THE METABOLISM OF ALBUMIN-BOUND C^{14}-LABELED UNESTERIFIED FATTY ACIDS IN NORMAL HUMAN SUBJECTS

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Recent studies have indicated that the unesterified fatty acids in plasma (UFA) may represent a major form in which fat is transported to tissues for utilization as a source of energy. The evidence indicating the metabolic importance of UFA includes a close correlation between plasma UFA concentration and nutritional state (1, 2), direct evidence of removal of UFA by the heart and liver in fasting humans (2, 3), and the early participation of UFA in chylomicron metabolism (4). These studies have indicated that plasma UFA turnover must occur at a rapid rate. This fact has been established in recent studies on dogs (4–6), humans (7, 8), rabbits (9), and rats (10). The purpose of this paper is to describe the measurement of the rates of plasma turnover and oxidation of albumin-bound C^{14}-labeled unesterified fatty acids in normal human subjects. A tentative scheme for UFA metabolism in man is also presented.

METHODS

The C^{14}-carboxyl labeled acids used in these studies included palmitic and linoleic acids obtained from the Nuclear Instrument and Chemical Corporation, Chicago, Ill., and oleic acid from Tracerlab, Inc., Boston, Mass. The palmitic and linoleic acids were subjected to 25 and 50 tube counter-current distribution, respectively, using the solvent systems of Ahrens and Craig (11). With both acids, 95 to 98 per cent of the radioactivity was distributed in a single symmetrical peak. In the case of the linoleic acid, the distribution of radioactivity corresponded exactly to that of added unlabeled linoleic acid, obtained from the Hormel Institute, and determined by titration. The remaining radioactivity was present in material with partition coefficients corresponding to very polar short chain acids. The method of Howton, Davis and Nevenzel (12) used for preparation of the linoleic acid has been found to yield as much as 15 per cent trans isomers. The amount of labeled linoleic acid available was too small to permit spectroscopic analysis for such isomers. The oleic acid was analyzed by vapor phase chromatography, through the courtesy of Dr. E. H. Ahrens. The radioactivity was distributed as follows: 89.5 per cent as oleic acid, 6.5 per cent as stearic, palmitic or longer chain acids, 2.1 per cent as palmitoleic, and the remaining 1.8 per cent as 12 to 15 carbon acids. The approximate specific activities of the three acids were: palmitic, 2.5 mc. per mM; linoleic, 4.46 mc. per mM; and oleic, 0.43 mc. per mM.

The labeled acids were prepared for injection in the following manner. The acids were dissolved in aqueous ethanol containing a molar equivalent amount of K_2CO_3 and heated briefly at 100° C. to evaporate the ethanol. While still warm, the aqueous solutions were added to approximately five volumes of a 25 per cent solution of human serum albumin, obtained from the American Red Cross. The final solutions, containing less than 1 mole added fatty acids per mole albumin, were optically clear. Individual doses were placed in sterile vials, cultured and kept frozen until use.

Subjects. Twenty-one male and two female patients at the Clinical Center, ranging in age from 18 to 76 years, were utilized as "normals" for these studies. Eighteen of these patients were "normal control volunteers," admitted for research studies only, who had no evidence of organic disease. The five remaining patients had been admitted for study of unrelated disorders, and had no clinical evidence of abnormal lipid metabolism. With the exception of one patient, whose serum triglycerides were 300 mg. per cent, all the subjects had normal levels of serum total and esterified cholesterol, phospholipids and triglycerides. Turnover studies were also made in several patients with idiopathic hyperlipemia, essential hypercholesterolemia with xanthoma tendinosum, diabetic acidosis, or the nephrotic syndrome. The data obtained in the latter patients were not utilized, however, in the formulation and interpretation of the normal metabolism of UFA in the human and are referred to only when specifically stated.

The nutritional status of the subjects was varied to achieve a wide range of plasma UFA concentration at the time of the test. In general, fasting or carbohydrate-loading was employed. The fasting subjects were usually permitted no food after the evening meal, roughly 16 hours before the injection of labeled substrate. Some of the subjects, including all upon whom measurements of radioactivity in blood and expired air were extended beyond three hours, were fasted a total of 20 to 24 hours before the test, and kept without food until the completion of observations up to 24 hours later. The "carbohydrate-loaded" subjects were fed approximately 250 grams

of carbohydrate as Karo® syrup diluted with water, in divided doses beginning two hours before and continuing during the period of observations. In several instances glucose was also administered intravenously during the test period. During the first hour of observations, the subjects rested on a table. An inlying needle was placed in a brachial artery to facilitate rapid collection of blood samples. A mouthpiece connected to a low-resistance, two-way respiratory valve permitted inspiration of room air and expiration into a train of Douglas bags.

After blood was removed for determination of plasma lipids and zero time plasma UFA concentration, the labeled UFA-C^{14}-albumin solution, varying from 3 to 15 ml. in volume, was rapidly injected intravenously. The dose administered varied from 0.001 to 0.005 mc. and represented 0.005 to 0.01 mEq. added UFA. Blood samples were then taken rapidly at 2, 3, 4, 5, 6, 8 and 10 minutes and varying intervals thereafter for 60 minutes. The arterial needle was then removed and subsequent blood samples taken at longer intervals from a peripheral vein. An additional blood sample was usually removed at 10 minutes for a second determination of UFA concentration. About 10 seconds were required to remove each of the 11 ml. blood samples.

