Utilization of ascites plasma very low density lipoprotein triglycerides by Ehrlich cells

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Abstract Much of the lipid present in the ascites plasma in which Ehrlich cells grow is contained in very low density lipoproteins (VLDL). Chemical measurements indicated that triglycerides were taken up by the cells during in vitro incubation with ascites VLDL. When tracer amounts of radioactive triolein were incorporated into the ascites VLDL, the percentage uptakes of glyceryl tri[1-^{14}C]oleate and triglycerides measured chemically were similar. The cells also took up [2-^{3}H]glyceryl trioleate that was added to VLDL, but the percentage of available ^{3}H recovered in the cell lipids was 30–40% less than that of ^{14}C from glyceryl tri[1-^{14}C]oleate. This difference was accounted for by water-soluble ^{3}H that accumulated in the incubation medium, suggesting that extensive hydrolysis accompanied the uptake of VLDL triglycerides. Radioactive fatty acids derived from the VLDL triglycerides were incorporated into cell phospholipids, glycerides, and free fatty acids, and they also were oxidized to CO_2. Triglyceride utilization increased as the VLDL concentration was raised. These results suggest that one function of the ascites plasma VLDL may be to supply fatty acid to the Ehrlich cells and that the availability of fatty acid to this tumor is determined in part by the ascites plasma VLDL concentration. Although Ehrlich cells incorporate almost no free glycerol into triglycerides, considerable amounts of [2-^{3}H]glyceryl trioleate radioactivity were recovered in cell triglycerides. This indicates that at least some VLDL triglycerides were taken up intact. The net uptake of VLDL protein and cholesterol was very small relative to the triglyceride uptake, suggesting that intact triglycerides are transferred from the ascites VLDL to the Ehrlich cells and that hydrolysis occurs after the triglyceride is associated with the cells.

Supplementary key words tumors · fatty acids · esterification · oxidation · hydrolysis · cholesterol

Ehrlich ascites tumor cells grow in the peritoneal cavity of male mice (1), and they are bathed in an ascites plasma that contains free fatty acids and lipoproteins (2). In vitro studies with radioactive free fatty acids have demonstrated that Ehrlich cells can take up and metabolize this substrate (3–5). Moreover, turnover studies have shown that the ascites plasma free fatty acid is a quantitatively important substrate for the growing cells (6). Since fatty acid esters are present in the lipoproteins of the ascites plasma, we wondered whether they, too, might be a potential source of lipid for the Ehrlich cell. Analysis of the ascites plasma revealed that most of the esterified fatty acid was contained in very low density lipoproteins (VLDL) in the form of triglycerides (2). In the present communication, we demonstrate that the ascites plasma VLDL triglycerides can be utilized by the Ehrlich cells during in vitro incubation. Some data concerning the mechanism of VLDL triglyceride uptake by these cells also are described.

METHODS

Triglyceride substrates

Glyceryl tri[1-^{14}C]oleate and [2-^{3}H]glyceryl trioleate were obtained from Amersham/Searle, Arlington Heights, Ill. The purity of the isotopes was greater than 98% as indicated by thin-layer chromatography with a solvent system containing hexane–diethyl ether–acetic acid–methanol 180:40:6:4 (7). Di- and monoglycerides were the major contaminants. After saponification, more than 98% of the glyceryl tri[1-^{14}C]oleate radioactivity was in the saponifiable fraction, and more than 98% of the [2-^{3}H]glyceryl trioleate radioactivity was in the aqueous layer.

Lipoprotein isolation and labeling

The Ehrlich ascites tumor was obtained from male CBA mice on days 12–14 after transplantation (8). Chilled tubes were employed to collect the tumor, and all centrifugations were done at 5°C. The ascitic suspension was centrifuged at 600 *g* for 5 min and then at 2000 *g* for 5 min to separate the ascites fluid from the tumor cells. At this point, 1 mg/ml EDTA and 25 μg/ml penicillin and streptomycin were added to the fluid. Chylomicrons were removed from the ascites plasma by centrifugation at

Abbreviations: VLDL, very low density lipoprotein.

