Metabolism of bis(monoacylglycero)phosphate in macrophages

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Abstract To further elucidate the role of bis(monoacylglycero)phosphate in lysosomes, its metabolism was assessed by incubation of intact and disrupted macrophages in the presence of labeled lipid precursors. In rabbit pulmonary macrophages bis(monoacylglycero)P accounted for 17.9% and acylphosphatidylglycerol for 2.6% of phospholipid phosphorus. Major fatty acids in bis(monoacylglycero)P were oleic (47%), linoleic (29%), and arachidonic (6.4%); those in acylphosphatidylglycerol were of similar distribution except for a high content of palmitic acid (20%). When homogenates of rabbit pulmonary and peritoneal macrophages, rat pulmonary macrophages, and human blood leukocytes were incubated with *sn*[^{14}C]glycerol-3-phosphate and CDP-diacylglycerol at pH 7.4, there was labeling of bis(monoacylglycero)P and acylphosphatidylglycerol that correlated with content of bis(monoacylglycero)P. When intact rabbit pulmonary macrophages were incubated for 60 min with [^{3}H]glucose and [^{32}P]orthophosphate, small amounts of label appeared in bis(monoacylglycero)P and only traces in acylphosphatidylglycerol. In contrast, incubation of intact cells with the ^{14}C-labeled fatty acid precursors palmitic, oleic, and arachidonic acids resulted in much greater labeling of the two lipids. Labeling of phospholipids was greatest with arachidonate as precursor and least with palmitate; after 60 min, labeling of bis(monoacylglycero)P with arachidonate was 10- and 50-fold greater than with oleate and palmitate, respectively, and was exceeded only by that of phosphatidylcholine. Calculated ratios of labeling of fatty acid to P, particularly those for arachidonate, were much greater for bis(monoacylglycero)P and for acylphosphatidylglycerol than for other phospholipids. This suggests a uniquely high turnover of fatty acids in bis(monoacylglycero)P and acylphosphatidylglycerol and thus a more specific role for these compounds in metabolism of complex lipids in the lysosome.—**Huterer, S., and J. Wherrett.** Metabolism of bis(monoacylglycero)phosphate in macrophages. *J. Lipid Res.* 1979. **20:** 966–973.

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Bis(monoacylglycero)phosphate is a phospholipid that is localized to secondary lysosomes (1–6). In certain kinds of lysosomal storage the cellular concentration of this lipid increases markedly, for example, in inherited deficiency of lysosomal hydrolase (7, 8), following the administration of cationic amphiphilic compounds (9–12), or following the administration of other compounds that become concentrated in secondary lysosomes (4, 13). Moreover, the synthesis of bis(monoacylglycero)P from phosphatidylglycerol in rat liver takes place maximally at low pH in secondary lysosomes (14). It should be noted that the mechanism of synthesis suggested by these experiments does not account for the stereochemical configuration of the glycerophosphoglycerol backbone (*sn*-1-glycerophospho-*sn*-1′-glycerol) determined for the majority of bis(monoacylglycero)P from rat liver and other tissues (15, 16) and confirmed by one of us in samples of bis(monoacylglycero)P from rat and human liver (17). In order to clarify the role of bis(monoacylglycero)P in phospholipid metabolism and in lysosomal function, and also the mechanisms responsible for alterations in its metabolism associated with cell storage, we have begun to examine aspects of its metabolism in cell systems. We report here studies of the incorporation of labeled lipid precursors into bis(monoacylglycero)P and other phospholipids on incubation with both intact and disrupted macrophages. The results demonstrate active synthesis of bis(monoacylglycero)P and acylphosphatidylglycerol in macrophages and suggest a strikingly high rate of fatty acid esterification in comparison to other phospholipid classes.

MATERIALS AND METHODS

Lipid precursors

D[1-^{3}H]Glucose (18 Ci/mmol), [^{32}P]orthophosphoric acid (2 mCi/ml), *sn*-[U-^{14}C]glycerol-3-phosphate (130

Abbreviations: APG, acylphosphatidylglycerol; BMP, bis(monoacylglycero)P, bis(monoacylglycero)phosphate; DPG, diphosphatidylglycerol; P, phosphate, PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; t, tentative; tr, trace.

