

**I**

**KINETICS OF THE AMINO ACID AND PEPTIDE CATALYZED  
DEALDOLIZATION OF DIACETONE ALCOHOL**

**II**

**DETERMINATION OF THE HYDROXYMETHYL GROUP  
IN SUGARS AND RELATED SUBSTANCES**

**III**

**KINETICS OF THE ACID AND BASE CATALYZED  
DEGRADATION OF THE TRIOSSES**

**By**

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## TABLE OF CONTENTS

|  | PAGE |
|--|------|
| <b>I. KINETICS OF THE AMINO ACID AND PEPTIDE CATALYZED DEALDOLIZATION OF DIACETONE ALCOHOL</b> |      |
| INTRODUCTION.....  | 1    |
| HISTORICAL BACKGROUND.....   | 2    |
| EXPERIMENTAL METHODS.....  | 5    |
| Materials.....   | 5    |
| Procedure.....   | 5    |
| RESULTS AND DISCUSSION.....  | 6    |
| SUMMARY.....   | 12   |
| LITERATURE CITED.....  | 13   |
| <br><b>II. DETERMINATION OF THE HYDROXYMETHYL GROUP IN SUGARS AND RELATED SUBSTANCES</b>       |      |
| INTRODUCTION.....  | 15   |
| HISTORICAL BACKGROUND.....   | 16   |
| EXPERIMENTAL METHODS.....  | 20   |
| Materials.....   | 20   |
| Apparatus.....   | 20   |
| Recommended Procedure.....   | 20   |
| Study of Experimental Conditions.....  | 22   |
| Identity of the dye.....   | 22   |
| Effect of oxidation in acid solution.....  | 22   |
| Effect of sulfuric acid concentration used in dye development.....                             | 23   |
| Effect of aeration.....  | 24   |
| Stability of formaldehyde-sulfite solution.....  | 26   |
| Stability of the dye.....  | 26   |
| RESULTS AND DISCUSSION.....  | 28   |
| SUMMARY.....   | 33   |
| LITERATURE CITED.....  | 34   |

**I**

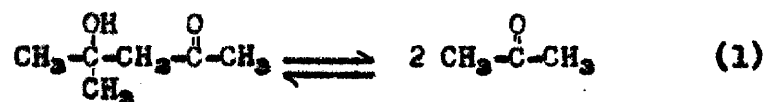
**KINETICS OF THE AMINO ACID AND PEPTIDE  
CATALYZED DEALDOLIZATION OF  
DIACETONE ALCOHOL**

## INTRODUCTION

Although catalysis of the aldol condensation by amino acids and peptides has been recognized for some time, the literature offers no precise data concerning the relative efficiencies of these substances as catalysts for aldolization (or dealdolization) or the nature of the catalytic species involved. Since such information was desired to substantiate recent work on the mechanism of the Maillard reaction, as well as to provide a possible insight into the mode of action of the enzyme, aldolase, the catalysis of the dealdolization of diacetone alcohol by buffered solutions of glycine, DL- $\alpha$ -alanine,  $\beta$ -alanine, and glycylglycine and their respective sodium salts has been examined kinetically. The effect of metallic ions on the glycine and glycylglycine catalyzed reactions has also been determined.

## HISTORICAL BACKGROUND

The dealdolization of diacetone alcohol (Equation 1) in alkaline solutions of both strong and weak bases has been widely investigated, and Koelichen (1) and Akerlof (2,3) have demonstrated catalysis of this reaction by the hydroxyl ion. Likewise, Bell (4) observed only an

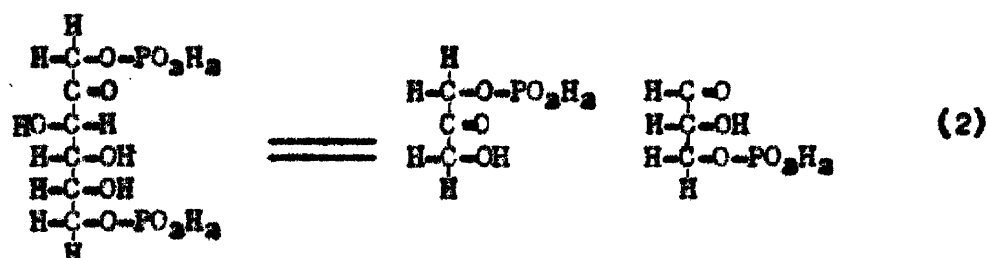


hydroxyl ion catalysis in kinetic studies of the aldol condensation. French (5) found that the phenol-sodium phenoxide system was active catalytically only to the extent of its hydroxyl ion concentration, thus ruling out the consideration of a generalized acid-base catalysis in the reaction. Akerlof (6) misinterpreted the effect of the weaker bases, and it remained for Miller and Kilpatrick (7) and Westheimer and Cohen (8) to demonstrate that dealdolization is specifically catalyzed by ammonia and the primary and secondary amines but not by tertiary amines. Thus, the catalyses involved are highly specific in nature.

Fischer and Marschall (9) produced aldol condensations in buffered solutions of amino acids (alanine), peptides (leucylleucine), and even proteins (egg white) but found acylated amino acids (hippuric acid) inactive. Likewise, Budnitskaya (10) observed an acceleration of the aldol condensation of acetaldehyde by glycine, alanine, and aspartic acid in that order while amines, amides, diketopiperazines, peptone, and egg

albumin were effective to a lesser extent. Finally, Langenbeck and Borth (11) investigated the effects of secondary amino acids and found sarcosine, N-ethyl glycine, N-methyl alanine, and phenyl-sarcosine catalytically active while N-ethyl alanine, N-benzyl alanine, and  $\alpha$ -methyl amino isobutyric acid were not catalysts.

The enzyme, aldolase, catalyzes the breakdown of hexose diphosphate to triose phosphates according to Equation 2:



In in vitro studies of this enzyme, Meyerhof, Lohmann, and Schuster (12) found catalysis of the condensation of dihydroxyacetone phosphate with aldehydes other than glyceraldehyde. The enzyme is therefore specific for dihydroxyacetone phosphate but not for the aldehyde involved.

The materials tested for aldolase activity have been nearly as numerous as the investigators. Typical sources have been muscle and yeast extracts (13), tumor extracts (14), nervous tissue (15), bacteria (16,17), blood serum (18), molds (19), higher plants (20,21), and bovine milk (22).

That the enzymes isolated from various sources are identical is a matter of conjecture. Only the variety from skeletal muscle has been crystallized (23,24), the others being prepared as extracts. The role



of metallic ions as activators or inhibitors of the aldolase system is rather confused. The enzyme isolated from muscle by Herbert, et al. (25) was not a metallo-protein and was inhibited by iodine and heavy metals but not by oxidized or reduced glutathione. Warburg and Christian (24) confirmed the reported absence of heavy metal ions from muscle aldolase as well as the lack of inhibition by substances such as pyrophosphate,  $\alpha, \alpha'$ -dipyridyl, cysteine, or glutathione, capable of forming complexes with the heavy metals. However, in studies on yeast extracts, the same investigators observed that cysteine produced an inhibition which was reversed by the addition of  $Zn^{++}$ ,  $Fe^{++}$ ,  $Co^{++}$ , or  $Cu^{++}$  suggesting a possible metallic ion activator for this particular species. Stumpf's pea aldolase (20) displayed no inhibition by  $Cu^{++}$ ,  $Hg^{++}$ , or  $Ag^+$  or by cysteine or  $\alpha, \alpha'$ -dipyridyl. Aldolase prepared from Clostridium perfringens (17) by Bard and Gunsalus was inhibited by  $\alpha, \alpha'$ -dipyridyl and 1, 10-phenanthroline, an effect reversed by  $Fe^{++}$  and  $Co^{++}$ . It would seem, therefore, that a species difference exists between the various enzymes, and in this work the role of metallic ions in simplified systems (glycine and glycylglycine) has been observed.

## EXPERIMENTAL METHODS

### Materials

Eastman Kodak Company diacetone alcohol was freshly distilled prior to each determination and boiled at 60-62° C/14 mm. Eastman Kodak Company glycine was twice recrystallized from a methanol-water mixture. Pfanstiehl C.P. DL- $\alpha$ -alanine and Eastman Kodak Company  $\beta$ -alanine were recrystallized from ethanol-water mixtures. Pfanstiehl C.P. glycylglycine was not further purified. Sodium chloride, cupric chloride, and magnesium sulfate were all C.P. reagents.

### Procedure

Unless otherwise indicated, the ionic strength was maintained at 1.0 M with sodium chloride. In some determinations, weighed samples of the amino acids were added to the reaction mixture followed by the amount of standard sodium hydroxide necessary to give the desired buffer ratio; in others, aliquots of standard buffer solutions of the amino acid and its sodium salt were employed.

Reaction velocities were measured dilatometrically at a temperature of 18.60 $\pm$ 0.001° C. In each case the initial diacetone alcohol concentration was 0.08 molar. The data followed a first order plot (Figure 1), and the pseudo constants were evaluated by the method of Guggenheim (26) using decadic logarithms throughout.

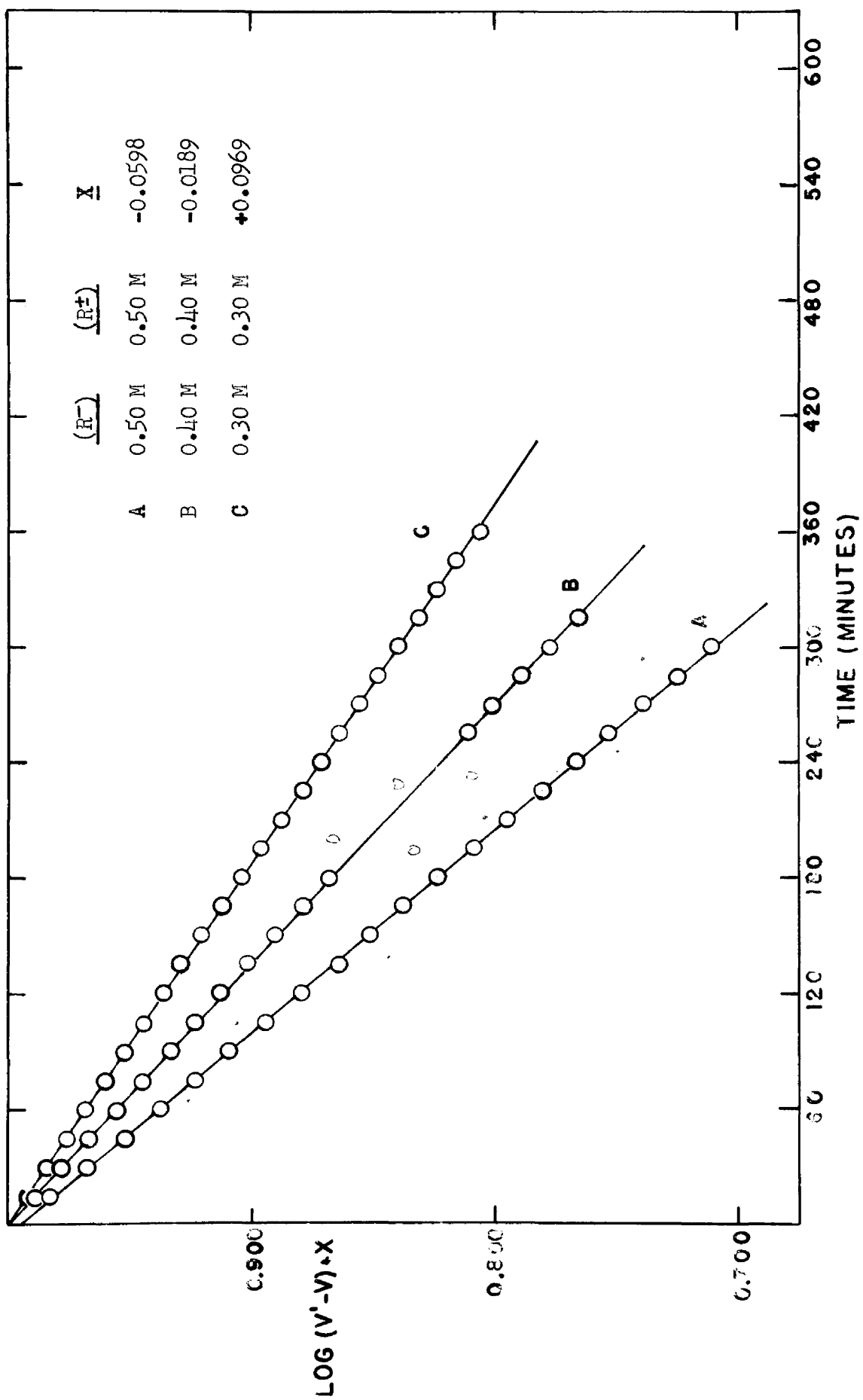


Figure 1. Typical rate curves for glycine systems.

## RESULTS AND DISCUSSION

First order pseudo constants obtained at constant buffer ratios and varying buffer concentrations for each system, and, in the case of glycine, at two buffer ratios, are shown in Table I.

In the amine systems studied by Westheimer and Cohen (8), the kinetics expression was found to be:

$$-\frac{dA}{dt} = k^i [A] = (k_{OH^-}[OH^-] + k_B[B]) [A] \text{ or}$$

$$k^i = k_{OH^-}[OH^-] + k_B [B] \text{ where}$$

[A] = concentration of diacetone alcohol at time  $t$

[OH<sup>-</sup>] = concentration of hydroxyl ion due to buffer

[B] = concentration of molecular primary or secondary amine

$k^i$  = experimentally observed first order pseudo constant

$k_{OH^-}$  and  $k_B$  = respective catalytic constants.

In the amino acid buffers employed in these determinations, the species present are  $R-CH(NH_3^+)-COO^-$  ( $R^+$ ) and  $R-CH(NH_2)-COO^-$  ( $R^-$ ). The concentration of  $R-CH(NH_2)-COOH$  ( $R$ ) may be neglected since  $pK_2 = \log R^+/R$  as reported by Edsall and Blanchard (27) are 5.42, 5.41, 5.53, and 4.61 for glycine,  $\alpha$ -alanine,  $\beta$ -alanine, and glycylglycine respectively.

Accordingly, the amino acid anion ( $R^-$ ) would be the predicted catalyst and the kinetics expression would become:

$$k^i = k_{OH^-}[OH^-] + k_{R^-}[R^-]$$

TABLE I  
PSEUDO CONSTANTS

| System                | $[R^-]$<br>moles/liter | $[R^+]$<br>moles/liter | $k^+ \times 10^5 \text{ min}^{-1}$ |
|-----------------------|------------------------|------------------------|------------------------------------|
| Glycine               | 0.10                   | 0.10                   | 18.1 <sup>a</sup>                  |
|                       | 0.25                   | 0.25                   | 44.2 <sup>a</sup>                  |
|                       | 0.30                   | 0.30                   | 53.3                               |
|                       | 0.40                   | 0.40                   | 73.7                               |
|                       | 0.50                   | 0.50                   | 94.9                               |
|                       | 0.20                   | 0.067                  | 36.8                               |
|                       | 0.40                   | 0.133                  | 74.0                               |
|                       | 0.45                   | 0.150                  | 86.0                               |
| DL- $\alpha$ -alanine | 0.20                   | 0.20                   | 15.5                               |
|                       | 0.30                   | 0.30                   | 25.3                               |
|                       | 0.40                   | 0.40                   | 32.5                               |
| $\beta$ -alanine      | 0.10                   | 0.10                   | 29.2                               |
|                       | 0.20                   | 0.20                   | 58.5                               |
|                       | 0.30                   | 0.30                   | 80.0                               |
| Glycylglycine         | 0.20                   | 0.05                   | 16.5                               |

<sup>a</sup> $\mu = 0.5$ ; for all others  $\mu = 1.0$ .

This has been realized experimentally, for a plot of psuedo constant versus amino acid anion concentration at constant buffer ratio (constant  $[\text{OH}^-]$ ) is strictly linear (Figure 2). In addition, the curves for glycine at different buffer ratios are indistinguishable. This confirms the amino acid anion as the active species, and from the slopes of these curves, the catalytic constant,  $k_R^-$ , for each anion has been calculated (Table II).

TABLE II  
CATALYTIC CONSTANTS

| Catalyst              | $k_R^- \times 10^4 \text{ mol}^{-1} \text{ min}^{-1}$ |
|-----------------------|---|
| $\beta$ -alanine      | 26.4  |
| Glycine               | 18.5  |
| DL- $\alpha$ -alanine | 8.1   |
| Glycylglycine         | 8.3   |

Due to the proximity of the intercepts to the origin, no attempt has been made to evaluate  $\text{pK}'$ s for the systems involved.

To test the effect of metallic ions on the amino acid catalyzed reaction, determinations were made using  $\text{Cu}^{++}$  and  $\text{Mg}^{++}$  in the presence of glycine buffers and  $\text{Cu}^{++}$  in the presence of the glycylglycine system. The results are shown in Table III. Albert (28) found that the metal ion complexes with glycine in order of decreasing stability constants were  $\text{Cu}^{++} > \text{Ni}^{++} > \text{Zn}^{++} > \text{Co}^{++} > \text{Cd}^{++} > \text{Fe}^{++} > \text{Mn}^{++} > \text{Mg}^{++}$ . The two extremes have therefore been examined.  $\text{Mg}^{++}$ , incapable of strongly complexing glycine,

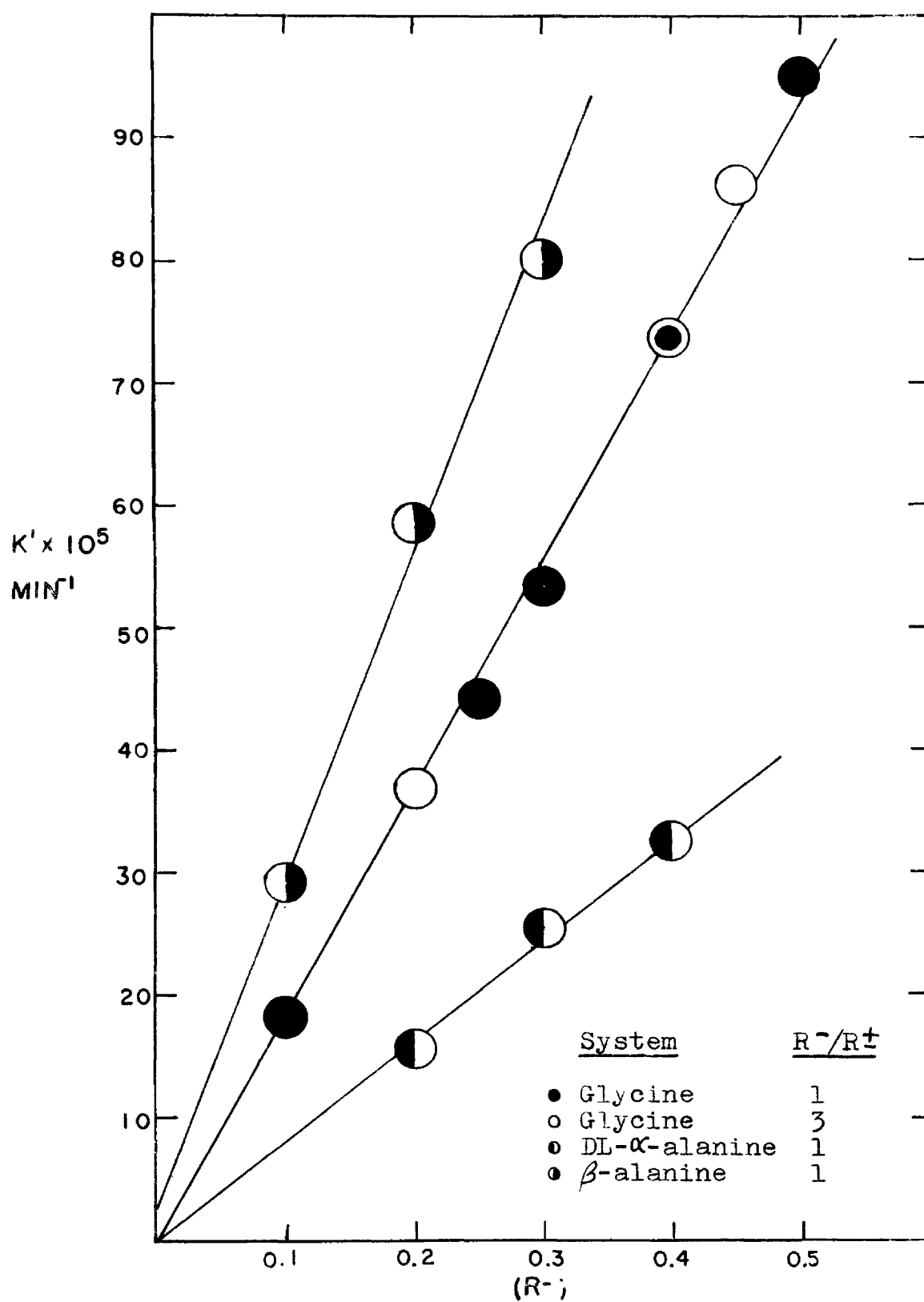


Figure 2. Relation of pseudo constant ( $k'$ ) to amino acid anion concentration ( $R^-$ ).

had little effect on the reaction while  $\text{Cu}^{++}$  produced inhibition in both systems. Assuming that  $\text{Cu}(\text{glycinate})^-$ , is formed (29) and is catalytically inactive, the calculated pseudo constant is  $48.0 \times 10^{-5} \text{ min}^{-1}$  which is in excellent agreement with the observed value of  $48.3 \times 10^{-5} \text{ min}^{-1}$ . The effect of  $\text{Cu}^{++}$  on glycylglycine is qualitatively the same, but the magnitude is somewhat greater than expected.

