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Induction of the Lactose Transport System in a Lipid-Synthesis-Defective Mutant of *Escherichia coli*

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In order to relate the biogenesis of the lactose transport system to lipid synthesis, a glycerol-requiring mutant of *Escherichia coli* K-12 with a specific defect in L-glycerol-3-phosphate synthesis was isolated and characterized. The defective enzyme is the biosynthetic L-glycerol-3-phosphate dehydrogenase [L-glycerol-3-phosphate: NAD (P) oxidoreductase, EC 1.1.1.8] which functions as a dihydroxyacetone phosphate reductase to provide L-glycerol-3-phosphate for lipid synthesis. In this mutant, removal of glycerol from the growth medium results in inhibition of the synthesis of protein, deoxyribonucleic acid, and phospholipid. Inhibition of phospholipid synthesis immediately follows glycerol removal, whereas the inhibition of deoxyribonucleic acid and protein synthesis is preceded by a short lag period. Glycerol starvation does not change the turnover pattern of previously synthesized phospholipids. The blocking of lipid synthesis by glycerol starvation causes a drastic decrease in inducibility of β-galactoside transport activity relative to β-galactosidase, indicating that induction of lactose transport requires de novo lipid synthesis.

The involvement of lipids in membrane function has been the object of intensive study (22). Of all the biochemical processes associated with membranes, transport is the only one requiring a membrane site of action. The lactose transport system in *Escherichia coli* is one of the best characterized systems both genetically (5, 10, 11) and biochemically (2, 6, 7, 12, 15, 19, 23). A previous report from this laboratory (4) described two sets of observations. (i) Studies on derepressed synthesis of *lac* operon proteins indicate that induction of a functional lactose transport system involves steps in addition to *y* gene translation. (ii) Studies with unsaturated fatty acid auxotrophs indicate that simultaneous synthesis of lipid containing unsaturated fatty acids is required for the induction of lactose transport. The availability of other lipid biosynthetic mutants would permit a closer examination on the functional role of lipid in transport system biogenesis. With this consideration in mind, a mutant defective in the synthesis of L-glycerol-3-phosphate, the first true intermediate in lipid synthesis, was isolated and characterized, and the induction of lactose transport was studied.

MATERIALS AND METHODS

Chemicals. Nonradioactive amino acids and thiamine hydrochloride were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio; *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, from Aldrich Chemical Co., Inc., Milwaukee, Wis.; acid-hydrolyzed casein, from Difco Laboratories, Detroit, Mich.; thymidine and reduced nicotinamide adenine dinucleotide phosphate, from PL Biochemicals, Milwaukee, Wis.; 2-mercaptoethanol, from Eastman Organic Chemicals, Rochester, N.Y.; triethanolamine hydrochloride, dithiothreitol, and dihydroxyacetone phosphate, from Calbiochem., Los Angeles, Calif.; pancreatic ribonuclease, from Worthington Biochemical Corp., Freehold, N.J.; *o*-nitrophenyl-β-D-galactopyranoside, from Pierce Chemical Co., Rockford, Ill.; isopropyl-1-thio-β-D-galactopyranoside (IPTG), methyl-1-thio-β-D-galactopyranoside (TMG), and β-D-galactosyl-1-thio-β-D-galactopyranoside (thiodigalactoside), from Mann Research Laboratories, New York, N.Y.; and ^{3}H-thymidine, ^{14}C-L-isoleucine, ^{14}C-TMG, and ^{32}P-inorganic phosphate, from New England Nuclear Corp., Boston, Mass. Chloramphenicol was the gift of Parke, Davis & Co., Detroit, Mich., and ^{3}H-*p*-nitrophenyl-α-D-galactopyranoside was a gift from E. P. Kennedy.

Media. The basal media used were medium A (3)

and medium LP with the following composition (in mg/liter): $FeSO_4$, 1; Na·citrate·$2H_2O$, 440; $MgSO_4 \cdot 7H_2O$, 100; $(NH_4)_2SO_4$, 1,000; with potassium phosphate buffer (*p*H 7) added at a final concentration of 1 mM. Medium AS and medium LPS are medium A and medium LP supplemented with succinate (0.4%), thiamine hydrochloride (2 μg/ml), and valine, lysine, leucine, isoleucine, methionine and threonine added at a concentration of 50 μg/ml of the L-isomer. Medium A was replaced by medium LP when a low-phosphate medium was required.

