JOURNAL OF NUCLEAR MEDICINE 6:210-219, 1965

Labeled Plasma Lipids After Ingestion of Radioactive Fats[1,2]

J. C. Sisson[3]

Ann Arbor, Michigan

Fat tolerance tests using triolein-^{131}I as an isotopic tracer for ingested lipid have been used to demonstrate gastrointestinal malabsorption (1) and abnormal lipid transport in patients with myocardial infarcts (2-4) and diabetes mellitus (5). In these tests, most of the plasma ^{131}I-lipid appeared to be in the triglyceride fraction of the plasma lipids (6) and in the chylomicron and very low density divisions of the plasma lipoproteins (7).

The work reported here has extended the investigations of incorporation of ingested labeled-fat into the individual fractions of plasma lipids and plasma lipoproteins. In selected subjects, the plasma levels of lipid-^{131}I were compared with those of lipid-^{14}C attained after the simultaneous administration of triolein-^{131}I and tripalmitin-carboxy-^{14}C or tristearin-carboxy-^{14}C.

MATERIALS AND METHODS

Subjects: The four individuals studied were men hospitalized in the Ann Arbor Veterans Administration Hospital. No patient had evidence of gastrointestinal disease.

No attempt was made to control the diets of the subjects before investigation, but none was on a diet more radical than a modest reduction in calories. Various medications were given to the subjects for their individual illnesses. There was no interruption of these drugs, but none was receiving heparin or hypocholesterolemic agents. Oral prothrombin lowering drugs were given to some of the patients with myocardial infarcts. Cigarette smoking (in subjects JC, FO, and WW—Table I) was not curtailed.

Meal: After an overnight fast the subject was given a meal containing 80 gm powered milk (% by weight: protein 35.6 carbohydrate 52.0, fat 1.0, and water

[1]This work was supported in part by grants from the Michigan Heart Association and the Michigan Memorial Phoenix Project (No. 210).

[2]This material was presented in part at the Annual Meeting of the Midwestern Section of the Society of Nuclear Medicine (October 20, 1963).

[3]Radioisotope Service, Ann Arbor Veterans Administration Hospital and Nuclear Medicine Unit and Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan.

3.5)[1], 36.4 gm (40 ml) olive oil, and approximately 100 μc of ^{131}I triolein (Raolein, Abbott Laboratories). This meal was homogenized with 250 ml of water and three drops of lemon extract in a Waring blender, and counted with a scintillation probe at a distance of two feet. The empty blender was washed several times with small amounts of water, and the washes were also drunk by the subject. The empty blender always contained less than 2 per cent of the radioactivity upon recounting. A standard of ^{131}I triolein was pipetted with the same pipette used for the meal and dissolved in chloroform. Tripalmitin-carboxy-^{14}C was purchased from the Nuclear-Chicago Corporation and tristearin-carboxy-^{14}C was obtained from New England Nuclear Corporation. The ^{14}C lipids were dissolved in absolute ethanol at 70°C and 35 μc were pipetted into the blender just before blending. Appropriate standards were pipetted at this time and also dissolved in chloroform. None of the radioactive compounds was rechromatographed after arrival from the source.

The meal was not varied for differences in the weight of the subjects. No food except the test meal was given until after the last blood specimen.

Analysis of ^{131}I lipid: Heparinized venous blood was obtained before the meal (10 ml), and at 2, 4, 6 and 8 hour intervals (30 ml) subsequent to the test meal. Hematocrits were performed on the fasting and 8-hour specimens. The blood samples were then centrifuged at 500 x g at 0°C for 20 minutes and the plasma removed. One milliliter plasma aliquots were counted in a well-type scintillation counter.

The plasma samples were later precipitated by trichloroacetic acid by the method of Seller *et al* (3), and the precipitate containing the lipoproteins was counted.

One milliliter plasma aliquots were used for triglyceride determinations by the method of Van Handel and Zilversmit (8). After the zeolite extraction procedure, 5 ml aliquots of the filtrate were evaporated under air, redissolved in 1 ml of chloroform and counted. Very little phospholipid and free fatty acids are present in this extract, and virtually no radioactivity was found in cholesterol esters after separation on a silicic acid column (see below), so this counting represented almost solely triglyceride radioactivity.

