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Flux of free fatty acids among host tissues, ascites fluid, and Ehrlich ascites carcinoma cells

Pierre Mermier[1] **and Nome Baker**

Radioisotope Research, Veterans Administration Wadsworth Hospital Center, Los Angeles, California 90073, and Department of Medicine, UCLA School of Medicine, Los Angeles, California 90024

Abstract The role of plasma free fatty acids (FFA) in the transport of fatty acids from host tissues to Ehrlich ascites carcinoma in mice was studied. [9,10-^{3}H]Palmitate complexed to mouse serum (albumin) was injected either intraperitoneally or intravenously into unanesthetized tumor-bearing mice. The incorporation of radioactivity into tumor extracellular fluid FFA, tumor cell FFA, neutral lipid, phospholipid, water-soluble material in cells and fluid, plasma FFA, host carcass total lipid fatty acids, and water-soluble (i.e., nonlipid) material was measured. In addition, the quantity of fatty acid in each of the above lipid fractions was determined. The data were analyzed by multicompartmental analysis (SAAM) using a digital computer, and fractional rate constants of FA movement within and out of the host–tumor system were calculated. These rate constants and pool size measurements were used to estimate the corresponding fluxes. Although FFA in the tumor's extracellular fluid were replaced rapidly, almost none of the newly formed fluid FFA was derived from plasma FFA. Moreover, the transfer of FFA from the tumor extracellular fluid FFA to plasma FFA was virtually negligible. We suggest that the net amount of FFA required to replace the fluid FFA utilized for tumor energy and growth may be derived from direct transfer of FFA from host tissues to the ascitic fluid and that plasma FFA is not an intermediate in this transport process. The transport of FFA from the host to tumor cell lipids through the tumor extracellular fluid was about 26-fold greater than that required to account for net lipid accumulation during growth.

Supplementary key words [9,10-^{3}H]palmitate · fatty acid–albumin complex · lipids · transport · multicompartmental analysis

Cancer growth, in vivo, is generally accompanied by a loss of lipid FA from the host's carcass (1, 2). This net loss of fat has been attributed to the tumor's capacity to accumulate and oxidize fat derived from the host (1, 2). The Ehrlich ascites carcinoma has been used extensively as a model to study this phenomenon (3–5). These studies have shown that ascites tumor FA are not derived to any large extent from de novo FA synthesis (3), a slow process in many neoplastic tissues (6), relative to fat accumulation during tumor growth.

Previous studies of FA flux into ascites tumor cells have been carried out after introducing labeled fatty acids into extracellular fluid (ascites fluid or buffer) either in vitro (4, 7) or in vivo (3). A rapid incorporation of labeled fatty acids into the tumor cell lipid occurs in both of these circumstances; however, this need not indicate a net inward movement of FA, because the extent of simultaneous efflux of unlabeled FA from the tumor lipids is not known. In this connection it is known that the tumor cells are capable of releasing FFA at a significant rate under specified in vitro conditions (5). Nevertheless, in view of the apparent inability of tumor cells to synthesize FA rapidly, the rapid uptake of FFA from the extracellular fluid by tumor cells, and the capacity of the tumor cells to oxidize a large fraction of the labeled FFA taken up (3), it is expected that a large net flux of FA occurs from the host tissues to the tumor extracellular fluid (3). It has been assumed that this flux of FA to the ascites fluid is a result of the movement into it of FFA from the circulating blood; however, such a flux has not been looked for experimentally.

In the present study the flux of FFA from the circulating blood to the ascites fluid and from the ascites fluid to the circulating blood have been measured in vivo. These fluxes have been compared with those of FFA into tumor cell lipids, into tumor and host oxidative pathways, and into host lipids. Our experiments show that most of the net movement of FFA from the host to the tumor must occur by a pathway that does not involve plasma FFA as an intermediate.

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Abbreviations: FA, fatty acids; FFA, free fatty acids; DG, diglycerides; TG, triglycerides; PL, phospholipids; SAAM, systems analysis and modeling (a digital computer program); NL, neutral lipids; TLFA, total lipid fatty acids.

[1] Present address: Département de Biochimie, Laboratoire du Professeur E. Stein, 30 Quai de l'Ecole de Médecine, 1211 Genève, Switzerland.

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MATERIALS AND METHODS

Cells

Lettré-Ehrlich hyperdiploid ascites tumor cells, transplanted from the subline maintained in Swiss-Webster mice by Dr. Ralph McKee, Biological Chemistry Department, UCLA School of Medicine, were used. The tumor, defined as the ascites fluid and the cells suspended therein, was harvested 6–10 days after a previous transplantation. For transplantation, the tumor was diluted with 5 vol of Krebs-Ringer bicarbonate buffer at pH 7.4 (8) and centrifuged 5 min at 4°C at approx. 10 *g*. The cells were washed with the buffer, centrifuged again (approx. 230 *g*), and resuspended in buffer, and 0.20 ml of the suspension, containing approximately 1.5×10^7 cells, was injected intraperitoneally into each host mouse.

Mice

Swiss-Webster mice (Hilltop Lab Animals, Inc., Los Angeles), 6–10 wk old, were fed Purina laboratory mouse chow and water ad lib., but food was removed at 9:00 a.m., 2 hr before the start of each experiment.

Fatty acids

[9,10-^{3}H]Palmitic acid, 500 mCi/mmole, obtained from Amersham/Searle Corp., was purified by drying and extracting from hexane into alkaline 40% isopropanol followed by acidification and reextraction into fresh hexane. The resulting fatty acid was found to be at least 98% pure by radio-thin-layer chromatography (9). A fatty acid–albumin complex was prepared by a slight modification of the method described by Friedberg et al. (10). The labeled palmitic acid was dissolved in a slight excess of KOH in methanol and the solution was dried; then, after addition of 0.85% NaCl, it was heated to 60–70°C until total solubilization of the palmitate. An eightfold volume of mouse serum was then added to the warm solution. The final molecular ratio of added (radioactive) palmitate/serum albumin was approximately 1, and the ratio of total (added and already present) FFA/albumin was approximately 2. In one study several other labeled complexes were prepared in a similar way using either extracted bovine serum albumin (Pentex, FFA poor, with or without added palmitate) or dog serum. The molar FFA/albumin ratios of these preparations are given in Table 5.

Injection and sampling

The complex was injected either into the tail vein (20 μl containing 2 μCi of ^{3}H and about 8 nmoles of FFA) or into the peritoneal cavity after a 10-fold dilution into saline (100 μl containing 1 μCi of ^{3}H and about 4 nmoles of FFA). For some mice, venous capillary blood samples were then drawn at various times from an ophthalmic sinus (11) at time intervals ranging from 5 sec to 90 min. This method of blood sampling has been used extensively in our laboratory and has proved highly reliable in kinetic tracer studies of mouse glucose turnover. Usually, samples can be obtained by an experienced technician within 2 sec of the desired time, and 50 μl of blood can be collected within a 5-sec period. Blood flow is not interrupted by the procedure. Only three blood samples, with a combined volume of 200 μl, were drawn from each mouse in our studies.

At the end of an experiment, the mice were killed, the abdomens were opened, and the tumors were collected as fast as possible into a centrifuge tube and chilled in an ice bath. The abdomens were rinsed with distilled water, and the washing was saved for measurement of its total radioactivity. The carcass was saponified in boiling aqueous 30% KOH. In some experiments the mice were quickly dissected after the removal of the tumor, and certain organs, as well as the "median" and the "extreme" carcass, were saponified separately. We define the median carcass as that part of the carcass surrounding the tumor, from the pelvis to the pleural cavity (anterior to the diaphragm); the extreme carcass is defined as the balance of the mouse anterior and posterior carcass, including the limbs.

The tumor cells were separated from the intraperitoneal fluid by either centrifugation or quick filtration. In the "centrifugation method," the tumor was kept at 0–4°C; an equal volume of Krebs-Ringer buffer at 0–4°C was added to it, and the centrifugation was done as described above. In the "quick filtration method," 2 ml of whole tumor fluid was reserved for analysis, and another 0.3 ml was added to 4.7 ml of Krebs-Ringer buffer at 0–4°C and the cells were separated from the diluted extracellular fluid by rapid Millipore filtration. The total time required for filtration was less than 1 min. In this procedure the fluid components and total components were determined by direct analysis, and the cell components were calculated by difference.

