A STUDY OF THE REDUCING SUBSTANCES AND LACTIC ACID FORMED IN THE CONDENSATION OF FORMALDEHYDE BY CALCIUM HYDROXIDE

by

RICHARD W. LEONG

A THESIS

Presented to the Department of Biochemistry
and the Graduate Division
of the University of Oregon Medical School
in partial fulfillment
of the requirements for the degree of
Master of Science

June 1942

APPROVED:

[redacted]

(Professor in Charge of Thesis)

April 25
1942

[redacted]

(For the Committee)

TABLE OF CONTENTS

INDEX OF THE TABLES

INDEX OF THE TABLES

INDEX OF THE GRAPHS

INTRODUCTION

THE CATALYTIC CONDENSATION OF FORMALDEHYDE IN THE PRESENCE OF AN ALKALINE AGENT

In the year 1859 Butlerow (1) discovered that trioxymethylene in the presence of lime water is condensed to a sweet tasting sugar-like syrup. Hoffman (2) later discovered formaldehyde and proved that trioxymethylene is a polymerized form of formaldehyde. In 1864 von Baeyer (3) from a consideration of the formation of sugar like substances from formaldehyde under the action of alkalies, postulated that formaldehyde is an intermediate product in the photosynthesis of carbohydrates by plants. His theory stimulated studies on the condensation of formaldehyde from both a photosynthetic and a non-photosynthetic viewpoint. Von Baeyer's theory has been changed somewhat by various schools of scientists, but in most modern theories of photosynthesis the role of formaldehyde still remains dominant.

The condensation of formaldehyde to sugar has opened several angles of interest for study. Among these are the alkaline agents of condensation, the effects of various catalysts, the products of condensation, the mechanism of condensation, and the process occurring in photosynthesis.

Much work has been done upon the various alkaline agents of condensation. Loew (4) was probably among the

first to explore this particular approach when he discovered that by using magnesium oxide instead of calcium hydroxide a fermentable condensation product was produced. The hydroxides of certain of the divalent metals have been found to be the most effective condensing agents. Lead hydroxide was found to be especially satisfactory as a condensing base when it is desirable to study the products of condensation since, due to its low alkalinity, it does not change the products after the completion of condensation as much as do the stronger alkaline hydroxides. Hydroxides of the monovalent metals are not satisfactory bases for condensation.

Schmalfuss (5) observed that the addition of glucose or fructose causes a marked acceleration of the condensation and his work started the study of the role of catalysts in the condensation of formaldehyde. Kusin (6) has done the most extensive work in this particular field. His work on the catalytic effect of monoses on formaldehyde condensation has led him to certain conclusions regarding the chemical structure responsible for the catalytic action. Because glycerol and mannit failed as catalysts, he ruled out any possible catalytic effect from the polyatomic alcohol structure. The non-catalytic effect of saccharose and the catalytic effect of glycolaldehyde eliminated the cyclic forms and the glucoside linkage as factors in the catalysis. The free aldo and keto groups were suspected. The nearly equal catalytic activity of fructose, glucose, maltose, and glycolaldehyde made this idea rather untenable and pointed

to the enediol group, which they all possess in alkaline solution. When he blocked enol formation by acetylation of the sugars prior to use and found that the catalytic effect was lost, Kusin concluded that the enediol structure probably represents the catalytically active group. It is interesting to observe that ascorbic acid and acetoin, two substances possessing the enediol structure and having excellent catalytic power, are abundantly present in plant life. Recently J. Van Bruggen (7) studied systematically many catalysts and found thatthose catalysts that furnish the enediols most readily are the most active catalysts and that many of them are simple aldo and keto sugars. He observed that aldonic acids are capable of catalyzing the condensation, though less efficiently than the sugars. Theoretically these aldonic acids may form enediol groups in alkaline solution. Their catalytic effect suggests that they probably do. Besides the enediols his work suggests that possibly the amineols and the peculiar structure of kojic acid are active catalysts.

The study of the compounds present in the syrupy product is not only interesting in itself, but it affords the chemist a basis for speculation as to the possible mechanism of condensation.

The eminent chemist Emil Fischer (8) first separated from the condensation product three different sugars, two of which he designated α-acrose and β-acrose and a third he found to be a ketopentose. Later he identified α-acrose to

be d-l fructose. β -acrose was identified by Küster and Schoder (9) to be d-l sorbose. The ketopentose was found by C. Neuberg (10) to be l-arabinoketose which was confirmed by H. E. Euler (11). An aldotetrose was discovered by E. Loew and was identified by Orthmer and Gerish to be d-l erythrose (12). Other workers have added d-l threose to the list. The compounds listed so far are the constituents of the product at the final condensation stage.

Euler (11) and Loew (4) found glycolaldehyde and dioxyacetone present in the early stages of formaldehyde condensation. Orthmer and Gerish stressed glycolaldehyde as the true primary product which condenses with formaldehyde to produce glyceroaldehyde, and a portion of the latter forms dioxyacetone through Lobry de Bruyn-Van Ekenstein phenomenon. They attributed the cold reducing action of the condensation product to the action of the primary products formed in the condensation.

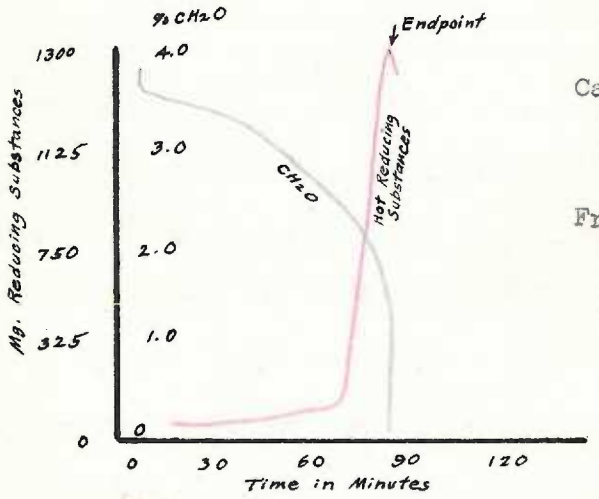
A study of the biochemical properties of the syrupy mass formed at the point of complete formaldehyde condensation has been made in our laboratory. It was discovered that the syrup mass does not form demonstrable liver glycogen when administered parentally or orally to white rats and that it is not practical as a diuretic because the syrup when administered intravenously to dogs showed toxic properties.

J. Van Bruggen in 1938 began the study of the condensation of four per cent formaldehyde containing four grams

calcium hydroxide per 100 milliliters at 40° C. with and without various catalysts. In each condensation reaction the concentrations of the formaldehyde and the hot reducing substances (reduction by Shaffer Hartman reagent #50 at 100° C.) were followed simultaneously during the reaction, and the two curves plotted together. The graph shows that about one half of the formaldehyde had disappeared before hot reducing substances were formed. As soon as the hot reducing substances began, the hot reduction curve showed a precipitous climb. At the same time the formaldehyde curve dropped equally precipitously as the formaldehyde was transformed into the sugar-like product. Van Bruggen believes that the first half of the formaldehyde curve represents the formaldehyde loss probably due to the Cannizzaro reaction and the formation of substances not oxidizable by the Shaffer Hartman reagent #50. He suggests that the part of the hot reducing curve showing initial rise probably represents the point where the concentration of the primary products of the condensation reaction has reached the stage where its autocatalytic effect passes into macroscopic proportions. The essential slope and shape of all formaldehyde curves are about the same, no matter what the catalyst used.

The graph on the following page illustrates a typical relationship between the formaldehyde and hot reduction curves. The endpoint of the condensation is conveniently

marked by the reaction mixture becoming pale yellow. At this point the hot reducing value is maximum. The hot reduction value rapidly decreases after the yellow endpoint is reached, due to further changes probably caused by action of the calcium hydroxide.



Catalysed By 0.11 mM. Glucose per 100 ml. of 4% CH_2O (Merk)

From J. B. Van Bruggen's Thesis "Catalysis of Formaldehyde Condensation", 1939.

The history of the condensation of formaldehyde by alkaline agents would not be complete without a review of some of the theories which attempted to explain how catalysts promote the condensation.

Kusin's theory of the action of the catalyst from the viewpoint of the enediol structure stands alone in the literature. Before Kusin's work on this particular phase of the condensation there had been no contribution to the subject except the report of Schmalfuss that reducing sugars are active catalyst. Kusin's series of papers remain entirely the only recent work published on catalytic action pertaining to the non-photosynthetic condensation of form-

aldehyde.

As has been mentioned there are present in plants compounds having a stable enediol structure such as ascorbic acid and acetoin. There are also compounds which in alkaline solution have the enediol structure. These compounds are the sugars with a free reducing group and some of the hydroxyl organic acids. The following equations illustrate the Lobry de Bruyn-Van Ekenstein phenomenon when a sugar is placed in an alkaline solution. It will be noted that the reactions are reversible and an enediol structure is one product.

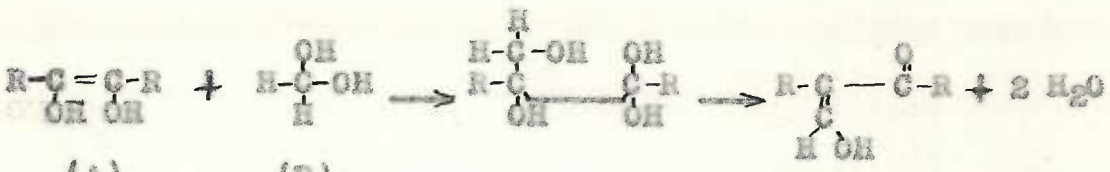
Aldehyde Form — Enediol Form — Keto Form

(R represents $CH_2OH(CHOH)_x$)

Kusin chose benzoin ($C_{14}H_{12}O_2$) in order to study the intermediate steps through which the enediol catalyst acts because benzoin has a free sugar group, and being a stable compound, it can be recovered quantitatively from the reaction mixture. The following equation expresses the tautomeric equilibrium of benzoin in an alkaline solution:

Kusin expressed his theory of the catalytic action of benzoin in the following two steps:

First Step

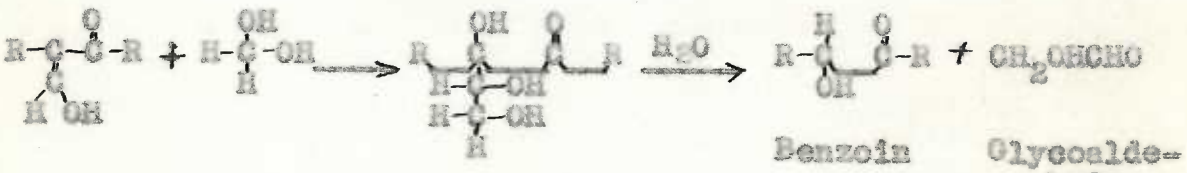


(A) (B) (Compound Known)

A------Enediol Form of Benzoin

B------Hydrated Form of Formaldehyde

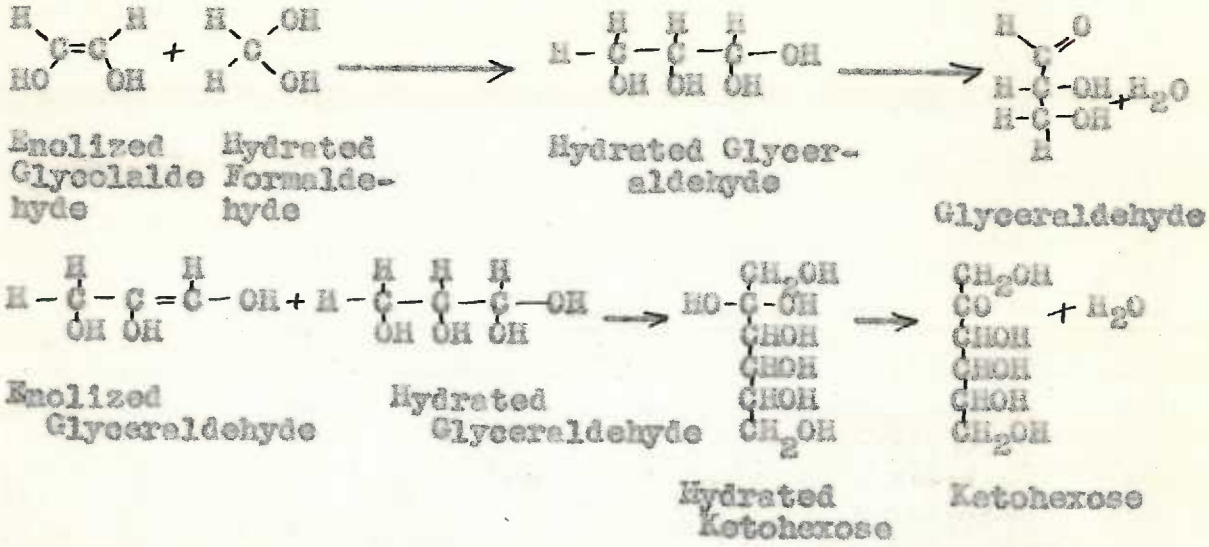
Second Step



Compound Unknown — Benzoin — Glycoaldehyde (An intermediate sugar)

(R is the phenyl group)

Although speculation on the actual mechanism of catalytic action is rare, the same is not true concerning the formation of the final sugar products (products appearing at the endpoint of the condensation). Many theories have been offered. Among them are Baeyer's stepwise type of condensation, Nef's condensation of formaldehyde by enolic combinations, and others based upon aldo condensation. Kusin carried his enediol idea over to the formation of the final condensation products. The following equations illustrate his viewpoint:



Two German chemists, Orthmer and Gerish have done the most recent and the most complete work concerning the condensation of intermediate to final products. They studied samples of the condensation product through the various stages of condensation, and identified the products by subjecting them to hydrogenation, fractional distillation, and then preparing benzoyl derivatives. These workers observed the presence of substances in the condensation product which reduce alkaline copper solution in the cold. At the point of maximum cold reduction they found a maximum concentration of short chain sugars, glycolaldehyde and dioxyacetone. At the completion of the condensation, two ketohexoses, an aldotetrose, and a ketopentose were found. From their work on the formation of straight chain hexoses, Orthmer and Gerish drew three conclusions. The first is that the stepwise combination of six formaldehyde molecules probably accounts for only a small portion of the condensation product because the intermediate small molecules formed

can readily condense with themselves. Secondly, the combination of tetroses and dioses to form straight chain hexose molecules is improbable because dioses have a greater tendency to combine with themselves to form tetroses than to combine with tetroses to form hexoses. Lastly, the most likely mode of formation of the hexoses in the condensation product is the combination of the dihydroxyacetone molecules with glyceraldehyde molecules. Since no branched chains were found in the condensation product, the possibility of molecules of dihydroxyacetone or of glyceraldehyde combining with themselves probably does not exist. The following equations illustrate the formation of branched chain sugars from the condensations of dioxyacetone molecules and of glyceraldehyde molecules:

$$CH_2OH-CO-CH_2OH + CH_2OH-CO-CH_2OH \longrightarrow$$

Dioxyacetone Dioxyacetone (Dihydroxyacetone)

$$HO-C(CH_2OH)(CH_2OH)-CHOH-CO-CH_2OH$$ (4 possibilities)

$$CHO-CHOH-CH_2O + CHO-CHOH-CH_2O \longrightarrow$$

Glyceraldehyde Glyceraldehyde

$$HO-C(CHO)(CH_2OH)-CHOH-CHOH-CH_2OH$$ (8 possibilities)

When molecules of glyceraldehyde and of dioxyacetone combine with each other, the reaction proceeds in the following fashion:

$CH_2OH-CHOH-CHO + CH_2OH-CO-CH_2OH \longrightarrow$

Glyceraldehyde Dioxyacetone

$CH_2OH-CO-(CHOH)_3-CH_2OH$

Straight Chain Ketohexose

The aldotetrose and ketopentose (ketoarabinose) are most likely formed respectively from the combination of two molecules of glycolaldehyde and from the combination of two molecules of glycolaldehyde and from the combination of glycolaldehyde and dioxyacetone.

The condensation of formaldehyde in vitro always makes one wonder about its relationship to the photosynthesis of sugar by plants. It is of interest to speculate how this particular chemical reaction might fit into the problem of the photosynthesis of sugar should such a process occur in photosynthesis by plants.

The condensation of formaldehyde would follow the production of formaldehyde generally assumed to occur through the photo-reduction of carbon dioxide. In vitro formaldehyde condensation has been studied from both the photosynthetic and the non-photosynthetic viewpoints, the latter especially in the presence of an alkaline agent. While it seems that the conditions within the environment of the plant favor the photo-condensation of formaldehyde to sugar, the study of this form of condensation in vitro has been found to be very inefficient. It is well known, however, that living cells acting through enzyme systems,

may smoothly and efficiently promote chemical reactions which are achieved with difficulty or not at all in vitro. Formaldehyde is readily condensed to sugar-like substances in the presence of certain alkaline agents and especially when catalytic substances containing the enediol group are present. It is certainly true that the reaction of plant juices is not alkaline as required for the in vitro formation of sugars from formaldehyde. Enediol compounds such as ascorbic acid and acetoin are present and may possibly function as catalysts for the process of photosynthesis with the aid of plant enzyme systems. No definite facts concerning such possibilities are known, and these hypotheses must be considered as entirely theoretical.

In a few last words to this introduction, I shall bring the subject of cold reduction to the foreground again, and the special interest toward which this research is directed.

Orthmer and Gerish first observed the cold reduction property of the condensation reaction and used this property to determine the point where the condensation must be stopped in order to obtain a syrup containing a maximum of primary sugars. In our laboratory Van Bruggen had followed the cold reduction curve of the condensation reaction of both an uncatalysed reaction and a catalysed reaction. He found that in both cases the maximum cold reduction precedes the endpoint of the reaction by several minutes.

As there has been no comparative work done on the cold reduction values of products obtained in condensation reactions induced by various types of catalysts, and their relation to the hot reduction values, it is the purpose of this research to investigate these unknown relationships.

The formation of saccharinic acids, such as lactic acid, as a result of the action of alkali upon sugars has been extensively investigated. Accordingly the production of lactic acid during the condensation of formaldehyde to sugars in alkaline solution was considered worthy of investigation. The results of this study are included in the thesis following the discussion of cold reduction.

EXPERIMENTAL

THE TECHNIQUE OF THE CONDENSATION EXPERIMENTS

Each condensation was carried out in a large pyrex tube (38 x 200) suspended in a constant temperature water bath regulated by an electric thermostat. The condensation tube held a white opaque mixture of 100 ml. of 4% HCHO in distilled water, 4 g. of $Ca(OH)_2$, and 0.27 mM. (millimol) of a catalyst (if used) in each experiment. An adjacent tube containing a mixture of 4 g. of $Ca(OH)_2$ in 100 ml. of distilled water was used for the color control of the end-point. The stirrers for the two tubes and the water bath were driven by a single pulley at a speed which kept the $Ca(OH)_2$ in a homogenous suspension. The pulley was driven by a ¼ h. p. motor. The water bath was illuminated by a blue light from a 100 watt Mazda lamp during each experiment. This allowed an accurate detection of the first instant of yellow color change in the condensation mixture at the endpoint.