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mCi/mmol), [1-^{14}C]palmitic acid (50.2 mCi/mmol), [1-^{14}C]oleic acid (48 mCi/mmol), and [1-^{14}C]arachidonic acid (51 mCi/mmol) were obtained from New England Nuclear, Quebec. The radioactive fatty acids were neutralized with NaOH and complexed with bovine albumin (fraction V) (Sigma) in a molar ratio of fatty acid to albumin of 5:1 (18). CDP-diacylglycerol was purchased from Serdary Research Laboratories, Inc., London, Ontario.

Preparation of macrophages

Pulmonary alveolar macrophages from unstimulated male New Zealand rabbits weighing 2–3 kg were obtained by lavage with 150 mM NaCl, collected by centrifugation, washed twice, and suspended in Krebs-Ringer phosphate medium, pH 7.4, containing 0.92 mM $CaCl_2$ and 4.5 mM glucose (19). To remove contaminating red cells, a hemolysis step was usually included in which cells were exposed to 0.2% NaCl for 60–90 sec. More than 90% of the cells in all preparations appeared to be intact as judged by exclusion of trypan blue. Electron microscopy of a preparation revealed cells 10–20 μm in diameter having the typical appearance of alveolar macrophages with numerous secondary lysosomes and small electron-lucent vacuoles.

Rabbit peritoneal macrophages were obtained by washing the peritoneal cavity through a trochar with 250 ml of Tyrode's solution. Macrophages were collected at 19 hr (preparation 1 in Table 3) and at 5 days (preparation 2 in Table 3) after intraperitoneal injection of 150 ml of 0.1% glycogen into 2-kg rabbits. At 19 hr, more than 70% and, at 5 days, more than 95% of the cells were identified as macrophages in smears stained with Leishman's stain. Human white cells were isolated (20) from a donation of 18 ml of fresh venous blood. Pulmonary macrophages obtained from rats by lavage (21) were donated by Dr. Jennifer Sturgess. Where homogenate was used, cells were suspended in 0.1 M Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose and disrupted with Polytron (Kinematica GmbH, Luzern) until more than 90% of the cells were broken as determined by phase microscopy.

Incubation conditions

For incubation of intact macrophages, 3.4 ml of suspension containing 3–5 × 10^7 cells were pipetted into plastic 25-ml Erlenmeyer flasks. To each flask was added 0.1 ml of labeled fatty acid–albumin complex containing 1–2 μCi of radioactivity and flasks were incubated for 10 and 60 min at 37°C in a shaking bath in an atmosphere of O_2–CO_2 95:5. At the end of incubation, cells were chilled, collected by centrifugation (480 g × 10 min), and washed twice with ice-cold 0.9% NaCl. Incubation of macrophages with labeled glucose occurred under the same conditions except that more radioactivity (50–100 μCi) was added and the final concentration of glucose was as indicated in Table 4. When labeled orthophosphate was used as precursor, the Krebs-Ringer phosphate medium was replaced with Krebs-Ringer HEPES buffer (pH 7.4) containing 4.5 mM D-glucose, 0.08 mM arachidonate complexed to albumin, 1.2 mM PO_4^{3-}, and 50 μCi of ^{32}P per incubation (22). Conditions for incubation of homogenates with labeled *sn*-glycerol-3-phosphate and CDP-diacylglycerol are given in the legend of Table 3.

