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INCORPORATION OF [1-^{14}C]LINOLEIC ACID INTO LIPIDS OF POLYMORPHONUCLEAR LEUKOCYTES

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SUMMARY

1. Polymorphonuclear leukocytes obtained from rabbit-peritoneal exudates, incubated *in vitro*, rapidly incorporate into cell lipids [1-^{14}C]linoleic acid, present in the medium as non-esterified fatty acid bound to albumin.

2. Almost all radioactivity recovered in association with cell lipids appears in the esterified form, both as phospholipid and as non-phospholipid.

3. Metabolic utilization of the [1-^{14}C]linoleic acid incorporated was evident from evolution of $^{14}CO_2$ and appearance of increasing amounts of water-soluble radioactivity in water washes of lipid extracts of the leukocytes.

4. Uptake of linoleic acid does not take place at low temperatures and is markedly diminished by inhibition of anaerobic glycolysis, whereas interference with oxidative metabolism does not inhibit accumulation.

5. The possibility is discussed that incorporation by the leukocytes of non-esterified fatty acids depends upon esterification and therefore upon the availability of glycerophosphate derived from anaerobic glycolysis.

INTRODUCTION

It has previously been demonstrated that polymorphonuclear leukocytes synthesize most fatty acids, but that they do not incorporate [1-^{14}C]acetate into linoleic, linolenic and arachidonic acid[1]. Since the fatty acid composition of the cell lipids does not change during conditions of active metabolism *in vitro*[1], it was concluded that the polymorphonuclear leukocyte either excluded these three essential fatty acids from metabolism or that transport from the outside medium maintained intracellular levels.

To distinguish between these alternatives, the transport and fate of one of the three fatty acids mentioned above, [1-^{14}C]linoleic acid, has been investigated in an *in vitro* system employing a suspension of rabbit polymorphonuclear leukocytes. The results indicate that [1-^{14}C]linoleic acid present in the medium is rapidly incorporated into the lipids of white blood cells and actively metabolized. A preliminary report of this investigation has been made[2].

MATERIALS AND METHODS

[1-^{14}C]Linoleic acid, with a specific activity of 3 mC/mmole, was obtained from Nuclear Chicago Company. On arrival, 50 μC of the labeled material were dissolved in 50 ml of

benzene and stored at −4°. Prior to each experiment 1 μC was pipetted into a glass-stoppered 40-ml tube, the benzene evaporated off under a nitrogen stream and the labeled fatty acid taken up in a 2-phase system containing isopropyl alcohol, heptane, water and sulfuric acid as described by DOLE[3]. Water-soluble radioactive material was retained in the lower, watery phase. The upper phase was transferred to a round bottom flask and the solvent evaporated off under a nitrogen stream. The thin film of labeled fatty acid produced in this manner was then taken up in a few millilitres of ascitic fluid or rabbit serum in which albumin served as a carrier. Uptake was facilitated by swirling of the flask in a water bath at approx. 50°. The major protein fractions in a sample of ascitic fluid containing label were separated by paper electrophoresis and scanned for radioactivity in a strip scanner (Nuclear Chicago). Most radioactivity was found as a single peak in association with the albumin fraction. The purity of the [1-^{14}C]linoleic acid was tested in the following manner: 0.5 μC of [1-^{14}C]linoleic acid was taken up in 5 ml of ascitic fluid as described above. After extraction of the mixture for lipid the total fatty acids were separated by gas–liquid chromatography, the individual fatty acids recovered and their radioactivity determined as described by FARQUHAR *et al.*[4]. Approx. 6 % of the radioactivity recovered occurred in association with acids other than linoleic acid.

