Utilization of free fatty acids complexed to human plasma lipoproteins by mammalian cell suspensions

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ABSTRACT The purpose of this study was to determine whether lipoprotein-bound free fatty acid could be utilized by isolated mammalian cells. Ehrlich ascites tumor cells were incubated in vitro with radioactive free fatty acids that were bound to human plasma lipoproteins. Under these conditions, lipoprotein-bound free fatty acids were readily taken up by the cells. After 2 min of incubation with free fatty acids bound to low density lipoproteins, most of the radioactivity that was associated with the cells was in the form of free fatty acids. As the incubation continued, increasing amounts of radioactivity were incorporated into CO_2 and cell lipids, particularly phospholipids. Most of the free fatty acid uptake was the result of fatty acid transfer from low density lipoproteins to the cell, not from irreversible incorporation of the intact free fatty acid–low density lipoprotein complex. Fatty acid uptake increased as the ratio of free fatty acid to low density lipoprotein was raised. When albumin was added to the medium, free fatty acid uptake decreased. A large percentage of the newly incorporated cellular radioactivity was released into the medium if the cells were exposed subsequently to a solution containing albumin. Most of the released radioactivity was in the form of free fatty acid. The results with this experimental model suggest that lipoprotein-bound free fatty acid, like albumin-bound free fatty acid, is readily available for uptake by isolated cells. The mechanism of free fatty acid utilization by the Ehrlich cell is similar when either low density lipoprotein or serum albumin serves as the fatty acid carrier.

SUPPLEMENTARY KEY WORDS Ehrlich ascites cells · transport · uptake · serum albumin · oxidation · esterification · release

THE PURPOSE of this study was to determine whether long-chain free fatty acids that are bound to plasma lipoproteins can be utilized by mammalian cells. In vitro studies have demonstrated that FFA can bind to plasma lipoproteins (2–4). Moreover, some FFA probably is transported as a complex with lipoproteins when the plasma FFA concentration is extremely high, such as after heparin injection in a patient with hypertriglyceridemia (5). Therefore, it was of interest to learn whether lipoprotein-bound FFA are readily available for uptake by mammalian cells and, if so, whether the mechanism of utilization is similar to that of albumin-bound FFA (6).

The present studies were performed with suspensions of Ehrlich ascites cells. Uptake and utilization of albumin-bound FFA have been investigated extensively with Ehrlich cells (7), and we thought that use of this model system would permit more definitive interpretation of the lipoprotein data. Our results indicate that lipoprotein-bound FFA are readily taken up by the Ehrlich cell. The metabolic fate of lipoprotein-bound FFA in this cell appears to be identical to that of albumin-bound FFA.

METHODS

The procedures for transplanting, harvesting, and washing the Ehrlich cells have been described (6). Suspensions of Ehrlich cells were prepared in a buffer solution containing 0.132 M NaCl, 0.0046 M KCl, 0.0012 M $MgSO_4$,

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Abbreviations: FFA, free fatty acid or acids; LDL, low density lipoproteins.

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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 containing approximately 10^8 cells/ml were employed for each experiment. Cell counts were obtained using a microscope and clinical hemocytometer.

Fatty acids of the highest purity available commercially were purchased from Hormel Institute, Austin, Minn., and radioactive isotopes were obtained from New England Nuclear Corp., Boston, Mass. The labeled fatty acids were dissolved in hexane and purified by extraction with alkaline ethanol (6). Thin-layer chromatography on Adsorbosil-5 in a solvent system containing hexane–diethyl ether–methanol–acetic acid 180:40:4:6 revealed that more than 99% of the added radioactivity migrated in the FFA zone. Aliquots of a hexane solution containing both labeled and carrier fatty acid were added to liquid scintillation counting vials and dried under a stream of N_2. The radioactivity contained in these samples was measured in a Packard Tri-Carb 3375B liquid scintillation spectrometer following addition of 18 ml of toluene–methanol 7:3 (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 0.5 mCi/mmole. The remainder of the hexane solution of the fatty acid was added to Celite that had been washed to remove acid-soluble material, and the hexane was evaporated under a stream of N_2 (8). In most preparations there was 1 μeq of fatty acid per 10 mg of Celite.

