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EFFECTS OF DL-GLYCERALDEHYDE 3-PHOSPHATE AND DL-3-HYDROXY-4-OXOBUTYL-1-PHOSPHONATE ON PHOSPHOLIPID METABOLISM IN E. COLI

by

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ABSTRACT

EFFECTS OF DL-GLYCERALDEHYDE 3-PHOSPHATE AND DL-3-HYDROXY-4 OXOBUTYL-1-PHOSPHONATE ON PHOSPHOLIPID METABOLISM IN E. COLI

by

Chu-Tay Tang

Adviser: Professor Burton E. Tropp

At a concentration of 2.5 mM DL-glyceraldehyde 3-phosphate is bactericidal, whereas its phosphonic acid analogue, DL-3-hydroxy-4-oxobutyl-1-phosphonate is bacteriostatic. The glycerol 3-phosphate (G3P) transport system is required for the entry of the biologically active L-enantiomer. L-glyceraldehyde must be phosphorylated by the cell to exert its full effect upon growth.

The addition of glyceraldehyde 3-phosphate to a culture of Escherichia coli caused no preferential inhibition of the accumulation of deoxyribonucleic acid, ribonucleic acid, or phosphoglycerides, the accumulation of proteins was less affected. There is no differential effect upon the accumulation of phosphatidylethanolamine and phosphatidylglycerol, although cardiolipin was not markedly affected. Pulse labeling studies revealed that the rate of deoxyribonucleic acid and phosphoglyceride synthesis were most affected by glyceraldehyde 3-phosphate. The rate of phosphatidylethanol-

amine synthesis was most severely inhibited during the one hour treatment with glyceraldehyde 3-phosphate. 3-Hydroxy-4-oxobutyl-1-phosphonate had a similar effect on the accumulation of deoxyribonucleic acid, ribonucleic acid, phosphoglyceride and protein to that described for glyceraldehyde 3-phosphate. However, the inhibition of phosphatidylglycerol accumulation was slightly stronger than that of phosphatidylethanolamine. Differences were also observed concerning the rate of phosphoglyceride synthesis. 3-Hydroxy-4-oxobutyl-1-phosphonate has its greatest inhibitory effect on the rate of phosphatidylglycerol synthesis at early times, whereas it is most inhibitory to the rate of phosphatidylethanolamine synthesis at later times. The inhibition of the rate of synthesis of macromolecules and phosphoglycerides is not due to a nucleotide inhibition caused by either glyceraldehyde 3-phosphate or 3-hydroxy-4-oxobutyl-1-phosphonate.

Studies with mutant strains ruled out aerobic G3P dehydrogenase, G3P synthase, and fructose 1,6-diphosphate aldolase as the primary sites of action. L-glyceraldehyde 3-phosphate and corresponding L-form of 3-hydroxy-4-oxobutyl-1-phosphonate are competitive inhibitors of sn-glycerol 3-phosphate in the reactions catalyzed by acyl coenzyme A:sn-glycerol 3-phosphate acyltransferase (apparent K_i of 0.55 mM and 1.25 mM, respectively) and cytidine 5'-diphosphate-diglyceride:sn-glycerol 3-phosphate phosphatidyltransferase (apparent K_i of 2.7 mM and 4.7 mM, respectively). A K_m

mutant for the former enzyme was susceptible to both inhibitors. Neither DL-glyceraldehyde 3-phosphate nor 3-hydroxy-4-oxobutyl-1-phosphonate affects acyl coenzyme A:lysophosphatidate acyltransferase activity.

Glyceraldehyde 3-phosphate "resistant" mutants were isolated by incubating the mutagenized cells in the presence of 2.0 mM DL-glyceraldehyde 3-phosphate and 2.5 mM L-glyceraldehyde to avoid G3P transport-negative mutants and glycerol kinase-negative mutants. One mutant was selected for study. Glyceraldehyde 3-phosphate was bacteriostatic to this mutant. The acyl CoA:sn-G3P acyltransferase and CDP-diglyceride:sn-G3P phosphatidyltransferase from this mutant were as sensitive to glyceraldehyde 3-phosphate as the enzymes isolated from the parent strain. The same was true of sensitivity to the phosphonic acid analogue. The possible mode of action of glyceraldehyde 3-phosphate and 3-hydroxy-4-oxobutyl-1-phosphonate as inhibitors of phospholipid biosynthesis are discussed.

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CHAPTER 1

INTRODUCTION

Phospholipids are ubiquitous components of the membrane of all cells. In Escherichia coli (E. coli) 10% of the dry weight of the cell is lipid consisting almost entirely of membrane phospholipids (Cronan & Vagelos, 1972). The cell envelope of E. coli is composed of two membranous components, the outer membrane and the cytoplasmic membrane. The phospholipid composition of the outer membrane is qualitatively similar to that of the cytoplasmic membrane (Osborn et al., 1974).

These phospholipids play extremely important roles in the enzymatic activity (Esfahani et al., 1972; Mavis & Vagelos, 1972), structural integrity, active transport, growth and macromolecular biosynthesis (Cronan & Vagelos, 1972; Cronan & Gelmann, 1975). Phospholipid participation in oxidative phosphorylation (Cunningham & Hager, 1971a; Cunningham & Hager, 1971b), carbohydrate transport (Kundig & Roseman, 1971; Milner & Kaback, 1970; Weisberg et al., 1975) and lipopolysaccharide synthesis (Rothfield & Romeo, 1971) have been studied.

A knowledge of the regulatory mechanisms controlling phospholipid metabolism is necessary for a complete understanding of the role of phosphoglycerides in membrane

structure and function. The isolation and characterization of mutants of E. coli defective in membrane phospholipid synthesis is one approach to this problem (Cronan & Gelmann, 1975; Silbert, 1975; Silbert & Vagelos, 1974). A complementary approach to this problem is a search for drugs that might influence the regulation of phosphoglyceride synthesis. Analogues of common intermediates offer considerable promise for such an approach. Phosphonic acid analogues of natural phosphates have been extensively studied for this purpose (Engel, R., 1977). For this reason, phosphonic acid analogues of glycerol 3-phosphate were synthesized (Kabak et al., 1972) and their effects on cell growth and phospholipid metabolism were studies (Shopsis et al., 1972; Shopsis et al., 1973). One compound which appears promising is 3,4-dihydroxybutyl-1-phosphonate (DHBP). This analogue inhibits the growth of E. coli and also has a marked effect upon phospholipid metabolism (Shopsis et al., 1972; Shopsis et al., 1973; Shopsis et al., 1974; Cheng et al., 1975; Tyhach et al., 1976).

The hydrated form of L-glyceraldehyde 3-phosphate may be considered to be an analogue of sn-glycerol 3-phosphate in which a hydroxyl group replaces a hydrogen at carbon-1. In aqueous solution, glyceraldehyde 3-phosphate exists as an equilibrium mixture of the geminal diol (hydrated form) and free aldehyde in a molar ratio of 29:1 (Trentham et al., 1969). The purpose of this investigation is to report on

the effects of L-glyceraldehyde 3-phosphate and its phosphonic acid analogue, 3-hydroxy-4-oxobutyl-1-phosphonic acid (Goldstein et al., 1974b) on phosphoglyceride metabolism in E. coli. The studies comprising this dissertation deal specifically with mutants of E. coli with defects in the glycerophosphate regulon (glp) and phospholipid metabolism. Therefore, it seems appropriate to review studies concerning: (i) metabolism of sn-glycerol 3-phosphate by E. coli, (ii) phosphoglyceride metabolism in E. coli and (iii) the effects of phosphonic acid analogues of sn-glycerol 3-phosphate on cell growth and phospholipid metabolism in E. coli.

I. sn-Glycerol 3-Phosphate Dissimilation and Regulation in E. coli

(A). Metabolic pathway

The metabolism of sn-glycerol 3-phosphate by E. coli has recently been reviewed by Lin (Lin, 1976). Most of the studies on sn-glycerol 3-phosphate (sn-G3P) metabolism were carried out with strains descended from E. coli E15 which is a derivative of K12 defective in alkaline phosphatase (Lin, 1976).

The network for the metabolism of glycerol and sn-G3P are presented in Figure 1. Cells of E. coli K12 can grow

on either glycerol or sn-glycerol 3-phosphate as the sole source of carbon and energy. The passage of glycerol across the cell membrane is governed by facilitated diffusion, an energy-independent process (Hayashi & Lin, 1965b). Once inside the cell, glycerol is converted into sn-glycerol 3-phosphate by the action of glycerol kinase which is dependent on ATP (Hayashi and Lin, 1967). The absence of a concentrative mechanism for glycerol uptake is indicated by the failure of all glycerol kinase-negative mutants to accumulate radioactive material when incubated with [^{14}C]glycerol. Furthermore, it was shown that the K_m of glycerol kinase (1.3 μM) corresponds fairly closely to the growth K_m (0.9 μM) (Hayashi & Lin, 1965a). On the contrary, the experiments with sn-glycerol 3-phosphate show that the K_m of aerobic G3P dehydrogenase (2 mM) is very different from the growth K_m (4 μM) (Hayashi & Lin, 1965a).

Glycerol kinase of E. coli is an inducible enzyme (Hayashi & Lin, 1965b) which has been purified and crystallized (Hayashi & Lin, 1967). It is subject to a feedback inhibition by fructose 1,6-diphosphate (FDP) in E. coli (Zwaig & Lin, 1966). A mutant which is resistant to fructose 1,6-diphosphate grows faster than its wild-type parent when glycerol serves as the sole carbon source (Zwaig et al., 1970). The glycerol kinase not only mediates the first reaction in the glycerol dissimilatory pathway, but also is responsible for the trapping of the substrate. Therefore,

the activity of glycerol kinase is a rate-limiting step in the metabolism of glycerol. Glycerol can neither serve as a carbon source nor act as an inducer for the remaining proteins of the glycerol system in cells lacking glycerol kinase activity (Cozzarelli & Lin, 1966).

sn-Glycerol 3-phosphate can be taken up directly from the medium by a specific active transport system (Lin, et al., 1962; Hayashi et al., 1964). A constitutive mutant strain for the glp system lacking alkaline phosphatase and the aerobic G3P dehydrogenase (Cozzarelli et al., 1965; Cozzarelli et al., 1968; Hayashi et al., 1964; Zwaig & Lin, 1966) was employed for the study of sn-G3P transport. The genetic markers were selected to avoid external hydrolysis and internal catabolism of the substrate. When [^{14}C]-labeled sn-G3P was incubated with the mutant, a 1,000-fold concentration of the substrate was readily achieved. Accumulated sn-G3P was rapidly released from the cells upon the addition of unlabeled substrate or metabolic inhibitors (Hayashi et al., 1964). Prior hydrolysis of sn-G3P to free glycerol is not responsible for the utilization of the substrate, since a mutant that lacks glycerol kinase and therefore can not grow on glycerol, nevertheless grows well on sn-G3P (Lin et al., 1962). Further, metabolism of sn-G3P is not required for transport (Lin et al., 1962) and sn-G3P is transported intact (Hayashi et al., 1964).

Glycerol 3-phosphate permease can serve as a port of

entry for several compounds including arsenate (Willsky et al., 1973), fosfomycin (Hendlin et al., 1969) and a phosphonic acid analogue of G3P, 3,4-dihydroxybutyl-1-phosphonate (Leifer et al., 1977). A mutant which has an impaired sn-G3P transport system can no longer utilize G3P as a carbon source (Hayashi et al., 1964).

sn-Glycerol 3-phosphate is subsequently oxidized in a reaction catalyzed by one of two distinct sn-glycerol 3-phosphate dehydrogenases: the aerobic G3P dehydrogenase and the anaerobic G3P dehydrogenase, neither of which is linked to pyridine nucleotides (Kistler et al., 1969; Kistler & Lin 1971). Aerobic catabolism of sn-G3P requires the aerobic G3P dehydrogenase which is tightly associated with the cell membrane and has noncovalently bound FAD as its coenzyme (Kistler et al., 1969). Aerobic cultures of mutants lacking this enzyme cannot utilize either glycerol or sn-G3P as carbon sources. Furthermore, their growth on casein hydrolysate or succinate can be inhibited by either compound (Koch et al., 1964). Derivatives of the aerobic G3P dehydrogenase-negative mutants become glycerol-resistant or G3P-resistant by losing their kinase or their permease, respectively (Cozzarelli et al., 1965; Cozzarelli et al., 1968).

sn-Glycerol 3-phosphate can also be catabolized by the action of the anaerobic G3P dehydrogenase which is distinguished from the aerobic enzyme by a slower rate of sedimentation during centrifugation in a sucrose gradient (Kistler

et al., 1969) and a requirement for added flavins for its in vitro assay (Kistler & Lin, 1972). Mutant cells lacking aerobic G3P dehydrogenase but possessing the anaerobic dehydrogenase can grow anaerobically with either glycerol or glycerol 3-phosphate as a carbon source and with fumarate or nitrate as the exogenous hydrogen acceptor. Nitrate is reduced to nitrite and fumarate to succinate (Kistler & Lin, 1971). Mutants lacking the anaerobic G3P dehydrogenase can not grow anaerobically.

When E. coli is cultured in the absence of exogenous glycerol or sn-G3P, sn-G3P is produced by the reduction of dihydroxyacetone phosphate (DHAP) by G3P synthase (Kito & Pizer, 1968; Kito & Pizer, 1969; Spector & Pizer, 1975). This enzyme was frequently referred to as the biosynthetic (or anabolic) G3P dehydrogenase. Since it acts as a reductase when it carries out its biosynthetic function, it has recently been termed G3P synthase (Lin, 1976). Mutants lacking G3P synthase are auxotrophic for glycerol or G3P (Bell, 1974; Hsu & Fox, 1970; Weisberg et al., 1975). G3P synthase was purified approximately 1000-fold from extracts of E. coli. The equilibrium strongly favors the formation of G3P. The K_m values for G3P, DHAP and NADPH are 210 μM, 170 μM and 10 μM, respectively. The kinetics of inhibition as a function of G3P concentration gave a sigmoid curve. At 2 mM DHAP, 50% inhibition occurs in the presence of 35 μM G3P (Kito & Pizer, 1969).

(B). The glp regulon

The structural genes coding for the transport systems and enzymes for the metabolism of glycerol and sn-G3P are found in three widely separated regions of the E. coli chromosome (Cozzarelli et al., 1968). The locations of these genes in the recalibrated chromosomal map (Bachmann et al., 1976) are shown in Figure 2.

The structural genes specifying the glycerol facilitator (glp F) and glycerol kinase (glp K) appear to be parts of a single operon located at minute 87 (Berman & Lin, 1971; Cozzarelli & Lin, 1966). The structural gene for the sn-G3P transport protein, permease (glp T) and the gene for the anaerobic G3P dehydrogenase (glp A) are situated at minute 48 and belong to a single operon (Cozzarelli et al., 1968; Kistler & Lin, 1971). Mutations affecting aerobic G3P dehydrogenase (glp D) map in another region at minute 74. This structural gene shows 98-99% cotransduction with the structural gene for the repressor (glp R) (Cozzarelli et al., 1968; Hofnung et al., 1971). Since a single repressor regulates the specific repression of all these proteins with G3P as the inducer, the glp system is thus a regulon comprising three operons (Cozzarelli et al., 1968).

In a mutant lacking aerobic G3P dehydrogenase, G3P nevertheless can induce other gene products in the glp regulon. It is suggested that G3P need not be metabolized

in order to act as an inducer (Cozzarelli et al., 1968). In contrast, glycerol cannot act as an inducer in mutants lacking glycerol kinase (Koch et al., 1964). Even the expression of the glp F,K operon can only be induced by G3P (Hayashi & Lin, 1965b).

There are three kinds of control mechanisms that govern the expression of all the proteins of the glp regulon in E. coli K12, specific repression by the product of glp R gene, catabolite repression, and respiratory repression (Freedberg & Lin, 1973). The operons of the glp system show different response to each control. The synthesis of aerobic G3P dehydrogenase (glp D product) is far more sensitive to specific repression than that of other members of the glp system including glycerol kinase (glp K product) and G3P transport (glp T product) (Cozzarelli et al., 1968; Freedberg & Lin, 1973). The apparent affinity of the glp D operon for the repressor is about an order of magnitude higher than that of either of the other two operons. It was suggested that the wasteful catabolism of the endogenous G3P was minimized in the absence of the inducer. In addition, once the external G3P or glycerol becomes available, a rapid accumulation of the inducer is assured by a higher activity ratio of glycerol kinase to aerobic G3P dehydrogenase or that of G3P permease to aerobic G3P dehydrogenase (Lin, 1976).

The pattern of differential sensitivity of the three operons to catabolite repression by glucose is opposite to

that observed with specific repression. In mutants lacking the regulator gene, the syntheses of glycerol kinase and G3P permease are more sensitive to catabolite repression than that of the aerobic G3P dehydrogenase (Freedberg & Lin, 1973; Koch et al., 1964). Although the glp D operon is relatively less sensitive to catabolite repression, the expression of this gene cannot continue if the supply of inducer is curtailed by the catabolite repression of the glp F,K and glp T,A operons since the glp D operon is extremely sensitive to the repressor (Lin, 1976).

Mutant missing the repressor and one of the two G3P dehydrogenases were used for studying the levels of the other dehydrogenase. It was shown that the levels of these two G3P dehydrogenases vary in opposite directions in response to differential exogenous hydrogen acceptors (Freedberg & Lin, 1973). Cyclic adenosine 3',5'-monophosphate (cAMP) reverses the catabolite repression of glycerokinase when cells grow aerobically on glucose. However, cAMP does not relieve the repression of the anaerobic G3P dehydrogenase in the same cell (Freedberg & Lin, 1973). Thus respiratory repression involves a third mechanism of control, independent of specific or catabolite repression.

II. Metabolism of Phospholipids in E. coli

(A). Metabolic pathway

The current scheme of phospholipid biosynthesis in E. coli is presented in Figure 3. Although a group of phospholipases is localized in the outer membrane of E. coli, all but one of the enzymes involved in phospholipid biosynthesis are found in the inner membrane (Bell et al., 1971; Osborn et al., 1974; White et al., 1971). The exception is phosphatidylserine synthetase which is predominantly associated with ribosomes (Raetz & Kennedy, 1972; Raetz & Kennedy, 1974; Raetz, 1976). In addition, evidence for translocation of newly synthesized phosphatidylethanolamine from the inner membrane to the outer membrane in vivo has been obtained by pulse chase experiments (Osborn et al., 1974).

There are two proposed pathways for the synthesis of phosphatidic acid from sn-G3P: the first involves acylation at position 1 of sn-glycerol 3-phosphate to form 1-acyl-sn-glycerol 3-phosphate followed by acylation at position 2 to form diacyl-glycerol 3-phosphate. The second involves the formation of 2-acyl-sn-glycerol 3-phosphate as an intermediate.

Vagelos and co-workers have found that 1-acyl-glycerol 3-phosphate accumulates when saturated acyl-CoA (palmitoyl-

CoA) is incubated with glycerol 3-phosphate in the presence of E. Coli acyltransferase. In contrast, 2-acyl-glycerol 3-phosphate was formed when unsaturated acyl-CoA (oleoyl-CoA, palmitoleoyl-CoA, or cis-vaccenoyl-CoA) were used (Ray et al., 1970; Sinensky, 1971; Van Den Bosch & Vagelos, 1970).

However, Wakil and co-workers noted that: (1) after incubations of G3P with acyl-CoAs, structural analysis of the monoacyl-glycerol 3-phosphate indicated that the major isomer is 1-acyl-glycerol 3-phosphate (2) the particulate preparation which catalyzes the acylation of monoacyl-glycerol 3-phosphate is specific for 1-acyl-glycerol 3-phosphate, and (3) during the acylation of radioactive G3P with unsaturated acyl-CoA, radioactive monoacyl-glycerol 3-phosphate is effectively trapped by the addition of unlabeled 1-acyl-G3P but not by the addition of unlabeled 2-acyl-G3P (Okuyama & Wakil, 1973). They conclude that 1-acyl-sn-glycerol 3-phosphate is the sole monoacyl intermediate in phosphatidic acid biosynthesis.

G3P acyltransferase and 1-acyl-sn-glycerol 3-phosphate acyltransferase utilize various saturated and unsaturated acyl-CoAs at comparable rates in vitro. However, the selectivities of both acyltransferases for acyl-CoAs appear dependent upon the concentration of acceptors (Okuyama et al., 1976). In the presence of both palmitoyl-CoA and oleoyl-CoA and at low concentrations of G3P comparable to those found in vivo, saturated fatty acids are preferentially

esterified at position 1 by membrane preparations, whereas unsaturated fatty acids are preferentially esterified at position 2. However, in the presence of saturating amounts of the acceptors, the acylation at position 1 and position 2 was less selective for the acyl-CoAs (Okuyama et al., 1976). Therefore, the positional specificities of various acyl-CoAs observed at lower concentrations of the acceptors in phosphatidic acid synthesis in vitro may explain the asymmetry fatty acids observed in vivo (Van Golde & Van Deenen, 1967; and Silbert, 1970).

G3P acyltransferase, the first enzyme of the phosphoglyceride biosynthetic pathway, was recently extracted with Triton X-100 and purified 40-fold. This enzyme is inactive in the detergent extracts but can be reconstituted by the addition of phospholipids (Snider & Kennedy, 1977). The enzyme is free from lysophosphatidate acyltransferase and makes only 1-acyl-sn-G3P (Snider & Kennedy, 1977). This result provides further support for 1-acyl-sn-G3P serving as the true intermediate for phosphatidic acid synthesis. Although acyl-acyl carrier protein (acyl-ACP) is probably the physiological donor in vitro, the thioester moiety of the acyl donor can be either coenzyme A (CoA) or ACP. It was demonstrated that the same acyltransferase catalyzes the incorporation of both thioester substrates into phospholipids (Ray & Cronan, 1975). G3P acyltransferase is inhibited by guanosine tetraphosphate (ppGpp) (Merlie & Pizer,

1973). It was later found that the guanosine nucleotide inhibits the acyltransferase only when acyl-CoA was the acyl donor. No inhibition was observed when the enzyme was assayed with acyl carrier protein in the presence of ppGpp (Lueking & Goldfine, 1975; Ray & Cronan, 1975).

Information concerning all of the reactions of phospholipid synthesis subsequent to the formation of phosphatidic acid is based upon the presence of following enzymatic activities in E. coli: cytidyltransferase (Carter, 1968), phosphatidylglycerol phosphate synthase (Chang & Kennedy, 1967a), cardiolipin synthase (Hirschberg & Kennedy 1972), phosphatidylserine synthetase (Raetz & Kennedy, 1974), CDP-diglyceride hydrolase (Raetz et al., 1976), and phosphatidylserine decarboxylase (Dowhan et al., 1974). Moreover, mutants of E. coli defective in phosphatidylserine synthetase (Ohta et al., 1974a; Ohta et al., 1974b; Raetz, 1976) and phosphatidylserine decarboxylase (Hawrot & Kennedy, 1975) have been isolated and characterized. These findings provide direct evidence for the involvement of phosphatidylserine as the intermediate in the synthesis of phosphatidylethanolamine.

The selectivities of acyl-CoA:G3P acyltransferase and acyl-CoA:monoacylglycerophosphate acyltransferase for saturated and unsaturated fatty acyl CoAs were described previously. Since all phospholipid species are derived from the phosphatidic acid, the fatty acid compositions of various phospholipid species are quite similar (Cronan & Vagelos,

1972).

The catabolism of the phospholipids has been studied to a limited extent in E. coli. The fatty acid moieties of the phospholipids are extremely stable in growing cells (Cronan, 1968). Although phosphatidylethanolamine phosphorus is very stable, the phosphate group of phosphatidylglycerol and cardiolipin turn over at a moderate rate (Cronan & Vagelos, 1972). The turnover of phosphatidylglycerol appears to be due to its conversion to cardiolipin and a "novel polysaccharide" (Hirschberg & Kennedy, 1972).

In E. coli the presence of phospholipase A_1, A_2 and C activities have been reported (Doi & Nojima, 1975; Proulx & Van Deenen, 1967a; Proulx & Van Deenen 1967b; Okuyama & Nojima, 1969; Scandella & Kornberg, 1971). The phospholipase A_1 has been purified approximately 5,000-fold to near homogeneity by solubilization with sodium dodecyl sulfate (SDS)-butanol, isoelectric precipitation, acetone fractionation, and SDS-acrylamide gel electrophoresis. The enzyme is stable in 3% sodium dodecyl sulfate and hydrolyzes the 1-acyl chain of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin at comparable rates (Scandella & Kornberg, 1971).

(B). Escherichia coli mutants in lipid metabolism

Many mutants for enzymes involved in lipid metabolism have been extensively studied. The locations of these genes are presented in Figure 2. This topic has recently been reviewed by Silbert (Silbert, 1975). According to the biochemical defects, these mutants are divided into four major groups: (1) fatty acid biosynthesis (2) phospholipid biosynthesis (3) fatty acid degradation (4) phospholipid degradation.

Fatty acid biosynthetic mutants of E. coli include fab A, fab B, fab C, fab D, fab E, cvc and vtr. Fab A and fab B are unsaturated fatty acid auxotrophs, and extracts from these cells cannot form unsaturated fatty acid products. Fab A strains are defective in the β-hydroxydecanoyl thioester dehydrase (Cronan et al., 1969; Cronan et al., 1972; Silbert & Vagelos, 1967), the enzyme that introduces the double bond of the unsaturates. Fab B strains are defective in the β-ketoacyl ACP synthetase I (Rosenfield et al., 1973). Fab E strains are defective in acetyl CoA-carboxylase (Bachmann et al., 1976). Cvc mutants are not unsaturated fatty acid auxotrophs. However, they have increased cis-Δ^9-16:1 and very little cis-Δ^{11}-18:1 in their phospholipid (Gelmann & Cronan, 1972). It was suggested that a reduction in β-ketoacyl ACP synthetase II may account for the cvc phenotype (Silbert, 1975). The counterpart of cvc is a mutation (vtr) described by Broekman (Silbert, 1975) which

have increased cis-Δ^{11}-18:1 and reduced cis-Δ^{9}-16:1 in their phospholipids. In vitro fatty acid synthetic studies have not been reported and the enzymatic basis of this mutation is not clear. Fab C is an unsaturated fatty acid-requiring strain and has not been fully characterized by in vitro studies (Broekman & Hoekstra, 1973).

Temperature-sensitive mutants affecting total fatty acid synthesis have been isolated by using a [^{3}H]acetate radiation suicide procedure (Harder et al., 1972). Fab D strains isolated by this procedure possess a themolabile malonyl CoA-ACP transacylase (Harder et al., 1974). Both saturated and unsaturated fatty acid synthesis are temperature sensitive in vivo and in vitro. A decrease in malonyl transacylase causes a preferential reduction in the long chain fatty acyl groups (16:0 and 18:0) of the phospholipid in vivo.

Two kinds of mutants affecting phospholipid synthesis have been isolated: mutations in the common portion of the phospholipid biosynthetic pathway leading to CDP-diglyceride, and mutations beyond the liponucleotide level. The former mutation results in the reduction of overall production of phospholipid. However, the second type of mutation selectively reduces the formation of one type of glycerophosphatide.

Two classes of E. coli mutants defective in membrane phospholipid synthesis have been selected as glycerol 3-

phosphate auxotrophs. Gps A strains are defective in G3P synthesis due to the absence of the G3P synthase (Bell, 1974; Cronan & Bell, 1974a; Hsu & Fox, 1970). Pls B strains are defective in utilization of endogenous G3P and contain a G3P acyltransferase activity with an apparent K_m for G3P 10 times higher than that of its parent (Bell, 1974; Cronan & Bell, 1974b; Kito et al., 1969; Pizer et al., 1974). The mutant G3P acyltransferase also differs from the wild type in at least six other characteristics including in vitro thermolability, sensitivity to detergents, dependence upon pH, Mg^{2+}, and salt (Bell, 1975).

Although most revertants, no longer requiring G3P for growth, regained normal apparent K_m for G3P, there are two revertants which retained abnormal G3P acyltransferase activty. The G3P synthase of these two novel revertants was about 20-fold less sensitive to feedback inhibition by G3P (Bell & Cronan, 1975). It was demonstrated that the feedback resistant G3P synthase phenotypically suppressed the G3P acyltransferase K_m lesion.

A second class of mutations, pls A, that effect acyltransferase activity have been isolated. In the pls A strains, the activity of glycerol 3-phosphate acyltransferase is thermolabile in vitro and lost when cells are placed at the restrictive temperature prior to conducting the in vitro measurements. However, recent data suggest that the pls A locus may lower intracellular ATP levels

(Glaser et al., 1975). More work is required to elucidate the enzymatic lesion in pls A strains.

The mutants which are specifically defective in the synthesis of phosphatidylethanolamine have been isolated by a [^{3}H] serine suicide selection, first described by Cronan (Cronan, 1972). Two kinds of mutants affecting phosphatidylethanolamine have been reported. Pss strains are defective in phosphatidylserine synthetase (Ohta et al., 1974a; Ohta et al., 1974b; Raetz, 1976). Psd strains possess a temperature sensitive phosphatidylserine decarboxylase (Hawrot & Kennedy, 1975).

E. coli contains enzymes catalyzing β-oxidation. Mutants in many steps in the pathway have been isolated and designated fad for fatty acid degradation. The mutations that effect the enzymes catalyzing β-oxidation include thiolase I (fad A), hydroxyacyl-CoA dehydrogenase (fad B) (Overath et al., 1969), acyl-CoA synthetase (fad D) (Overath et al., 1969; and Bachmann, et al., 1976). Fad E strains may be defective in the electron transport flavoprotein for acyl-CoA dehydrogenase (Klein et al., 1971). Although fad mutations do not affect the fatty acid composition of E. coli growing without fatty acid supplement, they prevent the degradation of exogenously supplied fatty acids prior to incorporation into phospholipids. In order to modify the membrane lipid, the fab fad double mutants are more useful than fab mutants (Overath et al., 1970).

A phospholipase A mutant in E. coli has been isolated (Ohki et al., 1972). The pld A strain has lost the detergent-resistant phospholipase A_1, phospholipase A_2 and lysophospholipase activities (Doi & Nojima, 1973). A second type of phospholipase A mutant has also been found (Nojima et al., 1972), which is defective in the detergent-sensitive, phosphotidylglycerol-specific, cytoplasmic phospholipase A (Albright et al., 1973; Doi et al., 1972).

(C). Manipulation of phospholipid composition in E. coli

The availability of unsaturated fatty acid (UFA) auxotrophs of E. coli has made possible the manipulation of the membrane phospholipids since the phospholipid fatty acid composition reflects the fatty acid provided in the culture medium (Esfahani et al., 1969; Silbert & Vagelos, 1974).

UFA auxotrophs can be supplemented with a wide variety of fatty acids including the cis-monoenoic acids synthesized by prototrophic strains, as well as fatty acids possessing unusual structural features with respect to chain length and the number, position or stereochemistry of the double bonds (Esfahani et al., 1971a; Esfahani et al., 1971b; Overath et al., 1970; Silbert & Vagelos, 1974). The auxotrophs were found to acylate exogenous UFA preferentially at position 2 of the phospholipids, indicating that specific acylation was not dependent on endogenous UFA synthesis

(Silbert, 1970).

It was noted that E. coli adjusts the fatty acid composition of its phospholipids in response to growth temperature. As the temperature of growth is lowered, the proportion of UFA (chiefly cis-vaccenic acid) in the membrane increases (Cronan & Vagelos, 1972). When cerulenin-treated cultures were grown with a mixture of [^{3}H]palmitate and cis-[^{14}C]vaccenate, at least 85% of the phospholipid fatty acyl moieties in these cultures were derived from these exogenously supplied fatty acids (Sinensky, 1971). It was also demonstrated that the ratio of exogenous saturated to unsaturated fatty acids incorporated into phospholipid increased with increasing growth temperature (Cronan, 1975). The ratio of saturated to unsaturated species appearing in the free fatty acid fraction of an E. coli double mutant (pls B, fad E) depends on the incubation temperature at the time of synthesis of these acids. Double mutants (pls B, fad E) cultured at 43 C accumulated free fatty acids which were at least 80% saturated, whereas those cultured at 15 C accumulated fatty acids which were less than 47% saturated (Cronan, 1975).