Two milliliters of plasma were extracted for lipids by the method of Dole (9). This extract was treated by the method of Borgstrom (10) to separate the FFA from triglycerides. The triglyceride fraction was evaporated under air and counted in 1 ml of chloroform. The ultracentrifugation procedure, using a Model L Beckman Spinco ultracentrifuge with a 40.3 rotor, followed a modification of that outlined by Cornwell *et al* (11). Separate one or two ml plasma samples were layered under normal saline, density 1.005 g/ml, and centrifuged at 9500 x g for 30 minutes for chylomicron separation, and at 100,000 x g for 18 hours to separate both chylomicrons and the very low density (vld) lipoproteins. In a density of 1.063 g/ml, separation of the low density lipoproteins along with

[1]The milk was a valuable agent in the homogenization of the lipid. The total makeup of the meal was not thought unphysiologic or greatly different from previous tests (2,3).

the above two fractions was accomplished by ultracentrifugation at 100,000 x g for 18 hours. After slicing the centrifuge tube below the visible lipoprotein fraction (using care not to disturb the layered lipid fractions), the lipoproteins were emulsified with a small amount of normal saline by drawing back and fourth through a No. 26 G needle. The lipoproteins were then washed into tubes and each ml of emulsified lipid was extracted with 15 ml chloroform and 7.5 ml methanol, and this extract was then washed three times with 1 ml of water. The lipid extracts were then evaporated under air and counted in 1 ml of chloroform. Very low density lipoprotein activities were determined by subtraction of the respective chylomicron activities associated therewith. Low density lipoprotein activities were obtained by subtraction of the contributions from both of the lighter fractions from the radioactivities in the lipid separated at 1.063 g/ml. The radioactivity in each lipoprotein fraction was expressed as per cent of activity in the zeolite triglyceride fraction of the same plasma specimen. Since the plasma lipid radioactivity was nearly all present in triglycerides, the individual lipoprotein fraction radioactivites were not further analyzed.

Analysis of ^{14}C lipid: The procedures were essentially those used for the ^{131}I lipid analysis, but plasma samples were not counted as such. Before separation of the triglycerides and FFA by the method of Borgstrom (10), this extract was evaporated to dryness, taken up in petroleum ether (BP 60-70°C), and placed on silicic acid columns. Separation of the lipid into cholesterol ester, triglyceride-FFA, and phospholipid fractions was then accomplished by the method of Hirsch and Ahrens (12). The triglycerides and FFA were then separated as above. The lipid fractions were evaporated in liquid scintillation vials and dissolved in a scintillation solution.[1] Counting of ^{14}C was performed in an automatic Packard Tri-Carb Counter. Internal standards were added later to correct for quenching. The scintillation vials containing the specimens to be counted were stored in a refrigerator at 0°C for seven weeks to permit decay of ^{131}I before counting ^{14}C. Attempts were made to assess the radioactivity contribution by lipid-^{131}I by establishing a decay curve of serial counting over a one week period. This proved to be less satisfactory than by storing the scintillation vials. There appeared to be no change in radioactivity by periodic counting over several weeks that did not follow the general pattern of ^{131}I decay, nor was quenching of the internal standards of a significant degree. These findings suggest that storage had made no appreciable change in the physical characteristics, of the scintillation solution.

Calculation: Plasma specimens were counted in duplicate for at least 2000 counts each, and all others for at least 1000 counts. Blood volume was considered to be 7.2 per cent of body weight (2) and the plasma volume = blood volume × (1- hematocrit). The results of radioactivity assays was expressed as per cent of dose in the plasma volume which is said to be a better method of presenting such data than by per cent of dose per liter of blood or plasma (13).

[1](0.5% 2, 5-diphenyloxazole (PPO) and 0.03% 1, 4-bis 2-) (5-pheny-loxazolyl)-benzene (POPOP) in toluene).

