PART I

THE LOBRY de BRUYN TRANSFORMATION OF D-GLUCOSE AND 3,4,6-TRIMETHYL-D-FRUCTOSE

PART II

KINETICS OF THE METAL ION CATALYZED DEGRADATION OF DL-GLYCERALDEHYDE

By

Don Shuso Miyada

A THESIS

Submitted to the School of Graduate Studies of Michigan
State College of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Chemistry

1953

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Don S. Miyada

THESIS ABSTRACT

The base catalyzed degradation of D-glucose was studied kinetically in sodium hydroxide solutions by two methods of analysis of the reaction mixtures involving specific oxidation and the optical rotation of such mixtures. The implications of the results are discussed.

The base catalyzed degradation of 3,4,6-trimethyl-D-fructose in dilute solutions of calcium, barium, and sodium hydroxides was investigated. There appeared to be no difference in the trend of formation of the methylated products with the three bases; however, in solutions containing alkaline earth bases, a steady increase in periodate consumption was observed which was probably caused by the demethylation of the methylated hexoses. A comparison of the effect by these bases in the enolization reaction indicated that hydroxyl ion catalysis predominates.

A new set of values for the catalytic constants of acetic acid and acetate ion was obtained for the general acid-base catalyzed degradation of glyceraldehyde, and the dependency of the reaction rate on ionic strength was determined. The effect of metal ion catalyzed degradation of glyceraldehyde was investigated using the following metal ions: lithium, calcium, barium and magnesium. An acceleration in the rate of reaction was observed in all cases except with magnesium ion where increased metal ion concentration produced a diminution of the pseudo first order rate constant. A mathematical treatment of the catalysis by calcium ion, based on (1) incomplete dissociation of the species $CaOAc^+$

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and (2) the limitation of catalysis by the calcium acetate system to calcium and acetate ions, was found to give pseudo first order rate constants which compared favorably with experimentally determined values as well as a reasonable dissociation constant for the species, $CaOAc^+$.

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HISTORICAL INTRODUCTION

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HISTORICAL INTRODUCTION

The reversible transformation of aldoses and ketoses occurring in dilute alkaline solution was first described by Lobry de Bruyn and Alberda van Ekenstein in 1895. (1,2) These investigators studied the interconversion of fructose, mannose, and glucose under the influence of dilute solutions of different metallic bases. Their later work included investigation of similar transformations of galactose, melibiose, maltose, and lactose. (3) Other investigators have studied the effects of the metallic hydroxides on glyceraldehyde, (4) lactose, (5) glucoheptose, (6) xylose and arabinose, (7) and cellobiose. (8)

Organic bases have also been used to catalyze the Lobry de Bruyn transformation. Fischer, Taube, and Baer converted glyceraldehyde into dihydroxyacetone using the base pyridine. (9) Danilov and co-workers reported that glucose was isomerized to fructose without any admixed mannose when heated with either pyridine or quinoline; however, in either aqueous pyridine or aqueous alcoholic quinoline, the rearrangement did not proceed so smoothly and was accompanied by acid and mannose formation. (10) Midorikawa and Takeshima confirmed these observations (11) and extended them to include quinaldine as a catalyst. (12) Anhydrous organic bases, especially pyridine, have been of value in the preparation of many ketoses from the corresponding aldoses. (13-20)

Lobry de Bruyn found that boiling water caused the isomerization of fructose. Garbutt and Hubbard demonstrated the interconversion of glucose, fructose, and mannose in boiling aqueous solutions buffered at

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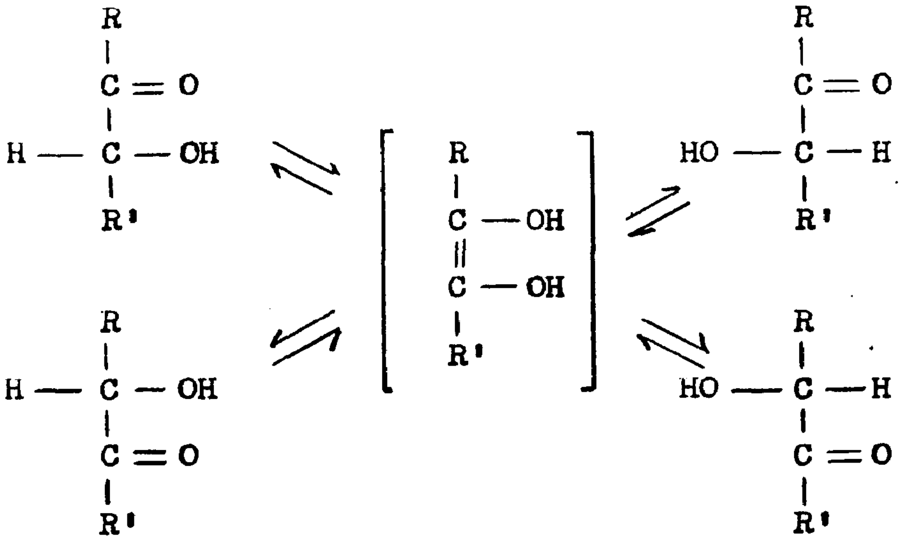
neutrality. (21) Spoehr and co-workers observed this transformation in presence of neutral and slightly acid phosphate buffers. (22,23) The number of investigators applying different conditions to this basic isomerization reaction is legion, and only a few are mentioned here.

Despite the large number of investigations of this reaction, precise data in the literature having to do with its kinetics or mechanism are strictly limited. This is probably due to the fact that the transformation is complicated by fragmentation reactions, acid formation, and rearrangements, all of which were recognized very early.

The generally accepted mechanism of the Lobry de Bruyn-van Ekenstein transformation postulates enediol intermediates and may be represented schematically as follows:



[R = H, CH_2OH, etc. ; R' = H, CH_2OH, etc.]

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As early as 1900, Wohl and Neuberg explained the conversion of glyceraldehyde to dihydroxyacetone in alkaline solutions on the basis of an enediol intermediate. (4) Lobry de Bruyn and, in his earlier writings, Nef, (24) assumed the enediol to be formed by the alternate addition and removal of the elements of water. Later in 1910, Nef (25) assumed a molecular shift as the basis for enediol formation, and the research of Lewis and co-workers with tetramethyl-hexoses and trimethyl-pentoses supported the simpler concept of enolization. (26-30) Since no ketoses were found, it was suggested that only the 1-2 enediol can form in these methylated sugars. In addition, high iodine absorbing substances believed to be the enediol intermediates were present in alkaline solution but rapidly disappeared on acidification. The investigations of Michaelis and Rona (31) and Groot (32) also implied enediol formation. In a series of investigations of carbohydrate oxidations, Evans and his co-workers repeated and extended the observations of Nef by conducting quantitative studies of products formed under more carefully controlled experimental conditions. (33-47) They accepted Nef's postulation of an equilibrium between the sugars and a series of enediols in alkaline solution.

Electroreduction of sugars in alkaline solutions by Wolfrom and co-workers gave isomeric polyols indicating the presence of 1,2- and 2,3-enediols. (49-52)

Deuterium analysis has produced conflicting results. Early studies by Fredenhagen and Bonhoeffer indicated no incorporation of carbon bound deuterium with D-glucose at 25° C. (53) On this basis, a "dimer

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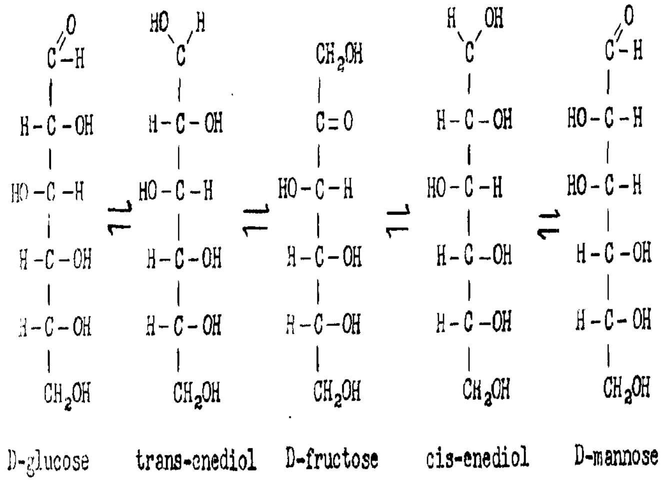
intermediate" was postulated. Goto reported similar results. (54) In search of an explanation for this reported isomerization without exchange of carbon bound hydrogen by deuterium, Bothner-By and Gibbs employed 1-C^{14}-D-glucose to test the possibility of carbon chain rearrangement during the reaction. (55) No such rearrangement was observed. Topper and Stetten in a reinvestigation of the reactions of D-glucose in heavy water observed deuterium exchange in agreement with the enediol mechanism. (56) From their results, they postulated the following mechanism.



Sowden and Schaffer (57) working with heavy water presented further evidence for deuterium exchange at 25°C. but concluded that fructose was not a necessary intermediate in the conversion of glucose to mannose. The consensus appears to indicate the general acceptance of an enediol intermediate brought about by an enolization of the sugars.

The nature and the mode of action of catalysts have received little attention. The majority of the reactions have been carried out in alkaline solutions, and the activity of the bases has been attributed to the hydroxyl ion concentration. Utilizing the initial rate method, Michaelis and Rona found that the rate of isomerization of D-glucose was directly proportional to the hydroxyl ion concentration. (31)

Spoehr and Strain studied the interconversion of glucose to fructose in a slightly acidic phosphate buffer. (58) Ashmarin and co-workers studied the effects of acetate, formate, and succinate buffers on glucose and fructose. (59-61) The latter investigators found that, in all instances, these anions acted as bases according to the Bronsted theory in catalyzing the transformation. Braun and Konnova reported similar findings with an acetate buffer. (62)

Using aqueous pyridine systems, Midorikawa observed that with increasing pyridine concentrations, the conversion of glucose to fructose increased to a maximum and subsequently decreased. In analogy with Lowry and Faulkner's results on the mutarotation of glucose, (63) Midorikawa proposed an acid-base catalysis. (64) Employing methods based on periodate scission, Forist and Speck have not only demonstrated acid-base catalysis in the interconversion of glyceraldehyde to dihydroxyacetone but have determined the kinetics of this transformation under the influence of acetate, formate, and trimethylacetate buffer systems. (64)

Several investigators have found variations in the Lobry de Bruyn transformation depending on the particular cationic species involved. Lobry de Bruyn and van Ekenstein reported that $Pb(OH)_2$ converted glucose to mannose with no detectable fructose, and that under these conditions fructose was not isomerized to the corresponding aldose. (3) Nef reported that enolization of hexose was not caused by calcium and lead acetates

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or by calcium chloride. In 1919, Murschauser, in a series of papers, (65) described the effects of alkaline earth carbonates on D-glucose and concluded that dextrose is converted to levulose and eventually into other levorotatory or weakly dextrorotatory sugars. In 1926, Kusin reported that the mechanism of isomerization shows cationic dependence in that different types of enolic intermediates are involved when alkalies containing, respectively, mono- or divalent cations are employed. (66, 67) A comparative study of the action of calcium hydroxide and sodium hydroxide on D-glucose and D-fructose at 25°C. indicated that with calcium hydroxide, the sugars showed reducing power which persisted for some time after acidification. Such results were not obtained with sodium hydroxide. Calcium hydroxide favored the formation of mannose from glucose, whereas sodium hydroxide favored the formation of fructose from glucose. Hence, Kusin postulated that, at low temperatures, calcium hydroxide produced a cyclic enol without rupture of the pyranose ring, and sodium hydroxide produced an acyclic enol. At higher temperatures, no difference in the action of the two bases was detectable.

Recently, Sowden and Schaffer have reported that the nature of the initial course of the isomerization of D-mannose is dependent on the cationic species present. (68) These investigators observed this effect with 0.5 N bases at 25°C.; however, at base concentrations of 0.035 N and at a temperature of 35°C., they found no differences when the cationic species were changed. Wind (69) and Ahlstrom and von Euler (70) reported that oxidations of glyceraldehyde in buffered solutions are catalyzed by heavy metals. Assuming the enediol as the oxidizable species, this may

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indicate increased ability for enediol formation in the presence of these ions.

A reaction which complicates the Lobry de Bruyn reaction is the dehydration of sugars; this was observed by Deniges (71) and Fischer and Taube (72) with dihydroxy acetone. However, again, little information is available concerning either the kinetics or the mechanism of the transformation of glyceraldehyde and dihydroxyacetone into pyruvaldehyde. Various investigators have suggested that a common enediol is involved in the conversion of the trioses to pyruvaldehyde. Among these were Evans and Cornthwaite, (40) Strain and Spoehr, (60) and Smith and Anderson. (73) In 1934, Dische and Robbins reported that the addition of phosphate or arsenate catalyzes the transformation of glyceraldehyde and dihydroxyacetone to pyruvaldehyde. (74) The addition of other ions in the form of calcium chloride, sodium fluoride, sodium citrate, sodium sulfate, copper sulfate, ferric chloride, or ferric sulfate in appropriate concentrations had no effect on this reaction in neutral solutions. Recently, Forist and Speck have shown that a general acid-base catalysis exists for this reaction also.

The present work is comprised of a reinvestigation of the alkaline degradation of reducing sugars, and the effects of metallic ions in the Lobry de Bruyn transformation by application of new analytical methods capable of a high degree of precision to these reaction mixtures. In this, hexoses were chosen for investigation of the reactions occurring in alkaline media for the reason that side reactions, such as aldolization and dehydration, appear to be slower relative to enolization than

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they are with simpler sugars. Moreover, the higher carboned reducing sugars possess large and well established specific rotations, thus making possible examination of the systems for stereospecific effects. In order to gain further insight to the Lobry de Bruyn transformation of a substance with which dehydration effects are minimal, the work has been extended to include examination of the isomerization of 3,4,6-trimethyl-D-fructose as catalyzed by sodium, calcium, and barium hydroxides.

The investigation of the effects of metal ions in the Lobry de Bruyn transformation under acidic conditions has been carried out with glyceraldehyde in acetate buffers. This triose reacts at conveniently measurable rates under these circumstances at moderate temperatures and concentrations, and dehydration proceeds to a definite product, pyruvaldehyde, which permits a detailed analysis of the system.

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EXPERIMENTAL

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PART I

The Lobry de Bruyn Transformation of D-glucose and 3,4,6-trimethyl-D-fructose.

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EXPERIMENTAL METHODS

Materials

The D-glucose (76) and D-fructose (77) employed in these measurements were prepared according to Bureau of Standards procedure. Matheson Company practical grade chromotropic acid was recrystallized from 50% ethanol. Periodic acid, perchloratoceric acid, and nitroferroin were obtained from G. Frederick Smith Chemical Company. Matheson's practical grade dimethylsulfate was vacuum distilled before using. Inulin was a Nutritional Biochemicals Corporation product. Pyridine and chloroform were Bakers analytical reagent grade chemicals. Acetic anhydride was an Eastman Kodak Company white label product and phenylhydrazine was an Eastman Kodak Company yellow label product. Acetone, oxalic acid, calcium carbonate, calcium hydroxide, barium carbonate, barium hydroxide, sodium hydroxide, sodium bicarbonate, sodium sulfite, hydrochloric acid, anhydrous sodium sulfate, anhydrous magnesium sulfate, phosphorous pentoxide, arsenious acid, potassium iodide, and iodine were all C. P. reagents. Sulfuric acid was Merck reagent grade.

Preparation of 3,4,6-trimethyl-D-fructose

In order to prepare 3,4,6-trimethyl-D-fructose, the general procedure has been to methylate inulin, a natural fructosan, hydrolyze the methylated product, hydrolyze the resulting methyl-fructoside and then isolate and purify the subsequently formed 3,4,6-trimethyl-D-fructose. There are

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two distinct methods for methylating inulin; they will be designated method A and method B. Method A was that described by Hirst, McGilvray, and Percival (78) and involves an initial methylation of inulin by dimethylsulfate followed by repeated methylations with methyl iodide and silver oxide.

In method B, the procedure of Haworth and Streight, (79) trimethyl-inulin is prepared by dimethyl sulfate methylation of triacetyl-inulin. Preliminary experiments indicated that the latter method was the more practicable. The following is a description of the slight modification of Haworth's method which was used for preparing trimethyl-fructose.

(1) Swelling of Inulin. One hundred grams of inulin was vigorously stirred with a liter of pyridine at 80°C. for approximately two hours. During this period, the mixture became a clear, yellowish-green solution. The solution was then cooled with continued stirring.

(2) Acetylation of Inulin. Two hundred milliliters of acetic anhydride was gradually added over a period of six hours to the above material, representing 100 g. of anhydrous inulin. During the addition, the reaction mixture was stirred vigorously and maintained at 20°C. This was followed by the gradual addition of 370 ml. of acetic anhydride with stirring at 20°C. The resulting solution was allowed to stand 12 hours. The supernatant liquid was then poured into 15 liters of ice water. A white precipitate of crude triacetyl-inulin formed immediately. This was filtered in portions and repeatedly washed with distilled water to

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remove pyridine and acetic anhydride. The product was dried in air and then placed over potassium hydroxide for further drying.* The yield of crude product was 202 g., 97 per cent of theory.

(3) Preparation of Trimethyl-inulin. Twelve grams of triacetyl-inulin was dissolved in 250 ml. of acetone in a two liter flask fitted with a Hirschberg stirrer. The temperature was kept at 55°C. during the gradual addition of 120 ml. of dimethylsulfate and 320 ml. of 30 per cent, aqueous sodium hydroxide. One-tenth of the volume of each reagent was added every ten minutes. After the second or third addition, an emulsion formed which persisted until the end of the reaction. During the formation of the emulsion, the temperature rose rapidly unless the reaction mixture was cooled. When the addition of dimethylsulfate and sodium hydroxide was complete, 100 ml. of water was added, and the temperature of the reaction mixture was raised to 75°C. for a period of 15 minutes to distil off the bulk of the acetone. During this operation, trimethyl-inulin precipitated in the form of pale yellow pellets. This solid product was separated from the warm reaction mixture by filtration. It was then digested three times for two hours with 70-ml. portions of boiling water. After trituration of the resulting product with acetone and ether, the methylated inulin was obtained as a fine, white product. Methylated inulin is soluble in acetone, hence only 5 ml. of acetone was used in this operation. Repeated trituration with 15-ml. portions of

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*Although recrystallization of triacetyl-inulin can be accomplished from hot methanol, it was found that the large losses involved made such purification impractical at this stage of the preparation.

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ether removed the colored impurities. The above procedure appears to give the best results, inasmuch as a batch using twice the amounts of reagents gave an incompletely methylated product. (79) The melting point of the product was 140°C. which is in agreement with values reported by previous investigators. (79) The average yield was 7.1 g. of trimethyl-inulin, 97 per cent of the theory based on triacetyl-inulin.

(4) Hydrolysis of Trimethyl-inulin. Trimethyl-inulin was hydrolyzed in 5-g. portions by digesting with 250 ml. of 70 per cent alcohol in which was dissolved 2.5 g. of oxalic acid. The digestions were carried out at 80°C. for from eleven to seventeen hours. The shorter period gave a poor yield, possibly due to incomplete hydrolysis. The oxalic acid solution was then neutralized with calcium carbonate and the solution was allowed to stand for several hours. The supernatant liquid was decanted through a fluted filter paper and the residual mixture was centrifuged. The liquids were combined and evaporated under reduced pressure (15 mm.) at 40°C. The resulting light syrup was extracted repeatedly with chloroform. The chloroform extracts were combined, and dried over anhydrous sodium sulfate and again reduced to a syrup. Because of a possible presence of a fructoside, the syrup was digested with 0.25 per cent, aqueous hydrochloric acid for seventy hours at 20°C. The mineral acid was neutralized with barium carbonate, and the solution was filtered to remove traces of an unidentified substance which was found to be soluble in chloroform but insoluble in liquid trimethyl-D-fructose. The filtrate was reduced to a syrup by distillation at

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reduced pressure, and the syrup was again extracted with chloroform. The extract was reduced again to a syrup. This syrup was dissolved in dry benzene and the benzene was distilled at reduced pressure in order to remove final traces of water. The residue was then distilled under reduced pressure, and the colorless fraction distilling at 115°C. at 0.2 mm. was collected. The average yield of trimethyl-D-fructose was 3.8 g. 76 per cent of the theory based on trimethyl-inulin. $[\alpha]_D^{20}$ = 23.1° in chloroform. (conc. = 1.02 g. per 100 ml. of solution.) $[\alpha]_D^{20}$ = 28.9° in water. (conc. = 1.02 g. per 100 ml. of solution) The osazone prepared according to the procedure of Haworth and Learner (80) was a yellow product melting at 80°-81°C. in close agreement with values reported by previous investigators.[*] (80,81)

The analysis based on the release of formaldehyde as a consequence of periodate scission, gave the expected result of one mole of formaldehyde per mole of 3,4,6-trimethyl-D-fructose. These results are shown in Table II. The results of periodate reduction, which also gave nearly the expected value for pure trimethyl-fructose are shown in Table I and Figure 1.

