RADIOTRIOLEIN REVISITED: A STUDY OF THE ^{131}I-TRIOLEIN ABSORPTION TEST USING RADIOCHEMICALLY PURE TRIOLEIN IN MAN

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In 1949 Stanley and Thannhauser introduced radioiodinated olive oil to study fat absorption (*1*). Subsequently, a number of reports supported the use of the radioiodinated triolein (RITO) absorption test in the detection of fat malabsorption (*2–9*). However, these radioactivity results were not correlated with simultaneous chemical fat balance studies. When such correlations were carefully done, it became apparent that the ^{131}I-triolein absorption test results were often misleading (*10–16*). Whole-blood or plasma radioactivity levels within the normal range have been reported in from 42 to 71% of subjects with steatorrhea (*10,11,16*). Fecal radioactivity excretion has in general correlated better with fat balance studies, but this correlation is especially poor in subjects with only moderate steatorrhea (*15,17*).

In 1960 Lakshminarayana showed that commercial ^{131}I-triolein was quite impure, with 46–80% of the ^{131}I label on nontriglyceride fractions such as monoglycerides, diglycerides, and monohydric alcohol esters (*18*). Subsequently, Tuna (*19*) and later Kennedy (*20*) demonstrated that only 30–60% of the label was on triglycerides.

Tuna, et al also made radiochemically purified ^{131}I-triolein and showed that four subjects with chemical steatorrhea but with normal blood radioactivity levels using commercial ^{131}I-triolein had clearly abnormal blood radioactivity levels using purified radiotriolein (*19*). These results suggested that the impurities present in ^{131}I-triolein might be responsible for the inaccuracy of this absorption test. To date, however, no further study of radiochemically pure ^{131}I-triolein in man has been reported.

The purpose of this study was to evaluate the triolein absorption test with radiochemically purified ^{131}I-triolein (PRITO) simultaneously with chemical fat balance studies in normal subjects and in patients with steatorrhea.

METHODS

Commercial ^{131}I-triolein, 3–5 mCi/ml, was obtained from Abbott Laboratories and was stored in redistilled hexane under nitrogen at 4°C. Aliquots were spotted on thin-layered silicic acid plates* and developed with 90/12/1 (vol/vol) hexane/diethyl ether/acetic acid. Lipid fractions were then identified by iodine vapor or H_2SO_4 charring and compared with neutral lipid standard mixtures†. Autoradiographs were made by exposure of the plate to standard x-ray film. The distribution of radioactivity on each chromatographic strip was quantitated by counting the areas identified by the autoradiograph in a sodium iodide scintillation crystal.

To obtain radiochemically pure radiotriolein (PRITO), aliquots of the hexane-^{131}I-triolein solution containing approximately 400 mg triolein and up to 1 mCi radioiodine were reduced under nitrogen to volumes of 3–5 ml and applied to a 50-gm silicic acid‡ column. The triglyceride fraction was eluted with 5% diethyl ether in redistilled hexane (*21*) with a flow rate of approximately 3 ml/min. Collection of appropriate radiolabeled fractions was monitored by a collimated detector at the effluent end of the column. The collected fractions were evaporated to dryness under nitrogen, dissolved in 10 ml of redistilled hexane, and rechromatographed to check for radiochemical purity. Subsequently each flask was stored under nitrogen at 4°C, and radiochemical purity was determined weekly.

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* Brinkmann precoated chromatoplates, Brinkmann Instruments Inc., Westbury, N.Y.

† TLC Standard Mix 1, Hormel Institute, Austin, Minn.

‡ 100-mesh, Mallinckrodt Chemical Works, St. Louis, Mo.