Generally before the hot and cold reduction curves were determined for a particular catalysed condensation, the endpoint of the condensation was first determined in a preliminary experiment so that the intervals for withdrawal of the samples could be planned. By this method it was possible to get a truer picture of the reduction curves--especially the cold reduction curve because a greater number of samples

could be withdrawn during the period of greatest reducing activity. From the beginning of marked reducing activity and through the period of the rapid loss of reduction, samples were withdrawn every 2 min. for the cold reduction curve. Samples preceding and following these periods were taken less often. The peak of each cold reduction curve appeared only momentarily, and a large number of samples taken during the period just preceding the yellow endpoint was necessary in order to catch the maximum cold reduction. The peak of each hot reduction was easily determined because it was marked by the endpoint of the condensation--the point when the reaction mixture turned yellow.

At the beginning of every condensation experiment, the HCHO solution with catalyst added was preheated to 40° C. This required 5 min. of heating in the constant temperature bath. The tubes and the water bath were kept stirred continuously from the period of preheating through the completion of the experiment. The electric timer was set as soon as the $Ca(OH)_2$ was added to the HCHO solution.

THE REAGENTS USED IN THE CONDENSATION

The 4% HCHO was made from Merk C. P. 40% HCHO and distilled water. A batch of 12 l. was prepared which was sufficient for all the experiments performed. The HCHO was stored in a large glass bottle from which the necessary quantity was drawn for each experiment by a siphon system. The concentration of the reagent was checked periodically by the Romijin iodometric method @ and found to remain constant.

Van Bruggen observed that $Ca(OH)_2$ from different chemical companies caused some variations in the length of the condensation reaction due to slight differences in the small amount of impurities present. On the basis of his findings, this laboratory is using Mallinckrodt C. P. $Ca(OH)_2$ as the standard lime. The lime in the laboratory is kept in a tightly rubber stoppered glass bottle to minimize absorption of moisture.

The catalysts employed in the experiments were all chemically pure substances.

@ Romijin (13) Iodometric Method of HCHO Determination: 5 ml. of HCHO solution containing not more than 50 mg. of HCHO were mixed with 40 ml. of N/10 iodine solution. Strong NaOH was added slowly by drops until the solution was light yellow. 10 min. were allowed for the complete conversion of the HCHO to HCOOH by the oxidizing action of iodine in the alkaline medium. Then the solution was acidified with strong HCl, and the liberated iodine was titrated with N/10 $Na_2S_2O_3$. Each ml. of N/10 iodine used by the reaction represents 0.0015 g. of HCHO.

EXPERIMENTAL WORK ON THE REAGENT FOR DETERMINATION OF REDUCING VALUES

The first phase of this particular research problem was concerned with the selection of a copper reagent suitable for making comparative studies of hot and cold reduction values. The approach to this phase was directed by several properties of the condensation product. They are as follows:

1. The molecular weight of the condensation product from the action of calcium hydroxide on formaldehyde averages about 180 (7).

2. The hexose sugars are probably straight chain molecules as shown by the evidence of Orthner and Gerish.

3. The evidence as a whole at present supports the keto sugar as the predominant form of the hexose sugars.

4. From Van Bruggen's work, the greatest concentration of the product obtained from the condensation of four per cent formaldehyde is less than two per cent.

It is desirable that the copper reagent selected should be relatively inert to the final products of the condensation while at the same time being reduced by the primary products. On the basis of the above properties, two per cent fructose in four per cent calcium hydroxide was selected as the standard by which the relative activities of several copper reagents toward the condensation product were studied. The reduction of each copper reagent by the

primary products was determined on samples withdrawn from a formaldehyde condensation mixture catalysed by 0.27 millimol glucose for one hour at 40° C.

Soxhlet's @ modification of Fehling's reagent was first tested for reduction by the standard fructose solution because both Orthner and Gerish and Van Bruggen used the reagent for cold reduction.

The technique was as follows:

1 ml. of the standard fructose solution at 40° C. was added to 2 ml. of Soxhlet's reagent (mixed solution containing both A and B components) and allowed to react for 30 min. (timed by an automatic clock) at room temperature (28° C.). The amounts of reduction expressed in terms of 0.005 N. $Na_2S_2O_3$ were:

31.3

33.5

The peak of the cold reduction curve which Van Bruggen obtained from the condensation of HCHO catalysed by 0.27 mM. glucose is only 30 ml. 0.005 N. thiosulfate. Orthner and Gerish state that only the short carbon chain sugars show cold reduction. Why fructose reduces in the cold in this

---

@ Soln. A--6.928 g. $CuSO_4.5H_2O$ in 100 ml. of H_2O.
Soln. B--34.6 g. Rochelle salt and 10 g. NaOH in 100 ml. H_2O.

After a ½ hr. of reduction 2.5 ml. of 25% H_2SO_4 was added along with 1 ml. of 20% KI. The liberated iodine was titrated with 0.05 N. $Na_2S_2O_3$. A blank was run simultaneously, and the difference between it and the sample was recorded.

case is not clear. The high pH factor of Soxhlet's Reagent is the most likely cause. Fragmentation of the sugar into substances possessing cold reducing properties must be considered.

The next copper reagent studied was Shaffer-Hartman reagent #60. From here on the temperature for cold reduction was standardized at 40° C. because room temperature is apt to be a variable factor, a higher temperature abbreviates the reduction time, and the thermostatically controlled water bath may be conveniently used. With this reagent (refer to Table C) the standard fructose solution reduced at a slower rate for the first ten minutes than did the condensation mixture, but by the fifteen minute period, there remained only a slight difference between the condensation and standard fructose reducing powers. It was obvious that Shaffer-Hartman reagent #60 was not satisfactory, and that a reagent of lowered pH should be tried.

Since Shaffer-Hartman reagent #50 is less alkaline with a pH of 9.37, it was deemed worthy of trial. The fructose curve showed much less reduction as was to be expected, but the condensation curve showed an initial slow reduction passing into a markedly accelerated reduction (Table A and B). Apparently the lowered pH is favorably to increased reduction by the condensate. Upon adding sufficient $NaHCO_3$ to Shaffer-Hartmann reagent #50, the pH decreased to 8.82. The two reduction curves were in general only slightly lower than for the preceding reagent (Table B and graph on page 23).

By making the ratio of $Na_2CO_3/NaHCO_3$ 7/60 (grams), a reagent having a pH of 8.27 was obtained. This reagent gave a very low reduction curve with fructose. Since by further altering the buffer ratio, it was not possible to decrease the pH to a lower value and yet have a buffer stable enough to maintain the pH constant for a convenient period, it was decided to use the reagent for our cold reduction experiments. The reduction time of 20 min. was set because at this point there is very little reduction by fructose. However, since the concentration of the $NaHCO_3$ in the reagent is not far from saturation, it was difficult to dissolve the compound. Equivalents of postassium salts were then substituted because $KHCO_3$ is much more soluble. The pH of the reagent so obtained, however, is slightly higher--8.46. The reduction curves are also slightly higher than the sodium (pH 8.27) reagent. To prevent $CuCO_3$ from precipitating out of the reagent, the Rochelle salt was increased from 25 to 37.5 g. This copper reagent buffered by postassium carbonate-bicarbonate to 8.46, and prepared as outlined below, was found suitable for following reduction values during the course of formaldehyde condensation.

PREPARATION OF THE COLD REDUCTION REAGENT

The following stock solutions were kept:

$CuSO_4.5H_2O$ ---------- 15%

Rochelle Salt ---------- 25% (kept in refrigerator)

KIO_3 ---------- 2.8536% (25 ml. = 0.7134 g.)

KI ---------- 10%

A liter of the reagent was made up at a time and was kept in a closed container with a siphon system connected to an automatic pipette. The system was kept closed so that the slow loss of CO_2 was minimized. Under this condition the pH remained constant at 8.46 for a long period. 9 g. of $KHCO_3$ were dissolved in about 500 ml. of distilled water. 50 ml. of $CuSO_4$ solution were mixed with 150 ml. Rochelle salt solution, and the mixture was introduced slowly into the buffer solution through a long stem funnel which projected underneath the surface of the liquid. As the mixture was being introduced, the buffer solution was gently rotated. 25 ml. KIO_3 solution were pipetted into the mixture, and lastly 10 ml. of 10% KI solution. The mixture was diluted to 1 l.

THE TECHNIQUE OF DETERMINING THE HOT AND COLD REDUCTION VALUES

Before a condensation reaction was started, a number of pyrex tubes (25 x 200) were filled with 5 ml. of the copper reagent measured with a precision automatic pipet. Each pair of tubes was labeled properly for the sample which it was to receive. Glass bulbs were used to cover the tubes. A series of 50 ml. Erlenmeyer flasks each containing 9 ml. of 4% $Ca(OH)_2$ was used for diluting the samples for hot reduction determinations.

At the proper interval, about 3 ml. of the condensation mixture were withdrawn by a special pipet. 1 ml. was pipetted into the cold reduction tube containing 5 ml. of copper reagent and supported in the 40° C. constant temperature bath. This tube had been preheated for a few minutes before the sample of the condensation mixture was taken. Another milliliter was diluted with 9 ml. of $Ca(OH)_2$. 1 ml. of this diluted sample was added to one of the tubes containing 5 ml. of copper reagent and put immediately into a boiling water bath. The reduction time was accurately controlled by an electric stop clock for 20 min. At the end of the reduction period, the tubes were cooled for 5 min. in tap water. 2 ml. of a mixture of 2.5% $K_2Cr_2O_4$ and 2% KI were added, and this was followed by 5 ml. of 2.5 N. H_2SO_4. The tubes were shaken thoroughly, allowed to stand

5 min., and titrated with freshly made 0.005 N. $Na_2S_2O_3$ using a West automatic sugar titration apparatus. A blank was run on the copper reagent, and the reduction values were recorded as ml. 0.005 N. thiosulfate titration differences.

The tables and graph which appear in the several pages following show the experimental data obtained in the experiments described above.

Reduction Time Curves at 40° C. Table A

Composition of Copper Reagent in Grams Per Liter

$CuSO_4.5H_2O$ ---------- 7.5
KIO_3 ---------- 0.7134
(Equivalent to 20 ml. N.)
KI ---------- 1.0
Rochelle Salt ---------- 25.0
Na_2CO_3 ---------- 25.0
$NaHCO_3$ ---------- 20.0
(Shaffer-Hartman Reagent #50)

pH of reagent ---------- 9.37
pH of mixture of 1 ml. condensate and 5 ml. reagent ---------- 9.58
(Readings by Beckman Glass Electrode)

ml. 0.005 N. $Na_2S_2O_3$ Titration Difference

Time Minutes	Fructose	Formaldehyde Condensation
5	0.05	4.00
10	0.85	10.25
15	1.35	15.35
20	3.45	19.45
30	5.65	over 19.75
45	9.20	over 19.75
60	12.45	over 19.75

Note:

Column 2 contains the values from the oxidation of 1 ml. 2% fructose, and column 3 the values from the oxidation of 1 ml. of the glucose catalysed condensate taken after 1 hr. of condensation.

Reduction Time Curves at 40° C. Table B

Composition of Copper Reagent in Grams Per Liter

$CuSO_4.5H_2O$	7.5
KIO_3	0.7134
(Equivalent to 20 ml. N.)	
KI	1.0
Rochelle Salt	25.0
Na_2CO_3	25.0
$NaHCO_3$	50.0
pH of reagent	8.82
pH of mixture of 1 ml. condensate and 5 ml. reagent (Readings by Beckman Glass Electrode)	9.02

ml. 0.005 N. $Na_2S_2O_3$ Titration Difference

Time Minutes	Fructose	Formaldehyde Condensate
5	0.25	4.75
10	0.95	10.45
15	1.70	14.10
20	2.50	16.60
30	3.95	over 19.65
45	6.45	over 19.65
60	9.85	over 19.65

Note: Same as Table A.

Reduction Time Curves at 40° C. Table C

Composition of Copper Reagent in Grams Per Liter

$CuSO_4.5H_2O$ ---------- 7.5
KIO_3 ---------- 0.7134
(Equivalent to 20 ml. N.)
KI ---------- 1.0
Rochelle Salt ---------- 25.0
Na_2CO_3 ---------- 40.0
(Shaffer-Hartman Reagent #60)

pH of reagent ---------- 10.12
pH of mixture of 1 ml. condensate and 5 ml. reagent ---------- 10.84
(Readings by Beckman Glass Electrode)

ml. 0.005 N. $Na_2S_2O_3$ Titration Difference

Time Minutes	Fructose	Formaldehyde Condensate
2		2.75
4		6.55
5	5.00	
6		7.85
8		9.35
10	6.45	10.50
15	12.30	12.80
30	18.45	18.95
45	over 19.80	over 19.85
60	over 19.80	over 19.85

Note: Same as for Table A.

Reduction Time Curves at 40° C. Table D

Composition of Copper Reagent in Grams Per Liter

$CuSO_4.5H_2O$	7.5
KIO_3	0.7134
(Equivalent to 20 ml. N.)	
KI	1.0
Rochelle Salt	37.5
K_2CO_3	0.1
$KHCO_3$	72.0
pH of reagent	8.46
pH of mixture of 1 ml. condensate and 5 ml. reagent	8.9-9.0

(Readings by Beckman Glass Electrode)

ml. 0.005 N. $Na_2S_2O_3$ Titration Difference

Time Minutes	Fructose	Formaldehyde Condensate
5	0	1.25
10	0.39	4.25
15	0.49	6.50
20	0.96	9.63
30	1.47	14.90
45	2.09	19.00
60	3.19	over 19.75

Note: Same as for Table A.

Reduction Time Curves at 40° C. Table E

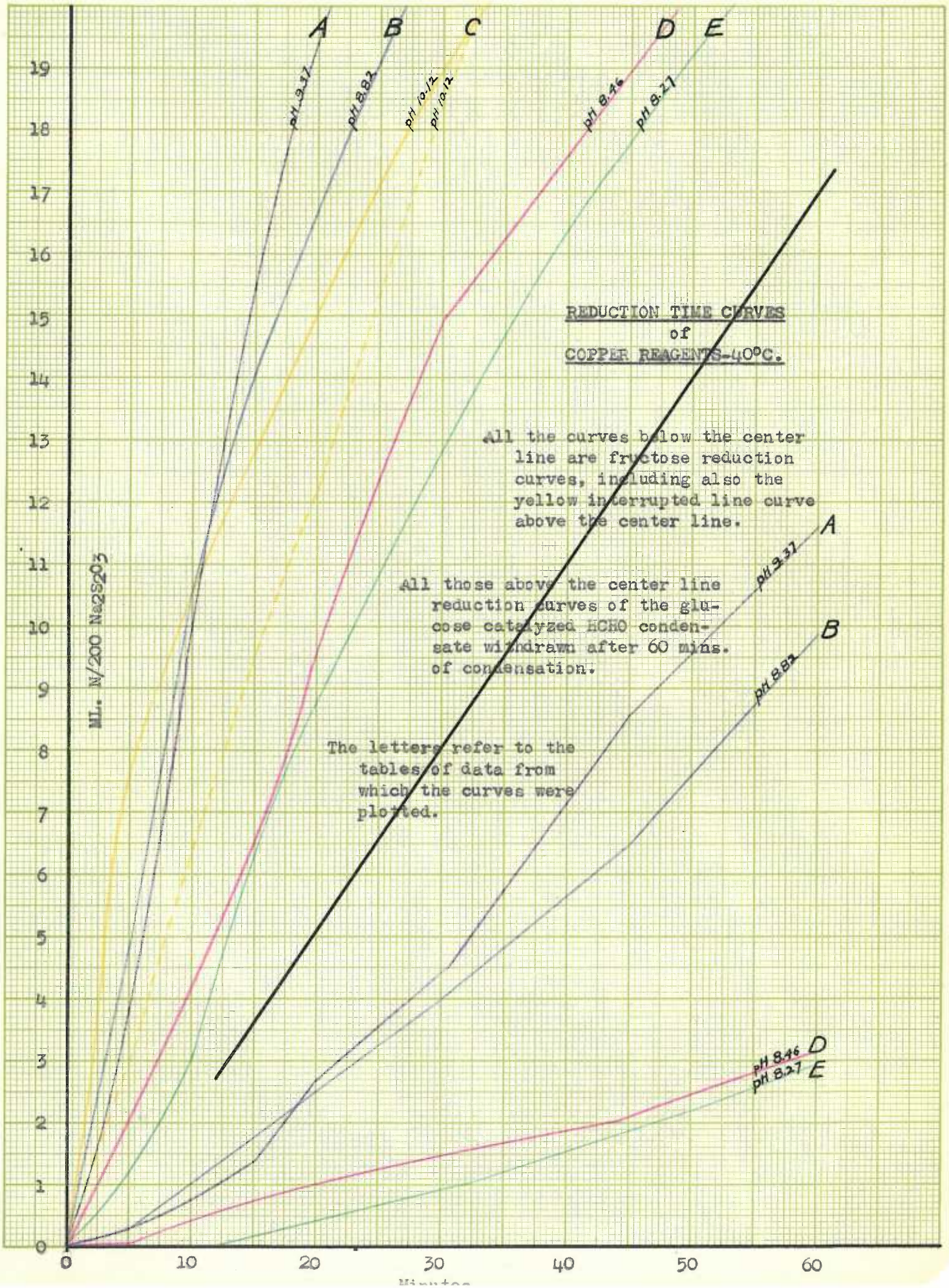
Composition of Copper Reagent in Grams Per Liter

$CuSO_4.5H_2O$	7.5
KIO_3	0.7134
(Equivalent to 20 ml. N.)	
KI	1.0
Rochelle Salt	37.5
Na_2CO_3	7.0
$NaHCO_3$	50.0
pH of reagent	8.26
pH of mixture of 1 ml. condensate and 5 ml. reagent	8.74-8.78
(Readings by Beckman Glass Electrode)	

ml. 0.005 N. $Na_2S_2O_3$ Titration Difference

Time Minutes	Fructose	Formaldehyde Condensate
5	0.00	1.25
10	0.00	3.00
15	0.18	6.30
20	0.45	8.75
30	0.95	12.80
45	1.92	17.80
60	2.92	over 19.45

Note: Same as for Table A.


A
B
C
D
E
pH 9.37
pH 8.82
pH 10.12
pH 10.12
pH 8.46
pH 8.27
REDUCTION TIME CURVES
of
COPPER REAGENTS-40°C.
All the curves below the center line are fructose reduction curves, including also the yellow interrupted line curve above the center line.
All those above the center line reduction curves of the glucose catalyzed HCHO condensate withdrawn after 60 mins. of condensation.
The letters refer to the tables of data from which the curves were plotted.
ML. N/200 Na2S2O3
Minutes
0
10
20
30
40
50
60
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19

DATA

The data in the following tables and the curves which they represent give a picture of the hot and cold reduction activities throughout the course of the condensation and the period following the condensation. Each table presents the same condensation reaction but catalysed by a different substance. In every case the concentration of the catalyst was 0.27 millimol per 100 milliliters of formaldehyde. Before an experiment was carried out to determine the reduction curves, a preliminary experiment was first done to determine the time of the condensation endpoint. It was necessary to repeat many of the experiments in order to determine accurately the reduction activities at particular time intervals in the condensations. The cold reduction curves presented most of the difficulties. Since the peak of each cold reduction curve exists momentarily, it is easily missed, especially if for some unknown reason, the endpoint of the condensation was off a minute or more. Difficulties of this nature were usual with the condensations catalysed by slow catalysts. The first set of reduction values for a particular condensation reaction often gave curves which were irregular. However, on repetition of the experiment, curves of normal contour were obtained. The data for any particular pair of curves may come from more than one experiment.

