Uptake of long-chain fatty acid methyl esters by mammalian cells

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ABSTRACT Albumin-bound long-chain fatty acid methyl esters (ME) were taken up and utilized by Ehrlich ascites tumor cells and slices of rat heart, liver, and kidney. Much more ME than albumin was taken up by the tumor cells, indicating that ME dissociated from the carrier protein during their uptake. 70–80% of the radioactivity associated with the cells after 1 min of incubation at 37°C remained as ME. The results of studies with metabolic inhibitors and glucose suggest that uptake of ME is an energy-independent process. Changes in incubation medium pH between 7.8 and 6.5 did not markedly alter uptake of ME. Cells incubated with FFA and methanol did not synthesize ME. These findings indicate that ME are taken up intact, and they suggest that the presence of an anionic carboxyl group is not essential for the binding of a long-chain aliphatic hydrocarbon to a mammalian cell.

When incubation with labeled ME was continued for 1 hr, increasing amounts of radioactivity were recovered in FFA, phospholipids, neutral lipid esters, and CO_2. ME radioactivity associated with the cells after a brief initial incubation was released in the form of ME and FFA when the cells were incubated subsequently in a medium containing albumin. If the second incubation medium contained no albumin, most of the ME radioactivity initially associated with the cells was incorporated into phospholipids, neutral lipid esters, and CO_2. These results suggest that much of the ME which is taken up, is hydrolyzed to FFA, and that the fatty acids derived from ME are available for further metabolism.

SUPPLEMENTARY KEY WORDS free fatty acid · uptake · Ehrlich ascites tumor cells

PLASMA FFA is a major source of lipid for many mammalian tissues. Therefore, the mechanism of FFA utilization is of interest and has been the subject of continuing study. The first step in FFA utilization is an energy-independent binding of fatty acid to the cell surface (1–5). Through the use of FFA of different chain lengths and structures, some understanding of the role of the hydrocarbon chain in FFA uptake has been obtained (3, 4, 6, 7). However, the question of whether the anionic carboxyl group is essential for uptake has not been investigated. Information concerning this point might provide insight into the nature of the cellular FFA binding sites and the FFA-site interaction. In an attempt to examine this question, we have studied the uptake of a long-chain hydrocarbon containing a nonionizable carboxyl terminus. Fatty acid methyl esters were chosen as the carboxyl-substituted FFA analogue for two reasons. First, ME bind to albumin (8), the physiological transport protein for FFA. Thus, the uptake of this analogue can be studied under the same experimental conditions that are used for FFA, that is, when it is presented to the cell as an albumin complex. Second, we thought it advisable to determine whether the analogue, like FFA (9), would be available for further metabolism after combining with the cell. Since mammalian tissues contain several different esterases, it was reasonable to assume that an ester might be more readily converted into a utilizable substrate than analogues containing other types of carboxyl–terminal modifications.

Most of the experiments were performed with suspensions of Ehrlich ascites tumor cells. FFA uptake has been studied extensively with Ehrlich cells (1, 3, 7), and we thought that the use of this model system would allow for more definitive interpretations of the data. Albumin-bound ME were taken up by these cells, and the fatty

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Abbreviations: ME, long-chain fatty acid methyl ester(s); FFA, free fatty acid(s).

* Research Career Development Awardee of the National Heart Institute (1-K4-HE-20,338).

acid moieties of the bound ME were available for further metabolism. A few additional studies with slices of rat heart, liver, and kidney demonstrated that ME also can be utilized by these tissues.

METHODS

The procedures for transplanting, harvesting, and washing the Ehrlich cells have been described (3). Suspensions of Ehrlich cells were prepared in a buffer solution containing 0.132 M NaCl, 0.0046 M KCl, 0.0012 M $MgSO_4$, and 0.016 M sodium phosphate, adjusted to pH 7.4 with 0.1 N HCl. This solution was used in most experiments and will be referred to in the text as "buffered salt solution." Freshly prepared suspensions which contained approximately 10^8 cells/ml were employed for each experiment. Cell counts were obtained using a microscope and clinical hemocytometer.

