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Metabolism of the Glucosyl Diglycerides and Phosphatidylglucose of *Staphylococcus aureus*

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A glucose containing lipid, phosphatidylglucose (probably 3-*sn*-phosphatidyl-1'-glucose) and a lipid tentatively identified as phosphatidylethanolamine have been characterized in the lipids of *Staphylococcus aureus*. These lipids together comprise less than 2% of the total phospholipids of exponentially growing *S. aureus* and accumulate to 14% of the total phospholipid in stationary-phase cells. These lipids lost no ^{32}P when cells grown with $H_3{}^{32}PO_4$ were transferred to nonradioactive medium during the exponential growth phase. This was in marked contrast to the other phospholipids which lost ^{32}P rapidly. The loss of ^{32}P from phosphatidic acid and cardiolipin in exponentially growing cells was biphasic, suggesting heterogeneity of phospholipid phosphate metabolism. The mono- and diglucosyl diglycerides showed a rapid loss of ^{14}C-glucose during growth in nonradioactive medium but no loss of ^{14}C from the fatty acids of these lipids. The ^{14}C in the glucose and fatty acids of the glucosyl diglycerides was derived from glucose.

Glucosyl diglycerides are characteristic of the lipids of gram-positive bacteria (9). In this study, a second type of glucose-containing lipid, phosphatidylglucose (PGL), has been characterized from the lipids of *Staphylococcus aureus*. Previously, PGL has been reported only in *Mycoplasma* (15). PGL together with a second minor lipid, phosphatidylethanolamine (PE), were unusual in that there was apparently no metabolism of phosphate during exponential growth. This was in sharp contrast to the phospholipid phosphate of the other lipids and the glucose of the glucosyl diglycerides which were metabolized actively during exponential growth.

MATERIALS AND METHODS

Materials. Glucose-*UL*-^{14}C was supplied by New England Nuclear Corp., Boston, Mass. Phospholipase C from *Bacillus cereus* was supplied by General Biochemicals, Chagrin Falls, Ohio. Sources of other materials were previously reported (11, 20-25).

Growth of S. aureus. The strain, culture conditions, methods of preservation, preparation of inocula, and harvesting procedures were previously described (22). The growth medium contained 0.3% Trypticase (Pancreatic Digest of Casein, BBL), 0.08% (w/v) yeast extract (Difco), 0.2 mM K_2HPO_4, 15 mM tris (hydroxymethyl) aminomethane (Tris), 4 mM glycerol, 5 mM sodium acetate, and 0.5 mM sodium gluconate made to *p*H 7.4; purines, vitamins, and iron were added after autoclaving as previously described (22). The manipulations involved in the pulse-chase experiments were those of White and Tucker (24).

Extraction and deacylation of the lipid. The bacterial culture was poured onto an equal volume of ice and acidified to *p*H 2 with 6 N HCl. The cells were collected by centrifugation, and the lipids were extracted from the packed cells by the procedure of Bligh and Dyer (1). This extraction procedure was shown to remove 96% of the total fatty acids from the cells of *S. aureus* (22, 23). Deacylation of the diacyl phospholipids was accomplished by mild alkaline methanolysis in 1.5 hr at 0 C. The methanolysis mixture was neutralized with the weak cationic resin, Biorex 70 (BioRad Corp., Richmond, Calif.) (20). By using this procedure, the fatty acid methyl esters were recovered from the hydrolysis mixture after extraction with three portions of diethyl ether, and the glycerol phosphate esters were recovered from the aqueous phase.

The lipids and glycerol phosphate esters derived from them by mild alkaline methanolysis are the following: PE and glycerol phosphorylethanolamine (GPE), PGL and glycerol phosphoryl glucose (GPGL), phosphatidylglycerol (PG) and glycerol phosphoryl glycerol (GPG), lysylphosphatidylglycerol (LPG) and GPG, phosphatidic acid (PA) and glycerol phosphate (GP), and cardiolipin (CL) and diglycerol phosphoryl glycerol (GPGPG). Two glucosyl diglycerides were identified in this strain of *S. aureus* (22): diglucosyl diglyceride (DG) when deacylated yields glycerol diglucoside (GDG) and monoglucosyl

diglyceride (MG) which yields glycerol monoglucoside (GMG) after deacylation.