Simultaneously with the injection of the labeled substrate, the collection of expired air was begun. Routinely, air was collected for 2 minute periods for 10 minutes, then for 5 minute periods for a further 50 minutes. Subsequent 5 to 10 minute samples were collected at intervals up to 24 hours. When the observations were extended for 24 hours, the subjects remained at rest during the day and were awakened several times during the night for collection of air and blood samples. Blood samples were collected in dry syringes, transferred to tubes containing heparin, mixed thoroughly and immediately placed at 0 to 4° C. until analytical procedures could be begun.

For measurement of UFA radioactivity and concentration, the chilled blood samples were centrifuged and the plasma lyophilized and extracted according to the method previously reported (3). It was determined initially that such extracts of plasma obtained 15 minutes and longer after administration of the tracer acids contained significant radioactivity in neutral lipids. The iso-octane extracts of plasmas taken after 10 minutes were therefore extracted with 0.1 N NaOH in 50 per cent ethanol according to the procedure of Borgström (13). The aqueous extracts were then acidified, and the fatty acids re-extracted with iso-octane. Separate aliquots of the final iso-octane phase were used for titration and for counting. UFA specific activity was expressed as counts per minute per mEq. titratable acidity. Radioactivity was also determined in the UFA-free extracts. In the early (2 to 10 minute) samples, the entire iso-octane extract was assayed for radioactivity. UFA specific activity was then calculated, using the mean of the separately determined zero time and 10 minute UFA concentrations.

An aliquot of the labeled fatty acid-albumin solution was extracted in acetone-ethanol 1 : 1, and aliquots of this were taken for measurement of the total radioactivity administered.

Carbon dioxide content, radioactivity and specific activity in the expired air samples were determined by a method previously described (14).

In the preparation of the samples for assay of radioactivity, the solvent extracts were evaporated at steam bath temperature. Twenty-five mg. of unlabeled palmitic acid was added to the vials containing UFA before evaporation to minimize loss of radioactivity through sublimation. Two ml. aliquots of the amine-carbonate solutions for $C^{14}O_2$ assay were placed directly in the counting vials. Fifteen ml. of phosphor solution (400 mg. per cent diphenyloxazole in toluene) was added to each vial. Assays were made at $-10°$ C., using a Packard Tri-Carb Scintillation spectrometer. To permit direct comparison with the assays of lipid radioactivity, the radioactivity in the $C^{14}O_2$ samples was corrected for quenching through use of an internal standard. The anticipated standard error in assay of radioactivity of plasma UFA rose from less than ± 1 per cent in the samples taken during the first 10 minutes to ± 10 to 25 per cent in the samples taken at seven hours. The maximum anticipated standard error for the $C^{14}O_2$ assays was ± 10 per cent.

All data were subsequently corrected by multiplying by the factor: $\frac{\text{body weight (in Kg.)} \times 10^5}{\text{radioactivity administered (in cpm)}}$. The results so expressed therefore correspond in each subject to those which would have been obtained at a constant dose of 10^5 cpm per Kg. body weight.

RESULTS

Disappearance of UFA-C^{14} from the plasma

The early disappearance of radioactivity from the plasma UFA after injection of palmitic acid-1-C^{14} is shown in Figure 1. In this subject blood samples were obtained by collecting sequential five second samples from the arterial needle for the first three minutes after injection. The concentration of radioactivity declined exponentially from the second to the eighth minute. Intravascular mixing appeared to be complete within the first two minutes. This was consistent with the known behavior of the albumin-bound dye, T-1824, immediately following its intravenous administration (15). In most subjects, a semilogarithmic plot of the concentrations of radioactivity against time began to deviate from linearity after the fourth or fifth minute. The disappearance curve of UFA radioactivity thereafter rapidly became more complex (Figure 3). As will be discussed later, much of this complexity appeared to repre-