TABLE I

FAT TOLERANCE TEST RESULTS

Subject	Age/ Race	Ht (in)/ Wt (lb)	Disease	Blood Glucose F	Blood Glucose 2H	Serum Cholesterol	Plasma TG mg/100 ml F	Plasma TG mg/100 ml 2	Plasma TG mg/100 ml 4	Plasma TG mg/100 ml 6	Plasma TG mg/100 ml 8	Plasma ^{131}I %/Dose 2	Plasma ^{131}I %/Dose 4	Plasma ^{131}I %/Dose 6	Plasma ^{131}I %/Dose 8	TCA ^{131}I % Plasma ^{131}I 2	TCA ^{131}I % Plasma ^{131}I 4	TCA ^{131}I % Plasma ^{131}I 6	TCA ^{131}I % Plasma ^{131}I 8
JC	61 W	67½ 115	Myocardial infarct Anemia		149	175	83	99	117	120	74	1.1	5.3	9.4	8.1	36	34	29	10
WL	65 W	68 182	Myocardial infarct	94	146	208	111	126	192	179	141	1.1	7.8	10.5	9.2	27	41	35	21
FO	55 W	65 177	Myocardial infarct	70	184	264	125	157	219	231	188	1.0	9.4	*11.8*	11.2	30	45	42	31
WW	50 W	64 128	Normal	76	105	249 244	105	171	251	198	91	3.0	10.1	*11.6*	8.1	*53*	*54*	43	16

*Italics indicates abnormally high values.

RESULTS

A. *Basic fat-^{131}I tolerance test (plasma and lipoprotein radioactivities) and triglycerides.*

Whole blood radioactivity of greater than 15 per cent (of the dose in the calculated blood volume) was considered to be abnormally high by Berkowith *et al* (14). Since some radioactivity (probably ^{131}iodide) is found in red cells (15), the corresponding upper limit of normal for radioactivity in the plasma volume will be less. After a comparison of plasma and whole blood radioactivities following a triolein-^{131}I meal in 15 patients, it was found that 11.5 per cent of the dose in the plasma volume corresponded to 15 per cent of the dose in the blood volume. Values greater than 11.5 per cent plasma radioactivity were arbitrarily considered abnormal to agree with the previous literature. Radioactivity in plasma lipoproteins precipitated by trichloroacetic acid (TCA) was considered abnormal when this value was greater than 50 per cent of the plasma radioactivity (16).

Tabulation of data is seen in Table I. Abnormalities, as defined above in plasma and TCA-^{131}I, were found in two of the subjects.

Triglyceride determinations had a standard error of $\pm$ 11 mg/100 ml. Fasting values of greater than 153 mg/100 ml (5.2 mEq/1) of plasma, and any subsequent value of greater than 256 mg/100 ml (9.0 mEq/1), were considered abnormal (17). With these criteria, none of the patients had values outside the normal range (Table I).

B. *Radioactivity in the plasma triglycerides.*

The TCA precipitate represents a nearly quantitative separation of lipoproteins from the soluble ^{131}iodide. The radioactivity in the triglycerides (TG) by the zeolite separation (Van Handel and Zilversmit (8)) constitutes an average of 90 per cent of the TCA activity (Table II). Triglyceride activity determined after the separation of free fatty acids (FFA) by the method of Borgstrom (10) correlated very closely with that of the zeolite method. Thus, the radioactivity in the TCA precipitated lipoproteins is due principally to labeled plasma triglycerides in all four subjects.

Initially it was hoped that an evaluation of the FFA would provide some clues to the defect in triglyceride transport and/or metabolism, but, unfortunately, the levels of FFA radioactivity are very low, and contamination by a small per cent of triglycerides would affect the FFA radioactivities to a great extent.

C. *Relationship to lipid-^{131}I to plasma lipoprotein fractions.*

Ultracentrifugal separation of lipoprotein fractions revealed that most of the radioactivity resided in the chylomicrons and very low density fractions as seen in Table III. Only the data obtained at four and six hours are shown, since the triglyceride and plasma radioactivity abnormalities were found principally at these times. The low levels of activity in the two hour plasma specimens

made precise counting difficult, but there appeared to be no great difference in the distribution of radioactivity in these isolated lipoproteins when compared to the samples obtained at other times. The eight hour specimens also demonstrated lipoprotein-^{131}I distribution patterns similar to the four and six hour plasmas.

The partition of radioactivity among the lipoprotein fractions was similar in all patients (Table III) when allowance was made for variations due to technique. The presence of abnormally high TCA and total plasma radioactivities was not associated with any apparent difference in the percentage of triglyceride radioactivity recovered in the individual lipoprotein moieties.

