Influence of pH of the medium on free fatty acid utilization by isolated mammalian cells

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ABSTRACT Studies with Ehrlich ascites tumor cells showed that small decreases in the pH of the incubation medium from 7.4 increase the magnitude of incorporation of free fatty acid (FFA) into the cells from an albumin solution. A similar effect occurred when rabbit erythrocytes, rat heart slices, or rat liver slices were incubated with FFA–bovine albumin solutions and when tumor cells were incubated with FFA in media containing human albumin, β-lactoglobulin, or rat plasma. The effect was not seen when the medium contained no protein.

When the pH of the albumin-containing medium was lowered from 7.4 to 6.6, oxidation of FFA to CO_2 by the tumor cells increased, esterification of the FFA (mostly into phospholipids and triglycerides) increased, and less esterified radioactive fatty acid was depleted from the cells. Hence, more fatty acid accumulated in the cells in more acid media.

These findings suggest that small changes in extracellular pH might regulate FFA utilization and lipid accumulation in mammalian tissues.

SUPPLEMENTARY KEY WORDS   uptake · oxidation · esterification · release · albumin · Ehrlich ascites tumor cells · erythrocytes · β-lactoglobulin

FREE FATTY ACID (FFA), the form in which fat is released from adipose cells, is a major source of lipid for many mammalian tissues (1). Mechanisms that regulate the uptake, utilization, and accumulation of FFA in tissues are of considerable interest, and cells from various tissues have been used extensively as models for detailed study of these processes (2–7).

Recently, Mackenzie, Mackenzie, and Reiss observed (8) that more lipid accumulated in both mouse fibroblasts and rat liver cells grown in tissue culture when the pH of the medium was 6.9–7.0 than when it was 7.4. Horse serum was present in these culture media, and FFA was the major source of fatty acid for the cells (9). From these observations Mackenzie et al. concluded (9) that FFA contained in the medium became more available for utilization when the pH was lowered and that the increased accumulation of lipid in the cells resulted from increased FFA incorporation.

The present report contains results from studies with isolated Ehrlich ascites tumor cells, an experimental model system, that are compatible with this interpretation. My studies indicate that the amount of exogenous FFA taken up increases progressively as the pH of the incubation medium is decreased below 7.4 and that this leads to a greater net accumulation of fatty acid in the cells.

METHODS

Radioactive FFA–albumin solutions were prepared by addition of an aqueous soap solution to albumin dissolved in buffered salt solution as described previously (10). All pH measurements were made with a Radiometer (Copenhagen) pH meter 27 equipped with an expanded scale for the pH range 6.8–8.2. Before each set of readings, the pH meter was adjusted to pH 7.0 with a solution of 0.05 M KH_2PO_4–NaOH, pH 7.00 ± 0.02 at 25°C, obtained from Fisher Scientific Company (Fairlawn, N. J.). This instrument, although found to be quite stable, was always adjusted several times with the standard buffer solution during each set of readings. The accuracy of determining the pH of FFA–albumin solutions is illustrated by the following data (mean ± SEM, 10 determinations at 23°C): pH 6.98 ± 0.0021, pH 7.42 ± 0.0010, pH 7.85 ± 0.0008. Solutions were adjusted to the required pH with either 0.1 M NaOH or 0.1 M HCl. Fraction V bovine albumin, obtained from Pentex, Inc.

Abbreviation: FFA, free fatty acid.
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(Kankakee, Ill.), contained 0.16 μeq of FFA per μmole of protein as determined by titration (11). Crystalline bovine albumin was purchased from Armour Pharmaceutical Co. (Chicago, Ill.) and was extracted with charcoal by the method of Chen (11) before it was used.

Ehrlich ascites tumor cells were harvested from the peritoneal cavity of tumor-bearing mice and washed as described previously (12). Cells were incubated with FFA–albumin solutions at 37°C with air as the gas phase. Unless stated otherwise, the incubation medium contained 0.116 M NaCl, 0.0049 M KCl, 0.0012 M MgSO$_4$, and 0.016 M Na$_2$HPO$_4$ and was adjusted to the required pH with either NaOH or HCl. Since the amounts of either Na$^+$ or Cl$^-$ added in the pH adjustments were small relative to those already present in the medium, corrections for differences in ionic strength were not made.

After incubation, the cells were isolated by centrifugation at 2000 g for 3 min at 0°C and washed twice with 30 ml of the buffered salt solution that was adjusted to the same pH as the medium in which the cells were incubated. The lipid contained in the sedimented cell pellet was extracted with 20 ml of chloroform–methanol 2:1 (13), and the chloroform phase was isolated after addition of 5 ml of 0.004 N HCl (12). Radioactivity contained in one aliquot of the chloroform solution was measured with a Packard Tri-Carb 3375 liquid scintillation spectrometer in a toluene scintillator solution (12). Quenching was monitored with the external standard.