Analysis

Lipids were extracted from the tumor into chloroform–methanol (12). The lipids of the cells isolated by centrifugation and of the extracellular fluid were extracted with either chloroform–methanol (12) or isopropanol–hexane. Extraction with isopropanol–hexane was a modification of techniques used to extract FFA and neutral lipids (13, 14). Our modified method, in contrast to that of Dole (13), did not exclude PL from the isopropanol–hexane phase. No difference in the relative concentration of tumor PL using isopropanol–hexane or chloroform–methanol was observed. The modified isopropanol–hexane extraction was carried out as follows. To 1.0-ml samples, the following were added: 2.0 ml of water, 4.0 ml of isopropanol-3 N H_2SO_4 39:1 (v/v), and 5.0 ml of hexane. The

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mixture was shaken for 60 sec and allowed to stand for 1 hr. A measured major portion of the upper phase was dried under N_2. Aliquots of the chloroform phase of Folch extracts (12) were similarly dried. In each case, the dried lipids were redissolved in 5.0 ml of hexane–ethyl ether 50:50 (v/v). One aliquot was used for measurement of radioactivity in the total lipids. Most of the remainder was treated with 16-hr-activated silicic acid to remove PL by adsorption (Ref. 15, modified to use only one exposure to a batch of activated silicic acid). A measured portion of the hexane–ethyl ether neutral lipid extract was removed and dried completely under N_2; the residue was dissolved in 5 ml of hexane. FFA were separated from glycerides and other nonpolar neutral lipids by extraction into 5 ml of 0.1 N Na_2CO_3 in 40% isopropanol, acidification, and reextraction into hexane (13, 15). Analyses of radioactivity and of lipid FA in the total lipid extract before removal of PL, after removal of PL, and in the FFA fraction were carried out as described below. These data were used to determine, in part by difference, the *relative* distribution of ^3H and the *relative* lipid FA contents of the various lipid classes. To determine the absolute amount of radioactive and nonradioactive total lipid fatty acids (TLFA), tumor and cells were digested in 10% KOH in 50% methanol; the saponified fatty acids were acidified and extracted with hexane. Radioactivity in the nonlipid fraction was also assayed ("water-soluble ^3H"). The total lipids from separate aliquots of cells that had been extracted with chloroform–methanol were saponified, and fatty acids were extracted into hexane. The latter two methods gave values for TLFA concentration that agreed closely.

After saponification of the carcass or organ lipids, elimination of the unsaponifiable lipids, and acidification, the fatty acids were extracted with petroleum ether (30–60°C boiling range). Radioactivity in the nonlipid fraction was also measured ("water-soluble ^3H"). Plasma lipids were extracted with isopropanol–hexane (13).

All the above fractions, as well as the nonlipid fraction, were assayed for ^3H in a Packard liquid scintillation spectrometer (16). Nonradioactive FFA and FA obtained after saponification of esterified FA fractions were assayed by the ^{63}Ni method (17).[2]

During the early time intervals studied in our experiments, plasma FFA radioactivity was derived from direct measurement of plasma total lipid ^3H. In some cases, plasma lipids were separated by thin-layer chromatography (9) on Eastman Chromagram sheets; more than 90% of the radioactivity was found to be associated with the FFA fraction.

[2] Modified according to Dr. Dana E. Wilson, Department of Medicine, University of Utah School of Medicine, Salt Lake City, Utah, to use 64% cesium chloride in place of the saturated aqueous solution of potassium sulfate ("working solution").

TABLE 1. Comparison of tumor cell and extracellular fluid FFA concentrations after cell separation by two methods

Fraction	Centrifugation Method	Quick Filtration Method
	nmoles/ml tumor (cells and fluid)	
Cell FFA	269 ± 85[a]	128 ± 23[b]
Fluid FFA	48 ± 11[a]	282 ± 56[b]
Cell FFA/fluid FFA	5.6	0.5
Total FFA	317 ± 82[a]	410 ± 59[b]

[a] Averages ± SD of five animals.
[b] Averages ± SD of four animals.

RESULTS

Measurement of tumor lipids

Two methods of separating cancer cells from extracellular fluid were compared: centrifugation vs. rapid Millipore filtration. Much more time elapses during cell separation by centrifugation; therefore, artifacts such as adsorption of FFA by the cells in vitro (7) or continued FA activation and oxidation by the cells (as evidenced by the formation of water-soluble radioactivity) are expected to be more pronounced.

As shown in **Table 1,** the mean ratio of cell FFA to fluid FFA was more than 10 times greater when the cancer cells were separated by centrifugation rather than by filtration. The total amount of tumor FFA, 317–410 nmoles/ml, was not significantly influenced by the method of cell separation. Because of the large transfer of extracellular fluid FFA to the cells during separation by centrifugation, we utilized only the FFA data obtained after rapid Millipore filtration.

As shown in **Table 2,** 96% of the tumor's total fatty acids are located in the cancer cells, and 99% of the cell fatty acids are esterified. The cell total fatty acids represent less than 1% of the wet weight of the cells. In contrast to the cells, over 50% of the total fatty acids in the extracellular fluid was in the form of FFA. This contrasts markedly with the results of Spector (3), who found that FFA accounted for only 3% of the extracellular fluid TLFA. The discrepancy is due to differences in TLFA rather than FFA measurements. Spector found 6.8 µmoles of TLFA/ml fluid (3), whereas we found only 0.4 µmoles of TLFA/ml fluid (Table 2). In an earlier study (18), using

TABLE 2. Lipid composition of tumor cells and fluid

Fraction	Cells	Fluid
	nmoles of fatty acid/ml tumor	
FFA	128 ± 23[a]	282 ± 56[a]
NL	2,000 ± 884[b]	33 ± 30[b]
PL	7,940 ± 2830[b]	110 ± 51[b]
Total	10,100	425

[a] Quick filtration method, averages ± SD of four animals.
[b] Centrifugation method, averages ± SD of five animals.

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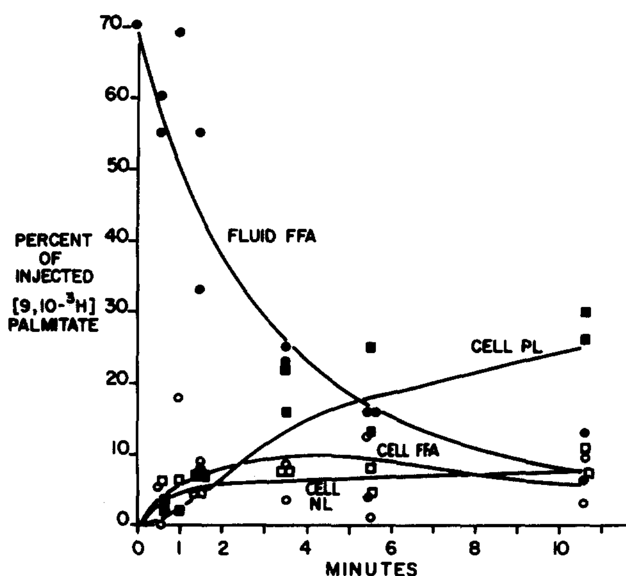

Fig. 1. Percentage of intraperitoneally injected [9,10-^{3}H]palmitate-albumin in extracellular fluid FFA and in lipid fractions of Ehrlich ascites carcinoma cells. Two mice were killed at each time point. The value at t_0 was derived from separate experiments on other mice (19). The curves are least square fits obtained by multicompartmental analysis of the data using a digital computer (SAAM program). Symbols: closed circles, fluid FFA; open circles, cell FFA; closed squares, cell PL; open squares, cell NL.