Polymorphonuclear leukocytes were obtained from rabbit-peritoneal exudates by methods previously described[5], with the following modification: only 50 ml instead of 300 ml of sterile saline containing 1 mg of glycogen/ml were injected. The smaller amount injected resulted in more concentrated cell suspensions and eliminated the need for centrifugation and resuspension in a smaller volume. Following collection by drainage the cell suspension was strained through a wire mesh to remove clumped cells and fibrin strands. A few millilitres of ascitic fluid or serum containing [1-^{14}C]linoleic acid were now added to the cell suspension. After thorough mixing the mixture was distributed in equal portions among several flasks which were incubated at 37° in a water bath under gentle agitation. The original ascitic fluid, in which the cells were incubated has a composition which closely resembles that of serum. It therefore provides a well buffered natural medium which contains lipid and protein in physiologic amounts. When inhibitors were used, these were added to the flasks prior to incubation. A pre-incubation sample was spun at 500 × *g* for 20 min to separate cells and medium. Incubated flasks were removed at suitable intervals, the contents transferred to centrifuge tubes and spun in a similar manner. The supernatant fluid was decanted and the cell pellet and the walls of the tube rinsed with 0.9 % saline to remove remaining ascitic fluid. The lipids of the cells and of a 5-ml aliquot of ascitic fluid were extracted with 20 volumes of chloroform–methanol (2:1, v/v) according to the procedure of FOLCH *et al.*[6]. Complete lipid extraction of the leukocytes was assured by breaking up the cell-buttons with a glass rod into fine particulate matter and by refluxing with the chloroform–methanol extraction mixture at 65° for 2 h. The white blood cell residue was then filtered off and washed three times with chloroform–methanol. The lipid extracts of both cells and medium were washed twice with 1/5 volume of water[6]. After separation of the chloroform and methanol plus water phases by centrifugation at 500 × *g* for 30 min, aliquots of the upper phase were taken for determination of water-soluble radioactivity. The second wash contained from 5 to 20 % of the radioactivity found in the first. The remainder of the upper phases was siphoned off and the lower, chloroform, phase was taken to dryness

under a nitrogen stream. The final extract was taken up in chloroform–methanol (2:1, v/v) and transferred through filter paper to a 25-ml volumetric flask. Determinations of radioactivity and total lipid content and fractionation of the lipids were carried out on aliquots of these final extracts. Radioactivity was counted in aluminum planchettes, using a gas-flow counter, Nuclear Chicago, Automatic Scaler with a micromil window. The amount of lipid material on the planchette was always so small that quenching was negligible.

Total lipid content of the final extract was determined gravimetrically[7] and/or colorimetrically[8]. The results of these two methods were in good agreement. Non-esterified fatty acid levels in the ascitic fluid were determined by the titration method of DOLE[3]. The double extraction procedure of this method was followed to eliminate titrable acidity contributed by organic acids other than fatty acids.

Major lipid classes were separated on small silicic acid columns as described by BORGSTRÖM[9]. Following addition of 2 g of silicic acid (Biorad) to the glass columns, these were baked overnight at 110°. After cooling the columns were washed successively with 5-ml washes of methanol, acetone, ethyl ether, petroleum ether (b.p. 30–60°) and finally with chloroform. The sample contained in a small amount of chloroform was placed on the column and glycerides, cholesterol, cholesterol esters and non-esterified fatty acids were eluted with 40 ml of chloroform. Phospholipids were eluted with 40 ml of methanol. The chloroform eluent was taken to dryness under a nitrogen stream and the remaining lipid redissolved in the two-phase system described by DOLE[3]. The non-esterified fatty acids were retained in the lower watery phase by the addition of 0.25 ml of 1 N NaOH. The upper phase was pipetted off and the lower phase washed 5 times with 5 ml of heptane to remove remaining esterified fat. The combined upper phase and heptane washes contained all the non-phospholipid except the non-esterified fatty acids. This latter fraction was recovered in three 5-ml petroleum ether (b.p. 30–60°) washes following acidification of the lower phase with 0.25 ml of 1 N HCl.

The radioactivity in the methanol eluent and in the two fractions obtained from the chloroform eluent was determined on planchettes and expressed as a percentage of the total radioactivity placed on the column. Recovery ranged from 95 to 105 %.