Analysis of lipids

After incubation, lipids from homogenates were extracted according to Bligh and Dyer (23) while those from whole cells were extracted using 19 volumes of chloroform–methanol 2:1 and then washed with 0.1 M KCl three times (24). Radioactive lipids were separated into "acidic" and "nonacidic" lipids on a DEAE-Sephadex column (25). Individual phospholipid classes were separated by two-dimensional thin-layer chromatography using the solvent systems chloroform–methanol-concentrated ammonia 65:25:4 (v/v) and chloroform–acetone–methanol–acetic acid–water 5:2:1:1:0.5 (v/v) as described previously (1). In the experiments of Table 3, nonradioactive bis(monoacylglycero)P and acylphosphatidylglycerol were added to lipid extracts of rabbit peritoneal macrophages and human white blood cells. The phospholipids were detected with I_2 vapor. The gel areas were collected by aspiration into short Pasteur pipettes plugged with glass wool from which the lipid was eluted with methanol. An aliquot of the eluate was taken for phosphate determination (26) and the rest was dried, dissolved in scintillation solution of Econofluor (New England Nuclear, Boston) –Triton X-100 2:1 (v/v), and radioactivity was determined in a scintillation counter (Mark I, Nuclear Chicago). Corrections for quenching were made using an external standard.

The fatty acid composition of individual phospholipids was determined by analysis of the fatty acid methyl esters (27) using gas–liquid chromatography. Analysis was carried out on a Hewlett-Packard 5840A gas chromatograph fitted with glass columns packed with 6% DEGS on Chromosorb W 80/100 mesh, and programmed from 165 to 185°C.

Identification of labeled lipids

The identity of the labeled phospholipids, on thin-layer chromatograms, tentatively designated as bis(monoacylglycero)P, acylphosphatidylglycerol, and

TABLE 1. Phospholipid composition of rabbit pulmonary macrophages

Phospholipid	Percent[a]
Phosphatidylcholine	36.3 ± 1.7
Phosphatidylethanolamine	18.8 ± 1.8
Sphingomyelin	10.0 ± 0.6
Phosphatidylinositol	6.2 ± 1.1
Phosphatidylserine	4.1 ± 0.8
Bis(monoacylglycero)phosphate	17.9 ± 2.2
Acylphosphatidylglycerol	2.6 ± 0.4
Phosphatidylglycerol	1.7 ± 0.6
Diphosphatidylglycerol	1.4 ± 0.5
Phosphatidic acid	0.5 ± 0.1

[a] The results are expressed as percent of total lipid phosphorus and include the mean ± SD of five different preparations.

phosphatidylglycerol according to chromatographic mobility in comparison to standards, was confirmed by mild alkaline hydrolysis and acetolysis. Labeled lipids were purified by column and thin-layer chromatography following extraction from a pulmonary macrophage homogenate that had been incubated with *sn*-[U-^{14}C]glycerol-3-P as described in Table 3. The purified lipids were subjected to mild alkaline hydrolysis (28) and water-soluble products were identified by cellulose thin-layer chromatography (1). The major labeled product from all three lipids cochromatographed with the major phosphate ester released from phosphatidylglycerol standard. Similarly, the three lipids were purified from macrophages after incubation with [1-^{14}C]arachidonate and were subjected to acetolysis (29). Thin-layer chromatography of the products revealed that the lipid designated bis(monoacylglycero)P gave only one radioactive spot which cochromatographed with monoacyldiacetylglycerol, whereas acylphosphatidylglycerol gave two radioactive spots cochromatographing with monoacyldiacetylglycerol and diacylmonoacetylglycerol, and phosphatidylglycerol gave one spot cochromatographing with diacylmonoacetylglycerol. The methyl esters of individual fatty acids from a sample of purified bis(monoacylglycero)P isolated from macrophages that had been incubated with [1-^{14}C]arachidonate were isolated by Dr. M. Khan using a Hewlett-Packard Model 4701 gas chromatograph. Radioactivity was present only in arachidonic acid methyl ester.