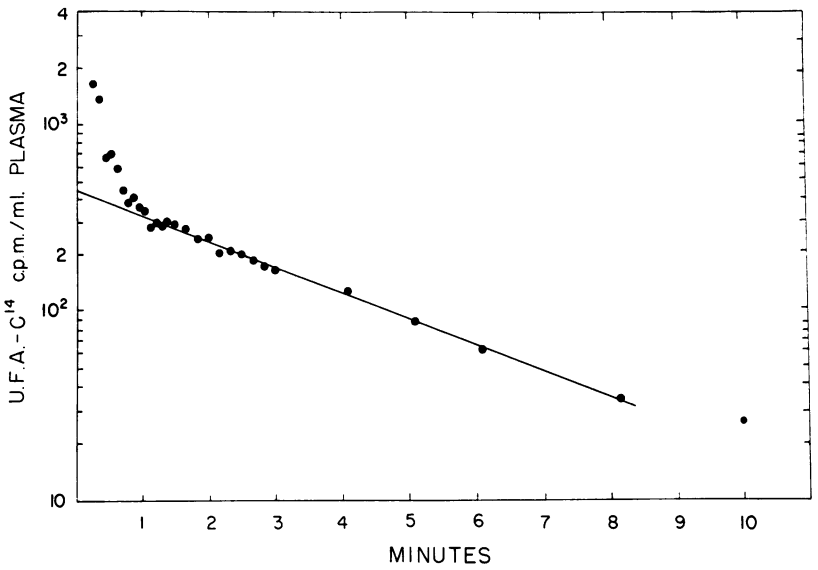

FIG. 1. THE DISAPPEARANCE OF PALMITATE-1-C^{14} FROM THE PLASMA IN A NORMAL SUBJECT FASTED FOR 42 HOURS

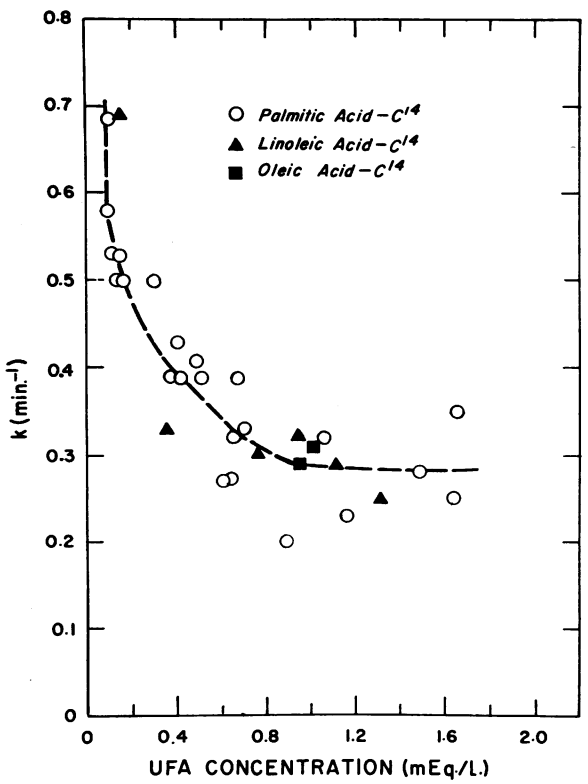

FIG. 2. COMPARISON OF THE FRACTIONAL TURNOVER RATE OF PLASMA UNESTERIFIED FATTY ACIDS (UFA) WITH PLASMA UFA CONCENTRATION

sent reflux of radioactive UFA back to the blood. The initial slope of this curve was felt to represent the most accurate measurement of the rate of disappearance of radioactive UFA from the plasma. Therefore, the straight line fitted to the semilogarithmic plot of the UFA specific radioactivity obtained at the second, third and fourth minutes was used to calculate the fractional turnover rate of plasma UFA. In 30 such determinations made in subjects whose plasma UFA concentration varied 19-fold (0.09 to 1.71 mEq. per L.), the half periods of decay ranged from 1.0 to 3.4 minutes. The relationship of the fractional turnover rate of plasma UFA to the plasma UFA concentration is shown in Figure 2. It was of interest that the rate constants calculated for the disappearance of palmitic and linoleic acids at various concentrations, and for oleic acid at the single concentration tested, were very similar. From Figure 2, it will be observed that the fraction of the plasma UFA removed per minute is approximately one-quarter of the total plasma pool at UFA concentrations greater than 1 mEq. per L. In one diabetic in moderate acidosis, not included in the data presented, the same fractional turnover rate was observed at a plasma UFA concentration of 2.09 mEq. per L. As plasma concentrations fell

below 1 mEq. per L. the fractional turnover rate appeared to be concentration dependent and increased sharply with decreasing UFA concentration. The quantity of UFA removed from the plasma per minute (flux of UFA from plasma, in mEq. per minute) was calculated by dividing the product of plasma UFA concentration and the estimated plasma volume of the subjects (taken as 3.5 per cent of body weight) by the fractional turnover rate. Fluxes so calculated varied from 0.14 to 1.50 mEq. per minute. These calculations included the assumption that the fractional turnover rates of all other unesterified fatty acids were similar to those of palmitic, oleic and linoleic acid.

The zero time mixing volume for the injected radioactive UFA was estimated from the intercept of the extrapolated two to four minute total radioactivity-time curve and the dose administered. These mixing volumes averaged 30 per cent greater than the estimated plasma volume. The discrepancy between zero time mixing volume and plasma volume was found to be of comparable magnitude in three subjects in whom plasma volume was experimentally determined by T-1824 dye dilution. The finding of a "mixing volume" greater than plasma volume was consistent with the rapid removal of UFA, since considerable loss of radioactivity would be expected to occur before mixing was complete.

The time course of plasma UFA specific activity for 22 hours after injection of labeled acids is shown in Figures 3 and 4. Figure 3 represents composite data from four subjects and Figure 4 from three subjects. The specific activity-time curves for UFA and $C^{14}O_2$ after injection of oleate-C^{14} as determined in two subjects were not significantly different from those obtained after palmitate-C^{14} (Figure 3). With these two acids,

