CATALYSIS OF THE FORMALDEHYDE CONDENSATION

by

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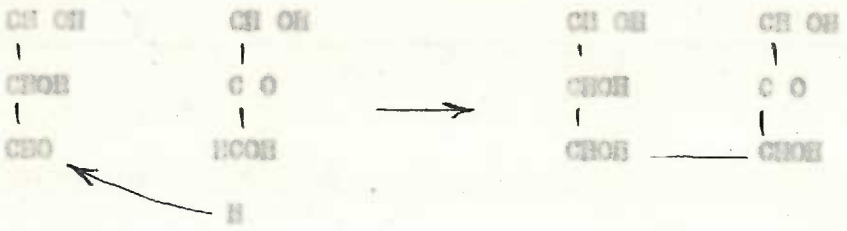
Historical

In 1859, Butlerow[1] condensed trioxymethylene by means of lime water to a syrup with sugar-like properties. Some ten years later Hoffman[2] discovered formaldehyde and showed that Butlerow's trioxymethylene was a polymerised form of formaldehyde. On the basis of the findings of these earlier workers, Baeyer[3] in 1884, postulated his Assimilation Theory, in which he made an attempt to explain, on a chemical basis, the process by which plants synthesise carbohydrates. In this theory of photosynthesis he assumed that the plant takes carbon dioxide from the air and combines it with water to give formaldehyde, which in turn is quickly changed or converted to carbohydrates. The production of formaldehyde as a photosynthetic intermediate is considered probable in various modern theories of photosynthesis. The in vitro condensation of formaldehyde to sugars, affording a chemical analogy to the second part of Baeyer's theory, has been studied by a number of workers. A few of the main points of interest will be reviewed in the following section.

Butlerow found the sweet yellow syrup obtained from his condensation of trioxymethylene to be optically inactive as well as non-fermentable. Loew[4] obtained a similar non-fermentable syrup which

he called methose, by treating formaldehyde with lime water. He later substituted magnesium oxide for the lime water and produced a syrup identical in many ways with those previously obtained but with the property of being somewhat fermentable by yeast. Because he considered it possible that glycerose is an intermediate product in the formaldehyde condensation, Emil Fischer[5] treated acrolein bromide and later glycerose with baryta and made syrups identical with those obtained by other workers. These various syrups were shown by Fischer and Rassmore3to be complex sugar mixtures from which could be isolated a fraction called α acrose. This fraction was separated by the use of phenylhydrazine. Acrose was later shown by Fischer and Tafel[7] to contain the inactive forms of two naturally occuring sugars, namely, glucose and fructose. The glycerose used by Fischer was an equilibrium mixture of glyceric aldehyde and dihydroxyacetone and according to Fischer could form a keto hexose by the following mechanism:



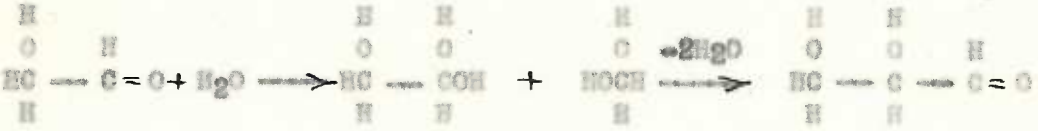
Glycerose Keto Hexose

Possible mechanisms of the formaldehyde condensation. Formaldehyde, postulated to exist in solution as a hydrate, can, according to Baeyer,

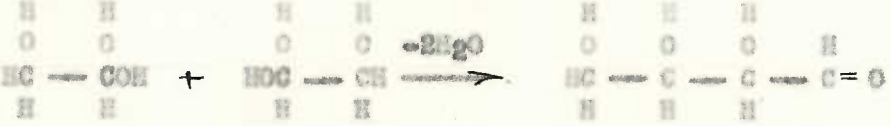
condense by the elimination of a molecule of water.

Hydrated Form

Such a condensation would yield a diose, which might then add on a molecule of formaldehyde to form a triose.



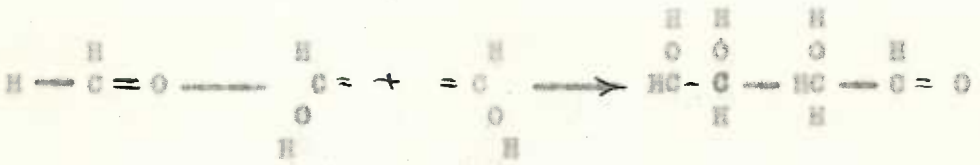
or might condense with another molecule of diose to form a tetrose.



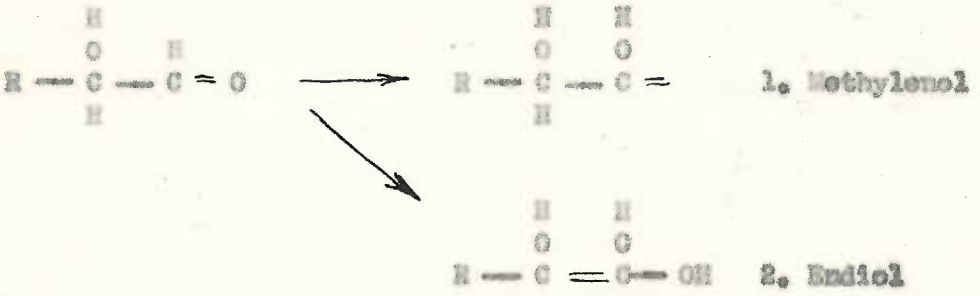
Successive condensations would yield pentoses, hexoses and possibly larger molecules.

As a result of his studies on the action of alkalies on aldehydes, Nef[8] suggested that the condensation occurred through the combination of two enolic molecules. Formaldehyde might exist in an enolic form

which could condense with itself to form a diose.

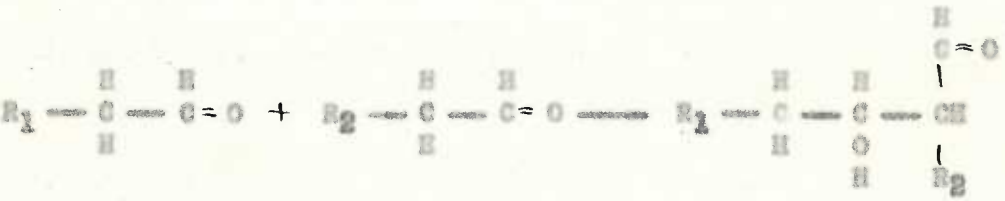


A diose so formed might also have an enolic form which could produce larger molecules by a series of condensations similar to those previously described. Condensation beyond the two carbon molecule stage is complicated by the fact that such molecules may exist in two enolic forms, either of which might conceivably condense.

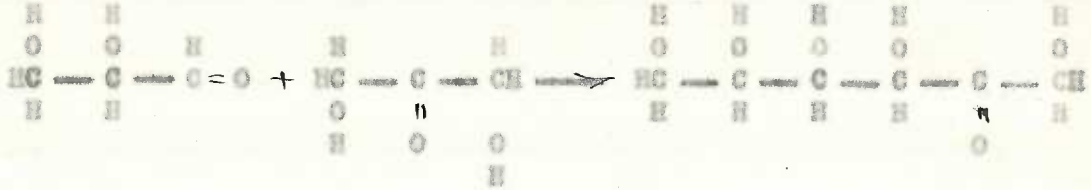


Enolic forms

Another possibility is the "aldol type" of condensation, a reaction that is quite general for aldehydes with alpha hydrogen atoms. Aldehydes with one or two carbon atoms would yield straight chain condensation products as shown below. Larger molecules on the other hand, would give branched chains.



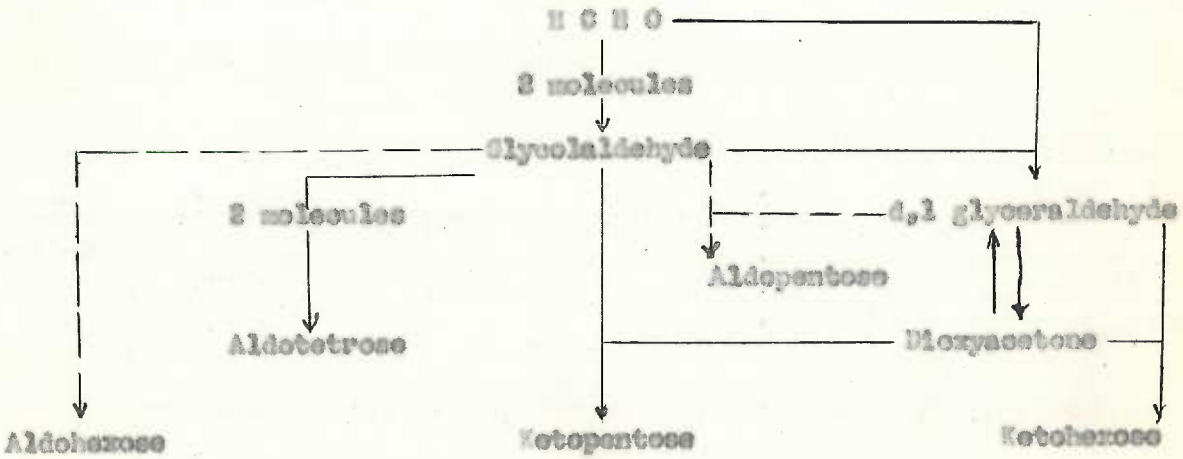
As will be explained in more detail later, workers have been unable to find any such branched chains in the condensation syrup. This type of condensation may yield large molecules with straight chains if one of the combining molecules is an aldehyde and the other a ketone.



Since many aldehydes in alkaline solution exist as an equilibrium mixture of aldehyde, enol, and keto forms, it is quite possible that the aldol condensation takes place to a significant degree.

Recent contributions to the problem. Although the valuable contributions of Emil Fischer gave definite evidence concerning the composition of the condensation syrup, his use of phenyl hydrazine was found of little further value for this problem, because of the difficulty of separating the osazones formed. A real contribution was made by Orthner and Gerish in 1933 when they introduced new methods of analysis, with which they were able to study in both a qualitative and quantitative way, the products of the condensation reaction. By catalytically hydrogenating the mixture of sugars contained in the syrup, formed by condensing formaldehyde with lead hydroxide at 100 degrees Centigrade, these workers obtained the corresponding alcohols. After the alcohols had first been partially separated by fractional distillation, they were identified through the benzol derivatives. Orthner and Gerish found no branched chain molecules of any kind in their product. Aldo-tetroses were present but only keto-pentoses and hexoses. In order to explain these observations, they postulated the following possibilities of reaction. In this scheme the most probable course of the reaction is indicated by heavy lines while the doubtful courses are indicated by broken lines.