Forty-four subjects were studied, 42 while on an 80-gm fat diet for 5 days, and two while on an 100-gm fat diet. Three to five drops of Lugol's solution were administered daily. On the morning of the third day, following an overnight fast, each subject ingested the purified ^{131}I-triolein in a liquid test meal (*22–24*). To make this meal, 70 gm of skimmed milk powder, 39 gm of dextrose, 1 gm of vanilla, and 210 cc of water were initially blended in a Waring blender. An aliquot of hexane containing 15–25 μCi of PRITO was evaporated under nitrogen in a flask, and 30 gm of fresh corn oil (Mazola) were added to this flask. After mixing, this radioactive lipid-oil mixture was added to the blender, and the contents were blended at low speed for 1 min. The test meal thus contained 75 gm of carbohydrate, 25 gm of protein, 30 gm of fat, and 15–25 μCi PRITO in a total volume of 325 cc. Meal radioactivity was counted in a plastic container and then ingested by the patient over a 5–10-min period. Immediately afterwards, the residue was washed from the sides of the cup with 60 cc of water and was also drunk by the patient. Following completion of ingestion, 325 cc of water were added to the plastic cup and the radioactivity present was compared with the initial count. In all cases more than 98% of the initial radioactivity in the cup was ingested by the patient.

Beginning 2 hr after ingestion, whole-blood samples were obtained hourly. Aliquots of these samples were counted immediately after each venepuncture, and blood collection was continued until the increasing blood radioactivity concentration leveled off or actually fell on two successive hourly determinations. Blood was thus collected for at least 6 hr in all subjects and in some for 7 hr. From each sample, 2 ml of whole blood and plasma were removed and counted in a sodium iodide well counter and compared to a ^{131}I standard. In addition, lipids were extracted from 2-ml plasma (*25*), redissolved in hexane, and counted similarly. Results were expressed as the percent administered dose per liter whole blood or plasma and as the percent administered dose which was lipid soluble per liter of plasma. The maximal (peak) concentration of radioactivity obtained was then used for correlation with chemical fecal fat results.

Urine was collected for the first 24 hr after isotope ingestion and compared to an ^{131}I standard. Results were expressed as the percent of the administered dose excreted per 24-hr urine.

Following completion of the blood sampling, the patient resumed his 80-gm fat diet, and 72-hr stools were carefully collected free of urine in weighed gallon paint cans. A stool diary was kept on each patient to insure 72-hr collections. If the first stool on the morning of the test occurred after ingestion of the isotope, this stool was collected separately, checked for radioactivity, and the 72-hr collection was then begun. In several cases, bowel evacuation was not possible at the termination of the 72-hr period, and diet and stool collections were continued for another day.

The stool cans were weighed; ethanol and water were added to a total dilution of 2,000 or 3,000 cc (*26*), homogenized on a paint can shaker, and counted and compared to ^{131}I standards diluted with water to similar geometry. Fecal radioactivity was expressed as the percent administered dose per 72-hr stool. After counting, chemical fat determinations were performed on weighed aliquots of these same 72-hr collections by the Jover and Gordon modification (*26*) of the method of van de Kamer (*27*). All determinations were done in duplicate by the same two research technicians. More than 92% of measured amounts of triglyceride (Mazola oil) added to several control 3-day fecal collections were recovered by this method. Stool fat was expressed as grams fat "excreted" per day and as the coefficient of fat absorption:

$$\text{Coefficient of fat absorption (\%)} = \frac{\text{gm fat ingested} - \text{gm fat "excreted"}}{\text{gm fat ingested}} \times 100.$$

RESULTS

Purified ^{131}I-triolein. Staining of the developed chromato-plates containing the commercial ^{131}I-triolein compared to a standard lipid mixture showed that the principal lipid present in the Abbott preparation was triglyceride (Fig. 1) with a faint spot of free fatty acid and several unknown spots closer to the origin. Autoradiography disclosed the presence of numerous radiochemical impurities. The principal radioactive spot coincided with the tail of the chemical triglyceride and was shown to be a triglyceride by elution from a silicic acid chromato-plate and determination of the fatty acid/glycerol molar ratio after alkaline hydrolysis. Two radioactive spots were always seen immediately after this principal radioactive triglyceride spot and were thought to be the result of different degrees of iodination of the double bonds present in the oleic acid side chains (*20*). Of all the batches of commercial RITO, from 50 to 82% of the total radioactivity applied to the chromato-plate was found in this principal radioactive spot, from 18 to 39% in the two immediately following spots, and from 7 to 27% in the several spots closer to the origin (Fig. 1). No significant radioactivity remained at the origin.