Apparatus

A Beckman Model B spectrophotometer equipped with matched Corex cells was used for optical density measurements. A Rudolph polarimeter

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[*] Optical rotation data do not compare as favorably with literature values. $[\alpha]_D^{20}$ = 23.1 in chloroform. $[\alpha]_D^{20}$ = 26.3° (82), 27.7° (81), 25° (80) in chloroform. $[\alpha]_D^{20}$ = 28.9° in water. $[\alpha]_D^{18}$ = 30.51° in water (83).

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TABLE I

PERIODATE OXIDATION OF 3,4,6 TRIMETHYL-D-FRUCTOSE

Oxidation Time (Hours)	Equivalents of IO_4^- Consumed Per Mole of Sugar**	Theoretical Consumption Of IO_4^- Per Mole of Sugar**
2	1.871	2.028
4	1.897	2.028
8	1.923	2.028
12	2.019	2.028
18	2.121	2.028

* All values are average of duplicate analysis.
** The word sugar refers to 3,4,6 trimethyl-D-fructose.

TABLE II

FORMALDEHYDE ANALYSIS OF 3,4,6 TRIMETHYL-D-FRUCTOSE

Oxidation Time (Hours)	Moles of Formaldehyde Found x 10^6	Theoretical Amount of Formaldehyde in Moles x 10^6
1.25	0.984	0.994
2.00	1.014	1.019

* All values are average of quadruplicate analysis.

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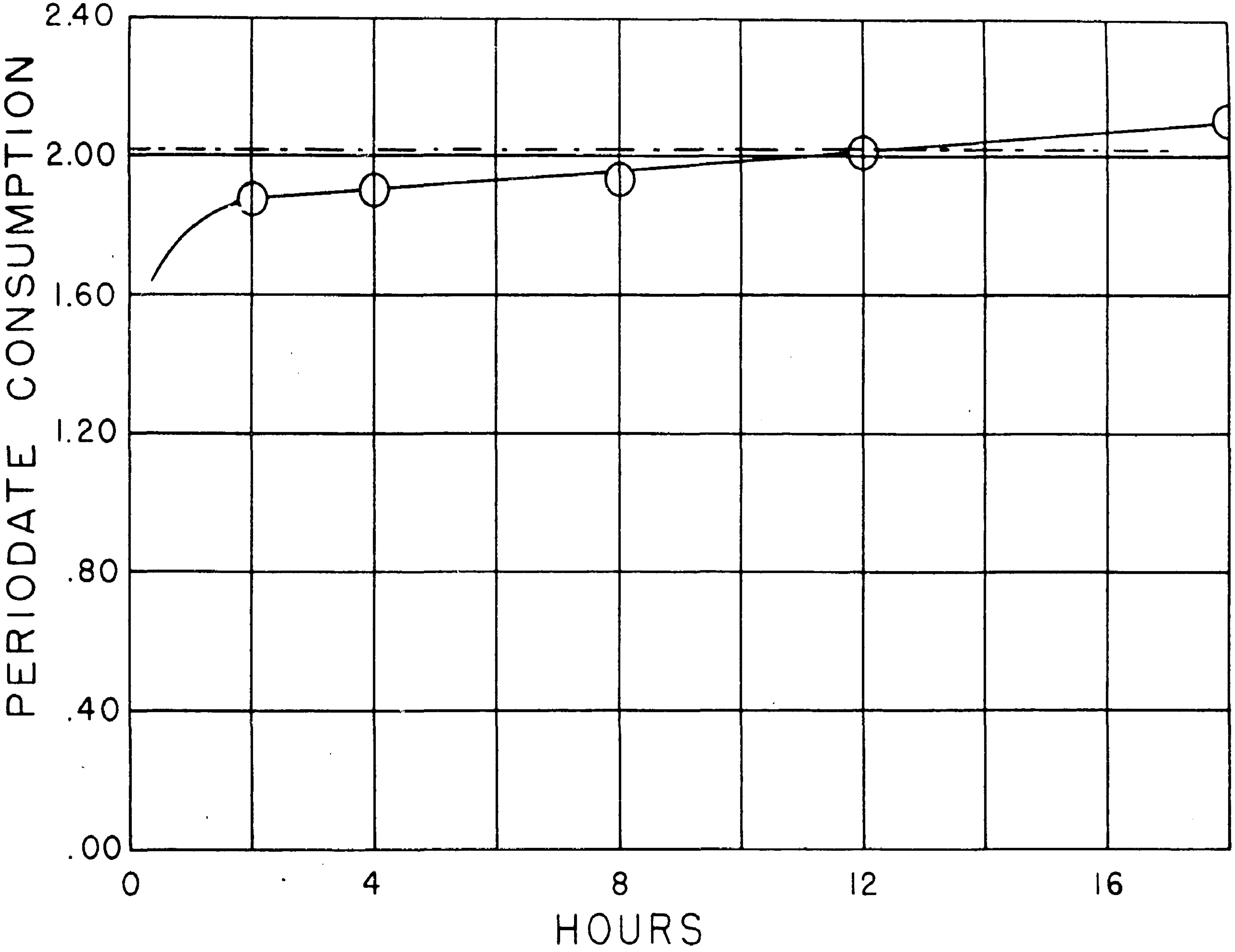

Figure 1. Periodate consumption per mole of 3,4,6-trimethyl-D-fructose in terms of equivalents of periodate per liter versus oxidation time.

equipped with a sodium lamp and a thermostat was employed for optical rotation measurements.

Quantitative Analysis of Reaction Mixture

In the Lobry de Bruyn reaction there are two principal products and the original carbohydrate in the reaction mixture. Such a mixture obviously requires three analytical methods or three independent equations for its description. Assuming that the starting hexose is transformed into only the other two hexoses, the stoichiometry of the reaction provides one equation. Two analytical methods are then required to estimate the concentrations of the three principal products. The first analytical method employed involved optical rotation measurements. The expression for the rotation of such a mixture is as follows.

$$\left[\frac{[\alpha]_D^{25} \cdot C \cdot d}{100}\right]_{\text{Glucose}} + \left[\frac{[\alpha]_D^{25} \cdot C \cdot d}{100}\right]_{\text{Mannose}} + \left[\frac{[\alpha]_D^{25} \cdot C \cdot d}{100}\right]_{\text{Fructose}} = \text{optical rotation of reaction mixture.}$$

By the substitution of 10c/M = moles per liter, the equation was reduced to

$$[.036]\,[G]\,[\alpha_G]_D^{25^\circ} + [.036]\,[M]\,[\alpha_M]_D^{25^\circ} + [.036]\,[F]\,[\alpha_F]_D^{25^\circ}$$

= optical rotation of reaction mixture or

$$[G]\,[\alpha_G]_D^{25^\circ} + [M]\,[\alpha_M]_D^{25^\circ} + [F]\,[\alpha_F]_D^{25^\circ} = \frac{\text{optical rotation}}{.036} \qquad (1)$$

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$[\alpha]_D^{25^\circ}$ = specific rotation of the sugar at 25°C. using sodium light. The subscripts within the brackets refer to the first letter of the hexose. The values for specific rotations are 52.7°, 14.2°, and -92.4° for glucose, mannose, and fructose respectively.

C = grams of hexose in 100 ml. of water at 25°C.

d = length of polarimeter tube in decimeters.

[G], [M] and [F] refer to the concentrations of glucose, mannose, and fructose respectively in moles per liter.

The additivity of optical rotations of dilute solutions of these sugars was tested, and in all instances, the added values were, within experimental error, in agreement with corresponding values calculated from specific rotation data. This principle has long been used in carbohydrate chemistry in the analysis of simple mixtures of sugars.

The second of the analytical methods involved the Malaprade reaction in which the uptake of periodate by the reaction mixture was measured. The equation employed follows:

$$[10.20]\ [G] + [10.20]\ [M] + [9.40]\ [F] = I \qquad (2)$$

I is equal to the periodate uptake of the reaction mixture per millimol of sugar. The value 10.20 represents the equivalents of periodate reduced per mole of glucose or mannose by the methods used in this experiment. This value is an average obtained from a large number of determinations and is assumed to be the value for mannose because both sugars give the same products in equal quantities on oxidation. Rechecks on

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the reagents and the method of analysis consistently gave the value of 10.20 instead of 10.00 milliequivalents of periodate reduced per millimol of sugar.

The value, 9.40, represents the equivalents of periodate reduced by one mole of fructose under the conditions of this experiment. During early experiments, oxidations made with periodic acid gave inconsistent results, and it was found that glyoxylic acid, a product of periodate oxidation of fructose, was oxidized subsequent to the addition of sodium bicarbonate in the determination of unreduced periodate. The oxidation of glyoxylic acid by bicarbonate buffered periodate is described by Chargaff and Sprinson. (84) Accordingly, all periodate oxidations of the hexoses were performed in bicarbonate buffer. The value, 9.40, is in good agreement with literature values. Reeves (85) obtained 1.7 moles of formaldehyde per mole of fructose, and Chargaff and Sprinson (84) reported a periodate uptake of 4.8 moles per mole of fructose. All of the concentration terms in the three simultaneous equations are in moles per liter.

An attempt was made to replace periodate oxidation by perchloratoceric acid oxidation as described by Forist, Speck, and Neely. (86) This appeared attractive since fourteen equivalents of Ce^{++} are required to oxidize one mole of fructose, whereas twelve equivalents of Ce^{++} are required to oxidize one mole of either glucose or mannose. However, this approach to the analysis of these mixtures gave results of a low order of reproducibility.

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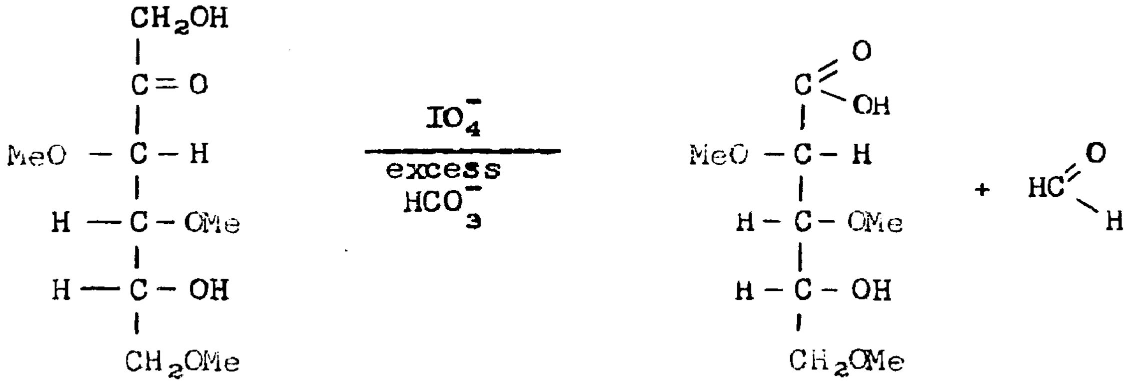
The methods of analysis of reaction mixtures containing 3,4,6-trimethyl-D-fructose as substrate are similar to the ones just described. 3,4,6-trimethyl-D-fructose affords a convenient analysis, for as indicated in the equation below, two equivalents of periodate are consumed per mole of trimethyl-fructose, and one mole of formaldehyde is released.



Hence by analyzing aliquots of the reaction mixture for formaldehyde, after periodate scission, the rate of disappearance of the starting fructose derivative could be determined. The principal products, assuming a Lobry de Bruyn type of reaction, are 3,4,6-trimethyl-D-glucose and 3,4,6-trimethyl-D-mannose. Neither of these substances release formaldehyde when oxidized with bicarbonate buffered periodate. However, both compounds consume two equivalents of periodate per mole, so that the integrity of the system: trimethyl-fructose-trimethyl-mannose-trimethyl-glucose could be conveniently checked by determination of its periodate consumption. The expression used for calculating the concentration of trimethyl fructose is given below.

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$$\frac{x}{a}\ (C) = F \qquad (3)$$

x = optical density ($\log I_0/I$) of the dye produced from the interaction of the reaction mixture with chromotropic acid.

C = initial concentration of trimethyl-fructose in the reaction mixture expressed as moles per liter.

a = optical density of the dye produced by C concentration of trimethyl-fructose with chromotropic acid.

F = concentration of trimethyl-fructose in moles per liter.

The concentrations of trimethyl-mannose and trimethyl-glucose were calculated by solving two simultaneous equations, one involving optical rotation measurements and the other involving a stoichiometric relationship between the concentrations of the three, methylated sugars. The equation involving optical rotation measurements was derived in a manner similar to that used for equation (1) and is given below.

$$[G]\ [\alpha_G]_D^{25^\circ} + [M]\ [\alpha_M]_D^{25^\circ} + [F]\ [\alpha_F]_D^{25^\circ} = \frac{\text{optical rotation}}{.0444} \qquad (4)$$

$[\alpha]_D^{25}$ = specific rotation of the sugar at 25°C. using the sodium D line. The values used for $[\alpha_G]_D^{25}$, $[\alpha_M]_D^{25}$, and $[\alpha_F]_D^{25}$ were 77.5°, 987) 8.2°, (88) and 28.9° respectively. The subscripts G, M, and F refers to methylated glucose, mannose, and fructose respectively.

[G], [M], and [F] refer to the concentrations of trimethyl-glucose, trimethyl-mannose, and trimethyl-fructose, respectively, expressed as moles per liter.

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The stoichiometric equation assumes that the total concentration of the methylated sugars is equal to the initial concentration of trimethyl-fructose. In equation form,

$$G + M + F = 1 \qquad (5)$$

The last two equations can be solved simultaneously for the concentrations of 3,4,6-trimethyl-D-glucose and 3,4,6-trimethyl-D-mannose by inserting the values for trimethyl-fructose which were calculated previously, from formaldehyde analysis. In this manner, the concentrations of the three components in the reaction mixture can be determined.

The base catalyzed degradation of D-glucose and 3,4,6-trimethyl-D-fructose were carried out in a blackened reaction flask which was placed in a $25^\circ \pm .01^\circ$C. water bath. Nitrogen, purified by passage through two gas washing bottles containing pyrogallol-sodium hydroxide solution and one containing water, was bubbled into the reaction mixture at a rate of approximately 100 ml. per minute. In this manner, photochemical effects and oxygen oxidation were minimized. Reaction samples were removed periodically and quenched by the addition of an equal volume of dilute, standard acid. This step eliminated the possible presence of an enediol at the time of periodate oxidation. Sodium-2,6-dichlorbenzenone-indophenol was the reagent employed in testing for enediols. Acid formation during the alkaline degradation of the hexose was determined by neutralizing an aliquot of reaction mixture with an excess of standard acid and back titrating with standard base. In the case of methylated sugars, the stability of the methylated products in aqueous alkaline

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solutions was tested by determining periodate consumption of aliquots of the mixture which were removed from time to time.

The concentrations of sodium, calcium, and barium hydroxide solutions were determined by titration with standard base to the bromthymol blue end point. The calcium ion concentration was also estimated gravimetrically as the monohydrated oxalate, whereas the barium ion concentration was determined by weighing the sulfate.

The following is a description of a typical experiment. The calculated amount of the sugar was weighed into a volumetric flask, and sufficient water was added to dissolve the substance. A volume of standard base was then added to give the required hydroxyl ion concentration. The solution was mixed, placed in the water bath for fifteen minutes, and then diluted to volume. Nitrogen gas was passed through the solution at the time of mixing and thereafter. Samples were removed periodically, quenched, and stored in the refrigerator. Usually, samples were removed at the beginning and at the end of the rate studies to test for acid and enediol formation.

Analytical Procedures

Formaldehyde Analysis

The method used was devised by Forist and Speck. (75) A 2-ml. aliquot of the quenched reaction mixture was transferred to a 100-ml. volumetric flask. Two milliliter of 0.3 M periodic acid was added to the flask followed by 2 ml. of 1 M sodium bicarbonate. Oxidation was allowed to proceed for one hour. At the end of this period, excess

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periodate was reduced by the addition of 5 ml. of 0.5 M sodium sulfite, and the solution was diluted to the mark. Duplicate 1-ml. aliquots of this solution were pipetted into 50-ml. volumetric flasks followed by 1 ml. of 5 per cent chromotropic acid and 5 ml. of 14 M sulfuric acid. The flasks were heated in a boiling water bath for 30 minutes, cooled, and diluted with approximately 40 ml. of distilled water. The flasks were allowed to cool to room temperature and then 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 determined against a reagent blank at a wavelength of 570 mμ using a Beckman Model B spectrophotometer and 1-cm. Corex cells for the measurement. The amount of formaldehyde was calculated from a standard curve prepared by a similar treatment of glucose.

Determination of Periodate Consumption

The oxidations were carried out in periodic acid solutions buffered with sodium bicarbonate. This was followed by determination of the periodate consumed according to the method of Fleury and Lange. (89) Five milliliters of the quenched reaction mixture was mixed with 20 ml. of standard 0.1500 N periodic acid and 10 ml. of saturated sodium bicarbonate. Oxidation was allowed to proceed for two hours at which time 10 ml. of standard 0.1000 N arsenite was added. The solutions were allowed to stand overnight and were then titrated with 0.0100 N iodine solution to the starch end point.

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Cerate Oxidation

The method used was described by Forist, Neely, and Speck. (86) A 5-ml. sample of the quenched reaction mixture was oxidized by 15 ml. of standardized 0.480 N Ce^{+4} in 6 M perchloric acid; the oxidation was allowed to proceed for exactly one hour. The excess Ce^{+4} was then reduced with 15 ml. of standard 0.1000 N arsenite solution. Two drops of osmium tetroxide in 0.10 M sulfuric acid was added to catalyze the reduction which was allowed to continue for eight minutes. The excess arsenite was then oxidized with 0.0120 N Ce^{+4} in 2 M perchloric acid to the nitroferroin end point.

Optical Rotation Measurements

Measurements were made on the quenched reaction samples at 25°C. with a Rudolph polarimeter equipped with a sodium lamp and a two decimeter, water jacketed polarimeter tube.

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RESULTS AND DISCUSSION

As a result of preliminary studies as well as the probability that side reactions possess a higher order of dependence on hydroxyl ion concentration than the enolization reaction being studied, it was decided to maintain the hydroxyl ion concentration in the vicinity of 0.01 N for the isomerization of D-glucose.

The data obtained using 0.020 N sodium hydroxide appear in Table III. Periodate consumption and the change in optical rotation with time are shown in Figures 2 and 3 respectively. The concentrations of the three sugars were calculated by taking points from these curves and applying the three simultaneous equations. The concentrations of the sugars at different times appear in Table IV and are also plotted in Figure 4. The values were not calculated beyond the 32nd hour, at which time a negative mannose concentration was obtained.

The results of one of two subsequent experiments with 0.010 N sodium hydroxide solutions illustrates the difficulty in obtaining reproducible data.

The data are listed in Tables V and VI and their plots are shown in Figures 5 and 6.

An alternate method of oxidation of the reaction mixture with perchlorato-ceric acid was attempted, and the results obtained from one of three runs are given in Table VII and Figures 7 and 8.

The data from analysis of glucose degradation mixtures by the technique of specific oxidation indicate that this approach cannot be

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TABLE III

THE LOBRY de BRUYN TRANSFORMATION OF D-GLUCOSE
[0.02 N NaOH, 0.10 M D-glucose, 25°C.]

Sample	Time Of Reaction	Meq. of IO_4^- Consumed Per Mmol. of Sugar	HCHO Formation Log I_0/I	Optical Rotation Readings, °
1	1/12 hr.	10.216	312	1.886
2	4 hrs.	10.194	-	1.700
3	9 hrs.	10.168	-	1.516
4	12 hrs.	10.149	330	1.368
5	21 1/3 hrs.	10.081	-	1.048
6	24 hrs.	10.087	352	0.958
7	28 hrs.	10.060	-	0.884
8	32 hrs.	10.046	-	0.784
9	36 hrs.	10.021	364	0.684
10	44 hrs.	9.971	-	0.558
11	48 hrs.	9.959	370	0.454
12	52 hrs.	9.932	-	-

* Optical rotation readings were taken using a 2-dm. polarimeter tube.

