

# **~ RADIOIODINATED TRIOLEIN IN MALABSORPTION STATES**

BY

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In the light of the difficulties and inadequacies of established methods in the investigation of intestinal malabsorption the report by Stanley and Thannhauser (1949) that radioiodinated olive oil might provide an index of fat absorption appeared to be of considerable interest. Subsequent investigators in America, using a pure neutral fat, triolein, similarly iodinated, have reported on the studies of levels of stool, urine, and blood radioactivity in various diseased states. Variations in technique and in the criteria for interpretation of results have resulted in conflicting claims regarding the relative value of blood and stool analysis after an oral dose of radioiodinated triolein.

The results therefore are reported here of an investigation undertaken to test the hypothesis put forward by the group of workers from the Duke University School of Medicine, North Carolina (Ruffin, Shingleton, Baylin, Hymans, Isley, Sanders, and Sohmer, 1956; Ruffin, Keever, and Cheers, 1957; Shingleton, Baylin, Isley, Sanders, and Ruffin, 1957a; Shingleton, Isley, Floyd, Sanders, Baylin, Postlethwait, and Ruffin, 1957b; Isley, Sanders, Baylin, Sharpe, Hymans, Ruffin, Shingleton, and Wilson, 1957; Ruffin, Keever, Cheers, Shingleton, Baylin, Isley, and Sanders, 1958) that an "invariable inverse relationship exists between the radioactive material in blood and stool" after an oral dose of radioiodinated triolein. The opportunity was also taken to study simultaneously the urinary radioactivity.

## **Materials and Methods**

**Patients.**—Patients in the present study consisted of two broad groups:

**Control Series.**—This consisted of 32 "normal" ward patients with no clinical evidence of gastrointestinal or renal disease and six known cases of idiopathic steatorrhoea. The diagnosis of steatorrhoea had been established on clinical and biochemical

evidence, including a conventional fat-balance study. All six patients had been on treatment with a gluten-free diet, with or without folic acid, for periods exceeding one year. Four were in complete clinical and haematological remission as evinced by their bowel rhythm, stool consistency, nutritional status, and blood levels. The remaining two patients had persistent but variable morning diarrhoea with some bowel urgency.

**"Blind" Series.**—Fourteen patients at another hospital formed a "blind" series. Their diagnosis was known to only one of us (R. H.), who carried out the tests in these cases and submitted the specimens to the other (S. D. M.) for assay of radioactivity and an opinion. Ten of the patients in this series were "normal" and the remaining four were cases of idiopathic steatorrhoea diagnosed according to criteria previously mentioned. All but one had been on treatment with a gluten-free diet, with or without folic acid, for periods exceeding four weeks. Two of these patients, the longest on therapy, namely six and eight months, were in complete clinical and haematological remission.

**Test Meal.**—The test meal was prepared with radioiodinated triolein† after the procedure outlined by Ruffin *et al.* (1956) with minor modifications (Fig. 1). Two emulsions of arachis oil with equal

### TEST MEAL OF <sup>131</sup>I TRIOLEIN

<i>Hot Emulsion (A)</i>	<i>Cold Emulsion (B)</i>
1 ml. <sup>131</sup> I triolein	200 ml. arachis oil
49 ,, arachis oil	200 ,, water
50 ,, water	15 ,, Tween 80
4 ,, Tween 80	

Test meal = Vol. of A with 20–25  $\mu$ c. <sup>131</sup>I  
 + Vol. of B  $\longrightarrow$   
 Final vol. of 1 ml./kg.

Fig. 1

volumes of water were prepared using commercial Tween 80 as an emulgent. One of these, emulsion "A," contained the radioiodinated triolein.

The test meal consisted of a volume of emulsion "A," containing 20–25  $\mu$ c. of <sup>131</sup>I with sufficient volume of emulsion "B" to give a final volume of 1 ml./kg. body weight, i.e., approximately 0.5 ml. arachis oil/body weight.

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†Supplied by Radiochemical Centre, Amersham.

**Procedure.**—Lugol's iodine, 10 minims t.i.d., was given by mouth for two days before the test to minimize thyroidal uptake of  $^{131}\text{I}$  which becomes split off *in vivo* after absorption of radioiodinated triolein.

After an overnight fast the patient ingested the test meal and the washings from two rinses of the container with tepid water. Residual activity in the container was extracted with petroleum ether and this was taken into consideration in the final assessment of total activity actually administered.

Venous blood samples were then withdrawn into oxalated containers at hourly intervals for six hours. Urine was collected in two 24-hour periods following the test dose. Stools were collected for 72 hours in 500 ml. waxed cardboard cartons.