D. *The relationship of triglyceride-^{131}I to triglyceride-^{14}C.*

Table IV demonstrates that the tripalmitin-^{14}C was associated with higher radioactivity levels in plasma triglycerides than was the triolein-^{131}I which was given simultaneously. Plasma ^{14}C radioactivity was greater than that of ^{131}I in all lipid fractions studied when tripalmitin was the ^{14}C triglyceride. However, the plasma radioactivities after tristearin-^{14}C ingestion closely approximated the activity levels of the simultaneously administered triolein-^{131}I.

Separation of triglycerides from other lipid fractions by silicic acid chromatography (with subsequent removal of FFA) resulted in values similar to those obtained by the simpler zeolite extraction. Cholesterol esters after silicic acid chromatographic separation contained but minute amounts of ^{14}C radioactivity when either tripalmitin-^{14}C or tristearin-^{14}C were used. Both the zeolite and the

TABLE II

COMPARISON OF TRIGLYCERIDE-^{131}I WITH TCA-PRECIPITABLE-^{131}I

Time after ^{131}I Meal (Hrs)	2	4	6	8
$\frac{\text{Zeolite Triglyceride-}^{131}\text{I}}{\text{TCA }^{131}\text{I}} \times 100$				
In 4 subjects (Mean ± SE)	90 ± 14	97 ± 13	86 ± 11	87 ± 3

TABLE III

ULTRACENTRIFUGAL LIPOPROTEIN DATA

(As % Cf Plasma Triglyceride-^{131}I)

Time After ^{131}I Meal	4 Hours				6 Hours			
Subject	*JC*	*WL*	*FO*	*WW**	*JC*	*WL*	*FO**	*WW**
Chylomicrons	23	33	22	34	18	19	14	29
Very Low Density (D 1.005 g/ml)	37	47	63	33	37	47	—	34
Low Density (D 1.005—1.063 g/ml)	4	0	2	4	15	3	—	14

*Abnormally high plasma or TCA activity at these times.

Dole (9) extraction methods remove a large proportion of the phospholipid fraction, and the total incorporation of ^{14}C labeled fatty acids into phospholipids cannot be determined here. Little or no lipid-^{131}I should be incorporated into phospholipid according to Lakshminarayana *et al* (6), and the small differences between total lipoprotein-^{131}I activities (TCA) and the triglyceride-^{131}I activities (Table II) support this observation.

Although data are not shown, the distribution patterns were similar for lipoprotein-^{131}I and lipoprotein-^{14}C. Tripalmitin-^{14}C produced higher levels of lipoprotein radioactivity (per dose administered) than triolein-^{131}I in each lipoprotein fraction, while tristearin-^{14}C and triolein-^{131}I labeled the lipoprotein fractions with approximately equal proportions of the administered isotope.

TABLE IV

COMPARISON OF ^{131}I & ^{14}C TRIGLYCERIDE RESULTS

% Dose as Triglyceride in Plasma Space

	^{131}I	^{14}C
	W.L.	
	Tripalmitin-carboxy-^{14}C & Triolein-^{131}I	
2 hr	0.3	0.6
4 hr	2.7	4.7
6 hr	2.6	3.8
8 hr	1.6	2.5
	W.W.	
	Tripalmitin-carboxy-^{14}C & Triolein-^{131}I	
2 hr	1.3	2.6
4 hr	5.8	9.4
6 hr	4.7	7.4
8 hr	1.1	2.3
	J.C.	
	Tristearin-carboxy-^{14}C & Triolein-^{131}I	
2 hr	0.3	0.4
4 hr	1.9	1.5
6 hr	2.5	2.1
8 hr	0.7	0.6
	F.O.	
	Tristearin-carboxy-^{14}C & Triolein-^{131}I	
2 hr	0.3	0.5
4 hr	3.6	4.3
6 hr	4.2	3.9
8 hr	3.1	2.7

DISCUSSION

Ninety per cent of the labeled plasma lipid was found in the form of triglyceride after the triolein-^{131}I meal. This figure is in agreement with that of Lakshminarayana *et al* who also recorded that the diglyceride and free fatty acid fractions contained the remaining 10 per cent of the lipid ^{131}I (6).