Bacterial strains. All strains are derivatives of *E. coli* K-12. The glycerol-requiring auxotroph (GR-1) used in these studies was derived from a β-glucoside-fermenting derivative of strain MH-1, which is $F^- B_1^-$ *str*^{r}, after treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (1). The mutagenized cells were grown on medium A supplemented with 0.4% glycerol to eliminate unwanted auxotrophs from the population. After penicillin selection (9) in medium A supplemented with 1.0% glucose, survivors were spread on medium A-glycerol-agar and replica-plated onto medium A-glucose-agar. In two independent trials of mutagenesis and selection, glycerol-requiring auxotrophs were obtained from the penicillin survivors at a frequency of approximately 5×10^{-3}. Spontaneous phenotypic variants of GR-1, no longer requiring glycerol for growth, were obtained at a frequency of $>10^{-6}$ by plating on medium A-glucose-agar. Transductants to growth on medium A-glucose-agar were obtained by using bacteriophage P1kc at a multiplicity of infection of 0.1. Strain MH-1 and a P1*kc* lysate derived from strain MH-1 were obtained from W. Epstein.

All cultures were grown aerobically at 37 C. Growth was measured turbidimetrically with a Klett colorimeter using a no. 42 filter. One Klett unit corresponds to approximately 5×10^6 cells per ml.

L-Glycerol-3-phosphate dehydrogenase assay. A 50-ml sample of a culture, grown to late exponential phase in medium AS supplemented with glycerol (0.4%), was centrifuged in a Sorvall RC-2B centrifuge (10,000 × *g*, 10 min) at 4 C. The cells were washed once by centrifugation and suspension in 10 ml of 10 mM triethanolamine hydrochloride buffer (*p*H 7.5) containing 2 mM dithiothreitol, and finally suspended at a concentration of 1.1×10^{10} cells/ml in the same buffer. The cells were disrupted by sonic irradiation. After centrifugation for 30 min at 100,000 × *g* in a Spinco ultracentrifuge, 0.1 ml of the supernatant fraction was assayed for L-glycerol-3-phosphate dehydrogenase activity by the method of Kito and Pizer (18).

Protein synthesis. The incorporation of amino acids into cell proteins was followed by supplementing cell cultures with ^{14}C-L-isoleucine (0.27 c/mmole) at 0.1 μc/ml. After incubation at 37 C for 15 min, duplicate 1-ml samples of the labeled culture were removed and mixed with 1 ml of ice-cold 10% trichloroacetic acid. The cells were then collected by filtration on membrane filters (DA, Millipore Corp., Bedford, Mass.) and washed with cold 5% trichloroacetic acid; the radioactivity was determined in a liquid scintillation spectrometer.

Deoxyribonucleic acid (DNA) synthesis. Cellular DNA synthesis was followed by supplementing cell cultures with ^{3}H-thymidine (3 c/mmole) at 0.27 μc/ml and ^{12}C-thymidine at 0.33 μg/ml. Samples (1 ml) were pipetted directly into 1 ml of ice-cold 10% trichloroacetic acid at 2, 4, and 6 min after thymidine addition. The cells were collected on membrane filters (DA) and washed with 5% trichloroacetic acid; the radioactivity was determined as described for protein synthesis. Incorporation of ^{3}H-thymidine into DNA was linear with time over the interval measured.

Separation of membrane and soluble fractions. Cells labeled with ^{14}C-L-isoleucine were harvested and suspended in 0.05 M tris(hydroxymethyl)aminomethane-hydrochloride buffer, *p*H 7.6, containing chloramphenicol at a concentration of 50 μg/ml. The cell suspension was subjected to sonic irradiation for 1.5 min. Ribonuclease and ethylenediaminetetraacetate were then added at final concentrations of 20 μg/ml and 5 mM, respectively. After incubation at 37 C for 30 min, the suspension was resolved into soluble and membrane fractions by centrifugation for 1 hr at 27,000 × *g*, and the acid-precipitable radioactivity was determined.