Thin-layer chromatography in hexane–diethyl ether–methanol–acetic acid 90:20:2:3 was employed to separate another aliquot of the cell lipid extract into five fractions: phospholipids, lower glycerides and cholesterol, FFA, triglycerides, and cholesteryl esters (12, 14). The lipids were made visible by exposure to iodine vapor, and after the iodine had sublimed, the outlined segments of silica gel were scraped directly into 15 ml of a dioxane-containing scintillator solution (15) for measurement of radioactivity content.

The cell lipid extract was saponified as follows: the dried residue from an aliquot of the chloroform solution was dissolved in an alcoholic KOH solution and heated for 1 hr at 65°C (16).

When only the medium was to be analyzed after incubation, the contents of the incubation flask were transferred directly to chilled plastic centrifuge tubes and the cells were sedimented at 0°C for 5 min at 6000 g (16). A 1 ml aliquot of the supernatant solution was extracted with 5 ml of isopropanol–isooctane–1 N H$_2$SO$_4$ 40:10:1 (16). Additional experimental details are included in the footnotes to the tables.

^{14}CO$_2$ evolution from cells was measured in flasks sealed with rubber serum stoppers and containing removable glass center wells (12). Radioactivity trapped in the KOH solution contained in the center well was measured in a toluene–methanol scintillator solution (12). In each experiment, duplicate incubations containing no cells were included, and the small amount of radioactivity recovered in the KOH in these incubations was subtracted from the total quantity that was recovered from the corresponding flasks containing cells. Measurements of O$_2$ consumption were made manometrically at 37°C as described previously (10, 17).

Palmitate-1-^{14}C was incorporated into the lipids of the Ehrlich cell during growth of the tumor by injection of the isotope intraperitoneally into tumor-bearing mice (18). These labeled cells were harvested, washed, and incubated as before (18).

Slices were cut from rat livers that had been perfused in situ with 50 ml of cold 0.15 M NaCl and from rat heart ventricular muscle with a Stadie–Riggs hand microtome (17). The slices were measured (wet weight), and then incubated for 2 min with palmitate-1-^{14}C–bovine albumin solutions at 37°C. The slices were recovered by pouring the contents of each incubation beaker through a gauze pad. They were washed with 20 ml of cold 0.15 M NaCl solution and transferred to a manual, all-glass tissue grinder containing 10 ml of chloroform–methanol. After homogenization, the contents of the tissue grinder were transferred to glass extraction tubes and the tissue grinder was rinsed with an additional 10 ml of chloroform–methanol. These lipid extracts were treated in the same way as those prepared from the tumor cells (12).

Freshly prepared rabbit erythrocytes were incubated for 2 min with palmitate-1-^{14}C–albumin and washed twice with 30 ml of cold 0.15 M NaCl solution. The lipid of these cells was extracted by procedure III of Ways and Hanahan (19).

RESULTS

Uptake of FFA in Unesterified Form

The tumor cells took up radioactive FFA rapidly in unesterified form from the incubation medium at each pH that was tested. The magnitude of FFA uptake depended upon both the molar ratio of the FFA–albumin solution and the pH of the incubation medium. After this rapid uptake of fatty acid in unesterified form, which occurred in less than 30 sec,[1] the cell content of unesterified radioactive fatty acid changed very little as the incubation proceeded. For example, when the cells were incubated

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[1] This and other incubation times refer only to the period of exposure of the cells to the medium at 37°C. Immediately after incubation, the contents of the flasks were transferred to tubes containing 25 ml of cold buffer solution, and the cells were sedimented by centrifugation at 0°C. Approximately 3 min elapsed between the end of the incubation and the removal of the supernatant solution from the sedimented cells.

with a palmitate–Fraction V bovine albumin solution of molar ratio 3.0, pH 7.4, only 8% more radioactivity *in unesterified form* was present in the cells after 10 min of incubation than after 30 sec, the shortest incubation period that was tested. Likewise, at pH 6.0, less than 1% more radioactivity in unesterified form was recovered from the cells after 10 min of incubation as compared with 30 sec. Hence, at each pH, a steady-state amount of radioactive FFA was associated with the cells, and this was taken up quickly after exposure to FFA–albumin.

The magnitude of FFA uptake as a function of extracellular pH is shown in Table 1. Uptake of each fatty acid that was tested (palmitate, oleate, linoleate, and laureate) increased as the pH was lowered from 8.0 to 6.0. This occurred when the FFA : albumin molar ratio was either low or high.

FFA uptake at pH 7.4 increased progressively as the molar ratio of FFA to Fraction V bovine albumin in the incubation medium was raised (Fig. 1 shows this for linoleate; similar curves were obtained for palmitate, oleate, and laurate). This is in agreement with results obtained previously (10, 20). A similar progressive increase in the magnitude of FFA uptake as a function of molar ratio occurred in media of lower pH (Fig. 1). The difference in linoleate uptake between pH 6.4 and pH 7.4 became greater as the molar ratio was raised.