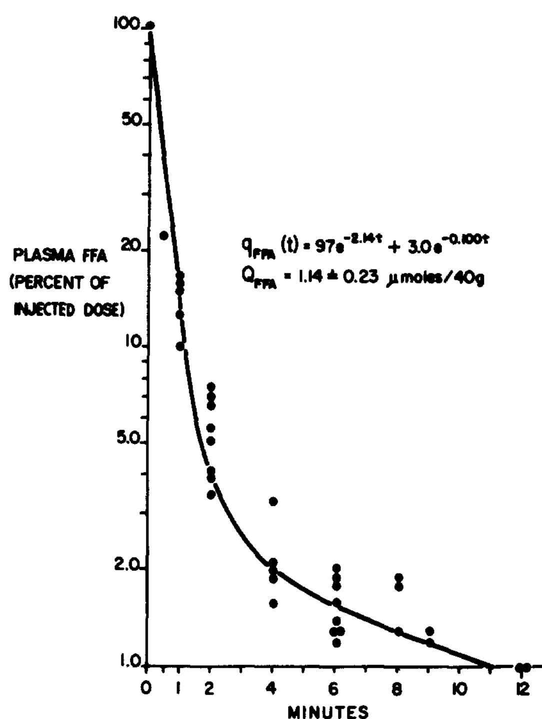

Fig. 2. Percentage of intravenously injected [9,10-^{3}H]palmitate-albumin in plasma FFA of mice bearing Ehrlich ascites carcinoma. Serial orbital samples were obtained from each of eight mice. The mean plasma FFA pool size (±SD) is also shown; the latter value was derived from concentrations of terminal blood samples.

quick filtration and gas-liquid chromatographic analysis of FA methyl esters, we reported values of 1 and 2 μmoles of TLFA/ml fluid in 12- and 5-day tumors, respectively. The presence of blood in tumors would be expected to influence the results considerably. Our values for extracellular fluid FFA are based upon analyses of nonbloody tumors exclusively.[3] The differences between the levels of esterified FA found in our studies and those in Spector's experiments (3) may represent mouse strain differences. Larger quantities of lipoproteins from the plasma may penetrate the capillary walls into the tumor extracellular fluid in the CDF_1 and CBA mice used by Spector than in our Swiss-Webster mice.

Uptake of FFA from extracellular fluid

The disappearance of labeled palmitate from tumor extracellular fluid and the incorporation of ^{3}H into cancer cell lipids at early times after intraperitoneal injection are shown in **Fig. 1.** As noted previously (19), only 70% of the injected radioactivity may be recovered from the tumor at zero time (5 sec) in tumors having a 5-ml volume, which was the mean volume of tumors used in the experiment shown in Fig. 1. Regarding the missing 30% of the injected ^{3}H, about 15% of the injected ^{3}H was recovered in the distilled water washes of the peritoneal cavity and 15% was apparently bound tightly to the host tissues within about 5 sec. 90% of the labeled palmitate that remained in the extracellular fluid at zero time (5 sec) disappeared from it within 10 min. Much of the ^{3}H activity was incorporated into tumor cell lipids in 10 min; however, 17% of the injected ^{3}H disappeared from the peritoneal cavity in this time. As shown in Fig. 1, the label appeared in the cells in 10 min as FFA (6%), as neutral lipids (8%), and as PL (28%); thus, in 10 min, 42% of the injected ^{3}H, about 15% of the injected ^{3}H was recovered reasonably well with Spector's (3) earlier observation that about 40% of intraperitoneally injected [1-^{14}C]palmitate was incorporated into cancer cell lipids in 10 min, at which time 70% of the injected palmitate disappeared from the fluid FFA. Spector also reported that most of the lipid FA ^{14}C was in PL.

The data in Fig. 1 were used for multicompartmental analysis, the results of which will be discussed in a subsequent section. However, even without mathematical analysis, our data clearly confirm Spector's (3) earlier in vivo observations that FFA in the tumor fluid are rapidly replaced and that a major part of the FFA leaving the fluid is incorporated into cancer cell lipids. Based upon Spector's work one may deduce that a significant part of the FFA efflux is also used for oxidation to CO_2 and H_2O (3). One would expect that a part of the FFA efflux not

[3] Dr. A. A. Spector has informed us that he found high concentrations of lipid esters in the tumor extracellular fluid regardless of the degree of bloodiness.

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TABLE 3. Transfer of radioactivity from tumor fluid to host tissues after intraperitoneal injection of [9,10-^{3}H]palmitate in tumor-bearing mice

Time after injection (min)	1	3	6	15	30	60
No. of animals	2		5	4	2	4
			% of injected dose, mean ± SD			
Median carcass, lipids	3.0 ±0.5		3.8 ±0.8	3.7 ±0.4	3.0 ±0.3	5.7 ±3
Median carcass, water-soluble	0.077 ±0.023		1.4 ±0.9	2.2 ±1	1.9 ±0.9	3.4[a] ±2
Extreme carcass, lipids	1.1 ±0.1		1.9 ±0.9	1.9 ±1	2.7 ±0.7	2.1 ±1
Extreme carcass, water-soluble	0.0		0.79 ±0.4	1.4 ±0.6	2.6 ±1	4.4 ±0.6
Liver, lipids[b]			1.1 ±0.4	1.1 ±0.2	1.3 ±0.1	1.6 ±0.1
Liver, water-soluble[b]			1.4 ±0.1	1.1 ±0.8	2.8 ±0.6	2.0 ±0.7
Plasma (% of injected ^{3}H/ml)[c]	0.013 ±0.013	0.044 ±0.003	0.130 ±0.023	0.277 ±0.051		

The mean tumor volume averaged approx. 9 ml.
[a] n = 3.
[b] Averages of two animals.
[c] n = 3. Results of a study using a fourth mouse were deleted because the values were higher than those from the other three mice. In the fourth mouse, the values at t = 1, 3, 6, and 15 min, respectively, were 0.072, 0.243, 0.302, and 0.554.

appearing in tumor cell lipids was transferred to host tissues; however, this has not been studied by previous workers.

Transfer of FFA from the tumor extracellular fluid to the host's tissues after intraperitoneal injection of labeled palmitate was studied using tumors of at least 8-ml volume because the poorly understood initial host uptake (at t = 5 sec) seen in 5-ml tumors is almost negligible in the animals bearing the larger tumors (19). As shown in **Table 3,** transfer of ^{3}H-labeled FFA from the tumor extracellular fluid to the host was clearly evident. By 60 min, 19% of the injected dose could be recovered as lipid ^{3}H and nonlipid ^{3}H in the host's tissues. One-third of this activity was present in tissues that were not in direct contact with the peritoneal cavity ("extreme carcass"). In fact, significant activity was present in these tissues within 60 sec; yet, almost no radioactivity appeared in the plasma during the first 3 min after intraperitoneal injection (Table 3), at which time only 30% of the injected [^{3}H]palmitate remained in the tumor extracellular fluid (Fig. 1). The measurements of blood plasma ^{3}H (Table 3) were obtained in a separate experiment designed for that purpose only. Radioactivity in the carcasses was not measured. Further analysis and discussion of these findings are presented below (multicompartmental analysis). In brief, the analysis indicates that very little [^{3}H]palmitate was transferred directly from the peritoneal fluid to the FFA of host blood plasma; one may surmise that the labeled lipids that appeared in the carcass were transported there either through lymph flow or by direct transfer to adjacent tissues in the case of the median carcass.

Transport of FFA from plasma to cancer

A separate study of the movement of labeled FFA from host blood plasma to the tumor was carried out by injecting tracer intravenously. As shown in **Fig. 2,** the plasma FFA was turning over so rapidly that 95% of the labeled FA was removed from the plasma within approximately 2 min. After this time interval, the fractional removal rate was markedly decreased, in agreement with earlier observations in rats (20). The plasma FFA pool size and an exponential function describing the fall in ^{3}H-labeled FFA are given in Fig. 2. From these values one may calculate that the irreversible disposal rate (mean ± SD) of plasma FFA (21) was 1.52 ± 0.20 μmoles/min/40 g mouse.

Almost none of the labeled FFA that left the plasma after intravenous injection of [9,10-^{3}H]palmitate appeared in the tumor in the first 15 min. As shown in **Table 4,** only 0.2% of the injected palmitate was found in the lipids of Ehrlich ascites carcinoma (cells plus extracellular fluid) at times during which 98% or more of the

TABLE 4. Uptake of radioactivity by the tumor after intravenous injection of [9,10-^{3}H]palmitate

Time after Injection	Tumor Lipids	Tumor, Water-soluble
min	*% of injected dose recovered*	
1	0.08 ± 0.05	0.07 ± 0.03
3	0.28 ± 0.03	0.41 ± 0.09
6	0.20 ± 0.03	0.61 ± 0.14
15	0.21 ± 0.01	0.97 ± 0.15

Each value is the mean of two animals (± range).

