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Consequences of Glycerol Deprivation on the Synthesis of Membrane Components in a Glycerol Auxotroph of *Staphylococcus aureus*

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In a glycerol auxotroph of *Staphylococcus aureus*, the deprivation of glycerol affected the formation of certain membrane components. (i) There was synthesis of fatty acids at the predeprivation rate even though the fatty acids synthesized accumulated as free fatty acids rather than as esterified fatty acids; (ii) there was a complete cessation of phospholipid and vitamin K isoprenologue biosynthesis; (iii) there was conservation of the glycerol esters of the complex phospholipids and glucolipids; (iv) there was an immediate decrease in the rate of synthesis of monoglucoslydiglyceride (30%) and diglucosyldiglyceride (60%); (v) there was a 50% decrease in the rate of synthesis of the polar and nonpolar carotenoids; (vi) there was synthesis of protoheme, heme *a*, and nonspecific membrane protein at the predeprivation rate; and (vii) there was an abrupt cessation in the formation of new, functional glycine transport activity.

Glycerol and oleate auxotrophs of several bacterial species have been used to study the structure and function of bacterial membranes (4, 7, 8, 10, 11, 14). These mutants have been particularly useful because glycerol is an essential component of both phospholipids and glycolipids, and these compounds are localized in the bacterial membranes (5, 20). We have extended the observations of Mindich (9, 11) in previous communications (8, 14) and have shown that when glycerol auxotrophs of *Bacillus subtilis* or *Staphylococcus aureus* are deprived of glycerol, there is an immediate cessation of net phospholipid synthesis, whereas protein synthesis continues for about two generations. It has also been reported (9, 10) that proteins are incorporated into the cell membrane in the absence of net phospholipid synthesis, resulting in an increase in the buoyant density of the membrane. This suggested that phospholipid synthesis exerted no apparent control over the incorporation of proteins into the membrane.

We have already shown (8, 14) that, although there is no net synthesis of phospholipids in the absence of glycerol, there is active metabolism of the individual phospholipids and a change in the overall phospholipid composition in these auxotrophs. Furthermore, as first noted by Mindich (*personal communication*), there is an increase in the free fatty acid content of the bacteria and a shift from the usual C-15 to C-17 iso- and anteiso-branched-chain fatty acids to C-19 or C-21 branched-chain fatty acids. Mindich subsequently showed (12) that in *B. subtilis* the fatty acid synthetase is not inhibited by the presence of free fatty acids and that the rate of fatty acid synthesis is the same when the cells are resuspended in the presence and absence of glycerol.

In this study, we confirm the results of Mindich concerning the synthesis of fatty acids in glycerol auxotrophs of *S. aureus* and show that the synthesis of neutral lipids (vitamin K isoprenologues, carotenoids, and glycolipids) of this strain are in some way regulated by the rate of phospholipid biosynthesis. It is shown that formation of a new transport system for the amino acid glycine is impaired during the deprivation of glycerol. The amino acid transport system in these cells involves the electron transport system (16).

MATERIALS AND METHODS

Growth of bacteria. The medium and the growth conditions used have been described previously (14). The bacteria were grown in synthetic medium containing glycerol until they reached mid-log phase. They were then harvested by filtration (14), washed with at least two volumes of medium without glycerol, and resuspended in medium, with or without

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glycerol, as indicated in the figure legends. The experiments described were carried out in 125 ml (total volume) of medium unless otherwise indicated.

Isolation and separation of neutral lipids. Total lipids were extracted by a modification of the Bligh and Dyer procedure (1, 21). Neutral lipids were isolated from the total lipid extract by silicic acid, column chromatography as previously described (15). A sample of the total lipid was applied to a 1 g silicic acid column. The neutral lipids were then eluted with 6 ml of chloroform-acetone (1:1, v/v), and the phospholipids were eluted with 10 ml of chloroform-methanol (1:1, v/v).

One-dimensional chromatography on Whatman silica gel-impregnated paper (SG-81) with chloroform-isooctane (2:1, v/v) was used to separate the nonpolar carotenoids (R_F 1.0) and the vitamin K isoprenologues (R_F 0.7) from the polar carotenoids and free fatty acids (R_F to 0.2) (6). After chromatography, the papers were placed on Kodak no-screen X-ray film. Areas on the chromatograms corresponding to dark spots on the film were cut out and counted as previously described (8).

