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Nonenzymatic Formation of "Energy-Rich" Lactoyl  
and Glyceroyl Thioesters from Glyceraldehyde and a Thiol

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### Summary

The "energy-rich" thioester, N-acetyl-S-lactoylcysteine, is formed under anaerobic conditions from glyceraldehyde and N-acetylcysteine at ambient temperature in aqueous solutions of sodium phosphate (pH 7.0). The conversion of glyceraldehyde to lactoyl thioester occurs at a rate of about 0.4% per day in reactions with 10 mM glyceraldehyde, 10 mM thiol, and 500 mM sodium phosphate (pH 7.0). Thioester formation proceeds at an estimated efficiency of 76%, since a similar reaction with 12.5 mM thiol yields 50.7% lactate at 6 months from only 66.5% of the glyceraldehyde (dihydroxyacetone). The formation of lactoyl thioester most likely occurs by the phosphate-catalyzed dehydration of glyceraldehyde to give pyruvaldehyde, which combines with thiol to form a hemithioacetal that rearranges to the thioester. A second "energy-rich" thioester, N-acetyl-S-glyceroylcysteine, is also produced from glyceraldehyde when these reactions are carried out in the presence of oxygen and to a limited extent in the absence of oxygen. In the presence of oxygen the formation of glyceroyl thioester continues until the thiol disappears completely by oxidation. The significance of these reactions to the energetics of the origin of life is discussed.

Key Words: Glyceraldehyde - Intramolecular rearrangement - Oxidation -  
Lactoyl thioester - Glyceroyl thioester - Prebiotic

Abbreviations: Ac-Cys, N-acetylcysteine; Ac-Cys(Lac), N-acetyl-S-lactoylcysteine; Ac-Cys(Glc), N-acetyl-S-glyceroylcysteine; TBDMS, tert-butyldimethylsilyl group.

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## Introduction

The maintenance of polypeptides and polynucleotides in an aqueous environment requires their continued synthesis that by in large balances their hydrolytic decomposition. A fairly stable source of energy, in a chemical form, is needed to continually drive this new synthesis. It is our contention that this energy was provided during the origin of life by nonenzymatic reactions that chemically resemble substrate-level phosphorylations. Since substrate-level phosphorylations frequently operate by the initial formation of an "energy-rich" thioester, which is used to drive the phosphorylation of ADP to ATP (Decker et al., 1970), we have studied related chemical reactions that produce thioesters by oxidation of aldehydes, and have examined the ability of thioesters to drive the synthesis of phosphoanhydrides, like pyrophosphate and phosphorylimidazole.

In earlier studies, we demonstrated the photochemical synthesis of acetyl thioester from acetaldehyde and a disulfide (Weber, 1981a), and showed lactoyl thioester synthesis from pyruvaldehyde (Weber, 1982a). More recently, we reported the thiol-dependent formation of lactate and glycerate from glyceraldehyde (Weber, 1983a). We now describe the synthesis from glyceraldehyde and N-acetylcysteine of the lactoyl thioester, N-acetyl-S-lactoylcysteine, and the glyceroyl thioester, N-acetyl-S-glyceroylcysteine.

N-acetylcysteine was used as the thiol in our reactions, since it resembles a cysteine residue in a prebiotic peptide and cysteine (cystine) has been synthesized under prebiotic conditions (Sagan and Khare, 1971; Hong and Becker, 1979). The prebiotic synthesis of glyceraldehyde is generally considered to have occurred by oligomerization of formaldehyde (Gabel and Ponnamperna, 1967; Reid and Orgel, 1967; Mizuno and Weiss, 1974). Formaldehyde is readily produced under presumed prebiotic conditions (Garrison et al., 1951; Miller, 1957; Getoff et al., 1960; Hubbard et al., 1971; Bar-Nun and Hartmann, 1978).

Although we have not yet investigated phosphoanhydride synthesis with lactoyl and glyceroyl thioesters, we have shown that acetyl thioester can act as a condensing agent in the synthesis of the phosphoanhydrides—pyrophosphate,



tripolyphosphate, and phosphorylimidazole (Weber, 1981b). Acetyl thioesters have also been shown to drive the synthesis of pyrophosphate on the abundant phosphate mineral, hydroxyapatite (Weber, 1982b). Earlier studies by others of prebiotic, phosphoanhydride synthesis have used thermal methods (Rabinowitz et al., 1968; Osterberg and Orgel, 1972; Handschuh et al., 1973) or chemical condensing agents other than thioesters (Steinman et al., 1964; Miller and Paris, 1964; Beck and Orgel, 1965; Ferris, 1968).

## Experimental

### Materials

N-acetyl-L-cysteine, 2,2'-dithiobis-(5-nitropyridine), D-glyceraldehyde, pyruvic acid (sodium salt), DL-glyceric acid (Hemi-calcium salt), pyruvaldehyde (methylglyoxal, 40% aqueous solution),  $\beta$ -D(-)fructose, L(-)sorbose, dihydroxyacetone, t-butyldimethylsilyl chloride, 14% boron trifluoride in methanol, and trifluoroacetic acid were obtained from Sigma Chemical Co.; hydroxylamine hydrochloride and methyl red from Matheson, Coleman and Bell; lactic acid from J. T. Baker Chemical Co.; glycolic acid and methyl DL-lactate from Fisher Scientific Co.; formic acid from Eastman Kodak Co.; DL-[ $^{14}\text{C}$ ]lactic acid (sodium salt) and [ $1\text{-}^{14}\text{C}$ ]glycerol from Amersham; [ $^{14}\text{C}$ ]formic acid (sodium salt) from ICN chemical and radioisotope division; [ $2\text{-}^{14}\text{C}$ ]pyruvic acid from New England Nuclear.

D-[ $^{14}\text{C}$ ]Glyceraldehyde was synthesized as described in our earlier publication (Weber, 1983a; Periin, 1962). Unlabeled D-Glyceraldehyde was purified with the thin-layer chromatography technique that was used for the purification of [ $^{14}\text{C}$ ]glyceraldehyde (Weber, 1983a); the concentration of unlabeled D-glyceraldehyde was measured by the method of Beck (1957). Dendroketose was prepared as described by Gutsche et al. (1967). N,N'-diacetylcystine was available from our earlier synthesis (Weber, 1981a). The procedure of Wedmid and Baumann (1977) was used to prepare methyl glycerate. The hydroxamates of lactic acid and glyceric acid were prepared as described by Thompson (1951).

