JOURNAL OF BACTERIOLOGY, Oct. 1971, p. 219-226
Vol. 108, No. 1

Printed in U.S.A.

Metabolism of Phosphatidylglycerol, Lysylphosphatidylglycerol, and Cardiolipin of *Staphylococcus aureus*

STEVEN A. SHORT AND DAVID C. WHITE

Biochemistry Department, University of Kentucky Medical Center, Lexington, Kentucky 40506

Received for publication 28 May 1971

Staphylococcus aureus accumulated cardiolipin (CL) and lost phosphatidylglycerol (PG) during the stationary phase of growth. The minor lipids, phosphatidylethanolamine and phosphatidylglucose, also accumulated, whereas the lysylphosphatidylglycerol (LPG) content of the membrane remained constant as stationary phase continued. During exponential growth, the proportions and total content of phospholipids per cell remained constant. The metabolism of the phospholipids was examined under these conditions. In pulse-chase experiments, the phospholipids lost ^{14}C from the glycerols slower than ^{32}P. When the phospholipids were labeled with ^{14}C glycerol, the unacylated glycerols of PG and LPG lost ^{14}C, whereas the diacylated glycerols either accumulated or did not lose ^{14}C. In all experiments, the PG showed a more rapid metabolism than the LPG. When staphylococcal CL was hydrolyzed by *Haemophilus parainfluenzae* CL-specific phospholipase D into phosphatidic acid (PA) and PG, the incorporation of ^{32}P into both of the phosphates of CL was found to be parallel at both the PG and PA ends of the molecule. However, the specific activity of the ^{32}P at the PA end was twice that at the PG end of the molecule. The PG end of the CL apparently came from a portion of the cellular PG pool with about 20% the specific activity of the total cellular PG. The turnover of two of the glycerols of the PG portion of CL was like that of the cellular PG. The diacylated glycerol of the PG and of CL and of the membrane PG showed neither turnover nor incorporation of ^{14}C. Half of the radioactivity was lost from the middle glycerol of CL and the free glycerol of the cellular PG in one bacterial doubling. The diacylated glycerol from the other end of the CL molecule (the PA end) lost radioactivity almost as rapidly as the middle glycerol for 10 min. After the initial rapid loss, the turnover slowed to a rate 10 times slower than the middle glycerol, indicating that the ^{14}C was actually accumulating at this end of the molecule. The phosphates and glycerols involved in the hydrolysis and resynthesis of the CL molecule during exponential growth in *S. aureus* apparently come from different pools of PG.

The phospholipids of strain U-71 of *Staphylococcus aureus* have been identified as phosphatidylglycerol (PG), lysylphosphatidylglycerol (LPG), phosphatidic acid (PA), cardiolipin (CL), with traces of phosphatidylethanolamine (PE) and phosphatidylglucose (PGL; references 17 and 20). These phospholipids, together with the carotenoids, vitamin K_2 isoprenologues, and glucolipids [monoglucosyldiglyceride (MG) and diglucosyldiglyceride (DG)] make up the major components of the staphylococcal membrane (20). The phospholipids total about 60 μmoles; the carotenoids, 0.1 μmoles; the vitamin K_2 isoprenologues, 0.2 μmoles; and the glucolipids, 10 μmoles per g of dry weight in exponentially growing cells (5, 8, 9, 17, 20).

To understand further the possible functions of the phospholipids in the staphylococcal membrane, the metabolism of these lipids was examined in detail. Earlier studies by other workers centered around the lipid LPG which was postulated to have a role in macromolecular synthesis (6). Van Deenen and his co-workers found an increase in the level of LPG in the membrane when the *p*H of the growth medium was lowered (11). The increased LPG represented both increased synthesis and slower catabolism (7). In studies from our laboratory, LPG has also been

shown to have a slow rate of ^{32}P loss during exponential growth at *p*H 7.4 (17).

In the gram-negative bacterium, *Haemophilus parainfluenzae*, it has been shown that different parts of the phospholipid molecule lose radioactivity at different rates (19, 21). The 2-linked fatty acids have a much more active metabolism than the 1-linked fatty acids (19). The polar portion of the phospholipids has a much more rapid metabolism than the diacylated glycerol or the 1-linked fatty acid (19). In the glucolipids of *S. aureus*, the total fatty acids lost no ^{14}C during exponential growth, but the glucose of MG and DG lost half of their ^{14}C in 30 min (one doubling; reference 17). Furthermore, the CL and PA of *S. aureus* have been shown to have a biphasic loss of ^{32}P in pulse-chase experiments (17). These results suggest that there are at least two classes of PA and CL in *S. aureus*. Heterogeneity in the metabolism of CL has recently been documented in *H. parainfluenzae* (Tucker and White, J. Bacteriol., *submitted for publication*). The present study extends our earlier work and documents that the glycerols and phosphate moieties of the complex lipids show differences in metabolism during growth conditions in which the lipid composition of the membrane remains constant.