The temperature-dependent change of state of the acyl moieties of membrane phospholipids from a solid, hexagonal close-packing to a fluid, more random array has been called phase transition or order-disorder transition (Engleman, 1970; Shechter et al., 1974). The transition temperature

(Tt) and the temperature range between initiation and completion of the phase transition are both dependent on the fatty acyl content and polar head group composition of the phospholipids.

The transition temperature of lipids acylated with saturated fatty acid is higher than when the lipids contain cis-monosaturated fatty acid. The presence of di- or polyunsaturated fatty acyl chains further decreases the transition temperature (Ladbrooke & Chapman, 1969). When an E. coli UFA auxotroph was cultured on oleic acid, the membrane lipid transition was lower than when the auxotroph was supplemented with elaidate (Esfahani et al., 1971b).

The manipulating of UFA contents of E. coli has been used to access the roles of membrane lipid in many complex functions: (1) transport systems such as lactose transport, β-glucoside transport (Wilson et al., 1970; Schairer & Overath, 1969) and amino acid transport (Esfahani et al., 1971b), (2) membrane-enzyme interactions such as glycerol 3-phosphate acyltransferase and glycerol 3-phosphate dehydrogenase (Mavis & Vagelos, 1972), (3) initiation of DNA synthesis (Fralick & Lark, 1973) and (4) cell integrity (Henning et al., 1969).

The Arrhenius plot (rate-temperature profile) of the lactose transport system could be altered by supplemention of UFA auxotrophs with various UFAs (Schairer & Overath, 1969; Wilson et al., 1970). The two line segment of the

biphasic Arrhenius plots can be extrapolated to an intersection point that is dependent on the UFA used to support the growth of UFA auxotroph. The inflection temperature observed varies from 7 C for linoleate to 30 C for elaidate. It was indicated that the Arrhenius plot inflection is an indication of a transition in the lipid environment of transport sites in the membrane (Schairer & Overath, 1969; Wilson et al., 1970).

The lipid phase properties of the membrane are of vital importance to E. coli which normally controls the physical properties of its membrane by manipulating either endogenous or supplemental fatty acids to maintain a relatively constant state in response to temperature changes (Cronan & Gelmann, 1975). It was demonstrated that both fluid and non-fluid phospholipid molecules are required for growth. The minimum amount of UFA required to support the growth of E. coli at 37 C is 15 to 20% (Cronan & Gelmann, 1973). If the membrane lipid contains less than one third the normal amount of either saturated or unsaturated acylated lipids, cell death will result (Cronan & Gelmann, 1975).

III. Effects of Phosphonic Acid Analogues of Glycerol 3-Phosphate on the Metabolism of Phospholipid in E. coli

Two phosphonic acid analogues of glycerol 3-phosphate, 3,4-dihydroxybutyl-1-phosphonic acid ($CH_2OHCHOHCH_2CH_2PO_3H_2$) and 2,3-dihydroxypropyl-1-phosphonic acid ($CH_2OHCHOHCH_2PO_3H_2$) were synthesized (Kabak et al., 1972) and their effects on the growth and metabolism of E. coli have been extensively studied.

3,4-Dihydroxybutyl-1-phosphonate is actively transported by the sn-glycerol 3-phosphate transport system of E. coli (Leifer et al., 1977) and inhibits the cell growth of E. coli possessing this transport system. The three-carbon phosphonate analogue does not appear to effect cell growth (Shopsis et al., 1972). Glycerol 3-phosphate inhibits the growth of strains of E. coli that are constitutive for the glycerol 3-phosphate transport system and that lack the aerobic G3P dehydrogenase (Cozzarelli et al., 1965). The growth inhibition caused by 3,4-dihydroxybutyl-1-phosphonate differs from that caused by the natural matabolite in that it is not offset by the presence of glucose or high concentrations of phosphate in the medium and occurs in the presence of the aerobic glycerol 3-phosphate dehydrogenase (Shopsis et al., 1972).

The incorporation of labeled precursors of lipid, protein, ribonucleic acid and deoxyribonucleic acid into

bacterial cells was measured in the presence of either glycerol 3-phosphate or one of its phosphonic acid analogues. It was found that 3,4-dihydroxybutyl-1-phosphonate strongly inhibits the incorporation of [^{33}P]phosphate into phospholipid, but 2,3-dihydroxypropyl-1-phosphonate only slightly affected phospholipid synthesis (Shopsis et al., 1973). Pulse labeling experiments with [^{32}P]phosphate revealed that phospholipid synthesis is inhibited to a much greater extent by 3,4-dihydroxybutyl-1-phosphonate than is the synthesis of DNA or RNA. In addition, the four-carbon phosphonate alters the distribution of labeled precursors into phospholipids. The major change was a rapid reduction in the rate of synthesis of phosphatidylglycerol fraction and a slower but almost equally pronounced inhibition of the rate of phosphatidylethanolamine synthesis. Cardiolipin is less significantly affected (Shopsis et al., 1973; and Shopsis et al., 1974). In contrast, glycerol 3-phosphate was found to have its strongest inhibitory effect on the incorporation of labeled uracil into RNA and little effect on the distribution of labeled acetate into the phosphoglycerides (Shopsis et al., 1973).

3,4-Dihydroxybutyl-1-phosphonate is both a competitive inhibitor (apparent K_i of 740 μM) and a substrate (apparent K_m of 450 μM) for the CDP-diglyceride: sn-glycerol 3-phosphate phosphatidyltransferase. The four-carbon phosphonate is also an inhibitor of the reduction of dihydroxyacetone

phosphate by glycerol 3-phosphate synthase (apparent K_i of 42 μM) (Cheng et al., 1975). However, neither the aerobic G3P dehydrogenase nor the acyl-CoA: glycerol 3-phosphate acyltransferase can use 3,4-dihydroxybutyl-1-phosphonate as a substrate. The four-carbon phosphonic acid analogue does not exhibit inhibitory activity for these two enzymes as well as CDP-diglyceride: serine phosphatidyltransferase.

3,4-Dihydroxy-[3-^{3}H]butyl-1-phosphonate was readily incorporated into a very polar lipid material by cultures of strain 8 (Shopsis et al., 1974; Cheng et al., 1975; and Tyhach et al., 1976) and in vitro by CDP-diglyceride:sn-glycerol 3-phosphate phosphatidyltransferase (Cheng et al., 1975; and Tyhach et al., 1976). The labeled lipids have been fractionated by DEAE-cellulose chromatographic column and identified as the phosphonic acid analogue of phosphatidylglycerolphosphate (Tyhach et al., 1976).

The in vitro examination of the enzymes involved in glycerol 3-phosphate metabolism helped to explain the effects of the four-carbon phosphonate on phosphoglyceride metabolism in vivo. It was suggested one possible site of action for the phosphonate is at the level of CDP-diglyceride:sn-glycerol 3-phosphate phosphatidyltransferase (Cheng et al., 1975).

Figure 1: Network for the metabolism of glycerol and sn-glycerol 3-phosphate in E. coli. It also lists the genes for coded proteins. Abbreviations used are as follows: DHAP for dihydroxyacetone phosphate; FDP for fructose-1,6-diphosphate; G3P for sn-glycerol 3-phosphate; GAP for glyceraldehyde 3-phosphate; F6P for fructose 6-phosphate. (Modified from Lin, 1976)

Figure 1

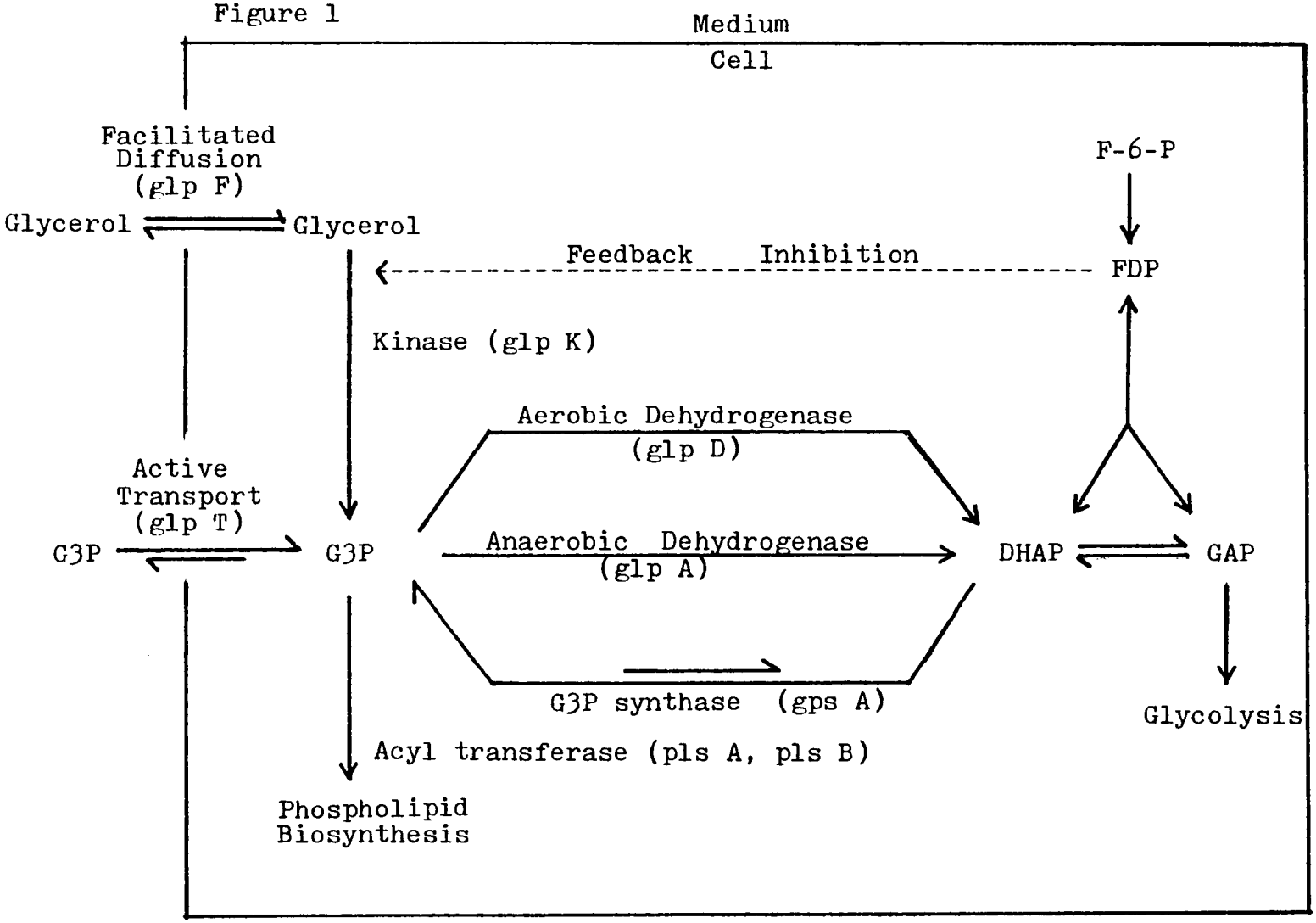

Medium
Cell
Facilitated
Diffusion
(glp F)
Glycerol
Glycerol
F-6-P
Feedback Inhibition
FDP
Kinase (glp K)
Aerobic Dehydrogenase
(glp D)
Active
Transport
(glp T)
G3P
G3P
Anaerobic Dehydrogenase
(glp A)
DHAP
GAP
G3P synthase (gps A)
Glycolysis
Acyl transferase (pls A, pls B)
Phospholipid
Biosynthesis

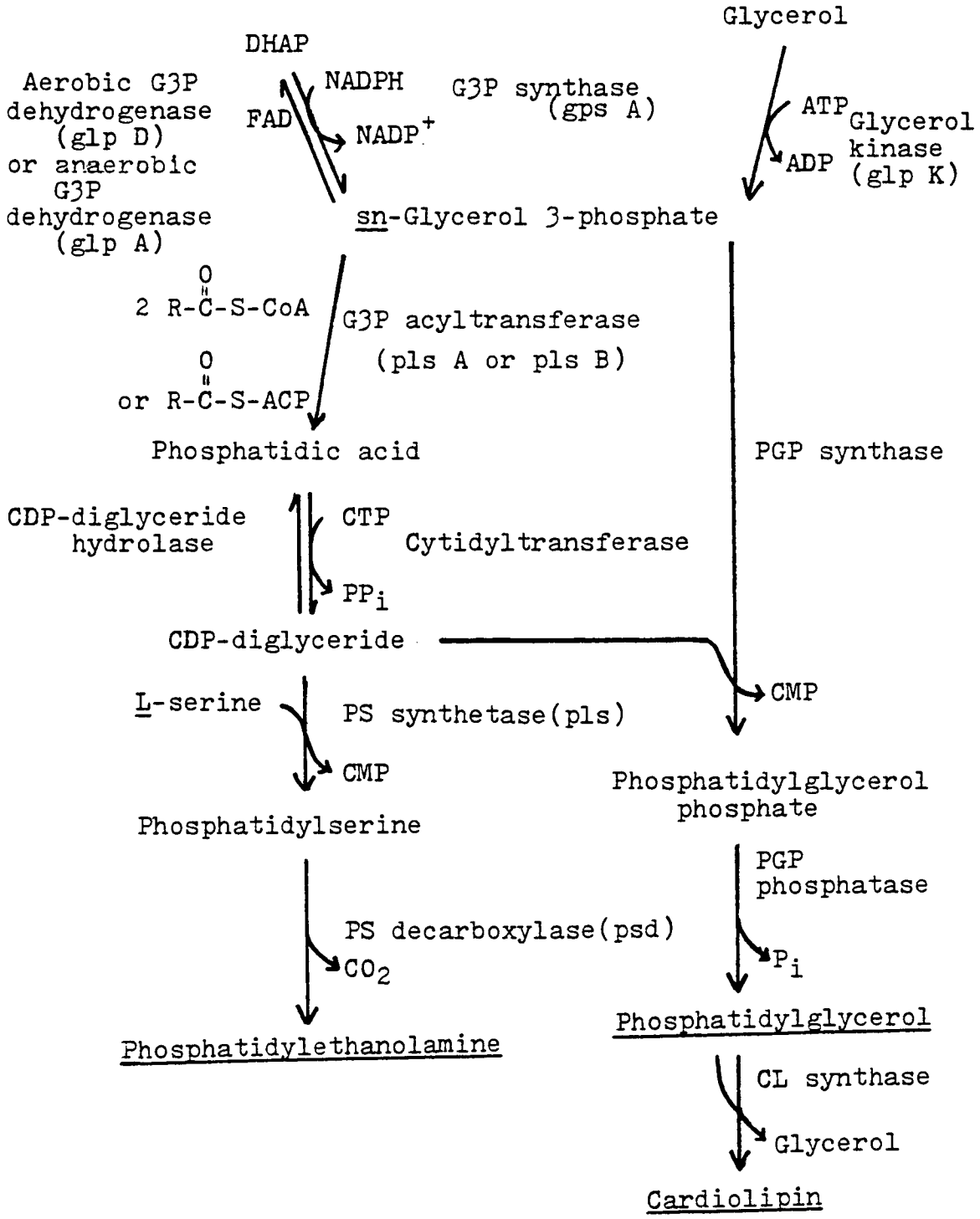
Figure 2: The genetic map of genes for the glp system and phospholipid metabolism. Genetic nomenclature is that of Bachmann et al (Bachmann et al., 1976). The structural genes for the glp system and phospholipid metabolism are located in the recalibrated chromosomal map (Bachmann et al., 1976). (Modified from Lin, 1976)

Figure 2

Genes of the glp system	Gene location (Minute)	Genes of the lipid metabolism
	5	fad E (Possibly electron transport flavoprotein for acyl CoA dehydrogenase(s))
	11	pls A (Temperature sensitive G3P acyltransferase)
	22	fab A (β-Hydroxydecanoyl-thioester dehydrase)
	24	fab D (Malonyl CoA-ACP transacylase)
	40	fad D (Acyl CoA synthetase)
	46	fab C (Biosynthesis of UFA)
glp T (G3P transport)		
glp A (Anaerobic G3P dehydrogenase)	48	
	49	pss (Phosphatidylserine synthetase)
	50	fab B (β-Ketoacyl ACP synthetase)
	62	fda (FDP aldolase)
	71	fab E (Acetyl CoA-carboxylase)
glp R (Repressor)		
glp D (Aerobic G3P dehydrogenase)	74	
	77	pls B (K_m defect in G3P acyltransferase)
	80	gps A (G3P synthase)
	84	pld A (Detergent resistant phospholipase A)
	85	fad A (Thiolase I)
		fad B (Hydroxyacyl-CoA dehydrogenase)
glp F (Glycerol facilitator)	87	
glp K (Glycerol kinase)		
	93	psd (Phosphatidylserine decarboxylase)

Figure 3: The biosynthesis of the phospholipids of E. coli. Abbreviations used are as follows: DHAP for dihydroxyacetone phosphate; FAD for flavin adenine dinucleotide; $NADP^+$ and NADPH respectively for the oxidized and reduced form of nicotinamide adenine dinucleotide phosphate; G3P for sn-glycerol 3-phosphate; ACP for acyl carrier protein; CMP for cytidine 5'-monophosphate; CDP for cytidine 5'-diphosphate; CTP for cytidine 5'-triphosphate; ADP for adenosine 5'-diphosphate; ATP for adenosine 5'-triphosphate; PS for phosphatidylserine; PGP for phosphatidylglycerol phosphate; CL for cardiolipin. (Modified from Raetz, 1977)

Figure 3


Glycerol
DHAP
Aerobic G3P dehydrogenase (glp D) or anaerobic G3P dehydrogenase (glp A)
NADPH
FAD
NADP+
G3P synthase (gps A)
ATP
ADP
Glycerol kinase (glp K)
sn-Glycerol 3-phosphate
2 R-C(=O)-S-CoA
or R-C(=O)-S-ACP
G3P acyltransferase (pls A or pls B)
Phosphatidic acid
PGP synthase
CDP-diglyceride hydrolase
CTP
Cytidyltransferase
PPi
CDP-diglyceride
CMP
L-serine
PS synthetase(pls)
CMP
Phosphatidylglycerol phosphate
Phosphatidylserine
PGP phosphatase
PS decarboxylase(psd)
CO2
Pi
Phosphatidylglycerol
Phosphatidylethanolamine
CL synthase
Glycerol
Cardiolipin

CHAPTER 2

MATERIALS AND METHODS

I. Chemicals

L-[^{3}H]isoleucine, sn-[^{14}C]glycerol 3-phosphate, and carrier-free [^{33}P]phosphate were purchased from the New England Nuclear Corp., Boston, Mass. Carrier-free [^{32}P] phosphate and sn-[^{14}C]glycerol 3-phosphate were obtained from ICN Corp., Irvine, Calif. The following materials were obtained from the Sigma Chemical Co., St. Louis, Mo.: DL-glyceraldehyde 3-phosphoric acid (diethyl acetal, monobarium salt); N,N-bis-(2-hydroxyethyl)glycine (Bicine); 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB); tris(Hydroxymethyl)aminomethane (TRIS); D- and L-glyceraldehyde; ethyl methanesulfonate; L-isoleucine; L-valine; GTP; CTP; UTP; ATP; octylphenoxypolyethoxyethanol (the nonionic detergent Triton X-100); casein hydrolysate; and bovine serum albumin. D-Glyceraldehyde 3-phosphoric acid (diethyl acetal, dicyclohexyl ammonium salt) was a product of Boehringer Mannheim Corp., New York, N.Y. DL-3,4-dihydroxybutyl-1-phosphonate, DL-3,4-dihydroxy[3-^{3}H]butyl-1-phosphonate and DL-3-hydroxy-4-oxobutyl-1-phosphonic acid (diethyl acetal, dilithium salt) were prepared by the method of Goldstein et al (Goldstein et al., 1974a; Goldstein et al., 1974b). Glyceraldehyde 3-phos-

phoric acid and 3-hydroxy-4-oxobutyl-1-phosphonic acid were generated from the corresponding acetals by using the procedure recommended by the Sigma Chemical Co. (0.50 mmoles of diethyl acetal of glyceraldehyde 3-phosphoric acid or its phosphonic acid analogue were dissolved in 7 ml of distilled water and mixed with 3.0 grams of Dowex 50, Hydrogen ion form. The mixture was placed in a boiling water bath for 3 min with constant shaking, then immediately chilled in ice and centrifuged. The Dowex 50 was washed once with 3 ml of distilled water. The combined supernatants containing 50 mM glyceraldehyde 3-phosphate or its phosphonic acid analogue were stored frozen until required).

1-Palmitoyl glycerol 3-phosphate (lysophosphatidic acid) and cytidine diphosphate-dipalmitin (CDP-dipalmitin) were purchased from Serdary Research Laboratories Inc., London Ontario, Canada. Oleoyl coenzyme A and palmitoyl coenzyme A (CoA) were obtained from P-L Biochemicals, Inc., Milwaukee, Wis. The bacterial phospholipid standards phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin were purchased from Supelco Inc., Bellefonte, Pa.

Silica Gel G thin-layer plates were obtained from Analabs, Inc., North Haven, Conn. Polyethyleneimine impregnated thin-layer plates, polygram CEL 300 PEI, were purchased from Brinkmann Instruments, Inc., Westbuty, N.Y. Eugon agar was a product of Fisher Scientific, Fair Lawn, N.J. 2-Mercaptoethanol and glycerol were purchased from Matheson, Coleman

and Bell, Norwood, Ohio. Bacto-Agar was a product of Difco Laboratories, Detroit, Mich. Formic acid was a product of J. T. Baker Chemical Co., Philipsburg, N.J. Membrane filter, HA 0.45 μ were purchased from Millipore Corp., Bedford, Mass. Kodak no-screen medical X-ray film; Kodak liquid X-ray developer and replenisher; Kodak rapid fixer with hardener were purchased from Eastmen Kodak Co., N.Y., N.Y. All other chemicals were of reagent grade.

II. Bacterial Strains and Culture Conditions

The bacterial strains studied are described in Table 1. The effect of glyceraldehyde 3-phosphate and related compounds upon growth was determined by monitoring growth turbidimetrically in a Klett-Summerson colorimeter with a 660-nm filter (1 Klett unit is equivalent to 5×10^6 cell/ml). Cells were cultured in 10 to 25 ml of medium in 250-ml Erlenmeyer flasks fitted with side arms. Incubations were in a New Brunswick Aquatherm G86 or Metabolyte G77 water bath shaker at 200 rpm. In all cases, several generations of exponential growth were allowed for the dilute starting cultures to attain the cell density at which the experiments were initiated. Viability studies were performed by diluting cultures and plating on Eugon agar. Unless a specific temperature is indicated, all incubations

were at 37 C.

Three types of minimal media were employed in growth studies. E. coli strains CY 115, 244 and 7 were cultured in Vogel and Bonner minimal medium (Vogel & Bonner, 1956) containing 0.5% glycerol as the sole carbon source and supplemented with thiamine-hydrochloride, 2 mg/liter; methionine, 50 mg/liter; and tryptophan, 50 mg/liter. E. coli strains 6, 8, BB #6, BB #7, BB26-36 R2, M2-3, M4-9, and 3C-1 were cultured in Garen and Levinthal minimal medium containing 0.6 mM phosphate (Garen & Levinthal, 1960) with 0.5% potassium succinate as the sole carbon source. E. coli strains K10 and NP 315 were cultured in the same medium described above except that 0.5% glycerol was supplemented as the sole carbon source. A Bicine-buffered medium was utilized in experiments involving the effects of glyceraldehyde on the growth of E. coli 8, 9 and M4-9 and the effects of glyceraldehyde 3-phosphate and its phosphonic acid analogue on the rate of synthesis of RNA, DNA, phosphoglycerides and proteins. This medium contained Bicine-hydrochloride, 100 mM (pH 7.4); NaCl, 90 mM; KCl, 40 mM; NH_4Cl, 200 mM; and all the other salts in concentrations required for the Garen and Levinthal synthetic medium (Garen & Levinthal, 1960). Cells used for the preparation of enzyme extracts were cultured in Garen and Levinthal minimal medium as described above and harvested in late logarithmic-phase.

III. Assay of Protein Synthesis

Cultures of E. coli strain 8 at a cell density of 15 to 20 Klett units were simultaneously mixed with various concentrations of DL-glyceraldehyde 3-phosphate, DL-3-hydroxy-4-oxobutyl-1-phosphonate or water and L-[^{3}H]isoleucine (0.45 μCi/ml, 3.93 mCi/mmole). Incorporation of label into protein was determined by a slight modification of the procedure of Byfield and Scherbaum (Byfield & Scherbaum, 1966; Tropp et al., 1970). Samples (0.1 ml) of the culture were removed at the indicated times and spotted on Whatman no. 3MM filter paper discs which were immediately immersed in 5% trichloroacetic acid. The old trichloroacetic acid was poured out 30 min after the last disc was collected, and the discs were washed for an additional 20 min with fresh 5% trichloroacetic acid. The process was repeated twice more, after which the filters were washed twice with acetone. Approximately 10 ml of 5% trichloroacetic acid were used for each of the discs in each wash and approximately half as much acetone was used. The dry discs were counted in toluene scintillation fluid (Tropp et al., 1970) by use of a Beckman model LS-200 scintillation counter.

IV. Accumulation of DNA, RNA and Phosphoglycerides

Cultures of E. coli strain 8 at a cell density of 15 to 20 Klett units were simultaneously mixed with various concentrations of DL-glyceraldehyde 3-phosphate, DL-3-hydroxy-4-oxobutyl-1-phosphonate or water and 1 to 3 μCi of [^{33}P]phosphate per ml. Samples of 1 ml were removed at the indicated times and then analyzed by a modification of the procedure of Lusk and Kennedy (Lusk and Kennedy, 1972; Shopsis et al., 1974). They were treated with 2.0 ml of cold 10% (W/V) trichloroacetic acid and 1 ml of cold carrier E. coli sonic extract (100 Klett units before sonification) was added. The precipitates were collected by centrifugation, washed two times with cold 5% trichloroacetic acid, and then suspended in 7.0 ml of chloroform-methanol-water (5:5:1) for an hour. The samples were then centrifuged and the supernatant fluid containing the phosphoglycerides was removed. To the supernatant was added 1.6 ml of water to separate the chloroform layer. The chloroform extract was then washed three times with 2 M KCl and once with water according to the procedure of Bligh and Dyer (Bligh & Dyer, 1959) as modified by Ames (Ames, 1968). A portion of these chloroform soluble extracts was evaporated and counted as described above. The remainder of the chloroform soluble extracts was retained for subsequent analysis. The pellets were washed

with chloroform-methanol-water (5:5:1), the wash was discarded, and the pellets were suspended in 2.0 ml of 0.5 N KOH. These suspensions were incubated overnight at 30 C to hydrolyze the RNA. The samples were chilled, 2.0 ml of 1 M perchloric acid added, and the samples centrifuged to sediment the DNA. A 1.0-ml aliquot of the supernatant fluid containing hydrolyzed RNA was counted in 10 ml of Patterson-Greene scintillation fluid (Patterson & Greene, 1965). The DNA pellets were dissolved in 2.0 ml of 1 N KOH, neutralized with HCl, and precipitated with 12% trichloroacetic acid. The new pellets were washed once with 5% trichloroacetic acid, dissolved in 1.0 ml of 0.2 N KOH, and counted in 10 ml of Patterson-Greene scintillation fluid (Patterson & Greene, 1965).

V. Rate of Synthesis of Macromolecules and Phosphoglycerides

The rate of synthesis of RNA, DNA, and phosphoglycerides was determined by pulse labeling with [^{32}P]phosphate. Cultures of E. coli strain 8 at a cell density of 15 to 20 Klett units were treated with 0.1 mM DL-glyceraldehyde 3-phosphate, DL-3-hydroxy-4-oxobutyl-1-phosphonate or water. At various time intervals after treatment, 2 ml of cells were incubated with 30 μCi of [^{32}P] phosphate for 10 min. They were treated with 3.0 ml of cold 10% trichloroacetic acid and 1 ml of cold

carrier E. coli sonic extract was added. The precipitates were collected, washed and separated into RNA, DNA and phosphoglycerides as described above.

The rate of protein synthesis was followed by measuring the conversion of isoleucine into trichloroacetic acid-insoluble material. Cultures of E. coli strain 8 at a cell density of 15 to 20 Klett units were treated with 0.1 mM DL-glyceraldehyde 3-phosphate, DL-3-hydroxy-4-oxobutyl-1-phosphonate or water. At various time intervals after treatment, 1 ml of cells were incubated with 5 µCi of L-[^{3}H]isoleucine and 15 µg of L-isoleucine for 10 min. Samples of 0.10 ml were removed and assayed as previously described.

VI. Analysis of Phosphoglycerides

Lipid extracts obtained in the experiments described above were analyzed by thin-layer chromatography. The solvent was evaporated and the samples were redissolved in chloroform. The chromatographic procedure was the two-step developing system with acetone-light petroleum ether (1:3) as the first solvent and chloroform-methanol-water (65:25:3) as the second solvent (Nunn & Tropp, 1972). After development of the thin layer chromatograms, the phospholipids were detected by exposure of the plates to iodine vapors. The identification of various lipids was established by the

simultaneous chromatography of known standards. Radioactivity of the individual spots was determined (Shopsis et al., 1974).

VII. Assay of ^{32}P Incorporation into Nucleotides

The intracellular nucleotide concentrations were determined by the procedure of Cashel (Cashel et al., 1969). Cultures of strain 8 and the glyceraldehyde 3-phosphate resistant mutant, strain 3C-1 were grown in Garen and Levinthal minimum medium containing 0.6 mM phosphate (Garen and Levinthal, 1960) supplemented with 0.5% potassium succinate. When a cell density of 20 Klett units was reached, 2 ml of cultures were simultaneously mixed with a drug (0.1 mM DL-glyceraldehyde 3-phosphate, 0.1 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate, 0.03 mM DL-3,4-dihydroxybutyl-1-phosphonate) or water and 250 µCi of [^{32}P] phosphate. The cultures were incubated at 37 C for 30 min, then samples were removed and filtered on a membrane filter. The filter and cells were then suspended in 2 ml of ice-cold 2 N formic acid. The nucleotide levels were determined by chromatographing an aliquot of these extracts on polyethyleneimine plates (Cashel et al., 1969). The plates were developed with 0.85 M potassium phosphate buffer (pH 3,4). ^{32}P-Labeled nucleotides were located by autoradiography and cochromato-

graphed with unlabeled nucleotide standards (ATP, CTP, GTP and UTP) which were in turn located with ultraviolet light. The autoradiograms were developed by using the procedure recommended by Eastman Kodak Co. (The autoradiograms were immersed in developer for 5 min at 68 F with intermittent agitation. Films were then removed with tongs and immersed in a 3.5% acetic acid stop bath for 1 min with constant agitation. They were then placed in fixer at 60-75 F for 10 min with intermittent agitation. Finally the autoradiograms were washed throughly with tap water for 30 min and allowed to dry. The Rf values of ATP, CTP, GTP and UTP were 0.31, 0.38, 0.15 and 0.50, respectively). In a control experiment, in which cell extracts were treated with activated charcoal before chromatography, insignificant amounts of label were found in the areas of the chromatgram corresponding to the locations of the nucleoside triphosphates.