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TABLE IV

THE LOBRY de BRUYN TRANSFORMATION OF D-GLUCOSE

[0.02 N NaOH, 0.10 N D-glucose, 25°C.]

Time of Reaction	Meq. of IO_4^- Consumed Per Mmol. of Sugar	Optical Rotation Readings, °	Concentrations, Mmol. x 10^1/Ml.		
			Glucose	Mannose	Fructose
0	10.216	1.886	1.000	0.000	0.000
4	10.195	1.704	0.933	0.013	0.054
8	10.173	1.528	0.881	0.066	0.053
12	10.151	1.368	0.844	0.074	0.082
16	10.130	1.220	0.811	0.081	0.108
20	10.108	1.084	0.787	0.078	0.135
24	10.086	0.972	0.783	0.054	0.163
28	10.064	0.860	0.779	0.031	0.190
32	10.042	0.768	0.788	Neg.	-

* Optical rotation readings were taken using a 2-dm. polarimeter tube.

TABLE V

THE LOBRY de BRUYN TRANSFORMATION OF D-GLUCOSE
[0.01 N NaOH, 0.10 M D-glucose, 25°C.]

Sample	Time of Reaction	Meq. of IO_4^- Consumed Per Mmol. of Sugar	Optical Rotation Readings, °
1	0 hr.	10.220	1.880
2	4 hrs.	10.187	1.740
3	9 hrs.	10.168	1.680
4	12 hrs.	10.155	1.630
5	20 hrs.	10.126	1.412
6	24 hrs.	10.108	1.360
7	28 hrs.	10.088	1.272
8	32 hrs.	10.074	1.184
9	36 hrs.	10.044	1.140
10	44 hrs.	10.015	1.042
11	48 hrs.	10.011	1.000
12	52 hrs.	9.979	0.952
13	56 hrs.	9.974	0.898
14	60 hrs.	9.968	0.850
15	68 hrs.	9.955	0.792
16	84 hrs.	9.917	0.644

* Optical rotation readings were taken using a 2-dm. polarimeter tube.

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TABLE VI

THE LOBRY de BRUYN TRANSFORMATION OF D-GLUCOSE
[0.01 N NaOH, 0.10 M D-glucose, 25°C.]

Time of Reaction	Meq. of IO_4^- Consumed Per Mmol. of Sugar	Optical Rotation Readings, °	Concentrations, Mmol. x 10^1/Ml.		
			Glucose	Mannose	Fructose
0	10.220	1.880	1.000	0.000	0.000
4	10.198	1.785	0.995	-0.023	0.028
8	10.178	1.693	0.995	-0.023	0.028
12	10.159	1.605	0.995	-0.023	0.028
16	10.140	1.517	0.995	-0.023	0.028
20	10.121	1.432	1.000	0.000	0.000
24	10.103	1.352	1.003	-0.145	0.148

* All optical rotation readings were taken using a 2-dm. polarimeter tube.

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TABLE VII

THE LOBRY de BRUYN TRANSFORMATION OF D-GLUCOSE
[0.01 N NaOH, 0.10 M D-Glucose, 25°C.]

Sample	Time of Reaction	Meq. of Ce^{+4} Reduced Per Mmol. of Sugar	Optical Rotation Readings, °
1	0 hr.	12.189	1.865
2	1 hr.	12.204	1.842
3	2 hrs.	12.207	1.830
4	3 hrs.	12.215	1.826
5	4 hrs.	12.228	1.787
6	5 hrs.	12.236	1.762
7	6 hrs.	12.279	1.747
8	7 hrs.	12.284	1.712
9	9 hrs.	12.293	1.694
10	11 hrs.	12.315	1.656

* Optical rotation readings were taken using a 2-dm. polarimeter tube.

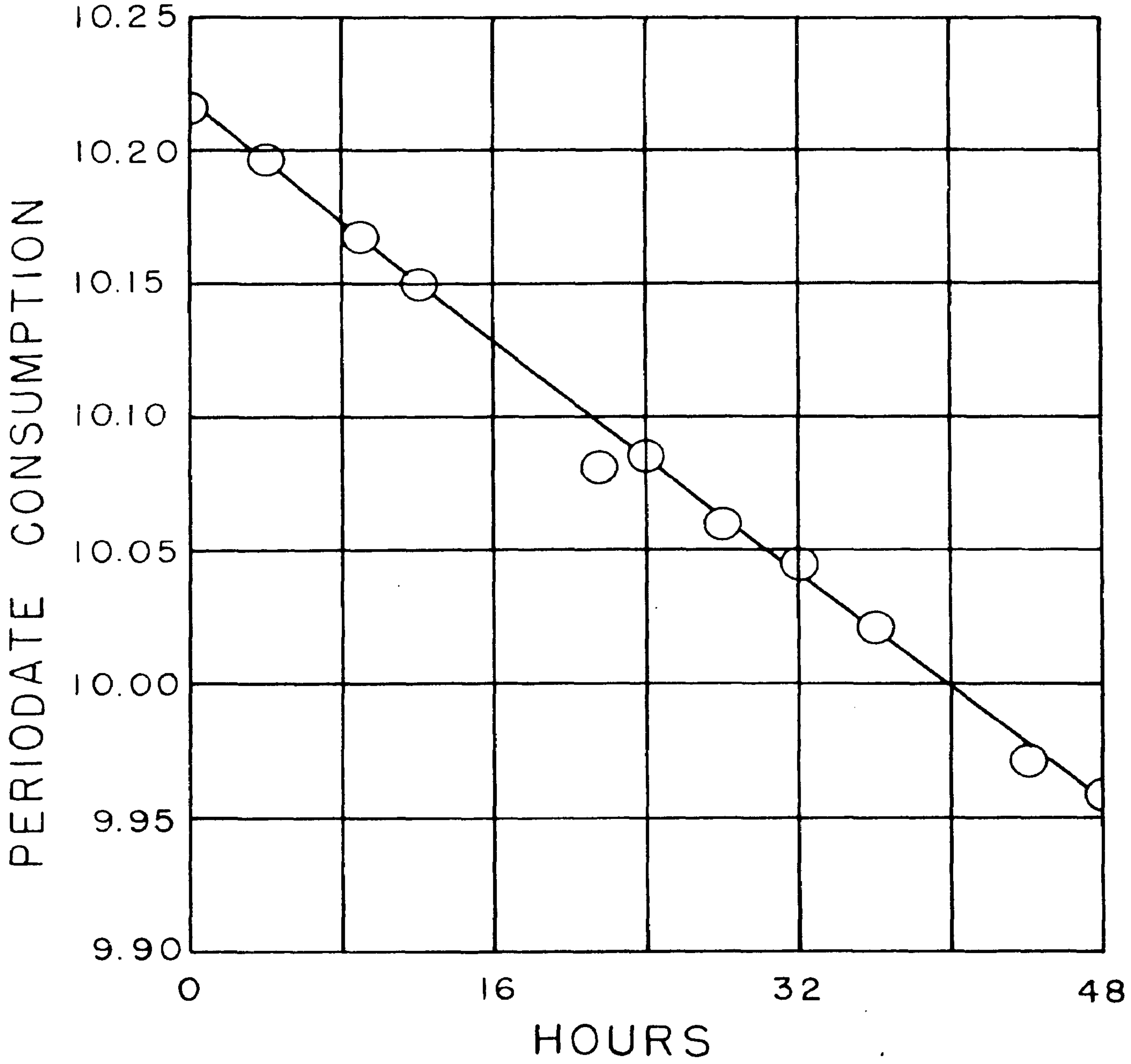

Figure 2. Periodate consumption in equivalents of periodate reduced per mole of hexose (based on initial glucose concentration) from a reaction mixture originally .100 M in D-glucose and .0200 M in sodium hydroxide versus reaction time.

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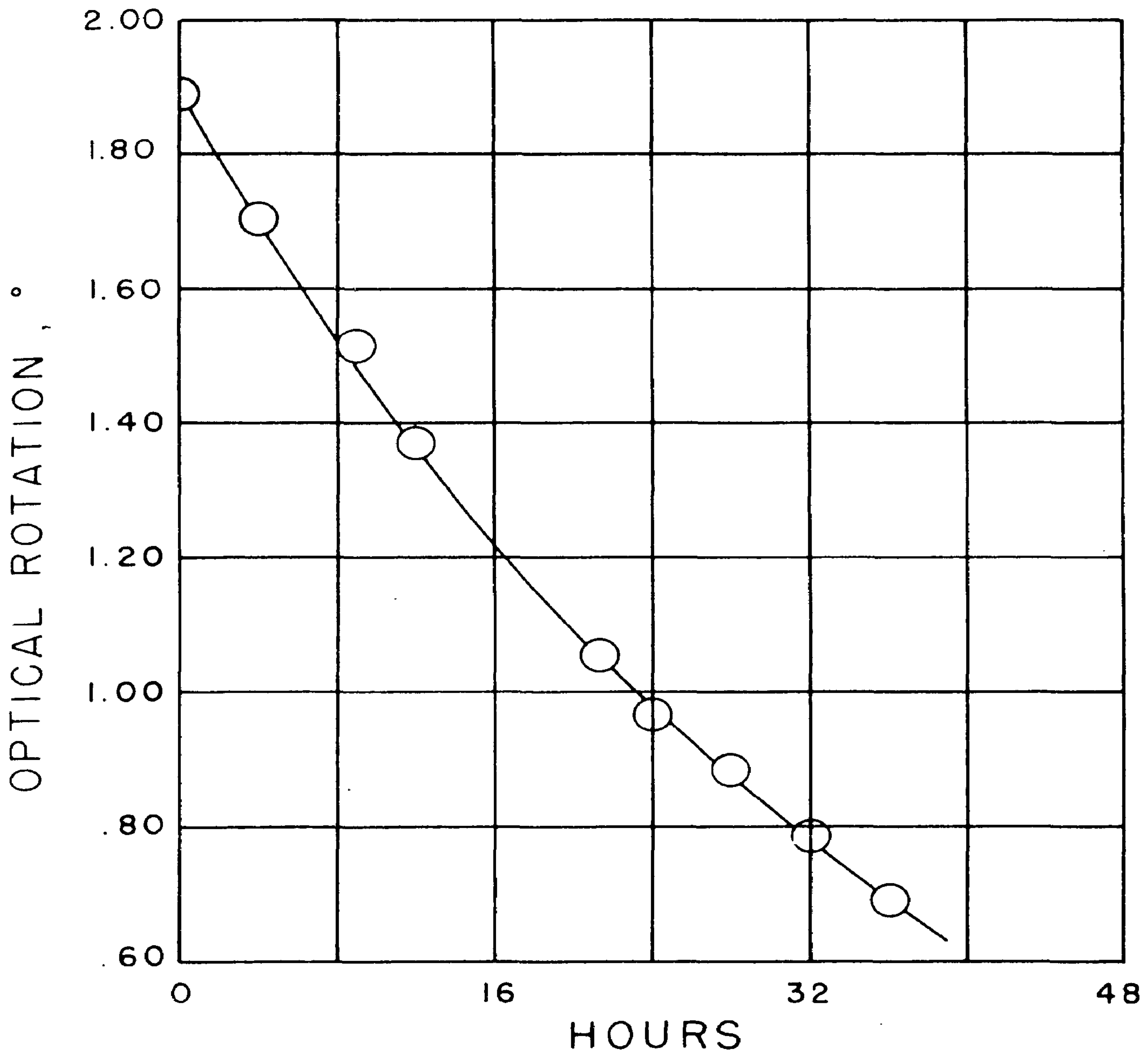

Figure 3. Optical rotation (2 dcm. tube) of a reaction mixture originally .100 M in D-glucose and .0200 N in sodium hydroxide versus reaction time.

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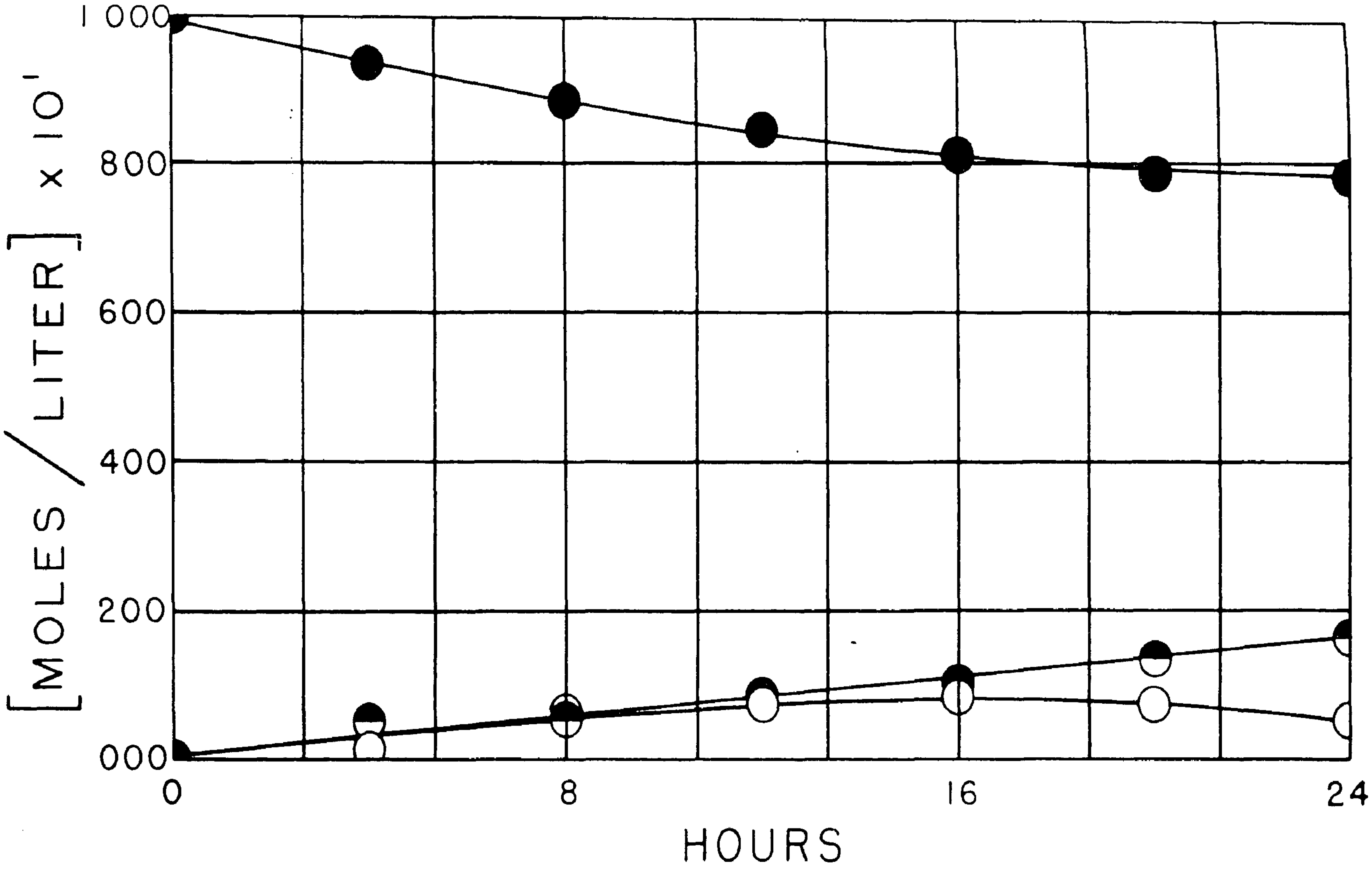

Figure 4. Concentrations of hexoses in moles per liter x 10^1 in reaction mixtures originally .100 M in D-glucose and .0200 N in sodium hydroxide versus reaction time. ●, ◓, and ○ refer to glucose, fructose, and mannose respectively.

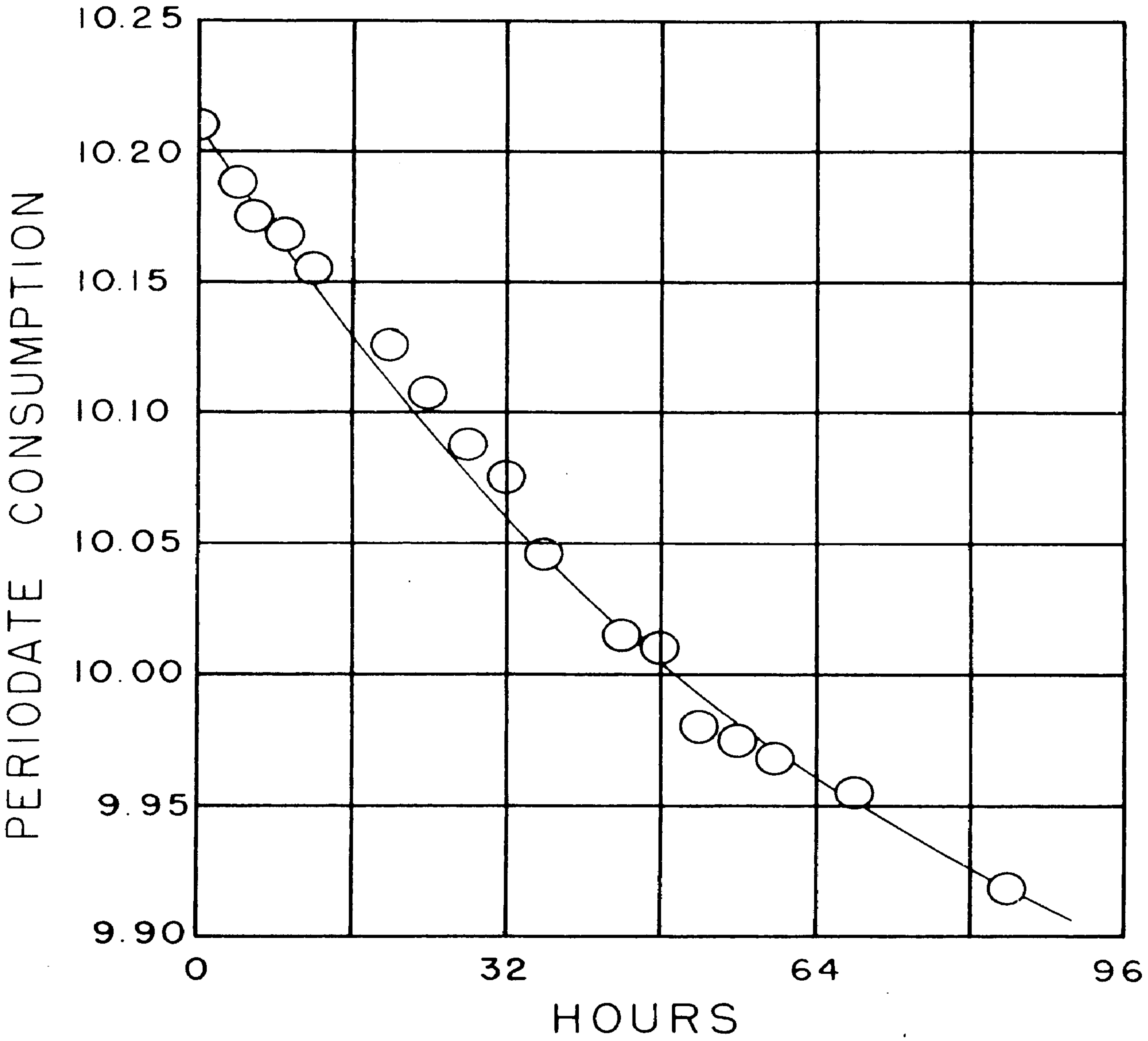

Figure 5. Periodate consumption in equivalents of periodate reduced per mole of hexose (based on initial glucose concentration) from a reaction mixture originally .100 M in D-glucose and .0100 N in sodium hydroxide versus reaction time.