RESULTS

Composition of phospholipids of pulmonary macrophages and their fatty acids

The phospholipid composition of rabbit pulmonary macrophages is given in **Table 1.** The values obtained, in particular the high content of bis(monoacylglycero)P (18%), are similar to those previously reported (2). In addition, acylphosphatidylglycerol (2.6% of total phospholipid phosphorus), phosphatidylglycerol (1.7%), and diphosphatidylglycerol (1.4%) were detected. The fatty acid compositions of individual phospholipids are given in **Table 2.** Major fatty acids of bis(monoacylglycero)P were oleic acid (47%), linoleic acid (29%), and arachidonic acid (6.4%), as found previously (2). Fatty acid composition of acylphosphatidylglycerol was similar to that of bis(monoacylglycero)P except for a higher percentage of palmitic acid. Of the other phospholipids, phosphatidylcholine and phosphatidylglycerol had a very high content of palmitic acid, whereas a high percentage of arachidonic acid was characteristic of phosphatidylethanolamine and phosphatidylinositol. In unstimulated rat pulmonary macrophages, bis(monoacylglycero)P comprised 6% of the phospholipid P.

TABLE 2. Fatty acid composition of phospholipids from rabbit pulmonary macrophages

Fatty acid	PC	PE	PI	PS	BMP	APG	PG	DPG
				area percent[a]				
16:0	48.1 ± 1.9	8.8 ± 0.9	10.2 ± 0.8	3.2 ± 0.4	4.0 ± 0.3	20.1 ± 2.7	38.6 ± 4.5	4.7 ± 1.5
16 DMA		11.1 ± 0.3						
16:1	4.3 ± 0.6	1.0 ± 0.3	0.5 ± 0.2	0.9 ± 0.1	0.9 ± 0.3	1.5 ± 0.4	3.9 ± 0.8	3.1 ± 1.6
18:0	6.7 ± 0.5	12.9 ± 0.4	33.0 ± 2.3	38.5 ± 3.2	6.0 ± 0.8	8.3 ± 0.9	8.6 ± 1.2	2.2 ± 0.7
18 DMA		4.4 ± 1.6						
18:1	16.8 ± 1.7	18.7 ± 0.7	21.6 ± 1.2	39.9 ± 1.7	46.8 ± 2.4	39.4 ± 0.9	34.1 ± 2.7	26.4 ± 2.4
18:2	16.7 ± 2.0	9.0 ± 1.2	7.7 ± 1.4	11.3 ± 5.0	29.1 ± 3.4	20.7 ± 1.8	8.6 ± 0.6	58.9 ± 5.2
18:3	1.0 ± 0.2	0.4 ± 0.1	0.7 ± 0.1	1.2 ± 0.7	1.3 ± 0.3	1.2 ± 0.3	1.1 ± 0.3	1.7 ± 0.2
20:3[t]	0.4 ± 0.1	0.6 ± 0.3	1.8 ± 0.5	0.5 ± 0.1	0.8 ± 0.2	0.4 ± 0.2	tr	1.1 ± 0.3
20:4	6.1 ± 1.0	24.7 ± 1.3	23.1 ± 1.2	2.2 ± 1.2	6.4 ± 0.8	4.7 ± 0.5	1.4 ± 0.2	2.0 ± 0.2
24:1	0.4 ± 0.1	2.4 ± 0.4	1.2 ± 0.7	1.5 ± 0.4	1.1 ± 0.2	0.9 ± 0.3		
22:5	1.0 ± 0.1	5.3 ± 0.4	tr	1.3 ± 0.2	1.7 ± 1.7	2.7 ± 1.0		
22:6	tr	0.5 ± 0.1			0.5 ± 0.2	tr		
unident.							3.7 ± 1.8	

[a] The results are presented as means ± SD of four different preparations.

TABLE 3. Formation of phospholipids in homogenates incubated in the presence of added *sn*-[U-^{14}C]glycerol-3-phosphate and CDP-diacylglycerol

Tissue	Phospholipid Formed		
	PG	BMP	APG
Rabbit pulmonary macrophages	197	45.5	3.1
Rabbit peritoneal macrophages (1)	569	10.6	4.1
Rabbit peritoneal macrophages (2)	407	9.5	1.0
Rat pulmonary macrophages	541	5.6	4.0
Human white blood cells	108	1.5	0.3
Rat liver crude mitochondria	955	3.3	tr

Incubation mixtures contained 0.1 M Tris-HCl, pH 7.4, 0.2 mM CDP-diacylglycerol, 5 mM glutathione, 50 U penicillin, 15 μM *sn*-[U-^{14}C]glycerol-3-phosphate, (sp act 130 mCi per mmol), and 2 mg protein in a volume of 0.5 ml. The same incubation mixture was used for crude mitochondria (30) except that the concentration of *sn*-[^{14}C]glycerol-3-phosphate was 216 μM. Incubation was carried out in a 37°C shaking incubator bath for 2 hr, followed by extraction of lipids as described in Methods. Under these conditions, formation of BMP and APG was linear up to 4 hr. Results are expressed as pmol of product formed per mg protein (40).

