Studies on the Role of Phospholipids in Phagocytosis*

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SUMMARY

The increased incorporation of $^{32}$P into phosphatidic acid and phosphatidylinositol in polymorphonuclear leukocytes undergoing phagocytosis is not secondary to an elevated specific activity of adenosine triphosphate during phagocytosis. The incorporation of labeled glycerol, oleic acid, and linolenic acid into the above phospholipids is not increased on stimulation of phagocytosis. Phosphatidic acid is synthesized directly from $\alpha$-glycerophosphate added to the incubation medium, but this synthesis is not increased on stimulation of phagocytosis. If it is assumed that the $\alpha$-glycerophosphate which enters the cell is accessible to all of the intracellular $\alpha$-glycerophosphate this result argues against the increased synthesis of phosphatidic acid being catalyzed by $\alpha$-glycerophosphate acylase. The formation of phosphatidic acid in homogenates of leukocytes from ATP and diglyceride or monoglyceride was, respectively, 20 and 12 times that from $\alpha$-glycerophosphate and palmitoyl coenzyme A plus oleyl coenzyme A. These results suggest that the increased synthesis of phosphatidic acid on induction of phagocytosis may be brought about by diglyceride kinase or possibly monoglyceride kinase plus lysophosphatidic acid acylase. The incorporation of labeled inositol into phosphatidylinositol is increased in cells undergoing phagocytosis, indicating that the increased incorporation of $^{32}$P into this phospholipid is not solely a result of a higher specific activity of phosphatidic acid, which may be the precursor for phosphatidylinositol in this system.

The electron microscopic observations of Goodman and Moore (1) illustrated rather clearly that the plasma membrane of leukocytes was involved in ingestion of particles. Sbarra and Karnovsky (2) and Karnovsky and Wallach (3) obtained evidence that lipids may participate in phagocytosis: acetate-$^{14}$C, uniformly labeled glucose-$^{14}$C, and orthophosphate-$^{32}$P were incorporated into phosphatidylinositol more rapidly than from $\alpha$-glycerophosphate added to the incubation medium. For enzymatic assays the cells, which had been washed in 0.9% NaCl, were homogenized for 2 min in 0.25 M sucrose to correspond to a 10% suspension of the original packed cells. For enzymatic assays the cells, which had been washed in 0.9% NaCl, were homogenized for 2 min in 0.25 M sucrose in an all glass homogenizer. Homogenates diluted with 0.25 M sucrose to correspond to a 10% suspension of the original packed cells.

Experimental Procedure

Leukocyte Preparations—Polymorphonuclear leukocytes were obtained by the method of Iyer, Islam, and Quastel (6). The packed cells were suspended in 4 volumes of Krebs-Henseleit bicarbonate medium (7) containing 5 mM calcium. Microscopic examination showed that these preparations contained more than 75% polymorphonuclear leukocytes. When $\alpha$-glycerophosphate-$^{32}$P incorporation or inositol-$^{2}$H incorporation was studied, leukocyte suspensions were prepared similarly except that Krebs phosphate medium (7) was used in place of bicarbonate medium. For enzymatic assays the cells, which had been washed in 0.9% NaCl, were homogenized for 2 min in 0.25 M sucrose in an all glass homogenizer. Homogenates diluted with 0.25 M sucrose to correspond to a 10% suspension of the original packed cells.

Incubations—Phagocytosis was initiated by the addition of starch (from Anamathus cruenthus), which was kindly provided by Dr. Bernard Hofreiter of the Norther Regional Laboratory, United States Department of Agriculture, Peoria, Illinois, or by inert polystyrene latex spherules (1305 μ diameter), kindly provided by the Dow Chemical Company. The polystyrene particles were dialyzed against distilled water.
Flasks containing the same amount of polystyrene particles were measured by following the fall in turbidity of the medium at the

collection. The supernatant fluids were pooled and made up to 50 ml with 0.9% sodium

kaonia by the method of Santiago-Calvo

developed on silicic acid-impregnated paper with phenol-ammonium acetate solution.
Separation of the polyphosphoinositides the chromatograms were

developed according to the method of Bartlett (13). Another aliquot of the sulfuric acid extract was treated by the method of Berenblum and Chain (14), and inorganic Pi was determined by counting aliquots of the 2-butanol phase. Protein was estimated by the method of Lowry et al. (15).