Isolation of fatty acids. Total fatty acids were obtained by saponifying samples of whole cells with 3 N KOH for 4 hr at 100 C (19). Neutral lipids were then recovered by extraction twice with 0.5 volumes of diethyl ether. The water phase was adjusted to *p*H 2.0 with concentrated HCl, and the fatty acids were extracted with diethyl ether as described above. Non-esterified fatty acids were isolated as follows. Before obtaining the two-phase system in the Bligh and Dyer procedure for extracting total lipids (14), $NaHCO_3$ was added to give a final concentration of 1 M. After obtaining two phases, the lipid phase was removed, and the aqueous phase was adjusted to *p*H 2 with concentrated HCl. This phase was then extracted twice with 0.5 volume of diethyl ether.

Chromatography of glycolipids. Glycolipids were separated from the total lipid extract by chromatography on Whatman silica-gel-impregnated paper (SG-81) by using the first and third solvents of Wuthier (22). Autoradiograms were prepared as described above. The areas of the chromatograms corresponding to the glycolipids were cut out and, in the case of those samples labeled with ^{14}C-glucose, were eluted from the paper (21) and deacylated (15) before counting. Samples which were labeled with ^{14}C-acetate were counted without further treatment.

Radioisotopes. The following radioisotopes were used in the experiments reported here: acetic-*1*-^{14}C acid (59.2 mCi/mmole); DL-mevalonic-*2*-^{14}C acid (6.33 mCi/mmole); glycerol-*1,3*-^{14}C (14.1 mCi/mmole); D-glucose-*U*-^{14}C (150 mCi/mmole); L-glycine-*U*-^{14}C (83 mCi/mmole); δ-aminolevulinic-*4*-^{14}C acid (24.5 mCi/mmole). All of the isotopes were purchased from New England Nuclear Corp. (Boston, Mass.), with the exception of δ-aminolevulinic acid which was supplied by Calbiochem (Los Angeles, Calif.).

RESULTS

Effect of glycerol deprivation on fatty acid biosynthesis. The glycerol auxotroph S-2 of *Staphylococcus aureus*, when deprived of glycerol, did not incorporate $H_3{}^{32}PO_4$ into its phospholipids (Fig. 1). During the first 75 min of glycerol deprivation, the incorporation of ^{14}C-acetate into its lipids continued at a rate of 50 to 80% of that in cultures supplemented with glycerol. When glycerol was re-added to the deprived cultures, the incorporation of both ^{14}C-acetate and $H_3{}^{32}PO_4$ increased at twice the predeprivation rate.

The increase in acetate incorporation during glycerol deprivation represented primarily the synthesis of free fatty acids (fatty acids not esterified to lipids) as shown in Fig. 2. These fatty acids that were synthesized in the absence of glycerol were four to six carbon atoms longer than the normal C-15 or C-17 branched-chain fatty acids (14). The free fatty acid content of the cells increased from the normal 1% level to 13% during the initial 60 min following the removal of glycerol.

When cells were grown with ^{14}C-acetate, in medium plus and minus glycerol, the majority of the radioactivity was localized in the free fatty acids in medium devoid of glycerol in contrast to esterified fatty acids in medium containing glycerol. After pulsing the mutant cells with ^{14}C-acetate for one and one-half generations in medium containing glycerol and

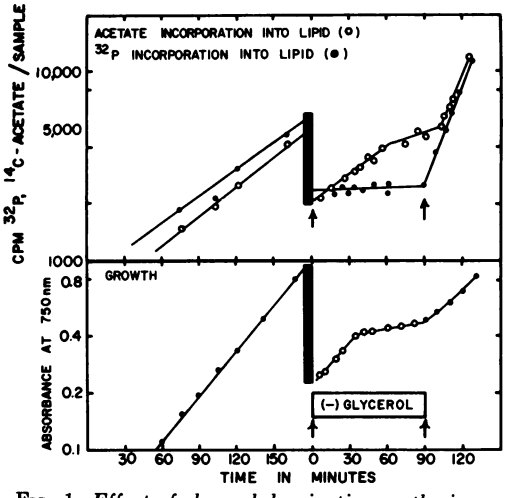

FIG. 1. *Effect of glycerol deprivation on the incorporation of ^{14}C-acetate and $H_3{}^{32}PO_4$ into the lipids of Staphylococcus aureus. The cells were grown, washed, and resuspended in medium containing constant-specific-activity ^{14}C-acetate and $H_3{}^{32}PO_4$. At the times indicated, 5-ml samples were withdrawn and the lipids were extracted (21). The neutral lipids were separated from the phospholipids on a 1 g silicic acid column by elution with 6 ml of chloroform-acetone (1:1); the phospholipids were eluted with 10 ml of chloroform-methanol (1:1).*