$^{14}CO_2$ evolved during incubation was collected in hyamine following the procedure described by ISSELBACHER[10]. The hyamine was quantitatively transferred with toluene into a 20-ml counting flask. A suitable phosphor was added (2,5-diphenyl oxazole and 1,4 bis-2(5-phenyl oxazole)-benzene in a ratio of 40:1) and the samples counted in a Packard tricarb liquid scintillation spectrometer. The counting efficiency was 40 %, as compared to 15 % for determination of radioactivity in the gas-flow counter.

Gas–liquid chromatography of methyl esters of fatty acids (prepared as described by STOFFEL *et al.*[11]) was carried out with an Apiezon stationary phase at 198° using argon as carrier gas and a ^{90}Sr ionization detector[4].

RESULTS

Uptake of [1-^{14}C]linoleic acid by polymorphonuclear leukocytes

The incorporation of [1-^{14}C]linoleic acid into cell lipids during incubation at 37° for 1 h is shown in Fig. 1A and the disappearance of radioactivity from the medium in Fig. 1B. In each experiment the radioactivity/mg cell lipid after 0, 30 and 45 min

has been expressed as a percent of the value found after 60 min of incubation and the radioactivity/mg medium lipid as a per cent of the amount present prior to incubation. This manner of presentation was selected because there was considerable variation in the radioactivity/mg lipid in different experiments. Variations in the volume of the medium and in its lipid content as well as in the amount of [1-^{14}C]linoleic acid taken up by the albumin in the ascitic fluid or serum can account for differences in the radioactivity of the incorporated lipid from experiment to experiment. Despite considerable scatter it may be noted that the radioactivity/mg medium lipid always fell during incubation (Fig. 1B).

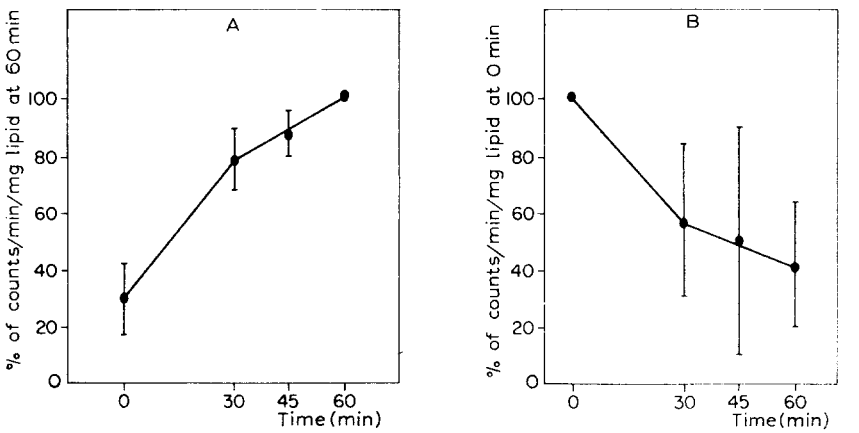

Fig. 1. Appearance (A) of radioactivity in cell lipids and disappearance (B) of radioactivity from the medium lipids during incubation of leukocytes at 37° in ascitic fluid containing [1-^{14}C]linoleic acid. In each experiment, counts/min/mg cell lipid after 60 min of incubation and counts/min/mg medium lipid at 0 min of incubation were taken as 100%. The standard deviation from the mean (black dots) is given by the lines through each dot.

The relatively high value of radioactivity/mg cell lipid even before incubation was initiated can be explained by the time required for processing of the first sample at room temperature. Approx. 10 min elapsed between mixing of the cell suspension with the labeled material and subsequent centrifugation. Evidently, considerable incorporation occurred during this time.

For the duration of the experiments the lipid content of the leukocytes remained essentially constant. The lipid content of the medium as a rule also did not change detectably, but showed a small and progressive decrease in some experiments.

Esterification of [1-^{14}C]linoleic acid taken up by leukocytes

Conclusive evidence of true incorporation of [1-^{14}C]linoleic acid into cell lipids was obtained by fractionation of the lipid extracts on silicic acid columns into phospholipids, "non-phospholipids" (including glycerides, cholesterol, and cholesterol esters) and non-esterified fatty acids.