Radioactivity was measured on 2 ml. and 5 ml. aliquots of whole blood and urine, respectively, in a well-type scintillation counter. Suitable dilutions of stock standard solutions, prepared simultaneously with the test meals, were also assayed. Results were expressed as percentage of dose administered per litre of blood and percentage of dose excreted in each 24-hour and cumulative 48-hour period from the test meal.

Stool collections were assayed for radioactivity by placing the cartons in a large well cut out in a lead shield placed over a thallium activated sodium iodide scintillation crystal. Suitable aliquots of the stock standard solutions were assayed by suspension in a 10% gelatin solution in similar cartons. Activity in stools was expressed as percentage of dose administered.

### Results

**Blood Tests.**—The observed levels of radioactivity in the 32 "normal" patients with the relevant statistical ranges are shown in Table I and Fig. 2. Results of duplicate blood tests on two patients, shown in Fig. 3, attest to the reproducibility of the characteristic pattern of blood radioactivity in the individual cases.

TABLE I

BLOOD RADIOACTIVITY AS PERCENTAGE OF DOSE PER LITRE OF RADIOIODINATED TRIOLEIN IN 32 NORMAL CONTROLS

	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours	6 Hours
$\bar{x}$	0.74	1.60	2.46	2.84	2.77	2.71
$\sigma$	$\pm 0.4$	$\pm 0.54$	$\pm 0.65$	$\pm 0.70$	$\pm 0.75$	$\pm 0.72$
$\bar{x} \pm \sigma$	0.34–	1.06–	1.81–	2.14–	2.02–	1.99–
	1.14	2.14	3.11	3.54	3.52	3.43
$\bar{x} \pm 2\sigma$	0–	0.52–	1.16–	1.44–	1.27–	1.27–
	1.54	2.68	3.76	4.24	4.37	4.15

$\bar{x}$  = Mean.  $\sigma$  = Standard deviation.

In Fig. 4 the levels of radioactivity in the six control cases of steatorrhoea are superimposed on the graph of mean levels and one standard deviation range observed in the "normal" control patients. It is apparent that two curves in this

group are indistinguishable from those of the "normal" controls.

Fig. 5 shows the blood radioactivity in four cases of steatorrhoea in the "blind" series. The remaining 10 "normal" patients in this series showed curves indistinguishable from those of the normal control subjects and are therefore not shown in the figure.

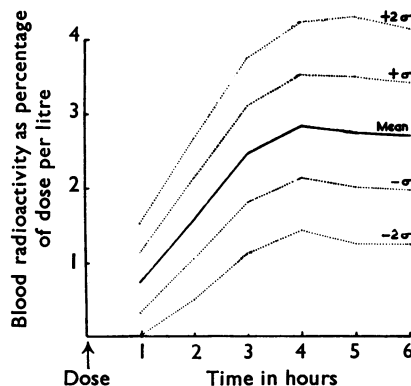


FIG. 2.—Radiiodinated triolein test in 32 "normal" controls.

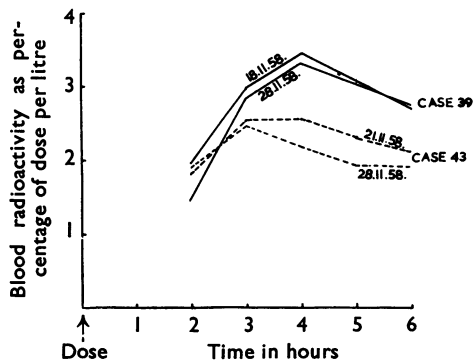


FIG. 3.—Duplicate tests in two patients showing reproducibility of blood curves.

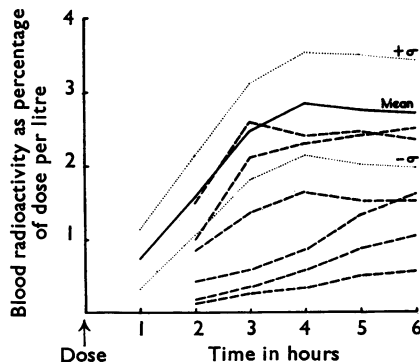


FIG. 4.—Radiiodinated triolein test in six controls with steatorrhoea

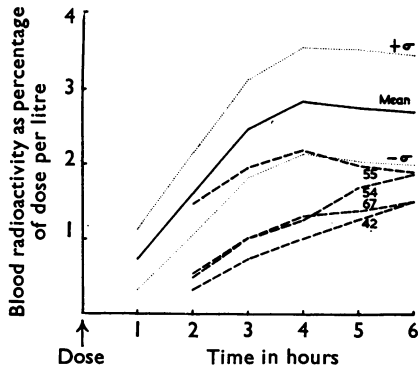


FIG. 5.—Radioiodinated triolein test in the “blind” series of four cases of steatorrhoea.