Phospholipid synthesis and turnover. Cells growing in medium LPS supplemented with 0.4% glycerol were labeled with ^{32}P-inorganic phosphate at 4 μc/ml. The labeled phospholipids were extracted with chloroform-methanol (2:1, v/v), and the nonlipid radioactivity was washed out with 2 M KCl (14). A sample of the chloroform extract was taken for the determination of radioactivity. Individual phospholipids were separated on silica gel thin-layer chromatograms (E. Merck AG, Darmstadt, Germany) with chloroform-methanol-water-acetic acid (65:25:4:2, v/v) used as the solvent system. The fractions were detected by autoradiography and quantitated by scraping the appropriate areas of silica gel into vials for determination of radioactivity. Reference lipid samples were the gift of J. H. Law.

Assay of β-galactosidase and thiogalactoside acetylase. IPTG-induced cells were harvested by centrifugation and suspended in a solution consisting of medium A, 80 μg of chloramphenicol per ml, and 20 mM 2-mercaptoethanol. For β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) assay (21), the suspended cells were toluenized for 45 min at 37 C with shaking. Thiogalactoside acetylase (acetyl-coenzyme A: galactoside 6-*O*-acetyltransferase, EC 2.3.1.18) was assayed by the method of Fox and Kennedy (8).

Assay of transport. Transport was measured by the uptake of TMG. The 0.7-ml system contained cells, medium A, and 0.5 mM ^{14}C-TMG, 10^6 counts per min per μmole. The system was brought to 28 C, and the reaction was initiated by the addition of labeled TMG. After incubating at 28 C for 1 min, 0.5-ml samples were collected on membrane filters (DA) and washed three times with medium A; the radioactivity was determined. The ^{14}C-TMG uptake obtained when 5

mM thiodigalactoside was included in control reactions was subtracted from these values.

Assay of M protein by binding with p-nitrophenyl-α-D-galactopyranoside (α-PNPG). This assay is based on a procedure which measures the equilibrium binding between a transport substrate and the M protein (M. K. Rumley, J. B. Armstrong, and E. P. Kennedy, Abstr. Lactose Operon Meeting, Cold Spring Harbor, N.Y., p. 26, 1969). The substrate exhibiting the highest affinity for M protein (5 μM) is α-PNPG. All operations are performed in SH-buffer (0.1 M potassium phosphate buffer at *p*H 6.3 containing 0.01 M 2-mercaptoethanol), and the temperature of solutions is maintained at ice-bath temperature unless otherwise stated. Membranes are collected from ultrasonically disrupted cells by 30 min of centrifugation at 100,000 × *g* and are washed once by suspension in SH-buffer followed by centrifugation. The membranes from each portion of cells (*see* Table 3) are then suspended in 17 ml of SH-buffer. The assay is run in duplicate, with each assay containing 4 ml of membrane suspension and 1 ml of SH-buffer containing 25 μM ^{3}H-α-PNPG. Control assays contain 2.5 mM thiodigalactoside to inhibit specific binding of α-PNPG to M protein. After 20 min of incubation at 28 C, the membranes are collected by 30 min of centrifugation at 100,000 × *g*, and the supernatant solution is discarded. Radioactivity not trapped in the pellet is washed away by careful rinsing with water. The pellet is then suspended by homogenizing in 5 ml of 5% Triton X-100, and 1.5-ml portions from each 5-ml sample are counted in triplicate in 20 ml of Patterson-Green counting solution containing 3:1 toluene-Triton X-100, 4 g of 2,5-diphenyloxazole and 50 mg of 1,4-bis-2-(5-phenyloxazolyl) per liter. The M protein-bound radioactivity is the difference between radioactivity bound in the absence and in the presence of thiodigalactoside.

RESULTS

Identification of the enzymatic defect. In *E. coli*, the first step in the dissimilation of intracellular glycerol requires a kinase for the formation of L-glycerol-3-phosphate (20). L-Glycerol-3-phosphate is either further catabolized or metabolized to phosphatidic acid, the first true lipid intermediate. In the absence of exogenous glycerol, L-glycerol-3-phosphate formation can be catalyzed by a pyridine nucleotide-linked L-glycerol-3-phosphate dehydrogenase (17, 18). It was expected that a requirement for glycerol which would block lipid synthesis could arise from a defect in this biosynthetic enzyme. This was found to be the case.