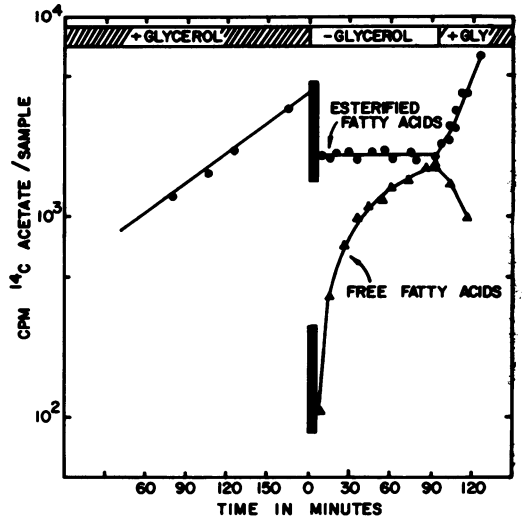

FIG. 2. *Effect of glycerol deprivation on fatty acid biosynthesis. The cells were grown in medium containing constant-specific-activity ^{14}C-acetate. At the times indicated 15-ml samples were withdrawn, and the total lipids were extracted by a modification of the Bligh and Dyer method (21). Free fatty acids were obtained by adding $NaHCO_3$ to a final concentration of 1 M in the one-phase system. After adding chloroform and buffer to make a two-phase system, the lipid phase was withdrawn and the free fatty acids were extracted with 0.5 volume of diethyl ether twice after adjusting the pH to 2.0 with concentrated HCl.*

resuspending them in medium plus and minus glycerol containing 0.1 M acetate, there was essentially no turnover of the fatty acids (Fig. 3B). This suggested that once fatty acids were esterified in this mutant, there was very little exchange or degradation of the fatty acids. However, when cells were pulsed with ^{14}C-acetate in medium devoid of glycerol, there was considerable turnover of the free fatty acids, a 40% decrease in the radioactivity in one generation (Fig. 3A). These results suggested that upon glycerol deprivation there was free fatty acid degradation mediated either by the induction or activation of a fatty acid oxidation pathway. More important, because the free fatty acids formed during the deprivation of glycerol were degraded, the actual rate of fatty acid synthesis in cells deprived of glycerol was much higher than indicated by the acetate incorporation. Because the decrease in free fatty acid content per generation amounted to 40% (Fig. 3A) and the average rate of free fatty acid synthesis during glycerol deprivation was 65% of the exponential rate, free fatty acid synthesis was at least equal to the predeprivation rate. This means that, even though free fatty acids accumulated up to 13% of the total, they did not appear to be feedback inhibitors of the fatty acid synthetase, as was also noted by Mindich in *B. subtilis* (12).

Effect of glycerol deprivation on the turnover of glycerol in the phospholipids and glucolipids. It was previously shown (8, 10, 14) that when cells were deprived of glycerol net phospholipid synthesis ceased immediately; however, there was turnover and metabolism of the polar portions of the phospholipids (8, 14). After pulsing the cells grown in medium containing ^{14}C-glycerol for two generations, the turnover of the glycerol in the individual lipids was measured in medium plus and minus glycerol (Fig. 4). In medium containing glycerol, the turnover of the ^{14}C-glycerol incorporated into the total lipids was about 50% faster than in cells deprived of glycerol (Fig. 4B). In medium supplemented with glycerol, 50% of the ^{14}C-glycerol was lost from phosphatidic acid (PA), 30% from phosphatidylglycerol (PG), and 10% from lysylphosphatidylglycerol (LPG) in one generation (Fig. 4C, F, and G). In medium devoid of glycerol, 50% of the radioactivity was lost from PG; however, LPG and PA accumulated the glycerol label (Fig. 4C, F, and G). The glucolipids, monoglucosyldiglyceride (MG) and diglucosyldiglyceride (DG), accumulated the ^{14}C-glycerol in both plus and minus glycerol media; however, in the absence of glycerol the accumulation was greater. This

