Phospholipid Synthesis in *Escherichia coli* Infected with T4 Bacteriophages

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After infection of *Escherichia coli* with T4 phage, phospholipid synthesis continued but at a reduced rate. The same phospholipid components were synthesized as in uninfected cells; however, the relative rates of $^{32}\text{P}_i$ incorporation into phosphatidylglycerol (PG) and phosphatidylethanolamine (PE) were altered. This alteration was most pronounced during the first 10 min after infection. Under these conditions, the isotope incorporated into PG equaled or exceeded that found in PG from uninfected cells. Chloramphenicol (CM) added before, but not 5 min after, infection inhibited the relative increase in PG synthesis, and CM added at different times after infection indicated that a protein synthesized between 3 and 6 min was required for this change to occur. Supplies of exogenous L-serine or L- α -glycerol-P failed to affect the relative rates of $^{32}\text{P}_i$ incorporation into PG and PE by infected or uninfected cells. Phospholipid synthesis was somewhat higher after infection with T4rII mutants than after infection with wild-type phage. After infection with these mutants or several amber mutants, the relative synthesis of PG and PE was characteristic of T4r⁺-infected cells. The phospholipid synthesized after infection did not rapidly turn over, but infection accelerated the loss of PG synthesized prior to infection.

When *Escherichia coli* is infected with T-even bacteriophages, biosynthesis of host deoxyribonucleic acid (DNA), ribonucleic acid, and enzymes ceases, and new biosynthetic reactions involved in phage production appear (8). Although polymer synthesis changes from bacterial to phage products, the reactions that provide the subunits and the energy for their activation are, for the most part, those used by the cell prior to infection. An actively metabolizing cell is a prime requirement for phage replication (7); although the cell wall and membrane are not known to contribute directly to the synthesis of phage, these structures must be maintained intact for phage production to proceed. There are several consequences of infection that suggest an effect on the cell membrane: a transient leakage of cell constituents (18, 19), increased permeability to acridines (21, 22), and the restriction of T4rII mutants in *E. coli* K-12 (λ) (2, 12, 20). The question of whether phage-directed processes play a role in the maintenance of the cell wall and membrane or whether these structures are altered has not been examined. As an initial approach to this question, we have studied the changes in phospholipid synthesis in *E. coli* infected with bacteriophage T4. Phospholipids were selected for this investigation because they constitute a large part of the cell membrane

(15), they can be easily isolated, and the two major components, phosphatidylglycerol (PG) and phosphatidylethanolamine (PE), can be readily separated by chromatographic procedures. The results of the experiments presented in this paper show that infection with T4 affects the rates at which these components are synthesized. The changes occurred soon after infection and correlate in time with the alteration in permeability previously observed.

MATERIALS AND METHODS

Bacteria and phages. *E. coli* B, obtained from S. S. Cohen, was the host strain for the majority of the experiments. K-10 strain 8, which is constitutive for L- α -glycerol-P transport and which lacks the catabolic L- α -glycerol-P dehydrogenase, was provided by E. C. C. Lin (13). The bacteriophages used were T4r⁺ from S. S. Cohen; T4rII 88H (deletion in A cistron) and T4rII 187 (deletion in B cistron) from S. Benzer via J. Flaks; amber mutants C290, N116, and N130 from R. S. Edgar; am E51 from J. S. Wiberg; and amber mutants B24 and N134 from F. Frankel.

Growth conditions. An overnight culture of *E. coli* was diluted 1:20 into fresh medium and was grown with aeration at 37°C in the minimal medium of Hershey (14), which contained 5, 15, or 50 μg of P_i per ml, glucose sterilized by filtration (final concentration, 1 mg/ml), and 0.1% casein hydrolysate. The latter was omitted in experiments in which serine-3- ^{14}C was used to label phospholipids. In the experiment that deter-

mined the fate of phospholipids synthesized prior to infection, an overnight culture was diluted 1:40 into medium containing 5 μg of $^{32}\text{P}_i$ per ml and 0.5 μC of $^{32}\text{P}_i$ per ml. When the culture reached 2×10^8 cells/ml, it was chilled and washed by centrifugation with medium containing 50 μg of $^{32}\text{P}_i$ per ml. Cells were then diluted to the original volume in the latter medium and grown to 2.7×10^8 cells/ml; the culture was divided and part of it was infected. Chloramphenicol (CM) was added at different times before, at the time of, or after infection, at a final concentration of 50 or 100 $\mu\text{g}/\text{ml}$. Growth was measured turbidimetrically with a Klett colorimeter (no. 42 filter) and by plating diluted samples on nutrient agar plates.