VIII. Assay of Acyl CoA:sn-glycerol 3-Phosphate Acyltransferase

Membranes were prepared from E. coli strain 8, BB26-36 R2 and 3C-1 as described by Cheng et al (Cheng et al., 1975). The activity of acyl CoA:sn-glycerol 3-phosphate acyltransferase of the membrane preparation was assayed by determining the incorporation of [^{14}C] glycerol 3-phosphate (24.8 mCi/mmol) into lipid. The reaction mixture (total volume of 0.35 ml) contained 35 µmol of Bicine-hydrochloride, pH 8.5; 2.45 µmol of $MgCl_2$; 150 µg of bovine serum albumin; 21 nmol of palmitoyl CoA or oleoyl CoA; 100-120 µg of enzyme;

and the indicated concentration of sn-[^{14}C]glycerol 3-phosphate. The assays were performed at 30 C and initiated by the addition of palmitoyl CoA or oleoyl CoA. The specific activity at maximum velocity of the strain 8 preparations was 2.4 nmol/min per mg. In some cases, the activity of acyl CoA:sn-glycerol 3-phosphate was also assayed by monitoring the release of CoA by measuring its chemical interaction with DTNB by a modification of the procedure of Cronan et al (Cronan et al., 1970). The assay mixture (total volume of 1 ml) contained 100 µmol of Bicine-hydrochloride, pH 8.5; 5 µmol of $MgCl_2$; 1 mg of bovine serum albumin; 50 nmol of palmitoyl CoA; 1.0 µmol of DTNB; 60 µg of enzyme; and 0.75 µmol of sn-glycerol 3-phosphate. The initial rates were monitored by measuring absorbance at 25 C in a Gilford model 250 spectrophotometer equipped with a multiple-sample absorbance recorder.

IX. Assay of Acyl CoA:lysophosphatidate Acyltransferase

The preparation of this particulate enzyme was the same as that of acyl CoA:sn-glycerol 3-phosphate acyltransferase. The acyl CoA:lysophosphatidate acyltransferase was assayed by a colorimetric procedure that was essentially the same as described above for acyl CoA:glycerol 3-phosphate acyltransferase (Cronan et al., 1970; Van Den Bosch

& Vagelos, 1970). The reaction mixture (total volume of 1 ml) contained 100 μmol of Bicine-hydrochloride, pH 8.5; 0.5 μmol of $MgCl_2$; 1 mg of bovine serum albumin; 60 nmol of oleoyl CoA; 1.0 μmol of DTNB; 60 μg of enzyme; and 25 nmol of 1-palmitoyl glycerol 3-phosphate.

X. Assay of CDP-diglyceride:sn-Glycerol 3-Phosphate Phosphatidyltransferase

The particulate fraction was prepared from E. coli strain 8 or 3C-1 as described previously (Cheng et al., 1975). The activity of CDP-diglyceride:sn-glycerol 3-phosphate phosphatidyltransferase was assayed by monitoring the conversion of [^{14}C]glycerol 3-phosphate into chloroform-extractable material as described previously (Cheng et al., 1975). The assay mixture (total volume of 0.25 ml) contained 62.5 μmol of Bicine-hydrochloride, pH 8.0; 20 μmol of CDP-dipalmitin; 2.5 μmol of $MgCl_2$; 1.25 μmol of 2-mercaptoethanol; 0.5 mg of Triton X-100; 55-87 μg of particulate enzyme; and the indicated concentrations of sn-[^{14}C]glycerol 3-phosphate (24.8 mCi/mmol or 100 mCi/mmol). The enzyme assay was performed at 37 C and initiated by the addition of enzyme preparation.

All enzyme preparations were prepared at 1 to 4 C. Protein concentrations were determined by the method of

Lowry et al (Lowry et al., 1951). The rates of reaction were linearly dependent upon enzyme concentration and time in the range reported.

XI. Isolation of Glyceraldehyde 3-Phosphate Resistant Mutants

Glyceraldehyde 3-phosphate (GAP) resistant mutants were isolated by the procedure of Miller (Miller, 1972). E. coli strain 8 were grown to 50 Klett units in glucose minimal medium. Cultures were washed and resuspended in half of the original volume of minimal medium containing 0.2 M Tris, pH 7.5. Two ml of resuspended cells were added into a solution of 0.4 ml ethyl methanesulfonate (EMS) and 7.6 ml 1 M Tris, pH 7.4. Vigorous shaking is then carried out for 5 minutes at 37 C. The mutagenized cells were diluted 10-fold in 1% casein hydrolysate, 0.5% NaCl, pH 7.4 and incubated at 37 C for phenotypic segragation. The overnight culture were diluted 25-fold and grown in the same casein hydrolysate medium containing 2.0 mM DL-glyceraldehyde 3-phosphate and 2.5 mM L-glyceraldehyde. GAP-resistant cells were streaked on Eugon agar plates. Colonies appearing on these plates were then plated on various agar plates and incubated overnight at 37 C for the selection of GAP-resistant mutants retaining characteristics of parent strain.

For this purpose, the following agar plates were prepared: 1.5% Difco agar in Garen and Levinthal minimal medium (Garen and Levinthal, 1960) supplemented with 0.6 mM phosphate and various compounds including (1) 2.5 mM DL-glycerol 3-phosphate and 0.5% succinate, (2) 0.5% glycerol 3-phosphate, (3) 2.5 mM DL-3,4-dihydroxybutyl-1-phosphonate and 0.5% glucose, (4) 2.0 mM DL-glyceraldehyde 3-phosphate, 2.5 mM L-glyceraldehyde and 0.5% succinate, (5) 0.5% glycerol, (6) L-valine, 100 µg/ml and 0.5% glucose.

The ability to transport glycerol 3-phosphate was examined by measuring the incorporation of DL-3,4-dihydroxy [3-^{3}H]butyl-1-phosphonate into lipid. E. coli strains 8, 6, M4-9 and the mutants to be tested were cultured on 36-39 Klett units, [^{3}H]DHBP (61 µCi/µmol) was added to a final concentration of 0.033 mM and incubated at 37 C. Samples of 0.6 ml were removed after 2 hr. To the aliquots, 2.25 ml of chloroform-methanol (1:2), 0.75 ml of chloroform and 0.75 ml of water were added sequentially. The chloroform extracts were then washed and counted as described above.

Table 1. Bacterial strains studied[a]

Strain	Mating Type	Genotype	Source and Reference
K10	Hfr	ton A22, T_2^R, rel-1	D. Fraenkel, Harvard Medical School (Bachmann, 1972)
6	Hfr C	pho A8, ton A22, T_2^R, glp T, rel-1 (λ)	E. C. C. Lin, Harvard Medical School (Hayashi et al., 1964)
7	Hfr C	glp R^c2, pho A8, ton A22, T_2^R, rel-1 (λ)	E. C. C. Lin, Harvard Medical School (Hayashi et al., 1967)
8	Hfr C	Same as 7, glp D3	J. Cronan Jr., Yale University (Hayashi et al., 1964)
9	Hfr C	Same as 8, glp K	E. C. C. Lin, Harvard Medical School (Hayashi et al., 1964)
244	Hfr C	Same as 7, glp K_i	E. C. C. Lin, Harvard Medical School (Berman & Lin, 1971)
BB #6	Hfr C	Same as 8, gps A^{FR}	R. Bell, Duke University Medical Center Personal Communication

Table 1. Bacterial strains studied[a] (continued)

Strain	Mating Type	Genotype	Source and Reference
BB #7	Hfr C	Same as 8, gps A^{FR}	R. Bell, Duke University Medical Center Personal Communication
BB26-36 R2	Hfr C	Same as 8, gps $A3^{FR}$, pls B26	R. Bell, Duke University Medical Center (Bell & Cronan, 1975)
CY 115	F^-	gps A20, met E70, trp E8, xyl-5, tsx-67, str-109	J. Cronan Jr., Yale University (Cronan & Bell, 1974)
NP 315	Hfr	Same as K10, fda^{ts}	F. C. Neidhardt, University of Michigan (Böck & Neidhardt, 1966)
M2-3	Hfr C	Same as 8, glp T	This Laboratory (Leifer et al., 1977)
M4-9	Hfr C	Same as 8, glp T	This Laboratory (Leifer et al., 1977)

[a] Genetic nomenclature is that of Bachmann et al (Bachmann et al., 1976). The allele numbers are those of the Coli Genetic Stock Center at Yale University.

CHAPTER 3

RESULTS AND DISCUSSION

I. The Biologically Active Form of DL-Glyceraldehyde 3-Phosphate and Its Phosphonic Acid Analogue, DL-3-Hydroxy-4-oxobutyl-1-phosphonate

At a concentration of 2.5 mM, DL-glyceraldehyde 3-phosphate and its phosphonate analogue, DL-3-hydroxy-4-oxobutyl-1-phosphonate, are potent inhibitors of the growth of E. coli strain 8 (Figure 1). The L-form of the phosphate must account for the inhibition since as shown in Figure 1, D-glyceraldehyde 3-phosphate does not influence cell growth. Neither L-glyceraldehyde 3-phosphate nor optically pure phosphonic acid analogue was available for study.

Figures 2A and 2B show the effects of various concentrations of DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate, respectively, upon the growth of E. coli strain 8. The two compounds exert their inhibitory effects within the same concentration ranges.

The data presented in Figure 3A reveal that 2.5 mM DL-glyceraldehyde 3-phosphate is bactericidal to E. coli strain 8 whereas the data presented in Figure 3B indicate that 2.5 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate is bacteriostatic.

A racemic mixture of glyceraldehyde 3-phosphate is

bactericidal (Figure 3A) whereas D-glyceraldehyde 3-phosphate does not affect cell growth (Figure 1). The hypothesis that the L-forms of both the phosphate, and phosphonate are the biologically active inhibitor is consistent with following data related to transport. The sn-glycerol 3-phosphate transport system is required for glyceraldehyde 3-phosphate and 3-hydroxy-4-oxobutyl-1-phosphonate to act as inhibitors. To study this possibility in greater detail, several independently isolated glycerol 3-phosphate transport-negative mutants have been examined. These mutants include E. coli strain 6 which has a defective glycerol 3-phosphate transport system (Hayashi et al., 1964) and strain M2-3, a 3,4-dihydroxybutyl-1-phosphonate-resistant mutant which simultaneously has lost the ability to transport 3,4-dihydroxybutyl-1-phosphonate and glycerol 3-phosphate (Leifer et al., 1977). When cultures of E. coli strain 6 and M2-3 were grown on low-phosphate synthetic medium (Garen & Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate, both strains were completely insensitive to DL-glyceraldehyde 3-phosphate and its phosphonic acid analogue (Figures 4A and 4B). In contrast, strain 8, the parent of strain M2-3 was quite sensitive to the aldehydes (Figure 1). Hayashi et al. have demonstrated that DL-glyceraldehyde 3-phosphate is an inhibitor of the sn-glycerol 3-phosphate transport system. The stereospecificity of the glycerol 3-phosphate transport system (Hayashi et al., 1964) and its

requirement for cell susceptibility strongly suggested that the L-form of the glyceraldehyde 3-phosphate and its phosphonic acid analogue are the biologically active inhibitors. Additional transport systems capable of recognizing glyceraldehyde 3-phosphate and 3-hydroxy-4-oxobutyl-1-phosphonate may also exist, for example, E. coli cells possessing the hexose phosphate transport system are susceptible to these compounds (Tropp, B., unpublished data).

We examined the question of whether L-glyceraldehyde 3-phosphate or its nonphosphorylated derivative was the true inhibitor. As evident from Figure 5A, L-glyceraldehyde has some inhibitory effect upon strain 9, a glycerokinase-deficient mutant of strain 8 (Hayashi et al., 1964). However, strain 8 was considerably more susceptible to L-glyceraldehyde (Figure 5B). Strain M4-9, a derivative of strain 8 which lacks the G3P transport system is also sensitive to L-glyceraldehyde (Figure 5C). The ability of glycerokinase to catalyze the phosphorylation of L-glyceraldehyde (Hsyashi & Lin, 1967) provides an explanation for the difference in susceptibility observed between strain 8 and strain 9. The slight inhibition observed in strain 9 may be due to leakiness of the mutation, a second kinase activity, or an effect of the nonphosphorylated aldehyde. We do not have sufficient data to distinguish among the possibilities. D-Glyceraldehyde has an equally slight effect upon strains 8 and 9 (Figures 5A and 5B), which was not

studied further. Since L-glyceraldehyde is a less potent inhibitor of cell growth than its phosphate ester (Figures 1 and 5B), it appears unlikely that L-glyceraldehyde 3-phosphate is hydrolyzed prior to exerting its inhibitory effect. Furthermore, strain 8 lacks alkaline phosphatase activity (Hayashi et al., 1964). These results clearly indicate that the phosphorylated derivative is important in the bactericidal effects. L-Glyceraldehyde is quite an effective inhibitor of cell growth in strains capable of phosphorylating this compound and the D-form is considerably less active (Figures 5A and 5B). In addition, a G3P transport-negative strain which is resistant to glyceraldehyde 3-phosphate nevertheless is sensitive to L-glyceraldehyde (Figure 5C). These results provide additional support for the hypothesis that the L-form of glyceraldehyde 3-phosphate and its phosphonic acid analogue are the biologically active inhibitors.

In aqueous solution glyceraldehyde 3-phosphate exists as the geminal diol (hydrated form) and free aldehyde in a molar ratio of 29:1 (Trentham et al., 1969). The L-glyceraldehyde 3-phosphate may therefore be considered to be an analogue of sn-glycerol 3-phosphate in which a hydroxyl group replaces a hydrogen atom at carbon-1. 3-Hydroxy-4-oxobutyl-1-phosphonate must also exist primarily as the geminal diol in aqueous solution. This structural feature may explain the ability of DL-glyceraldehyde 3-phosphate and its phosphonic acid analogue to be transported into and

subsequently inhibit the growth of E. coli.

It is of interest that, although D-glyceraldehyde 3-phosphate and sn-glycerol 3-phosphate are ubiquitous metabolic intermediates, L-glyceraldehyde 3-phosphate is not a naturally occurring biological compound. The close structural relationship between glyceraldehyde 3-phosphate and glycerol 3-phosphate may be responsible for the evolution of optically unrelated intermediates for carbohydrate and phosphoglyceride metabolism.

II. Effects of DL-Glyceraldehyde 3-Phosphate and Its Phosphonic Acid Analogue, DL-3-Hydroxy-4-oxobutyl-1-phosphonate on the Accumulation and the Rate of Synthesis of Macromolecules and Phospholipids

One method for delineating the site of action of a drug is to examine the various concentrations of the drug on macromolecular and lipid synthesis. The effects of various concentrations of DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate upon the accumulation of protein, DNA, RNA and phospholipid are depicted in Figures 6 to 10. This approach has been applied with some success to the delineation of the target site for another glycerol 3-phosphate analogue, 3,4-dihydroxybutyl-1-phosphonate (Shopsis et al., 1973; Shopsis et al., 1974). However, it

failed to reveal a single most sensitive site of action for glyceraldehyde 3-phosphate and its phosphonic acid analogue (Figures 6 to 11; Tables 1 and 2). Among the pathways examined, protein synthesis is least affected by either of the aldehydes.

The phosphoglycerides accumulated during a one hour incubation of E. coli strain 8, cultured in the presence of DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate at each of the concentrations tested in Figure 9 were analyzed. The phosphatidylglycerol and phosphatidylethanolamine accumulations were significantly inhibited by DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate. Cardiolipin which accounts for less than 5% of the phosphoglyceride fraction of these early logarithmic-phase cultures was not markedly affected by either aldehyde inhibitor (Figures 10A and 10B). Although phosphatidylglycerol and phosphatidylethanolamine accumulations were inhibited to the same extent by DL-glyceraldehyde 3-phosphate (Figure 10C), the inhibition of phosphatidylglycerol accumulation was slightly stronger than that of phosphatidylethanolamine during the one hour treatment with 3-hydroxy-4-oxobutyl-1-phosphonate (Figure 10D).

Both DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate inhibit macromolecular and phospholipid synthesis, however, glyceraldehyde 3-phosphate is a more potent inhibitor than is its phosphonic acid analogue

(Figures 6 to 11; Tables 1 and 2). Comparison amongst the inhibitory effects of 3,4-dihydroxybutyl-1-phosphonate, glyceraldehyde 3-phosphate and 3-hydroxy-4-oxobutyl-1-phosphonate reveals several differences concerning the accumulation of macromolecules and phospholipids in the presence of drugs. Studies on the incorporation of labeled precursors into DNA, RNA, protein and lipid show that 3,4-dihydroxybutyl-1-phosphonate inhibits phospholipid accumulation most effectively (Shopsis et al., 1973). The major change was a reduction in the percentage of radioactivity in the phosphatidylglycerol fraction (Shopsis et al., 1973). There is no preferential inhibition of the accumulation of deoxyribonucleic acid, ribonucleic acid or phospholipids by either glyceraldehyde 3-phosphate or 3-hydroxy-4-oxobutyl-1-phosphonate, protein synthesis is less affected (Tables 1 and 2). However, the slight preferential inhibition of the accumulation of phosphatidylglycerol caused by 3-hydroxy-4-oxobutyl-1-phosphonate is somewhat similar to that caused by 3,4-dihydroxybutyl-1-phosphonate (Figure 10D and Shopsis et al., 1973).

Pulse-labeling studies were designed to obtain further information about the perturbation of macromolecular and phosphoglyceride metabolism in E. coli caused by DL-glyceraldehyde 3-phosphate and its phosphonic acid analogue at a concentration of 0.10 mM. This concentration was previously demonstrated to strongly inhibit phospholipid synthesis

(Figures 9 and 10) but to have only a mild effect upon cell growth (Figure 2). The effect of these compounds on the rate of synthesis of protein, DNA, RNA and phospholipid have been examined (Figures 12 to 16). Cultures of E. coli strain 8 were pulsed with [^{32}P]phosphate at various times after the addition of the inhibitors. The rate of synthesis of DNA, RNA and phosphoglycerides is severely inhibited by glyceraldehyde 3-phosphate at the earliest time point. However, only DNA and phosphoglyceride synthesis were inhibited to a great extent by glyceraldehyde 3-phosphate at later time points (Figures 12 to 15, 17 and Table 3). The rate of synthesis of protein is the least affected during the one hour treatment with glyceraldehyde 3-phosphate (Figure 17A and Table 3). 3-Hydroxy-4-oxobutyl-1-phosphonate inhibits the rate of synthesis of macromolecules and phospholipids more strongly at later than at earlier times (Figures 12 to 14, 16, 17 and Table 4). Phosphoglyceride and DNA were most affected by the phosphonate.

Figures 15 and 16 present the results of an analysis of the phospholipid synthesized during the pulse. The rate of synthesis of phosphatidylethanolamine is most strongly inhibited by glyceraldehyde 3-phosphate, although phosphatidylglycerol is also severely affected (Figure 17D). The inhibition of the rate of the synthesis of phospholipids becomes less pronounced during the one hour treatment with glyceraldehyde 3-phosphate (Figure 17D). 3-Hydroxy-4-oxobutyl-1-

phosphonate caused a quite different effect on the rate of phospholipid synthesis. The rate of synthesis of phosphatidylglycerol is severely inhibited during the one hour treatment with the phosphonic acid analogue. The strongest inhibition of phosphatidylethanolamine is at the later times (Figure 17E) and this is consistent with the result that a slight preferential inhibition of the accumulation of phosphatidylglycerol caused by 3-hydroxy-4-oxobutyl-1-phosphonate (Figure 10D). The rate of synthesis of cardiolipin was only mildly affected by the presence of these compounds (Figure 17, D and E).

Comparison amongst the effects of 3,4-dihydroxybutyl-1-phosphonate, glyceraldehyde 3-phosphate and 3-hydroxy-4-oxobutyl-1-phosphonate on the rate of synthesis of macromolecules and phospholipids reveals several differences. Glyceraldehyde 3-phosphate inhibits the rate of synthesis of macromolecules and phosphoglycerides more strongly at early rather than late times. On the contrary, its phosphonic acid analogue inhibits more strongly at late times than at the early times (Figure 17, A to E). The rate of synthesis of DNA and phosphoglycerides are severely inhibited by treatment with 0.10 mM DL-glyceraldehyde 3-phosphate or DL-3-hydroxy-4-oxobutyl-1-phosphonate for 65 min (Tables 3 and 4). However, pulse labeling studies using [^{32}P]phosphate have indicated that phospholipid synthesis is inhibited to a much greater extent by 3,4-dihydroxybutyl-1-phosphonate

than is the synthesis of DNA and RNA (Shopsis et al., 1974). Like 3,4-dihydroxybutyl-1-phosphonate, 3-hydroxy-4-oxobutyl-1-phosphonate severely inhibits the rate of phosphatidylglycerol synthesis at the earliest time point (Figure 17E and Shopsis et al., 1974). The rate of synthesis of phosphatidylglycerol and phosphatidylethanolamine were strongly inhibited by 3-hydroxy-4-oxobutyl-1-phosphonate at later time points. The inhibition of the rate of synthesis of phospholipids by glyceraldehyde 3-phosphate is different from that by 3,4-dihydroxybutyl-1-phosphonate and 3-hydroxy-4-oxobutyl-1-phosphonate, since the rate of synthesis of phosphatidylethanolamine was most affected by glyceraldehyde 3-phosphate at all times (Figure 17D). The inhibition of the rate of synthesis of each class of phospholipids: phosphatidylethanolamine, phosphatidylglycerol and cardiolipin parallels that of total phospholipids during the one hour treatment with glyceraldehyde 3-phosphate (Figure 17D). Therefore the patterns of inhibition of the rate of phosphoglyceride synthesis caused by 3,4-dihydroxybutyl-1-phosphonate, glyceraldehyde 3-phosphate and 3-hydroxy-4-oxobutyl-1-phosphonate are quite different from one another (Shopsis et al., 1974; Figure 17, D and E).

It was shown that 0.1 mM DL-glyceraldehyde 3-phosphate or DL-3-hydroxy-4-oxobutyl-1-phosphonate inhibits the rate of macromolecular and lipid synthesis (Figures 12 to 16) and DL-3,4-dihydroxybutyl-1-phosphonate, at a concentration of

0.03 mM, caused an immediate 50% decrease in the rate of phosphatidylglycerol synthesis (Shopsis et al., 1974). Under these conditions, the analogues had only a slight effect upon cell growth (Figure 2, A and B; Shopsis et al., 1973). When strain 8 was cultured on low phosphate minimum medium (Garen & Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% succinate, the addition of 0.1 mM DL-glyceraldehyde 3-phosphate, 0.1 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate or 0.03 mM DL-3,4-dihydroxybutyl-1-phosphonate did not significantly affect the intracellular concentrations of nucleoside triphosphates (Table 5). These results suggested that none of these glycerol 3-phosphate analogues causes strain 8 to become leaky or lose its energy supply. Therefore, the inhibition of rate of synthesis of macromolecules and phosphoglycerides observed is not due to a nucleotide limitation.

Studies with mutants (Bell, 1974) and inhibitors (Nunn, 1975; Nunn & Tropp, 1972; Shopsis et al., 1973; Shopsis et al., 1974) indicate that a block in phosphoglyceride synthesis is frequently accompanied by an inhibition of macromolecular synthesis. If, as appears possible, the primary target of L-glyceraldehyde 3-phosphate is at the level of phosphoglyceride biosynthesis, this compound should prove to be an important research tool in the study of phosphoglyceride metabolism. This is particularly so, since the non-phosphorylated derivative should be quite permeable to many

cell membranes and, therefore, not require a special transport system. Once inside the cell, glycerol kinase can activate the L-glyceraldehyde as shown for E. coli (Figure 5B). Preliminary experiments with Bacillus subtilis and yeast revealed that the former is quite susceptible to L-glyceraldehyde and DL-glyceraldehyde 3-phosphate, whereas the latter is not (Klein, D., Engel, R., and Tropp, B. unpublished data).

III. Effects of DL-Glyceraldehyde 3-Phosphate and Its Phosphonic Acid Analogue, DL-3-Hydroxy-4-Oxobutyl-1-Phosphonate on the Growth of Various E. coli Mutants

A second approach to the site of action involves a study of the effects of glyceraldehyde 3-phosphate and its phosphonic acid analogue on various E. coli mutants lacking enzymes involved in phospholipid and carbohydrate metabolism. The present studies were performed with E. coli strain 8 to facilitate a comparison with previous data concerning the effects of another sn-glycerol 3-phosphate analogue, 3,4-dihydroxybutyl-1-phosphonate on this strain (Cheng et al., 1975; Shopsis et al., 1972; Shopsis et al., 1973; Shopsis et al., 1974). The lack of aerobic G3P dehydrogenase activity in strain 8 is not related to the inhibitory effects observed, since strain 7 and other cells exhibiting this

activity were quite susceptible to glyceraldehyde 3-phosphate and its phosphonic acid analogue (Figure 18).

Several enzymes involved in carbohydrate and lipid metabolism should be considered as potential target sites for the aldehyde inhibitors. One possible site of activity for these compounds is G3P synthase (sn-G3P: NADP oxidoreductase) studied by Kito and Pizer (Kito & Pizer, 1969). This enzyme is inhibited by 3,4-dihydroxybutyl-1-phosphonate (Cheng et al., 1975). Strains BB #6 and BB #7 possess a G3P synthase which was resistant to feedback inhibition by glycerol 3-phosphate. As shown in Figure 19, A and B, they were sensitive to glyceraldehyde 3-phosphate and 3-hydroxy-4-oxobutyl-1-phosphonate. Furthermore, glyceraldehyde 3-phosphate is also bactericidal to strains BB #6 and BB #7 and the phosphonic acid analogue is bacteriostatic to them (Table 6). These results are similar to those obtained with strain 8 (Figure 3A), although strains BB #6 and BB #7 are more sensitive to glyceraldehyde 3-phosphate than strain 8. If the anabolic G3P dehydrogenase is the sole physiological site of inhibition then a strain such as E. coli CY 115 (Cronan & Bell, 1974) lacking this activity, cultured in minimum medium containing glycerol as the sole carbon source, should be resistant to glyceraldehyde 3-phosphate, 3-hydroxy-4-oxobutyl-1-phosphonate, and 3,4-dihydroxybutyl-1-phosphonate. The data presented in Figure 20 indicate this is definitely not the case. Thus each of these compounds must exert its

inhibitory effect upon some site other than or in addition to the G3P synthase. These results are noteworthy in another respect. They show that E. coli is sensitive to the inhibitors in a high phosphate medium.

E. coli strain 244 possesses a glycerol kinase which is insensitive to feedback inhibition by fructose 1,6-diphosphate (Berman & Lin, 1971). When strain 7 and 244 are cultured on the minimum medium (Garen & Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% glycerol as the carbon source, strain 244 is expected to have a larger internal pool of glycerol 3-phosphate than its wild-type parent strain 7. It was shown that strain 244 is far less sensitive to 3,4-dihydroxybutyl-1-phosphonate than strain 7 which is wild type for glycerol kinase (Tyhach et al., 1976). Both strains, however, were totally inhibited by 3,4-dihydroxybutyl-1-phosphonate when cultured on 0.5% glucose as carbon source. This effect is not due to glycerol 3-phosphate competing with 3,4-dihydroxybutyl-1-phosphonate for transport into the cell, since alkaline phosphatase was added to the growth medium (Tyhach et al., 1976). Strain 244 is also considerably less sensitive to glyceraldehyde 3-phosphate and 3-hydroxy-4-oxobutyl-1-phosphonate than is strain 7 (Figures 18 and 21). These results suggest that these three glycerol 3-phosphate analogues act directly upon enzymes involved in G3P metabolism and the high intracellular levels of glycerol 3-phosphate reverse the inhibitory effect

by these analogues.

L-Glyceraldehyde 3-phosphate might also serve as a substrate of fructose 1,6-diphosphate aldolase. In this case, L-sorbose 1,6-diphosphate would be formed, which might then be the true inhibitor. The experimental results depicted in Figures 22 and 23 serve to eliminate fructose 1,6-diphosphate aldolase from this category. E. coli strain NP 315 (Böck & Neidhardt, 1966), a temperature-sensitive fructose 1,6-diphosphate aldolase mutant, is quite sensitive to glyceraldehyde 3-phosphate and its phosphonic acid analogue at either 30 C or the nonpermissive temperature, 42 C. Strain K10 and NP 315 are more sensitive to 3-hydroxy-4-oxobutyl-1-phosphonate when cultured at 42 C than at 30 C (Figures 22 and 23). Wild-type E. coli K10 is more sensitive to the phosphonate at 30 C than was strain NP 315 at 30 C (Figures 22A and 23A). One possible interpretation of these results is that the altered aldolase has a different substrate recognition pattern which permits it to detoxify 3-hydroxy-4-oxobutyl-1-phosphonate at 30 C. The other glycolytic enzymes are not likely targets because of their greater stereospecificity for D-glyceraldehyde 3-phosphate.

Table 7 indicates the effects of 2.5 mM DL-glyceraldehyde 3-phosphate and 2.5 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate on the growth of various E. coli strains. Among the various mutants examined, glycerol 3-phosphate transport-negative cells were the only strains which are completely

insensitive to DL-glyceraldehyde 3-phosphate or DL-3-hydroxy-4-oxobutyl-1-phosphonate (Table 7).

E. coli strains which lack G3P synthase (CY 115), possess a temperature-sensitive fructose 1,6-diphosphate aldolase (NP 315), or a feedback resistant G3P synthase (BB #6 and BB #7) were strongly inhibited by 2.5 mM DL-glyceraldehyde 3-phosphate or 2.5 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate (Table 7). These results suggested that any one of these enzymes alone can not be the primary target. However, they do not exclude the possibility that some of these defective enzymes may contribute to the inhibitory effect observed in cells treated with the aldehyde inhibitors, since either glyceraldehyde 3-phosphate or 3-hydroxy-4-oxobutyl-1-phosphonate may have multiple target sites in E. coli (see following discussion).

IV. Effects of DL-Glyceraldehyde 3-Phosphate and Its Phosphonic Acid Analogue, DL-3-Hydroxy-4-Oxobutyl-1-Phosphonate on the Enzymes Involved in Phospholipid Synthesis

A third approach to the site of action is to determine whether the durg inhibits a specific enzymatic activity in an in vitro assay. Acyl CoA:glycerol 3-phosphate acyltransferase, the first enzyme of the phosphoglyceride biosynthetic pathway is one of the potential target sites. When assayed

by the radioactive method as shown in Figure 24, A and B, DL-glyceraldehyde 3-phosphate is a competitive inhibitor of the acyltransferase. D-Glyceraldehyde 3-phosphate does not inhibit the acyltransferase. With either oleoyl CoA or palmitoyl CoA as the acyl donor, the apparent K_i for L-glyceraldehyde 3-phosphate is 0.55 mM. Figure 25, A and B, show that 3-hydroxy-4-oxobutyl-1-phosphonate was also a competitive inhibitor of the acyltransferase in the presence of either oleoyl CoA or palmitoyl CoA as substrate. The apparent K_i for the corresponding L-form of 3-hydroxy-4-oxobutyl-1-phosphonate is 1.25 mM. The spectrophotometric assay confirmed that glyceraldehyde 3-phosphate and its phosphonic acid analogue inhibited acyltransferase activity. Furthermore, this assay revealed that neither DL-glyceraldehyde 3-phosphate nor its phosphonic acid analogue stimulates the hydrolysis of either palmitoyl CoA or oleoyl CoA and is therefore not the substrate for the acyltransferase.

It appeared possible that K_m mutants for the G3P acyltransferase, by virture of an inability to recognize L-glyceraldehyde 3-phosphate, might be resistant to this compound and its phosphonic acid analogue. Figure 26 shows that strain BB26-36 R2 is very sensitive to glyceraldehyde 3-phosphate and 3-hydroxy-4-oxobutyl-1-phosphonate. In addition, 2.5 mM DL-glyceraldehyde 3-phosphate is bactericidal to strain BB26-36 R2. In agreement with the report of Bell and Cronan (Bell & Cronan, 1975), it was found that the G3P acyltrans-

ferase of BB26-36 R2 had a K_m of 577 μM for sn-glycerol 3-phosphate. In the presence of 32.3 μM sn-glycerol 3-phosphate, 2 mM DL-glyceraldehyde 3-phosphate and 2 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate inhibited mutant acyltransferase by 41.8% and 37.5%, respectively (Table 8). The acyltransferase of BB26-36 R2 therefore recognizes glyceraldehyde 3-phosphate and its analogue and the susceptibility of mutant acyltransferase to the drugs was similar to that of the strain 8 enzyme (Table 8).

Acyl CoA:lysophosphatidate acyltransferase, the second enzyme of phosphoglyceride biosynthesis, was not sensitive to either DL-glyceraldehyde 3-phosphate or DL-3-hydroxy-4-oxobutyl-1-phosphonate. This is significant, since it indicates that all the sensitivity observed in the sn-glycerol 3-phosphate:acyl CoA acyltransferase assay is specific for this enzyme.