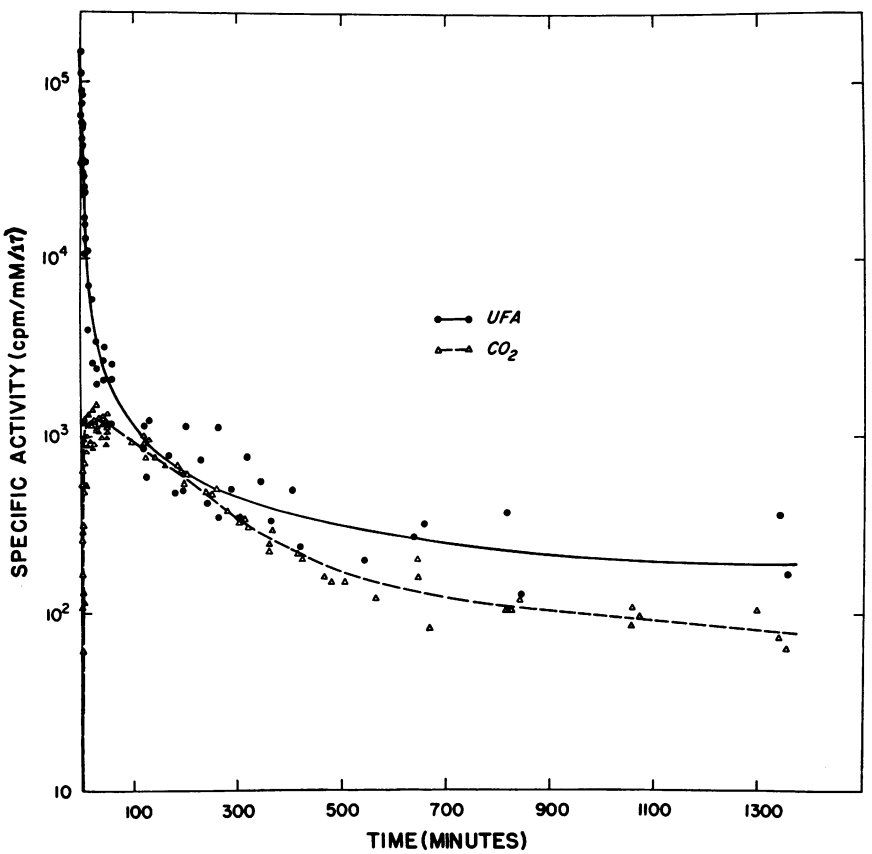

FIG. 3. SPECIFIC ACTIVITY IN PLASMA UFA AND EXPIRED CO_2 AFTER INTRAVENOUS INJECTION OF ALBUMIN-BOUND PALMITIC ACID-1-C^{14} IN FOUR FASTING SUBJECTS
The UFA specific activities only have been divided by 17 (see text).

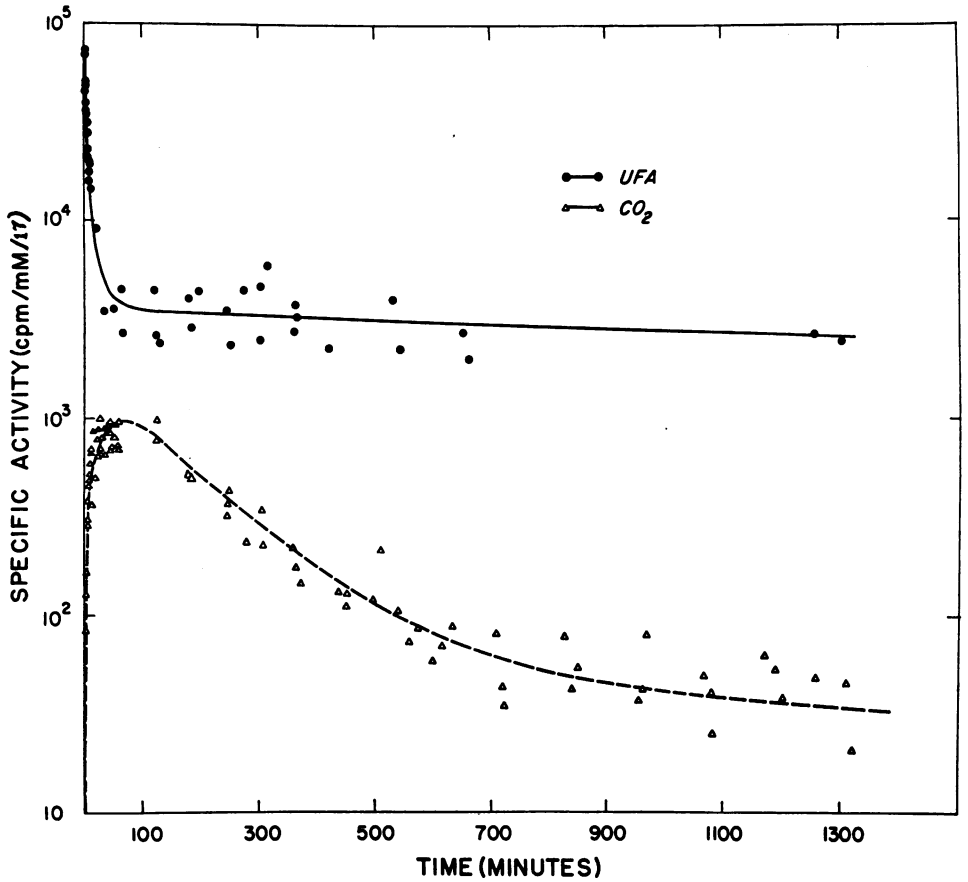

FIG. 4. SPECIFIC ACTIVITY IN PLASMA UFA AND EXPIRED CO_2 AFTER INTRAVENOUS INJECTION OF ALBUMIN-BOUND LINOLEIC ACID-1-C^{14} IN THREE FASTING SUBJECTS

The UFA specific activities only have been divided by 17 (see text).

the concentration of UFA-C^{14} in the plasma declined rapidly in 30 to 60 minutes to a concentration of about 1 per cent of that at zero time. This was followed by progressively slower rates of decline. The general form of the plasma UFA specific activity-time function was that of a sum of exponential decay processes. By graphic subtraction, at least five exponential terms could be obtained from the UFA specific activity curve shown in Figure 3, having rate constants varying from 0.27 to 0.00032 minute^{-1}. Because of the inaccuracy in measurement of UFA radioactivity after five to seven hours, only the earlier portion of the curve was used for kinetic analysis (Figure 5). The later portion of the UFA specific activity-time curve derived from linoleic acid-C^{14} (Figure 4) had a different form from that obtained with either palmitic or oleic acid. After injection of linoleic acid there was a rapid decline to about a level of 1 per cent of the zero time specific activity, following which further decay proceeded extremely slowly.