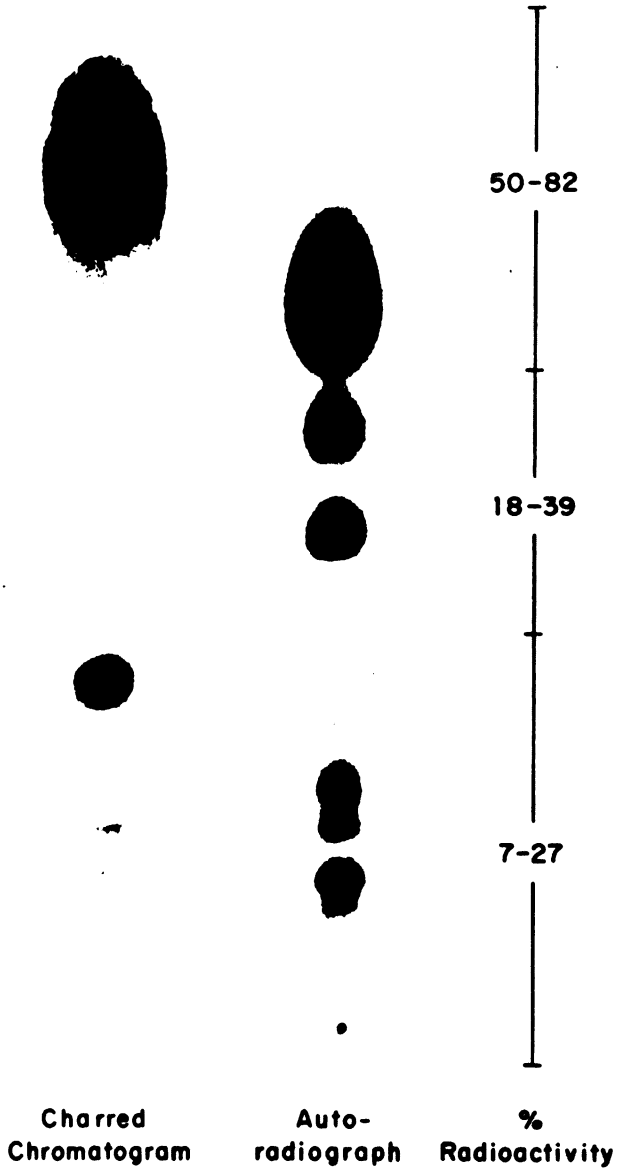

FIG. 1. Comparison of charred chromatogram with autoradiograph of commercial triolein. Developed chromato-plate drawn on left shows spots of triglyceride, fatty acid, and several unidentified spots closer to origin. Numerous radiochemical impurities may be seen on autoradiograph on right with principal radioactive spot coinciding with tail of chemical triglyceride spot. Percentages listed at far right represent ranges of percentages of total radioactivity applied to plates which were found in three areas indicated. Only material from principal radioactive spot (shown chemically to be a triglyceride) was used in study.

After column purification only fractions with more than 98% of their radioactivity in this principal radioactive spot were used subsequently for patient studies because of the lack of absolute certainty of the nature of the other labeled spots.

Storage of the purified radiotriolein for up to 4 weeks showed no significant radiochemical change.

Absorption of purified ^{131}I-triolein. The 44 subjects studied were separated into three different groups on the basis of their chemical fat balance studies (Table 1). Group I included 15 normal volunteers (12 males, three females, ages 22–35) with no history of intestinal disease and normal chemical fat absorption. Group II included ten subjects with previously documented gastrointestinal disease but who had normal chemical fat absorption when we studied them. Group III consisted of 19 patients with chemical fat malabsorption (13 males, six females) of various etiologies (Table 1).

The carbohydrate-rich test meal was well tolerated by the majority of the subjects studied. One patient with a vagotomy and pyloroplasty had symptoms of dumping. A patient with cirrhosis, who had been slightly nauseated prior to the study, vomited 1 hr after ingestion of the test meal, but the radioactive emesis was counted and the study was completed. Two other subjects (one from Group I and one from Group II) complained of transient postprandial epigastric discomfort.