Figure 27 shows DL-glyceraldehyde 3-phosphate is a competitive inhibitor of CDP-diglyceride:sn-glycerol 3-phosphate phosphatidyltransferase. The D-enantiomer was without effect. 3-Hydroxy-4-oxobutyl-1-phosphonate is also a competitive inhibitor of the phosphatidyltransferase (Figure 28). The apparent K_i's for L-glyceraldehyde 3-phosphate and the comparable stereochemical form of the phosphonic acid analogue are 2.7 mM and 4.7 mM, respectively. The physiological significance of these enzymatic studies were demonstrated by observing the effect of DL-glyceraldehyde 3-

phosphate and 3-hydroxy-4-oxobutyl-1-phosphonate on the accumulation of phosphoglycerides (Figure 10, A and B) and the rate of synthesis of phosphoglycerides (Figure 17, D and E) previously described.

Both aldehydes are competitive inhibitors for acyl CoA:sn-glycerol 3-phosphate acyltransferase, the first enzyme of the phosphoglyceride biosynthetic pathway (Figures 24 and 25). The apparent K_i's for DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate are 1.1 mM and 2.5 mM, respectively. This is strikingly different from 3,4-dihydroxybutyl-1-phosphonate which is not recognized by the acyltransferase (Cheng et al., 1975). Although all three glycerol 3-phosphate analogues are recognized by CDP-diglyceride:sn-glycerol 3-phosphate phosphatidyltransferase, the K_i for DL-3,4-dihydroxybutyl-1-phosphonate is 740 μM (Cheng et al., 1975) which is much lower than the K_i's for L-glyceraldehyde 3-phosphate and S-3-hydroxy-4-oxobutyl-1-phosphonate, which are 2.7 mM and 4.7 mM, respectively (Figures 27 and 28). Thus the phosphatidyltransferase is considerably more susceptible to 3,4-dihydroxybutyl-1-phosphonate, than it is to glyceraldehyde 3-phosphate or 3-hydroxy-4-oxobutyl-1-phosphonate. These results plus the fact that acyl CoA:sn-glycerol 3-phosphate acyltransferase is more sensitive to DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate (apparent K_i of 1.1 mM and 2.5 mM, respectively) (Figures 24 and 25) than is CDP-

diglyceride:sn-glycerol 3-phosphate to DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate (apparent K_i of 5.4 mM and 9.4 mM, respectively) (Figures 27 and 28) helped to explain the different effects of these three glycerol 3-phosphate analogues on phospholipid synthesis. Glyceraldehyde 3-phosphate and 3-hydroxy-4-oxobutyl-1-phosphonate strongly inhibited the accumulation and the rate of synthesis of both phosphatidylethanolamine and phosphatidylglycerol (Figures 10A to 10D, 17D and 17E), while 3,4-dihydroxybutyl-1-phosphonate specifically inhibited the accumulation of phosphatidylglycerol (Shopsis et al., 1973) and severely and immediately inhibited the rate of synthesis of phosphatidylglycerol during the one hour treatment (Shopsis et al., 1974).

V. The Isolation of GAP "Resistant" Mutants

A fourth approach to the site of action is to isolate a mutant which is resistant to DL-glyceraldehyde 3-phosphate. Since DL-glyceraldehyde 3-phosphate is bactericidal to strain 8 (Figure 3A) and L-glyceraldehyde strongly inhibits the growth of strain 8 (Figure 5B), the mutagenized cells were incubated in the presence of 2.0 mM DL-glyceraldehyde 3-phosphate and 2.5 mM L-glyceraldehyde to avoid selecting G3P transport-negative mutants or glycerol kinase-negative

mutants. In order to isolate the glyceraldehyde 3-phosphate resistant mutant retaining phenotypic markers of strain 8, mutant cells were checked on Difco agar plates and found to have the following properties:(1) sensitivity to 2.5 mM DL-glycerol 3-phosphate, (2) inability to use 0.5% glycerol 3-phosphate as the sole carbon source, (3) sensitivity to 2.5 mM DL-3,4-dihydroxybutyl-1-phosphonate, (4) resistance to 2.0 mM DL-glyceraldehyde 3-phosphate and 2.5 mM L-glyceraldehyde, (5) inability to use 0.5% glycerol as the sole carbon source, (6) sensitivity to L-valine (100 μg/ml).

All isolated mutants which incorporated 3,4-dihydroxy-[3-^{3}H]butyl-1-phosphonate into phospholipids in vivo were susceptible to 3,4-dihydroxybutyl-1-phosphonate. On the contrary, those which could not incorporate 3,4-dihydroxy-[3-^{3}H]butyl-1-phosphonate into phospholipid were resistant to 3,4-dihydroxybutyl-1-phosphonate and probably belong to the class of G3P transport-negative mutants.

Strain 3C-1 is one of the isolated glyceraldehyde 3-phosphate "resistant" mutants. It has a doubling time of 1.8 hr when cultured in Garen and Levinthal medium (Garen & Levinthal, 1960) containing 0.6 mM phosphate with 0.5% potassium succinate as the sole carbon source (Figure 29). While under the same condition, strain 8 has a doubling time of 2.3 hr (Figure 1). Although the growth of strain 3C-1 is susceptible to 2.5 mM DL-glyceraldehyde 3-phosphate, as can be seen, this mutant was less sensitive to glyceralde-

hyde 3-phosphate than is the parent strain (Figures 29 and 30). Furthermore, 2.5 mM DL-glyceraldehyde 3-phosphate is bacteriostatic to strain 3C-1 (Figure 31). This finding is in striking contrast to strain 8, to which the phosphate is bactericidal (Figure 3A). Both strains, however, were totally inhibited by 2.5 mM DL-3,4-dihydroxybutyl-1-phosphonate. In addition, this mutant possessed a glycerol 3-phosphate transport system, since the growth of strain 3C-1 was completely inhibited by 3,4-dihydroxybtuyl-1-phosphonate (Figure 29). Therefore, the absence of a bactericidal effect of glyceraldehyde 3-phosphate on strain 3C-1 is not due to a missing transport system. The kinetic studies also show that strain 3C-1, like its parent strain, was sensitive to 2.5 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate, 2.5 mM DL-glycerol 3-phosphate and L-valine (100 μg/ml) (Figures 29 and 30). Moreover, when strain 3C-1 cultured on low phosphate medium (Garen & Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% succinate, neither glyceraldehyde 3-phosphate nor 3-hydroxy-4-oxobutyl-1-phosphonate significantly affects the intracellular concentrations of nucleoside triphosphates (Table 11). Therefore, both strain 3C-1 and its parent strain do not become leaky or lose the energy supply upon the treatment with glyceraldehyde 3-phosphate or its phosphonic acid analogue (Tables 5 and 11).

Since acyl CoA:sn-glycerol 3-phosphate acyltransferase and CDP-diglyceride:sn-glycerol 3-phosphate phosphatidyl-

transferase of strain 8 were competitively inhibited by glyceraldehyde 3-phosphate (Figures 24 and 27), it appeared possible that these two enzymes prepared from the glyceraldehyde 3-phosphate "resistant" mutant might be insensitive to this compound. Therefore, sn-G3P acyltransferases were prepared from strain 8 and strain 3C-1. The enzyme activities were assayed by the radioactive assay method. As shown in Table 9, DL-glyceraldehyde 3-phosphate and 3-hydroxy-4-oxobutyl-1-phosphonate inhibited sn-G3P acyltransferases with either oleoyl CoA or palmitoyl CoA as substrate. In the presence of 32.3 μM sn-glycerol 3-phosphate, 4 mM glyceraldehyde 3-phosphate inhibited strain 8 and 3C-1 acyltransferases by 69.4% and 63.1%, respectively, when palmitoyl CoA served as the acyl donor. Under the same conditions, 4 mM 3-hydroxy-4-oxobutyl-1-phosphonate inhibited strain 8 and the mutant acyltransferases by 43.8% and 30.3%, respectively. Thus both acyltransferases were comparably inhibited by either glyceraldehyde 3-phosphate or its phosphonic acid analogue (Table 9). CDP-Diglyceride:sn-glycerol 3-phosphate phosphatidyltransferase, the second enzyme which was sensitive to DL-glyceraldehyde 3-phosphate (Figure 27) was prepared from strain 8 and 3C-1. In the presence of 16 μM sn-glycerol 3-phosphate, 3 mM glyceraldehyde 3-phosphate inhibited strain 8 and strain 3C-1 phosphatidyltransferases by 38.9% and 34.8%, respectively (Table 10). When 3 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate served as the inhibitor,

the phosphatidyltransferases from strain 8 and 3C-1 were inhibited by 14.9% and 19.7%, respectively (Table 10). Therefore, the susceptibility of these phosphatidyltransferases to either glyceraldehyde 3-phosphate or its phosphonic acid analogue was not much different. These results suggested that, in addition to the acyltransferase and phosphatidyltransferase, glyceraldehyde 3-phosphate and its phosphonic acid analogue may have some additional unknown target sites.

Since the specific activity of mutant acyltransferase is higher than that of strain 8 acyltransferase under the conditions used (Table 9), it would be interesting to compare the apparent k_m and maximum velocity (Vmax) of mutant acyltransferase with those of strain 8 acyltransferase in the future. In order to obtain further information about the mode of action of glyceraldehdye 3-phosphate and its phosphonic acid analogue, studies concerning the effect of these compounds on macromolecular and phospholipid metabolism in E. coli strain 3C-1 is strongly recommended. Furthermore, by using radioactive labeled L-glyceraldehyde 3-phosphate and comparable stereochemical form of its phosphonic acid analogue in the future, it should be possible to further probe the metabolic fate of these compounds in E. coli. It has been found that 3,4-dihydroxy[3-^{3}H]butyl-1-phosphonate is incorporated into a large negative charge lipid by cultures of E. coli strain 8 and in vitro by CDP-diglyceride:

glycerol 3-phosphate phosphatidyltransferase (Cheng *et al.*, 1974; Tyhach *et al.*, 1976). This labeled lipid was identified as the phosphonic acid analogue of phosphatidylglycerol phosphate (Tyhach *et al.*, 1976). It would be interesting to investigate the possible formation of lipid analogues in the presence of labeled glyceraldehyde 3-phosphate or its phosphonic acid analogue by the cultures of *E. coli*, since such a new lipid may prove useful in revealing the possible effects of this lipid analogue on the activity of membrane-bound enzymes *in vitro* or membrane structure *in vivo*. The availability of a glyceraldehyde 3-phosphate "resistant" mutant further introduces a new variable into the study in this respect.

Table 1. Effect of various concentrations of DL-glyceraldehyde 3-phosphate on macromolecular and lipid synthesis[a]

Macromolecular synthesis	DL-glyceraldehyde 3-phosphate			
	0.05 mM		0.25 mM	
	Total cpm per ml of treated cells	% of untreated cultures	Total cpm per ml of treated cells	% of untreated cultures
DNA	2,780	85	1,380	42
RNA	52,800	83	25,200	40
Protein	9,550	101	6,600	69
Phosphoglyceride	28,300	66	15,800	37

[a] DNA, RNA, protein, and phospholipid synthesis in E. coli strain 8 cultured on Garen and Levinthal medium (Garen and Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate were monitored as a function of different concentrations of DL-glyceraldehyde 3-phosphate. DL-glyceraldehyde 3-phosphate and the labeled precursors of DNA, RNA and phospholipid ([^{33}P]phosphate, 1 to 3 μCi/ml) or the precursor of protein (L-[^{3}H]isoleucine, 0.45 μCi/ml, 3.93 mCi/mmole) were simultaneously added to cultures of E. coli strain 8 when the cell density reached 15 to 20 Klett units. The cells were then incubated for 60 minutes. The incorporation of labeled precursors was determined as described in Materials and Methods.

Table 2. Effect of various concentrations of DL-3-hydroxy-4-oxobutyl-1-phosphonate on macromolecular and lipid synthesis[a]

Macromolecular synthesis	DL-3-hydroxy-4-oxobutyl-1-phosphonate			
	0.05 mM		0.25 mM	
	Total cpm per ml of treated cells	% of untreated cultures	Total cpm per ml of treated cells	% of untreated cultures
DNA	2,474	77	1,875	58
RNA	58,224	91	40,160	63
Protein	12,100	103	10,200	87
Phosphoglyceride	32,900	77	25,800	60

[a] DNA, RNA, protein, and phospholipid synthesis in E. coli strain 8 cultured on Garen and Levinthal medium (Garen and Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate were monitored as a function of different concentrations of DL-3-hydroxy-4-oxobutyl-1-phosphonate. DL-3-hydroxy-4-oxobutyl-1-phosphonate and the labeled precursors of DNA, RNA and phospholipid ($[^{33}P]$phosphate, 1 to 3 μCi/ml) or the precursor of protein (L-$[^{3}H]$ isoleucine, 0.45 μCi/ml, 3.93 mCi/mmole) were simultaneously added to cultures of E. coli strain 8 when the cell density reached 15 to 20 Klett units. The cells were then incubated for 60 minutes. The incorporation of labeled precursors was determined as described in Materials and Methods.

Table 3. Effects of DL-glyceraldehyde 3-phosphate on the rate of macromolecular and lipid synthesis[a]

Macromolecular synthesis	Time of addition of the inhibitor			
	6 min		65 min	
	Total nmoles incorporation per ml of untreated cells	% of untreated cultures	Total nmoles incorporation per ml of untreated cells	% of untreated cultures
DNA	0.18	25.5	0.30	33.3
RNA	7.9	25.3	11.7	68.4
Protein	4.9	54.3	6.0	88.1
Phosphoglyceride	4.2	15.9	8.1	39.5

[a] Cultures of E. coli strain 8 were grown in Bicine-buffered medium supplemented with 0.6 mM phosphate and 0.5% potassium succinate. When a cell density of 15 to 20 Klett units was reached, DL-glyceraldehyde 3-phosphate was added to a final concentration of 0.10 mM. At various times after addition of the drug, 2 ml of culture was removed and incubated with labeled precursors of DNA, RNA, and phospholipid ([^{32}P] phosphate, 15 μCi/ml) or the precursor of protein (L-[^{3}H]isoleucine, 5 μCi/ml, 43.7 mCi/mmole) for 10 minutes. The incorporation of labeled precursors was determined as described in Materials and Methods.

Table 4. Effects of DL-3-hydroxy-4-oxobutyl-1-phosphonate on the rate of macromolecular and lipid synthesis[a]

Macromolecular synthesis	Time of addition of the inhibitor			
	6 min		65 min	
	Total nmoles incorporation per ml of untreated cells	% of untreated cultures	Total nmoles incorporation per ml of untreated cells	% of untreated cultures
DNA	0.18	66.7	0.30	26.7
RNA	7.9	75.9	11.7	42.0
Protein	4.9	88.6	6.0	78.2
Phosphoglyceride	4.2	64.3	8.1	22.2

[a] Cultures of E. coli strain 8 were grown in Bicine-buffered medium supplemented with 0.6 mM phosphate and 0.5% potassium succinate. When a cell desity of 15 to 20 Klett units was reached, DL-3-hydroxy-4-oxobutyl-1-phosphonate was added to a final concentration of 0.10 mM. At various times after addition of the drug, 2 ml of culture was removed and incubated with labeled precursors of DNA, RNA, and phospholipid ([^{32}P]phosphate, 15 μCi/ml) or the precursor of protein (L-[^{3}H]isoleucine, 5 μCi/ml, 43.7 mCi/mmole) for 10 minutes. The incorporation of labeled precursors was determined as described in Materials and Methods.

Table 5. Effects of DL-glyceraldehyde 3-phosphate, DL-3-hydroxy-4-oxobutyl-1-phosphonate and DL-3,4-dihydroxybutyl-1-phosphonate on the intracellular levels of nucleoside triphosphates in strain 8[a]

Addition	^{32}P incorporated into nucleoside triphosphates (cpm/5 µl culture)			
	GTP	ATP	CTP	UTP
1. None	1,954	4,670	1,361	1,249
2. GAP, 0.1 mM	2,046	4,167	1,409	1,108
3. HOBP, 0.1 mM	1,883	4,359	1,456	1,143
4. DHBP, 0.03 mM	1,519	3,636	1,342	1,456

[a] The intracellular nucleotide concentrations were determined by the incorporation of [^{32}P]phosphate into nucleoside triphosphates. E. coli strain 8 was cultured on Garen and Levinthal medium (Garen and Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate. When a cell density of 20 Klett units was reached, the indicated concentration of drug and 250 µCi of [^{32}P]phosphate were added to 2 ml of culture. Control cultures received an equal volume of distilled water instead of drug in addition to labeled phosphate. The cultures were incubated at 37 C for 30 min. Cell extracts were prepared as described in Materials and Methods and the nucleotide levels were determined by chromatographing an aliquot of cell extracts on polyethyleneimine plates (Cashel et al., 1969). GTP, ATP, CTP, UTP, guanosine, adenosine, cytidine, and uridine 5'-triphosphates, respectively.

Table 6. Effects of 2.5 mM DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate on the viability of E. coli strains 8, BB #6 and BB #7[a]

Strains	Treatment	Klett Units	Cells/ml
8	GAP	20	1.8×10^7
	HOBP	26	2.0×10^8
BB # 6	GAP	20	1.3×10^6
	HOBP	21	1.4×10^8
BB # 7	GAP	19	1.5×10^6
	HOBP	19	1.3×10^8

[a] E. coli strains 8, BB #6 and BB #7 were cultured on Garen and Levinthal medium (Garen and Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate. Strain 8 was treated with inhibitors for 5 hours, however, BB # 6 and BB #7 were treated for 4 hours. The determination of cell viability was described in Materials and Methods. Untreated strain 8 culture has 1.35×10^8 cells/ml at 17 Klett units.

Table 7. Effects of DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate on the generation time of various E. coli strains[1]

Strains	Phenotypic trait affected	carbon source of growth	Generation time		
			untreated	2.5 mM GAP	2.5 mM HOBP
6	Lacks G3P transport	succinate(a)	2.6	2.6	2.6
8	Lacks aerobic G3P dehydrogenase	succinate(a)	2.3	> 15.0	> 15.0
M2-3	Lacks G3P transport	succinate(a)	2.0	2.0	2.0
7	Constitutive for glp regulon	glycerol (b)	1.8	> 15.0	4.4
244	Glycerol kinase is insensitive to feedback inhibition by FDP	glycerol (b)	1.2	5.3	1.5
CY 115	Lacks G3P synthase	glycerol (b)	2.0	9.0	6.0
K10	Prototrophic strain and the parent of strain NP 315	glycerol (a)	(30C) 2.4	> 15.0	9.4
			(42C) 1.6	15.0	15.0
NP 315	Temperature sensitive FDP aldolase	glycerol (a)	(30C) 3.3	> 15.0	5.8
			(42C) 2.0	> 15.0	12.0
BB #6	G3P synthase is resistant to feedback inhibition by G3P	succinate(a)	1.8	> 15.0	12.0
BB #7	Same as BB #6	succinate(a)	2.2	> 15.0	12.0
BB26-36 R2	Same as BB #6 and K_m defect in acyltransferase	succinate(a)	2.0	> 15.0	> 15.0
3C-1	GAP-resistant	succinate(a)	1.8	11.0	> 15.0

[1] See Materials and Methods for details. All incubations were at 37 C unless otherwise indicated. Two types of minimal media were employed in growth studies. (a) Garen and Levinthal medium (Garen and Levinthal, 1960) supplemented with 0.6 mM phosphate (b) Vogel and Bonner minimum medium (Vogel and Bonner, 1956).

Table 8. Effect of DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate on acyl-CoA: sn-glycerol 3-phosphate acyltransferase from E. coli strain 8 and BB26-36 R2[a]

Addition	G3P acyltransferase activity (%)	
	strain 8	strain BB26-36 R2
GAP, 2 mM	51.6	58.2
GAP, 4 mM	30.6	39.4
HOBP, 2 mM	67.7	62.5
HOBP, 4 mM	56.2	44.0

[a] The activity of G3P acyltransferase was assayed by determining the incorporation of [^{14}C]glycerol 3-phosphate into lipid. The assay conditions are described in Materials and Methods. The reaction mixture contained 60 μM palmitoyl CoA, 32.3 μM sn-[^{14}C]glycerol 3-phosphate (24.8 mCi/mmol) and varying concentrations of DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate. Acyltransferase from strain 8 and strain BB26-36 R2 incorporated 0.110 and 0.0893 nmol of G3P per min per mg of protein, respectively.

Table 9. Effects of DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate on acyl-CoA: sn-glycerol 3-phosphate acyltransferase from E. coli strain 8 and 3C-1[a]

Acyl donor	Addition	G3P acyltransferase activity (%)	
		strain 8	strain 3C-1
oleoyl-CoA	GAP, 2 mM	37.2	41.1
	GAP, 4 mM	19.2	23.2
	HOBP, 2 mM	46.5	45.5
	HOBP, 4 mM	29.3	37.6
palmitoyl-CoA	GAP, 2 mM	51.6	58.4
	GAP, 4 mM	30.6	36.9
	HOBP, 2 mM	67.7	87.8
	HOBP, 4 mM	56.2	69.7

[a] The activity of G3P acyltransferase was assayed by determining the incorporation of [^{14}C]glycerol 3-phosphate into lipid. The assay conditions are described in Materials and Methods. The reaction mixture contained 60 μM palmitoyl CoA and 32.3 μM sn-[^{14}C]glycerol 3-phosphate or 60 μM oleoyl CoA and 64.6 μM sn-[^{14}C]glycerol 3-phosphate and varying concentrations of DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate. When palmitoyl CoA served as acyl donor, acyltransferase from strain 8 and strain 3C-1 incorporated 0.110 and 0.176 nmol of G3P per min per mg of protein, respectively. When oleoyl CoA served as acyl donor, acyltransferase from strain 8 and strain 3C-1 incorporated 0.038 and 0.099 nmol of G3P per min per mg of protein, respectively.

Table 10. Effects of DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate on CDP-diglyceride: glycerol 3-phosphate phosphatidyltransferase from E. coli strain 8 and 3C-1[a]

Addition	Phosphatidyltransferase activity (%)	
	strain 8	strain 3C-1
GAP, 3 mM	61.1	65.2
GAP, 6 mM	36.7	59.4
HOBP, 3 mM	85.4	80.3
HOBP, 6 mM	45.8	63.5

[a] The assay conditions are described in Materials and Methods. The reaction mixture contained 16 μM sn-[^{14}C] glycerol 3-phosphate (100 mCi/mmol) and varying amounts of DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate. Phosphatidyltransferase from strain 8 and strain 3C-1 incorporated 3.16×10^{-2} and 2.54×10^{-2} nmol of G3P per min per mg of protein, respectively.

Table 11. Effects of DL-glyceraldehyde 3-phosphate, and DL-3-hydroxy-4-oxobutyl-1-phosphonate on the intracellular levels of nucleoside triphosphates in strain 3C-1

Addition	^{32}P incorporated into nucleoside triphosphates (cpm/5 µl culture)			
	GTP	ATP	CTP	UTP
1. None	2,353	4,539	1,169	1,285
2. GAP, 0.1 mM	2,237	3,913	1,190	1,324
3. HOBP, 0.1 mM	2,317	4,475	1,299	1,313

[a] The intracellular nucleotide concentrations were determined by the incorporation of [^{32}P]phosphate into nucleoside triphosphates. E. coli strain 3C-1 was cultured on Garen and Levinthal medium (Garen and Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate. When a cell density of 20 Klett units was reached, the indicated concentration of drug and 250 µCi of [^{32}P] phosphate were added to 2 ml of culture. Control cultures received an equal volume of distilled water instead of drug in addition to labeled phosphate. The cultures were incubated at 37 C for 30 min. Cell extracts were prepared as described in Materials and Methods and the nucleotide levels were determined by chromatographing an aliquot of cell extracts on polyethyleneimine plates (Cashel et al., 1969). GTP, ATP, CTP, UTP, guanosine, adenosine, cytidine, and uridine 5'-triphosphates, respectively.

Figure 1: Effects of DL-glyceraldehyde 3-phosphate, DL-3-hydroxy-4-oxobutyl-1-phosphonate and D-glyceraldehyde 3-phosphate on the growth of E. coli strain 8 cultured in low-phosphate synthetic medium (Garen & Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate. The inhibitors were added to early logarithmic-phase cultures at the start of the experiment. The additions were as follows: ■ , 2.5 mM DL-glyceraldehyde 3-phosphate; □ , 2.5 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate; ▲ , 1.25 mM D-glyceraldehyde 3-phosphate; and ○ , untreated.

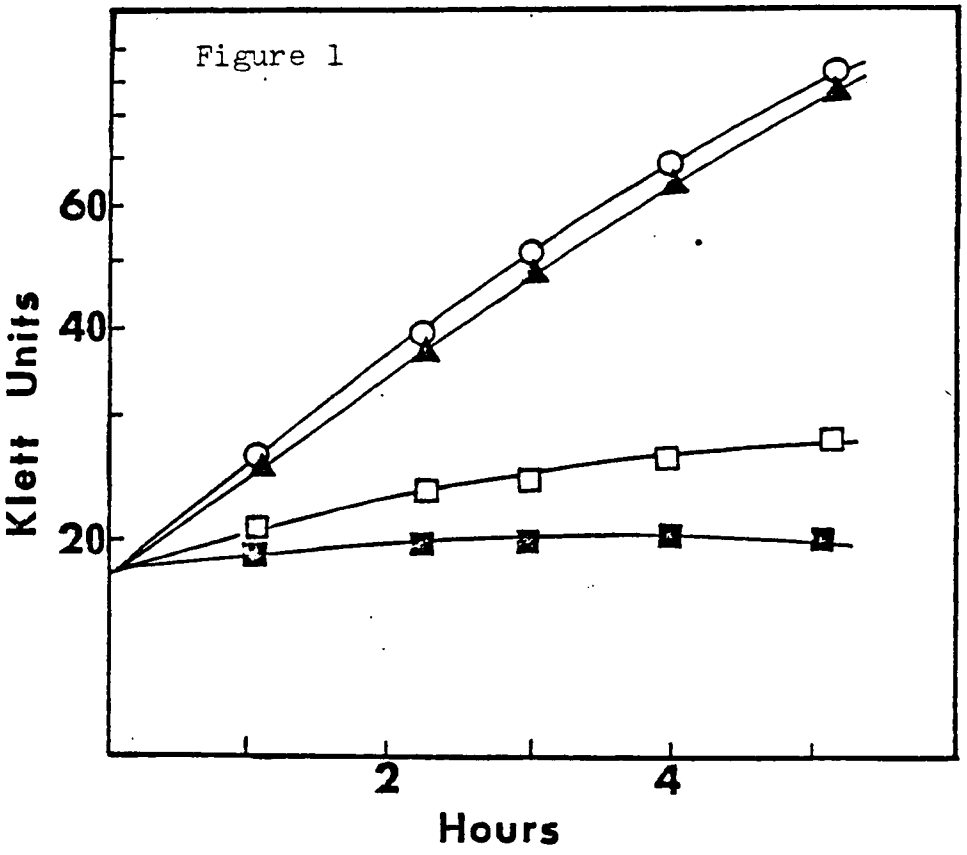


Figure 1
60
40
20
Klett Units
2
4
Hours

Figure 2: Effects of various concentrations of (A) DL-glyceraldehyde 3-phosphate and (B) DL-3-hydroxy-4-oxobutyl-1-phosphonate on the growth of E. coli strain 8 cultured in low-phosphate synthetic medium (Garen & Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate. At the time indicated by the arrow, the inhibitors were added to the following final concentrations: ○, 2.5 mM; ●, 1.0 mM; □, 0.5 mM; ■, 0.25 mM; △, 0.10 mM; and ▲, untreated.

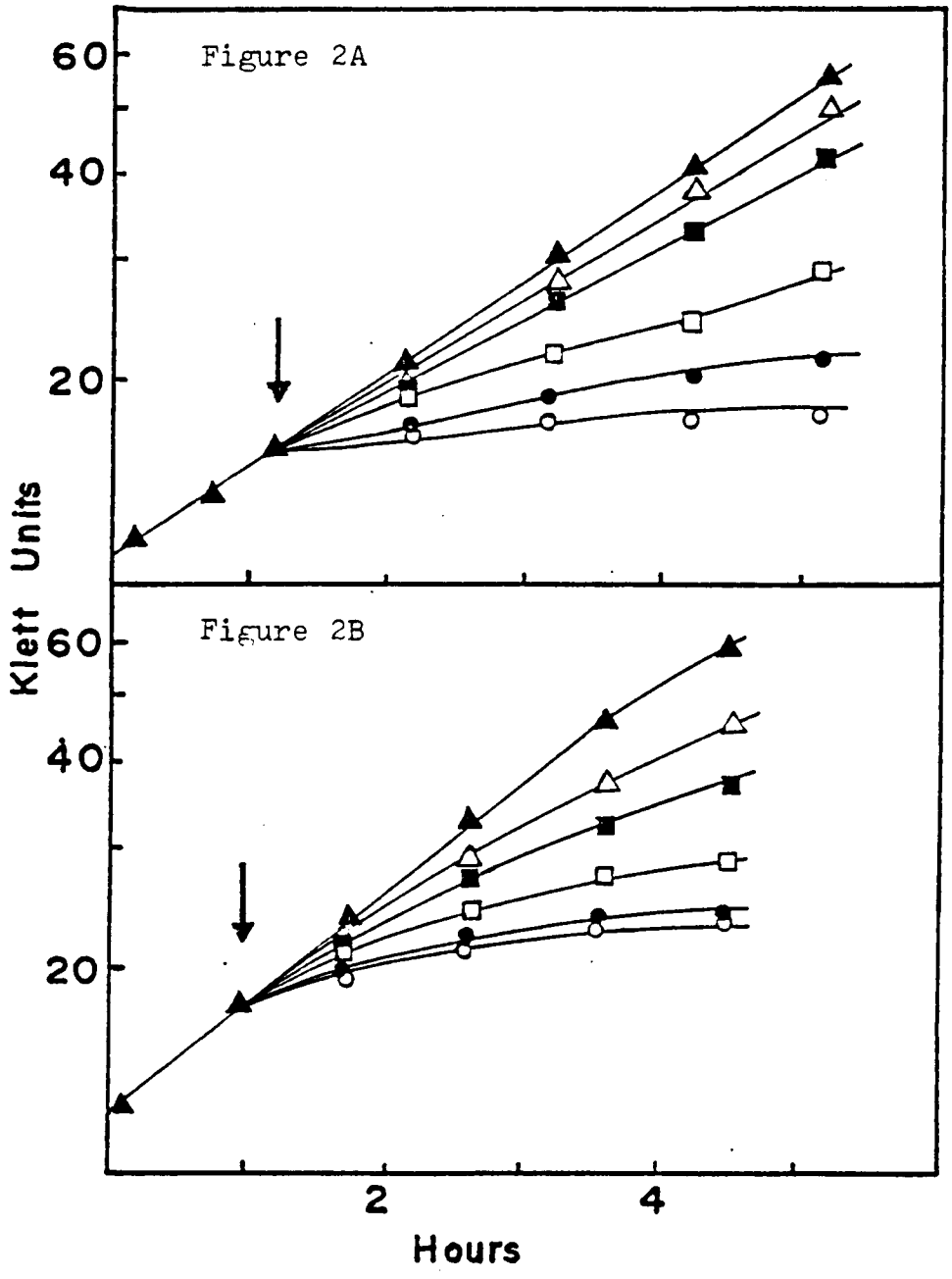


Figure 2A
60
40
20
Figure 2B
60
40
20
Klett Units
2
4
Hours

Figure 3: Effects of (A) 2.5 mM DL-glyceraldehyde 3-phosphate and (B) 2.5 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate on the viability of E. coli strain 8 cultured in low-phosphate synthetic medium (Garen & Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate. The inhibitors were added at the time indicated by the arrow. Cell viability was determined as described in Materials and Methods. Symbols: ▲ , cell turbidity in Klett units of untreated cultures; ■ , cell turbidity in Klett units of treated cultures; and ● , cell viability of treated cultures.

