Remodeling of Granulocyte Membrane Fatty Acids During Phagocytosis

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ABSTRACT During phagocytosis, new phospholipid is synthesized from triglyceride fatty acid and may be utilized to form the membranes of phagocytic vesicles. In addition, hydrogen peroxide, which can peroxidize unsaturated fatty acids, is generated. Since both of these processes could change membrane fatty acid composition during the conversion of cytoplasmic granules and plasma membranes to phagosomes, the lipid compositions of these structures were examined. Phagocytic vesicles were prepared by density gradient centrifugation of polystyrene latex particles after phagocytosis. Granule and plasma membrane fractions were isolated by density gradient and differential centrifugation. Phospholipids and fatty acids were analyzed by thin-layer chromatography and gas-liquid chromatography.

While whole cells, granules, plasma membranes, and phagosomes were all similar in phospholipid composition, phagosome fatty acids were significantly more saturated than those of the other fractions. This was primarily due to reduced oleic and arachidonic acids and increased palmitic acid in the phagocytic vesicle lipids. Plasma membrane was also more saturated in comparison to whole cells and granules. However, this difference was not sufficient to explain the marked comparative saturation of the phagosomes. The observed increase in fatty acid saturation in these lipids may have been induced by a combination of either peroxidative destruction of polyunsaturated fatty acids or phospholipase activity, coupled with reacylation mechanisms favoring saturated fatty acids.

INTRODUCTION

Phospholipid turnover in polymorphonuclear leukocytes is markedly increased during phagocytosis, suggesting that new lipid membranes may be formed during phagocytosis (1–5). Reacylation of lysophosphatides is also accelerated during phagocytosis (3–5), and there is evidence that the fatty acids of cellular triglycerides are used for this purpose (6, 7). Since the terminal fatty acids of mammalian triglycerides are predominantly saturated (8), and since this process of reacylation may be related to the action of a lipase which like pancreatic lipase (9) might be likely to preferentially attack those terminal fatty acids of the triglycerides, saturated fatty acids might be expected to preferentially take part in the lysophosphatide reacylation. In addition, hydrogen peroxide is generated during phagocytosis (10–13). This compound is known to attack polyunsaturated fatty acids (14–17). Thus, both the preferential utilization of triglyceride fatty acid for reacylation and the generation of hydrogen peroxide in the white cell during phagocytosis could produce a net increase in the percentage of saturated fatty acids in the membranes of these cells. Previous studies in model membrane systems have shown that such increased saturation of membrane fatty acid may reduce membrane permeability (18–20).

In the studies reported here, we have performed phospholipid and fatty acid analyses on whole human leukocytes, leukocyte granular membranes, and "plasma membranes" before phagocytosis. We have also performed similar assays on phagocytic vesicle membranes after phagocytosis. In general, we have found that phagocytic vesicles obtained after phagocytosis differ markedly in phospholipid fatty acid composition from either the lysosomes or the plasma membrane from which they were presumably derived. In particular, we have found that these vesicle membranes contained very low proportions and absolute amounts of arachidonic acid, which is the fatty acid most susceptible to peroxidation. Conversely, the concentration and absolute amount of saturated pal-

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mitic acid was found to be elevated in phagosomes as compared to lysosomes and plasma membranes.

In addition, we present kinetic data from leukocytes doubly prelabeled with saturated and unsaturated fatty acids showing a preferential incorporation of saturated triglyceride fatty acids into apparently newly formed leukocyte phosphatidylcholine during phagocytosis.

We suggest that these changes in fatty acid composition and disposition may decrease the permeability of phagocytic vesicle membranes. Lowered phagosome permeability may serve to increase the efficiency of intracellular killing by maintaining high concentrations of bactericidal agents and their cofactors within the vesicle and may also serve to limit the dissemination of such potentially deleterious agents within the whole cell.

METHODS

Materials. EGSS-X (an ethylene glycol succinate polyester combined with a silicone), Gas-Chrom P, and 10% boron trifluoride in methanol were purchased from Applied Science Labs, Inc., State College, Pa. Polystyrene latex beads, 1.099 μ in diameter, were purchased from the Dow Chemical Company, and butylated hydroxytolulene, or BHT (2,6-*tert*-butyl-*p*-cresol),[1] was a gift from the Shell Chemical Corporation, New York. All other solvents and reagents used were reagent grade. Thin-layer plates coated with Silica Gel F-254 for phospholipid separations were obtained from Brinkmann Instruments, Inc., Westbury, New York.