MATERIALS AND METHODS

Materials. $H_3{}^{33}PO_4$ was supplied by New England Nuclear Corp., Boston, Mass. It contained less than 5% ^{32}P. Other materials were supplied as described (17, 20).

Growth of S. aureus. The medium, harvesting procedures, strain, and cultural conditions were previously described (17).

Extraction and analysis of the lipids. The lipids were extracted by the modified Bligh and Dyer method as described (2, 17, 20). The lipids were separated on silica gel-impregnated paper in two dimensions, located by autoradiography, and quantitatively eluted (17). In some experiments the lipids were deacylated by mild alkaline methanolysis, and the water-soluble glycerol phosphate esters were determined by chromatography on aminocellulose paper (16).

Enzymatic hydrolysis of the lipids. PG and LPG were hydrolyzed with cabbage phospholipase D, and the products were separated and analyzed as described (21). CL was hydrolyzed with a purified sample of the CL-specific phospholipase D from *H. parainfluenzae* supplied by Y. Ono (13, 14). The CL was suspended in 2% (w/v) Triton X-100 with vigorous shaking and then diluted so the final Triton concentration was 0.7% before hydrolysis. The hydrolysis of *S. aureus* CL was complete in 30 min at 25 C. The PG isolated from the CL was then hydrolyzed with the cabbage phospholipase D.

Analysis of radioactivity. ^{33}P and ^{32}P were determined simultaneously in the scintillation spectrometer as described (Tucker and White, J. Bacteriol., *submitted for publication*). ^{3}H and ^{14}C were determined simultaneously in a solution of Aquaflour (New England Nuclear Corp., Boston, Mass.) under conditions where the ^{14}C channel = 0.002 ^{3}H + 0.534 ^{14}C and the ^{3}H channel = 0.475 ^{3}H + 0.047 ^{14}C. Autoradiograms were prepared as described (16).

RESULTS

Incorporation of ^{14}C and ^{32}P into the phospholipids. *S. aureus* incorporated 28% of the ^{14}C from glycerol-*1,3*-^{14}C (8.8 μCi per 50 ml of media) of which 2.6% was recovered in the phospholipids in an incorporation period of two bacterial doublings. The distribution of ^{14}C between the fatty acids and glycerols of the phospholipids is illustrated in Table 1. No ^{14}C from glycerol was incorporated into the lysine of LPG, the ethanolamine of PE, or the glucose of PGL during this period. *S. aureus* incorporated 0.2% of the ^{32}P in the medium (specific activity of 62 nCi per μmole of phosphate) into the phospholipids per bacterial doubling.

Proportions of the phospholipids during the aerobic growth cycle. During the progression from exponential to stationary aerobic growth, the total phospholipid content of the cells remained constant at 60 to 65 μmoles per g of dry weight (Fig. 1). In the late exponential and early stationary growth phases, the proportion of PG decreased from 76 to 38% of the lipid phosphate, and the proportion of CL increased from 5 to 30%. The proportion of LPG remained constant at 14% throughout the growth cycle. The proportion of PGL increased from 0.7 to 11% and that of PE increased from 0.3 to 7% of the total phospholipids. Exponentially growing cells at cell densities of less than 0.17 mg of dry weight per ml had a relatively constant phospholipid content. The proportions of the lipids during exponential growth was reexamined in detail (Fig. 2). In this experiment, the total content of phospholipid and the proportions did not change during a period of 2.2 bacterial doublings. The metabolism of phospholipids in the remainder of this study was examined during this period so that any changes in metabolism occurred under conditions when the amount of each phospholipid per cell remained constant.

Turnover of ^{32}P and ^{14}C in the phospholipids during exponential growth. The turnover of the phospholipids labeled with both ^{32}P and ^{14}C is illustrated in Fig. 3. The culture was grown with 100 μCi of glycerol-*1,3*-^{14}C and 1 mCi of $H_3{}^{32}PO_4$ per 750 ml of culture for two bacterial doublings (1 hr). The culture was then centrifuged, washed in nonradioactive medium, and resuspended in 3,500 ml of nonradioactive medium. These operations were performed at 37 C and took about 15 min. During growth in the nonradioactive medium, 500-ml samples were taken

TABLE 1. *Incorporation of glycerol-1,3-^{14}C into the phospholipids of Staphylococcus aureus*

Lipid[a]	Glycerol phosphate ester[b]	Fatty acids
Phosphatidylglycerol	53	47
Lysylphosphatidylglycerol	64	36
Cardiolipin	55	45
Phosphatidic acid	50	50

[a] Bacteria were grown with glycerol-*1,3-^{14}C* for 2 doublings, the lipids extracted, separated chromatographically, recovered, and deacylated by mild alkaline methanolysis (17). The ^{14}C in the fatty acids and the glycerol phosphate esters was then determined.

[b] Per cent of ^{14}C in phospholipid backbone.