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30,000 *g* for 30 min (9). VLDL was isolated from the infranatant solution by preparative ultracentrifugation at 100,000 *g* for 16 hr at plasma density (1.006 g/ml) (2). Each VLDL preparation was washed once by flotation through a NaCl solution at density 1.006. A tube-slicing device was used to isolate the lipoproteins after each ultracentrifugation (10). The purity of lipoprotein preparations was checked either by cellulose acetate (11) or agarose gel electrophoresis (12) using an oil red O stain. Each ascites plasma VLDL preparation migrated as a single band in the alpha region as defined by the mobilities of the lipoproteins present in normal human plasma (2). Most of the lipid in the Ehrlich ascites plasma was recovered in the VLDL: 88 ± 5% of the total plasma triglycerides and 60 ± 9% of the total plasma cholesterol (means ± SEM of six determinations). The physiological triglyceride concentration in the ascites plasma was 2270 ± 520 nmoles/ml, and the cholesterol concentration was 1088 ± 233 nmoles/ml.

Radioactive triglycerides were incorporated into the VLDL solution by incubation of the isolated lipoproteins with triglyceride-coated Celite. The method was similar to that used to incorporate radioactive cholesterol (13), triglycerides (14), and free fatty acid (15) into human plasma lipoproteins. Radioactive triglyceride was dissolved in hexane and added to Celite (Johns-Manville, L 665-A, 7–9 μm). 0.75–1.0 μCi of ^{14}C and 2–3 μCi of ^{3}H were added per mg of Celite, and the hexane was evaporated under N_2. The specific activities of the ^{14}C- and ^{3}H-labeled trioleins were 36 mCi/mmole and 195 mCi/mmole, respectively. For labeling, VLDL preparations varying in triglyceride content from 450 to 700 nmoles/ml were incubated with the triglyceride-coated Celite for 2 hr at 23°C. These incubations contained 1 mg of Celite/ml of the VLDL solution. After incubation, the suspension was centrifuged at 10,000 *g* for 10 min at 4°C to sediment the Celite: the supernatant solution was passed through a Millipore filter of 1.2 μm pore size to ensure that all Celite particles were removed. Under these conditions, approximately 5% of the available radioactivity was incorporated into VLDL. The distribution of radioactivity in the loaded VLDL was determined by thin-layer chromatography; triglyceride comprised 93–97% of the label in all preparations used in these experiments. If triglycerides contained less than 93% of the radioactivity, the VLDL was purified by washing with bovine serum albumin (5 nmoles/ml of VLDL), and the VLDL was reisolated by preparative ultracentrifugation. After this wash, the triglycerides contained at least 93% of the total radioactivity present in the VLDL solution.

Ascites VLDL was also labeled in vivo with L-[^{3}H]valine. Mice were fasted for 12 hr and then injected intraperitoneally with 20 μCi of generally labeled L-[^{3}H]valine (1.3 Ci/mmole, New England Nuclear, Boston, Mass.). The ascitic suspension was collected 4 hr after injection of the isotope, and the ascites plasma was separated from the tumor cells by centrifugation as described above. The ascites plasma was dialyzed against 0.15 M NaCl for 24 hr. The NaCl solution was changed four times during the dialysis. VLDL then was isolated by ultracentrifugation. After washing, aliquots of the VLDL were precipitated with 10% trichloroacetic acid. All of the radioactivity contained in the aliquot was recovered in the precipitate, indicating the absence of free [^{3}H]valine. Furthermore, chloroform–methanol extraction of aliquots of the VLDL revealed that only 15–20% of the valine radioactivity was present in lipids. The [^{3}H]valine-labeled VLDL was used to study the uptake of VLDL protein during cellular incubations. Therefore, it was necessary to remove the radioactivity present in VLDL lipids prior to assay of the incubation medium. This was done by delipidating aliquots of the VLDL-containing medium with ethanol–ether 3:1 for 16 hr at 4°C (16). The protein precipitate was then solubilized with Soluene-100 (Packard Instrument Co., Downers Grove, Ill.) and assayed for radioactivity.