All the catalysts were used in concentrations of 0.27 mM. per 100 ml. HCHO. The following catalysts and their endpoints were used:

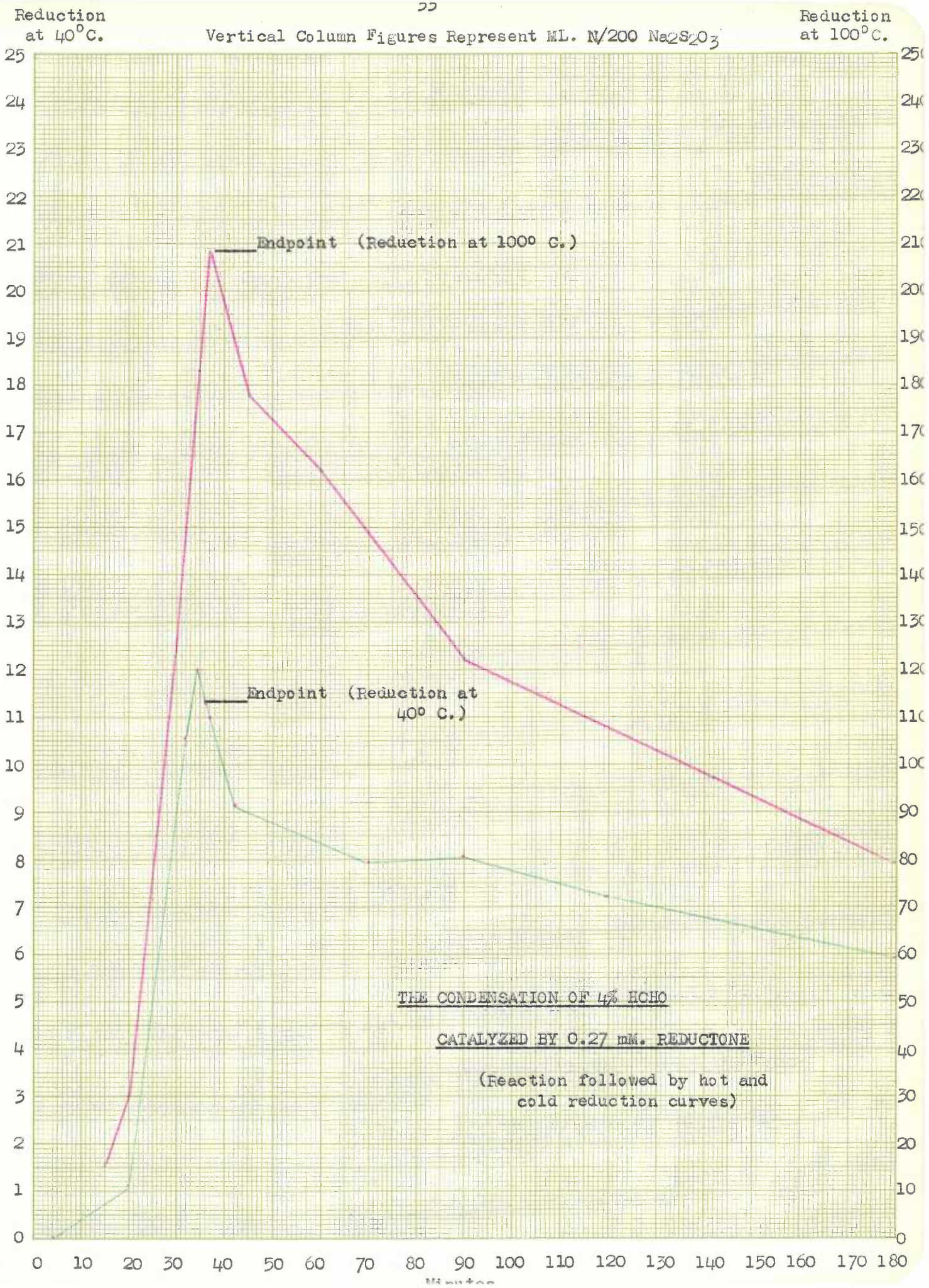
Table	Catalyst	Endpoint (Minutes)
1	Reductone	37
2	Fructose	44
3	Xylose	54
4	Maltose	58.5
5	Galacturonic Acid	64.5
6	Glucose	64
7	Lactose	72
8	Kojic Acid	80
9	Glucosamine	79.5
10	Calcium Mannonate	82.5
11	Calcium Gluconate	93
12	Tartaric Acid	163.5
13	Control Reaction (Blank)	207

Hot and Cold Reducing Values — Table 1

The Condensation of 4% HCHO at 40° C. Catalysed By 0.27 mM. (0.0244%) Reductone

Time Minutes	ml. 0.005 N. $Na_2S_2O_3$ Titration Difference Reduction at 40° C.	Reduction at 100° C.
4	0.11	----
15	----	16.5
20	1.14	31.0
25	----	60.5
30	8.56	124.5
32	10.06	-----
34	12.02	-----
35	-----	184.0
36	11.26	-----
37 (E.P.)	11.01	208.0
42	9.33	-----
45	----	178.0
60	8.04	163.0
70	8.16	-----
90	8.00	133.0
180	----	79.5

Note: Refer to Table 2


Reduction at 40°C.
Vertical Column Figures Represent ML. N/200 $Na_2S_2O_3$
Reduction at 100°C.
Endpoint (Reduction at 100° C.)
Endpoint (Reduction at 40° C.)
THE CONDENSATION OF 4% HCHO
CATALYZED BY 0.27 mM. REDUCTONE
(Reaction followed by hot and cold reduction curves)
Minutes

Hot and Cold Reducing Values — Table 2

The Condensation of 4% HCHO at 40° C. Catalysed By 0.27 mM. (0.050%) Fructose

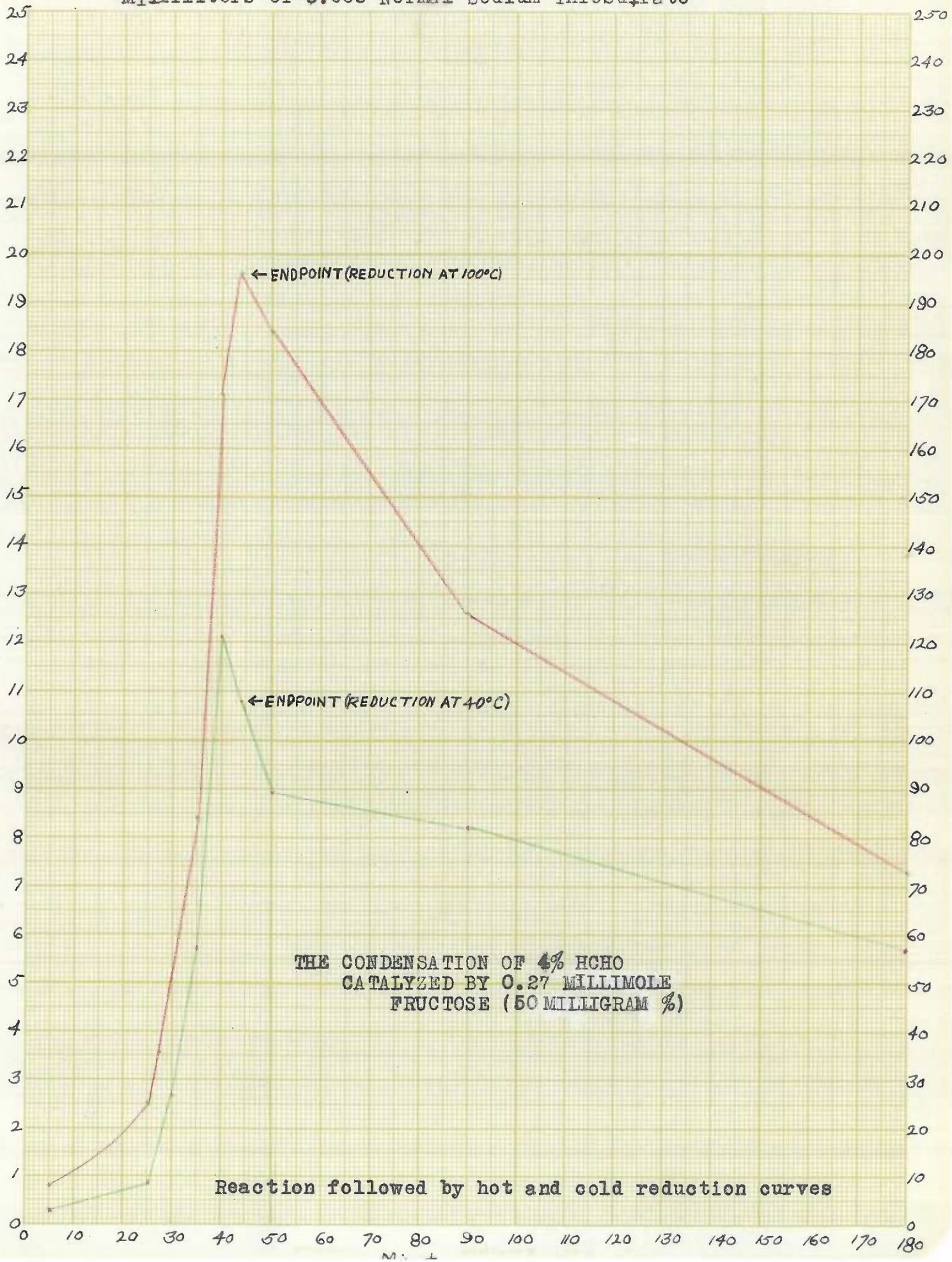
Time Minutes	ml. 0.005 N. $Na_2S_2O_3$ Titration Difference: Reduction at 40° C.	Reduction at 100° C.
5	0.30	8.0
25	0.85	25.7
30	2.70	47.9
35	5.70	84.0
40	12.10	171.3
44 (E.P.)	10.78	196.0
50	8.95	184.2
90	8.22	126.3
180	5.68	72.9

Note: Columns 2 and 3 contain the reduction values from 1 ml. of the condensate taken at the various time intervals.

duction
t 40 C

Vertical Column Figures Represent
Milliliters of 0.005 Normal Sodium Thiosulfate

Reduction
at 100 C



Hot and Cold Reducing Values — Table 3

The Condensation of 4% HCHO at 40° C. Catalysed by 0.27 mM. (0.0417%) Xylose

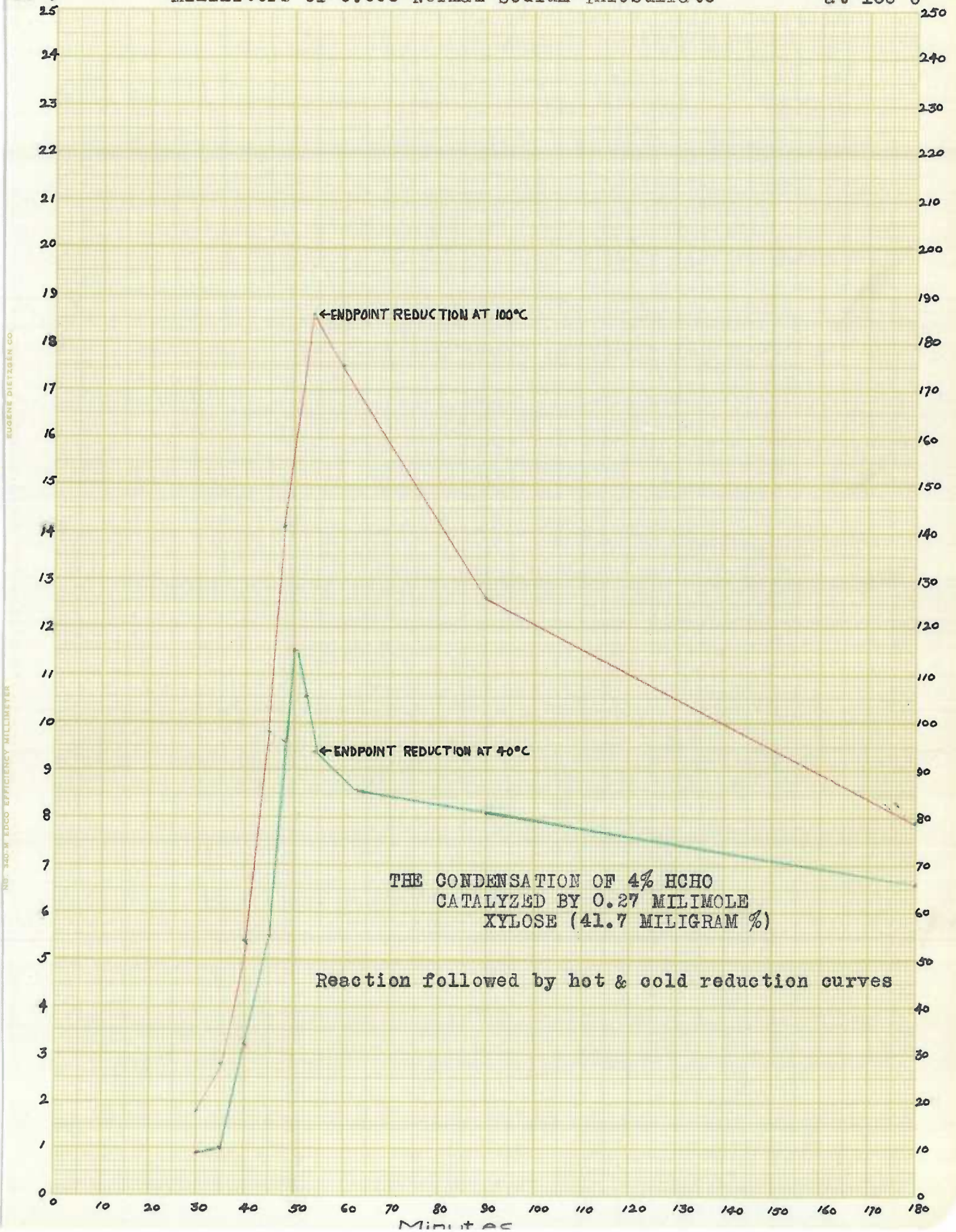
Time Minutes	ml. 0.005 N. $Na_2S_2O_3$ Titration Difference: Reduction at 40° C.	Reduction at 100° C.
30	0.90	17.0
35	1.00	28.0
40	3.25	54.7
45	5.50	107.5
48	9.65	141.0
50	11.52	-----
52	10.55	-----
54 (E.P.)	9.42	186.0
60	-----	175.0
63	8.55	-----
70	-----	161.5
73	-----	-----
90	8.05	126.2
93	-----	-----
120	-----	111.8
180	6.60	78.5

Note: Refer to Table 2

ıction
40 C

Vertical Column Figures Represent
Mililiters of 0.005 Normal Sodium Thiosulfate

Reduction
at 100 C



Hot and Cold Reducing Values — Table 4

The Condensation of 4% HCHO at 40° C. Catalysed By 0.27 mM. (0.100%) Maltose

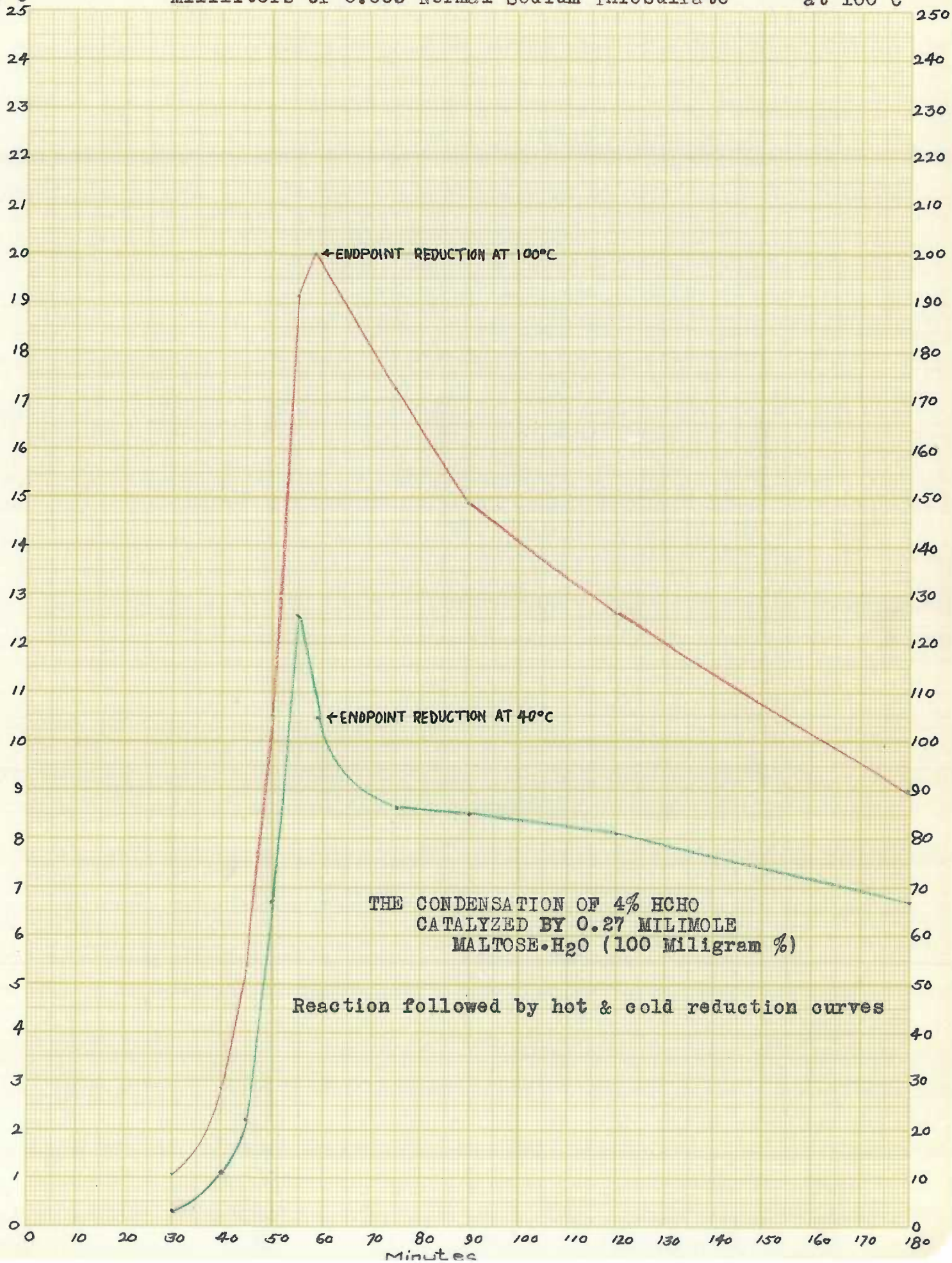
Time Minutes	ml. 0.005 N. $Na_2S_2O_3$ Titration Difference Reduction at 40° C.	Reduction at 100° C.
30	0.30	10.8
40	1.15	28.8
45	2.22	52.8
50	6.70	105.0
55	12.55	191.3
58.5 (E.P)	10.47	200.0
75	8.67	172.5
90	8.50	149.0
120	6.10	126.2
180	6.70	90.0