Measurement of Radioactivity—Radioactivity of the polyphosphoinositides was determined by measuring the specific activity of the terminal phosphates in ATP. The specific activity of the terminal phosphates in the trichloracetic acid extracts was determined according to the method of Crane and Lipmann (12) with the following modifications. Sulfuric acid, 1 N, was used in place of 1 N HCl to recover the labile phosphate from charcoal. Labile phosphorus in the sulfuric acid was measured by the method of Bartlett (13). Another aliquot of the sulfuric acid extract was treated by the method of Berenblum and Chain (14), and inorganic Pi was determined by counting aliquots of the 2-butanol phase. Protein was estimated by the method of Lowry et al. (15).
of ATP would be formed per mg, dry weight, per min. Since the peritoneal exudate cells contained 8.3 nmoles of ATP per mg, dry weight (see below), it would require about one-third of a minute for the terminal phosphate of ATP to reach isotopic equilibrium with the intracellular orthophosphate. It is probable that at 90 min the penultimate phosphate of ATP would be equilibrated isotopically with intracellular orthophosphate. Therefore, with the use of the specific activity of the ATP as a measure of the specific activity of the intracellular orthophosphate it would appear that the rate of isotopic equilibration of the intracellular orthophosphate with extracellular orthophosphate is slow, being about 10% in 90 min. It can be calculated from the data of Wu, Sessa, and Hamerman (23) that in chicken leukocytes incubated in Krebs-Henseleit bicarbonate medium containing physiological concentrations of orthophosphate about one-quarter to one-third of the intracellular orthophosphate exchanged with extracellular orthophosphate in 1 hour.

The time course in labeling of the individual phospholipids was also followed and in general agreed with the data of Kornovsky and Wallach (3). In addition the labeling of diposphoinositol and triphosphoinositol was followed and was shown to exhibit kinetic curves very similar to that of phosphatidic acid.

On the average the leukocyte preparations contained 0.83 µmole (range, 0.71 to 0.95) of ATP and 8.8 µmoles (range, 6.2 to 10.9) of phospholipid phosphorus per 100 mg of cells, dry weight. During the incubation period no significant changes in the amounts of ATP or total phospholipid phosphorus have been noted.

Effect of Phagocytosis on Phospholipid Synthesis and Specific Activity of ATP—Substances which enter cells either slowly or not at all in the absence of particles may enter at appreciable rates during particle uptake (5). If orthophosphate-32P and particles are offered to leukocytes at the same time, phosphate from the medium might gain more rapid entry into the phagocytizing cell, thus raising the specific activity of intracellular orthophosphate as compared to controls. Stimulation of 32P incorporation into various substances could thus be an indirect effect. Such a phenomenon should be readily detected by measuring the specific activity of the ATP.

As shown in Table I the specific activity of ATP from phagocytizing cells incubated in bicarbonate medium for 30 min agreed within 2% with the specific activity of ATP of control cells. On the other hand, the incorporation of 32P into phosphatidic acid and phosphatidylinositol was increased 71% and 128%, respectively. The stimulation in incorporation of 32P at 30 min in cells phagocytizing starch in three separate experiments averaged 57% (range, 32 to 77%) and 89% (range 75 to 128%) in phosphatidic acid and phosphatidylinositol, respectively. These results indicate that if orthophosphate from the incubation medium entered the cell more rapidly during phagocytosis it was not in contact with the metabolic machinery of the cell (it was possibly sequestered in vesicles) and that the increased incorporation of 32P into the phospholipids was not due to a higher specific activity of ATP.

The data in Table II show that, in bicarbonate medium and at times ranging from 2 to 12 min after adding starch to leukocytes which had previously been incubated with 32P for 30 min, the specific activities of ATP in phagocytizing cells agreed within 10% with those in control cells. A significant rise in the radioactivity in phosphoryl acid was observable between 6 and 12 min. Phosphatidylinositol did not show an appreciable increase in radioactivity over the time period measured. Similar results were obtained with polystyrene particles, except that the maximum phospholipid effect and particle uptake occurred at much shorter intervals, usually within 5 min. When the incubations were carried out in Krebs phosphate medium, increases in specific activity of ATP up to 20% have been noted (see Table IV).