Cell incubations

The Ehrlich cells that were isolated from the ascites were washed as described previously (8). Suspensions of the cells were incubated with VLDL in a buffer solution containing 0.058 M NaCl, 0.0025 M KCl, 0.006 M $MgSO_4$, 0.085 M Na_2HPO_4, and 0.011 M glucose adjusted to pH 7.4 with 1 N HCl. The total volume of the incubation medium was 5.5 ml, and it contained 1.4–2.0 × 10^8 cells and 310–3500 nmoles of triglyceride. The ratio of VLDL triglycerides to the quantity of cells in these media was in the physiological range in all experiments concerned with triglyceride utilization. Incubations were performed at 37°C in a temperature-controlled water bath with shaking. Air served as the gas phase. Incubations were terminated by pouring the contents of each flask into chilled polypropylene centrifuge tubes and sedimenting the cells at 600 *g* for 5 min at 4°C. The cells were washed by resuspension in 10 ml of 0.15 M NaCl and centrifugation at 600 *g* for 5 min. Sedimentation and washing were repeated twice to remove any loosely adsorbed material. After washing, the cells were extracted with chloroform–methanol 2:1 (v/v) (17). The chloroform phase of the extract was isolated, and aliquots were taken for measurement of total lipid radioactivity and for thin-layer chromatography in the hexane–diethyl ether–acetic acid–methanol system. Lipid standards obtained from Applied Science Laboratories, Inc., State College, Pa., were added to each chromatogram. Lipids were made visible with I_2. After sublimation of the I_2, the radioactivity present in segments of the silica gel containing (*a*) phospholipids, (*b*) monoglycerides and diglycerides, (*c*) free fatty acid, (*d*) triglyceride, and (*e*) cholesteryl esters was

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TABLE 1. Comparison of triglyceride and total cholesterol depletion from ascites plasma VLDL during incubation with Ehrlich ascites tumor cells

Incubation Time	Decrease in Medium Content[a]	
	Triglycerides	Total Cholesterol
min	%	
15	12.3 ± 0.8	4.9 ± 0.4
45	15.0 ± 1.1	3.9 ± 0.6
75	16.9 ± 0.4	3.9 ± 0.6

[a] Each value is the mean of four determinations ± SE. The medium initially contained 2.60 μmoles of triglyceride and 1.10 μmoles of cholesterol. Each flask contained 1.5×10^8 cells.

measured. The aqueous-methanol phase of the lipid extract was evaporated to dryness and also assayed for radioactivity.

For analysis of the medium, the contents of the incubation were centrifuged first at 600 *g* for 5 min at 4°C. The fluid was decanted and recentrifuged at 2000 *g* for 10 min. Aliquots of the supernatant solution were extracted with chloroform–methanol 2:1, and the lipid and aqueous phases were separated with 0.04 N HCl. Both phases were evaporated to dryness, and their radioactivity content was measured in a 2425 Packard Tri-Carb refrigerated liquid scintillation spectrometer. A dioxane-containing scintillation solution was used (18), and quenching was monitored with the external standard. In all experiments, labeled VLDL was incubated without cells as a control. Lipid extracts of these control media were checked by thin-layer chromatography for triglyceride hydrolysis. No radioactivity was lost from the incubation media, and no hydrolysis of the labeled triglycerides was observed in these cell-free controls.

Collection of $^{14}CO_2$ was made using incubation flasks with removable center wells containing 0.2 ml of 2 N KOH (19). In order to assay for radioactivity in these CO_2 experiments, the center wells were added to 18 ml of toluene–methanol scintillation solution (20).

In order to evaluate the leakage of enzymes from the cells, lactate dehydrogenase activity was measured in the cells prior to incubation and in the medium after 30 min of incubation with VLDL. Lactate dehydrogenase activity was measured by the method of Hsieh and Vestling (21). Immediately after the cells were added to the VLDL solution, 2% of the lactate dehydrogenase activity was recovered in the supernatant fluid after removal of the cells by centrifugation. After 30 min of incubation, 2.2% of the activity was recovered in the supernatant solution. Therefore, almost no lactate dehydrogenase was released from the cells during incubation with the medium containing VLDL. In additional experiments, we found that no lipolytic activity was released from the cells during 60 min of incubation. The substrate for these lipase assays was ascites plasma, and lipolytic activity was measured as free fatty acid by titration (22).

Chemical methods

Triglycerides were measured in an aliquot of the chloroform–methanol extract by the automated method of Kessler and Lederer (23). Cholesterol was determined by the method of Abell et al. (24). Protein was analyzed by a modification of the Lowry procedure (25).

RESULTS

Uptake of VLDL lipids

Table 1 shows the time course of VLDL triglyceride and total cholesterol depletion from the medium during incubation with Ehrlich cells. Most of the decrease in medium triglycerides occurred during the first 15 min of incubation. However, there was a slower but continuing depletion of medium triglycerides as the incubation continued, and the amount remaining in the medium after 75 min was significantly smaller than after 15 min ($P < 0.01$). A decrease in the medium cholesterol content also occurred during the first 15 min, but there was no further change in the total cholesterol content as the incubation progressed. In control experiments without cells, no change in either medium triglyceride or cholesterol content occurred during 75 min of incubation.