### Chromatography and Electrophoresis

Paper chromatography was carried out by descending elution on Whatman

3MM paper in System I with the upper phase from ethyl acetate, pyridine, water (2:1:2 v/v); in System II with tert. butyl alcohol, formic acid, water (7:1.5:1.5 v/v); in System III with the upper phase from n-butanol, 89% phenol, acetic acid, water (2.5:5:1:5 v/v); in System IV with n-butanol, pyridine, water (6:4:3 v/v). High-voltage paper electrophoresis in System V used Whatman 3MM paper with a 0.03M potassium phosphate buffer (pH 7.1). Thin-layer chromatography was performed on Silica Gel G (or GF) plates (1500 micron layer) from Analtech Inc. Table 1 lists the chromatographic and electrophoretic mobilities of the substances studied. The products formed from [ $^{14}\text{C}$ ]glyceraldehyde were located by running the chromatograms and electrophoretograms through a Baird RSC-363 radio-chromatographic scanner. The areas of the paper that contained the radioactive products were cut out, placed in vials that contained 20 ml of scintillator made with Liquifluor from New England Nuclear, and counted in a Beckman Scintillation Counter. Reaction products were identified by co-chromatography with commercially available radioactive and non-radioactive standards, whenever possible. Thiols were visualized with the 2,2'-dithiobis-(nitro pyridine) spray (Grasset, and Murray, 1969). Disulfides and thioesters were seen as dark spots under ultraviolet light. Organic acids were detected by spraying with methyl red in a borate buffer (Lederer and Lederer, 1957). Pyruvaldehyde, glyceraldehyde, dihydroxyacetone and sugars were visualized by spraying with p-anisidine (Putnam, 1957). The rhodamine B spray (Stahl, 1965) and phosphomolybdic acid spray (Gasparic and Churacek, 1978) were used to visualize tert-butyldimethylsilylated compounds.

#### Preparation of N-Acetyl-S-glyceroylcysteine (Ac-Cys(Glc))

The method of Corey and Venkateswarlu (1972) was used to synthesize 2,3-di-O-TBDMS-glyceric acid methyl ester. Methyl glycerate (6.0 g, 50 mmole), imidazole (19.0 g, 280 mmole) and TBDMS chloride (21.0 g, 140 mmole) were added to 25 ml of N,N-dimethylformamide and reacted 24 h at ambient temperature. Twenty-five milliliters of ethyl ether and 200 ml of water were added to the reaction mixture and the pH of the aqueous phase was adjusted to 4.5 by adding solid citric acid to the stirred mixture. The acidified solution was extracted once

with 100 ml of ethyl ether. The ether extract was washed with 20 ml of water and concentrated in vacuo to 25 ml. 2,3-di-O-TBDMS-Glyceric acid was purified by preparative thin-layer chromatography of one-half of the crude preparation on 11 Silica Gel G plates (1500 micron-layer) from Analtech Inc. ( $R_f=0.56$ ; developing solvent (A), petroleum ether ( $30^{\circ}$ - $60^{\circ}$ C)-ethyl ether, 9:1, v/v). The adsorbent layer that contained 2,3-di-O-TBDMS-glyceric acid methyl ester was scraped off, and the methyl ester was eluted with ethyl ether, which was removed in vacuo. The yield of 2,3-di-O-TBDMS-glyceric acid methyl ester was 5.5 g or 63% based on methyl glycerate.

2,3-di-O-TBDMS-Glyceric acid methyl ester (2.7 g, 7.8 mmole) was dissolved in a solution of 20 ml of ethanol and 5 ml water. The methyl ester was hydrolyzed by adding 1.0 ml portions of 2M NaOH every 45 min until the NaOH totaled 9.0 mmole. Hydrolysis was continued for 2 h beyond the final NaOH addition. The reaction solution was concentrated in vacuo, 10 ml of water and 8 ml of ethyl ether were added, and the pH of the aqueous phase was adjusted at  $0^{\circ}$ C to pH 2.5 by adding solid citric acid to the stirred mixture. The organic phase was removed, and the aqueous phase extracted three additional times with 8 ml of ethyl ether. The four ether extracts were combined and washed with 2.0 ml of water. The ether was evaporated in vacuo and 2.0 ml of toluene was added and removed three times in vacuo in order to remove water. The yield of 2,3-di-O-TBDMS-glyceric acid was 2.35 g or 90% based on 2,3-di-O-TBDMS-glyceric acid methyl ester. Analytical thin-layer chromatography on Silica Gel G (developing solvent (B), petroleum ether ( $30^{\circ}$ - $60^{\circ}$ C)-ethyl ether-acetic acid, 70:24:6, v/v) showed that the preparation of 2,3-di-O-TBDMS-glyceric acid ( $R_f=0.65$ ) contained small amounts of 2,3-di-O-TBDMS-glyceric acid methyl ester and suspected 2(3)-O-TBDMS-glyceric acid. This preparation was used without purification in the synthesis of thioester.

The thioester, N-acetyl-S-(2,3-di-O-TBDMS-glyceroyl)cysteine was prepared by the imidazolidine method (Weber and Orgel, 1979). 2,3-di-O-TBDMS-glyceric acid (100 mg, 0.30 mmole) was dissolved in 250  $\mu$ l of tetrahydrofuran. 1,1'-Carbonyldiimidazole (68 mg, 0.42 mmole) was added to the solution and allowed to react 10 min. The solution of imidazolidine was added to N-acetylcysteine (39 mg, 0.24 mmole) dissolved in a mixture of 125  $\mu$ l of tetrahydrofuran and 50  $\mu$ l

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of N,N'-dimethylformamide and reacted 1.5 h at ambient temperature. Five milliliters of water was added to the reaction solution. The pH of this solution was adjusted at 0°C to 2.5 with solid citric acid and extracted three times with 5 ml of ethyl acetate. The ethyl acetate extract, which contains the thioester, was concentrated to 0.5 ml in vacuo. N-Acetyl-S-(2,3-di-O-TBDMS-glyceroyl)-cysteine was purified by preparative thin-layer chromatography on one plate of Silica Gel GF ( $R_f=0.72$ ; developing solvent (C), chloroform-methanol-acetic acid, 85:10:5, v/v). The adsorbent layer that contained the thioester, which was visible under ultraviolet light, was scraped off. The thioester was eluted with methanol, and the methanol was removed in vacuo.