Cell collection and preparation. Peripheral venous blood was obtained in 50-ml plastic syringes from human patients with chronic myelogenous leukemia. 3 U/ml of heparin was added to prevent coagulation. The syringe needles were smoothly bent 90° to the axis of the syringe, and the syringes were then supported vertically with the plunger handle down for 1–2 h at room temperature. In the presence of the extremely elevated sedimentation rates of these patients, this gravity sedimentation procedure produced a distinct greenish-white cell supernatant suspension which was easily separated from most of the underlying red cells by expressing the supernatant through the bent needles while still keeping the syringes vertical. By conventional Giemsa-stained preparations, these suspensions were found to contain 80–90% mature polymorphonuclear leukocytes or bands. The remaining white cells were immature forms. In addition, there was approximately one red cell for every three white cells at this stage of the preparation. Subsequent washing reduced this red cell contamination to less than one red cell for seven white cells. (In terms of lipid mass, the maximal red cell contamination was less than 4% of the leukocyte lipids.) The cells were then washed three times and resuspended in Krebs-Henseleit buffer (KHB) (21), consisting of 123 mM NaCl, 4.9 nM KCl, 3.1 nM $CaCl_2$, 1.2 mM KH_2PO_4, 2.5 mM $MgSO_4$, and 52 mM $NaHCO_3$, pH 7.4.

Isolation of granules. A minor modification of the method of Cohn and Hirsch (22), as used by Baehner, Gilman, and Karnovsky (11), was employed.

Isolation of plasma membrane. The "Tris method" of Warren, Glick, and Nass (23) was used. All solutions used in this procedure contained 5 mM $MgCl_2$. Approximately 1 ml of packed cells was suspended in three volumes of 0.05 M Tris-HCl, pH 7.4, and homogenized with 20 strokes of a tight-fitting pestle. An equal volume of 20% sucrose was added to the homogenate, which was then layered on the top of a discontinuous sucrose density gradient consisting of 4 ml each of 50% and 30% sucrose solutions. The gradient was centrifuged at 1,600*g* for 15 min, and the material at the 30%/50% interface was collected. 4 ml of 30% sucrose was added to this material, which was then centrifuged at 5,860*g* for 20 min; the resulting pellet was saved and suspended in 2 ml of 30% sucrose. A discontinuous gradient was formed using 1.8 ml each of 65, 55, 50, 45, and 40% sucrose solutions, and the resuspended pellet was layered on the top. This gradient was centrifuged at 90,000*g* for 5 h in a Spinco SW 40 rotor. Plasma membrane fractions were collected at the 40%/45% and 45%/50% interfaces.

Isolation of phagocytic vesicles. A modification[2] of the method of Wetzel and Korn (24) was used as follows: white cells were suspended and washed twice in Krebs-Ringer phosphate (KRP) buffer, consisting of 120 mM NaCl, 4.8 mM KCl, 2.6 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 15.6 mM Na_2HPO_4, pH 7.4, with 200 mg/100 ml glucose. Final resuspension was achieved with 3.5 volumes of KRP-glucose. The cell suspension was placed in a 25 ml siliconized flask, and polystyrene latex beads were added to a concentration of 1 mg/ml. The mixture was incubated for 35 min at 37°C and centrifuged in plastic tubes at 700*g* for 5 min. The precipitated white cells were washed twice in cold, 0.02 M Tris-HCl, pH 6.8, and resuspended in 1.5 volumes of 60% sucrose. The mixture was then homogenized with 10 strokes of a tight-fitting Teflon pestle. The homogenate was transferred to a cellulose nitrate ultracentrifuge tube and layered with 3.2 ml each of 25, 20, and 10% sucrose solutions. This gradient was then centrifuged for 90 min at 26,000 rpm in a Spinco L-65 centrifuge (SW 40 rotor, brake off). Phagocytic vesicles were harvested from the 10%/20% interface. This fraction was either lipid-extracted directly with 100% ethanol, or it was sonicated at 100 W·s for 1 min in 5 volumes of deionized water. The sonicate was centrifuged at 5,000*g* for 30 min, and the resulting supernate (free of polystyrene latex beads) was centrifuged at 57,000 rpm in a Spinco type 65 rotor for 75 min. The final pellet was considered to be a highly purified phagocytic vesicle membrane preparation.