Crude extracts prepared from the parent strain and mutant strain GR-1 were examined for their ability to catalyze the dihydroxyacetone phosphate-dependent oxidation of reduced nicotinamide adenine dinucleotide phosphate. Such activity is present in extracts prepared from parent strain cells but is missing in those from the mutant strain (Table 1). The combination of parent strain and mutant strain extracts yields activity comparable to the parent strain extract alone, making it unlikely that the inactivity of the mutant extract is due to the presence of an inhibitor. A glycerol-requiring auxotroph obtained after an independent mutagenesis and selection procedure had the same enzymatic defect as strain GR-1.

Extracts prepared from 10 transductants of GR-1 selected for growth on medium A-glucose-agar were tested and found to have wild-type levels of L-glycerol-3-phosphate:NAD(P) oxidoreductase activity. Six spontaneous mutants of GR-1 selected for growth on medium A-glucose-agar were also tested and were devoid of activity. Since these prototrophs arose at a frequency of about 10^{-6}, it is likely that the detection of true revertants was obscured by another more frequent mutation(s), perhaps giving rise to an alternative pathway for L-glycerol-3-phosphate biosynthesis.

Growth properties and macromolecule synthesis in the mutant during glycerol starvation. The mutant has a strict requirement for glycerol for growth. It can grow on glycerol as the sole carbon source but fails to grow on other carbon sources (succinate, glucose, acid-hydrolyzed casein) unless glycerol is present. The doubling time is 80 min in medium AS and 95 min in medium LPS, when the media are supplemented with 0.4% glycerol.

Upon removal of glycerol, growth (assayed turbidimetrically) continued at a reduced rate and stopped in less than one doubling time. Concomitant decreases were observed for the rates of macromolecule synthesis (Fig. 1). Cells do not lose their viability during a 6-hr period of glycerol starvation. To test the coordination of synthesis of *lac* operon proteins with other cellular macromolecules during glycerol starvation, β-galactosidase synthesis was measured together with the rates of ^{14}C-isoleucine incorporation and of ^{32}P-phospholipid formation (Fig. 2). Glycerol removal depresses equally the synthesis of both β-galactosidase and cellular proteins. It is noteworthy, but not unexpected, that the decrease of

TABLE 1. *NADP-linked L-α-glycerophosphate dehydrogenase activity of cell extracts from mutant and parent strains*

Strain	Dihydroxyacetone phosphate reduced
	nmoles/min
Parent (MH-1)	6.7
Mutant (GR-1)	<0.1

phospholipid synthesis precedes the decrease of either protein or DNA synthesis (Fig. 1 and 2).

Incorporation of proteins into membrane during glycerol starvation. A cessation of lipid synthesis could lead to general defects in membrane formation, such as the failure to incorporate newly synthesized proteins into membranes or a selective repression of membrane protein synthesis. These possibilities were tested in an experiment in which the distribution of newly synthesized protein in membrane and supernatant fractions was compared in the mutant in the presence or absence of glycerol (Table 2). Glycerol starvation produced no pronounced decrease in the per cent of labeled protein incorporated into membrane.

Induction of the lac operon during glycerol starvation. A report from this laboratory suggested that lipid synthesis might be required for de novo formation of functional lactose transport sites on the membrane (4). To further test this hypothesis, induction of the *lac* operon was followed in the mutant strain with glycerol either present in or absent from the medium (Fig. 3). The ratio of induced transport activity to β-galactosidase activity remained nearly constant when glycerol was present. In the absence of glycerol, however, the ratio was subject to a pronounced decrease of over 10-fold, confirming the hypothesis that de novo lipid synthesis is required for *lac* transport induction. This decrease in ratio is not due to a polarity effect in transcription or translation, since the ratio of thiogalactoside acetylase to β-galactosidase induction was measured and found not to be altered significantly by glycerol starvation. The decreased ratio of transport to β-galactosidase induction obtained during the later periods of glycerol starvation is not increased by subsequent incubation in the presence of glycerol after removal of inducer. This indicates that the synthesis of lipid and transport protein must be simultaneous if functional transport sites are to be formed.