The TBDMS protecting groups were removed by reacting the N-acetyl-S-(2,3-di-O-TBDMS-glyceroyl)cysteine with a solution of 2.0 ml of trifluoroacetic acid and 0.20 ml water for 4 h at ambient temperature. The trifluoroacetic acid was removed in vacuo and the residue was taken up on 0.40 ml of methanol. Ac-Cys(Glc) was purified by preparative thin-layer chromatography on one plate of Silica Gel GF ( $R_f=0.42$ , developing solvent (D), chloroform-methanol-acetic acid, 70:25:5, v/v). The adsorbent layer that contained Ac-Cys(Glc), which was visible under ultraviolet light, was scraped off. The thioester was eluted with methanol and the methanol was removed in vacuo. The residue was redissolved in methanol that was again removed in vacuo. The Ac-Cys(Glc) preparation was dissolved in 0.5 ml of water and the pH adjusted to 5 with 1.0M sodium bicarbonate. The yield was 74  $\mu$ moles or 31% based on N-acetylcysteine. Ac-Cys(Glc) was characterized by hydrolysis (pH 12, 30 min) to give glyceric acid hydroxamate (Stadtman, 1957). The absorption spectrum (200-300 nm) of Ac-Cys(Glc) resembled that of other thioesters (Stadtman, 1957 and references therein). At the wavelength of maximum absorbance (238 nm) the molar-extinction coefficient was found to be 3890 by relating the absorbance at 238 nm of Ac-Cys(Glc) to the concentration of thiol released by its ammonolysis (Lynen, 1951). Thiol concentration was measured with 5,5'-dithiobis-(2-nitrobenzoic acid) (Zahler and Cleland, 1968).

#### Preparation of N-Acetyl-S-lactoylcysteine (Ac-Cys(Lac))

The method of preparation of Ac-Cys(Lac) was similar to that described

for Ac-Cys(Glc). The following description presents the details of the Ac-Cys(Lac) synthesis. 2-O-TBDMS-Lactic acid methyl ester was prepared from methyl lactate (10.4 g, 100 mmole), imidazole (19.0 g, 280 mmole) and TBDMS chloride (21.0 g, 140 mmole) in 25 ml N, N'-dimethylformamide. Thin-layer chromatography in solvent (A) was used to purify 2-O-TBDMS-lactic acid methyl ester ( $R_f=0.67$ ). The yield of 2-O-TBDMS-Lactic acid methyl ester was 6.7 g or 59% based on methyl lactate. This methyl ester was hydrolyzed by the addition of 1.0 ml portions of 2M NaOH until the NaOH totaled 10.5 mmole. The yield of 2-O-TBDMS-lactic acid was 2.1 g or 87% based on 2-O-TBDMS-lactic acid methyl ester. Analytical thin-layer chromatography in solvent (B) showed that the preparation of 2-O-TBDMS-lactic acid ( $R_f=0.54$ ) contained small amounts of 2-O-TBDMS-lactic acid methyl ester and lactic acid.

N-Acetyl-S-(2-O-TBDMS-lactoyl)cysteine was prepared by reaction of 2-O-TBDMS-lactic acid (102 mg, 0.50 mmole) in 250  $\mu$ l of tetrahydrofuran with 1, 1'-carbonyldiimidazole (113 mg, 0.70 mmole) to give the imidazolide, which was subsequently added to a solution of N-acetylcysteine (65 mg, 0.40 mmole) in 125  $\mu$ l of tetrahydrofuran and 50  $\mu$ l of N, N'-dimethylformamide. Thin-layer chromatography in solvent (C) was used to purify the thioester, N-acetyl-S-(2-O-TBDMS-lactoyl)cysteine ( $R_f=0.65$ ). The thioester was deprotected in a 1 h reaction with trifluoroacetic acid and water at ambient temperature. Ac-Cys(Lac) was purified by thin-layer chromatography on Silica Gel GF ( $R_f=0.57$ , developing solvent (D)). The yield of Ac-Cys(Lac) was 169  $\mu$ moles or 42% based on N-acetylcysteine. Hydrolysis and hydroxylaminolysis yielded the expected products. At the wavelength of maximum absorbance (237 nm) the molar extinction coefficient was found to be 4050.

#### Reactions with D-[ $^{14}$ C]Glyceraldehyde

Reaction solutions were prepared under a nitrogen blanket from solutions of substrates that had been flushed for 20 min with nitrogen in order to remove oxygen. In a typical reaction, 125  $\mu$ l of 1.0M sodium phosphate (pH 7.0), 5  $\mu$ l of 0.50M N-acetylcysteine, 20  $\mu$ l of water, 25  $\mu$ l of 0.10M D-glyceraldehyde and 75  $\mu$ l of D-[ $^{14}$ C]glyceraldehyde were added with gastight Hamilton syringes to a test tube that had been flushed with nitrogen. The resulting solution was

flushed 5 min with nitrogen and then degassed in a desiccator for 5 min at 2/mm Hg pressure. Nitrogen was admitted to the desiccator and the reaction solutions sterilized by filtration through autoclaved Millipore FG filters (13 mm, 0.2  $\mu$ m pore size) in Swinnex filter holders. In reactions carried out in the absence of oxygen twenty microliter aliquots of these reaction solutions and 5  $\mu$ l of toluene were transferred with sterilized Drummond microcap disposable pipettes into sterilized reaction tubes, which were sealed at 1 mm Hg pressure. In reactions carried out in the presence of oxygen, the sterilized reaction solution was bubbled with oxygen for 10 min every 24 h; the reaction tube that contained the solution was kept open to air. All reactions were carried out at ambient temperature in the dark. Reactions were ended by removing and freezing at  $-80^{\circ}\text{C}$  the 20  $\mu$ l solutions in the sealed reaction tubes or 20  $\mu$ l aliquots of the reaction solution that was open to air. Five microliter aliquots were analyzed by chromatography and electrophoresis in Systems I-V that were described earlier. Analysis of organic acids and thioesters involved electrophoresis in System V followed by chromatography in System II to separate the unresolved thioesters, Ac-Cys(Lac) and Ac-Cys(Glc), and the organic acids, lactate and glycerate. The values reported for Ac-Cys(Lac) and Ac-Cys(Glc) have been corrected for their respective losses of 8% and 13% during electrophoresis and chromatography. Chromatography in System I was used to separate glyceraldehyde and dihydroxyacetone.

The half-life ( $t_{1/2}=8.7$  h) of Ac-Cys(Lac) in the reaction depicted in Fig. 4 was calculated from the rate constant( $k$ ) of Ac-Cys(Lac) disappearance with the expression,  $t_{1/2}=0.693/k$ , which assumes first order kinetics. The rate constant( $k$ ) was calculated by substituting the corrected, steady-state Ac-Cys(Lac) concentration (0.25%, 22 h-3d) into the expression,  $k[\text{Ac-Cys(Lac)}]=0.48\%/d$ , which assumes that Ac-Cys(Lac) disappearance follows pseudo first order kinetics (rate= $k[\text{Ac-Cys(Lac)}]$  and its rate is equal to the rate of lactate formation (0.48%/d, 22h-3d).