TABLE III  
METALLIC ION EFFECTS

| System        | $[\text{R}^-]^*$ | $[\text{R}^+]^*$ | Ion              | $[\text{M}^{++}]^*$ | $k^* \times 10^5 \text{ min}^{-1}$ |
|---------------|------------------|------------------|------------------|---------------------|------------------------------------|
| Glycine       | 0.30             | 0.30             | ----             | ----                | 53.3                               |
|               | 0.30             | 0.30             | $\text{Mg}^{++}$ | 0.01                | 51.5                               |
|               | 0.30             | 0.30             | $\text{Cu}^{++}$ | 0.01                | 48.3                               |
| Glycylglycine | 0.20             | 0.05             | ----             | ----                | 16.5                               |
|               | 0.20             | 0.05             | $\text{Cu}^{++}$ | 0.01                | 9.8                                |

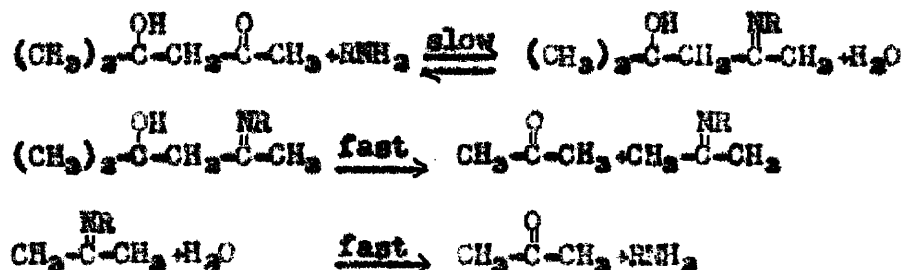
\* All concentrations are in moles/liter.

These observations of the role of metal ions in the simplified "aldolase-like" systems employed are similar to those made by Herbert, et al. (25) with  $\text{Ag}^+$ ,  $\text{Zn}^{++}$ , and  $\text{Cu}^{++}$  and the aldolase enzyme from skeletal muscle. The fact that muscle aldolase exhibits maximum activity at pH 7.2 (30), above its isoelectric point of 6.05 (23), coupled with the knowledge that the basic amino acids comprise approximately 20% of the total amino acid content of the protein (31), strongly suggests that



the action of the enzyme may be one of specific amine catalysis. The metals mentioned by Herbert, *et al.* (25) are all readily complexed by amines and their inhibitory action may be due to removal of the active center as a result of complex formation such as was realized experimentally in the case of glycine and glycyglycine with  $\text{Cu}^{++}$ .

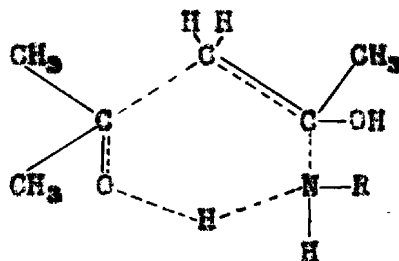
The mechanism of the amine catalyzed dealdolization has never been elucidated. Westheimer and Jones (32) showed that the rate of the methylamine catalyzed dealdolization of diacetone alcohol in aqueous methanol and ethanol was independent of the dielectric of the medium thus ruling out the decomposition of a dipolar ion as the rate determining step. The formation of a Schiff's base type intermediate between the amine and diacetone alcohol has been suggested. On the basis of the data of Westheimer and Jones (32) and the observation that the velocity constants for the n-butylamine catalyzed reaction are the same in water and in 50% water-dioxane mixtures, Koob, Miller and Day (33) have concluded that the formation of such a Schiff's base is the rate determining step and have written the following equations:



Such a mechanism seems unlikely since it fails to account for the catalysis by secondary amines where a dehydration of the intermediate is impossible.

The discovery by these investigators that the dealdolization of diacetone alcohol in anhydrous ethylene glycol as catalysed by a large number of amines proceeds at five to fifteen times the corresponding rate in aqueous solution was explained by attributing powers of "activation" to the glycol.

It has been shown that a generalized acid catalysis operates in the formation of Schiff's bases such as semi-carbazones (34), phenylhydrazones, and oximes (35). The fact that, in amine catalysed dealdolizations, the conjugate acid of the base involved is inactive catalytically, as shown by Miller and Kilpatrick (7), Westheimer and Cohen (8), and the present investigation, would support the belief that if the first step in the reaction is the formation of a Schiff's base, it is not the rate determining step. It is strongly suspected that the rate determining step may be the disproportionation of a cyclic intermediate such as:



## SUMMARY

1. The catalysis of the dealdolization of diacetone alcohol in buffered solutions of glycine, DL- $\alpha$ -alanine,  $\beta$ -alanine, and glycoylglycine and their respective sodium salts has been studied kinetically.
2. The amino acid anion has been shown to be the catalytically active species, the order of activity being  $\beta$ -alanine > glycine > DL- $\alpha$ -alanine-glycoylglycine.
3. Metallic ions have been shown to have no accelerating effect on the reaction; ions capable of forming complexes with amines inhibit the reaction.
4. A possible mode of action of the enzyme, aldolase, is suggested.
5. The mechanism of amine catalysed dealdolization is discussed.

## LITERATURE CITED

- (1) K. Koelichen, Z. physik. Chem., 31, 129 (1900).
- (2) G. Akerlof, J. Am. Chem. Soc., 48, 3046 (1926).
- (3) G. Akerlof, J. Am. Chem. Soc., 49, 2955 (1927).
- (4) R. P. Bell, J. Chem. Soc., 1937, 1637.
- (5) C. C. French, J. Am. Chem. Soc., 51, 3215 (1929).
- (6) G. Akerlof, J. Am. Chem. Soc., 50, 733 (1928).
- (7) J. G. Miller and M. Kilpatrick, Jr., J. Am. Chem. Soc., 53, 3217 (1931).
- (8) F. H. Westheimer and H. Cohen, J. Am. Chem. Soc., 60, 90 (1938).
- (9) F. O. Fischer and A. Marschall, Ber., 64B, 2825 (1931).
- (10) E. V. Budnitskaya, Biokhimiya, 6, 146 (1941).
- (11) W. Langenbeck and G. Borth, Ber., 75B, 951 (1942).
- (12) O. Meyerhof, K. Lohmann, and P. Schuster, Biochem. Z., 286, 301, 319 (1936).
- (13) O. Meyerhof and K. Lohmann, Biochem. Z., 271, 89 (1934).
- (14) E. Boyland and M. E. Boyland, Biochem. J., 29, 1910 (1935).
- (15) C. Malaguzzi-Valeri, Arch. sci. biol. (Italy), 22, 77 (1936).
- (16) M. F. Utter and C. H. Werkman, J. Bact., 42, 665 (1941).
- (17) R. C. Bard and I. C. Gunsalus, J. Bact., 52, 387 (1950).
- (18) O. Warburg and W. Christian, Biochem. Z., 314, 399 (1943).
- (19) F. Lynen and H. P. Hoffman-Walbeck, Ann., 559, 153 (1948).
- (20) P. K. Stumpf, J. Biol. Chem., 176, 233 (1948).
- (21) S. Tewfik and P. K. Stumpf, Am. J. Botany, 36, 567 (1949).

- (22) B. D. Polis and H. W. Shaukier, J. Dairy Sci., 33, 619 (1950).
- (23) J. F. Taylor, A. A. Green, and G. T. Cori, J. Biol. Chem., 173, 591 (1948).
- (24) O. Warburg and W. Christian, Biochem. Z., 314, 149 (1943).
- (25) D. Herbert, H. Gordon, V. Subrahmanyam, and D. E. Green, Biochem. J., 34, 1108 (1940).
- (26) E. A. Guggenheim, Phil. Mag., [7], 2, 538 (1926).
- (27) J. T. Edsall and M. H. Blanchard, J. Am. Chem. Soc., 55, 2337 (1933).
- (28) A. Albert, Biochem. J., 47, 531 (1950).
- (29) R. M. Keefer, J. Am. Chem. Soc., 68, 2329 (1946).
- (30) A. L. Dounce, S. R. Barnett, and G. T. Beyer, J. Biol. Chem., 185, 769 (1950).
- (31) S. F. Velick and E. Ronzoni, J. Biol. Chem., 173, 627 (1948).
- (32) F. H. Westheimer and W. A. Jones, J. Am. Chem. Soc., 63, 3283 (1941).
- (33) R. P. Koob, J. G. Miller, and A. R. Day, J. Am. Chem. Soc., 73, 5775 (1951).
- (34) J. B. Conant and P. D. Bartlett, J. Am. Chem. Soc., 54, 2881 (1932).
- (35) G. H. Stempel, Jr., and G. S. Schaffel, J. Am. Chem. Soc., 66, 1158 (1944).

## **II**

### **DETERMINATION OF THE HYDROXYMETHYL GROUP IN SUGARS AND RELATED SUBSTANCES**

## INTRODUCTION

In the course of an investigation of the degradation of glyceraldehyde, it became necessary to devise an analytical scheme for determining total sugars (glyceraldehyde plus dihydroxyacetone) in the presence of pyruvaldehyde. A method combining accuracy with speed and ease of manipulation was needed for use in kinetics studies involving large numbers of samples.

A procedure has been developed which consists of an oxidation of the sugars in a periodic acid-sodium bicarbonate buffer, destruction of the excess periodate and iodate by addition of sodium sulfite, and subsequent spectrophotometric determination of the formaldehyde present in this solution by means of the chromotropic acid reaction. The method has shown general applicability in the determination of sugars and related substances.

## HISTORICAL BACKGROUND

Malaprade (1,2) first established that the hydroxymethyl group of sugars and polyols was oxidized to formaldehyde by periodate. This reaction was later extended to the  $\alpha$ -amino alcohols by Nicolet and Shinn (3). The formaldehyde so formed has been found useful in the determination of many substances, and, as a result, many schemes have been devised for its measurement.

In early studies of the action of periodic acid on polyhydroxylated compounds, Fleury and Lange (4) determined formaldehyde by means of Nesbiter's reagent according to the method of Bougault and Gros (5), and also gravimetrically with Vorländer's dimedon reagent (6).

The precipitation of formaldehyde with dimedon, 5,5-dimethyl-1,3-cyclohexanedione, as modified by Ionescu and Bodea (7) and by Yoe and Reed (8) has been widely and effectively used. Karrer and Pfäffli (9) removed the formaldehyde formed during periodate oxidation by steam distillation at reduced pressure and precipitated the dimedon derivative from the distillate. As many investigators have observed, low results were obtained when the oxidations were carried out in acid solution. Reeves (10), in determining a series of sugars, precipitated the dimedon derivative directly from the reaction mixture after destruction of the excess periodate and iodate with sodium arsenite. This investigation showed that the theoretical amount of formaldehyde was produced and recovered if the periodate oxidation was carried out in solutions



buffered at pH 7.5 by sodium bicarbonate. This was confirmed by Jeanlos (11) who, however, was unable to obtain the theoretical amount of formaldehyde from dihydroxyacetone by this procedure. In a modification of Reeves' scheme, Bell (12) and Bell, Palmer, and Jones (13) conducted the periodate oxidations in phosphate buffers at pH 7.4-7.5. This was satisfactory for simple sugars but with highly methylated derivatives gave somewhat low and erratic results. Dimedon precipitation of formaldehyde has likewise been employed following periodate oxidation of serine in bicarbonate solution by Nicolet and Shinn (14) and in phosphate buffer by Rees (15), while Mead and Bartron (16) utilized a similar procedure in determining formaldehyde produced on oxidation of a series of hydroxyamino alkanes.

Polarographic determination of formaldehyde distilled from solution following periodate oxidation was used by Boyd and Pambach (17) in the determination of serine and by Warshawsky and Elving (18) in the analysis of mixtures of ethylene and 1,2-propylene glycols.

In determining mixtures of glycols, Desmuelle and Naudet (19) precipitated the excess periodate by the addition of potassium nitrate and measured the formaldehyde in the supernatant by the reaction with phenylhydrazine hydrochloride and potassium ferricyanide. Tannenbaum and Bricker (20) recently made a critical study of this method for determining formaldehyde and found that the operations must be very closely timed, a necessity which greatly curtails its usefulness.

The reaction of formaldehyde with chromotropic acid (1,8-dihydroxy naphthalene-3,6-disulfonic acid) in sulfuric acid solution, first

described by Egrive (21), has been developed into a very sensitive and excellent colorimetric method for formaldehyde by MacFadyen, Watkins, and Anderson (22), and by Bricker and Johnson (23). Boyd and Logan (24) measured serine by distilling formaldehyde from an oxidation mixture and condensing it with chromotropic acid. Bricker and Roberts (25) utilized a permanganate oxidation, followed by a periodate oxidation, and a formaldehyde determination on the distillate in measuring terminal unsaturation in organic compounds.

In determining mannitol in plasma and urine, Corcoran and Page (26) oxidized the sample with periodic acid for eight to ten minutes, destroyed excess oxidant with standard stannous chloride, and developed the chromotropic acid-formaldehyde dye directly on the reaction mixture. Under these conditions, glucose was converted to about five per cent of the theoretical formaldehyde.

Lambert and Neish (27) determined glycerol in fermentation solutions by a five-minute oxidation with periodic acid, reduction of excess periodate and iodate to iodide by a large excess of sodium arsenite, and a direct measure of formaldehyde in the oxidation mixture by the color reaction with chromotropic acid. Glucose gave about five per cent of the theoretical formaldehyde.

Of the many methods described, only the last two are attractive as being applicable to large numbers of samples. In both cases, an acid oxidation is used which was found slow and unsatisfactory in this investigation. The use of stannous chloride is undesirable since it is unstable. Its concentration must be known accurately because an excess

of stannous chloride produces a loss of formaldehyde due to reduction. In this investigation, use of arsenite to destroy excess periodate and iodate has invariably led to a copious evolution of iodine upon addition of sulfuric acid to the solution. Since iodine interferes with the dye formation (28), this is highly undesirable.

## EXPERIMENTAL METHODS

### Materials

The D-glucose used in these experiments was National Bureau of Standards Dextrose (Lot #4560). D-xylose, D-mannitol, and maltose were all Pfanstiehl C.P. reagents. Dihydroxyacetone was a Nutritional Biochemicals product while DL-glyceraldehyde was synthesized by the method of Fischer and Baer (29). Pfanstiehl C.P. DL-serine was recrystallized from 50% ethanol. Victor Chemical Works glycolic acid was recrystallized from methyl ethyl ketone. Matheson Company practical chromotropic acid was recrystallized from 50% ethanol. The periodic acid was a G. Frederick Smith Chemical Company product while the arsenious oxide was a Baker's C.P. analysed material. The sulfuric acid was Merck reagent grade and the sodium bicarbonate, sodium sulfite, potassium iodide, and iodine were all C.P. reagents.

### Apparatus

Beckman Model B and Model DU spectrophotometers equipped with Corex cells were used for optical density measurements.

### Recommended Procedure

To a mixture of 2.0 ml. of 0.3 M periodic acid and 2.0 ml. of 1.0 M sodium bicarbonate in a 100 ml. volumetric flask is added 1.0 ml. of solution containing from 0.01 to 0.10 millimole of the  $\text{-CHOH-CH}_2\text{OH}$  group

and capable of reducing no more than 1.0 milliequivalent of periodate. The oxidation is allowed to proceed for a period of one hour. At the end of that time, 5.0 ml. of 0.5 M sodium sulfite solution is added to the mixture and the resulting formaldehyde-sulfite solution diluted to 100 ml. A blank is prepared containing the same amounts of sodium bicarbonate, periodic acid, and sodium sulfite.

One milliliter aliquots of the formaldehyde-sulfite solution and of the blank are then pipetted into separate 50 ml. volumetric flasks equipped with glass stoppers. To each is added 0.5 ml. of 10% chromotropic acid and 5 ml. of 1M sulfuric acid (7 parts sulfuric acid to 2 parts water by volume). The flasks are immersed in a boiling water bath, the stoppers inserted, and the heating continued for 30 minutes. At the end of this time the flasks are cooled and approximately 40 ml. of distilled water added to each flask. The flasks are again cooled and the solutions diluted to 50 ml. with distilled water. Excess sulfur dioxide is then removed by bubbling air saturated with water vapor through the solutions at a rate of 750-1000 ml. of air per minute. The optical density of the unknown is measured against the blank at 570  $m\mu$  using the Beckman Model B spectrophotometer.

A calibration curve is prepared by determining the optical densities obtained by the above procedure with solutions of known glucose content. Such a curve is strictly linear over the recommended concentration range (Figure 1).

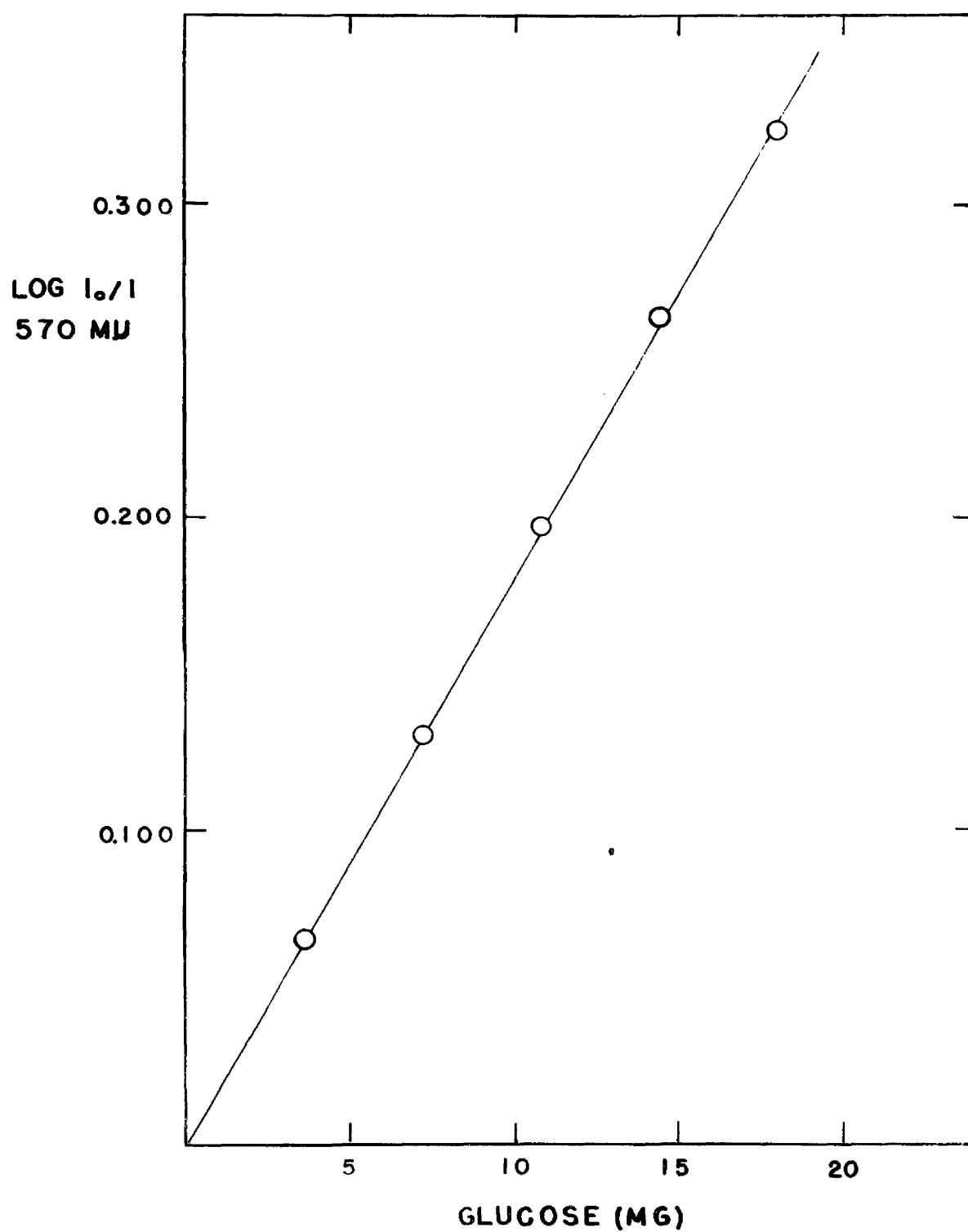


Figure 1. Calibration curve.

### Study of Experimental Conditions

Identity of the dye. The spectrum of the dye produced from 0.1 millimole of dihydroxyacetone by the recommended procedure was measured using the Beckman Model DU spectrophotometer and compared with that found by Speck (30) for the formaldehyde-chromotropic acid dye (Figure 2). The two spectra were identical.

Effect of oxidation in acid solution. To test the effect of an acid oxidation on formaldehyde production, samples of D-glucose, D-xylose, and DL-glyceraldehyde were oxidized by 2.0 ml. of 0.3 M periodic acid in the absence of sodium bicarbonate, and formaldehyde was determined by the recommended procedure. In addition, periodate consumption was measured by a modification of the method of Fleury and Lange (31) as follows: One milliliter aliquots of the sugar solutions were mixed with 2.00 ml. of 0.3 M periodic acid, the oxidation allowed to proceed for the indicated times, the solutions neutralized with solid sodium bicarbonate, 2.0 ml. of 5% potassium iodide followed by 5.00 ml. of 0.13142 N sodium arsenite solution added, and the excess arsenite titrated with 0.01 N iodine to a starch endpoint. The results are shown in Table I.

Since about four hours is required for complete oxidation to the theoretical amount of formaldehyde in acid solution while this is accomplished in one hour or less in buffered solution at pH 7.5, the periodic acid-sodium bicarbonate buffer described in the recommended procedure has been employed throughout this investigation.