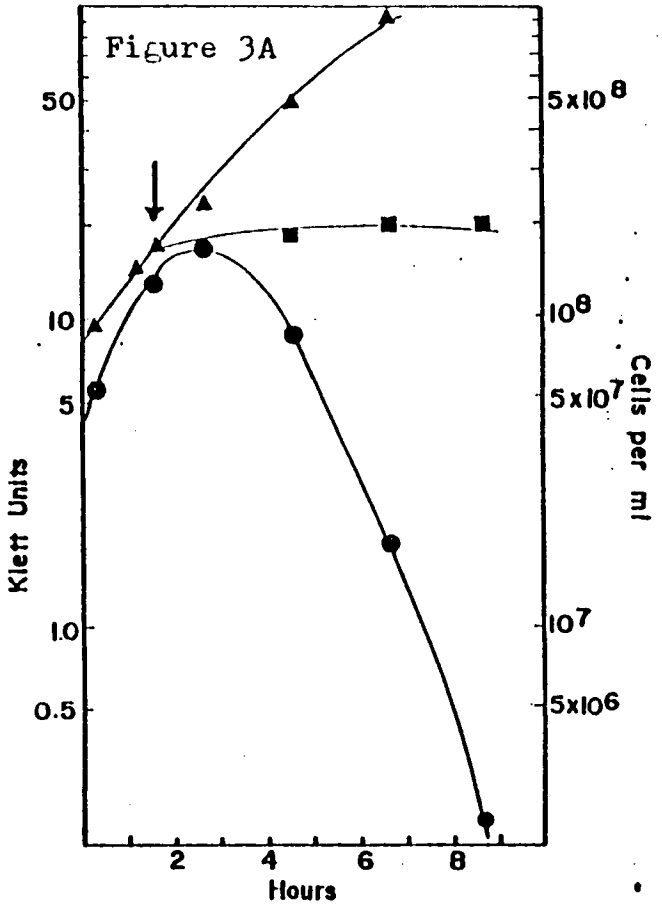


Figure 3A
50
10
5
1.0
0.5
Klett Units
5x10^8
10^8
5x10^7
10^7
5x10^6
Cells per ml
2
4
6
8
Hours

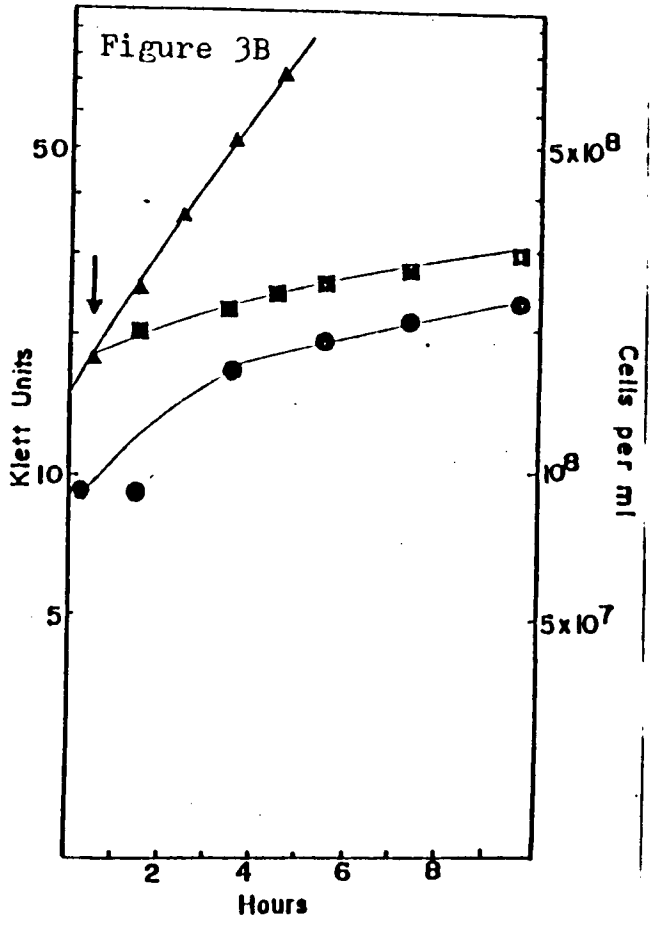


Figure 3B
50
10
5
Klett Units
5x10^8
10^8
5x10^7
Cells per ml
2
4
6
8
Hours

Figure 4: Effects of DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate on the growth of E. coli strains M2-3 and 6 cultured in low-phosphate minimal medium (Garen & Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate. The inhibitors were added at the time indicated by the arrow. Symbols: (A) strain M2-3: ■ , 2.0 mM DL-glyceraldehyde 3-phosphate; □ , 2.0 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate; and ● , untreated. (B) strain 6: ● , 2.5 mM DL-glyceraldehyde 3-phosphate; △ , 2.0 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate; and ○ , untreated.

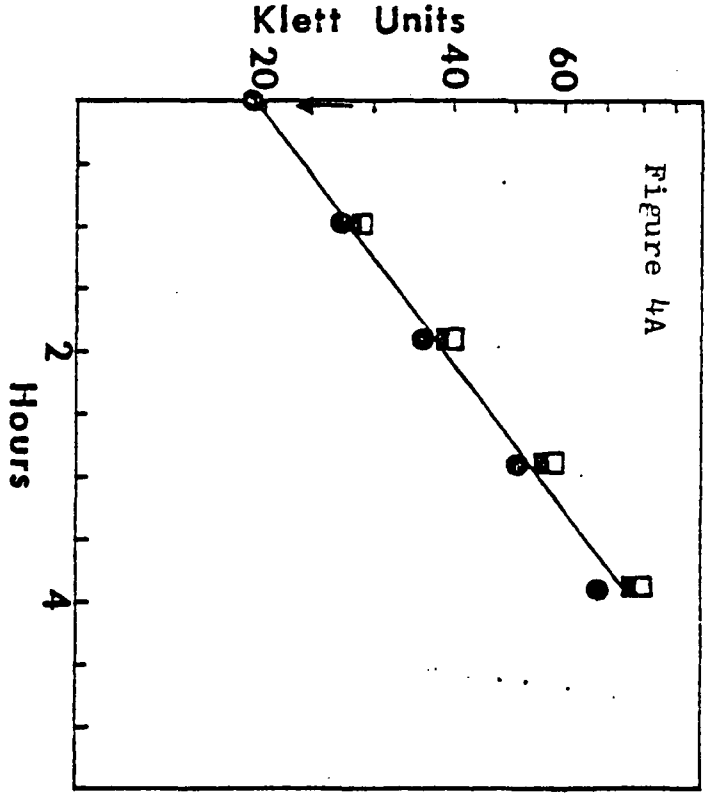


Figure 4A

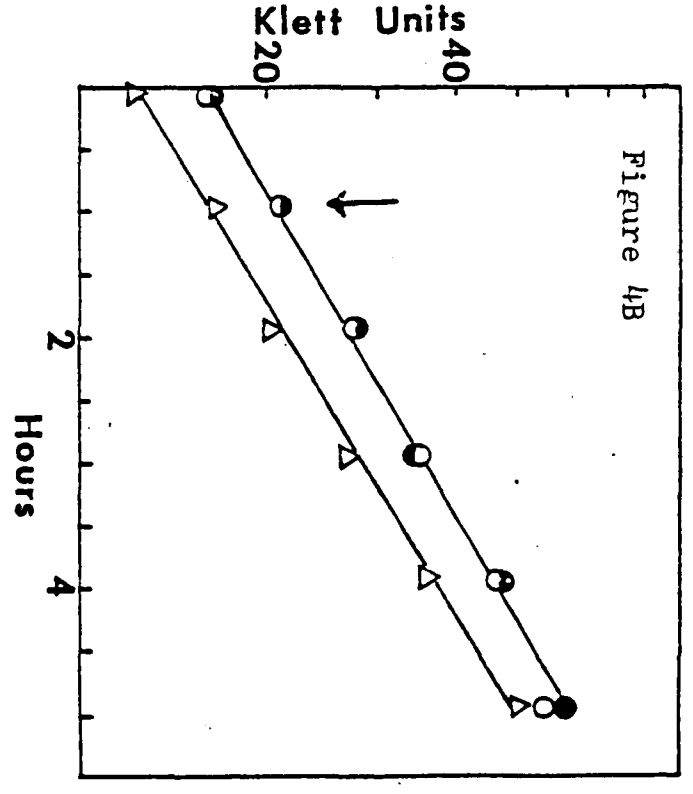


Figure 4B

Figure 5: Effects of L- and D-glyceraldehyde on the growth of E. coli strains 8, 9 and M4-9 in Bicine-buffered medium as described in Materials and Methods supplemented with 0.6 mM phosphate and 0.5% potassium succinate. L- or D-glyceraldehyde was added to a final concentration of 2.5 mM to early logarithmic-phase cultures at the start of the experiment. Symbols: (A) strain 9: ● , untreated; ■ , D-glyceraldehyde; and ▲ , L-glyceraldehyde. (B) strain 8: ○ , untreated; □ , D-glyceraldehyde; △ , L-glyceraldehyde. (C) strain M4-9: ▽ , L-glyceraldehyde; and ▼ , untreated.

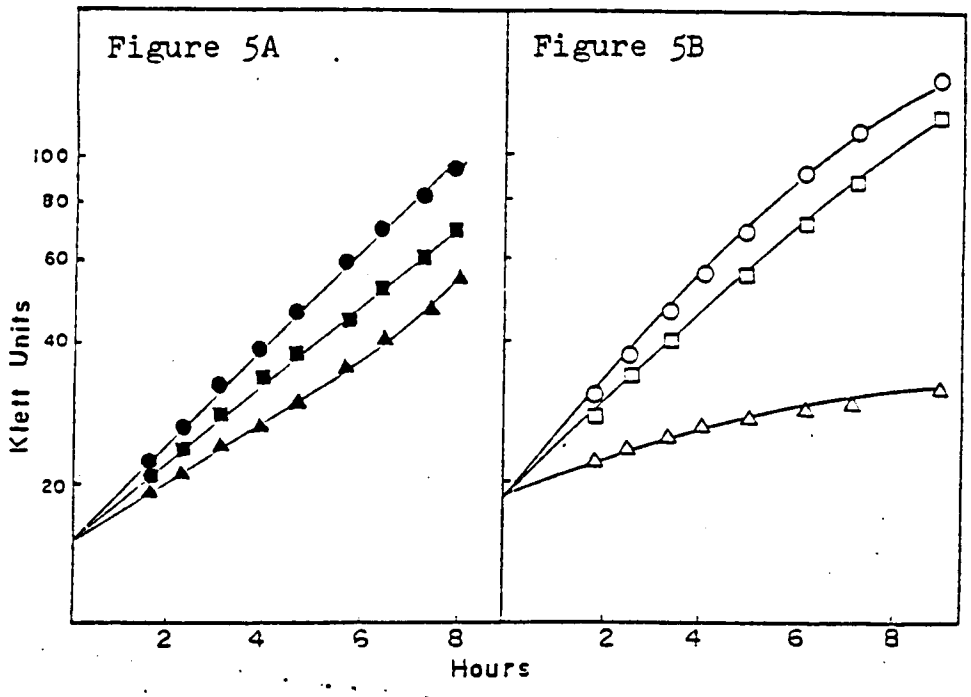


Figure 5A
Figure 5B
100
80
60
40
20
Klett Units
2
4
6
8
Hours

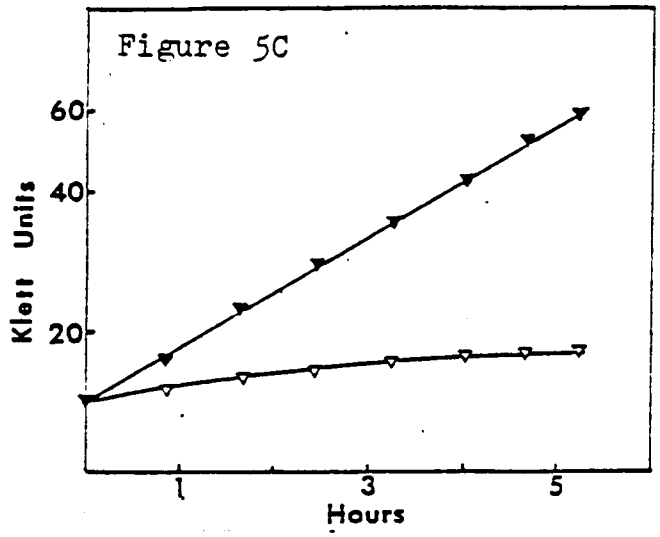


Figure 5C
60
40
20
Klett Units
1
3
5
Hours

Figure 6: Protein synthesis by E. coli strain 8 cultured in low-phosphate synthetic medium (Garen and Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate was monitored as a function of different concentrations of (A) DL-glyceraldehyde 3-phosphate and (B) DL-3-hydroxy-4-oxobutyl-1-phosphonate. The drug and [^{3}H]isoleucine (0.45 μCi/ml, 3.93 mCi/mmole) were simultaneously added to cultures of E. coli strain 8 when the cell density reached 15 to 20 Klett units. Incorporation of label was determined as described in Materials and Methods. The concentrations of inhibitors used were as follows: ▲, 0.5 mM; □, 0.25 mM; ■, 0.10 mM; ●, 0.05 mM; and ○, untreated.

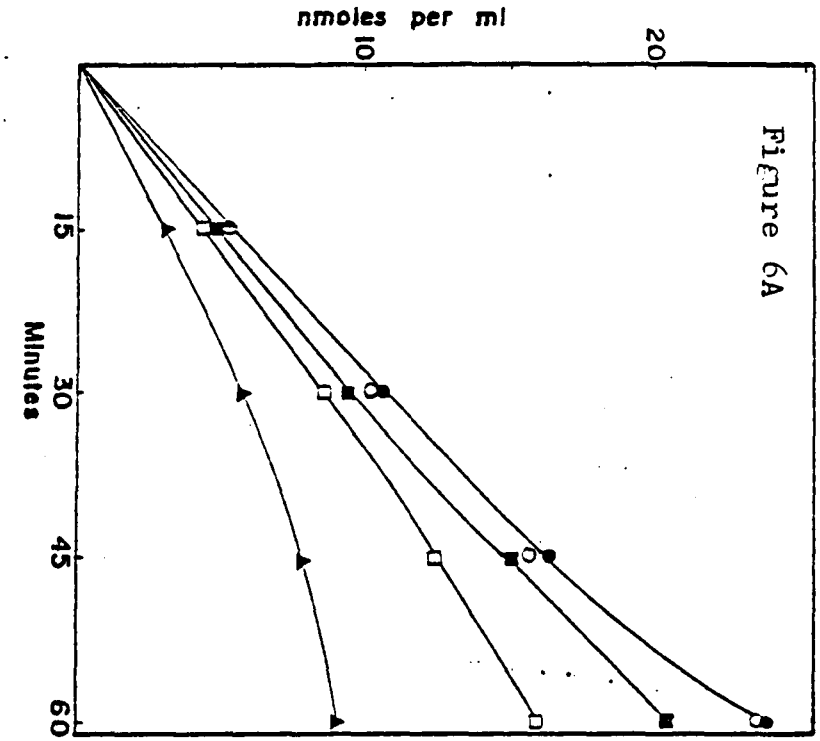


Figure 6A

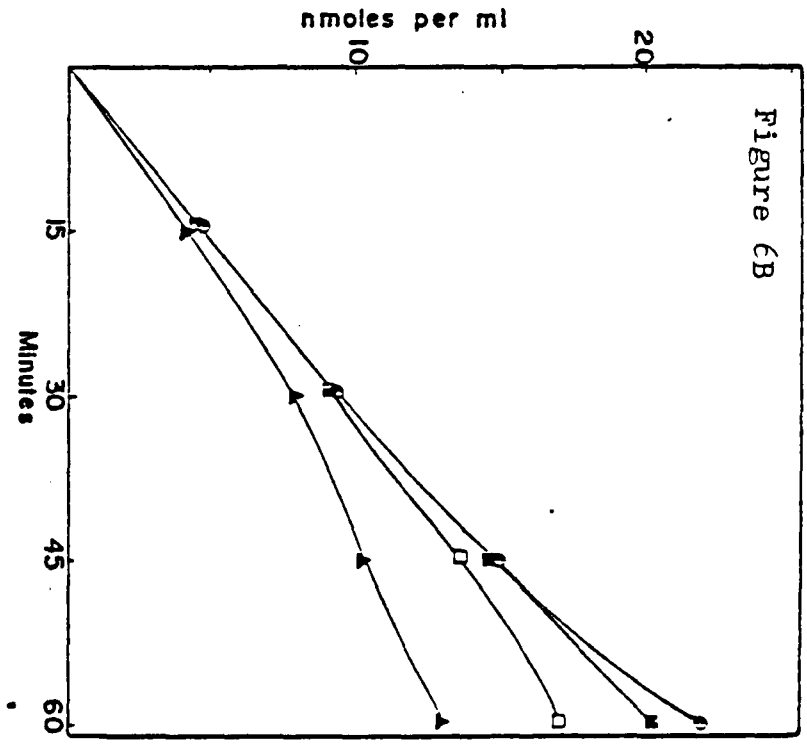


Figure 6B

Figure 7: DNA synthesis by E. coli strain 8 cultured in low-phosphate synthetic medium (Garen and Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate was monitored as a function of different concentrations of (A) DL-glyceraldehyde 3-phosphate and (B) DL-3-hydroxy-4-oxobutyl-1-phosphonate. The drug and [^{33}P]phosphate (1 to 3 μCi/ml) were simultaneously added to cultures of E. coli strain 8 when the cell density reached 15 to 20 Klett units. Incorporation of label was determined as described in Materials and Methods. The concentrations of inhibitors used were as follows: ▲ , 0.5 mM; □ , 0.25 mM; ■ , 0.10 mM; ● , 0.05 mM; and ○ , untreated.

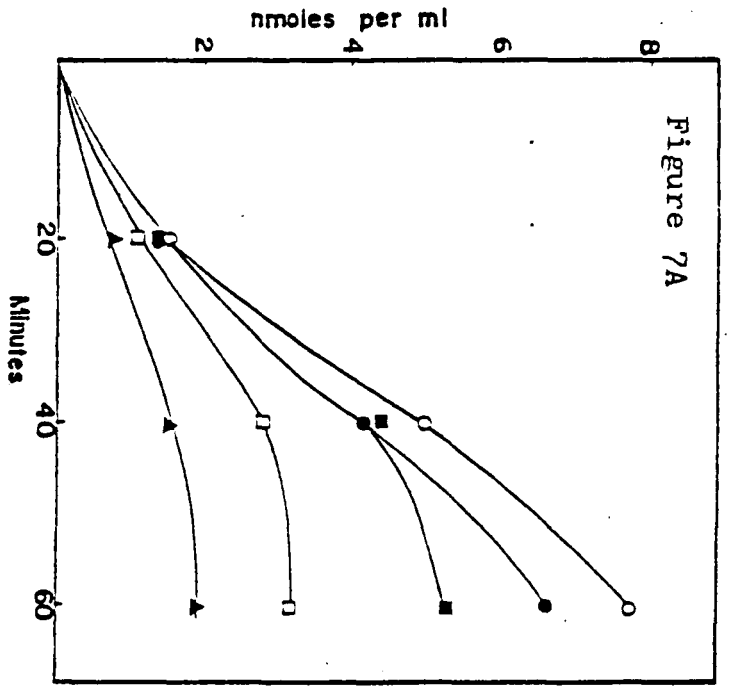
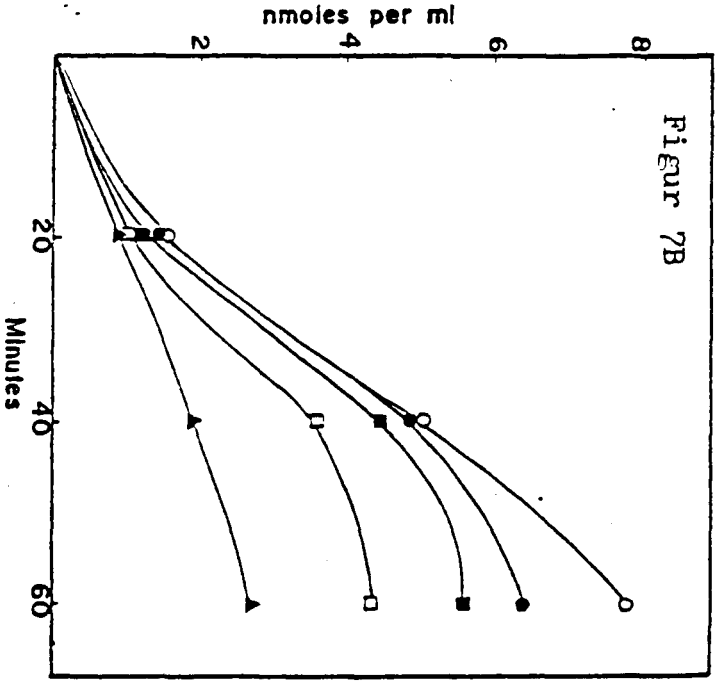


Figure 7A



Figur 7B

Figure 8: RNA synthesis by E. coli strain 8 cultured in low-phosphate synthetic medium (Garen and Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate was monitored as a function of different concentrations of (A) DL-glyceraldehyde 3-phosphate and (B) DL-3-hydroxy-4-oxobutyl-1-phosphonate. The drug and [^{33}P]phosphate (1 to 3 μCi/ml) were simultaneously added to cultures of E. coli strain 8 when the cell density reached 15 to 20 Klett units. Incorporation of label was determined as described in Materials and Methods. The concentrations of inhibitors used were as follows: ▲ , 0.5 mM; □ , 0.25 mM; ■ , 0.10 mM; ● , 0.05 mM; and ○ , untreated.

Figure 8A

Figure 8B

Figure 9: Phospholipid synthesis by E. coli strain 8 cultured in low-phosphate synthetic medium (Garen and Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate was monitored as a function of different concentrations of (A) DL-glyceraldehyde 3-phosphate and (B) DL-3-hydroxy-4-oxobutyl-1-phosphonate. The drug and [^{33}P]phosphate (1 to 3 μCi/ml) were simultaneously added to cultures of E. coli strain 8 when the cell density reached 15 to 20 Klett units. Incorporation of label was determined as described in Materials and Methods. The concentrations of inhibitors used were as follows: ▲ , 0.5 mM; □ , 0.25 mM; ■ , 0.10 mM; ● , 0.05 mM; and ○ , untreated.

Figure 9A

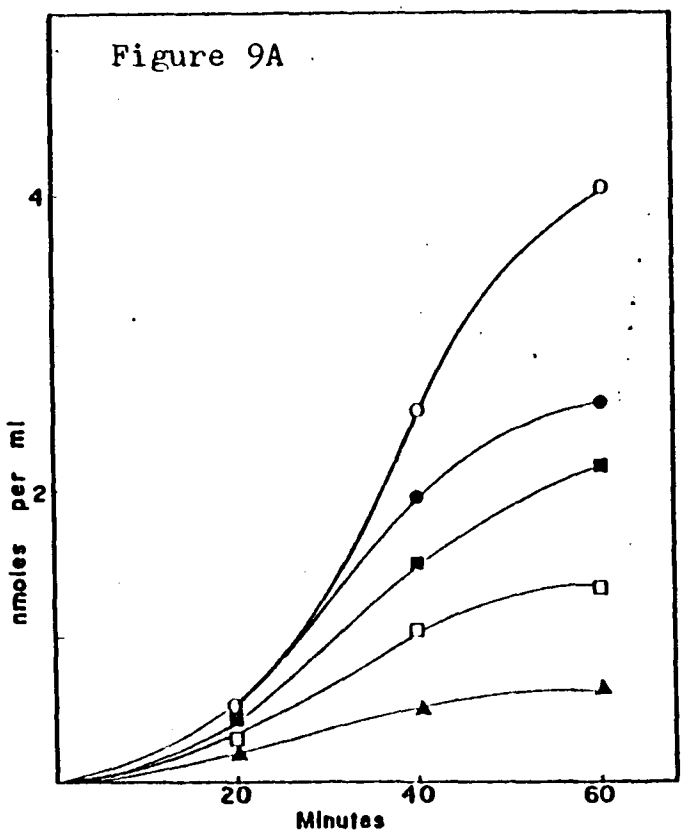


Figure 9B

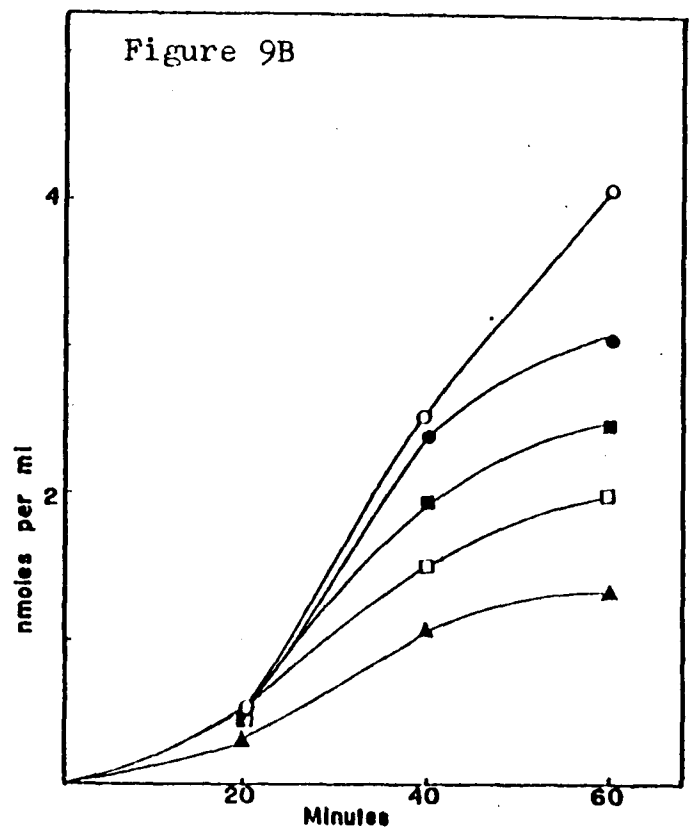
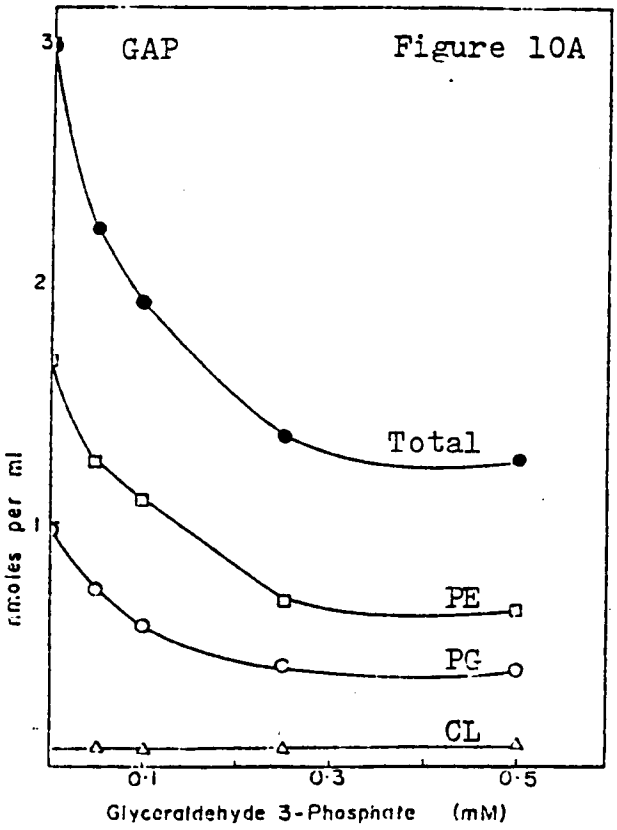
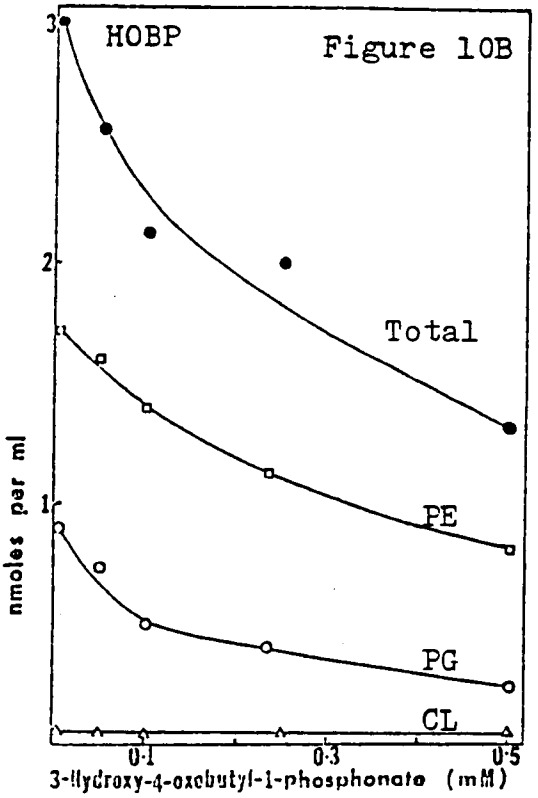
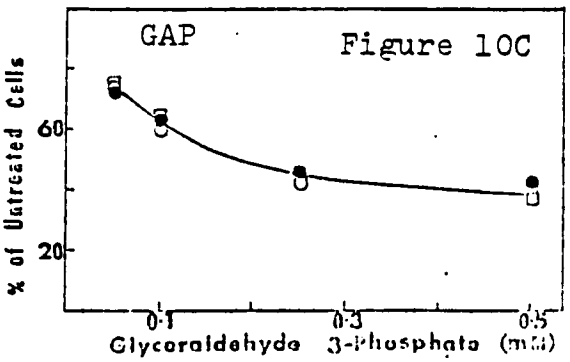


Figure 10: Effects of various concentrations of DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate on the distribution of [^{33}P]phosphate into the phospholipids of E. coli strain 8 cultured in low-phosphate synthetic medium (Garen and Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate. The drug and [^{33}P]phosphate (1 to 3 μCi/ml) were simultaneously added to cultures of E. coli strain 8 when the cell density reached 15 to 20 Klett units. The cells were then incubated for 60 minutes. Phospholipids were extracted and chromatographed as described in Materials and Methods. Symbols: ● , total phospholipids; □ , phosphatidylethanolamine; ○ , phosphatidylglycerol; and △ , cardiolipin. (A) and (C) DL-glyceraldehyde 3-phosphate, and (B) and (D) DL-3-hydroxy-4-oxobutyl-1-phosphonate.


GAP
Figure 10A
nmoles per ml
Total
PE
PG
CL
0·1
0·3
0·5
Glyceraldehyde 3-Phosphate (mM)


HOBP
Figure 10B
nmoles per ml
Total
PE
PG
CL
0·1
0·3
0·5
3-Hydroxy-4-oxobutyl-1-phosphonate (mM)


GAP
Figure 10C
% of Untreated Cells
60
20
0·1
0·3
0·5
Glyceraldehyde 3-Phosphate (mM)

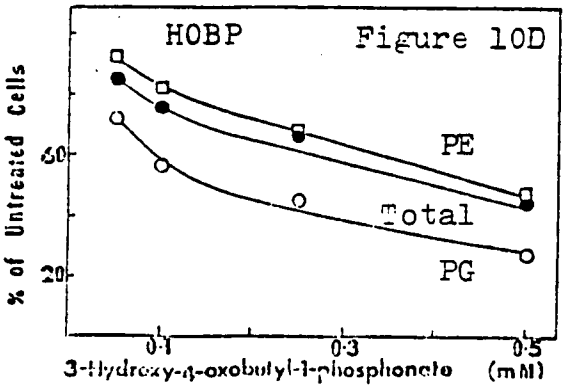

HOBP
Figure 10D
% of Untreated Cells
PE
Total
PG
0·1
0·3
0·5
3-Hydroxy-4-oxobutyl-1-phosphonate (mM)

Figure 11: Protein, DNA, RNA and phospholipid synthesis in E. coli strain 8 cultured on low-phosphate synthetic medium (Garen and Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate were monitored as a function of different concentrations of (A) DL-glyceraldehyde 3-phosphate and (B) DL-3-hydroxy-4-oxobutyl-1-phosphonate. A drug and the labeled precursors of DNA, RNA and phospholipid ([^{33}P]phosphate, 1 to 3 μCi/ml) or the precursor of protein (L-[^{3}H]isoleucine, 0.45 μCi/ml, 3.93 mCi/mmole) were simultaneously added to cultures of E. coli strain 8 when the cell density reached 15 to 20 Klett units. The cells were then incubated for 60 minutes. The incorporation of labeled precursors was determined as described in Materials and Methods. Symbols: ▲ , protein; ● , DNA; ■ , RNA and △ , phospholipid.