Correlation between Particle Uptake and Stimulation of 32P Incorporation into Phosphatidic Acid—Leukocytes were incubated in one set of duplicate flasks for 30 min with 32P, and phagocytosis was then initiated by adding polystyrene particles. Samples were withdrawn at each of the time periods indicated. Another set of flasks was similarly treated, except that 32P was omitted; particle uptake was determined in this set. The results of this experiment are shown in Fig. 2. The polystyrene

![Graph](image-url)
particles were ingested rapidly during the first 5 min (about 260 μg per mg, dry weight); phagocytosis was rather slow during the next 5 min (about 70 μg of polystyrene was taken up per mg, dry weight). The time course for uptake of polystyrene particles was very similar to that previously reported (9). The stimulation of 32P incorporation into phosphatidic acid paralleled particle uptake; i.e. there was a burst of incorporation in the phagocytizing cells during the first 5 min, after which the incorporation was about the same as in the control cells. In another experiment the incorporation of 32P into phosphatidic acid in control cells was linear up to 30 min and showed a slope almost identical with that shown in Fig. 2. The kinetics of incorporation suggests that during active phagocytosis there is increased synthesis of phosphatidic acid, which ceases when particle uptake is complete. In separate experiments in which leukocytes were allowed to ingest particles for 30 min, and 32P was then added, there was no significant stimulation of 32P incorporation into phosphatidic acid in cells that had completed particle ingestion. The molecules newly synthesized as a result of stimulation of phagocytosis are presumably not lost, since there was no loss in radioactivity when phagocytosis ceased. This is in contrast to the situation with protein secretion in pancreas slices (24, 25). It should be noted that in Table II where starch was used to stimulate phagocytosis, there was a lag of 4 to 6 min after adding starch before a definite stimulation in 32P labeling in phosphatidic acid was observed. Karnovsky and Wallach (3) observed an initial lag in the stimulation of 32P incorporation in phosphatidic acid after initiation of phagocytosis with starch, and they noted a leveling off in stimulation after 30 min.

Incorporation of Glycerol-1-14C, Linolenic Acid-1-14C, and Oleic Acid-1-14C into Lipids of Leukocytes—Elsbach (26) reported that palmitic, stearic, oleic, and linoleic acids account for 85% of the total fatty acids of polymorphonuclear leukocytes. Acetate-14C incorporated into the fatty acids of neutral lipids and phospholipids was higher in phagocytizing cells; there was a slight inhibition of the incorporation of linoleic-1-14C into the total lipids during phagocytosis (27). Karnovsky (28) reported that glycerol-14C was incorporated only very poorly into the total lipids. In neither of these studies was the incorporation of 14C-labeled precursors into the individual phospholipids studied. We found that with oleic acid-1-14C as precursor, lecithin, phosphatidylethanolamine plus phosphatidylserine, phosphatidic acid, and phosphatidylinositol incorporated about 50, 20, 20, and 10% of the radioactivity, respectively. Linolenic acid-1-14C gave similar results. With glycerol-1-14C, lecithin accounted for about 75% of the total incorporated radioactivity, the other phosphatides incorporating about equal amounts of the remaining 25%. There was no increase in incorporation of radioactivity from any of the 14C-labeled precursors into any of the phosphatides when leukocytes were incubated with polystyrene particles.

Incorporation of Inositol-2-3H into Lipids of Polymorphonuclear Leukocytes during Phagocytosis—Table III shows that phagocytosis, induced either with starch or with polystyrene particles, was associated with an increased incorporation of inositol-2-3H into the total lipids. Studies with slices of pancreas (8), brain cortex (29), salt gland, and sympathetic ganglia (18) have shown that virtually all of the inositol-2-3H incorporated into the total lipid extract can be accounted for in phosphatidylinositol. There was a greater stimulation of inositol-2-3H incorporation associated with starch ingestion than with polystyrene ingestion. This parallels the effects of these two types of particles on 32P incorporation into phosphatidylinositol.
Phagocytizing cells. Phosphate medium rather than bicarbonate when orthophosphate-3P was used as precursor. With a-
incorporation into phosphatidic acid and phosphatidylinositol a-glycerophosphate-3P. There was the usual stimulation of 32p into phospholipids in nonphagocytizing and incorporation of 32p from orthophosphate-32P and a-glycerophosphate- 32P there is an incorporation of 32p into the
above phospholipids, but there is no stimulation by acetyl-
a-glycerophosphate- 32 P is not involved in the stimulation of 32p incorporation by