Three of the patients with steatorrhoea can be readily identified, but the fourth case with levels of radioactivity similar to those of the “normal” control patients was missed on this analysis.

**Urine Tests.**—Table II shows the levels of radioactivity in the individual 24- and cumulative 48-hour urine collections with the relevant statistical ranges as observed in 24 of the “normal” control patients in whom the urinary radioactivity was studied.

TABLE II  
URINARY EXCRETION OF RADIOACTIVITY AS PERCENTAGE OF DOSE OF RADIOIODINATED TRIOLEIN IN 24 NORMAL CONTROLS

Time (hr.)	Mean	Standard Deviation (σ)	Mean ± σ	Mean ± 2σ
0-24 (16 cases)	44.4	± 12.37	32.03-56.77	19.66-69.14
24-48 (16 cases)	16.5	± 6.3	10.2-22.8	3.9-29.1
0-48 (24 cases)	55.3	± 12.9	42.4-68.2	29.5-81.1

Tables III and IV show the excretory pattern in the six control patients with steatorrhoea and all the 14 patients in the “blind” series, respectively.

TABLE III  
URINARY EXCRETION OF RADIOACTIVITY AS PERCENTAGE OF DOSE OF RADIOIODINATED TRIOLEIN IN CONTROLS WITH STEATORRHOEA

Patient	0-24 hr.	24-48 hr.	0-48 hr.
22	47.5	16.9	64.4
24	o+ 14.2 o++	15.9	o+ 30.1
26	—	—	—
32	—	—	o+ 26.0 o++
37	34.8	o+ 8.0	42.8
60	o+ 9.69 o++	o+ 0.55 o++	o+ 10.24 o++

o+ Abnormal at 1 S.D. Range of normal excretion.  
o++ Abnormal at 2 S.D. Range of normal excretion.

TABLE IV  
URINARY EXCRETION OF RADIOACTIVITY AS PERCENTAGE OF DOSE OF RADIOIODINATED TRIOLEIN IN “BLIND” SERIES

Patient	0-24 hr.	24-48 hr.	0-48 hr.
42	o+ 22.4	10.4	o+ 32.8
43	40.2	11.25	51.45
44	o+ 29.4	17.8	47.2
45	36.2	17.9	54.1
46	58.2	19.05	77.25
53	47.4	22.9	70.3
54	47.2	16.3	63.5
55	66.0	18.8	84.8
57	—	—	—
61	—	—	—
63	—	—	—
64	65.2	20.9	86.1
65	68.4	o+ 4.14	72.54
67	45.5	12.2	57.7

o+ = Abnormal at 1 S.D. range of normal excretion.  
o++ = Abnormal at 2 S.D. range of normal excretion (none).  
Numbers set in bold type show patients with steatorrhoea.

Those considered abnormal at the various statistical levels of accuracy are delineated in the tables and it is apparent that the urine tests form an even less satisfactory index than the blood tests for distinguishing the abnormal patients.

**Stool Tests.**—Table V shows the results of stool radioactivity analysis in the entire series of cases. Twenty of the 32 “normal” patients on whom stool tests were successfully completed show a statistical range of excretion of 0 to 2.8%.

TABLE V  
RADIOACTIVITY AS PERCENTAGE OF DOSE IN 72 HOURS. STOOL COLLECTIONS AFTER RADIOIODINATED TRIOLEIN

Subjects	No.	Observed Values	Mean ± 3σ
Control cases:			
“Normal”	20	0.13-2.2	(0.88 ± 3 × 0.64)
Steatorrhoea	6	4.1-37.6	0-2.8
“Blind” series:			
“Normal”	10	0-2.35	(0.68 ± 3 × 0.69)
Steatorrhoea	4	44.2 5.8 0.9 4.3	0-2.75

σ = Standard deviation.

The six control patients with steatorrhoea excreted radioactivity in amounts varying from 4.1 to 37.6% of the dose, which distinguished all six patients from the normal controls.

Radioactivity in the stools of the 10 “normal” patients in the “blind” series, namely 0 to 2.75%, was comparable to that of the “normal” patients in the control series.

The excretion of radioactivity in the stools of four patients with steatorrhoea in the “blind” series is shown individually and of these three

can be separated from the "normal" controls. The fourth patient excreted radioactivity in amounts consistent with a satisfactory fat absorption. This was the same patient who was missed in the blood tests.