Conditions of infection. When the bacterial culture reached 2×10^8 cells/ml, the culture was divided, tryptophan was added (50 $\mu\text{g}/\text{ml}$), and bacteriophages were added at an input multiplicity of between 3 to 10 phages per bacterium. Phage titers, infectious centers, and survivors were assayed as described by Adams (1). Antiserum was prepared by standard methods. Survivors usually comprised 0.1 to 1.0% of the culture.

Conditions of radioactive labeling. $^{32}\text{P}_i$ was added either immediately after phage or 5 min later. In pulse-labeling experiments, $^{32}\text{P}_i$ was added at different times after infection and the sample was subsequently precipitated by trichloroacetic acid; in all experiments, $^{32}\text{P}_i$ was carrier-free and at a final concentration of 0.5 or 1.0 $\mu\text{C}/\text{ml}$. The specific activity of ^{14}C -serine and ^{14}C -L- α -glycerol-P is given in the text.

Chemicals and analytical procedures. Phosphatide standards were obtained from Mann Fine Chemicals, Inc. (New York, N.Y.) and from Pierce Chemical Co. (Rockford, Ill.), $^{32}\text{P}_i$ from E. R. Squibb & Sons (New York, N.Y.), and serine-3- ^{14}C from Calbiochem (Los Angeles, Calif.). Glycerol-1,3- ^{14}C was purchased from International Chemical and Nuclear Corp. (City of Industry, Calif.) and was enzymatically converted to L- α -(1,3- ^{14}C)-glycerol-P (5). The L- α -(1,3- ^{14}C)-glycerol-P was purified by chromatography on a Dowex-formate column. DL- α -Glycerol-P was purchased from Sigma Chemical Co. (St. Louis, Mo.). CM was a gift of Parke-Davis & Co. (Detroit, Mich.). WB-2 resin-impregnated paper was purchased from H. Reeve Angel & Co. (Clifton, N.J.).

Total phosphate was determined by the method of Bartlett (4). To assay radioactivity, duplicate samples on phospholipid were plated on stainless-steel planchets, dried, and counted in a windowless, gas-flow counter (Nuclear-Chicago Corp., Des Plaines, Ill.). Planchets were counted for a total of 2,000 or 20,000 counts. The data presented in the tables are expressed as total radioactivity in the phospholipid extracted from the cells.

Phospholipid extraction. Ten or 25-ml samples of cell suspensions were precipitated by trichloroacetic acid at a final concentration of 5% (w/v). The precipitate was centrifuged and the supernatant fluid was poured off. Phospholipids were extracted and washed by the method of Kanfer and Kennedy (16). Samples were either plated and counted for radioactivity, or the phosphate content was measured.

Fractionation of phospholipids. Samples of washed

or unwashed phospholipids were chromatographed on WB-2 resin-impregnated paper with diisobutylketone-acetic acid-water (8:5:1) as the solvent system (6). Up to 2 ml of lipid extract was placed in a 2.5-cm strip, and the chromatogram was developed from 7 to 9 hr. During this time, the solvent reached the end of the paper (45 cm) and dripped off. The water-soluble, deacylated derivatives were also separated by chromatography. The washed chloroform solution of phospholipids was deacylated by the procedure of Dawson (9) or by a modification of the procedure described by Ballou et al. (3). The aqueous solution of the deacylated phospholipid was evaporated by a stream of air at less than 50 C and was taken up in 100 μliters of water; 50- μliter samples were spotted on Whatman no. 1 paper. Descending paper chromatography with a solvent composed of phenol (48 g), water (12 ml), and concentrated ammonium hydroxide (0.2 ml) was used to separate the compounds.