Unlabeled ME and radioactive ME containing ^{14}C in the carboxyl carbon atom of the fatty acid moiety were purchased from Applied Science Laboratories Inc., State College, Pa. The ME were dissolved in hexane, and the solution was extracted with an alkaline ethanol solution (3) in order to remove any FFA impurity. Thin-layer chromatography on Silica Gel G in a solvent system containing petroleum ether–diethyl ether–acetic acid 180:20:2 revealed that more than 99% of the radioactivity migrated in the methyl ester zone. Aliquots of the hexane solution were added to liquid scintillation counting vials, dried under a stream of N_2, and counted in a Packard Tri-Carb 3380 liquid scintillation spectrometer following addition of 18 ml of a toluene–methanol 70:30 (v/v) scintillator solution containing 0.3% 2,5-diphenyloxazole and 0.01% 1,4-bis [2-(4-methyl-5-phenyloxazolyl)] benzene. Quenching was monitored with the external standard. The specific radioactivity of each preparation was approximately 0.05 Ci/mole. The hexane solution of ME was added to Celite that had been washed to remove acid-soluble material, and the hexane was evaporated under a stream of N_2 (10). In most preparations, there was 1 μmole of ME per 100 mg of Celite.

"FFA-free" bovine serum albumin was purchased from Miles Laboratories, Inc., Elkhart, Ind. These albumin preparations contained less than 0.02 μEq of long-chain FFA per μmole of protein as determined by titration with 0.02 N NaOH (11). Albumin was dissolved in 100 ml of buffered salt solution and dialyzed with mechanical stirring for 16 hr at 4°C against 1 liter of the same solution. The albumin solution was adjusted to pH 7.4, and aliquots of it were incubated at 23°C with ME-coated Celite (10). The uptake of ME by the albumin reached a maximum value within 2 hr, and was independent of the amount of ME–Celite that was used. However, for routine preparation, a 30 min incubation time was employed. Mixing was accomplished with a magnetic stirrer. The Celite was sedimented by centrifugation at 10,000 *g* for 10 min at 0°C. After the resulting supernatant solution was centrifuged for a second time, it was passed through a Millipore filter of 1.2 micropore size (10). The protein content and radioactivity present in the solution were measured (8), and the ME content was calculated from the specific radioactivity of the ME preparation. Enough buffered salt solution was added so that the final albumin concentration was 0.5 μmole/ml. The maximum ME–albumin molar ratio that could be obtained by this method of preparation was 1.5. In most preparations, the ME content was between 0.05 and 0.65 μmole/ml. More than 99% of the radioactivity contained in the albumin solutions migrated as ME when a lipid extract was analyzed by thin-layer chromatography. Each solution was readjusted to pH 7.4 before use. In one preparation, methyl palmitate-1-^{14}C was added to ^{131}I-labeled albumin which was prepared and purified by Dr. Frank Cheng of the Nuclear Medicine Section, University of Iowa. ^{131}I radioactivity was measured in a well scintillation spectrometer.

In most experiments, the cells were incubated at 37°C with buffered salt solution containing albumin-bound ME. The gas phase was air. In experiments with glucose, the incubation medium was modified in order to prevent pH changes resulting from excessive lactate production (12, 13). Both the glucose-free and glucose-containing media in these experiments contained 58 mM NaCl, 2.5 mM KCl, 0.6 mM $MgSO_4$, and 85 mM sodium phosphate, pH 7.4. All incubations were done in a temperature-controlled water bath with shaking. The incubation was terminated by pouring the contents of each flask into a glass-stoppered centrifuge tube containing 30 ml of cold buffered salt solution. Following centrifugation at 1500 *g* for 3 min at 0°C, the supernatant solution was siphoned off, and the cells were dispersed in 25 ml of fresh buffered salt solution. Sedimentation and washing were repeated twice. The final cell pellet was extracted with 20 ml of chloroform–methanol 2:1 (v/v) for 15 hr; the centrifuge tubes were shaken vigorously at intermittent intervals (3). Separation of the extract into two phases was obtained in a separatory funnel after addition of 5 ml of 0.04 N HCl; the chloroform phase was removed and dried under N_2. The lipid residue was dissolved in 2 ml of fresh chloroform. One aliquot of the chloroform solution was added to a counting vial and dried under N_2; radioactivity was measured in the liquid scintillation spectrometer following addition of 18 ml of the toluene–methanol scintillator solution. The lipids contained in a second aliquot of the chloroform solution were separated by thin-layer chromatography as described above. Lipid standards (Applied Science Laboratories Inc.) were

added to each chromatogram. The lipid spots were made visible by exposure of the plates to I_2 vapor; the areas were outlined, and the I_2 was allowed to sublime. The outlined segments of silica gel were scraped directly into liquid scintillation counting vials containing a dioxane–water scintillator solution (14), and the radioactivity was measured.