Radioactivity in red cells after injection of UFA-C^{14}

Red cells from blood samples removed from subjects one hour after administration of labeled palmitic and oleic acids contained only 1 per cent of the radioactivity contained in an equivalent volume of plasma. These findings were consistent with similar measurements made in the dog (16).

The excretion of UFA carboxyl carbon-14 in the expired CO_2

The oxidation of labeled fatty acids introduced into the plasma proceeded rapidly. Specific ac-

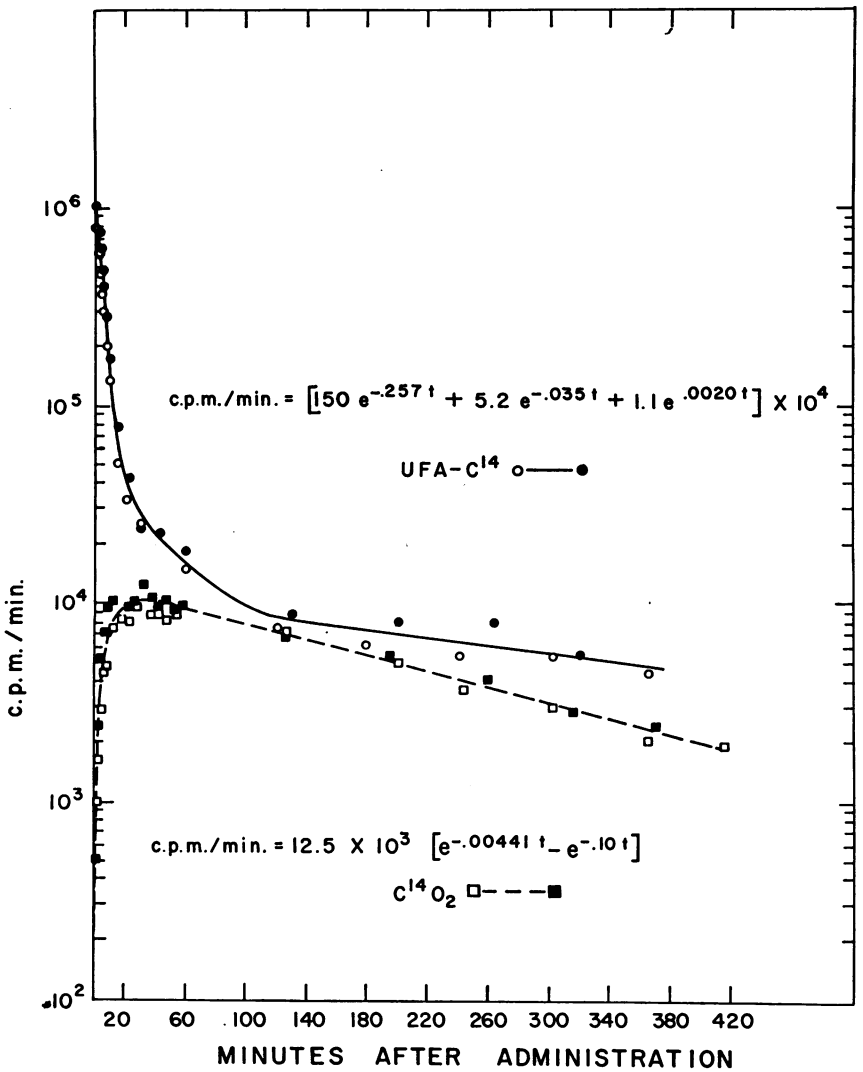

FIG. 5. FLUX OF LABELED CARBOXYL CARBON OF PALMITIC ACID-1-C^{14} FROM PLASMA AS UFA AND IN EXPIRED CO_2 IN TWO FASTING SUBJECTS

Open symbols represent data from one subject; solid symbols, data from the second subject.

tivity-time curves for the expired CO_2 after injection of palmitic acid also appear in Figure 3. The composite data from two subjects given oleic acid were practically identical. Maximum specific activity was reached in about 30 minutes. After injection of linoleic acid, the maximum specific activity was reached after about one hour and the peak specific activity was somewhat lower (Figure 4). The specific activity-time function for all three acids could be expressed in the general form: cpm/mM = $Ae^{-at} + Be^{-bt} + Ce^{-ct}$. The rate constant b, predominating from the first to eighth hours, was 0.0069 $minute^{-1}$ for palmitic acid (calculated from the curve fitted to the composite data from four patients, Figure 3) and 0.0065 $minute^{-1}$ for linoleic acid (composite data from three patients, Figure 4). The total fraction of radioactive carboxyl carbon excreted by one hour after injection is shown in Table I. In the seven patients in whom extended measurements were made, the fraction excreted by 22 hours ranged from 31 to 64 per cent of the injected

TABLE I

The appearance of carboxyl carbon-14 in expired air after injection of labeled unesterified fatty acids (UFA)

Acid	Nutritional status	No. of subjects	Per cent dose C^{14} excreted in 1 hr.
Palmitic	Fasting	11	9.4 (5.7–13.3)
Palmitic	Carbohydrate-fed	3	4.7 (2.7–6.0)
Oleic	Fasting	2	10.0 (8.2–11.8)
Linoleic	Fasting	5	6.3 (5.3–8.2)

dose. In the one patient given both palmitic and linoleic acids with measurement of CO_2 excretion for as long as 22 hours, the total amount of radioactivity excreted following injection of linoleic acid was two-thirds of that obtained from palmitic acid.