**TABLE III**

**Incorporation of inositol-2-3H into phosphatidylinositol during phagocytosis**

The cells were incubated for 40 min in Krebs phosphate me-
dium (in place of bicarbonate medium). Other additions were as described under "Experimental Procedure." Each flask con-
tained 0.81 μmole or 124 μC of inositol-2-3H.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Radioactivity in lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dpm/μg, dry wt</td>
</tr>
<tr>
<td>Without starch</td>
<td>8,680</td>
</tr>
<tr>
<td>With starch</td>
<td>15,550</td>
</tr>
<tr>
<td>Without polystyrene</td>
<td>9,950</td>
</tr>
<tr>
<td>With polystyrene</td>
<td>12,100</td>
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</tbody>
</table>

**TABLE IV**

**Incorporation of α-glycerophosphate-32P into lipids during phagocytosis induced by starch**

The cells were incubated for 30 min. Krebs phosphate me-
dium was used in place of bicarbonate medium. Other additions were as described under "Experimental Procedure." Counts are corrected to 106 cpm per μg of inorganic phosphorus or α-glycero-
phosphate phosphorus. The concentration of α-glycerophosphate-32P was 2.7 mM and it had a specific activity of 1.3 × 106 cpm per μmole.

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Specific activity in ATP</th>
<th>Radioactivity in phospholipids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm/μg, acid-labile P</td>
<td>cpm/μg, dry wt</td>
</tr>
<tr>
<td>Orthophosphate-32P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without starch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With starch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Glycerophosphate-32P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without starch</td>
<td></td>
<td></td>
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<tr>
<td>With starch</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*PA, phosphatidic acid; PI, phosphatidylinositol; PC, phosphatidylethanol.

or microsomal fractions are incubated with ATP-γ-32P or with orthophosphate-32P (the latter under conditions of oxidative phosphorylation) there is a stimulation by acetylene of 32P incorporation into phosphatidic acid and phosphatidylinositol (30, 31). On the other hand if the source of radioactive is α-glycerophosphate-32P there is an incorporation of 32P into the above phospholipids, but there is no stimulation by acetyl-
ol. Similar results were obtained with pancreas slices when the incorporations of orthophosphate-32P and α-glycerophosphate-32P were compared. These results suggest that the synthesis of phosphatidic acid by acylation of α-glycerophosphate (32) is not involved in the stimulation of 32P incorporation by acetylene in brain and in pancreas. Table IV shows the incorporation of 32P from orthophosphate-32P and α-glycerophosphate-32P into phospholipids in nonphagocytizing and phagocytizing cells. Phosphate medium rather than bicarbonate medium was used in these experiments in order to dilute any orthophosphate-32P which might be derived from hydrolysis of α-glycerophosphate-32P. There was the usual stimulation of 32P incorporation into phosphatidic acid and phosphatidylinositol when orthophosphate-32P was used as precursor. With α-glycerophosphate-32P as precursor there was about the same incorporation of 32P into phosphatidic acid, but there was no increase in incorporation on stimulation of phagocytosis. (It should be pointed out that the observed radioactivities in the phospholipids were corrected to a specific activity of 106 cpm per μg of P for the precursor.) These observations indicate that phosphatidic acid can be formed in leukocytes by acylation of α-glycerophosphate but they suggest that the increased incor-
poration of 32P from orthophosphate-32P in phagocytizing cells may not involve a pathway with α-glycerophosphate as an intermediate (Karnovsky (28) has already shown that the specific activity and pool size of α-glycerophosphate are not increased in phagocytizing cells).

Relative to the phosphatidic acid there was very much less incorporation of 32P into phosphatidylinositol when α-glycerophosphate-32P was used as precursor than when orthophosphate-
32P was used as precursor. This is of interest in view of the fact that phosphatidic acid can be a precursor for phosphatidyl-
inositol.

Under conditions in which the same amount of radioactivity was recovered in phosphatidic acid the specific activity of ATP with α-glycerophosphate-32P as precursor was only 5 to 10% of that when orthophosphate-32P was used as precursor. This observation, in conjunction with the fact that different patterns of labeling were obtained in the various phosphatides with the two precursors, indicates that α-glycerophosphate-32P was di-
rectly incorporated into phosphatidic acid. If all of the incor-
poration of α-glycerophosphate-32P were due to prior hydroly-
sis to orthophosphate-32P the labeling in phosphatide acid would have borne a constant ratio to the specific activity of the ATP, irrespective of the precursor, and the pattern of labeling in the three phosphatides would have been the same with either pre-
cursor.