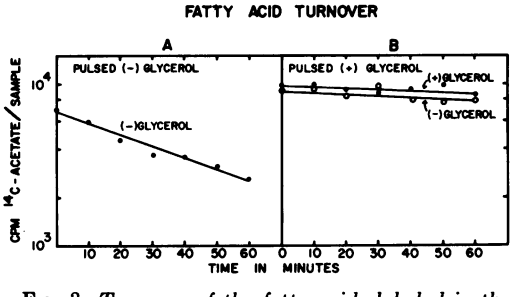

FIG. 3. *Turnover of the fatty acids labeled in the presence and absence of glycerol. The cells were grown in 100 ml of medium containing glycerol to an absorbancy of 0.45 at 750 nm, washed with 200 ml of medium devoid of glycerol, and resuspended in 100 ml of medium plus and minus glycerol, each containing 100 μCi of ^{14}C-acetate. After 1.5 generations (ca 60 min) the cells were filtered, washed as described above, and resuspended in medium plus and minus glycerol containing nonradioactive acetate (0.1 M). At the times indicated, 15-ml samples were removed and the total fatty acids were extracted as before (14).*

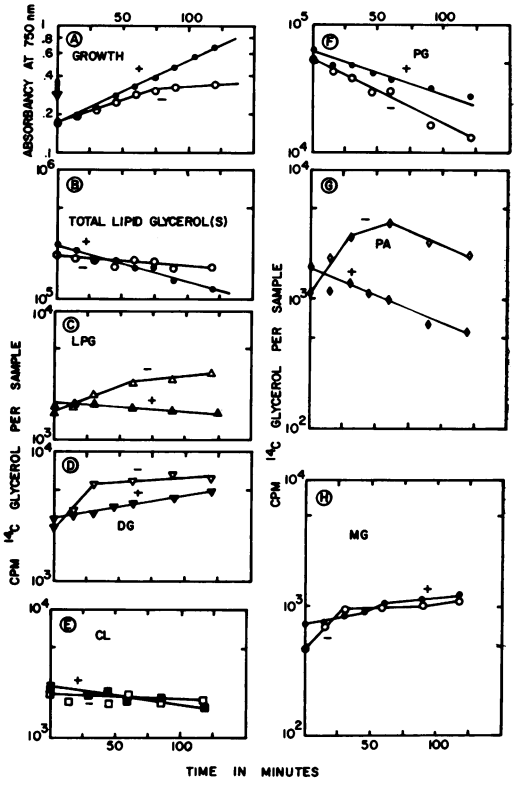

FIG. 4. *Turnover of the lipid glycerol in the presence and absence of glycerol. The turnover of ^{14}C-glycerol was measured by growing the cells in 100 ml of medium containing 250 μCi of ^{14}C-glycerol for two generations. After harvesting by filtration, the cells were washed with medium devoid of glycerol and resuspended in medium plus (30 μg/ml) and minus glycerol. At the times indicated, samples were extracted as described (21), and the individual lipids were separated by chromatography on Whatman SG-81 paper by using the first and third solvents of Wuthier (22). LPG refers to lysylphosphatidylglycerol (C), DG refers to diglucosyldiglyceride (D), CL refers to cardiolipin (E), PG refers to phosphatidylglycerol (F), PA refers to phosphatidic acid (G), and MG refers to monoglucosyldiglyceride (H).*

suggested that once these two compounds were synthesized they were metabolically stable. Figure 4B illustrates that the turnover of the ^{14}C-glycerol moiety in the phospholipids and glucolipids was slower in medium devoid of glycerol and that the glycerol moiety, once incorporated into lipid, was conserved when the cells were deprived of glycerol.

In Fig. 4, the zero time points for cells incubated with and without glycerol were not exactly equal, for the volumes of the original culture collected on the membrane filter and washed into the medium were not exactly equal.

Effect of glycerol deprivation on glucolipid synthesis. The synthesis of the two glucolipids of *S. aureus*, MG and DG, was measured by the incorporation of ^{14}C-acetate into the esterified fatty acids and ^{14}C-glucose into the polar portion of the glucolipids, as shown in Fig. 5. Studies with the isolated glucolipids have established that in the glycerol auxotroph 95% of the ^{14}C-acetate was incorporated into the fatty acids, and the ^{14}C-glycerol exclusively labeled the glycerol portion of the glucolipids. In the deacylated esters derived from the glucolipids, ^{14}C-glucose labeled only the glucose of these deacylated lipids. Upon glycerol deprivation, the rate of ^{14}C-acetate incorporation into DG immediately decreased to 40% of the normal rate (Fig. 5A) and ceased 60 min after deprivation. The incorporation of acetate into MG decreased to 70% of the normal rate. Similar results were obtained for both glucolipids when uniformly labeled glucose incorporation was measured in the absence of glycerol (Fig. 5B).