Lipid extraction. Whole leukocytes, plasma membrane, granules, and sonicated phagocytic vesicle membrane preparations were extracted by the method of Folch, Lees, and Sloane Stanley (25) with the exception that 2:1 chloroform-methanol solutions contained BHT as antioxidant (1 mg/100 cc solvent). Phagocytic vesicle preparations which had not been sonicated were extracted by 20 volumes of ethanol with 1 mg/100 ml BHT at room temperature for 12 h. As expected from the previous work of Wetzel and Korn (24), both isolation and extraction methods for granule membranes gave equal amounts of lipid phosphorus for equal aliquots of the same granule preparation.

Phosphorus assay. Lipid phosphorus was determined by a minor modification of the colorimetric method of Lowry, Roberts, Leiner, Wu, and Farr (26).

Thin-layer chromatography. A minor modification of the method of Skipski, Peterson, and Barclay (27) was

[1] *Abbreviations used in this paper:* BHT, 2,6-*tert*-butyl-*p*-cresol; GLC, gas-liquid chromatography; KHB, Krebs-Henseleit buffer; KRP, Krebs-Ringer phosphate buffer; PV, phagocytic vesicle.

[2] Cohen, P., and R. L. Baehner. Personal communication.

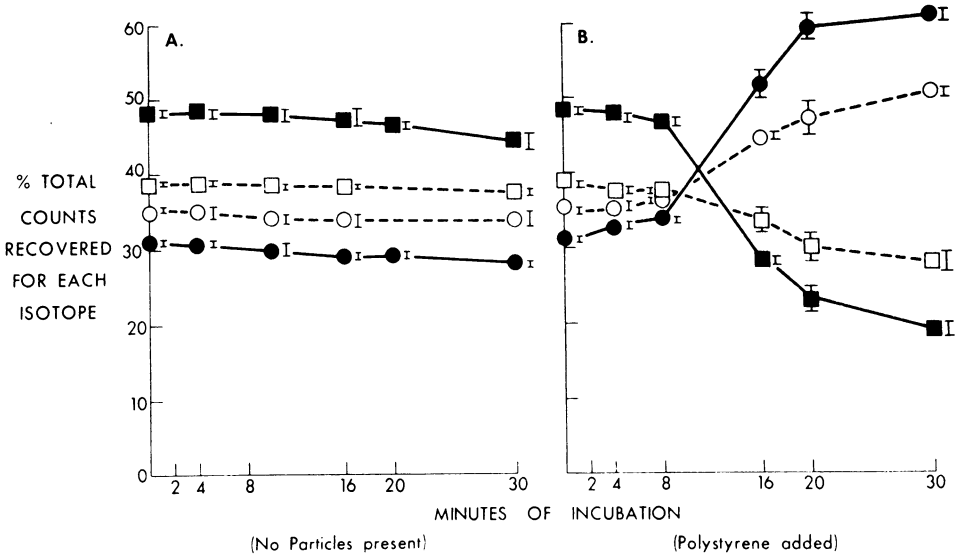

FIGURE 1 (A) Disposition of fatty acids in granulocytes during incubation of doubly prelabeled cells without phagocytosis. (B) Disposition of fatty acids in granulocytes after phagocytosis of doubly prelabeled cells. See text for further details. ●——●, palmitic acid in phosphatidylcholine; ■——■, palmitic acid in triglyceride; ○——○, linoleic acid in phosphatidylcholine; □——□, linoleic acid in triglyceride. Vertical bars indicate range of triplicate determinations.

used. The developing solution contained 50 mg/100 ml BHT and development proceeded under nitrogen.

Preparation of methy ester derivatives. The esterification procedure presented by Dodge and Phillips (28) was employed, except that heating was done at 75°–85°C for 30 min and extraction was performed with 1.5-ml aliquots of a mixture of isomeric hexanes (Fisher Scientific Co., Fairlawn, N. J.) with 1 mg/100 ml BHT.

Gas-liquid chromatography (GLC). GLC was performed on fatty acid methyl ester samples dissolved in hexanes with a Jarrel-Ash instrument, model 28-710, equipped with an 8 ft glass column packed with 7% EGSS-X on Gas-Chrom P, 100–120 mesh, and a hydrogen flame ionization detector. The carrier gas-flow rate was approximately 30 ml/min. The column temperature was maintained at 190°C, the injector at 215°C, and the detector at 180°C. Retention times were determined daily through the use of a known mixture of fatty acid methyl esters as reference standards. Sample volumes of no more than 3 μl were used. Peak areas were calculated by triangulation and were assumed to be proportional to the molecular weights of the component fatty acids. Corrections were applied to yield fatty acid proportions in terms of mole percentages.