Lipoproteins were isolated from human plasma by preparative ultracentrifugation according to the method of Havel, Eder, and Bragdon (9). Initially, each specimen of plasma was centrifuged at 4°C for 30 min at 10,000 *g* in order to remove chylomicrons. This step was followed by the three ultracentrifugations described by Havel et al. (9), using a Spinco L3-40 ultracentrifuge and either 50 Ti or 60 Ti rotors. Each of the three lipoprotein fractions, very low density (1.006), low density (1.006–1.063), and high density (1.063–1.21), were washed once by ultracentrifugation for 16 hr at 100,000 *g* through a solution of the appropriate maximum density. A tube-slicing device was employed to isolate the lipoproteins following ultracentrifugation (9). Each lipoprotein solution was dialyzed for 48 hr at 4°C against 4 liters of the buffered salt solution. The lipoproteins were loaded with labeled FFA by incubation with FFA-coated Celite at 23°C for 30 min. This method has been employed to load plasma and albumin solutions with FFA (8). After the pH of the lipoprotein–FFA solution was adjusted to 7.4, protein (10), FFA (11), FFA radioactivity (8), total cholesterol (12), and triglyceride (13) were measured. Each of the washed lipoprotein preparations migrated as a single band on paper electrophoresis that was performed according to the procedure of Lees and Hatch (14). When the radioactive FFA content of the electrophoretogram was to be determined, albumin was omitted from the buffer solution. Additional electrophoretic studies were done to examine the effect of added FFA on the mobility of LDL, the lipoprotein employed in most of the cell experiments. When small quantities of FFA were added to LDL (e.g., 0.34 μeq/mg of LDL-protein), the electrophoretic mobility did not change. However, when more than 0.5 μeq of FFA per mg of LDL-protein was added, the electrophoretic mobility increased, there was decreased staining in the region of the main LDL band, and there was smearing of lipid-staining material for 2–3 cm ahead of the main band. The inherent FFA content of all of the LDL preparations that were used was extremely low (< 0.05 μeq/mg of LDL-protein).

In most experiments, the cells were incubated with buffered salt solution containing lipoprotein-bound radioactive FFA. Air served as the gas phase. All incubations were done in a 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. After 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) (6). 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 this chloroform solution was dried under N_2 and counted in the liquid scintillation spectrometer after 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 obtained from Applied Science Laboratories Inc., State College, Pa., were added to each chromatogram. The lipids were made visible by exposure of the TLC plates to I_2 vapor; the spots 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 (15), and the radioactivity contained in each segment was measured.

CO_2 collections were made in special flasks containing removable center wells (6). 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 the center well were added to 18 ml of the toluene–methanol scintillator solution for measurement of radioactivity. In each experiment, duplicate incubations containing 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 containing cells.

In one group of experiments, the cells were exposed briefly to an LDL–palmitate-1-^{14}C solution, washed thoroughly, 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. In some of these experiments, the release of radioactivity into the second incubation medium also was followed. In these incubations, the cells were sedimented from the second medium at 7,000 *g* for 5 min at 0°C, and a 1-ml aliquot of the supernatant solution was extracted with 20 ml of chloroform–methanol solution. The content and distribution of radioactivity present in the chloroform phase were determined.

Precipitation of lipoproteins was done with heparin and 1 M $MnCl_2$ as described by Fredrickson, Levy, and Lees (16).

RESULTS

Characterization of LDL-bound Radioactive FFA

Table 1 shows the distribution of radioactivity following electrophoresis of LDL-bound palmitate-1-^{14}C using an albumin-free barbital buffer, pH 8.6. A single lipoprotein band was detected in each of the four electrophoretograms. This band was much more diffuse than in corresponding electrophoretograms run in the albumin-containing buffer (14), but all of the lipid-staining material still was located in the region of the strip where β-lipoprotein is known to migrate. More than 99% of the radioactivity recovered from each of the electrophoretograms was present in the zone containing the lipid-staining material. A maximum of 0.04% of the radioactivity present in these samples was recovered from the albumin zone. In contrast, when Fraction V human serum albumin containing palmitate-1-^{14}C was subjected to electrophoresis in this system, only 1.1% of the radioactivity was recovered in the β-lipoprotein zone, and 95% of it was located in the albumin zone.