For purposes of comparison, the plasma UFA specific activities shown in Figures 3 and 4 were divided by 17, representing an approximation of the average number of carbon atoms per UFA molecule in the plasma (17). The assumption that a labeled carboxyl carbon in the injected acids will undergo an "obligatory dilution" of 17-fold is based on the observations in rats of Weinman and associates (18) that carbons 1, 6 and 11 of injected palmitic acid emerge at the same rate in the expired CO_2. Such an approximation does not allow for any undetermined amount of recycling of fragments of the UFA molecule which may occur. For palmitic and oleic acids the "corrected" specific activities of plasma UFA and expired $C^{14}O_2$ were similar for about two to six hours, following which the UFA specific activity again became greater. Following injection of linoleic acid the UFA specific activity was always many times greater than that of the expired CO_2.

Recycling of plasma UFA

Inspection of the plasma UFA specific activity curves shown in Figures 3 and 4 suggests that labeled carboxyl carbon from the injected C^{14} acids is returning to the plasma after its disappearance. More meaningful computations to ascertain this point are obtainable by converting the observed specific activity measurements into the form of flux of C^{14} from the plasma as UFA or into the expired air as $C^{14}O_2$. For this purpose, the plasma UFA specific activity (in cpm per mEq.) at time t may be multiplied by the experimentally estimated flux of UFA from plasma (in mEq. per minute) as defined above, to obtain the flux of C^{14} (in cpm per minute). The mean output of CO_2 (in mM per minute) is likewise used to convert the observed $C^{14}O_2$ specific activities (in cpm per mM) to flux of C^{14} in expired air.

The changes in isotopic flux with time calculated for two subjects receiving palmitic acid-1-C^{14} are plotted semilogarithmically in Figure 5. A single curve has been fitted to the combined values for UFA-C^{14} and for $C^{14}O_2$ from each patient and an analytical expression, consisting of a sum of exponential terms, derived for the curves. These expressions may be integrated over the period of observation to obtain the total flux during this period. Such total fluxes for several subjects receiving different labeled acids are summarized in Table II.

TABLE II

Isotope data in six fasting patients

Patient	Status	C^{14}-acid	Plasma UFA at outset	Plasma UFA flux	CO_2 output	Amount C^{14} administered	Isotopic flux* in Plasma UFA	Isotopic flux* in CO_2
			mEq./L.	*mEq./min.*	*mM/min.*	*10^6 cpm*	*10^6 cpm*	
M. B.	Fasting	Palmitic	0.61	0.42	8.2	7.2	10.5†	2.3†
L. J.	Fasting	Palmitic	1.16	0.74	9.2	7.8		
P. R.	Fasting	Oleic	0.95	0.63	10.2	7.0	10.1	2.7
B. S.	Fasting	Linoleic	0.95	0.67	13.0	7.6		
G. G.	Fasting	Linoleic	1.32	0.87	15.2	7.5	26.4†	2.6†
Q. W.	Fasting	Linoleic	1.12	0.81	8.3	7.2		

* Flux of $C^{14} = \int_0^t \gamma\, dt$; where t = 420 minutes, γ = the sum of exponential terms fitted to the curves for the isotopic flux in plasma UFA ($\gamma = \gamma_a$), or in expired $C^{14}O_2$ ($\gamma = \gamma_b$), observed for the first 420 minutes following administration of the tracer acid.

† Data from these subjects were combined and fitted with a single curve from which γ_a and γ_b, respectively, were derived.

From inspection of these data, it is obvious that within seven hours after injection of labeled palmitic and oleic acids to fasted subjects, about 50 per cent more radioactivity than was administered has passed through the plasma. Since the plasma UFA still contains slowly declining radioactivity at 22 hours (Figure 3), it is certain that, were an analytical expression (γ_a, Table II) for isotopic flux in plasma UFA available which contained sufficient exponential terms adequately to describe this late decay, the integrated flux, $\int_0^\infty \gamma_a\, dt$, would be several times the injected dose. This assumes that the fractional turnover rate of plasma UFA remains the same and that the radioactivity in the plasma UFA is contained in the acid in which it was injected. It is reasonable to assume that some recycling of labeled carboxyl carbon may occur in acids other than the original labeled substrate, and this factor is being evaluated by chromatographic techniques.

As was previously mentioned, linoleic acid-1-C^{14} behaves differently from palmitic and oleic acid, undergoing far more recycling into the plasma compartment (Table II). It will be noted that two of the three subjects given this acid had unusually high total CO_2 outputs. Observations on a slightly larger group (Table I) indicate that linoleic acid carboxyl carbon is excreted more slowly than that from palmitic and oleic acids.

Effect of nutritional state on plasma turnover and oxidation of UFA

Since there is considerable evidence indicating the precedence of glucose oxidation over that of fat both in humans and other animals (19), it was considered pertinent to compare UFA metabolism in fasting and carbohydrate-loaded subjects. The flux of plasma UFA in fasted and carbohydrate-loaded subjects is shown in Table III.

Although the mean plasma UFA concentration in the carbohydrate-fed subjects was one-sixth of that in the fasted subjects, the greater fractional turnover rate of plasma UFA at the lower plasma UFA concentrations tended to equalize the two groups with respect to UFA flux. The total flux of plasma UFA in carbohydrate-fed subjects averaged about one-third of that in individuals fasted an average of 16 hours. The oxidation of UFA, once introduced into the plasma, proceeds more slowly after carbohydrate loading, but is by no means eliminated (Table I). It has been shown in rats (20, 10) that carbohydrate feeding virtually abolishes oxidation of intravenously injected palmitic acid. It is of interest that in man it was not found possible to prevent appreciable oxidation of plasma UFA by supplying an excess of calories as carbohydrate. One subject received a high-calorie diet containing over 300 Gm. of carbohydrate daily for three days, plus an intravenous infusion of 100 Gm. of glucose with insulin during the first two hours after injection of palmitic acid-1-C^{14}. His total output of radioactivity within seven hours was about one-third of that expired by fasted subjects receiving palmitic acid.