BHT in the samples emerged with a retention time similar to that of methyl myristate, and myristic acid was thus not quantified in experiments in which BHT was used. When carried through the methylation procedures, BHT also gave rise to additional peaks with retention times similar to those expected for methyl heptadecanoate and methyl nonadecanoate. Neither of these artifactual peaks was included in the calculated values presented below.

An unsaturation index (UI) was calculated for each lipid sample as the sum of the number of double bonds in each fatty acid species multiplied by its mole percentage (as determined by GLC).

Radioactive fatty acid disposition studies. In order to evaluate the possible role of triglyceride fatty acid in the new assembly of phagosome phospholipids, white cells were preincubated with fatty acids to label their triglycerides and then, after appropriate washing, allowed to phagocytize polystyrene beads.

The methods and sources of materials were identical with those previously described for preincubation with fatty acid followed by phagocytosis but without added lysolecithin (7). Two sources of fatty acid were used, however, ([^{14}C]linoleic acid and [^{3}H]palmitic acid). After preincubation but before phagocytosis, aliquots were removed for determination of the average triglyceride fatty acid specific activity for both isotopes. After phagocytosis, triglycerides and phosphatidylcholine were isolated and assayed for ^{3}H and ^{14}C specific radioactivity as previously described (7). For clarity in the presentation of the results (Fig. 1) the raw ^{14}C data has been standardized to the ^{3}H data to give equivalent starting specific activities in the initial preincubation sample (raw ^{14}C counts multiplied by 2.3 throughout).

Statistical analysis. The Student's *t* test was used to determine the statistical significance of the data in Tables I through IV. When calculating the cumulative standard deviations of the unsaturation indices, we used the expression

$$(SD)^2 = \Sigma a^2 \cdot b^2$$

where, for each fatty acid, a is the number of double bonds and b is the standard deviation corresponding to the content of this fatty acid in the complete lipid sample. Differences are considered significant if $P < 0.01$.

RESULTS

Description of cell fractions. Granules appeared as light-green pellets, and contained about 14% of the

total cell phospholipid. This lipid yield was comparable to that reported by Cohn and Hirsch (22). Six samples were prepared.

Plasma membrane preparations were isolated as white bands in the continuous sucrose gradients. Under the polarized light microscope, they appeared as small closed vesicles which displayed birefringence. Yields were usually in the range of 5–20 μg of lipid phosphorus per 1–3 ml of packed cells, for a recovery of 1–2% of the total cell lipid. Six plasma membrane samples were prepared. By phase microscopy these fractions appeared to be a moderately uniform preparation of vesicles from 2 to 4 μ in diameter. These structures did not stain with methyl green pyronine or phosphotungstic acid hematoxalin (29). Moreover, studies on three of these preparations have shown an average of a 16-fold increase in 5′-nucleotidase activity (30) per milligram protein in this fraction in comparison to whole cells and an analogous 4-fold diminution of acid phosphatase activity (31).

Six phagocytic vesicle fractions ("PV") were prepared. Four of these samples were extracted with ethanol and subjected to phospholipid analysis. The other two preparations were sonicated (see Methods), and the polystyrene latex-free vesicle membranes were lipid-extracted by the Folch procedure (25). Along with three of the ethanol-extracted samples, these preparations were analyzed for fatty acid distributions. Fatty acid proportions were, on the average, very similar for both extraction methods; however, considerable variation did occur among the five samples, probably due to the small quantities of lipid available (3–5% of total cell lipids). This resulted in comparatively large standard deviations about the mean fatty acid percentages. Since these variations were not correlated with the extraction method, all five determinations were averaged together.

Phospholipids of the cell fractions. Table I lists the results of phospholipid analyses on the cell fractions. The values for whole cell phospholipid content were in close agreement with those reported by Gottfried (32) and Schwandt, Birk, and Ehrhart (33). Granules differed from whole cell and plasma membrane lipid composition by containing significantly large proportions of phosphatidylethanolamine and less phosphatidylcholine. In comparison to the whole cell, plasma membranes had somewhat decreased levels of phosphatidylcholine and high proportions of phosphatidylinositol/phosphatidylserine and sphingomyelin. Phagocytic vesicle phospholipids had a lower content of phosphatidylinositol/phosphatidylserine than these of the whole cells and plasma membrane.