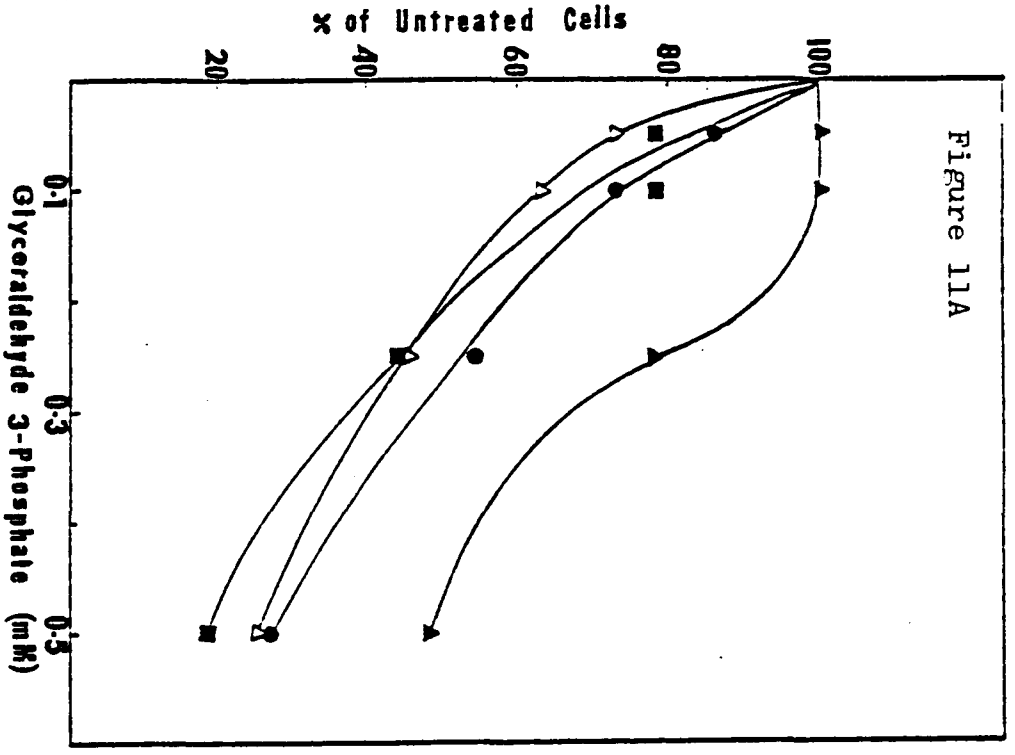


Figure 11A

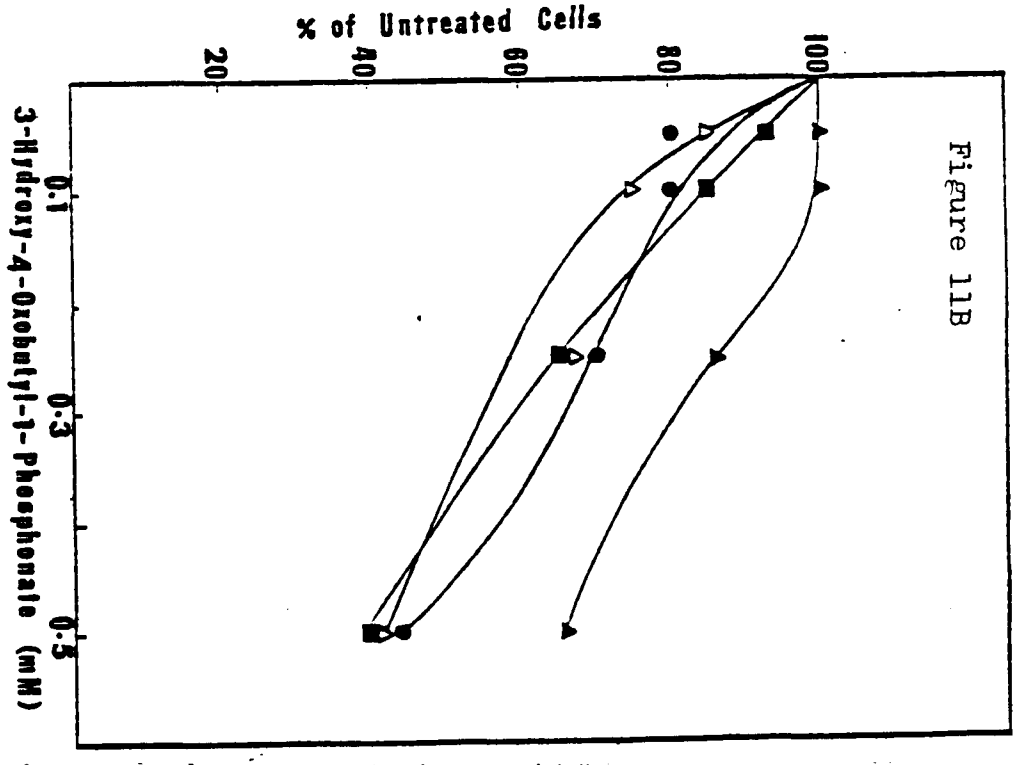


Figure 11B

Figure 12: Effects of (A) DL-glyceraldehyde 3-phosphate and (B) DL-3-hydroxy-4-oxobutyl-1-phosphonate on the rate of protein synthesis. E. coli strain 8 was cultured on Bicine-buffered medium supplemented with 0.6 mM phosphate and 0.5% potassium succinate. When a cell density of 15 to 20 Klett units was reached, the cultures were treated with 0.10 mM DL-glyceraldehyde 3-phosphate or DL-3-hydroxy-4-oxobutyl-1-phosphonate. At various time intervals after treatment, 2 ml of culture were removed and incubated with L-[^{3}H]isoleucine (5 µCi/ml, 43.7 µCi/mmole) for 10 minutes. Incorporation of label was determined as described in Materials and Methods. Zero time indicates the time of addition of the inhibitor. Symbols: (A) 0.10 mM DL-glyceraldehyde 3-phosphate: ▲, untreated cultures; and ●, treated cultures. (B) 0.10 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate: ▲, untreated cultures; and ■, treated cultures.

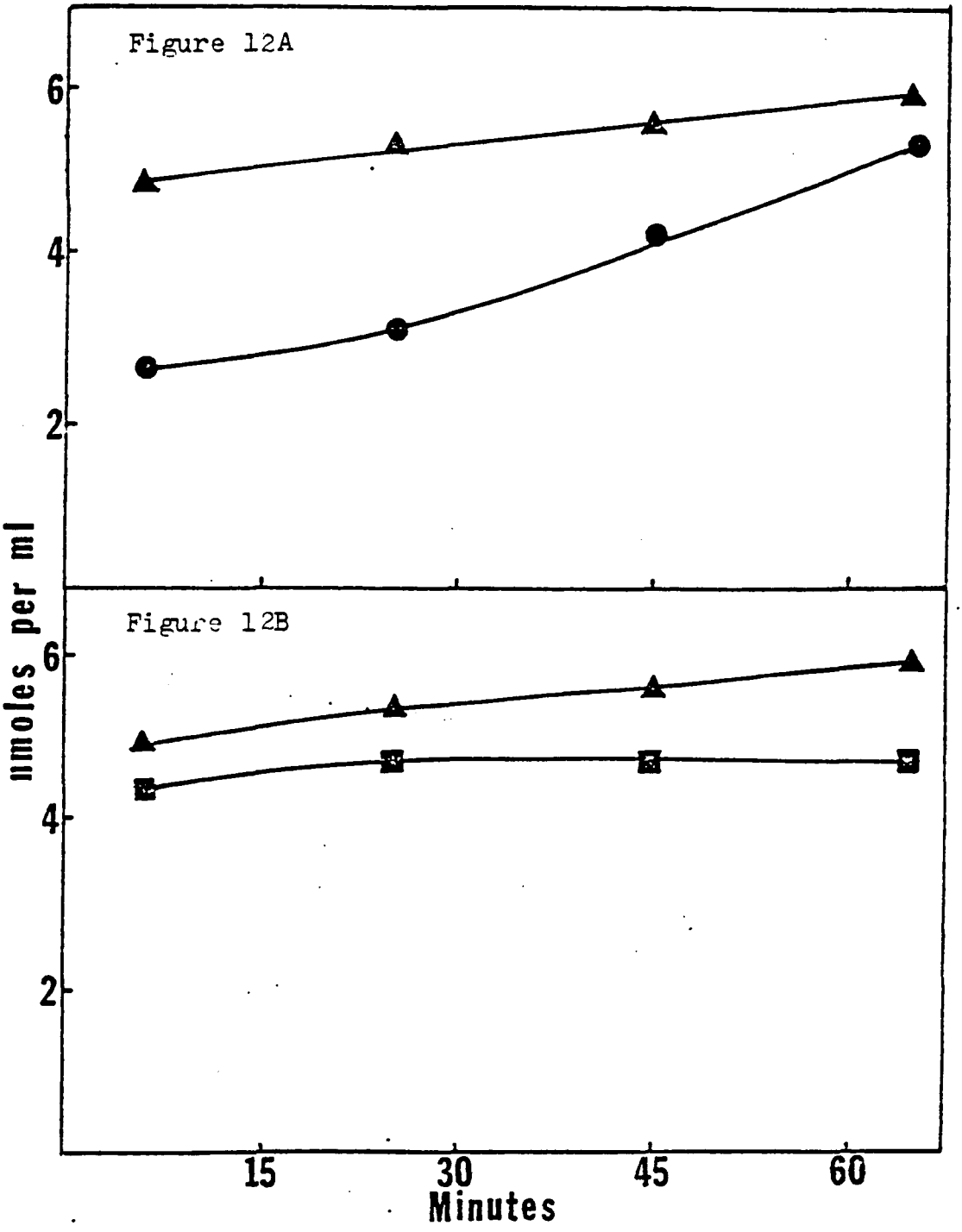


Figure 12A
6
4
2
Figure 12B
6
4
2
umoles per ml
15
30
45
60
Minutes

Figure 13: Effects of (A) DL-glyceraldehyde 3-phosphate and (B) DL-3-hydroxy-4-oxobutyl-1-phosphonate on the rate of DNA synthesis. E. coli strain 8 was cultured on Bicine-buffered medium supplemented with 0.6 mM phosphate and 0.5% potassium succinate. When a cell density of 15 to 20 Klett units was reached, the cultures were treated with 0.10 mM DL-glyceraldehyde 3-phosphate or DL-3-hydroxy-4-oxobutyl-1-phosphonate. At various time intervals after treatment, 2 ml of culture were removed and incubated with [^{32}P]phosphate (15 μCi/ml) for 10 min. Incorporation of label was determined as described in Materials and Methods. Symbols: (A) 0.10 mM DL-glyceraldehyde 3-phosphate: ▲, untreated cultures; and ●, treated cultures. (B) 0.10 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate: ▲, untreated cultures; and ■, treated cultures.

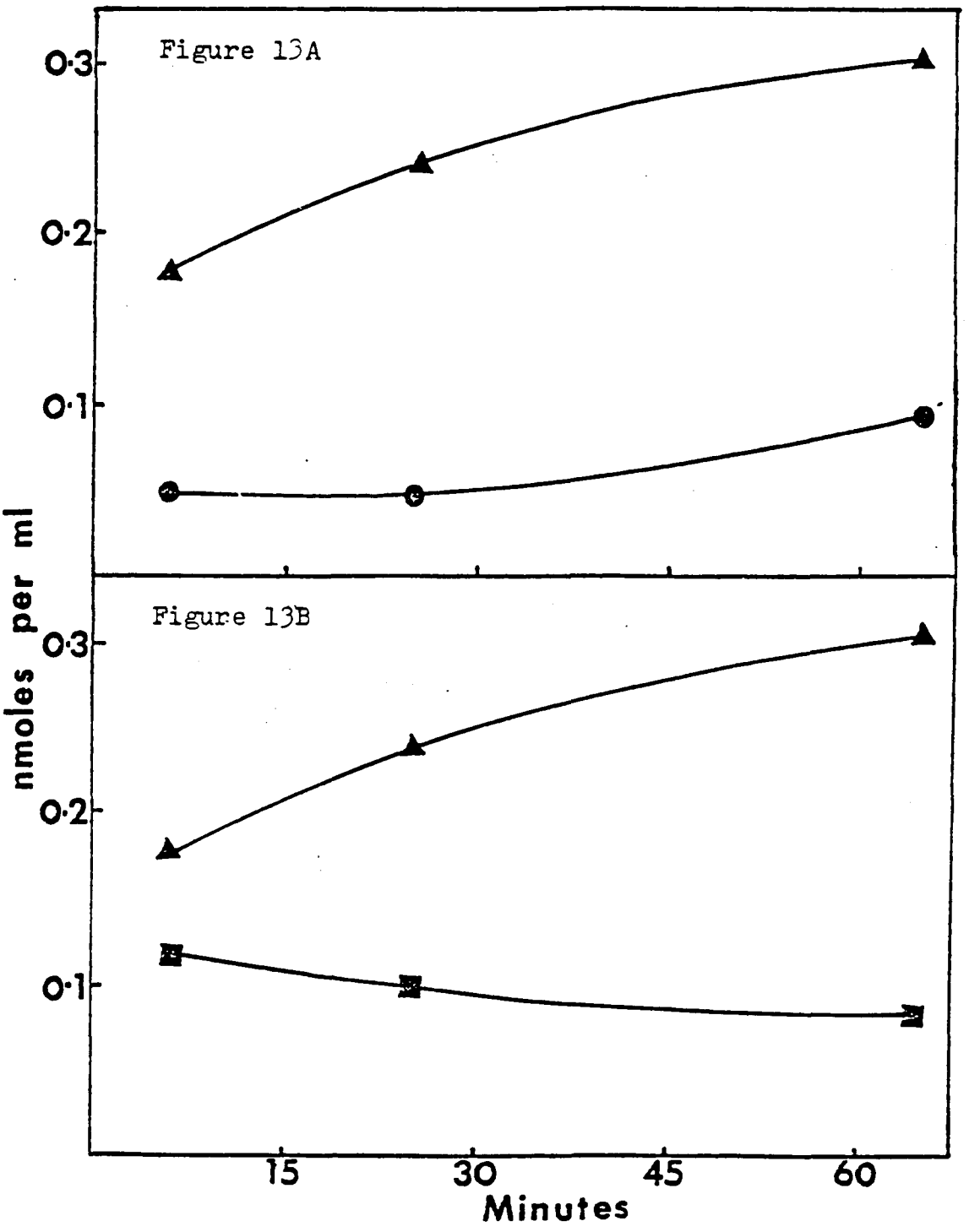


Figure 13A
0·3
0·2
0·1
Figure 13B
0·3
0·2
0·1
nmoles per ml
15
30
45
60
Minutes

Figure 14: Effects of (A) DL-glyceraldehyde 3-phosphate and (B) DL-3-hydroxy-4-oxobutyl-1-phosphonate on the rate of RNA synthesis. E. coli strain 8 was cultured on Bicine-buffered medium supplemented with 0.6 mM phosphate and 0.5% potassium succinate. When a cell density of 15 to 20 Klett units was reached, the cultures were treated with 0.10 mM DL-glyceraldehyde 3-phosphate or DL-3-hydroxy-4-oxobutyl-1-phosphonate At various time intervals after treatment, 2 ml of culture were removed and incubated with [^{32}P]phosphate (15 μCi/ml) for 10 min. Incorporation of label was determined as described in Materials and Methods. Symbols: (A) 0.10 mM DL-glyceraldehyde 3-phosphate: ▲ , untreated cultures; and ● , treated cultures. (B) 0.10 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate: ▲ , untreated cultures; and ■ , treated cultures.

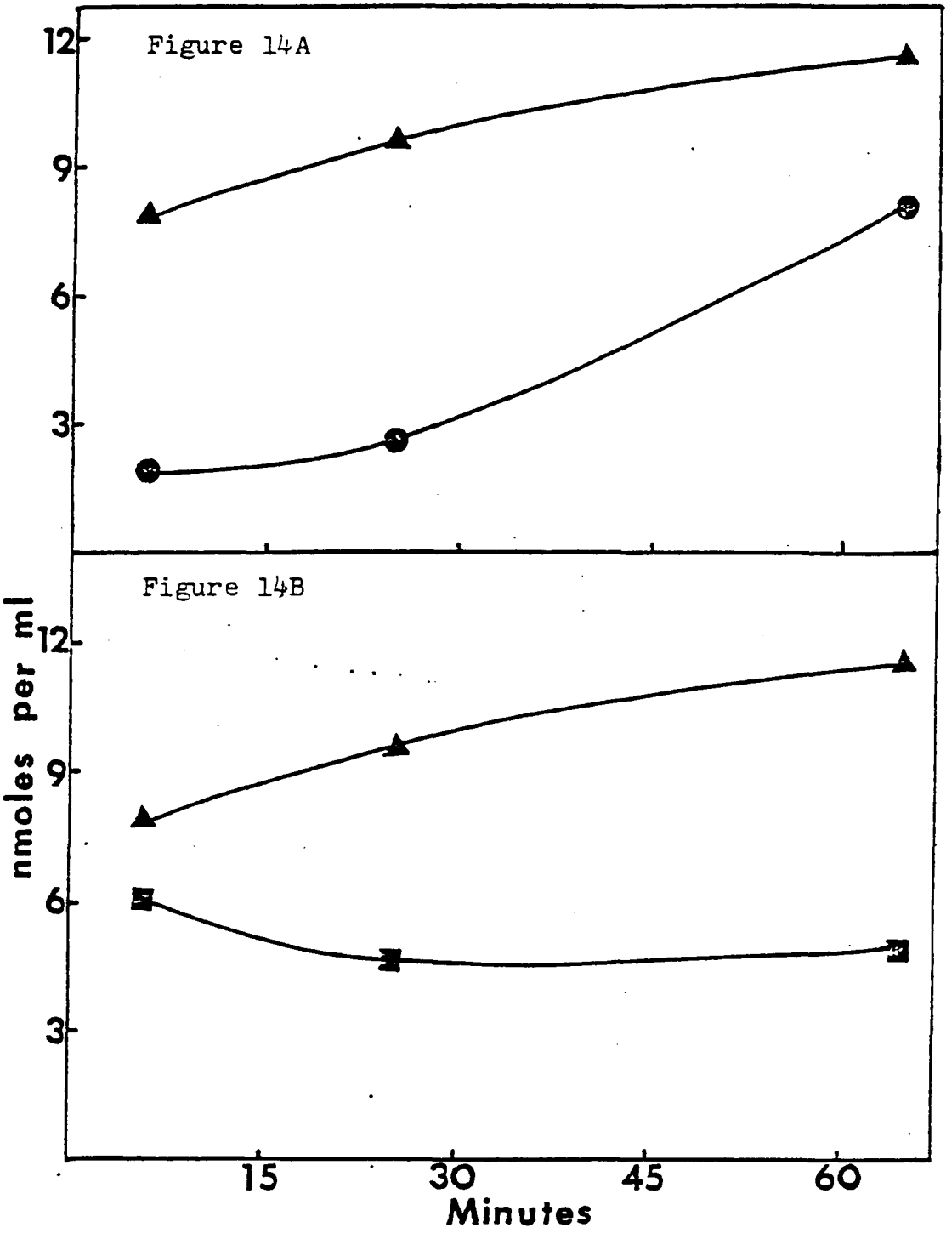


Figure 14A
12
9
6
3
Figure 14B
nmoles per ml
12
9
6
3
15
30
45
60
Minutes

Figure 15: Effects of DL-glyceraldehyde 3-phosphate on the rate of phospholipid synthesis. E. coli strain 8 was cultured on Bicine-buffered medium supplemented with 0.6 mM phosphate and 0.5% potassium succinate. When a cell density of 15 to 20 Klett units was reached, the cultures were treated with 0.10 mM DL-glyceraldehyde 3-phosphate. At various time intervals after treatment, 2 ml of culture were removed and incubated with [^{32}P]phosphate (15 µCi/ml) for 10 minutes. Incorporation of label was determined as described in Materials and Methods. Zero time indicates the time of addition of the inhibitor. Symbols: ▲ , untreated cultures; and ● , 0.10 mM DL-glyceraldehyde 3-phosphate-treated cultures. (A) total phospholipids; (B) phosphatidylethanolamine; (C) phosphatidylglycerol; and (D) cardiolipin.

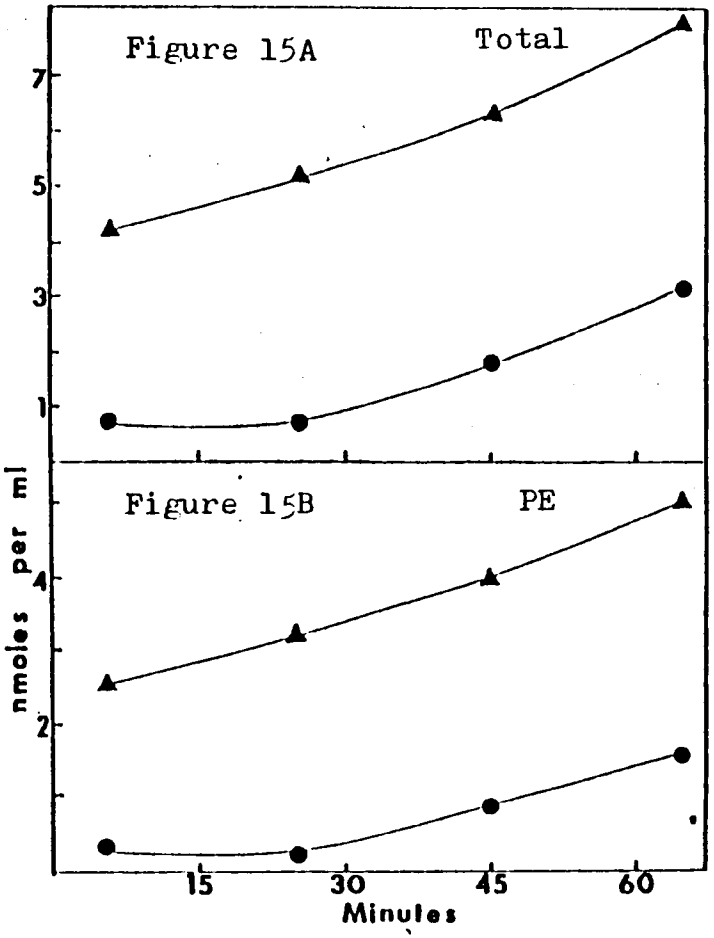


Figure 15A
Total
Figure 15B
PE
nmoles per ml
7
5
3
1
4
2
15
30
45
60
Minutes

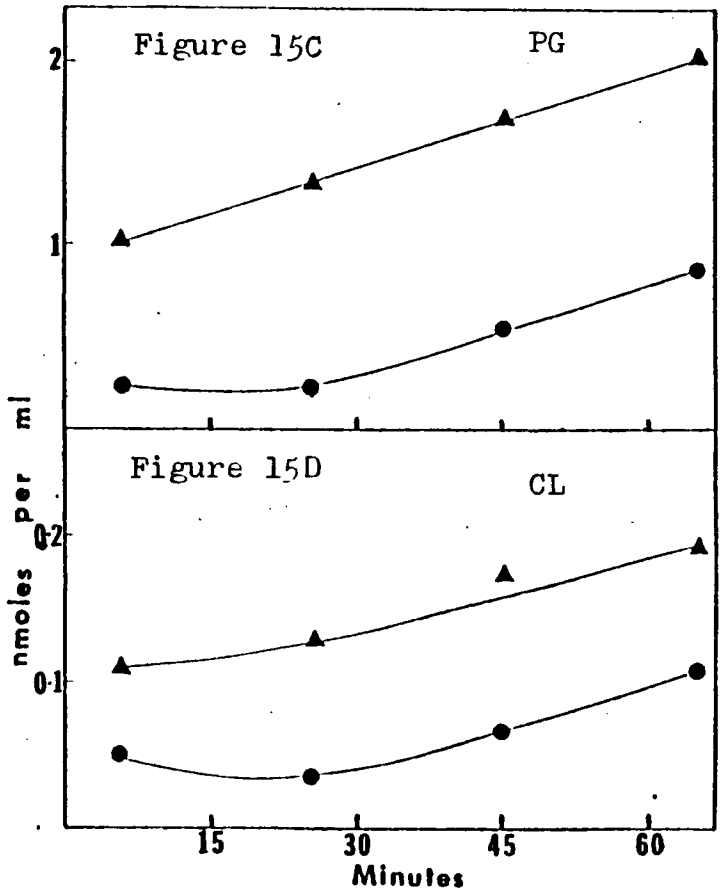


Figure 15C
PG
Figure 15D
CL
nmoles per ml
2
1
0·2
0·1
15
30
45
60
Minutes

Figure 16: Effects of DL-3-hydroxy-4-oxobutyl-1-phosphonate on the rate of phospholipid synthesis. E. coli strain 8 was cultured on Bicine-buffered medium supplemented with 0.6 mM phosphate and 0.5% potassium succinate. When a cell density of 15 to 20 Klett units was reached, the cultures were treated with 0.10 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate At various time intervals after treatment, 2 ml of culture were removed and incubated with [^{32}P]phosphate (15 μCi/ml) for 10 minutes. Incorporation of label was determined as described in Materials and Methods. Zero time indicates the time of addition of the inhibitor. Symbols: ▲ , untreated cultures; and ■ , 0.10 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate-treated cultures. (A) total phospholipids; (B) phosphatidylethanolamine; (C) phosphatidylglycerol; and (D) cardiolipin.

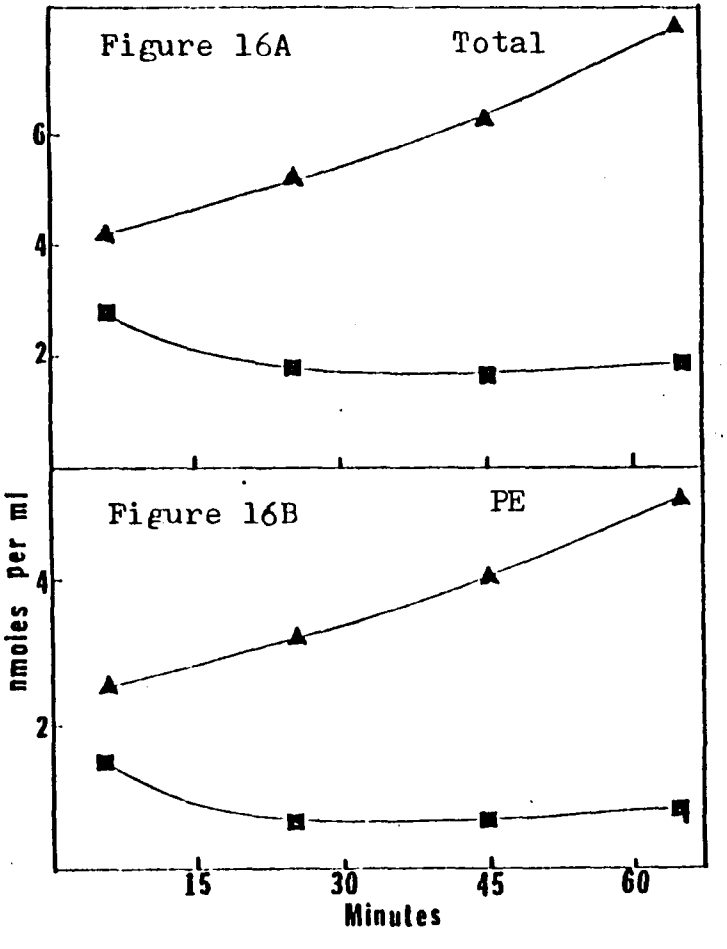


Figure 16A
Total
6
4
2
Figure 16B
PE
nmoles per ml
4
2
15
30
45
60
Minutes

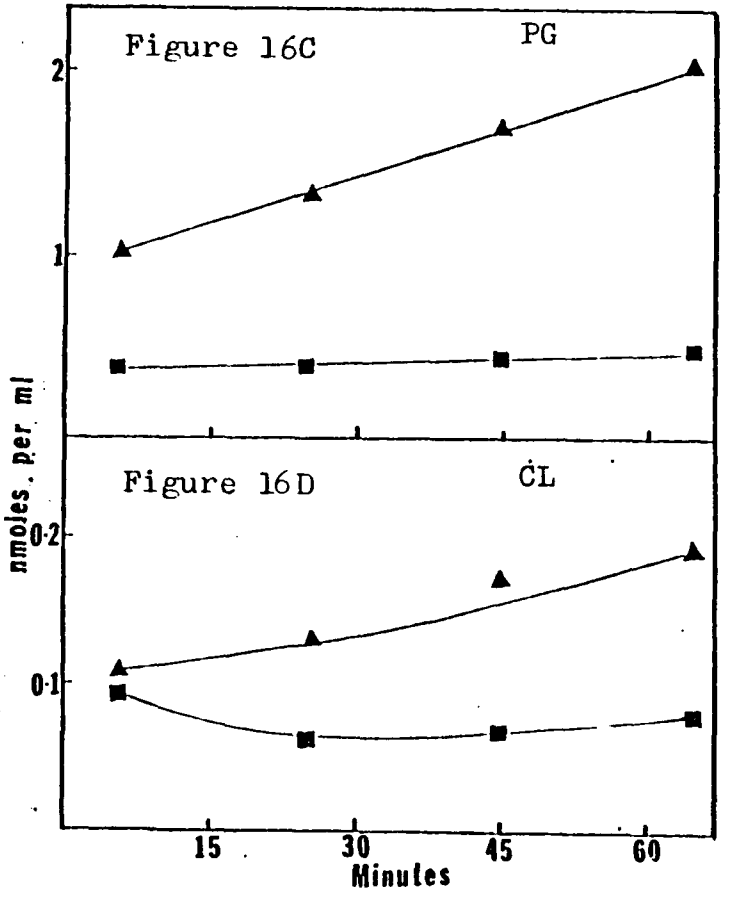


Figure 16C
PG
2
1
Figure 16D
CL
nmoles per ml
0·2
0·1
15
30
45
60
Minutes

Figure 17: Effects of DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate on the rate of protein, DNA, RNA and phospholipid synthesis. Cultures of E. coli strain 8 were grown in Bicine-buffered medium supplemented with 0.6 mM phosphate and 0.5% potassium succinate. When a cell density of 15 to 20 Klett units were reached, DL-glyceraldehyde 3-phosphate or 3-hydroxy-4-oxobutyl-1-phosphonate was added to a final concentration of 0.10 mM. At various times after addition of the drug, 2 ml of culture was removed and incubated with labeled precursors of DNA, RNA and phospholipid ([^{32}P]phosphate, 15 μCi/ml) or the precursor of protein (L-[^{3}H]isoleucine, 5 μCi/ml, 43.7 mCi/mmole) for 10 minutes. The incorporation of labeled precursors was determined as described in Materials and Methods. Symbols: (A) protein synthesis, (B) DNA synthesis, and (C) RNA synthesis: ▲ , 0.10 mM DL-glyceraldehyde 3-phosphate-treated cultures; and ● , 0.10 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate-treated cultures. (D) phospholipid synthesis of 0.10 mM DL-glyceraldehyde 3-phosphate-treated cultures: □ , cardiolipin; ○ , phosphatidylglycerol; △ , phosphatidylethanolamine; and ▽ , total phospholipids. (E) phospholipid synthesis of 0.10 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate-treated cultures: ■ , cardiolipin; ● , phosphatidylglycerol; ▲ , phosphatidylethanolamine; and ▼ , total phospholipids.