The location of the phospholipids or their deacylated derivatives was determined by exposing the chromatograms to X-ray film. After development of the radioautograms, the compounds were qualitatively eluted from the resin-impregnated paper with chloroform-methanol-NH₄OH (20:5:1) or from the phenol chromatogram with water. Duplicate samples of each component were plated, dried, and counted.

The technique of separating phospholipids on resin-impregnated paper became available after many of the experiments described in this paper had been performed with chromatography of the water-soluble, deacylated phospholipid derivatives. Essentially, the results obtained were the same with both techniques; however, the ion-exchange paper had the advantage that fewer manipulations were involved; e.g., KCl washing was not necessary. Occasionally, the separation of the deacylated derivatives on the phenol chromatogram was not considered satisfactory and the experiment had to be repeated. The chromatography on the resin-impregnated paper was at all times reproducible.

Thin-layer chromatography was performed on silica gel F₂₅₄ with chloroform-methanol-NH₄OH as the solvent (6:3.5:0.5).

Fractionation of cell components. After the phospholipids were extracted from the cells by the method described above, nucleic acids were extracted with 5% (w/v) trichloroacetic acid for 15 min at 90 C. The insoluble material was collected by centrifugation, washed with 5% trichloroacetic acid, and then treated with 1.5 N NH₄OH. The material made soluble by NH₄OH was considered to be protein. The radioactivity in samples of each fraction was measured, and the distribution of isotope in the three fractions was calculated.

RESULTS

Identification of phospholipids synthesized after infection with T4r⁺. *E. coli* was infected with T4r⁺ and $^{32}\text{P}_i$ was added to the medium; 45 min after infection, the cells were precipitated with trichloroacetic acid and the phospholipids were extracted and chromatographed. Chromatography was performed on the resin-impregnated

paper with and without KCl washing, and with the phenol-water system after washing and deacylation.

Radioautograms showed the same phospholipid compounds in infected bacteria as in the control culture of uninfected bacteria. Figure 1 shows the separation of the lipids obtained with the resin system. Washing the phospholipids with KCl removed the nonlipid material that remained at the origin of the chromatogram (not shown), and concentrated the phospholipid in the organic layer by removing the methanol present in the extract. Figure 2 shows the radioautogram of the deacylated phospholipids. R_f values for these compounds from the infected culture were 0.09,

0.15, 0.32, and 0.60. These values were in close agreement with those reported by Kanfer and Kennedy (16) for the deacylated phospholipids of uninfected *E. coli* B: glycerophosphate, 0.09; glycerophosphorylserine, 0.17; glycerophosphorylglycerol (GPG), 0.37; and glycerophosphoryl-ethanolamine (GPE), 0.65. Standard phosphatidyl-L-serine and PE, subjected to the same procedures, gave the R_f values reported. The standard phosphatides were detected with a ninhydrin spray. The two major phospholipid components on the resin paper were eluted and identified by paper chromatography of their deacylated derivatives and by thin-layer chromatography. The minor components, which

FIG. 1. Radioautogram of the phospholipids separated by chromatography on ion-exchange paper. Washed and unwashed extracted phospholipid (0.4 ml) from infected cells was chromatographed with 0.4 ml of unwashed phospholipid from uninfected cells.

FIG. 2. Radioautogram of the deacylated phospholipids separated by paper chromatography.

contained approximately 10% of the $^{32}\text{P}_i$ incorporated into phospholipid, were not identified.

Kinetics of phospholipid synthesis measured by cumulative and pulse uptake of $^{32}\text{P}_i$ and serine- $3\text{-}^{14}\text{C}$. As suggested by the radioautograms (Fig. 1 and 2), the rate of $^{32}\text{P}_i$ uptake into phospholipids decreased after infection as compared with uninfected bacteria (Fig. 3), but isotope accumulated in phospholipids as late as 75 min after infection. In several experiments, phosphate analyses were performed on samples of unfractionated phospholipids obtained at different times after infection. Phosphate present in lipid

increased about 42% between 5 and 75 min after infection, and about 160% in uninfected bacteria during the same time interval. These results show that the uptake of isotope reflects net synthesis of phospholipids.

