Biosynthesis of Phosphatidic Acid, Lysophosphatidic Acid, Diglyceride, and Triglyceride by Fatty Acyltransferase Pathways in Escherichia coli*  

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SUMMARY

Fatty acyl coenzyme A-glycerol 3-phosphate, fatty acyl coenzyme A-lysophosphatidic acid, fatty acyl coenzyme A-mono- and diglyceride acyltransferase activities were found in a particulate fraction of broken cell preparations of Escherichia coli. The synthesis of phosphatidic acid and lysophosphatidic acid catalyzed by these enzymes was demonstrated to be dependent upon the presence of glycerol 3-phosphate, fatty acyl-CoA, and magnesium ion. Magnesium ion was partially replaceable by calcium ion. Isolated, radiochemically pure lysophosphatidic acid served as a substrate in the synthesis of phosphatidic acid in an enzymatic reaction with fatty acyl-CoA. This observation suggests that lysophosphatidic acid functions as an intermediary in the synthesis of phosphatidic acid from glycerol 3-phosphate and fatty acyl-CoA. Under similar conditions of incubation both monoglyceride and diglyceride functioned as acceptors of the fatty acyl moiety from fatty acyl-CoA.

Glycerol, however, did not serve as a substrate in the acylation reaction. Glycerol was incorporated into phospholipid only when adenosine triphosphate was added to an incubation system capable of acylating glycerol 3-phosphate.

Lauryl, myristyl, palmityl, stearyl, oleyl, linoleyl, linolenyl, cis-vaccenyl, and trans-vaccenyl coenzyme A derivatives were tested and found to be active substrates in vitro in the acylation of glycerol 3-phosphate. However, lauric, stearic, linoleic, linolenic, and trans-vaccenyl acylates have not been reported to exist as constituents of the phospholipids of E. coli. This finding suggests that the acyl transferase reaction does not possess the specificity required for excluding certain fatty acids which do not naturally occur in the phospholipids of E. coli.

E. coli B were grown, harvested, and disrupted under conditions previously described (4). The crude homogenate was centrifuged at 3,000 × g for 10 min in a Servall refrigerated centrifuge. The supernatant fluid was then centrifuged at 30,000 × g for 30 min. Both residues were resuspended in a solution containing 0.01 M dibasic sodium phosphate and 0.1% cysteine hydrochloride (final pH 7.0), and the centrifuging process repeated again. Except where indicated, only the washed 30,000 × g residue was saved and resuspended in cysteine-phosphate in a concentration of about 0.5 mg protein per 0.1 ml. The enzymatic activity diminished slightly over a 2-week period of storage at −20°C. In the experiment dealing with the fractionation of the enzyme, the washed 3,000 × g residue and the 30,000 × g residue each was suspended in a volume of cysteine-phosphate equal to the final volume of the

Phosphatidic acid (in the coenzyme form, cytidine diphosphate-diglyceride) has been shown thus far to serve as a precursor of at least two other bacterial phospholipids (1-3). These findings suggest that phosphatidic acid will occupy the key position of a common precursor in bacterial lipid metabolism as it does in animal metabolism. Because of the importance of phosphatidic acid to bacterial lipid metabolism, studies have been carried out in this laboratory to provide a better understanding of the pathways for the biosynthesis of this phospholipid in bacteria. Pieringer and Kunnes (4) have demonstrated that a heat-stable particulate enzyme preparation from Escherichia coli readily catalyzed the synthesis of phosphatidic acid from ATP and 1,2-diglyceride and the synthesis of lysophosphatidic acid from ATP and monoglyceride. The results to be presented in this paper demonstrate that lysophosphatidic acid and phosphatidic acid can also be formed in Escherichia coli by acyl transferase pathways. Particulate preparations from E. coli catalyze the transfer of the fatty acyl moiety of fatty acyl coenzyme A to glycerol 3-phosphate to form lysophosphatidic acid, which in turn reacts with another molecule of fatty acyl coenzyme A to form phosphatidic acid. Similar enzyme preparations also catalyzed the formation of diglyceride from monoglyceride and fatty acyl-CoA; and triglyceride from diglyceride and fatty acyl-CoA. A portion of this work has been presented in a preliminary communication (5). Recent short communications by Althaud and Vagelos (6) and Goldfine (7) contribute significant data to the study of the acylation of glycerol 3-phosphate in bacteria.