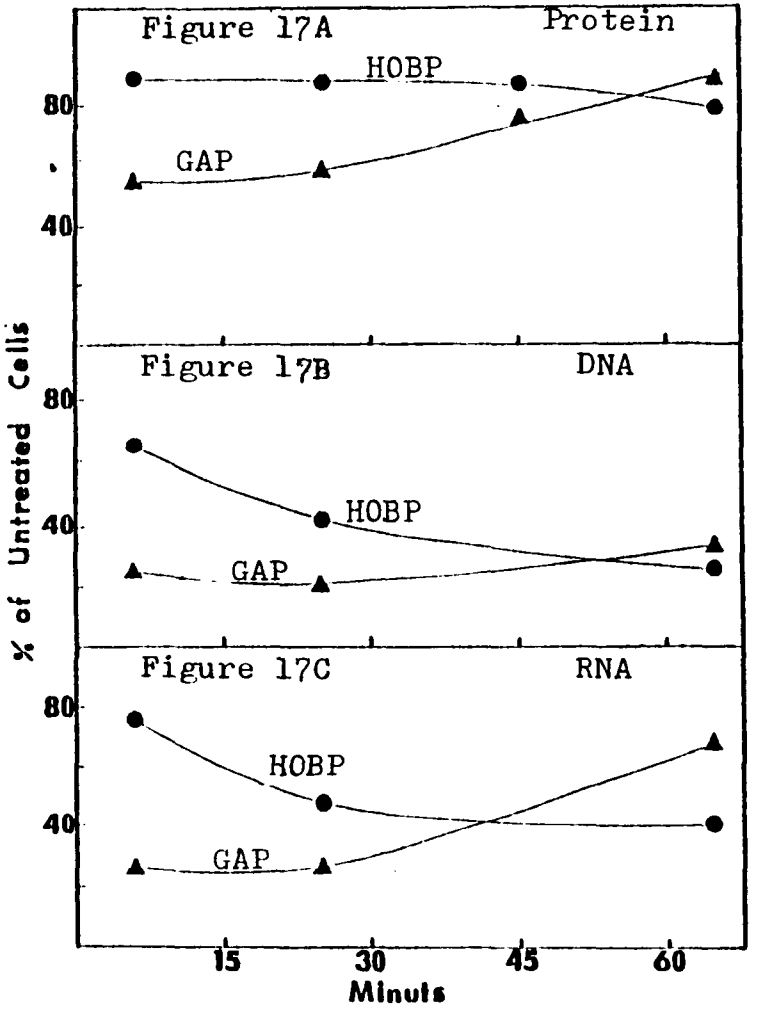


Figure 17A
Protein
HOBP
GAP
Figure 17B
DNA
HOBP
GAP
Figure 17C
RNA
HOBP
GAP
% of Untreated Cells
80
40
15
30
45
60
Minuts

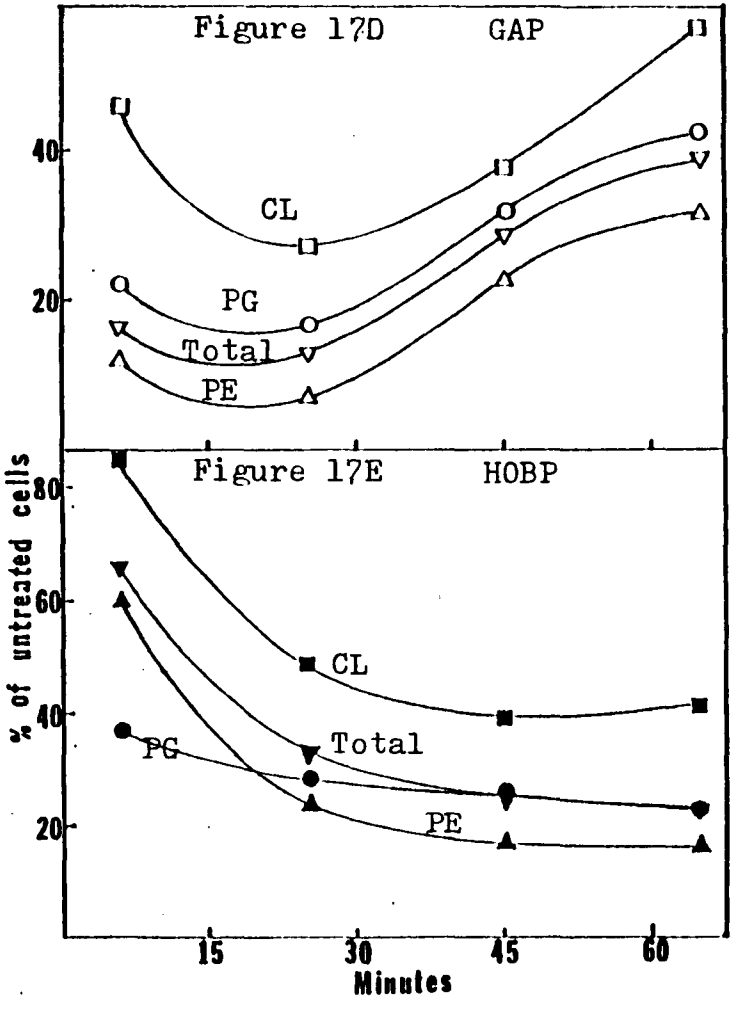


Figure 17D
GAP
CL
PG
Total
PE
Figure 17E
HOBP
CL
Total
PG
PE
% of untreated cells
40
20
80
60
15
30
45
60
Minutes

Figure 18: Effects of glyceraldehyde 3-phosphate and related compounds on the growth of E. coli strain 7 cultured in Vogel and Bonner minimal medium (Vogel & Bonner, 1956) supplemented with 0.5% glycerol; thiamine-HCl, 2 mg/l; methionine, 50 mg/l; and tryptophan, 50 mg/l. The inhibitors were added at the time indicated by the arrow. The additions were as follows: ■ , 2.5 mM DL-glyceraldehyde 3-phosphate; ● , 2.5 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate; ○ , 2.5 mM DL-3,4-dihydroxybutyl-1-phosphonate; and ▲ , none.

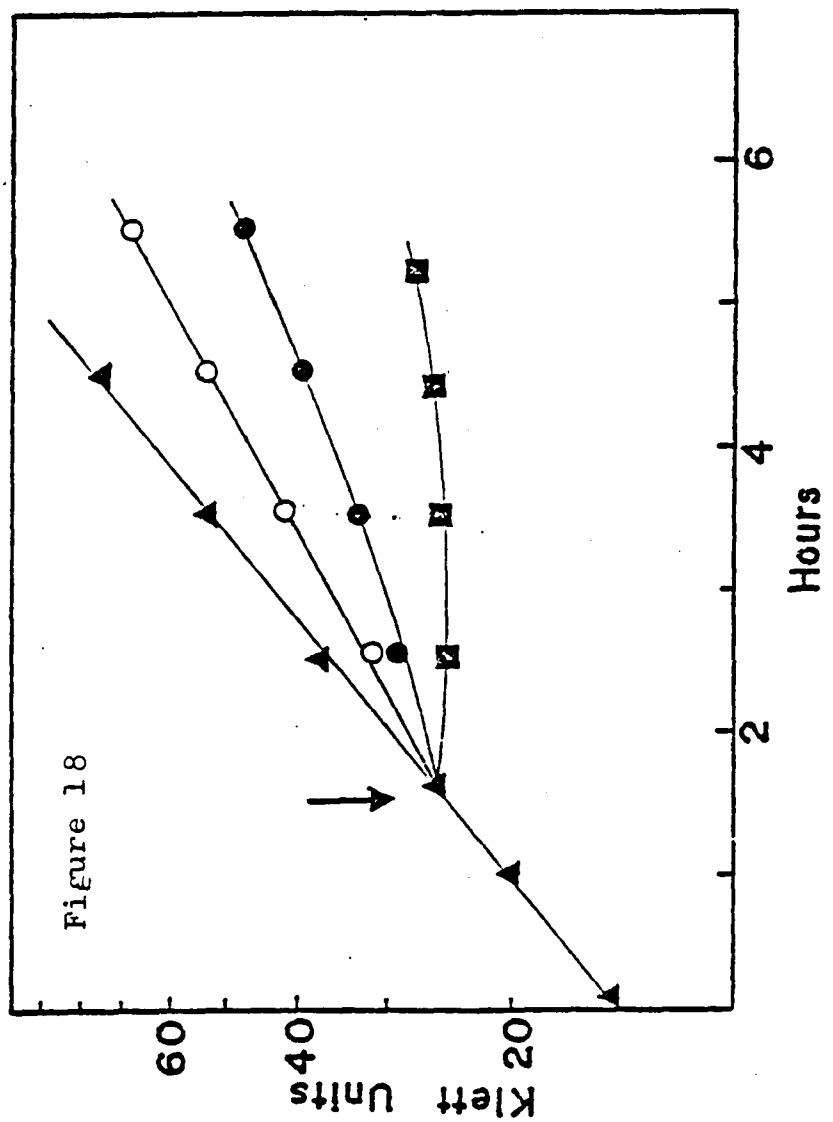


Figure 18

Figure 19: Effects of DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate on the growth of E. coli strains BB #6 and BB #7 cultured in low-phosphate minimal medium (Garen & Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate. The inhibitor were added to early logrithmic-phase cultures at the start of the experiment. Symbols: (A) strain BB #6: ▲, 2.5 mM DL-glyceraldehyde 3-phosphate; △, DL-3-hydroxy-4-oxobutyl-1-phosphonate; and ●, untreated. (B) strain BB #7: ○, 2.5 mM DL-glyceraldehyde 3-phosphate; ■, DL-3-hydroxy-4-oxobutyl-1-phosphonate, and △, untreated.

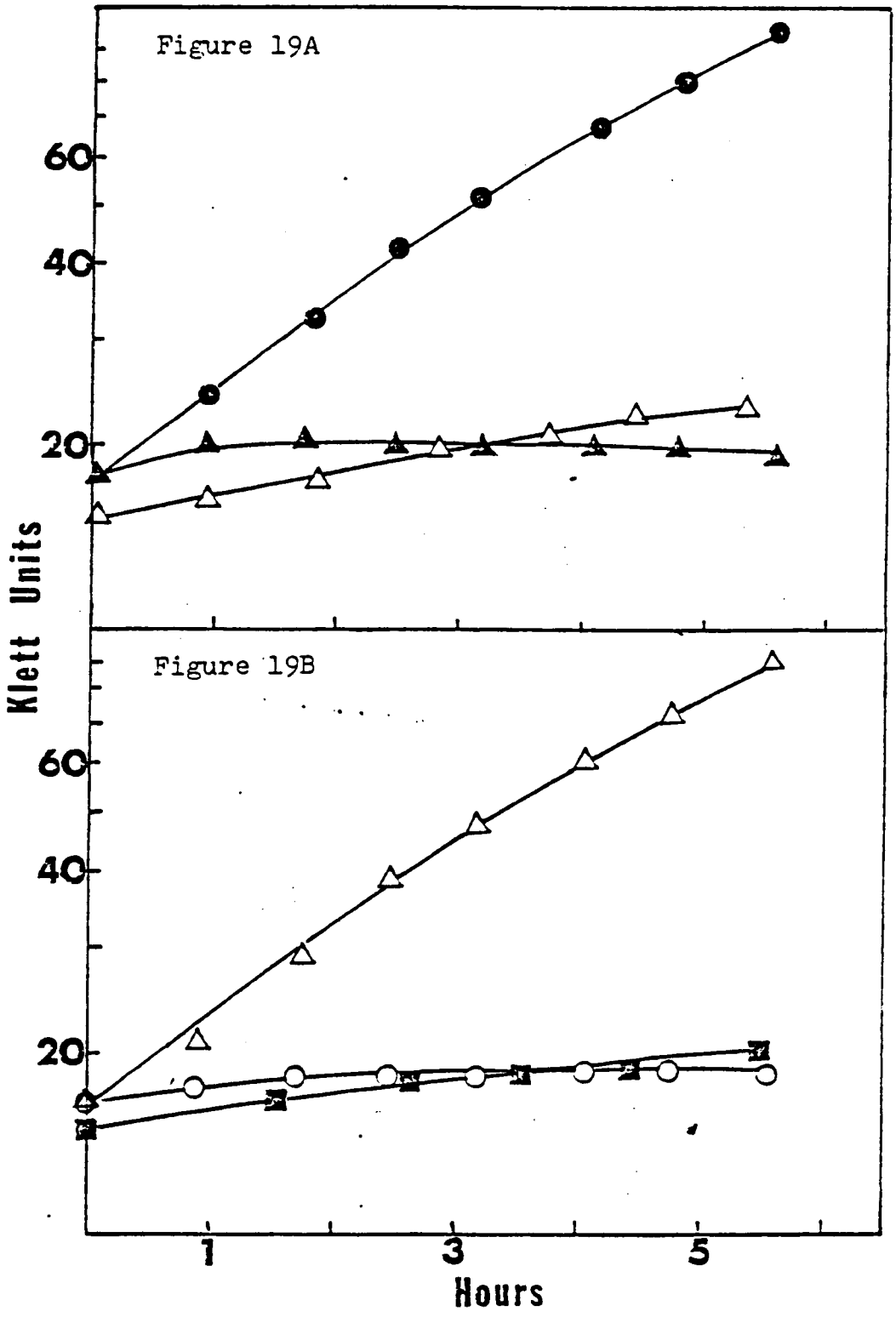


Figure 19A
60
40
20
Klett Units
Figure 19B
60
40
20
1
3
5
Hours

Figure 20: Effects of glyceraldehyde 3-phosphate and related compounds on the growth of E. coli strain CY 115 cultured in Vogel and Bonner minimal medium (Vogel & Bonner, 1956) supplemented with 0.5% glycerol; thiamine-HCl, 2 mg/l; methionine, 50 mg/l; and tryptophan, 50 mg/l. The inhibitors were added at the time indicated by the arrow. The additions were as follows: ■ , 2.5 mM DL-glyceraldehyde 3-phosphate; ● , 2.5 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate; ○ , 2.5 mM DL-3,4-dihydroxybutyl-1-phosphonate; and ▲ , none.

Figure 20

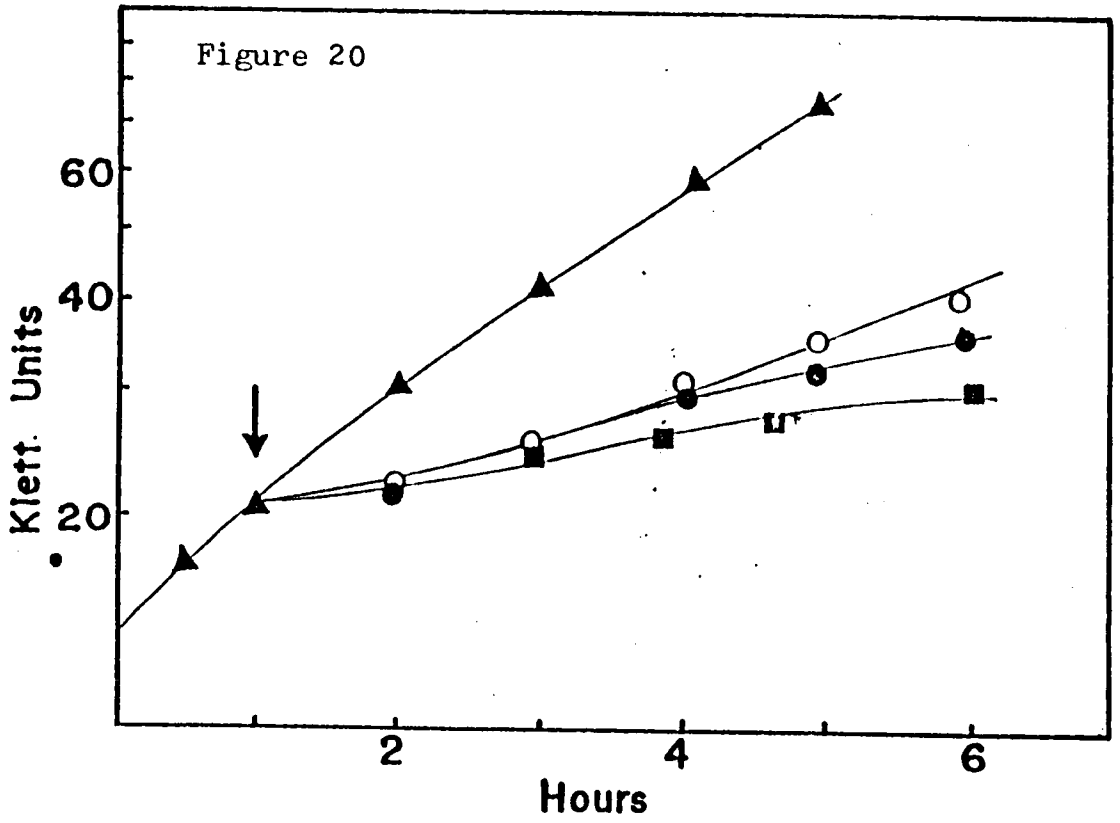


Figure 21: Effects of DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate on the growth of E. coli strain 244 cultured in Vogel and Bonner minimal medium (Vogel & Bonner, 1956) supplemented with 0.5% glycerol; thiamine-HCl, 2 mg/l; methionine, 50 mg/l; and tryptophan, 50 mg/l. The inhibitors were added to early logrithmic-phase cultures at the start of the experiment. Symbols: ■ , 2.5 mM DL-glyceraldehyde 3-phosphate; ● , 2.5 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate; and ▲ , untreated.

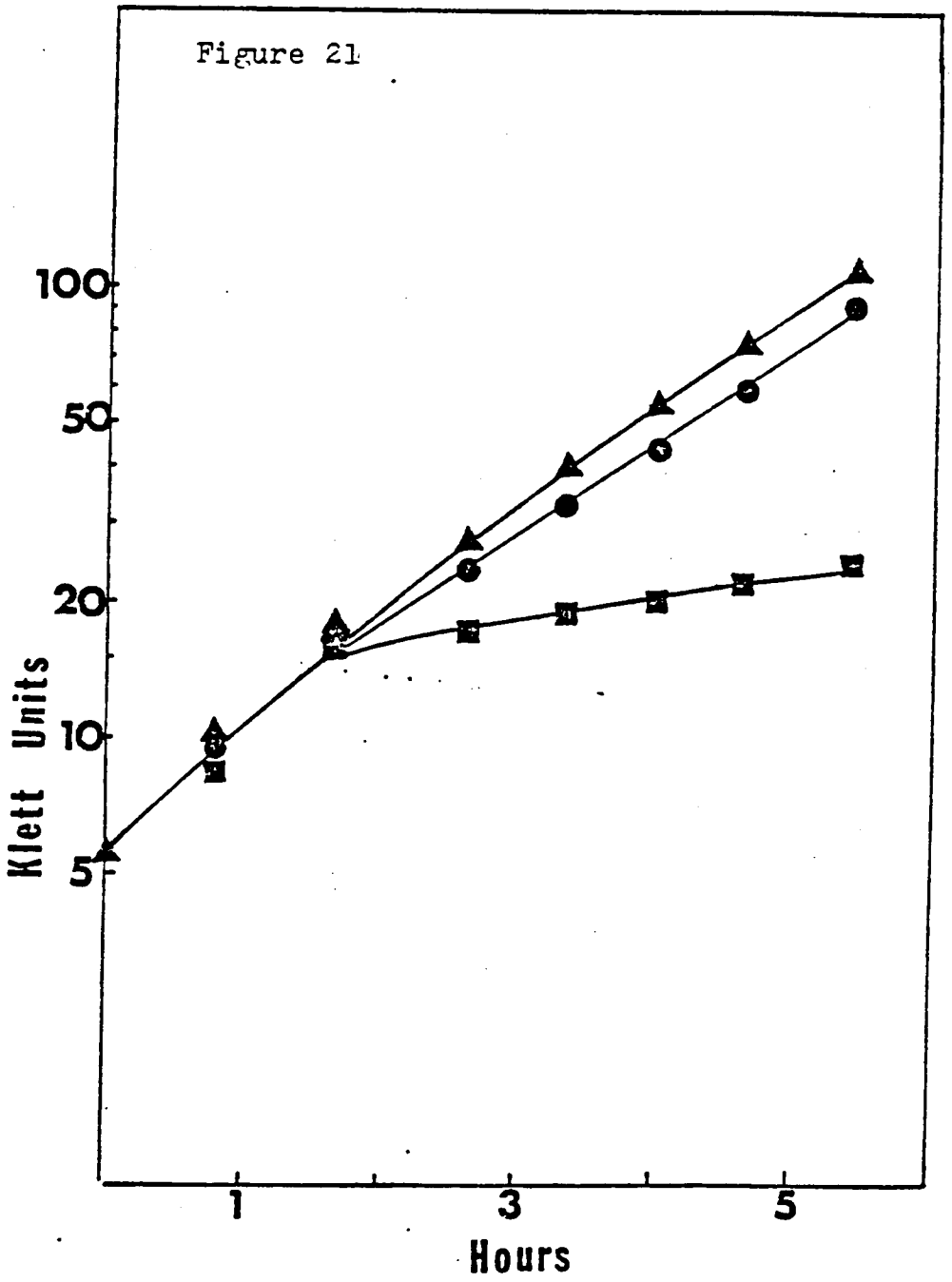


Figure 21
100
50
20
10
5
Klett Units
1
3
5
Hours

Figure 22: Effects of DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate on the growth of E. coli strain K10 cultured in low-phosphate minimal medium (Garen & Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% glycerol. The inhibitors were added at the time indicated by the arrow. (A) the temperature remained at 30 C throughout the duration of the experiment. (B) the temperature increased from 30 C to 42 C at the time indicated by the arrow. Symbols: ■ , 2.5 mM DL-glyceraldehyde 3-phosphate; ○ , 2.5 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate; and ● , untreated.

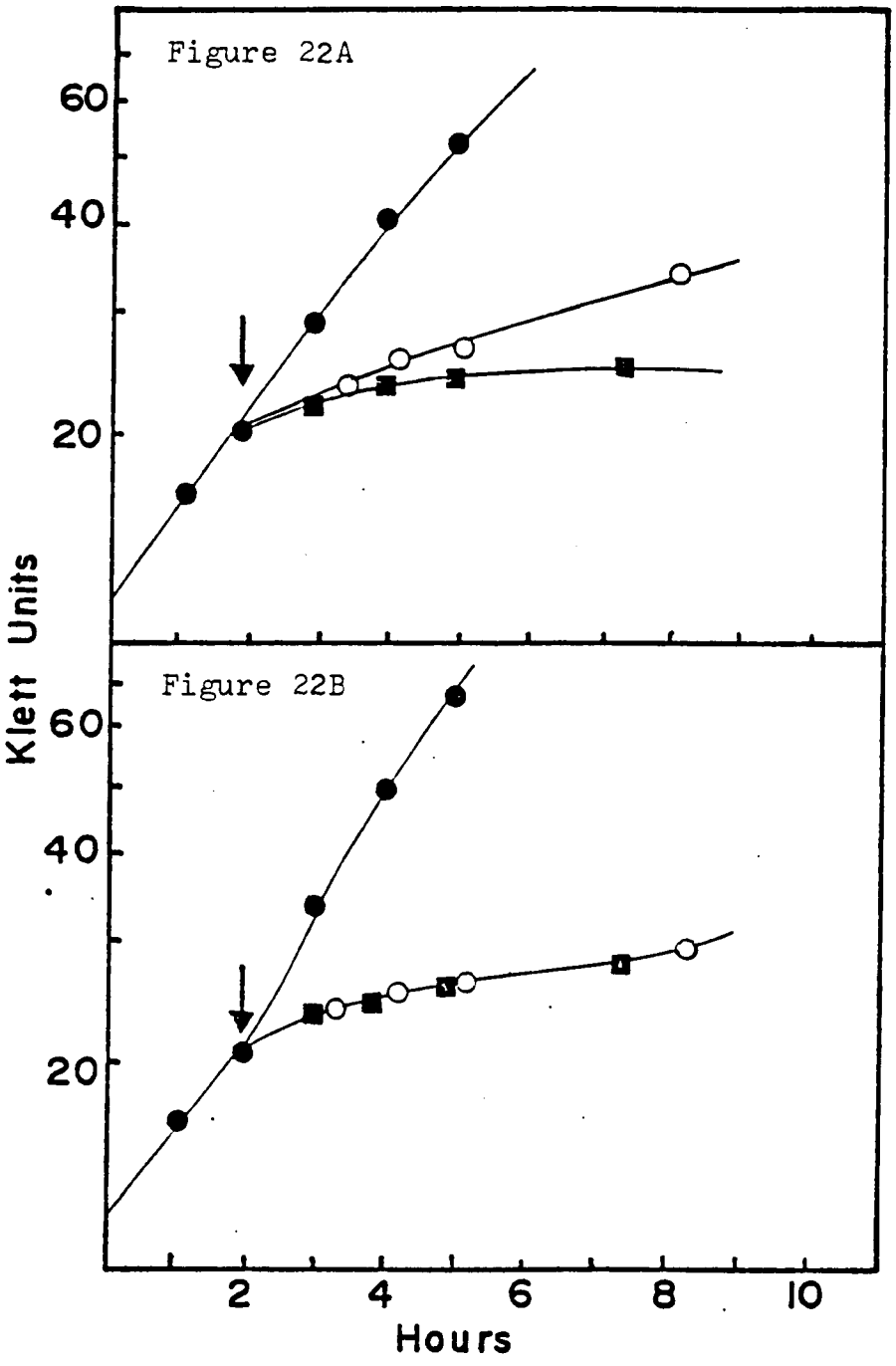


Figure 22A
60
40
20
Figure 22B
60
40
20
Klett Units
2
4
6
8
10
Hours

Figure 23: Effects of DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate on the growth of E. coli strain NP 315 cultured in low-phosphate minimal medium (Garen & Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% glycerol. The inhibitors were added at the time indicated by the arrow. (A) The temperature remained at 30 C throughout the duration of the experiment. (B) The temperature increased from 30 C to 42 C at the time indicated by the arrow. Symbols: ■ , 2.5 mM DL-glyceraldehyde 3-phosphate; ○ , 2.5 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate; and ● , untreated.

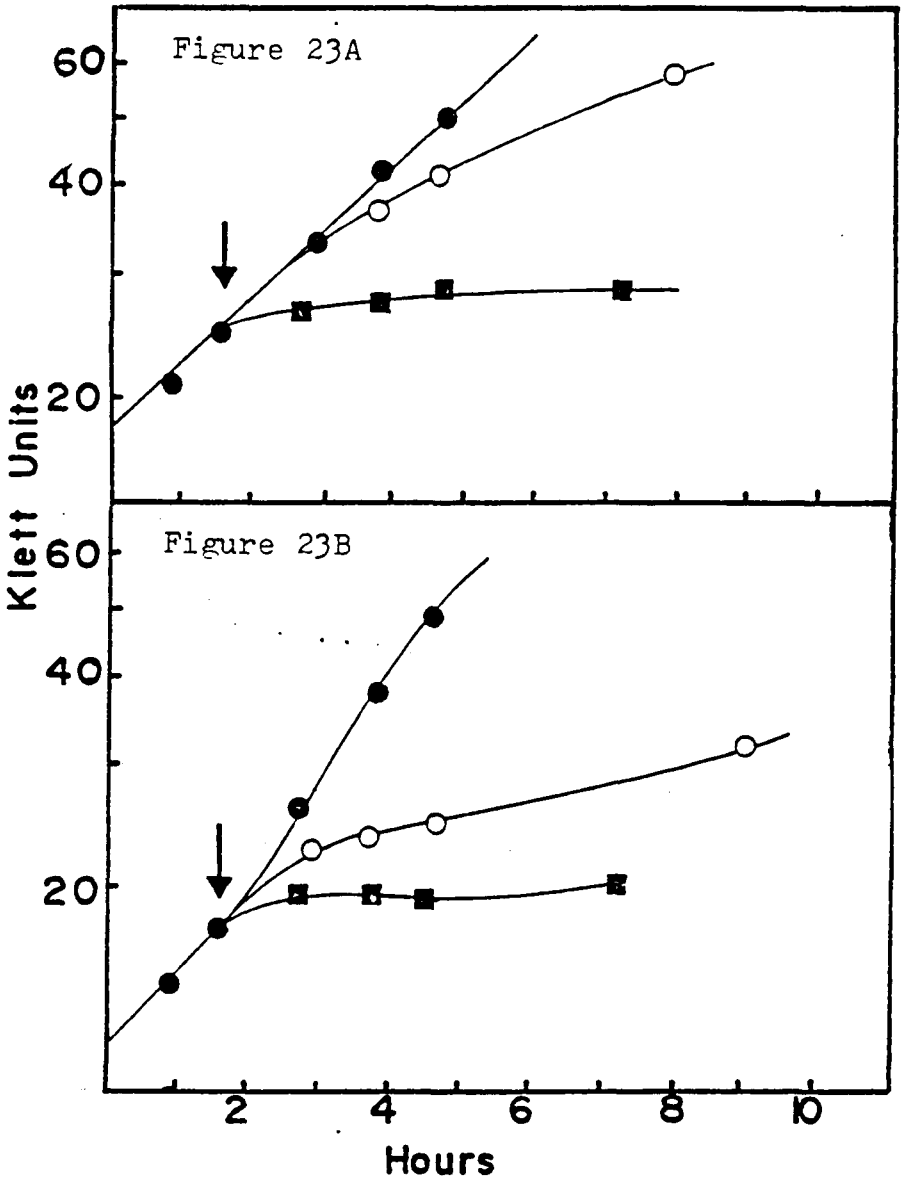


Figure 23A
60
40
20
Klett Units
Figure 23B
60
40
20
2
4
6
8
10
Hours

Figure 24: Determination of the apparent K_i of DL-glyceraldehyde 3-phosphate for acyl CoA:sn-glycerol 3-phosphate acyltransferase by the Dixon method (Dixon & Webb, 1964). The assay conditions are described in Materials and Methods. The reaction mixtures were ■, 64.6 μM, and ●, 32.3 μM in sn-[^{14}C]glycerol 3-phosphate (24.8 mCi/mmol) and contained varying concentrations of DL-glyceraldehyde 3-phosphate. (A) Palmitoyl CoA served as acyl donor, and (B) oleoyl CoA served as acyl donor.