Table I shows that almost all radioactivity found in association with cell lipids appears in the esterified form both as phospholipid and as non-phospholipid. The radioactivity in each of the three fractions has been expressed as a percentage of the total radioactivity recovered. Esterification was so rapid that less than 10% of the

radioactivity incorporated during the time required for processing of the preincubation sample remained in the non-esterified fatty acid fraction. It may be noted that the distribution of radioactivity between phospholipids and glycerides was remarkably constant while [1-^{14}C]linoleic acid accumulated during incubation for 1 h.

Various fractions of the non-phospholipids were separated by thin-layer chromatography and the radioactivity associated with each fraction determined as indicated in the legend of Table II. The percentages given represent only approximate values because recovery of the radioactivity applied to the thin-layer plates was only 80 %.

TABLE I

INCORPORATION OF [1-^{14}C]LINOLEIC ACID INTO PHOSPHOLIPIDS, NON-PHOSPHOLIPIDS AND FREE FATTY ACIDS OF LEUKOCYTE LIPIDS DURING INCUBATION AT 37°

Washed lipid extracts of the leukocytes were separated on silicic acid columns as described in the section on methods. The radioactivity in the indicated fractions has been expressed as a percentage of the total radioactivity recovered from the column. The results are given as the mean ± one standard deviation. The figure between parentheses in the first column indicates the number of observations.

Minutes of incubation	*Percent of total counts/min in cell lipids*		
	Phospholipids	*Non-phospholipids*	*Free fatty acids*
0 (4)	51.4 ± 2.2	41.2 ± 4.9	6.95 ± 3.0
30 (3)	55.0 ± 2.6	41.5 ± 1.3	3.4 ± 1.35
60 (8)	54.6 ± 2.0	42.8 ± 3.2	3.0 ± 3.2

TABLE II

DISTRIBUTION OF RADIOACTIVITY AMONG VARIOUS FRACTIONS OF NON-PHOSPHOLIPIDS OF LEUKOCYTES INCUBATED WITH [1-^{14}C]LINOLEIC ACID FOR 60 MIN

The results are shown of three independent experiments. The non-phospholipids of whole lipid extracts of the leukocytes were separated from the phospholipids on silicic acid columns by elution with chloroform. The eluent was reduced in volume under a nitrogen stream and transferred with a micro pipette onto a silica gel thin-layer chromatography plate[18]. Separation into the indicated fractions was achieved using a solvent system consisting of petroleum ether (b.p. 30–60°)–ethyl ether–glacial acetic acid (79:20:1, v/v). The fractions were visualized by exposure to iodine vapors and then scraped off the plate onto filter paper and eluted from the silica gel with ethyl ether and chloroform–methanol (2:1, v/v) into counting flasks which were counted in a Packard liquid scintillation counter.

	Radioactivity in each fraction as percentage of total recovered		
Free fatty acids	3.9	1.1	5.3
Monoglycerides	5.2	0.6	6.0
Diglycerides	5.2	4.9	3.3
Triglycerides	81.8	90.1	81.3
Cholesterol esters	5.8	3.2	4.3

It may be seen, however, that more than 80 % of the radioactivity occurred in the triglycerides and less than 10 % in the cholesterol ester fraction. Of the total lipid of the leukocytes approx. 60 % is phospholipid and 20 % triglyceride[1]. Since most of the radioactivity in the non-phospholipid fraction was associated with the triglycerides,

it may be calculated that at the end of one hour the triglycerides had incorporated from 2 to 2.5 times more [1-^{14}C]linoleic acid/mg than the phospholipids.

Lack of evidence for release of radioactive lipid previously taken up by polymorphonuclear leukocytes

Lipid extracts of cell-free ascitic fluid to which had been added [1-^{14}C]linoleic acid were fractionated into phospholipids, non-phospholipids and non-esterified fatty acids. Approx. 3 % of the radioactivity recovered was associated with the phospholipid and 2 % with the neutral fat fraction. The remainder was found with the non-esterified fatty acids. When the [1-^{14}C]linoleic acid was run through a silicic acid column with chloroform prior to addition to the ascitic fluid there was no significant change in the percentage of radioactivity found in the methanol eluent of the subsequently fractionated medium lipids. This suggests that the radioactivity in the ester fractions can at least in part be attributed to incomplete separation of the lipid classes.

