

A SCREENING TEST FOR STEATORRHOEA USING ¹³¹I-LABELLED TRIOLEIN

BY

M. LUBRAN AND J. D. PEARSON

From the West Middlesex Hospital, Isleworth, and the Physics Department, Guy's Hospital, London

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During recent years there has been increasing interest in the study of fat absorption, particularly in the investigation of conditions associated with steatorrhoea. The need for a single diagnostic test for steatorrhoea, suitable for use with both out-patients and in-patients, has become apparent. Ideally, such a test should be easy to carry out for the patient and nursing staff as well as for the laboratory technician, and should distinguish, without equivocation, between steatorrhoea and other conditions. So far, no such test has been published, but the procedure described in this paper goes a long way towards meeting these requirements, being simple, easy, and convenient to carry out, and, on the whole, giving a definite answer about the presence or absence of steatorrhoea. In addition, there is given a convenient method for the preparation of neutral fat or fatty acid labelled with ¹³¹I.

Principle of the Test

The patient, on a normal diet, is given a tracer dose of radioactive fat in milk, and faeces are collected for three or four days. The percentage of the administered dose excreted in the faeces is determined. Values over 6% are consistent with steatorrhoea, but it is better to regard 5% as the upper limit of normality and to carry out a chemical fat determination on the faeces when values between 5% and 7% occur. This can be done on the faeces used for the determination of radioactivity, thus wasting no extra time and calling for no extra effort on the part of the patient or nursing staff. It should be emphasized that this test is presented as a screening test for steatorrhoea, to replace conventional chemical procedures in most cases. It will not, in itself, distinguish the various causes of steatorrhoea from one another.

Technique of the Test

Preparation of ¹³¹I-labelled Fat.—The reagents required are the following:

1. Potassium iodide solution, 1 mg./ml.
 2. Potassium iodate solution, 2 mg./ml.
 3. 2N sulphuric acid.
 4. Iodide-thiosulphate solution. Dissolve 5 g. potassium iodide and 5 g. sodium thiosulphate in distilled water to give a volume of 100 ml.
 5. Ether (anaesthetic grade).
- All other reagents should be analytical reagent grade.

Preparation of Iodinating Solution.—To 1–2 ml. of a solution containing 1 mc. of carrier-free ¹³¹I as sodium iodide,* in a 10 ml. glass-stoppered test-tube, add 1 ml. of potassium iodide solution, layer 3 ml. of ether on to the aqueous phase and then add 0.5 ml. of potassium iodate solution and 0.2 ml. of sulphuric acid. Stopper the tube and shake gently until all the liberated iodine has passed into the ether layer. Remove the lower, colourless layer with a Pasteur pipette and discard. Add drop-wise to the remaining ether layer a freshly prepared solution of chlorine in ether (made by treating potassium permanganate with concentrated hydrochloric acid and passing the resulting chlorine into ether until the ether is distinctly yellow) until the brown colour of the iodine has disappeared. As a rule, only one drop of chlorine solution is needed.

Iodination of Fat.—Dissolve 1 ml. of triolein in 3 ml. of ether in a 15-ml. glass-stoppered tube. Using a clean Pasteur pipette, add the iodinating solution prepared above, stopper, mix by tapping, and allow to stand at room temperature for at least one hour. The preparation may be left overnight at this stage, without harm. Then add an equal volume of iodide-thiosulphate solution, shake gently, allow the mixture to separate into two layers (centrifuge, if necessary, to break any emulsion) and remove the lower layer with a Pasteur pipette. Repeat the washing process with iodide-thiosulphate and then, twice more, with equal volumes of distilled water. Transfer the washed ethereal solution of iodinated fat to a 150 ml. glass-stoppered Erlenmeyer flask, evaporate the ether on a water-bath at about 60° C., add about 0.5 ml. of "Tween 80" (Solmedia Ltd.†) and 100 ml. of distilled

* Supplied by the Radiochemical Centre, Amersham.
† Solmedia Ltd., 35, Orford Road, London, E. 17.

water, stopper and shake well until an even suspension is produced.