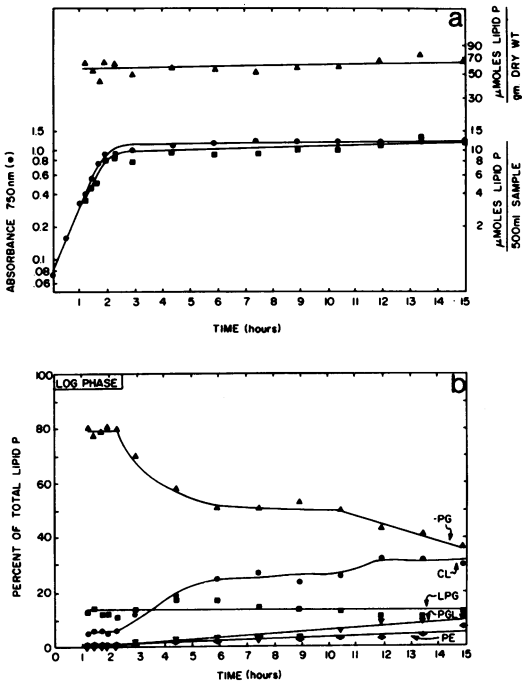

FIG. 1. *Proportions of the phospholipids in S. Aureus during the growth cycle. a, Changes in bacterial density measured as the absorbancy at 750 nm (●), the micromoles of lipid phosphate per 500-ml sample (■), and the micromoles of phospholipid per gram of dry weight (▲). Lipids from cells grown with $H_3{}^{32}PO_4$ for nine doublings were separated chromatographically (17), located by radioautography, recovered, and deacylated. b, Proportions of ^{32}P and phosphate for each phospholipid determined and plotted: PG (▲), CL (●), LPG (■), PGL (▼), and PE (◆). The pH of the medium was 7.4 at the start and 7.3 at the end of the experiment.*

into 500 ml of crushed ice; the lipids were extracted and deacylated, and the radioactivity in each glycerol phosphate ester was determined. In all of the esters, the loss of ^{32}P was more rapid

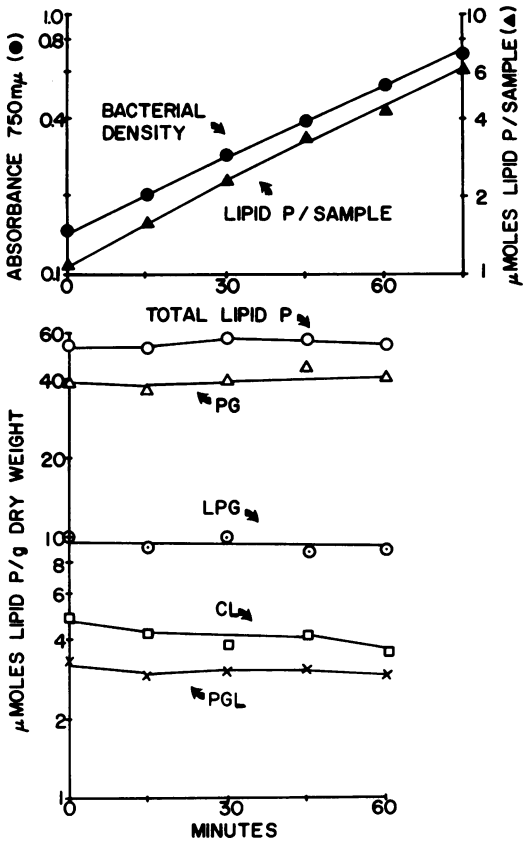

FIG. 2. *Phospholipid composition of S. aureus during the aerobic exponential growth phase. Phospholipids were analyzed as in Fig. 1.*

than the loss of ^{14}C. The time required for the loss of half of the radioactivity from each of the glycerol phosphate esters was: half of the doubling time for ^{32}P and three doubling times for the glycerol in glycerolphosphorylglycerol (GPG) derived from PG; three doubling times for ^{32}P and four doubling times for the ^{14}C in the GPG derived from LPG; 1.4 doubling times for the ^{32}P and 1.8 doubling times for the ^{14}C in the diglycerolphosphorylglycerol derived from the CL; and 1.8 doubling times for the ^{32}P and about 5.3 doubling times for the ^{14}C from the GP derived from PA. There was no turnover of the ^{14}C or ^{32}P from the glycerol phosphate ester derived from PGL. ^{32}P accumulated in this lipid and in the PE during pulse-chase experiments (17).