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TABLE 5. Effect of varying the type of FFA–albumin complex on the relative incorporation of radioactivity into tumor and host tissues after intravenous injection of [9,10-^{3}H]palmitate

	Bovine Albumin	Bovine Albumin	Mouse Serum	Dog Serum
FFA/albumin	0.33	1.35	2	2
No. of animals	4	4	4	8
Time (min)	8	8	6–15	5–15
		% of injected dose, mean ± SD		
Incorporation into tumor lipid	0.24 ± 0.09	0.45 ± 0.47	0.20 ± 0.03	0.24 ± 0.05
Uptake into carcass				
As lipids	56 ± 7.0	60 ± 6.6	52 ± 5.6	56 ± 4.9
As nonlipids			24 ± 2	35 ± 8.1
Plasma lipids, 2 min after injection (% of injected dose/ml of plasma)	2.4 ± 0.4[a]	3.4 ± 0.1[a]	2.4 ± 0.68	1.9 ± 0.3[a]

[a] Mean of two mice ± range.

injected ^{3}H-labeled FFA had been removed from the plasma by the host's tissues.

Water-soluble ^{3}H accumulated for at least 15 min in the tumor (Table 4) and reached values considerably higher than the ^{3}H of the tumor lipids. However, only 1% of the injected dose was recovered in the water-soluble fraction at 15 min.

Because the transfer of FFA from the circulation to the peritoneal fluid was almost negligible (0.007 μmole of FFA/min/40 g mouse compared with 1.51 μmoles of FFA/min/40 g mouse transferred to the host tissues), we considered the possibility that our observations were due to an artifact linked to the nature of the albumin–palmitate complex that was used (22). Therefore, [9,10-^{3}H]palmitate was complexed to albumin from sources other than mouse serum, and different molar ratios of FFA to albumin were studied. As shown in **Table 5,** in each experiment less than 0.5% of the intravenously injected labeled palmitate was incorporated into the tumor lipids. In every case, more than 95% of the labeled FA was removed from the plasma within 2 min, and, after this time interval, the disappearance rate of ^{3}H-labeled lipids from the plasma was the same regardless of the type of complex injected. Almost all (80–95%) of the radioactivity that disappeared from the plasma was found in the carcass in the sum of the lipid and water-soluble fractions (Table 5).

Description of the multicompartmental analysis

The techniques used in multicompartmental analysis have been described (23, 24). Briefly, the simplest model that, tentatively, seemed compatible with both the data and previously published information was formulated. Based upon an initial set of estimated values for fractional rates of flow in our model, the total radioactivity in each compartment was calculated (numerical solution to differential equations) by the SAAM 23 program (23) using an IBM 360/91 digital computer. Then, the values for the fractional rates were altered by successive iterations until a satisfactory least squares fit between the calculated and observed curves was obtained.

Models and assumption

In the present work the model describing the tumor and host lipid interchanges was constructed from two separate models shown in **Figs. 3** (tumor) and **4** (host). In each case the site of injection of [9,10-^{3}H]palmitate is shown by an asterisk and a curved arrow, and the fraction of the

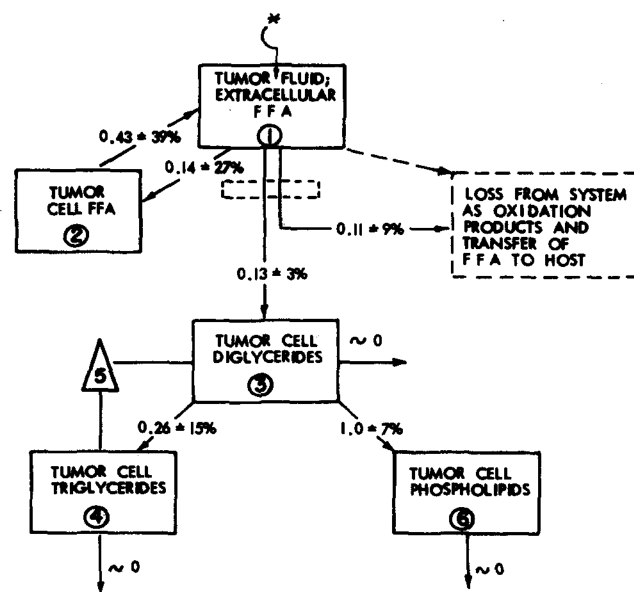

Fig. 3. Simplified model of FFA movement from extracellular FFA into Ehrlich ascites carcinoma cell lipids. The model is discussed in the text. Fractional rate constants (min^{-1} ± percent SD) were derived from the computer analysis of data shown in Fig. 1. The broken rectangle indicates rapidly turning over intermediates involved in both the FFA esterification and oxidation pathways. A broken arrow is shown to indicate that FFA transfer to the host does not occur by way of intermediates common to esterification and oxidation pathways. This broken arrow was not included in the multicompartmental analysis. Compartment 5 is a "summer" compartment; it represents the cell neutral lipid fraction. Separate data for DG and TG were not obtained.

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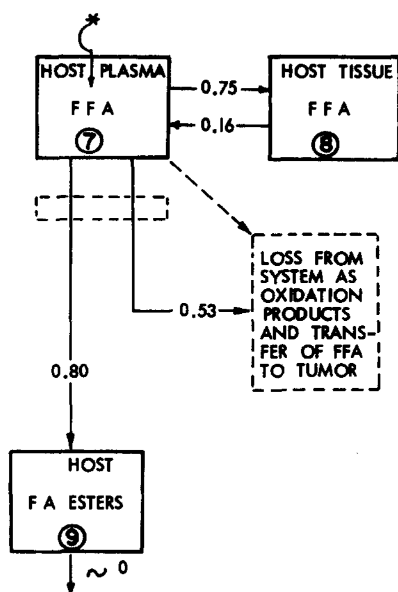

Fig. 4. Simplified model of FFA exchange, esterification, and oxidation by the host tissues and transfer to tumor lipids in mice bearing Ehrlich ascites carcinomas. Fractional rate constants (min^{-1}) were derived from the data shown in Fig. 2 and were obtained by the solution of Skinner et al. (see Ref. 24). The broken rectangle and broken arrow are explained in Fig. 3.

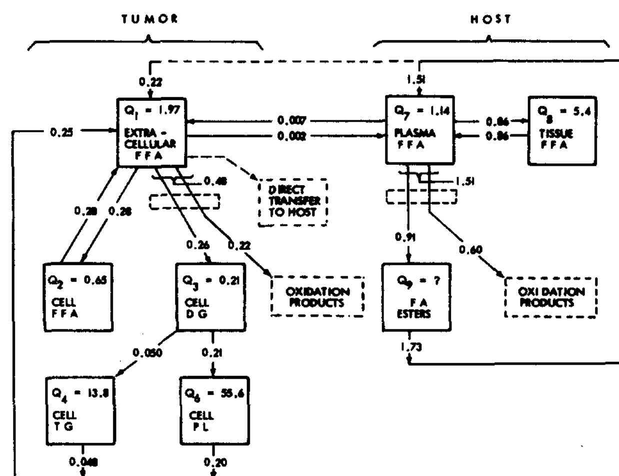

Fig. 5. Combined model of tumor and host systems showing FFA transport rates (μmoles of FA/min) and pool sizes. The following pool sizes were estimated from actual measurements: Q_1, $(Q_3 + Q_4)$, Q_6, and Q_7. Q_2 was also measured and found to be approximately equal to the estimated value. Transport rates were calculated on the basis of the fractional rate constants shown in Figs. 3 and 4, the measured pool sizes, and steady state (or non-steady state) considerations. Additional information was required to estimate k_{17}, k_{71}, and the rates of oxidation; see text for details. Values are standardized to 7 ml of whole tumor in a 40-g mouse. Fluxes out of Q_4 and Q_6 were calculated on the assumption that each pool was increasing in constant proportion at the previously estimated (18) rate of 0.010 μmole of FA/min (Q_4 plus Q_6).

material in each compartment transferred per minute in each direction is indicated by a straight arrow. The complete tumor–host system is shown in **Fig. 5.** Also presented in this figure are the fluxes of material from each compartment and the compartment sizes. The parameters in Figs. 3–5 will be discussed in a later section (Analysis); however, we shall first describe the models and summarize the assumptions upon which the models are based.