Note: Refer to Table 2

uction
40 C

Vertical Column Figures Represent
Mililiters of 0.005 Normal Sodium Thiosulfate

Reduction
at 100 C

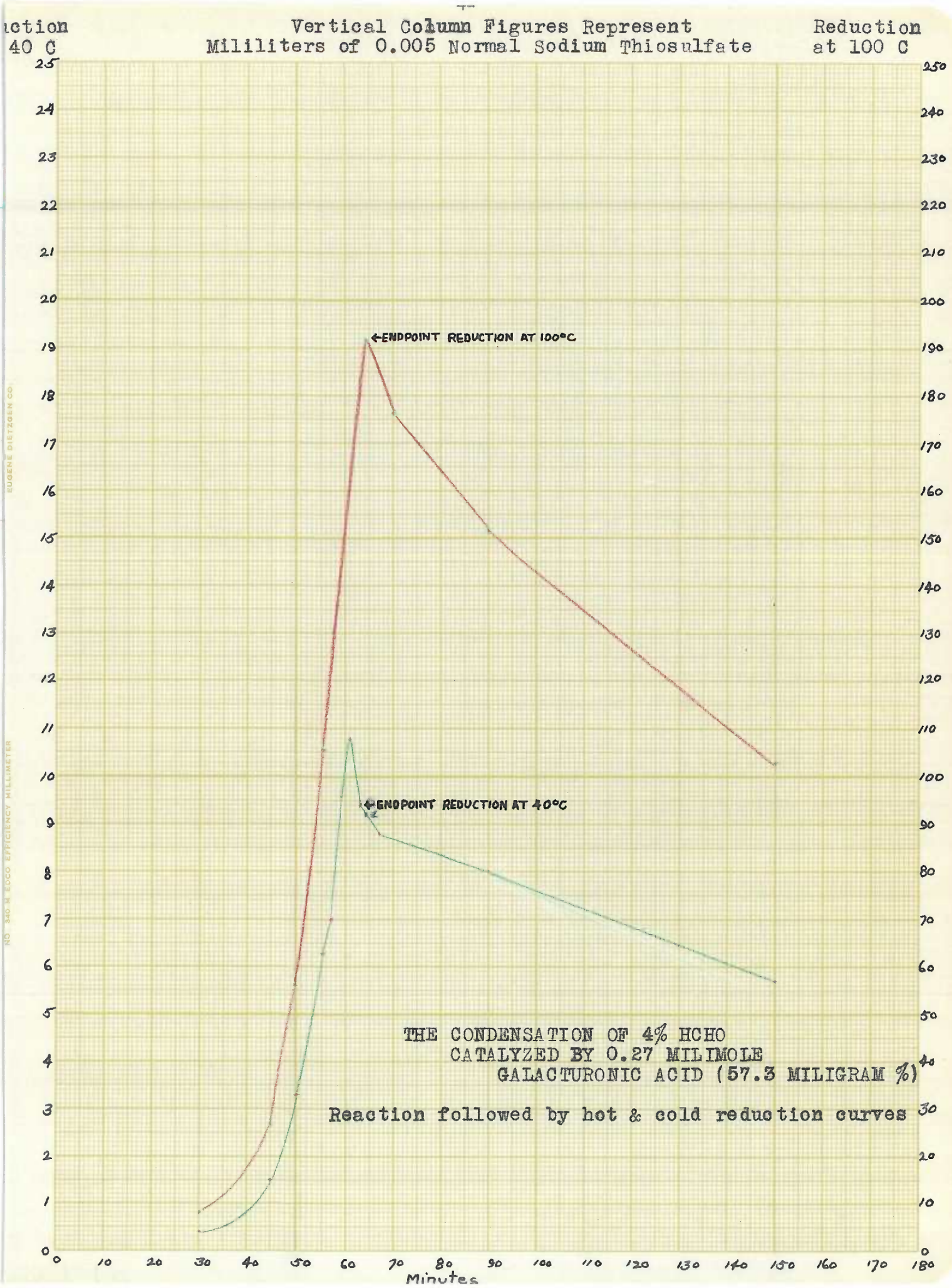


Hot and Cold Reducing Values — Table 5

The Condensation of 4% HCHO at 40° C. Catalysed By 0.27 mM. (0.057%) Galacturonic Acid

Time Minutes	ml. 0.005 N. $Na_2S_2O_3$ Titration Difference Reduction at 40° C.	Reduction at 100° C.
30	0.40	07.6
45	1.56	27.5
50	3.30	56.8
55	6.20	----
56	----	105.8
57	7.03	----
59	9.67	----
61	10.82	----
63	9.45	----
64½ (E.P.)	----	191.5
67	8.77	----
70	8.69	177.3
90	8.04	151.5
150	5.68	102.8

Note: Refer to Table 2


ıction
40 C
Vertical Column Figures Represent
Milliliters of 0.005 Normal Sodium Thiosulfate
Reduction
at 100 C
←ENDPOINT REDUCTION AT 100°C
←ENDPOINT REDUCTION AT 40°C
THE CONDENSATION OF 4% HCHO
CATALYZED BY 0.27 MILIMOLE
GALACTURONIC ACID (57.3 MILIGRAM %)
Reaction followed by hot & cold reduction curves
Minutes

Hot and Cold Reducing Values — Table 6

The Condensation of 4% HCHO at 40° C. Catalysed By 0.27 mM. (0.050%) Glucose

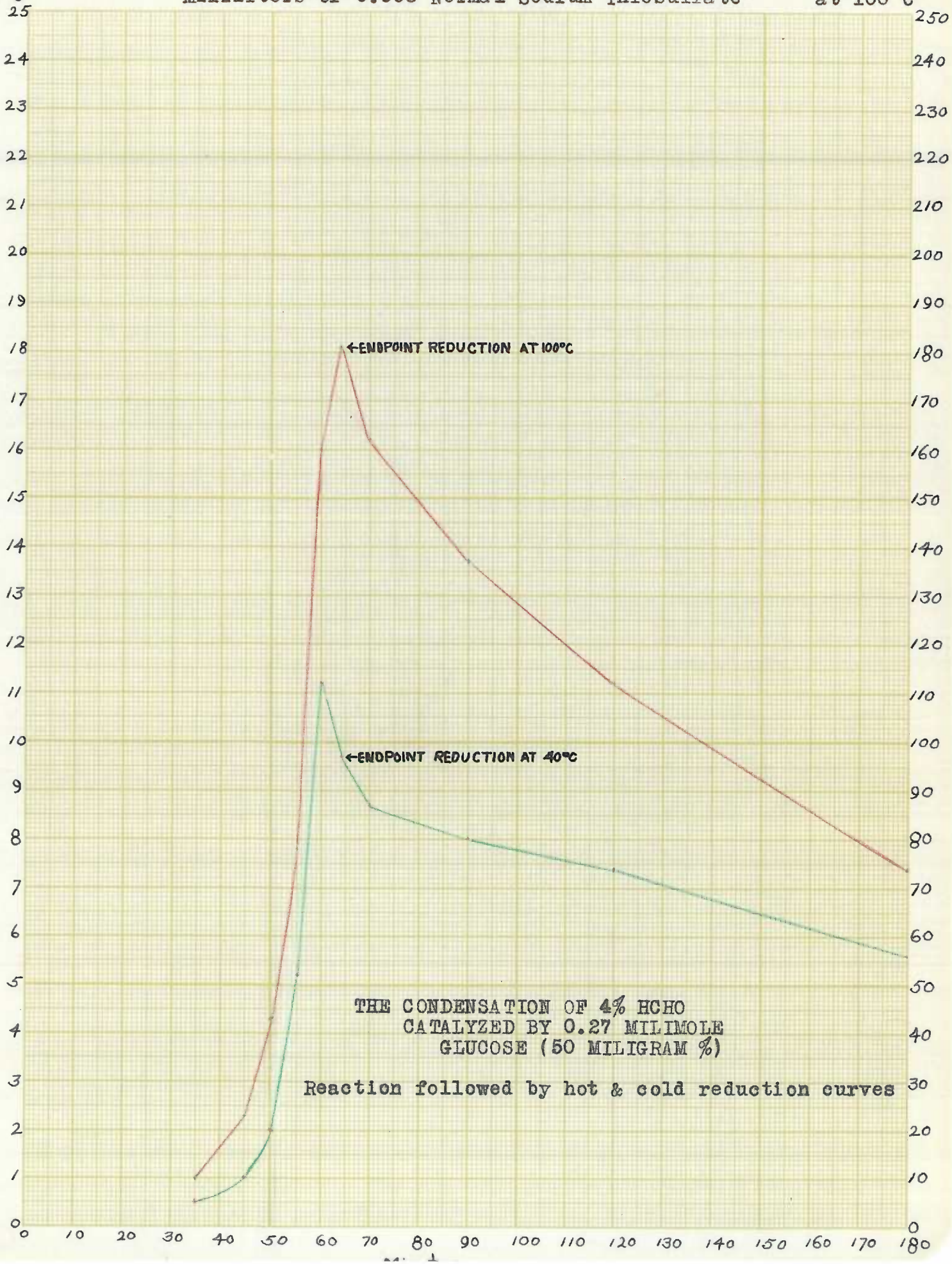
Time Minutes	ml. 0.005 N. $Na_2S_2O_3$ Titration Difference: Reduction at 40° C.	Reduction at 100° C.
35	0.45	09.5
45	0.95	23.2
50	2.10	45.5
55	5.20	76.7
60	11.24	160.0
64 (E.P.)	9.68	161.5
70	8.68	161.8
90	8.00	136.9
120	7.43	112.2
180	5.62	73.8

Note: Refer to Table 2

ction
40 C

Vertical Column Figures Represent
Mililiters of 0.005 Normal Sodium Thiosulfate

Reduction
at 100 C



THE CONDENSATION OF 4% HCHO
CATALYZED BY 0.27 MILIMOLE
GLUCOSE (50 MILIGRAM %)

Reaction followed by hot & cold reduction curves

Hot and Cold Reducing Values — Table 7

The Condensation of 4% HCHO at 40° C. Catalysed By 0.27 mM. (0.100%) Lactose·Hg_2O

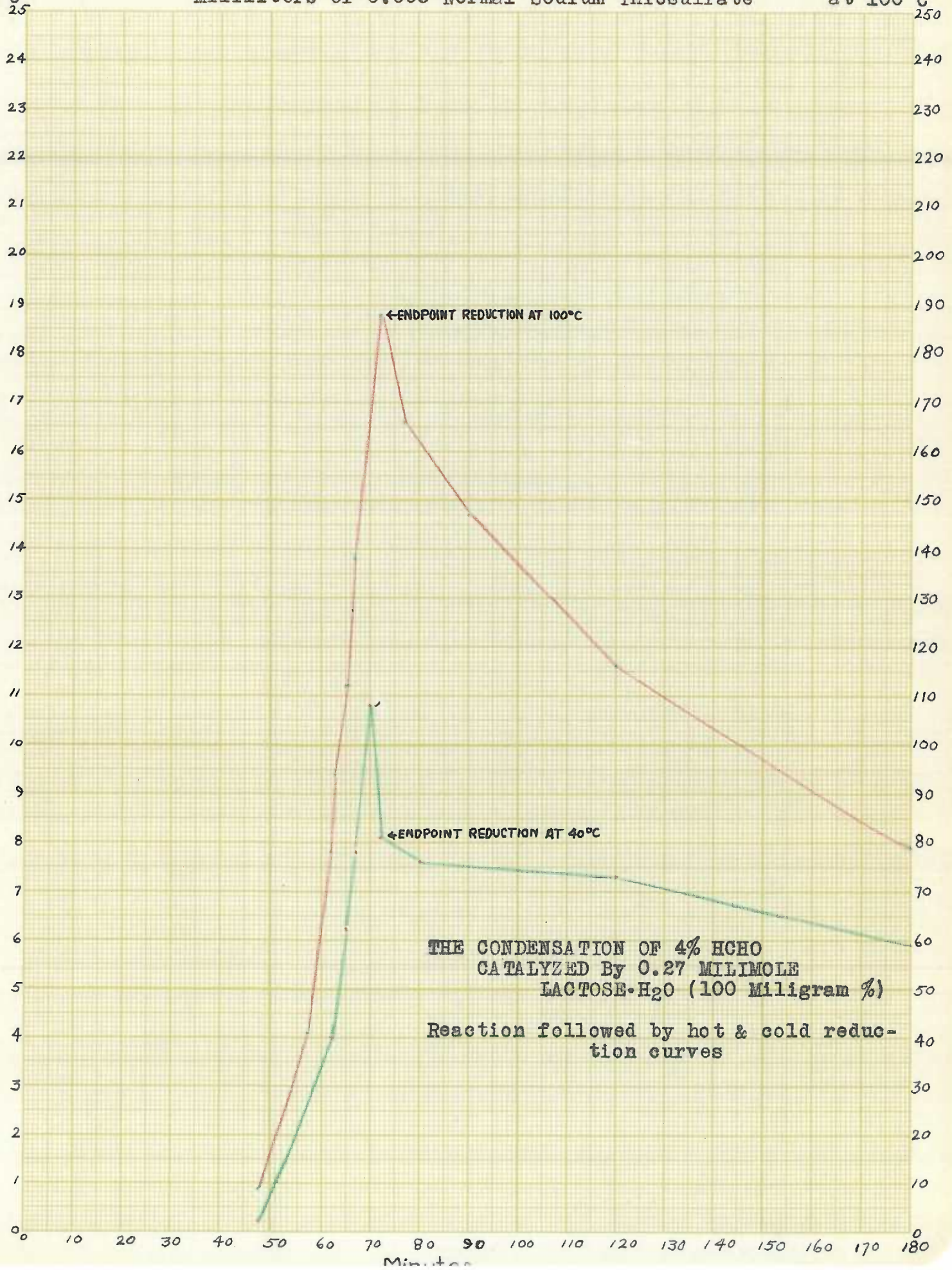
Time Minutes	mL. 0.005 N. $Na_2S_2O_3$ Titration Difference Reduction at 40° C.	Reduction at 100° C.
48	0.22	14.2
56.5	----	41.8
61.5	----	78.5
62	4.07	----
64.5	----	112.8
65	6.25	----
66.5	----	128.6
67	7.80	----
70	10.85	----
72 (E.P.)	8.17	187.8
76.5	----	165.5
80	7.67	----
86.5	----	145.1
90	6.52	147.2
120	7.32	116.1
185	5.86	76.4

Note: Refer to Table 2

ction
40 C

Vertical Column Figures Represent
Milliliters of 0.005 Normal Sodium Thiosulfate

Reduction
at 100 C



Hot and Cold Reducing Values — Table 8

The Condensation of 4% HCHO at 40° C. Catalyzed By 0.27 Mn. (0.0384%) Kojic Acid

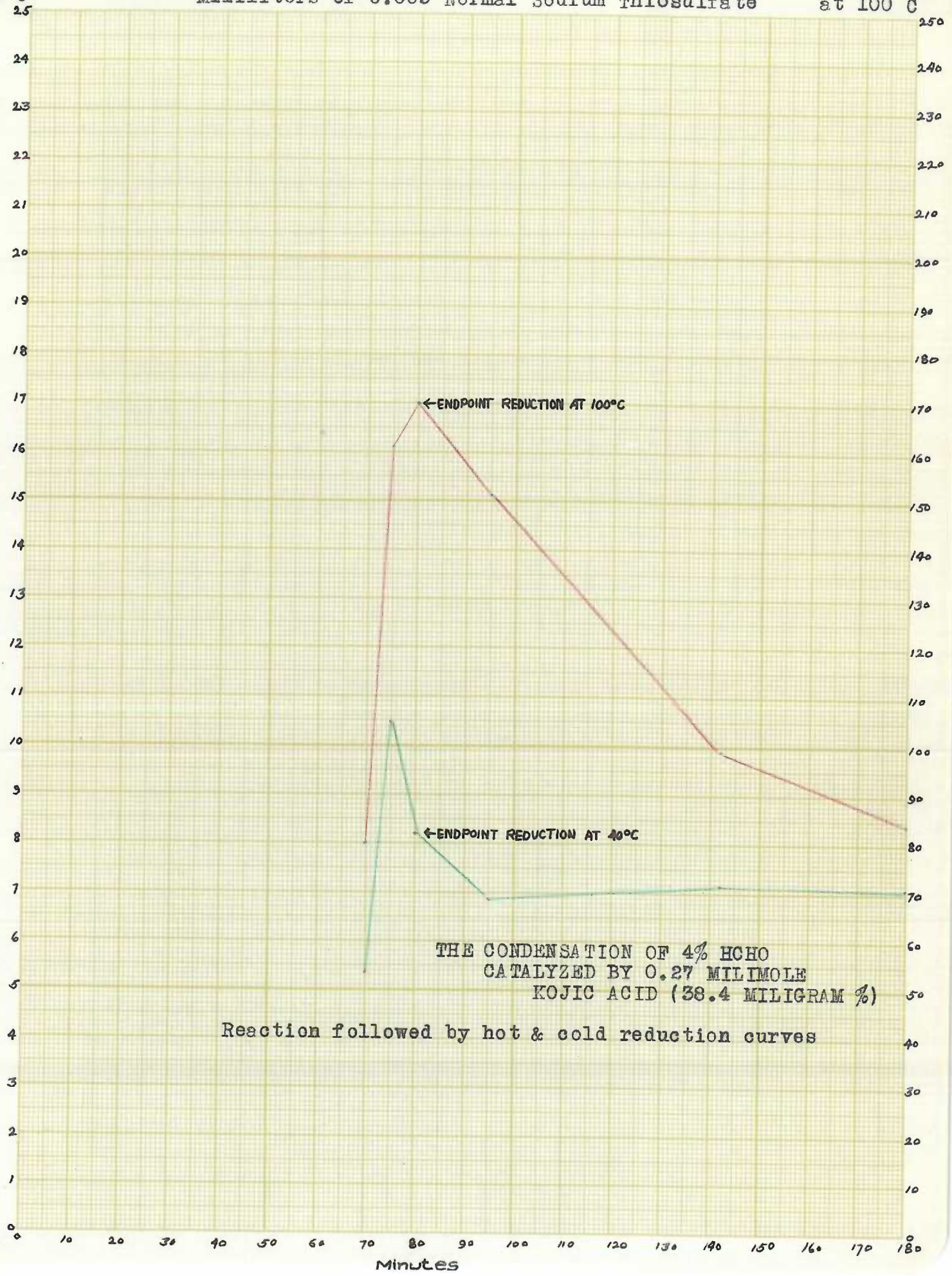
Time Minutes	ml. 0.005 N. $Na_2S_2O_3$ Titration Difference Reduction at 40° C.	Reduction at 100° C.
70	5.37	79.7
75	10.47	161.2
80 (E.P.)	8.22	169.7
95	6.87	151.2
142	7.17	99.2
180	7.12	84.2