Turnovers of the glycerols of PG and LPG. PG and LPG were recovered from cells growing exponentially during the chase period of an experiment similar to that described in Fig. 3. The PG and LPG were treated with cabbage phospholipase D, and the resulting PA and unacy-

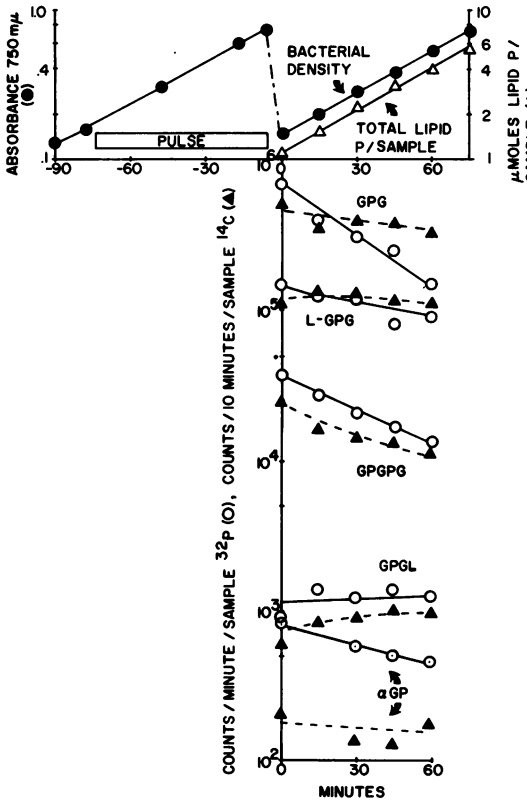

FIG. 3. *Turnover of* ^{32}P *and* ^{14}C *in the glycerol phosphate esters derived from the phospholipids of S. aureus. The cells were grown with 100 μCi of glycerol-1,3-*^{14}C *and 1 mCi of* $H_3{}^{32}PO_4$ *per 750 ml for two doublings, centrifuged, and resuspended in 3,500 ml of nonradioactive medium as illustrated in the upper graph. Bacterial density was measured as absorbancy at 750 nm (●) and the total lipid phosphate per sample (△). During the growth in nonradioactive medium, 500-ml samples were withdrawn; the lipids were separated chromatographically, recovered, and deacylated; and the* ^{32}P (O) *and* ^{14}C (▲) *in each glycerol phosphate ester was determined.*

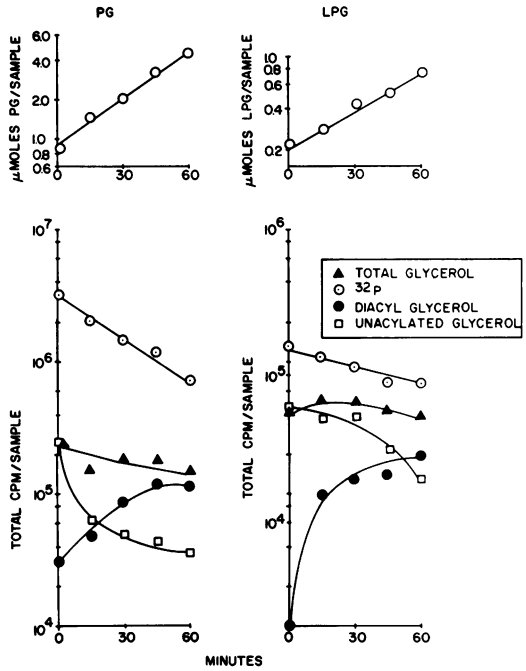

FIG. 4. *Turnover of the* ^{14}C *in the unacylated and diacylated glycerols and* ^{32}P *in the PG and LPG of S. aureus. Cells were grown with glycerol-1,3-*^{14}C *and* $H_3{}^{32}PO_4$ *as for the experiment illustrated in Fig. 3, and the PG and LPG were recovered. The increase in these lipids during exponential growth is shown in the upper graphs. The LPG and PG were hydrolyzed with cabbage phospholipase D, the free or unacylated glycerol was extracted, the PA was deacylated, and the radioactivity was determined.*

lated glycerols were recovered. The ^{14}C in the free glycerol was compared with that in the diacylated glycerol and with the ^{32}P (Fig. 4). In both lipids, the ^{32}P and ^{14}C in the unacylated glycerol were lost, whereas the diacylated glycerols of PG and LPG accumulated ^{14}C. In lipids labeled with glycerol-*1,3-*^{14}C, the position of the ^{14}C in the glycerols was determined after cleaving the lipids with phospholipase D, recovering the PA, and then deacylating to GP. The GP and unacylated glycerol were then cleaved with periodate. If no change occurred in the position of the ^{14}C from the glycerol-*1,3-*^{14}C, 100% of the ^{14}C should be recovered from the unacylated glycerol as formaldehyde and 50% from the C-1 position of GP as formaldehyde. In an incorporation experiment lasting for 2.2 doublings there was no evidence of randomization of the ^{14}C (Fig. 5).