Stability of the lac transport system during glycerol starvation. An alternative explanation for the decreased ratio of induced transport activity to galactosidase activity states that transport activity may become highly labile under conditions of glycerol starvation. The experiment in Fig. 4 shows that transport activity is subject to a slow inactivation, but that the rate of inactiva-

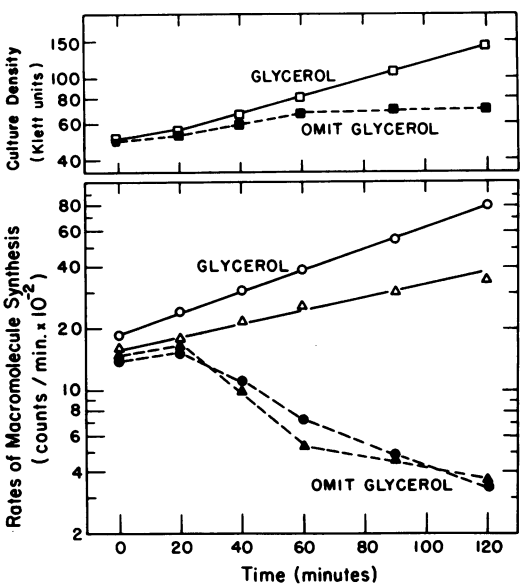

FIG. 1. *Macromolecule synthesis in the mutant in the absence of glycerol. Cells were grown in exponential phase to approximately 80 Klett units in medium AS supplemented with 0.4% glycerol. The cells were harvested, washed once by centrifugation and suspension in medium A at 37 C, suspended at 50 Klett units in medium AS, and divided into two equal portions. The two portions were incubated at 37 C with or without glycerol (0.4%). At the times indicated, samples were removed, and the rates of DNA (△, ▲) and protein (○, ●) synthesis were determined from the radioactivity incorporated. Growth (□, ■) was expressed in Klett units. Incubation with glycerol added is indicated by solid lines and open symbols (△, ○, □); incubation without glycerol is indicated by dashed lines and filled symbols (▲, ●, ■).*

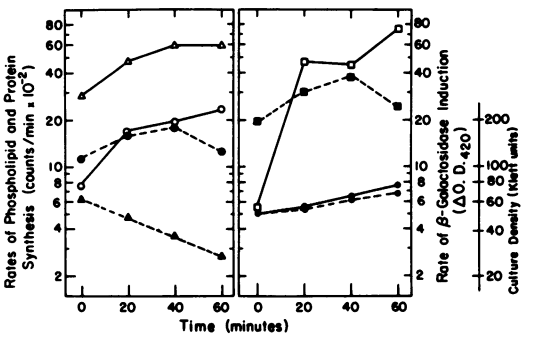

FIG. 2. *Synthesis of protein, phospholipids, and β-galactosidase in the mutant in the absence of glycerol. The culture conditions are identical to those given in Fig. 1, except that medium LPS supplemented with 0.4% glycerol was used as growth medium and medium A was replaced by medium LP in the washing procedure. Samples were withdrawn from cultures incubated in the presence or absence of 0.4% glycerol at 20-min intervals and processed for the measurement of protein synthesis (¹⁴C-isoleucine incorporation) (○, ●), phospholipid synthesis (△, ▲), and IPTG-induced β-galactosidase synthesis (□, ■). Culture density is expressed in Klett units. Incubation with glycerol is indicated by solid lines, and without glycerol by dashed lines.*

TABLE 2. *Incorporation of ^{14}C-isoleucine into membrane protein*

Incubation condition[a]	Time of ^{14}C-isoleucine addition[b]	Total protein as membrane protein[c]
	min	%
Glycerol	0	16.8
	20	18.0
	40	16.9
	60	15.5
Glycerol omitted	0	15.7
	20	15.9
	40	14.6
	60	13.0

[a] The culture conditions are identical to those given in Fig. 1.

[b] At the stated times, samples were withdrawn from cultures of the mutant incubated in the presence or absence of 0.4% glycerol, and ^{14}C-isoleucine incorporation into membrane and soluble protein fractions during a 15-min period was determined.

[c] The sum of radioactivity recovered from soluble and membrane fractions is taken as 100%.