EXPERIMENTAL PROCEDURE

E. coli B were grown, harvested, and disrupted under conditions previously described (4). The crude homogenate was centrifuged at 3,000 × g for 10 min in a Servall refrigerated centrifuge. The supernatant fluid was then centrifuged at 30,000 × g for 30 min. Both residues were resuspended in a solution containing 0.01 M dibasic sodium phosphate and 0.1% cysteine hydrochloride (final pH 7.0), and the centrifuging process repeated again. Except where indicated, only the washed 30,000 × g residue was saved and resuspended in cysteine-phosphate in a concentration of about 0.5 mg protein per 0.1 ml. The enzymatic activity diminished slightly over a 2-week period of storage at −20°C. In the experiment dealing with the fractionation of the enzyme, the washed 3,000 × g residue and the 30,000 × g residue each was suspended in a volume of cysteine-phosphate equal to the final volume of the

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‡ Temple University School of Medicine Fellow.
30,000 × g supernatant fluid. A portion of the original crude homogenate was diluted to a volume equivalent to the volume of the supernatant fluid.

Radioactive glycerol 3-phosphate (labeled with either 32P or 14C) was prepared from glycerol (if radioactive, Positions 1 and 3 were labeled) and ATP (if radioactive, the γ-phosphorus atom was labeled) in the presence of purified glycerokinase of *Candida* *meyoderna* (8) purchased from Boehringer Mannheim Corporation, New York. The radioactive product was purified by ion exchange chromatography (9). Glycerol 3-phosphate of lower specific radioactivity was prepared by mixing pure glycerol 3-phosphate of high specific radioactivity with nonradioactive, racemic glycerol 3-phosphate and glycerol 1-phosphate. The presence of glycerol 1-phosphate did not appear to have a detrimental effect on the function of 14C-glycerol 3-phosphate as a substrate. 32P-ATP was synthesized enzymatically by the method of Penefsky *et al.* (10). Glycerol-1,3-14C was purchased from New England Nuclear.

Fatty acyl-CoA derivatives were prepared from acid chlorides according to the method of Seubert (11), except for the following modification. When unsaturated fatty acyl-CoA derivatives were synthesized, three layers were observed to form at the step in Seubert's procedure (11) in which the tetrahydrofuran is removed from the perchoric acid-treated reaction mixture. The upper layer was an oil (presumably nonesterified unsaturated fatty acid), the middle layer was an aqueous solution, and the lower layer was a semisolid precipitate, which contained unsaturated fatty acyl-CoA derivatives as a precipitate. The precipitate was separated from the oil and almost all of the aqueous solution by centrifuging and removing the upper two layers carefully with a Pasteur pipette. The precipitate was dried under vacuum, washed with several portions of diethyl ether, and extracted six times with 0.5 ml of water. To the combined water extract were added 1.8 ml of 5% perchloric acid. The precipitate was separated from almost all of the soluble material again by centrifuging and removing the supernatant solution with a pipette. The precipitate was washed with acetone, and with several portions of diethyl ether, and extracted six times with 0.5 ml of water. To the combined water extract were added 1.8 ml of 5% perchloric acid. The precipitate was separated from almost all of the soluble material again by centrifuging and removing the supernatant solution with a pipette. The precipitate was washed with acetone, and with several portions of diethyl ether. After drying under vacuum, the residue was immediately dissolved in a known volume of 0.01 M sodium phosphate buffer, pH 6.5. The acyl-CoA derivatives were analyzed by a Cary 15 recording spectrophotometer and were found to have the expected maximum absorption at 262 nm. Based on spectrophotometric and hydroxamate (12) determinations, the purity of the fatty acyl-CoA derivatives was 80 to 85%. The most active enzyme preparations of *E. coli* converted 94% of palmityl-CoA into phospholipid. Lauryl, myristyl, stearyl, oleyl, linoleyl, and linolenyl chlorides, and cis- and trans-vaccenic acids were purchased from the Hormel Foundation, Austin, Minnesota and were more than 99% pure. Palmityl chloride was checked by gas liquid chromatography of the methyl ester derivative. More than 90% of the material appeared in the peak that had the retention time of methyl palmitate. A fraction of a percentage of the material appeared in peaks having the retention times of methyl myristate and methyl stearate. cis- and trans-vaccenyl chlorides were synthesized from the respective acids and oxalyl chloride (13). Coenzyme A was a product of the Boehringer Mannheim Corporation, New York.