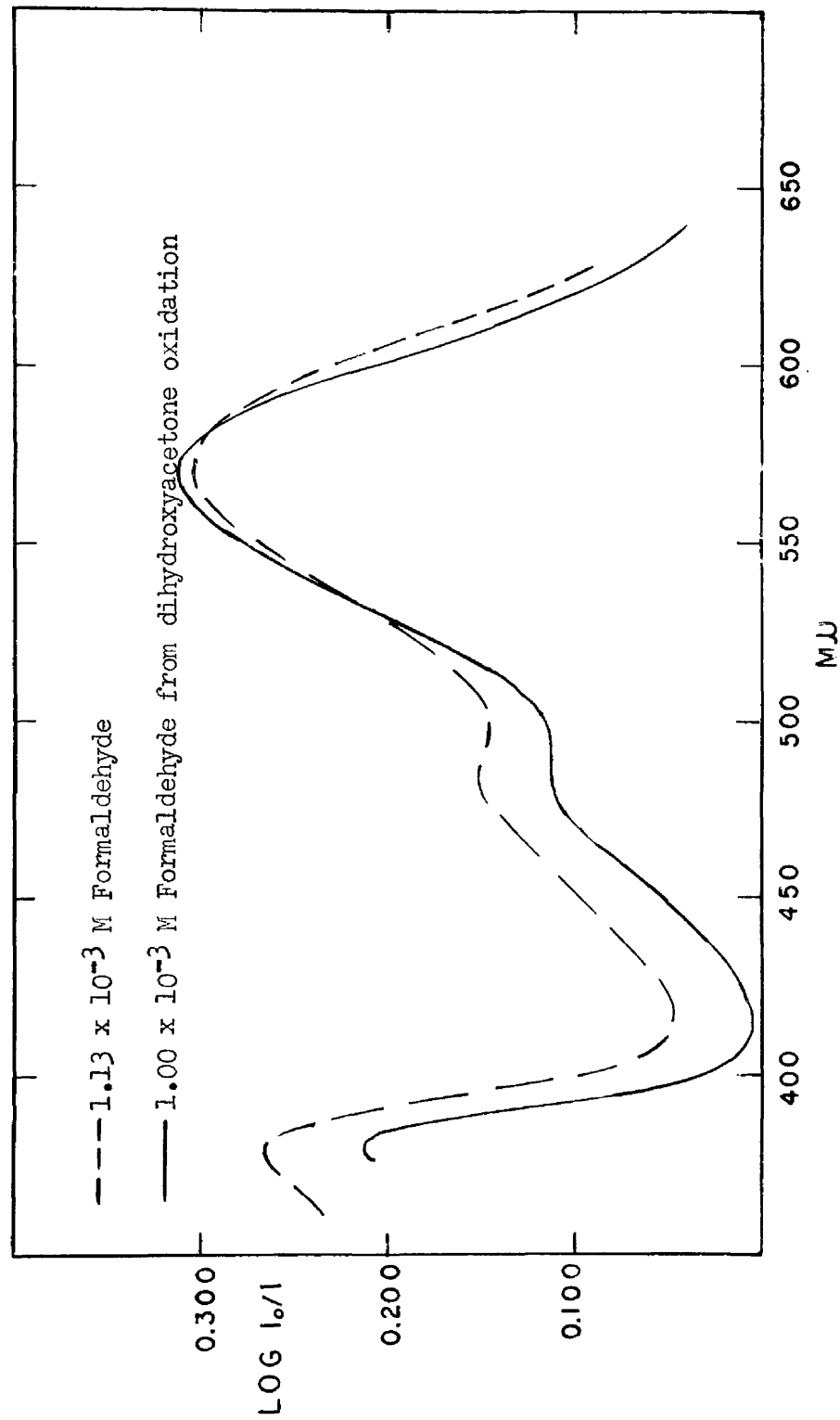


Figure 2. Comparison of spectra.



TABLE I  
EFFECT OF ACID OXIDATION ON FORMALDEHYDE  
PRODUCTION AND PERIODATE CONSUMPTION

| Oxidation<br>Time<br>(hours) | <u>D-glucose</u> |                    | <u>D-xylose</u> |                    | <u>DL-glyceraldehyde</u> |                    |
|------------------------------|------------------|--------------------|-----------------|--------------------|--------------------------|--------------------|
|                              | HCHO*            | IO <sup>-</sup> ** | HCHO*           | IO <sup>-</sup> ** | HCHO*                    | IO <sup>-</sup> ** |
| 1                            | 63               | 99.0               | 59              | 98.4               | 91                       | ----               |
|                              | 63               | 99.2               |                 | 98.4               |                          |                    |
|                              |                  |                    |                 | 98.1               |                          |                    |
| 2                            | 88               | 99.2               | --              | ----               | --                       | ----               |
|                              | 88               | 99.3               |                 |                    |                          |                    |
| 3                            | 97               | 99.4               | --              | ----               | 100                      | ----               |
|                              | 97               | 99.4               |                 |                    |                          |                    |
| 4                            | 100              | 99.4               | --              | ----               | 100                      | ----               |
|                              | 100              | 100.0              |                 |                    |                          |                    |

\* Per cent of theoretical formaldehyde formed.

\*\* Per cent of theoretical periodate consumed.

Affect of sulfuric acid concentration used in dye development. In preliminary work, the chromotropic acid-formaldehyde dye was developed in concentrated sulfuric acid (18 M) as prescribed by Bricker and Johnson (23). Since glycolic acid is an end product of the oxidation of dihydroxyacetone, it was tested as a possible interference. Table II shows the effect of heating 1.0 ml. of 0.001 M glycolic acid (the amount present after oxidation of 0.1 millimole of dihydroxyacetone by the recommended procedure) with 0.5 ml. of 10% chromotropic acid and 5 ml. of 18 M sulfuric acid for the indicated times. Subsequent to this observation, an analytical method for glycolic acid based on this color formation was described by Fleury, Courtois, and Perles (32).

TABLE II  
PRODUCTION OF DYE BY GLYCOLIC ACID

| Time of Heating<br>(minutes) | Log $I_0/I$ at 570 $m\mu$ |
|------------------------------|---------------------------|
| 30                           | 0.043                     |
| 60                           | 0.077                     |
| 150                          | 0.128                     |

Speck (30) had previously suggested that concentrated sulfuric acid oxidized diacetyl to produce formaldehyde, and it was thought that a similar oxidation occurred with glycolic acid. Therefore, the effect of heating 0.001 millimole of glycolic acid with 0.5 ml. of 10% chromotropic acid and 5 ml. of sulfuric acid of varying concentrations for thirty minutes was measured. This is shown in Table III.

Since the glycolic acid interference could be eliminated by using 1*M* sulfuric acid, samples of DL-glyceraldehyde were carried through the recommended procedure and the optical densities compared with those obtained using 18 *M* sulfuric acid, Table IV. It is obvious that the sensitivity is unimpaired.

Effect of aeration. It was observed early in this investigation that the presence of excess sulfur dioxide in solution resulted in considerable erratic bleaching of the characteristic dye. Utilization of the evaporation technique of Bricker and Vail (28) failed to give reproducible results. It was therefore decided to attempt aeration of the samples.

TABLE III  
EFFECT OF SULFURIC ACID CONCENTRATION  
ON GLYCOLIC ACID DYE FORMATION

| Sulfuric acid Concentration | Log $I_0/I$ at 570 $m\mu$ |
|-----------------------------|---------------------------|
| 18 M                        | 0.043                     |
| 16                          | 0.005                     |
| 14                          | 0.000                     |
| 12                          | 0.000                     |

TABLE IV  
EFFECT OF SULFURIC ACID CONCENTRATION ON INTENSITY OF  
CHROMOTROPIC ACID-FORMALDEHYDE DYE

| DL-glyceraldehyde<br>(mg. oxidized) | Log $I_0/I$ at 570 $m\mu$ * |                |
|-------------------------------------|-----------------------------|----------------|
|                                     | 18 M $H_2SO_4$              | 14 M $H_2SO_4$ |
| 9.0                                 | 0.300                       | 0.301          |
| 7.2                                 | 0.239                       | 0.238          |
| 5.4                                 | 0.179                       | 0.179          |
| 3.6                                 | 0.120                       | 0.121          |

\* Average of three samples.

Table V shows the effect of time of aeration on the dye produced from 0.1 millimole of D-glucose by the recommended procedure. A thirty minute aeration produces maximum optical density.

TABLE V  
EFFECT OF AERATION TIME ON INTENSITY OF DYE

| Aeration Time<br>(minutes) | Log $I_0/I$ at 570 m $\mu$ |
|----------------------------|----------------------------|
| 0                          | 0.175                      |
| 10                         | 0.270                      |
| 20                         | 0.289                      |
| 30                         | 0.301                      |
| 45                         | 0.305                      |
| 60                         | 0.302                      |
| 180                        | 0.301                      |

Stability of formaldehyde-sulfite solution. To test its stability, 1.0 ml. aliquots of the formaldehyde-sulfite solution from the oxidation of 0.1 millimole of D-glucose were removed at the times indicated in Table VI and carried through the recommended procedure. Such a solution is perfectly stable for at least forty-eight hours.

Stability of the dye. The dye produced from 0.1 millimole of D-glucose by the recommended procedure was stored in the dark and the optical density measured at the times indicated in Table VII. The dye, also, is stable for at least forty-eight hours.

TABLE VI  
STABILITY OF FORMALDEHYDE-SULFITE SOLUTION

| Age of Solution<br>(hours) | Log $I_0/I$ at 570 m $\mu$ |
|----------------------------|----------------------------|
| 0                          | 0.323; 0.324               |
| 24                         | 0.325; 0.325               |
| 48                         | 0.323; 0.324               |

TABLE VII  
STABILITY OF DYE

| Age of Dye<br>(hours) | Log $I_0/I$ at 570 m $\mu$ |
|-----------------------|----------------------------|
| 0                     | 0.323                      |
| 24                    | 0.325                      |
| 48                    | 0.323                      |

## RESULTS AND DISCUSSION

The procedure herein described has been successfully applied to the determination of D-glucose, D-xylose, DL-glyceraldehyde, dihydroxyacetone, D-mannitol, and maltose. The results are indicated in Table VIII. In the analysis of thirty-eight samples of D-glucose, the precision was  $\pm 1.1\%$ .

In the case of DL-serine, low results were always obtained (Table IX). Extended oxidations produced no higher formaldehyde values thus eliminating the possibility of incomplete reaction. Van Slyke, Hiller, and MacFadyen (33) in studying the periodate oxidation of serine, obtained low values for ammonia at neutral to slightly alkaline pH's and believed this due to a reaction between ammonia and formaldehyde. This may account for the low and reproducible values obtained in this investigation.

To test the recommended procedure on a disaccharide, maltose was selected. This sugar was oxidized within thirty minutes to produce one mole of formaldehyde per mole of disaccharide with no further oxidation after four hours (Table X). This indicates that no hydrolysis occurred during oxidation in a neutral buffer and suggests the possibility of applying this procedure to end group determinations on high molecular weight polysaccharides.

The method presented here is superior to those of Corcoran and Page (26) and Lambert and Neish (27) since by utilizing oxidation at pH 7.5, the more slowly oxidized sugars may be determined as well as rapidly oxidized materials such as glycerol and mannitol. Use of sodium sulfite

TABLE VIII  
RESULTS OBTAINED BY RECOMMENDED PROCEDURE

| Compound          | Taken<br>(mg.) | Found<br>(mg.)         |
|-------------------|----------------|------------------------|
| D-glucose         | 18.0           | 18.0; 17.9; 18.0; 18.0 |
|                   | 14.4           | 14.5; 14.4; 14.4; 14.4 |
|                   | 10.8           | 10.9; 10.9; 11.0; 10.9 |
|                   | 7.2            | 7.5; 7.4; 7.4; 7.4     |
|                   | 18.0           | 18.1; 18.1; 18.0       |
|                   | 14.4           | 14.5; 14.4; 14.9       |
|                   | 10.8           | 10.8; 10.8; 11.0       |
|                   | 7.2            | 7.0; 7.1; 7.3          |
|                   | 18.0           | 17.7; 17.8             |
|                   | 14.4           | 14.5; 14.5             |
|                   | 10.8           | 10.9; 10.8             |
|                   | 7.2            | 7.1; 7.1               |
|                   | 3.6            | 3.6; 3.5               |
| D-xylose          | 15.0           | 14.9; 15.0; 15.0       |
|                   | 12.0           | 11.9; 12.0; 12.0       |
|                   | 9.0            | 8.9; 9.0; 9.0          |
|                   | 6.0            | 5.9; 5.9; 6.1          |
| DL-glyceraldehyde | 9.0            | 9.0; 9.0; 9.0          |
|                   | 7.2            | 7.2; 7.2; 7.2          |
|                   | 5.4            | 5.4; 5.3; 5.4          |
|                   | 3.6            | 3.6; 3.6; 3.6          |
| Dihydroxyacetone  | 9.0            | 9.0; 9.0; 9.0          |
| D-mannitol        | 9.1            | 9.0; 9.0; 9.0          |
|                   | 4.6            | 4.5; 4.5; 4.6          |
| Maltose           | 36.0           | 35.0; 35.1             |
|                   | 32.5           | 33.3; 33.3; 33.2       |

TABLE IX  
DETERMINATION OF DL-SERINE

| Taken<br>(mg.) | Found<br>(mg.)       | Per cent of Theory |
|----------------|----------------------|--------------------|
| 10.5           | 9.5<br>9.5<br>9.4    | 91                 |
| 5.25           | 4.83<br>4.80<br>4.80 | 93                 |

TABLE X  
FORMALDEHYDE PRODUCTION FROM MALTOSE

| Time of Oxidation<br>(hours) | Moles HCHO/mole Maltose |      |
|------------------------------|-------------------------|------|
| 0.5                          | 1.02                    | 0.97 |
| 1.0                          | 1.02                    | 0.98 |
| 1.5                          | 1.03                    | -    |
| 2.0                          | 1.02                    | -    |
| 4.0                          | -                       | 0.97 |



to destroy excess periodate and iodate eliminates the necessity of a standard reagent such as stannous chloride and avoids interference by iodine produced when arsenite is used. The additional aeration step necessitated by the use of sodium sulfite is not a serious drawback since large numbers of samples may be aerated simultaneously. The stability of the formaldehyde-sulfite solution coupled with the stability of the dye allows extreme flexibility of operation. A complete analysis requires about 2 1/2 hours, and many samples may be run concurrently.

Obviously any material which interferes with the formaldehyde determination by chromotropic acid constitutes an interference in this method. In addition, any substances producing formaldehyde on periodate oxidation will interfere. The glycolic acid interference has been eliminated by the choice of sulfuric acid concentration used during the dye development. Diacetyl (30) and pyruvaldehyde (34) which are known to react with chromotropic acid cause no interference since they are cleaved by periodate.

The results obtained by an "acid oxidation" are shown in Table I. At least four hours is required for complete reaction as measured by formaldehyde production. However, periodate consumption as measured by the method of Fleury and Lange (31) is complete in one hour. Hughes and Nevell (35) observed similar results in a study of the oxidation of glucose by periodate. They found periodate consumption as measured by the method of Fleury and Lange to be very rapid while formic acid production was much slower. If, however, periodate consumption was measured by the addition of acidified potassium iodide and a titration of the liberated iodine with sodium thiosulfate, it then paralleled formic acid

production. These investigators considered that the oxidation proceeded by way of intermediates which were more stable in alkaline than in acid solutions. Our data would agree with this hypothesis. Apparently periodate reacts rapidly with the reducing sugars both in acid and in neutral media. The intermediates so formed must react only slowly with arsenite in neutral solution or, in other words, behave as iodate, thus accounting for the apparent complete consumption of periodate. In acid solution, however, this intermediate breaks down in the presence of iodide and allows measurement of unreduced periodate. Therefore, the statement of Hughes and Nevell regarding the relative stability of the intermediates in acid or alkali is true only in the presence of iodide. In the absence of iodide, the intermediates would appear to be less stable in alkaline solution since the products of the reaction are released more rapidly. It is possible that disproportionation of the periodate-reducing sugar complex involves participation of the hydroxyl ion.

## SUMMARY

1. A new method for determining the hydroxymethyl group in sugars and related substances is described which consists of a periodate oxidation in neutral solution buffered by bicarbonate, destruction of excess periodate and iodate with sodium sulfite, and subsequent determination of formaldehyde directly on the reaction mixture by the reaction with chromotropic acid.
2. The effects of (a) acidity of the oxidation medium, (b) sulfuric acid concentration during dye development, and (c) time of aeration have been investigated.
3. The stability of the formaldehyde-sulfite solution and of the dye has been determined.
4. The method has been successfully applied to the determination of D-glucose, D-xylose, DL-glyceraldehyde, dihydroxyacetone, D-mannitol, and maltose.
5. DL-serine has been found to give low yields of measurable formaldehyde.
6. Maltose has been shown to yield one mole of formaldehyde per mole of disaccharide suggesting use of this method in end group analysis of polysaccharides.
7. The method is compared with similar procedures.
8. The anomalous "acid oxidation" is discussed.

## LITERATURE CITED

- (1) L. Malaprade, *Compt. rend.*, 186, 382 (1928).
- (2) L. Malaprade, *Bull. soc. chim.*, 43, 683 (1928).
- (3) B. H. Nicolet and L. A. Shinn, *J. Am. Chem. Soc.*, 61, 1615 (1939).
- (4) P. Fleury and J. Lange, *J. Pharm. Chim.*, 17, 196 (1933).
- (5) J. Bougault and R. Gros, *J. Pharm. Chim.*, 26, 5 (1922).
- (6) D. Vorlander, C. Ihle, and H. Volkhols, *Z. anal. Chem.*, 77, 321 (1929).
- (7) M. V. Ionescu and C. Bodea, *Bull. soc. chim.*, 47, 1408 (1931).
- (8) J. H. Yoe and L. C. Reed, *Ind. Eng. Chem., Anal. Ed.*, 13, 238 (1941).
- (9) P. Karrer and K. Pfäelker, *Helv. Chim. Acta*, 17, 766 (1934).
- (10) R. E. Reeves, *J. Am. Chem. Soc.*, 63, 1476 (1941).
- (11) R. Jeanlos, *Helv. Chim. Acta*, 27, 1509 (1944).
- (12) D. J. Bell, *J. Chem. Soc.*, 1948, 992.
- (13) D. J. Bell, A. Palmer, and A. T. Jones, *J. Chem. Soc.*, 1949, 1536.
- (14) B. H. Nicolet and L. A. Shinn, *J. Biol. Chem.*, 139, 687 (1941).
- (15) M. W. Rees, *Biochem. J.*, 40, 632 (1946).
- (16) J. F. Mead and E. A. Bartron, *J. Am. Chem. Soc.*, 70, 1286 (1948).
- (17) M. J. Boyd and K. Bamach, *Ind. Eng. Chem., Anal. Ed.*, 15, 314 (1943).
- (18) B. Warshowsky and P. J. Elving, *Ind. Eng. Chem., Anal. Ed.*, 18, 253 (1946).
- (19) P. Desnuelle and M. Naudet, *Bull. soc. chim.*, 12, 871 (1945).
- (20) M. Tannenbaum and C. E. Bricker, *Anal. Chem.*, 23, 354 (1951).

- (21) E. Eegriwe, Z. anal. Chem., 110, 22 (1937).
- (22) D. A. MacFadyen, H. D. Watkins, and P. R. Anderson, J. Biol. Chem., 158, 107 (1945).
- (23) C. E. Bricker and H. B. Johnson, Ind. Eng. Chem., Anal. Ed., 17, 400 (1945).
- (24) J. M. Boyd and M. A. Logan, J. Biol. Chem., 146, 279 (1942).
- (25) C. E. Bricker and K. H. Roberts, Anal. Chem., 21, 1331 (1949).
- (26) A. C. Corcoran and J. H. Page, J. Biol. Chem., 170, 165 (1947).
- (27) M. Lambert and A. C. Neish, Can. J. Research, 28B, 83 (1950).
- (28) C. E. Bricker and W. A. Vail, Anal. Chem., 22, 720 (1950).
- (29) H. O. L. Fischer and E. Baer, Helv. Chim. Acta, 18, 514 (1935).
- (30) J. C. Speck, Jr., Anal. Chem., 20, 647 (1948).
- (31) P. Fleury and J. Lange, J. Pharm. Chim., 17, 107 (1933).
- (32) P. Fleury, J. Courtois, and R. Perles, Mikrochemie ver. Mikrochim. Acta, 36/37, 863 (1951).
- (33) D. D. Van Slyke, A. Hiller, and D. A. MacFadyen, J. Biol. Chem., 141, 681 (1941).
- (34) B. J. Thornton and J. C. Speck, Jr., Anal. Chem., 22, 899 (1950).
- (35) G. Hughes and T. P. Nevell, Trans. Faraday Soc., 44, 941 (1948).

### **III**

#### **KINETICS OF THE ACID AND BASE CATALYZED DEGRADATION OF THE TRIOSSES**

## INTRODUCTION

Although considerable effort has been devoted to an elucidation of the mechanism of the Lobry de Bruyn-van Ekenstein transformation in alkaline solution, little information is available concerning the occurrence of this reaction in dilute acid solution or the exact nature of the catalyses involved. Likewise, pyruvaldehyde production from the trioses (as well as from the higher sugars) in both acid and alkaline solutions has long been recognized, yet little is known of the catalysts for this reaction or its mechanism.

Knowledge of the catalyses operative in these processes was considered necessary to the study of the mechanism of reducing sugar degradation in amino acid systems (Maillard reaction). Therefore, the interconversion of DL-glyceraldehyde and dihydroxyacetone, as well as the simultaneous dehydration to pyruvaldehyde, has been examined kinetically in buffered solutions of formic, acetic, and trimethylacetic acids and their respective sodium salts. The effect of calcium ion on these reactions has also been observed.