**Diglyceride Kinase, Monoglyceride Kinase, and α-Glycerophosphate Acylase in Homogenates of Leukocytes—**Diglyceride kinase and monoglyceride kinase have been shown in a variety of mammalian tissues (19, 34, 35) and in *Escherichia coli* (36). The lysophosphatic acid which is a product of the monoglycer-
ide kinase reaction is rapidly acylated (37, 36). Diglyceride kinase appears to be involved in the increased turnover of phosphatidic acid in salt gland in connection with stimulated sodium transport (38). The results presented above suggest that the stimulation of 32P incorporation from orthophosphate-
32P into phosphatidic acid might involve either the diglyceride kinase or the monoglyceride kinase reaction. It was therefore decided to assay leukocyte homogenates for all three enzymatic mechanisms of phosphatidic acid synthesis.

Fig. 3 shows the rates of formation of phosphatidic acid from diolen and monoolein. When the lipid extracts were chroma-
tographed by the method of Marinetti et al. (10) it was found that the only lipid formed from diolen was phosphatidic acid. In the case of monoolein the radioactivity was found in lys-
ophosphatic acid and phosphatidic acid, the ratio of total radioactivities being 1:2, respectively. The initial rates of phosphorylation of diglyceride and monoolein were found to be 34.0 and 22.5 mpmoles per mg of protein per hour. Diglyceride kinase and monoglyceride kinase are therefore active in leukocyte homogenates. The enzyme which acylates lysophos-
phatic acid also appears to be active. Since no fatty acyl-CoA was added the acyl groups must have been provided from som endogenous source, or possibly from the added monoolein b

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C. M. Redman and L. E. Hokin, unpublished observations.
The formation of phosphatidic acid from α-glycerophosphate without prior breakdown to orthophosphate suggests that α-glycerophosphate acylase is also present. To determine the relative activities of the three reactions a leukocyte homogenate was assayed for the three enzymes under comparable conditions (Table V). The monoglyceride kinase and the diglyceride kinase showed similar activities. α-Glycerophosphate was acylated, and this acylation was stimulated about 7-fold if ATP, CoA, and fatty acids were added and about 50-fold if ATP and acyl-CoA were added. However, the maximum rate of formation of phosphatidic acid from α-glycerophosphate was only about 5% of that from diglyceride and 8.8% of that from monoglyceride.

There is evidence that monoglyceride kinase and diglyceride kinase are the same enzyme. However, the maximum rate of formation of phosphatidic acid from α-glycerophosphate has never been detected in intact cells incubated with orthophosphate-32P; we favor the view that diglyceride is the natural substrate.

**DISCUSSION**

A comment should be made about the stimulations in 32P incorporation reported here and those reported by Karnovsky and Wallach (3). With the same species, similar incubation times, and similar technique of initiation of phagocytosis in four separate experiments the latter investigators observed stimulations of 1230, 130, 260, and 190% in the phosphatidic acid fraction, 440, 220, 160, and 50% in the phosphatidylinositol fraction, 510, 220, 150, and 20% in the phosphatidylethanolamine fraction, and 110, 30, 270, and 120% in the phosphatidylethanolamine fraction. In experiments not shown here we found no significant stimulation in the phosphatidylethanolamine plus phosphatidylerine spot on stimulation of phagocytosis with either starch or polystyrene particles. We cannot account for the greater stimulations observed by Karnovsky and Wallach (3), but their data and ours suggest that the stimulation in their first experiment was not typical, and this single experiment contributed considerably to the high average stimulation which they report. Both groups of investigators are in agreement that stimulation of phagocytosis caused no significant increase in the incorporation of 32P into phosphatidylcholine.

During phagocytosis of particles, soluble substances in the incubation medium are taken up at an increased rate. The data of Wu et al. (23) and those presented here indicate that the entry of orthophosphate into the cell is slow. It is obvious that if any process were to accelerate the entry of extracellular orthophosphate before isotopic equilibrium of intracellular orthophosphate with the extracellular orthophosphate the specific activity of the intracellular orthophosphate and the ATP would be increased. The fact that when the cells were incubated in bicarbonate medium the specific activity of the ATP was not increased during stimulation of incorporation of 32P into phosphatidic acid and phosphatidylinositol argues against the phospholipid effects being secondary to an increased rate of entry of orthophosphate into the cell or to an increased specific activity of ATP due to other causes. This interpretation is fortified by the observation that the incorporation of inositol-2-3H into the lipids was also increased on stimulation of phagocytosis.