Incorporation of *sn*-glycerol-3-phosphate in macrophage homogenates

In initial experiments, homogenates of macrophages from different sources were incubated with [U-^{14}C]-*sn*-glycerol-3-phosphate and CDP-diacylglycerol at pH 7.4 (30). Results are given in **Table 3.** Incorporation of label into bis(monoacylglycero)P and acylphosphatidylglycerol was greater in all macrophage preparations than in the active subcellular fraction from rat liver. Incorporation was readily detected in the mixed population of cells isolated from human blood.

Incubation of macrophages with [^{3}H]glucose and [^{32}P]orthophosphate

Macrophages suspended in modified Krebs-Ringer medium were incubated with [^{3}H]glucose and [^{32}P]-orthophosphate and the label appearing in phospholipids was measured after 10 and 60 min. The results are shown in **Table 4.** There was a small uptake of glucose radioactivity (0.7%) into the lipids of macrophages and a slightly higher uptake of [^{32}P]orthophosphate (1.8%). Among the phospholipids most of the radioactivity was incorporated into phosphatidylcholine. Small amounts of label appeared in bis(monoacylglycero)P which increased between 10 and 60 min. Amounts of label found in acylphosphatidylglycerol were close to background.

Incubation of macrophages with 1-^{14}C-labeled palmitic, oleic, and arachidonic acids

The incorporation of radioactivity into macrophage phospholipids after 10 and 60 min of incubation with fatty acid precursors is shown in **Table 5.** Results of individual experiments are shown in which different concentrations of precursors were added. Fatty acid precursors were rapidly taken up by macrophages; in every experiment, more than 70% of radioactivity was recovered in the cells after 10 min of incubation. Labeling of phospholipids was greatest when arachidonate was the precursor and least when palmitate was added. Labeling was maximum when precursors were added in a concentration of 0.08 mM. A higher concentration inhibited labeling of phospholipids other than phosphatidylglycerol and acylphosphatidylglycerol. The differences in degree of labeling by the three precursors may reflect differences in the sizes of intracellular pools of the respective fatty acids.

Bis(monoacylglycero)P was readily labeled; when oleic and arachidonic acids were the precursors, it was the most highly labeled phospholipid after 60 min other than phosphatidylcholine. When arachidonate was the precursor, labeling of bis(monoacylglycero)P after 60 min was 10- and 50-fold greater than that observed with oleate and palmitate, respectively, and the increase was greater than that occurring in other bone of phospholipids. Thus, the ratio of fatty acid to P radioactivities incorporated into intact cells will

TABLE 4. Incorporation of radioactive phosphate and glucose into phospholipids of pulmonary macrophages

Concentration of Substrate	Incubation Time	PC	PE	PI	BMP	APG	PG	PA
mM	*min*			*[^{32}P]orthophosphate*				
1.2	10	93	7	83	0.3	tr	3	123
	60	3706	51	239	1.5	tr	14	488
				[^{3}H]glucose				
0.005	10	1.3	0.09	0.28	0.11	tr	0.05	
	60	9.2	0.28	0.24	0.17	tr	0.09	

Results are expressed as pmol [^{32}P]orthophosphate and pmol [^{3}H]-labeled radioequivalent of glucose incorporated per 10^7 cells and represents the means of two experiments with different preparations where similar values were obtained.