The individual results of this study are given in Table 1. The coefficient of fat absorption in the Group I subjects ranged from 94.2 to 98.7% with a mean of 97.6%. Peak whole-blood radioactivity levels in this normal group are shown in Fig. 2. Mean peak whole-blood radioactivity was 2.8% of the administered dose per liter whole blood, and the 2-standard deviation lower limit was 1.9% dose/liter. Three-day fecal radioactivity measurements in this group ranged from 5.1 to 0.4% of the administered dose (Fig. 3), indicating an absorption of from 94.9 to 99.6% of the radioactive label. Since fecal radioactivity levels were not normally distributed*, calculation of the standard deviations were of no value, and the "lower limit of normal" absorption of radioactive label was 94.9% of the administered dose.

The coefficient of fat absorption in the 19 abnormal subjects (Group III) ranged from 27 to 90% (Table 1). Peak whole-blood radioactivity levels of two subjects (11%) in this group fell within normal limits (Fig. 2). One (4%) of these abnormal subjects absorbed 98.9% of the radioacitve label, but all others absorbed less than 93% (Fig. 3).

The coefficient of fat absorption in the ten subjects in Group II ranged from 93.5 to 97.3% (Table 1). The peak whole-blood level in one (10%) of these ten subjects was clearly below the normal range (Fig. 2), but his absorption of radioactive label by fecal determination was normal. Three (30%) of the ten had excessive fecal radioactivity, absorbing less than 92% of the radioactive label

* Rankit Test. Bliss CI: *Statistics in Biology*. vol I, New York, McGraw-Hill, 1967

(Fig. 3 and Table 1). In each of these three cases, however, whole-blood radioactivity levels were within the normal range.

Results obtained using the combination of peak whole-blood and total fecal radioactivity are given in Fig. 4. When both blood and fecal radioactivity levels were normal, steatorrhea was never seen. When both of these parameters were abnormal, steatorrhea was always present. When one or the other of these parameters were abnormal, the patient either had steatorrhea or a gastrointestinal disease in remission at the time of this study.

To determine whether the number of blood specimens taken could be reduced, whole-blood radioactivity concentrations at the fifth and at the sixth hours were evaluated separately. Using the 6-hr

TABLE 1. SUMMARY OF CHEMICAL AND RADIOACTIVE RESULTS OF 44 SUBJECTS STUDIED WITH PURIFIED RADIOTRIOLEIN

Patient No.	Diagnosis	CFA*	Radioactivity: % label absorbed	Peak WB† (% dose/liter)	Peak plasma (% dose/liter)	Peak lipid (% dose/liter)	24-hr urine (% dose/liter)
Group I: Normal Volunteers							
1		97.9	97.3	3.7	5.7	3.4	—
2		98.5	99.1	2.9	4.1	1.5	—
3		95.6	94.9	2.9	4.2	2.2	—
4		97.8	98.5	3.3	5.4	2.9	—
5		94.2	99.6	2.4	3.4	1.1	—
6		98.4	98.4	2.4	3.2	1.0	—
7		97.8	98.6	2.3	3.0	0.7	55
8		98.0	99.4	2.6	5.5	1.4	55
9		97.1	98.8	3.6	4.7	1.4	61
10		97.9	99.5	2.7	3.5	1.1	—
11		98.2	98.9	3.2	4.0	0.9	59
12		97.9	98.9	2.1	2.7	0.5	68
13		98.6	99.6	2.6	3.6	1.1	63
14		98.7	97.9	2.7	3.8	1.6	66
15		97.6	97.0	2.7	3.0	1.2	44
Group II: Disease in Remission							
16	Pancreatitis	97.3	97.7	4.1	7.4	3.7	—
17	Celiac sprue	95.0	97.1	2.1	2.9	0.8	71
18	Celiac sprue	96.6	98.2	3.1	4.3	2.0	—
19	Celiac sprue	96.3	96.1	2.8	3.8	1.4	—
20	Celiac sprue	96.7	98.6	1.0	1.2	—	—
21	Celiac sprue	93.5	79.9	2.9	3.6	1.5	55
22	Celiac sprue	96.7	96.9	2.8	3.8	1.8	48
23	Celiac sprue	95.3	91.3	2.1	3.2	2.0	62
24	Celiac sprue	95.4	96.1	2.3	2.8	1.1	28
25	Short bowel	94.7	91.7	2.2	2.9	1.3	43
Group III: Steatorrhea							
26	Pancreatic insufficiency	27	62	0.5	0.6	0.3	—
27	Collagenous sprue	39	0	0.1	0.1	0.1	—
28	Cirrhosis	85	24	0.6	0.8	0.3	—
29	Pancreatic insufficiency	90	76	1.6	2.4	1.1	—
30	Celiac sprue	80	91.8	0.5	0.7	0.2	—
31	Short bowel	42	11	0.2	0.2	0.2	—
32	Celiac sprue	57	52	0.2	0.2	0.1	26
33	Blind loop	81	53	2.1	3.2	1.6	—
34	Non tropical sprue	90	33	0.5	0.7	0.5	16
35	Celiac sprue	56	76	0.9	1.0	0.6	35
36	Pancreatic insufficiency	86	92.7	1.6	2.4	1.0	54
37	Giardiasis & hypogammaglobulinemia	88	85.5	1.5	1.7	0.6	56
38	Pancreatic insufficiency	82	98.9	1.1	1.6	0.6	24
39	Dermatitis herpetiformis	73	89.2	1.3	1.5	0.4	48
40	Ileo-colonic shunt	40	14.3	0.1	0.2	—	2
41	Pancreatic insufficiency	54	58.2	1.4	1.9	0.9	33
42	Non tropical sprue	89	80.4	2.2	2.9	1.0	53
43	Pancreatic insufficiency	67	85	1.4	1.8	0.6	34
44	Pancreatic insufficiency	48	47	1.4	1.9	1.1	23

