

Relationship between fatty acid and glucose utilization in Ehrlich ascites tumor cells

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ABSTRACT Glucose greatly increased total free fatty acid (FFA) esterification by Ehrlich ascites tumor cells. However, the FFA concentration of the cells was not altered. Less exogenous FFA was oxidized to CO₂ at any given extracellular FFA:albumin molar ratio when glucose was available, but increasing amounts of radioactive CO₂ were produced as the FFA:albumin molar ratio was raised, even in the presence of glucose. It is suggested that glucose, by providing either energy or an excess of triose acceptor for fatty acid esterification, stimulated FFA uptake only indirectly, by increasing the utilization of FFA subsequent to initial uptake from the medium, i.e., by increasing the turnover rate of the cellular FFA pool.

Availability of glucose decreased the oxidation of endogenous lipid radioactivity and the depletion of endogenous lipid ester radioactivity. Most of the radioactivity utilized was derived from phospholipids, and depletion of phospholipid radioactivity was spared when glucose was available. Depletion of cellular total lipid ester also was spared in the presence of glucose.

Availability of FFA did not decrease total glucose uptake or its oxidation to CO₂. Glucose utilization by these cells appears not to be regulated by FFA availability in the manner that Randle and coworkers described for muscle.

KEY WORDS free fatty acids · palmitate · glucose uptake · oxidation · esterification · phospholipids · neutral lipids · esters · saponification · Ehrlich ascites tumor cells · albumin

THE RELATIONSHIP between fatty acid and glucose metabolism in tumor cells has been previously investigated (2-5). Recent developments suggested

A preliminary report of this work was presented before the Council on Arteriosclerosis of the American Heart Association, Bal Harbour, Fla., 13 October 1965 (1).

Abbreviations: FFA, free fatty acid(s); \bar{v} , the approximate FFA:albumin molar ratio obtained by dividing total FFA concentration by total albumin concentration.

that further study of this relationship was warranted. First, the processes involved in transfer of free fatty acid (FFA) from FFA-albumin complex to cell surface have been extensively studied with the use of suspensions of Ehrlich ascites tumor cells as a model system (6, 7). This offered an opportunity to examine in more detail the nature of the well-known effects of glucose on utilization of FFA (8, 9) and on utilization of endogenous lipid stores. Second, FFA have been shown to inhibit glucose uptake in non-tumor tissues (10), and it was of interest to determine whether this regulatory mechanism was operative in tumor cells.

MATERIALS AND METHODS

Methods for the preparation, incubation, and analysis of Ehrlich ascites tumor cells and the preparation of all solutions are described in the preceding paper (11). Uniformly labeled glucose-¹⁴C was obtained from Nuclear-Chicago Corporation (Des Plaines, Ill.). When glucose-¹⁴C served as substrate, its specific radioactivity was determined by liquid scintillation counting in a scintillator solution containing 30% methanol (6), and all radioactive measurements, including those of the cell lipid extract, were made with this scintillator. The lipid ester content of cells was determined by hydroxamic acid formation (12) as described previously (7). Endogenous lipid of the cell was labeled by injection of palmitate-1-¹⁴C into the peritoneal cavity of tumor-bearing mice prior to harvesting of the tumor cells (11).

To determine the amounts of water-soluble radioactive materials released by the cell during incubation with palmitate-1-¹⁴C in vitro, we transferred the total contents of the incubation flask without dilution to plastic tubes, and the cells were sedimented by centrifugation at 0°C for 3 min at 10,000 × *g*. One milliliter of the