14C-1-Palmityl-CoA (21,300 cpm per pmole) was synthesized from 14C-1-palmitic acid (supplied by Nuclear-Chicago) by the enzymatic procedure of Kornberg and Pricer (14) as modified by Weiss, Kennedy, and Kiyasu (15). The presence of a small amount of a radiochemical impurity (about 1.5% of the total counts) was detected by silicic acid paper chromatography (16). The impurity had the chromatographic properties of phosphatidic acid, a by-product that one might expect to detect in this enzymatic method of preparation of palmityl-CoA of relatively high specific radioactivity. In experiments employing 14C-palmityl-CoA as a substrate, the impurity was accounted for through the use of zero time controls.

Radioactive lysophosphatidic acid, employed as a substrate in the experiments demonstrating the conversion of lysophosphatidic acid to phosphatidic acid, was prepared from 32P-ATP and α-monopalmitin by the monoglyceride phosphokinase reaction of heat-treated particulate preparations of *E. coli* (4). Paper chromatography was employed to check that heat-treated preparations of *E. coli* produced only radiochemically pure lysophosphatidic acid.

14C-Monopalmitin and 14C-dipalmitin were synthesized from palmityl chloride and glycerol-1,3-14C (molar ratio of 2:1, respectively) in the presence of dry pyridine. After standing 1 day at room temperature the mixture was diluted with chloroform-methanol (1:1, v/v). The chloroform layer, formed after the addition of water, was washed extensively with water. The heterogeneous mixture of radioactive neutral glycerides (obtained in 81% yield) in the chloroform phase were separated and characterized by thin layer chromatography (silica gel plates developed with *n*-hexane-ethyl ether-acetic acid, 80:20:1, v/v). The mixture of glycerides was found to be composed of 48.8% 14C-monopalmitin (Rf = 0.06), 44.7% 14C-tripalmitin (Rf = 0.28), and 6.8% 14C-nilvalpin (Rf = 0.62). Cochromatography in two other systems (*n*-hexane-ethyl ether-acetic acid, 90:10:3, v/v) and diisobutyl ketone-acetic acid, 40:25:5, v/v) was also carried out. No further separation of the isomers of monopalmitin and dipalmitin was attempted. Based on the work of others the monopalmitin was assumed to be composed of approximately 90% 1 and 3 isomers and 10% 2 isomer, and the dipalmitin was assumed to be about 30% 1, 2, and 3 isomers, and 70% 1, 3 isomer (17).

The methods of Hokin and Hokin (18) were employed to isolate, purify, and measure the radioactive lipids formed in the enzymatic reactions. In order to ensure complete precipitation of radioactive phospholipid product, approximately 5 mg of bovine serum albumin were added to each incubation mixture just prior to the addition of trichloracetic acid. Protein was determined by the method of Lowry *et al.* (19). The detergent Cutsan (diisobutylphenoxyethyleneoxyethanol) was purchased from Fisher Scientific Company, Philadelphia.