Effect of glycerol deprivation on neutral lipid biosynthesis. To study the relationship between phospholipid and neutral lipid biosynthesis, cells were grown in complete medium with constant-specific-activity ^{14}C-acetate, filtered, washed, and resuspended in medium devoid of glycerol. As in other experi-

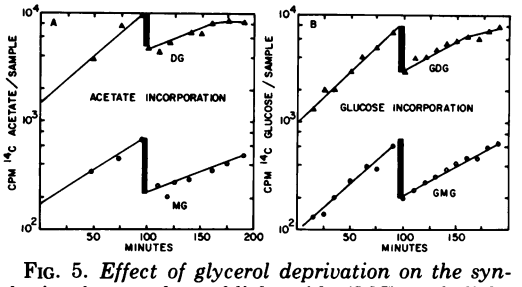

FIG. 5. *Effect of glycerol deprivation on the synthesis of monoglucosyldiglyceride (MG) and diglucosyldiglyceride (DG). The cells were grown, washed, and resuspended in medium containing either ^{14}C-acetate or ^{14}C-glucose at the same specific activities in medium plus or minus glycerol. At various times after glycerol deprivation, samples were withdrawn and the lipids were extracted. The individual lipids were isolated by two-dimensional chromatography as described (22). The glucolipids labeled with ^{14}C-acetate were counted without further treatment. The glucolipids labeled with ^{14}C-glucose were deacylated by mild alkaline methanolysis (21), and the water-soluble fraction was dried under air and counted. The rate of glucolipid synthesis in cells filtered and resuspended in medium with glycerol continued at the pretreatment exponential rate without lag.*

ments, there was no acetate incorporation into the fatty acids of the phospholipids in the period of glycerol deprivation (Fig. 6B). The incorporation of ^{14}C-acetate into the nonpolar carotenoids (mainly phytoene) and the polar carotenoids (mainly rubixanthin) was about half the rate of synthesis by cells grown in the presence of glycerol (Fig. 6C and D). There was a complete cessation of vitamin K isoprenologue biosynthesis during glycerol deprivation (Fig. 5D). The immediate and complete cessation of vitamin K_2 biosynthesis was also detectable when ^{14}C-mevalonate, a direct precursor for the synthesis of the isoprenologue side chain, was utilized as a measure of continued synthesis (Fig. 7).

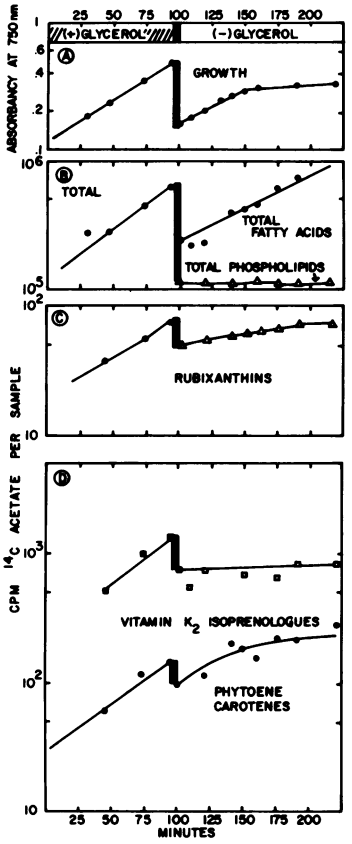

FIG. 6. *Effect of glycerol deprivation on the synthesis of neutral lipids. The cells were grown in medium containing ^{14}C-acetate, filtered, and washed as before (14). After washing, the cells were resuspended in medium devoid of glycerol and containing the same-specific-activity ^{14}C-acetate. At the times indicated, 5-ml samples were removed, and the lipids were extracted as described (21). After the lipids were extracted from each sample, the neutral lipids and phospholipids were separated by silicic acid column chromatography as described (15). The nonpolar carotenoids (R_F 0.9 to 1.0), vitamin K isoprenologues (R_F 0.7 to 0.8), and polar lipids (fatty acids and polar carotenoids, R_F 0 to 0.2) were separated by Whatman SG-81 paper chromatography by using a solvent of chloroform-isooctane (2:1).*