The tumor model (Fig. 3) was used to analyze the data of Fig. 1 and Table 3. The model shows a reversible flow of material between the FFA of extracellular fluid and a pool of tumor cell FFA. Most of the cell-associated FFA pool was considered not to be on the path between extracellular fluid FFA and the tumor cell lipids. This was tentatively established by utilizing trial models in which various proportions of the total flow between extracellular fluid FFA and the tissue lipids were routed through the tissue FFA pool. These trials showed that the data of Fig. 1 could be generated satisfactorily by a model in which none of the extracellular fluid FFA passed through the bulk of the tumor cell FFA pool. Although our model does not show any cell FFA as an intermediate in all lipid ester formation, we assume that there is, in fact, a small intermediate FFA pool that turns over so rapidly that it may be ignored for practical purposes.

The flow of fatty acids from the tumor extracellular fluid FFA pool to the tumor cell lipids in Fig. 3 was assumed (25) to be a one-way flow through a diglyceride pool to triglycerides and phospholipids. This is an oversimplification because the model ignores the possible hydrolysis of DG and the reverse transfer of FA to the extracellular FFA pool. It also ignores the Lands pathway (26), by which FFA (FA–CoA) could be incorporated into lipid esters, and it fails to take into account the known presence of alkyl glyceryl ether esters (27) and of cholesteryl esters in the neutral lipid fraction (28). Our data do not provide useful information regarding the quantitative significance of these various pathways, and nothing would be gained by including them in our tentative model. However, our calculations of FFA flux into the "end products" (NL and PL) are relatively unaffected by the choice of model (16).

It was known from previous findings (Ref. 20 and unpublished observations by the present authors) that the fractional removal rate of fatty acids from the very large triglyceride and phospholipid pools would be so small that the loss of ^{3}H-labeled fatty acids from these compartments would be negligible in the short time period studied. Hence, all losses from the initially labeled extracellular FFA pool that could not be accounted for by ^{3}H present in cell FFA, neutral lipids, and phospholipids were modeled as a loss of extracellular ^{3}H-labeled FFA from the system (Fig. 3). The known components of this loss are oxidation of fatty acids and the transfer of FFA to the host.

We assume that the same activated fatty acid intermediates that form lipid esters intracellularly are also interme-

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diates in FA oxidation. For consistency with previous assumptions, we must also assume, tentatively, that the bulk of the tumor cell FFA does not mediate the conversion of extracellular FFA to oxidation products. The broken rectangle in Fig. 3 designates the intracellular activated FA intermediates. Because these intermediates are present in trace amounts, they do not have an essential characteristic (time delay in release of inflowing ^{3}H) of a separate compartment. In the operation of the model, arrows through the broken rectangle are equivalent to direct outflow of [^{3}H]palmitate from the extracellular FFA compartment, whether to oxidation products, to diglyceride, or to the host as transferred FFA.

The host model (Fig. 4) was used to analyze the data of Fig. 2 and Table 4. This model is essentially the same as that used earlier (20) in an analysis of plasma FFA flux in rats, so it will not be described further here. Like Fig. 3, it includes the fractional flows of material from the compartments in the directions shown by the straight arrows, and material balance as required for steady state operation is not represented.

The model shown in Fig. 5 has the same compartments as the models of Figs. 3 and 4; it differs from these in that compartment sizes are given (the Q's), and fluxes (transfer rates in μmoles/min), rather than fractional outflows, are given alongside the arrows.

The compartment sizes assigned to tumor extracellular fluid FFA (Q_1), plasma FFA (Q_7), and tumor cell phospholipids (Q_6) are measured values. The sum of tumor cell diglyceride FA (Q_3) and tumor cell triglyceride FA (Q_4) is a measured value, i.e., the measured amount of tumor cell neutral lipid FA. The apportionment of the total size between Q_3 and Q_4 was determined by the fractional inflow from Q_1, a compartment of known size (Fig. 3), into Q_3, together with the steady state requirement for unchanging values for Q_3 and Q_4 at the fractional flow rates shown in Fig. 3. The compartment sizes for tumor cell FFA (Q_2) and for host tissue FFA (Q_8) were determined by the fractional flow rates into each from a compartment of known size, as shown in Figs. 3 and 4, together with the steady state requirement for unchanging compartment size in each case.

The flow of material shown in Fig. 5 between tumor extracellular fluid FFA and host plasma FFA is based on considerations described below.

The flow of fatty acids shown in Fig. 5 from tumor cell triglycerides (Q_4) and phospholipids (Q_6) is the amount required to keep these compartments at constant size despite the continuous influx of FA into these from diglycerides. These values have been corrected for the observed small increment (10 nmoles/min/7 ml of tumor) in tumor lipids that was observed in separate experiments to be associated with tumor growth (18). It was assumed that this accretion can be treated as net deposition of PL and TG in proportion to the relative rates of synthesis of these materials as developed in the analysis. The efflux of relatively unlabeled fatty acids from these compartments is shown entering extracellular FFA directly without passage through the cellular FFA pool. Obviously this is incorrect; however, we have no information as to whether newly formed FFA in the tumor cells pass through the bulk of the cell-associated FFA (Q_2) or whether these hydrolysis products pass out of the cell by way of a much smaller intracellular FFA pool. In either case, this uncertainty does not influence any of the conclusions reached in our analysis.

The amount of FFA entering the extracellular fluid pool of the tumor from the tumor lipids was found to be inadequate to maintain the constant size of the pool. Since the transfer of FFA from the host plasma to the extracellular fluid FFA was observed to be insignificant, a separate pathway must exist whereby FFA (of a kind not labeled early after [^{3}H]palmitate is injected into host plasma) is transferred by an unknown mechanism to the tumor extracellular FFA, from the host tissues, without traversing the host plasma FFA pool. This movement is shown by a broken line in Fig. 5. Flow in the opposite direction, i.e., from tumor extracellular fluid FFA to host tissue lipids, also occurs by an unknown mechanism not involving the traversal of the host plasma FFA pool. This flow, represented by a broken line in Fig. 5, is known to exist because of the shape of the ^{3}H vs. time curve of the host tissue lipids after [9,10-^{3}H]palmitate is injected into the tumor extracellular fluid; this is discussed further below.

In addition, a number of other assumptions implicit in most tracer studies have been made (e.g., instantaneous mixing of tracer with tracee in the compartment into which the label is introduced and equivalence in the biological handling of tracer and tracee molecules). Moreover, we have made the following five assumptions on the basis of published information.

(*1*) Fatty acid synthesis in tumor cells from two-carbon fragments is negligible relative to the rate of extracellular FFA incorporation into tumor lipids (3). (*2*) Release of FFA from tumor lipids to tumor extracellular fluid occurs (5) and is assumed to be responsible in part for maintaining the steady state amount of FFA in the intracellular fluid. (*3*) Host plasma FFA exchanges with an appreciable part of host tissue FFA that is not on the pathway between plasma FFA and the intermediates of FFA metabolism in host tissue cells (20, 29). (*4*) The percentage of nontumor cells present in the peritoneal cavities of our tumorous mice was so low (30) that we may consider lipid metabolism of the total cell population to be representative of that in the tumor cells. (*5*) Although palmitate represents only about one-fourth of the FFA in ascites fluid (3), Spector and Steinberg (4) have evidence that labeled pal-

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mitate is a representative tracer for all tumor extracellular fluid FFA.