### Table V

**Rates of synthesis of phosphatidic acid by various pathways in leukocyte homogenates**

<table>
<thead>
<tr>
<th>Additions</th>
<th>Amount of phosphatidic acid synthesized (μmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 5.8</td>
</tr>
<tr>
<td>ATP</td>
<td>0.041</td>
</tr>
<tr>
<td>ATP + monoolein</td>
<td>0.202</td>
</tr>
<tr>
<td>ATP + monoolein + acyl-CoA</td>
<td>0.366</td>
</tr>
<tr>
<td>ATP + diolein</td>
<td>0.082</td>
</tr>
<tr>
<td>α-Glycerophosphate-32P</td>
<td>0.0053</td>
</tr>
<tr>
<td>α-Glycerophosphate-32P + ATP + CoA</td>
<td>0.0025</td>
</tr>
<tr>
<td>α-Glycerophosphate-32P + ATP + acyl-CoA</td>
<td>0.0050</td>
</tr>
</tbody>
</table>

\* P. R. Galsworthy and L. E. Hokin, unpublished observations.

**Fig. 3.** Formation of phosphatidic acid from diglyceride and monoglyceride in leukocyte homogenates. The incubation vessels contained 4.8 μmoles of MgCl₂, 4.8 μmoles of KCl, 3.2 μmoles of NaF, 0.088 μmole of ADP, 1.20 μmoles of carbamyl phosphate-32P, 0.4 unit of carbanate kinase, 90 μmoles of imidazole buffer (pH 6.5), 0.05 ml of 0.135 M diolein or monoolein as deoxycholate dispersions, or 0.05 ml of 0.3% deoxycholate solution, and 0.05 ml of leukocyte homogenate. The times of incubation were 5 min with the ATP system and 30 min with α-glycerophosphate-32P.
If it is assumed that there is a single pool of \( \alpha \)-glycerophosphate, the increased incorporation of \( ^{32}P \) into phosphatidic acid during phagocytosis is unlikely to be brought about by an increased acylation of glycerophosphate, since there was no increase in phosphatidic acid formation from \( \alpha \)-glycerophosphate on induction of phagocytosis. Since there was ample diglyceride kinase activity in the polymorphonuclear leucocytes this enzyme may be responsible for the stimulated incorporation of \( ^{32}P \) into phosphatidic acid. This could also account for the failure to observe stimulation of glycerol-1-\( ^{14}C \) incorporation into phosphatidic acid on induction of phagocytosis and the failure to observe increased incorporation of fatty acids, although here the problem is complicated by the very likely possibility of exchange of fatty acids on preformed phosphatidic acid which would obscure an increased synthesis of phosphatidic acid.

Paulus and Kennedy (39) have shown that phosphatidic acid is a precursor for phosphatidylinositol synthesis; the phosphate in the phosphatidic acid is retained. It could be argued that the increased incorporation of \( ^{32}P \) into phosphatidylinositol is secondary to that in phosphatidic acid and that phosphatidylinositol synthesis or turnover is not increased. The fact that inositol incorporation into phosphatidylinositol was also increased in phagocytizing cells makes this explanation unlikely.

When the secretion of substances ranging in size from sodium chloride to proteins is stimulated in a variety of eocine and endocrine glands there is a marked stimulation of incorporation of \( ^{32}P \) into certain phospholipids, notably phosphatidic acid and phosphatidylinositol (40). In some instances the incorporation of other precursors into these phosphatides is also stimulated. Karnovsky and Wallach (3) have suggested that the phospholipid effect in the pancreas may be related to the process of zymogen extrusion, which electron micrographs (41) suggest involves a fusion of the membrane of the zymogen granule with the plasmalemma, a parting of the membrane at the point of fusion, and a discharge of the zymogen granule contents into the acinar lumen. Recent studies in our laboratory argue against this explanation of the phospholipid effect in pancreas (24, 25, 42). If calcium is omitted from the incubation medium acetylcholine fails to elicit amylase extrusion from pancreas slices; yet the magnitude of the phospholipid effect in pancreas is many times greater than that in leukocytes (16, 24). Yet, judging from observations with the phase contrast microscope of leucocytes ingesting polystyrene particles and from cytological studies of the number of zymogen granules extruded from the pancreas on stimulation, the activity would appear to be greater in the leucocytes.

It is of interest that \( \alpha \)-glycerophosphate entered intact leucocytes. This was also found to occur in pancreas slices. This somewhat unusual behavior of a phosphate ester makes it rather useful for studying the mechanism of the phospholipid effects which, with the exception of brain cortex (30), require intact cells.

REFERENCES

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