Effect of glycerol deprivation on the incorporation of protein into the membrane. In order to investigate the effects of glycerol deprivation on membrane function, the incorporation of protein into the membrane and the transport of the amino acid glycine was measured. The measurement of protein incorporation into the membrane was assayed by the incorporation of ^{14}C-serine into nonspecific membrane protein and by the incorporation of ^{14}C-δ-aminolevulinic acid, a precursor of the prosthetic groups of the cytochromes, into membrane-bound protein. δ-aminolevulonic acid was shown to be a precursor of protoheme and heme *a* which are the prosthetic groups of the cytochromes in the membrane of *S. aureus* (17). From both assays it was clear that proteins were incorporated into the membrane at an undiminished rate for 90 min after glycerol deprivation (Fig. 8). These results were consistent with the continuation of total protein synthesis which accumulated ^{14}C-serine at the

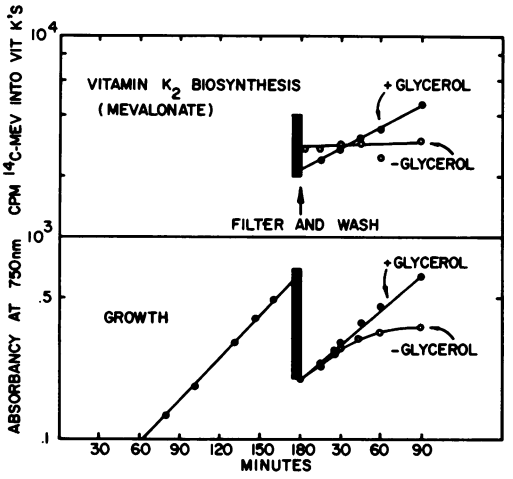

FIG. 7. *Effect of glycerol deprivation on the synthesis of vitamin K isoprenologues. The cells were grown, washed, and resuspended in medium plus and minus glycerol containing the same specific activity of ^{14}C-DL-mevalonic acid (MEV). At the times indicated, 5-ml samples were withdrawn and the lipids were extracted. The vitamin K isoprenologues were separated from the phospholipids, free fatty acids, and carotenoids by chromatography on Whatman SG-81 paper by using a solvent of chloroform-isooctane (2:1). The vitamin K isoprenologues were measured, without further purification or separation, into their individual isoprenologues.*

predeprivation rate for 90 min and then ceased (14).

In order to study the effect of glycerol deprivation on the functionality of this membrane, the rate of transport of the amino acid glycine into the free amino acid pool of whole cells was measured (Fig. 9). Amino acids can be transported into the free amino acid pool of whole cells in the presence of chloramphenicol (2). When glycerol auxotrophs were deprived of glycerol, the specific activity of the glycine transport, instead of remaining constant as was the case for cells grown in the presence of glycerol, decreased as a function of time, as seen in Fig. 9. Thus, when cells were deprived of glycerol some component of the membrane involved in glycine transport was either not synthesized or, if synthesized, was nonfunctional.

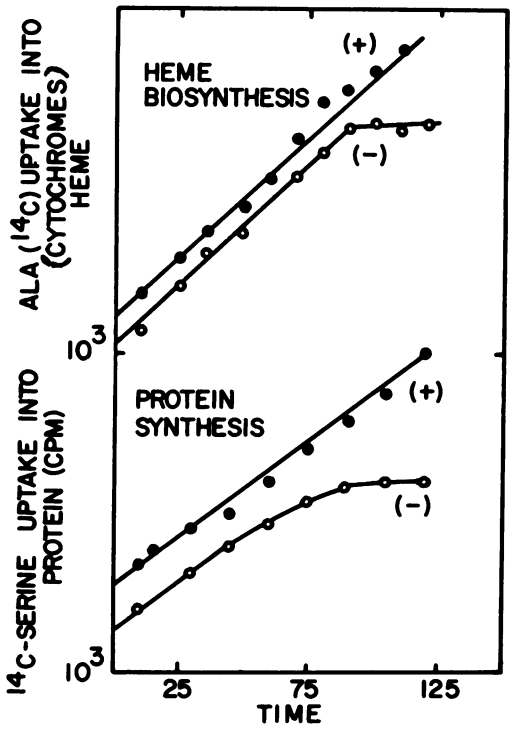

FIG. 8. *Incorporation of ^{14}C-δ-aminolevulinic acid (ALA) and ^{14}C-serine into cell membrane protein. In both experiments the cells were grown with the isotope indicated, filtered, washed with medium devoid of glycerol, and resuspended in medium plus and minus glycerol containing the same specific activity of the isotope (ALA, 0.8 μCi/ml; serine, 1.2 μCi/ml). At the times indicated, 2-ml samples were withdrawn and centrifuged. The samples were resuspended in 2 ml of 50 mM phosphate buffer (pH 7.4) containing lysostaphin (8 μg/ml), deoxyribonuclease (20 μg/ml), and ribonuclease (20 μg/ml). After incubation for 30 min at 37 C, 5 ml of buffer was added, and the membranes were centrifuged at 17,000 × g for 30 min. The membranes were resuspended in 50 mM phosphate buffer, and samples were taken for the determination of radioactivity.*