Separation of the lipids. The diacyl phospholipids were separated by two-dimensional chromatography on silica gel-impregnated paper (Whatman SG-81) by using solvent systems 1 and 3 described by Wurthier (26). The radioactive lipids were located by autoradiography and quantitatively eluted from the paper with a solvent of chloroform-methanol-water-concentrated ammonium hydroxide (300:300:15:0.2, v/v; references 11, 20, 25). The glycerol phosphate esters obtained after mild alkaline methanolysis were separated by two-dimensional chromatography on either acid-washed aminocellulose paper (Whatman AE-81; references 20, 25), or on cellulose thin-layer plates (Eastman 6064; reference 11). The esters were quantitatively recovered from aminocellulose paper by soaking in several portions of 2 N ammonium hydroxide (1.5 hr) and then by rinsing in water. The glycerol phosphate esters were also identified by their elution volumes from long Dowex-1 columns with an ammonium formate gradient containing sodium borate (11, 20). This chromatographic system is very reproducible, and the elution volumes of many phosphate esters were determined (R. L. Lester, *manuscript in preparation*).

Phospholipase C-hydrolysis. PGL was treated with *B. cereus* phospholipase C as described by Smith (14) except that 5 mM Tris-maleate buffer (*p*H 7.2) was used. The hydrolysis was terminated after 72 hr, the diethyl ether was removed in a stream of nitrogen, and 5 volumes of methanol and 2.5 volumes of chloroform were added. The one-phase mixture was shaken and allowed to stand for 2 hr. Sufficient chloroform and water were then added to complete the Bligh and Dyer extraction (1), and the chloroform and aqueous phases were separated.

Phospholipase D hydrolysis. Phospholipase D treatment was performed by the method of White and Tucker (24).

Analysis of the lipid. The deacylated product of PGL, GPGL, was hydrolyzed in 100 mM HCl for 5 min at 100 C, the HCl was removed in a stream of nitrogen, and the hydrolysis products were identified chromatographically. Glucose was determined with the anthrone reagent and glycerol by the release of formaldehyde after acid hydrolysis (3, 22). Glucose, galactose, ethanolamine, and serine release less than 1% of the formaldehyde obtained from glycerol when the assay is performed under these conditions (3). Fatty acyl esters were determined by the ferric hydroxymate method (3). Phosphate was determined colorimetrically after perchloric acid digestion (22).

The radioactivity of the lipid or lipid products was determined with a Packard scintillation spectrometer (20).

RESULTS

Identification of PGL. The phospholipid designated as PGL in Fig. 1 was recovered from several chromatograms and partitioned against chloroform-methanol-water in proportions used by Bligh and Dyer to remove any contaminating