## Results

Fig. 1(a) and Fig. 1(b) show, respectively, the formation of organic acids and thioesters from glyceraldehyde in the presence of the thiol, Ac-Cys, without oxygen. As seen in Fig. 1(a), the rate of formation of lactate increases in the



first day from 0.14%/d(0h-5h) to 0.32%/d(5h-22h), and finally reaches a constant value of about 0.41%/d(22h-4d). Fig. 1(b) shows that the concentration of the lactoyl thioester, Ac-Cys(Lac) increases during the first day and then stabilizes at roughly 0.2% or 20  $\mu$ M. The fact that the increase and stabilization of the Ac-lys(Lac) concentration coincides with the increase and stabilization of the rate of lactate formation indicates that Ac-Cys(Lac) is an intermediate in the synthesis of lactate from glyceraldehyde. Alkaline hydrolysis of pooled, radioactive Ac-Cys(Lac) product from the 1d-3d reaction solutions yields radioactive lactate (85%), glycolate (10%), and glycerate (5%). The yields of organic acids in a similar reaction that used 12.5 mM instead of 10 mM Ac-Cys at 6 months of reaction are lactate (50.7%), glycerate (2.9%), glycolate (0.62%), and formate (0.44%). The formation of lactate probably continues beyond 6 months, because glyceraldehyde (6.58%) and dihydroxyacetone (26.96%) are still available in the reaction solution. A small amount of hexose (approx. 6%), which includes dendroketose, is also present in the reaction solution at 6 months.

As shown in Fig. 1(a), glycerate formation from glyceraldehyde occurs mostly in the first day of reaction. Fig. 1(b) shows that the synthesis of glyceroyl thioester, Ac-Cys(Glc), is also limited to the first day. The rates of synthesis and decomposition of Ac-Cys(Glc) appear to be fast enough for it to be an intermediate in the synthesis of glycerate from glyceraldehyde. The small amount of oxidant that is responsible for the synthesis of Ac-Cys(Glc) has not been identified. The oxidant may be oxygen, since bubbling oxygen through this reaction solution at 4 days increases the Ac-Cys(Glc) concentration from 0.22% to 2.54% five hours later. The formation of Ac-Cys(Glc) from glyceraldehyde in the presence of oxygen is discussed later in more detail. Alkaline hydrolysis of pooled, radioactive Ac-Cys(Glc) product from the 2 h and 5 h reaction solutions yields radioactive glycerate (>95%).

Fig. 2(a)(b) depicts the formation of products from glyceraldehyde in the absence of thiol without oxygen. Fig. 2(a) shows that lactate synthesis is negligible without thiol. A comparison of Fig. 2(a) with Fig. 1(a) reveals that the rate of glycerate production is slower in the absence of thiol; whereas, the rates of formation of formate and glycolate are faster. Fig. 2(b) shows a

FIGURE 1

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small but regular increase in the radioactivity that migrates with non-radioactive, synthetic Ac-Cys(Lac) and Ac-Cys(Glc) during analysis by electrophoresis and chromatography. The identity of the radioactive product that is responsible for this increase is unknown. Thioesters are eliminated as candidates, since the reaction solution does not contain thiol. The insert at the top of Fig. 2(a), shows that isomerization of glyceraldehyde to dihydroxyacetone occurs readily in the absence of thiol. The rate of isomerization is comparable to that observed in the presence of thiol, is shown in Fig. 1(a).

FIGURE 2

Fig. 3(a)(b) depicts the formation of products from glyceraldehyde in the presence of both thiol and oxygen. Fig. 3(a) shows that oxygen increases the rate of formation of glycolate, formate, and initially glycerate, but not lactate. Lactate and glycerate production slow down dramatically and nearly stop on the second day of reaction. This event is preceded by a substantial decrease in the concentration of Ac-Cys(Lac) and Ac-Cys(Glc) between the first and second day. These changes near the second day of reaction are probably due to rapid disappearance of Ac-Cys, that is almost gone by the second day, as shown in the insert at the top of Fig. 3(a). Without Ac-Cys the thioesters cannot be synthesized and the formation of glycerate and lactate eventually stop. This explanation was tested by adding new Ac-Cys(10 mM) to the reaction solution at 4 days of reaction. The reaction solution was examined 5 hours later for new synthesis of thioesters, glycerate, and lactate. Ac-Cys(Glc) was found to increase from 0.15% to 6.56%, glycerate from 15.05% to 16.36%, and lactate from 1.31% to 1.46%. Ac-Cys(Lac) could not be measured because a new product overlapped Ac-Cys(Lac) on the final chromatogram. This new product, which migrates close to Ac-Cys(Lac) during electrophoresis and chromatography, is thought to be N-acetyl-S-glycoloylcysteine(1.66% yield), because it hydrolyzes in alkaline solution to give glycolate. This product probably is formed from glyceraldehyde and not glycolaldehyde, since very little glycolaldehyde(< 1.8%) is present in the reaction solution.

FIGURE 3

Fig. 4 shows the formation of lactate, glycerate, and their thioesters with 5mM glyceraldehyde instead of 10mM glyceraldehyde that was used in earlier reactions. The corrected Ac-Cys(Lac) values in the figure are obtained by measurement of the radioactive products that are formed by hydrolysis of



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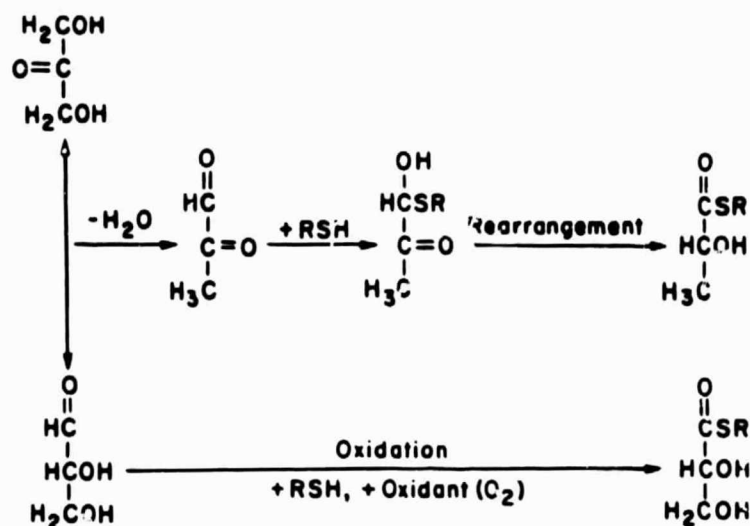
radioactive Ac-Cys(Lac) product, which is isolated by the standard analytical procedure. This hydrolysis of Ac-Cys(Lac) gives glycolate and glycerate in addition to lactate; a result that indicates a small contamination of Ac-Cys(Lac) with Ac-Cys(Glc) and suspected N-acetyl-S-glycoloylcysteine. The percent error caused by this contamination is high early in the reaction (0h-2h) when the Ac-Cys(Lac) concentration is low, but much less later in the reaction when the Ac-Cys(Lac) concentration is higher.