supernatant solution was transferred to 5 ml of isopropanol-isooctane- $N H_2SO_4$ 40:10:1 and vigorously shaken. After 30 min, 3 ml of isooctane and 2 ml of 0.1 $N H_2SO_4$ were added, and the isooctane phase was removed after shaking. The acidic isopropanol phase was extracted three more times with isooctane, and the isooctane extracts were pooled and reextracted with 10 ml of 0.005 $N H_2SO_4$. Residual protein was sedimented from the acidic isopropanol phase by centrifugation, and the supernatant solution was made alkaline by the addition of NaOH. Saturated $Ba(OH)_2$ (0.2 ml) was added for the purpose of removing any residual $^{14}CO_2$ and the solution was recentrifuged. Aliquots of the supernatant isopropanol solution and the dilute H_2SO_4 extract were analyzed for radioactivity in the methanol-containing scintillator, and the sum of the radioactivity in these phases was taken as the amount of palmitate oxidized to water-soluble compounds. Palmitate- $1-^{14}C$ solutions not incubated with cells contained no appreciable amounts of water-soluble radioactivity when similarly extracted.

Total glucose uptake from the incubation medium was measured with the aid of commercially available glucose oxidase reagents (Worthington Biochemical Corporation, Freehold, N.J.).

RESULTS

Effect of Glucose on FFA Utilization

FFA is rapidly taken up in unesterified form by Ehrlich ascites tumor cells in amounts dependent on the medium FFA:albumin molar ratio (\bar{v}), and this cellular FFA concentration is maintained relatively constant as

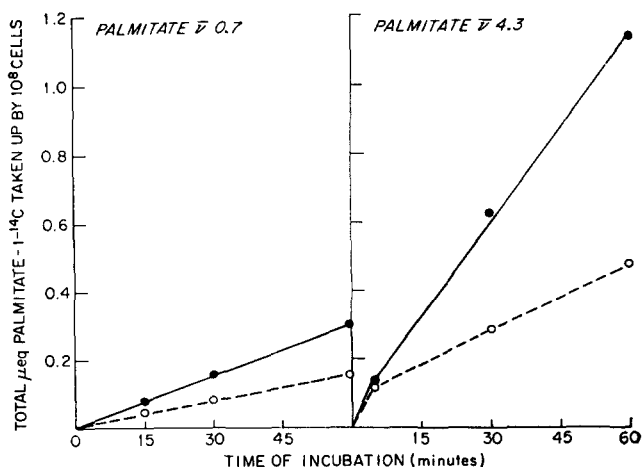


FIG. 1. Effect of 4.4×10^{-3} M glucose on the total uptake of palmitate- $1-^{14}C$ from the incubation medium during a 1 hr incubation. Two experiments with widely differing palmitate- $1-^{14}C$:albumin molar ratios are shown; \bar{v} 0.7 on the left side of the figure and \bar{v} 4.3 on the right side. The solid curve represents incubations in the presence of glucose; the dashed curve, incubations in the absence of glucose.

TABLE 1 EFFECT OF UNLABELED GLUCOSE ON INCORPORATION OF PALMITATE- $1-^{14}C$ INTO CELLULAR ESTERIFIED LIPID AFTER 60 MIN INCUBATION

Lipid Ester	Palmitate- $1-^{14}C$ Incorporated*		Increase Due to Glucose
	No Glucose	Glucose†	
	$\mu\text{eq/hr per } 10^8 \text{ cells}$		%
Phospholipid	0.046	0.166	361
Neutral lipid	0.017	0.094	553

* Mean of 2 determinations.

† 5×10^{-3} M.