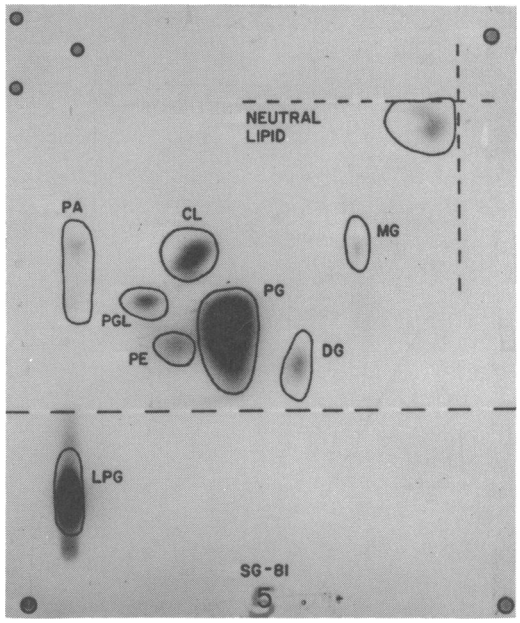

FIG. 1. *Autoradiogram of the lipids of S. aureus. The lipid was extracted from cells grown with glucose-UL-^{14}C and $H_3{}^{32}PO_4$ and chromatographed on silica gel-impregnated paper (Whatman SG-81). Ascending chromatography was performed with a solvent of chloroform - methanol - diisobutylketone - acetic acid - water (23:10:45:25:4, v/v) to a line 14 cm above the origin. The paper was then cut 3.5 cm above the origin as indicated by the dotted lines, and the upper portion was rotated 90° and chromatographed in a solvent of chloroform - methanol - diisobutylketone - pyridine - 0.5 M ammonium chloride buffer, pH 10.4 (30:17.5:25:35:6, v/v). The chromatogram was then dried in air; the lower portion was attached and placed on Kodak no-screen X-ray film.*

paper or silica gel. On mild alkaline methanolysis, this lipid was quantitatively deacylated in 1.5 hr at 0 C. Two-dimensional chromatography of the ^{32}P-labeled glycerol phosphate ester revealed distinctive mobilities for this compound on cellulose thin-layer plates (Fig. 2) and on aminocellulose paper. The chromatographic mobility on aminocellulose paper for the unknown ester relative to the mobilities of the glycerol phosphate esters derived from known phospholipids is illustrated in Fig. 3.

The unknown phospholipid was found to react with the anthrone reagent, suggesting the presence of a carbohydrate moiety in the lipid. After growth of *S. aureus* with glucose-*UL*-^{14}C, the lipid was collected and deacylated; the resulting glycerol phosphate ester was hydrolyzed in 3 N HCl for 50 min at 100 C. A single carbohydrate moiety was recovered which had the

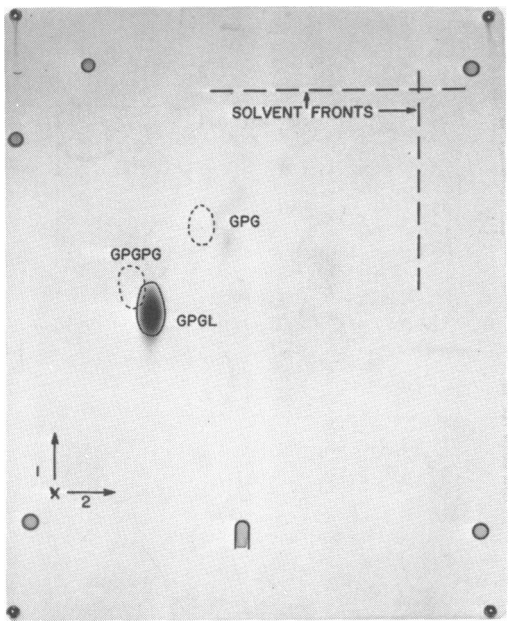

FIG. 2. *Autoradiogram of GPGL on cellulose thin-layer plates. Eastman cellulose chromograms 6064 were spotted with G^{32}PGL, authentic GPGPG and GPG and chromatographed with 3.8 mM ethylenediaminetetraacetic acid and 0.7 M ammonium bicarbonate in 90 mM ammonium hydroxide containing 67% (v/v) ethyl alcohol in the first dimension and isobutyric acid-water-concentrated ammonium hydroxide (66:33:1, v/v) in the second dimension. The authentic lipids were detected by a molybdate spray (2).*

chromatographic mobility of glucose in two-solvent systems (Table 1). When the deacylated lipid containing 0.178 μmole of phosphate was reacted with the anthrone reagent (using glucose as standard), this ester was found to contain 0.189 μmole of glucose for a glucose to phosphate molar ratio of 1.06 to 1.00. The fatty acyl ester to phosphate molar ratio for the diacyl phospholipid was found to be 1.99 to 1.00 (1.866 μmoles of fatty acyl ester to 0.938 μmole of phosphate). A second portion of the diacyl lipid was hydrolyzed in 2 N HCl in sealed glass vials, and the formaldehyde released from the glycerol was measured under conditions in which glucose is unreactive (3). The glycerol (using glycerol phosphate as standard) to phosphate molar ratio obtained was 1.14 to 1.00 (1.08 μmoles of glycerol to 0.938 μmole of phosphate). When authentic phosphatidylglycerol was used as a control for these analytical methods, the molar ratios for fatty acyl ester-glycerol-phosphate were 2:2:1.