FIGURE 4

Fig. 4 also shows that Ac-Cys(Glc) disappears between 5 h and 2 d with a half-life estimated to be equal to or less than 13 h. This rate is much faster than the hydrolytic half-life of 193 h for Ac-Cys(Glc) in 500mM sodium phosphate (pH 7.0). Likewise, the calculated half-life of 8.7 h for Ac-Cys(Lac) in the reaction solution is much faster than its hydrolytic half-life of 142 h in 500mM sodium phosphate (pH 7.0) (see Experimental: Reactions with D-[<sup>14</sup>C]glyceraldehyde for calculation). The rapid disappearance of Ac-Cys(Glc) and Ac-Cys(Lac) in the reaction solution indicates that glyceraldehyde catalyzes the decomposition of these thioesters. Indeed, the half-life of synthetic 0.4mM Ac-Cys(Glc) decreases from 193 h to 2.2 h when 10mM glyceraldehyde is added to the 500mM sodium phosphate buffer (pH 7.0). Ac-Cys(Lac) also shows a comparable decrease from 142 h to 2.2 h under the same conditions. The mechanism of this effect by glyceraldehyde is being studied in more detail at this time.

### Discussion

We have shown the anaerobic formation of lactoyl thioester and the aerobic synthesis of glyceroyl thioester from glyceraldehyde. Synthesis of these "energy-rich" thioesters occurs at ambient temperature in an aqueous solution of sodium phosphate (pH 7.0). The scheme below shows reactions that we believe are involved in the formation of lactoyl and glyceroyl thioesters. The first step in the production of lactoyl thioester is shown to be the phosphate-catalyzed dehydration of glyceraldehyde and/or dihydroxyacetone to give



pyruvaldehyde (Riddle and Lorenz, 1968; Fedoronko and Konigstein, 1969). In the second step, pyruvaldehyde rapidly forms a hemithioacetal with the thiol, N-acetylcysteine. Finally, pyruvaldehyde hemithioacetal undergoes a phosphate-catalyzed rearrangement to give lactoyl thioester (Baal et al., 1978; Weber, 1982a), which ultimately hydrolyzes to lactate. Lactoyl thioester formation from glyceraldehyde is considered an irreversible process with a free energy change of about -17 kcal/mole, which is estimated from the free energy involved in the hydrolysis of a thioester (Jencks, 1976) and the formation of lactate from glyceraldehyde (Decker et al., 1970). The synthesis of lactoyl thioester can probably occur with concentrations of glyceraldehyde and thiol that are lower than we have examined, since the formation of pyruvaldehyde hemithioacetal occurs rapidly (Davis and Williams, 1969; Vince and Wadd, 1969) and has a favorable equilibrium  $K_{\text{diss}}$  equals  $[\text{pyruvaldehyde}][\text{glutathione}]/[\text{hemithioacetal}] = 3 \times 10^{-3} \text{ M}$ , Vander Jagt et al., 1972; Kanchuger and Byers, 1979).

Our studies show that the concentration of lactoyl thioester rises in the beginning of the reaction, but it soon reaches a constant (steady-state) value at which its rate of formation equals its rate of hydrolysis to lactate. In the steady-state, the rate of formation of thioester, which equals lactate formation,

proceeds at a relatively slow rate of roughly 0.4%/d from 10mM glyceraldehyde and 10mM thiol in the presence of 500mM sodium phosphate. However, thioester synthesis can be maintained for a long time at an efficiency that exceeds 76%, since a similar reaction with 12.5 mM thiol yields 50.7% lactate at 6 months from only 66.5% of the glyceraldehyde(dihydroxyacetone). Lactoyl thioester concentration in the steady-state is less than anticipated because glyceraldehyde significantly increases the rate of hydrolysis of the thioester.

The dehydration of glyceraldehyde to give pyruvaldehyde is considered the slowest step in the pathway, since the production of lactoyl thioester from glyceraldehyde is much slower than its formation from pyruvaldehyde (Weber, 1982a). This dehydration or beta elimination, like that of sugars under alkaline conditions (Speck, 1958; Pigman and Anet, 1972), probably involves a 1,2-enediol ionized at C-1, as an intermediate derived from either glyceraldehyde or dihydroxyacetone. The dehydration of glyceraldehyde is also slower than the isomerization of glyceraldehyde to dihydroxyacetone that reaches equilibrium at a 5 to 1 ratio of dihydroxyacetone to glyceraldehyde (Weber, 1983a). Fedoronko and Konigstein (1969) also report that the phosphate-catalyzed isomerization of glyceraldehyde is more rapid than its dehydration, and they give a value of 5 to 1 for the equilibrium ratio of dihydroxyacetone to glyceraldehyde. However, Speck (1958) reports that this ratio is 17, and the generally accepted equilibrium ratio of dihydroxyacetone phosphate to glyceraldehyde-3-phosphate is approximately 22 (Reynolds et al., 1971 and references therein).

The reaction scheme also depicts the synthesis of glyceroyl thioester from glyceraldehyde. Glyceroyl thioester formation occurs in the presence of oxygen and to a limited extent in the apparent absence of oxygen. The oxidant in the second case has not been identified. Although the mechanism of glyceroyl thioester formation is not known, the oxidation of glyceraldehyde in the absence of thiol to give formate and glycolate (Weber, 1983a) resembles the alkaline degradation of aldoses by oxygen that yields formate and an aldonic acid with one less carbon (Warshowsky and Sandstrom, 1952; Green, 1980). This alkaline degradation is generally thought to begin by the addition of oxygen to the enolate anion of the sugar (De Wilt and Kuster, 1971; Isbell, 1976).

The formation of lactoyl thioester is considered a plausible prebiotic energy source for several reasons. First, it operates in the presence of water with simple substrates at concentrations of glyceraldehyde and thiol as low as 2mM (Weber, 1983b). Although the 500mM sodium phosphate concentration is high, the reaction proceeds at lower phosphate concentration at a reduced rate (Weber, 1982a). Also, the reaction is not restricted to phosphate as catalyst, since imidazole catalyzes thioester formation (Weber, 1983b). Second, lactoyl thioester formation from glyceraldehyde is efficient ( $> 78\%$ ) and appears to operate as long as glyceraldehyde is available. The prebiotic formation of glyceraldehyde is thought to have occurred by oligomerization of formaldehyde (Gabel and Ponnamperna, 1967; Reid and Orgel, 1967; Mizuno and Weiss, 1974), which was readily produced on the early Earth (Garrison et al., 1951; Miller, 1957; Getoff et al., 1960; Hubbard et al., 1971; Bar-Nun and Hartman, 1978). The formation of hexoses from glyceraldehyde that accompanies lactoyl thioester synthesis suggests that under the mild conditions of our reactions, formaldehyde might be incorporated into sugars by aldol condensation. Also, the interconversion of glyceraldehyde (dihydroxyacetone) and larger sugars by aldolization and dealdolization may allow the reversible storage of glyceraldehyde in the form of more stable, larger sugars (Konigstein and Fedoronko, 1975; Gutsche et al., 1967; Degani and Halmann, 1968; Mizuno and Weiss, 1974). Finally, the chemical similarity between lactoyl thioester synthesis and glycolysis suggests that this chemical process has the potential to develop into glycolysis in a fairly direct manner. Glycolysis, like our chemical pathway, operates under anaerobic conditions and produces chemical energy initially in the form of a thioester from energy provided by the rearrangement of glyceraldehyde to lactic acid. The formation of lactoyl thioester is simpler than glycolysis in that the acyl group of the thioester is generated by an intramolecular rearrangement and does not require the presence of a redox carrier like NADH. The importance of thioesters in anaerobic energy-metabolism is further emphasized by the observation that anaerobically grown bacteria most frequently synthesize the pyrophosphate bonds of ATP with energy derived from thioesters (Thauer, 1977).