## HISTORICAL BACKGROUND

### The Lobry de Bruyn-van Ekenstein Transformation

The reversible transformation of aldoses into ketoses occurring in dilute alkaline solution was first described by Lobry de Bruyn and van Ekenstein (1, 2) and bears the names of these investigators. Glucose, fructose, and mannose were each converted to the others in dilute solutions of sodium, potassium, or ammonium hydroxide, calcium or magnesium oxide, or sodium or potassium carbonate. In addition, non-fermentable substances (3-ketohexoses?) were formed. These studies also included the similar transformations of galactose, maltose, lactose and melibiose. Other researchers have applied this isomerization to glyceraldehyde and dihydroxyacetone (3), lactose and lactulose (4), glucoheptose and glucoheptulose (5), xylose and arabinose (6), and cellobiose (7). Calcium hydroxide has been the usual catalyst.

In most cases, the simple interconversion of sugars has been complicated by fragmentation reactions and acid formation. Gottfried and Benjamin (8) recently conducted an exhaustive study of the formation of ketoses, organic acids, unfermentable substances, and color during treatment of glucose with bases and arrived at a series of empirical expressions which permitted calculation of each product.

Utilizing improved techniques involving radioisotope dilution analyses for glucose and fructose and a corrected phenylhydrazone precipitation procedure for mannose, Sowden and Schaffer (9) have



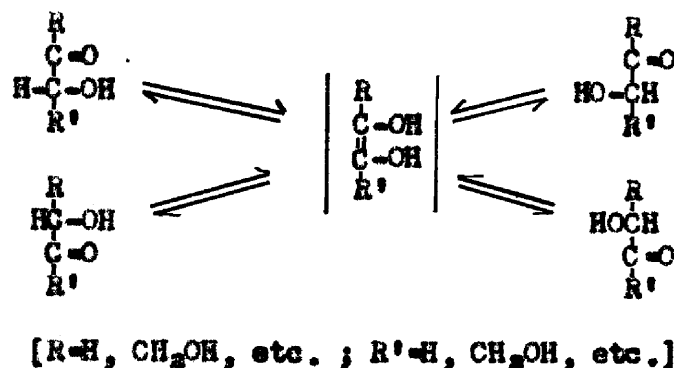
reinvestigated the reactions of these sugars in 0.035 N sodium hydroxide at 35° C. After extended reaction times, summation of analyses for these three sugars accounted for about eighty percent of the starting material; the remainder had been converted to a mixture of non-fermentable sugar products of unknown nature.

Garbutt and Hubbard (10) demonstrated interconversion of glucose, fructose, and mannose in boiling aqueous solution and in boiling solutions buffered at neutrality while Murschhauser (11) reported the isomerization of glucose on boiling with alkaline earth carbonates. Spoehr and co-workers observed similar conversions in the presence of neutral (12) and slightly acid (13) phosphate buffers.

That organic bases could produce the Lobry de Bruyn-van Ekenstein reaction was shown by Fischer, Taube, and Baer (14) who converted glyceraldehyde into dihydroxyacetone by boiling with anhydrous pyridine. Danilov, et al. (15), found that glucose was isomerized to fructose without mannose production by anhydrous pyridine. Mannose was formed, however, when aqueous pyridine and aqueous alcoholic quinoline were employed. Midorikawa and Takeshima (16) confirmed these observations and extended the reaction to include quinaldine (17). Anhydrous pyridine has been a valuable tool in the synthesis of many ketoses (18-25).

The mechanism of the Lobry de Bruyn-van Ekenstein transformation has been the subject of much experimental work. In 1900, Wohl and Neuberg (3) explained the conversion of glyceraldehyde to dihydroxyacetone in alkaline solution on the basis of an enediol formation, and this

mechanistic concept is still the accepted one. In general form, this may be pictured schematically as follows:



On the basis of the fragmentation products obtained on oxidation of various sugars in alkaline solution, Nef (26) postulated the formation of a series of enediols capable of being split by the oxidants. The spontaneous rearrangement of an "aldosate" anion suggested as the mechanism by Michaelis and Rona (27) and by Groot (28) also implied previous enediol formation. In an exhaustive series of investigations of carbohydrate oxidations, Evans and co-workers (29-43) were led to the conclusion that an equilibrium existed between the sugars and a series of enediols in alkaline solution.

Gustus and Lewis (44) examined the oxidation of tetramethyl glucose in alkaline solution and obtained results indicating the presence of a 1,2-enediol. Extension of these studies to the alkaline rearrangement of tetramethyl glucose (45), tetramethyl mannose (46), trimethyl xylose (47), and trimethyl arabinose (48) showed, in each case, formation of the corresponding epimeric methylated aldose with no ketose production. In addition, high iodine absorbing substances, believed to be the enediol

intermediates, were present in alkaline solution but rapidly disappeared on acidification. Under similar conditions, 3-methyl glucose (49) was converted to 3-methyl fructose with no detectable enediol concentration (also true for glucose).

Electroreduction of sugars in alkaline solution by Wolfrom and co-workers (50-54) gave isomeric polyols indicating the presence of 1,2- and 2,3-enediols.

On the other hand, early studies of the Lobry de Bruyn-van Ekenstein transformation in alkaline deuterium oxide solutions produced results contrary to the predictions of the enediol mechanism. Fredenhagen and Bonhoeffer (55) reported no incorporation of carbon-bound deuterium with glucose at 25° C while at higher temperatures non-reproducible data were obtained. On the basis of these results, a "dimer intermediate" mechanism was proposed for the low temperature reaction and a keto-enol mechanism at higher temperatures. Goto (56) reported comparable findings. In search of an explanation for this reported isomerization without exchange of carbon-bound hydrogen by deuterium, Bothner-By and Gibbs (57) employed 1-C<sup>14</sup>-D-glucose to test the possibility of carbon chain rearrangement during the reaction. No such rearrangement was observed.

Topper and Stetten (58), in a reinvestigation of the reactions of glucose in heavy water, observed deuterium exchange at both 25° and 35° C in agreement with the enediol mechanism. These results also indicated that the mannose produced when glucose was treated with saturated lime water was derived exclusively from fructose, and the following scheme was presented:

glucose  $\rightleftharpoons$  trans-enediol  $\rightleftharpoons$  fructose  $\rightleftharpoons$  cis-enediol  $\rightleftharpoons$  mannose

Sowden and Schaffer (59) have recently presented further evidence of deuterium exchange at 25° C but concluded that fructose was not a necessary intermediate in the conversion of glucose to mannose.

Although the enediol mechanism for the Lobry de Bruyn-van Ekenstein transformation has apparently been well established, the nature and mode of action of the catalysts involved have received considerably less attention. Most studies have been conducted in alkaline solutions and Michaelis and Rona (27) reported glucose decomposition proportional to the hydroxyl ion concentration. Various bases including the alkali and alkaline earth hydroxides and carbonates as well as phosphate systems have been employed, but the activity has been attributed to the hydroxyl ion present in these solutions.

Little information is available concerning sugar interconversions in acid solutions under the influence of bases other than the hydroxyl ion. A conversion of fructose into glucose in slightly acid phosphate systems was reported by Spoehr and Strain (13). Ashmarin and co-workers (60-62) examined the reactions of glucose and fructose in formate, acetate, and succinate buffers and found an apparent catalysis of the interconversion (and also of hydroxymethyl furfural formation) by the anions of the acids involved. A similar acetate ion catalyzed isomerization of arabinose was reported by Braun and Konnova (63). In each case, a bisulfite fractionation scheme (64) was employed to separate aldoses from ketoses, and the isomer produced was identified as the osazone of the starting material. Utilizing aqueous pyridine systems,

Midorikawa (65) observed that with increasing pyridine concentrations, the conversion of glucose to fructose increased to a maximum value and subsequently decreased. Due to the similarity of these results to those obtained by Lowry and Faulkner (66) in the mutarotation of glucose, an acid-base catalysis was proposed.

Several investigators have reported apparent variations in the nature of the Lobry de Bruyn-van Ekenstein transformation dependent on the particular cationic species involved. Lobry de Bruyn and van Ekenstein (67) found that lead hydroxide converted glucose to mannose with no detectable fructose, and that, under those conditions, fructose was not isomerized to the corresponding aldoses. Nef (26) reported that calcium and lead acetates and lead chloride caused no enolization of the hexoses. A comparative study of the action of calcium and sodium hydroxides on glucose at 25° C was conducted by Kuzin (68). With calcium hydroxide, a substance (believed to be the enol), capable of reducing iodine and dichlorophenolindophenol, was present which disappeared on acidification. Under similar conditions, sodium hydroxide produced no such result. Mannose formation was favored by the calcium hydroxide; fructose formation by sodium hydroxide. From these observations, Kuzin concluded that at low temperatures, calcium hydroxide produced a cyclic enol without rupture of the pyranose ring whereas a straight chain enol was formed in the presence of sodium hydroxide. At higher temperatures, no difference in the action of the two bases was detectable. Sowden and Schaffer (9) have recently confirmed that the initial course of isomerization is different in mono- and divalent bases

(calcium and barium hydroxides) of 0.5N concentration. At 0.035N concentrations, however, these investigators found no apparent difference. Wind (69) observed that oxidation of glyceraldehyde and dihydroxyacetone in phosphate solutions was catalyzed by heavy metals, especially copper. Ahlstrom and von Euler (70) likewise found that oxygen consumption by buffered solutions of the trioses was catalyzed by cupric, ferric, and ferrous sulfates. Assuming the enediol as the oxidizable species, these data may indicate increased ability for enediol formation in the presence of these metal ions.

#### Pyruvaldehyde Formation

Pyruvaldehyde has been indicated as a degradation product of carbohydrates when subjected to a wide variety of conditions.

Neuberg and Oertel (71) obtained pyruvaldehyde from glucose, fructose, and mannose by warming with dilute sodium carbonate as did Fernbach and Schoen (72). Fischler (73) and Fischler and Lindner (74) found pyruvaldehyde in the distillates from dilute alkaline solutions of glucose, fructose, galactose, maltose and lactose. Triose formation and subsequent dehydration to pyruvaldehyde was postulated. Neuberg and Rewald (75) reported pyruvaldehyde formation on heating arabinose, xylose, rhamnose, glucose, fructose, galactose, and glucosamine with ten per cent ammonia. Bernbauer and Wolf (76) heated glucose with calcium and magnesium carbonates and obtained pyruvaldehyde, supposedly through a triose intermediate. Evans and co-workers found that alkaline degradation resulted in pyruvaldehyde production from glucose and galactose (31),

fructose (35), mannose (37), arabinose and xylose (39), maltose (38), cellobiose, lactose, melibiose, and gentiobiose (40), and rhamnose (42) as well as glyceraldehyde (33) and dihydroxyacetone (34). Again, triose formation was considered the first step in the degradation of the higher sugars.

Enders and Marquardt (77) observed pyruvaldehyde in the distillates from aqueous solutions of glucose and xylose. Cameron (78) obtained pyruvaldehyde from glucose in the presence of acetic acid and benzylamine while Taufel and Burmeister (79) found that after extended acid hydrolyses sucrose solutions contained pyruvaldehyde. Enders (80) investigated the production of pyruvaldehyde from maltose over a wide range of pH values. A minimum was observed at pH 1. Increased acidity increased the conversion until a maximum was reached in twenty per cent sulfuric acid. As the pH was raised, the conversion increased to a second maximum at pH 11-12. Above that point conversion of pyruvaldehyde to lactic acid became more rapid than pyruvaldehyde formation.

Pyruvaldehyde was usually identified as its osazone. The observations that acetol was produced along with pyruvaldehyde (same osazones) on distillation of hexoses in phosphate (81-84) and in dilute sodium carbonate solutions (85,86) as well as on the distillation of aqueous glucose and maltose solutions (87) cast doubt on some of the earlier results. However, utilizing paper partition chromatography, Speck (88), has recently unequivocally identified pyruvaldehyde as a product in distillates from amino acid-reducing sugar reaction mixtures as well as in distillates from potassium hydroxide solutions of glucose.

The ready conversion of the trioses to pyruvaldehyde has long been known, and, as noted previously, many workers have considered the trioses as precursors of pyruvaldehyde in its production from the higher sugars. Demiges (89) reported the formation of pyruvaldehyde by the action of sulfuric acid on dihydroxyacetone, and Neuberg, et al. (90) utilized this reaction in determining the trioses. Evans and co-workers observed pyruvaldehyde production from glyceraldehyde (33) and dihydroxyacetone (34) in potassium hydroxide solutions. Fischer and Taube (91) were able to dehydrate dihydroxyacetone to pyruvaldehyde by a distillation with phosphorous pentoxide.

Little information is available concerning the mechanism of the dehydration of the trioses or the catalyses involved. Dische and Robbins (92) reported an acceleration of pyruvaldehyde formation in neutral glyceraldehyde or dihydroxyacetone solutions on addition of phosphate or arsenate.  $\text{Ca}^{++}$ ,  $\text{Cu}^{++}$ ,  $\text{Fe}^{++}$  and  $\text{Fe}^{+++}$  had no apparent effect. Evans and Cornthwaite (34) have suggested that pyruvaldehyde arises from the enediol common to the trioses. In a study of the effect of amines on the conversion of trioses into pyruvaldehyde, Strain and Spoehr (93) found that several weakly basic amines catalysed this reaction in dilute acetic acid solutions. These investigators also thought that a common enediol modification of the trioses might be essential to the dehydration. Analysis of their data indicates that the amines which were active were weak bases such that, at the acidities employed, they existed largely in the form of the free amine. This suggests a base catalysis and perhaps a specific amine effect. Smith and Anderson (94) have recently



investigated the dehydration of the 3-C-phenyltrioses in dilute acetic acid. The aldotriose required the presence of a primary amine for dehydration, while this was unnecessary for the ketotrioses. An enediol intermediate was proposed. Speck (88) has demonstrated the presence of pyruvaldehyde in distillates from glyceraldehyde-amino acid mixtures.

The present investigation was designed (a) to confirm the occurrence of the Lobry de Bruyn-van Ekenstein transformation in acid media, (b) to test the possibility of a generalized acid-base catalysis in this reaction and in the simultaneous formation of pyruvaldehyde, (c) to examine the effects of divalent cations on these processes, and (d) to gain as much information as possible concerning the reaction mechanisms involved. DL-glyceraldehyde and dihydroxyacetone were chosen for this study since, in each case, there is only one possible isomerization or dehydration product, and, in addition, the number of disturbing side reactions is few.

## EXPERIMENTAL METHODS

### Materials

The DL-glyceraldehyde employed in these measurements was synthesized by the method of Fischer and Baer (95); the dihydroxyacetone was a Nutritional Biochemicals product. The acetic acid was Baker's C.P. analyzed. Eastman Kodak Company practical formic acid was fractionally distilled and the fraction boiling at  $99.5^{\circ}\text{C}$  used in this investigation. The trimethylacetic acid was an Eastman Kodak Company product. Standard sodium perchlorate was prepared by neutralizing standard 2 M perchloric acid solution to pH 7.0 with standard sodium hydroxide solution. Calcium perchlorate and calcium acetate were prepared in solution by neutralization of weighed samples of Baker's C.P. calcium carbonate with the respective acids. The n-butanol, n-heptanol, and hydroxylamine hydrochloride were Eastman Kodak Company products. Periodic acid, perchloric acid, and nitroferroin were obtained from the G. Frederick Smith Chemical Company. Sodium oxalate, arsenious oxide, and perchloric acid were Baker's C.P. analyzed materials. Matheson Company practical chromotropic acid was recrystallized from 50% ethanol. Sulfuric acid was Merck reagent grade. Silver nitrate, nickelous sulfate, sodium acetate, sodium sulfite, sodium bicarbonate, potassium iodide, iodine, ammonium hydroxide, and sodium hydroxide used in this investigation were all C. P. reagents.

### Apparatus

A Beckman Model DU spectrophotometer equipped with quartz cells was used for ultraviolet spectral measurements while a Beckman Model B spectrophotometer with corex cells was employed for measurements in the visible range. A Beckman pH meter with an outside glass electrode was used for pH determinations.

### Identification of Products

Glyceraldehyde yields pyruvaldehyde in acid solution (89, 90). In preliminary studies utilizing the cerate oxidation technique described below, degradation of glyceraldehyde in 2.5 M perchloric acid at 50° C indicated pyruvaldehyde as the only product. Therefore, pyruvaldehyde was anticipated as the predominant product of glyceraldehyde degradation in mildly acid buffer systems such as acetate at 50° C. The possibility of the transformation of glyceraldehyde to dihydroxyacetone was not overlooked in view of the interconversion of hexoses (60-62) and pentoses (63) reported to occur under similar conditions.

Either oxidation with periodic acid and subsequent determination of the periodate consumed by the method of Fleury and Lange (96) or oxidation with perchlorate cerate in 4 M perchloric acid followed by determination of the tetravalent cerium consumed by the procedure of Speck, Forist, and Neely (97) would permit determination of glyceraldehyde and pyruvaldehyde in mixtures of these substances provided the initial glyceraldehyde concentration is known. In a periodate oxidation,

glyceraldehyde and pyruvaldehyde consume four and two equivalents of oxidant per mole respectively whereas with cerate the consumption is six and two equivalents per mole. At the beginning of this investigation, the cerate method was chosen due to the greater differential in equivalents of oxidant consumed.

#### Cerate oxidation studies.

If pyruvaldehyde were the only product, cerate consumption should decrease with time. Therefore, preliminary studies of the degradation of glyceraldehyde in acetate buffers at 50° C were made by measuring cerate consumed by aliquots of the reaction mixture at various times as follows: 2.00 ml. aliquots of the reaction mixture (initially 0.2 M in glyceraldehyde) were withdrawn at definite times, added to 20.0 ml. of 0.2687 N perchlorate cerate in 4 M perchloric acid, and the oxidation allowed to proceed for at least four hours. At the end of that time, 25.0 ml. of 0.1825 N sodium oxalate in 0.1 M perchloric acid was added and the excess oxalate titrated with 0.02703 N cerate in 2 M perchloric acid to a nitro-ferroin end point.

In preliminary experiments, quantitative oxidation of pure glyceraldehyde had been observed in a period of four hours. However, the reaction mixtures containing acetate buffers were incompletely oxidized after four hours (low values at zero time). On the other hand, over oxidation occurred if the oxidations were allowed to proceed for longer periods. The presence of acetate may have caused a reduction in the specificity of the oxidant, or acetate and formate may have been slowly

oxidized. This led to the discarding of this method for quantitative purposes. Notwithstanding this, the cerate oxidation studies contributed valuable qualitative information. Instead of the expected drop in cerate consumption as degradation of the triose in acetate buffer proceeded, there was a rapid rise followed by a gradual leveling off and, after prolonged reaction times, a decrease. Although quantitative calculations could not be made from these data, it was apparent that some product, capable of a greater cerate consumption than glyceraldehyde was being formed. It seemed probable that this substance was dihydroxyacetone. The eventual drop in cerate consumption suggested a slow conversion of one or both of the trioses to pyruvaldehyde.

#### Ultraviolet spectral studies.

Cerate oxidation studies indicated dihydroxyacetone as well as pyruvaldehyde as reaction products. In an effort to further identify these or other products the following experiment was carried out:

A typical reaction mixture was prepared which was 0.20 M in DL-glyceraldehyde and 0.40 M each in acetic acid and sodium acetate. This solution was placed in a thermostat at 50° C, and, at definite times, 2.0 ml. aliquots were removed and diluted to 10 ml. The absorption spectra of these samples in the region 240-330 mμ were then measured using the Beckman Model DU spectrophotometer.

Figure 1 shows the absorption spectra of pure DL-glyceraldehyde, dihydroxyacetone, and pyruvaldehyde whereas Figure 2 gives the results of the above experiment over a period of twenty-five hours. The initial glyceraldehyde spectrum changes fairly rapidly with a strong peak

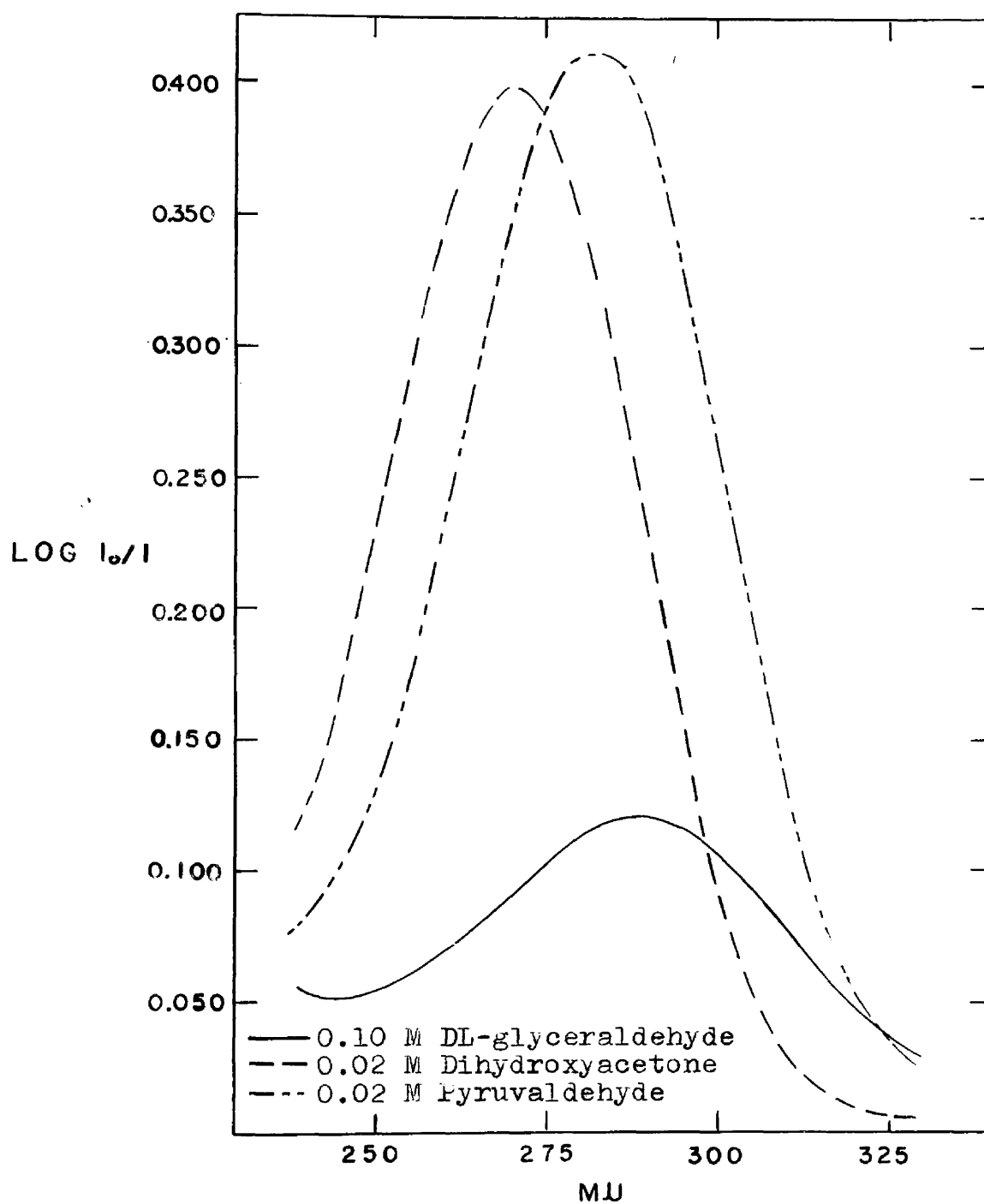


Figure 1. Ultraviolet absorption spectra.