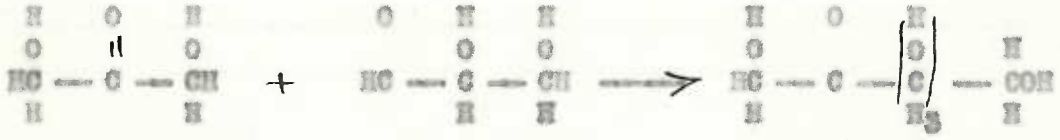
Figure 1



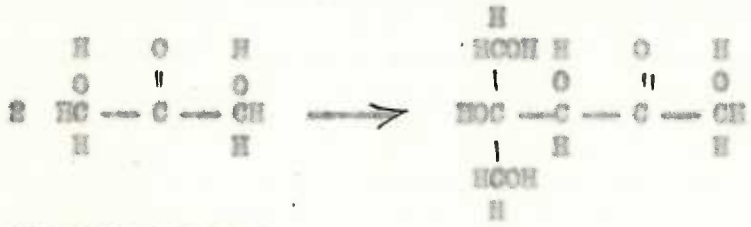
The straight chained hexoses could be formed in several possible ways. 1. The stepwise combination of six formaldehyde molecules very likely does not account for much of the final product due to the fact that the intermediate compounds of such a condensation could easily combine with themselves. In doing so they would more quickly furnish hexose products than could the reaction between the larger number of formaldehyde molecules. 2. The addition of glycolaldehyde to a tetrose is not likely to account for much of the hexoses, for at the time, as found by Orthmer and Gerish, when appreciable quantities of tetroses are present, the concentration of glycolaldehyde is low. 3. The most likely method of hexose formation is the union of one molecule of glyceraldehyde with one of dihydroxyacetone. As has been stated previously, the absence of branched chains rules out the possibility of two molecules of either glyceraldehyde or dihydroxyacetone condensing

to form hexoses. Possible modes of aldol combinations between molecules of the trioses are:

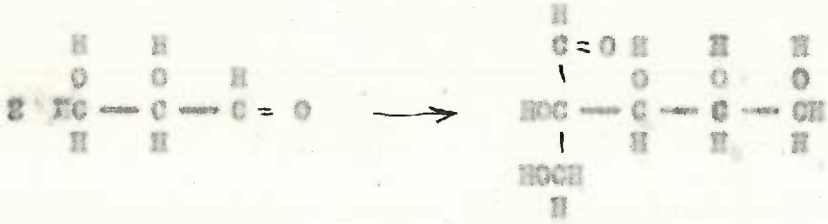
Figure 2



Dihydroxyacetone Glyceraldehyde Ketohexose

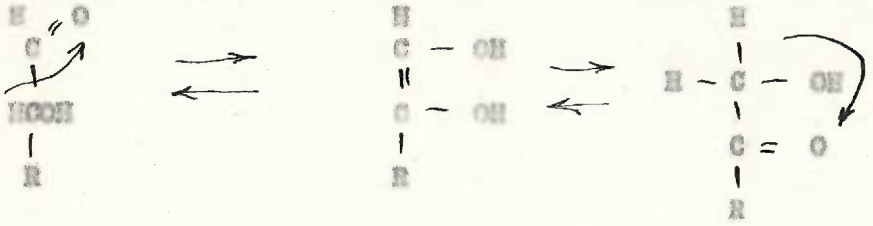


Dihydroxyacetone



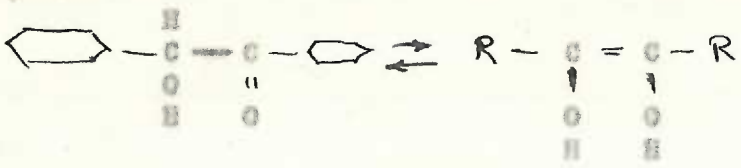
Glyceraldehyde

Catalysis of the condensation. Schmalfuss[10] (1927) noticed that certain sugar molecules could markedly catalyze the condensation reaction, that is, they were able to reduce the time required for the formaldehyde to be converted to sugars. In 1935, Ruzin[11] further investigated this catalytic effect and found that the active catalyst is the endiol group of the sugar. Such a group is easily formed by an aldose or ketose sugar in the process of enolisation in alkaline solution.



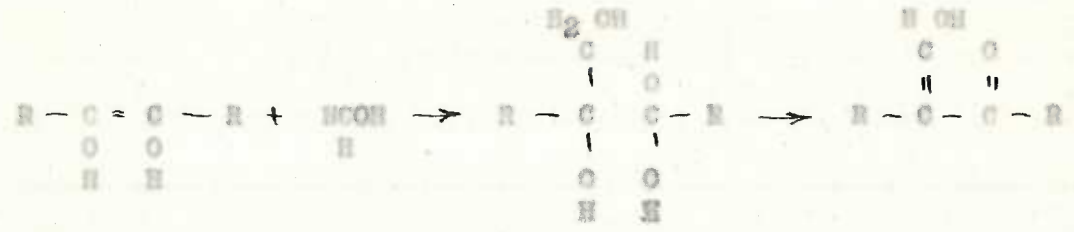
Aldehyde form Endiol form Keto form

In an attempt to determine the mechanism of the catalysis Ruzin used the compound benzoin as the catalytic agent. Benzoin is known to acquire the endiol group in alkaline solution by the following tautomeric change.

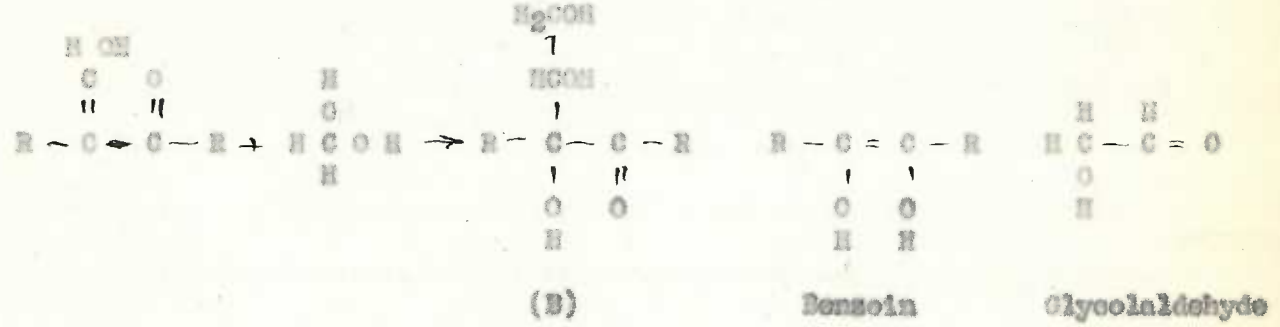


From the condensation mixture he was able to isolate an addition compound of benzoin and at a later stage of the condensation he recovered the original benzoin. With this information he postulated the following

mechanism for the catalytic action of benzoin.



Enol form of Benzoin | Formaldehyde (hydrated) | Known addition compound (A)



(B) | Benzoin | Glycolaldehyde

If the reaction does follow some course similar to the one outlined above, the catalyst is effective for several reasons. Its primary effect, that of the formation of intermediate products such as glycolaldehyde, tends to shorten the time of condensation. These products can not only further condense, but can exert a secondary catalytic effect when they enolize in an analagous manner to benzoin.

In an attempt to better understand the mechanism of the catalyzed formaldehyde condensation we have investigated the relative catalytic activities of a number of compounds that furnish structures related to

the endiol group. In addition to these qualitative studies we have also made an attempt to understand the reaction from a quantitative standpoint. This has been done by studying the quantitative relations of formaldehyde and reducing substances during the course of the reaction. In the concluding section of this paper there will be some discussion of the properties of the syrup isolated from the condensation mixture.

Experimental

Standardization of conditions and reagents. In order to compare and contrast the relative effectiveness of the catalysts of the formaldehyde condensation, it was necessary to adopt some characteristic of the reaction that would serve as a standard of comparison. Such a basis for comparison was found to be furnished by the reaction itself when at a certain stage of the condensation, a color change takes place. Although this color change is more fully discussed later, it should be mentioned that the change takes place when all formaldehyde has disappeared and reducing substances have reached a maximum. The condensation mixture under our conditions of work, is white in color because of an excess of lime present. At the time when the formaldehyde concentration is zero, certain changes take place in the reducing substances that have been formed, that cause the solution to turn straw yellow and later deep brown in color. In the discussion to follow this time will be called the "end point", and such an end-point in a reaction in which no catalyst has been used will be called the "blank end point."

Condensing agents. Although a number of reagents such as magnesium oxide, lead hydroxide and calcium hydroxide will bring about the condensation of formaldehyde at elevated temperatures, only one of these was found to be of use at low temperatures. Both magnesium

oxide and lead hydroxide require a temperature of 100 degrees Centigrade to effectively condense formaldehyde. Calcium hydroxide was found to be effective at a temperature of 40 degrees Centigrade. Since a temperature of 40 degrees Centigrade was more compatible with the various factors influencing our work, we chose calcium hydroxide as the condensing reagent. Early in the work it became apparent that various commercial brands of lime were quite different in their condensing qualities. Although each reagent used, and its make and concentration, will be discussed in detail later, it should be stated that in all this work a reaction mixture consisted of 100 milliliters of a 4.0 per cent solution (water) of formaldehyde, with 4.0 grams of calcium hydroxide. The reaction of such a mixture is spoken of as a blank reaction. When catalyst was added the reaction is referred to as a catalysed reaction. The reagents in the proportions described above were put into 1 x 8 inch pyrex test tubes, immersed in a water bath at 40 degrees Centigrade and mechanically stirred to produce a constant slow mixing which was just sufficient to keep the lime suspended.

The following table compares the condensing qualities of lime obtained from three different sources. The condensing effect of calcium hydroxide was found to be related to factors other than pH. Solutions of sodium and potassium hydroxide with the same pH as the calcium

hydroxide produced no sugar condensation.

Table I

	pH	Blank Reaction
Lilly's Lime (Medicinal Grade)	11.04	130 minutes
Mallinckrodt Lime (Analytical Reagent)	11.06	100 minutes
Baker Lime	11.02	10 - 12 hours
Sodium Hydroxide	11.02	No condensation
Potassium Hydroxide	11.02	No condensation

From certain experiments, listed in a following table, it was found that the amount of lime used in the reaction was an important factor. Such results suggested that the size of the particles of the suspended lime might vary. Smears of the various limes were viewed under a measuring microscope. In all cases the unit particle size was found to be between 0.9 and 1.2 micra.

In order to determine whether or not the purity of the reagent was a factor in determining the speed of the condensation, the following experiments were done.

Preparation of pure calcium oxide. A solution of calcium nitrate with a maximum of limit of metallic impurities of 0.02 per cent, was treated with oxalic acid to precipitate calcium oxalate. The washed and dried salt so prepared was thoroughly burned in an electric furnace to produce pure calcium oxide. This calcium oxide when used in equivalent amounts in the condensation reaction, gave the solution a pH of 11.30 and caused a blank reaction to be complete in 117 minutes.

Preparation of known impure calcium oxide. The lime was prepared as above, except that to the wet calcium oxalate precipitate there was added one milligram each of twelve known metals. The metals used were all possible impurities of lime and included the following: iron, nickel, silicon, sulphur, magnesium, sodium, potassium, lead, zinc, barium, cobalt, and cadmium. The product yielded a slightly colored solution when added to the formaldehyde -- however, it was still possible to observe a color end point. Although this lime gave a pH similar to the other limes, its blank reaction time was greater than ten hours.

The following table lists the various limes tried and shows their action in a blank reaction as well as in reactions catalysed by 0.27 millimoles of glucose.