When G^{32}PGL was hydrolyzed in 0.1 N HCl for 5 min at 100 C, approximately 4% of the G^{32}PGL was lost, and 10% of the ^{32}P originally present in the G^{32}PGL was recovered in G^{32}P. This G^{32}P co-chromatographed with authentic nonradioactive GP on aminocellulose paper in two dimensions (as in Fig. 3). The G^{32}P was detected by autoradiography and the standard GP by periodate and *o*-tolidine (22).

Neither the PGL nor the GPGL react with aniline-phthalate (8) or silver nitrate (17). The lack of reactivity with aniline-phthalate indicates that no free reducing group was present in the lipid or its deacylated product.

Phospholipase C hydrolysis. The unknown lipid which appears to be phosphatidylglucose (PGL) was isolated from *S. aureus* grown with glucose-*UL*-^{14}C and $H_3{}^{32}PO_4$ and recovered from silica gel-impregnated paper after chromatography as in Fig. 1. Traces of silica gel and paper were removed after partitioning the lipid in a system containing chloroform-methanol-water (1, 1, 0.9, v/v). A total of 0.497 μmole of PGL was hydrolyzed with phospholipase C from *B. cereus*, and 17.2% of the ^{32}P was recovered in the aqueous portion after extraction of the hydrolysis mixture. The aqueous portion obtained after hydrolysis was mixed with authentic glucose-1-phosphate (G-1-P) and glucose-6-phosphate (G-6-P) and applied to a Dowex-1 column. A total of 96% of both the ^{14}C and ^{32}P

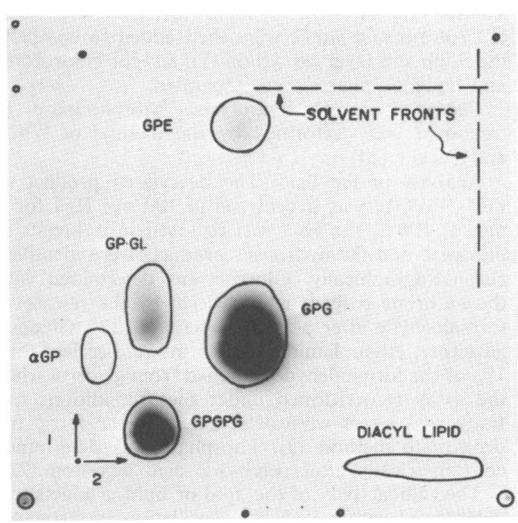

FIG. 3. *Autoradiogram of the glycerol phosphate esters of the phospholipids from S. aureus grown with $H_3{}^{32}PO_4$. Aminocellulose paper (Whatman AE-81) was chromatographed in 3 M formic acid containing 0.4% (v/v) pyridine in the first dimension and 1.15 M ammonium acetate containing 11.8 mM ethylenediaminetetraacetic acid made to pH 5.0 with acetic acid and diluted 3:7 (v/v) with 95% ethanolic 0.26 M ammonium hydroxide in the second dimension.*

in the water-soluble product of phospholipase C hydrolysis was eluted with authentic G-1-P (Fig. 4). This chromatographic system clearly indicates that the phospholipase C hydrolysis product was not G-6-P.

The organic phase of the hydrolysis mixture contained two components. One had the chromatographic mobility of the starting material PGL. The other lipid contained no ^{32}P and had the chromatographic mobility of diglyceride in three-solvent systems (Table 2).

Phospholipase D hydrolysis. A portion of the lipid from cells grown with $H_3{}^{32}PO_4$ was treated with plant phospholipase D. After 24 hr, 90% of the ^{32}P from the control ^{32}PG was found in the phosphatidic acid (identified by its migration on silica gel-impregnated paper as in Fig. 1). However, only 3.5% of the ^{32}P from ^{32}PGL was recovered in phosphatidic acid.