### Discussion

The use of  $^{131}\text{I}$  as a tag for triolein depends on the assumption that the chemical bond between the two substances is stable *in vitro* during the process of digestion and absorption. That this assumption is probably correct is suggested by the findings of several workers who have studied the metabolism of radioiodinated triolein in man and in laboratory animals (Hoffman, 1953; Shingleton, Wells, Baylin, Ruffin, and Sanders, 1955; McCandless and Zilversmit, 1955; Ruffin *et al.*, 1956; Turner, 1956; Malm, Reemtsma, and Barker, 1956; Beres, Wenger, and Kirsner, 1957; Reemtsma, Malm, and Barker, 1957; Van Handel and Zilversmit, 1957; and Duffy and Turner, 1958). We have confirmed that the major part of  $^{131}\text{I}$  is in the lipid fraction of blood in the early stages of absorption and cannot be dialysed but can be precipitated with trichloroacetic acid. Radioactivity in the precipitate is not water soluble but can be extracted with fat solvents. We have also been able to confirm that the stool radioactivity is not dialysable but can be extracted with ether. Radioiodinated triolein is stable after six hours' incubation with gastric juice, 25% HCl, and trichloroacetic acid. The emulsion used for the test meals was found to be stable when examined over a period of four weeks (unpublished data).

Variations in the amount and nature of vehicles used for the administration of radioiodinated triolein, insistence on a fasting state of the stomach before the test meal or otherwise, and the diverse criteria used in the evaluation of results manifest in the various reported investigations on the use of radioiodinated triolein, make it difficult to compare the results of any two series with confidence. The method suggested by Ruffin *et al.* (1956) for the test procedure seems to be least subject to criticism. It ensures the use of a known amount of nutrient of constant composition, namely 0.5 ml. of arachis oil per kilogram body weight, and ensures a fasting state of the stomach before and during the initial six hours of the test, i.e., the period of the blood test. The need for prior preparation of the patient with Lugol's iodine has recently been disputed by Grossman and Jordan (1958). They found that the omission of this precaution "does not introduce

any significant difference in the blood or faecal levels of radioactivity." Using such prior preparation with iodine, however, we have found no significant difference in the levels of radioactivity over the neck and thigh in three of the normal control patients examined during the six-hour period of the blood test. Malm *et al.* (1956) report no appreciable uptake by the thyroid gland in patients so prepared. The use of preliminary iodine medication seems desirable, at least to protect the thyroid from any unnecessary irradiation, however small.

The pattern of blood radioactivity observed in the "normal" control patients suggests that the blood curve is characteristic and reproducible in the same patient (Fig. 3). This is in agreement with the findings of Baylin, Sanders, Isley, Shingleton, Hymans, Johnston, and Ruffin (1955) and Beres, Wenger, and Kirsner (1957). McKenna, Bourne, and Matzko (1957) have, however, concluded otherwise, but it is suggested that their experience may have been due to the patient having breakfast before the test meal.

Of the six known cases of idiopathic steatorrhoea in the control series, only four showed results below the normal limits of minimal "absorption." The remaining two cases, even on the statistically unsatisfactory criteria of one standard deviation range of the normal levels, show curves indistinguishable from the "normal" control patients. Both these patients were in full clinical and haematological remission as, indeed, were two of the four patients with abnormal results. These results are not surprising when it is considered that the instantaneous blood radioactivity is a function of many diverse and, as yet, ill-understood factors. Thus the rate and amount of absorption, utilization, storage, and extra-vascular sequestration of fat, the completeness of the thyroid block, the renal clearance of free  $^{131}\text{I}$  split off *in vivo*, and the rate of gastric emptying would all affect the observed blood levels of radioactivity. It is of interest to note that Peters and Van Slyke (1946), reviewing the data of chemical determinations of serum lipids in the post-operative state in normal subjects, observed a wide range. They suggest that this is due, in some measure, to the inclusion in most reported series of stray maximum and minimum figures obtained from ostensibly normal persons. Similar extreme readings are to be noted in almost all adequately documented series on radioiodinated triolein.

The observed levels of blood radioactivity in the control series were also assessed by alternative

criteria suggested, such as the four-hour radioactivity levels (based on the fact that most of the normal patients show peak levels of blood radioactivity at this hour), and the average of four-, five-, and six-hour levels of radioactivity (Baylin *et al.*, 1955; Ruffin *et al.*, 1956; Shingleton *et al.*, 1957a and b; Berkowitz and Sklaroff, 1957; and Kaplan, Edidin, Fruin, and Baker, 1958). In addition, as seven of our normal control subjects had peak levels of radioactivity at three hours, five at five hours, and three as late as six hours, assessment was also made in terms of peak levels of radioactivity alone. Results of these alternative assessments, however, showed no significant improvement in identification of the abnormal cases on the method described. Findings in three normal subjects of peak levels of blood radioactivity as late as the sixth hour suggested that the blood radioactivity should be studied for more prolonged periods. A small number of patients were studied for seven to eight hours, but this was not found to give any additional information of value.