Table II

Lime	Grade	Time of End Point	
		Blank	Catalysed
Lilly	Medicinal	130 minutes	64 minutes
Synthetic	Pure	117 minutes	60 minutes
Mallinckrodt	A.R.	100 minutes	68 minutes
Synthetic	Impure	11 hours	72 minutes
Baker	U.S.P.	11 hours	76 minutes

As seen from the above, it is quite evident that the presence of small amounts of metallic impurities exert a negative catalytic effect upon the formaldehyde condensation.

As has been previously stated, an amount of lime is used that is much more than is necessary for a saturated solution. The reason for this is obvious when the following table is considered. The reactions in column A were all catalyzed by 0.55 millimoles of glucose. Column B compares blank reactions

Table III

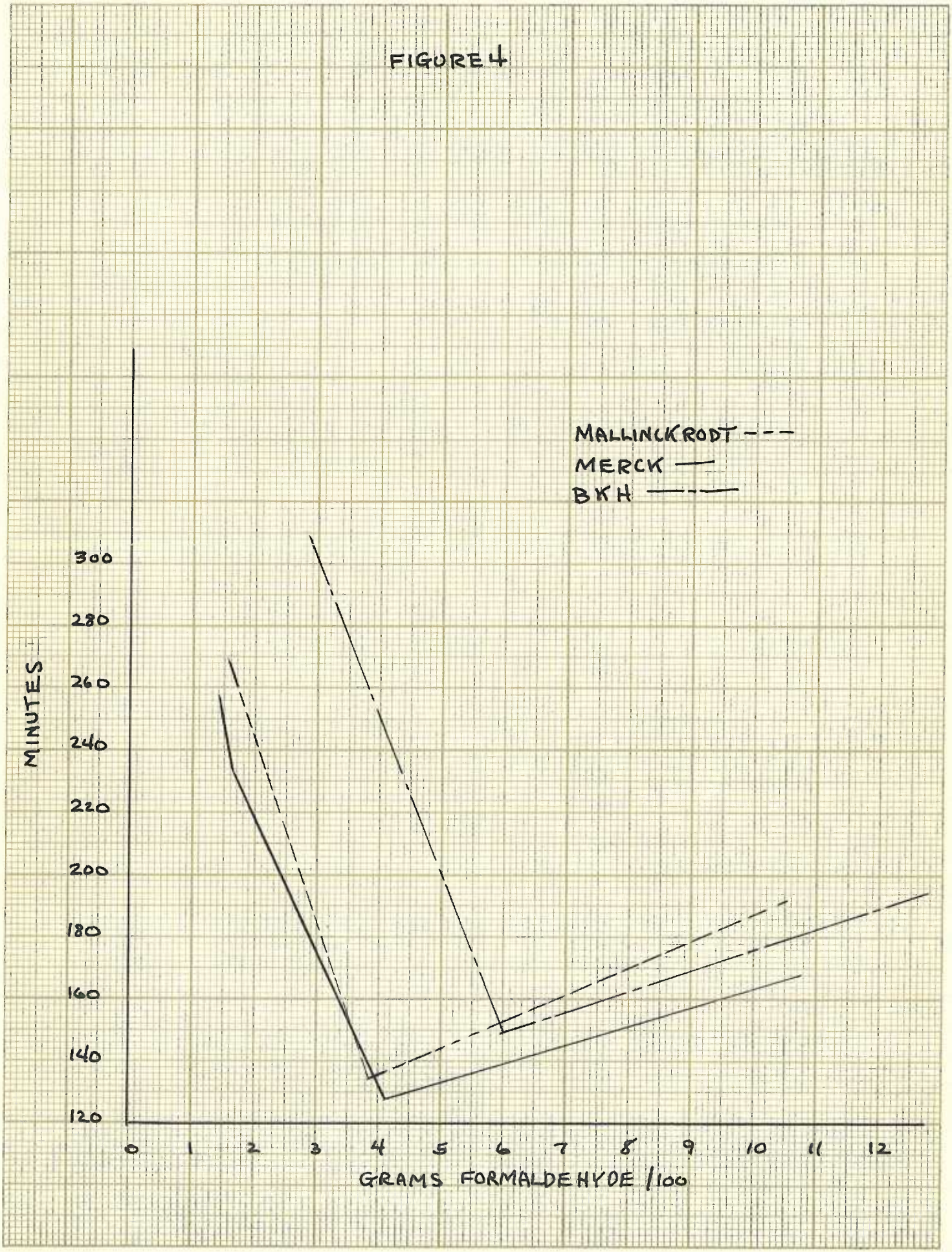
	A	B
2 grams Lilly's lime	60 minutes	180 minutes
4 grams Lilly's lime	54 minutes	127 minutes
6 grams Lilly's lime	60 minutes	165 minutes
4 grams Lilly's lime --- filtered*	90 minutes	

*This solution was thoroughly mixed at 40 degrees Centigrade and then quickly filtered on a sintered glass filter, the filtrate then being returned to the water bath. The pH of this solution was 11.94.

Because of the short time required for the four grams of lime per 100 milliliters of formaldehyde, this concentration was chosen as our standard.

Formaldehyde. The following graph shows the relationship between formaldehyde concentration and time of blank condensation. The various points were determined by condensing 50 milliliters of the appropriate concentration of formaldehyde with two grams of Lilly's lime at 40 degrees Centigrade.

FIGURE 4


MALLINCKRODT - - -
MERCK —
BKH —·—
MINUTES
300
280
260
240
220
200
180
160
140
120
0
1
2
3
4
5
6
7
8
9
10
11
12
GRAMS FORMALDEHYDE /100

Because of the conveniently low point of the Merck formaldehyde curve at a concentration of four per cent, this reagent (reagent grade) was used to make up the solutions used in all of our later work. When Mallinckrodt lime was used with this four per cent formaldehyde solution, the blank reaction required 180 minutes for completion.

The following table shows that the amount of a given lime used with a definite brand and concentration of formaldehyde is related to the time of the blank reaction. It is interesting to observe that excess lime may greatly retard the rate of reaction.

Table IV

9.9% Merck Formaldehyde	6 g. Lilly's Lime	245 minutes
9.9% Merck Formaldehyde	4 g. Lilly's Lime	165 minutes
4.03% Merck Formaldehyde	6 g. Lilly's Lime	153 minutes
4.03% Merck Formaldehyde	4 g. Lilly's Lime	127 minutes
0.97% Mallinckrodt	4 g. Lilly's Lime	331 minutes
0.97% Mallinckrodt	2 g. Lilly's Lime	245 minutes

Methods of analysis. Formaldehyde concentrations of the reaction mixtures were determined by the use of dimethyl dihydroresorcinol (dimedon). This reagent forms a water insoluble complex with formaldehyde according to the following equation:

$$CH_2O + 2C_8H_{12}O_2 \longrightarrow CH_2(C_8H_{12}O_2)_2 + H_2O$$

A formaldehyde solution must be diluted to contain no more than 40 milligrams of formaldehyde per sample. It is customary to have the size of the sample below 10 milliliters. Such a solution is made slightly acid with hydrochloric acid and mixed with 100 milliliters of a saturated solution of dimedon. The mixture is warmed on a water bath and allowed to stand over night. The precipitate is collected on a weighed Gooch crucible which is then dried at 90 degrees Centigrade for several hours and reweighed. The weight of precipitate times 0.1027, gives the weight of formaldehyde in the sample.

Pure formaldehyde solutions, such as our four per cent stock solutions are more easily determined by an iodimetric method described by Romijn[12]. In this method formaldehyde is quantitatively oxidized to formic acid by remaining in contact with iodine in alkaline solution for a short time. In our application of this method, five milliliters of formaldehyde solution containing not more than fifty milligrams of formaldehyde were mixed with 40 c.c. N/10 iodine solution. Strong NaOH was added drop by drop until the solution was light yellow.

After ten minutes, the solution was acidified with strong hydrochloric acid. The liberated iodine was then titrated with thiosulfate and the formaldehyde concentration was calculated: 1 ml. N/10 iodine = 0.00150 gm. formaldehyde.

Reducing substances formed from the condensation were determined by the use of a modified Shaffer-Hartman reagent, described by Somogyi[15], the values being reported as milligrams of glucose reducing equivalent per 100 milliliters of solution. With this reagent the solution to be analyzed must not contain more than two milligrams or less than 0.5 milligrams of glucose in five milliliters. For analysis of the condensation mixture, one milliliter of solution was carefully removed by pipette and neutralized to phenol red with 0.5 N HCl. Samples taken before reducing substances appeared in appreciable amounts were diluted to a final volume of 25 milliliters. Later samples were diluted to fifty milliliters. Five milliliters of the dilutions and five milliliters of reagent were pipetted into 8" x 1" test tubes, mixed well, and the tubes heated in a boiling water bath for fifteen minutes. During the determination the tubes were covered with glass bulbs. After cooling to room temperature, one milliliter of a solution containing four grams of KI and five grams of $KgCgO_4$ per 100 milliliter was added. Five milliliters of normal $MgSO_4$ were now blown in rapidly and after five minutes the tubes titrated with freshly prepared 0.005 normal thiosulfate, starch being used as an indicator. A blank value was

run with each series of tubes and the milliliter titration difference between the blank and the sample was multiplied by 0.115 to give the milligrams of glucose reducing equivalent in the five milliliter sample.

The cold reducing power of the condensation mixture was measured by Soxhlet's modification of Fehlings reagent.* Two milliliters of the mixed reagent and one milliliter of the undiluted condensation mixture were allowed to stand at room temperature for one-half hour. Two and five tenths milliliters of twenty-five per cent $HgSO_4$ were added along with one milliliter of a twenty per cent solution of KI. The liberated iodine was titrated with 0.05 N thiosulfate. A blank was run simultaneously and the titration difference between it and the sample was recorded.

In preliminary work on this problem, samples of the condensation mixture to be analysed for sugar were first treated with dimedon to precipitate any formaldehyde present. The excess dimedon was then removed by precipitation with $Fe_2(SO_4)_3$ — $BaCO_3$. Reducing values obtained using such a procedure were quite variable and the method was unsatisfactory. In another attempt to separate the excess dimedon, a procedure was devised in which the dimedon was extracted with benzene in the apparatus shown below.