Prior to incubation, the molar ratio of triglycerides to total cholesterol in the VLDL was 2.3. The molar ratio of triglycerides to cholesterol taken up by the cells, as calculated from the decreases in the incubation medium, was 5.8 at 15 min and 9.4 at 75 min. Therefore, even at 15 min, not all of the triglyceride taken up by the cells can be explained by binding or incorporation of intact VLDL. There was no detectable change in the lipoprotein electrophoretic pattern of the medium after incubation of the VLDL with the cells.

Uptake of VLDL proteins

In all of the studies reported in this communication, VLDL was the only protein added to the extracellular medium. During incubations with cells, we could demonstrate no statistically significant change in the VLDL protein content of the medium. In a typical experiment, 1.160 ± 0.020 mg of VLDL protein was incubated for 10 and 60 min with the cells. After 10 min, the medium contained 1.120 ± 0.017 mg of VLDL protein; after 60 min, it contained 1.160 ± 0.063 mg of VLDL protein (means ± SE of four determinations). These values for VLDL protein were obtained after subtraction of the amount of protein that was released from the Ehrlich cells during the incubation. Based upon these experiments, only a small percentage (<3%) of the VLDL could be bound intact to

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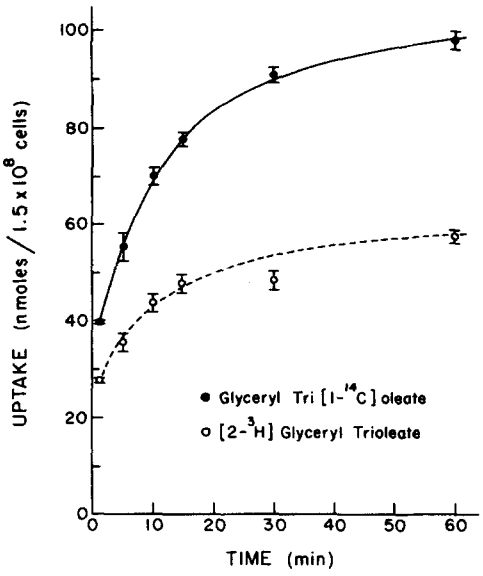

Fig. 1. Kinetics of glycerol tri[1-^{14}C]oleate and [2-^{3}H]glyceryl trioleate uptake by Ehrlich cells. The radioactive triglycerides were incorporated into ascites plasma VLDL. Each flask contained 1650 nmoles of VLDL triglycerides in a total volume of 5.5 ml. Incubation was at 37°C with air as the gas phase. Each point is the mean of four determinations ± SE.

the cells. Because of the uncertainties associated with these protein determinations, estimates of VLDL protein uptake also were made using L-[^{3}H]valine-labeled VLDL (**Table 2**). In both experiments, only a very small decrease (<2%) was noted in the VLDL protein radioactivity present in the medium after 1 and 60 min of incubation. Therefore, these data are in complete agreement with the results obtained from the experiments in which VLDL protein was measured chemically. Taken together, they indicate that most of the VLDL triglyceride uptake is not due to incorporation or irreversible binding of intact VLDL.

Comparison of chemical and isotopic triglyceride uptake

VLDL was labeled with glyceryl tri[1-^{14}C]oleate, and the depletion of VLDL triglycerides and radioactivity from the incubation medium was compared (**Table 3**).

TABLE 2. Incubation of [^{3}H]valine-labeled VLDL with Ehrlich ascites cells

Expt. No.	Medium Triglyceride Content	Incubation Time	n	Protein Radioactivity in the Medium[a]	
				Without Cells	With Cells
	μmoles	*min*		*cpm*	
1	9.66	1	2	2175	2132
		60	2	2175	2153
2	11.60	60	4	2441 ± 55	2443 ± 44

[a] Each flask contained 2 × 10^{8} cells.

The percentage decreases of chemically measured and radioactive triglycerides were not significantly different at any of the three VLDL concentrations or time intervals tested. Therefore, within experimental limits, glyceryl tri[1-^{14}C]oleate appears to be a valid tracer for measuring the uptake of inherent VLDL triglycerides in this model system.