The percentages of radioactivity in the ester fractions were not exceeded in samples of ascitic fluid from cell suspensions incubated up to 1 h indicating that no detectable radioactive esterified fat had been released by the cells into the medium.

Table III further shows that little radioactivity leaves the cells after incorporation of labeled fatty acid had occurred. Leukocytes were incubated as usual with [^{14}C]linoleic acid in the medium. After 1 h the suspension was removed from the bath, spun at low speed, the supernatant fluid decanted and the cells resuspended in ascitic fluid not containing isotope and incubated again at 37° for an additional hour.

TABLE III

EFFECT OF REINCUBATION OF LEUKOCYTES IN ASCITIC FLUID NOT CONTAINING LABEL, ON RELEASE OF RADIOACTIVITY PREVIOUSLY TAKEN UP DURING INCUBATION AT 37° FOR 1 H

		Reincubated in "cold" medium after 60 min	
Minutes of incubation	*60*	*120*	*120*
	37°	*37°*	*4°*
Counts/min/mg medium lipid	1200	300	200
Counts/min/mg cell lipid	8200	6200	8300

The results demonstrate that little radioactivity appeared in the medium, particularly in view of the fact that some "hot" medium remained behind after decantation. This was inevitable because the cells were spun at low speed and not too well packed in order to facilitate resuspension and to minimize damage by centrifugation. It is of interest that following reincubation the radioactivity/mg cell lipid was higher at 4° than at 37°, while the radioactivity of the medium lipid was similar at both temperatures. This suggested that metabolic utilization of the labeled fat at 37° caused a decrease in the radioactivity of the cell lipids.

Approximate magnitude of incorporation of linoleic acid

An estimate of the magnitude of the transport of linoleic acid into the leukocytes was based upon the amount of linoleic acid present in the medium as non-esterified

fatty acid and the amount removed by the cells. The non-esterified fatty acid level in ascitic fluid in 9 experiments ranged from 135 to 485 μequiv/l (mean: 245). Gas–liquid chromatographic analysis of the fatty acid composition of this fraction shows that linoleic acid constitutes about 10 % of the non-esterified long-chain fatty acids present. Therefore, 1 ml of ascitic fluid contains from 0.01 to 0.05 μequiv of linoleic acid or from 3 to 15 μg. The disappearance of radioactivity from the medium was used to calculate the amount of linoleic acid removed by the cells, assuming that no hydrolysis of esterified fat in the medium took place during incubation. It could thus be computed in 9 experiments that from 4 to 14 μg (mean: 7.5) of linoleic acid/mg of cell lipid/h are taken up by the cells. Since linoleic acid represents 30 % of all fatty acids of the rabbit leukocytes[1], this means that from 1.0 to 4.5 % of the linoleic acid of the cell are replaced in 1 h by transport from the outside medium.

Dependence of uptake of [1-^{14}C]linoleic acid on glycolysis

The effect of inhibition of metabolism on the incorporation of [1-^{14}C]linoleic acid into cell lipids is shown in Table IV. At 4° no uptake of isotope occurred over that observed at T_0 ($P > 0.3$). A marked inhibitory effect on uptake was produced by inhibition of glycolysis by either sodium iodoacetate or sodium fluoride. In contrast, interference with oxidative metabolism by the addition of 2,4-dinitrophenol or potassium cyanide to the medium did not affect incorporation. It may be noted that although inhibition of oxidative metabolism did not prevent the accumulation of radioactivity in the cell lipids, the disappearance of radioactivity from the ascitic

TABLE IV

EFFECT OF INHIBITION OF METABOLISM UPON THE INCORPORATION OF [1-^{14}C]LINOLEIC ACID INTO CELL LIPIDS AND UPON THE DISAPPEARANCE OF THE LABEL FROM THE ASCITIC FLUID