**RESULTS**

*Identification of Radioactive Products Formed from Glycerol 3-Phosphate and Palmityl-CoA as Lysophosphatidic Acid and Phosphatidic Acid—Incubation of glycerol 3-phosphate-32P, palmityl-CoA, and magnesium ion in the presence of an enzyme preparation of *E. coli* produced two radioactive phospholipids that were readily extracted into chloroform and were easily separated by chromatography of the chloroform extract on silicic acid-impregnated paper (16) as shown in Figure 1, Lane 3. When a mixture of the two phosphatides labeled with 32P was treated with dilute methanolic alkali according to the procedure.*

1 Based on data presented by B. Serdairevich and K. K. Carroll at the 40th Fall Meeting of the American Oil Chemists’ Society.
of Dawson (20), only one radioactive compound was produced, as shown by chromatography in three different paper systems (4). The radioactive compound produced from the mild alkaline hydrolysis of the two 32P-labeled lipids cochromatographed with glycerol 3(1)-phosphate in all three systems: $R_f = 0.3$ in System A, Whatman No. 1 developed with 1 M ammonium acetate (pH 7.5)-ethanol (30:70); $R_f = 0.22$ in System B, Whatman No. 3MM developed with 1 butanol acetic acid-water (5:3:1). The $R_f$ of the 32P-compound and $R_f$ of glycerol 3(1)-phosphate were 0.15, $R_f$ of glycerol 2-phosphate was 0.06 in System C, Whatman No. 1 developed with 3 N ammonium hydroxide-methyl Cellosolve-methyl ethyl ketone (3:7:2), all saturated with borax acid. Because the two phospholipids synthesized from glycerol 3-phosphate and palmityl-CoA produce only glycerol 3(1)-phosphate on mild alkaline hydrolysis, the two lipids must differ only in the number of fatty acid groups esterified to the glycerol 3(1)-phosphate “backbone” of the lipid. Identical results were obtained on alkaline hydrolysis of phosphatidic acid and lysophosphatidic acid synthesized by the diglyceride and monoglyceride phosphokinase pathways, respectively, of E. coli (4). The phosphatides produced in the acyltransferase system were further identified by cochromatography on silicic acid paper according to Marinetti, Erbland, and Kochen (16).

The dependence of incorporation of glycerol a-phosphate into the lower phosphatides produced from glycerol 3-phosphate and palmityl-CoA with the radioactivities of authentic phosphatidic acid and lysophosphatidic acid, respectively, of E. coli (4). 32P-labeled spots on the chromatogram were counted directly in a gas-flow Geiger counter. The radioactivities of the standard phospholipids produced from glycerol 3-phosphate and palmityl-CoA with the radioactivities of authentic phosphatidic acid and lysophosphatidic acid synthesized by the diglyceride and monoglyceride phosphokinase reactions of E. coli (4). Lane 1, phosphatidic acid synthesized by the diglyceride phosphokinase reaction, and the unknown phosphatides could be summed. These summation data confirm the conclusion deduced from the visual evaluation of the autoradiogram that the phosphatides produced in the acyltransferase reaction are phosphatidic acid and lysophosphatidic acid.

**Table I**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Lyosphatidic acid and phosphatidic acid formed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg mole/mg protein/hr</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
</tr>
<tr>
<td>Complete system</td>
<td>27.7</td>
</tr>
<tr>
<td>Palmitoyl-CoA omitted</td>
<td>0.18</td>
</tr>
<tr>
<td>Magnesium omitted</td>
<td>17.4</td>
</tr>
<tr>
<td>Zero time control (enzyme omitted)</td>
<td>0.09</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
</tr>
<tr>
<td>Complete system</td>
<td>2061</td>
</tr>
<tr>
<td>Glycerol 3-phosphate omitted</td>
<td>757</td>
</tr>
</tbody>
</table>

With the structurally known phosphatidic acid and lysophosphatidic acid synthesized by glyceride phosphokinase pathways of E. coli, as shown in Fig. 1. Autoradiography revealed that the radioactive phosphatide having the higher $R_f$ in Lane 3 chromatographed exactly with phosphatidic acid produced in the diglyceride phosphokinase reaction and the phosphatide having the lower $R_f$ in Lane 3 chromatographed exactly with lysophosphatidic acid produced in the monoglyceride phosphokinase reaction. The radioactive spots of the standard phospholipids (Lanes 1 and 2, Fig. 1) and the unknown phosphatides (Lane 8, Fig. 1) chromatographed either separately (Lanes 1, 2, and 3, Fig. 1) or together in certain mixtures (Lanes 4 and 6, Fig. 1) were cut out and quantitated in a gas-flow Geiger counter. The data of Fig. 1 demonstrate that the radioactivities of the standards and the unknown phosphatides could be summed. These summation data confirm the conclusion deduced from the visual evaluation of the autoradiogram that the phosphatides produced in the acyltransferase reaction are phosphatidic acid and lysophosphatidic acid.