DISCUSSION

In a glycerol auxotroph of *S. aureus* designated S-2, the deprivation of glycerol effected certain changes in the formation of the membrane components. (i) There was synthesis of fatty acids at the predeprivation rate; however, these fatty acids accumulated as free fatty acids, not as esters. (ii) There was a complete cessation of phospholipid and vitamin K biosynthesis. (iii) There was conservation of the glycerol esters of the complex lipids. (iv) There was a decrease in the rate of synthesis of the polar and nonpolar carotenoids. (v) There was

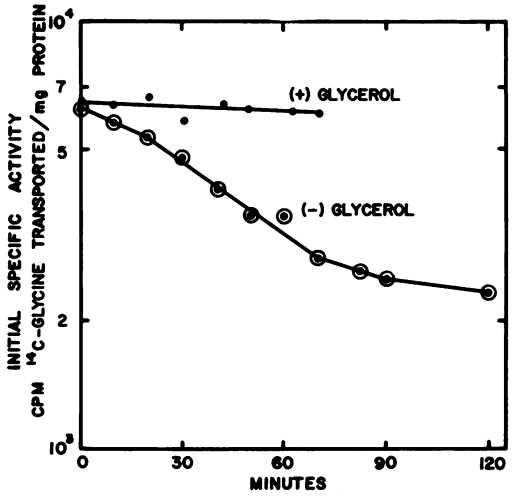

FIG. 9. *Effect of glycerol deprivation on the transport of ^{14}C-glycine into whole cells. The cells were grown to an absorbancy of 0.45 in 100 ml of medium, washed with 150 ml of medium devoid of glycerol, and resuspended in 100 ml of medium minus glycerol. At the times indicated, 10 ml samples were withdrawn and centrifuged at 10,000 × g for 2 min at 37 C. The pellets were resuspended in 2 ml of medium, devoid of glycerol and containing 50 μg of chloramphenicol per ml, and incubated at 37 C. After 1 min, 0.1 μCi of ^{14}C-glycine was added, and samples were withdrawn after 0, 2, 5, 7, 10, and 15 min of incubation. Samples of 0.2 ml were diluted with 2.0 ml of cold 50 mM phosphate buffer (pH 7.4), filtered, and washed with 5 ml of buffer. The filters were dried and counted. Samples were also taken for the determination of protein. Specific activities of uptake measurements were calculated after 2 min of incorporation. When uptake was linear with time (16), the logarithm of the transport activity was plotted on the abcissa.*

synthesis of protoheme, heme *a*, and membrane protein at the predeprivation rate. (vi) There was an abrupt cessation in the formation of new, functional glycine transport activity.

When exponentially growing cells of *S. aureus* were deprived of glycerol, net phospholipid synthesis ceased immediately; however, growth, RNA synthesis, and protein synthesis continued for 30 to 90 min (14). During this period of glycerol deprivation, apparent fatty acid synthesis continued at 65% of the normal rate for 90 min (Fig. 1). The fatty acids that were synthesized during glycerol deprivation appeared as free fatty acids containing four to six more carbon atoms than the normal fatty acids (14). During glycerol deprivation there was very little esterification of fatty acids into phospholipids or glucolipids (Fig. 1 and 2). Because the apparent rate of fatty acid synthesis was about 65% of the normal rate immediately after glycerol deprivation and because the free fatty acids accumulated to 13% of the total fatty acids, the free fatty acids possibly exerted a feedback inhibitory effect on the fatty acid synthetase, as has been suggested (3). However, Mindich (12) has shown with a glycerol auxotroph of *B. subtilis* that the actual rate of fatty acid synthesis was the same in medium plus and minus glycerol, even though in the *Bacillus* system free fatty acids accumulate to 20% of the total in the absence of glycerol. The free fatty acids, which were synthesized in the absence of glycerol, have a much faster turnover rate than the esterified fatty acids synthesized in the presence of glycerol. The maintenance of an apparent rate of fatty acid biosynthesis of 65% of the predeprivation rate, when the free fatty acids were catabolized at a rate of 40% of the total per generation (Fig. 3A), indicated that the actual rate of fatty acid biosynthesis was at least equal to the predeprivation rate. Thus, there was no evidence for feedback inhibition of the fatty acid synthetase by free fatty acids, as also noted by Mindich (12). Also, there was no loss of radioactivity from the fatty acids of cells resuspended in medium, either with or without glycerol, if these fatty acids were esterified to the complex lipids (Fig. 3B). This suggested that there was very little exchange or transesterification of the esterified fatty acids, even in the absence of glycerol where the free fatty acids account for 13% of the total. If there had been exchange, there would have been turnover.