Whole lipid fatty acids. Table II shows the results of fatty acid analyses on the whole lipid extracts of the cell fractions. Whole cell fatty acid proportions differed from the results reported by Chiarioni, Nardi, and Valentino (34), who found higher proportions of oleic, arachidic, and linolenic acids, and lower levels of stearic and arachidonic acids. No antioxidants were used in these earlier investigations, which may well have resulted in a loss of arachidonic acid and a relative increase in the proportions of other fatty acids.

TABLE I
*Phospholipid Proportions**

	Whole cells (n = 6)	Granules (n = 8)	"Plasma membranes"‡ (n = 6)	Phagocytic vesicles (n = 4)
PE				
%	29.8	39.2	26.9	27.5
SD	3.1	3.3	1.7	2.8
PI/PS				
%	17.4	15.1	22.6	12.0
SD	2.7	1.2	2.2	1.9
PC				
%	38.8	32.1	30.8	43.4
SD	3.6	2.9	0.9	4.8
SM				
%	14.0	12.9	18.3	16.5
SD	2.6	1.1	0.9	4.4

Percentage of various phosphatides in whole C.M.L. cells and cell fractions (see Methods). Results from six whole cell, eight granule, six plasma membrane, and four phagocytic vesicle samples. The four areas of the thin-layer plate were arbitrarily assumed to contain 100% of the phospholipids present. Total phosphorus content of residual Silica gel accounted for less than 5% of the recovered lipids. Abbreviations used: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI/PS, phosphatidylinositol plus phosphatidylserine; SM, sphingomyelin; %, mean molar percentage (moles per 100 moles lipid) of phospholipid or fatty acid; SD, standard deviation about the mean mole percentages.

* In this and subsequent tables the data have been rounded off to two figures. Accordingly, total percentages may not add precisely to 100.

‡ Isolated by method of Warren, Glick, and Nass (23) with reservations on purity as noted in the text.

Granules were found to have a fatty acid distribution similar to that of the whole cell although their arachidonic acid levels and saturation indices were somewhat higher. In contrast, plasma membrane samples contained less oleic and arachidonic acids and more palmitic acid than whole cells. These differences gave the plasma membrane lipids an unsaturation index lower than those for either whole cells or granules.

Phagocytic vesicle lipids were considerably less unsaturated than any of the other fractions. In particular, the proportion of palmitic acid was significantly higher than in whole cells. Slightly higher levels of pentadecanoic, arachidic, and eicosadienoic acids were also observed in these vesicles. The percentages of linoleic,

TABLE II

Whole Lipid Extracts

n.....	6 Whole cells	6 Granules	6 "Plasma membrane"	5 Phagocytic vesicles	n.....	6 Whole cells	6 Granules	6 "Plasma membrane"	5 Phagocytic vesicles
12:0					18:2				
%	0.7	—	2.8	—	%	8.6	6.6	10.4	4.3
SD	0.3		0.6		SD	1.4	0.6	1.3	1.2
14:1					18:3				
%	1.5	1.8	1.7	—	%	1.5	0.9	3.3	3.0
SD	0.6	0.5	0.4		SD	0.9	0.4	0.5	1.1
15:0					20:0				
%	1.4	1.0	4.0	4.1	%	0.6	—	1.3	6.1
SD	0.6	1.0	0.3	0.9	SD	0.2		0.2	2.2
16:0					20:2				
%	19.7	16.4	28.0	30.9	%	0.6	0.5	1.3	5.0
SD	2.6	1.1	5.6	6.1	SD	0.1	0.4	0.3	2.8
16:1					20:4				
%	—	1.3	—	—	%	13.6	17.2	10.7	5.0
SD		0.5			SD	1.6	0.9	1.5	0.6
18:0					22:0				
%	19.2	19.1	16.7	21.9	%	1.4	3.2	1.0	2.6
SD	1.6	0.3	2.0	5.7	SD	0.5	0.2	0.2	0.3
18:1									
%	31.3	36.7	20.4	17.1	UI	109.7	124.0	100.2	64.9
SD	2.4	0.7	1.9	4.8	SD	7.7	4.2	7.5	8.7