**Conditions Required for Biosynthesis of Phospholipid**—The incorporation of glycerol 3-phosphate-14C into phosphatidic acid and lysophosphatidic acid is completely dependent upon the presence of enzyme and fatty acyl-CoA and is stimulated significantly by exogenous magnesium ion (Table I, Experiment 1). The dependence of incorporation of glycerol 3-phosphate into...
lipid upon fatty acyl-CoA implies that the latter compound is the other substrate of the reaction. This implication has been confirmed by the demonstration of a stimulation of incorporation of 14C-1-palmitoyl-CoA into phosphatidic and lysophosphatidic acids by glycerol 3-phosphate (Table I, Experiment 2).

The degree of dependence of the reaction on magnesium ion varied somewhat from enzyme preparation to preparation. This variation may be caused by differences in endogenous concentrations of magnesium. Magnesium ion, which is partially replaceable by calcium ion (approximately 50% as effective), is strikingly inhibitory above the optimum concentration of 0.015 M. Other divalent cations (Cd++, Mn++, Zn++, Ca++, Mg++, and Cu++) were tested and were found to have little or no activity as cofactors. The reaction is also extremely sensitive to the concentration of the fatty acyl-CoA substrate as is exemplified by the very sharp optimum concentration curve of palmitoyl-CoA (Fig. 2). The inhibition observed at higher concentrations of palmitoyl-CoA cannot be attributed to the presence of unesterified palmitic acid, because palmitic acid when added in concentrations between 0.040 and 0.91 mM to an incubation system containing optimum concentrations of palmitoyl-CoA had no inhibitory effect on the reaction. The acyltransferase specific activity was highest in particulate fractions of disrupted cells of E. coli. The 30,000 X g residue fraction (minus the 3,000 X g residue) employed in these studies had a specific activity of 43.2 (nanomoles per mg of protein per hour of glycerol 3-phosphate incorporated into phosphatidic acid and lysophosphatidic acid) compared with a value of 2.3 for the 30,000 X g supernatant fraction.

Use of Different Fatty Acyl-CoA Derivatives as Substrates—Substitution of other fatty acyl-CoA derivatives at the optimum concentration of each derivative for palmitoyl-CoA at its optimum concentration in general was found to result in a diminution of the amount of glycerol 3-phosphate incorporated into phosphatidic acid and lysophosphatidic acid (Table II). With the exception of oleyl-CoA, palmitoyl-CoA was consistently more active as a substrate in every experiment carried out than the other coenzyme A derivatives tested. Interestingly laurly, stearly, linoleyl, linolenyl, and trans-vaccenyl-CoA are active substrates in the transferase reactions even though these fatty acids have not been detected in the phospholipids of E. coli (21, 22). Experiments also were carried out in which mixtures of oleoyl-CoA and palmitoyl-CoA in varying concentration ratios were compared with the same CoA derivatives above over the same concentration range. These experiments failed to demonstrate any difference between the mixtures and the individual unsaturated and saturated fatty acyl-CoA derivatives with respect to the amount of synthesis or to the ratio of lysophosphatidic acid to phosphatidic acid formed. There was no demonstrable preference for either the saturated or unsaturated CoA derivative in these acyltransferase reactions.

**pH Optimun**—The enzyme was most active at pH between 7.5 and 8.5 with slightly higher activity at a final pH of about 8.

**Time Course of Incorporation of Glycerol 3-phosphate**—The formation of phosphatidic acid and lysophosphatidic acid from glycerol 3-phosphate proceeded at a linear rate for about 10 min and diminished rapidly until completion in 20 min. There was no loss of product from the incubation even though the reaction was carried out for longer periods of time.