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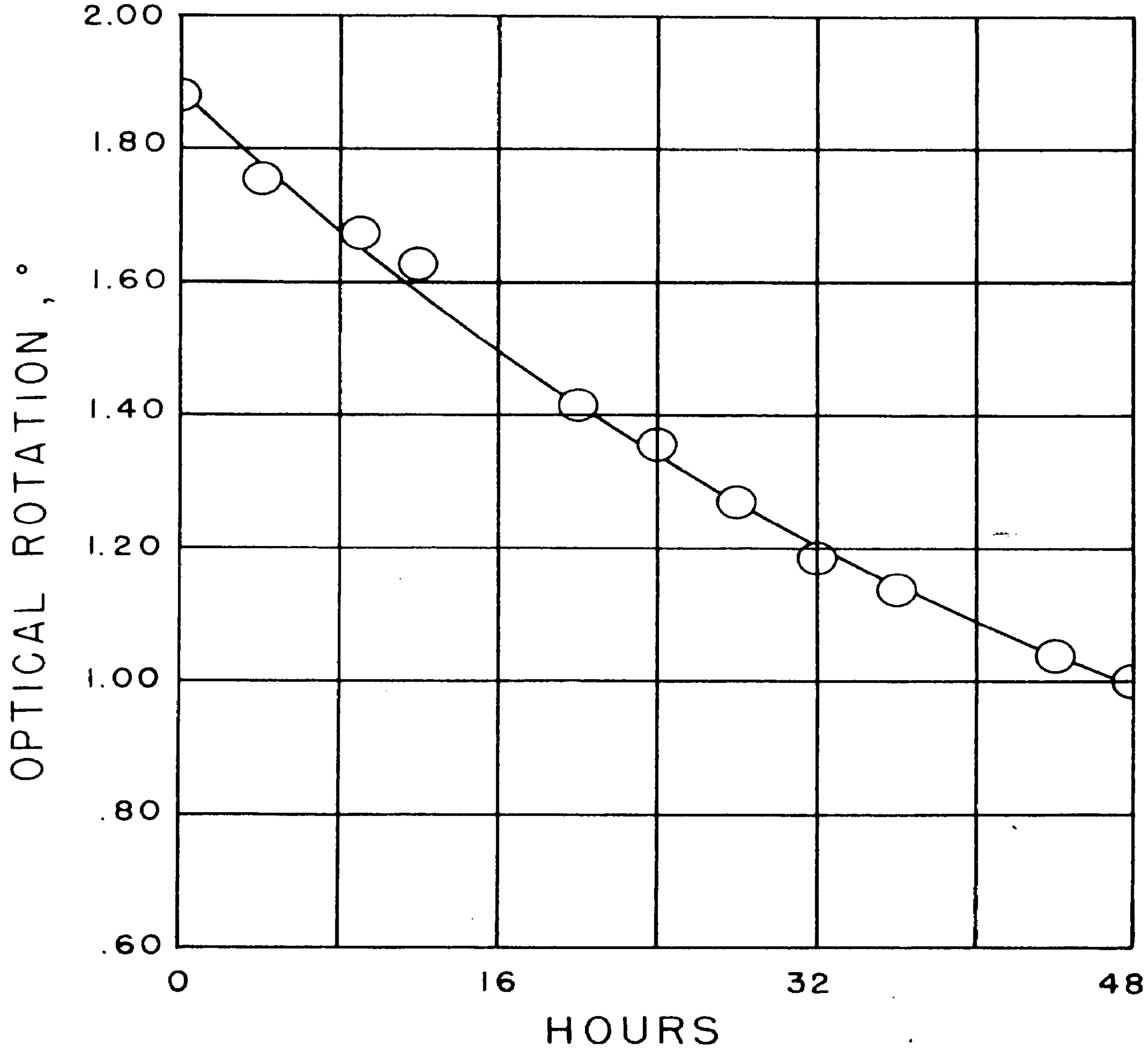

Figure 6. Optical rotation (2 dcm. tube) of a reaction mixture originally .100 M in D-glucose and .0100 N in sodium hydroxide versus reaction time.

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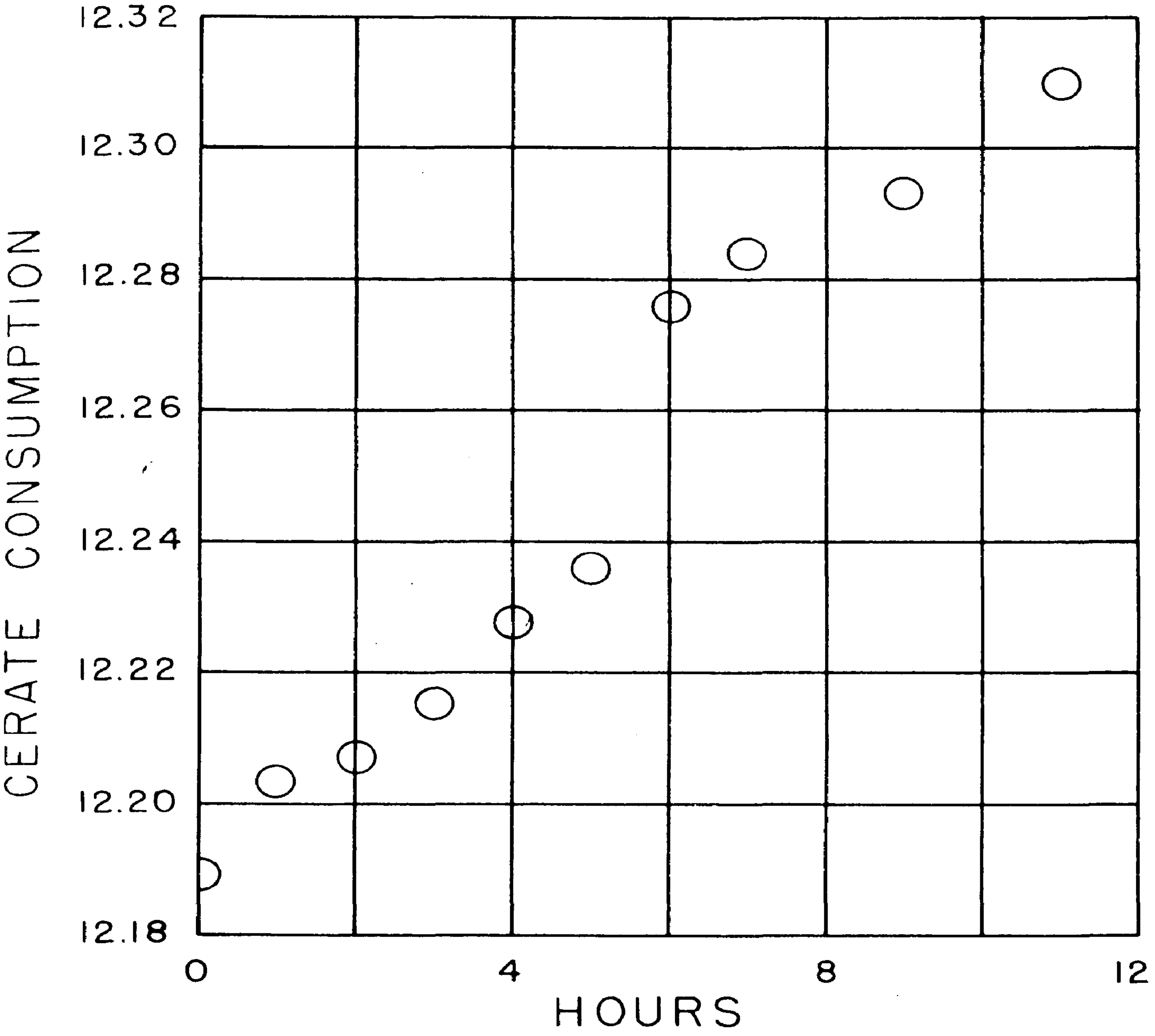

Figure 7. Cerate consumption in equivalents of cerate reduced per mole of hexose (based on initial glucose concentration) from a reaction mixture originally .100 M in D-glucose and .0100 N in sodium hydroxide versus reaction time.

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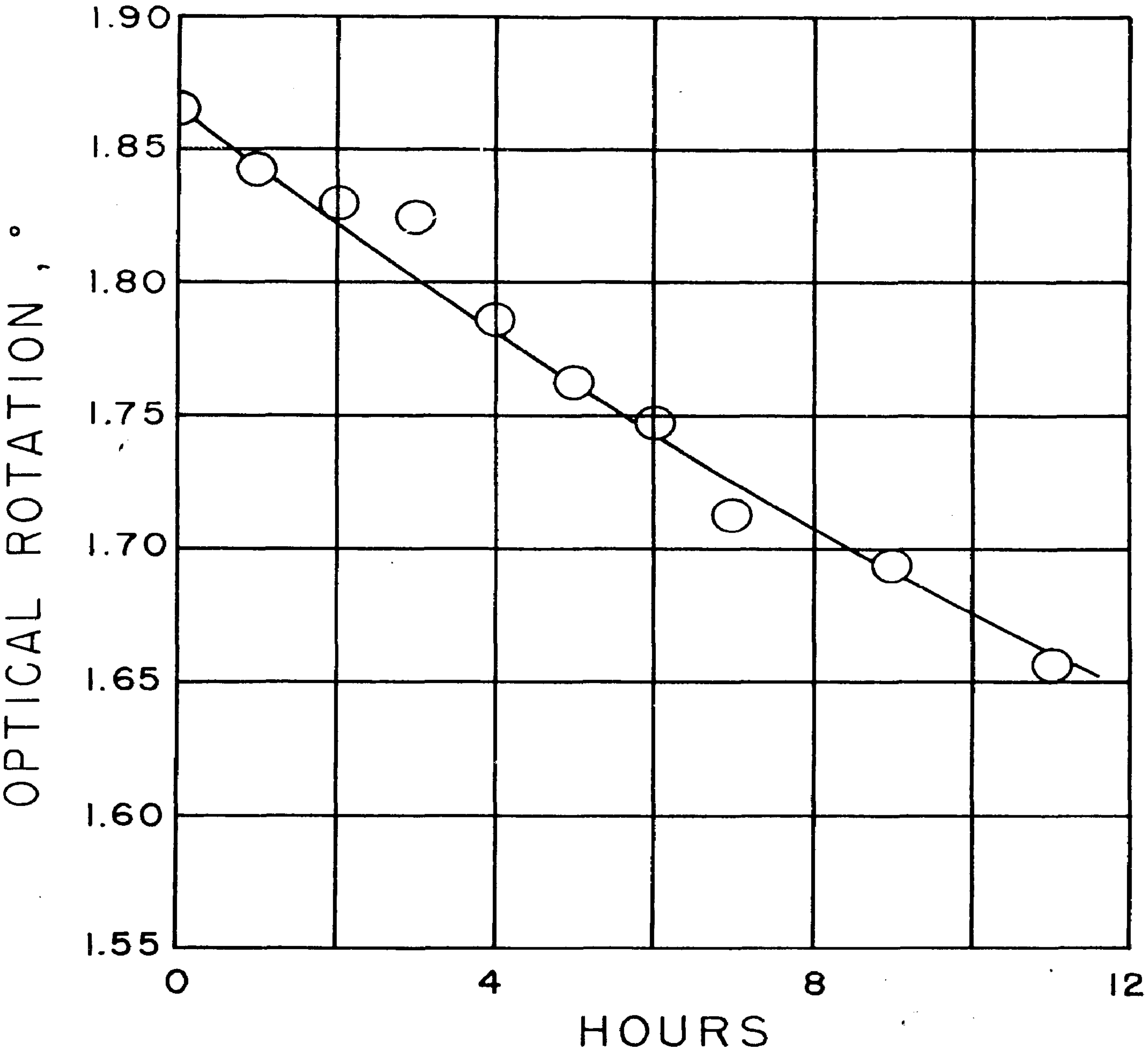

Figure 8. Optical rotation (2 dcm. tube) of a reaction mixture originally .100 M in D-glucose and .0100 N in sodium hydroxide versus reaction time.

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employed as a means for following the course of this reaction beyond its earliest stages. Hence, its usefulness is greatly limited in any attempt to obtain precise information concerning the mechanism of this particular reaction. Notwithstanding this, the method does indicate quite clearly that the degradation of glucose, even at very low hydroxyl ion concentrations, is far more complex than was implied in earlier investigations including that of Wolfrom and Lewis. (27) This is in agreement with results obtained recently by Sowden and Schaffer. (68)

The results obtained with glucose led to the investigation of 3,4,6-trimethyl-D-fructose which was expected to undergo side reactions less readily than do the unmethylated hexoses and which is ideally adapted to analysis by periodate scission.

The results of the Lobry de Bruyn transformation of 3,4,6-trimethyl-D-fructose are shown in Tables VIII, IX, and X and Figures 9-17. From a comparison of the data obtained with calcium, sodium, and barium hydroxides, the principal effect appears to be that of hydroxyl ion catalysis, and in each case the ratios of the initial rate of trimethylglucose formation to that of the corresponding mannose derivative are nearly identical. However, the reaction is, in reality, far more complex in the presence of calcium or barium hydroxides than it is when catalyzed by sodium hydroxide. Evidence for this is the considerable increase in the periodate titers of the reaction mixtures containing calcium or barium ions, shown in Figure 18, as well as the fact that these mixtures became permanently colored much more rapidly than those containing

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TABLE VIII

THE SODIUM HYDROXIDE CATALYZED LOBRY de BRUYN TRANSFORMATION OF 3,4,6-TRIMETHYL-D-FRUCTOSE

[0.0200 N NaOH, 0.10 M Trimethyl-fructose, 25°C.]

Time of Reaction	Optical Rotation Readings, °	HCHO Formation Log I_0/I	Concentrations, Mmol. x 10^{1}/Ml.		
			Glucose	Fructose	Mannose
0	1.290	321	0.000	1.000	0.000
1	1.364	298	--	--	--
2	1.434	279	0.087	0.870	0.043
3	1.482	262	--	--	--
4	1.552	250	0.155	0.782	0.063
5	--	--	--	--	--
6	1.590	232	0.200	0.720	0.080
7	1.650	225	--	--	--
8	1.692	217	0.234	0.670	0.096
9	--	--	--	--	--
10	1.720	202	0.259	0.629	0.112

* All optical rotation readings were taken using a 2-dm. polarimeter tube.

TABLE IX

THE CALCIUM HYDROXIDE CATALYZED LOBRY de BRUYN TRANSFORMATION OF 3,4,6-TRIMETHYL-D-FRUCTOSE

[0.0169 N $Ca(OH)_2$, 0.10 M Trimethyl-fructose, 25°C.]

Time of Reaction	Optical Rotation Readings, °	HCHO Formation Log I_0/I	Concentrations, Mmol. x 10^1/Ml. Glucose	Fructose	Mannose
0	1.252	320	0.000	1.000	0.000
1	1.312	302	--	--	--
2	1.368	288	0.068	0.903	0.029
3	1.424	277	--	--	--
4	1.478	267	0.120	0.834	0.046
5	1.534	257	--	--	--
6	1.546	250	0.156	0.784	0.060
7	1.560	244	--	--	--
8	1.562	238	0.177	0.740	0.083
9	--	--	--	--	--
10	1.560	223	0.184	0.706	0.110

* All optical rotation readings were taken using a 2-dm. polarimeter tube.

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TABLE X

THE BARIUM HYDROXIDE CATALYZED LOBRY de BRUYN TRANSFORMATION OF 3,4,6-TRIMETHYL-D-FRUCTOSE
[0.0184 N $Ba(OH)_2$, 0.10 M Trimethyl-fructose, 25°C.]

Time of Reaction	Optical Rotation Readings, °	HCHO Formation Log I_0/I	Concentration, Mmol. x 10^1/Ml.		
			Glucose	Fructose	Mannose
0	1.321	320	0.000	1.000	0.000
1	1.390	303	--	--	--
2	1.455	288	0.075	0.894	0.031
3	1.497	273	--	--	--
4	--	--	0.125	0.822	0.053
5	1.550	255	--	--	--
6	--	--	0.159	0.763	0.078
7	1.598	237	--	--	--
8	1.602	230	0.180	0.717	0.103
9	1.604	224	--	--	--
10	1.600	219	0.189	0.682	0.129

* All optical rotation readings were taken using a 2-dm. polarimeter tube.

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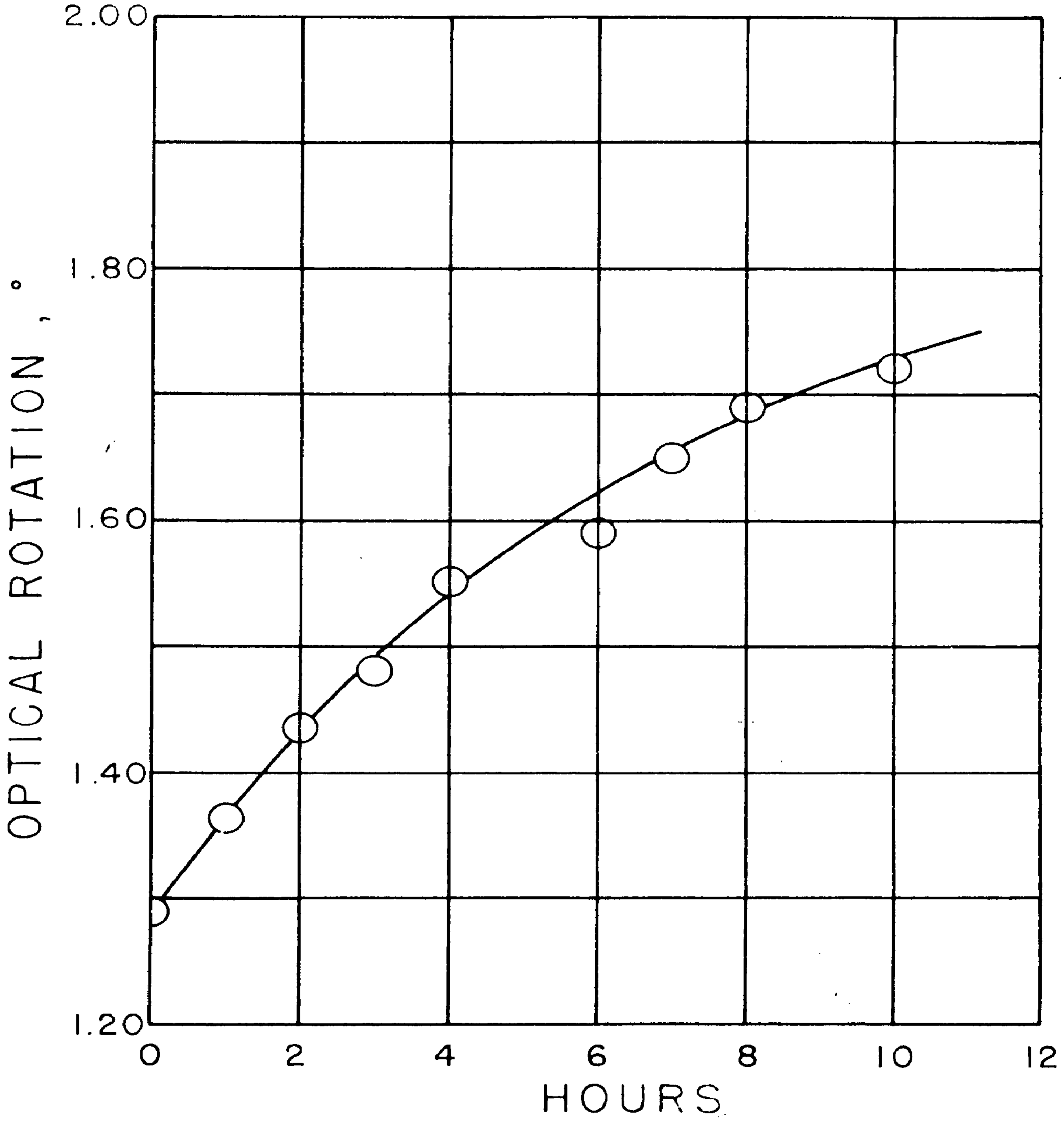

Figure 9. Optical rotation (2 dcm. tube) of a reaction mixture originally .100 M in 3,4,6-trimethyl-D-fructose and .0200 N in sodium hydroxide versus reaction time.