Assay of Radioactivity of Fat.—This may be carried out by any convenient method. In this work the multitube γ -ray counting apparatus of Veall and Baptista (1954) has been used, the fat suspension being diluted 1 in 10. The excreta counter described below has also been used, after calibration with 1 μ c. of ^{131}I .

Preparation of Patient before Test.—In order to make possible the determination of the faecal fat by chemical methods, if this should prove necessary, on the specimens used for measuring radioactivity, the patient should be instructed to consume, for two days before the test starts and during the period of faecal collection, 2 pints of milk and 1 oz. of butter or margarine in addition to the other constituents of his usual diet. This will provide an intake of about 100 g. of fat daily. In addition, thyroid absorption of iodine should be blocked by the administration of 10 minims of Lugol's iodine t.d.s., or 10 mg. of potassium iodide (as tablets) t.d.s., for the two days preceding the test and the day of the test itself.

Administration of Dose.—The dose is most conveniently given about two hours after the patient has had his usual breakfast. An accurately measured volume (usually 2 ml., containing 5–10 μ c. ^{131}I) of the freshly shaken fat suspension is delivered into about 100 ml. of milk in a cup. After the dose has been completely drunk, the patient drinks two further 100 ml. portions of milk, which are added to the cup. There is no significant radioactivity remaining in the cup, if this procedure is followed. For young children, the dose should be 1 μ c. for each stone of body weight. The stock radioactive fat suspension may be kept at least two weeks at room temperature without deterioration or liberation of free iodine.

Collection of Faeces.—Patients should be instructed to collect all faeces, without waste and without contamination by urine, passed from the moment of drinking the dose until the end of four clear days, including the fourth day up to the time of administration of the dose. The collection period may be reduced by one day, with a risk of introducing small errors (Tables II and III).

Faeces are best collected in non-disposable spun-aluminium cans, with tight-fitting lids,* made to fit snugly into the excreta counter and to hold about 2 litres. They may be easily cleaned with detergent and hot water. The patient should be instructed to pass his faeces directly into the can, after first emptying his bladder. Both male and female patients have, as a rule, found no difficulty in using the cans in this way. Several days' collections may be made in the same can, but, if the patient objects to this, one container may be used for each day, in which case the cans must be labelled with the date of collection. The main advantages of this method of collection are that

it is almost impossible to contaminate the faeces with urine (which would completely invalidate the results) and that there is no waste of faeces. If it is impossible to collect the faeces in this way, then more conventional methods may be used, care being taken to transfer the faeces quantitatively and to avoid urine contamination. Waxed cartons, although suitable for well-formed stools, have in practice not proved suitable for liquid or semi-formed stools and have, in addition, insufficient capacity for the bulky stools, often in excess of 1 kg., passed by patients with steatorrhoea or diarrhoea.

Measurement of Radioactivity.—This may be conveniently carried out using the excreta counter of Veall and Vetter (1952), which consists of a ring of Geiger tubes. The can should be raised so as to bring it into the sensitive zone of the tubes. At least 3,000 counts above background should be recorded, using conventional electronic apparatus or a clinical monitor.* This equipment is the same as that used in many laboratories for the urinary excretion test of thyroid function of Fraser, Hobson, Arnott, and Emery (1953). The activity of a standard must be determined at the same time as that of the faeces. The standard (usually 20% of the dose) is accurately pipetted into a can containing about 1 litre of warm 10% gelatin. This is allowed to set, the fat being stirred in with a glass rod left *in situ*. This procedure gives an even dispersal of the radioactive fat and prevents spilling of the standard and accidental contamination of the counter. Activity of the faeces is expressed as a percentage of the dose given.

Chemical Fat Determination.—Where necessary, this may be quickly carried out by the method of Kamer, Huinink, and Weyers (1949) on the same faecal specimens.