Fatty acid proportions in the whole lipid extracts of whole cells and cell fractions (see Methods and Table I legend) Results from six whole cell, six granule, six plasma membrane, and five phagocytic vesicle samples. In the abbreviations of the fatty acids, the digits to the left of the colon state the number of carbon atoms in the fatty acid chain, while the number to the right states the number of carbon-carbon double bonds in that chain. The fatty acids and the abbreviations used are given below:

12:0, lauric acid; 14:1, myristoleic acid; 15:0, pentadecanoic acid; 16:0, palmitic acid; 16:1, palmitoleic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid; 20:0, arachidic acid; 20:2, eicosadienoic acid; 20:4, arachidonic acid; 22:0, behenic acid; UI, unsaturation index (sum of the individual mole percentages for each fatty acid multiplied by the number of carbon-carbon double bonds in that fatty acid); — indicates that this particular fatty acid was not detected in any sample examined. Standard deviations are not reported when the fatty acid was detected in only one sample. The unsaturation indices differed from each other at a significance level of $P < 0.01$ in all combinations with the single exception of whole cells versus plasma membranes. Arachidonic acid levels were significantly different between all fractions.

arachidonic, and, in particular, oleic acids were considerably lower than in whole cells. All of these differences resulted in an unsaturation index which was significantly ($P < 0.01$) lower than those for whole cells, granules, or plasma membranes.

Because polystyrene latex beads were present in isolated phagocytic vesicles, an ethanol extract of the quantity of spherules employed in these experiments was prepared to rule out the possibility of contamination from the latex itself. This extract contained no measurable fatty acid.

Fatty acids of phospholipid fractions. Tables III and IV show the analyses of phosphatidylethanolamine and phosphatidylcholine in the various cell fractions. For both of these phosphatides, granules had a fatty acid distribution and unsaturation index similar to those of the whole cell; plasma membrane had a lower unsaturation index due to decreased proportions of oleic and arachidonic acids and increased levels of palmitic acid. Phagocytic vesicles were less unsaturated for the same reasons. Notably, in the phosphatidylethanolamine fraction, phagocytic vesicles were much less saturated than plasma membranes (P for saturation indices < 0.01). The fatty acids of the quantitatively less important phosphatidylinositol/phosphatidylserine, neutral lipid, and sphingomyelin fractions were also analyzed (data not shown), and similar trends were observed; i.e., plasma membrane and phagocytic vesicle preparations had simi-

TABLE III
Phosphatidylethanolamine

n.....6	Whole cells	5 Granules	6 "Plasma membrane"	5 Phagocytic vesicles
12:0				
%	3.4	—	1.9	1.1
SD	1.8		0.7	1.6
14:1				
%	1.3	3.7	2.8	2.5
SD		0.7	2.1	3.5
15:0				
%	4.4	6.1	2.1	1.2
SD	2.1	0.3	1.1	1.6
16:0				
%	12.0	6.7	26.2	42.9
SD	2.3	0.6	2.7	8.5
16:1				
%	—	0.9	—	—
SD		0.1		
18:0				
%	18.6	17.1	18.5	14.8
SD	3.6	1.8	3.9	3.3
18:1				
%	24.8	26.4	8.4	23.0
SD	2.9	2.8	3.3	4.8
18:2				
%	6.0	6.6	9.8	1.1
SD	1.6	0.3	0.6	
18:3				
%	1.1	1.6	8.2	1.6
SD	0.7	0.2	1.1	2.2
20:0				
%	1.3	0.7	2.1	—
SD	0.3	0.7	0.6	
20:2				
%	1.7	2.4	2.4	1.2
SD	1.5	0.2	0.3	
20:4				
%	23.6	24.2	15.4	9.8
SD	3.4	1.8	2.2	6.2
22:0				
%	1.7	3.0	1.1	0.9
SD	0.7	0.2	0.3	
UI	139.3	150.6	121.8	73.8
SD	14.6	7.9	10.3	26.4

Fatty acid proportions in the phosphatidylethanolamine of whole cells and cell fractions (see Methods and legends for Tables I and II). Results from six whole cell, five granule, six plasma membrane, and five phagocytic vesicle samples.

lar compositions and tended to be more saturated than whole cells or granules.

Prelabeled triglyceride fatty acid disposition studies. Figs. 1 A and B show the results of one representative experiment of three similar studies showing the comparative disposition of saturated ([^{3}H]palmitic) and unsaturated ([^{14}C]linoleic) fatty acid in leukocytes after phagocytosis.