**Affinity for Substrates**—When the concentration of glycerol 3-phosphate was varied and the palmitoyl-CoA concentration held constant at 8.4 x 10^-5 M, half of the maximal rate was attained at a glycerol 3-phosphate concentration of 1.8 x 10^-4 M. At a glycerol 3-phosphate concentration of 3 x 10^-4 M, the half maximal rate of biosynthesis occurred at a concentration of palmitoyl-CoA of approximately 2.5 x 10^-5 M.

**Synthesis of Phosphatidic Acid from Lysophosphatidic Acid and Palmitoyl-CoA**—The data thus far have shown that both phosphatidic acid and lysophosphatidic acid are produced from glycerol 3-phosphate and fatty acyl-CoA in an enzymatic reaction (Fig. 1, Lane 8). These results and the fact that the two phospholipids differ from one another by only one fatty acid ester moiety suggest that lysophosphatidic acid is an intermediary in the formation of phosphatidic acid. As an intermediary it should function as a substrate in a reaction with fatty acyl-CoA. When lysophosphatidic acid (1-palmitoyl glycerol 3-phosphate) labeled with 32P (prepared from a-monopalmitin and ATP-32P by the monoglyceride phosphokinase

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**Figure 2.** Concentration curve of palmitoyl-CoA. Incubation conditions were similar to those of Table II, except that the palmitoyl-CoA concentration was varied as indicated.

**Table II**

<table>
<thead>
<tr>
<th>Acyl-CoA</th>
<th>Optimum concentration</th>
<th>Glycerol 3-phosphate incorporated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauryl-CoA</td>
<td>0.066</td>
<td>10.2</td>
</tr>
<tr>
<td>Myristyl-CoA</td>
<td>0.046</td>
<td>28.4</td>
</tr>
<tr>
<td>Palmitoyl-CoA</td>
<td>0.038</td>
<td>43.2</td>
</tr>
<tr>
<td>Stearoyl-CoA</td>
<td>0.030</td>
<td>15.6</td>
</tr>
<tr>
<td>Oleoyl-CoA</td>
<td>0.126</td>
<td>43.6</td>
</tr>
<tr>
<td>Linoleyl-CoA</td>
<td>0.176</td>
<td>36.0</td>
</tr>
<tr>
<td>Linolenyl-CoA</td>
<td>0.290</td>
<td>6.7</td>
</tr>
<tr>
<td>cis-Vaccenyl-CoA</td>
<td>0.11</td>
<td>14.5</td>
</tr>
<tr>
<td>Trans-Vaccenyl-CoA</td>
<td>0.17</td>
<td>26.0</td>
</tr>
</tbody>
</table>
reaction of E. coli (4)) was allowed to react with palmityl-CoA in the presence of magnesium ion and a particulate enzyme from E. coli, radioactivity was found at an Rf on silicic acid-impregnated paper (16) that coincided with the Rf of phosphatidic acid. Mild alkaline hydrolysis of this compound yielded glycerol 3(1)-phosphate as identified by paper chromatography, indicating that the new radioactive phospholipid product is phosphatidic acid. Omission of palmityl CoA or magnesium ion reduced the amount of synthesis to lower levels (Table III). The amount of synthesis of phosphatidic acid from lysophosphatidic acid under the conditions of incubation employed is undoubtedly less than maximum, because only small amounts of radiochemically pure lysophosphatidic acid were available. These data demonstrate the presence of a fatty acyl-CoA-lysophosphatidic acid acyltransferase in particulate enzyme preparations of E. coli.

Incompetence of Glycerol as Substitute Substrate for Glycerol 3-Phosphate—Incubation of glycerol with palmityl-CoA in the presence of a whole homogenate enzyme preparation of E. coli under conditions that readily incorporate glycerol 3-phosphate into lysophosphatidic acid acyltransferase in particulate enzyme preparations of E. coli.