The assumption of instantaneous mixing of tracer with tracee in the injection compartment deserves special comment because it is almost surely incorrect in the case of intraperitoneally administered tracer. Although care was taken to massage the abdomen so as to facilitate postinjection mixing, the relative uptake of dose by the tumor and by the host was found to be seriously influenced by the degree of mixing and the localization of the injected bolus (19). On the other hand, we have observed no difference in the fractional rates of FFA removal from the peritoneal cavity after 5 sec regardless of the volume of the tumor or the extent to which the tracer was transferred to the host within the first 5 sec. This observation is consistent with the possibility that, even when mixing is incomplete within the total fluid, the bolus may be distributed similarly within a restricted area of the tumor fluid in the case of large and small tumors. Thus, there may be a fairly homogeneous subsystem within a heterogeneous larger compartment. If the fractional uptake of FFA by tumor cells within the subsystem is similar to that of the rest of the tumor, then our analysis would yield a good estimate of the fractional turnover of the FFA by the entire heterogeneous system. It is imperative in such a system that single animals be used for each time point (rather than aspiration of serial, small samples of tumor fluid) to avoid the complications and variations that could arise from the withdrawal of samples from the heterogeneous system.

Analysis of fractional rate constants

The fractional rate constants for the tumor and host systems are shown in Figs. 3 and 4, respectively. 38% of the FFA in the tumor extracellular fluid was removed each minute (Fig. 3), 14%/min was transferred to an exchangeable pool of FFA in (or on) the ascites tumor cells, 11%/min was removed by oxidative pathways or was transferred to the host, and 13%/min was converted to tumor cell lipid esters. For every molecule of FFA incorporated into TG, four molecules were incorporated into tumor cell PL (Fig. 3). As shown in Fig. 4, FFA was removed from plasma at a fractional rate of over 200%/min. 75%/min of the plasma FFA pool exchanged with an extraplasma FFA pool and 133%/min was converted to host lipids, oxidized, and/or transferred to the tumor. About 80%/min appeared in the host lipids. Thus, 53%/min was either oxidized or transferred to the tumor.

In each of the models shown in Figs. 3 and 4, arrows are shown leaving the system, but crucial information regarding FFA movement between the tumor and host has not been included. The latter relationships are, of course, the major aims of the present study; i.e., we wish to determine how much of the FFA leaving the tumor's extracellular fluid FFA was transferred to the host's plasma FFA compartment each minute, and vice versa. The fractional rate of FFA transfer from tumor extracellular fluid FFA to host plasma FFA (λ_{71}) and the reverse flow rate (λ_{17}) were extremely small and not accurately determined. Maximal limits for these rates were obtained as follows: λ_{17} was estimated by a computer analysis using the model shown in Fig. 5. In this analysis, the initial conditions were set at 100% in compartment 7, with no activity in any other compartment at zero time. All fractional transfer rates shown in Figs. 3 and 4 were held constant, and λ_{17} was allowed to vary until it reached a value that would give rise to the following observed relationship, 5 to 15 min after intravenous injection of various labeled [9,10-^{3}H]palmitate–albumin complexes into the host (see Table 5):

$$q \text{ (tumor lipids)}/q \text{ (host lipids)} = 0.005 \text{ (range } 0.004\text{–}0.0075)$$

where q (tumor lipids) = % of injected ^{3}H in tumor lipids, and q (host lipids) = % of injected ^{3}H in host lipids. The estimated value of λ_{17} computed in this fashion was 0.006/min. Only about half of the FFA transferred from plasma to tumor extracellular fluid FFA is incorporated into tumor lipids. Thus, one may also calculate that only about 2% of the irreversibly disposed plasma FFA ($[0.003/1.33] \times 100$) was transferred to tumor lipids. This value is considered maximal because of low counts in tumor lipids, some of which might represent contamination or spurious background counts.

λ_{17} was estimated by comparing the total ^{3}H radioactivity in plasma as a function of time after *intraperitoneal* injection of [9,10-^{3}H]palmitate with the values, predicted by computer simulation, on the assumption that λ_{01} in Fig. 3 is transferred irreversibly to plasma FFA. The resulting model is a coupling of that shown in Fig. 3 with the model shown in Fig. 4 in which λ_{71} becomes 0.11/min, or some other arbitrarily fixed value, and λ_{01} is set at $0.11 - \lambda_{71}$. Values for q_7, the radioactivity in plasma FFA, could then be predicted. The latter simulation is shown in **Fig. 6.** As shown in Fig. 6, the maximum radioactivity in plasma FFA should have been reached within 1 min. At that time almost 2.5% of the injected [9,10-^{3}H]palmitate would have been found in plasma FFA, if 11%/min of the tumor extracellular FFA entered the plasma FFA compartment directly. In contrast, the observed total radioactivity in plasma was only about 1% of the predicted value for plasma FFA at 1 min (Fig. 6); furthermore, the observed plasma radioactivity did not reach a maximum value until at least 15 min after the time of intraperitoneal injection. This analysis was based upon the plasma ^{3}H data for the three mice shown at the bottom of Table 3. Values for a fourth mouse were deleted (Table 3, footnote *c*). However, the conclusion is in no

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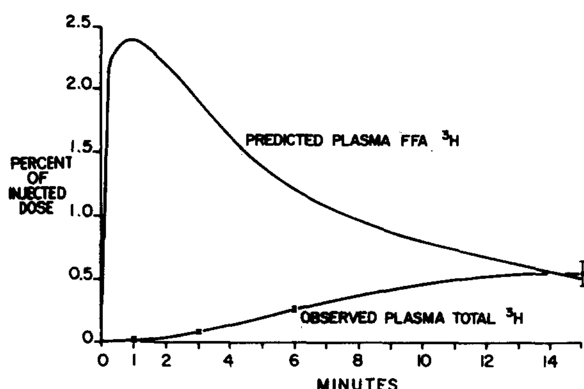

Fig. 6. Predicted and observed values for appearance of radioactivity in plasma FFA after intraperitoneal injection of [9,10-^{3}H]palmitate in tumor-bearing mice. The predicted curve is based upon the assumption that all of the radioactivity leaving the tumor fluid FFA compartment each minute (out of the whole tumor system) was transported to the host's plasma FFA compartment. Note that the observed curve is that of total radioactivity in plasma and probably includes labeled compounds in addition to FFA (e.g., ^3H_2O). The discrepancy between predicted and observed values is so great as to indicate that almost none of the fluid FFA was being transferred directly to plasma FFA. Vertical bars are ±SD (n = 3). See text and figures for further details of the simulation.

way affected by the inclusion of data from the fourth mouse. Clearly, less than 1% of the radioactivity leaving the tumor extracellular fluid FFA compartment each minute (i.e., <1% of 0.11/min) could be transferred to plasma FFA. On this basis, a maximum value of 0.001/min was assigned to λ_{71}. It is worth noting that, in the simulation shown in Fig. 6, an excellent correspondence between predicted and observed values was obtained, at one point in time (t = 15 min), assuming that all of the radioactivity in plasma at that time was associated with FFA, which is unlikely. A serious error in interpretation could have resulted had a comparison been made at that one point in time.

Quantitative evaluation of the importance of plasma FFA in relation to FFA metabolism in Ehrlich ascites carcinoma

The transport of FFA from the plasma to the extracellular fluid (μmoles/min/7 ml tumor) in relation to various other values for FFA flux, oxidation, and lipid FA ester formation in tumor and host is summarized in Fig. 5. These values were derived from the fractional rate constants, measured pool sizes of plasma FFA (1.14 μmoles) and of tumor extracellular fluid FFA (1.97 μmoles), steady state considerations, and our independently measured estimate (18) of the net rate of tumor lipid accumulation during tumor growth (0.010 μmole/min). Additional values for NL and PL contents of tumor cells were also available; however, these parameters were not used in any of the flux estimates.

Values for FA flux (transport rates) are shown in Fig. 5. As shown in this figure, 0.48 μmole of FA enters the tumor extracellular FFA compartment each minute. Only 0.25 μmole of FA/min can be derived from tumor cell lipolysis. Therefore, 0.23 μmole of FA/min must come from other tissues; less than 0.01 μmole of FA/min is transferred directly from plasma FFA. It follows that 0.22 μmole of FA/min is derived directly from host tissues. Of the 0.48 μmole of FA leaving the tumor extracellular FFA compartment each minute, a negligible portion is transferred to plasma FFA; almost equal portions are oxidized (or transferred directly to host tissues) and esterified by way of small, rapidly turning over intermediate compartments. The flux of FA into tumor cell lipid esters is 26-fold greater than the net rate of FA ester accumulation in the tumor. Thus, for every 0.26 μmole of FA esterified, 0.25 μmole is hydrolyzed and 0.010 μmole accumulates each minute.