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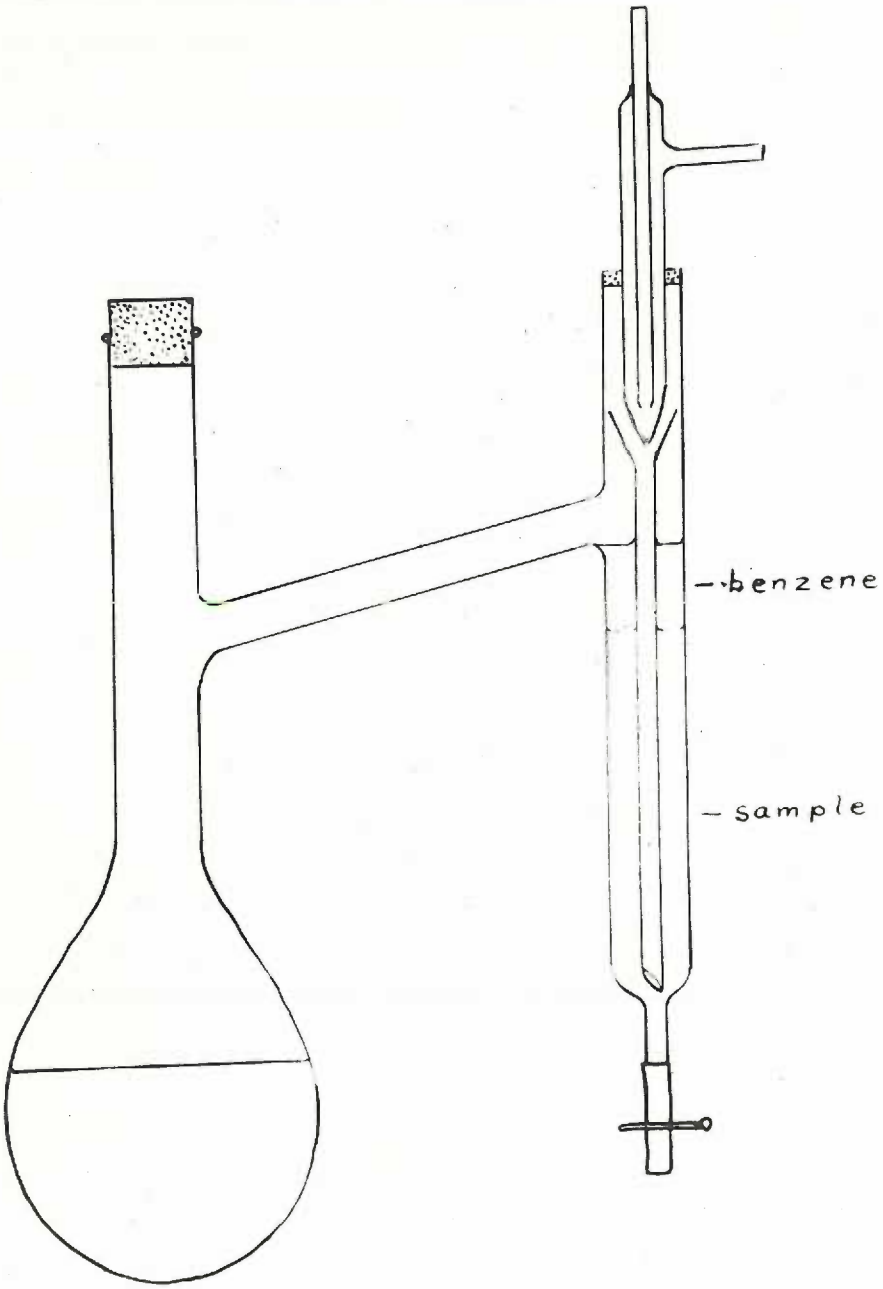
* Solution A — 6.928 grams $CuSO_4 \cdot 5H_2O$ in 100 milliliters H_2O
Solution B — 34.6 grams Rochelle salts and 10 grams NaOH in 100 cubic centimeters H_2O

FIGURE 5



Although this method gave rather consistent results, it was wasteful of both time and materials. To determine whether any treatment of the condensation sample was necessary, the following experiment was done. A one milliliter sample was taken from a condensation reaction, just previous to the end point, a time at which the formaldehyde concentration was almost zero. This sample was neutralized to phenol red with hydrochloric acid and diluted to fifty milliliters. Two five milliliter portions were withdrawn for analysis and placed in tubes. Five milliliters of water was mixed with one sample and five milliliters of water containing four milligrams of formaldehyde with the other. The second mixture contained formaldehyde equivalent to that present in a sample prepared from a condensation solution of four per cent formaldehyde concentration. Sugar was determined as previously described. The reduction value on the tube with added water was equivalent to 1230 milligrams glucose per 100 milliliters, while the tube containing added formaldehyde gave a reduction equivalent to 1200 milligrams of glucose per 100 milliliters, a difference of only eleven milligrams or an error of about one per cent. Because of the negligible error due to the presence of formaldehyde it was not removed in later work.

The following table gives a comparison of values found by the above methods. Each set of values represents samples taken from mixtures

catalysed by 0.11 millimoles of glucose

Table V

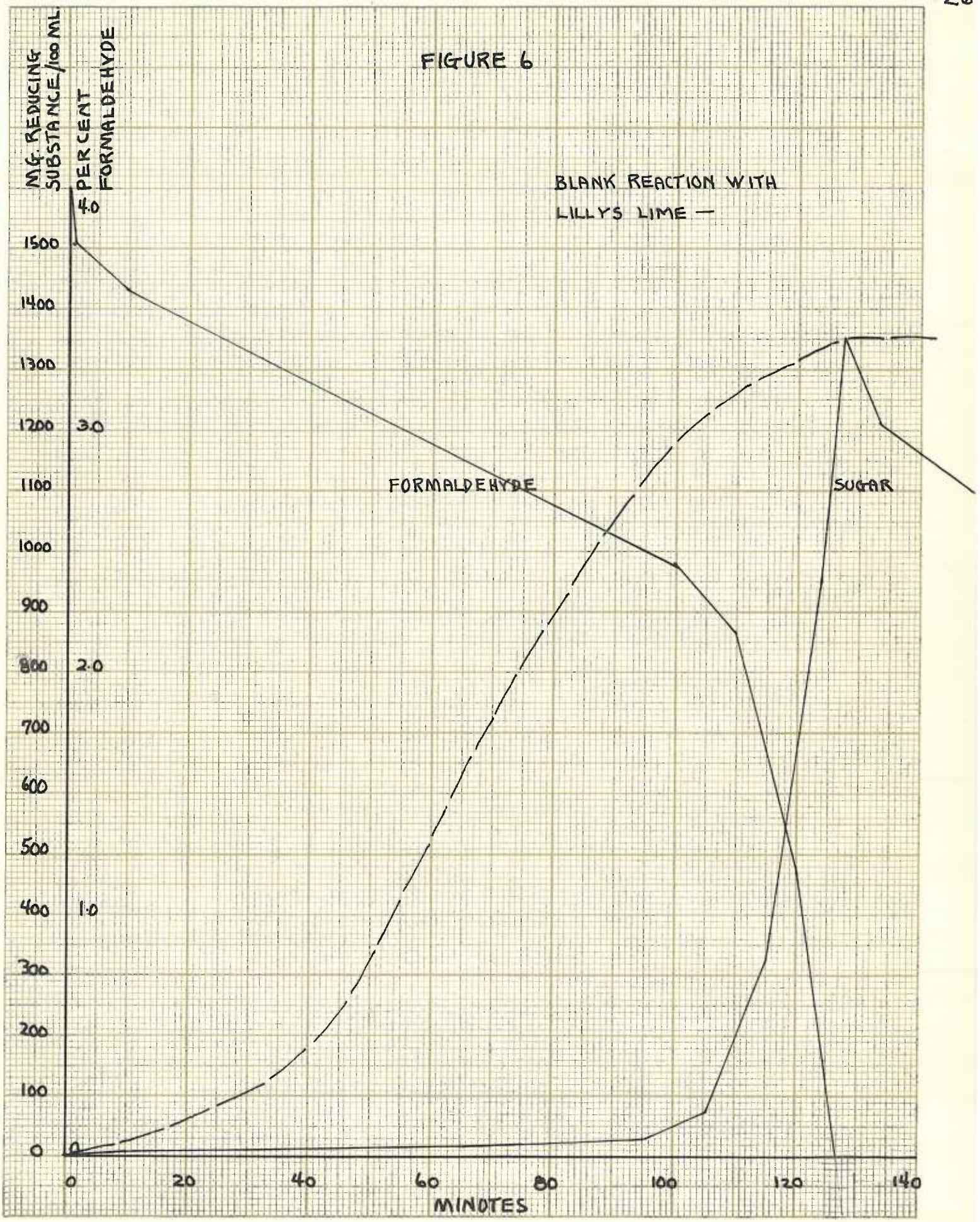
Time	Mg. Reducing Substance Given By		
	Precipitation	Extraction	No Treatment
5	30.0	12.9	0.0
10	------	29.2	10.0
30	11.0	------	12.0
65	------	27.0	17.0
90	30.0	281.0	28.0
120	230.0	737.0	542.0
127	206.0	926.6	1560.0
140	168.0	696.0	1048.0

Data and Discussion

Quantitative Aspects of the Problem. Previous to the work of Orthmer and Gerish[9] there had been little quantitative study of the condensation reaction. These workers, however, followed the reaction by determining reducing substances formed in the condensation, as well as by methods previously described. Their sugar determinations showed the appearance of gradually increasing amounts of reducing substances until a maximum had been reached. After this point reducing values decreased. Since the conditions of our work were quite different from those used by these workers, we also made quantitative studies of the condensation. We have studied not only reactions involving the condensation of pure formaldehyde, but also those catalyzed by various molecules.

Figure VI shows a comparison of formaldehyde and reducing substances determined on a blank reaction. On this graph one set of figures on the ordinate, represents the concentration of reducing substances in glucose equivalents while the other represents the concentration of formaldehyde. The abscissa shows the times at which samples were withdrawn for analysis, the zero point being the time at which the calcium hydroxide was added to the formaldehyde. It is seen that shortly after the lime was added, the formaldehyde concentration fell rapidly to about 3.5 per cent. A portion of this decrease

FIGURE 6


MG. REDUCING SUBSTANCE/100 ML
PER CENT FORMALDEHYDE
4.0
3.0
2.0
1.0
0
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
20
40
60
80
100
120
140
MINUTES
BLANK REACTION WITH LILLYS LIME —
FORMALDEHYDE
SUGAR

may be due to the Cannizzaro reaction in which some of the formaldehyde is changed to methyl alcohol and formic acid.

After this initial drop, the concentration falls more slowly until it reaches a point some 20-30 minutes before the end point. The curve now falls away sharply until the concentration is zero. It is interesting to notice that the formaldehyde concentration is reduced to almost half its initial value before any appreciable amounts of sugars are formed. As the formaldehyde enters into this phase of rapid condensation, reducing substances appear in appreciable amounts. The long induction period as shown by the slowly rising sugar curve, would seem to indicate that no appreciable condensation is taking place, or it may mean that condensation products are being formed that do not reduce the reagents used for their determination. When reducing sugars have risen to a certain level, the reaction seems to become autocatalytic for the formaldehyde is now quickly condensed and sugar values rise until maximum is reached. At this point the color change previously described, occurs. After this, the sugar curve is lowered rather steeply for a while, but later levels off. It is probable that during the preliminary phase of the reaction, there is a slow production of molecules which can enolize and serve as catalysts to speed the reaction. In such a manner the products of the condensation progressively exert increasing catalytic effect and toward the end cause it to proceed at a very rapid rate. The sharp decrease in both hot and cold reduction after the yellow end point must

be attributed to further changes in the condensation products. The fact that the maximum concentration of small sugar molecules (dihydroxy acetone, glyceric aldehyde and glycolaldehyde) as shown by cold reduction occurs sometime before the hot reduction maximum suggests that condensation of the smaller molecules to larger ones occurs during the interval between cold and hot reduction maxima. Orthmer and Gerish[9] using lead hydroxide as a condensing agent and a reaction temperature of 100 degrees Centigrade obtained a sugar curve different from ours in several respects. The broken line curve on figure VI approximates the determinations made by these workers. Their reaction appears to have a shorter induction period and a slower rise in reducing values. Orthmer and Gerish ran no formaldehyde curves. These workers have suggested that the reducing values represented on the lower part of their curve are due to the presence of the primary products of the condensation, namely, glycolaldehyde and dihydroxyacetone. They found it necessary to use strongly alkaline copper reagents for estimating these substances by their cold reducing power. Cold reduction determinations made by us with Soxhlet's reagent which was also used by Orthmer and Gerish gave results comparable to theirs.