Figure 24A

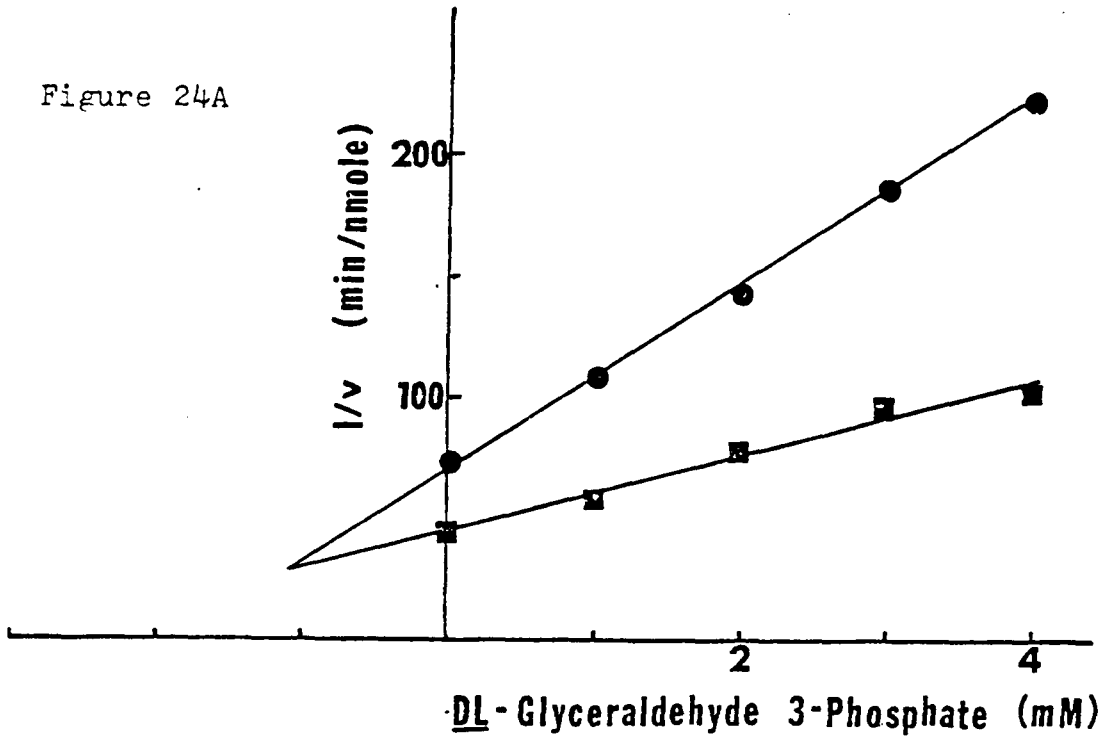


Figure 24B

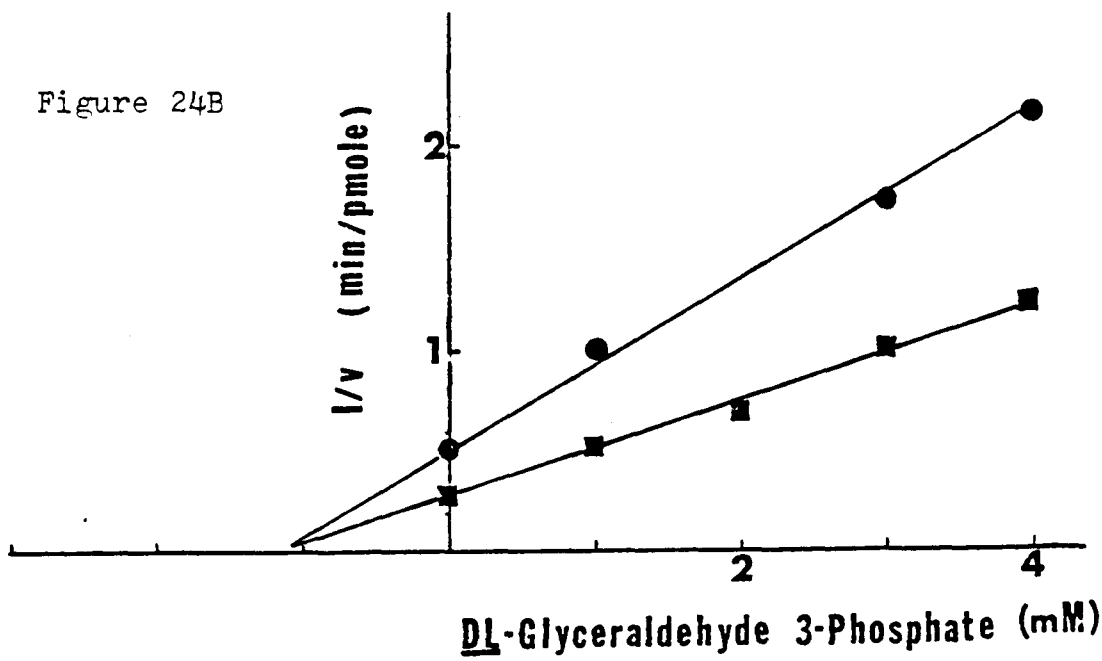


Figure 25: Determination of the apparent K_i of DL-3-hydroxy-4-oxobutyl-1-phosphonate for acyl CoA:sn-glycerol 3-phosphate acyltransferase by the Dixon method (Dixon & Webb, 1964). The assay conditions are described in Materials and Methods. The reaction mixtures were ■ , 64.6 μM, and ● , 32.3 μM in sn-[^{14}C]glycerol 3-phosphate (24.8 mCi/mmol) and contained varying concentrations of DL-3-hydroxy-4-oxobutyl-1-phosphonate. (A) Palmitoyl CoA served as acyl donor and (B) oleoyl CoA served as acyl donor.

Figure 25A

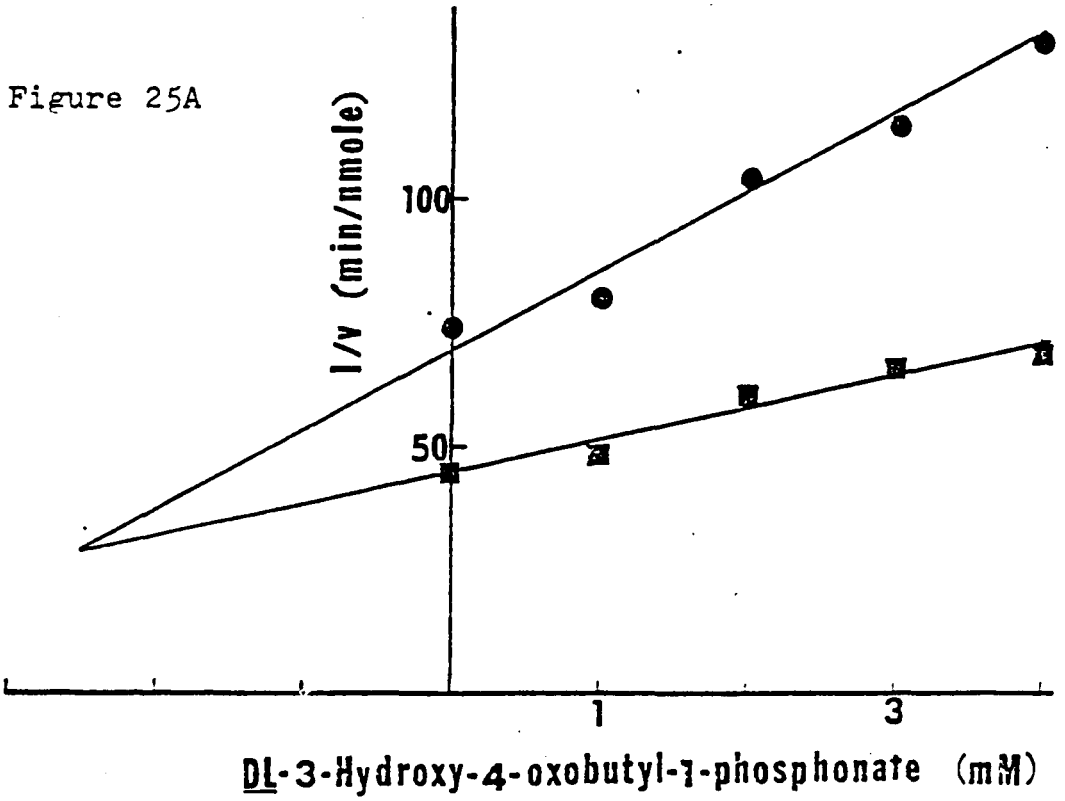


Figure 25B

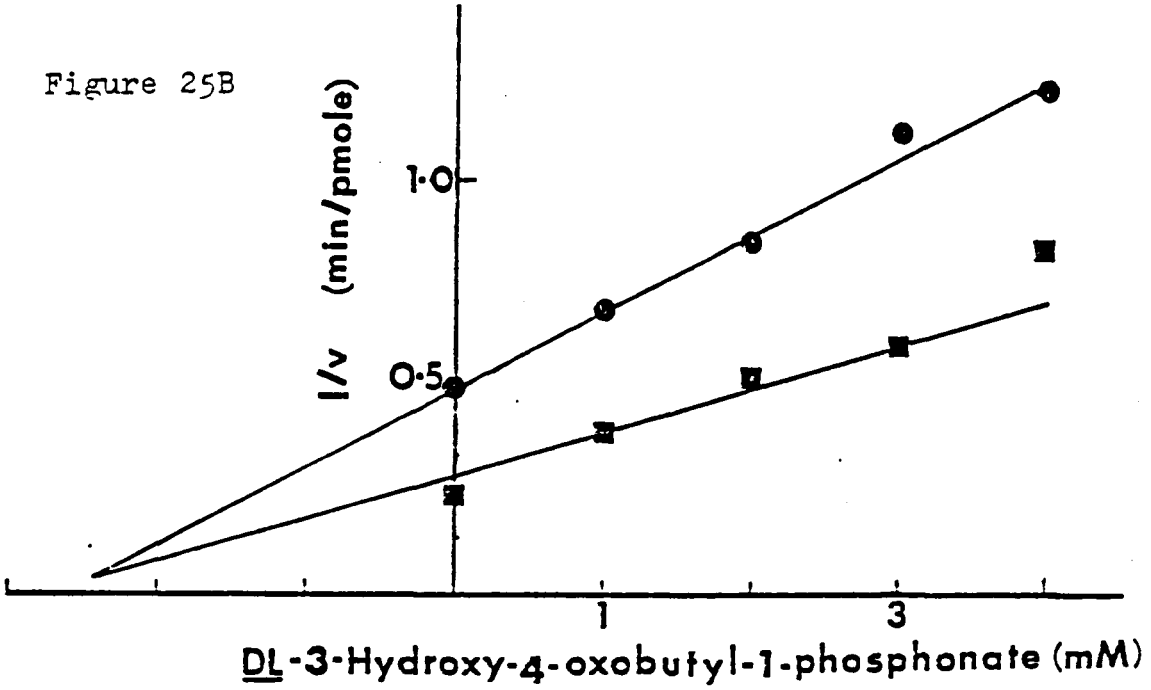


Figure 26: Effects of DL-glyceraldehyde 3-phosphate and DL-3-hydroxy-4-oxobutyl-1-phosphonate on the growth of E. coli strain BB26-36 R2 cultured in low-phosphate minimal medium (Garen & Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate. The inhibitor were added at the time indicated by the arrow. Symbols: ■ , 2.5 mM DL-glyceraldehyde 3-phosphate; ● , 2.5 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate; and ▲ , untreated.

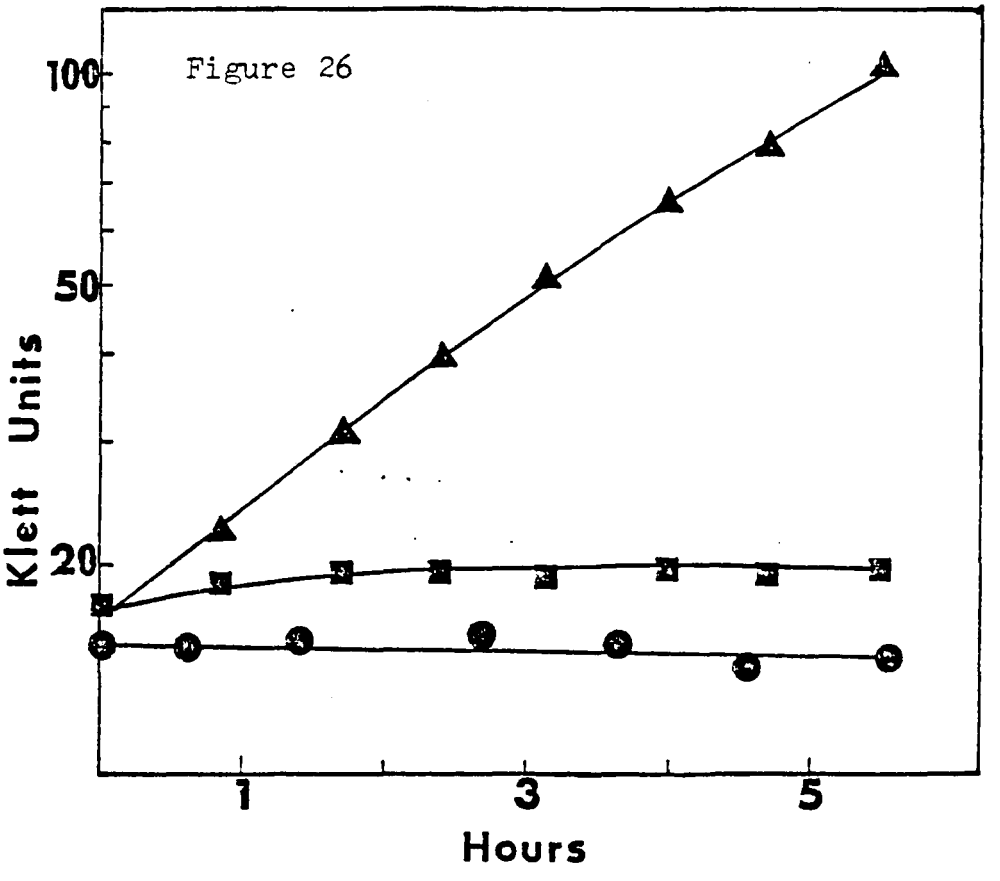


Figure 26

Figure 27: Determination of the apparent K_i of DL-glyceraldehyde 3-phosphate for CDP-diglyceride:sn-glycerol 3-phosphate phosphatidyltransferase by the Dixon method (Dixon & Webb, 1964). The assay conditions are described in Materials and Methods. The reaction mixtures were □, 64.6 µM, and ○, 32.3 µM, in sn-[^{14}C]glycerol 3-phosphate (24.8 mCi/mmol) and contained varying amounts of DL-glyceraldehyde 3-phosphate.

Figure 27

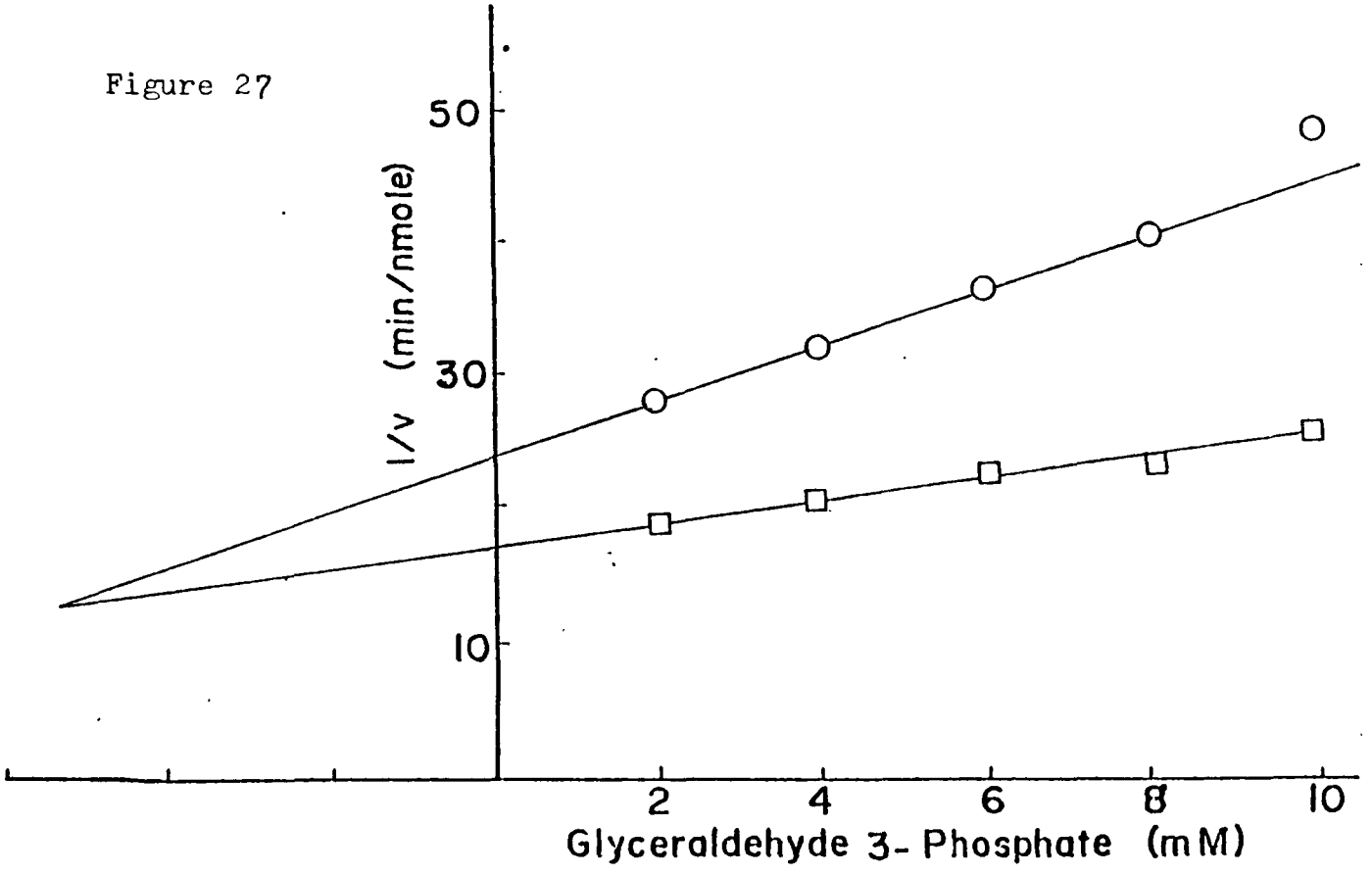


Figure 28: Determination of the apparent K_i of DL-3-hydroxy-4-oxobutyl-1-phosphonate for CDP-diglyceride:sn-glycerol 3-phosphate phosphatidyltransferase by the Dixon method (Dixon & Webb, 1964). The assay conditions are described in Materials and Methods. The reaction mixtures were ■ , 16 μM, and ● , 8 μM, in sn-[^{14}C]glycerol 3-phosphate (100 mCi/mmol) and contained varying amounts of DL-3-hydroxy-4-oxobutyl-1-phosphonate.

Figure 28

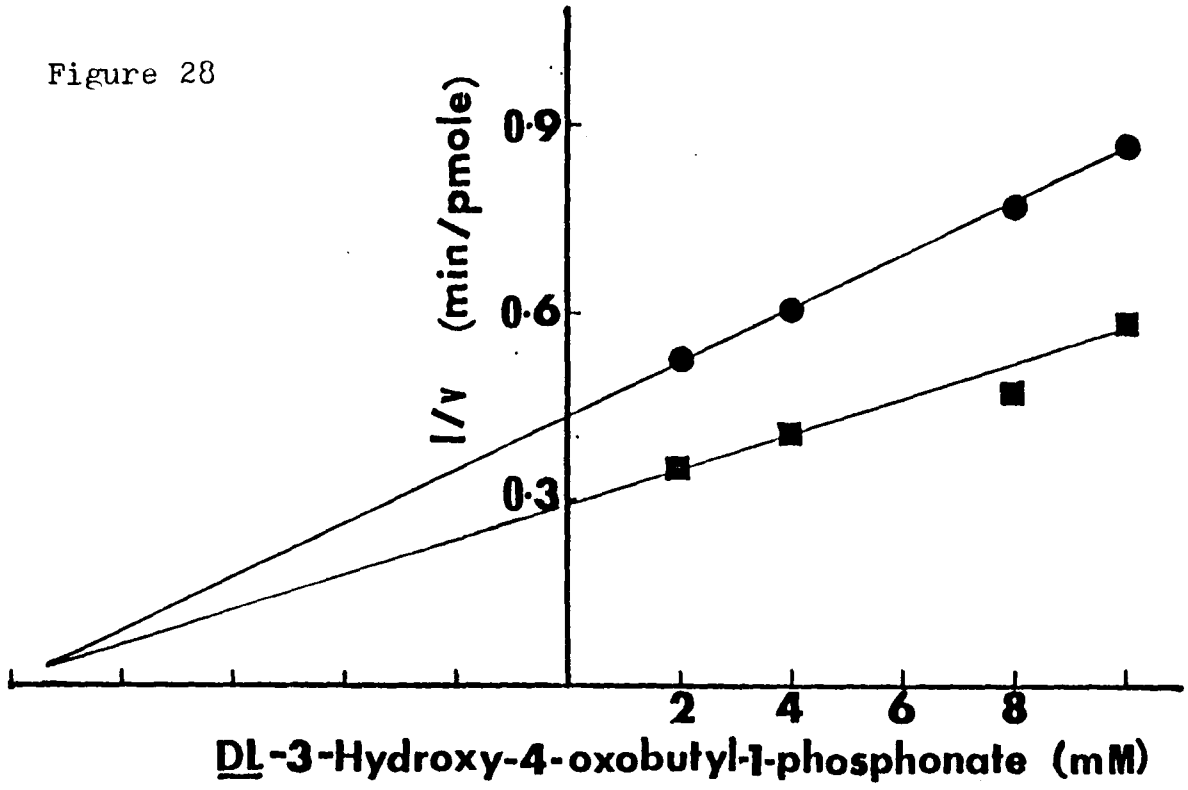

0·9
0·6
0·3
1/v (min/pmole)
2
4
6
8
DL-3-Hydroxy-4-oxobutyl-1-phosphonate (mM)

Figure 29: Effects of five inhibitors on the growth of E. coli strain 3C-1 cultured in low-phosphate synthetic medium (Garen & Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate. The inhibitors were added to early logrithmic-phase cultures at the start of the experiment. Symbols: □ , 2.5 mM DL-3,4-dihydroxybutyl-1-phosphonate; ▲ , 2.5 mM L-valine; ○ , 2.5 mM DL-glyceraldehyde 3-phosphate; ■ , 2.5 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate; △ , 2.5 mM DL-glycerol 3-phosphate; and ● , untreated.

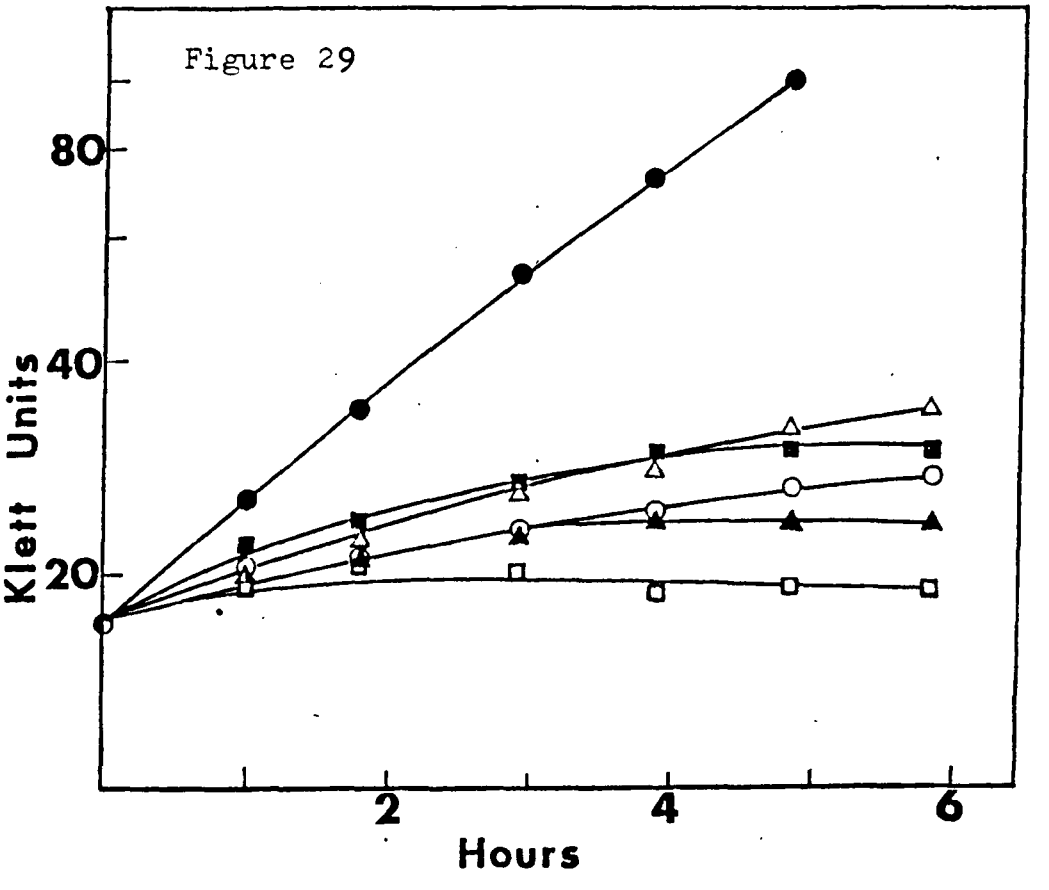


Figure 29
80
40
20
Klett Units
2
4
6
Hours

Figure 30: Effects of five inhibitors on the growth of E. coli strain 8 cultured in low-phosphate synthetic medium (Garen & Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate. The inhibitors were added to early logrithmic-phase cultures at the start of the experiment. Symbols: □ , 2.5 mM DL-3,4-dihydroxybutyl-1-phosphonate; △ , 2.5 mM L-valine; ○ , 2.5 mM DL-glyceraldehyde 3-phosphate; ■ , 2.5 mM DL-3-hydroxy-4-oxobutyl-1-phosphonate; ▲ , 2.5 mM DL-glycerol 3-phosphate; and ● , untreated.

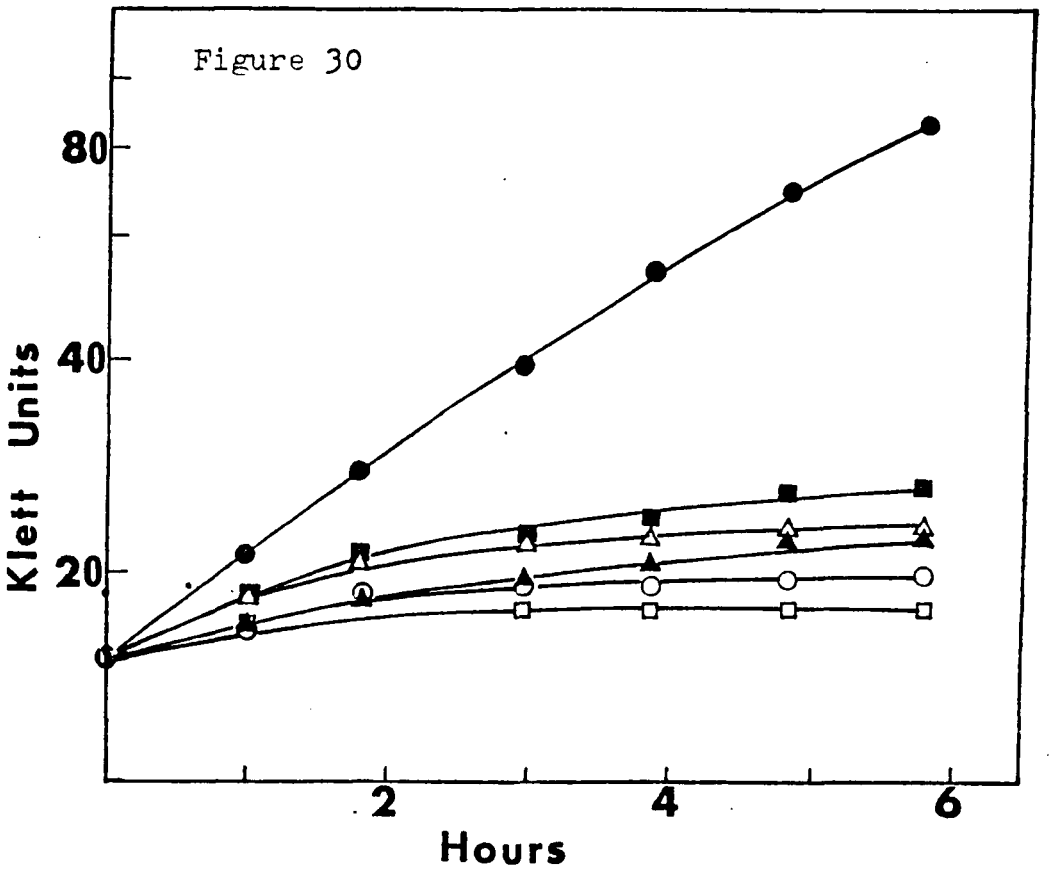
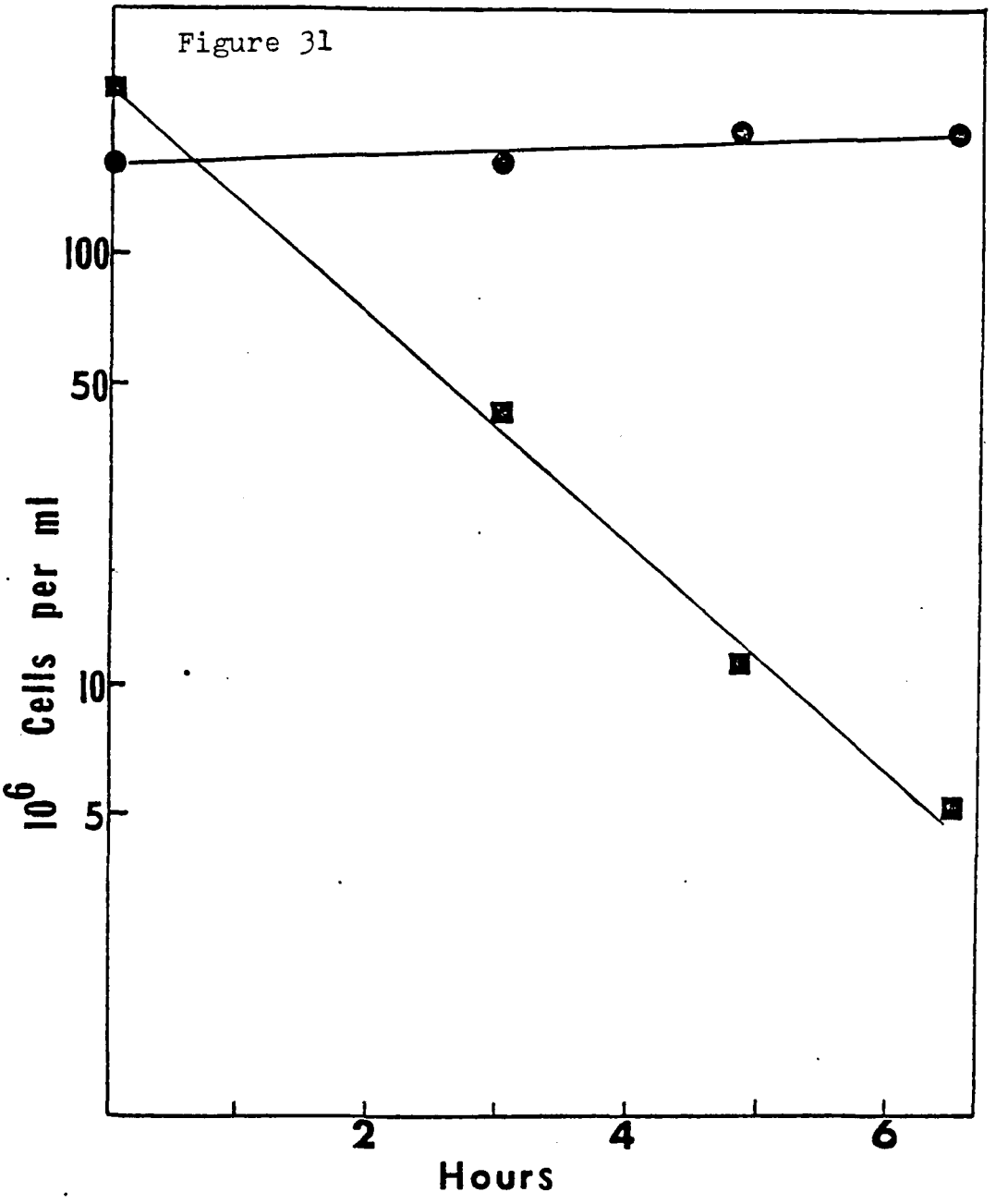


Figure 30
80
40
20
Klett Units
2
4
6
Hours

Figure 31: Effects of 2.5 mM DL-glyceraldehyde 3-phosphate on the viability of E. coli strains 3C-1 and 8 cultured in low-phosphate minimal medium (Garen & Levinthal, 1960) supplemented with 0.6 mM phosphate and 0.5% potassium succinate. The inhibitor was added to early logrithmic-phase cultures at 16 Klett units at the start of the experiment. Cell viability was determined as described in Materials and Methods. Symbols: ● , strain 3C-1; and ■ , strain 8.

Figure 31



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