Note: Refer to Table 2

ction
40 C

Vertical Column Figure Represent
Mililiters of 0.005 Normal Sodium Thiosulfate

Reduction
at 100 C



THE CONDENSATION OF 4% HCHO
CATALYZED BY 0.27 MILIMOLE
KOJIC ACID (38.4 MILIGRAM %)

Reaction followed by hot & cold reduction curves

Hot and Cold Reducing Values — Table 9

The Condensation of 4% HCHO at 40° C. Catalysed By 0.27 mN. (0.0562%) Glucosamine

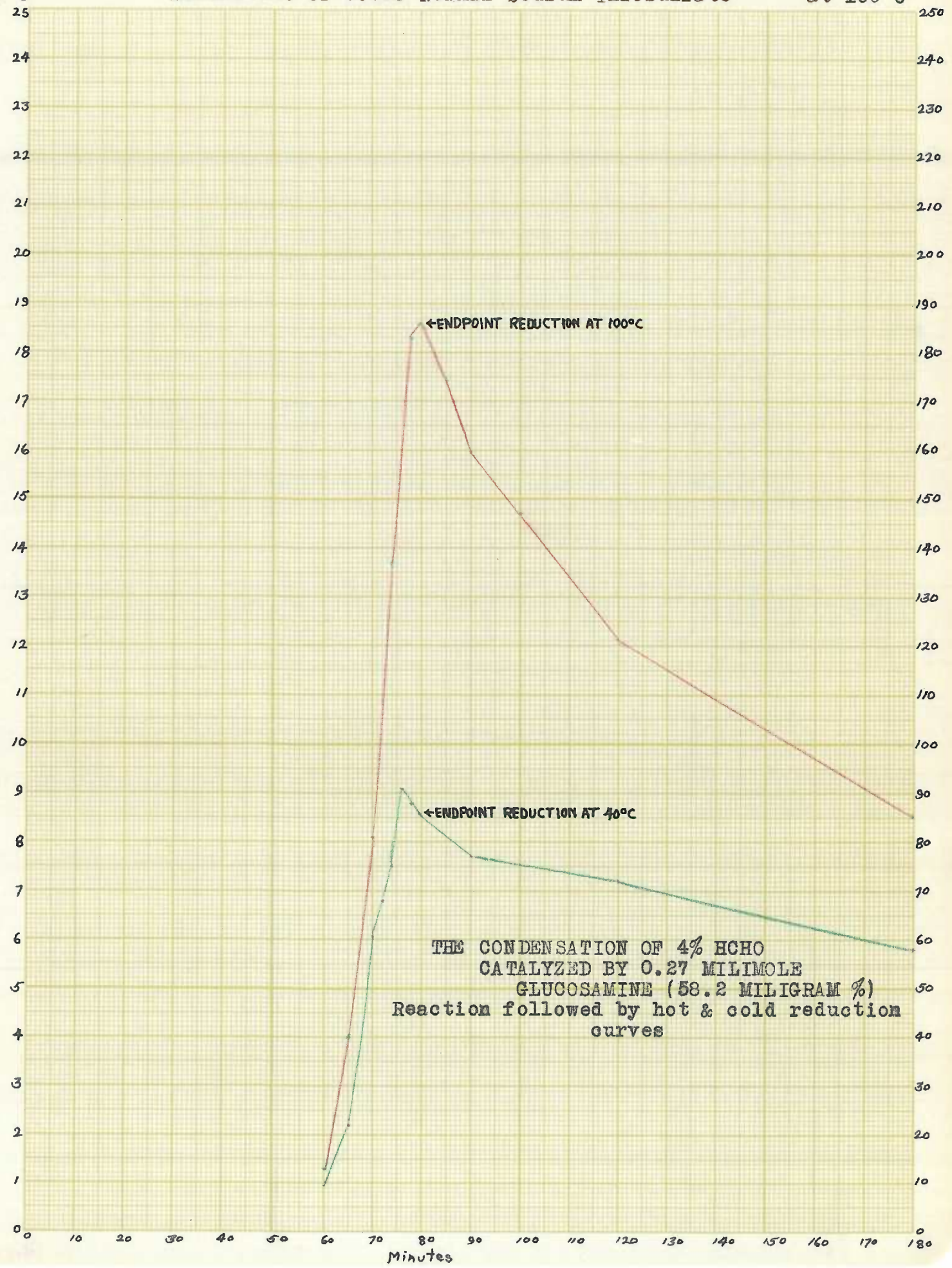
Time Minutes	ml. 0.005 N. $Na_2S_2O_3$ Titration Difference Reduction at 40° C.	Reduction at 100° C.
60	0.95	12.7
65	2.18	39.7
70	6.10	81.0
72	6.79	-----
74	7.50	137.0
76	9.11	-----
78	8.79	183.9
79.5 (E.P.)	8.65	186.0
85	8.92	174.0
90	7.68	159.6
100	6.59	146.6
120	7.24	121.4
180	5.85	85.5

Note: Refer to Table 2

ıction
40 C

Vertical Column Figures Represent
Milliliters of 0.005 Normal Sodium Thiosulfate

Reduction
at 100 C



THE CONDENSATION OF 4% HCHO
CATALYZED BY 0.27 MILIMOLE
GLUCOSAMINE (58.2 MILIGRAM %)
Reaction followed by hot & cold reduction
curves

Hot and Cold Reducing Values — Table 10

The Condensation of 4% HCHO at 40° C. Catalysed By 0.27 mM. (0.1162%) Calcium Mannonate

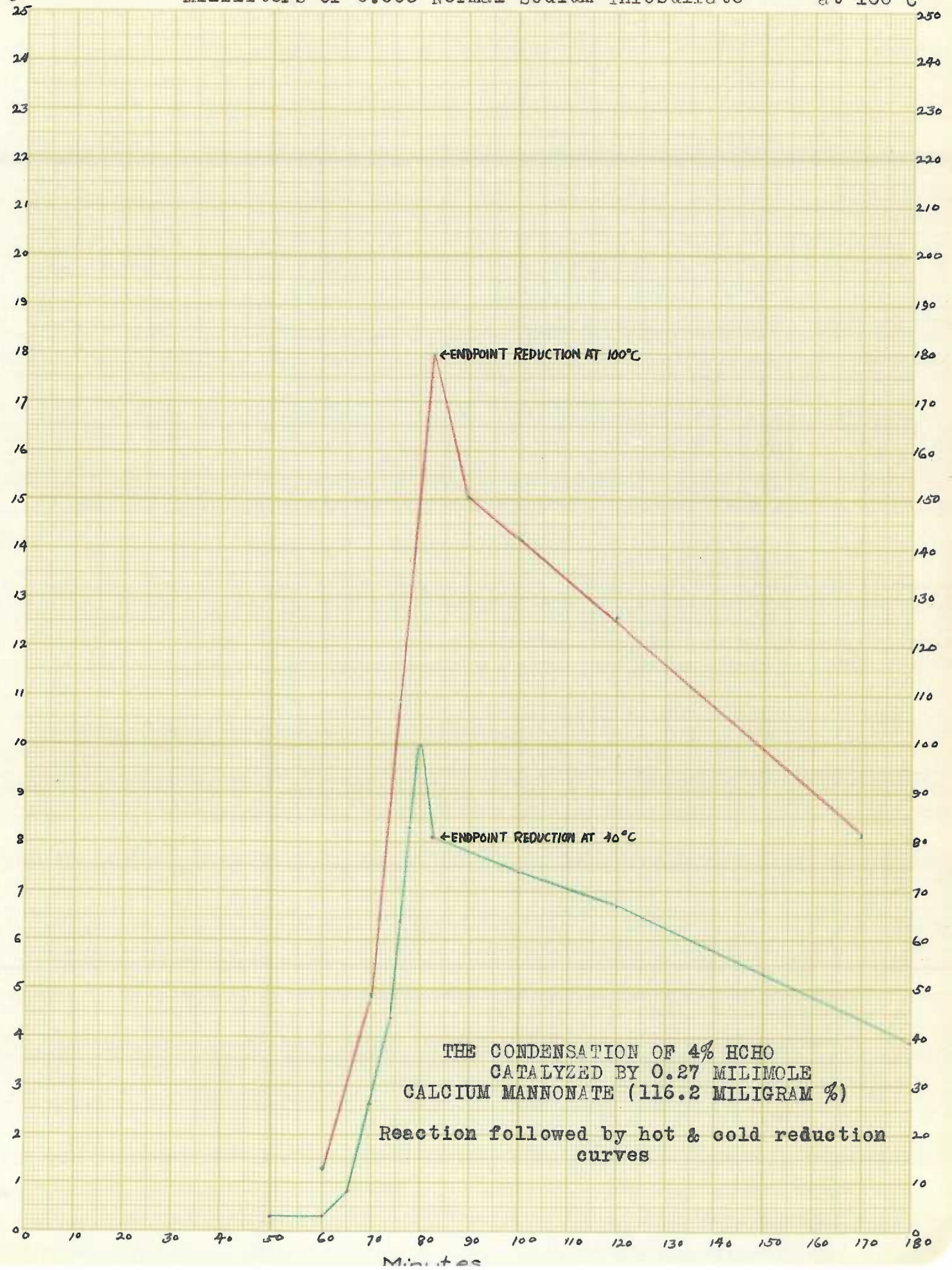
Time Minutes	ml. 0.005 N. $Na_2S_2O_3$ Titration Difference Reduction at 40° C.	Reduction at 100° C.
50	0.27	14.7
60	0.30	13.3
65	0.60	----
70	2.65	48.7
74	4.40	----
76	6.35	109.0
78	8.32	-----
80	10.04	-----
82½ (E.P.)	8.09	179.5
100	7.44	142.3
120	6.65	125.9
180	3.92	61.0

Note: Refer to Table 2

ction
40 C

Vertical Column Figures Represent
Milliliters of 0.005 Normal Sodium Thiosulfate

Reduction
at 100 C



Hot and Cold Reducing Values — Table 11

The Condensation of 4% HCHO at 40° C. Catalysed By 0.27 mM. (0.1162%) Calcium Gluconate

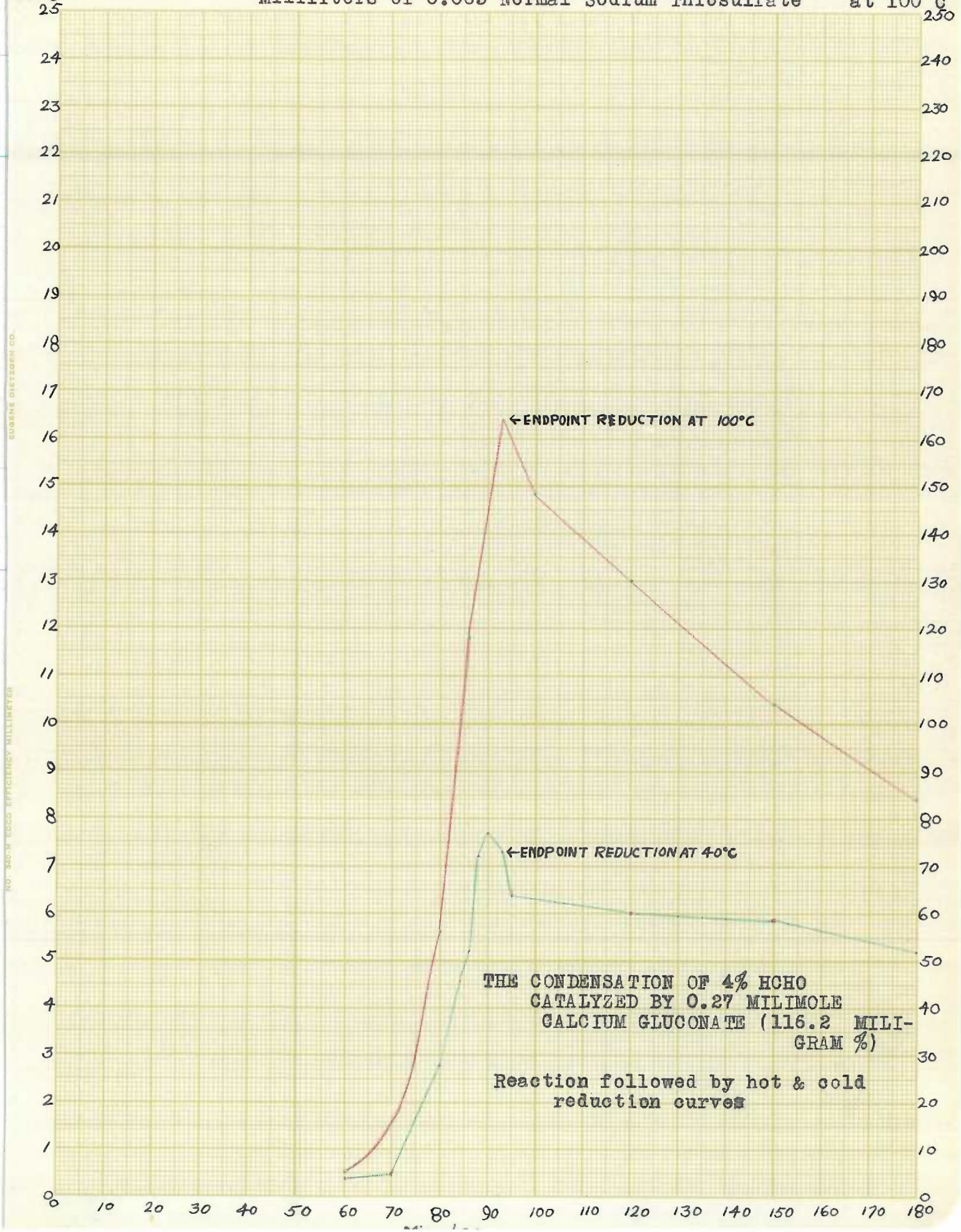
Time Minutes	ml. 0.005 N. $Na_2S_2O_3$ Titration Difference	
	Reduction at 40° C.	Reduction at 100° C.
55	0.37	04.9
70	0.52	16.4
80	2.82	56.1
84	4.57	-----
86	5.17	117.7
88	7.25	-----
90	7.70	-----
93 (E.P.)	7.26	164.2
95	6.37	-----
100	6.86	148.4
120	6.02	129.7
150	5.89	104.2
180	5.16	84.2

Note: Refer to Table 2

Vertical Column Figures Represent
Milliliters of 0.005 Normal Sodium Thiosulfate

uction
40 C

Reduction
at 100 C

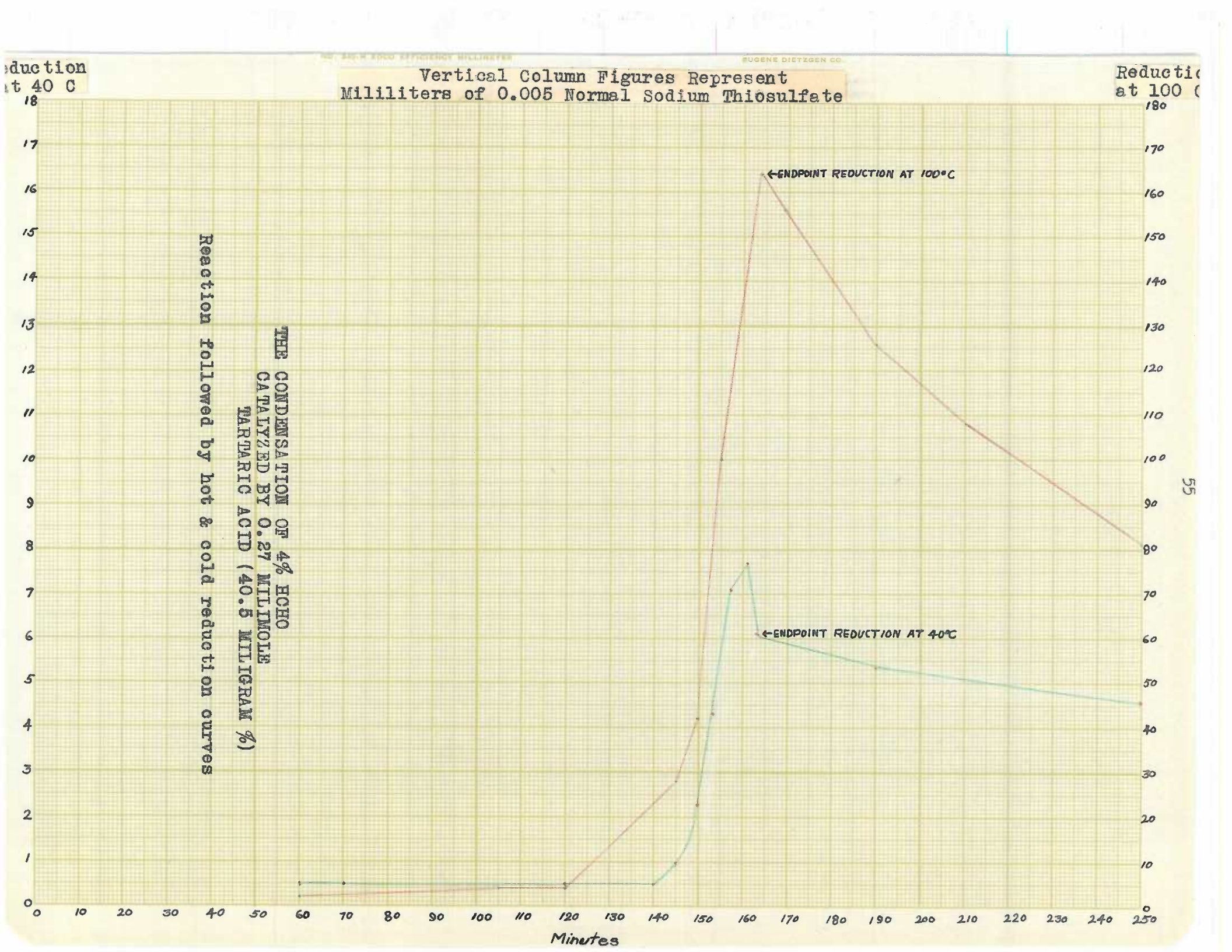


Hot and Cold Reducing Values — Table 12

The Condensation of 4% HCHO at 40° C. Catalysed By 0.27 mM. (0.0405%) Tartaric Acid

Time Minutes	ml. 0.005 N. $Na_2S_2O_3$ Titration Difference Reduction at 40° C.	Reduction at 100° C.
60	0.50	1.8
120	0.50	4.0
140	0.50	----
145	0.98	28.5
150	2.29	51.7
153.5	4.30	----
155	----	100.5
155.5	5.20	----
157.5	7.13	----
160.5	7.75	----
163.5 (E.P.)	6.10	164.6
169	----	156.0
190	5.35	126.0
255	4.55	78.5