* Coefficient of fat absorption = % of daily fat intake absorbed.
† Whole blood.

radioactivity values alone gave essentially the same results as with the overall peak values, except that one (7%) of the 15 normal subjects in Group I would have fallen below the normal range. Using the single 5-hr radioactivity values alone, considerable overlap was seen between these three groups.

Results obtained using peak plasma radioactivity levels were similar to those using peak whole-blood radioactivity levels. Plasma always contained the highest concentration of radioactivity. The percent of total plasma activity which was lipid soluble was extremely variable, ranging from 8 to 100% (mean 40%). There were no significant differences in the percent of plasma activity which was lipid bound between these three groups.

Peak lipid radioactivity levels and 24-hr urinary radioactivity determinations were of no value in separating Groups I and III because of the marked overlap between these two groups (Table 1).

DISCUSSION

There are at least three major problems inherent in the radioiodinated triolein absorption test. First, variable gastric emptying may result in equally variable blood radioactivity levels (*2*).

Second, the process of iodinization of triolein may produce an abnormal fat molecule, the extent of the abnormality depending on how many of the three double bonds are labeled. Such a chemically abnormal triglyceride might behave abnormally during any or all of the phases of the digestive process. Iodinated 2-mono-oleate or oleic acid might be less soluble in the micellar phase than the ordinary "parent" compounds. Further, iodinated fatty acids and monoglycerides might cross the intestinal mucous membrane less well. Three of our ten subjects with intestinal disease (Group II) but with normal chemical fat absorption on an 80-gm fat diet absorbed less radioactive label than normal. Perhaps any abnormal behavior of iodinated triolein might be magnified in the setting of minor abnormalities of the luminal and/or mucosal phases of fat absorption.

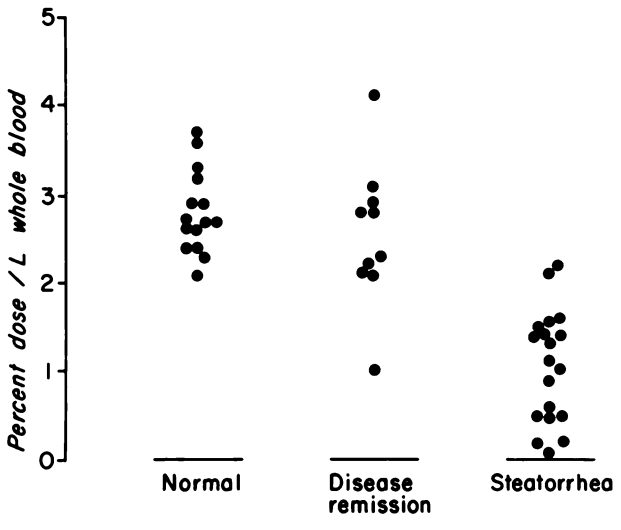

FIG. 2. Peak whole-blood radioactivity levels in 44 subjects studied. Mean peak whole-blood radioactivity in 15 normal subjects was 2.8% dose/liter whole blood, and 2-standard deviation lower limit 1.9 dose/liter.