The LDL and albumin solutions also were incubated with heparin and $MnCl_2$ (16). Approximately 99% of the radioactivity was precipitated in the LDL samples, but only 3.1% of the radioactivity was precipitated in the sample containing albumin. Taken together, these results indicate that the palmitate-1-^{14}C added to solutions containing LDL actually was associated with the lipoprotein.

TABLE 1 CHARACTERIZATION OF THE LDL-BOUND RADIOACTIVE FFA

Sample	Percentage of Radioactivity: Migrating with Lipoprotein*	Percentage of Radioactivity: Precipitated by Heparin and $MnCl_2$†
	%	
LDL 1	99.9	99.5
LDL 2	99.9	99.6
LDL 3	99.8	99.4
LDL 4	99.8	98.9
Albumin	1.1	3.1

* Palmitate-1-^{14}C, 2 mCi/mmole, was added to four samples of LDL and to Fraction V human serum albumin. Two aliquots of each solution were subjected to electrophoresis using an albumin-free barbital buffer, pH 8.6. One of each pair of LDL electrophoretograms was stained with Oil Red O, and one of the albumin-containing strips was stained with bromthymol blue. The stained electrophoretograms served as references to locate the lipoprotein and albumin zones on the unstained strips. The latter were cut into segments, and the ratioactivity present in each zone was measured in a liquid scintillation spectrometer using a toluene–methanol scintillator solution. Each electrophoretogram contained 2–3 × 10^4 dpm.

† The LDL and albumin solutions were incubated with heparin and $MnCl_2$ (16). Additional aliquots of these solutions were incubated with heparin and distilled water, and these samples served as the controls. After centrifugation, the radioactivity present in an aliquot of each supernatant solution was measured after addition to a toluene–methanol scintillator solution. The radioactivity that was precipitated in the samples containing $MnCl_2$ was calculated by difference.

Utilization of Lipoprotein-bound FFA by Cell Suspensions

The kinetics of fatty acid incorporation during a 1-hr incubation of lipoprotein-bound FFA with Ehrlich ascites cells is shown in Table 2. Lipoproteins were the only proteins present in these incubation media. In each experiment there was a rapid uptake of radioactive FFA by the cells, and 41–70% of the radioactivity initially present in the medium was incorporated by the cells after only 2 min of incubation. The rate of FFA incorporation was much slower between 2 and 60 min, probably because much less labeled FFA was available in the medium for uptake. After 60 min, 50–75% of the available radioactivity was taken up by the cells. This "total" uptake refers to all of the labeled FFA incorporated by the cells, i.e., that present in the cells as well as that oxidized to CO_2.

In experiments 1 and 2, different cell preparations were incubated with aliquots of the same LDL–palmi-

TABLE 2 UPTAKE AND UTILIZATION OF LIPOPROTEIN-BOUND RADIOACTIVE FREE FATTY ACID BY THE CELLS

Expt. No.	Fatty Acid		Lipoprotein		Duration of Incubation†	Total Fatty Acid Uptake by the Cells‡	Utilization of Radioactive Fatty Acid			
	Type	Amount	Type*	Amount of Protein			CO_2	Phospholipids	Neutral Lipid Esters	FFA
		μmole		*mg*	*min*	*μmole/10⁸ cells*	*nmole/10⁸ cells*			
1	Palmitate-1-^{14}C	2.4	LDL	1.9	2	1.0	0	54	12	960
					30	1.2	110	290	61	740
					60	1.4	310	400	91	640
2	Palmitate-1-^{14}C	2.4	LDL	1.9	2	0.98	0	27	13	940
					30	1.1	100	160	36	820
					60	1.2	180	210	67	740
3	Palmitate-1-^{14}C	0.8	LDL	1.6	2	0.40	0	30	40	330
					30	0.49	110	86	59	230
					60	0.58	240	130	44	160
4	Oleate-1-^{14}C	0.3	LDL	2.0	2	0.14	0	16	14	110
					30	0.15	32	60	21	34
					60	0.18	71	59	24	22
5	Palmitate-1-^{14}C	0.2	VLDL	0.1	2	0.14	0	27	9	110
					30	0.15	38	70	21	17
					60	0.15	45	72	22	10
6	Palmitate-1-^{14}C	1.2	HDL	2.2	2	0.59	0	21	5	560
					30	0.68	92	96	30	460
					60	0.72	190	90	54	390

* The abbreviations used are: LDL, human low density lipoproteins; VLDL, human very low density lipoproteins; HDL, human high density lipoproteins.