In a previous study (7) the ^{131}I fatty acids were found principally in the chylomicrons and the very low density lipoproteins ($D<1.005$) with very little activity in the beta or low density lipoproteins ($D<1.063$). The incorporation of ^{14}C fatty acids into low density lipoproteins appeared to proceed more slowly than into the very low density lipoproteins (18). It was not surprising then to find nearly all of the lipid-^{131}I associated with the chylomicrons and the very low density lipoproteins, and low levels of activity in the low density lipoproteins of this study.

Although differences in metabolism and transport between tripalmitin and tristearin were expected, the relationship of triolein-^{131}I (actually ^{131}iodo-chloro-tristearin) to the metabolism and transport of normal triglycerides was a major consideration in this study. Labeled tripalmitin resulted in higher radioactivity levels than did the simultaneously ingested triolein-^{131}I, while tristearin-^{14}C and the triolein-^{131}I were quite comparable in radioactivity levels.

The reason for the difference between tripalmitin-^{14}C and tristearin-^{14}C plasma radioactivities when each is compared with the simultaneously administered triolein-^{131}I is not clear. Individual triglycerides may have differences in digestion and in the subsequent absorption, incorporation into the various plasma lipoproteins, and metabolism of the digested lipid components to account for higher tripalmitin-^{14}C radioactivities than comparable tristearin-^{14}C levels. Olivecrona *et al* (19) suggest that the prompt and greater incorporation of chylomicron tristearin fatty acids into phospholipids may account for a higher level of plasma tripalmitin after both triglycerides are injected as chylomicrons.

Since the fatty acids of both triolein-^{131}I and tristearin-^{14}C are saturated 18-carbon chains, the body tissues may not distinguish any difference between them, and this would account for the nearly equal proportions of these two labeled triglycerides in the plasma. However, the close association between plasma triolein-^{131}I and tristearin-^{14}C may be one of chance rather than of similar pathways, since the transport and metabolism of the two lipids may differ, while the resultant plasma triglyceride radioactivities are the same. The plasma lipid-^{131}I content is possibly affected by the heterogeneity of the commercial triolein-^{131}I (20). It is also possible that some deiodination of triglyceride-^{131}I occurs during absorption (21). Further, the incorporation of stearic-^{14}C acid, but not oleic-^{131}I acid, into plasma phospholipids may be appreciable (19, 21-23).

The general uniformity of results in the proportion of radioactivities in the lipoprotein fractions and in the relationships of the triglycerides labeled with ^{14}C to those tagged with ^{131}I suggests that the diseases in certain subjects (diabetes mellitus and atherosclerosis) played no major role in these parts of the study.

ACKNOWLEDGEMENTS

The author is indebted to Drs. P. Willis and W. Weber for help with patients, to Drs. W. H. Beierwaltes and J. Matovinovic for helpful criticism and advice, and to Mr. G. Patton and Mrs. S. Serfontein for technical aid.