To obtain a more sensitive measurement of phospholipid synthesis, the uptake of $^{32}\text{P}_i$ or serine- $3\text{-}^{14}\text{C}$ into phospholipids was measured during 5-min intervals at various times after infection. Isotope incorporation (Fig. 4) confirmed that synthesis occurred as late as 75 min after infection.

Characteristics of phospholipid synthesis after

FIG. 3. Rate of synthesis of phospholipids and turbidity changes in infected and uninfected *Escherichia coli* B cells. The bacteria were infected with an input multiplicity of 4 phages per bacterium when the concentration of bacteria was 3×10^8 cells/ml. Four per cent of the cells survived infection, and 60% of the cells were recovered as infectious centers. Symbols: ●, uninfected cells; ■, infected cells.

FIG. 4. Cumulative and pulse uptake of ³²P_i and serine-3-¹⁴C into phospholipids of *Escherichia coli* B infected with T4r⁺. In the cumulative experiment, serine was at a concentration of 0.5 mM and a specific activity of 50,000 counts per min per μ mole; in the pulse experiment, the concentration was 0.05 mM and the specific activity was 500,000 counts per min per μ mole. ³²P_i uptake is designated by solid lines (●) and solid bar graphs; serine-3-¹⁴C by dashed lines (▲) and shaded bar graphs.

infection of *E. coli* B with T4r⁺ or T4rII. To determine whether phospholipid synthesis after infection was dependent on the specific infectious agent, we compared T4r⁺ with 2 T4rII mutants. These mutants were selected because previous

studies suggested that the rII function involved the cell envelope (2, 12, 20). Since *E. coli* B infected with T4rII lyses 25 to 30 min after infection, these studies were terminated at 20 min. The rate of isotope incorporation into phospholipid was higher when *E. coli* B was infected with T4rII than when it was infected with T4r⁺ (Fig. 5). Both T4rII mutants tested (88H and 187) gave the same results.

To obtain a more precise picture of the effect of phage infection on phospholipid synthesis, isotope incorporation into the phospholipid components of *E. coli* was examined quantitatively after chromatography with or without prior deacylation.

The radioautogram of a chromatogram (Fig. 6) showed greater incorporation of ³²P_i into the T4rII-infected cells than into cells infected with T4r⁺, and suggested that after infection with either phage the incorporation into PG and PE was approximately equal. PG and PE or GPG and GPE were eluted from chromatograms and counted. The results (Table 1) show that infection altered the relative incorporation of ³²P_i into the major phospholipid components. At 10 min after infection with either T4r⁺ or T4rII, the isotope incorporation into PG was the same or higher than that obtained with uninfected cells (Table 1). After infection with either T4r⁺ or T4rII, isotope incorporation into PE was lower than in uninfected cells.

A convenient way to express the relative incorporation of ³²P_i into the major phospholipid components is as a ratio. When the ratios of

GPG/GPE from various experiments were calculated and averaged, the ratio in uninfected *E. coli* B was 0.35; in T4r⁺-infected cells, 0.70; in T4rII-infected cells, 0.80. The average ratio of PG/PE was 0.38 for uninfected *E. coli* B and 0.90 when these cells were infected with T4r⁺.

The minor phospholipid components contained 10% of the ³²P_i incorporated into phospholipid under all conditions of infection, and the distribution of isotope was the same in infected and in uninfected cells.

The possibility existed that the reduction in PE synthesis after infection was the result of a

reduced supply of serine. This hypothesis was tested by supplementing the medium with serine. The results of an experiment with T4rII (Table 2) showed that exogenous serine failed to increase PE synthesis significantly. Similar results were obtained with T4r⁺. These experiments eliminated the possibility that a defective serine supply was the cause of the observed change in PE synthesis and suggested that the relative increase in PG synthesis after infection was a specific response to the phage.

Effect of CM on phospholipid synthesis after infection. We wanted to determine whether phage-directed protein synthesis was required to produce the changes in phospholipid synthesis observed after infection. CM was used to stop protein synthesis prior to or after infection, and the extent and character of the phospholipid synthesized was determined. Figure 7A shows that CM reduced phospholipid synthesis in uninfected bacteria and, when added prior to infection, reduced the rate of synthesis below that normally found after infection. A similar experiment with T4rII showed that CM added prior to infection reduced the rate of ³²P_i incorporation (Fig. 7B) and prevented the change in distribution of the isotope (Table 3). When CM was added 5 min after infection, the characteristic change in distribution of ³²P_i between PG and PE occurred.