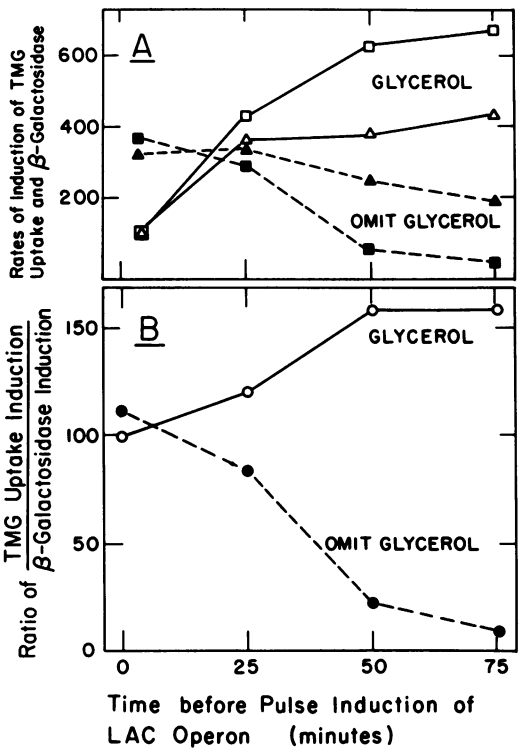

FIG. 3. *Requirement of glycerol for induction of transport. The culture conditions are identical to those in Fig. 1. Samples were withdrawn at 25-min intervals from cultures incubated in the presence or absence of 0.4% glycerol, pulse-induced for 15 min with 2×10^{-4} M IPTG, and processed for the assay of ^{14}C-TMG uptake (□, ■) and β-galactosidase activity (△, ▲). Data are expressed as per cent of the activity of the culture induced in the presence of 0.4% glycerol at time-zero. Induction with glycerol added is indicated by solid lines, and with glycerol omitted by dashed lines. (A) IPTG-induced synthesis of transport (TMG uptake) and β-galactosidase activity. (B) Ratio of transport to β-galactosidase.*

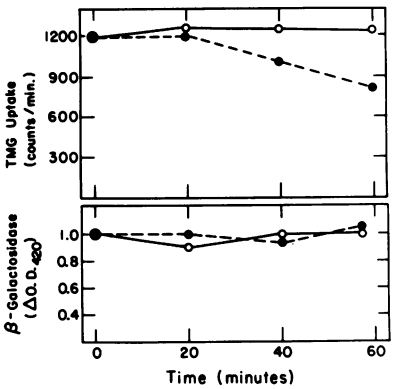

FIG. 4. *Effect of incubation in the absence of glycerol on the stability of transport activity and β-galactosidase activity. Cells of the mutant growing in exponential phase in medium AS supplemented with glycerol were pulse-induced for 15 min with 2×10^{-4} M IPTG. The cells were collected by centrifugation at 37 C, washed once by suspension in medium A, centrifuged, and suspended in medium AS. The cells were equally divided into two portions, one of which was supplemented with glycerol at 0.4%. The cultures were incubated at 37 C with shaking, and samples were withdrawn at 20-min intervals for assay of transport and β-galactosidase. The presence of glycerol is indicated by solid lines and absence of glycerol by dashed lines.*

tion is not sufficient to account for the reduced transport/β-galactosidase activity ratios noted in Fig. 3. In an experiment in which glycerol was added to a culture partly inactivated for transport as described in Fig. 4, no restoration of activity was observed.

Synthesis of M protein during glycerol starvation. The product of the *lac y* gene, the M protein, can be quantitated by an assay which measures the specific binding of a substrate for transport. The M protein is coded for by the middle of the three structural genes of the *lac* operon. Thus the ratio of induced synthesis of M protein/β-galactosidase should be unaltered by glycerol starvation since the ratio of thiogalactoside transacetylase/β-galactosidase induction is not affected by this condition. Since the shutting-off of lipid synthesis brought on by glycerol starvation could lead to a defect in folding of the

M protein, its synthesis was studied using the binding assay. The observation that the M protein synthesized during glycerol starvation is not inactive in galactoside binding (Table 3) shows that the defect in transport induction during glycerol starvation is due to events subsequent to translation.

Phospholipid turnover during glycerol starvation. The fate of lipid ^{32}Pi was followed in experiments in which lipid was labeled either for a short period or for several generations prior to glycerol removal. The only alteration in phospholipid composition was the selective loss of phosphatidylglycerol, but this occurred both in the presence and in the absence of glycerol (Table 4). In agreement with others, no significant turnover of total lipid phosphorus was observed over the time interval studied (13, 14).