Note: Refer to Table 2

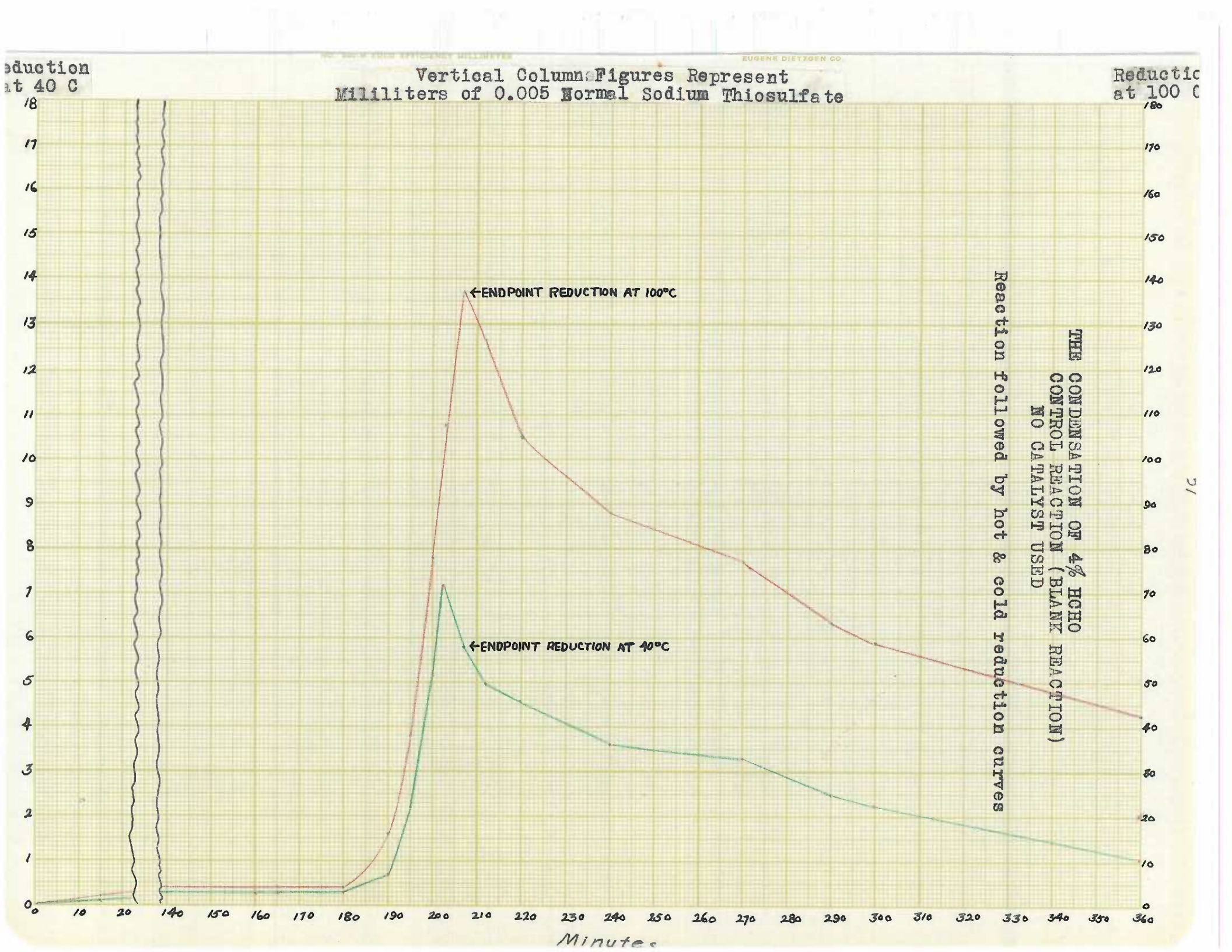

Vertical Column Figures Represent
Milliliters of 0.005 Normal Sodium Thiosulfate
THE CONDENSATION OF 4% HCHO
CATALYZED BY 0.27 MILIMOLE
TARTARIC ACID (40.5 MILIGRAM %)
Reaction followed by hot & cold reduction curves
←ENDPOINT REDUCTION AT 100°C
←ENDPOINT REDUCTION AT 40°C
Minutes
55

Hot and Cold Reducing Values — Table 13

Control Reaction (Blank)

Time Minutes	Reduction at 40° C.	Reduction at 100
15	0.14	2.4
45	0.13	3.4
75	0.16	4.7
105	0.50	3.5
135	0.29	3.4
165	0.24	3.6
180	0.26	3.9
190	0.67	16.5
195	2.20	38.7
200	5.17	78.2
202.5	7.19	107.7
207 (B.P.)	5.80	137.2
212	4.97	127.2
220	4.67	105.2
240	3.57	88.2
270	3.32	77.7
300	2.27	58.9
360	1.02	42.2

Note: Refer to Table 2


Reduction at 40 C
Vertical Column Figures Represent
Milliliters of 0.005 Normal Sodium Thiosulfate
Reduction at 100 C
←ENDPOINT REDUCTION AT 100°C
←ENDPOINT REDUCTION AT 40°C
THE CONDENSATION OF 4% HCHO
CONTROL REACTION (BLANK REACTION)
NO CATALYST USED
Reaction followed by hot & cold reduction curves
Minutes

DISCUSSION

To bring certain relationships to light, all the reduction curves are plotted on a single graph, and the values for similar parts of each curve tabulated in tables. The values were obtained from a study of the data and of the plotted curves. None of these values are to be considered absolute values for any particular pair of reduction curves because absolute values are not significant enough to demand the additional work necessary to establish them. Rather they are of importance for comparative studies of several relative relationships.

In table one, the time periods during which the hot and cold reducing activities become maximal in each of the condensation reactions appear arranged in the order of the speed of the catalysts.

The time periods for the hot reduction maxima are very accurate because they are marked by the endpoint color change. Those for the cold reduction curves are not marked by any signs during the course of the condensation experiment, and their accuracy depends on the numerous samples taken near the endpoint of the reaction, which may or may not happen to include the moment of the greatest cold reduction. However, the cold reduction maxima determined by the samples cannot have more than one minute of error.

It is of interest to observe that the period following the cold reduction maximum before the hot reduction maximum

is reached is somewhat constant for each of the catalysed condensation reactions and does not vary much from the average 3.3 minutes.

A study of the time interval between the point of initial rise of each cold reduction curve and its peak shows a remarkably constant range of which the average is 22 minutes. This seems to indicate that the influence of the catalyst works only to shorten the latent period preceding the beginning of the cold reduction curve. The catalyst itself does not modify the rate of formation of substances which are responsible for the cold reducing power. The work of Orthmer and Gerish furnished strong evidence that the cold reduction curve describes the concentrations of the short carbon chain primary products. It seems probable that the action of a catalyst hastens the formation of these short carbon chain molecules until they reach the concentration capable of producing an autocatalytic reaction.

The peak of each hot reduction curve always follows the peak of the cold reduction curve by a fairly constant time as already mentioned. The endpoint always appears on the abrupt descent of the cold reduction curve. Orthmer and Gerish found that the maximum of short sugar molecules is formed at the cold reduction peak and that these short sugar molecules condensed further to larger molecules. On the basis of this finding, the cold reduction curve should fall precipitously to practically no reducing activity as the hot reduction curve reaches its peak. The cold reduction curve

does not follow this pattern. Each cold reduction curve characteristically shows two phases of declination. During the first several minutes there is an abrupt declination of reduction. It is during this phase that the hot reduction climbs to its peak. Following the brief initial phase, the rest of the cold reduction curve falls away in a gradual slope of declination.

A study of the rates of declination in the two phases of declination was made (Table VI, page 72). The initial phase shows a declination rate fourteen times greater than the second phase.

Like the cold reduction curves, but to a lesser degree, the hot reduction curves are so similar that one hot reduction curve can almost be superimposed on another hot reduction curve.

The period of the condensation when the hot reduction curve indicates beginning formation of hot reducing molecules coincides with the initial formation of cold reducing molecules. The time duration intervening between the initial rise of the hot reduction curve to its peak, like that of the cold reduction curve, is rather constant--the average being 25.3 minutes (refer to tables I and II, pages 67-68).

Of interest, but probably not of particular importance, is the rate of declination of the hot reduction curve following the peak of hot reduction. The declination is not sharply marked into two phases as that of the cold reduction

curve. However, early declination is rapid. In order to have a numerical expression of the average hot reduction declination curve, the rates of decrease during the first half hour was compared with that of the second half hour (Table V, page 71). The loss of hot reducing activity during the first half hour is practically twice the rate existing during the second half hour. The subsequent slope of decline shows roughly little change for each of the curves (graph, page 66)

The consideration of the relationships existing between the general hot and cold reduction curves can well be indicated at this stage of the discussion.

The time relationship between the formation of the hot and cold reducing substances have been correlated during the earlier portion of this discourse. The comparative rates of formation was studied (Table IV, page 70). The ratio of the rate-formation of hot reduction to that of cold reduction may be expressed by the number thirteen.

The following ratios comparing the rates of declination of two average hot and cold reduction curves are of interest:

$$\frac{\text{Average Rate of Drop For Hot Reduction the First Half Hour}}{\text{Average Rate of Cold Reduction Gradual Drop}} = 56$$

$$\frac{\text{Average Rate of Drop For Hot Reduction Second Half Hour}}{\text{Average Rate of Cold Reduction Gradual Drop}} = 30.7$$

The figures are of no particular significance except to indicate abstractly the relationship of the two declination rates following the endpoint of the condensation.

There are two interesting correlations concerning the reducing values present at the peaks of the hot and cold reduction curves.

A graphical presentation of hot and cold reduction curves plotted according to the speed of condensation indicates a definite relationship existing between the speed of the catalyst and the heights of the reduction peaks (Table III, page 69, and graph, page 66). In general the very active catalysts produce very high reduction peaks; the less active catalysts produce proportionally lower reduction maxima. The uncatalysed condensation reaction (blank condensation) is the slowest condensation reaction and has the lowest pair of reduction maxima. That the amount of reducing substances that can be formed varies inversely with the time of a condensation can logically be attributed to two factors. Of the two factors, the Cannizarro reaction in which formaldehyde is lost through conversion to methyl alcohol and formic acid is undoubtedly the more important. Then also, the factor of evaporation must be considered to play some part in the loss of formaldehyde.

A correlative study of the ratios between the reducing values at the hot and cold reduction peaks for each catalysed condensation reaction presents a narrow range of variation with the average ratio of eighteen. This quantitatively rather constant relationship is evidence favorable toward the assumption that the chemistry of the condensation reaction

during the condensing stage is uninfluenced by the catalyst.

The discussion up to the present has consisted of a detailed analysis of the reduction curves. The most significant finding is that regardless of the influence on the speed of the condensation by catalytic action, all hot and cold reduction curves are identical within experimental errors both in the form characteristic to each of the two curves and in their inter-relationships. The only catalytic influence on the reduction curves is the variation of the heights of maximal reduction, the latter being directly proportional to the speed of the catalysis.

Based on the nature of the reduction curves, it is considered fitting to conclude the discussion with an interpretation of the probable mechanism involved in the condensation of formaldehyde by calcium hydroxide.

The condensation of formaldehyde begins with a latent period during which time no reducing activity is present. It is conceivable that an infinitesimal number of formaldehyde molecules combined to form two and three carbon chains. These molecules probably exist partially as enediols due to the alkaline reaction. The concentration of enediols slowly rises until it reaches the level of autocatalytic action. Atthis point formaldehyde is converted rapidly into two and three carbon chain molecules. Simultaneously these intermediate chains are condensed to form predominantly hexoses and some tetroses and pentoses. The reduction curves at

this stage of the formaldehyde condensation rise rapidly. At the peak of the cold reduction curve the test for formaldehyde becomes negative and the maximal concentration of intermediate products is present (12). This is also the point of greatest catalytic activity, for any formaldehyde introduced now is rapidly condensed into sugars (7). In the next three minutes, the cold reduction curve has traversed approximately one half of its first phase of rapid declination. This portion of the first phase is caused by continued condensation of the intermediate products to the final products bringing the hot reduction curve to its peak. The rest of the cold reduction abrupt declination can be due to the destruction of the uncondensed two and three carbon chain molecules and possibly other unstable enediols by action of the alkali. However, it is possible that the entire phase of abrupt declination may represent the continued condensation of the intermediate products to the final products and that the true endpoint of the condensation may be at the point where the gradual declination phase of the cold reduction curve begins. If this view is followed, the peak of the hot reduction curve, which coincides with the instant of color change to yellow, may be only the apparent endpoint of the condensation. The interpretation (as expressed in the last two sentences) is based on the idea that the decomposition of the final sugars of condensation does not begin abruptly at the point

when the condensation of formaldehyde is complete, but that the decomposition reaction accelerates as the concentration of the final sugar molecules increases until at the apparent endpoint, the equilibrium of the chemical balance moves to favor the decomposition reaction. While the exact point of complete condensation can be a subject of speculation, the instant of color change and maximum hot reduction has such a definite quantitative relationship with the time of condensation for each experiment that this instant was accepted as the endpoint to facilitate a more accurate comparison of the many relationships studied. The gradual declination phase of the cold reduction curve probably represents the reducing action of the fragmenting sugars, the disintegration of which is consistent with the rapid fall of the hot reduction curve. With the forms and the inter-relationships of the reduction curves being identical, the action of the catalyst appears confined to the latent period where it speeds the formation of the intermediate molecules to a concentration capable of autocatalytic action.

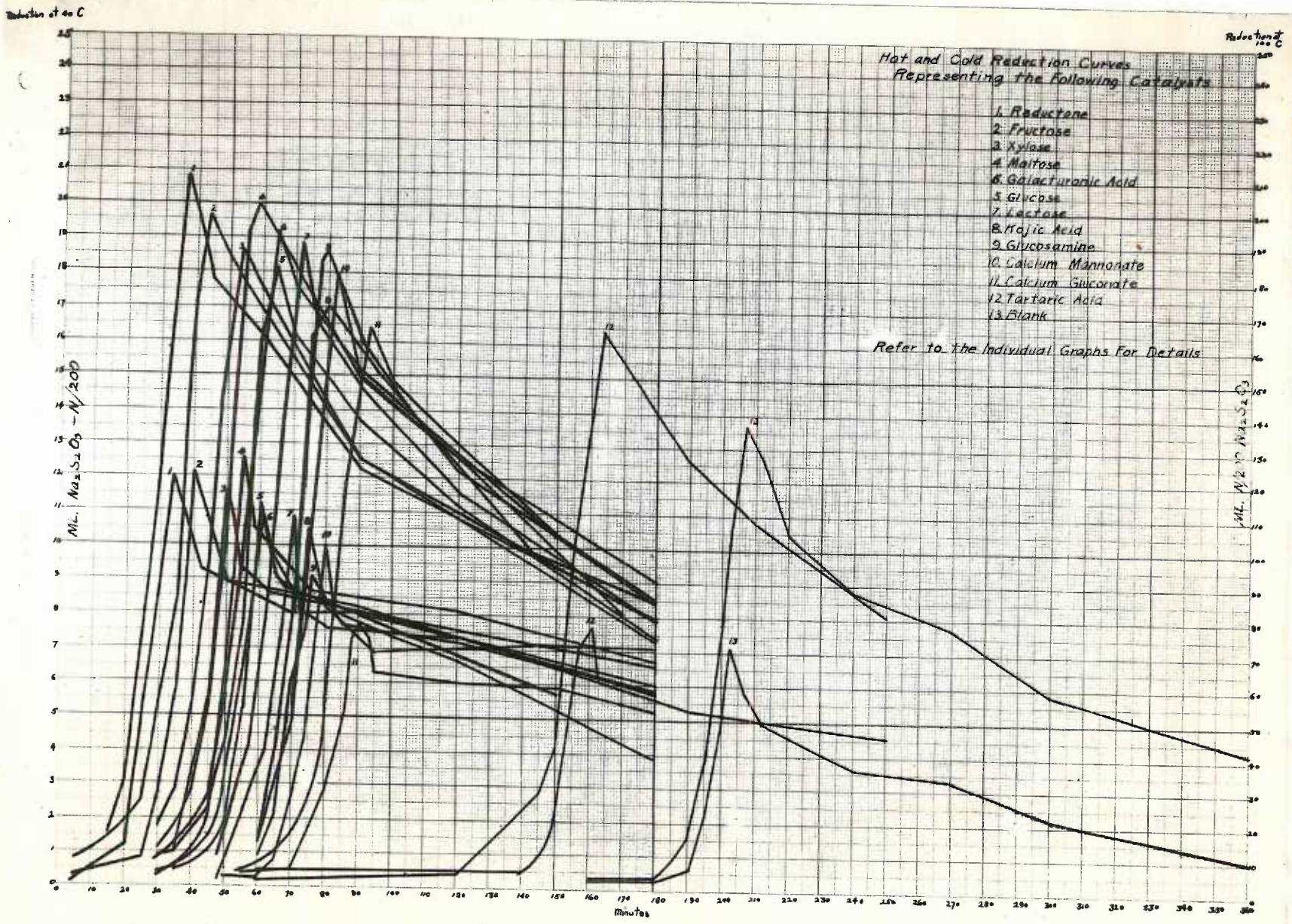

Reduction at 40 C
Reduction at 100 C
Hot and Cold Reduction Curves
Representing the Following Catalysts
1. Reductone
2. Fructose
3. Xylose
4. Maltose
6. Galacturonic Acid
5. Glucose
7. Lactose
8. Malic Acid
9. Glycosamine
10. Calcium Mannonate
11. Calcium Gluconate
12 Tartaric Acid
13 Blank
Refer to the Individual Graphs For Details
Ml. Na2S2O3 - N/200
Minutes

Table I A Comparison of the Time Values for the Hot Reduction Maxima and the Cold Reduction Maxima of the Various Catalysed Condensation Reactions

Catalyst	Hot Red. Maximum	Hot Red. Maximum	Difference
1. Reductone	37 Mins.	34 Mins.	3 Mins.
2. Fructose	44	40	4
3. Xylose	54	52	2
4. Maltose	58.5	55	3.5
5. Glucose	64	60	4
6. Galacturonic Acid	64.5	61	3.5
7. Lactose	72	70	2
8. Kojic Acid	80	75	5
9. Glucosamine	79.5	76	3.5
10. Calcium Mannonate	82.5	80	2.5
11. Calcium Gluconate	93	90	3
12. Tartaric Acid	163.5	160.5	3
13. Blank (No catalyst)	207	202.5	4.5
Average			3.3

Note: The catalysts are arranged in order of the time of the cold reduction peak. The hot reduction values are in the same order except for Glucosamine. The figures under hot reduction maximum and cold reduction maximum represent the time duration of condensation before the respective maximum was reached.

Table II A Comparison of the Time Intervals Between the Initial Rise and Peaks of each Cold Reduction Curve.

Catalyst	Minutes
1. Reductone	22
2. Fructose	25
3. Xylose	22
4. Maltose	25
5. Glucose	25
6. Galacturonic Acid	31
7. Lactose	22
8. Kojic Acid	
9. Glucosamine	21
10. Calcium Mannonate	20
11. Calcium Gluconate	20
12. Tartaric Acid	20.5
13. Blank (No Catalyst)	22.5
Average	22

Note: The average does not include Galacturonic Acid because insufficient samples were taken near the commencement of cold reduction activity to determine with any accuracy the initial rise of cold reduction.