Radioactivity in the urine collections was almost completely accounted for by inorganic  $^{131}\text{I}$ , which was totally extractable by a Cl phase ion exchange resin (amberlite resin, IRA-400 (Cl), B.D.H.). The 24- and 48-hour urinary radioactivity is, therefore, even less satisfactory than the blood tests as it represents the end-result of the various processes determining the blood radioactivity as described above.

In the assessment of stool radioactivity, it was feared that errors might be introduced in the results through contamination of the stools with significant amounts of radioactivity in the urine, a contingency difficult to avoid with certainty, especially in the female patients. The more stringent levels of statistical accuracy of three standard deviation ranges were therefore adopted. With these criteria, all six patients with idiopathic steatorrhoea were readily differentiated from the "normal" control patients. It was also noted that all six patients excreted radioactivity in amounts greater than 4% of the dose. For added safety, the 4% figure was accepted as an arbitrary upper limit of normal and it was decided to regard any results between 2.8 and 4% as possibly due to urinary contamination.

The results of the control series, therefore, failed to confirm the claim put forward by the North Carolina group of workers that an "invariable inverse relationship exists between the radioactive material in blood and stool."

Conclusions were further tested in a group of 14 "blind" cases as described. In this series, however, both the blood and stool tests identified three of the four patients with idiopathic steatorrhoea. The probability of such differentiation obtaining by chance is 1 in 25, which renders the results of both tests highly significant in this group. It should be noted that the curves of normal limits of blood radioactivity are computed at one standard deviation range, which excludes as many as 16% of normal subjects at the lower end of the normal curve. Results of the blood tests are, therefore, inferior to the stool tests as concluded in the control series. The similarity in the range of faecal radioactivity in the 10 "normal" patients in the blind series and 30 similar patients in the control series would seem to suggest that the dangers of cross-contamination with urinary radioactivity can be over-emphasized when proper precautions have been taken to eliminate, or at any rate to minimize, this hazard.

The results in the fourth patient with idiopathic steatorrhoea in the "blind" series are indistinguishable from those of "normal" control subjects on either test. This patient was in complete clinical and haematological remission, as indeed were two of the remaining three who gave abnormal results. A final reassessment of fat absorption with chemical balance studies in the one patient with anomalous results was unfortunately not possible as the patient was unwilling to re-enter hospital. The exact state of fat absorption in this patient must, therefore, remain in doubt. It is of interest to note, however, that her urinary excretion of 85% of the dose over the 48-hour period was the second highest result recorded in the entire series.

Within the limits of this trial, it is concluded that the stool test with radioiodinated triolein may be of value in establishing the diagnosis of steatorrhoea. (Lubran and Pearson (1958), using a similar technique, came to the same conclusion regarding its usefulness as a screening test in this condition.) Finding low curves of blood radioactivity would suggest steatorrhoea, but normal levels do not exclude the presence of steatorrhoea. These findings are in agreement with those reported by McKenna *et al.* (1957) and Grossman and Jordan (1958). Urinary radioactivity does not provide a satisfactory diagnostic index and may in fact introduce error in the stool tests if rigid precautions to avoid cross-contamination of stools with urine are not observed.

### Summary

Results of blood, urine, and stool tests with radioiodinated triolein in a control series of 32 "normal" and six known cases of idiopathic steatorrhoea under therapy are reported.

Comparable studies in a "blind" series of 10 "normal" patients and four patients with idiopathic steatorrhoea (three treated) are described.

Of the 10 cases of steatorrhoea in the series, the blood tests identified seven as abnormal (one untreated, six treated), whereas the stool tests differentiated nine cases (one untreated, eight treated). One treated case in the "blind" series, in full clinical remission, was missed on both tests, but excreted radioactivity in urine in amounts at the upper limits of the normal range. Results of urine tests were much less satisfactory.

Within the limits of this trial it is concluded that the stool tests with radioiodinated triolein may be of value in establishing the diagnosis of steatorrhoea. Finding low levels of blood radioactivity would suggest steatorrhoea (within the statistical limitations described), but normal levels do not exclude the presence of steatorrhoea. Analysis of urine for radioactivity is much less satisfactory.

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