The table below lists hot and cold reduction values for a blank reaction and for one catalysed by 0.27 millimoles of glucose. Since the hot and cold determinations were made with different reagents, for one of which (cold reduction) we have no glucose conversion factor,

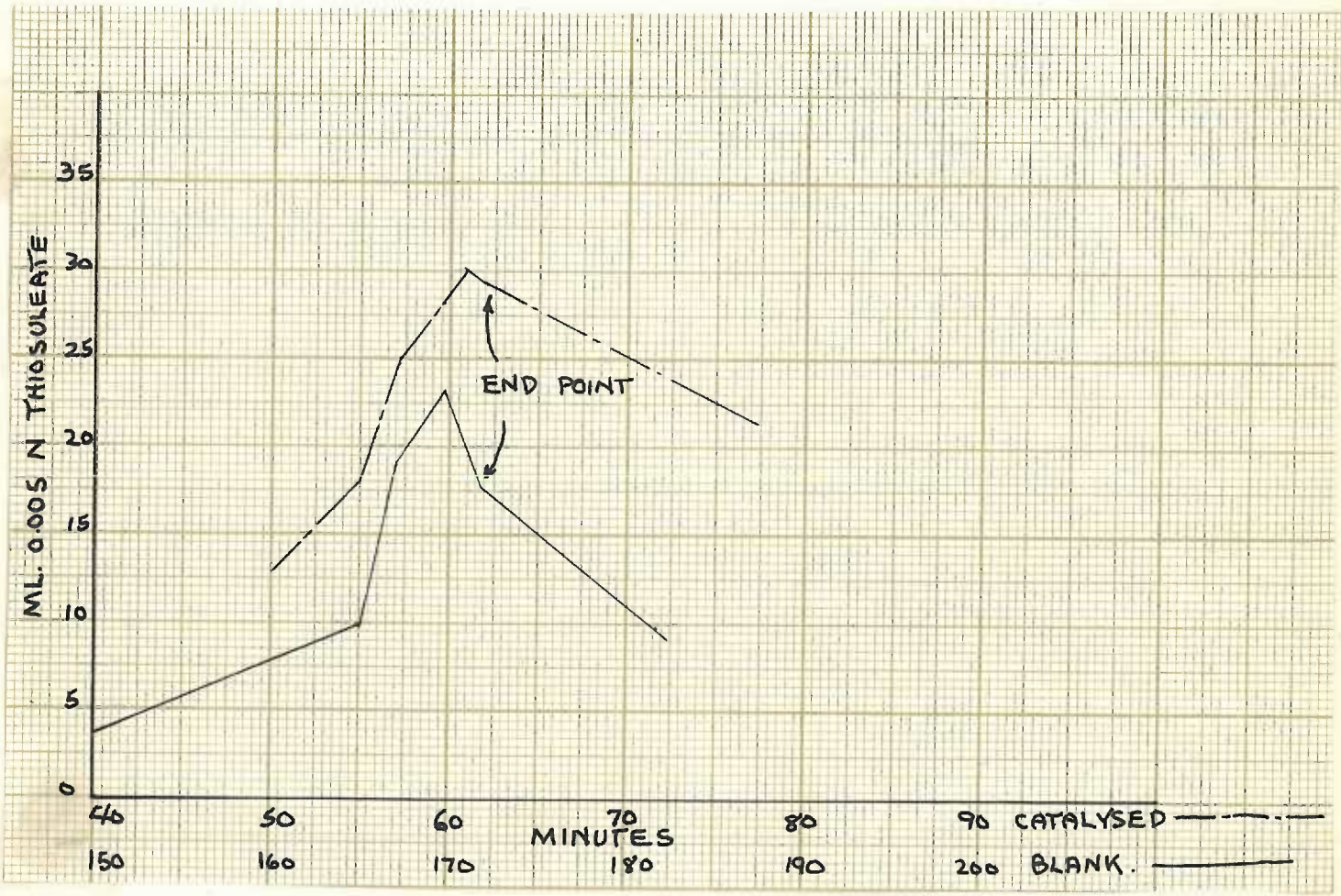
the values reported are in terms of milliliters of 0.005 N thiosulfate. Because of this, the figures should be compared only in a qualitative manner.

Table VI

Time	Blank Reaction Reduction in ml. 0.005 N thiosulfate Hot	Cold	Time	Catalysed Reaction Reduction in ml. 0.005 N thiosulfate Hot	Cold
0	0	0	0	0	0
30	0	0	30	2.0	0
60	0	0	45	3.3	3.0
75	0.3	0	50	5.7	15.5
120	0.4	0	55	----	18.0
135	0.45	1.0	57	6.6	24.5
150	0.50	4.0	59	9.5	26.0
165	8.12	10.0	61	11.8	30.0
167	----	10.0	63	14.1 End Point	29.0
169	10.80	23.0	65	15.1	27.0
172	11.40 End Point	16.0	75	10.8	25.0
180	8.75	13.0			

Mallinckrodt's lime and Merck's formaldehyde used.

The cold reduction relations in table VII are better shown by the curves below:



It is apparent that whatever condensation products are responsible for the cold reduction, they reach a maximum concentration before the color end point. It is possible that the condensation of these smaller molecules into larger ones is responsible for the continued rise of the hot reduction curve.

Reducing substances determined on catalysed reactions, show curves quite similar to that of the blank reaction. A series of typical curves showing variations in formaldehyde and sugar during catalysed condensation reactions is shown in figures VII - XI inclusive. In all cases, however, the curve is shifted toward the zero point because of the shortening of the time of the end point by the catalyst. It is easily seen that both the formaldehyde and sugar curves are of the same general shape, in all cases the formaldehyde concentration dropping off sharply as the sugar concentration rises.

Although the sugar curves have the same slope, the height to which they go varies. Column A of Table VIII below gives the yellow end point times for a series of condensation catalysts, while column B shows reducing values at this point. As noted above, the reducing values are maximal at the end point.

Table VIII

Catalyst (0.11 M./100)	A End Point	B Mg. Reducing Sugars
Sorbose	56	1913
Reductone	62	1769
Levulose	67	1697
Galactose	70	1565
Maltose	73	1536
Glucose	78	1547
Mannose	79	1455
Cellobiose	84	1136
Mannoketoheptose	85	1600
Glucoheptose	85	1676
Mannoheptose	86	1600
Lactose	92	1441
Ascorbic Acid	98	1564
Galaheptose	100	1508
Blank	130	1350

FIGURE 7

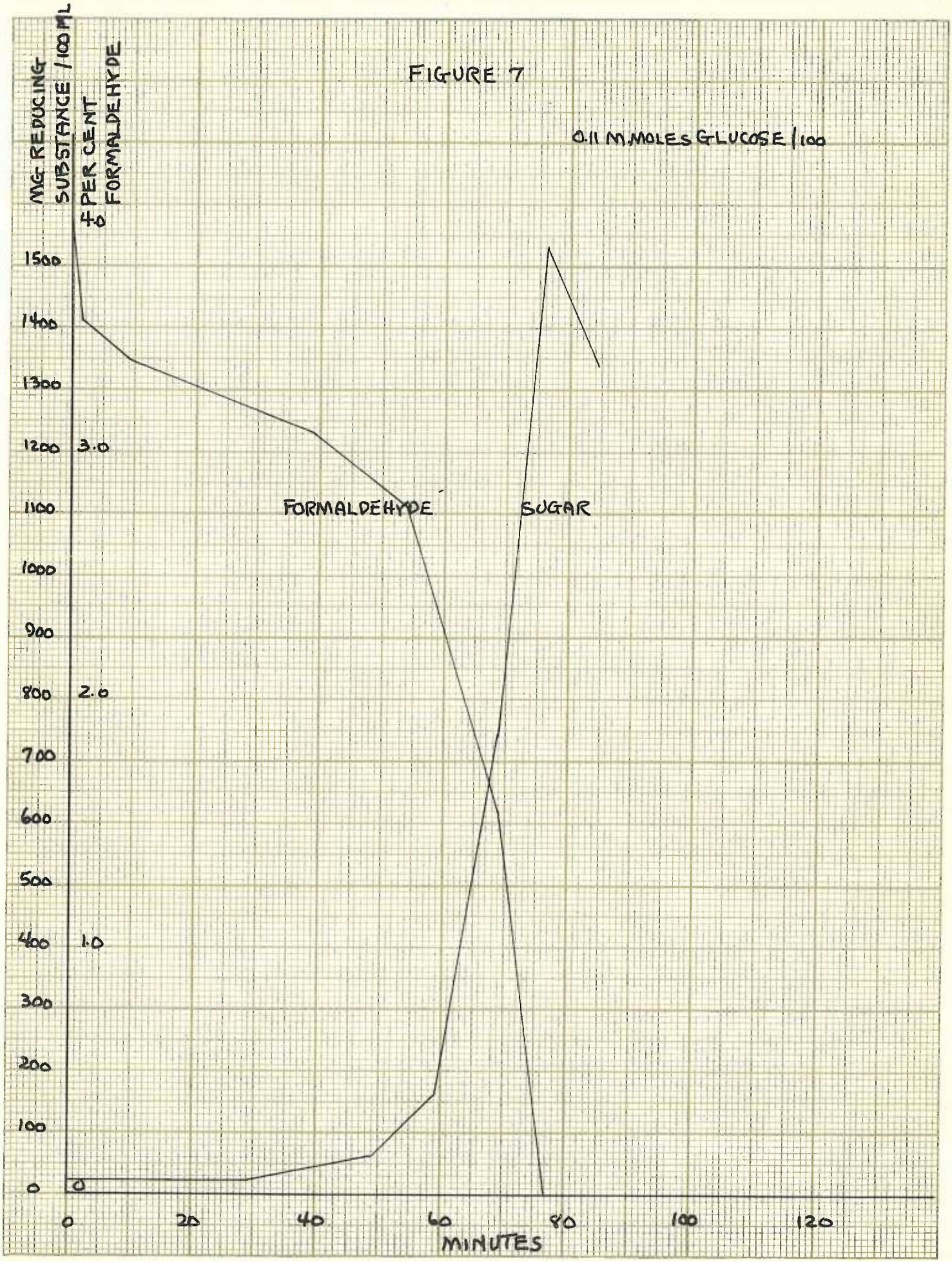

MG REDUCING SUBSTANCE / 100 ML
PER CENT FORMALDEHYDE
0.11 M. MOLES GLUCOSE / 100
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
3.0
2.0
1.0
FORMALDEHYDE
SUGAR
0
20
40
60
80
100
120
MINUTES