Even though net phospholipid synthesis ceased immediately after glycerol deprivation, glucolipid synthesis continued at a reduced rate for 60 to 90 min. The continued synthesis of MG and DG can be accounted for by the turnover of the ^{14}C-glycerol from the phospholipids as shown in Fig. 4. When cells were pulsed with ^{14}C-glycerol and resuspended in medium plus and minus nonradioactive glycerol, the rate of turnover of the lipid glycerol was 50% faster in cells resuspended in medium containing glycerol. The radioactive label accumulated in the glucolipids both in the presence and absence of glycerol; however, in the absence of glycerol the rate of accumulation was faster (Fig. 4). This suggested a precursor role for the diglyceride portion of the phospholipid molecule in the synthesis of the glucolipids, as has been indicated by Peringer (13). The turnover of ^{14}C-glycerol from the phospholipids in cells deprived of glycerol was consistent with changes in the proportions of the individual phospholipids during glycerol deprivation (14). The content of PG decreased with a concomitant increase in the content of LPG. During glycerol deprivation the cells conserved the lipid glycerol to a much greater extent than cells supplemented with glycerol.

The synthesis of the neutral lipids was affected to various degrees by the deprivation of glycerol. The carotenoids were continually synthesized at a slower rate after glycerol deprivation throughout the experiment (Fig. 6). The synthesis of the vitamin K isoprenologues was drastically affected by the deprivation of glycerol. By utilizing both ^{14}C-acetate and ^{14}C-mevalonate to measure the synthesis of vitamin K (Fig. 6 and 7), it was shown that immediately after deprivation synthesis ceased.

During glycerol deprivation it has been shown that total protein synthesis continued unabated for 90 min, even though net phospholipid synthesis stopped immediately (14). In Fig. 8 it was shown that protein incorporation into the cytoplasmic membrane continued at the predeprivation rate for 90 min. During the induction of the membrane-bound electron transport system in *S. aureus*, it was previously shown that there was a simultaneous increase in the rate of synthesis of phospholipids, polar carotenoids, vitamin K_2 isoprenologues, glucolipids, hemes, and cytochromes (5). However, even though carotenoid biosynthesis was slowed and vitamin K_2 biosynthesis ceased abruptly, the incorporation of δ-aminolevulinic acid into the hemes of the cytochromes continued at predeprivation rate (Fig. 6, 7, and 8). The synthesis of these components of the electron transport system (18) did not

appear to be coordinately linked under these experimental conditions.

Mindich (10) has shown that in a glycerol auxotroph of *B. subtilis* the buoyant density of the membrane increased upon deprivation and has suggested that these findings place some constraint on models of membrane assembly, because it appeared that proteins were not incorporated into the cytoplasmic membrane as lipoprotein subunits. To look at the functionality of these lipid-poor membranes, the transport of the amino acid glycine was investigated. These results, shown in Fig. 9, suggest that upon glycerol deprivation some important component of the glycine transport system is either not synthesized or, if synthesized, is not functional, because the specific activity of the transport process decreased with the time of glycerol deprivation. The results obtained from the transport of glycine in cells deprived of glycerol might be accounted for by the cessation of vitamin K biosynthesis, because Short et al. (16) have shown that the electron transport system was obligatory for amino acid transport in vesicles. These results could also be explained by the lack of the proper environment supplied by the phospholipids for the individual components involved with amino acid transport. Preliminary results with vesicles derived from this glycerol auxotroph have shown that the substrate for oxygen utilization and amino acid transport is L-lactate and that the substrate available for amino acid transport is not lacking during glycerol deprivation.

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