Kinetics of VLDL triglyceride utilization

In order to gain insight into the metabolism of VLDL triglycerides by the cells, additional studies were performed with VLDL containing tracer amounts of radioactive triglycerides. Glyceryl tri[1-^{14}C]oleate and [2-^{3}H]glyceryl trioleate were incorporated into the same VLDL preparations, and triglyceride utilization was followed by measuring the radioactivity incorporated into cell lipids. The time course of uptake of these labeled triglycerides is presented in **Fig. 1.** Glyceryl tri[1-^{14}C]-oleate uptake was significantly greater than that of [2-^{3}H]glyceryl trioleate at each time point ($P < 0.001$). In an attempt to explain these differences in uptake between fatty acid- and glycerol-labeled triolein, additional experiments were done in which the aqueous soluble radioactivity present in the incubation medium and in the cells was measured. As seen in **Table 4,** appreciable quantities of aqueous soluble metabolites were recovered in the incubation medium and in the cells. The release of aqueous soluble ^{3}H into the medium increased as the incubation progressed. In addition, the aqueous extract of the cells contained a large quantity of ^{3}H radioactivity after 1 min and 10 min of incubation. At the later time points, however, the cellular aqueous soluble ^{3}H radioactivity decreased. In contrast to the amount of ^{3}H radioactivity, relatively small quantities of aqueous soluble ^{14}C were recovered from the cells and media. If these quantities of aqueous

TABLE 3. Comparison of VLDL triglyceride and glyceryl tri[1-^{14}C]oleate depletion from the medium during incubation with Ehrlich ascites tumor cells

Expt. No.	Incubation Time	Medium Triglyceride Content	Percentage Decrease in Medium Content[a]	
			Triglycerides	Glyceryl Tri[1-C^{14}]oleate
	min	*μmoles*	%	
1	1	1.49	4.4 ± 1.0	6.6 ± 0.7
	10	1.49	11.4 ± 1.4	10.5 ± 0.4
	30	1.49	16.9 ± 1.9	14.9 ± 0.4
2	60	1.82	16.2 ± 2.1	17.8 ± 0.7
	60	2.57	15.0 ± 1.9	15.5 ± 0.5
	60	3.51	15.5 ± 1.1	14.9 ± 1.0

[a] Each value is the mean of four determinations ± SE. There are no statistically significant differences between any of the comparisons of triglyceride mass and glyceryl tri[1-^{14}C]oleate. Each flask contained 1.5 × 10^{8} cells. The specific activities of VLDL triglycerides were 90 and 36 dpm/nmole for experiments 1 and 2, respectively.

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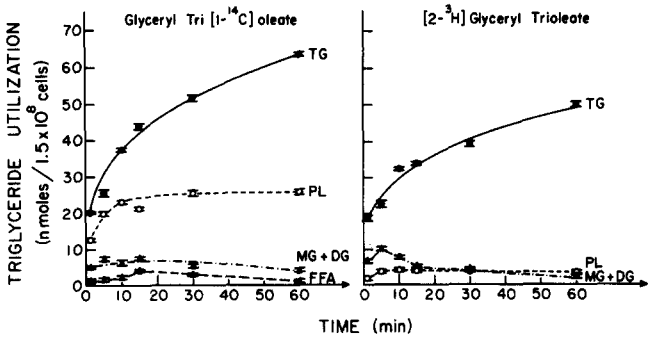

Fig. 2. Utilization of glyceryl tri[1-^{14}C]oleate and [2-^{3}H]glyceryl trioleate by Ehrlich cells. The conditions of incubation are the same as those listed in Fig. 1. Each point is the mean of four determinations ± SE. The abbreviations used are TG, triglyceride; PL, phospholipid; MG + DG, mono- and diglycerides; FFA, free fatty acid.

soluble ^{3}H are added to the ^{3}H recovered in cell lipids, the sum corresponds closely to the total uptake observed with glyceryl tri[1-^{14}C]oleate.

The metabolism of glyceryl tri[1-^{14}C]oleate and [2-^{3}H]glyceryl trioleate during the 1-hr incubation is shown in **Fig. 2.** With both labeled compounds, most of the radioactivity recovered from the cell lipids remained as triglyceride 27–30% of the glyceryl tri[1-^{14}C]oleate radioactivity was recovered in phospholipids. Small amounts of the ^{14}C uptake also were recovered in free fatty acids and lower glycerides. By contrast, very little of the [2-^{3}H]glyceryl trioleate radioactivity was recovered in phospholipids. Small amounts of ^{3}H were found in lower glycerides, but no ^{3}H was recovered in free fatty acids.

As shown in **Table 5,** $^{14}CO_2$ was evolved when the cells were incubated with VLDL containing glyceryl tri[1-^{14}C]oleate. The amount of glyceryl tri[1-^{14}C]oleate oxidized to $^{14}CO_2$ increased as the incubation progressed. At 1 hr, approximately 6% of the total glyceryl tri[1-^{14}C]oleate taken up by the cells was recovered as $^{14}CO_2$.