FIGURE 8

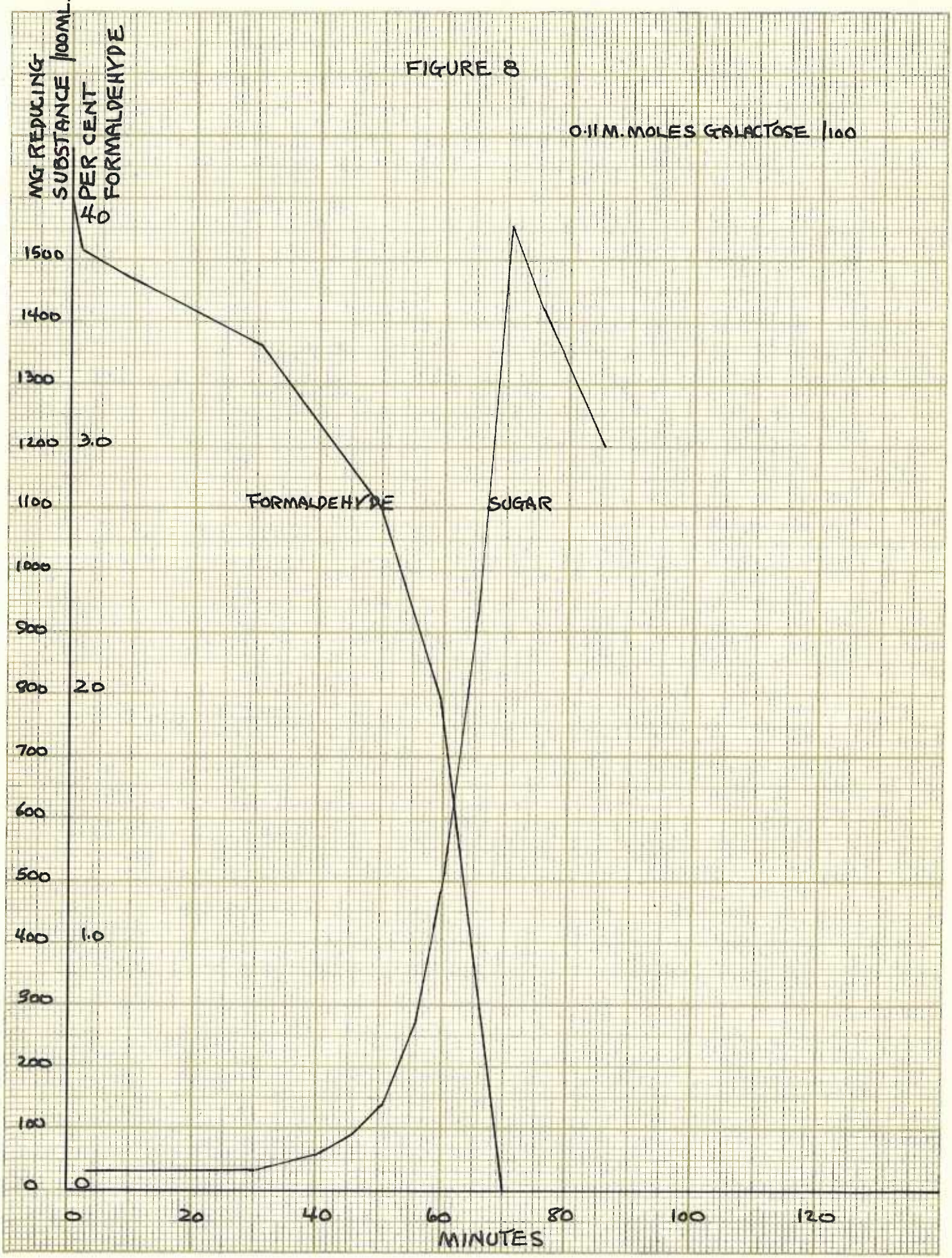
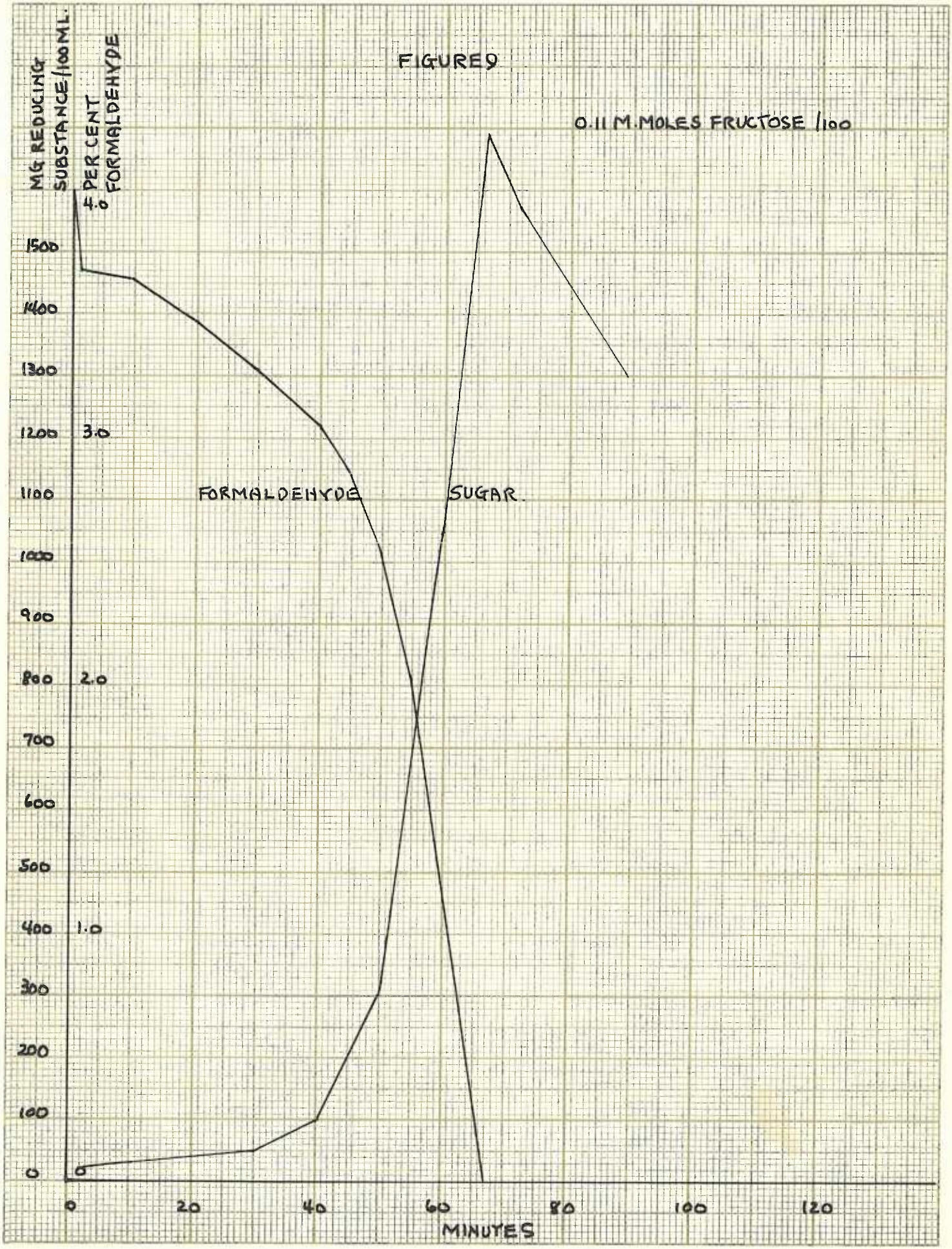

MG REDUCING SUBSTANCE /100ML.
PER CENT FORMALDEHYDE
0.11 M. MOLES GALACTOSE /100
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
4.0
3.0
2.0
1.0
0
FORMALDEHYDE
SUGAR
0
20
40
60
80
100
120
MINUTES

FIGURE 9


MG REDUCING SUBSTANCE/100 ML.
PER CENT FORMALDEHYDE
0.11 M. MOLES FRUCTOSE /100
FORMALDEHYDE
SUGAR
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
4.0
3.0
2.0
1.0
0
0
20
40
60
80
100
120
MINUTES