In Fig. 1 A, prelabeled leukocytes incubated without phagocytic stimulus show a minimal symmetric loss of both fatty acids with little redistribution of radioactivity between neutral lipids and phospholipids for 30 min of incubation. In Fig. 1 B, redistribution of radioactivity after phagocytosis, as has been previously described (7), is seen. Notably, in terms of the prelabeled triglyceride fatty acid, there is a considerably greater loss of labeled saturated fatty acids than unsaturated fatty acids. Conversely, there is a less striking increase of unsaturated fatty acid versus saturated fatty acids in the phosphatidylcholine fatty acids.

DISCUSSION

Purity of the cell fractions. Both granule and phagocytic vesicle preparations were assumed to be pure. These fractions were obtained by well established methods which had previously been tested for purity both morphologically and enzymatically.

Granules were extracted directly, without isolating the membrane itself. This fraction contained low proportions of lipid, and it has been shown that most of this lipid is in the membrane (35). Remotely, some lipid may have been present within the interior of the granules, which may have been included in the extract. If this were the case, secondary lysosomes (phagocytic vesicles) should have contained this lipid once fusion with the granules had occurred; however, whole vesicles (ethanol extracted) and vesicle membrane (sonicated) showed no differences in fatty acid distributions. Since extraction removed all of the lipid and sonication removed only surface membranes of these vesicles, it appeared that the only lipid present in significant quantities in the granules was contained in the membranes.

The purity of the plasma membrane preparation has not been unequivocally established; preliminary experiments indicate that these preparations are 16-fold enriched with 5′-nucleotidase in comparison to whole cells and that they have only moderate acid phosphatase contamination. Likewise, the morphology and histochemistry of these fractions suggest that they are enriched with membrane. However, we must emphasize that the identification of these samples as uncontaminated plasma membrane is tentative.

Warren, Glick, and Nass have reported several means of isolating membrane from mammalian cells (23). The

TABLE IV
Phosphatidylcholine

n.....6	Whole cells	5 Granules	5 "Plasma membrane"	5 Phagocytic vesicles
12:0				
%	—	—	—	1.8
SD				0.3
14:1				
%	—	0.3	—	—
SD		0.1		
15:0				
%	1.7	0.3	—	3.5
SD	1.7	0.1		0.4
16:0				
%	33.2	40.2	47.4	51.5
SD	4.9	3.2	1.4	8.1
16:1				
%	—	0.4	—	—
SD		0.2		
18:0				
%	14.1	7.6	14.4	13.0
SD	3.7	0.2	0.9	2.6
18:1				
%	34.5	36.5	24.0	15.6
SD	7.0	3.4	2.6	5.9
18:2				
%	6.9	8.2	6.2	4.2
SD	1.3	0.4	0.3	2.5
18:3				
%	1.7	2.0	1.8	2.9
SD	0.3	0.1	0.1	0.3
20:0				
%	1.7	0.5	3.2	1.9
SD	1.7	0.1	0.2	1.2
20:2				
%	1.4	0.7	1.4	2.9
SD	0.7	0.1	0.6	0.8
20:4				
%	3.5	3.2	1.3	2.6
SD	0.3	0.1	0.2	1.5
22:0				
%	0.7	0.6	0.2	—
SD	0.2	0.1	0.1	
UI	70.4	73.8	48.8	49.0
SD	7.8	3.6	3.1	10.0

Fatty acid proportions in the phosphatidylcholine of whole cells and cell fractions (see Methods and legends for Tables I and II). Results from six whole cell, five granule, five plasma membrane, and five phagocytic vesicle samples.

"Tris method" was selected for use in these experiments because it was expected to result in the least chemical damage to the membrane. Woodin and Wieneke have also reported isolating rabbit leukocyte membranes (36). Unfortunately, our attempts to prepare plasma membrane using this latter technique did not yield sufficient material for analysis.

Experiments similar to these have been reported by Mason, Stossel, and Vaughan (37) for the guinea pig polymorphonuclear leukocyte. These investigators found that while the phagocytic vesicles tended to be more saturated than the whole cells, the various fractions differed little in phospholipid composition. The different trends in lipid composition observed between the guinea pig and human leukocytes used in these studies can probably be attributed to species variations, since these cells differ markedly in enzymatic and protein composition (38, 39). There may also be further differences between these cells due to the collection procedures themselves. The guinea pig granulocytes were prepared from peritoneal exudates induced by inflammatory challenge. In contrast, the human cells were collected from the peripheral blood of relatively asymptomatic patients. Accordingly, both the milieux in which the cells developed and their basal state of biochemical responsiveness might be expected to be markedly different.