† Incubations were done at 37°C with air as the gas phase.

‡ Refers to the total FFA radioactivity incorporated by the cells, i.e., the sum of the lipid radioactivity present in the cells and the cumulative CO_2 radioactivity evolved. There were 0.83–0.96 × 10^8 cells per flask present in these experiments.

tate-1-^{14}C solution. The small quantitative differences in uptake that were noted probably resulted from variability in the two preparations of cells. Qualitatively similar results were obtained when the palmitate-1-^{14}C concentration was either high or low, when palmitate was replaced by oleate, and when LDL was replaced by either very low or high density lipoproteins. However, the amount of fatty acid incorporated by the cells was dependent upon the amount of FFA available in the incubation medium and the ratio of FFA to lipoprotein.

The distribution of the FFA radioactivity incorporated into cell lipids also is shown in Table 2. In each experiment there was a progressive increase in the amount of radioactive FFA oxidized to $^{14}CO_2$. Likewise, much more radioactivity was present in cell lipid esters after 30 min than after 2 min and, in most cases, the cell lipid ester radioactivity continued to increase between 30 and 60 min. In contrast, the maximum amount of cell FFA radioactivity was present at the earliest time tested, 2 min. The amount of cell FFA radioactivity decreased as the incubation continued.

In additional experiments, lipoproteins present in the mouse ascites plasma in which the Ehrlich cells were grown were employed as the FFA carrier. The cells readily took up palmitate-1-^{14}C from these lipoproteins also, and the distribution of the incorporated radioactivity was generally similar to that observed when human plasma lipoproteins served as the FFA carrier.

Table 3 shows the specific radioactivity of the medium FFA during the course of a 1-hr incubation with cells. The specific radioactivity was essentially unchanged after 2 min of incubation, but it decreased markedly thereafter. No changes were noted when the incubations were done in the absence of cells. Therefore, the decrease in medium FFA specific radioactivity was cell-mediated, probably resulting from either lipolysis of intracellular lipids or lipolysis of LDL lipid esters by a lipase released from the cells.

TABLE 3 CHANGES IN MEDIUM FFA SPECIFIC RADIOACTIVITY DURING INCUBATION WITH CELLS*

Duration of Incubation	Medium FFA Specific Radioactivity	
	Expt. 1	Expt. 2
min	*dpm/nmole*	
0	104	103
2	102	99
30	82	68
60	64	54

* The incubation media contained 0.33 μmole of palmitate-1-^{14}C, LDL equivalent to 1.48 mg of LDL-protein, and 10^8 cells. Incubation was at 37°C with air as the gas phase. Each value is the mean of two determinations.

The uptake of radioactive FFA and LDL-protein is compared in Table 4. Much more of the available FFA than LDL-protein was taken up by the cells. Therefore, most of the FFA uptake was the result of transfer of fatty acid from LDL to the cells, not from irreversible incorporation of the FFA–lipoprotein complex.

As illustrated in Fig. 1, palmitate-1-^{14}C and oleate-1-^{14}C uptake increased markedly as the ratio of FFA to LDL was raised. LDL concentration was measured as LDL-protein. In these experiments, the LDL concentration of each incubation medium was the same, and the ratio was varied by adding different quantities of FFA. A similar increase in FFA uptake as a function of increasing FFA–LDL ratio was noted when the FFA concentration was held constant and the LDL concentration was changed.

FFA uptake decreased markedly when FFA-poor serum albumin was added to the incubation medium. Data from an experiment with LDL-bound palmitate-1-^{14}C are shown in Fig. 2. The amount of palmitate-1-^{14}C taken up by the cells in 2 min was 97% less when the medium contained 2 μmoles of albumin as compared with an albumin-free medium.

Utilization of FFA Previously Incorporated into the Cells

In the following experiments, cells were exposed for 5 min to LDL-bound palmitate-1-^{14}C, isolated, washed thoroughly, and then incubated in a second medium. In this way, the utilization of the radioactivity taken up by the cells during the initial exposure could be followed.