A pH-dependent increase in the magnitude of radioactive FFA uptake by the tumor cells also occurred when FFA was bound to proteins other than Fraction V bovine albumin (Table 2 shows results for palmitate).

In contrast to the above results, uptake of oleate-1-^{14}C did *not* increase when the pH was lowered in an incuba-

TABLE 1   Uptake of 1-^{14}C-Labeled FFA in Unesterified Form by Ehrlich Ascites Tumor Cells

pH of Incubation Medium	No Glucose*						Glucose Present†	
	Palmitate			Oleate	Linoleate	Laurate	Palmitate	
	$\bar{v}$ 0.8‡	$\bar{v}$ 1.5	$\bar{v}$ 4.5	$\bar{v}$ 5.4	$\bar{v}$ 2.3	$\bar{v}$ 5.0	$\bar{v}$ 1.3	$\bar{v}$ 3.5
				mμeq/10⁸ cells				
8.0		10	120	140	6.9	23		
7.7		12	110	140	7.3	16		
7.4	4.3	13	130	130	6.4	17	4.6	58
7.1	5.8	15	140	200	8.1	18	5.7	77
6.7	7.0	15	200	250	12	32	8.9	107
6.4		17	250	300	14	34	11.8	129
6.0		22	290	360	16			

A 2 min incubation under air at 37°C was employed. Total volume of the incubation medium, 5.5 ml. Specific radioactivities of the FFA were approximately 10⁶ cpm/μeq. After incubation and washing, the cells were extracted with chloroform–methanol 2:1 and the lipids contained in the chloroform phase were separated by thin-layer chromatography. The radioactivity present in the zone corresponding to FFA was measured.

* In addition to labeled FFA, this medium contained 0.3 mM Fraction V bovine albumin, 0.116 M NaCl, 0.0049 M KCl, 0.0012 M MgSO₄, and 0.016 M Na₂HPO₄ and was adjusted to the required pH with either HCl or NaOH.

† This medium contained labeled FFA, 0.3 mM Fraction V bovine albumin, 0.011 M glucose, 0.05 8 M NaCl, 0.0025 M KCl, 0.0006 M MgSO₄, and 0.085 M sodium phosphate.

‡ $\bar{v}$, the molar ratio of total FFA to total albumin present in the medium before the start of incubation.

TABLE 2   Effect of pH of Medium on Initial Uptake of Palmitate-1-^{14}C Into Total Cell Lipid

Tissue	Medium*	No. of Determinations	Palmitate-1-^{14}C Uptake (Ratio pH 6.7 : pH 7.4)†	$P <$
Tumor cells	Bovine albumin	4	1.61	0.05
Tumor cells	Bovine albumin plus rat plasma‡	4	1.63	0.01
Tumor cells	Human albumin	4	1.62	0.01
Tumor cells	β-Lactoglobulin	4	1.80	0.01
Rabbit erythrocytes	Bovine albumin	4	1.13	0.01
Rat heart slices	Bovine albumin	5	1.39	0.05
Rat liver slices	Bovine albumin	5	1.51	0.01

* All media contained the 16 mM phosphate salt solution described under Table 1. The molar ratio of FFA to protein was 4.

† Ratio of the radioactivity present in the total lipid extract of the tissue incubated for 2 min at pH 6.7 as compared with pH 7.4.

‡ Equal volumes of bovine albumin dissolved in phosphate-buffered salt solution were mixed with freshly prepared rat plasma, and the solutions were adjusted to the required pH.
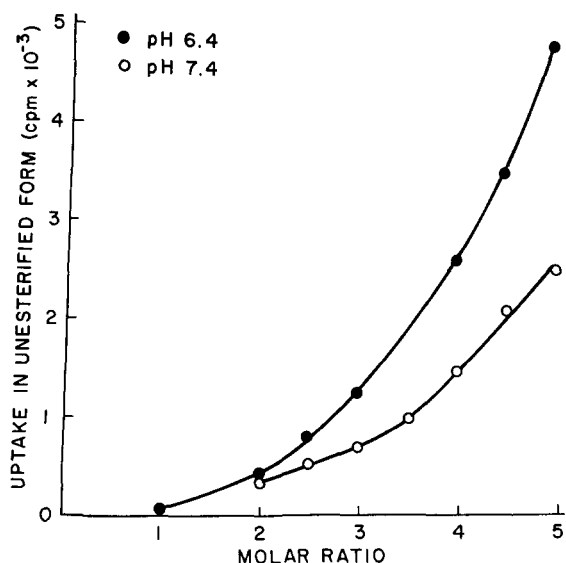
FIG. 1. Effect of pH of medium on uptake of linoleate-1-^{14}C as a function of the FFA:albumin molar ratio. The procedure was the same as that described in Table 1.

tion medium containing no protein (Table 3); on the contrary, it decreased. In these experiments, the oleate was added to the medium as the potassium soap, and the final oleate concentration was approximately 10^{-5} M, at least 10 times lower than in media containing albumin. There was no visible evidence of a decrease in oleate solubility when the pH of these protein-free media was lowered from 8.0 to 6.0.