Metabolism of each phosphate of CL. When a culture of *S. aureus* was grown with $H_3{}^{33}PO_4$ for 10 doublings and then pulsed with $H_3{}^{32}PO_4$, the ratio of ^{32}P to ^{33}P in the CL could be used as a measure of the specific activity of the phospholipid phosphates. The CL was then isolated from the *S. aureus* and hydrolyzed with the CL-specific phospholipase D from *H. parainfluenzae*. The enzyme completely hydrolyzed the *S. aureus* CL to PG and PA in 30 min as shown by chromatography of the reaction products on silica gel-impregnated paper in two dimensions. The reaction was apparently specific for the phosphate bond hydrolyzed, since significant differences were demonstrated in the ratios of fatty acids between the PA and PG ends of the molecule. The PA end of the CL molecule had a larger proportion of fatty acids with carbon

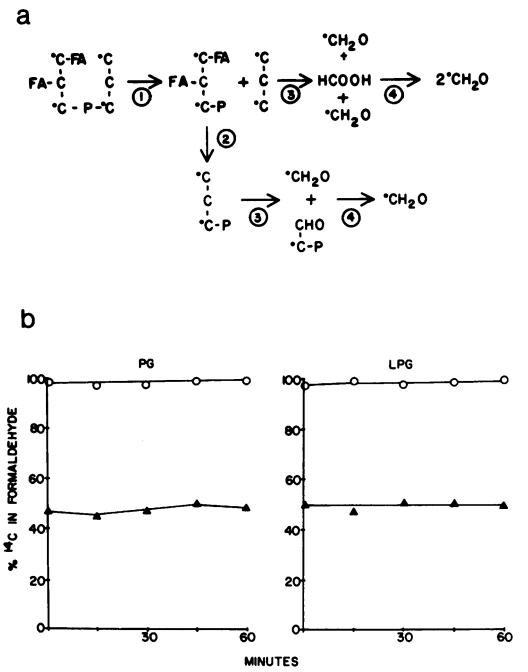

FIG. 5. *Determination of the position of ^{14}C in the glycerol of PG and LPG of Staphylococcus aureus grown with 100 μCi of glycerol-1,3-^{14}C/750 ml. The manipulations involved in these experiments are illustrated in part a: 1, treatment with cabbage phospholipase D; 2, deacylation of the phosphatidic acid after separation from the unacylated glycerol by extraction with chloroform; 3, periodate treatment; 4, column chromatography on Dowex-1 with recovery of the formaldehyde from the glycerol. The *C indicates the position of the ^{14}C. b, Percentage of the total ^{14}C obtained after step 3 that was present as formaldehyde; (○), for the top sequence of reactions corresponding to the free glycerol; (▲), for the sequence of reactions corresponding to diacylated glycerol.*

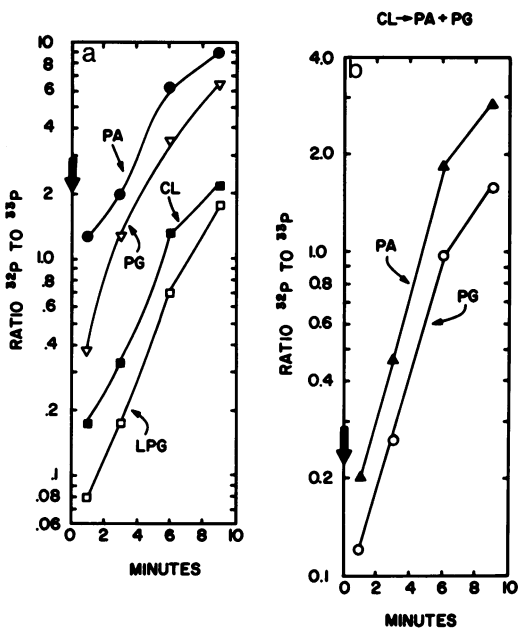

FIG. 6. *Asymmetric metabolism of the phosphates of CL. S. aureus was grown for eight doublings in the presence of 314 μCi of $H_3{}^{33}PO_4$ per 851 ml to a density of 0.15 mg of dry weight per ml, at which time 2 mCi of $H_3{}^{32}PO_4$ was added. After 1, 3, 5 and 9 min, 200-ml samples (0.1 to 0.13 μmoles of phospholipid) were removed within 10 sec into an equal volume of crushed ice and centrifuged. The cell pellet was then extracted, and the lipids were separated by two-dimensional chromatography on silica gel-impregnated paper (17). A portion of the CL was recovered from the paper, emulsified in 2% (v/v) Triton X-100, and hydrolyzed by the cardiolipin-specific phospholipase D of H. parainfluenzae (13, 14). The reaction was complete in 30 min, and the products were extracted and separated by paper chromatography as above. The ratio of ^{32}P to ^{33}P in the products of the enzymatic hydrolysis of CL, PG, and PA are plotted in a. The ratio of ^{32}P to ^{33}P in the PA, PG, CL, and LPG are illustrated in b. The radioactivity was determined in a scintillation spectrophotometer.*

chains longer than 18 carbon atoms and shorter than 14 carbon atoms than the PG end. The fatty acids were determined by gas-liquid chromatography. The $^{32}P/^{33}P$ ratio for the PG and PA obtained from the enzymatic hydrolysis of CL showed the PA end of the molecule had nearly twice the specific activity of the PG end of the CL molecule (Fig. 6b). The initial specific activity of the total cellular PG was two to four times greater than the initial specific activities of either phosphate from the CL molecule (Fig. 6).