long as the extracellular \bar{v} does not change appreciably (6, 7). The rate of exogenous FFA utilization is a function of the steady state concentration of FFA in the cells and, hence, the extracellular \bar{v} (7). Exogenous palmitate utilization increases as the cellular FFA concentration increases to $0.02 \mu\text{eq}/10^8$ cells, beyond which point the substrate no longer appears to be rate-limiting. This unesterified palmitate content of the cell is reached at an extracellular \bar{v} of 2.0 (7). Fig. 1 shows the effect of glucose on the utilization of palmitate- $1-^{14}C$ by the cell over 1 hr at $\bar{v} = 0.7$ (i.e., when steady state FFA content of the cells is rate-limiting), and at $\bar{v} = 4.3$ (i.e., when it is not). In either case, the presence of 4.4×10^{-3} M glucose greatly increased total utilization of exogenous palmitate. Identical results were obtained when glucose was present at higher concentration (1×10^{-2} M). The slightly higher initial rate of palmitate uptake at \bar{v} 4.3, both in the presence and in the absence of glucose, reflects the previously demonstrated rapid initial uptake of FFA in unesterified form (6, 7). At this high value of \bar{v} , about $0.1 \mu\text{eq}$ of palmitate is taken up in unesterified form within 1 min, after which the cellular content of unesterified palmitate remains constant throughout the remainder of the incubation. No initial rapid component of utilization is noted at \bar{v} 0.7 (Fig. 1) because the unesterified palmitate content of the cell at this low \bar{v} is relatively small, less than $0.01 \mu\text{eq}/10^8$ cells.

The glucose-induced stimulation of palmitate utilization at a given value of \bar{v} was shown not to be associated with an increase in the initial uptake of palmitate in unesterified form. Measurements of the cellular concentration of unesterified palmitate were made after 2 min of incubation which, as discussed above, yields values not significantly different from those obtained at any later time up to at least 1 hr. As shown in Fig. 2, over a wide range of values of \bar{v} , the cellular content of unesterified palmitate in the presence of 1.4×10^{-2} M glucose was either the same as or slightly less than in the absence of glucose.

As shown in Table 1, glucose strongly stimulated incorporation of palmitate- $1-^{14}C$ into both phospholipids and neutral lipids. More exogenous palmitate was in-

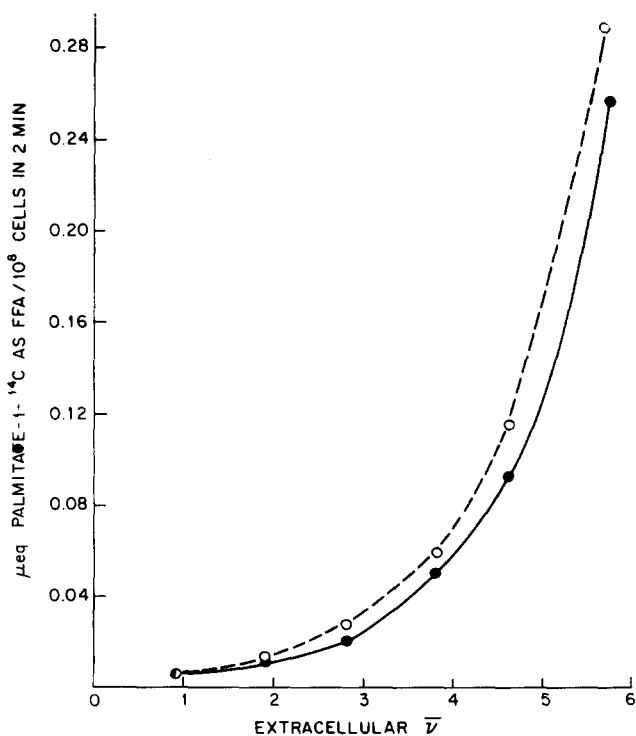


FIG. 2. Effect of 1.4×10^{-2} M glucose on the cellular unesterified palmitate concentration as a function of the palmitate- $1\text{-}^{14}\text{C}$: albumin molar ratio (\bar{v}) of the incubation medium. The solid curve represents incubation done in the presence of glucose; the dashed curve those without glucose.