Identification of PE. A lipid which co-chromatographed in two dimensions with authentic PE in the silica gel-impregnated paper chromatographic system (Fig. 1) has been detected in *S. aureus* U-71. After mild alkaline methanolysis, the glycerol phosphate ester derived from this lipid co-chromatographed with authentic GPE in a two-dimensional cellulose thin-layer system like that illustrated in Fig. 2 and in the amino cellulose paper system illustrated in Fig. 5. This ester was also eluted with authentic GPE from a Dowex-1 column by using 20 mM ammonium formate, *p*H 9.0 (see reference 11,

TABLE 1. *Identification of the sugar of glycerol phosphoryl glucose from the lipid of Staphylococcus aureus*[a]

Component	System 1[b] (mm from origin)	System 2[c] (R_F value)
Galactose	170	0.41
Mannose	210	0.55
Glycerol	300	0.76
Inositol	80	
GPGL	26	0.25
Glucose	181	0.46
Unknown	181	0.46

[a] *S. aureus* was grown with glucose-*UL*-^{14}C, the lipids were extracted and deacylated by mild alkaline methanolysis, the glycerol phosphate esters were separated chromatographically as in Fig. 3, and the GPGL was recovered and hydrolyzed in 3 N HCl at 100 C for 50 min. The HCl was removed in a stream of nitrogen and the sugar extracted three times with pyridine. After chromatography the ^{14}C was located by autoradiography or by cutting the chromatogram in 5-mm strips which were assayed in the scintillation spectrometer. The standards were located with silver nitrate or periodate *o*-tolidine stain (17, 22).

[b] Descending chromatography with a solvent of isopropanol–water (4:1, v/v) on Whatman no. 4 paper (12).

[c] Ascending chromatography on Eastman cellulose thin-layer plates (no. 6064) with a solvent of *n*-butanol–pyridine–water (6:4:3, v/v; reference 16).

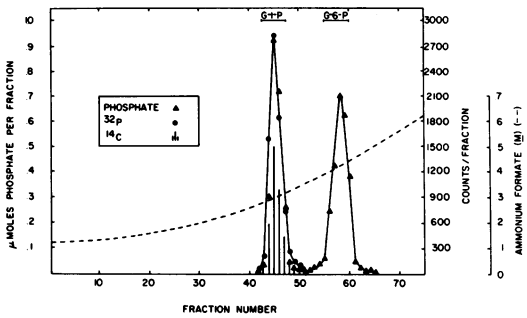

FIG. 4. *Chromatography of the water-soluble products of phospholipase C hydrolysis of PGL. Columns (6 mm in diameter and 81 cm long) of Dowex-1-8X, 200 to 400 mesh in the formate form were eluted with the ammonium formate gradient illustrated. The elution mixture contained 0.20 M sodium borate and was pH 9.50. The elution volume of authentic glucose-1-phosphate (G-1-P) and glucose-6-phosphate (G-6-P) are indicated at the top of the figure.*

TABLE 2. *Identification of diglyceride from phospholipase C hydrolysis of phosphatidylglucose from Staphylococcus aureus*[a]

Component	System 1[b]	System 2[c]	System 3[d]
Tripalmitin	0.53	0.94	0.67
Monopalmitin	0.00	0.14	0.15
Phospholipid	0.00	0.00	0.00
Dipalmitin	0.09	0.67	0.37
Unknown	0.09	0.67	0.37

[a] PGL was hydrolyzed with phospholipase C as in Fig. 4, and the organic phase was recovered and chromatographed. Standards were detected with rhodamine-6-G (22), the phospholipids by autoradiography (^{32}P), and the unknown by autoradiography (^{14}C). Numbers represent R_F values. Phospholipid "standard" represents a total phospholipid sample from *S. aureus*.

[b] Ascending chromatography on silica gel G thin-layer plates with hexane–diethylether–acetic acid (80:20:1, v/v).

[c] Ascending chromatography on silica gel G thin-layer plates with hexane–diethylether–acetic acid (30:20:1, v/v).

[d] Ascending chromatography on silica gel-impregnated paper (Whatman SG-81) with hexane–diisobutylketone–acetic acid (85:15:1, v/v).