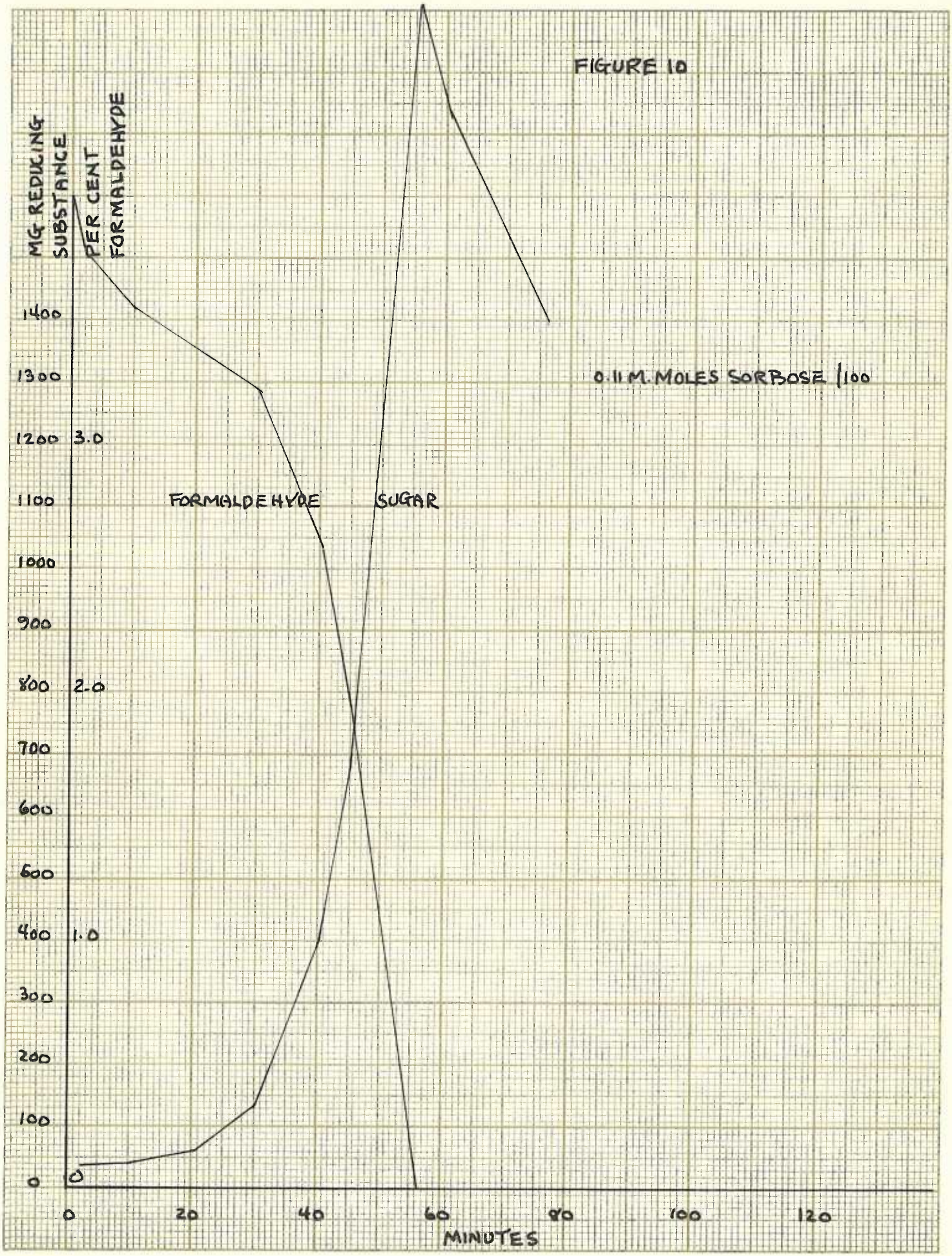
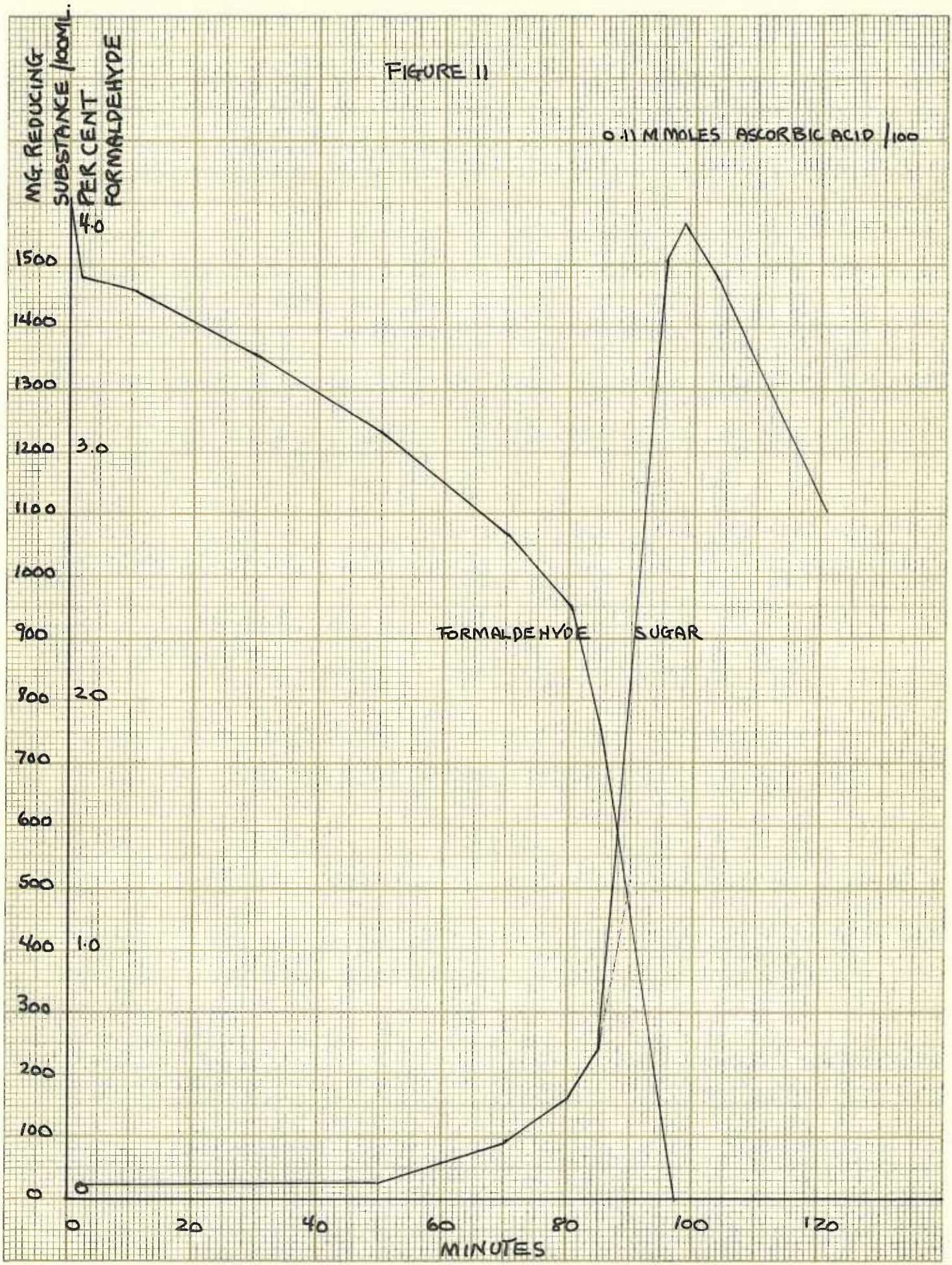


FIGURE 10
MG. REDUCING SUBSTANCE
PER CENT FORMALDEHYDE
0.11 M. MOLES SORBOSE /100
FORMALDEHYDE
SUGAR
1400
1300
1200
1100
1000
900
800
700
600
600
400
300
200
100
0
3.0
2.0
1.0
0
0
20
40
60
80
100
120
MINUTES

FIGURE 11


MG. REDUCING SUBSTANCE /100ML.
PER CENT FORMALDEHYDE
0.11 M MOLES ASCORBIC ACID /100
FORMALDEHYDE
SUGAR
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
4.0
3.0
2.0
1.0
0
0
20
40
60
80
100
120
MINUTES

It is evident that in certain cases there is a relationship between the time of catalysis and the amount of sugars formed. Since this seems to apply to only about half of the compounds investigated it is difficult to explain such a relationship. If in a series of reactions, the kind of catalyst is kept constant, and the time of condensation shortened by using progressively larger amounts of catalyst, results are obtained that are comparable to those in table IX.

Table IX

Millimoles Glucose per 100 ml.	Time	Mg. Sugar per 100 ml.
0.27	65	1638
0.11	78	1547
0 (blank)	130	1350

Although the relation of the color end point to the formation of a maximum amount of reducing substances has been discussed, it should be kept in mind that such a point is not reached until the formaldehyde has been completely condensed. The dependence of the color end point upon this minimum concentration of formaldehyde is shown by the material to follow.

Under the conditions of our work, 0.27 millimoles of glucose causes 100 milliliter of formaldehyde to condense in sixty-four minutes. For this study, three condensation reactions were started, each of the

three tubes containing 100 milliliters of formaldehyde, four grams of lime and 0.27 millimoles of glucose. All the tubes should have shown an end point in 64 minutes. To the first tube five milliliters of four per cent formaldehyde was added four minutes before the end point. Such treatment delayed the end point two minutes. Five milliliters of four per cent formaldehyde was added to the second tube just after the end point, causing a fading of the yellow color. Another end point, however, appeared ten minutes later. The third tube was treated with a like amount of formaldehyde just as the end point appeared. In this case, the end point was delayed until four minutes later. The above experiment is tabulated below.

	End Point
1. 5 ml. HCHO added at 60 minutes	66 minutes
2. 5 ml. HCHO added at 65 minutes	74 minutes
3. 5 ml. HCHO added at 64 minutes	68 minutes

The reagent added to tube number one was added at a time when the condensation mechanisms were very active, the excess reagent being used up in two minutes. Tube number three shows that at the end point the active process had begun to slow down, the excess formaldehyde requiring four minutes for condensation. This fact is substantiated by tube two to which the formaldehyde was added after the color change. In this case, ten minutes were required to condense the excess formaldehyde.

The observation, that during a certain stage of the reaction,

formaldehyde is very effectively condensed, led to certain special studies to be discussed in a section of this thesis entitled "Preparation and Properties of the Condensation Syrup."

Catalysis of the Condensation. Schmalfuss[10] discovered that certain sugars catalyse the formaldehyde condensation. Kusin later showed that compounds having an enediol group or capable of forming this group by the tautomeric shift of a hydrogen atom are catalysts of the condensation. In an attempt to better understand the relationship between molecular structure and catalytic function, we have investigated the relative activities of a number of compounds. In table X, the amount of compound used as catalyst in the condensation of fifty milliliters of four per cent formaldehyde by two grams of lime, is listed at the head of each column of figures. The time represents the number of minutes elapsed from the moment at which the lime was added to the formaldehyde, until a color and point appeared. Before the lime was added, the catalyst was dissolved in the formaldehyde, and the solution warmed in the water bath to the reaction temperature (40 degrees Centigrade). The catalysts have been arranged in the order of activity, as judged by their action at the highest concentrations. In this work, an attempt was made to use compounds of high purity. Several of the substances were prepared in our laboratory. Some of these preparations are referred to below.

Table X

	Milligrams per 100 ml.	1000	800	600	400	200	100	50	20	10	4	2	1
	Millimoles per 100 ml.	5.55	4.44	3.32	2.22	1.11	0.55	0.27	0.11	0.055	0.022	0.011	0.001
	Catalyst	Time in Minutes											
1	l Sorbose	12		16		25		34	45	45	60	65	81
2	Reductone	19	20	21	25	27	33	38	46	58	72	85	
3	Levulose	22	26	26	31	37	40	40	49	60	69	81	89
4	Glycolaldehyde	24	26	30	32	36	41	57	65	72	98	116	127
5	Xylose	25	25	28	31	36	43	51	66	73	87	99	111
6	d Arabinose	29	29	31	36	41	47	57	69	79	95	106	122
7	l Arabinose	29	29	34	42	38	56		67	75	91	102	120
8	α d Glucoheptose			32	35	38			65	73	89	103	114
9	d Galactose	32	32	33	34	38		51	68	77	91	102	115
10	d Glucose	30	30	30	42	47	65	63	79	88		110	
11	d Mannose	36	42	42	44	48	52	76	84	93	108	124	131
12	Maltose	38	38	38	42	46	51	86	68	77	90	106	117
13	Ascorbic acid	40	40	40	40			54	75	85	103	125	136
14	α d Mannoheptose			46		52		61	77	86	102	114	128
15	Glucurone	40											
16	Glyoxal	40	45	47	56	69	92						
17	Calcium Gluconate	45		44				96					
18	Kojic acid	42	42	45	49	56	67						
19	α d Mannoketo-heptose			51		56		62	68	74	99	107	121
20	Galacturonic acid	45			46			56					
21	Glucose amine	45	47	52	58	67	73						
22	Cellobiose	46	46	50	63	59	65	73	84	101	115	124	135
23	Lactose	47	47	49	55	59	66	70	80	98	109	121	129
24	Calcium Mannonate	58			65			91					
25	Cystine	80				95							
26	Erythronic acid lactone	72	78	79	88	116	129						
27	Calcium Galactonate*	74			73			88					
28	2,3 Di-OH Butyric acid lactone*	76	84	89	99	110	122						
29	Galaheptose												
30	Aspartic acid	80				95							

Table X
(Continued)

	Catalyst	Time in Minutes											
Milligrams per 100 ml.		1000	800	600	400	200	100	50	20	10	4	2	1
Millimoles per 100 ml.		5.55	4.44	3.32	2.22	1.11	0.55	0.27	0.11	0.055	0.033	0.011	0.001
31	Mucic acid	84			84			84					
32	Maleic acid*	98				116							
33	Erythro 1,2 di-OH butyric acid	93	99	108	113	128	135						
34	Glycollic acid	94		106		115							
35	Succinic Acid	93				112							
36	1,3 di-OH butyric acid lactone	98	107	112	118	129	182						
37	Cinnamaldehyde	99											
38	N-tetra methyl glucose	99				136							
39	α methyl glucose	100		109			157						
40	Calcium Lactate	102			129		169						
41	Sucrose	103		116			166						
42	Three 1-2 di-OH butyric acid	108		107	111	121	130						
43	Mandelic acid	108				131							
44	Acetyl acetone	106					126						
45	Lactic acid	107											
46	Glutaric acid	110				135							
47	Glycine	120											
48	Glycerine	129			142		149						
49	Phthalic acid	130											
50	Calcium levulinate	135		146			165						
51	Calcium Propionate	138	138	136	139								
52	Salicilic acid	155				112							
53	Benzoic acid	156											
54	Tartaric acid	138	138	140		137	140						
55	Calcium butyrate	158		155		139							
56	Hippuric acid	159											
57	Acetic acid	160		156		156							
58	Citric acid	169											

*Lilly's Line

Hydroxy methelene glycolaldehyde (reductone) was prepared by the method of Euler and Martius[14]. The behavior of this and other compounds in alkaline solution, will be discussed later.