### Table III

Conversion of lysophosphatidic acid to phosphatidic acid

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Phosphatidic acid synthesised (µmol/mg protein/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1 Complete</td>
<td>3.06</td>
</tr>
<tr>
<td>Palmityl-CoA omitted</td>
<td>0.75</td>
</tr>
<tr>
<td>Experiment 2 Complete</td>
<td>2.27</td>
</tr>
<tr>
<td>Magnesium omitted</td>
<td>0.47</td>
</tr>
</tbody>
</table>

### Table IV

Incompetence of glycerol as a substrate

The incubation tubes contained the following reagents in various combinations as indicated: 0.16 mM 14C-glycerol (5,075 cpm per µmol); 0.071 mM palmitoyl-CoA; 7.4 mM ATP; 7.4 mM carrier glycerol 3(1)-phosphate; 2.25 mM 14C-glycerol 3-phosphate (112 cpm per µmol) with 2.2 mM glycerol 1-phosphate; 37 mM Tris-HCl buffer, pH 9.1; 37 mM magnesium chloride; and 0.37 mg of protein of a whole homogenate preparation of E. coli suspended in 0.05 ml of 0.1% cysteine hydrochloride-0.01 M sodium phosphate, pH 7.0, in a final volume of 0.27 ml. The incubation was carried out at 37° for 15 min.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Lipid synthesised (µmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14C-Glycerol</td>
<td>1.9</td>
</tr>
<tr>
<td>14C-Glycerol; palmitoyl-CoA</td>
<td>11.2</td>
</tr>
<tr>
<td>14C-Glycerol; ATP</td>
<td>5.5</td>
</tr>
<tr>
<td>14C-Glycerol; palmitoyl-CoA; ATP</td>
<td>455</td>
</tr>
<tr>
<td>14C-Glycerol; palmitoyl-CoA; ATP; glycerol 3(1)-phosphate</td>
<td>11.2</td>
</tr>
<tr>
<td>14C-Glycerol 3-phosphate; glycerol 1-phosphate</td>
<td>53</td>
</tr>
<tr>
<td>14C-Glycerol 3-phosphate; glycerol 1-phosphate; palmityl-CoA</td>
<td>16,500</td>
</tr>
</tbody>
</table>

### Table V

Acylation of monoglyceride and diglyceride with palmityl-CoA

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Diglyceride</th>
<th>Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1 (14C-monopalmitin) Complete system</td>
<td>1.35</td>
<td>0.02</td>
</tr>
<tr>
<td>Cutseum omitted</td>
<td>0.04</td>
<td>0.005</td>
</tr>
<tr>
<td>Magnesium omitted</td>
<td>0.87</td>
<td>1.2</td>
</tr>
<tr>
<td>Experiment 2 (14C-dipalmitin) Complete system</td>
<td>6.1</td>
<td>1.2</td>
</tr>
</tbody>
</table>

* None detected.

Significant quantities of glycerol are incorporated into lipid only when ATP in addition to palmityl-CoA is present. However, the amount of glycerol incorporated under these conditions is reduced to a very great extent if nonradioactive glycerol 3(1)-phosphate is added to the incubation system. These results suggest that, prior to becoming incorporated into lipid, glycerol must first be phosphorylated to glycerol 3-phosphate before participating as a substrate in the acyltransferase reaction. Allahand and Vagelos (6) also have stated that glycerol did not serve as a substrate in the acylation reaction.

**Acylation of Monopalmitin and Dipalmitin**—Incubation of 14C-monopalmitin (labeled in carbon atoms 1 and 3 of the glycerol moiety) with palmitoyl-CoA, Cutseum, magnesium ion, and a particulate preparation from E. coli resulted in the formation of two radioactive products, which were easily separated from each other and from 14C-monopalmitin by chromatography on thin layers of Silica Gel G developed with petroleum ether (Skelly B)-ethyl ether-acetic acid (80:20:1, v/v). The radioactive products cochromatographed with standard dipalmitin (Rf = 0.28) and tripalmitin (Rf = 0.62). The conversion of monopalmiton to dipalmiton and smaller amounts of tripalmiton was dependent on the presence of palmityl-CoA, the detergent Cutseum, and to some extent on magnesium ion (Experiment 1, Table V). Substitution of 14C-dipalmitin for 14C-monopalmitin in the reaction mixture resulted in the formation of 14C-triglyceride, which was identified as above by chromatography (Experiment 2, Table V). This reaction was also dependent on palmityl-CoA. Thus, the 30,000 × g particulate preparations of E. coli are capable of catalyzing the acylation of monoglyceride and diglyceride as well as glycerol 3-phosphate and lysophosphatidic acid.
Fatty Acyltransferase Pathways in E. coli