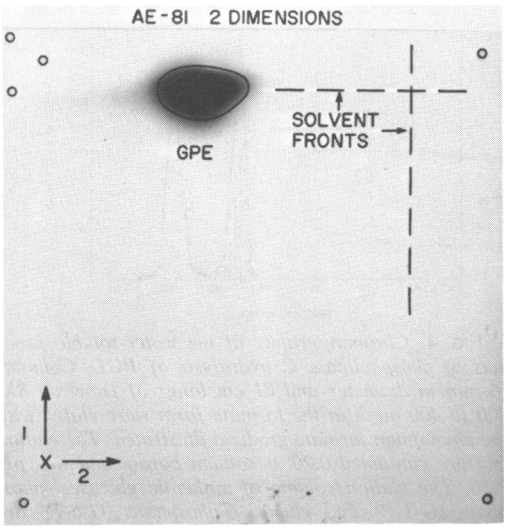

FIG. 5. *Autoradiogram of $G^{32}PE$ isolated from S. aureus. Chromatography of $G^{32}PE$ from the PE of S. aureus combined with authentic PE on aminocellulose paper as in Fig. 3. Authentic PE was located with the periodate, o-tolidine dip (22).*

Fig. 7). The *S. aureus* lipid is thus presumed to be PE. Identification of ^{14}C-labeled ethanolamine after hydrolysis would be necessary to confirm the identification of PE. Labeled ethanolamine from the PE has not been recovered from *S. aureus* grown with ^{14}C-labeled serine, glucose, glycerol, acetate, or amino acid mixtures in the complex medium needed for growth.

Incorporation of glucose into the glucosyl diglycerides. Both monoglucosyl and diglucosyl diglycerides have been characterized from this strain of *S. aureus* (5, 22). The bacteria were grown with glucose-*UL*-^{14}C for several generations, and the lipids were isolated. These glucosyl diglycerides were separated chromatographically (Fig. 1) and recovered from several papers as previously described (11). The glucolipids were then deacylated by mild alkaline methanolysis and the position of the ^{14}C in the glycerol glucosides determined. The GDG derived from DG and the GMG derived from MG were hydrolyzed in 3 N HCl at 100 C for 60 min after which the HCl was removed in a stream of nitrogen. In reconstruction experiments, 16% of added glycerol-*1,3*-^{14}C was lost during the hydrolysis and removal of HCl by our evaporation procedure. The hydrolysis products were separated by ascending chromatography on cellulose thin-layer plates with a solvent of *n*-butanol-pyridine-water (6:4:3, v/v), and the lipids were located by autoradiography with standards of labeled glycerol (R_F 0.76) and glucose (R_F 0.46). All of the ^{14}C from GDG and GMG was found at the R_F value of glucose, and none was detectable with that of glycerol. A total of 91% of the ^{14}C in GDG and 90% of the ^{14}C in GMG present before hydrolysis was recovered at the R_F of glucose after hydrolysis and chromatography, indicating destruction of about 10% of the glucose during acid hydrolysis.

Metabolism of the phospholipids of S. aureus. *S. aureus* was grown with glucose-*UL*-^{14}C and $H_3{}^{32}PO_4$ for 1 hr, harvested by centrifugation, and resuspended into medium containing no radioactivity. During the exponential growth that followed, samples were withdrawn and the lipids were isolated. The growth of the bacteria under these conditions is illustrated in the upper portion of Fig. 6. During the exponential growth period, the content of phospholipid per cell remained constant as the cells grew with a doubling time of 33 min. In both GDG and GMG derived from DG and MG, the glucose lost 50% of its ^{14}C in about one doubling although the fatty acids from these lipids did not lose ^{14}C throughout 2.2 doublings (Fig. 6, A and B). During the chase period, PGL, measured as GPGL, accumulated ^{32}P, whereas the major phospholipids were actively losing this label (Fig. 6, C). Under the growth conditions of this experiment, no ^{14}C was incorporated into the glycerol phosphate backbone of the phospholipids from the glucose-*UL*-^{14}C label. The GP derived from PA showed a biphasic loss of ^{32}P (Fig. 6, D). In the rapid phase, half of the ^{32}P label was lost in 1.0 bacterial doubling; and in the second phase, half of the ^{32}P was lost in 1.6 bacterial doublings. PG, measured as GPG, and LPG, also measured as GPG, had turnover rates of 1.0 and 4.0 doubling times, respectively (Fig. 6, E). The lipid tentatively identified as PE, measured as GPE, accumulated ^{32}P during exponential growth in the chase period (Fig. 6, F). The turnover of CL, measured as GPGPG, was also found to be biphasic (Fig. 6, F). In the first or rapid phase, half of the ^{32}P was lost in 1.0 bacterial doubling, whereas half of the ^{32}P was lost during the second phase in 2.0 doublings.