No changes could be observed by the eye or the light microscope in the cells after exposure to media of pH 6–8 as compared with cells incubated in media of pH 7.4.

FFA Release from Cells

Much of the newly incorporated radioactive FFA is released to the medium when labeled Ehrlich cells are in-

TABLE 3 UPTAKE OF OLEATE-1-^{14}C IN UNESTERIFIED FORM
AS A FUNCTION OF THE pH OF A PROTEIN-FREE MEDIUM

	Oleate-1-^{14}C Uptake	
pH of Medium	Expt. 1	Expt. 2
	cpm	
8.0	16,900	13,300
7.5	20,500	13,500
7.0	19,400	12,700
6.5	16,900	9,000
6.0	8,000	4,100

The conditions of incubation were the same as those described in Table 1, except that the total volume of the incubation medium was 40 ml. The initial oleate-1-^{14}C concentration was approximately 0.01 mM, added as the potassium salt. The radioactivity present in the cell FFA fraction was determined.

cubated with albumin solutions at pH 7.4 (10, 12). FFA release also occurred when labeled cells were exposed to albumin-containing media of pH less than 7.4. Over the pH range of 8.0 to 5.3, maximum FFA release occurred during the 1st min of incubation. Thus, like FFA uptake, FFA release occurred very rapidly.

When the medium contained relatively high concentrations of albumin (that is, when the molar ratio of albumin to labeled cell FFA before incubation was 5 or more), the magnitude of FFA release was not changed as the pH was lowered. However, when lower concentrations of albumin were used, less cell FFA was released to the medium at pH values lower than 7.4 (Fig. 2). The medium (4 ml) contained 0.1 μmole of albumin, and the molar ratio of albumin to the initial cell content of radioactive palmitate was approximately 0.67. In making these calculations, I assumed that the specific radioactivity of the palmitate released by the cell was identical with that of the palmitate-1-^{14}C to which the cells were exposed initially.

Oxidation of FFA to CO$_2$

The amount of exogenous palmitate-1-^{14}C oxidized to ^{14}CO$_2$ increased somewhat when the medium was made[1] lightly acid. At pH 7.5, 18,940 ± 160 cpm was recovered as ^{14}CO$_2$ after a 30 min incubation; at pH 6.8, 21,260 ±
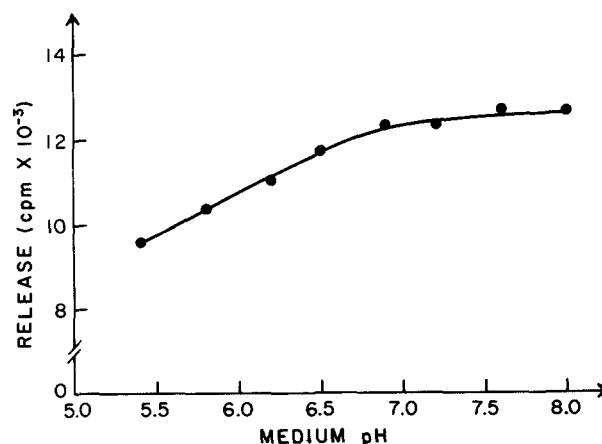

FIG. 2. Effect of pH of medium on the release of radioactive FFA from labeled cells. Cells were loaded with palmitate-1-^{14}C in vitro by exposure for 1.5 min at 37°C to 20 ml of a pH 7.4 solution containing 2 μeq of palmitate-1-^{14}C and 0.4 μmole of albumin per ml. The cells were isolated from the medium by centrifugation at 0°C, washed three times with cold buffered salt solution of pH 7.4, and dispersed in fresh buffered salt solution. 0.5-ml aliquots of cells were added to 4 ml of medium containing bovine albumin (0.025 mM) dissolved in buffer (Table 1), adjusted to the required pH with NaOH or HCl. Incubation, 5 min at 37°C in air. The cells were sedimented at 5,000 g for 5 min at 0°C, and 1 ml of the supernatant solution was analyzed for FFA radioactivity. Before the incubation was started, additional 0.5-ml aliquots of the labeled cells were added to 20 ml of chloroform–methanol solution so that the initial radioactivity content of the cells could be determined.