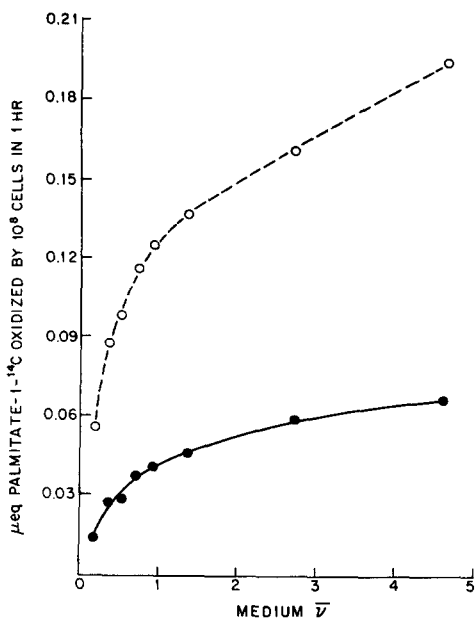


FIG. 3. Effect of 2.8×10^{-2} M glucose on the quantity of palmitate- $1\text{-}^{14}\text{C}$ oxidized to $^{14}\text{CO}_2$ as a function of the extracellular palmitate: albumin molar ratio. The solid curve represents incubations done in the presence of glucose; the dashed curve those without glucose.

TABLE 2 EFFECT OF GLUCOSE ON RELATIVE INCORPORATION OF PALMITATE- $1\text{-}^{14}\text{C}$ INTO CO_2 AND WATER-SOLUBLE PRODUCTS RELEASED FROM CELLS DURING A 1 HR INCUBATION*

Unlabeled Substrate	Radioactivity Converted to:†		
	CO_2	Water-Soluble Products	Ratio, $\text{CO}_2/\text{Water-Soluble}$
None	<i>cpm/hr</i>		
None	250,050	30,200	8.3
Glucose‡	99,700	17,100	5.8

* $2.5 \mu\text{eq}$ of palmitate- $1\text{-}^{14}\text{C}$ and $0.5 \mu\text{mole}$ of albumin, \bar{v} 5.0, in a total volume of 3 ml.

† Mean of 2 determinations.

‡ 2.5×10^{-2} M glucose.

incorporated into phospholipids than into neutral lipids, whether or not glucose was available. In the absence of glucose, the ratio of incorporation into phospholipids relative to neutral lipids was 2.7. With glucose, this was decreased to 1.8, which indicates that relatively more palmitate was channeled into neutral lipid esters when glucose was available.

Oxidation of palmitate- $1\text{-}^{14}\text{C}$ to $^{14}\text{CO}_2$ was greatly depressed at all values of \bar{v} when glucose was present (Fig. 3). Over the range of \bar{v} values studied (0.3 to 4.9) only about one-third as much $^{14}\text{CO}_2$ was produced in the presence of 2.8×10^{-2} M glucose. In the course of these oxidation studies, it was observed that a small but significant quantity of radioactivity appeared in the medium in nonlipid form, i.e., was present in the acidic isopropanol phase after extraction of the incubation medium and removal of the isooctane phase (Table 2). Since the medium was extracted at acid pH and $\text{Ba}(\text{OH})_2$ was added, the radioactivity could not represent carbonate. Repeated extractions of the isopropanol phase with isooctane did not remove the radioactivity, and the amounts found were not closely correlated with the quantity of palmitate- $1\text{-}^{14}\text{C}$ present in the incubation medium. In view of the recent work of Hepp, Prusse, Weiss, and Wieland (13), it seems likely that some of this water-soluble radioactivity represents acetate. In any case, the amount relative to the quantity of palmitate- $1\text{-}^{14}\text{C}$ converted to $^{14}\text{CO}_2$ is small and does not alter the qualitative conclusions regarding the effects of glucose on fatty acid oxidation. As shown in Table 2, even when palmitate utilization was maximal ($\bar{v} = 5$), the water-soluble radioactivity produced was only one-eighth that appearing as $^{14}\text{CO}_2$. Addition of glucose suppressed oxidation of palmitate to CO_2 and also decreased the yield of water-soluble metabolite. Thus, the inhibition of $^{14}\text{CO}_2$ production in the presence of glucose does not simply reflect diversion of the labeled palmitate into an alternative degradative pathway.