Fig. 3 illustrates the results of an experiment in which a protein-free medium was employed for the second incubation. Initially, 75% of the cell radioactivity was in the form of FFA. As the incubation progressed, there was a marked fall in cell FFA radioactivity. This was accompanied by the production of increasing quantities of

TABLE 4 COMPARISON BETWEEN RADIOACTIVE FFA AND LDL-PROTEIN UPTAKE BY THE CELLS*

Expt. No.	Duration of Incubation	Medium Content	
		FFA Radioactivity	LDL-protein
	min	*dpm* × 10^{-2}	*mg*
1	0	159	1.24
	2	64	1.18
	30	22	1.09
2	0	116	1.56
	2	68	1.42
	30	35	1.50

* The incubation media in experiment 1 contained 0.2 μmole of palmitate-1-^{14}C and 1.1 × 10^8 cells. In experiment 2, the media contained 0.31 μmole of palmitate-1-^{14}C and 0.9 × 10^8 cells. Incubation was at 37°C with air as the gas phase. Each value is the mean of two determinations.

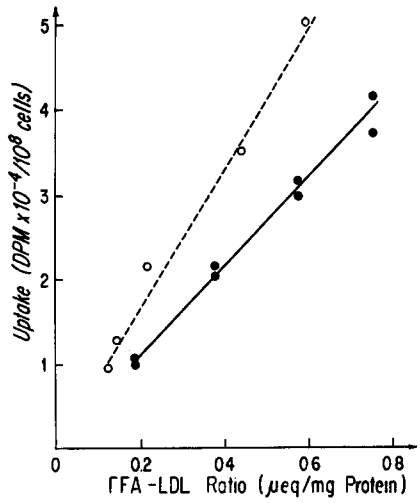

FIG. 1. Effect of ratio of FFA to LDL-protein on FFA uptake, The solid curve illustrates the results of an experiment with palmitate-1-^{14}C; the dashed curve represents an experiment with oleate-1-^{14}C. Incubation was for 2 min at 37°C with air as the gas phase. Each palmitate incubation medium contained 9.4 × 10^7 cells and LDL equivalent to 1.35 mg of LDL-protein in a total volume of 3 ml. The palmitate-1-^{14}C content of the media varied from 254 to 1020 nmoles. Each oleate incubation medium contained 9.6 × 10^7 cells and LDL equivalent to 3.58 mg of LDL-protein in a total volume of 3 ml. The oleate-1-^{14}C content of the media varied from 444 to 2130 nmoles. Each experiment was done with a different cell and LDL preparation.

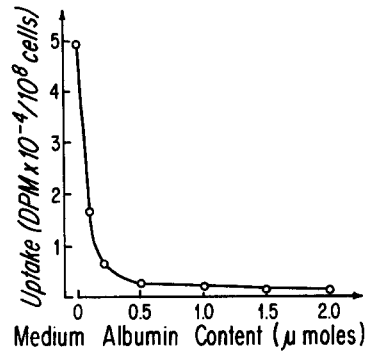

FIG. 2. Effect of medium albumin concentration on the uptake of LDL-bound FFA. Incubation was for 2 min at 37°C with air as the gas phase. Each medium contained 6.8 × 10^7 cells, LDL equivalent to 1.55 mg of LDL-protein, and 1.1 μmoles of palmitate-1-^{14}C (9.6 × 10^4 dpm). The total volume of each incubation medium was 5 ml.

$^{14}CO_2$. Small increases in the cell phospholipid and neutral lipid ester radioactivity also occurred as the incubation continued. The total amount of radioactivity recovered at 60 min was 7% greater than the radioactivity contained in the cells at the start of the second incubation. The reason for this discrepancy was not investigated, but it probably results in part from the presence in the cells of radioactive water-soluble intermediates at the start of the second incubation (17).