The formation of glyceroyl thioester from glyceraldehyde and oxygen does not seem to be a likely prebiotic reaction, since the level of oxygen in the prebiological atmosphere was probably very low (Canuto et al., 1982; Levine and Augustsson, 1982). However, the possibility remains that oxygen and reduced carbon were produced from water and carbon dioxide in the aqueous prebiotic environment by a simple, non-enzymatic, photosensitized reaction. Also, the oxidative formation of thioesters may not be restricted to glyceraldehyde and oxygen. Other substrates, like formaldehyde or glycolaldehyde, with oxidants, such as disulfides or aldehydes, may give thioesters. Formyl thioester formation from formaldehyde and a thiol with a second molecule of formaldehyde as the oxidant is probably the simplest possibility.

The prebiotic synthesis of thioesters is thought to have provided a stable source of useful chemical energy for the synthesis of phosphoanhydrides, like pyrophosphate, tripolyphosphate and phosphorylimidazole (Weber, 1981b; Weber, 1982b). These phosphoanhydrides, in turn, acted as phosphorylating agents and condensing agents in biopolymer synthesis (Oro and Stephen-Sherwood, 1976; Hulshof and Ponnampereuma, 1976).

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Table 1. Chromatographic and electrophoretic mobilities (Rm)<sup>a</sup>

	System I (Rm)	System II (Rm)	System III (Rm)	System IV (Rm)	System V (Rm)
Lactic acid	1.00	1.00	1.00	-	1.00
Glyceric acid	0.71	0.74	0.30	-	1.04
Glycolic acid	0.74	0.89	0.66	-	1.21
Formic acid	0.87	-	-	-	1.63
Pyruvic acid	1.35	-	-	-	1.22
Lactic acid hydroxamate	-	0.81	-	-	-
Glyceric acid hydroxamate	-	0.54	-	-	-
Ac-Cys(Lac)	1.53	1.04	1.18	-	0.61
Ac-Cys(Glc)	-	0.86	-	-	0.61
Ac-Cys	1.38	1.03	1.33	-	0.89
N,N'-diacetylcystine	0.68	0.97	0.86	-	1.05
Glyceraldehyde	1.33	-	-	1.00	0.05
Dihydroxyacetone	2.47	-	-	0.98	0.14
Fructose	1.68	-	-	0.65	0.05
Sorbose	1.68	-	-	0.65	0.05
Dendroketose	2.01	-	-	0.76	0.07
Glucose	-	-	-	0.61	-
Glycolaldehyde	1.43	-	-	1.13	0.05
Pyruvaldehyde	3.05	-	-	1.28	0.04

<sup>a</sup> Chromatographic and electrophoretic mobilities in Systems I, II, III, and V are given relative to lactic acid and in System IV are given relative to glyceraldehyde.

Figure 1. Formation of products from 10 mM [ $^{14}\text{C}$ ]glyceraldehyde in the absence of oxygen in 500 mM sodium phosphate (pH 7.0) at ambient temperature with 10 mM N-acetylcysteine.

Figure 2. Formation of products from 10mM [ $^{14}\text{C}$ ]glyceraldehyde in the absence of oxygen in 500 mM sodium phosphate (pH 7.0) at ambient temperature without N-acetylcysteine.

Figure 3. Formation of products from 10 mM [ $^{14}\text{C}$ ]glyceraldehyde in the presence of oxygen in 500 mM sodium phosphate (pH 7.0) at ambient temperature with 10 mM N-acetylcysteine.

Figure 4. Formation of lactate, glycerate and their thioesters from 5 mM [ $^{14}\text{C}$ ] glyceraldehyde in the absence of oxygen in 500 mM sodium phosphate (pH 7.0) at ambient temperature with 10 mM N-acetylcysteine. Not shown are the yields of glycolate 0.10%(0h), 0.12%(2h), 0.07%(5h), 0.14%(1d), 0.53%(2d), 0.50%(3d), 0.51%(8d) and formate (0.06%(0h), 0.15%(2h), 0.15%(5h), 0.24%(1d), 0.20%(2d), 0.20%(3d), 0.44%(8d).