Effect of Glucose on the Utilization of Endogenous Lipid

By intraperitoneal injection of palmitate- $1\text{-}^{14}\text{C}$ into

TABLE 3 EFFECT OF UNLABELED GLUCOSE ON OXIDATION OF ENDOGENOUSLY LABELED LIPID DURING A 90 MIN INCUBATION

Unlabeled Substrate	Radioactivity Recovered as $^{14}\text{CO}_2^*$
	<i>cpm</i>
Albumin†	39,700
Albumin + glucose‡	18,100
Albumin + palmitate§	18,400
Albumin + palmitate + glucose	13,500

Cellular lipid was labeled in vivo by intraperitoneal injection of palmitate- ^{14}C -albumin into tumor-bearing mice. Prior to incubation, the cells contained 1250 cpm as CO_2 . This was subtracted from the total $^{14}\text{CO}_2$ recoveries noted after 90 min of in vitro incubation, and the differences are shown in the table.

* Mean of 2 determinations.

† 3.3×10^{-4} M albumin.

‡ 1.4×10^{-2} M glucose.

§ 1.7×10^{-3} M palmitate.

tumor-bearing mice, it was possible to incorporate radioactivity predominantly into esterified lipids of the tumor cell and then to study the utilization of the endogenous lipid during subsequent in vitro incubation. As shown in Table 3, the presence of 1.4×10^{-2} M glucose in the incubation medium reduced the oxidation of endogenous lipid radioactivity to $^{14}\text{CO}_2$ by 50% in a medium containing FFA-extracted albumin. When the medium contained a large amount of unlabeled palmitate ($\bar{v} = 5$), which in itself decreased endogenous $^{14}\text{CO}_2$ production by about 50%, the addition of glucose further reduced endogenous $^{14}\text{CO}_2$ formation, but only by about 15%. Availability of glucose in the incubation medium also conserved endogenous lipid radioactivity, as shown in Table 4. Prior to incubation, much more radioactivity was present in phospholipids than in neutral lipid esters. After 90 min of in vitro incubation without glucose, 16% of the endogenous esterified radioactivity was depleted, and essentially all of this was lost from the phospholipid fraction. In contrast, only 4% of the total lipid ester radioactivity was depleted when 1.8×10^{-2} M glucose was present, approximately equal amounts being lost from the phospholipid and neutral lipid fractions. The glucose-induced conservation of endogenous lipid radioactivity is consistent with the reduction in oxidation of

TABLE 4 EFFECT OF GLUCOSE ON UTILIZATION OF ENDOGENOUS LIPID LABELED IN VIVO WITH PALMITATE- ^{14}C

Incubation Medium	Time of Incubation	Lipid Radioactivity*		
		Total	Phospholipid	Glyceride
	<i>min</i>			
	0	332,700	237,500	70,600
Buffer	90	279,100	201,900	69,000
Buffer + glucose†	90	319,100	231,500	65,600

* Mean of 2 determinations.

† 1.8×10^{-2} M glucose.

TABLE 5 CHANGES IN TOTAL CELLULAR LIPID ESTER IN THE PRESENCE OF GLUCOSE

Substrate	Time of Incubation	Cellular Lipid Ester*					
		Expt. 1		Expt. 2		Expt. 3	
		Total	Average Decrease	Total	Average Decrease	Total	Average Decrease
	<i>hr</i>	$\mu\text{eq}/10^8 \text{ cells}$					
None	0	8.32	—	8.14	—	7.32	—
		8.42		8.26		7.34	
				8.06		7.52	
None	2	7.92	0.54	7.66	0.57	6.73	0.64
		7.75		7.66		6.65	
				7.43		6.86	
Glucose†	2	8.15	0.38	7.94	0.13	7.20	0.19
		7.82		8.10		7.23	
				8.02		7.17	

* Duplicate determinations were made with the same cell preparation in Expt. 1, and triplicate determinations were made in Expts. 2 and 3. The individual values and the average decrease are listed.

† 1.8×10^{-2} M glucose.

endogenous lipid radioactivity noted above.