Pulse labeling of phospholipids. The previous experiment suggested that within 5 min after infection a protein was synthesized that was required for the change in phospholipid synthesis. To determine with greater precision when this protein was synthesized, CM was added to samples of an infected culture at different times after infection, ³²P_i was added to the CM-treated cells 1 min later, and after an additional 10 min the infection was terminated with trichloroacetic acid. The distribution of isotope between PG and PE is shown in Table 4, experi-

FIG. 5. Uptake of ³²P_i into phospholipid of *Escherichia coli* B uninfected or infected with T4r⁺ or T4rII. Cells were infected with T4r⁺ and T4rII 88H at multiplicities of 4.4 and 6.7, respectively. After infection with T4r⁺, 80% of the cells were recovered as infectious centers and 6.2% as survivors. After infection with T4rII 88H, the infectious centers were 110% and the survivors 0.07%.

TABLE 1. Radioactivity in major phospholipid components of infected and uninfected *Escherichia coli*

Expt ^a	Phospholipid	Radioactivity (counts/min)					
		Uninfected cells		T4r ⁺ -infected cells		T4rII-infected cells	
		10 min ^b	20 min	10 min	20 min	10 min	20 min
I	PG	10,200	30,200	10,600	22,200	10,209	24,900
	PE	24,300	70,000	10,900	21,000	10,050	25,900
II	GPG	4,030	11,810	3,305	3,830	8,180	15,820
	GPE	11,430	34,220	4,330	6,225	8,940	19,820

^a In experiment I, unwashed phospholipids extracted from infected or uninfected *E. coli* B were chromatographed on resin-impregnated paper. In experiment II, the phospholipids from *E. coli* B were deacylated and the water-soluble derivatives were chromatographed.

^b Minutes after infection.

FIG. 6. Radioautogram of phospholipid from $T4rII$ - and $T4r^+$ -infected cells of *Escherichia coli* B. Samples were taken 20 min after infection, and 0.4 ml of the lipid extract was chromatographed without KCl washing.

ment I. These data indicate that a protein synthesized between 3 and 6 min was required to produce the PG/PE ratio characteristic of infected cells, and that the largest changes in phospholipid synthesis occurred early after infection. By 16 min after infection, the rate of isotope incorporation into PG was reduced but the PG/PE ratio remained higher than in uninfected cells. A similar pulse-labeling experiment performed in the absence of CM confirmed this result (Table 4, experiment II).

Certain amber mutants were selected to represent early and late functions and to include mutations in genes mapping near the rII region (11).

The results of the experiment with the amber mutants are summarized in Table 5. In all cases, the incorporation of $^{32}P_i$ into phospholipids was somewhat greater than when the infection was performed with $T4r^+$, and the PG/PE ratio characteristic of infected cells was observed.

Effect of infection on preformed phospholipid. The effect of infection on lipids of the uninfected cell was determined by prelabeling with $^{32}P_i$ and measuring the loss of isotope after infection. As shown in Fig. 8A, by 80 min, the uninfected cell lost 38 and 21% of the label initially present in PG and PE, respectively, whereas the infected cell lost 68 and 32%, respectively. When the

TABLE 2. Radioactivity in phospholipid after infection in the presence or absence of serine

Expt ^a	Phospholipid	Radioactivity (counts/min)					
		Uninfected cells		T4rII-infected cells			
				Without serine		With serine ^b	
		10 min ^c	20 min	10 min	20 min	10 min	20 min
I	PG	9,720	19,200	10,960	19,720	8,920	17,600
	PE	17,000	48,000	10,400	18,800	8,520	19,680
II	GPG	11,910	20,800	14,280	33,650	13,820	28,080
	GPE	41,000	86,300	19,600	47,400	20,550	53,400

^a In experiment I, the phospholipids were chromatographed, whereas in experiment II the deacylated derivatives were chromatographed.

^b The serine concentration was initially 3 mM. T4rII 88H was used.

^c Minutes after infection.