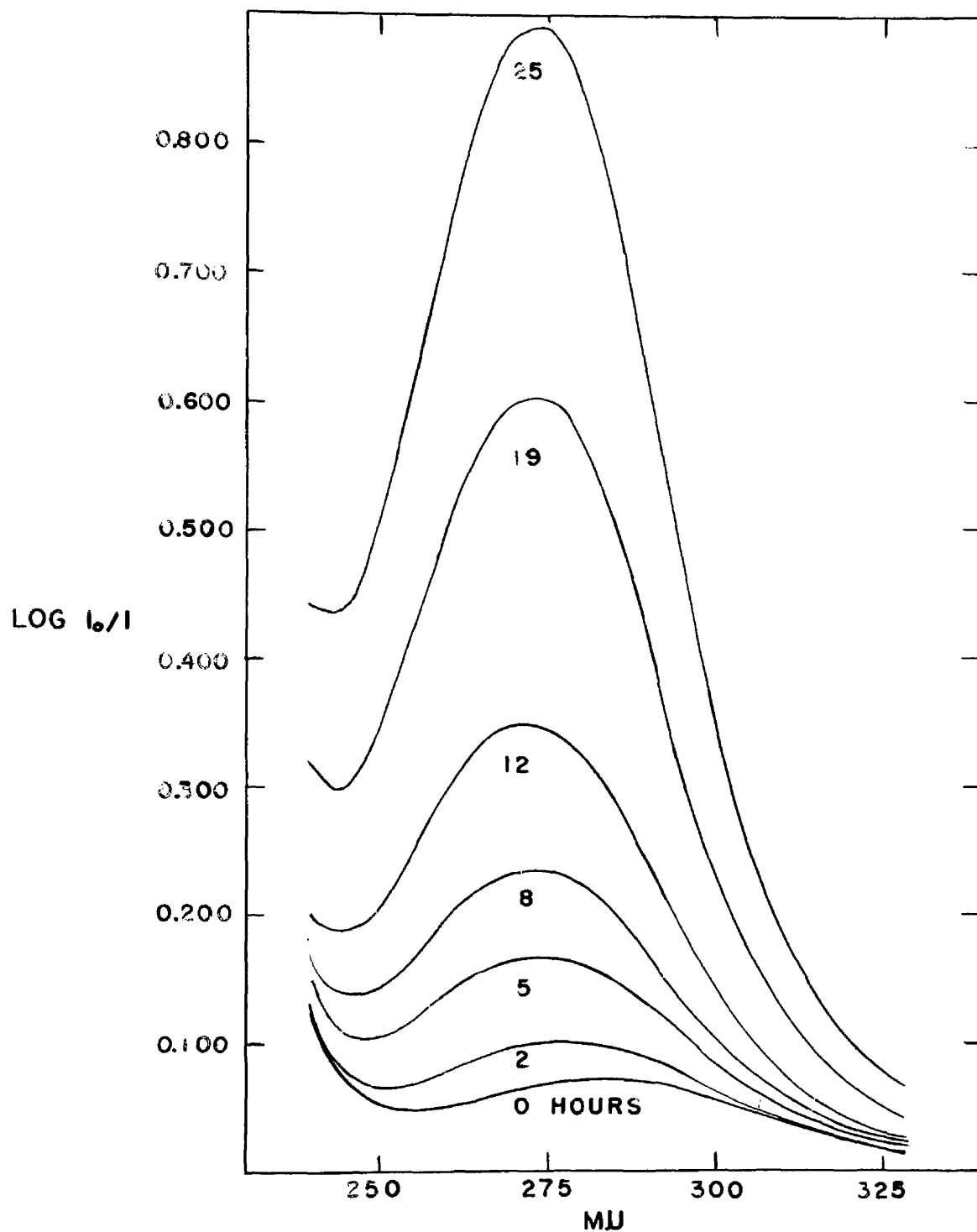


Figure 2. Spectral changes during the degradation of DL-glyceraldehyde in acetate buffer at 50° C.

developing near 270 mμ after twelve hours. On further reaction, there is a slow shift toward the longer wave lengths, the maximum occurring at 275 mμ after twenty-five hours. Comparison of these curves with those of Figure 1 suggests a rapid formation of dihydroxyacetone (maximum at 270 mμ) with a slow conversion to pyruvaldehyde (maximum at 280 mμ).

Identification of dihydroxyacetone by paper chromatography.

The reaction mixture employed for the spectral studies was used for chromatography. The sample was removed for this experiment after twenty-four hours. Two drops of this solution were placed on filter paper (Whatman No. 1) and an ascending chromatogram developed using n-butanol saturated with water as the solvent (98). Simultaneously, pure samples of DL-glyceraldehyde, dihydroxyacetone, pyruvaldehyde, and a synthetic mixture of these three were chromatographed under identical conditions. After thirty-two hours, the chromatograms were removed, the solvent front marked, and the sheets dried before an electric fan. They were then sprayed with a solution containing equal volumes of 5 M ammonium hydroxide and 0.1 M silver nitrate, dried at 110° C for about five minutes, washed with distilled water, and dried once more (99). The spots were circled and  $R_f$  values calculated. The results are shown in Table I. In every case, glyceraldehyde produced a poor chromatogram and tailed considerably. Dihydroxyacetone gave a sharp, well defined spot and was easily seen even when partially overlapping the glyceraldehyde tail. It was obvious that pyruvaldehyde could not be detected by this procedure, probably due to its volatility. The third component of low  $R_f$



found after twenty-four hours in the reaction mixture was not identified but is believed to be a polymer formed from pyruvaldehyde. Anomalous periodate consumption by pyruvaldehyde in acetate buffers after extended times (cited below) supports this point of view.

Dihydroxyacetone was therefore established as a product of the degradation of glyceraldehyde under the conditions of this investigation.

Identification of pyruvaldehyde by paper chromatography.

Pyruvaldehyde was identified by chromatography of its dioxime according to the method of Speck (88) as outlined below.

TABLE I  
IDENTIFICATION OF DIHYDROXYACETONE

| Material Chromatographed | $R_f$       | Quality of Spot                           |
|--------------------------|-------------|---|
| DL-glyceraldehyde        | $\sim 0.35$ | Very diffuse; difficult to estimate $R_f$ |
| Dihydroxyacetone         | 0.31        | Well defined                              |
| Pyruvaldehyde            | --          | No spot                                   |
| Known Mixtures:          |             |   |
| DL-glyceraldehyde        | $\sim 0.36$ | Poorly defined                            |
| Dihydroxyacetone         | 0.33        | Well defined                              |
| Pyruvaldehyde            | --          | No spot                                   |
| Reaction Mixtures:       |             |   |
| DL-glyceraldehyde        | $\sim 0.36$ | Diffuse                                   |
| Dihydroxyacetone         | 0.33        | Well defined                              |
| Unknown                  | 0.05        | Well defined                              |

Forty-eight ml. of a reaction mixture, identical with the one described above, was removed after fifty-four hours, cooled, and mixed with 50 ml. of solution containing 1 gm. of hydroxylamine hydrochloride and 2 gm. of sodium acetate trihydrate. After standing overnight, the solution was saturated with sodium chloride and extracted six times with 25 ml. portions of diethyl ether. The combined ether extracts were dried over anhydrous sodium sulfate, filtered, and the ether removed on the water pump. The small amount of residue remaining was equilibrated with 2 ml. of distilled water, the solution filtered, and six drops placed on filter paper (Whatman No. 1). An ascending chromatogram was developed using n-heptanol saturated with water as the solvent. Simultaneously, a known sample of methylglyoxime was chromatographed under identical conditions. After thirty-six hours, the chromatograms were removed and dried before an electric fan in the absence of heat. They were then sprayed with a 3% solution of nickelous sulfate hexahydrate containing 0.1 ml. of 28% ammonia per 100 ml. and once more dried. The results are shown in Table II. Thus, both dihydroxyacetone and pyruvaldehyde were established as reaction products.

TABLE II  
IDENTIFICATION OF PYRUALDEHYDE

| Material Chromatographed | R <sub>F</sub> | Quality of Spot                        |
|--------------------------|----------------|--|
| Methylglyoxime           | 0.61           | Well defined; orange with rose center. |
| Reaction mixture oxime   | 0.60           | Well defined; orange with rose center. |

### Quantitative Analysis of Reaction Mixtures

Since two products were being formed, two analytical methods were necessary in order to estimate the three components, glyceraldehyde, dihydroxyacetone, and pyruvaldehyde, at any time. It was hoped that cerate oxidations could be employed, but, due to the anomalous oxidations discussed above, this method was discarded. A second attractive procedure consisted of oxidation with periodic acid and subsequent determination of periodate consumed by the method of Fleury and Lange (96). To test the applicability of this method to the problem at hand, samples of DL-glyceraldehyde and dihydroxyacetone of concentrations expected to be encountered were oxidized for varying times by approximately 0.1 N periodic acid and the periodate consumption determined. The results are given in Table III.

TABLE III  
PERIODATE OXIDATION OF TRIOSSES

| Oxidation Time<br>(Hours) | Equivalents $\text{IO}_4^-$ consumed per mole triose* |                  |
|---------------------------|---|------------------|
|                           | DL-glyceraldehyde                                     | Dihydroxyacetone |
| 1                         | <u>DL-glyceraldehyde</u>                              | 1.987            |
| 2                         | 3.941   | 1.992            |
| 3                         | 3.956   | 1.996            |
| 4                         | 3.956   | 2.008            |
| 6                         | 3.962   | 2.008            |
| Theory                    | 4.000   | 2.000            |

\* Average of duplicate analyses.

From these data, it is seen that periodate consumption by both sugars is complete after three hours under the conditions employed. Thornton and Speck (100), showed that pyruvaldehyde was completely oxidized by periodic acid in one hour. Therefore, in this investigation, periodate oxidations have been allowed to proceed for at least four hours, thus insuring complete reaction.

On periodate oxidation, glyceraldehyde consumes four equivalents of oxidant per mole whereas dihydroxyacetone and pyruvaldehyde each consume two equivalents per mole.

If G, D, and P represent millimoles of glyceraldehyde, dihydroxyacetone, and pyruvaldehyde respectively per ml. of solution analyzed,  $G_0$  represents initial glyceraldehyde concentration, and I represents milliequivalents of periodate consumed by one ml. of reaction mixture, the following equations may be written:

$$G + D + P = G_0 \quad (1)$$

$$4G + 2D + 2P = I \quad (2)$$

From these expressions, equation (3) may be obtained.

$$G = I/2 - G_0 \quad (3)$$

Therefore, beginning with pure glyceraldehyde, G at any time may be calculated from  $G_0$  and the periodate consumption, I. However, this method offers no distinction between D and P, giving only their sum.

Since G could be determined at any time, a second method allowing determination of total trioses would permit an estimation of all three components. The method selected was devised for this purpose and is described in Part II of this thesis.

The operating procedure employed is best described by outlining a typical experiment.

The calculated quantity of standard acid (or a weighed sample in the case of trimethylacetic acid) was mixed in a 50 ml. volumetric flask with sufficient standard sodium hydroxide to provide the desired buffer ratio and concentration. To this was added enough standard sodium perchlorate solution to bring the ionic strength to 0.4. This solution was placed in the constant temperature bath operating at  $50^{\circ} \pm 0.01^{\circ} \text{C}$  and brought to the bath temperature.

A sample of pure DL-glyceraldehyde (usually 0.9008 gm.) was weighed on an analytical balance, dissolved in redistilled water at the bath temperature, and quantitatively transferred to the reaction flask. The solution was mixed, brought nearly to the mark with redistilled water at the bath temperature, and returned to the bath for about fifteen minutes. It was then diluted to the mark, thoroughly mixed, returned to the bath, and the first samples removed.

In most experiments, solutions 0.20 M in glyceraldehyde were used and 1.00 ml. aliquots were removed at the desired times for analysis.

To determine periodate consumption, a 1.00 ml. aliquot of the reaction mixture was transferred to a 300 ml. erlenmeyer flask containing 15.00 ml. of 0.108 N periodic acid and the oxidation allowed to proceed for at least four hours. The solution was then neutralized and saturated with solid sodium bicarbonate and 2 ml. of 5% potassium iodide followed by 10.00 ml. of 0.13142 N arsenite added. After about fifteen minutes, the excess arsenite was titrated with 0.01 N iodine solution to a starch

endpoint. Milliequivalents of periodate consumed may be calculated from the expression:

$$I = 15 \times N_{IO_4^-} - 10 \times 0.13142 \times \text{ml. } I_2 \times N_{I_2} \quad (4)$$

To determine total trioses, a 1.00 ml. aliquot of the reaction mixture was transferred to a 200 ml. volumetric flask containing 4 ml. of 1 M sodium bicarbonate and 4 ml. of 0.3 M periodic acid, and the oxidation allowed to proceed for one hour. At the end of that time, 10 ml. of 0.5 M sodium sulfite was added and the solution diluted to the mark. Duplicate 1.00 ml. aliquots of this solution were pipetted into 50 ml. volumetric flasks, followed by 0.5 ml. of 10% chromotropic acid and 5 ml. of 11 M sulfuric acid. The flasks were heated in a boiling water bath for thirty minutes. They were then removed, cooled, and about 40 ml. of distilled water added. The flasks were cooled once more, and the solutions diluted to the mark. Excess sulfur dioxide was removed by bubbling air saturated with water through these solutions at a rate of 750-1000 ml. per minute. The optical densities of these solutions were then measured against a reagent blank at 570 mμ using the Beckman Model B spectrophotometer. The total triose values were calculated from a standard curve prepared by similar treatment of either D-glucose or DL-glyceraldehyde.

## RESULTS AND DISCUSSION

## Order of the Reaction

Preliminary studies indicated that pyruvaldehyde was not completely stable in buffers of the type employed in this investigation. A fresh solution of pyruvaldehyde (obtained by distillation of a sulfuric acid solution of DL-glyceraldehyde) was prepared in a buffer 0.40 M in sodium acetate and 0.133 M in acetic acid. This solution was placed in the 50° C thermostat and one ml. aliquots removed periodically for periodate oxidation. At the same times, similar aliquots were tested for formaldehyde producing material by the total triose analysis. The results are shown in Table IV. The solution was colored after eighteen hours, and some material capable of a greater periodate reduction than pyruvaldehyde itself was formed.

TABLE IV  
INSTABILITY OF PYRUVALDEHYDE IN ACETATE BUFFER

| Time of Standing<br>(Hours) | Meq. $\text{IO}_4^-$<br>Consumed* | Apparent [P]<br>(Moles/l) | HCHO Producing<br>Material |
|-----------------------------|-----------------------------------|---------------------------|----------------------------|
| 0                           | 0.2679                            | 0.1340                    | None                       |
| 18                          | 0.2852                            | 0.1426                    | None                       |
| 24                          | 0.2930                            | 0.1465                    | None                       |
| 53                          | 0.2958                            | 0.1479                    | --                         |
| 72                          | 0.3047                            | 0.1524                    | --                         |
| 336                         | 0.3944                            | 0.1972                    | --                         |

\* Average of duplicate analyses

In most of the reactions studied, the starting material was pure DL-glyceraldehyde, and its concentration at any time could be calculated from a knowledge of the periodate consumed by an aliquot of the reaction mixture as outlined above. Due to the anomalous periodate consumption by pyruvaldehyde when present in appreciable concentrations and in order to disregard the reverse reaction in the interconversion of the trioses, only "initial velocity" data has been used. Reactions were usually followed to the disappearance of 20-45% of the initial glyceraldehyde. A plot of the logarithm of glyceraldehyde concentration versus time was linear (Figure 3) indicating a first order disappearance of glyceraldehyde. In over forty velocity determinations, no deviations from this order were observed.

If a reaction is of the first order, the pseudo constant calculated from the slope of the log G versus time curve is independent of initial reactant concentration. Therefore, velocity determinations were made at different initial glyceraldehyde concentrations in buffers 0.40 M each in acetic acid and sodium acetate. The results are shown in column two of Table V.

TABLE V  
EFFECT OF INITIAL GLYCERALDEHYDE CONCENTRATION

| [Go]<br>(Moles/l) | $k' \times 10^3 \text{ min}^{-1}$ | (D/P) <sub>1</sub> |
|-------------------|-----------------------------------|--------------------|
| 0.10              | 233                               | 5.9                |
| 0.20              | 205*                              | 5.0**              |
| 0.40              | 167                               | 5.3                |

\* Average of four determinations  
\*\* Average of two determinations



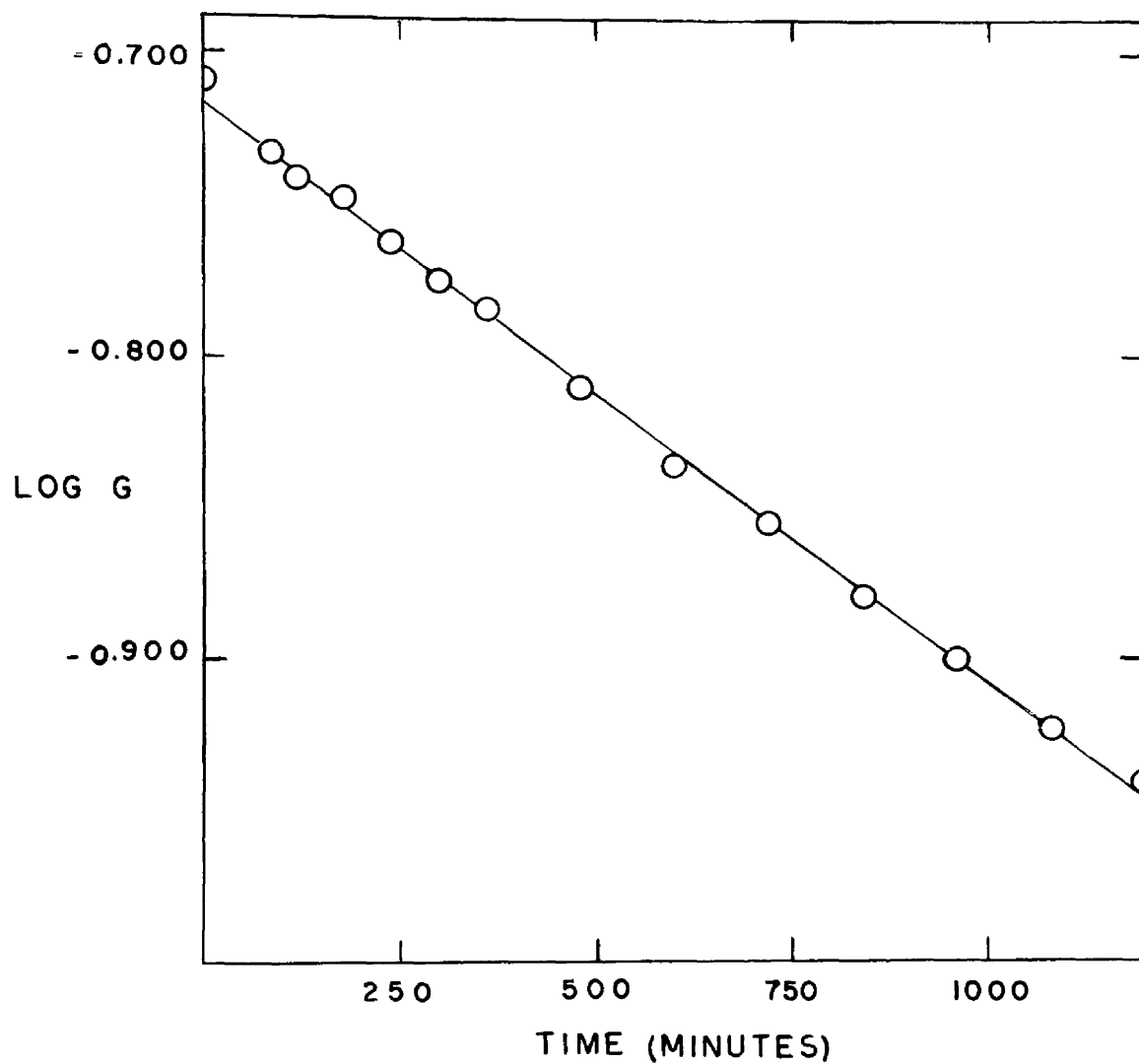


Figure 3. Typical rate curve (Experiment 11, Table VI).