Suspensions of leukocytes in ascitic fluid containing [1-^{14}C]linoleic acid were incubated for 1 h with or without the indicated inhibitors. The results, counts/min/mg lipid in either cells or ascitic fluid, are expressed as a percentage of the value found after 60 min of incubation at 37° in the case of the cells and of the value found prior to incubation in the case of the ascitic fluid. The results are given as mean ± one standard deviation. The figure between parentheses indicates the number of observations. Sodium iodoacetate used in a final concentration of 0.001 M[13], NaF used in a final concentration of 0.02 M, KCN used in a final concentration of 0.0025 M, 2,4 dinitrophenol used in a final concentration of 0.0025 M. The effects of the inhibitors iodoacetate and NaF were identical and are shown under one heading as is the case for KCN and 2,4-dinitrophenol.

Minutes of incubation		*Cells*	*Ascitic fluid*
0		30.2 ± 12.5 (20)	100 (18)
60 4°		36.0 ± 14.9 (6)	94.8 ± 12.0 (5)
37°		100 (16)	41.0 ± 22.0 (14)
	+ Iodoacetate or NaF	54.2 ± 19.5 (15)	84.4 ± 11.8 (15)
	+ KCN or 2,4-dinitrophenol	110.1 ± 13.7 (8)	64.5 ± 4.4 (6)

fluid was somewhat reduced ($P < 0.02$). This latter finding may be explained by decreased breakdown of lipid during incubation with cyanide or 2,4-dinitrophenol (see below), apparently resulting in a decrease in the total amount of radioactive fatty acid removed by the cells.

Metabolic utilization of [1-^{14}C]linoleic acid incorporated into lipids of leukocytes

Evidence that the [1-^{14}C]linoleic acid incorporated was also broken down by the cells stems from the findings that $^{14}CO_2$ was released during incubation and that increasing amounts of radioactivity appeared in water washes of the lipid extracts. In three experiments the radioactivity released as CO_2 in 1 h represented 4.2, 6 and 8 % of the radioactivity of the cell lipids after this time of incubation, while in 14 experiments from 1.5 to 8.8 % (mean: 3.3) appeared in the water washes of the cell-lipid extracts.

Table V shows the effect of inhibition of metabolism upon the release of $^{14}CO_2$ and water-soluble radioactivity. The results have been expressed as a percentage of the value obtained after 60 min of incubation in each experiment. At 4° no $^{14}CO_2$ could be detected and the radioactivity of the water washes of the cell lipid extract did not differ from that present before incubation. Inhibition of glycolysis by either iodoacetate or NaF did not significantly suppress the release of $^{14}CO_2$ and only moderately the appearance of water-soluble radioactivity, despite the smaller quantity of [1-^{14}C]-linoleic acid incorporated (Table IV). Inhibition of oxidative metabolism abolished $^{14}CO_2$ release but only partly inhibited the release of water-soluble radioactivity.

TABLE V

EFFECT OF INHIBITION OF METABOLISM UPON RELEASE OF $^{14}CO_2$ AND WATER-SOLUBLE RADIOACTIVITY DURING INCUBATION OF LEUKOCYTES WITH [1-^{14}C]LINOLEIC ACID

Suspensions of leukocytes in ascitic fluid containing [1-^{14}C]linoleic acid were incubated with or without the indicated inhibitors. The results are expressed as a percentage of the value found after 60 min of incubation at 37° in each experiment. Inhibitors were used in the concentrations shown in the legend of Table IV. The figure between parentheses indicates the number of observations. Water-soluble radioactivity refers to radioactivity found in water washes of the cell lipid extracts.