Previous studies in our laboratory demonstrated the synthesis of lysocephatidic acid and phosphatidic acid by glyceride phosphokinase pathways in E. coli (4). The work reported here and by Ailhaud and Vagelos (6) provides evidence for the synthesis of these two phospholipids also by fatty acyl-CoA-glycerol 3-phosphate acyltransferase and fatty acyl-CoA-lysocephatidic acid acyltransferase reactions in E. coli. From the combined results of our studies it is concluded that phosphatidic acid can be formed in E. coli from three different metabolites by the following reaction scheme.

\[
\text{Glycerol 3-phosphate + fatty acyl-CoA} \rightarrow \text{phosphatidic acid} \quad (1)
\]

\[
\text{Monoglyceride + ATP} \rightarrow \text{lysocephatidic acid} \quad (2)
\]

\[
1,2-\text{Diglyceride} + \text{ATP} \rightarrow \text{phosphatidic acid} \quad (3)
\]

The data demonstrating the inability of glycerol to be acylated directly to monoglyceride and diglyceride suggest that only the acyltransferase reaction participates in the biosynthesis of phosphatidic acid de novo. The function of the glyceride phosphokinase reactions in E. coli at this time is not clear. One can only speculate that the relatively high activity and the absolute specificity for the 1,2 isomer of diglycerides (23) should warrant the glyceride phosphokinase(s) a role of some significance; possibly one of scavenging for glycerides formed from degradative processes and converting them back to phospholipids. The need for glycerol to be in the phosphorylated state prior to its incorporation into lipids would appear to be as essential in E. coli and probably other bacteria as it is in animals (24, 25). However, once glycerol has become incorporated into lipid, the presence of the phosphate moiety is no longer required for acyltransferase activity, as is indicated by the ability of monoglyceride and diglyceride to function as substrates.

Although laurie, stearic, linoleic, linolenic, and trans-vaccenic acids were not detected either by Kaneshiro and Marr (21), or by Hildebrand and Law (22) as constituents of phospholipids of E. coli, all of these fatty acids in the form of derivatives of coenzyme A as well as four other fatty acid derivatives which have been found in E. coli serve as substrates in the fatty acyl-CoA-glycerol 3-phosphate acyltransferase reaction. The inability to detect linoleic and linolenic acids in E. coli by these workers is supported by the report of Scheuerbrandt and Bloch (26), who failed to find any polyunsaturated fatty acids even in trace amounts in E. coli. The participation of a fatty acid, which does not appear to exist in the phospholipids of E. coli, as a substrate in vivo in the fatty acyl-CoA-glycerol 3-phosphate acyltransferase reaction indicates that regulation of the entrance of fatty acids into the phosphatides of E. coli does not reside at the glycerol 3-phosphate acyltransferase site, but probably at some other enzymatic step(s) in the scheme of lipid metabolism of E. coli.

Ailhaud and Vagelos (6) have found that both palmitoyl-CoA and palmitoyl-acyl carrier protein function as substrates in the enzymatic synthesis of lipids from glycerol 3-phosphate in E. coli. This finding suggests that the glycerol 3-phosphate acyltransferase or acyltransferases of E. coli exhibit a relatively low specificity for the nonlipid moiety as well as the fatty acid portion of the fatty acid thioester substrate.

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Biosynthesis of Phosphatidic Acid, Lysophosphatidic Acid, Diglyceride, and Triglyceride by Fatty Acyltransferase Pathways in *Escherichia coli*

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