Metabolism of the glycerols of PG, LPG, and CL. PG, LPG, and CL were isolated from *S. aureus* after growth with glycerol-2-^3H at constant specific activity and a pulse with glycerol-1,3-^{14}C. The CL was quantitatively hydrolyzed to PG and PA by the CL-specific phospholipase D of *H. parainfluenzae*. The PA from the CL was deacylated to GP, and the $^{14}C/^3H$ ratio was determined. The PG recovered from the other end of the CL molecule was hydrolyzed with cabbage phospholipase D as for the experiment illustrated in Fig. 4, the free glycerol was extracted, and the PA was deacylated to GP before the radioactivity was determined. The PG and LPG were treated as in Fig. 4. If there was neither turnover nor incorporation, the change in the $^{14}C/^3H$ ratio would fall at the rate illustrated by the dotted line in the upper part of Fig. 7. Changes in the ratio with greater slopes than the dotted line indicate turnover. The times necessary for a 50% decrease in the $^{14}C/^3H$ ratio in

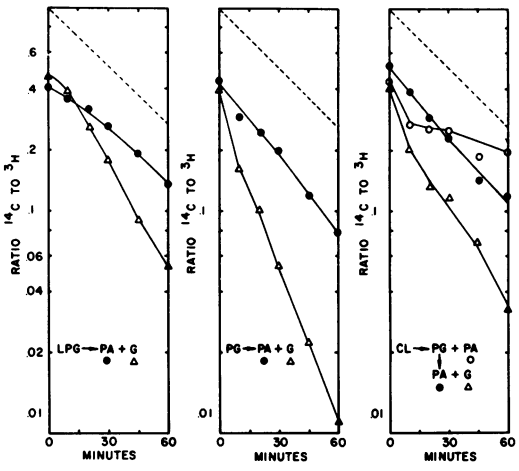

FIG. 7. *Asymmetry in the metabolism of the glycerols of LPG, PG, and CL in S. aureus. The growth medium contained 2.5 mCi of glycerol-2-^{3}H per 2,050 ml, which was divided into a 350-ml portion and a 1,700-ml portion after thorough mixing. The 350-ml portion was inoculated, and the cells were allowed to grow through 10 doublings to a density of 0.03 mg of dry weight per ml, at this time 200 μCi of glycerol-1,3-^{14}C was added, and the cells were incubated for 45 min. The cells were collected on a membrane filter (Millipore Corp.), and then resuspended into the 1,700-ml portion of the medium which contained no ^{14}C but contained glycerol-2-^{3}H at the same specific activity as before. Samples containing 0.1 to 1.2 μmoles of phospholipid were withdrawn, and the lipids were separated chromatographically (17). The LPG, PG, and CL were recovered. The LPG and PG were analyzed as Fig. 4. The CL was hydrolyzed to PA and PG by the CL-specific phospholipase D as in Fig. 6. The PG derived from the CL was further hydrolyzed by cabbage phospholipase D to PA plus the free glycerol. The free glycerol and the PA were treated as in Fig. 4. The dotted line indicates the rate of change in the $^{14}C/^{3}H$ ratio if neither turnover nor incorporation occurred in exponentially growing cells. The doubling time was 30 min throughout this experiment.*

the free glycerols of LPG and PG and the middle glycerol of CL were 19, 11, and 15 min, respectively. The times necessary for a 50% reduction in the $^{14}C/^{3}H$ ratio from the diacylated glycerol of LPG, PG, and the part of the CL molecule that became PG after enzymatic hydrolysis were 41, 24, and 27 min, respectively. The time necessary for a 50% reduction of the $^{14}C/^{3}H$ ratio from the diacylated glycerol in the PA end of the CL molecule was 116 min after an initial rapid change lasting 10 min. If there were neither turnover nor incorporation, the ratio should decrease by 50% in 30 min. The initial specific activity in terms of the $^{14}C/^{3}H$ ratio for all the glycerols from these lipids was 0.40 to 0.42 except for the diacylated glycerol from the PG end of the CL molecule which was 0.56.

DISCUSSION

The data reported in this study indicated that *S. aureus* accumulated CL and lost PG in the membrane during the shift from exponential to stationary growth (Fig. 1). CL accumulation in the stationary growth phase has been detected in several gram-negative species (4, 15). CL also seems to accumulate when the bacteria are stressed as by the addition of colicines (3), by the deprivation of oleate (10) or glycerol (12) in auxotrophic mutants, or by the addition of antibiotics (Tucker and White, J. Bacteriol., *submitted for publication*) or inhibitors (Ono and White, J. Bacteriol., *submitted for publication*) to the culture. PG has been shown to be the sole precursor of CL in this strain of *S. aureus* (S. A. Short and D. C. White, Bacteriol. Proc., p. 151), and studies with inhibitors suggest that the catabolism of CL contributes to the PG pool as in other bacteria (19). Shifts in the PG $\rightleftharpoons$ CL equilibrium suggest that these changes could be related to some important function which depends on lipid metabolism.