Measurements of net changes in lipid ester confirmed the "sparing" effect of glucose on endogenous lipid. As is shown in Table 5, the cellular stores of lipid ester were depleted by an average of 0.29 $\mu\text{eq}/\text{hr}$ per 10^8 cells during incubation without exogenous substrate, but only by 0.12 $\mu\text{eq}/\text{hr}$ per 10^8 cells in the presence of glucose. These decrements correspond, respectively, to only 3.6 and 1.5% of the initial cellular lipid ester content, a much smaller percentage decrease than that calculated from the radioisotope data. This disparity suggests either (a) that the lipid ester pool is not uniformly labeled, a fraction of higher specific radioactivity contributing a major part of the fatty acid utilized, or (b) that esterified palmitate is turning over more rapidly than most of the other esterified fatty acids.

Effect of FFA on Glucose Utilization

Between 16 and 22 μmoles of glucose were taken up from the incubation medium by 10^8 cells in 1 hr (Table 6). The rate of glucose uptake was slightly higher during the first 10 min, after which it remained linear during the remainder of the 1 hr incubation. Essentially no difference in total glucose uptake was noted from addition of either albumin or palmitate-albumin, $\bar{v} = 5$, to the medium.

The oxidation of glucose- ^{14}C to $^{14}\text{CO}_2$ is shown in Table 7. $^{14}\text{CO}_2$ production was linear over 1 hr, and about the same amount of glucose radioactivity was oxidized to CO_2 at glucose concentrations of 2.5×10^{-3} and 1×10^{-2} M. Addition of unlabeled palmitate at either $\bar{v} 1$ or 5 had very little effect on the quantity of glucose radioactivity oxidized to CO_2 ; the small and variable differences noted

TABLE 6 EFFECT OF PALMITATE ON THE UPTAKE OF GLUCOSE (5.5×10^{-3} M)

Addition	Glucose Uptake	
	Expt. 1*	Expt. 2*
	<i>μmoles/hr per 10⁸ cells</i>	
None	16.8	21.6
Albumin (\bar{p} 0)†	16.5	19.6
Albumin (\bar{p} 0.4)‡	15.7	21.6
Palmitate-albumin (\bar{p} 5.0)§	16.5	21.6

0.5×10^8 cells were incubated at 37°C with glucose, and the additional substrates listed, in a total volume of 4 ml. The incubation mixture was centrifuged at $4000 \times g$, and 0.2 ml of supernatant solution was analyzed for glucose content by the glucose oxidase method.

* Mean of 2 determinations.

† FFA-extracted albumin (0.125 μmole/ml) containing no titratable acidity.

‡ Unextracted albumin (0.125 μmole/ml) containing 0.42 μeq of titratable acidity per μmole of protein.

§ 0.625 μeq/ml of palmitate and 0.125 μmole/ml of albumin.

are at the limits of reproducibility of the procedures used.

Table 8 shows the effect of FFA on the incorporation of glucose-¹⁴C radioactivity into cellular esterified lipids. When exogenous palmitate ($\bar{p} = 4$) was available, total incorporation of glucose radioactivity into cellular lipid esters was doubled, with more being incorporated into neutral lipid esters relative to phospholipids than in an FFA-free medium. In an identical experiment, the total lipid extract from the cells was saponified and the radioactivity present in lipid and water-soluble components was measured. As seen in Table 9, the presence of exogenous palmitate again doubled the total incorporation of glucose radioactivity into lipid. However, the amount of radioactivity incorporated into the fatty acid fraction was about the same whether or not unlabeled palmitate was available, whereas the label appearing in water-soluble components (glycerol and water-soluble derivatives of the phospholipids) was doubled. It should be noted that the combined amount of glucose oxidized to ¹⁴CO₂ and incorporated into lipid represented only 2–4% of the total glucose taken up by the cells.