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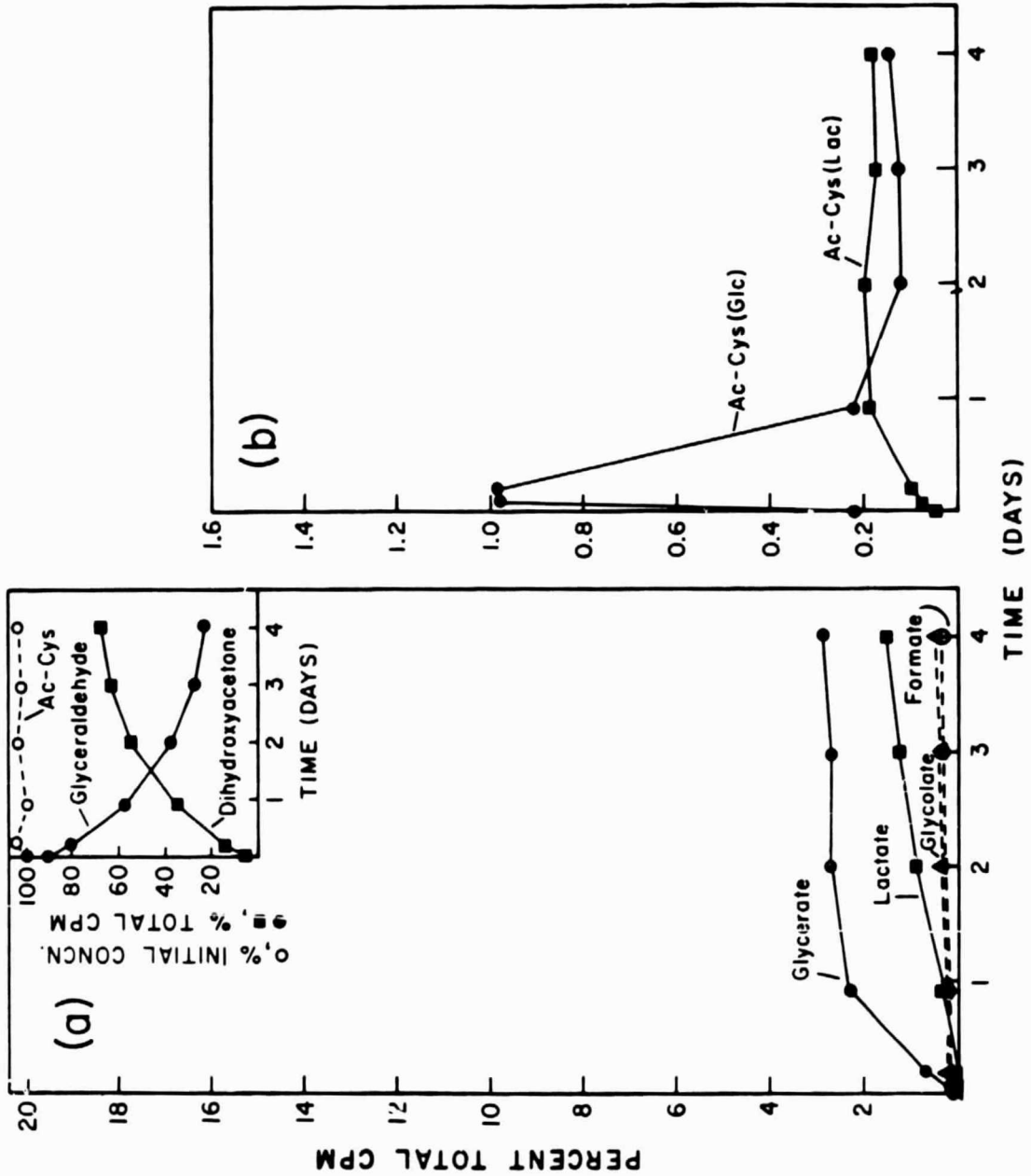


Figure 1

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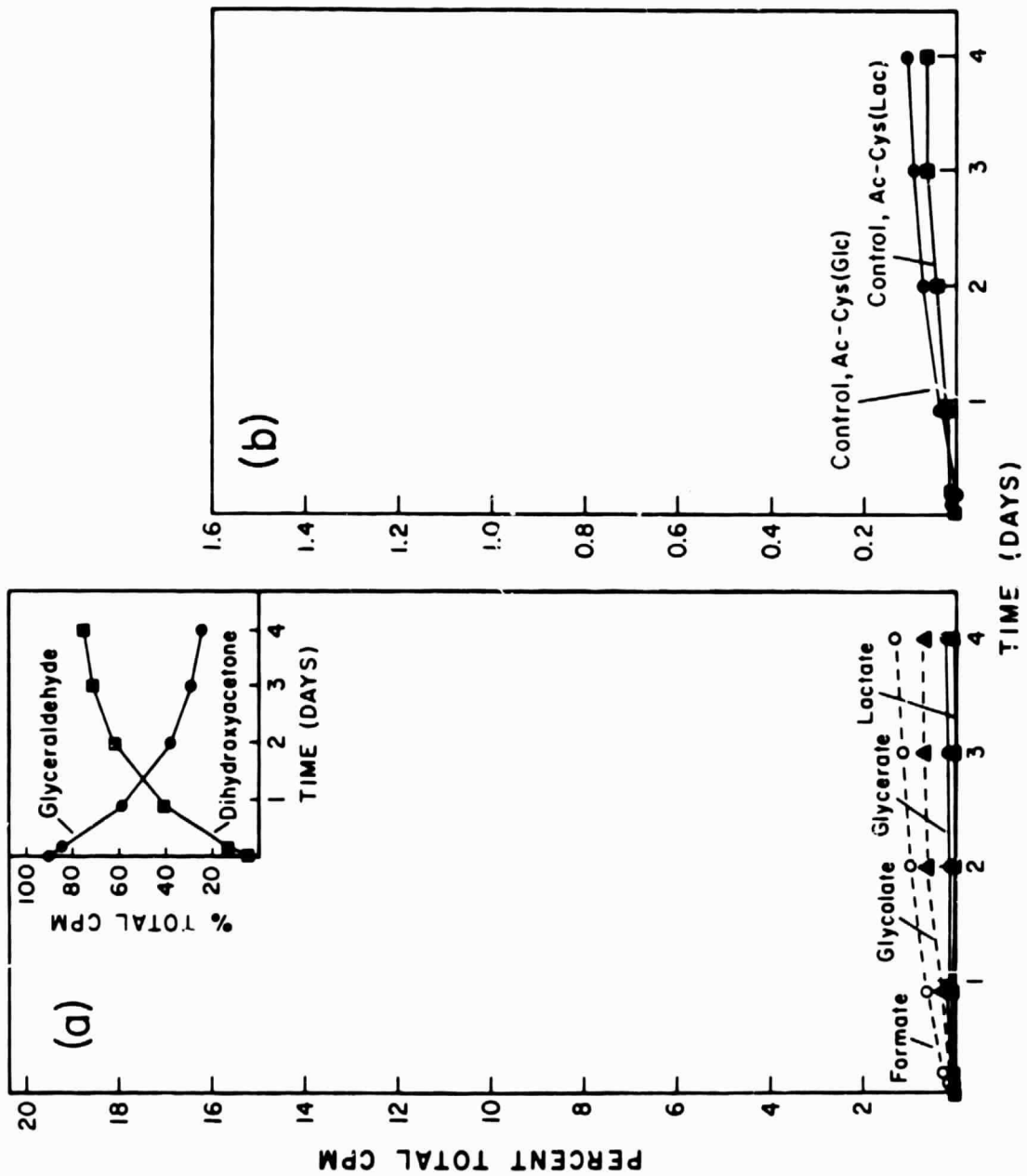