TABLE III

Effect of nutritional state on flux of palmitic acid-1-C^{14} from the plasma

Status	No. of subjects	Plasma UFA		
		Concentration	$T_{\frac{1}{2}}$	Flux
		mEq./L.	*min.*	*mEq./min.*
Fasting	18	0.79 (0.30–1.65)	2.2 (1.4–3.4)	0.66 (0.32–1.50)
Carbohydrate-fed	6	0.13 (0.09–0.16)	1.3 (1.0–1.4)	0.23 (0.14–0.49)

Comparison of the rate of CO_2 excretion (in mM CO_2 per minute ÷ 17) with the total flux of UFA from the plasma was also made (Figure 6) for fasting and carbohydrate-loaded subjects. In fasted subjects, the flux of UFA from the plasma was approximately equivalent to the rate of CO_2 excretion. As will be discussed presently, this does not imply that the only source of carbon dioxide during fasting is the oxidation of plasma UFA. In the carbohydrate-loaded subjects, the rate of CO_2 excretion was severalfold greater than the flux of UFA from the plasma.

Radioactivity in the neutral lipid fraction

The combined extraction procedure used also permits assay of radioactivity in a UFA-free extract. The neutral lipids include all of those normally present in plasma with the exception of phospholipids, only a few per cent of which are recovered.

Approximately one hour after injection of labeled UFA, the radioactivity (expressed as cpm

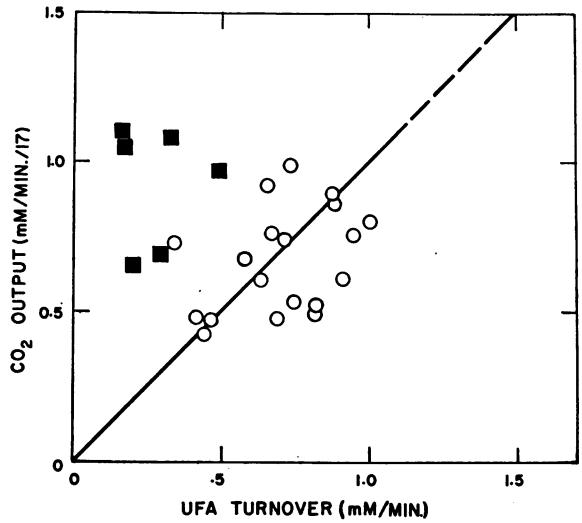

FIG. 6. COMPARISON OF THE FLUX OF UFA FROM THE PLASMA (UFA TURNOVER) WITH THE RATE OF CO_2 EXCRETION (mM $CO_2 \div 17$) IN FASTING AND CARBOHYDRATE-FED SUBJECTS

Open circles represent fasting subjects; solid squares, carbohydrate-fed subjects.

per ml. plasma) in the neutral lipid fraction exceeded that in the UFA and remained one to five times greater during the remainder of the observations. Serum taken from patients one hour after injection of linoleic and palmitic acids was extracted and passed over an ion-exchange column (21). The amount of radioactivity retained by the column (unesterified fatty acid) was comparable to that obtained in the UFA fraction by the solvent extraction procedures.

DISCUSSION

The data obtained from studies with labeled UFA described here may be combined with other available information to develop a hypothetical scheme for plasma UFA metabolism. Such a scheme can be described at the present time only in terms of a greatly simplified system of "compartments" (Figure 7). A similar hypothesis has been advanced by Dole (9).

Let A (Figure 7) represent the sources of plasma UFA. The evidence indicating that adipose tissue triglycerides probably represent the major source of plasma UFA includes: *1*) the finding of negative A-V differences for (unlabeled) UFA across areas rich in adipose tissue during times when other tissues such as the heart, liver and skeletal muscle are extracting UFA from the blood (3); and, *2*) the observation that release of UFA by rat adipose tissue *in vitro* is influenced in analogous fashion by several factors which alter plasma UFA concentrations *in vivo* (22). Should plasma UFA come largely from adipose tissue, it appears that the movement of UFA from plasma back to the adipose tissue is considerably less than the forward reaction. It has been shown that UFA-C^{14} administered intravenously to rats has little tendency to enter the adipose tissue (23), and comparison of the specific activities of plasma UFA and expired $C^{14}O_2$ obtained in these experiments (Figure 3) indicates that plasma UFA is not diluted in a pool the size of the body adipose depots before oxidation. The possibility has not been excluded, however, that there may be a significant backward flux limited to only a small fraction of the adipose fatty acids.

The flux of UFA from the plasma (B, Figure 7) to extravascular sites (C, E, Figure 7) has been measured experimentally. The half-times of disappearance for the labeled fatty acids of the order of one to three minutes are comparable to those previously reported for the dog (4–6). Laurell has also recently reported comparable findings in man for the first 10 minutes after the injection of palmitic and oleic acids (8). The high fractional turnover rates, ranging from 0.27 to 0.70 $minute^{-1}$, imply a very rapid diffusion rate across vascular membranes. Such rates are remarkable in consideration of the extremely high association constants between albumin and fatty acids (24). It is obvious from turnover studies with labeled albumin (25) that the albumin molecule does not leave the blood at a rate comparable to the rate with which UFA molecules leave. Extravascular albumin, sites on cell membranes having an equal affinity for UFA, or both, must assist in the removal of UFA from the plasma.