The studies in this paper together with earlier studies (18) establish that in the gram-positive *S. aureus* there are differences in the metabolism of the various phospholipids (Fig. 4) and differences in the rates of metabolism of various portions of the lipid molecules (Fig. 4, 6, 7). In *S. aureus*, the unacylated glycerol has a much more rapid metabolism than the diacylated glycerols of both PG and LPG (Fig. 4). Similar findings have been reported for *H. parainfluenzae* (21) and *Escherichia coli* (1). Since the rate of loss of radioactivity from a lipid during the chase period in a pulse-chase experiment represents the minimum rate at which the radioactive portion of the molecule was replaced by the nonradioactive component, any recycling of the radioactivity into the lipid would result in an apparent rate slower than the true rate. The experiment illustrated in Fig. 5 indicated that, if reincorporation of the glycerol did occur, it did so without changing the position of the ^{14}C in the glycerol-*1,3-^{14}C* (21).

LPG had much slower metabolism of its glycerols and phosphate than PG (Fig. 3 and 4) and seemed to remain at a constant proportion of the phospholipid in the membrane throughout the growth cycle (Fig.1), as long as the *p*H of the growth medium was maintained between 7.4 and 7.3. The proportion of the LPG was constant throughout the growth cycle although the proportions of the other phospholipids changed

markedly (Fig. 1). Incubation of the glycerol auxotroph of *Bacillus subtilis* in the absence of glycerol produced changes in the proportions of the PG, PE, PA, and CL, but the proportion of the LPG remained constant (12). Apparently, the LPG content of the membrane primarily reflects the *p*H of the growth medium (11).

The trace lipids PE and PGL in *S. aureus* accumulate ^{32}P (and ^{14}C in the PGL) during exponential growth (Fig. 3; reference 18). The proportions of these two lipids increase significantly as the cells age (Fig. 1).

The examination of CL metabolism in exponentially growing *S. aureus* by use of the CL-specific phospholipase D isolated from *H. parainfluenzae* established that (i) the enzyme was specific for one end of *S. aureus* CL (Fig. 6 and 7) and (ii) the metabolism of the glycerols and phosphates of the *S. aureus* CL molecule were different during growth under conditions in which the concentration of CL in the membrane remained constant (Fig. 2, 6, and 7). Incorporation of ^{32}P into the CL molecule of *S. aureus* involved different pools (Fig. 6). The phosphates of both the PA and PG end of the CL molecule were incorporated at nearly the same rates, although the specific activity of the PA end was twice that of the PG end (Fig. 6). In *H. parainfluenzae*, the phosphate forming the PG end of the CL showed much more rapid incorporation but apparently came from a pool with lower specific activity than the phosphate that formed the PA end of the CL molecule (19). Our studies have established that CL is synthesized from two molecules of PG with the loss of an unacylated glycerol in this strain of *S. aureus* (S. A. Short and D. C. White, Bacteriol. Proc. p. 151, 1971). From the data in Fig. 6, the PG pool from which the CL was formed was a portion of the total PG pool with 25% of the specific activity of the total PG pool. The unacylated glycerol of PG and the middle glycerol of CL had the same initial specific activity but the diacylated glycerol from the PG end of the CL had a higher initial specific activity than the diacylated glycerol of the total PG in the cells (Fig. 7). The diacylated glycerols of the PA and the PA end of the CL molecule had essentially the same initial specific activities (Fig. 7). The total cellular PA pool could only be indirectly related to CL synthesis since the direct precursor of CL in this organism is PG. In *H. parainfluenzae*, the PA and PG ends of the CL molecule come from portions of the total PG pool with one-half of the initial specific activities of the total PG and PA in the cells (19).

No enzyme that degrades CL has been isolated from *S. aureus*. In *H. parainfluenzae*, the CL-specific phospholipase D apparently acts in vivo to yield equimolar amounts of PA and PG (14; Ono and White, J. Bacteriol., *submitted for publication;* Tucker and White, J. Bacteriol., *submitted for publication*). In *S. aureus*, the diacylated glycerol and the middle glycerol from the PG end of the CL molecule have a metabolism essentially identical to the total cellular PG. The middle glycerol lost radioactivity about twice as rapidly as the diacylated glycerol of the PG end of the CL molecule (Fig. 7). In this experiment, the change in specific activity was about what would be expected if there were neither incorporation nor turnover. The PA end of the CL molecule showed a biphasic loss of ^{14}C from the diacylated glycerol. The rapid phase paralleled the loss of ^{14}C from the middle glycerol followed by a slow phase in which ^{14}C apparently accumulated. Perhaps this reflects the biphasic loss of ^{32}P from the CL molecule shown earlier (17). The slow rate of loss of ^{14}C from the diacylated glycerol of the PA end of the CL molecule either represented recycling of the ^{14}C glycerol from a pool with high specific activity only at that end of the CL molecule or that the glycerol at the PA end of the CL remained relatively stable in the membrane as the other glycerols were hydrolyzed and resynthesized. In exponentially growing *H. parainfluenzae*, the CL molecule lost ^{32}P and ^{14}C from the middle glycerol, but the average of the two diacylated glycerols accumulated ^{14}C (21).