REFERENCES

1. Pimparkar, B. D., Tulsky, E. G., Kalser, M. H. and Bockus, H. L.: Correlation of radioactive and chemical fecal fat determinations in various malabsorption syndromes. II. Results in idiopathic steatorrhea and diseases of the pancreas. *Am. J. Med.* **30**:927, 1961.
2. Likoff, W., Berkowitz, D., Woldow, A., Jacobs, A. G. and Sklaroff, D. M.: Radioactive fat absorption patterns: Their significance in coronary artery atherosclerosis. *Circulation* **18**:1118, 1958.
3. Seller, R. H., Brachfeld, J., Sandberg, H. and Bellet, S.: Use of I^{131}-labeled fat in the study of lipid handling in patients with coronary artery disease. *Am. J. Med.* **27**:231, 1959.
4. Brown, D. F., Heslin, A. S. and Doyle, J. T.: Postprandial lipemia in health and in ischemic heart disease: A comparison of three indexes of fat absorption and removal and their modification by systemic heparin administration. *New England J. Med.* **264**:733, 1961.
5. Sandberg, H., Min, B. S., Feinberg, L. and Bellet, S.: I^{131} triolein tolerance curves in patients with diabetes mellitus: Their similarity to those observed in myocardial infarction. *A.M.A. Arch. Int. Med.* **105**:866, 1960.
6. Lakshminarayana, G., Kruger, F. A., Cornwell, D. G. and Brown, J. B.: Chromatographic studies on the composition of commercial samples of triolein-I^{131} and oleic acid-I^{131}, and the distribution of the label in human serum lipids following oral administration of these lipids. *Arch. Biochem. Biophys.* **88**:318, 1960.
7. Kruger, F. A., Cornwell, D. G., Hamwi, G. J. and Brown, J. B.: Investigation of serum lipoprotein metabolism in man with I^{131}-labeled triolein. *Am. J. Clin. Nutrition* **8**:44, 1960.
8. Van Handel, E. and Zilversmit, D. B.: Micromethod for the direct determination of serum triglycerides. *J. Lab. Clin. Med.* **50**:152, 1957.
9. Dole, V. P., and Meinertz, H.: Microdetermination of long-chain fatty acids in plasma and tissues. *J. Biol. Chem.* **235**:2595, 1960.
10. Borgström, B.: Investigation on lipid separation methods. Separation of cholesterol esters, glycerides and free fatty acids. *Acta Physiol. Scandinav.* **25**:111, 1952.
11. Cornwell, D. G., Kruger, F. A., Hamwi, G. J. and Brown, J. B.: Studies on the characterization of human serum lipoproteins separated by ultracentrifugation in a density gradient. I. Serum lipoproteins in normal, hyperthyroid and hypercholesterolemic subjects. *Am. J. Clin. Nutrition* **9**:24, 1961.
12. Hirsch, J., and Ahrens, E. H., Jr.: The separation of complex lipide mixtures by the use fo silicic acid chromatography. *J. Biol. Chem.* **233**:311, 1958.
13. Sanders, A. P., James, J. M., Sharpe, K. W., Shingleton, W. W. and Baylin, G. J.: On the method of expressing blood levels in gastrointestinal absorption studies. *J. Nucl. Med.* **1**:285, 1960.
14. Berkowitz, D., Croll, M. W. and Likoff, W.: The effects of sitosterol, nicotinic acid and triparanol on fat tolerance. *Am. J. Clin. Nutrition* **10**:107, 1962.
15. Turner, D. A.: The absorption, transport, and deposition of fat. *Am. J. Digest Dis.* **3**:594, 1958.
16. Berkowitz, D., Sklaroff, D., Woldow, A., Jacobs, A. G. and Likoff, W.: Blood absorptive patterns of isotopically-labeled fat and fatty acid. *Ann. Int. Med.* **50**:247, 1959.
17. Brown, D. F., Heslin, A. S. and Doyle, J. T.: Fasting and postprandial serum triglyceride levels in healthy young Americans. *Am. J. Clin. Nutrition* **13**:1, 1963.

18. HAVEL, R. J.: Conversion of plasma free fatty acids into triglycerides of plasma lipoprotein fractions in man. *Metabolism* **10**:1031, 1961.
19. OLIVECRONA, T., GEORGE, E. P. AND BORGSTRÖM, B.: Chylomicron metabolism. *Fed. Proc.* **20**:928, 1961.
20. TUNA, N., MANGOLD, H. K. AND MOSSER, D. G.: Re-evaluation of the I^{131}-triolein absorption test: Analysis and purification of commercial radioiodinated triolein and clinical studies with pure preparations. *J. Lab. & Clin. Med.* **61**:620, 1963.
21. VAN HANDEL, E. AND ZILVERSMIT, D. B.: Limitation of radioiodine as a label for fat. *J. Lab. & Clin. Med.* **52**:831, 1958.
22. BLOMSTRAND, R., DAHLBACK, O. AND LINDER, E.: Asymmetric incorporation of linoleic acid-l-C^{14} and stearic acid-I-C^{14} into human lymph lecithins during fat absorption. *Proc. Soc. Exper. Biol. & Med.* **100**:768, 1959.
23. WHYTE, M., KARMEN, A. AND GOODMAN, DEW, S.: Fatty acid esterification and chylomicron formation during fat absorption: 2. Phospholipids. *J. Lipid Res.* **4**:322, 1963.

ERRATA—November, 1964.

J. G. McAfee, G. F. Fueger, H. S. Stern, H. N. Wagner, and T. Migita. Tc-99m Pertechnetate for Brain Scanning, *Journal of NUCLEAR MEDICINE*, **5**:811, November, 1964.

Page 814:

Figure 1A was printed upside down.

Page 825, second paragraph, second sentence should read:

"When Tc-99m has decayed completely, 10 mc become less than 0.5 x 10^{-4}uc Tc-99."