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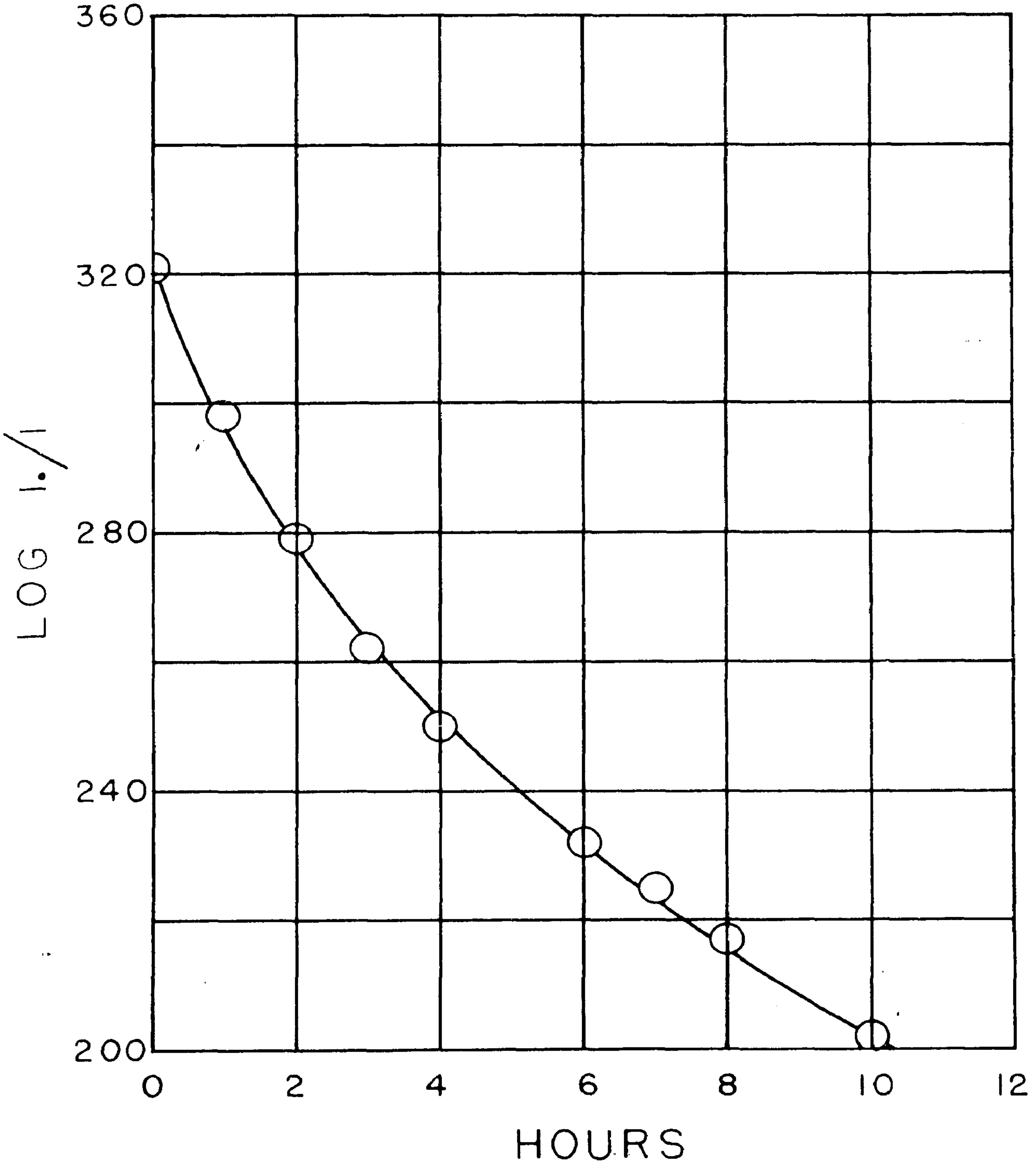

Figure 10. Log $^{I_0}/I$, optical density of formaldehyde-chromotropic acid dye obtained from a reaction mixture originally .100 M in 3,4,6-trimethyl-D-fructose and .0200 N in sodium hydroxide versus reaction time.

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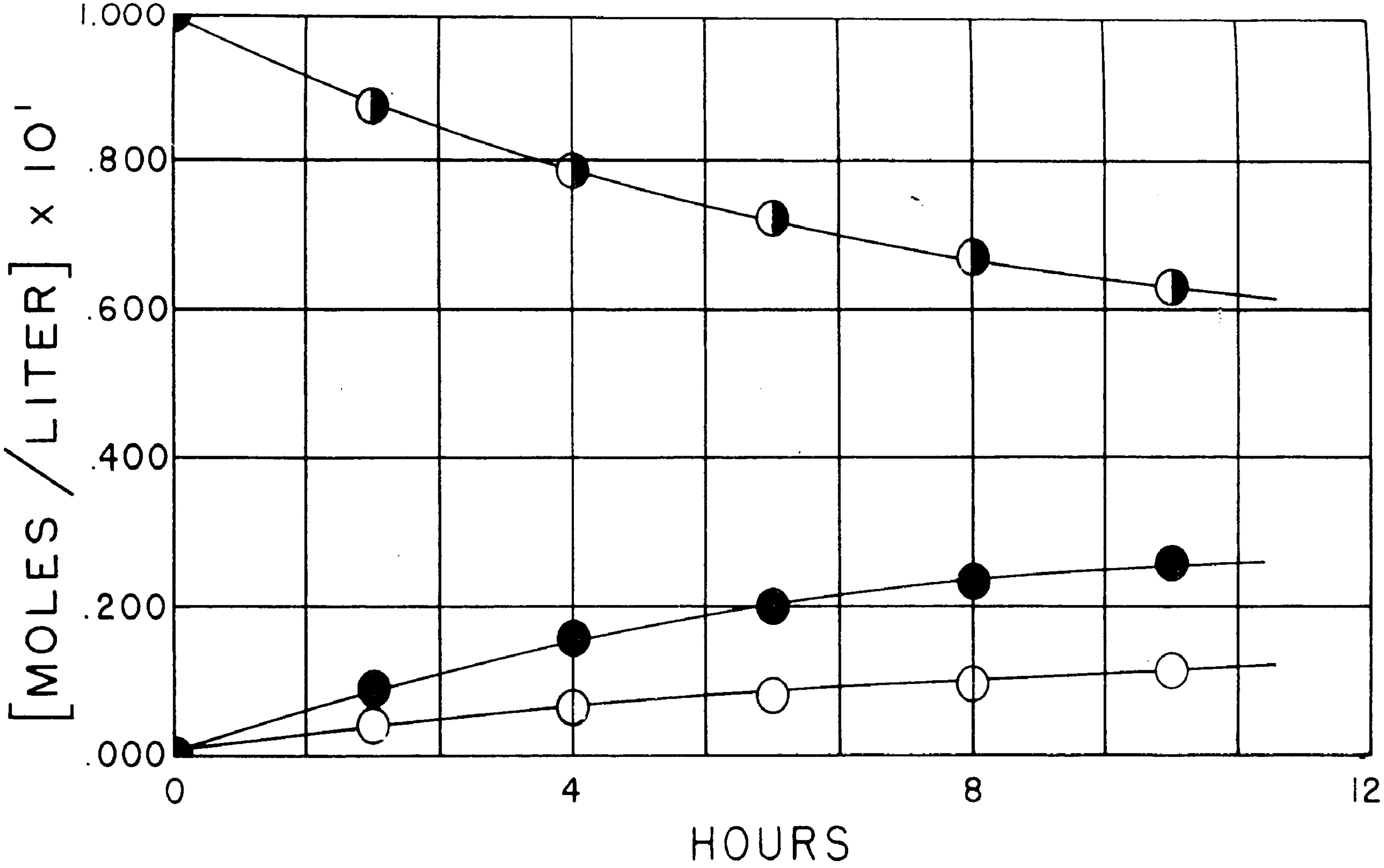

Figure 11. Concentrations of trimethyl hexoses in moles per liter x 10^1 from a reaction mixture originally .100 M in 3,4,6 trimethyl-D-fructose and .0200 N in sodium hydroxide versus reaction time. ●, ◑, and ○ refer to 3,4,6-trimethyl-D-(glucose, fructose, and mannose respectively.

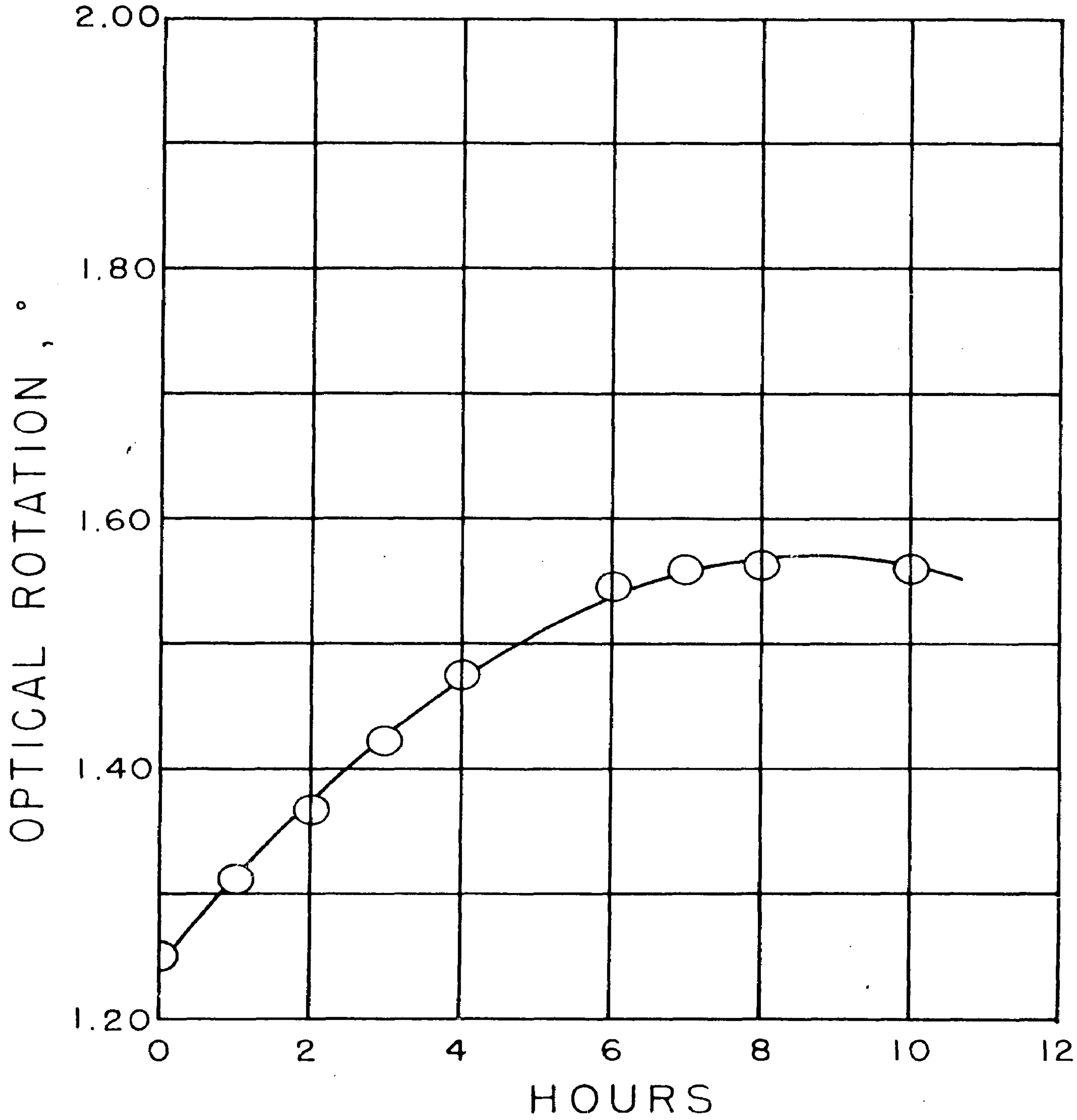

Figure 12. Optical rotations (2 dcm. tube) of a reaction mixture originally .100 M in 3,4,6-trimethyl-D-fructose and .0169 N in calcium hydroxide versus reaction time.

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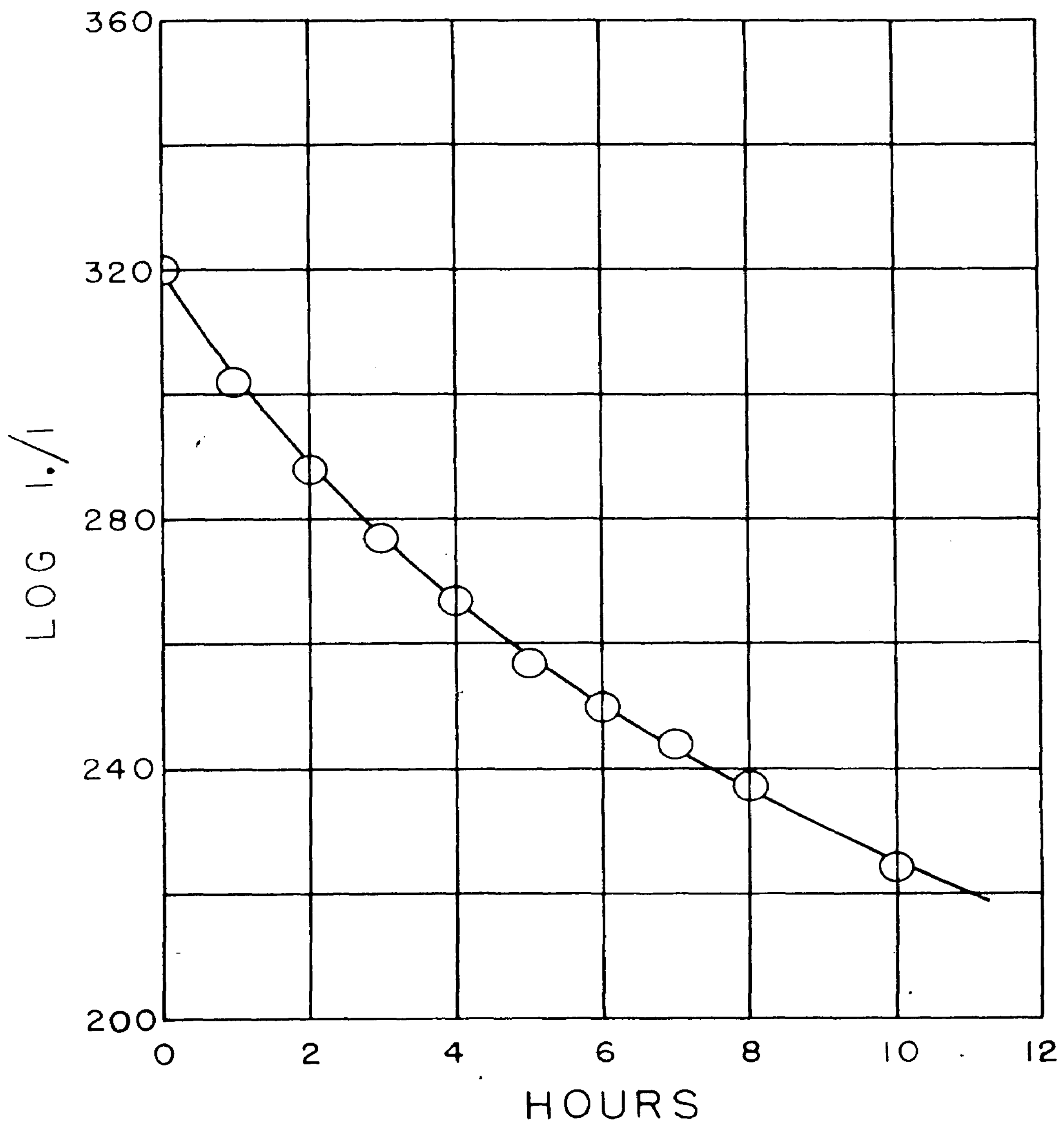

Figure 13. Log $^{I}\cdot/I$, optical density of formaldehyde-chromotropic acid dye obtained from a reaction mixture originally .100 M in 3,4,6-trimethyl-D-fructose and .0169 N in calcium hydroxide versus reaction time.

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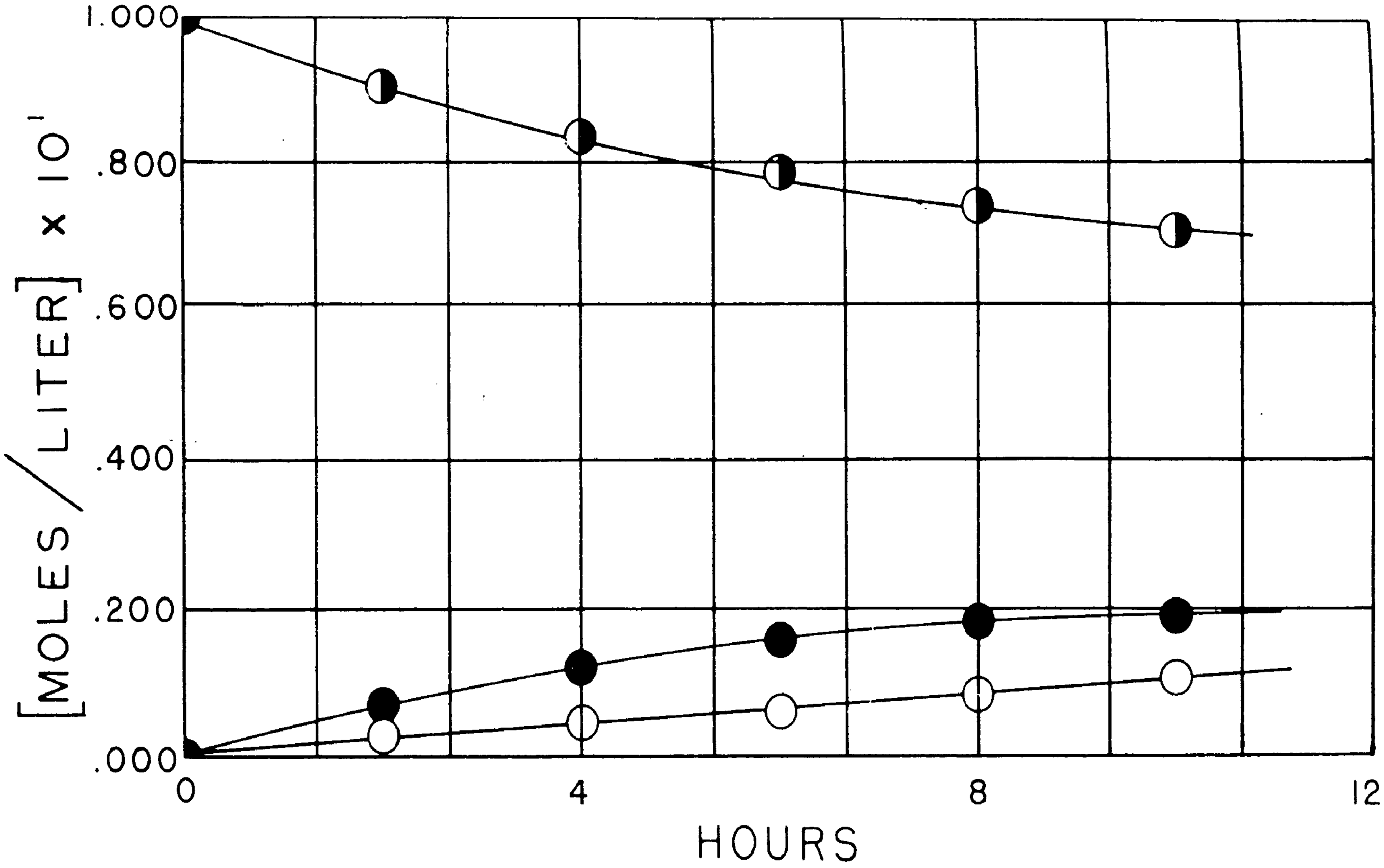

Figure 14. Concentrations of trimethyl hexoses in moles per liter x 10^1 from a reaction mixture originally .100 M in 3,4,6 trimethyl-D-fructose and .0169 N in calcium hydroxide versus reaction time. ●, ◑, and ○ refer to 3,4,6-trimethyl-D-(glucose, fructose, and mannose) respectively.