260 cpm (SEM for n = 7, P for difference < 0.01). As shown in Fig. 3, more $^{14}CO_2$ was produced as the pH of the incubation medium was lowered from 7.5 to 6.9. These differences were found at each time point that was examined during a 1 hr incubation. Palmitate-1-^{14}C–albumin was present in the incubation medium during the course of these incubations.

In contrast, there was no pH-dependent increase in the oxidation of FFA that had been taken up by the cells during a prior incubation (Table 4). Cells were loaded with palmitate-1-^{14}C, washed, and then incubated in protein-free media of pH 7.5 or 6.9. No difference was noted in the amount of $^{14}CO_2$ produced in either of these media. After 23 min of incubation, only 24% of the initial cell content of palmitate-1-^{14}C was converted to $^{14}CO_2$. These media did not contain glucose, and only a small proportion of the initial cell radioactive FFA content was esterified during the incubation period. Hence, failure to note a pH-dependent increase in $^{14}CO_2$ production under these conditions was not due to depletion of the substrate, namely radioactive cell palmitate.

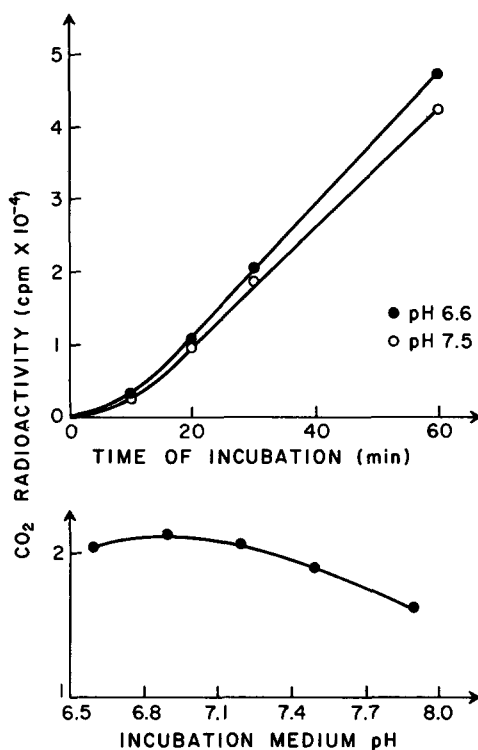The O_2 consumption of Ehrlich cells suspended in

TABLE 4 EFFECT OF pH OF MEDIUM ON RADIOACTIVITY RECOVERED AS $^{14}CO_2$ FROM LABELED CELLS DURING INCUBATION IN AN ALBUMIN-FREE MEDIUM

Time of Incubation	$^{14}CO_2$ Recovery*	
	pH 7.5	pH 6.9
min	*cpm $\times$ 10^{-2}*	
4	4.2	4.2
8	10.7	11.3
12	18.2	18.3
17	28.9	27.8
23	39.2	38.1

The cells were labeled with palmitate-1-^{14}C and washed as described in the legend to Fig. 2. Before incubation, 0.5 ml of the cell suspension contained 24,500 cpm, 67% of which was present as FFA. 0.5-ml aliquots of cells were incubated at 37°C under air with 4 ml of a solution containing 0.116 M NaCl, 0.0049 M KCl, 0.0012 M MgSO$_4$, and 0.016 M disodium phosphate. The medium was adjusted to either pH 7.5 or pH 6.9 with either NaOH or HCl. A 0.5 ml aliquot of cells contained 51 cpm as $^{14}CO_2$ before incubation in buffer and this value was subtracted from the total $^{14}CO_2$ recovered at each of the time points.
  * Each value is the mean of two determinations.

media of pH 6.8–7.6 remained constant during 2 hr of incubation. However, as compared with pH 7.4, 13% less O_2 was taken up in 2 hr at pH 6.4 and 20% less at pH 6.0. When the palmitate content of the medium was high (molar ratio 5.0), there was no difference in the quantity of O_2 consumed in media of pH 7.4 or pH 6.8 after 30 min of incubation. However, as the incubation proceeded, progressively less O_2 was taken up at pH 6.8 than at pH 7.4.

FFA Esterification

The quantity of labeled FFA incorporated into cell lipid esters after 30 min of incubation increased as the pH of the incubation medium was lowered from 7.4 to 6.6 (Table 5). This occurred whether palmitate, laurate, or linoleate served as substrate and at each of the FFA:albumin molar ratios tested. A similar pH-dependent increase in FFA esterification was found when glucose was omitted from the medium, but the magnitude of the effect was smaller.

Table 6 shows the distribution of the palmitate-1-^{14}C radioactivity incorporated into lipid esters. Similar results were obtained with the labeled FFA substrates. At each pH most of the radioactivity was incorporated into phospholipids, and the largest pH-dependent increase in exogenous FFA esterification occurred in this fraction. More FFA radioactivity also was incorporated into glycerides as the extracellular pH was lowered, especially into triglycerides. No pH-dependent increase in incorporation of labeled FFA into cholesteryl esters was found with any of the fatty acids tested.