Results

The test was carried out on six healthy males (laboratory staff) and 20 patients suffering from anaemia or disorders of the gastro-intestinal tract or pancreas, collection of faeces being made for three to six days. In order to determine the validity of the test, a chemical fat determination was made on the same sample of faeces used for measuring radioactivity in all patients, who consumed about 100 g. fat daily. No chemical fat determination was carried out in the case of the healthy normals, who were on their customary diet, absence of steatorrhoea being presumed.

In the six healthy males, excretion of radioiodinated fat did not exceed 4% of the dose administered (Table I) in three days. The results obtained with the patients fell into two groups, on the basis of the average daily excretion of fat, the dividing line being at 6 g. fat, corresponding to approximately 94% absorption of fat.

* Made by W. Still and Co., 29–31, Greville Street, London, E.C.1.

* Type 1294 DY, Dynatron Radio Ltd., Maidenhead, Berks.

TABLE I
EXCRETION OF ¹³¹I IN FAECES OF NORMAL SUBJECTS

% Dose Faecal ¹³¹ I Excreted in 3 Days*
4.0
3.5
3.4
3.0
3.1
1.7

* No simultaneous fat determination carried out.

In the group excreting below 6 g. fat daily (Table II), all 11 patients showed an iodine excretion not greater than 6%, in eight the excretion being below 5%, and in seven lower than 4%. On the basis of the fat excretion, all these patients must be considered as not showing steatorrhoea at the time of carrying out the test. Of the three patients with faecal

TABLE II
EXCRETION OF ¹³¹I IN FAECES OF PATIENTS WITHOUT STEATORRHOEA

Disease	Days of Collection	Faecal Fat (g./day)	Faecal ¹³¹ I Excretion (% Dose)	
			Total	After 3rd Day
Hypochromic anaemia ..	4	5.3	3.9	<0.2
Diarrhoea ..	6	3.5	4.2	0.4
Hypochromic anaemia ..	3	3.5	0.6	—
Diarrhoea ..	4	2.9	3.7	0.4
Ulcerative colitis ..	4	2.7	5.6	0.2
Diverticulosis of small intestine ..	3	2.5	6.0	—
Hypochromic anaemia ..	4	2.5	0.5	<0.2
Megaloblastic anaemia ..	4	2.2	5.1	<0.2
Post-gastr. my * ..	6	2.2	1.2	1.2
Diarrhoea ..	4	0.7	0.6	<0.2
Post-gastroctomy + megaloblastic anaemia ..	3	0.6	0.2	—

* No stools passed in first three days.

radio-iodine excretions of more than 5%, two were elderly women who were unable to use the cans directly for collecting faeces and it is probable that some urinary contamination occurred. The third patient had severe ulcerative colitis. It is possible that, in this disease, there may be some leakage of iodide from the blood into the bowel lumen.

In the group excreting above 6 g. fat daily (Table III), radio-iodine excretion exceeded 6% in eight of the nine patients and exceeded 7% in six cases. In one instance, the radio-iodine excretion was just under 5%. In this patient, who had a gluten-sensitive steatorrhoea, the excretion of faecal radio-iodine was 1.6% of the dose in the first two days, 3.3% on the third day, and none on the remaining two days. This pattern of excretion is anomalous and is consistent with some of the stool not having been collected on the important first two days.

From these results it may be concluded that radio-iodine excretions below 5% exclude steator-

TABLE III
EXCRETION OF ¹³¹I IN FAECES OF PATIENTS WITH STEATORRHOEA

Disease	Days of Collection	Faecal Fat (g./day)	Faecal ¹³¹ I Excretion (% Dose)	
			Total	After 3rd Day
Chronic pancreatitis ..	5	50.7	29.5	0.4
Post-gastroctomy ..	4	48.4	80.3	<0.2
Resection of most of small intestine ..	4	30.8	22.7	0.2
Post-gastroctomy ..	6	18.7	7.2	1.0
" ..	5	12.2	6.9	0.2
Tropical sprue ..	6	11.2	6.1	<0.2
Idiopathic steatorrhoea ..	4	9.5	13.5	0.3
" ..	3	7.6	13.9	—
Gluten-sensitive steatorrhoea ..	5	6.8	4.9	<0.2

rhoea and above 7% are consistent with steatorrhoea, in those cases where the test has been correctly carried out. With excretions between these values and whenever there is doubt about urinary contamination or completeness of collection of the faeces, a chemical determination on the same specimens of faeces will be necessary to decide the point.