FIG. 7. Effect of chloramphenicol (CM) on uptake of $^{32}\text{P}_i$ into phospholipid of *Escherichia coli* B cells, uninfected or infected with T4r⁺ or T4rII. (A) Infection was with T4r⁺. (B) Infection was with T4rII 88H. Symbols: ●, uninfected; ○, uninfected plus CM; ■, infected; □, infected plus CM at +5 min; △, infected plus CM at -5 min.

data in Fig. 8A were plotted as a first-order decay curve (Fig. 8B), the half-life for PG in uninfected cells was approximately 120 min and the half-life for PG in infected cells was 60 min.

Effect of α -glycerol-P on phospholipid synthesis. To test the possibility that the increase in PG synthesis relative to PE that occurred after infection was due to an increase in the availability of α -glycerol-P, we investigated the effect of supplying this compound exogenously. Because *E. coli* B was impermeable to phosphorylated compounds, *E. coli* K-10 strain 8, which is constitutive for the transport of L- α -glycerol-P, was used. Table 6 shows the distribution of ^{14}C -L- α -glycerol-P in the constituents of the cell.

Approximately 90% of the radioactivity was present in phospholipid; when L- α -glycerol-P was initially present at 0.1 mM, uptake was proportional to the growth of the culture for at least 40 min. The effect of adding unlabeled α -glycerol-P on the incorporation of $^{32}\text{P}_i$ into lipids is shown in Fig. 9. The marked reduction in $^{32}\text{P}_i$ incorporation was observed in infected and uninfected cells when α -glycerol-P was added either at the same time as $^{32}\text{P}_i$ or 15 min later. The relative incorporation of $^{32}\text{P}_i$ into PG and PE in the presence or absence of α -glycerol-P was the same (Table 7) in infected and in uninfected cells.

TABLE 3. *Effect of chloramphenicol (CM) on the distribution of $^{32}\text{P}_i$ in the major phospholipids after T4rII infection^a*

Time after infection (min)	Uninfected (counts/min)		CM added 5 min before infection (counts/min)		CM added 5 min after infection (counts/min)		Infected, no CM (counts/min)	
	PG	PE	PG	PE	PG	PE	PG	PE
10	8,100	17,700	3,300	8,400	13,200	12,900	13,200	10,500
20	20,600	51,900	7,200	18,000	15,600	24,000	23,600	30,600

^a Phospholipid components in 0.4-ml portions were separated on resin-impregnated paper. T4rII 88H was used to infect the culture.

TABLE 4. *Pulse labeling of phospholipid after infection^a*

Expt	Time of CM addition (min)	Time of $^{32}\text{P}_i$ addition or pulse time ^b (min)	PG (counts/min)	PE (counts/min)	PG/PE
I	0	1	5.9×10^3	17.9×10^3	0.33
	2	3	7.1×10^3	19.2×10^3	0.37
	5	6	10.5×10^3	14.9×10^3	0.71
	5	16	5.2×10^3	8.4×10^3	0.62
	15	16	7.6×10^3	11.3×10^3	0.68
	None ^c	0	10.2×10^3	27.9×10^3	0.36
II	0 ^c	0	5.4×10^3	16.4×10^3	0.33
		0-10	4.4×10^4	5.4×10^4	0.81
		10-20	2.0×10^4	3.6×10^4	0.55
		20-40	7.4×10^4	12.5×10^4	0.61

^a Samples of the uninfected culture were taken just prior to the addition of phage (time 0).

^b Times in experiment I refer to $^{32}\text{P}_i$ addition; times in experiment II refer to pulse time.

^c Uninfected cells used.

TABLE 5. *Radioactivity in the major phospholipids of Escherichia coli B infected with amber mutants of T4*

Type of phage ^a	Defective gene	Radioactivity in lipid (counts/min)		Radioactivity in lipid components at 20 min (counts/min)	
		10 min	20 min	PG	PE
Uninfected cells.....	—	5,750	17,700	2,500	6,360
T4r ⁺	None	4,040	8,750	2,050	2,730
B24 (dHMP kinase ⁻).....	1	4,800	7,800	3,800	3,890
E51 (dCTPase ⁻).....	56	5,800	9,650	3,317	4,172
N130 (DNase ⁻).....	46	5,000	8,450	3,745	3,275
N134 (DNA maturation ⁻).....	33	5,800	9,500	3,840	5,670
N116 (near rII).....	39	4,700	10,300	2,709	2,963
C290 (tail fibers ⁻).....	38	5,450	11,750	3,973	4,514

^a Abbreviations: dHMP kinase, deoxyhydroxymethylcytidylate kinase; dCTPase, deoxycytidine triphosphatase; DNase, deoxyribonuclease.