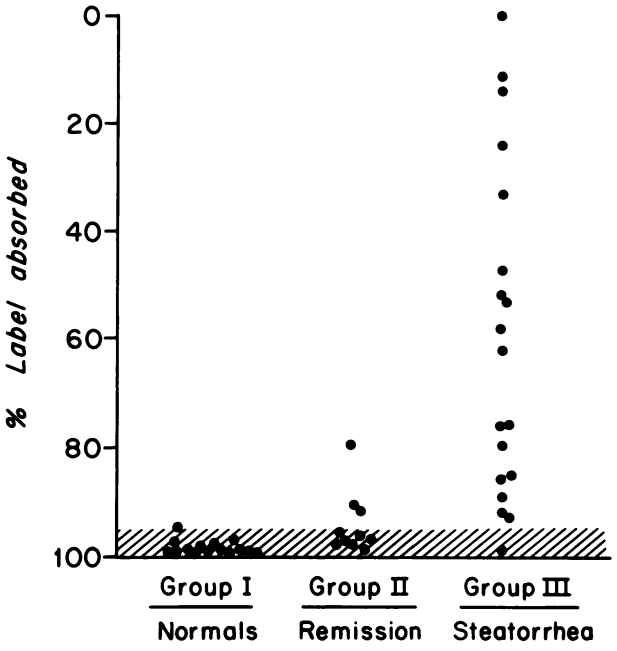

FIG. 3. Percent of total ^{131}I label absorbed as determined by 72-hr fecal radioactivity measurements after ingestion of radiochemically purified radiotriolein. Shaded area indicates normal range for fecal radioactivity as determined in our 15 normal volunteers.

Third, it has been clearly shown that unpurified RITO (*1,28–30*) is de-iodinated following oral ingestion. In our own study the mean maximum ^{131}I lipid radioactivity averaged 40% of the total ^{131}I plasma activity. Just where this de-iodinization occurs is less clear but possibly within the wall of the small intestine (*31*).

Despite the fact that radioiodinated triolein has chemical differences from natural fat, no attempts have been made to study the purified material in a clinical setting.

The radioactivity results in these 44 subjects correlate with chemical fat balance determinations better than do radioactivity results previously reported with unpurified triolein. Using peak blood radioactivity levels alone, falsely normal results were seen in 11% of the patients with steatorrhea. Using fecal radioactivity levels alone, only 4% of patients with steatorrhea had a falsely normal result. On the other hand, 30% of the ten subjects with gastrointestinal disease in remission without steatorrhea had exces-

sive fecal radioactivity. When peak whole-blood radioactivity levels were used together with fecal radioactivity determinations of label absorption, a more clear-cut separation of subjects with and without steatorrhea was possible. When both blood and fecal radioactivity levels were normal, steatorrhea was never present. When both these parameters were abnormal, steatorrhea was always present. Three subjects (16%) with steatorrhea had only one or the other parameter abnormal, however, and such findings would therefore necessitate a chemical fat balance. Since 40% of the ten Group II subjects also had one or the other of these two parameters abnormal, such findings in the absence of steatorrhea on an 80-gm fat diet may warrant either (A) repeat chemical fat balance with an intake of 100–120 gm fat per day, or (B) a search for intestinal disease in remission. This is especially true if it is the fecal radioactivity parameter which is abnormal.

It would appear then that radiochemical purification of ^{131}I-triolein definitely improves the accuracy of this test of fat absorption. Optimally for the routine hospital isotope laboratory, this radiochemical purification should be carried out by the commercial manufacturer. Given the availability of purified radioiodinated triolein, however, there are still significant objections to the use of this material as a screening test for steatorrhea:

1. The amount of radioactive label absorbed did not correlate well with chemical fat absorption in the 19 subjects with steatorrhea (correlation coefficient = 0.54, Fig. 5). Wide fluctuations in short-term chemical fat balance results may be seen where the coefficient of fat absorption is low (<80%) (*32*), but in the present study the correlation was poor even at coefficients of absorption between 80–90%. Thus fecal radioactivity determinations cannot be used to quantitatively assess fat malabsorption.
2. One should use both blood and fecal radioactivity determinations for optimal use of PRITO as a test for steatorrhea. Even though blood collection can be simplified by drawing a single 6-hr specimen, the unpleasant 3-day collection of feces cannot be obviated since use of blood specimens alone did not provide suitable accuracy.