CO_2 collections were made in special flasks containing removable center wells (3). The flasks were sealed with rubber serum stoppers, and CO_2 was trapped in 0.2 ml of 1 N KOH placed in the center well. The incubation was terminated by injection of 0.3 ml of 4 N H_2SO_4 into the medium, and the flasks were shaken for an additional 2 hr period. After the stoppers were removed, the contents of each center well were added to a counting vial which contained 18 ml of the toluene–methanol scintillator solution, and the radioactivity was measured. In each experiment, there were duplicate incubations in which no cells were included, and the small amount of radioactivity recovered in the KOH in these incubations was subtracted from the total quantity that was recovered from the corresponding flasks which contained cells.

In one group of experiments, the cells were exposed briefly to labeled ME, washed, suspended in fresh medium, and then incubated for various times in order to follow changes in the content and distribution of the cell lipid radioactivity. Aliquots of the cell suspension also were added to chloroform–methanol solution in order to determine the content and distribution of radioactivity prior to the start of the second incubation. The release of radioactivity into the second incubation medium also was followed. In these incubations, the cells were sedimented at 7000 *g* for 5 min at 0°C, and a 1 ml aliquot of the supernatant solution was extracted with 20 ml of chloroform–methanol. The content and distribution of radioactivity present in the chloroform phase was determined.

Slices of rat liver, heart, and kidney were prepared with a hand microtome as described previously (15). After the wet weight was measured, the slices were incubated at 37°C in 6 ml of buffered salt solution containing methyl palmitate-1-^{14}C and 0.5 μmole of albumin. The palmitate–albumin molar ratio was 0.8. Four slices of tissue having a total wet weight of 0.3–0.4 g were added to each flask. The time of incubation was 60 min; 95% O_2 and 5% CO_2 constituted the gas phase. The tissue, which was recovered by pouring the contents of each flask through a gauze pad, was washed with 20 ml of cold buffered salt solution and then homogenized in a tissue grinder containing 10 ml of chloroform–methanol. The tissue grinder was rinsed with an additional 10 ml of chloroform–methanol, and this solution was added to the homogenate. These tissue lipid extracts were treated in the same way as those prepared from the tumor cells.

RESULTS

Uptake and Utilization of ME

Fig. 1 illustrates the kinetics of utilization of methyl oleate-1-^{14}C by Ehrlich ascites tumor cells. Increasing amounts of methyl oleate were utilized as the incubation continued. At each time point, appreciable amounts of radioactivity were recovered from the cells in the form of ME. After 1–2 min of incubation, 75 ± 2% of the total uptake was present as ME (mean ± SEM, three experiments). The content of radioactive ME of the cells increased as the incubation progressed, and increasing quantities of radioactivity appeared in CO_2, phospholipids, and neutral lipid esters. Some radioactivity also was present in the cells as FFA. Qualitatively similar results were obtained with methyl palmitate-1-^{14}C. After 1–5 min of incubation, 73 ± 4% of the radioactivity contained in cells incubated with methyl palmitate was present as ME (mean ± SEM, seven experiments).

When cells were incubated for 5 min with media containing methyl palmitate-1-^{14}C and albumin-^{131}I, the ME uptake was much greater than that of albumin (Table 1). These media contained a low initial ME content (0.5 μmole), and 20–23% of the available methyl palmitate was taken up by the cells. In contrast, only 0–0.96% of the available albumin was taken up. Before incubation, the ^{14}C/^{131}I ratio of the medium was 0.094–0.098; after incubation, it was 0.074–0.080. These find-