TABLE 7 EFFECT OF UNLABELED PALMITATE ON THE OXIDATION OF GLUCOSE-¹⁴C

Time of Incubation	Glucose Concentration of Medium	Glucose- ¹⁴ C Converted to ¹⁴ CO ₂		
		Buffer	Palmitate-Albumin* (\bar{p} 1)	Palmitate-Albumin† (\bar{p} 5)
		<i>μmole/10⁸ cells</i>		
30	2.5×10^{-3}	0.25	0.23	0.21
	1×10^{-2}	0.23	0.25	0.27
60	2.5×10^{-3}	0.59	0.55	0.54
	1×10^{-2}	0.55	0.56	0.58

* 1 μeq of palmitate + 1 μmole of albumin, \bar{p} 1.0.

† 5 μeq of palmitate + 1 μmole of albumin, \bar{p} 5.0.

TABLE 8 INCORPORATION OF GLUCOSE-¹⁴C INTO CELLULAR LIPID

Unlabeled Substrate	Glucose- ¹⁴ C Incorporated into:*		
	Total Lipid	Phospholipid	Neutral Lipid
	<i>μmole/hr per 10⁸ cells</i>		
None	0.074	0.057	0.017
Palmitate-albumin†	0.143	0.085	0.059

* Mean of 2 determinations. The glucose-¹⁴C concentration in the incubation medium was 1.6×10^{-2} M.

† 4 μeq of palmitate + 1 μmole of albumin; \bar{p} 4.0.

TABLE 9 INCORPORATION OF GLUCOSE-¹⁴C INTO LIPID FRACTIONS (AFTER SAPONIFICATION)

Unlabeled Substrate	Glucose- ¹⁴ C Incorporated into:*			
	Total	Nonsaponifiable Lipid	Saponifiable Lipid	Water Soluble
	<i>μmole/hr per 10⁸ cells</i>			
None	0.071	0.002	0.023	0.046
Palmitate-albumin†	0.133	0.003	0.022	0.108

* Mean of 2 determinations. The incubation medium contained 8.9×10^{-3} M glucose-¹⁴C.

† 4.0 μeq of palmitate + 1 μmole of albumin, \bar{p} 4.0.

DISCUSSION

The present results show that glucose increases the rate of utilization of exogenous palmitate-1-¹⁴C, markedly increasing the quantity of labeled FFA incorporated into cell lipid esters, although simultaneously depressing FFA oxidation to ¹⁴CO₂. The glucose-induced stimulation of FFA utilization occurs at all levels of \bar{p} , without affecting the cell FFA concentration. Therefore, glucose appears to stimulate FFA utilization by increasing the turnover rate of the cellular FFA pool without altering its size. Presumably, then, the rate of transfer of FFA from albumin to cell is increased indirectly by stimulation of FFA esterification from the cellular FFA pool. These observations suggest that two factors regulate FFA utilization: the FFA content of the cell, an equilibrium concentration determined by the availability of FFA in the exogenous medium; and the turnover rate of the cellular FFA pool. A similar mechanism of FFA utilization was proposed by Goodman from studies on unesterified palmitate uptake by erythrocytes (14). Glucose presumably stimulates the rate of FFA incorporation into esters by supplying energy and (or) being converted to glycerophosphate, thus providing an excess of fatty acid acceptor for esterification.

The availability of glucose not only stimulates the synthesis of esterified lipids but also decreases the net depletion of endogenous esterified fatty acid. Both effects are responsible for the net accumulation of cellular lipid

ester that occurs when glucose and FFA are available to this tumor cell (7). The mechanism for "sparing" of endogenous lipid when glucose is present probably is increased reesterification of lipolyzed fatty acid, less FFA being available for either oxidation or release from the cell (11). A glucose-induced decrease in oxidation of esterified lipid to CO₂ has been observed previously by Medes and Weinhouse (2). The present data show that glucose conserves both endogenous lipid radioactivity and ester bonds (hydroxamic acid positive material), which indicates that a true net conservation of endogenous esterified lipid occurs and not simply a decrease in turnover of a small ester pool of very high specific radioactivity.