Finally, although it has been repeatedly shown that chronic myelogenous leukemia cells are quite similar to normal human granulocytes in terms of gross biochemical composition (32, 40) and probably in terms of biological function (41), the possibility that these neoplastic cells have fine differences from the normal in terms of fatty acid metabolism cannot be ruled out by the current studies. With the development of relatively atraumatic methods for collecting large quantities of normal human granulocytes (42), it may soon be possible to define this question more precisely.

Evidence for remodeling of granulocyte membranes. Since the phagosomes is presumably derived from the plasma membrane and the lysosome membrane (43, 44), and since we have found that both of these membranes are much less saturated than the phagosome membrane, the data suggest that phagocytosis induces an increase in vesicle membrane fatty acid saturation through some remodeling of the parent membranes.

It is unlikely that the observed changes were due to membrane auto-oxidation during the in vitro experimental manipulations since plasma membrane preparations, which took more than twice as long as the phagosome membranes to isolate, still contained comparatively large proportions of arachidonic acid. It is also unlikely that these changes were solely due to peroxidative loss of arachidonic acid in vivo, since large increments of palmitic acid contributed to the saturation of the vesicle membrane. These were not merely relative percentage

differences since the lower levels of polyunsaturatd fatty acids cannot arithmetically account for the elevated proportion of palmitic acid: some actual mass increase of the latter occurred. In addition, phagocytic vesicles also showed greatly decreased proportions of oleic acid. One would not expect that this mono-unsaturated fatty acid would be much more susceptible to peroxidation than saturated fatty acids (15, 45, 46).

These results suggest that beyond the simple peroxidative loss of polyunsaturated fatty acids, a reacylation system which, overall, favors palmitic acid is involved. There is evidence that mechanisms for such a process exist in mammalian cells. First, the terminal fatty acids of triglycerides are predominantly saturated fatty acids in mammalian cells (8), and it would seem likely from the pancreatic lipase analog (9) that these would be the first fatty acids used for acylation if lipase-activated cleavage of triglycerides followed by reacylation of lysophosphatidylcholine were a mechanism for new phospholipid synthesis during phagocytosis. Secondly, vitamin E-deficient human erythrocytes exposed to a peroxidative threat, undergo reacylation processes which favor saturated fatty acids (47). Thirdly, the direct data in Fig. 1 show first that there is a larger percentage of triglyceride labeling from serum with saturated fatty acids in comparison to unsaturated fatty acids before phagocytosis. This may represent a preferential uptake of saturated fatty acid into granulocyte triglyceride at rest. It may also simply reflect the fact that two sites are available for saturated acid labeling and only one is available for unsaturated labeling. Finally, prelabeled triglyceride palmitic acid is transferred to phosphatides more readily than triglyceride linoleic acid, directly demonstrating the preferential net incorporation of saturated fatty acids into newly synthesized phospholipid after phagocytosis.

In the current analytic studies, it appears that oleic and arachidonic acids were removed from their phospholipid backbones. Removal of these unsaturated fatty acids may have proceeded by either peroxidative destruction or phospholipase activity (or a combination of both). Whatever the mechanism, the net result was probably a population of lysophosphatides which were then rapidly reacylated. The data are consistent with the hypothesis that polyunsaturated fatty acids remained at ongoing risk of peroxidation, whereas saturated fatty acids did not. Therefore, a net accumulation of the saturated species, particularly palmitic acid, ensued.

The present data do not allow us to define whether phospholipase activity or peroxidative destruction is predominantly operative in the initial stages of this lipid saturation process. However, phagocytosis does eventually produce a remodeled lipid membrane surrounding the phagosome which is markedly different from the lysosomal and plasma membranes from which it was derived. From previous studies, these differences in fatty acid saturation between the remodeled and the parent membranes might be expected to reduce phagocytic vesicle membrane permeability (18–20). Such a change would be doubly advantageous for the phagocytic cell in that it would tend to maintain lethal concentrations of hydrogen peroxide and lysosomal enzyme cofactors surrounding the ingested organism, and it would also tend to inhibit cell autodigestion after bacterial killing had been initiated.

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