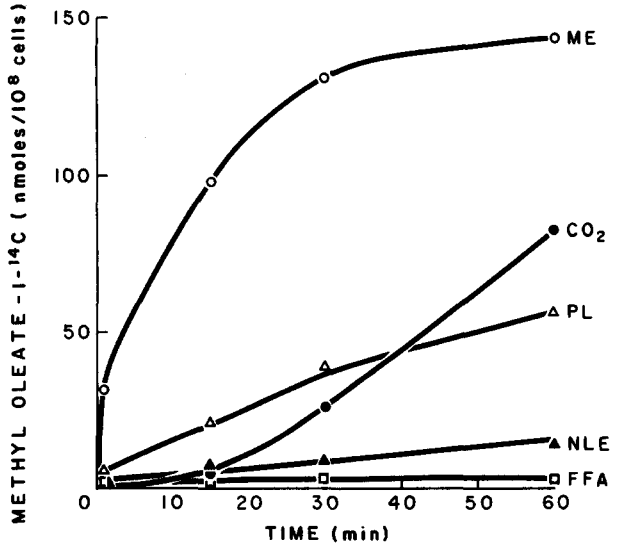

FIG. 1. Time course of methyl oleate-1-^{14}C uptake and utilization. Incubation was done at 37°C with air as the gas phase. The medium contained 1.5 μmoles of methyl oleate, and the molar ratio of methyl oleate to albumin was 1.5. After 1 hr of incubation, approximately 20% of the methyl oleate initially present in the medium was taken up by the cells. The following abbreviations are used: *ME*, methyl esters; *CO_2*, carbon dioxide; *PL*, phospholipids; *NLE*, neutral lipid esters; *FFA*, free fatty acid.

ings indicate that most of the ME taken up by the cells must have dissociated from albumin.

The content of radioactive ME of the cells reached a steady-state value after 30–60 min of incubation at all concentrations of medium ME that were tested. However, the steady-state amount of radioactive ME associated with the cells increased linearly as the ME concentration was raised. When the ME–albumin molar ratio was raised from 0.25 to 1.25, methyl palmitate uptake increased 9.6-fold and methyl oleate uptake increased 9.2-fold (Table 2). The magnitude of uptake of methyl palmitate and methyl oleate was similar at both the low and the high molar ratio. The quantity of ME radioactivity which was incorporated into other cellular components also increased as the medium ME concentration was raised, but the magnitude of increase was much smaller. For example, when the molar ratio of methyl palmitate-1-^{14}C–albumin was raised from 0.25 to 1.25, the radioactivity incorporated into substances other than ME increased as follows: phospholipids, 22%, neutral lipid esters, 29%; FFA, 89%; CO_2, 144%.

As shown in Table 3, uptake of methyl palmitate-1-^{14}C as ME was not affected by the presence of 0.05 M NaF and 0.001 M NaCN in the incubation medium. In these experiments, the cells were exposed to the inhibitors for 10 min prior to addition of the labeled ME, and the inhibitors remained in the medium during a subsequent 5 min incubation with the ME.

Table 4 shows the effect of changes in the incubation medium pH between 7.8 and 6.5 on uptake of methyl palmitate-1-^{14}C as ME. There was no significant difference between the mean values obtained at pH 7.4 and pH 7.8. However, small increases in uptake as ME (compared with that noted at pH 7.4) did occur when the medium pH was lowered to 7.0 or 6.5.

When 0.011 M glucose was present in the incubation medium, the total amount of ME incorporated into the cells was increased considerably (Table 5). This was due primarily to greater incorporation of ME radio-

TABLE 1 COMPARISON BETWEEN UPTAKE OF METHYL PALMITATE-1-^{14}C AND ALBUMIN-^{131}I

Expt. No.	Sample	Incubation Medium: FFA-^{14}C	Incubation Medium: Albumin-^{131}I	Incubation Medium: Ratio (^{14}C/^{131}I)
			dpm × 10^{-4}	
1	Preincubation	2.00	21.2	0.094
	Postincubation	1.60	21.1	0.076
2	Preincubation	1.96	20.2	0.097
	Postincubation	1.51	20.4	0.074
3	Preincubation	2.04	20.8	0.098
	Postincubation	1.64	20.6	0.080

Before incubation with cells, samples of the incubation media were taken in triplicate for analysis of the FFA-^{14}C and albumin-^{131}I content. The medium was then incubated with 1 ml of cells for 5 min at 37°C with air as the gas phase. After the cells were removed by sedimentation at 8000 *g* for 10 min at 0°C, samples of the resulting supernatant solution were taken in triplicate for analysis of the ^{14}C and ^{131}I. Each value is the mean of the three closely agreeing determinations. For these calculations corrections were made for the volume of buffered salt solution added with the cells.