Whatever the true story of CL metabolism may be, the evidence from this study indicates that the two diacylated ends of the CL molecule were equilibrated with different pools during exponential growth and after an initial loss of glycerol from the PA end of the molecule there was essentially no turnover. It is hoped that further studies with membrane systems can elucidate the possible functions of CL in the membrane.

ACKNOWLEDGMENTS

This study was supported by contract 12-14-100-10310(73) from the Agricultural Research Service, U. S. Department of Agriculture, administered by the Eastern Utilization Research and Development Division, Philadelphia, Pa., and grant GB-17984 of the Metabolic Biology Section of the National Science Foundation.

LITERATURE CITED

1. Ballesta, I. P. G., and M. Schaechter. 1971. Effect of shift-down and growth inhibition on phospholipid metabolism of *Escherichia coli*. J. Bacteriol. **107**:251-258.
2. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**:911-917.
3. Cavard, D., C. Rampini, E. Barbu, and J. Polonovski. 1968. Activité phospholipasique et autres modifications du métabolisme des phospholipides consécutives a l'action des colicines sur *E. coli*. Bull. Soc. Chim. Biol. **50**:1455-1471.

4. Cronan, J. E. 1968. Phospholipid alterations during growth of *Escherichia coli*. J. Bacteriol. **95**:2054–2061.
5. Frerman, F. E., and D. C. White. 1967. Membrane lipid changes during formation of a functional electron transport system in *Staphylococcus aureus*. J. Bacteriol. **94**: 1868–1874.
6. Gale, E. F., and J. P. Folkes. 1965. The incorporation of glycerol and lysine into the lipid fraction of *Staphylococcus aureus*. Biochem. J. **94**:390–400.
7. Gould, R. M., and W. J. Lennarz. 1970. Metabolism of phosphatidylglycerol and lysyl phosphatidylglycerol in *Staphylococcus aureus*. J. Bacteriol. **104**:1135–1144.
8. Hammond, R. K., and D. C. White. 1969. Formation of vitamin K_2 isoprenologues by *Staphylococcus aureus*. J. Bacteriol. **100**:573–578.
9. Hammond, R. K., and D. C. White. 1970. Carotenoid formation by *Staphylococcus aureus*. J. Bacteriol. **103**: 191–198.
10. Henning, U., G. Dennert, K. Rehn, and G. Deppe. 1969. Effects of oleate starvation in a fatty acid auxotroph of *Escherichia coli* K-12. J. Bacteriol. **103**:410–416.
11. Houtsmuller, U. M. T., and L. L. M. Van Deenen. 1964. On the accumulation of amino acid derivatives of phosphatidyl glycerol in bacteria. Biochim. Biophys. Acta **84**: 96–98.
12. Lillich, T. T., and D. C. White. 1971. Phospholipid metabolism in the absence of net phospholipid synthesis in a glycerol, requiring mutant of *Bacillus subtilis*. J. Bacteriol. **108**:790–797.
13. Ono, Y., and D. C. White. 1970. Cardiolipin-specific phospholipase D activity in the bacterium *Haemophilus parainfluenzae*. J. Bacteriol. **103**:111–115.
14. Ono, Y., and D. C. White. 1970. Cardiolipin-specific phospholipase D of *Haemophilus parainfluenzae*. II. Characteristics and possible significance. J. Bacteriol. **104**:712–718.
15. Randle, C. L., P. W. Albro, and J. C. Dittmer. 1969. The phosphoglyceride composition of gram-negative bacteria and the changes in composition during growth. Biochim. Biophys. Acta **187**:214–220.
16. Short, S. A., D. C. White, and M. I. H. Aleem. 1969. Phospholipid metabolism in *Ferrobacillus ferrooxidans*. J. Bacteriol. **99**:142–150.
17. Short, S. A., and D. C. White. 1970. Metabolism of the glucosyl diglycerides and phosphatidyl glucose of *Staphylococcus aureus*. J. Bacteriol. **104**:126–132.
18. Stárka, J., and J. Moravová. 1970. Phospholipids and cellular division of *Escherichia coli*. J. Gen. Microbiol. **60**: 251–257.
19. White, D. C. 1971. The involvement of lipids in the modification of the bacterial membrane. Proc. 10th Int. Congr. Microbiol., Mexico City, p. 113–119.
20. White, D. C., and F. E. Frerman. 1967. Extraction, characterization and cellular localization of the lipids of *Staphylococcus aureus*. J. Bacteriol. **94**:1854–1867.
21. White, D. C., and A. N. Tucker. 1969. Phospholipid metabolism during bacterial growth. J. Lipid Res. **10**:220–233.