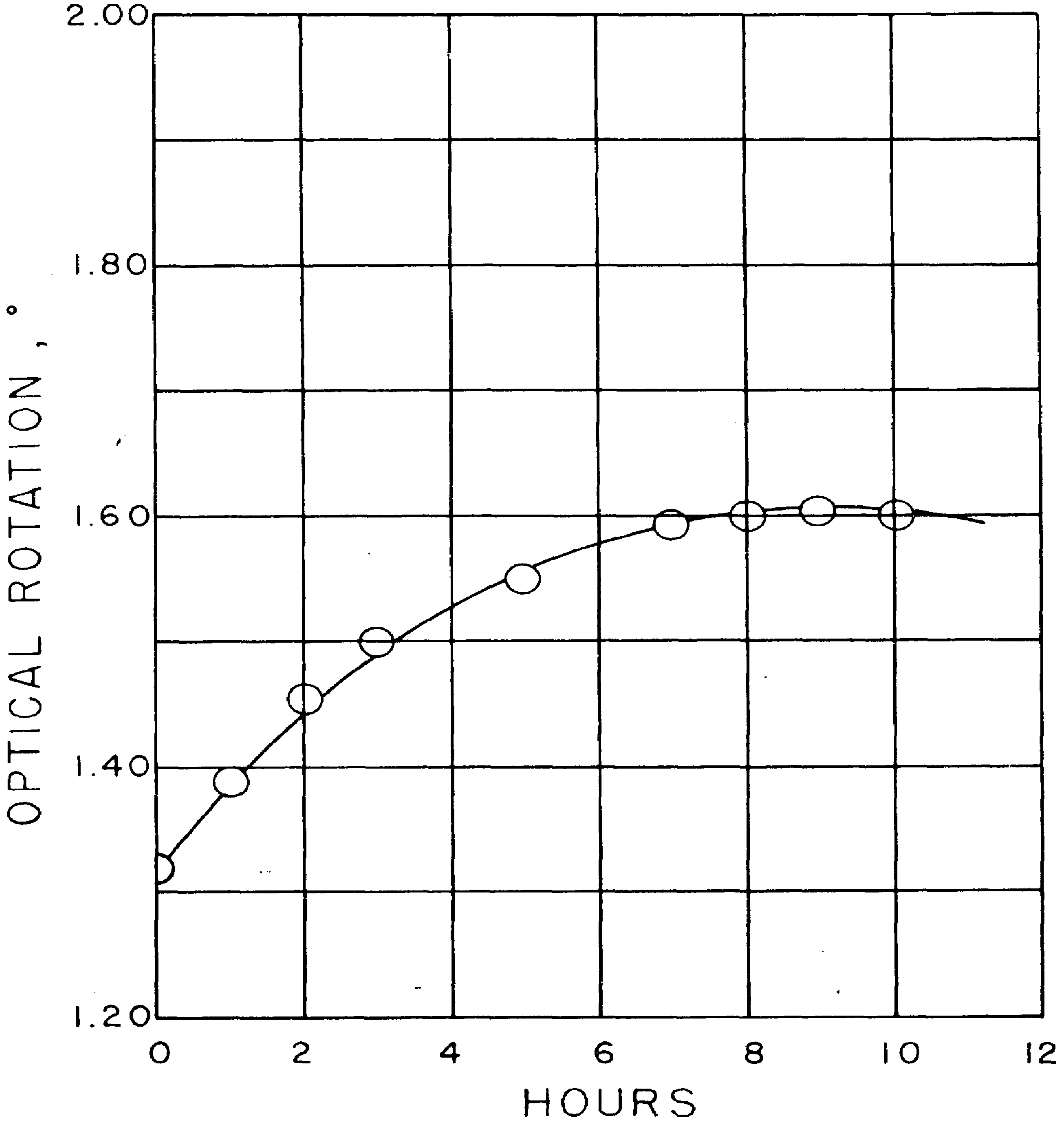

Figure 15. Optical rotation (2 dcm. tube) of a reaction mixture originally .100 M in 3,4,6-trimethyl-D-fructose and .0184 N in barium hydroxide versus reaction time.

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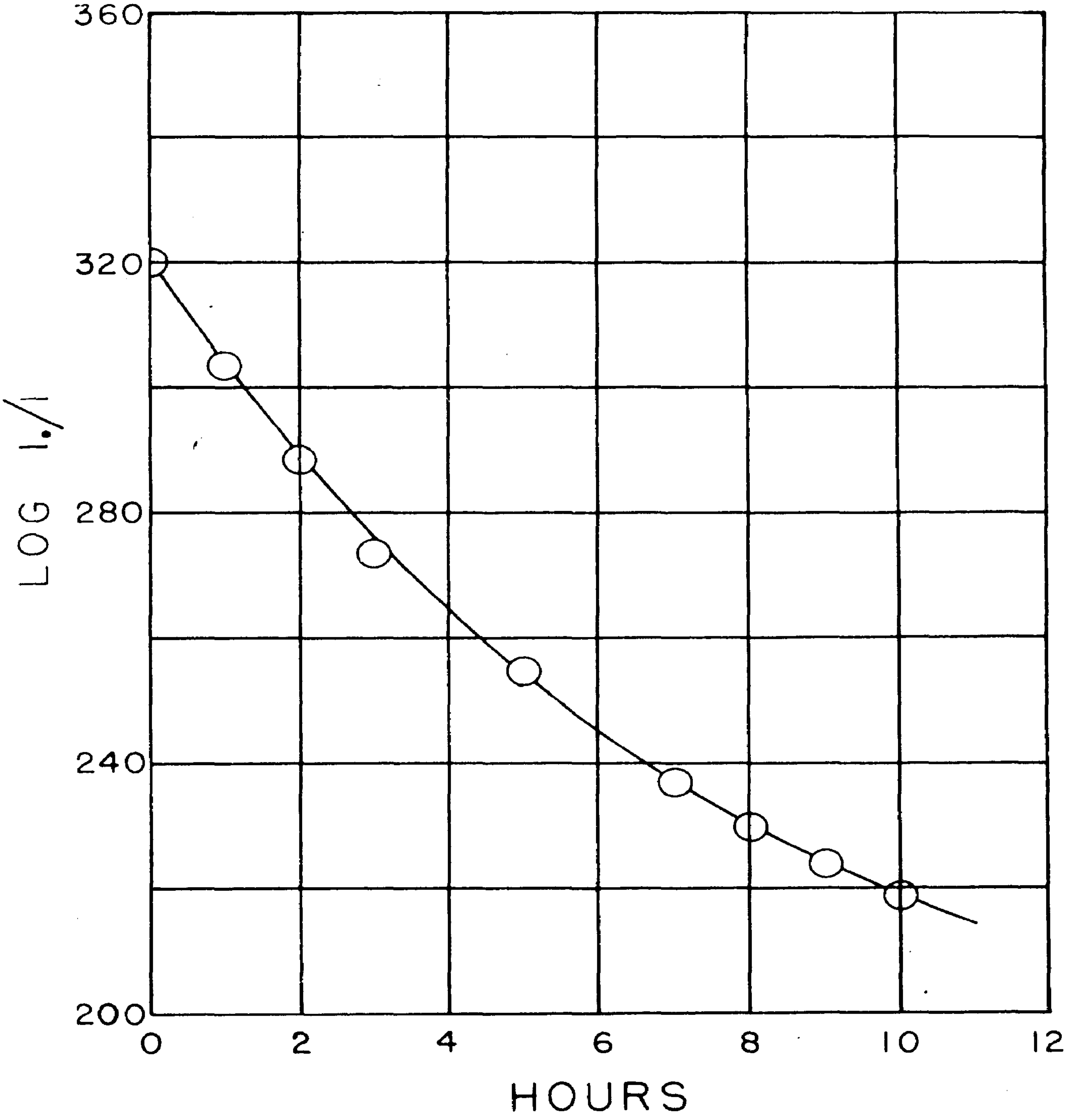

Figure 16. Log $^{I_\circ}/I$, optical density of formaldehyde-chromotropic acid dye obtained from a reaction mixture originally .100 M in 3,4,6-trimethyl-D-fructose and .0184 N in barium hydroxide versus reaction time.

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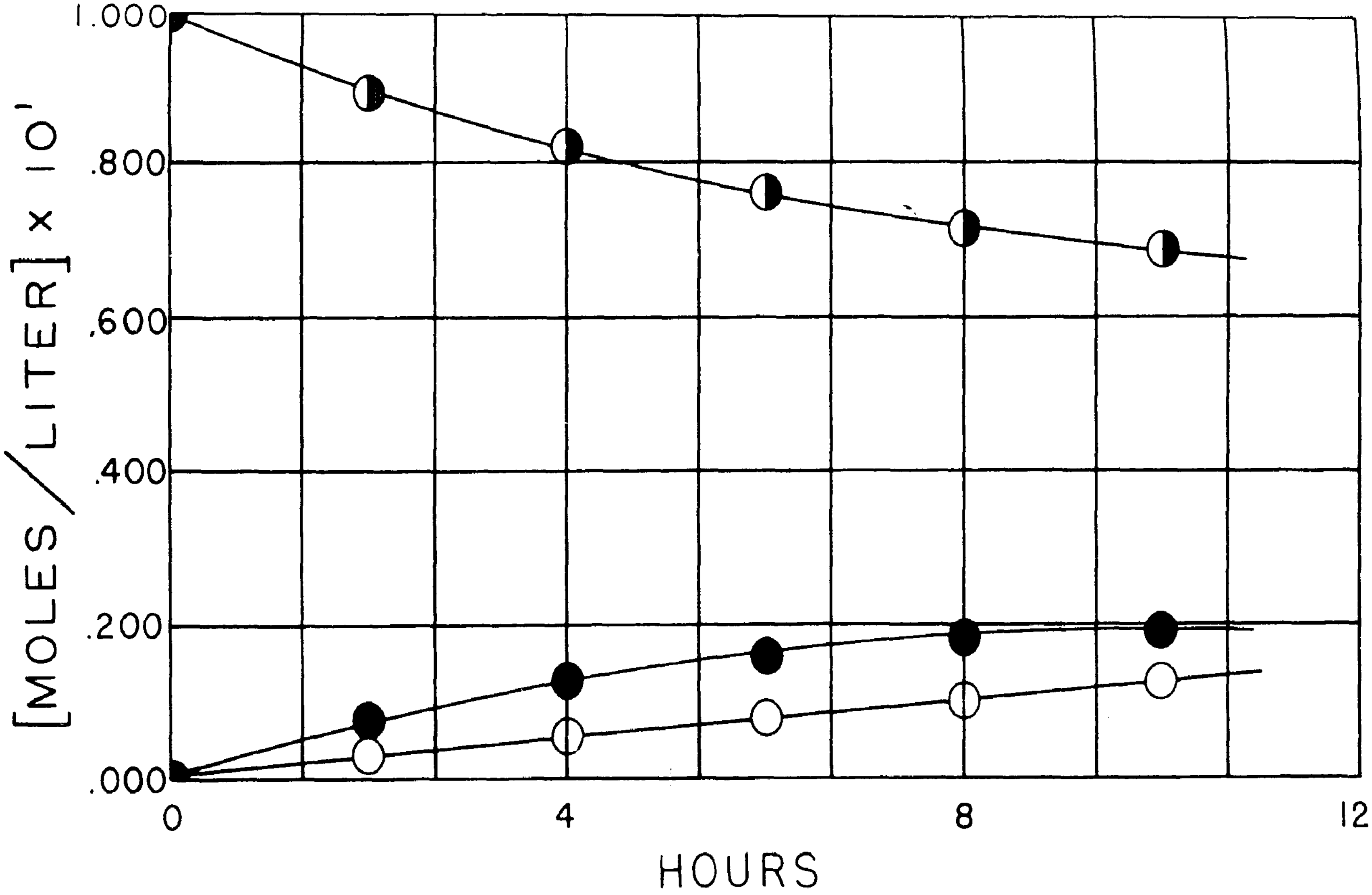

Figure 17. Concentrations of trimethyl hexoses in moles per liter x 10^1 from a reaction mixture originally .100 M in 3,4,6-trimethyl-D-fructose and .0184 N in barium hydroxide versus reaction time. ●, ◑, and ○ refer to 3,4,6 trimethyl-D-(glucose, fructose and mannose) respectively.

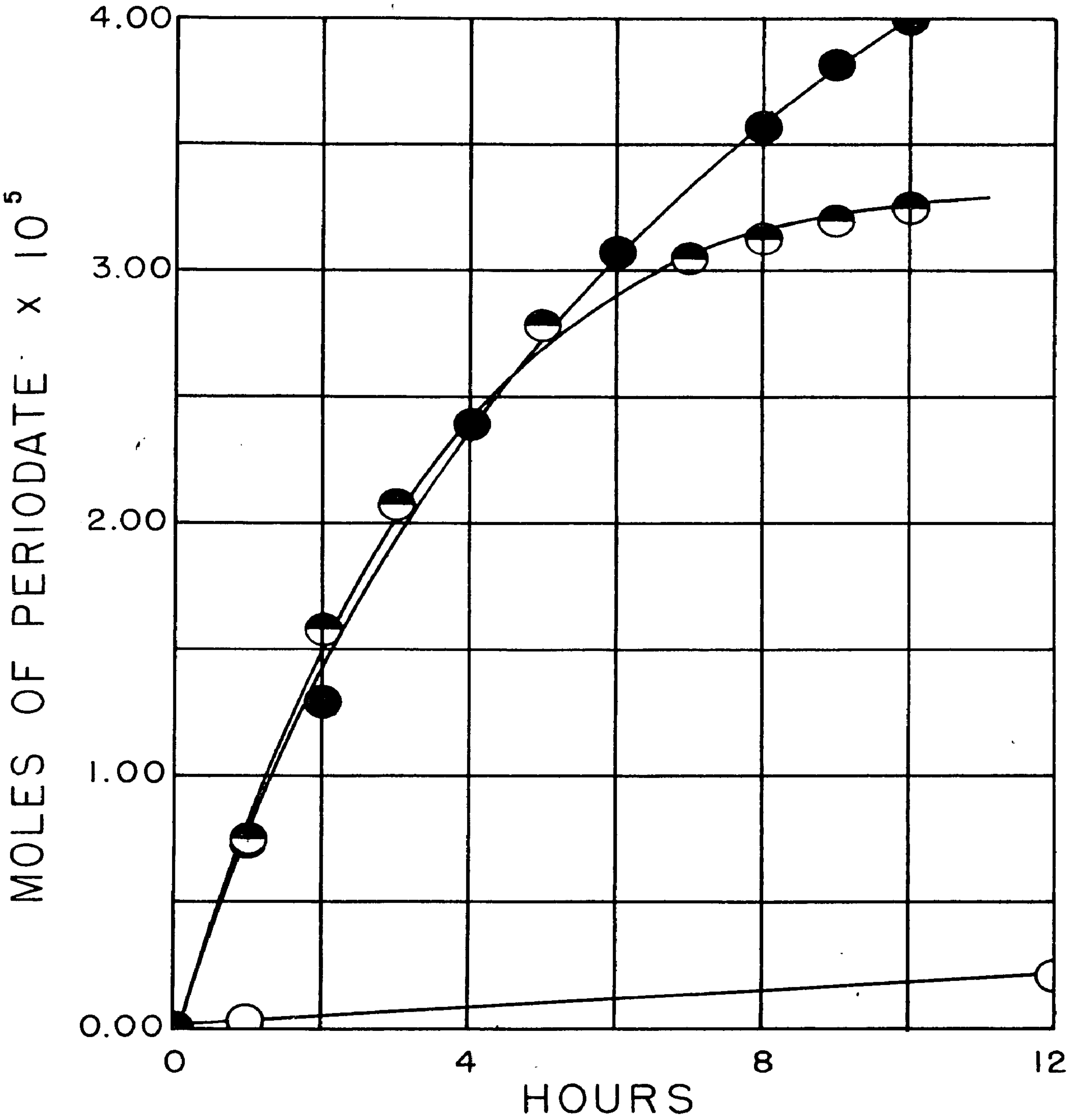

Figure 18. Increase in periodate consumption in moles per liter x 10^5 of one ml. samples of reaction mixture versus reaction time. Molarity of trimethyl hexose was .100 M. Molarity of base was approximately .0200 N. ●, ⊖, and ○ refer to transformations in the presence of calcium hydroxide, barium hydroxide, and sodium hydroxide respectively.

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sodium ion.* Barium hydroxide is in its effect intermediate between that by sodium and calcium hydroxides. The increase in periodate consumption is probably the result of demethylation and is a striking instance of catalysis by alkaline earth metal ions. It should be pointed out that the change in periodate consumption complicates the analysis of the mixtures and may indicate that the results obtained by this scheme are not valid beyond the point where the periodate titer becomes greater by 5 per cent than that for the trimethyl-hexoses.

These results obtained with 3,4,6-trimethyl-D-fructose do not agree with reports of previous investigations (66,68) of the isomerization of unmethylated hexoses as regards the implication of stereospecific effects by metal ions. The data also substantiate that obtained with glucose in indicating that simple equilibria among reducing sugars are seldom realized in alkaline solutions.

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* A yellowish-green indicator was formed which was decolorized after acidification in the early samples of the reaction mixture; later the coloration remained even after acidification.

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SUMMARY

An attempt was made to follow the Lobry de Bruyn transformation of D-glucose by a new scheme involving two methods of analysis and three simultaneous equations. The results indicated that side reactions occurred to a degree which greatly limited the usefulness of this approach. They also showed that alkaline glucose solutions are far more complex than was implied in the reports of many previous investigations.

The course of the isomerization of 3,4,6-trimethyl-D-fructose under the influence of the bases, sodium hydroxide, calcium hydroxide, and barium hydroxide, was followed by analyzing for formaldehyde after periodate oxidation and by optical rotation measurements. The results indicate no significant differences in the isomerization of 3,4,6-trimethyl-D-fructose in dilute solutions of these bases at 25°C.

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PART II

Kinetics of the Metal Ion Catalyzed Degradation of Glyceraldehyde

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EXPERIMENTAL METHODS

Materials

The DL glyceraldehyde employed in these measurements was synthesized by the method of Fisher and Baer. (90) Periodic acid was obtained from G. Frederick Smith Chemical Company. Matheson practical grade chromotropic acid was recrystallized from 50% ethanol. Standard 1 M sodium perchlorate was prepared by neutralizing standard 2 M perchloric acid to pH 7.0 with a standard 2 M sodium hydroxide solution. Calcium, barium, lithium, and magnesium acetate solutions were prepared by neutralization of weighed samples of C.P. metal carbonates with acetic acid. Arsenious acid, sodium carbonate, sodium bicarbonate, sodium sulfite, potassium iodide, iodine, sodium hydroxide, acetic acid, and perchloric acid were all C.P. reagents. Sulfuric acid was Merck reagent grade.

Apparatus

A Beckman Model B spectrophotometer equipped with matched Corex cells was used for optical density measurements. A Beckman pH meter was used for pH measurements.

Quantitative Analysis of Reaction Mixture

The method of analysis is that devised by Forist and Speck. (75) If G, D, and P represents millimols of glyceraldehyde, dihydroxyacetone and pyruvaldehyde respectively per milliliter of solution analyzed,

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G_o represents initial glyceraldehyde concentration, and I represents milliequivalents of periodate consumed by one milliliter of reaction mixture, then the following equations may be written.

$$G + D + P = G_o \qquad (6)$$

$$4G + 2D + 2P = I \qquad (7)$$

consequently,

$$G = I/_2 - G_o \qquad (8)$$

Hence, beginning with pure glyceraldehyde, the glyceraldehyde concentration, G, at any time may be calculated from the initial glyceraldehyde concentration and the periodate consumption, I. Since G can be determined at any time, a second method which would analyze for total trioses would permit an estimation of all three components. Total triose concentration was determined by formaldehyde analysis; by subtracting G, the glyceraldehyde concentration, from the total triose concentration, one obtains the concentration of dihydroxyacetone, D. The pyruvaldehyde concentration, P, is then calculated from equation (6).

A typical experiment is described below. The calculated quantity of standard 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 sufficient sodium perchlorate to give an ionic strength of 0.4 M in the final solution. This solution was placed in a water bath which was maintained at $50^\circ \pm .01^\circ C$. A sample of pure DL glyceraldehyde was weighed, dissolved in redistilled water, and transferred to the reaction flask. The solution was mixed, diluted nearly to the mark, and replaced in the water bath for fifteen minutes. It was

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then diluted to the mark, thoroughly mixed, and returned to the bath. Samples were removed periodically to determine periodate consumption and formaldehyde formation.

Analytical Methods

Determinations of periodate consumption and formaldehyde formation have been described previously in Part I of this thesis. The only difference is in the amounts of the reagents used.