TABLE 2 EFFECT OF ME–ALBUMIN MOLAR RATIO ON ME UPTAKE

Expt. No.	Substrate	Uptake by the Cells as ME: Molar Ratio 0.25	Uptake by the Cells as ME: Molar Ratio 1.25
		nmoles/10^8 cells	
1	Methyl palmitate-1-^{14}C	33 ± 0.2	329 ± 8.3
2	Methyl palmitate-1-^{14}C	25 ± 0.7	232 ± 4.2
3	Methyl oleate-1-^{14}C	31 ± 1.3	269 ± 6.2
4	Methyl oleate-1-^{14}C	32 ± 0.3	306 ± 3.1

Cells were incubated with the labeled ME–albumin medium for 30 min at 37°C with air as the gas phase. The cells were isolated, washed three times, and extracted with chloroform–methanol 2:1 (v/v). After the lipids were separated by thin-layer chromatography, the radioactivity contained in the ME zone of the chromatogram was determined. Each value is the mean of four determinations ± SEM. The differences between the mean values in each experiment are statistically significant ($P < 0.01$).

TABLE 3 EFFECT OF METABOLIC INHIBITORS ON ME UPTAKE

Expt. No.	ME–Albumin Molar Ratio	Uptake by the Cells as ME: No Inhibitor	Uptake by the Cells as ME: NaCN + NaF
		nmoles/10^8 cells	
1	0.50	45 ± 1.2	49 ± 3.4
2	0.73	78 ± 1.3	80 ± 8.5

Cells were incubated for 10 min at 37°C with either buffered salt solution or the same solution containing 0.05 M NaF plus 0.001 M NaCN. Methyl palmitate-1-^{14}C–albumin solution was added, and the incubation was allowed to continue for an additional 5 min. The cells were removed by sedimentation and washed three times. After the lipids were extracted with chloroform–methanol 2:1 (v/v), they were separated by thin-layer chromatography. The radioactivity in the ME zone of the chromatogram was determined. Each value is the mean of four determinations ± SEM. The differences between the means are not significant ($P > 0.1$).

TABLE 4 EFFECT OF CHANGES IN THE INCUBATION MEDIUM pH ON ME UPTAKE

Incubation Medium pH	Uptake by the Cells as ME
	nmoles/10^8 cells
7.8	131 ± 9.4
7.4	125 ± 4.2
5.0	149 ± 3.7
6.5	154 ± 11

Cells were incubated with methyl palmitate-1-^{14}C–albumin of molar ratio 0.7 for 30 min at 37°C with air as the gas phase, and the radioactivity incorporated into the cells as ME was determined. Each value is the mean of six determinations ± SEM. The mean values at pH 7 ($P < 0.01$) and pH 6.5 ($P < 0.05$) are significantly different from the value obtained at pH 7.4.

TABLE 5 EFFECT OF GLUCOSE ON ME UPTAKE AND UTILIZATION

Expt. No.	Substrate	Glucose Concentration	ME Utilization	
			Total Incorporation	Uptake as ME
		$M \times 10^2$	*nmoles/10^8 cells*	
1	Methyl oleate-1-^{14}C	0	191 ± 5	105 ± 5
		1.1	307 ± 11	117 ± 10
2	Methyl oleate-1-^{14}C	0	137 ± 3	96 ± 3
		1.1	208 ± 5	112 ± 3
3	Methyl palmitate-1-^{14}C	0	171 ± 2	119 ± 2
		1.1	262 ± 3	152 ± 5
4	Methyl palmitate-1-^{14}C	0	203 ± 2	147 ± 2
		1.1	280 ± 2	166 ± 3

Cells were incubated with either methyl palmitate-1-^{14}C– or methyl oleate-1-^{14}C–albumin of molar ratio 0.7 for 60 min at 37°C with air as the gas phase. The medium contained 0.058 M NaCl, 0.0025 M KCl, 0.0006 M $MgSO_4$, and 0.085 M sodium phosphate, pH 7.4. Glucose in a final concentration of 0.011 M was added to certain of the media. After incubation, the cells were sedimented by centrifugation at 4000 *g* and washed three times with buffered salt solution. Lipids were extracted from the cells with chloroform–methanol 2:1 (v/v), and the radioactivity contained in one aliquot of the isolated chloroform phase was measured. The lipids present in another aliquot of the chloroform phase were separated by thin-layer chromatography, and the radioactivity contained in each lipid component was determined. Each value is the mean of four determinations ± SEM.

activity into phospholipids and glycerides rather than to greater uptake as ME. For example, the total uptake of methyl oleate-1-^{14}C in 60 min was an average of 57% greater when glucose was contained in the incubation medium. The radioactivity present in cellular phospholipids and glycerides increased 128% when glucose was present, whereas that recovered in ME increased only 14%. Likewise, the presence of glucose increased total uptake of methyl palmitate-1-^{14}C by an average of 49%. The radioactivity present in phospholipids and glycerides increased 108% when glucose was available, but that present as ME increased only 21%.