Minutes of incubation		$^{14}CO_2$	*Water-soluble radioactivity*
0		—	12.3 ± 5.4 (10)
60	4°	0 (2)	15.0 ± 9.6 (7)
	37°	100 (3)	100 (11)
	+ Iodoacetate or NaF	81.0 ± 34.9 (6)	60.2 ± 25.0 (17)
	+ KCN or 2,4-dinitrophenol	4.5 ± 3.1 (3)	58.0 ± 13.9 (8)

Comparison of uptake of [1-^{14}C]linoleic acid by resting and phagocytic leukocytes

Phagocytosis is accompanied by stimulation of many parameters of metabolism such as glucose and oxygen uptake, lactate production, glycogen synthesis and lipid turnover[1,14,15]. It was considered of interest to study uptake of fatty acids under conditions of enhanced metabolism. Heat-killed streptococci of the strain S 43G (kindly made available by Dr. R. M. KRAUSE of the Rockefeller Institute) in a concentration of approx. 10 bacteria to 1 leukocyte were added to a suspension of white blood cells as previously described[1]. The results of two such experiments appear in Table VI. It is clear that phagocytizing cells did not incorporate more [1-^{14}C]-linoleic acid than the resting cells.

Table VI also shows that the lipid content of the medium was not less in the presence of engulfing leukocytes, except in one sample (Expt. 2, after 30 min of in-

cubation). The absence of stimulation by the phagocytic process of incorporation of radioactive linoleic acid therefore appears not due to a greater transport of unlabeled fat.

TABLE VI

INCORPORATION OF [1-^{14}C]LINOLEIC ACID INTO CELL LIPIDS OF LEUKOCYTES INCUBATED AT 37° FOR 30 AND 60 MIN WITH (+ STREP.) AND WITHOUT (CONTROL) HEAT-KILLED STREPTOCOCCI

In each of the 2 experiments shown 1 μC of [1-^{14}C]linoleic acid in 3 ml of rabbit serum was added to the cell suspension which was divided into 5 equal portions. To two of these 0.5 ml of heat-killed streptococci of the strain S 43G in physiologic saline was added to yield approx. 10 bacteria per leukocyte. To the remaining flasks 0.5 ml of saline was added.

Minutes of Incubation	*Ascitic fluid*				*Leukocytes*			
	Lipid content/5 ml (mg)		*Counts/min/mg lipid*		*Lipid content (mg)*		*Counts/min/mg lipid*	
Expt.	1	2	1	2	1	2	1	2
0	1.60	3.75	8500	6250	6.80	4.83	2500	1040
30 (control)	1.57	4.25	1900	5000	7.20	4.17	6800	3600
30 (+ strep.)	1.54	3.55	3400	5700	6.87	5.08	6200	2760
60 (control)	1.40	3.80	1600	4900	6.10	4.17	8000	4900
60 (+ strep.)	1.63	3.85	2400	5200	6.37	4.58	7300	3700

DISCUSSION

The results of these studies indicate that [1-^{14}C]linoleic acid, present in the medium as non-esterified fatty acid bound to albumin, is rapidly incorporated into the lipids of the leukocytes.

More than 90% of the [1-^{14}C]linoleic acid incorporated into the lipids of the leukocytes was recovered in the esterified form. Esterification of a large portion of isotopically labeled fatty acids taken up from the medium or perfusing fluid has been shown for many cell types. Among the tissues that incorporate labeled fatty acids into cell lipids are liver[16,17], heart[18], extremities[19,20], intestinal mucosa[21], adipose tissue[22], diaphragmatic muscle cells[23] and mouse ascites tumor cells[24,25]. The only exception thus far appears to be the mammalian erythrocyte which binds labeled palmitate without metabolic incorporation[26].

The distribution of radiocarbon between phospholipid and non-phospholipid esters remained constant during 1 h of incubation. The glycerides incorporated approx. 2.5 times more [1-^{14}C]linoleic acid/mg than the phospholipids. A comparable value has been found for the relative incorporation of [1-^{14}C]acetate into glycerides and phospholipids[1,15]. Little [1-^{14}C]linoleic acid appeared as cholesterol ester, confirming reports on the slow turnover of this fraction in other tissues[17,27].