The greater incorporation of palmitate-1-¹⁴C into phospholipids during the *in vivo* labeling procedure and the greater depletion of radioactivity from this fraction after *in vitro* incubation of the labeled cells indicate that the phospholipid fraction, or a component of it, is turning over more rapidly than the bulk of the cell lipid. Studies with rat diaphragm and heart also indicate that phospholipids supply relatively more endogenous lipid substrate than triglyceride (15, 16). The subcellular location of the phospholipids undergoing degradation remains to be determined, but it is known that 95% of the cell lipid phosphorus is isolated with the particulate fractions of the Ehrlich ascites tumor cell (17). Because phospholipids are known to play an essential role in maintaining the functional integrity of mitochondria, and probably of other cell particles and membranes, attrition of these phospholipids in cells starved for substrate might be one of the mechanisms involved in deterioration and death of the cell.

Since relatively large amounts of glucose radioactivity are incorporated into the glycerol moiety of lipid esters in the absence of an exogenous supply of fat (0.05 μmole/hr per 10⁸ cells), it appears that esterification continues actively, even when only endogenously supplied fatty acid is available. The conversion of glucose to fatty acids is much too small to supply the amounts required (0.10–0.15 μmole/hr per 10⁸ cells), and the endogenous FFA content at the beginning of the incubation is also very small (6, 11). Hence, the endogenous fatty acid used for lipid ester synthesis under these conditions must be derived by lipolysis, which indicates that a constant turnover of cellular esterified lipid occurs. Just as it stimulates esterification of exogenous FFA taken up by the cell, glucose is thought to stimulate the reesterification of endogenous FFA produced by lipolysis, thereby conserving endogenous lipid. This is compatible with the currently accepted hypothesis of glucose action in adipose tissue (18).

No evidence for inhibition of glucose utilization in the presence of FFA is apparent, which indicates that, in the Ehrlich ascites tumor cell, glucose metabolism is not

regulated to any appreciable extent by FFA availability. Indeed, in the presence of FFA, twice as much glucose was converted to lipid. However, since only about 2% of glucose taken up was converted to lipid even in the presence of FFA, this stimulation would not be expected to significantly influence over-all glucose utilization. About 4% of the glucose utilized was oxidized to CO₂ and, as reported previously by Scholefield, Sato, and Weinhouse (4), this was not influenced by availability of exogenous FFA. These data indicate that most of the glucose taken up is converted to metabolites other than CO₂ and lipid, consistent with the known high rates of aerobic glycolysis in tumors (19, 20). Previous work has shown that 60–90% of the glucose taken up by the Ehrlich ascites tumor cell is catabolized to lactate under aerobic conditions (19, 21, 22). The FFA-induced inhibition of glucose uptake in muscle (10, 23) is thought to be secondary to accumulation of intracellular citrate (24–27). When citrate accumulates, phosphofructokinase is inhibited and glycolysis is slowed. In the Ehrlich cell, as in other tissues, the phosphofructokinase reaction appears to be rate-limiting in glycolysis (28). Thus, it will be important to determine whether citrate accumulates in this cell when both FFA and glucose are available and whether Ehrlich cell phosphofructokinase is regulated by citrate concentration.

Does the apparent absence of FFA regulation of glucose utilization in these cells reflect special properties of tumor tissue? Or does it reflect the properties of the tissue of origin, probably mammary epithelium (29, 30)? There is reason to believe that it may be the latter. Allen, Friedmann, and Weinhouse (31) were unable to demonstrate FFA-induced regulation of glucose oxidation in normal kidney and liver slices. Holt, Haessler, and Isselbacher (32) have shown that in intestinal slices, glucose utilization was actually stimulated by availability of unlabeled FFA. Thus, it is possible that the FFA-induced inhibition of glucose utilization may be a regulatory mechanism that is operative only in muscle.

Manuscript received 15 February 1966; accepted 18 May 1966.

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