TABLE 5. Incorporation of radioactive fatty acids into phospholipids of pulmonary macrophages

Preparation	Fatty Acid Concentration	Incubation Time	PC	PE	PI	BMP	APG	PG	PA
	mM	*min*			[^{14}C]*palmitate*				
A	0.0075	10	299	13	36	3	3	2	26
		60	1685	41	58	10	9	5	4
B	0.023	10	677	56	68	7	10	14	188
		60	3610	144	204	29	43	21	101
					[^{14}C]*oleate*				
A	0.01	10	498	46	101	42	4	7	28
		60	1155	39	41	76	8	7	4
B	0.08	10	2795	222	491	133	29	13	245
		60	5739	295	297	439	69	16	44
C	0.54	10	478	33	77	10	46	12	12
		60	181	21	40	11	91	16	19
					[^{14}C]*arachidonate*				
A	0.008	10	1867	196	171	236	8	11	46
		60	1952	567	98	553	18	18	13
B	0.08	10	8010	958	1299	1150	42	22	247
		60	14147	2755	510	3236	122	23	82
C	0.70	10	1067	49	44	52	26	12	52
		60	507	60	40	50	106	18	15

Conditions of incubation are described under Methods. Total amount of radioactivity in the incubation medium was 2 μCi and the concentration of fatty acid in medium is given above. Results are expressed as pmol fatty acid incorporated/10^7 cells. Different macrophage preparations used for these experiments are indicated. Incorporation of fatty acid added in the lowest concentrations shown was determined in three preparations of cells of which the experiment shown is representative. Other results represent single experiments.

phospholipids. Acylphosphatidylglycerol became labeled and this increased between 10 and 60 min and also was greatest when arachidonate was the precursor. Labeling in both bis(monoacylglycero)P and acylphosphatidylglycerol exceeded that in phosphatidylglycerol. Sphingomyelin, phosphatidylserine, and diphosphatidylglycerol were poorly labeled when all three precursors were used and the results are omitted.

The specific radioactivities of phospholipids labeled after 10 and 60 min, which were calculated from one experiment, are plotted in **Fig. 1.** This experiment was typical of five experiments in which the amount of radioactivity added was between 1 and 3 μCi. As expected, the highest specific radioactivities were found in phosphatidic acid and phosphatidylinositol after 10 min; these values were greatly diminished after 50 min of further incubation. With the three precursors, both bis(monoacylglycero)P and acylphosphatidylglycerol were found to have appreciable specific radioactivities after 60 min. With arachidonate the specific radioactivity of bis(monoacylglycero)P after 60 min had become greater than all other classes except phosphatidylcholine.

DISCUSSION

Analysis of phospholipids in pulmonary alveolar macrophages from both rabbit and rat revealed high content of bis(monoacylglycero)P, a finding reported previously for rabbit, but not rat, macrophages (2). On chromatograms of peritoneal macrophage lipids, the spots corresponding to bis(monoacylglycero)P and acylphosphatidylglycerol were much less intense than those on chromatograms of pulmonary macrophage lipids. Only traces of these lipids were detected on chromatograms of lipids from human white blood cells. The fatty acid composition found for bis(monoacylglycero)P of rabbit pulmonary macrophages confirms the earlier finding (2) and is similar to that found for bis(monoacylglycero)P accumulating in liver and spleen of patients afflicted with storage disease (31). A different fatty acid composition was found for bis(monoacylglycero)P isolated from "tritosomes" (32) of rat liver (33) and from livers of rats and humans with drug-induced lipidosis (6, 9). In these analyses, docosahexenoic acid comprised as much as 70% of total fatty acid.

The related phospholipid, acylphosphatidylglycerol,

was first detected in rabbit lung (34) and we find that it is concentrated in alveolar macrophages where it comprises 2.6% of total phospholipid. The fatty acid composition of acylphosphatidylglycerol in rabbit macrophages is very similar to that reported for whole lung (34).