When the incubation medium did not contain added FFA, there was no pH-dependent increase in glucose-^{14}C incorporation into cell lipids (Table 7). However, the

FIG. 3. Effect of pH of medium on the oxidation of palmitate-1-^{14}C to $^{14}CO_2$. Washed cells (0.5 ml) were incubated at 37°C under air with 5 ml of medium containing 0.24 μeq/of palmitate-1-^{14}C and 0.24 μmole of bovine albumin per ml, 0.116 M NaCl, 0.0049 M KCl, 0.0012 M MgSO$_4$, and 0.016 M sodium phosphate, and adjusted to the required pH with either NaOH or HCl. Each point represents the mean of two determinations. Upper part, time course of incubation at pH 6.6 and 7.5; lower part, incubation for 30 min over a pH range.

TABLE 5 EFFECT OF pH OF MEDIUM ON THE INCORPORATION OF 1-^{14}C-LABELED FFA INTO CELL LIPID ESTERS

FFA	FFA: Albumin Molar Ratio	Glucose Present	FFA Incorporated Into Esters in 30 min*			
			pH 7.4	7.1	6.8	6.6
			$m\mu eq/10^8$ cells			
Palmitate	1.3	Yes†	57	79	110	120
	2.2	Yes	96	80	140	160
	3.5	Yes	170	200	220	230
	1.8	No‡	51	62	75	
Linoleate	2.3	Yes	141	174	217	248
Laurate	5.0	Yes	53	74	120	159

The media contained 0.2 mM bovine albumin (total volume, 10 ml).
* Each value is the mean of two determinations.
† This medium contained the glucose solution described in Table 1.
‡ This medium was the same as the medium without glucose described in Table 1.

TABLE 6 EFFECT OF pH ON THE INCORPORATION OF PALMITATE-1-^{14}C INTO THE VARIOUS CLASSES OF CELL LIPID ESTERS

Lipid Ester Class	Palmitate Incorporated			
	pH 7.4	7.1	6.8	6.6
	$m\mu eq/10^8$ cells			
Phospholipids	121	150	162	171
Triglycerides	28.3	34.0	38.1	38.1
Mono- and diglycerides	12.9	14.2	15.6	16.0
Cholesteryl esters	3.1	3.3	2.8	2.4

Incubation was for 30 min. The medium was a glucose solution as described under Table 1. The molar ratio of palmitate to albumin was 3.5. Each value is the mean of two determinations.

incorporation of glucose-^{14}C radioactivity into cell lipids was increased when the pH of media containing unlabeled FFA–albumin was lowered from 7.4 to 6.7. At each pH, most of the glucose radioactivity was incorporated into material that was water-soluble after saponification and extraction with isooctane. The small mean difference in incorporation of glucose radioactivity into the combined saponifiable and nonsaponifiable fractions at pH 6.7 as compared with pH 7.4 was not statistically significant ($P > 0.1$), but that in the water-soluble fraction following saponification was significantly different ($P < 0.01$). About 75% of the glucose radioactivity incorporated into lipid was recovered in phospholipids at each pH. Significantly more glucose radioactivity was incorporated into this fraction at pH 6.7 than at pH 7.4 ($P < 0.05$). Taken together, these results suggest that synthesis de novo of lipid esters, particularly phosphatides, is greater when the pH of the media containing FFA–albumin and glucose is decreased slightly below 7.4. Much of the fatty acid required for this synthesis appears to be supplied by the medium FFA,

TABLE 7 EFFECT OF pH OF MEDIUM ON THE INCORPORATION OF GLUCOSE-^{14}C INTO CELL LIPIDS

Fraction	Additions to Medium*	Glucose Radioactivity Incorporated in 30 min†		
		pH 7.4	pH 6.8	P
		cpm		
Before saponification				
Total lipid	None	921 ± 87	813 ± 65	N.S.
Total lipid	Palmitate–albumin‡	742 ± 57	979 ± 76	<0.05
Phospholipids	Palmitate–albumin	557 ± 56	755 ± 50	<0.05
After saponification				
Saponifiables plus non-saponifiables	Palmitate–albumin	35 ± 8	51 ± 12	N.S.
Water-soluble	Palmitate–albumin	725 ± 36	920 ± 26	<0.01

N.S., not significant.
* All media contained 0.01 M glucose-^{14}C, 0.058 M NaCl, 0.0025 M KCl, 0.0006 M MgSO$_4$, and 0.085 M sodium phosphate (total volume, 10 ml).
† Mean ± SEM; n = 4.
‡ 0.6 mM unlabeled palmitate, 0.2 mM bovine albumin.

and the glycerol moiety probably is derived from glucose.