A regular variation was observed which was not considered indicative of an order other than the first. The decrease in pseudo constant with increasing initial glyceraldehyde concentration was believed the result of decreased activity of the glyceraldehyde due to the many polar hydroxyl groups present in solution.

#### Calculation of Constants

Since the over-all disappearance of glyceraldehyde was of the first order, this may be expressed as

$$-dG/dt = k [G] \quad (5)$$

which on integration yields

$$-\ln G = kt + \text{constant} \quad (6)$$

$$\text{or} \quad \log G = -k't + \text{constant} \quad (7)$$

The pseudo constants ( $k'$ ), employing decadic logarithms, were evaluated at varying buffer concentrations and buffer ratios for formate, acetate, and trimethylacetate systems. The results are indicated in column seven of Tables VI, VII, and VIII.

The observed variations in pseudo constants with various buffer concentrations at constant buffer ratio, as well as at different buffer ratios, suggested an acid and base catalyzed reaction. If  $[HA]$  and  $[A^-]$  represent concentrations of the acid and conjugate base respectively of the buffer system involved, such a catalysis may be represented by the expression

$$-dG/dt = [k_H + [H^+] + k_{HA}[HA] + k_{A^-}[A^-]] [G] \quad (8)$$

TABLE VI  
PSEUDO CONSTANTS -- ACETATE SYSTEMS

| Expt.   | [Go] | [CH <sub>3</sub> COOH] | [CH <sub>3</sub> COONa] | [NaClO <sub>4</sub> ] | $\frac{A^-}{HA}$ | $k' \times 10^6$<br>min <sup>-1</sup> | (D/P) <sub>1</sub> |
|---|------|------------------------|-------------------------|-----------------------|------------------|---------------------------------------|--------------------|
| 18  | 0.20 | --                     | --                      | 0.40                  | --               | 0                                     | --                 |
| 4   | 0.20 | 0.20                   | 0.20                    | 0.20                  | 1                | 98                                    | --                 |
| 5   | 0.20 | 0.20                   | 0.20                    | 0.20                  | 1                | 99                                    | --                 |
| 10  | 0.20 | 0.20                   | 0.20                    | 0.20                  | 1                | 101                                   | 4.6                |
| 14  | 0.20 | 0.20                   | 0.20                    | 0.20                  | 1                | 100                                   | 4.5                |
| 15  | 0.20 | 0.30                   | 0.30                    | 0.10                  | 1                | 152                                   | 4.8                |
| 6   | 0.20 | 0.40                   | 0.40                    | --                    | 1                | 208                                   | --                 |
| 7   | 0.20 | 0.40                   | 0.40                    | --                    | 1                | 198                                   | --                 |
| 8   | 0.20 | 0.40                   | 0.40                    | --                    | 1                | 208                                   | 5.4                |
| 16  | 0.20 | 0.40                   | 0.40                    | --                    | 1                | 207                                   | 4.6                |
| 13  | 0.20 | 0.067                  | 0.20                    | 0.20                  | 3                | 96                                    | 4.6                |
| 12  | 0.20 | 0.10                   | 0.30                    | 0.10                  | 3                | 139                                   | 4.8                |
| 11  | 0.20 | 0.133                  | 0.40                    | --                    | 3                | 193                                   | 5.0                |
| Average (D/P) <sub>1</sub> = 4.8 <sup>±</sup> 0.2 |      |                        |                         |                       |                  |                                       |                    |

All concentrations in moles/l.

TABLE VII  
PSEUDO CONSTANTS -- FORMATE SYSTEMS

| Expt.                                  | [Co] | [HCOOH] | [HCOONa] | [NaClO <sub>4</sub> ] | $\frac{A^-}{HA}$ | $k' \times 10^6$<br>min <sup>-1</sup> | (D/P) <sub>1</sub> |
|--|------|---------|----------|-----------------------|------------------|---------------------------------------|--------------------|
| 23                                     | 0.20 | 0.20    | 0.20     | 0.20                  | 1                | 35                                    | 3.5                |
| 24                                     | 0.20 | 0.30    | 0.30     | 0.10                  | 1                | 60                                    | 3.5                |
| 26                                     | 0.20 | 0.40    | 0.40     | --                    | 1                | 71                                    | 2.4                |
| 29                                     | 0.20 | 0.067   | 0.20     | 0.20                  | 3                | 33                                    | 2.5                |
| 28                                     | 0.20 | 0.10    | 0.30     | 0.10                  | 3                | 51                                    | 2.6                |
| 27                                     | 0.20 | 0.133   | 0.40     | --                    | 3                | 62                                    | 4.4                |
| Average (D/P) <sub>1</sub> = 3.2 ± 0.7 |      |         |          |                       |                  |                                       |                    |

All concentrations in moles/l.

TABLE VIII  
PSEUDO CONSTANTS -- TRIMETHYLACETATE SYSTEMS

| Expt.                                  | [Co] | [(CH <sub>3</sub> ) <sub>3</sub> CCOOH] | [(CH <sub>3</sub> ) <sub>3</sub> CCOONa] | [NaClO <sub>4</sub> ] | $\frac{A^-}{HA}$ | $k' \times 10^6$<br>min <sup>-1</sup> | (D/P) <sub>1</sub> |
|--|------|---|--|-----------------------|------------------|---------------------------------------|--------------------|
| 34                                     | 0.20 | 0.05                                    | 0.10                                     | 0.30                  | 2                | 84                                    | 4.3                |
| 32                                     | 0.20 | 0.10                                    | 0.20                                     | 0.20                  | 2                | 177                                   | 6.1                |
| 35                                     | 0.20 | 0.025                                   | 0.10                                     | 0.30                  | 4                | 81                                    | 4.4                |
| 33                                     | 0.20 | 0.05                                    | 0.20                                     | 0.20                  | 4                | 166                                   | 6.8                |
| Average (D/P) <sub>1</sub> = 5.4 ± 1.1 |      |   |  |                       |                  |                                       |                    |

All concentrations in moles/l.

where the  $k$ 's are the respective catalytic constants. No term for spontaneous water catalysis has been included since, in the absence of buffer systems, no reaction occurred (Experiment 18, Table VI). The pseudo constant becomes

$$k' = [k_H + ] [H^+] + k_{HA}[HA] + k_A-[A^-] \quad (9)$$

or

$$k' = [k_H + ] [H^+] + \alpha [HA] \quad (10)$$

where

$$\alpha = k_{HA} + k_A- [A^-/HA] \quad (11)$$

Therefore, at constant buffer ratio (constant  $[H^+]$ ), a plot of  $k'$  versus  $[HA]$  should be linear. This was observed experimentally for the systems studied (Figures 4, 5, and 6). In every case the intercept was zero indicating negligible catalysis by the hydronium ion as well as water.

A plot of the slopes ( $\alpha$ ) of these curves against the buffer ratio  $[A^-/HA]$  should be linear. Moreover, the slopes and intercepts should equal the catalytic constants for the bases and acids involved respectively. Such a plot is shown in Figure 7, and the catalytic constants so calculated are given in Table IX.

From these data, it is seen that the over-all disappearance of glyceraldehyde is subject to an acid and base catalysis. The basic catalysis by trimethylacetate, acetate, and formate ions is in qualitative agreement with their relative strengths as Bronsted bases. The catalytic constants for the respective acids do not follow the expected pattern. However, the error in their estimation may be considerable due to the proximity of the intercepts to the origin. No explanation is evident for the apparent failure of the hydronium ion to function as a catalyst.

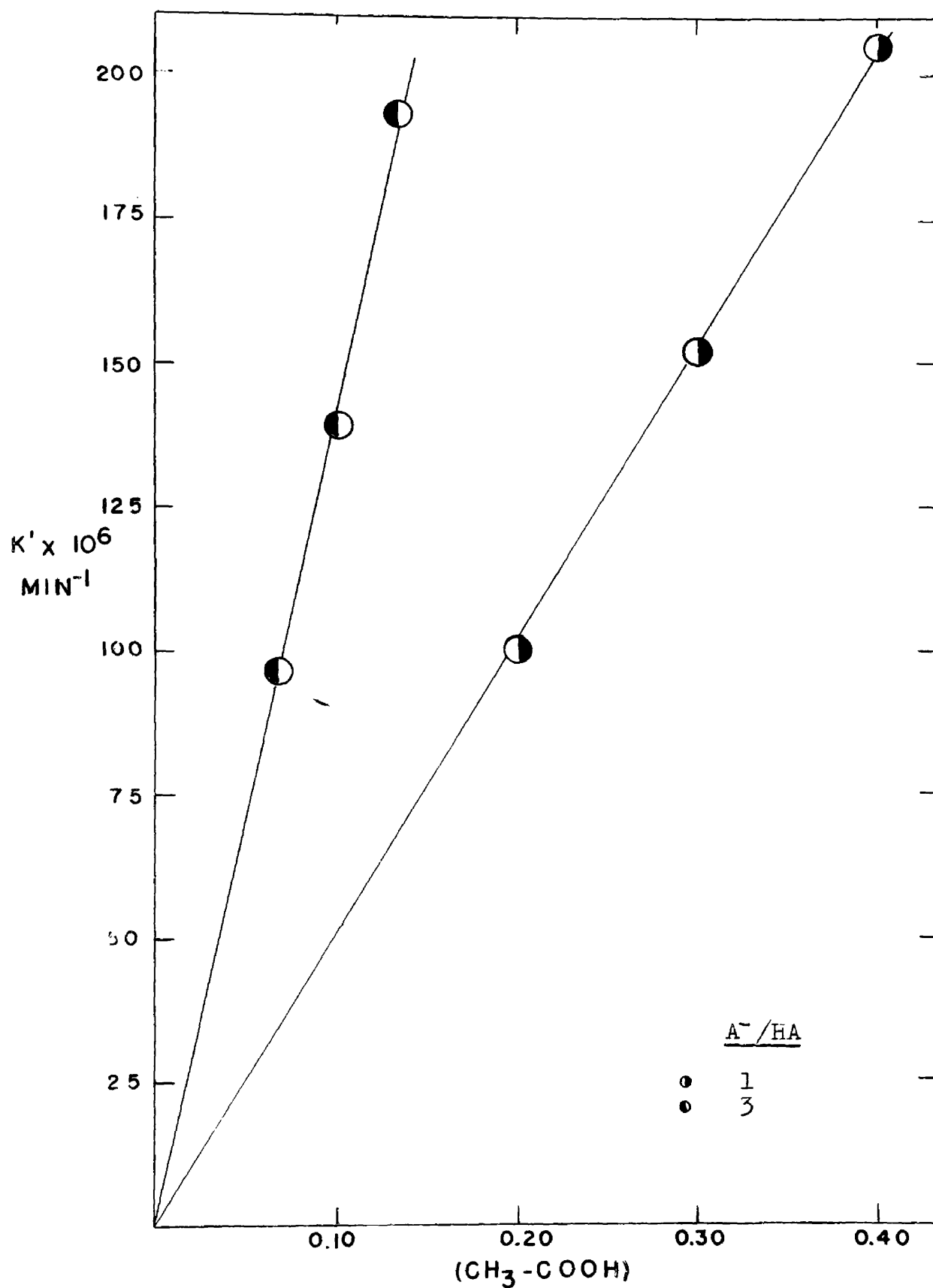


Figure 4. Relation of pseudo constant ( $k'$ ) to acetic acid concentration ( $\text{CH}_3\text{-COOH}$ ).

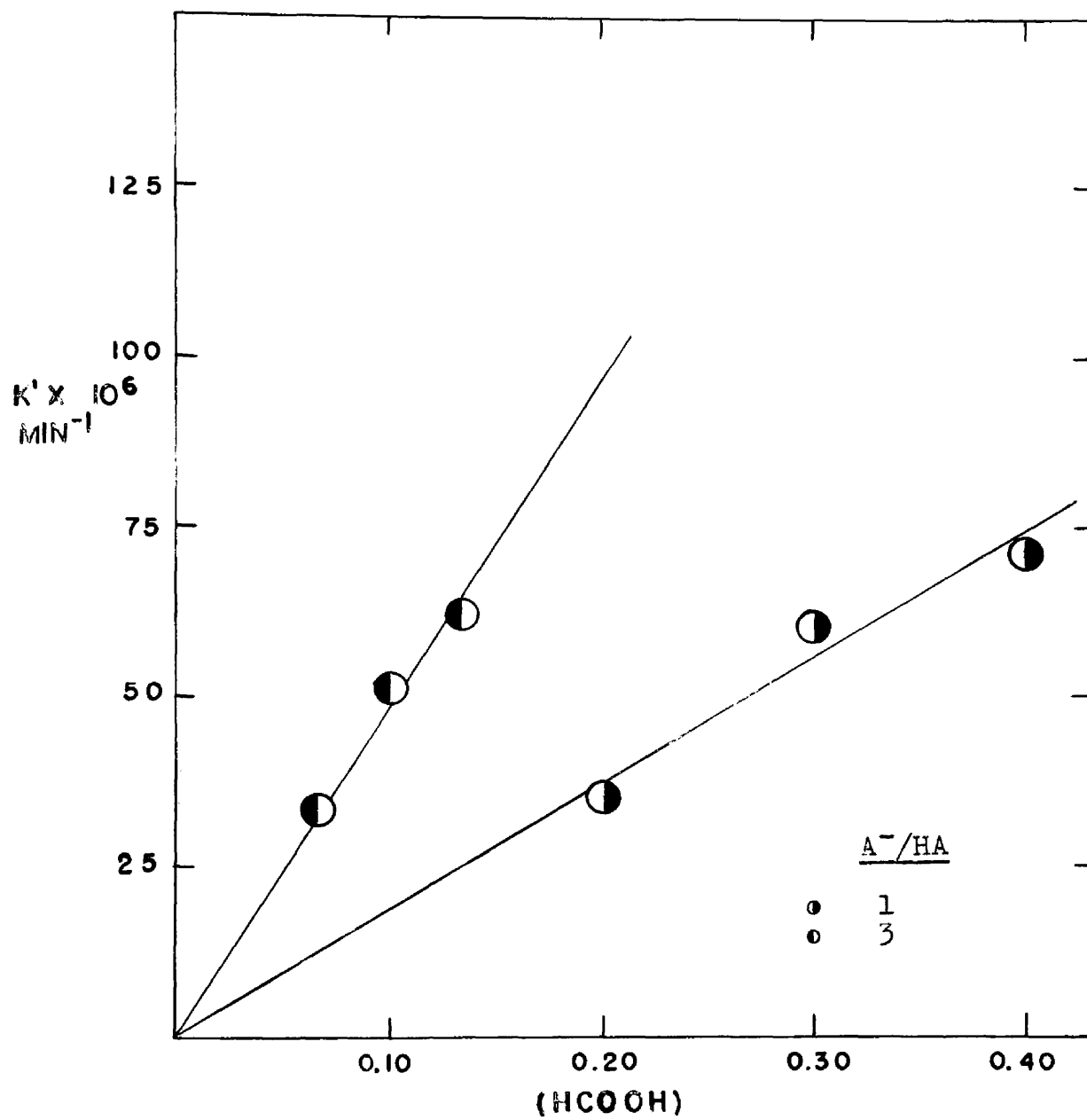


Figure 5. Relation of pseudo constant ( $k'$ ) to formic acid concentration ( $\text{HCOOH}$ ).

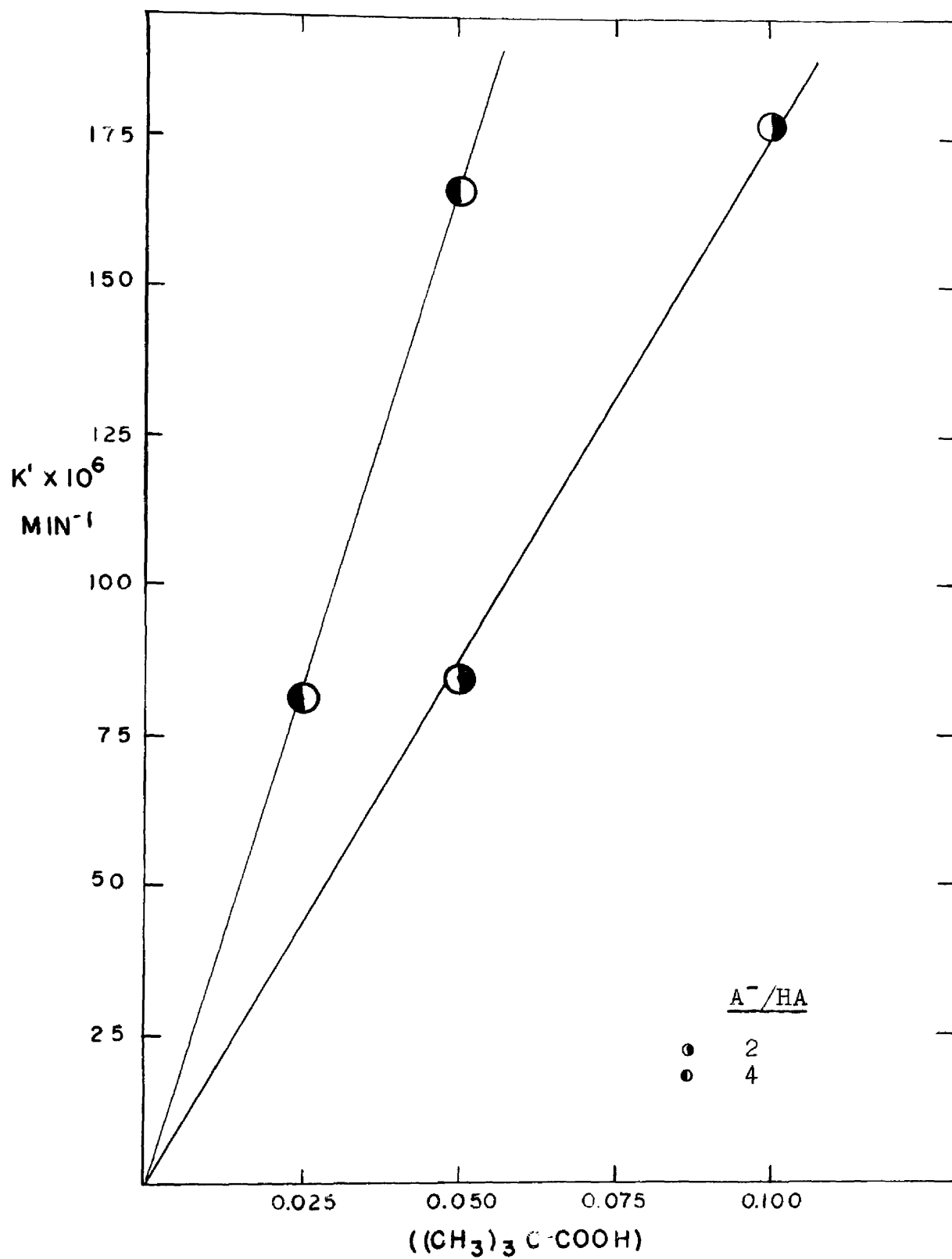


Figure 6. Relation of pseudo constant ( $k'$ ) to trimethylacetic acid concentration  $((\text{CH}_3)_3\text{C-COOH})$ .



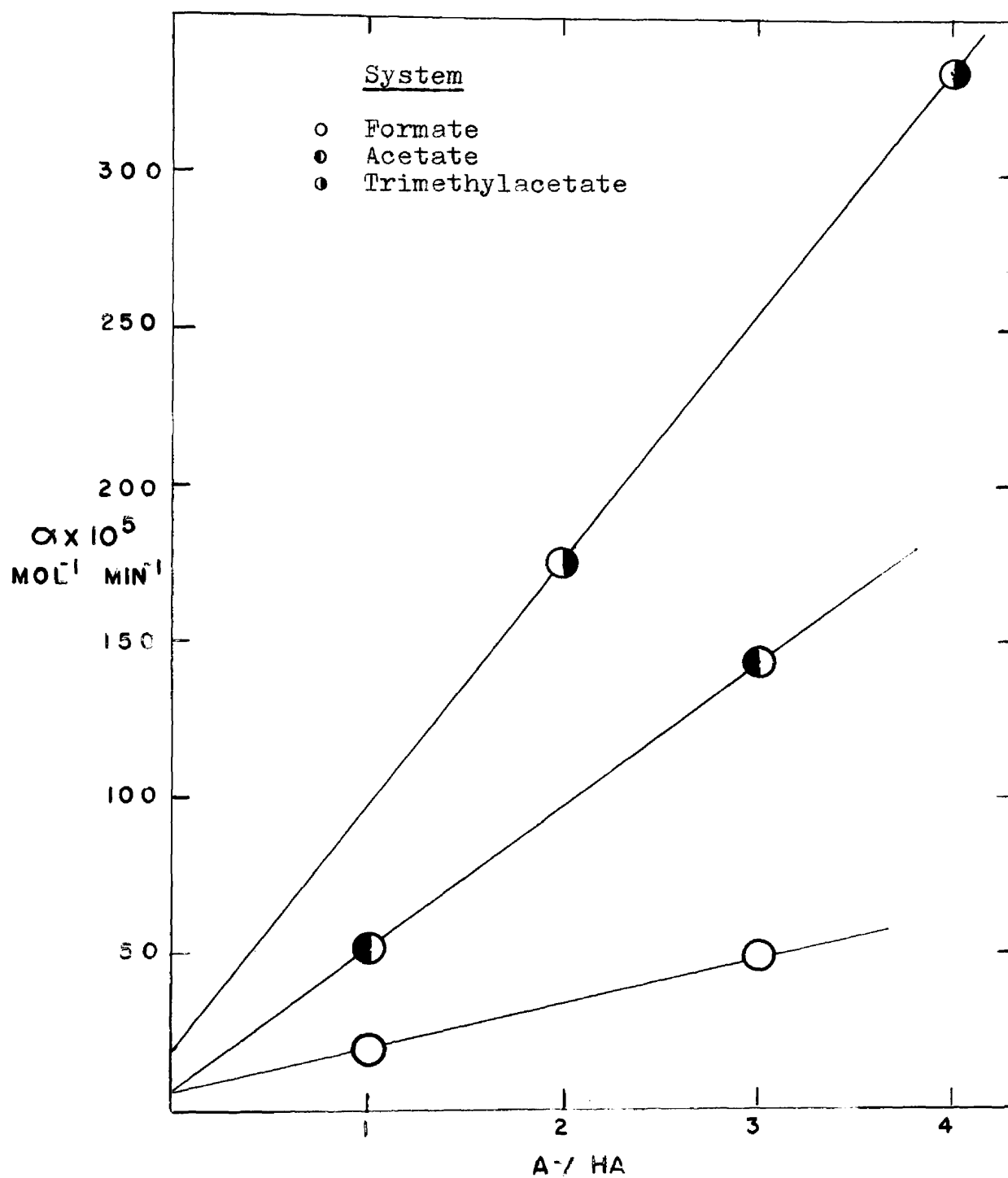


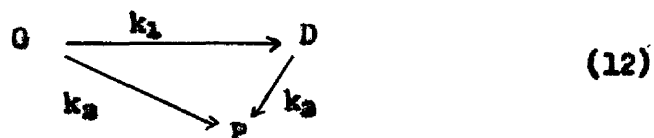
Figure 7. Relation of  $\alpha$  to buffer ratio ( $A^- / HA$ ).