DISCUSSION

The cessation of lipid synthesis after omission of glycerol from the growth medium for a mutant strain lacking L-glycerol-3-phosphate: NAD(P) oxidoreductase shows that the oxidoreductase catalyzes L-glycerol-3-phosphate biosynthesis in *E. coli*. It is likewise an indication that the other two known glycerophosphate dehydrogenases of this organism cannot supply L-glycerol-3-phosphate for lipid synthesis under the described conditions (16, 20).

The striking similarities between the decreased rates of DNA and protein synthesis upon prolonged glycerol starvation suggest a common cause. Whether this inhibitory phenomenon is mediated by a control mechanism or simply represents a decrease in energy reserves for macromolecule synthesis is not evident from this study.

The fact that incorporation of newly synthesized proteins into membrane does not require de novo lipid synthesis is in agreement with the recent findings of Mindich (J. Mol. Biol., *in press*), who has studied the properties of a glycerol-requiring auxotroph of *Bacillus subtilis*. Mindich also reports that the membrane-bound

TABLE 3. *Synthesis of the M protein during glycerol starvation*[a]

Growth condition during induction	β-Galactosidase	Transport (TMG uptake)	M protein (α-PNPG bound)
	%	%	%
Omit glycerol	43	5	42

[a] Activity induced with glycerol present is taken as 100%. A 1-liter culture of strain GR-1 was grown to 5×10^8 cells per ml in medium AS supplemented with 0.4% glycerol. The cells were collected by centrifugation at room temperature and washed once with sterile medium AS (glycerol omitted). The cells were suspended to the original volume in medium AS and incubated with glycerol omitted for 50 min at 37 C. At this point, the culture was divided into two equal portions, and each was diluted with an equal volume of medium AS containing 0.2 mM IPTG. Glycerol was added at 0.4% to one portion, and the portions were incubated for an additional 45 min. Chloramphenicol was then added at 50 μg/ml, and each portion was collected by centrifugation, washed once with SH-buffer containing chloramphenicol, and suspended in 40 ml of the same buffer. A 2.5-ml sample was removed from each portion for assay of β-galactosidase and TMG uptake, and the remainder of the portions was processed for the assay of M protein.

TABLE 4. *Phospholipid turnover in the mutant during incubation in the presence and absence of glycerol*[a]

Sample	Incubation conditions	Incubation time	Per cent of total radioactivity[b]			
			PS	PG	PE	CL
		min				
Prepn A		0	1.2	10.4	88.0	0.5
	Glycerol	20	0.7	10.7	88.0	0.5
		40	0.8	8.4	90.2	0.4
		60	0.5	7.8	91.6	ND
	Omit glycerol	20	0.9	11.7	87.0	0.6
		40	1.6	8.9	88.0	1.1
		60	1.0	6.3	91.5	1.0
Prepn B		0	1.9	10.9	86.9	0.4
	Glycerol	20	1.5	9.4	88.6	0.6
		40	1.5	9.0	89.0	0.5
		60	1.4	7.6	90.4	0.6
	Omit glycerol	20	1.5	11.6	86.3	0.6
		40	1.6	12.4	85.4	0.6
		60	1.5	9.0	89.0	0.6

[a] Cultures growing in exponential phase in medium LPS supplemented with 0.4% glycerol were labeled by exposure to ^{32}Pi for 0.2 generations of growth (preparation A) and for four generations of growth (preparation B). The cells were harvested, washed twice with medium A, suspended in medium AS (zero-time sample was removed at this point), and divided equally. One portion was incubated in the presence, and the other in the absence, of 0.4% glycerol. Samples were withdrawn at 20-min intervals and processed for phospholipid extraction. The sum of radioactivities in each individual phospholipid is taken as the total.

[b] Abbreviations: PS, phosphatidylserine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; CL, cardiolipin; ND, no detectable radioactivity.

succinic dehydrogenase formed during glycerol starvation is functional in an in vitro assay which does not require a functionally intact electron transport chain. In our study, induction of proteins of the *lac* operon, including M protein assayed by a substrate-binding technique, proceeds at the same relative rate during glycerol starvation. The synthesis during glycerol starvation of M protein which is membrane bound and binds galactosides but is nonfunctional for galactoside uptake indicates that certain species of membrane protein require simultaneous de novo lipid synthesis for assembly into fully functional ordered structures.

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