In additional experiments, cells were incubated for up to 60 min at 37°C with palmitic acid-1-^{14}C–albumin in the presence of 10^{-3}–10^{-1} M methanol. The total amount of radioactivity taken up by the cells was the same in the presence of 10^{-3} M methanol as in methanol-free media. Uptake decreased only slightly as the methanol concentration was raised. In no case was radioactivity recovered from the cells as ME.

Slices of rat myocardium, liver and kidney also took up and metabolized methyl palmitate-1-^{14}C. Uptakes at 60 min were 6.8, 3.5 and 6.2 nmoles/100 mg wet weight for heart, liver, and kidney, respectively (each value is the mean of three determinations). These uptakes do not include any methyl palmitate that was oxidized to CO_2, for this was not measured. 6–58% of the incorporated radioactivity was recovered as ME, and 10–21% as FFA. Phospholipids and neutral lipid esters contained 32–73% of the tissue radioactivity. The results of the individual incubations were quite variable, and we did not make a sufficient number of determinations to allow for precise quantitative comparisons among the three tissues.

Utilization of ME Previously Incorporated into the Cells

In the following experiments, cells were exposed briefly to a radioactive ME–albumin solution, isolated, washed thoroughly, and then incubated in a second medium so that the utilization of the radioactivity taken up during the initial exposure could be followed. Fig. 2 illustrates the results from an experiment in which 85% of the methyl palmitate-1-^{14}C taken up by the cells in the first incubation was present as ME. A protein-free medium was utilized for the second incubation. There was a progressive decrease of radioactive ME in cells as the second incubation continued. After 90 min of incubation, the cells contained only 18% of the initial content of radioactive ME content. The cellular FFA radioactivity

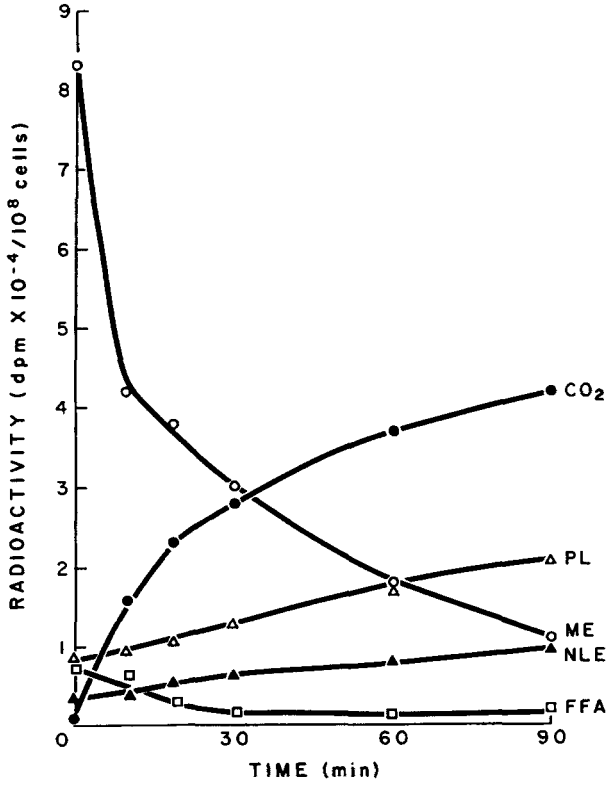

FIG. 2. Changes in the ^{14}C content of cells previously loaded with methyl palmitate-1-^{14}C. The cells were incubated initially with a methyl plamitate-1-^{14}C–albumin medium for 2 min at 37°C. After being washed three times with 10 volumes of buffer solution, the content and distribution of radioactivity in the cells were measured. Other aliquots of the cell suspension were incubated for up to 90 min at 37°C in a protein-free medium, and the content and distribution of radioactivity in the cells were followed. Each point is the mean of two determinations. The abbreviations are the same as those used in Fig. 1.

also decreased markedly. These decreases were accompanied by progressive increases of radioactivity in CO_2, phospholipid, and neutral lipid ester. It is evident that the bulk of the radioactivity that was incorporated into these fractions during the second incubation was derived from the radioactive ME of the cells. In this experiment, 90% of the radioactivity initially present in the cells was recovered after 90 min of incubation. In two other experiments, the recoveries after 90 min were 86 and 103%.