Table III The Following Table Presents For Each Catalysed Reaction the Reduction Values Expressed in Terms of Ml. 0.005 N. Sodium Thiosulfate For the Cold Reduction Maximum and the Hot Reduction Maximum, the Ratio Between the Two Maxima, and the Difference Between Them.

Catalyst	Hot Red. Max.	Cold Red. Max.	Ratio	Diff.
1. Reductone	208.0	12.01	17.4	196.0
2. Fructose	196.0	12.10	16.8	183.9
3. Xylose	186.0	10.55	17.5	175.4
4. Maltose	200.0	12.55	16.0	187.4
5. Glucose	181.5	11.24	16.2	170.3
6. Galacturonic Acid	191.5	10.82	17.7	180.7
7. Lactose	187.8	10.85	17.2	176.9
8. Kojic Acid	169.7	10.47	16.2	159.2
9. Glucosamine	186.0	9.11	20.4	177.9
10. Calcium Mannonate	179.5	10.04	17.8	169.5
11. Calcium Gluconate	164.2	7.70	21.3	156.5
12. Tartaric Acid	164.6	7.75	21.1	156.8
13. Blank (No Catalyst)	137.2	7.19	19.1	130.0
Average			18.0	

Note For Table IV (Following Page): The rates for the hot reduction rise were determined on the respective graphs from the stretch of each curve between the levels of 80 and 130 ml. thiosulfate. That of the cold reduction rise were determined from the stretch between the levels of 2 and 7 ml. thiosulfate. # Levels of 80 and 160 ml. used.

Table IV The Average Rates of Rise For the Hot and Cold Reduction Curve of the Various Catalysed Condensations Expressed in Terms of Ml. 0.005 N. Sodium Thiosulfate Per Minute.

Catalyst	Hot Red. Rate	Cold Red. Rate
1. Reductone	9.30	0.80
2. Fructose	8.55	0.63
3. Xylose	8.70	0.54
4. Maltose	9.17	0.90
5. Glucose	8.77	0.90
6. Galacturonic Acid	8.20	0.47
7. Lactose	8.77	0.57
8. Kojic Acid	16.00 #	0.92
9. Glucosamine	10.00	0.50
10. Calcium Mannonate	11.63	0.55
11. Calcium Gluconate	8.20	0.44
12. Tartaric Acid	7.30	0.78
13. Blank (No Catalyst)	8.47	0.65
Total Range	7.30--16.00	0.44--0.92
Average Range	8.20--10.00 (78.6%)	
Average Based On The Average Range	8.84	0.67
Ratio $\frac{\text{Hot Red. Rate}}{\text{Cold Red. Rate}}$	= 13.2	

Note: See note on the preceding page.

Table V. The Rates of Drop For the Hot Reduction Curves of the Various Catalysed Condensations Expressed in Terms of Ml. 0.005 N. Sodium Thiosulfate Per Minute.

Catalyst	First ½ Hour	Second ½ Hour
1. Reductone	1.96	1.33
2. Fructose	1.55	1.03
3. Xylose	1.62	0.76
4. Maltose	1.63	0.78
5. Glucose	1.58	0.80
6. Galacturonic Acid	1.47	0.80
7. Lactose	1.61	0.84
8. Kojic Acid	1.18	1.09
9. Glucosamine	1.75	0.83
10. Calcium Mannonate	1.59	0.86
11. Calcium Gluconate	1.25	0.82
12. Tartaric Acid	1.40	0.79
13. Blank (No Catalyst)	1.62	0.89
Range	1.18--1.96	0.89--1.33
Average Range	1.40--1.96 (84.6%)	0.76--1.09 (84.6%)
Average	1.64	0.86 (Based on Av. Range)

Table VI The Rates of Drop For the Cold Reduction Curves of the Various Catalysed Condensations Expressed in Terms of Ml. 0.005 N. Sodium Thiosulfate Per Minute.

Catalyst	Cold Red. Abrupt Drop Minutes	Abrupt Drop Rate	Average Rate of Cold Red. Gradual Drop (Based on 1 hr. following the abrupt dron)
1. Reductone	8	0.34	0.025
2. Fructose	10	0.32	0.023
3. Xylose	4	0.53	0.029
4. Maltose	3.5	0.59	0.039
5. Glucose	10	0.25	0.032
6. Galacturonic Acid	6.7	0.30	0.037
7. Lactose	2	1.33	0.020
8. Kojic Acid	5	0.46	0.019
9. Glucosamine	14	0.10	0.020
10. Calcium Mannonate	2.7	0.70	0.038
11. Calcium Gluconate	5	0.28	0.010
12. Tartaric Acid	2.5	0.66	0.020
13. Blank (No Catalyst)	12	0.19	0.029
Range	2--14	0.10--1.33	0.010--0.039
Average Range		0.10--0.70 (92%)	0.018--0.039 (92%)
Average	6.5	0.39 (Based on Av. Range)	0.028 (Based on Av. Range)

Ratio Abrupt Drop/Gradual Drop = 14

CONCLUSION

The reduction curves of formaldehyde condensation by calcium hydroxide was investigated in a series of experiments. The results proved that catalysts have little or no influence on the forms and inter-relationships of the hot and cold reduction curves. The presence of a catalyst acts to shorten the period of condensation preceding the initial formation of reducing substances. The more active catalysts are associated with greater reducing values present at the peaks of the reduction curves. That a catalyst directly affects the quantitative formation of reducing substances is a possible presumption. However, it is more likely that the faster catalysts allow the production of more reducing substances by virtue of shorter latent periods which limit the loss of formaldehyde through the Canizzarro phenomenon and vaporization.

The formation of the hot and cold reduction curves begin simultaneously following a period of latency. In the average experiment, the peak of cold reduction is reached 22 minutes following the initial indication of reducing activities. The peak of hot reduction appears 3.3 minutes later (average) during which time the cold reduction curve has traversed about one half of its short phase of rapid declination, the cold reduction curve passes into a gradual

loss of reducing power while the hot reduction curve drops rapidly away.

The relationships of the reduction curves in an average experiment is graphically presented on the following page.

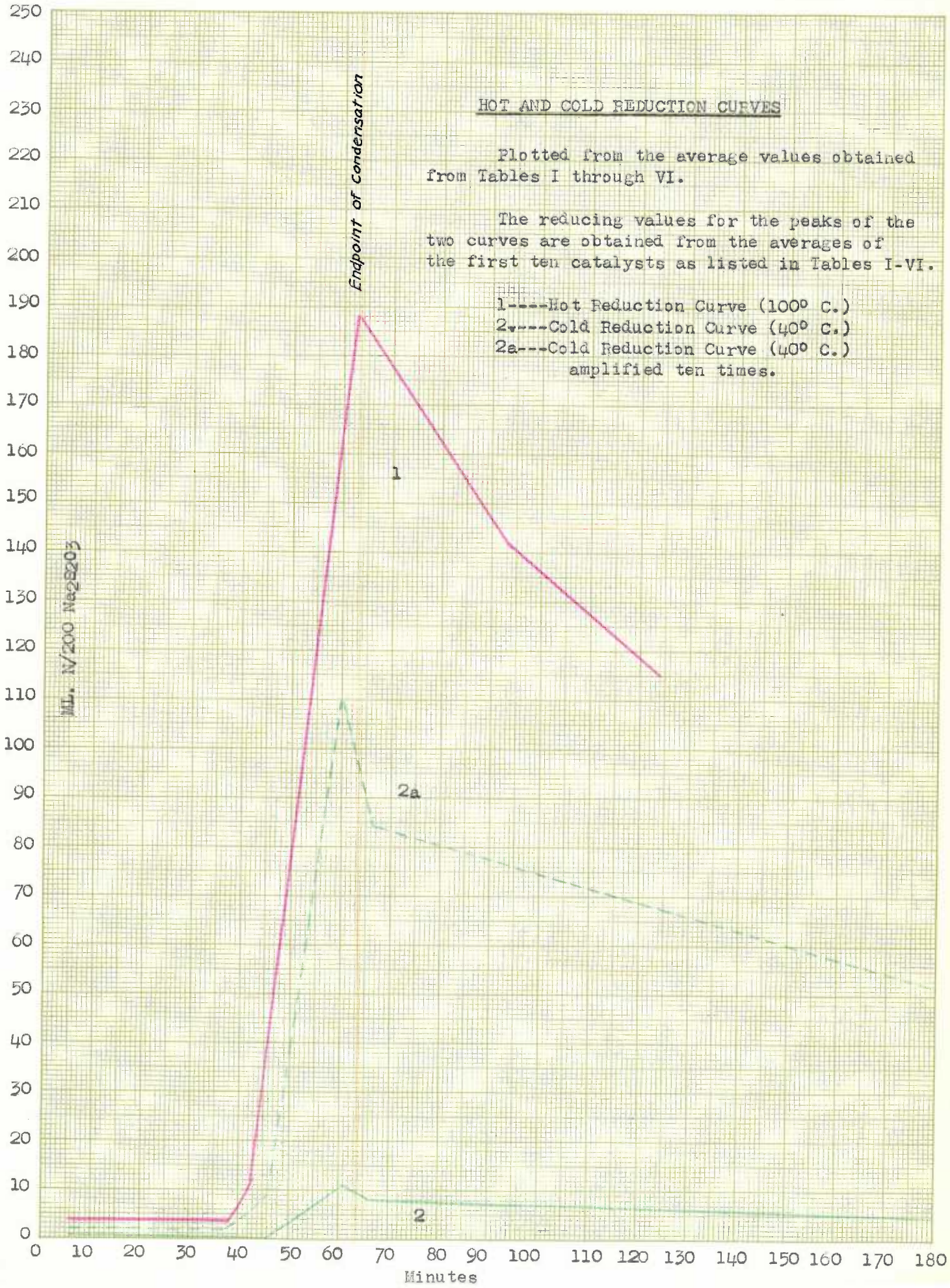

HOT AND COLD REDUCTION CURVES
Plotted from the average values obtained from Tables I through VI.
The reducing values for the peaks of the two curves are obtained from the averages of the first ten catalysts as listed in Tables I-VI.
1----Hot Reduction Curve (100° C.)
2----Cold Reduction Curve (40° C.)
2a---Cold Reduction Curve (40° C.) amplified ten times.
Endpoint of Condensation
1
2a
2
ML. N/200 Na2S2O3
Minutes
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250

Table of Values Used In the Plotting of the Hot and Cold Reduction Curves Based on Average Values (Refer to Tables I Through VI)

Maximum Cold Reduction Value----------10.97 ml.
0.005 N. Thiosulfate

Maximum Hot Reduction Value----------188.60 ml.
0.005 N. Thiosulfate

Time of the Maximum Cold Reduction----------60 min.

(The above three are the averages taken from the first ten catalysts as listed in Tables I-VI.)

Time Elapse Between the Cold Reduction Peak and the Hot Reduction Peak--------3.3 min.

Time Interval From the Initial Point of Rise to the Peak of the Cold Reduction Curve--22.0 min.

Cold Reduction Rate of Rise Per Minute----------0.67 ml.
0.005 N. Thiosulfate

Hot Reduction Rate of Rise Per Minute----------8.84 ml.
0.005 N. Thiosulfate

Cold Reduction Rate of Abrupt Drop Per Minute------0.39 ml.
0.005 N. Thiosulfate

Cold Reduction Length of Abrupt Drop ----------6.5 min.

Cold Reduction Rate of Gradual Drop Per Minute----0.028 ml.
0.005 N. Thiosulfate

Hot Reduction Rate of Drop the First Half Hour (Per Minute)----1.64 ml.
0.005 N. Thiosulfate

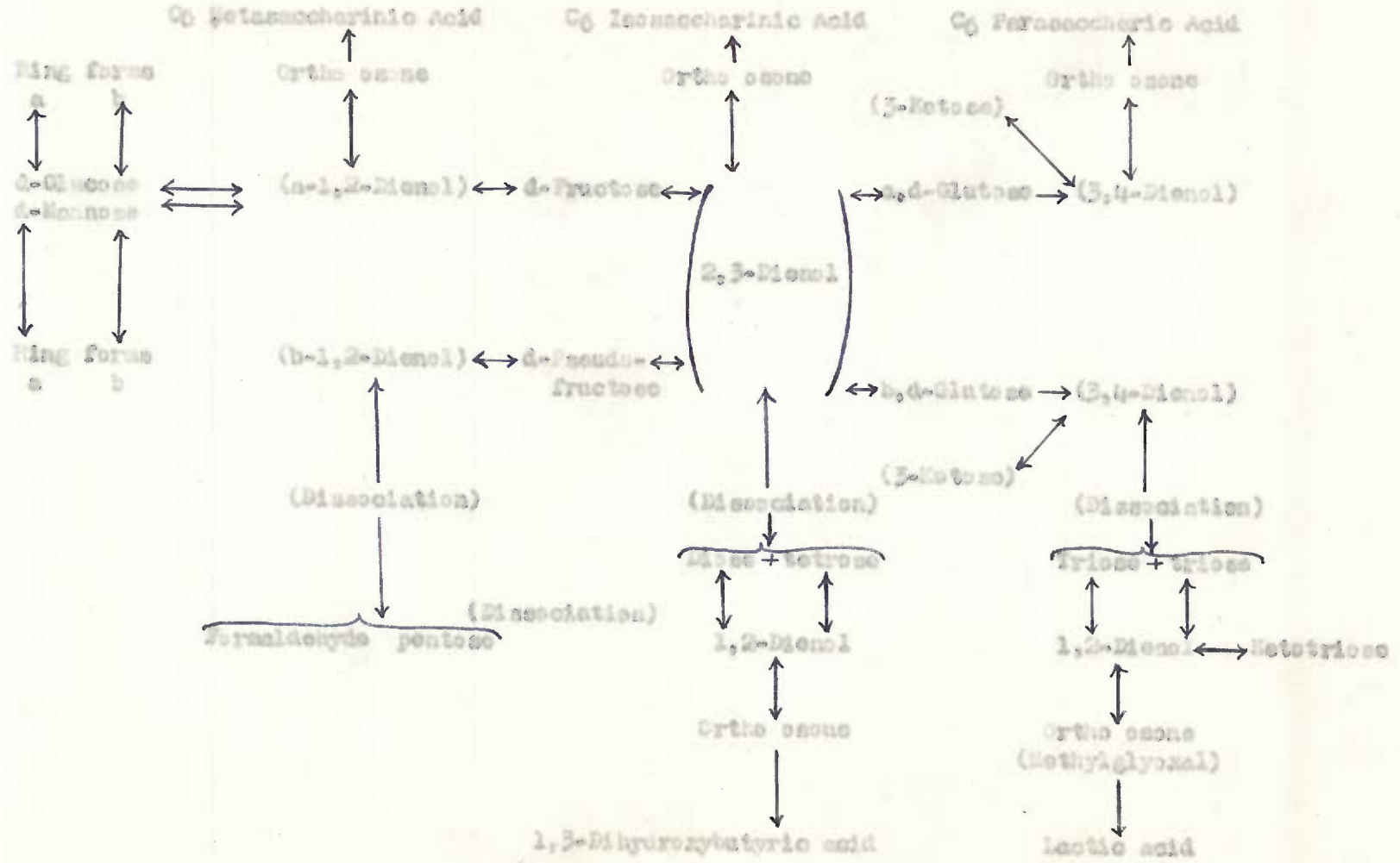
Hot Reduction Rate of Drop the Second Half Hour (Per Minute)----0.86 ml.
0.005 N. Thiosulfate

LACTIC ACID FORMATION IN THE CONDENSATION OF FORMALDEHYDE BY CALCIUM HYDROXIDE

INTRODUCTION

The chemistry of the sugars in alkaline solution has been a field for considerable research. C. A. Lobry de Bruyn and W. A. Ekenstein are pioneers in this field. They presented in 1895 their enediolic theory of sugar interconversion to explain the relationship of glucose, fructose, and mannose in alkaline solution. Other workers have since then found this theory applicable to all simple sugars studied. In 1928 M. L. Wolfrom and W. L. Lewis (14), through their study of the action of dilute alkali on tetramethyl glucose, found experimental verification of the Lobry de Bruyn and Van Ekenstein enediol theory of sugar interconversion.

During the years 1904-1914, Nef and his students made admirable contributions to the chemistry of the action of alkali upon sugars. They made further studies of the 1,2-enediol theory of Lobry de Bruyn and Van Ekenstein and also postulated the 2,3- and 3,4-enediols. Nef and his students made extensive studies on the saccharinic acids and other decomposition products of the various sugar enediols under different conditions. The following outline of relationships gives a general theory of the reactions that Nef has formulated from his studies (15).


Ring forms
a b
Ortho osone
(a-1,2-Dienol)
(b-1,2-Dienol)
(Dissociation)
Formaldehyde pentose
Ortho osone
2,3-Dienol
(Dissociation)
1,2-Dienol
Ortho osone
1,3-Dihydroxybutyric acid
Ortho osone
(3-Ketose)
(3,4-Dienol)
(3-Ketose)
(Dissociation)
1,2-Dienol
Ortho osone
(Methylglyoxal)
Lactic acid

In recent times W. L. Evans and his students have extensively continued Nef's brilliant work. Their present attack consists in preparing the theoretical intermediate products and comparing the action of these products in alkaline solutions with that of the parent sugar (16).

In the study of the chemistry of sugars in alkaline solutions, the study of lactic acid production has served as a very convenient index to the type of decomposition present because lactic acid can be readily and rather accurately determined quantitatively. The production of lactic acid by the action of a saturated solution of calcium hydroxide on different sugars under various experimental conditions has been reported. No work, however, has been published on the study of lactic acid in relation to the condensation formaldehyde by calcium hydroxide. Therefore it was considered that such a study would be an interesting addition to the chemistry of formaldehyde condensation by an alkaline agent.

EXPERIMENTAL

THE METHOD OF LACTIC ACID DETERMINATION

The modified Clausen method (19) was employed in the determination of lactic acid because it is simple and reliable. The basis of this method is the oxidation by potassium permanganate of lactic acid to acetaldehyde in boiling dilute sulfuric acid. The oxidation is catalysed by manganous sulfate, and the acetaldehyde formed is aerated into a large excess of sodium bisulfite solution. The combined bisulfite is titrated with iodine.

TECHNIQUE OF THE DETERMINATION

As in many determinations, the question of interfering substances arose. The determinations of samples for lactic acid in the condensation mixture during the course of an experiment were complicated by the presence of HCHO and the products of condensation. Therefore it was necessary to aerate off the HCHO and to precipitate out the sugar products before doing the lactic acid determination. A control was always run to make corrections for interfering substances such as HCHO, CH_3CHO, and CH_3COCH_3, which may be present in the air.