DISCUSSION

Phospholipid synthesis continued after infection with T4, as measured by $^{32}\text{P}_i$ uptake or increase in phosphate, but the rate of total phospholipid synthesis was reduced and the relative amounts of $^{32}\text{P}_i$ incorporated into the

major compounds was altered. These results raise the question of whether these changes relate to the infectious process or to the mechanisms which control the rates of PG and PE synthesis. A partial answer to this question was obtained from studies with the antibiotic CM. The experi-

FIG. 8. Effect of infection on prelabeled phospholipids. The unwashed lipid extract (1.0 ml), extracted from infected and uninfected cells, was separated into PG and PE by chromatography on resin-impregnated paper. Each point represents the phospholipids from an independent sample of bacteria carried through the extraction and chromatographic procedure. $T4r^+$ was added to half the culture at zero-time, which was 15 min after the culture resumed growth in medium containing $^{32}\text{P}_i$. Symbols: $\bullet$, PE from uninfected cells; $\blacksquare$, PE from infected cells; $\circ$, PG from uninfected cells; $\square$, PG from infected cells.

ments with this antibiotic showed that the protein synthesis which occurred between 3 and 6 min after infection was required for the characteristic change in the ratio of PG to PE synthesized, but that continued protein synthesis after 6 min was not necessary. Since it is generally accepted that synthesis of host proteins stops after infection, it appears that "early" viral protein is involved in or related to the changes in phospholipid synthesis observed. Whether these

changes are produced by viral enzymes or by a redirection of host activities is uncertain. A possible approach to this question and to the role of phospholipid synthesized after infection would be the study of phage mutants that fail to produce the characteristic changes. At present, all mutants tested showed the relative increase in PG synthesis. Cells infected with the $T4rII$ mutants, which were chosen for special attention because of the proposed relationship of the rII function with permeability, synthesized more lipid than cells infected with $T4r^+$ and usually showed a small increase in PG synthesis when compared to cells infected with wild-type phage. This increase in PG was also observed when *E. coli* K-12 (λ), the nonpermissive host, was infected with $T4rII$ (5a). The significance of the difference between $T4rII$ and $T4r^+$ is questionable in view of similar results obtained with the amber mutants which were defective in a variety of functions.

Although it cannot be stated definitely that the observed phospholipid synthesis relates to the infectious process, the correlation in time between the largest changes in the PG/PE ratio (0 to 10 min) and the changes in the permeability of the cell that occur soon after infection (2, 12, 18–22) make such a relationship plausible. The bulk of the phospholipid is in the cell membrane, and it appears reasonable to postulate that the early lipid synthesis is involved with resealing the cell membrane which presumably stops the leakage of cellular constituents (17, 18).

TABLE 6. Distribution of ^{14}C - α -glycerol-P in the cellular components of *Escherichia coli* K-10 strain 8

Expt	Time (min)	Phospholipid (counts/min) ^a	Nucleic acid (counts/min) ^a	Protein (counts/min) ^a
I	10	68,400	3,000	5,160
	20	114,900	6,720	9,490
	40	144,000	7,390	11,750
	60	123,900	10,940	17,160
II	10	18,600	1,650	2,900
	20	40,800	3,530	6,800
	40	93,600	9,500	13,800
	60	114,600	10,800	21,320

^a Total radioactivity in the samples is presented. Radioactive L- α -glycerol-P was added when the cells reached a concentration of 2×10^8 /ml, and 2 ml of the culture was removed for fractionation at the times indicated. In experiment I, the initial L- α -glycerol-P concentration was 20 μM , and the specific activity was 6 $\mu\text{C}/\mu\text{mole}$. In experiment II, the initial L- α -glycerol-P concentration was 100 μM , and the specific activity was 1.2 $\mu\text{C}/\mu\text{mole}$.