To compare synthetic activities in various tissues, CDP-diacylglycerol-stimulated incorporation of labeled *sn*-glycerol-3-P into bis(monoacylglycero)P and related acidic phospholipids was measured at neutral pH. Active labeling of bis(monoacylglycero)P, acylphosphatidylglycerol, and phosphatidylglycerol was found in macrophages from both rabbit and rat and in white cells from human blood. The formation of bis(monoacylglycero)P in 2 hr was considerably greater in cells with high contents of bis(monoacylglycero)P. For example, rabbit pulmonary macrophages formed four times as much as peritoneal macrophages and, although not quantified in peritoneal cells, it was clearly apparent from chromatograms that the content of bis(monoacylglycero)P was much greater in pulmonary cells. This indicates that increased cellular content of bis(monoacylglycero)P can be associated with increased synthesis. Therefore, increases of bis(monoacylglycero)P found in certain states of intracellular storage may result from increased synthesis rather than from decreased catabolism, and thus they represent a cellular adaptation to a load of substrate imposed upon the lysosomal system.

In an earlier study (2), the inability of intact rabbit pulmonary alveolar macrophages to incorporate either glycerol or phosphorus into bis(monoacylglycero)P in vitro was noted. However, cells in culture incorporate [^{32}P]orthophosphate into bis(monoacylglycero)P (35). The results in Table 4 demonstrate incorporation of glucose label and of P into phospholipids, in keeping with previous reports (36, 37), but they also demonstrate labeling of bis(monoacylglycero)P, acylphosphatidylglycerol, and phosphatidylglycerol with both precursors, which was less than that of other phospholipids. In a separate experiment, incorporation of labeled glycerol into bis(monoacylglycero)P and acylphosphatidylglycerol was also examined and was much lower than that incorporated into the other phospholipids.

In marked contrast to glucose, glycerol, and orthophosphate was the active incorporation of fatty acid precursors, particularly arachidonic acid, into bis(monoacylglycero)P. This is equally apparent when the data are calculated as specific radioactivity as in Fig. 1. When labeled oleic acid and arachidonic acid were incubated with a macrophage homogenate, incorporation of label into lipids was only 1% of that observed with intact cells. In contrast to the turnover of fatty acids in individual phospholipids, there is no evidence for independent turnover of P except in phosphatidylinositol where cleavage of inositol phosphate has been demonstrated (38).

Other enzymes with phospholipase C activity have not been found in mammalian tissues except for phosphatidate phosphohydrolase. It may be assumed, therefore, that the incorporation of P by intact cells represents de novo formation of the glycero-P back-

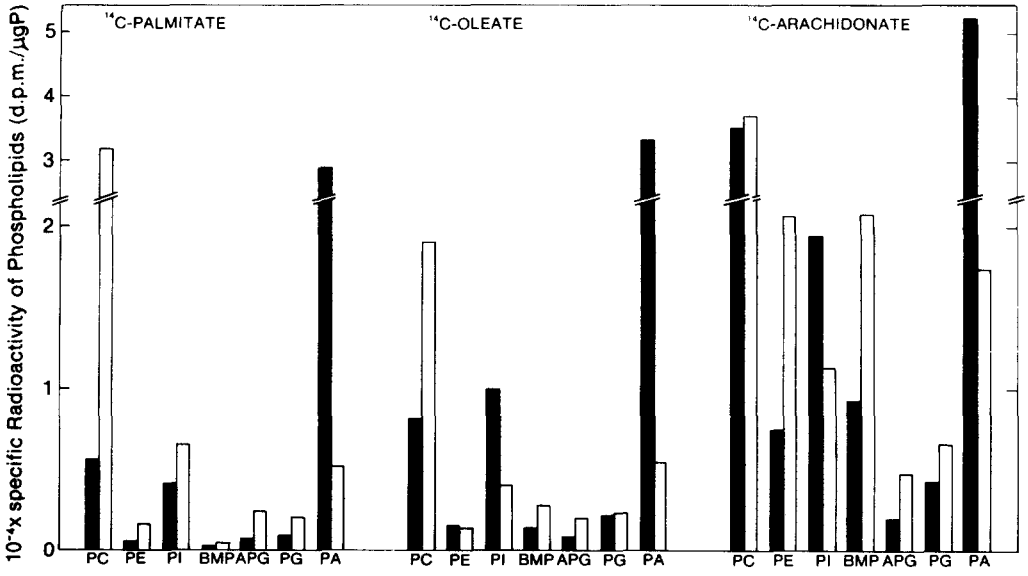

Fig. 1. The specific radioactivities of the individual phospholipids from pulmonary macrophages expressed as dpm/μg phospholipid P are from one representative experiment in which 2 μCi of fatty acid radioactivity was added to the incubation medium. Shaded bars represent values at 10 min and open bars values at 60 min of incubation.