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RESULTS AND DISCUSSION

Reactions were usually followed to the disappearance of 25 per cent of the initial glyceraldehyde concentration. A plot of the logarithm of the glyceraldehyde concentration versus time indicated a first order reaction confirming the results of Forist. (75) See Figure 19. Thus, neglecting the formation of glyceraldehyde from dihydroxyacetone, the rate of the reaction may be expressed as,

$$-\frac{d(G)}{dt} = k(G) \qquad (9)$$

which on integration yields,

$$-\ln G = kt + \text{constant}$$

$$\text{or} \qquad \log G = -k't + \text{constant} \qquad (10)$$

The pseudo constant, <u>k'</u>, embodies all of the catalytic species and in an acid buffer system, may be represented as follows:

$$k' = [\, k_{H^+}(H^+) + k_{HA}(HA) + k_{A^-}(A^-)\,] \qquad (11)$$

No terms for spontaneous water catalysis were included because there was no detectable reaction in the absence of buffer systems.

$$\text{Allowing, } \alpha = k_{HA} + k_{A^-} \quad (A^-/HA) \qquad (12)$$

$$k' = k_{H^+}(H^+) + \alpha\,(HA) \qquad (13)$$

At constant buffer ratio, i.e., constant hydrogen ion concentration, a plot of <u>k'</u> versus <u>HA</u> should be linear. This was observed for the systems

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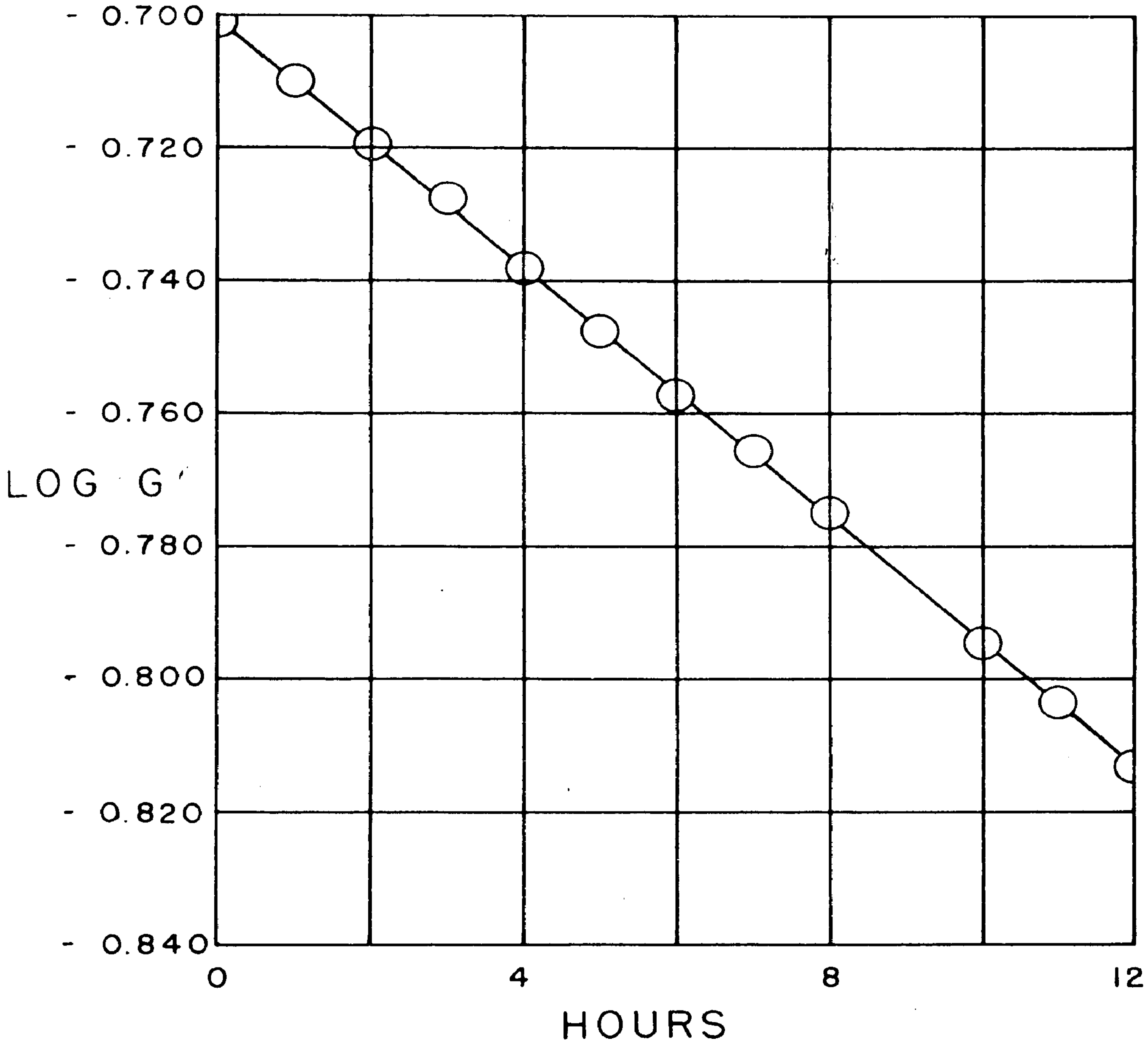

Figure 19. Typical rate curve. Logarithm of glyceraldehyde concentration in moles per liter versus reaction time.

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studied. In every case, the intercept was zero indicating negligible hydrogen ion and water catalysis.

A plot of the slopes of these curves, α, against the buffer ratio, A^-/HA, should be linear. The slopes and intercepts should equal the catalytic constants of the acids and bases involved respectively. Such plots have been given, and the catalytic constants k_1 and k_2 for acetic acid and acetate ions were found by Forist to be 6 x 10^{-5} mol^{-1} min.$^{-1}$ and 45 x 10^{-5} mol^{-1} min.$^{-1}$ respectively.

Inasmuch as pseudo constants calculated from the above constants varied somewhat from those obtained experimentally in the present work, a series of experiments were made in order to revise the values of the catalytic constants. In the first series, the acetate ion concentration and the ionic strength of the solution were kept constant, and the acetic acid concentration was varied. Results are shown in Table XI and Figure 20. In another series of experiments, the acetic acid concentration and the ionic strength were kept constant, and the acetate ion concentration was varied. The results are shown in Table XI and Figure 21. A new set of catalytic constants for acetic acid and acetate ion were calculated from these data and were found to be 2 x 10^{-5} mol^{-1} min.$^{-1}$ and 49 x 10^{-5} mol^{-1} min.$^{-1}$ respectively.

However, a subsequent experiment with only acetic acid as the catalytic species revealed that the catalytic constant for acetic acid is approximately equal to only 1 x 10^{-5} mol^{-1} min.$^{-1}$. Hence, the term, k_3 [HOAc] [OAc$^-$] was included in the revised rate expression. In assuming catalysis by this pair, the value, 5 x 10^{-5} mol^{-1} min.$^{-1}$ is obtained

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TABLE XI

PSEUDO CONSTANTS -- ACETATE SYSTEMS
(T = 50°C., μ = .40)

Expt.	$[G_0]$	$[CH_2COOH]$	$[CH_3COONa]$	$[NaClO_4]$	$k' \times 10^6$ min.$^{-1}$
19	0.20	0.40	0.40	--	210
29	0.20	0.40	0.25	0.15	136
32	0.20	0.40	0.15	0.25	82
31	0.20	0.25	0.40	--	207
28	0.20	0.133	0.40	--	201
26	0.20	0.20	0.20	--*	101
44	0.20	0.40	--	0.40	4
25,27	0.20	0.10	0.30	0.10	155
37	0.20	0.30	0.30	0.10	159

* This experiment was run at an ionic strength of .20 M.
** All concentrations in moles per liter.

for the catalytic constant, $\underline{k}_3$. The added term, k_3 [HOAc] [OAc$^-$] fits very well into the rate expression at different concentrations of acetic acid and acetate ion, giving values for $\underline{k}'$ which are in agreement, within the limits of error, with experimental values. Thus, the expression for the pseudo first order rate constant in acetate buffers will for the remainder of this discussion be written as follows:

$$k' = k_1\,[\mathrm{HOCa}] + k_2\,[\mathrm{OAc}^-] + k_3\,[\mathrm{HOAc}]\,[\mathrm{OAc}^-] \qquad (14)$$

where the values of $\underline{k}_1$, $\underline{k}_2$, and $\underline{k}_3$ are 1×10^{-5}, 49×10^{-5}, and 5×10^{-5} $\mathrm{mol}^{-1}\ \mathrm{min.}^{-1}$ respectively.

An experiment was performed to determine the effect of ionic strength on the rate. Instead of the usual ionic strength of 0.40 M, a solution with an ionic strength of 0.20 M was used, and no difference in the value of the pseudo rate constant was observed. The result is given in Table XI.

The Effect of Calcium Ion

The data on the effect of calcium ion were obtained in extending the work of Forist who made the observation that calcium ion catalyzes the conversion of glyceraldehyde to dihydroxyacetone and pyruvaldehyde. Forist's investigation revealed that this catalysis, which was first order in calcium ion, occurs only in the presence of bases, such as acetate ion; there was no measurable effect by solutions of calcium perchlorate. Since this reaction is catalyzed by both acetate ion and acetic acid in acetate buffers and since the calcium ion catalysis is acetate ion dependent in these solutions, the most reasonable expression

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for the pseudo rate constant is as follows:

$$k' = k_1 [HOAc] + k_2 [OAc^-] + k_3 [HOAc] [OAc^-] + k_4 [Ca^{++}] [OAc^-] \quad (15)$$

Forist's data were obtained by varying the calcium ion concentration in an acetate buffer, the acetate and acetic acid concentrations of which were each 0.2 molar. The present data, which are listed in Table XIIi, are the pseudo constants obtained at other acetate and acetic acid concentrations, and from them, the catalytic constant k_3 may be evaluated.

Plots of the variation of pseudo constant with calcium ion concentration at different acetate ion concentration are shown in Figure 23. From this it will be seen that the slopes of the lines obtained over a one and one-half fold change in acetate ion concentration are nearly identical. It might thus appear that although acetate ion is necessary for the catalysis by calcium ion, this effect is independent of variations in the acetate ion concentration over the range investigated. However, the catalysis by calcium ion is complicated by incomplete dissociation of calcium acetate. This is taken into account in the following mathematical treatment of the data which appears to afford a satisfactory explanation of these observations as well as to permit calculation of the magnitude of k_3.

The assumptions which are made in this analysis are,

(a) The dissociation of $Ca(OAc)_2$ to $CaOAc^+$ and OAc^- is virtually complete, whereas the dissociation of $CaOAc^+$ is not. This appears to be justified by previous investigation of the

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TABLE XII

PSEUDO CONSTANTS -- METAL ION SYSTEMS
(T = 50°C., μ = .40 M)

Expt.	$[G_0]$	$[CH_3COOH]$	$[CH_4COONa]$	$[NaClO_4]$	Metal Ion	$k' \times 10^6$ $min.^{-1}$
11	0.20	0.40	0.40	--	0.40 Li^+	209
12	0.20	0.40	0.40	--	0.30 Li^+	210
13	0.20	0.40	0.40	--	0.20 Li^+	212
14	0.20	0.40	0.40	--	0.10 Li^+	218
15	0.20	0.40	0.40	--	0.06 Li^+	216
17	0.20	0.40	0.40	--	0.02 Li^+	214
19	0.20	0.40	0.40	--	--	210
2,3	0.20	0.20	0.20	0.10	0.10 Mg^{++}	94
41	0.20	0.20	0.20	0.14	0.06 Mg^{++}	97
42	0.20	0.20	0.20	0.17	0.03 Mg^{++}	97
*	0.20	0.20	0.20	0.20	--	101

* Values obtained from the thesis of A. A. Forist.
** All concentrations in moles per liter.

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TABLE XIII

PSEUDO CONSTANTS -- METAL ION SYSTEMS (CONTINUATION)
(T = 50°C., μ = .40 M)

Expt.	$[G_0]$	$[CH_3COOH]$	$[CH_3COONa]$	$[NaClO_4]$	Metal Ion	k' x 10^6 min.$^{-1}$
39	0.20	0.30	0.30	--	0.10 Ca^{++}	181
38	0.20	0.30	0.30	0.04	0.06 Ca^{++}	170
43	0.20	0.30	0.30	0.07	0.03 Ca^{++}	165
37	0.20	0.30	0.30	0.10	--	159
21	0.20	0.10	0.30	--	0.10 Ca^{++}	175
24	0.20	0.10	0.30	0.04	0.06 Ca^{++}	167
23	0.20	0.10	0.30	0.07	0.03 Ca^{++}	162
25	0.20	0.10	0.30	0.10	--	155
5,6	0.20	0.20	0.20	0.10	0.10 Ba^{++}	120
7,8	0.20	0.20	0.20	0.14	0.06 Ba^{++}	111
10	0.20	0.20	0.20	0.17	0.03 Ba^{++}	106

* All concentrations in moles per liter.

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TABLE XIV

A COMPARISON OF EXPERIMENTAL AND CALCULATED VALUES FOR THE PSEUDO CONSTANTS

Buffer Ratio $[A^-/HA]$	Calcium Ion Concentration	$k' \times 10^6$ min.$^{-1}$	
		[Experimental]	[Calculated]
.20/.20	0.00	101*	102
.20/.20	0.05	111*	111
.20/.20	0.10	120*	120
.30/.10	0.00	155	150
.30/.10	0.05	165**	160
.30/.10	0.10	175	169
.30/.30	0.00	159	155
.30/.30	0.05	170**	165
.30/.30	0.10	180	174

* Values obtained from the thesis of A. A. Forist
** Values obtained from Figure 23.
*** All concentrations in moles per liter.

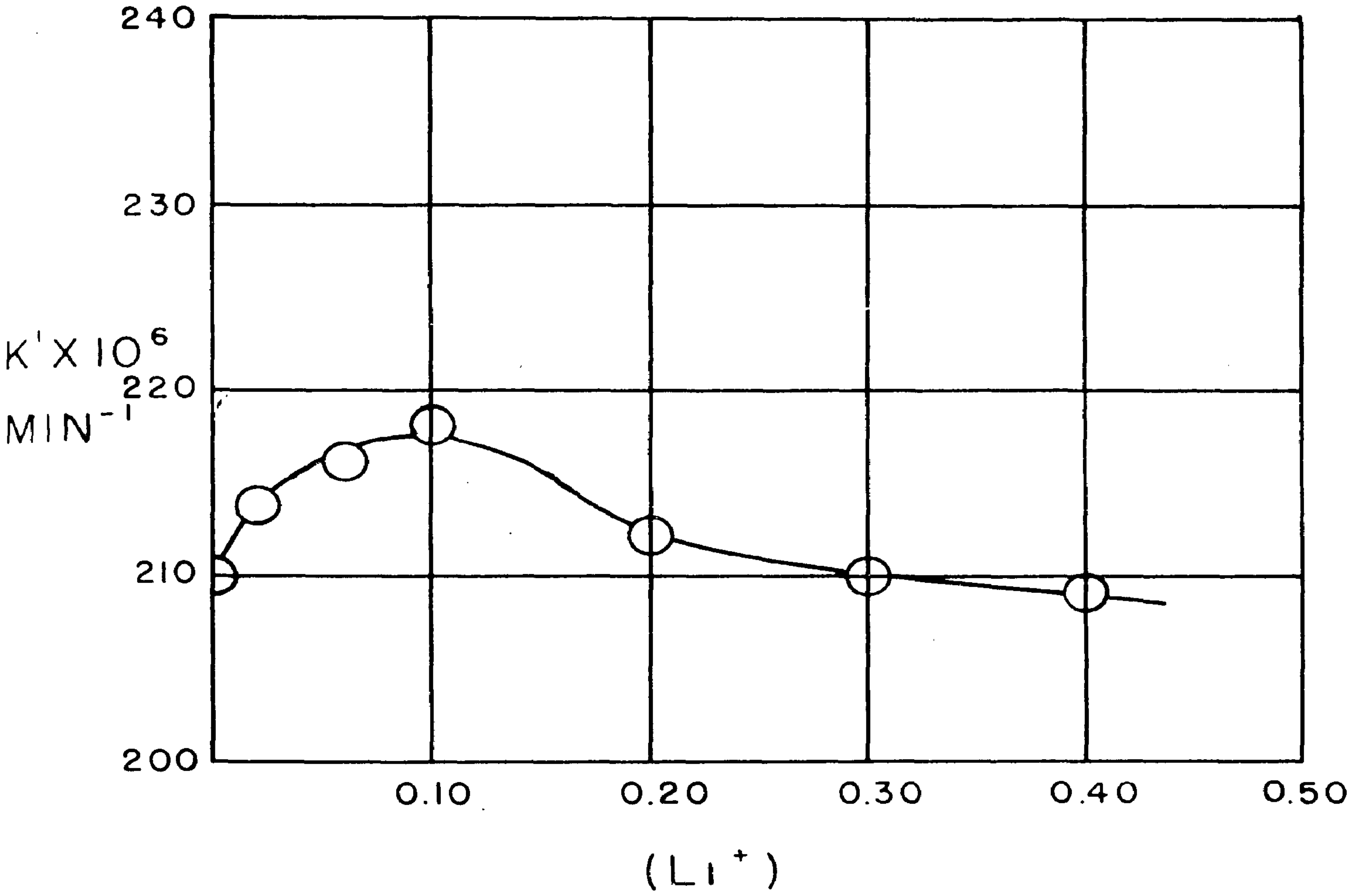

Figure 22. Relation of pseudo constant (k') to lithium ion concentration ((Li^+)) in moles per liter.

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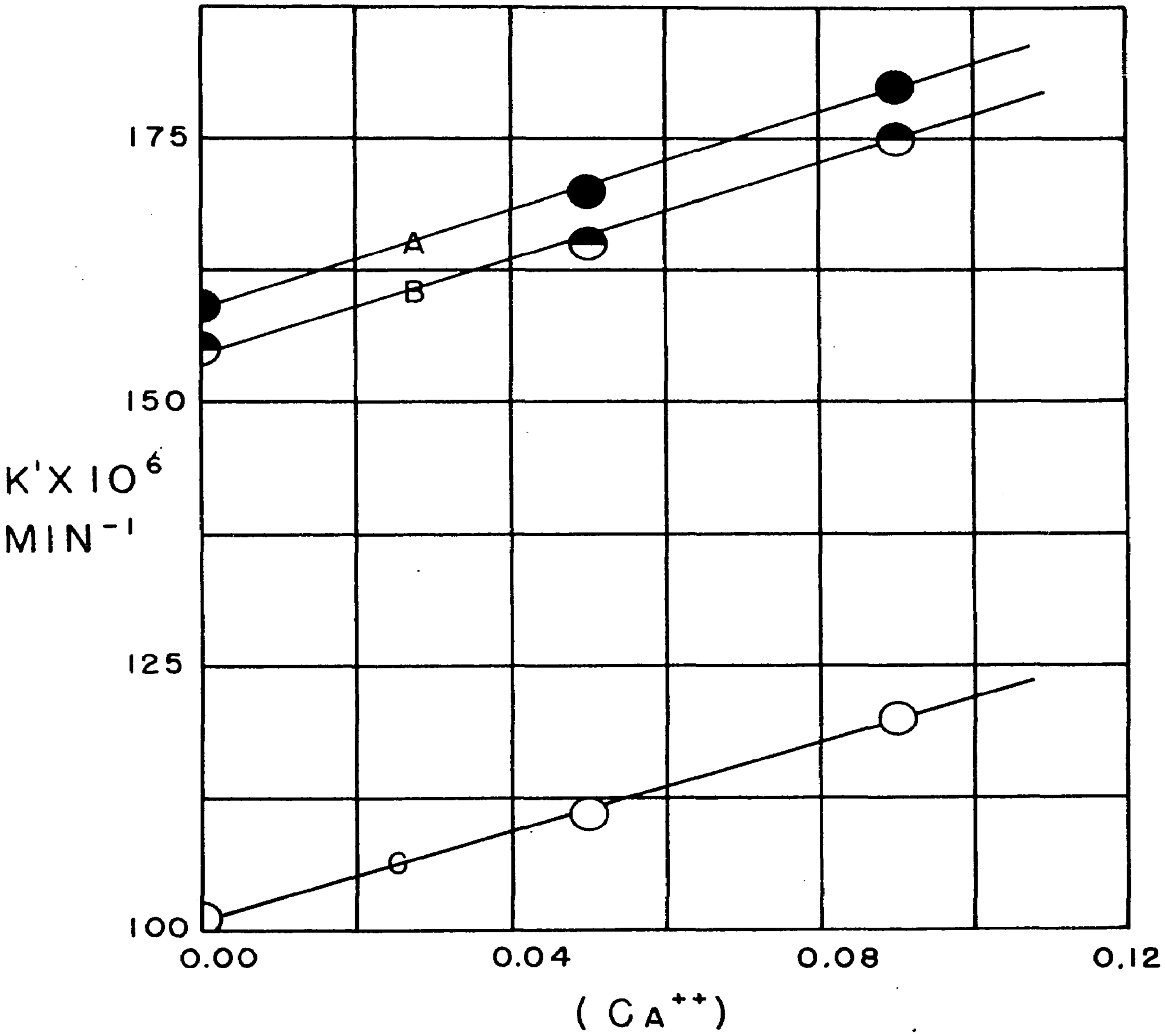

Figure 23. Relation of pseudo constant (k') to calcium ion concentration (Ca^{++}) in moles per liter. A, HOAc: OAc^- = .30:.30 ; B, HOAc:OAc^- = .10:.30 ; C, HOAc:OAc^- = .20:.20.