By 15 min after infection, PG synthesis was reduced and the PG/PE ratio dropped from between 0.8 and 1.0 to about 0.6. There are turbidity changes in the infected culture that correspond to these changes in phospholipid synthesis. As shown by Doermann (10), early after infection the turbidity of the culture initially drops and then regains the value it had just prior to infection. Subsequently, in cultures subject to lysis inhibition, the turbidity continues to rise.

FIG. 9. Incorporation of $^{32}\text{P}_i$ into phospholipid of *Escherichia coli* K-10 strain 8. Time of addition of phage is considered zero-time. Symbols: ●, uninfected cells; ○, uninfected cells with α -glycerol-P added at zero-time; ◐, uninfected cells with α -glycerol-P added at 15 min; ■, cells infected with T4r^+ ; □, cells infected with T4r^+ and given α -glycerol-P at zero-time; ◑, cells infected with T4r^+ and given α -glycerol-P at 15 min.

The same changes in turbidity were observed under the experimental conditions used for this investigation (Fig. 3B). The lipid synthesized early may be involved in resealing the cell, whereas lipid synthesized later may meet a requirement for the enlargement of the cell envelope.

That we are indeed dealing with synthesis rather than turnover of phospholipid is suggested by the phosphate analyses and the stability of material synthesized after infection. This stability was inferred from the experiment with *E. coli* K-10 strain 8, in which the $^{32}\text{P}_i$ incorporated into phospholipid after infection remained in this material after α -glycerol-P was added to stop further incorporation of isotope. The PG present in the cell prior to infection underwent accelerated decay after infection. The loss of this material would be expected to increase the demand for PG if a fixed ratio of PG/PE is required in the cell envelope.

Knowledge of the mechanism responsible for the changes in phospholipid synthesis after infection may have to await an understanding of the process that maintains the balance between PG and PE in the uninfected cell. The results of the nutritional experiments rule out the possibility that the amount of each phosphatide depends on the supply of the substrates L-serine and L- α -glycerol-P. In these experiments, phospholipids were synthesized from excess exogenous serine or L- α -glycerol-P, and the relative amounts of PG and PE were the same as when endogenous substrates were utilized. A more likely explanation for the different rates of PG and PE synthesis after infection is that the enzymatic makeup of the cell changes, or that the control process which regulates the activity of the enzymes responsible for phosphatide synthesis is altered. Since both PG and PE arise from the common precursor, cytidine diphosphate-diglyceride (17), reactions that involve this compound are a likely point for such controls to act.

Another possibility to be considered is that the

TABLE 7. Radioactivity in the major phospholipids of *Escherichia coli* K-10 strain 8 in the presence and absence of α -glycerol- P^a

Time after infection (min)	Uninfected (counts/min)		Uninfected plus α -glycerol- P^a (counts/min)		Infected (counts/min)		Infected plus α -glycerol- P^a (counts/min)	
	PG	PE	PG	PE	PG	PE	PG	PE
20	33,870	65,757	5,400	9,700	6,500	6,257	1,385	1,436
40	68,000	193,100	12,615	32,080	12,018	13,165	2,037	2,900

^a L- α -Glycerol-P, at an initial concentration of 0.2 mM, was added at the same time as $^{32}\text{P}_i$ and T4r^+ . The compounds were separated on resin-impregnated paper.

rate of PG and PE synthesis depends on the cell's requirements for those compounds. These requirements may differ in different regions of the cell envelope. Infection, by altering the relationships between the components of the envelope, could alter the rate of biosynthesis of PG and PE. Consistent with this hypothesis is the observation that different physiological conditions, such as the phase of growth of the culture, change the ratio of PG/PE. The ratio of PG/PE synthesized in stationary-phase cells is 0.90 (Pizer and Pylkas, *unpublished data*), as compared to 0.30 to 0.40 in log-phase cells. The similarity between infected and stationary-phase cells may be simply fortuitous, but it points out the difficulty in interpreting changes in complex structures. Although phospholipids may not be the components of the membrane initially affected by infection, the ease in labeling, isolating, and characterizing these compounds makes them a suitable probe for the detection of changes in the cell surface.

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