Discussion

The use of ¹³¹I-labelled triolein in the study of fat absorption is not new. Stanley and Thannhauser (1949) administered olive oil containing 100 μc. ¹³¹I and studied blood levels of radioactivity. They did not report any observations on the radioactivity of the faeces. Since then, most workers have endeavoured to find some relationship between blood radioactivity and disease, following doses of iodinated fat ranging from 25 to 100 μc. (Baylin, Sanders, Isley, Shingleton, Hymans, Johnston, and Ruffin, 1955; Malm, Reemtsma, and Barker, 1956; Sanders, Isley, Sharpe, Baylin, Shingleton, Hymans, Ruffin, and Reeves, 1956; Ruffin, Shingleton, Baylin, Hymans, Isley, Sanders, and Sohmer, 1956; Shingleton, Baylin, Isley, Sanders, and Ruffin, 1957; McKenna, Bourne, and Matzko, 1957; Beres, Wenger, and Kirsner, 1957). In a few cases, radioactivity measurements were made on the faeces and in still fewer cases parallel determinations of radioactivity and fat were carried out. Baylin and others (1955) reported a faecal excretion of less than 2% of the dose during a 48-hour collection in six normal subjects after 50 μc. of ¹³¹I-labelled triolein. Sanders and others (1956) and Shingleton and others (1957) found less than 1.4% of the dose in 24 normals in a 48-hour collection after 25-50 μc. of radioactive fat, with greatly increased values in diseases clinically associated with steatorrhoea (chemical

fat determinations were not carried out). Ruffin and others (1956) found less than 2% of the dose in a 48-hour collection of faeces after 25 μc . of labelled fat in normals. McKenna and others (1957) reported a faecal excretion of less than 1.5% of the dose (50–100 μc .) in five normals in 48 hours, but found values up to 4% in seven normals in 72 hours, and increased values in diseases with steatorrhoea (confirmed chemically, however, in only two cases). Beres and others (1957) measured a three-day faecal collection, both for radioactivity and fat, after 40–100 μc . ^{131}I -labelled triolein. In eight subjects with fat absorption coefficients above 94%, faecal radio-iodine excretion was less than 1.6%, while in five patients with fat absorption of 90% or less, excretion ranged from 3.5 to 53.5% of the dose.

The level of excretion of labelled fat is lower in subjects without steatorrhoea in most of the published data than is reported here. This difference is probably related to the longer collecting period employed in our test. In normal subjects, who usually have one bowel action daily, a 48-hour collection period may not include the second stool if there is bowel irregularity during the course of the test, a not infrequent event. Further, it is well known to those who carry out daily faecal fat estimations on patients with and without steatorrhoea that there is a wide variation in the amount of fat excreted daily. To get an accurate picture of fat excretion, it is necessary to take the mean of several days' excretion. Examination of the results of daily faecal radioactivity measurements in our subjects shows that, while in many cases excretion is essentially complete in 48 hours, there are several cases where only about two-thirds of the excretion has taken place in this time. Usually, excretion is complete in four days, but a three-day collecting period may be used with only a little error. However, when the patient is constipated, a six-day collection period may be necessary. As it may be unreliable to carry out a chemical fat determination on a 48-hour collection of faeces, it is recommended that the collection period in this test should be a minimum of three days and preferably four days.