A portion of the UFA leaving the plasma compartment is rapidly oxidized. The resulting carbon dioxide eventually expired after equilibrating with the body "bicarbonate pool" (D, Figure 7) presumably represents an irreversible step in UFA metabolism. In unpublished experiments we have determined that the single injection of 5 μc. of C^{14}-labeled bicarbonate is not accompanied by any

significant labeling of plasma UFA. While the extravascular site, C, where UFA is oxidized, may not yet be precisely defined, several of its features may be surmised. It is certainly intracellular, since mitochondrial enzymes are apparently essential for fatty acid oxidation, and the immediate volume in which plasma UFA is diluted (Figure 3) is greater than extracellular fluid. The representation of the site of oxidation, C, as a single compartment is an obvious oversimplification, since effective blood flow and oxidation of fatty acids are not identical for all tissue cells. The tissue distribution of injected UFA is extensive (9, 23). Dole has reported that about 90 per cent of labeled palmitate found in tissues after injection of UFA is found in triglycerides, phospholipids and cholesterol esters (9). The neutral lipid in the plasma has been shown to become radioactive rapidly in these experiments, suggesting return of labeled fatty acids, in ester form, from the extravascular "pool" in equilibrium with plasma UFA.

The evidence that linoleic acid undergoes much more recycling into plasma, seemingly without a comparable effect on its oxidation, raises the possibility that this acid may be in equilibrium with another "compartment" not concerned with oxidation, represented by E in Figure 7. The possibility that palmitic and oleic acids also are involved in similar distribution, but with less effect on recycling, is, of course, present. While the nature of E is not known, it is pertinent that the amount of radioactivity found in the nonphospholipid-containing neutral lipid fraction was not greater after linoleic acid than after palmitic or oleic acids. It is not unlikely that E, like C, is located within tissue cells and that the difference between E and C is one of function, rather than one of anatomical location. Transfer of labeled fatty acids between E and C, therefore, may or may not require the reappearance of the molecule in the plasma compartment, B.

The contribution of plasma UFA to total energy production is a problem of special interest. This contribution could be calculated if the fraction of the expired CO_2 which is derived from plasma UFA were known. The flux of fatty acids from the plasma can be calculated, and this has been found to approximate the total CO_2 carbon excretion in the fasting human (Figure 6). However, in order for plasma UFA to account for all of the CO_2 output under the conditions observed here, the flux of UFA back into the plasma would obviously have to be negligible in the steady state. The isotopic measurements showing significant recycling of the plasma UFA indicate that this is not so (Table II). For at least one acid, linoleic, which appears to be a lesser constituent of UFA under normal conditions (17) recycling is prominent. Therefore, although the flux of plasma UFA in the human fasted 16 to 24 hours (Table III) would appear to represent the equivalent of about 2,400 calories per day, an estimation of the directness with which this UFA is oxidized is required to convert plasma turnover to actual utilization. Palmitic and oleic acids have been shown to comprise nearly 50 per cent of the UFA in some fasting humans (17). Since about half of the labeled carboxyl carbon from injected palmitic or oleic acid is shown to reappear in the plasma within seven hours (Table II), it would seem that not more than half of the quantity of these two acids which leaves the plasma is directly oxidized. Hence, the flux of UFA from the plasma may be at least twice as great as the rate at which these acids are being oxidized for energy. Undoubtedly, the relationship of plasma flux to UFA utilization is a function which depends heavily upon nutritional state, exercise and other factors affecting caloric demand. A more precise estimation of the caloric contribution of plasma UFA, based on further mathematical analysis of these and similar data obtained in dogs (16), is in preparation (26). The measurement of plasma UFA metabolism in diabetes, various disorders associated with abnormal blood lipids, and certain other abnormal metabolic states will also be described subsequently.

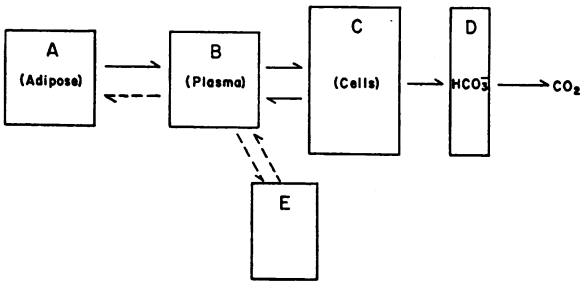

FIG. 7. HYPOTHETICAL DIAGRAM FOR PLASMA UFA METABOLISM

SUMMARY AND CONCLUSIONS

The metabolism of plasma unesterified fatty acids (UFA) in man has been studied by injection of albumin-bound carboxyl-labeled palmitic, oleic and linoleic acids. Twenty-one normal human subjects, ranging in age from 18 to 76 years, were used. The removal of labeled UFA carboxyl carbon from plasma and its excretion in expired CO_2 was measured. The flux of UFA from the plasma was found to vary with nutritional state and ranged from 0.09 to 1.5 mEq. per minute. Approximately 10 per cent of the labeled carboxyl carbon from palmitic and oleic acid was recovered as CO_2 in the first hour after injection. Calculation of isotopic flux from the plasma suggested that a large proportion of the labeled acids recycled one or more times in the plasma before oxidation, indicating that flux of UFA from the plasma may be considerably greater than the utilization of these acids for energy. Recycling of labeled linoleic acid was greater than that for oleic and palmitic acids. Labeled UFA also appeared quickly in plasma neutral lipids. A general scheme for plasma UFA metabolism is discussed.

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