TABLE 4. Effects of incubation time and VLDL triglyceride concentration on the conversion of glyceryl tri[1-^{14}C]oleate and [2-^{3}H]glyceryl trioleate to aqueous soluble metabolites[a]

Expt. No.	Incubation Time	Medium Triglyceride Content	Release of Aqueous Soluble Metabolites into Medium[b]		Aqueous Soluble Metabolites in Cells[c]	
			^{3}H	^{14}C	^{3}H	^{14}C
	min	*μmoles*	*nmoles*			
1	1	1.50	4.8 ± 0.5	0	9.3	1.9
	10	1.50	12.1 ± 0.7	1.9 ± 0.3	12.7	2.1
	30	1.50	21.8 ± 1.3	3.0 ± 0.5	6.8	1.6
	60	1.50	31.7 ± 0.8	3.1 ± 0.2	3.3	0.7
2	60	0.37	10.6 ± 0.4	1.4 ± 0.3	1.1	0.3
	60	0.75	16.6 ± 0.1	1.6 ± 0.2	2.1	0.5
	60	1.12	22.7 ± 1.1	3.3 ± 0.1	3.6	0.8

[a] Aqueous soluble triglyceride metabolites were calculated by dividing the dpm recovered in the aqueous-methanol layer of the chloroform–methanol extract by the VLDL triglyceride specific activity (90 dpm/nmole, ^{14}C; 240 dpm/nmole, ^{3}H). Each flask contained 1.5 × 10^{8} cells.

[b] Each value is the mean of four determinations ± SE.

[c] Each value is the mean of two determinations.

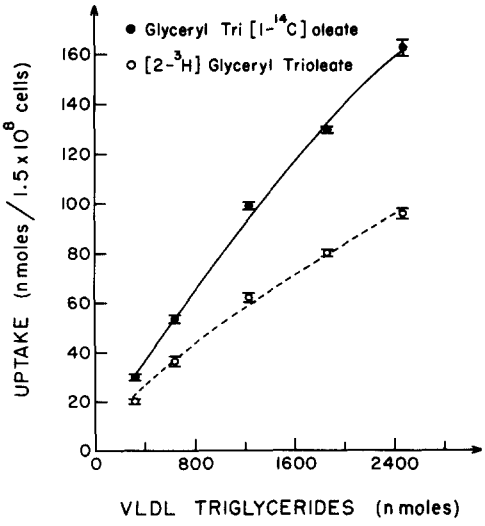

Fig. 3. Effect of VLDL triglyceride concentration on the uptake of glyceryl tri[1-^{14}C]oleate and [2-^{3}H]glyceryl trioleate by Ehrlich cells. The time of incubation was 1 hr at 37°C, and the total volume of the incubation medium was 5.5 ml. Each point is the mean of four determinations ± SE.

Effect of VLDL concentration on triglyceride utilization

Fig. 3 illustrates the effect of VLDL concentration on the uptake of radioactive triglycerides by the cells. Both glyceryl tri[1-^{14}C]oleate and [2-^{3}H]glyceryl trioleate were incorporated into the VLDL preparations used in these experiments. The amount of each labeled lipid taken up by the cells after 60 min of incubation increased as the VLDL concentration increased. At each VLDL concentration, the quantity of ^{14}C recovered in the cell lipids was considerably greater than that of ^{3}H ($P < 0.01$).

TABLE 5. Effects of time and VLDL triglyceride concentration on the oxidation of glyceryl tri[1-^{14}C]oleate to $^{14}CO_2$

Medium Triglyceride Content	Incubation Time	Triglyceride Oxidized to CO_2[a]
μmoles	*min*	*nmoles*
2.60	15	3.68 ± 0.12
2.60	30	8.59 ± 0.23
2.60	60	11.90 ± 0.47
1.95	60	10.10 ± 0.18
1.30	60	6.90 ± 0.23

[a] Each flask contained 2 × 10^{8} cells. Triglyceride oxidation was calculated by dividing the dpm recovered in the KOH by the VLDL triglyceride specific activity. Each value is the mean ± SE of four determinations.

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This difference again was attributable to the formation of aqueous soluble metabolites containing ^{3}H. Table 4 shows that the quantity of aqueous soluble ^{3}H produced increased as the amount of VLDL triglyceride available to the cells was raised. Again, the sum of the aqueous soluble ^{3}H and the ^{3}H in cell lipids corresponded closely to the total uptake observed with glyceryl tri[1-^{14}C]oleate.