	NB[a] NF[b]	AB[c] NF	NB AF[d]	AB AF	Total
Group I Normals	15	0	0	0	15
Group II Remission	6	1	3	0	10
Group III Steatorrhea	0	1	2	16	19
Total	21	2	5	16	44

FIG. 4. Use of combination of whole blood and fecal radioactivity levels in diagnosis of steatorrhea. a = normal peak blood radioactivity levels; b = normal total fecal radioactivity (i.e., absorption of label); c = abnormal peak blood radioactivity (below normal range); and d = abnormal total fecal radioactivity.

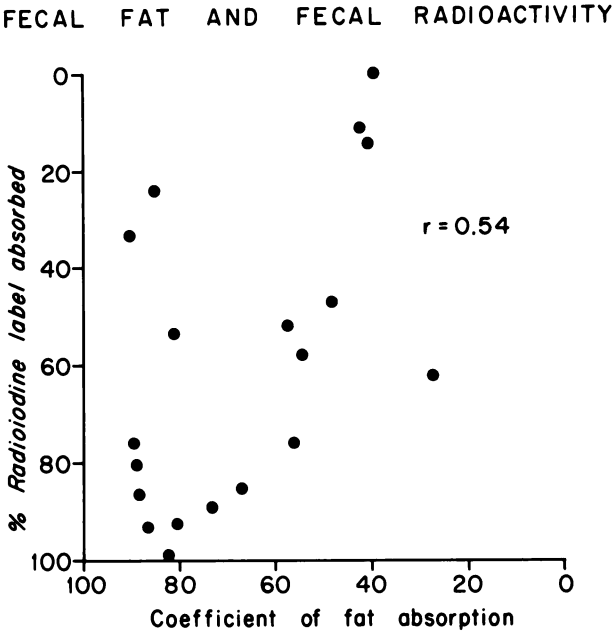

FIG. 5. Correlation of chemical fat absorption and absorption of purified radiolabel in 19 subjects with steatorrhea.

SUMMARY

Results of the ^{131}I-triolein absorption test correlate poorly with chemical fecal fat balances. It has been shown that numerous radiochemical impurities are present in radioiodinated triolein. To test the hypothesis that these impurities are responsible for this poor correlation, commercial radioiodinated triolein was purified to greater than 98% radiochemical purity and fed to 44 subjects during 3-day chemical fat-balance studies. These subjects included 15 normal volunteers (Group I), ten subjects with intestinal disease but without chemical fat malabsorption (Group II), and 19 subjects with steatorrhea of various etiologies (Group III). Whole-blood, plasma, plasma lipid, urine, and fecal radioactivities were measured after ingestion of the labeled test meal. Results obtained with radiochemically purified ^{131}I-triolein (PRITO) were better than previously reported results using unpurified radioiodinated

triolein (RITO). Blood radioactivity levels within the normal range were seen in 11% of patients with steatorrhea, but normal fecal radioactivity levels were found in only 4% of this same group. The 15 normal volunteers had both normal peak levels of whole blood radioactivity and normal fecal radioactivity. Sixteen of the 19 subjects with malabsorption had both excessive fecal radioactivity and low levels of whole blood radioactivity. Thus when both parameters were normal, chemical fat absorption was normal; when both parameters were abnormal, steatorrhea was present. The remaining three subjects in Group III and four subjects in Group II had one or the other of these two parameters abnormal. Whole-blood and plasma radioactivity determinations gave identical results; plasma lipid and urinary radioactivity levels were of no value because of the wide overlap between groups. There was no correlation between the amount of ^{131}I and the amount of chemical fat excreted in the stool. It is concluded that the radiochemical purification of radioiodinated triolein improves the accuracy of this absorption test of fat malabsorption. However, the inability to quantitate fat malabsorption and the need for a 3-day collection of stool limit the usefulness of this absorption test in screening patients for suspected steatorrhea.

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