Quite different results were noted when the second incubation medium contained albumin. The cell radioactivity decreased much more rapidly than during incubation in an albumin-free medium. ME and FFA radioactivity was depleted, but there was very little increase in cell phospholipids or neutral lipid ester radioactivity. Likewise, very little $^{14}CO_2$ was produced. Instead, much of the radioactivity initially contained in the cells was released into the incubation medium (Fig. 3). In cells loaded with methyl palmitate-1-^{14}C, from 73 to 85% of the initial cell lipid radioactivity was recovered in the extracellular fluid after 10 min of incubation at 37°C. The total albumin content of this medium was 1.0 μmole. After only 1 min of incubation, 53% of the initial cell lipid radioactivity was released to the medium. As shown on the right side of Fig. 3, raising the albumin content of the incubation medium to 2 μmoles did not cause any greater release of cell lipid radioactivity. When the cells were loaded with methyl oleate-1-^{14}C under similar conditions, 25% of the cell lipid radioactivity was released after 1 min and 53% after 15 min of incubation at 37°C.

Table 6 shows the distribution of the released lipid-soluble radioactivity. ME plus FFA accounted for 95–99% of the released radioactivity. In the methyl palmitate experiment, 51% of the radioactivity released after 1 min was present as FFA. After 10 min of incubation, 66% of the released radioactivity was recovered as FFA. In the methyl oleate experiment, FFA contained 37 and 34% of the radioactivity released after 1 and 10 min, respectively. The amount of radioactivity released as FFA in both experiments was greater than the radioactive FFA content of the cells at the start of the incubation. The radioactivity recovered from the medium as ME represented only 9–24% of the radioactive ME content of the cells at the start of the incubation.

DISCUSSION

These data demonstrate that albumin-bound ME can be utilized by mammalian cells and tissues. The studies with the tumor cells indicate that ME dissociates from albumin and that the intact ME combines with the cell. Hence, it appears that the presence of an anionic group is not necessary for the association of a FFA-like long-chain hydrocarbon with a mammalian cell. The mechanisms of ME and FFA uptake are similar in many respects. In both cases, (*a*) uptake is energy-independent, (*b*) the magnitude of uptake is dependent upon the quantity of ligand that is bound to albumin, and (*c*) much of the initial uptake is released if the cells are resuspended in albumin (3). Further, the maximum uptakes of ME that were obtained with the tumor cells were of the same order of magnitude as the maximum FFA uptakes that we have obtained previously in this system (7). However, at a given molar ratio, much more ME and FFA was taken up by the cells. This may be explained in part by