The effect of VLDL concentration on the metabolism of glyceryl tri[1-^{14}C]oleate and [2-^{3}H]glyceryl trioleate is shown in **Fig. 4.** At each concentration, the largest amount of cell lipid radioactivity was recovered in triglycerides. With glyceryl tri[1-^{14}C]oleate, a considerable quantity of radioactivity also was present in phospholipids, and the amount of phospholipid radioactivity increased markedly as the VLDL concentration was raised. Only small amounts of radioactivity were recovered in lower glycerides and free fatty acids, but some increase in these fractions also occurred as the VLDL concentration was raised. With [2-^{3}H]glyceryl trioleate, the radioactivity recovered in phospholipids and lower glycerides was small at all of the VLDL concentrations tested. However, as with the ^{14}C-labeled tracer, some increase in the radioactivity contained in these metabolites occurred as the VLDL concentration was raised.

There was a 70% increase in the amount of glyceryl tri[1-^{14}C]oleate oxidized to $^{14}CO_2$ as the VLDL concentration in the incubation medium was raised from 1.3 to 2.6 μmoles of triglyceride (see Table 5).

DISCUSSION

These studies indicate that triglycerides present in the Ehrlich ascites plasma VLDL can be utilized by the Ehrlich cells during in vitro incubation. This finding suggests the possibility that one function of the ascites plasma VLDL may be to supply fatty acids to the growing tumor cells, the fatty acids being transported from the host to the tumor predominantly in the form of triglycerides. During the course of tumor growth, there is considerable variation in the VLDL triglyceride concentration of the ascites plasma. The amounts of VLDL added to the incubation media relative to the quantity of cells were in the same range as those that are present in the ascites plasma during tumor growth (2). As seen in Figs. 3 and 4, the uptake and metabolism of triglycerides by the cells in vitro increased as the VLDL triglyceride concentration was varied within this range. Taken together, these results suggest that the supply of fatty acid from the host to the tumor probably is determined in part by the amount of VLDL delivered into the ascites plasma.

Free fatty acids also are present in the ascites plasma that bathes the Ehrlich cells in the mouse peritoneal cavity (6). Studies with radioactive tracers have led to the conclusion that most of the fatty acid required by these tumor cells for growth and respiration is derived from the ascites plasma free fatty acids (6). Therefore, it was somewhat surprising to find that Ehrlich cells also have the ability to utilize relatively large amounts of extracellular triglycerides. Like Ehrlich cells, mammalian cells in culture utilize lipids that are present in their surrounding medium (26–30). Although free fatty acids are by far the more important substrate in culture systems, several cultured cell lines are also able to utilize lipoprotein triglycerides (29–32). The rates at which Ehrlich cells utilized lipoprotein triglycerides, however, are much greater than those reported for triglyceride utilization by cells in culture. A possible explanation for this quantitative difference concerns the fact that the Ehrlich cells were exposed to homologous VLDL, whereas most of the tissue culture studies were done with heterologous serum containing a mixture of lipoproteins. In this context, studies with WI-38 cells in culture indicate that triglyceride utilization is reduced markedly when free fatty acids are added to the medium (30). The only substrate besides VLDL lipids that was available in the present incubations was glucose, a substance that is known to increase fatty acid esterification in Ehrlich cells (33). Glucose is transferred from the blood plasma to the peritoneal cavity of mice bearing the Ehrlich ascites carcinoma. It is utilized so rapidly by the cells, however, that no glucose can actually be detected in the ascites plasma between days 4 and 12 of tumor growth (34). Therefore, it is possible that the rates of VLDL triglyceride utilization noted in the present work may be considerably higher than those occurring in the intact ascites plasma.

Most of the tissue culture studies indicate that triglycer-

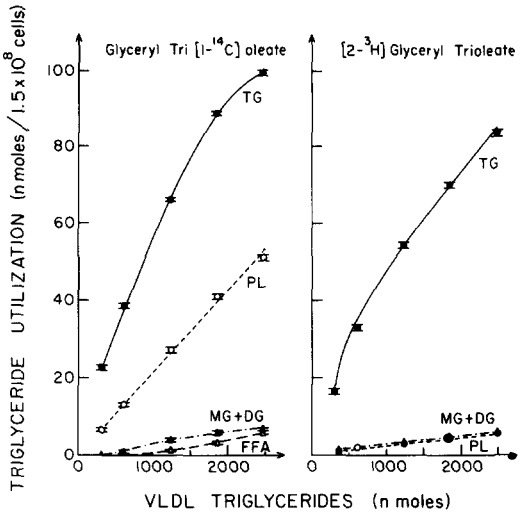

Fig. 4. Effect of VLDL triglyceride concentration on the utilization of glyceryl tri[1-^{14}C]oleate and [2-^{3}H]glyceryl trioleate by Ehrlich cells. The conditions of incubation and the abbreviations are the same as those listed in Fig. 2.