Quite different results were observed when the second incubation medium contained albumin. The cell radio-

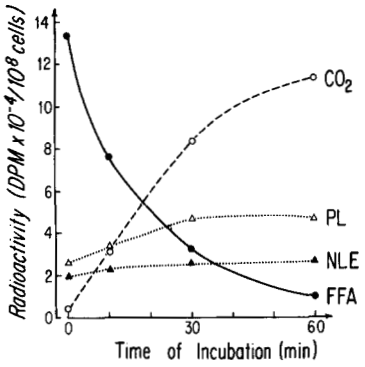

FIG. 3. Utilization of FFA deposited in the cells during a prior incubation. Initially, 2×10^9 cells were incubated for 5 min at 37°C in 20 ml of a solution containing palmitate-1-^{14}C bound to LDL. This medium contained 25.4 mg of LDL-protein and 7.5 μmoles of palmitate-1-^{14}C (8.2×10^4 dpm/μmole). The cells were isolated after incubation, washed three times, and then resuspended in buffer solution. Prior to the second incubation, 1-ml aliquots of the cell suspension were extracted with chloroform–methanol in order to determine the initial content and distribution of the cell lipid radioactivity. These "loaded" cells contained 0.21 μmole of radioactive fatty acid per 10^8 cells. Additional 1-ml aliquots of the cells were suspended in a protein-free medium, and the cell lipid and CO_2 radioactivity were determined during the course of a 1-hr incubation. The abbreviations used are: PL, phospholipids; NLE, neutral lipid esters.

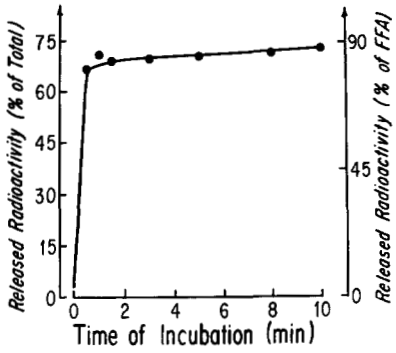

FIG. 4. Release of cell radioactivity to the incubation medium. The percentage of the cell total lipid radioactivity that was released into the medium is shown on the left ordinate; the percentage of the cell FFA radioactivity released into the medium is shown on the right ordinate. Methods similar to those described in Fig. 3 were employed. Initially, 2.5×10^9 cells were incubated for 5 min at 37°C with 20 ml of palmitate–LDL solution containing 14.8 mg of LDL-protein and 22.9 μmoles of palmitate-1-^{14}C (9.5×10^4 dpm/μmole). Prior to the second incubation, the cells contained 0.34 μmole of labeled fatty acid, 81% of which was present as FFA. Aliquots of the labeled cells were incubated with 2 μmoles of albumin for 0.5 to 10 min at 37°C. The total volume of the incubation medium was 3 ml, and air served as the gas phase. After incubation, the cells were removed by centrifugation, and the amount and distribution of the lipid-soluble radioactivity present in the supernatant solution was determined.

activity content decreased much more rapidly than in the protein-free medium. However, there was almost no increase in cell phospholipid and neutral lipid ester radioactivity, and very little $^{14}CO_2$ was produced. Instead, as shown in Fig. 4, much of the radioactivity initially contained in the cells was released into the incubation medium. Almost all of the release occurred during the first 30 sec of incubation in albumin. The released radioactivity was equivalent to 72% of the initial cell total lipid radioactivity and 88% of the initial cell FFA radioactivity. Thin-layer chromatography revealed that 91 ± 1.0% (mean ± SE, n = 10) of the released radioactivity was in the form of FFA. Only the cell FFA fraction contained sufficient radioactivity to account for the amount that was released. Taken together, these data indicate that much of the radioactivity initially taken up by the cells remained as FFA and was exchangeable with medium FFA. However, even when a large excess of albumin was present, a small amount of the cell FFA radioactivity was not released during a single exposure to the second incubation medium. Some nonexchangeable cell FFA radioactivity also was noted when cells were loaded with albumin-bound palmitate instead of LDL-bound palmitate (6, 7).

DISCUSSION

These data demonstrate that lipoprotein-bound FFA is readily available for uptake by a suspension of Ehrlich ascites cells. Much of the newly incorporated FFA could be removed from the cells by exposure to a solution containing albumin. When Ehrlich ascites cells are incubated with FFA–albumin solutions, a large percentage of the FFA uptake also is exchangeable (6). The amount of LDL-bound FFA that was taken up by the cells was dependent upon the FFA–protein ratio, a finding that also was noted with albumin-bound FFA (6). Moreover, the distribution of FFA radioactivity among phospholipids, neutral lipid esters, and CO_2 was similar in the present experiments, as it was in those where albumin served as the FFA carrier (7). These results suggest that lipoprotein-bound FFA is utilized by Ehrlich cells in a manner that is similar to albumin-bound FFA.

