SMALL GUT

All five cases of bacterial colonization contained organisms in the upper small intestine in numbers

ranging from 10^5 to 10^{10} organisms/ml, *E. coli* and *Bacteroides* being predominant. The results showed impaired uptake of glyceryl tripalmitate ($P < 0.001$) with normal, or near normal, uptake of palmitic acid. After treatment with tetracycline in two patients the glyceryl tripalmitate levels rose from 55 to 130 and from 28 to 57.

CORRELATION WITH FAECAL FAT EXCRETION

The results for glyceryl tripalmitate are plotted against mean daily faecal fat excretion in 42 patients (fig 3). The points in brackets denote an interval of greater than two weeks between the test and stool collection. Patients with results below 60 always had a mean daily faecal fat excretion greater than 6g. A negative linear correlation was found ($r = -0.45$, $P < 0.003$).

Fig 3 Daily faecal fat excretion plotted against absorption of glyceryl tripalmitate (GTP) assessed from breath maximal specific activity. The normal range for faecal fat is indicated at less than 6g/day, and for GTP is greater than 60% of the administered dose/m.mole $\text{CO}_2 \times 10^{-4}$. Figures in brackets indicate that the figures for faecal fat and glyceryl tripalmitate were obtained at an interval greater than two weeks.

MISCELLANEOUS CONDITIONS

Twenty-six patients with a variety of conditions were studied but no useful trends were observed, apart from two patients with obstructive jaundice (fig 1), who showed impaired absorption of glyceryl tripalmitate (41 and 42) compared with a more normal absorption (70 and 56) of palmitic acid.

Five patients with a Polya partial gastrectomy all had normal, or near normal, palmitic acid results. In three cases there was a reduced uptake of glyceryl tripalmitate, two having bacterial overgrowth in the blind loop. The remaining two patients had normal results.

This group also included Crohn's disease (three cases), cardiac failure (two cases), intestinal resection (two cases), diabetes (one case). One patient on neomycin did show a grossly deficient uptake of glyceryl tripalmitate (GTP 2; PA 72) which reverted to normal (GTP 84) on stopping the drug.

Discussion

We have assessed the glyceryl tripalmitate absorption test by comparison with faecal fat excretion, and we have examined the combination of glyceryl tripalmitate with palmitic acid tests to see if this contributes to the investigation of patients with malabsorption. A significant correlation between faecal fat excretion and glyceryl tripalmitate was obtained, and we have shown that a result for glyceryl tripalmitate below 60 indicates the presence of steatorrhoea (fig 3). A precise correlation should not be expected between faecal fats, which reflect both absorption and endogenous loss, and the glyceryl tripalmitate test which depends on the rate of gastric emptying, digestion, absorption, and utilization of fat.

The tests proved most useful in the diagnosis of pancreatic steatorrhoea, where all the cases studied showed a glyceryl tripalmitate : palmitic acid ratio of less than 0.4, whereas only one patient with coeliac disease had a similar ratio. We consider that the low result for glyceryl tripalmitate is due to a marked reduction in intraluminal pancreatic lipase which is necessary for the breakdown of glyceryl tripalmitate before absorption. Palmitic acid absorption is normal, since no hydrolysis is required.

We have also found a difference between the results for glyceryl tripalmitate and palmitic acid in obstructive jaundice and bacterial overgrowth in the small intestine, but in these cases the ratio was greater than 0.4. It may be expected that in these patients the intraluminal bile salt concentration was reduced, either because of reduced flow of bile or due to increased deconjugation (Tabaqchali, Hatzioannu, and Booth, 1968). It is of interest that in one patient receiving neomycin a reduction in the ratio of glyceryl tripalmitate to palmitic acid was observed. Neomycin is known to inhibit mixed micelle formation (Asatoor, Chamberlain, Emmerson, Johnson, Levi, and Milne, 1965). The explanation of these results requires further study.

Abnormal results might be expected after a Polya partial gastrectomy, possibly because of associated blind loop syndrome or due to inadequate mixing of the food with pancreatic juice (Cooke, Lennard-Jones, Sherif, and Wiggins, 1968). The glyceryl tripalmitate was reduced in three cases but the effect was not as marked as in cases of pancreatic exocrine insufficiency. The reduction in uptake may

not, therefore, indicate the exact mechanism of malabsorption in a patient after Polya partial gastrectomy.

Several factors could influence the results of these tests. Variation in the rate of gastric emptying could affect the time of the peak $^{14}\text{CO}_2$ level and its magnitude. Long-chain fatty acids and mono-glycerides are reconstituted in the intestinal cell to form triglycerides and are then transported as chylomicra in the lymphatic system; obstruction or congestion of the lymphatic pathways might delay excretion of the ^{14}C label. This is perhaps responsible for the low results for both tests in a case of chronic lymphatic leukaemia (glyceryl tripalmitate 30; palmitic acid 32). Low values (glyceryl tripalmitate 45; palmitic acid 39) were obtained for a patient with hypertriglyceridaemia (Fredrickson type 5) which could be explained by the excessive dilution of the labelled fat in the expanded plasma triglyceride pool. The rate of metabolism of fat is known to be reduced by carbohydrate meals (Kaihara and Wagner, 1968). For this reason our patients are tested while fasting. Finally, retention of CO_2 creates an increased pool into which the label would be diluted so that errors could occur in patients with respiratory failure. These points require further study.

We conclude that the ^{14}C fat absorption test is a useful clinical investigation. It causes no discomfort to the patient other than fasting and is safe. It can distinguish pancreatic steatorrhoea from coeliac disease in the majority of cases. It may also prove useful in the study of the blind loop syndrome.

We acknowledge gratefully the advice and assistance of Dr R. J. Bayly of the Radiochemical Centre, Amersham. We should like to thank Mr A. E. Wyman, who helped to set up the technique in Southampton, Mrs Denise Rickman, who carried out many of the tests, and Dr J. Middleton whose department estimated the faecal fats. We should like to thank our colleagues who kindly referred cases for investigation.

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