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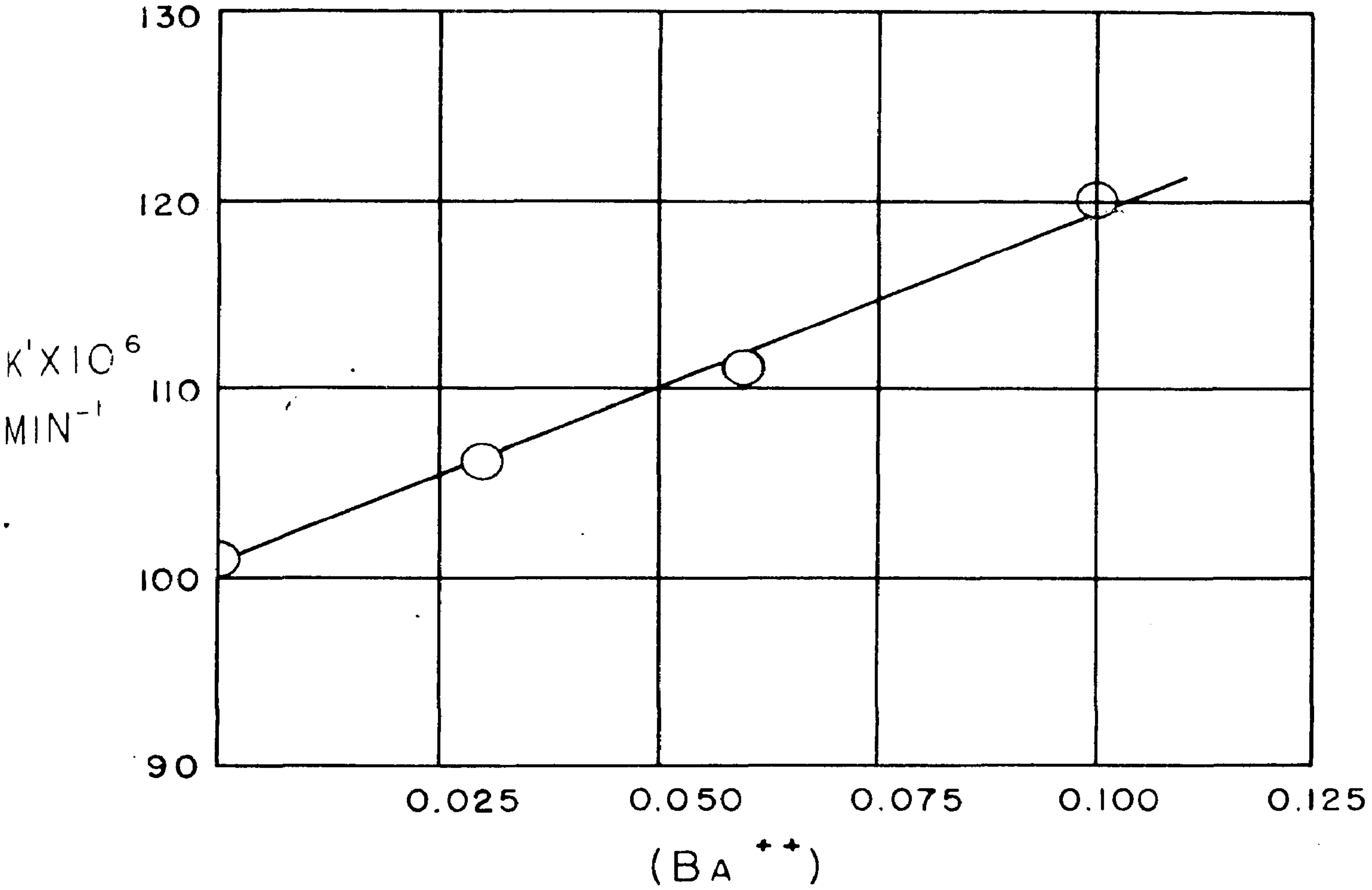

Figure 24. Relation of pseudo constant (k') to barium ion concentration (Ba^{++}) in moles per liter.

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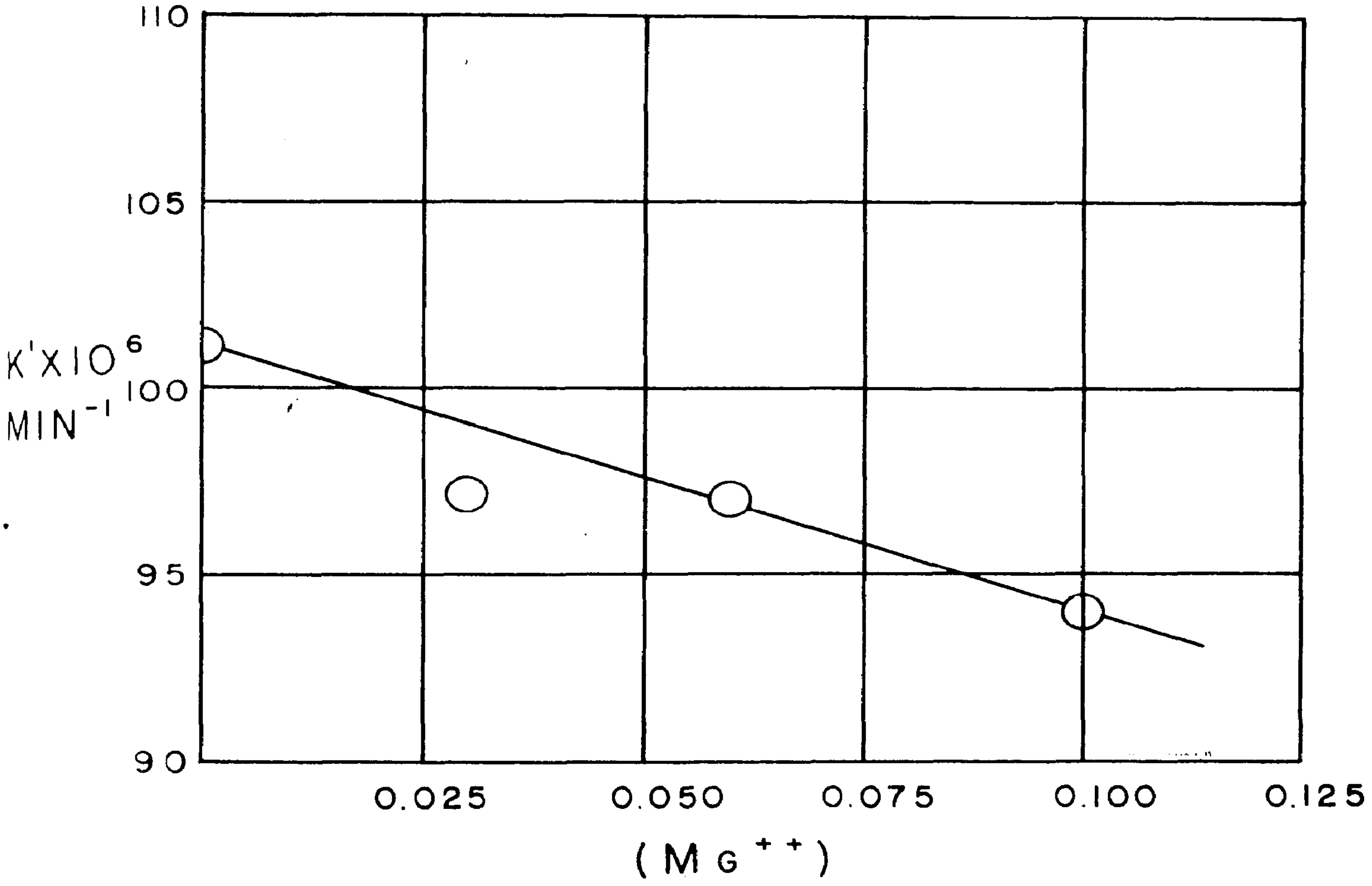

Figure 25. Relation of pseudo constant (k') to magnesium ion concentration (Mg^{++}) in moles per liter.

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dissociation of this substance. (91,92)

(b) The catalysis by the species $CaOAc^+$ is not measurable under these conditions. (93-96)

(c) The expression for the pseudo first order rate constant is that given by equation (14).

The symbols $\underline{a}$ and $\underline{y}$ represent the stoichiometric concentrations of acetate ion and calcium ion respectively, whereas $\underline{x}$ refers to the concentration of $CaOAc^+$. Thus, the expression for the dissociation constant may be written,

$$K_d = \frac{(a-x)\,(y-x)}{x} \tag{16}$$

From this,

$$x = \frac{a + y + K_d - [\,(a+y+K_d)^2 - 4\,ay]^{1/2}}{2} \tag{17}$$

and the expression for the pseudo constant may be rewritten as follows:

$$k' = k_1\,[HOAc] + k_2\,[a-x] + k_3\,[HOAc]\,[a-x] + k_4\,[a-x]\,[y-x] \tag{18}$$

Substituting the value for $\underline{x}$ into equation (18),

$$\begin{aligned} k' = {} & k_1\,[HOAc] + k_2\,a - \frac{k_2}{2}\,[\,a + y + K_d - \sqrt{(a + y + K_d)^2 - 4ay}\,] \\ & + k_3\,[HOAc]a - k_3\,\frac{[HOAc]}{2}\,[a + y + K_d - \sqrt{(a + y + K_d)^2 - 4ay}\,] \\ & + \frac{k_4 K_d}{2}\,[a + y + K_d - \sqrt{(a + y + K_d)^2 - 4ay}\,] \end{aligned} \tag{19}$$

This gives an expression in which the pseudo rate constant is a function of the original metal ion concentration.

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Differentiating,

$$\frac{dk'}{dy} = -\frac{k_2}{2} + \frac{k_2}{2}\left[\frac{y + K_d - a}{\sqrt{(a + y + K_d)^2 - 4ay}}\right]$$

$$-\frac{k_3\,[\mathrm{HOAc}]}{2} + \frac{k_3\,[\mathrm{HOAc}]}{2}\left[\frac{y + K_d - a}{\sqrt{(a + y + K_d)^2 - 4ay}}\right]$$

$$+\frac{k_4K_d}{2} - \frac{k_4K_d}{2}\left[\frac{y + K_d - a}{\sqrt{(a + y + K_d)^2 - 4ay}}\right] \qquad (20)$$

Equation (20) is employed in the calculation of K_d. In the process, two approximations are made, (1) the slopes of the lines representing different buffer ratios are equal at the point $y = 0.10$, and (2) the terms k_3 [HOAc] /2 are equal for the two different buffer ratios. By equating the slopes of the lines, the following expression is obtained,

$$\frac{y + K_d - a}{\sqrt{(a + y + K_d)^2 - 4ay}} = \frac{y + K_d - a'}{\sqrt{(a' + y + K_d)^2 - 4a'y}} \qquad (21)$$

in which a and a' are equal to 0.2 M and 0.3 M respectively. By the substitution of appropriate values for a, a', and y into equation (21), K_d for the species $CaOAc^+$ was found to be 0.15, and k_4 was calculated from equation (19) and found to be 570×10^{-6} mol^{-2} min.$^{-1}$. Substitution of this value for k_4 into equation (18) gave the following values for k' which are listed in Table XIII.

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The value of 0.15 for the dissociation constant of $CaOAc^+$ is in the same order of magnitude as 0.15 and 1.00 which were determined previously by McDougall and Larson (92) and Davies (91) respectively. The present value represents the concentration dissociation constant of $CaOAc^+$ at 50°C., whereas the value of Davies represents the corresponding constant at 25°C.; the value of McDougall and Larson represents the activity dissociation constant at 25°C.

The close agreement of calculated pseudo constants with the corresponding experimental values is an indication of the validity of the above treatment of the data.

The Effect of Barium Ion

The results obtained in the barium hydroxide catalyzed degradation of glyceraldehyde are shown in Table XIII. The results are similar to those observed with calcium hydroxide catalysis; consequently, there is no reason to believe that either the nature or the explanation of the catalysis is any different from that found for calcium hydroxide. The plot illustrating the effect of barium ion concentration on the pseudo rate constant is given in Figure 20.

The Effect of Magnesium Ion

The results are given in Table XII and again a linear relationship was obtained between the metal ion concentration and the pseudo rate constant. The plot is shown in Figure 25. The best straight line

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through the points has a negative slope, but the nature and explanation of this catalysis is probably similar to that found for calcium hydroxide.

The Effect of Lithium Ion

In testing the effect of lithium ion, the results shown in Table XII and Figure 22 were obtained by varying the lithium ion concentration in a system 0.40 M in acetic acid and 0.40 M in acetate ion. The plot shows an increase in the value of the pseudo rate constant through a maximum with increasing lithium ion concentration. Hence, the kinetics of the lithium ion catalyzed reaction appear to be more involved than that catalyzed by calcium hydroxide. Neely observed incomplete dissociation of lithium acetate in his work on the kinetics of mutarotation of aldoses in the presence of metal ions. (97)

However, the following expression which is analogous to that used to explain the calcium ion effect, cannot be applied in describing this effect by lithium ion

$$k' = k_1 [HOAc] + k_2 [OAc^-] + k_3 [HOAc] [OAc^-] + k_4 [Li^+] [OAc^-] \qquad (22)$$

since it does not permit a maximum or a minimum in the plot of <u>k</u>' against the stoichiometric concentration of the metal ion. This fact may be readily shown by rewriting the above expression in the same form as equation (19),

$$k' = k_1 [HOAc] + k_2 [a-x] + k_3 [a-x] [HOAc] + k_4 K_d \, x \qquad (23)$$

and setting the first derivative equal to <u>o</u>, which leads to the expression

$$k_2 + k_3\,[HOAc] = k_4K_d \qquad (24)$$

The terms of equation (24) are all constant. Substitution of $k_2 + k_3$ [HOAc] for k_4K_d in equation (23) gives the following expression in which k' is independent of the lithium ion concentration, an obvious absurdity.

$$k' = k_1\,[HOAc] + k_2\,[a-x] + k_3\,[HOAc]\,[a-x] + (k_2 + k_3\,[HOAc]\,)\,x \qquad (25)$$

The following expression does permit a maximum or a minimum in the plot of k' versus lithium ion concentration.

$$k' = k_1\,[HOAc] + k_2\,[OAc^-] + k_3\,[HOAc]\,[OAc^-] + k_4\,[Li^+]\,[OAc^-] + k_5\,[Li^+] \qquad (26)$$

However, evaluation of k_4 and the dissociation constant for lithium acetate requires determination of the value for k_5, and this was not attempted in the present work.

Mechanism of Metal Ion Catalysis

The fact that catalysis by a metal ion, such as calcium ion, involves a third order term in the expression for the pseudo first order rate constant indicates that the metal ion affects the loss of a proton from glyceraldehyde to a nucleophilic species and suggests the following as possible mechanisms for the catalysis (but does not serve to distinguish between them). (a) the catalysis requires a rapid initial formation of a more or less stable complex between glyceraldehyde and the metal ion

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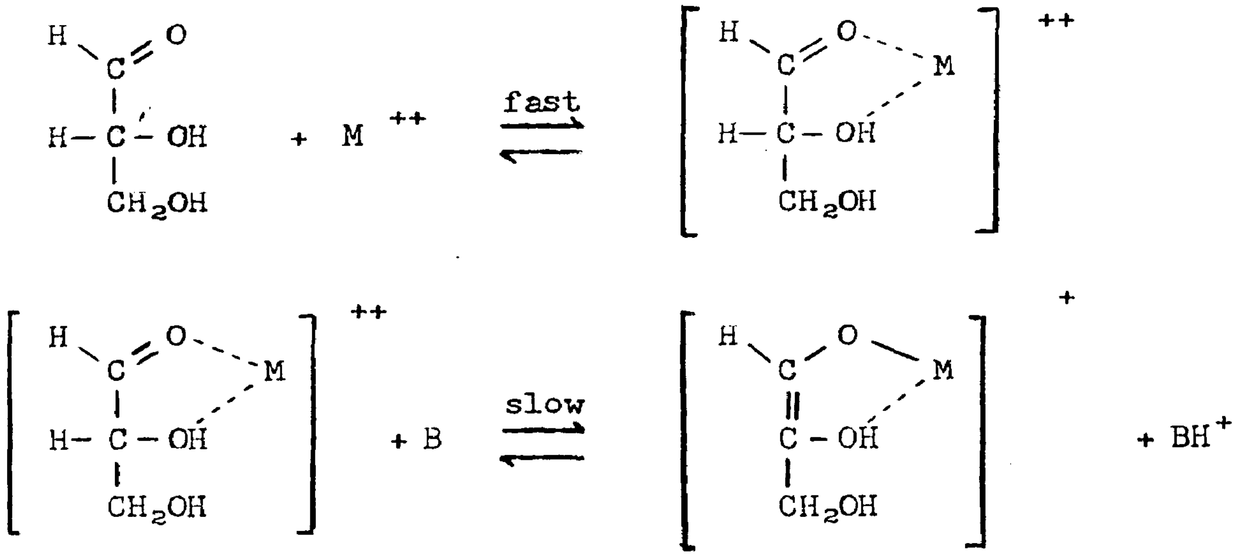
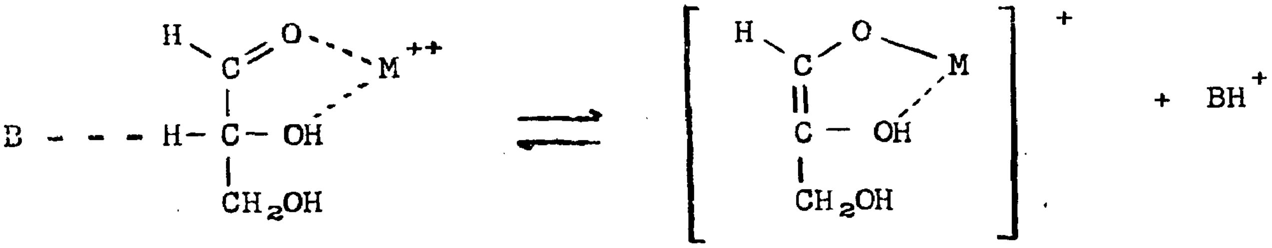
(this may actually involve chelation) followed by capture of a proton attached to carbon 2 of glyceraldehyde by a base such as acetate ion; or (b) the catalysis involves the nearly simultaneous attack of the electrophilic metal ion and the nucleophilic species on glyceraldehyde resulting in loss of a proton from the central carbon atom of the substrate. Both mechanisms are shown by the following equations.

(a)



(b)



In either event, it is not difficult to visualize that an attack on the carbonyl group of glyceraldehyde by a metal ion increases the acidity of the hydrogen attached to carbon 2 thus rendering it more susceptible to removal by a base. Although it appears to be quite certain that stable complexes between the ions of the alkaline earth metals and reducing

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sugars exist in alkaline solutions, there is no published evidence which points to their existence under the conditions of the present experiments. Hence, if the first of these mechanisms is correct, it probably does not involve the accumulation of such a complex, and the argument reduces to a consideration of a sequential or a concerted attack by these catalysts.

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SUMMARY

The catalytic constants of the species acetic acid and acetate ion in the degradation of glyceraldehyde were re-evaluated and found to be 1 x 10^{-5} and 49 x 10^{-5} per mole per minute, respectively. In addition, the value, 5 x 10^{-5} per mole per minute, was assigned to $\underline{k}_3$ in a new term, $k_3\,[HOAc]\,[OAc^-]$, in the expression for the pseudo first order rate constant on the basis of rate measurements in acetic acid solution. The effect of ionic strength on the rate of the reaction was determined.

It was observed that ions of lithium, calcium, and barium increase the rate of the glyceraldehyde degradation in acetate buffers, whereas the presence of magnesium ion produced an inhibitory effect. These data are explained as resulting from incomplete dissociation of the acetates of these metals as well as an electrophilic attack by the metal ions on glyceraldehyde.

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