TABLE 6. Ratio of fatty acid to phosphorus radioactivity incorporated into phospholipids of intact macrophages

Phospholipid	Labeling Ratio (^{14}C-labeled Fatty Acid/[^{32}P]Phosphate)					
	[^{14}C]Palmitate		[^{14}C]Oleate		[^{14}C]Arachidonate	
	10 min	60 min	10 min	60 min	10 min	60 min
PC	7	1	30	2	86	4
PE	8	3	32	6	137	54
PI	1	1	6	1	16	2
BMP	23	19	443	293	3833	2157
PG	5	2	4	1	7	2
PA	1	0.2	2	0.1	2	0.2

Values were calculated from data in Tables 4 and 5 for 10 and 60 min of incubation.

indicate the rates of fatty acid esterification for each phospholipid class relative to phosphate ester formation. Ratios calculated for the three fatty acid precursors are given in **Table 6** for 10- and 60-min incubations. The ratios found for bis(monoacylglycero)P are much higher than those calculated for any other phospholipid. This suggests a uniquely high rate of turnover in bis(monoacylglycero)P of certain fatty acids, at least. If it is assumed that P turns over independently of the fatty acids, it could be argued that the high ratios in Table 6 for bis(monoacylglycero)P merely reflect slow turnover of P relative to that in other phospholipids. However, calculation of specific activities shown in Fig. 1, particularly for arachidonic acid, indicates that relatively slow turnover of P in bis(monoacylglycero)P cannot account fully for the high labeling ratios. The apparent high turnover of fatty acids in bis(monoacylglycero)P may reflect the strict localization of this phospholipid to lysosomes. Equally rapid turnover of fatty acids in the lysosomal compartment of other more widely distributed phospholipids may be obscured by slow turnover occurring in the nonlysosomal compartments. It is apparent from Table 2 that the high ratios in bis(monoacylglycero)P cannot be attributed to the fatty acid composition of various phospholipid classes. These observations, taken in conjunction with the strict localization of bis(monoacylglycero)P to secondary lysosomes, suggest an important physiological role for this phospholipid in recycling fatty acids in the cell that are derived from other complex lipids during disassembly of lipoproteins and membranes in the lysosome.

A particular role in fatty acid metabolism is also supported by the demonstration that lysosomes that contain large amounts of triglyceride and cholesteryl ester may also have a high content of bis(monoacylglycero)P (5). Some evidence for a trans-acylation reaction between phosphatidylinositol and bis(monoacylglycero)P has been reported recently (39). The apparent high rate and specificity of incorporation of fatty acid precursors into a minority lipid class is evidence against the suggestion that bis(monoacylglycero)P is formed in the lysosome through random recombination (15).

The metabolic relationships of acylphosphatidylglycerol to bis(monoacylglycero)P in eukaryotic cells are unknown (14). The fatty acid composition of acylphosphatidylglycerol is similar to that of bis(monoacylglycero)P except that it is much richer in palmitic acid. Because the levels of radioactivity detected in acylphosphatidylglycerol after incubation of macrophages with ^{32}P were close to background and accurate counting was not performed, the ratio of fatty acid to P radioactivity could not be calculated for Table 6. However, a comparison of the labeling of bis(monoacylglycero)P and acylphosphatidylglycerol indicates that the ratios would be high, particularly for palmitic and oleic acids. This suggests that acylphosphatidylglycerol might be involved in lysosomal metabolism of fatty acid classes different than those for bis(monoacylglycero)P.

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