Figure 2

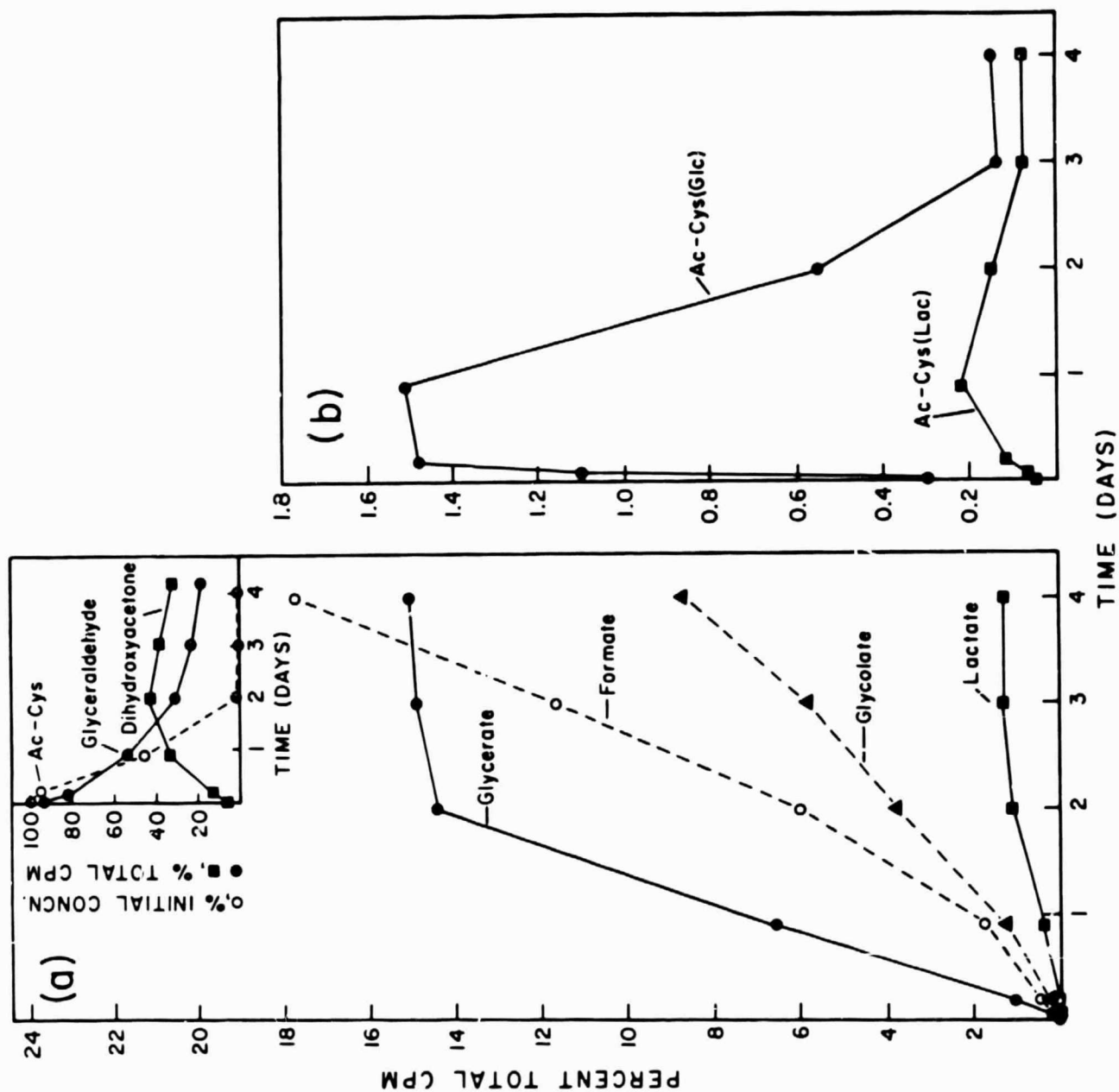
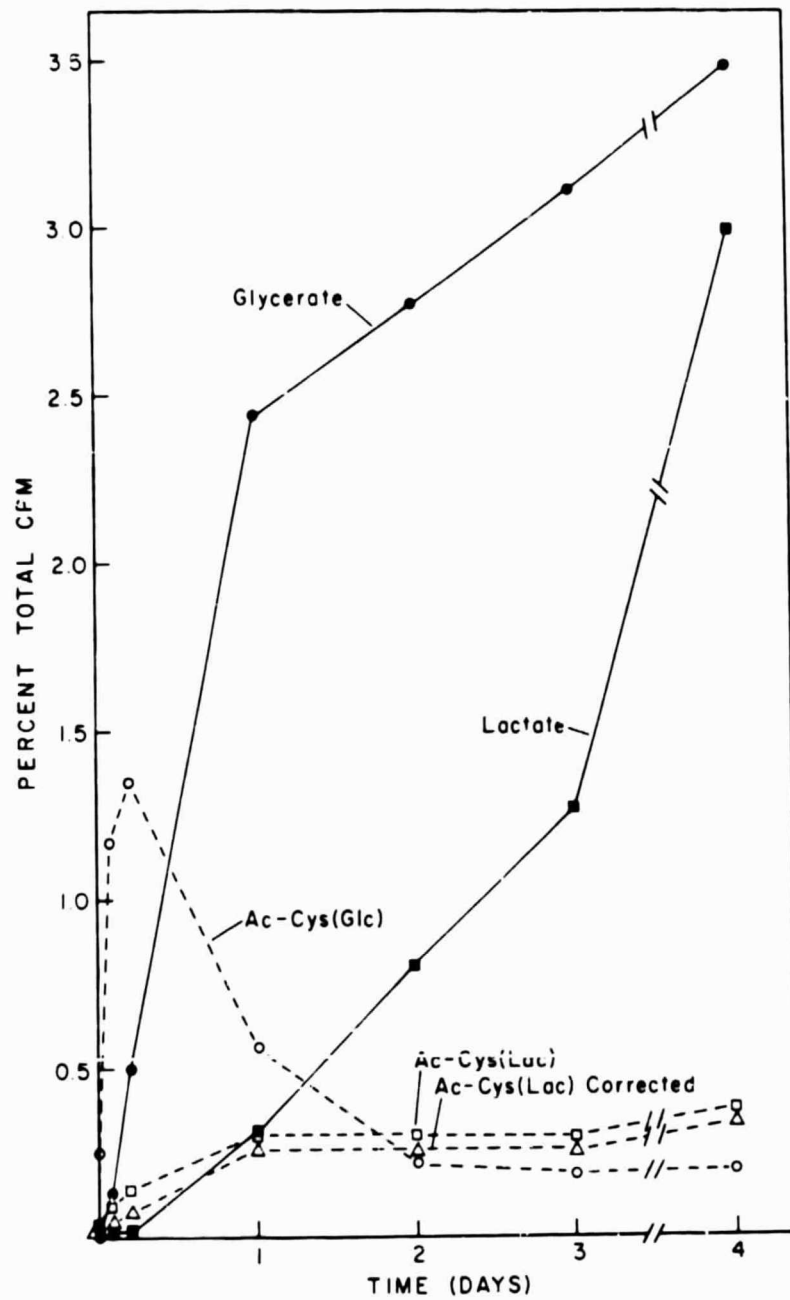


Figure 3

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Figure 4