There are three possible mechanisms for the uptake of lipoprotein-bound FFA: interaction between the lipoprotein and the cell membrane, dissociation of the FFA into an unbound intermediate, or release of a carrier substance from the cells which shuttled FFA from the lipoprotein to the cell. The present results do not distinguish among these mechanisms. However, they exclude the possibility that an appreciable amount of the FFA uptake was secondary to irreversible incorporation of the intact FFA–lipoprotein complex.

The possibility that small amounts of albumin adsorbed on the cell surface are involved in the transfer of FFA from lipoprotein to cell also cannot be excluded. Indeed, the ascites plasma in which the Ehrlich cells were grown contained albumin. However, in preparing the

cells for in vitro incubation, they were exposed briefly to a hypotonic solution and then washed repeatedly with large quantities of protein-free buffered salt solution (6). Moreover, addition of even very small amounts of albumin to the incubation medium (final concentration of 2×10^{-5} M) inhibited FFA uptake (see Fig. 2). Since the binding of FFA to albumin is much stronger than to either LDL (3) or the cell membrane (18–20), it is likely that albumin competed for the LDL-bound FFA more effectively than the cells and thus reduced its availability for uptake. A 2-min incubation was employed in this experiment. Under these conditions, FFA uptake decreases markedly as the FFA–albumin molar ratio is lowered (6). Initially, the LDL contained 1.1 μmoles of palmitate-1-^{14}C. The percentage of available palmitate that associated with albumin no doubt depended on the amount of albumin added to the medium. However, if one makes the simplifying assumption that all of the palmitate was associated with the albumin, the palmitate–albumin molar ratio was approximately 5 when 0.2 μmole of albumin was present and 0.5 when 2 μmoles of albumin were present. One would expect that the amount of FFA taken up by the cells in 2 min would be much larger when the FFA–albumin molar ratio was 5 than when it was 0.5 (6). Therefore, the observed decrease in palmitate uptake as the medium albumin content was raised can be explained by the fact that progressively more of the FFA was associated with strong albumin binding sites.

We have shown previously that FFA can be taken up by Ehrlich cells from a protein-free medium (6). However, the design of these experiments was limited because of the very low solubility of FFA in aqueous solutions. Only FFA binding to the cells was examined, and adequate quantitative measurements of FFA utilization were not made. In other experiments, we observed that FFA bound to β-lactoglobulin could be readily utilized by mammalian tissues (21). The interpretation of this finding also was not completely clear with respect to the cellular FFA transport mechanism, for β-lactoglobulin is similar enough in structure to albumin to possibly substitute for the latter in the uptake process. This possibility almost certainly does not apply to the plasma lipoproteins, particularly LDL, which has a grossly different structure from that of serum albumin (22). Hence, the demonstration that LDL-bound FFA is readily utilized in the absence of medium albumin supports the view that albumin merely serves to solubilize FFA in the plasma and extracellular fluid and has no specific role in transferring FFA into the cell.

One difference between the present results and those obtained with albumin is the marked decrease in cell FFA radioactivity during the course of the 1-hr incubation (see Table 2). When albumin was used as the FFA carrier, the cell FFA content remained constant as the incubation progressed (7). There are two likely causes for the decrease noted with LDL-bound FFA. First, a much larger percentage of the extracellular FFA was utilized by the cells when LDL served as the transport vehicle. If the cell FFA radioactivity represents a steady-state amount that is dependent upon the medium FFA content (7), then one would expect a larger decrease in the presence of LDL because of the greater depletion of labeled FFA from the medium. In addition, the decrease in medium FFA specific radioactivity in the LDL experiments (see Table 3) was much larger than in similar albumin experiments (23). This probably was due in part to a cell-mediated hydrolysis of LDL triglycerides. The ratio of triglyceride to total cholesterol in the LDL preparations was 0.22 ± 0.014 (mean ± SE, 13 determinations). Previous work indicates that Ehrlich cells can hydrolyze long-chain fatty acid esters that are present in the extracellular fluid (24). Therefore, the more rapid decline in cell FFA radioactivity during incubations with LDL can be explained adequately and probably does not constitute a fundamental difference in the utilization of LDL-bound and albumin-bound FFA.

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