An estimate of the amount of linoleic acid transported would appear to indicate a fairly rapid turnover of this fatty acid in the cell lipids; this was also suggested by the rapid metabolic utilization of the labeled fat. The amount of radioactivity evolved as CO_2 and released as water-soluble radioactivity during 1 h of incubation comprised approx. 10% of the radioactivity incorporated into cell lipids during this period. The extent to which the different lipid fractions that incorporated [1-^{14}C]linoleic acid contributed to this turnover cannot be determined from the available data. The constancy of distribution of radioactivity between the major fractions during the course of the experiment seems to indicate that differences in breakdown of [1-^{14}C]-

linoleic acid associated with each fraction were small. The possibility has not been excluded that rapid breakdown of labeled fat may be restricted to a small fraction, for example the non-esterified portion.

The observation that phagocytosis did not stimulate uptake of fatty acids and in fact suppressed incorporation, is unexpected, because it might have been anticipated that the increased metabolic activity of the phagocytic state would have resulted in an increase in transport of substrate in the form of non-esterified fatty acids, as well as of carbohydrate.

It has been suggested that phagocytosis or pinocytosis may represent a means of removal of exogenous lipid from the circulation by various tissues[28,29].

Although a process of engulfment may contribute to the transport of fat by the leukocyte, it does not appear likely that in the system studied phagocytosis or pinocytosis of fat constitutes an important factor. The radioactivity of the medium lipids always decreased during incubation, while in many experiments the lipid content of the medium did not change. These findings suggest a selective uptake of a small fraction of the total lipid in the medium. Since experiments with other 1-^{14}C-labeled long chain fatty acids (palmitic, stearic and oleic acid[30]) also showed a decrease in radioactivity of medium lipids it seems reasonable to assume that this small fraction represents the non-esterified fatty acids.

Release of unlabeled fat by the leukocytes also could result in diminishing radioactivity of medium lipids. It has previously been shown that little or no newly synthesized lipid appears in the medium while active synthesis is in progress[1]. Moreover, it appears from Table III, that labeled fatty acids incorporated by the cells remain in the cells. This suggests that fatty acid transport occurs in an inward direction only. Therefore, if lipid fractions other than the non-esterified fatty acids are taken up by the leukocytes, perhaps through a process of pinocytosis, such uptake must occur at a much slower rate.

The apparent unidirectional transport of fatty acid under the conditions of these experiments contrasts with observations on whole human blood by MARKS *et al.*[31] and JAMES *et al.*[32]. According to these investigators as much as 50 % of radioactivity incorporated into lipids from [1-^{14}C]acetate by mixed white blood cells appeared in plasma lipids in the course of 3 h. An explanation for this discrepancy is not readily available. Mononuclear cells of whole blood, which are not present in the peritoneal exudates to any appreciable extent, conceivably might release radioactive lipid found in plasma whereas polymorphonuclear leukocytes do not. Further studies are needed to resolve these divergent findings.

The observations on the effects of metabolic inhibitors on uptake of [1-^{14}C]linoleic acid perhaps provide a clue concerning the mechanism of transport. Remarkable was the unimpeded incorporation noted during interference with oxidative metabolism, whereas inhibition of glycolysis suppressed transport. The polymorphonuclear leukocyte derives about 75 % of its energy production from the utilization of glucose by anaerobic glycolysis[33]. It is therefore possible that the inhibitory effect on uptake of [1-^{14}C]linoleic acid observed in the presence of sodium iodoacetate and sodium fluoride is merely a consequence of decreased availability of metabolic energy. However, the finding that labeled linoleic acid in the cell lipids appeared almost exclusively in the esterified form, suggests an alternative explanation; namely that the effect of inhibition of glycolysis on uptake of non-esterified fatty acids may be specifically due to de-

creased production of glycerol derivatives. The number of triosephosphates representing potential ester sites may determine the rate of incorporation of fatty acids. Whether esterification, perhaps at the cell surface, provides the means by which transport of non-esterified fatty acid takes place remains to be determined. Recently much attention has been given to the possible role of phosphatidic acid as a carrier in transport processes[34,35]. Such a function of phosphatidic acid and/or its precursors can easily be visualized in the case of transport of non-esterified fatty acids. Of interest in this respect is the demonstration by SHAW AND STADIE of two glycolytic pathways in the muscle cell of rat diaphragm, one of which was postulated to occur as a complete enzyme system on the cell surface[36].

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