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ides are hydrolyzed in the medium prior to uptake by the cells (30–32). Recent work with L-strain mouse fibroblasts, however, indicates that these cells can take up intact triglycerides (35). Additional evidence for intact triglyceride uptake has been obtained from perfusion studies with aorta and adipose tissue (36, 37). The present studies with Ehrlich cells are also compatible with a mechanism involving at least some uptake of intact triglycerides even though about 50–60% of the total triglycerides taken up underwent hydrolysis. Hydrolysis of triglycerides in the medium did not occur when the ascites plasma VLDL was incubated without cells, and the only lipase so far isolated from Ehrlich cells was bound firmly to subcellular particles (38). This enzyme might be expected to hydrolyze triglycerides after they are associated with the cells. We cannot exclude the possibility that some of the triglycerides were hydrolyzed by a lipase released from the cells and that some free fatty acids and partial glycerides actually were taken up. Our studies, however, indicate that a lipase of high activity is not released from Ehrlich cells under the present conditions of incubation. Moreover, only negligible amounts of lactate dehydrogenase activity were released, indicating that the cell membrane did not become excessively porous to high molecular weight cytoplasmic proteins during these incubations. Taken together, these data make it appear unlikely that extracellular lipolysis can account for much of the triglyceride fatty acid that was taken up by the Ehrlich cells in these in vitro experiments.

The amounts of labeled triglyceride recovered in the cell lipids far exceeded the amounts that would be expected as a result of de novo triglyceride biosynthesis (5), supporting the view that some VLDL triglycerides were transferred intact to the cells. In addition, when Ehrlich cells are incubated with radioactive free fatty acids, either as a complex with plasma albumin or lipoproteins, more radioactivity is always incorporated into phospholipids than into triglycerides (5, 15). By contrast, when the cells were incubated with fatty acid-labeled triglycerides in the present work, much more radioactivity was incorporated into triglycerides than into phospholipids. The studies involving triglycerides labeled in the glycerol moiety also support an uptake mechanism involving at least some transfer of intact triglycerides to the cells. In these experiments, large amounts of labeled glycerol from triolein were recovered in cell triglycerides. Yet, almost no glycerol radioactivity is incorporated into triglycerides by Ehrlich cells when they are incubated with free fatty acids and labeled glycerol.[2] Therefore, it is likely that the [^{3}H]glycerol radioactivity that was recovered from the cells in triglycerides was the result of intact triglyceride uptake. As shown in Figs. 2 and 4, more fatty acid radioactivity (^{14}C) than glycerol radioactivity (^{3}H) was recovered from cellular triglycerides under all conditions of incubation. Assuming that the glyceryl tri[1-^{14}C]oleate and [2-^{3}H]glyceryl trioleate added to the VLDL are equally available to the cells, the difference between the ^{14}C and ^{3}H incorporation into cellular triglycerides represents triglyceride biosynthesis by the cells. Based on this assumption and the labeled fatty acids recovered in phospholipids, CO_2, and other metabolites, we calculated that 50–60% of the VLDL triglycerides taken up by the Ehrlich cells underwent hydrolysis.

One possibility is that the uptake of intact triglycerides is secondary to binding of VLDL to the Ehrlich cell membrane. Studies with chylomicrons and isolated liver cells have shown that binding of large lipoproteins to cell surfaces can occur (39). On the other hand, the data relating to the uptake of VLDL protein radioactivity, VLDL protein content, and VLDL cholesterol content indicate that the binding of intact VLDL to the Ehrlich cell is not the major mechanism of triglyceride uptake. These data are compatible, however, with the uptake mechanism proposed for the L cell, in which triglycerides are transferred from the lipoprotein to the cell membrane (35). According to this interpretation, lipoprotein triglycerides are hydrolyzed by cell suspensions after they are transferred to the cells, not while they are still a part of the lipoprotein complex.

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[2] Spector, A. Unpublished observations.

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