TABLE IX  
CATALYTIC CONSTANTS

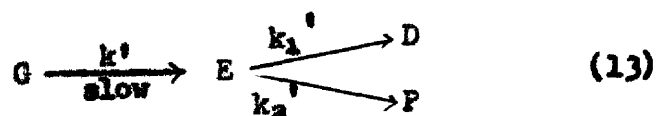
| System           | $k_{HA} \times 10^6$<br>$\text{mol}^{-1} \text{min}^{-1}$ | $k_A^- \times 10^6$<br>$\text{mol}^{-1} \text{min}^{-1}$ |
|------------------|---|--|
| Formate          | 6   | 14   |
| Acetate          | 6   | 45   |
| Trimethylacetate | 18  | 79   |

Dihydroxyacetone -- Pyruvaldehyde Ratios

Since an acid and base catalysis was established for the gross disappearance of glyceraldehyde, the formation of dihydroxyacetone and pyruvaldehyde must be subject to such catalysis. However, these data do not permit a choice between two possible pathways: (a) formation of dihydroxyacetone and pyruvaldehyde by two separate first order processes



or (b) by the breakdown of a common intermediate



Reverse processes are ignored since only initial velocity data have been employed. In an effort to decide between these possibilities, the relative rates of formation of dihydroxyacetone and pyruvaldehyde were determined.

Concentrations of these products were calculated as follows. Values for  $[G]$  from periodate consumption data and for  $[G + D]$  from total triose analyses were plotted against time and the best smooth curves drawn through these points (Figure 8). Corrected values for  $[G]$  and  $[G + D]$  were then calculated from these curves. From these corrected values,  $[D]$  and  $[P]$  were calculated at various times. Assuming that during initial stages of the reaction all the pyruvaldehyde comes from glyceraldehyde, a plot of  $[D]$  versus the corresponding  $[P]$  should be linear at low concentrations of products. This was observed experimentally (Figure 9) and the slopes of these curves, designated as  $(D/P)_i$ , are shown in column three of Table V and column eight of Tables VI, VII, and VIII.

Although the variations in  $(D/P)_i$  are large in some cases, these ratios are essentially constant for a given acid-base system and are independent of buffer ratio and concentration. If equation (12) represents the reaction process,  $(D/P)_i = k_1/k_2$  and it is implied that the catalytic constants for the acids and bases involved in the two reactions are always proportional to each other. This seems highly unlikely. The reaction indicated in equation (13), in which glyceraldehyde is slowly converted to an intermediate E which rapidly produces dihydroxyacetone and pyruvaldehyde seems more plausible. The value  $(D/P)_i$  would then represent the ratio of two fast processes essentially insensitive to amounts and ratios of catalysts present.

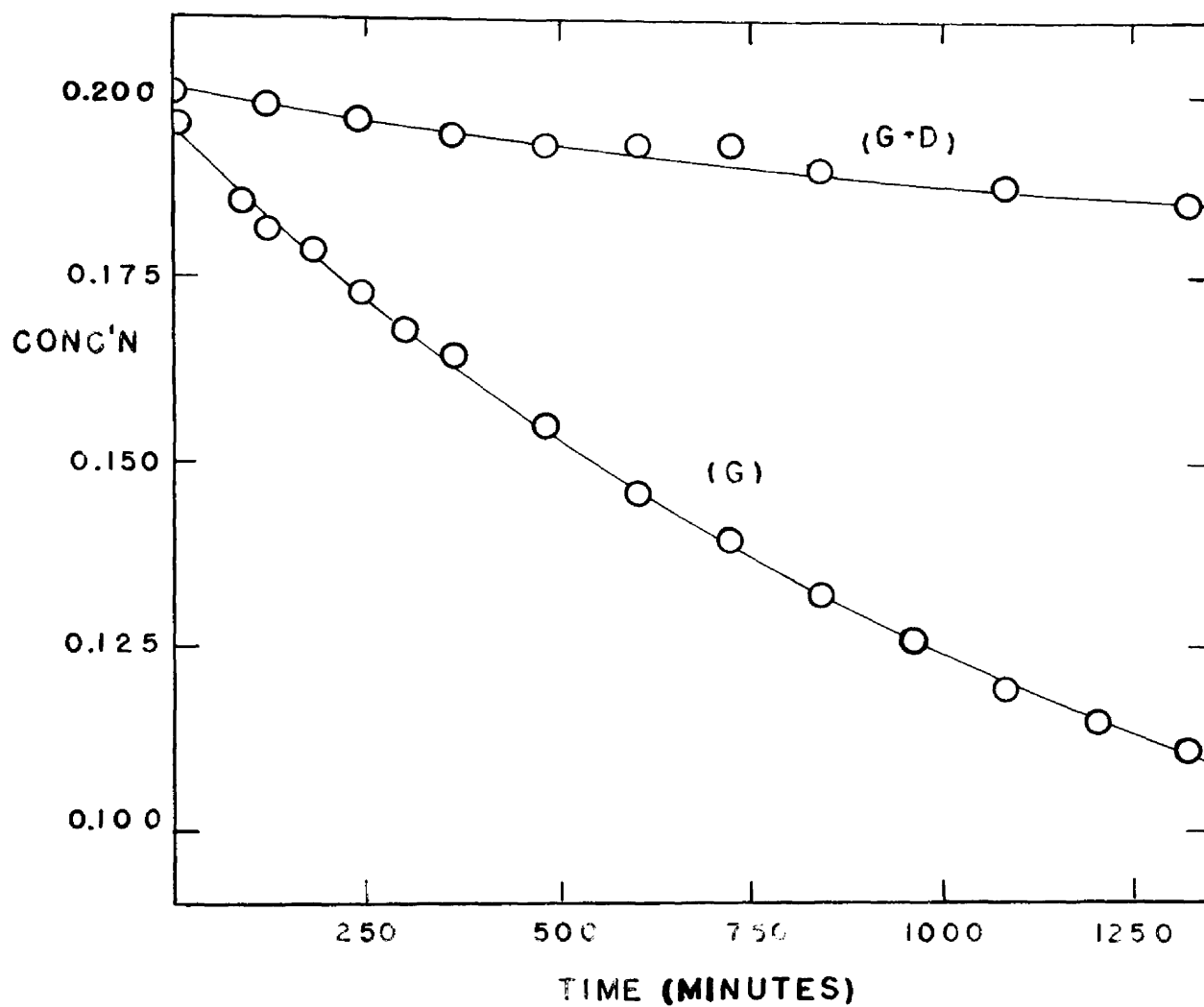


Figure 8. Typical concentration versus time curves (Experiment 11, Table VI).

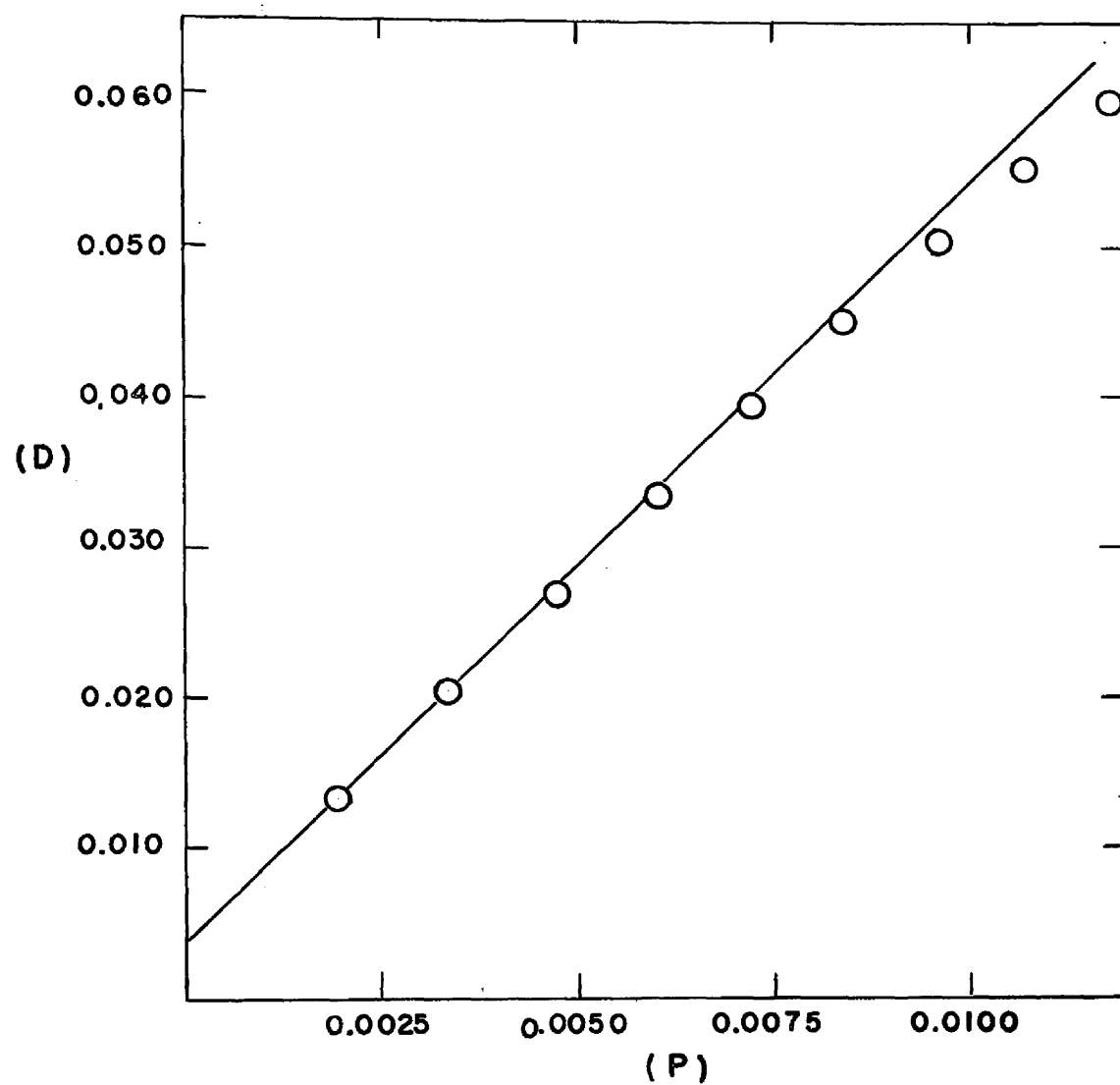


Figure 9. Typical (D) versus (P) curve  
(Experiment 11, Table VI).

### Glyceraldehyde -- Dihydroxyacetone Equilibrium

Efforts to evaluate an equilibrium constant for the reaction between glyceraldehyde and dihydroxyacetone were not entirely successful. The equilibrium was well in favor of the ketose. Experiments in which glyceraldehyde was the starting material gave results that were invalid due to the anomalous periodate uptake by the concentrated solutions of pyruvaldehyde present at or near equilibrium. Alternatively, pure dihydroxyacetone (0.10 M) was allowed to react for twenty-four hours in a buffer 0.30 M each in acetic acid and sodium acetate at 50° C. The usual analyses and calculations were made. A plot of  $[D]/[G]$  versus time was made and is shown in Figure 10. This curve approaches a value of 17 which does not compare unfavorably with values of about 20 reported for the corresponding triose phosphates (101,102).

### Effect of Calcium Ion

Since divalent cations reportedly produce anomalous reactions in the interconversion of the hexoses (see above),  $Ca^{++}$  was tested for its effect on the reactions of glyceraldehyde. Varying concentrations of  $Ca^{++}$  were added to reaction systems 0.20 M each in acetic acid and acetate ion. The results are indicated in Table X.

In neutral solution, calcium perchlorate produced no reaction, whereas in an acetate buffer the presence of  $Ca^{++}$  produced an augmentation of rate proportional to  $Ca^{++}$  concentration over the range investigated. The  $(D/P)_i$  ratio was not affected. The role of  $Ca^{++}$  as an accelerator of

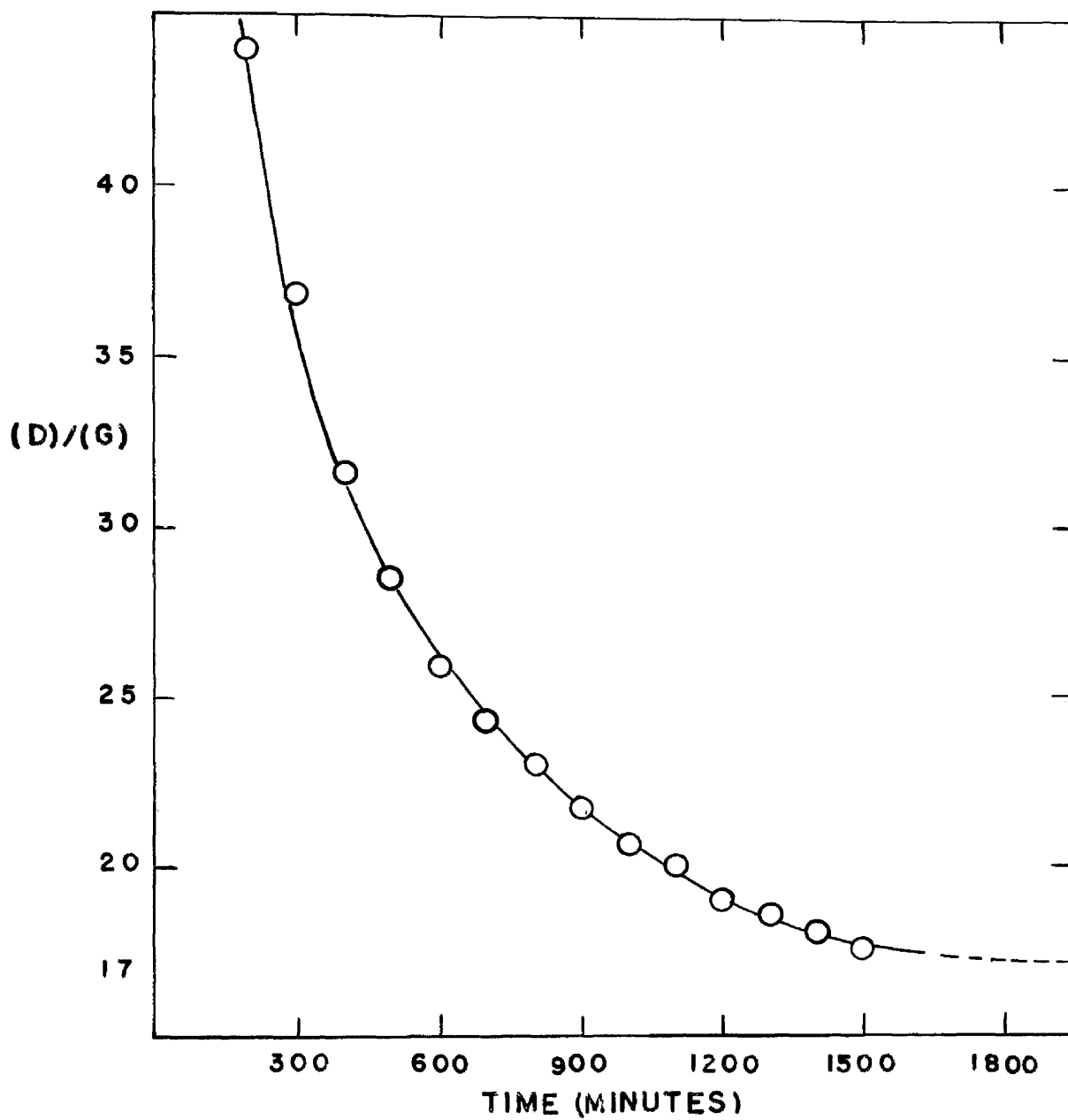


Figure 10. Evaluation of Dihydroxyacetone-Glyceraldehyde equilibrium constant.

TABLE X  
EFFECT OF CALCIUM ION

| Expt. | [Co] | [CH <sub>3</sub> COOH] | [CH <sub>3</sub> COO <sup>-</sup> ] | [Ca <sup>++</sup> ] | k' x 10 <sup>5</sup><br>Min <sup>-1</sup> | (D/P)1 |
|-------|------|------------------------|-------------------------------------|---------------------|---|--------|
| 18    | 0.20 | --                     | --                                  | --                  | 0   | --     |
| 39    | 0.20 | --                     | --                                  | 0.10*               | 0   | --     |
| 10    | 0.20 | 0.20                   | 0.20                                | --                  | 101                                       | 4.6    |
| 14    | 0.20 | 0.20                   | 0.20                                | --                  | 100                                       | 4.5    |
| 41    | 0.20 | 0.20                   | 0.20                                | 0.05**              | 111                                       | 4.6    |
| 40    | 0.20 | 0.20                   | 0.20                                | 0.10**              | 120                                       | 4.5    |

All concentrations in moles/l; u maintained at 0.4 by addition of NaClO<sub>4</sub>.

\* Calcium perchlorate

\*\* Calcium acetate

the reaction probably involves a complex between glyceraldehyde and Ca<sup>++</sup> which forms rapidly and which is capable of a more rapid reaction with acid or base than is free glyceraldehyde. The increased reactivity of metal ion complexes has been demonstrated by Steinberger and Westheimer (103) in the decarboxylation of dimethylaloacetic acid and by Kroll (104) in the hydrolysis of amino acid esters.