Sodium phosphate was present in high concentration (75 mM) in media containing glucose in order to maintain the extracellular pH essentially constant during incubation (21). The maximum change noted in pH of the medium after the 30 min incubation was a decrease of 0.13 unit; this occurred when the initial pH was 7.4. Incubation in media of high phosphate content did not alter the pattern of palmitate-1-^{14}C incorporation into the cell lipids as compared with that observed previously in media containing 16 mM sodium phosphate (22).

Additional experiments were designed to determine whether radioactive FFA already present in the cells would be incorporated into lipid esters to a greater extent or more rapidly when the pH of the incubation medium was lowered. A procedure similar to that described in Table 4 was employed. Cells were exposed briefly to a palmitate-1-^{14}C–albumin solution, washed, and incubated in a medium containing neither FFA nor albumin. Under these conditions there was little difference in the depletion of cell FFA radioactivity or in the increase in cell lipid ester radioactivity after incubation in a medium containing glucose of pH 7.4 as compared with pH 6.7.

Utilization of Endogenous Fatty Acid

Cells labeled with radioactive FFA before they were harvested from the mouse (18) were used to determine whether changes in the pH of the extracellular fluid might influence the utilization of fatty acid contained in tissue lipid esters. Palmitate-1-^{14}C was incorporated into the lipids of the Ehrlich cells by intraperitoneal injec-

TABLE 8 Effect of pH of Medium on Lipid Utilization by Cells Labeled In Vivo

| Medium | Fractional Decrease in Endogenous Lipid Radioactivity | | Difference | P |
	pH 7.4	pH 6.8		
	%		%	
Buffer*	7.3 ± 1.3	5.7 ± 0.75	1.6 ± 1.5	N.S.
Albumin†	8.2 ± 0.5	4.0 ± 1.1	4.2 ± 1.2	<0.05
Palmitate–albumin‡	8.6 ± 1.1	5.1 ± 1.2	3.5 ± 1.6	<0.1
Palmitate–albumin and glucose§	7.7 ± 0.4	3.5 ± 1.1	4.2 ± 1.2	<0.05

Cells were labeled with palmitate-1-^{14}C by intraperitoneal injection of the isotope into tumor-bearing mice. More than 98% of the radioactivity present in the cell lipids was contained in esterified forms. 1 ml of cells was incubated with 10 ml of medium for 30 min at 37°C with air as the gas phase. Percentage decreases are shown as means ± sem, n = 4, as are the differences between the decreases. N.S., not significant.

* The medium contained 0.116 M NaCl, 0.0049 M KCl, 0.0012 M MgSO₄, and 0.016 M sodium phosphate, and was adjusted to pH 7.4 or 6.8 by addition of either NaOH or HCl.

† In addition to the above salts, the medium contained albumin (0.2 mM). The fatty acid content of the albumin was 0.16 μeq/ml.

‡ Unlabeled palmitate, 3 μeq/μmole albumin, was added to the medium described in †.

§ The medium contained 0.6 mM unlabeled palmitate, 0.2 mM albumin, 0.058 M NaCl, 0.0025 M KCl, 0.0006 M MgSO₄, and 0.085 M sodium phosphate.

tion of the isotope into tumor-bearing mice. The labeled cells were then harvested, washed, and incubated in vitro (18). In no case was *more* endogenous lipid radioactivity depleted when the labeled cells were incubated in media of pH 6.8 as compared with those of pH 7.4 (Table 8). In fact, less endogenous lipid radioactivity was utilized at pH 6.8 than at pH 7.4 when the medium contained albumin, unlabeled palmitate–albumin, or glucose plus unlabeled palmitate–albumin.

Direct chemical determinations of the changes in the total cell content of fatty acid were not attempted because the pH-induced differences, as calculated from these isotopic data, were judged to be too small to be measured accurately. However, tentative conclusions concerning the net fatty acid content of the cells can be drawn from the isotopic data. When the pH of the extracellular fluid was lowered, more FFA present in the incubation medium was taken up by the cell and less endogenous fatty acid was utilized. These results indicate that a relative net increase in total fatty acid content almost certainly occurred when cells were incubated in media that were slightly acid as compared with media of pH 7.4.

DISCUSSION

These data indicate that the uptake and utilization of FFA are greater when the medium pH is decreased

slightly below 7.4. The pH dependence of FFA uptake is not unique to the mouse Ehrlich ascites tumor cell or to FFA complexed to bovine albumin, for a similar effect occurred with rat heart slices, rat liver slices, and rabbit erythrocytes and with media in which bovine album'n was replaced by human albumin or β-lactoglobulin (Table 2). The phenomenon also occurred when plasma was added to the incubation medium.