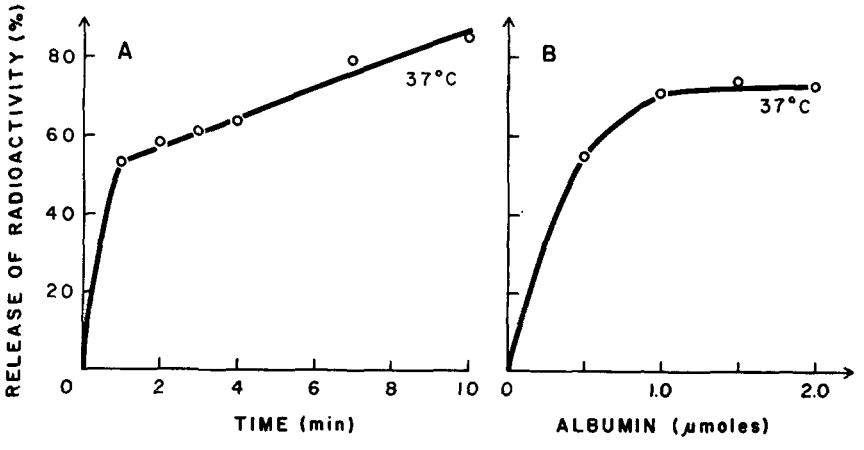

FIG. 3. Release of lipid-soluble radioactivity from the cells to the incubation medium. Cells were loaded with methyl palmitate-1-^{14}C, washed, and analyzed as described in Fig. 2. Aliquots of the labeled cell suspension were incubated in a solution containing albumin at 37°C, and the release of lipid-soluble radioactivity to the incubation medium was measured. In the experiment *A*, the cells were incubated with 1.0 μmole of albumin in buffered salt solution, pH 7.4, for 1–10 min. In the experiment *B*, the time of incubation was 10 min, and the albumin content of the medium was 0.5–2.0 μmoles. The results are expressed as the percentage of the initial cell lipid radioactivity that was released into the incubation medium.

TABLE 6 DISTRIBUTION OF RADIOACTIVITY RELEASED FROM THE CELLS

Substrate	Time of Incubation	Radioactivity Released		ME Release Relative to Initial Cell ME Content	FFA Release Relative to Initial Cell FFA Content
		ME	FFA		
	min	*dpm*		%	
Methyl palmitate-1-^{14}C	1	5,600	6,700	28	160
	10	4,800	11,000	24	260
Methyl oleate-1-^{14}C	1	650	410	14	120
	10	1,500	820	17	240

A procedure similar to that described in Fig. 2 was employed. The cells were loaded with radioactive ME. The second incubation was done at 37°C in a medium containing 1 μmole of albumin. Prior to incubation in albumin, a 1 ml aliquot of cells loaded with methyl palmitate-1-^{14}C contained 20,300 dpm with the following distribution: 71% ME, 21% FFA, 8% other lipid esters. The cells loaded with methyl oleate-1-^{14}C contained 4770 dpm with the following distribution: 74% ME, 7% FFA, 19% other lipid esters.

the fact that albumin binds FFA more tightly than ME (8). One difference between ME and FFA uptake concerns the time required to attain a steady-state content in the cell. This occurs more rapidly with FFA than with ME (7). However, this difference may be due to reasons other than cellular uptake, for steady-state concentrations also are attained more quickly when albumin is incubated with FFA-coated Celite (10) than with ME-coated Celite. The fact that ME can be taken up by mammalian tissues and that many aspects of ME and FFA uptake are similar suggests that the anionic carboxyl group may not be an important factor in the association of FFA with a cell. As a corollary, it follows that the major forces involved in FFA uptake probably are hydrophobic interactions between the fatty acid hydrocarbon chain and nonpolar components of the cell membrane. This hypothesis is supported by the fact that cellular FFA uptake decreases markedly as the length of the fatty acid hydrocarbon chain is shortened (7) even though binding to albumin also decreases (8, 16).

Whether the intact ME only binds to the cell surface or can actually penetrate into the cell cannot be determined from these data. Esterified long-chain fatty acid can enter certain cells without first undergoing hydrolysis. For example, monoglycerides penetrate into intestinal mucosal cells (17, 18), and the intact 2-monoglyceride is utilized for triglyceride synthesis (17, 19, 20). The ether analogue of a 2-monostearin also enters intestinal mucosal cells (18). On the other hand, triglycerides can bind to the cell surface, but they cannot enter the cell without first undergoing hydrolysis (21–23). The present results suggest that like triglycerides, most of the intact ME associated with the Ehrlich cells remains at the cell surface and that ME uptake actually represents binding to the cell membrane. A binding mechanism is consistent with the observation that uptake of the intact ME appears to be energy-independent. Moreover, a considerable percentage of the radioactive ME which was taken up was released when the cells were reexposed to albumin. The radioactivity available for release probably is located in such a way as to be accessible to the extracellular fluid, that is, it is present on or within the cell membrane. Much of the released radioactivity was recovered as FFA, suggesting that some hydrolysis of ME also takes place near the cell surface. Taken together, these findings are compatible with a mechanism in which the ester is bound at the cell surface and hydrolyzed at or near this location. The fatty acid that is produced then enters the cell as FFA and is available for utilization. A similar mechanism has been proposed for triglyceride utilization (21–23).

Excellent technical assistance was provided by Janice M. Soberoff and Betsy Imig.

This work was supported by research grants from the National Institutes of Health (HE-11,485 and MO1-FR-59) and the Iowa Heart Association.

Manuscript received 2 March 1970; accepted 8 June 1970.

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