It will be noticed that, while chemical fat and radio-iodine excretion are correlated, the values for each may be markedly different in the same patient. It is not possible to convert radio-iodine excretion into faecal fat excretion by means of a factor, with any accuracy. This is because the two tests do not quite measure the same thing. The labelled fat test measures the ability of the gut to absorb a particular triglyceride and its

products of digestion, while the chemical fat test measures the ability of the gut to absorb a mixture of fats (and derivatives) of varying molecular weight and degree of saturation. It is by no means certain that all fats, both saturated and unsaturated, can be absorbed equally well, particularly in disease. There is, therefore, no reason to expect that percentage fat excretion and percentage radio-iodine excretion should be identical. However, the experimental results reported here and by other workers show that there is a correlation between steatorrhoea and radio-iodine excretion.

It is theoretically possible that some of the radioactive iodine in the faeces may be derived from inorganic iodide resulting from metabolism of the labelled fat after absorption and subsequently excreted into the intestine; or iodine might be liberated from labelled fat in the intestine by enzymes or bacteria and thus be treated differently from the fat. However, Ruffin *et al.* (1956) have shown that the radioactive material in the stool is non-dialysable and that all the radioactivity is found in the fat fraction obtained by ether extraction. Further, Hoffman (1953) has shown, in animal experiments, that the isotope-fat bond is stable to enzyme action during absorption from the gut. It is unlikely, therefore, except perhaps in cases of severe diarrhoea or ulcerative colitis, that diffusion of radioactive iodine from the blood into the gut will be of any significance.

No attempt has been made in this paper to correlate levels of radio-iodine excretion with the cause of the steatorrhoea, as the amount of fat in the faeces is not diagnostic of any particular disease. The test is presented as a screening test, to be carried out instead of a fat balance, to select for further investigation those patients suffering from steatorrhoea, and those whose excretion lies in the border zone of 5 to 7%, thus necessitating a chemical determination. The test should prove of particular value in excluding steatorrhoea, especially in anaemia associated with absorption defects.

The test can also be carried out on infants and children, provided it is possible to keep the faeces free from urinary contamination. Napkins can be rolled up in a waterproof bag and the activity of the faeces in them read in the excreta counter. Because of the small dose given to infants, a long counting time is necessary.

The advantages of the test described in this paper are that it is easy to carry out and can be used for out-patients; an accurately known diet is not essential; the dose is given in a palatable

form; the laboratory techniques involved are quick, easy, and accurate; no blood samples are required. Tests of fat absorption depending on blood radioactivity often require three or more blood samples, taken several hours after the administration of the dose, which has to be large. This makes these tests uncomfortable and tedious for the patient, besides adding to the labour of the technician. Further, blood tests are almost impossible for very young children. By relying only on faecal radioactivity measurements, it has been possible to use a very small dose of ^{131}I , thus reducing considerably the radiation hazard, an important consideration when dealing with children.

The disadvantages of our test are that faeces have to be collected accurately; care has to be taken to prevent contamination by urine; on a few occasions a chemical fat determination might prove necessary. However, this causes extra work only for the technician. Perhaps the most serious disadvantage is that it requires the use of radioactive material and equipment to measure radioactivity. Those laboratories equipped to carry out urinary radio-iodine excretion tests, however, have all the apparatus necessary for the faecal labelled fat test.

Summary

1. A convenient method for labelling triolein (or oleic acid) with ^{131}I is described, and, also, the preparation of an emulsion suitable for oral administration.

2. A technique is given for administering 5–10 μc . of this fat and assaying the activity in stools collected for three or four days.

3. The results of carrying out this test on six normals and 20 patients, nine with steatorrhoea

proved chemically, show that steatorrhoea may be excluded when there is an excretion of radio-iodine less than 5% of the dose. Values over 7% are consistent with the presence of steatorrhoea. For intermediate values, faecal fat may be determined by chemical methods, on the same faecal specimens, without the need to carry out another test.

4. The test will not diagnose the cause of steatorrhoea, but is presented as a screening test for steatorrhoea, to be carried out in place of the conventional fat balance.

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