Mackenzie et al. have suggested (8, 9) that the increased accumulation of fat in cells grown in tissue culture media of pH 6.9–7.0 as compared with pH 7.4 is a consequence of greater incorporation of exogenous FFA at the lower pH. The present isotopic data are compatible with this interpretation. However, I did not attempt to confirm the pH-dependent relative increase in cell fatty acid content by chemical measurements for the following reasons. Ehrlich ascites tumor cells contain approximately 8.5 μeq of total fatty acid per 10⁸ cells (18). From the present isotopic results, one can calculate that the expected increment in fatty acid content when cells are incubated for 1 hr in media of pH 6.8 as compared with pH 7.4 is between 0.2 and 0.3 μeq. This represents a difference of only about 3%, a change that is within the limits of error of these chemical measurements. However, the relative increase in cell fatty acid content would be expected to become appreciable in long-term incubations such as those employed in the tissue culture systems (8, 9).

Most of the pH-induced increment in uptake of radioactive FFA by the Ehrlich cells resulted from greater incorporation of radioactivity into the cell FFA and phospholipid fractions (Tables 1 and 6). In contrast, most of the pH-induced increase in fatty acid content of the cultured fibroblasts and liver cells was due to an accumulation of triglyceride (8). This discrepancy probably reflects variations in the metabolic pattern of the cell types that were studied rather than differences in the underlying mechanism through which extracellular pH influences the fatty acid content of the tissues. For example, much more palmitate-1-^{14}C is incorporated into phospholipids than into glycerides by Ehrlich cells during incubation in media of pH 7.4 (10) and after intraperitoneal injection of the isotope into tumor-bearing mice (18, 23); but more palmitate-1-^{14}C is incorporated into glycerides than into phospholipids at pH 7.4 by rat liver slices (24, 25), perfused rat liver (26), and perfused rat heart (27). I suspect that the cultured fibroblasts and liver cells may fit into the second metabolic pattern.

Decreases in the pH of the incubation medium will produce corresponding decreases in the intracellular pH of the Ehrlich cell (28), and the possibility that the increase in exogenous FFA utilization results from changes in intracellular pH must be considered. There was, how-

ever, no pH-dependent change in the rate of utilization of labeled FFA already present in the cell; that is, of labeled fatty acid taken up in unesterified form by the cells during a previous incubation in a radioactive FFA–albumin solution (Table 4). Therefore, small changes in pH appear not to affect the utilization of *cell* FFA. Likewise, there was no pH-dependent increase in FFA uptake from a protein-free medium; in fact, less labeled FFA was taken up from a protein-free medium when the pH was reduced from 7.5 to 6.0 (Table 3). In addition, a pH-dependent decrease in the utilization of endogenous fatty acid did not occur when the cells were incubated in an albumin-free medium (Table 8). Taken together, these results suggest that the pH-dependent changes in fatty acid uptake and utilization do not result primarily from changes in the cells.

A more likely explanation is that changes in medium pH alter FFA utilization by affecting either the affinity of albumin for fatty acid of the mechanism through which protein-bound fatty acid is incorporated into the cell. I have noted in separate experiments (29) that, at a given FFA : albumin molar ratio, the *total unbound* FFA concentration becomes larger as the medium pH is decreased below 7.4. The magnitude of FFA uptake from an albumin solution of pH 7.4 is dependent upon the concentration of unbound fatty acid in equilibrium with the FFA–protein complex (12); in fact, unbound fatty acid probably is the species that is taken up by the cell (5, 12).

The rate of oxidation and esterification of labeled FFA at pH 7.4 depends upon the magnitude of the cell content of labeled FFA (5, 10). Since the rate of utilization of labeled FFA already contained in the cell was not altered by small changes in the medium pH, one would predict that the pH-induced increase in cell FFA uptake might result in a greater rate of FFA oxidation and esterification, as was found experimentally. Hence, the observed pH-induced changes in cellular FFA metabolism can be explained satisfactorily by assuming a primary effect of pH on the binding of FFA to albumin.

The pH-dependent increase in incorporation of glucose carbons into phosphatides and glycerides (Table 7) probably is a consequence of the greater uptake of FFA. Addition of glucose increases FFA esterification in the Ehrlich cell, presumably because glycerophosphate is made available through glycolysis (22). A direct effect of pH on glucose uptake has been demonstrated in certain tissues (30). However, it is unlikely that the observed increase in lipid ester formation results from a primary effect of pH on glucose utilization, for Poole has shown (28) that glycolysis in the Ehrlich cell is not stimulated by acidification.

Independently of the mechanisms involved, the empirical observation that small changes in the pH of the incubation medium will affect FFA utilization in isolated mammalian cells is potentially significant, for it suggests the possibility that pH changes in the extracellular fluids of the intact animal could affect FFA metabolism and the accumulation of fatty acids in tissues. Whether such a mechanism actually is of any physiological or pathological significance remains to be determined.

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