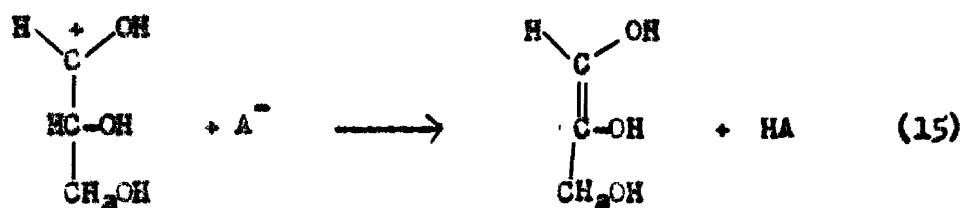
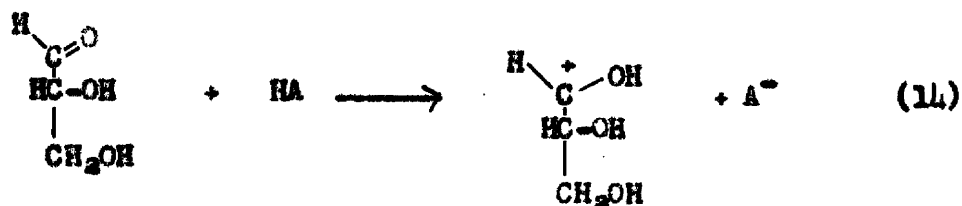
#### Mechanism of Reaction

It has been found that the over-all disappearance of glyceraldehyde is subject to an acid and base catalysis. Constant (D/P)1 values for a given acid-base system strongly suggest formation of a common intermediate capable of yielding both dihydroxyacetone and pyruvaldehyde. The nature of the catalyses support the formation of the enediol common to

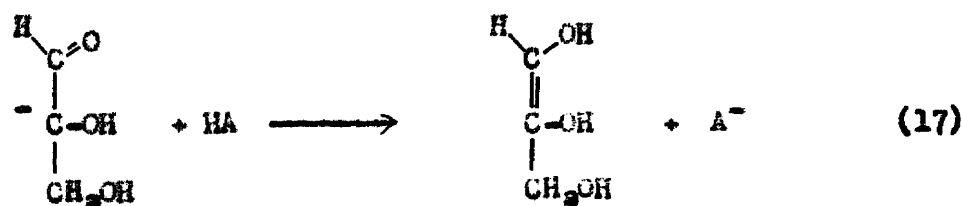
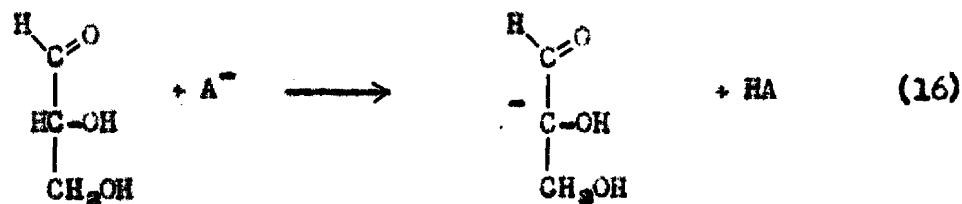


the trioses as the rate determining step according to the following schemes:

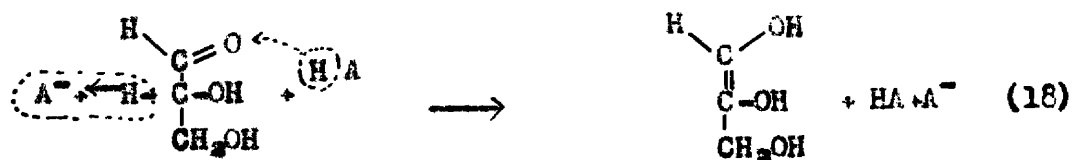
Acid catalysis —



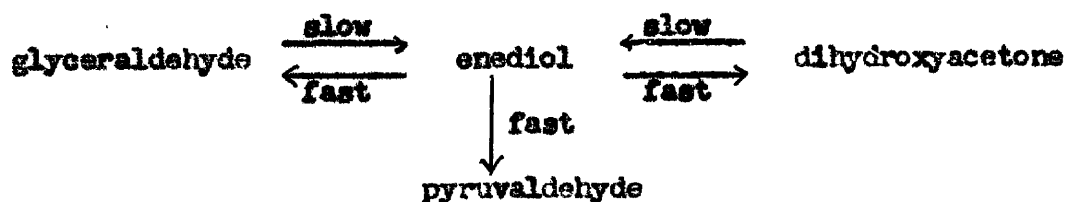
Base catalysis —



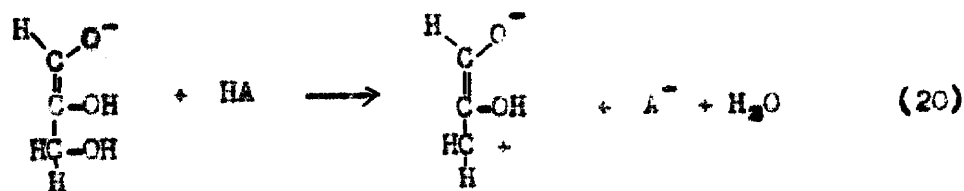
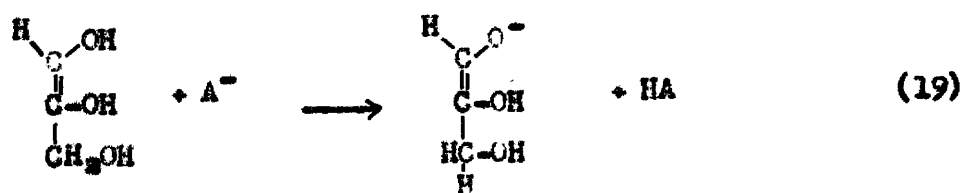
It is also possible that these processes are not separate but occur by a concerted mechanism as suggested by Swain (105).

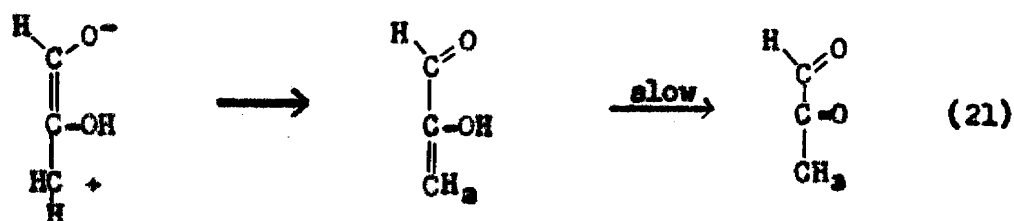


The value  $(D/P)_1$  is believed to be a measure of the relative rates of two rapid processes by which the enediol is converted to dihydroxyacetone and pyruvaldehyde respectively. The data do not permit an evaluation of the role of acids and bases in these processes although it is suspected that these substances do participate. The fact that a different  $(D/P)_1$  value is obtained in different acid-base systems implies their participation in the rapid steps. The constancy of the  $(D/P)_1$  value for a given system, independent of buffer ratio and concentration, may also imply participation of acid and/or base in the rapid steps. The degradation of glyceraldehyde may be considered as occurring as follows:



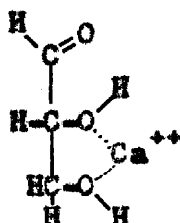
The transformation of enediol to yield pyruvaldehyde may involve a process such as:





This sort of mechanism is supported by the fact that pyruvaldehyde is apparently released as its enol. Freshly prepared pyruvaldehyde (by distillation of glyceraldehyde in sulfuric acid) displays a strong absorption maximum at approximately 250 mμ indicative of an enolic grouping. This peak disappears on standing and the curve given in Figure 1 is that of an equilibrated solution.

The apparent increased rate of enediol formation in the presence of  $\text{Ca}^{++}$  is probably the result of a glyceraldehyde- $\text{Ca}^{++}$  complex such as



It is conceivable that the electrostatic effect produced by an electrophilic  $\text{Ca}^{++}$  would increase the acidity of the hydrogen attached to the central carbon atom and thus render it more easily attacked by a basic catalyst.

### SUMMARY

1. The interconversion of glyceraldehyde and dihydroxyacetone, as well as the simultaneous conversion to pyruvaldehyde has been examined kinetically in buffered solutions of formic, acetic, and trimethylacetic acids and their respective sodium salts.
2. The degradation of glyceraldehyde has been found first order in the triose.
3. Glyceraldehyde degradation has been shown subject to an acid and base catalysis.
4. The relative rates of dihydroxyacetone and pyruvaldehyde formation have been found independent of buffer ratio and concentration for a given system; the relative rates are different in different acid-base systems.
5. The equilibrium constant for the reaction between glyceraldehyde and dihydroxyacetone has been evaluated.
6. An increase in the rate of glyceraldehyde degradation in the presence of  $\text{Ca}^{++}$  in acetate buffers has been observed; the increase was proportional to  $\text{Ca}^{++}$  concentration.
7. The possible mechanisms of the processes involved are discussed.

## LITERATURE CITED

- (1) C. A. Lobry de Bruyn, *Rec. trav. chim.*, 14, 156 (1895).
- (2) C. A. Lobry de Bruyn and W. A. van Ekenstein, *Rec. trav. chim.*, 14, 203 (1895); 16, 257, 262, 274, 282 (1897); 18, 147 (1899); 19, 1 (1900).
- (3) A. Wohl and C. Neuberg, *Ber.*, 33, 3099 (1900).
- (4) E. M. Montgomery and C. S. Hudson, *J. Am. Chem. Soc.*, 52, 2101 (1930).
- (5) W. C. Austin, *J. Am. Chem. Soc.*, 52, 2106 (1930).
- (6) W. C. Austin, C. J. Smalley, and M. I. Sankstone, *J. Am. Chem. Soc.*, 54, 1933 (1932).
- (7) S. M. Danilov and P. T. Pastukhov, *J. Gen. Chem. (U.S.S.R.)*, 16, 923 (1946).
- (8) J. B. Gottfried and D. G. Benjamin, *Ind. Eng. Chem.*, 44, 141 (1952).
- (9) J. C. Sowden and R. Schaffer, *J. Am. Chem. Soc.*, 74, 499 (1952).
- (10) H. R. Garbutt and R. S. Hubbard, *Proc. Soc. Exptl. Biol. Med.*, 33, 276, 274 (1935).
- (11) H. Murschauser, *Biochem. Z.*, 97, 97 (1919); 99, 190 (1919); 101, 74 (1919).
- (12) H. A. Spoehr and P. C. Wilber, *J. Biol. Chem.*, 69, 421 (1926).
- (13) H. A. Spoehr and H. H. Strain, *J. Biol. Chem.*, 85, 365 (1929).
- (14) H. O. L. Fischer, C. Taube, and E. Baer, *Ber.*, 60B, 479 (1927).
- (15) S. Danilov, E. Venus-Danilova, and P. Shantarovich, *Ber.*, 63B, 2269 (1930).
- (16) H. Midorikawa and T. Takeshima, *Repts. Sci. Research Inst. (Japan)*, 24, No. 5, Chem. Sect., 158 (1948).
- (17) H. Midorikawa, *Repts. Sci. Research Inst. (Japan)*, 25, No. 2/3, Chem. Sect., 33 (1949).

- (18) P. A. Levene and D. W. Hill, *J. Biol. Chem.*, 102, 563 (1933).
- (19) O. T. Schmidt and R. Treiber, *Ber.*, 668, 1765 (1933).
- (20) O. T. Schmidt and K. Haintz, *Ann.*, 515, 77 (1934).
- (21) M. Steiger and T. Reichstein, *Helv. Chim. Acta*, 19, 184 (1936).
- (22) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, 115, 731 (1936).
- (23) J. Barnett and T. Reichstein, *Helv. Chim. Acta*, 20, 1529 (1937).
- (24) K. Iwadare and B. Kubota, *Sci. Papers, Inst. Phys. Chem. Research (Tokyo)*, 34, 183 (1938).
- (25) Y. Khouvine, G. Arragon, and Y. Tomoda, *Bull. soc. chim.*, [5], 6, 354 (1939).
- (26) J. U. Nef, *Ann.*, 357, 214 (1907); 376, 1 (1910); 403, 204 (1914).
- (27) L. Michaelis and P. Rona, *Biochem. Z.*, 47, 447 (1912).
- (28) J. Groot, *Biochem. Z.*, 146, 72 (1924); 180, 341 (1927).
- (29) W. L. Evans, C. A. Buehler, C. D. Looker, R. A. Crawford, and C. W. Holl, *J. Am. Chem. Soc.*, 47, 3085 (1925).
- (30) W. L. Evans and C. A. Buehler, *J. Am. Chem. Soc.*, 47, 3098, 3102 (1925).
- (31) W. L. Evans, R. H. Edgar, and G. P. Hoff, *J. Am. Chem. Soc.*, 48, 2665 (1926).
- (32) W. L. Evans and C. E. Waring, *J. Am. Chem. Soc.*, 48, 2678 (1926).
- (33) W. L. Evans and H. B. Haas, *J. Am. Chem. Soc.*, 48, 2703 (1926).
- (34) W. L. Evans and W. R. Cornthwaite, *J. Am. Chem. Soc.*, 50, 486 (1928).
- (35) W. L. Evans and J. E. Hutchman, *J. Am. Chem. Soc.*, 50, 1496 (1928).
- (36) W. L. Evans, W. D. Nicoll, G. C. Strouse, and C. E. Waring, *J. Am. Chem. Soc.*, 50, 2267 (1928).
- (37) W. L. Evans and D. C. O'Donnell, *J. Am. Chem. Soc.*, 50, 2543 (1928).
- (38) W. L. Evans and M. P. Benoy, *J. Am. Chem. Soc.*, 52, 294 (1930).
- (39) W. L. Evans and R. F. Conway, *J. Am. Chem. Soc.*, 52, 3680 (1930).

- (40) W. L. Evans and R. C. Hockett, J. Am. Chem. Soc., 52, 4065 (1930);  
53, 4384 (1931).
- (41) W. L. Evans and C. C. Clark, J. Am. Chem. Soc., 54, 698 (1932).
- (42) G. F. Nadeau, M. R. Newlin, and W. L. Evans, J. Am. Chem. Soc., 55,  
4957 (1933).
- (43) K. G. A. Busch, J. W. Clark, L. B. Gemung, E. F. Schroeder, and  
W. L. Evans, J. Org. Chem., 1, 1 (1936).
- (44) E. L. Gustus and W. L. Lewis, J. Am. Chem. Soc., 49, 1512 (1927).
- (45) M. L. Wolfrom and W. L. Lewis, J. Am. Chem. Soc., 50, 837 (1928).
- (46) R. D. Greene and W. L. Lewis, J. Am. Chem. Soc., 50, 2813 (1928).
- (47) C. E. Gross and W. L. Lewis, J. Am. Chem. Soc., 53, 2772 (1931).
- (48) H. T. Neher and W. L. Lewis, J. Am. Chem. Soc., 53, 4411 (1931).
- (49) D. J. Loder and W. L. Lewis, J. Am. Chem. Soc., 54, 1040 (1932).
- (50) M. L. Wolfrom, M. Honigsberg, F. B. Moody, and R. M. Goepf, Jr.,  
J. Am. Chem. Soc., 68, 122 (1946).
- (51) M. L. Wolfrom, F. B. Moody, M. Konigsberg, and R. M. Goepf, Jr.,  
J. Am. Chem. Soc., 68, 578 (1946).
- (52) M. L. Wolfrom, B. W. Lew, and R. M. Goepf, Jr., J. Am. Chem. Soc.,  
68, 1443 (1946).
- (53) M. L. Wolfrom, B. W. Lew, R. A. Hales, and R. M. Goepf, Jr.,  
J. Am. Chem. Soc., 68, 2342 (1946).
- (54) M. L. Wolfrom, W. W. Binkley, C. C. Spencer, and B. W. Lew,  
J. Am. Chem. Soc., 73, 3357 (1951).
- (55) H. Fredenhagen and K. F. Bonhoeffer, Z. physik. Chem., A181, 392  
(1938).
- (56) K. Goto, J. Chem. Soc. Japan, 63, 217 (1942).
- (57) A. A. Bothner-By and M. Gibbs, J. Am. Chem. Soc., 72, 4805 (1950).
- (58) Y. J. Tepper and D. Stetten, Jr., J. Biol. Chem., 189, 191 (1951).
- (59) J. C. Sowden and R. Schaffer, J. Am. Chem. Soc., 74, 505 (1952).

- (60) P. A. Ashmarin and Y. S. Belova, Arch. sci. biol. (U.S.S.R.) 42, No. 3, 53 (1936).
- (61) P. A. Ashmarin and A. D. Braun, Arch. sci. biol. (U.S.S.R.), 42, No. 3, 61 (1936).
- (62) P. A. Ashmarin and A. D. Braun, Byull. Eksptl. Biol. Med., 4, No. 4, 374 (1937).
- (63) A. D. Braun and R. K. Konnova, Biokhimiya, 5, 497 (1940).
- (64) A. D. Braun, Biokhimiya, 2, 801 (1937).
- (65) H. Midorikawa, Repts. Sci. Research Inst. (Japan), 26, No. 1, 22 (1950).
- (66) T. M. Lowry and I. J. Faulkner, J. Chem. Soc., 127, 2883 (1925).
- (67) C. A. Lobry de Bruyn and W. A. van Ekenstein, Rec. trav. chim., 15, 92 (1896).
- (68) A. Kuzin, Ber., 69B, 1041 (1936).
- (69) F. Wind, Biochem. Z., 159, 58 (1925).
- (70) L. Ahlstrom and H. von Euler, Z. physiol. Chem., 200, 233 (1931).
- (71) C. Neuberg and W. Oertel, Biochem. Z., 55, 494 (1913).
- (72) A. Fernbach and M. Schoen, Compt. rend., 158, 976 (1914).
- (73) F. Fischler, Z. physiol. Chem., 157, 1 (1926); 165, 53 (1927).
- (74) F. Fischler and A. F. Lindner, Z. physiol. Chem., 175, 237 (1928).
- (75) C. Neuberg and B. Rewald, Biochem. Z., 71, 144 (1915).
- (76) K. Bernhauer and H. Wolf, Biochem. Z., 212, 443 (1929).
- (77) C. Enders and R. Marquardt, Naturwissenschaften, 29, 46 (1941).
- (78) C. N. Cameron, J. Am. Chem. Soc., 49, 1759 (1927).
- (79) K. Taufel and H. Burmeister, Z. anal. Chem., 129, 352 (1949).
- (80) C. Enders, Biochem. Z., 312, 349 (1942).
- (81) R. Nodzu, Bull. Chem. Soc. Japan, 10, 122 (1935).



- (82) R. Nodzu and K. Matsui, *Bull. Chem. Soc. Japan*, 10, 467 (1935).
- (83) R. Nodzu, K. Matsui, R. Goto, and S. Kunichika, *Mem. Coll. Sci., Kyoto Imp. Univ.*, 20A, 197 (1937).
- (84) R. Goto, *Bull. Chem. Soc., Japan*, 15, 103 (1940).
- (85) R. Nodzu and R. Goto, *Bull. Chem. Soc. Japan*, 11, 361 (1936); 15, 209 (1940).
- (86) R. Goto, *J. Chem. Soc. Japan*, 64, 999, 1054, 1183 (1943).
- (87) L. Sattler and F. W. Zerban, *J. Am. Chem. Soc.*, 70, 1975 (1948).
- (88) J. C. Speck, Jr., unpublished data.
- (89) G. Denigès, *Compt. rend.*, 148, 422 (1909).
- (90) C. Neuberg, A. Levite, and E. Schwenk, *Biochem. Z.*, 83, 244 (1917).
- (91) H. O. L. Fischer and C. Taube, *Ber.*, 57B, 1502 (1924).
- (92) Z. Dische and S. S. Robbins, *Biochem. Z.*, 274, 42 (1934).
- (93) H. H. Strain and H. A. Spoehr, *J. Biol. Chem.*, 89, 527 (1930).
- (94) L. I. Smith and R. H. Anderson, *J. Org. Chem.*, 16, 963 (1951).
- (95) H. O. L. Fischer and E. Baer, *Helv. Chim. Acta*, 18, 514 (1935).
- (96) P. Fleury and J. Lange, *J. Pharm. Chim.*, 17, 107 (1933).
- (97) J. C. Speck, Jr., A. A. Forist, and W. B. Neely, unpublished results.
- (98) W. G. Berl and C. E. Feazel, *J. Am. Chem. Soc.*, 73, 2054 (1951).
- (99) S. M. Partridge and R. O. Westfall, *Biochem. J.*, 42, 238 (1948).
- (100) B. J. Thornton and J. C. Speck, Jr., *Anal. Chem.*, 22, 899 (1950).
- (101) H. F. Utter and C. H. Werkman, *J. Bact.*, 42, 665 (1941).
- (102) C. Meyerhof and R. Junowicz-Kocholaty, *J. Biol. Chem.*, 149, 71 (1943).
- (103) R. Steinberger and F. H. Westheimer, *J. Am. Chem. Soc.*, 73, 429 (1951).
- (104) H. Kroll, *J. Am. Chem. Soc.*, 74, 2036 (1952).
- (105) C. G. Swain, *J. Am. Chem. Soc.*, 72, 4576 (1950).

I

KINETICS OF THE AMINO ACID AND PEPTIDE CATALYZED  
DEALDOLIZATION OF DIACETONE ALCOHOL

II

DETERMINATION OF THE HYDROXYMETHYL GROUP  
IN SUGARS AND RELATED SUBSTANCES

III

KINETICS OF THE ACID AND BASE CATALYZED  
DEGRADATION OF THE THIOSES

By

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AN ABSTRACT

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THESIS ABSTRACT

The catalysis of the dealdolization of diacetone alcohol in buffered solutions of glycine, DL- $\alpha$ -alanine,  $\beta$ -alanine, and glycylglycine and their respective sodium salts was examined kinetically by a dilatometric technique at 18.60° C. The amino acid or peptide anion was the catalytically active species, the order of activity being  $\beta$ -alanine > glycine > DL- $\alpha$ -alanine-glycylglycine. Metallic ions did not accelerate the reaction. Ions capable of forming amine complexes inhibited the reaction. The disproportionation of a cyclic intermediate involving the amine catalyst and diacetone alcohol was postulated as the rate determining step in the process.

A new method for determining the hydroxymethyl group in sugars and related substances was developed which consists of a periodate oxidation in neutral solution buffered by bicarbonate, destruction of excess periodate and iodate by addition of sodium sulfite, and subsequent spectrophotometric determination of formaldehyde in the reaction mixture after reaction with chromotropic acid. Glycolic acid interference was eliminated without reduction in sensitivity by using 1M sulfuric acid for development of the dye. A thirty minute aeration was sufficient to remove excess sulfur dioxide from the dye solution. Both the formaldehyde-sulfite solution and the formaldehyde-chromotropic acid dye were stable for at least forty-eight hours. D-glucose, D-xylose, DL-glyceraldehyde, dihydroxyacetone, D-mannitol, and maltose were successfully determined by this procedure. DL-serine gave consistently low yields of measurable formaldehyde. Maltose produced one mole of formaldehyde per mole of disaccharide suggesting use of this method in end group analysis of polysaccharides.

DL-glyceraldehyde was found to produce dihydroxyacetone and pyruvaldehyde in acidic buffers. These reactions of DL-glyceraldehyde were investigated kinetically in buffered solutions of formic, acetic, and trimethylacetic acids and their respective sodium salts at 50° C. The reaction components were determined by simultaneous measurement of periodate consumption and estimation of total trioses (the latter by means of the method described above). The degradation was of the first order in the triose and was subject to an acid and base catalysis. The relative rates of dihydroxyacetone and pyruvaldehyde formation were independent of buffer ratio and concentration for a given system but were different in different acid-base systems. The equilibrium constant for the conversion of glyceraldehyde to dihydroxyacetone was approximately 17. Calcium ion increased the rate of glyceraldehyde degradation, in proportion to the calcium ion concentration. The formation of an enediol was suggested as the rate determining step in the degradation of DL-glyceraldehyde. This enediol was thought to react by two separate, rapid processes to yield dihydroxyacetone and pyruvaldehyde. Formation of a triose-calcium ion complex more reactive than the free sugar was postulated.