These quantitative statements are not intended to serve as absolute values, and the reader should be reminded of the numerous assumptions and oversimplifications involved in the analysis. The following additional experimental and theoretical factors could have influenced our conclusions: (*1*) complications associated with the mixing of the tracer with the tracee in the tumor extracellular fluid, a process that is surely incomplete and capable of introducing serious errors in the analysis; (*2*) the unintentional use of tumors of varying sizes when there is evidence that the transfer of FFA from tumor to host tissues may be dependent on tumor volume (19); (*3*) the use of serial venous capillary blood samples obtained from an orbital sinus and the assumption that the FFA specific activity in each sample equaled that of arterial blood FFA; (*4*) the possibility that the drawing of blood samples from the orbital sinus influenced various aspects of FFA metabolism; (*5*) the presentation of a maximal value for direct FFA oxidation rate by the tumor when, in fact, no measurements of FA oxidation were made and no information was obtained regarding the extent to which FFA passes through small intermediary FA ester pools before the FA are oxidized to CO_2 and H_2O (our model implies that a large fraction of the tumor extracellular fluid FFA is oxidized after prior incorporation into large lipid pools; however, since oxidation rates were not directly measured, our estimated rates of esterification, hydrolysis, and subsequent oxidation of tumor FA should only be taken as crude estimates); and (*6*) the complete lack of data regarding pathways by which FFA could have been transferred from host tissues to tumor or vice versa. Thus, FFA could have been transferred from plasma to tumor by way of large FA pools in host tissues. Such a pathway, which now seems a highly likely possibility, was not considered in our model (see Fig. 5). The flux shown in Fig. 5 for tumor FFA "oxidation" was estimated by difference. Part of this flux represents direct transfer of FFA from tumor fluid to the liver and other host tissues (Fig. 5). Although

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no estimates of the values for rates of direct FFA transfer from tumor fluid to host tissues are given in our model (Fig. 5), we obtained evidence that such a transfer may occur, even with tumors of large volume (Table 3). Further studies are required in order to refine our estimates and to provide more information regarding our proposed model.

DISCUSSION

We have confirmed and extended the earlier work of others that showed that FFA is rapidly taken up by Ehrlich ascites carcinoma cells (3, 4, 7, 29) and by other neoplastic tissues (31). Thus, there must be a rapid, continual replacement of the small FFA pool in the tumor's extracellular fluid (3, 4). Since plasma FFA complexed to albumin is known to be the primary intermediate in the transport of FA through the blood from the host's fat depots to other tissues such as muscle and liver (see references in Ref. 20), one might assume (3) that plasma FFA is also the major source of the FFA that continuously flow into the tumor's interstitial fluid. However, our experiments do not support this hypothesis. Thus, we found that the rate of plasma FFA transport into the tumor's extracellular fluid was less than 2% of the total FFA flux from the tumor extracellular fluid into the tumor cells. Nevertheless, there is a net flux of FFA from some host source into tumor cells that represents nearly half of the total flux of FFA from the tumor extracellular fluid into the tumor cells. This contradicts the view that plasma FFA are a major source of the FA that are used as a metabolic fuel and as precursors of the cell lipids of Ehrlich ascites carcinoma cells. Two other major sources of tumor extracellular fluid FFA are to be considered. One is the lipid esters of tumor cells themselves (5). The other, a hypothetical source, is the lipid esters of those host tissues that are either in the peritoneal cavity or have lymph drainage into the peritoneal cavity.[4] Of these two sources, only the host tissues can supply the FFA that are consumed by the tumor in net amount. The direct transfer of FA from host to tumor without passage through the blood's transport system (FFA–albumin) seems plausible on the basis of several facts. First, the peritoneal cavity contains a considerable quantity of adipose tissue that bathes in the same fluid that encompasses the Ehrlich ascites carcinoma cells. Second, when adipose tissue is placed in a buffer similar in composition to that of extracellular fluid, a net release of fatty acids into the medium may occur (32). Third, other tissues that have lipase activity, including muscle (33) and liver (34), are potentially capable of releasing FFA into extracellular fluid and lymph. One wonders whether the ascites tumor produces agents that induce the release of FFA from surrounding host tissues into the ascites fluid and whether FFA flow through peritoneal fluid occurs normally in the absence of cancer cells. Also, one wonders whether a similar direct transfer of FFA between cells by way of extracellular fluid occurs in the case of normal tissues and solid tumors, in which case this form of FA transport may be an important general phenomenon.

An earlier study by Medes, Paden, and Weinhouse (35) suggests that dietary fatty acids may be incorporated into tumor tissue more slowly and by more indirect pathways than into host tissues. These authors fed [1-^{14}C]palmitic acid dissolved in carrier olive oil to mice bearing Lettré-Ehrlich ascites carcinoma and measured the subsequent incorporation of the labeled fatty acid into tumor and host lipids. All of the labeled palmitate was absorbed from the gastrointestinal tract within 6 hr. At that time, 20% of the fed dose of ^{14}C was recovered in expired CO_2, 6.5% in liver lipids, 72% (our calculation, by difference) in the host's extrahepatic tissues, and 0.0016% in the early Lettré-Ehrlich ascites carcinoma (0.24 g of cells). On a per gram of tissue basis, the rate of labeled FA incorporation into liver was 800-fold greater than the early uptake by the cancer cells. Moreover, the slow time course of the subsequent incorporation of the fed ^{14}C into lipids of various types of tumors (maximum at about 18 hr after the labeled FA had been absorbed from the gastrointestinal tract) strongly suggests that the exogenous fatty acids, which would have entered the circulation as chylomicron TG, were first incorporated into the host's tissues and subsequently transferred to the tumor cells. On the basis of our present experiments, the latter transfer probably occurred without mediation of plasma FFA.

Several other facets of our study warrant brief discussion even though the observations are incomplete and still poorly understood. We have observed both a very rapid transfer of [9,10-^{3}H]palmitate from tumor fluid to host tissues (19) and a much slower transfer, neither of which seemed to have been mediated by plasma FFA. Especially interesting was the rapid appearance of label in remote host tissues, i.e., those not bordering the tumor. We also obtained data on water-soluble ^{3}H that was presumably formed from the oxidation of the labeled substrate. Our analysis is consistent with earlier observations that appreciable quantities of fatty acid were oxidized by the tumor cells. Although a careful multicompartmental analysis of the $^{3}H_2O$ in tumor and host was not carried out, such an analysis may prove informative. Another point worthy of further study is the exchangeable FA pool that was associated with the tumor cells. In our first multicompartmental

[4] Still another conceivable source of tumor fluid FFA is FA derived from the lipolysis of lipoprotein lipids present in the extracellular fluid (Spector, A. A., and D. E. Brenneman. 1973. Fed. Proc. **32:** 672Abs). We wish to thank Dr. A. A. Spector for calling this omission to our attention.

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analysis, we assumed that the entire cell FFA pool was an obligatory intermediate in the conversion of extracellular FFA into all tumor lipids. This assumption was found to be incompatible with our data. In fact, our results were compatible with a model in which only a very small fraction of the total tumor cell FFA would be an intermediate in lipid ester formation; the bulk of the cell FFA appeared to exist as a pool that was in rapid equilibrium with extracellular FFA but was not on the path of tumor lipid ester synthesis. Although the existence of membrane-bound exchangeable FFA in tumor cells has been postulated by earlier workers (4, 7, 29), it still is not clear whether this pool actually exists in vivo, and, if so, what its physiological significance is. Because of technical difficulties associated with adsorption of FA to the tumor cell membrane and because our data at early times are limited, a separate study is required to evaluate the size and turnover rate of the tumor cell FFA pool that is an obligatory intermediate in lipid ester formation and in FA oxidation.

Our present analysis provides a basis for reevaluating the earlier conclusion of Spector (3) that FFA uptake by tumor cells is equal to the net rate of FA accumulation in Ehrlich ascites carcinoma cells during growth. We have previously noted (18) that the uptake of FFA by Ehrlich ascites tumor cells, as measured by Spector, must have been at least an order of magnitude faster than the net rate of tumor lipid FA accumulation. In the present analysis we have estimated that the gross flux of FFA into Ehrlich ascites carcinoma cells was 26-fold greater than the estimated (18) net rate of total lipid FA accumulation in the tumor cells. This relationship is of very great importance, for it permits one to calculate the following: (*1*) the approximate rate of tumor lipid lipolysis; (*2*) the approximate rate at which FA in the tumor's extracellular fluid may be derived from the tumor cells in vivo; and (*3*) the fraction of the fluid FFA that was derived from the host's tissues (approximately one-half).