DISCUSSION

Glucose containing phospholipids are rare in nature. Phosphatidylglucose has been reported to be a major lipid in *Mycoplasma laidlawii* strain *B* (15). A glucose containing phospholipid, probably a phosphatidylglycerol diglucoside has been reported in *Pseudomonas diminuta* (19) and in streptococci (4, 6). In this study, PGL has been identified in the lipids of *S. aureus* U-71. The lipid contains 2 moles of fatty acid, 1 mole of glucose, and 1 mole of glycerol per mole

of phosphate. Phospholipase C liberates diglyceride and a phosphorylated sugar with the chromatographic mobility of glucose-1-phosphate. Partial acid hydrolysis of GPGL liberates GP, indicating that the structure of the lipid may very likely be 3-*sn*-phosphatidyl-1′-glucose. PGL and GPGL from *S. aureus* do not contain a free reducing group, again suggesting the structure is 3-*sn*-phosphatidyl-1′-glucose. The PGL of *M. laidlawii* has been reported to be 3-*sn*-phosphatidyl-6′-glucose (15).

The phosphate of PGL has an unusual metabolism in *S. aureus* in that during exponential growth there was no loss of ^{32}P in a pulse-chase experiment (Fig. 6). Instead ^{32}P accumulated in the lipid. ^{14}C in the glycerol portion of the lipid also accumulated during exponential growth (Short and White, *unpublished data*). In exponentially growing cells, the PGL represented about 1% of the lipid phosphate. However, PGL accumulated during the growth cycle so that in the late-stationary-phase PGL accounted for 10% of the phospholipid (Short and White, *unpublished data*). A second trace lipid tentatively identified as PE was also found to increase in amount during the growth cycle (from 0.6% in exponential phase to 4% in stationary phase) and to accumulate ^{32}P during pulse-chase experiments (Fig. 6).

The metabolism of PGL in *S. aureus* contrasts sharply with its metabolism in one study of *M. laidlawii* in which both ^{14}C- and ^{32}P-glucose were lost at a rapid rate that correlated with the rate of glucose uptake for the cell (13). In these experiments, exponentially growing cells were incubated in Tris-buffer with the isotopes for a short time and then returned to nonradioactive growth medium. In the other major lipid in *M. laidlawii*, PG, neither ^{14}C nor ^{32}P showed any evidence of turnover during growth in nonradioactive medium (10, 13). This again contrasts with *S. aureus* in which the PG lost half its ^{32}P in one bacterial doubling time (Fig. 6). In a second study with *M. laidlawii*, cells were grown with labeled fatty acids, glucose, or $H_3{}^{32}PO_4$ during exponential growth, washed, and resuspended into nonradioactive medium. In this study, there was no loss of radioactivity from any of the lipids during growth in the nonradioactive medium (7). However there was a period of about 60 min between the exponential growth with the isotopes and the resuspension into nonradioactive medium that involved the washing and centrifugation of the cells. This long period could have obscured a short period of rapid turnover (*see below*).