The following procedure was standardized for all condensation reactions in which lactic acid studies were made:

A 5 ml. sample of condensation mixture was withdrawn during a particular period of the reaction and placed in a centrifuge tube. The first stage of the determination consisted of removing the interfering sugars. This was done by the $CuSO_4$-$Ca(OH)_2$ treatment outlined by Van Slyke (18). 4 ml. of 15% $CuSO_4.5H_2O$ and 1 ml. of 15 % $Ca(OH)_2$ were added to the sample. The amount of $CuSO_4$ used was calculated according to Van Slyke's figures (19) of 4.33 molecules of the salt to each molecule of sugar. The reagents added are sufficient to precipitate out sugar equivalent to slightly more than 2% glucose. After the two reagents were added, the centrifuge tube was fitted with a tight rubber stopper, and the contents thoroughly mixed by an inverting motion. After 30 min., 3 ml. of the supernatant liquid were withdrawn for the lactic acid determination. In the glucose catalysed condensation reaction from which samples for lactic acid determination were taken before the endpoint of the reaction, it was necessary to eliminate the HCHO present. In this case the 3 ml. supernatant liquid from the $CuSO_4$-$Ca(OH)_2$ treatment were diluted with 60 ml. of distilled H_2O and acidified as for the lactic acid determination. The mixture was boiled with rapid aeration in the lactic acid apparatus for 30 min. Glass beads were added to promote even boiling. At the conclusion of this treatment, only a slight trace of

HCHO was detectable by the dimedon test. The procedures for lactic acid determination were then carried out. Samples taken at intervals following the completion of the condensation did not require the above treatment since no HCHO is present.

The procedures of the lactic acid determination began with the addition of 10 ml. of a 10% $MnSO_4$ in 10 N. H_2SO_4 to the supernatant fluid (treated or untreated for HCHO) and diluted with distilled H_2O to a volume of 80 to 100 ml. in the lactic acid flask. Glass beads were added to promote even boiling. The lactic acid flask was then connected to a reflux condenser to which a suction pump was attached. The contents of the flask were boiled vigorously and aerated rapidly for 2 min. into an empty receiving flask. Then the heat and aeration were discontinued while another receiving flask containing 10 ml. of 1% $NaHSO_3$ was put in place. The aeration and heating were continued, and N/100 $KMnO_4$ was dropped into the solution at a rate which kept the boiling liquid colorless or nearly so. The complete oxidation of lactic acid to CH_3CHO was marked by a persistent pink color in the solution for 1 min. and by the separation of MnO_2. At this stage addition of $KMnO_4$ was discontinued, and the boiling continued for 5 min. more to sweep out all the CH_3CHO. Then the bisulfite tower was rinsed with distilled H_2O. The excess bisulfite was titrated with N/100 iodine using

starch indicator. The bound bisulfite was then liberated by addition of solid $NaHCO_3$ and titrated with N/100 iodine until a blue endpoint persited for 15 sec. upon the addition of more bicarbonate. The iodine required for this second titration represents the bisulfite combined with CH_3CHO. The factor by which the volume of N/100 iodine used in titration of the combined bisulfite may be converted to milligram per cent of lactic acid was derived as follows: Each ml. of N/100 iodine is equivalent to 4.5 mg. of lactic acid. The dilution factor of the condensation mixture was 2, and 3 ml. of the supernatant liquid were used in the lactic acid determination.

$$\text{Factor} = \frac{2 \times 4.5 \times 100}{3} = 300$$

A blank determination was run with every experiment using 3 ml. of distilled H_2O to correct for interfering substances present in the air and in the reagents.

DATA

Lactic Acid Values During the Formaldehyde Condensation Reaction

Table 1. Reaction Catalyzed By 0.27 mM. Glucose

Time of Sample In Minutes	Milligram Per Cent Lactic Acid
20	0
50	0
60	0
64 (Endpoint)	0

Table 2. Reaction Catalyzed By 0.27 mM. Glucose

Time of Sample In Minutes	Milligram Per Cent Lactic Acid
69½ (Endpoint)	----
70	0
85	53.14
100	82.02
115	84.26
130	107.10
145	122.40
160	151.56
175	168.30
190	183.60

Lactic Acid Values During the Formaldehyde Condensation Reaction

Table 3. Reaction Catalysed By 0.27 mM. Fructose

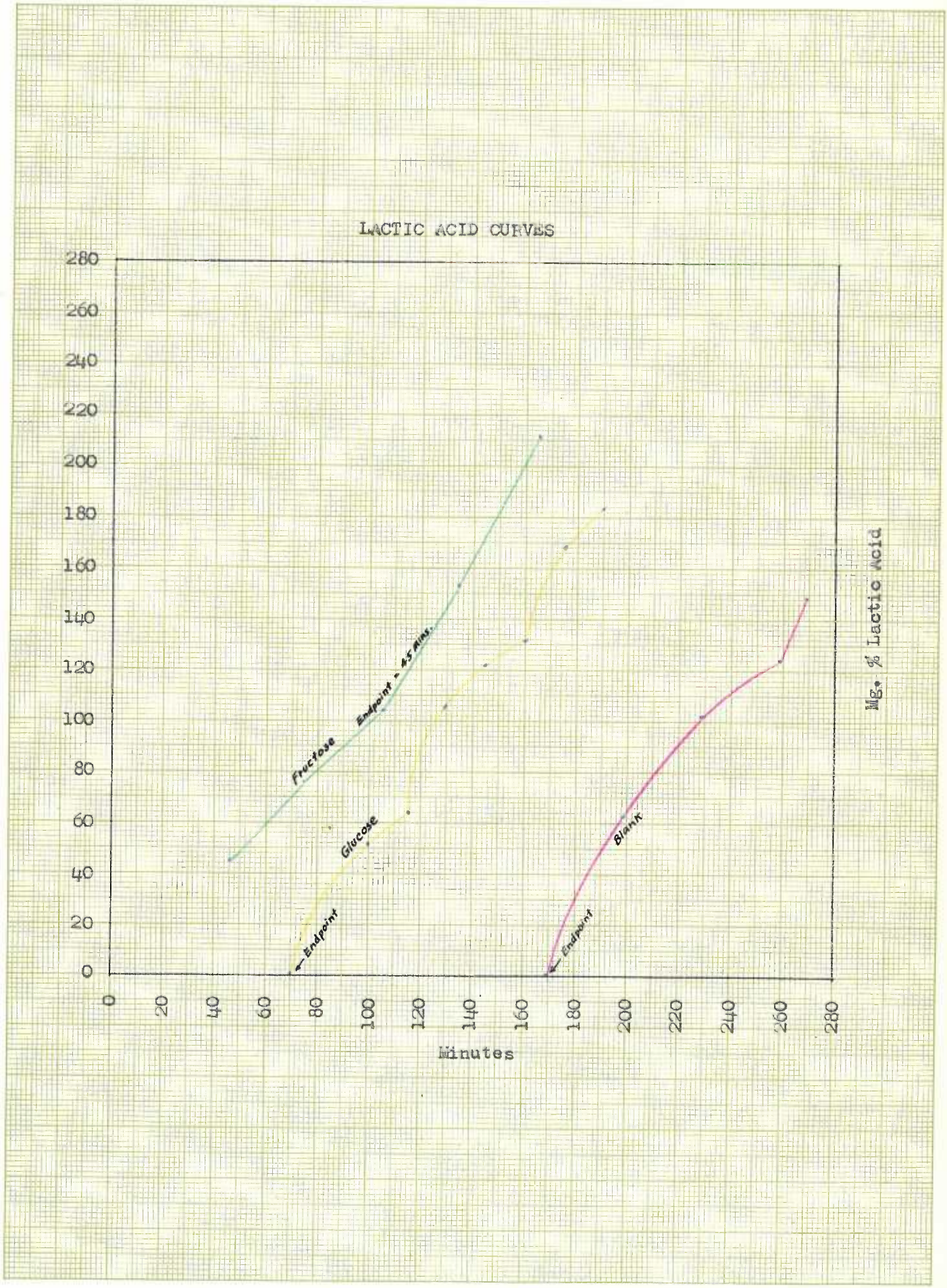
Time of Sample In Minutes	Milligram Per Cent Lactic Acid
45 (Endpoint)	----
47	45.90
75	76.50
105	104.04
135	153.00
165	211.14

Table 4. Non-catalysed Reaction (Blank Reaction)

Time of Sample In Minutes	Milligram Per Cent Lactic Acid
169 (Endpoint)	0
199	63.29
229	102.48
259	123.57
289	147.69

DISCUSSION

No lactic acid was detectable before the completion of the condensation reaction catalysed by 0.27 millimole glucose. This finding was anticipated since formaldehyde and the products of the Cannizzaro reaction (formic acid and methyl alcohol) which are the only known substances, do not form lactic acid in alkaline solutions. The sugars present during the rapid rise of the reducing action of the condensation mixture are mainly two and three carbon sugars. Glycoaldehyde does not form lactic acid in alkaline solutions. Glyceraldehyde and dihydroxyacetone once formed are undoubtedly too rapidly condensed to six carbon sugars before the slow lactic acid reaction can produce detectable quantities of the acid. Up to the endpoint of the glucose catalysed condensation reaction, no lactic acid was found. The sample taken several minutes later showed a minute amount of lactic acid. The amount of lactic acid gradually increased as shown by each succeeding sample. Similar experiments were carried out on a non-catalysed and a fructose catalysed condensation reaction. Since the glucose catalysed reaction showed no lactic acid before the endpoint, it was assumed no lactic acid would be found in the blank and the fructose catalysed reactions. Therefore no samples were taken before the endpoints of those two condensation reactions because the elimination of the formaldehyde made


LACTIC ACID CURVES
Mg. % Lactic Acid
Minutes
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
Fructose
Endpoint = 45 Mins.
Glucose
Endpoint
Blank
Endpoint

the determinations too laborious to be justified. The graph of the lactic acid curves is shown on the following page. The curves are practically identical. Of the three, the fructose catalysed reaction showed the greatest lactic acid formation, and the non-catalysed the least.

The formation of lactic acid following the endpoint of the condensation reaction is in accord with the expectation that once the reaction of condensation is completed, the newly formed products in the alkaline solution become engaged in a new reaction--a decomposition reaction. In this reaction unstable enediols are formed, which according to Evans and also P. A. Shaffer and T. E. Friedemann (15) exist in the form of salts. These unstable salts may either dissociate, rearrange into more stable structures, or both. Lactic acid is one of the products formed. Since the fructose catalysed reaction is the most rapid condensation reaction, it produces the most product upon which the alkaline solution may act to convert a portion into lactic acid.

What are the sources of lactic acid and through what mechanisms may its formation occur? Molecular weight studies of the syrup product obtained from the condensation of formaldehyde by calcium hydroxide were carried out in our laboratory, and the results indicate the product to be in form of hexose units. The nature of the hexose units is vague. Various workers, however, have reported the

presence of fructose, sorbose, and glucose. Pentoses and tetroses have also been found in various condensation products--namely, araboketose, erythrose, and threose--but these are undoubtedly present in very small amounts. The hexoses then represent the main sources for lactic acid formation in our condensation products. The formation of lactic acid from sugar and related compounds in an alkaline solution is dependent upon the presence of the unstable enediolic structure. The general theory postulated by Nef and by Evans attributes the formation of lactic acid to the cleavage of the 3, 4-enediol. In recent times, the modern concepts of bond strength as developed by the experimental methods of physical chemistry offer serious objections to the classical theory. Carbon to carbon double bonds require nearly twice the energy of single bonds to break them. F. W. Upson and associates (20) have reported several papers on their study of the reaction of barium hydroxide and monobasic sugar acids. It is their opinion that the cleavage of the sugar molecule at the 3, 4 position is due to the 1, 2-enediol and that this structure and not the 3, 4 enediol is the precursor of lactic acid. Upson explained the cleavage of the carbon chain based on Otto Schmidt's (21) "Double Bond Rule", which states that the double bonds strengthen the first following single bond and weaken the second following single bond. After the cleavage of the sugar molecules at the 3, 4 position, the aldotrioses

are converted to lactic acid by the way of the ortho-osone and methyglyoxal--an intramolecular oxidation-reduction.

Since the experiments were not conducted under anaerobic conditions, the presence of oxygen modifies the production of lactic acid. Oxygen tends to oxidize the cleavage fragments of sugar. Oxidation of the triose fragments will, of course, lower the amount of lactic acid produced. Then there will also be the tendency to form aldonic acid. Upson found that aldonic acids produce a great deal more lactic acid than the parent sugars. Using gluconic acid as an example, Upson (20) found that it yielded 65 per cent lactic acid compared with 25 per cent which Evans obtained from glucose. The presence of the carboxyl group in place of the aldehyde group is thought to stabilize the fragment to which it is attached. The fragment is then efficiently converted to lactic acid by a process involving intermolecular oxidation-reduction. The aldonic acids formed from erythrose and threose will yield a large amount of lactic acid whereas the parent sugars themselves cannot be converted to lactic acid by anaerobic action of alkaline solutions. The presence of oxygen can affect the results through one reaction to decrease lactic acid yield and through another reaction to increase lactic acid. There is no way of predicting the resultant effect of oxygen on lactic acid production under the conditions of the condensation experiment. Its effect can be determined,

however, by repeating the condensation experiments performed in this section under anaerobic conditions.

CONCLUSION

The study of the lactic acid curve during the course of a formaldehyde condensation reaction by calcium hydroxide was considered worthwhile as this phase of investigation has never been undertaken. Lactic acid studies were made of the following three condensation experiments: the non-catalysed reaction, the fructose catalysed reaction, and the glucose catalysed reaction. The curves are generally similar in form. The glucose catalysed condensation reaction showed no lactic acid in samples taken before or at the endpoint of the reaction. The first sign of lactic acid was found several minutes following the color change of the condensation, and thereafter succeeding samples showed progressively increased lactic acid present. The hexose sugars of the condensation product undoubtedly furnish the main source of lactic acid. The formation of the enediol structures in the alkaline solution leads to fragmentation of the hexose chains at the 3, 4 position. These fragments are converted to lactic acid.

The fact that the experiments were not conducted under anaerobic conditions must modify the formation of lactic acid. The presence of oxygen probably oxidizes a portion of the readily oxidizable sugar fragments thereby reducing lactic acid formation. Aldonic acids, however, can conceivably be present from oxidation of the aldehyde group by

by oxygen. Aldonic acids are more efficiently converted to lactic acid in alkaline solutions than the parent sugars. This will tend to increase lactic acid formation. The over-all effect of the presence of oxygen on lactic acid production during the course of the condensation reactions can be determined by further experiments in which oxygen is excluded.

BIBLIOGRAPHY

1. Butlerow, A. M. Ann. Chem., vol. 120, pp. 295--, 1861. Ref. cited from Ney, L. F. The condensation of formaldehyde and the reaction of formaldehyde with ascorbic acid, pg. 1, 1937. (Thesis presented to the Dept. of Biochemistry, University of Oregon Medical School.)

2. Spoehr, H. A. Photosynthesis, The Chemical Catalog Co. Inc., New York, 1926.

3. Baeyer, A. Ber., vol. 3, pp. 63--, 1864. Ref. cited from Van Bruggen, J. T. Catalysis of formaldehyde condensation, pg. 1, 1939. (Thesis presented to the Dept. of Biochemistry, University of Oregon Medical School.)

4. Loew, O. Zur Condensation des Formaldehyds Berichtigung. Ber., vol. 39, pp. 1592-3, 1906.

5. Schmalfuss, H. The effect of monoses and magnesium ions on the formation of sugar from formaldehyde. Biochem. Z., vol. 185, pp. 70-85, 1927.

6. Kusin, A. Über die katalytische Wirkung von Monosen auf die Formaldehyd-Kondensation. Ber., vol. 68B, pp. 619-24, 1935. II. Mitteil. Ber., vol. 68B, pp. 1494-9. 1935. III. Mitteil. Die Zwischenprodukte der Reaktion. Ber., vol. 68B, pp. 2169-73, 1935.

7. Van Bruggen, J. T. Catalysis of formaldehyde condensation. (Thesis presented to the Dept. of Biochemistry, University of Oregon Medical School, 1939.)

8. Fischer, E. Über die Verbindungen des Phenylhydrazins mit den Zuckerarten. Ber., vol. 21, pp. 988-91, 1888.

Fischer, E. and Passmore, F. Bildung von Acrose aus Formaldehyd. Ber., vol. 22, pp. 359-60, 1889.

Fischer, E. Synthese der Mannose and Lävulose. Ber., vol. 23, pp. 370-84, 1890.

9. Kuster, W., and Schoder, F. The formation of sorbose in the condensation of formaldehyde. Z. physiol. Chem., vol. 141, pp. 110-31, 1924.

10. Neuberg, C. Ueber die Isolirung von Ketosen II. Ber., vol. 35, pp. 2626-33, 1902.

11. Euler, H., and Euler, A. Ueber die Bildung von l-Arabinoketose aus Formaldehyd. Ber., vol. 39, pp. 45-51, 1906.

12. Orthner, L., and Gerish, E. The primary stages in the condensation of formaldehyde. Biochem. Z., vol. 259, pp. 30-52, 1933.

13. Romijn, J. Z. Ann. Chem., vol. 36, pp. 19--, 1897. Ref. cited from Treadwell, F. P., and Hall, W. T. Analytical Chemistry, 8th ed., John Wiley and Sons, Inc., New York, 1935.

14. Wolfrom, M. L., and Lewis, W. L. The action of dilute alkali on tetramethylglucose. J. Am. Chem. Soc., vol. 50, pp. 837-54, 1928.

15. Shaffer, P. A., and Friedemann, T. E. Formation of lactic acid and saccharinic acids. J. Biol. Chem., vol. 86, pp. 345-74, 1930.

16. Evans, W. L. Mechanism of carbohydrate oxidation. Chem. Reviews, vol. 6, pp. 281-315, 1929.

Evans, W. L., Nadeau, G. F., and Newlin, M. R. Mechanism of carbohydrate oxidation (XVI): Action of aqueous solutions of potassium hydroxide on l-rhamnose. J. Am. Chem. Soc., vol. 55, pp. 4957-63, 1933.

Evans, W. L., Gehman, H., and Kreider, L. C. Mechanism of carbohydrate oxidation (XXIII): Contribution to the alkaline hydrolysis of oligosaccharides. J. Am. Chem. Soc., vol. 58, pp. 2386-95, 1936.

16. Evans, W. L., and Plunkett, R. J. Mechanism of carbohydrate oxidation (XXIV): The action of aldehydo-d-glucose and aldehydo-d-galactose in alkaline solution. J. Am. Chem. Soc., vol. 60, pp. 2847-52, 1938.

Evans, W. L., Nichols, S. H., Jr., and McDowell, H. D. Synthesis of certain trisaccharides and a study of their behavior in alkaline solution. J. Am. Chem. Soc., vol. 62, pp. 1754-8, 1940.

17. Friedemann, T. E., Cotonio, M., and Shaffer, P. A. The determination of lactic acid. J. Biol. Chem., vol. 73, pp. 335-38, 1927.

18. Van Slyke, D. D. The determination of B-hydroxybuteric acid, acetoacetic acid, and acetone in urine. J. Biol. Chem., vol. 32, pp. 455-65, 1917.

19. Clausen, S. W. A method for the determination of small amounts of lactic acid. J. Biol. Chem., vol. 52, pp. 263-80, 1922.

20. Upson, F. W., Noyce, W. N., and Albert, W. D. Action of barium hydroxide on the monbasic sugar acids. J. Am. Chem. Soc., vol. 61, pp. 779-86, 1939.

21. Schmidt, O. The dissociation of carbon bonds. Chem. Reviews, vol. 17, pp. 137-54, 1935.

Miss June Dove Wong
(Typist)