We have ignored lipogenesis from two-carbon fragments as a possible important source of fatty acids on the basis of reports by other workers that de novo fatty acid synthesis from glucose in tumor cells is very slow relative to the rate of FFA uptake (3, 6). However, we consider that further studies of fatty acid synthesis in cancer cells are necessary in view of evidence that the lipogenic capacity of a tissue may be overlooked unless measurements are carried out during the time food is being absorbed from the gastrointestinal tract (36, 37) and in view of reports of high enzymatic potential for synthesizing fatty acids in hepatomas (38).

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REFERENCES

1. Mider, G. B., C. D. Sherman, Jr., and J. J. Morton. 1949. The effect of Walker carcinoma 256 on the total lipid content of rats. *Cancer Res.* **9:** 222–224.
2. Haven, F. L., and W. R. Bloor. 1956. Lipids in cancer. *Advan. Cancer Res.* **4:** 237–314.
3. Spector, A. A. 1967. The importance of free fatty acid in tumor nutrition. *Cancer Res.* **27:** 1580–1586.
4. Spector, A. A., and D. Steinberg. 1967. The effect of fatty acid structure on utilization by Ehrlich ascites tumor cells. *Cancer Res.* **27:** 1587–1594.
5. Spector, A. A., and D. Steinberg. 1966. Release of free fatty acids from Ehrlich ascites tumor cells. *J. Lipid Res.* **7:** 649–656.
6. Medes, G., A. Thomas, and S. Weinhouse. 1953. Metabolism of neoplastic tissue. IV. A study of lipid synthesis in neoplastic tissue slices in vitro. *Cancer Res.* **13:** 27–29.
7. Fillerup, D. L., J. C. Migliore, and J. F. Mead. 1958. The uptake of lipoproteins by ascites tumor cells. The fatty acid–albumin complex. *J. Biol. Chem.* **233:** 98–101.
8. Umbreit, W. W., R. H. Burris, and J. F. Stauffer, eds. 1957. Manometric Techniques and Tissue Metabolism. Rev. ed. Burgess, Minneapolis. 149.
9. Baker, N., and M. C. Schotz. 1964. Use of multicompartmental models to measure rates of triglyceride metabolism in rats. *J. Lipid Res.* **5:** 188–197.
10. Friedberg, S. J., W. R. Harlan, Jr., D. L. Trout, and E. H. Estes, Jr. 1960. The effect of exercise on the concentration and turnover of plasma nonesterified fatty acids. *J. Clin. Invest.* **39:** 215–220.
11. Riley, V. 1960. Adaptation of orbital bleeding technic to rapid serial blood studies. *Proc. Soc. Exp. Biol. Med.* **104:** 751–754.
12. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
13. Dole, V. P. 1956. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. *J. Clin. Invest.* **35:** 150–154.
14. Schotz, M. C., A. S. Garfinkel, R. J. Huebotter, and J. E. Stewart. 1970. A rapid assay for lipoprotein lipase. *J. Lipid Res.* **11:** 68–69.
15. Rose, H., M. Vaughan, and D. Steinberg. 1964. Utilization of fatty acids by rat liver slices as a function of medium concentration. *Amer. J. Physiol.* **206:** 345–350.
16. Baker, N., and R. J. Huebotter. 1972. Compartmental and semicompartmental approaches for measuring glucose carbon flux to fatty acids and other products in vivo. *J. Lipid Res.* **13:** 716–724.

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17. Ho, R. J. 1970. Radiochemical assay of long-chain fatty acids using ^{63}Ni as tracer. *Anal. Biochem.* **36:** 105–113.
18. Baker, N., P. Mermier, and L. Wilson. 1973. Net increase of lipid fatty acid in Ehrlich ascites carcinoma during growth. *Lipids.* **8:** 433–436.
19. Mermier, P., and N. Baker. 1973. Volume-dependent transfer of FFA from Ehrlich ascites carcinoma to host tissues. *Lipids.* **8:** 534–535.
20. Baker, N., and M. C. Schotz. 1967. Quantitative aspects of free fatty acid metabolism in the fasted rat. *J. Lipid Res.* **8:** 646–660.
21. Baker, N., and H. Rostami. 1969. Effect of glucose feeding on net transport of plasma free fatty acids. *J. Lipid Res.* **10:** 83–90.
22. Kessler, J. I., M. Demeny, and H. Sobotka. 1967. Rates of tissue uptake of palmitic acid-1-^{14}C complexed with albumin by two different procedures. *J. Lipid Res.* **8:** 185–190.
23. Berman, M. 1965. Compartmental analysis in kinetics. *In* Computers in Biomedical Research. Vol. 2. Stacey, R. W., and B. D. Waxman, editors. Academic Press, New York. 173–201.
24. Baker, N. 1969. The use of computers to study rates of lipid metabolism. *J. Lipid Res.* **10:** 1–24.
25. Weiss, S. B., E. P. Kennedy, and J. Y. Kiyasu. 1960. The enzymatic synthesis of triglyceride. *J. Biol. Chem.* **235:** 40–44.
26. Lands, W. E. M. 1960. Metabolism of glycerolipids. II. The enzymatic acylation of lysolecithin. *J. Biol. Chem.* **235:** 2233–2237.
27. Snyder, F., and R. Wood. 1968. The occurrence and metabolism of alkyl and alk-1-enyl ethers of glycerol in transplantable rat and mouse tumors. *Cancer Res.* **28:** 972–978.
28. Yamakawa, T., N. Ueta, and R. Irie. 1962. Biochemistry of lipids of neoplastic tissue. I. Lipid compositon of ascites tumor cells of mice. *Jap. J. Exp. Med.* **32:** 289–296.
29. Spector, A. A., D. Steinberg, and A. Tanaka. 1965. Uptake of free fatty acids by Ehrlich ascites tumor cells. *J. Biol. Chem.* **240:** 1032–1041.
30. Klein, G., and L. Révész. 1953. Quantitative studies on the multiplication of neoplastic cells in vivo. I. Growth curves of the Ehrlich and MClM ascites tumors. *J. Nat. Cancer Inst.* **14:** 229–277.
31. Weinhouse, S., A. Allen, and R. H. Millington. 1953. Metabolism of neoplastic tissue. V. Fatty acid oxidation in slices of transplanted tumors. *Cancer Res.* **13:** 367–371.
32. Gordon, R. S., Jr., and A. Cherkes. 1958. Production of unesterified fatty acids from isolated rat adipose tissue incubated in vitro. *Proc. Soc. Exp. Biol. Med.* **97:** 150–151.
33. Randle, P. J., P. B. Garland, C. N. Hales, and E. A Newsholme. 1963. The glucose–fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet.* **1:** 785–789.
34. Mayes, P. A., and J. M. Felts. 1968. The functional status of lipoprotein lipase in rat liver. *Biochem. J.* **108:** 483–487.
35. Medes, G., G. Paden, and S. Weinhouse. 1957. Metabolism of neoplastic tissues. XI. Absorption and oxidation of dietary fatty acids by implanted tumors. *Cancer Res.* **17:** 127–133.
36. Baker, N., and R. J. Huebotter. 1972. Rapid activation and inactivation of fatty acid synthesis from glucose in vivo. *J. Lipid Res.* **13:** 329–337.
37. Favarger, P., and J. Gerlach. 1958. Research on the synthesis of fats from acetate or glucose. IV. Importance of the liver in lipogenesis: experimental analysis. *Physiol. Pharmacol. Acta.* **16:** 188–200.
38. Sabine, J. R., S. Abraham, and I. L. Chaikoff. 1967. Control of lipid metabolism in hepatomas: insensitivity of rate of fatty acid and cholesterol synthesis by mouse hepatoma BW7756 to fasting and to feedback control. *Cancer Res.* **27:** 793–799.

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