In both studies of *M. laidlawii*, there was no loss of radioactivity from the glucosyl diglycerides (7, 13). This again contrasts sharply with the metabolism of the glucose portion of the glucosyl diglycerides of *S. aureus*. These lipids lost half the ^{14}C-glucose in about one bacterial doubling (Fig. 6). There was no loss of ^{14}C from the fatty acids

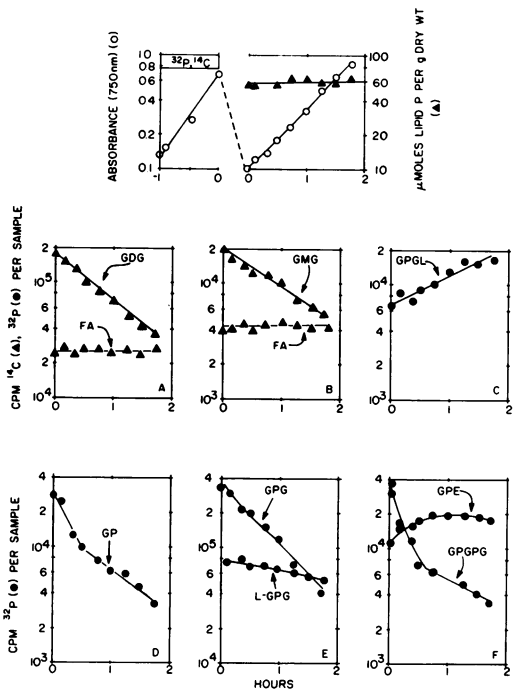

FIG. 6. *Metabolism of the lipids of S. aureus. S. aureus was grown with 500 μCi of glucose-UL-^{14}C and 300 μCi of $H_3{}^{32}PO_4$ in 500 ml for 1 hr and then centrifuged, resuspended in nonradioactive medium, and added to 3.5 liters of nonradioactive medium at 37 C as illustrated in the top portion of the figure. The culture was aerated with 6 liters of air per min. Samples of 500 ml (200 ml for the later points) were withdrawn, added to an equal volume of ice, brought to pH 2.0 with 6 N HCl, and centrifuged. The lipids were extracted from the cells, a portion was deacylated by mild alkaline methanolysis, and the glycerol phosphate esters were separated as in Fig. 3 after removal of the LPG by chromatography in the first dimension as in Fig. 1. The ^{32}P was determined in the GPGL derived from PGL (C), GP derived from PA (D), GPG derived from PG (E), GPE derived from PE (F), and GPGPG derived from CL (F). A second portion of the lipid was separated in the system illustrated in Fig. 1, and the glucosyl diglycerides and LPG were recovered from the paper. MG and DG were then deacylated to GMG and GDG, and the ^{14}C in the glucose of the glycerol glycosides and in the fatty acid methyl esters (FA) was determined (A and B). The LPG was deacylated, and the ^{32}P in the GPG was determined (E, labeled as L-GPG). In the top graph, cell growth was measured as the absorbance at 750 nm in 13-mm round test tubes (22). Absorbance values between 0.05 and 0.65 correspond linearly to dry weights between 0.017 and 0.19 mg (dry weight) per ml.*

of these molecules in 2.2 bacterial doublings (Fig. 6). Different rates of turnover in different portions of the same phospholipid molecule have been documented in the phospholipids of *Haemophilus parainfluenzae* (24), suggesting that hydrolysis followed by resynthesis of the lipid in situ could be an important part of membrane metabolism.

A biphasic loss of ^{32}P from the GP and GPGPG was detected in exponentially growing *S. aureus* (Fig. 6). The rapid initial phase was seen more clearly when the time between the exponential growth with the isotopes and the growth in nonradioactive medium was very short. In the experiment illustrated in Fig. 6, the period between the pulse and the chase was 17 min (0.38 bacterial doubling). The biphasic nature of the phospholipid phosphate metabolism was even more clearly shown when the culture growing in $H_3{}^{32}PO_4$ was filtered and immediately resuspended in nonradioactive medium (Short and White, *unpublished data*). This biphasic loss of radioactivity from CL and PA suggested that at least some portion of these lipids exist in positions in the membrane different from the bulk of the lipid. This view is consistent with the fact that heterogeneity exists in the membrane phospholipid distribution in *H. parainfluenzae* (18).

ACKNOWLEDGMENTS

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