Glyoxal prepared by a method described by Houben was almost identical in properties, with a commercial sample (Kahlbaum).

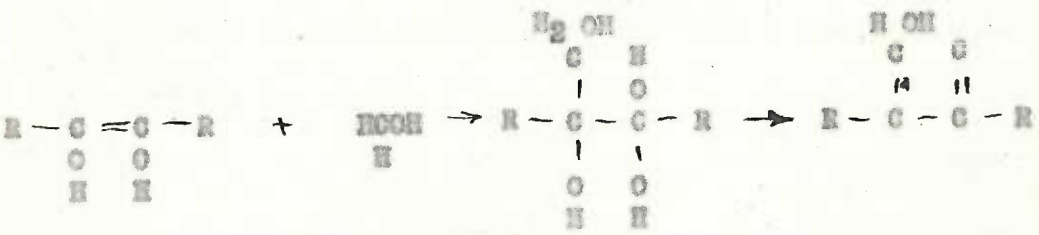
Glycolaldehyde was prepared from dihydroxymaleic acid as described by Fenton[16]. When dihydroxymaleic acid is heated in a water solution to 60 degrees Centigrade, an equivalent amount of glycolaldehyde is formed. Standard solutions of the acid were made up and heated to yield the glycolaldehyde, which was then added to the condensation tubes from a micro burette.

Aldonic acids were prepared by the electrolytic method of Isbell[17]. In this procedure, the appropriate aldose sugar is oxidised to the corresponding acid, by hypobromite ion formed when a current is passed through the solution of the sugar and suitable bromide salt. This method eliminates the use of excessive amounts of bromine, a serious fault of earlier procedures. Repeated recrystallisations of aldonic acids prepared by this method gave compounds of a high degree of purity.

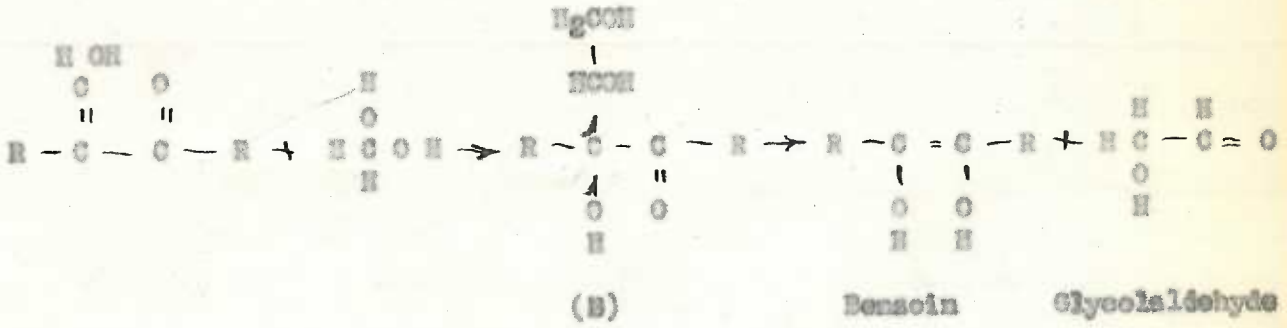
Galacturonic acid and glucuronic acid were furnished by certain other experiments in which the writer was actively interested. The galacturonic acid was prepared from pectin by enzyme hydrolysis and the glucuronic acid from borneol glucuronate according to Quick[18].

We are grateful to Dr. C. S. Hudson of the National Institute of Health for the samples of heptose sugars, to Dr. J. W. E. Glattfeld of the University of Chicago for the hydroxybutyric acids and to Dr. D. L. Smith of Kansas State College for the kojic acid. ~~he furnished.~~

According to Rusin, a substance acts as a catalyst in the formaldehyde condensation because it forms intermediate products with first one and then two molecules of formaldehyde. In his scheme, the following changes are postulated:



Enol form of Benzoin — Formaldehyde (hydrated) — Known addition compound (A)



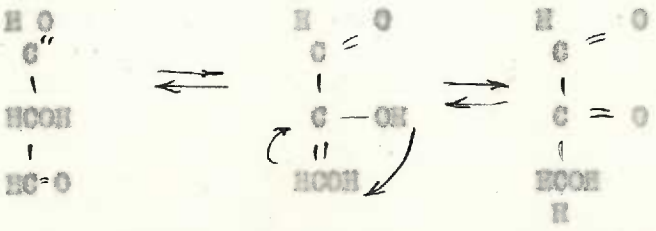
(B) — Benzoin — Glycolaldehyde

If catalysis is effected according to such a scheme, it would seem likely that those compounds which most easily furnish true endiol groups would have the greatest activity. On the other hand, compounds with one or both of the hydroxyl groups replaced with less liable groups would

be considerably less active.

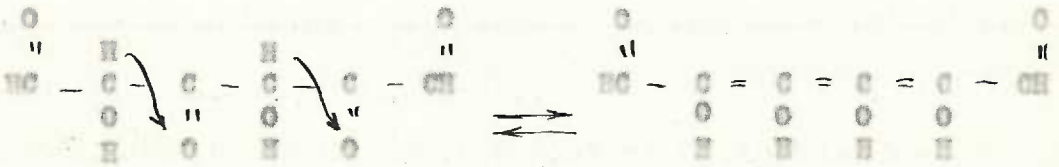
From a study of compounds listed in table X it is evident that they may be divided into three general groups. The first or most active group includes the first 23 molecules. Most of these are known to easily furnish endiol groups, many of the compounds being simple aldo ketone sugars. There are, however, several exceptions to the simple sugars included in the first group. Possible enol forms of these substances will be briefly discussed.

Reductone, as studied by Euler and Martius, may have any of the three following forms in solution.

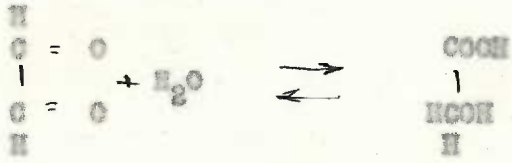


It is difficult to postulate a mechanism of catalysis for a compound known to have such a variety of forms. However, in alkaline solution the endiol form should be favored. Another molecule in this first group that may exist in several forms is glyoxal. In alkaline solution, the compound may polymerise to a trimeric or polymeric form. The trimeric form does not reduce Fehling reagent but does give a silver mirror with ammonical silver solutions. The polymeric form on the other hand does reduce Fehling's solutions. The glyoxal which we prepared

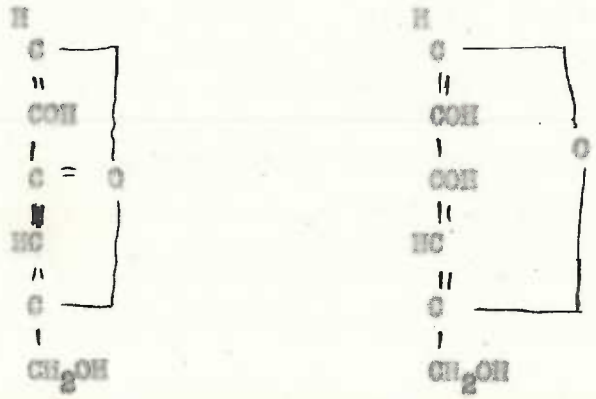
by oxidizing paraldehyde, gave both reactions, indicating that it was probably a mixture of the two. A possible structure for the trimeric molecule as suggested by Whitmore is:



The position of glyoxal in the list of catalysts seems to rule out the possibility of an appreciable concentration of any such enolic molecule. Glyoxal may possibly undergo a Cannizzaro reaction to give the less active glycollic acid as:



The kojic acid molecule has a structure that gives it a unique form when and if it enolizes.



Kojic acid

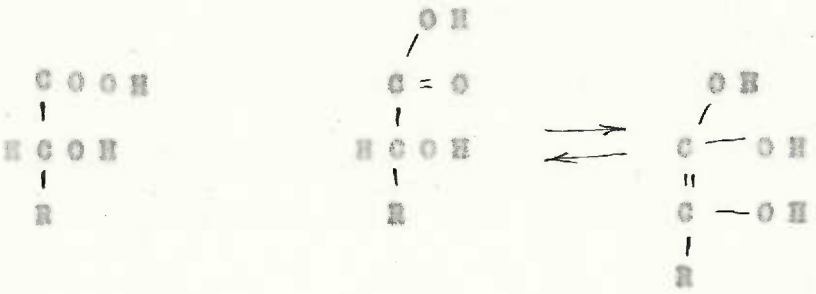
Enol form

Although the above enolisation would not seem to fit into the theory postulated by Kuzin, the compound is rated among the most rapid catalysts. It is possible that under the influence of the strong alkali further enolisation furnishes more reactive groups.

Ascorbic acid, having an endiol structure even in acid solution would at first seem to be an ideal catalyst. Its position in the list does not bear this out. Destruction of the acid would be expected to occur under the conditions of the experiment, thereby lowering its effective catalytic activity. This possibility seems of less moment when the relative activities of various concentrations of ascorbic acid are considered. Glucosamine, through enolisation, should furnish an amino-enol:

Although this structure does not fit well into Kuzin's scheme, it is possible that the continued action of alkali upon the catalyst causes a wandering of the double bond down the chain and the production of an endiol structure. Of course the amino-enol structure may act directly as a catalyst.

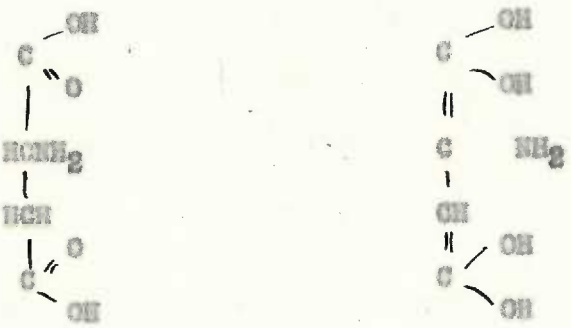
The second group of catalysts begins with calcium mannonate and includes the next nineteen compounds, down to and including gluteric acid. The majority of these molecules are acids with a hydroxyl group adjoining the carboxyl groups. Such compounds in alkaline solution might furnish endiols by the enolization shown below



In the condensation reactions such acids would be combined with dibasic calcium, the enolization then furnishing two endiols per molecule of salt:

It should be pointed out that calcium gluconate presents anomalous behavior in that its catalytic activity places it in the group of most active catalysts. Apparently the configuration of this compound favors enolization to a greater extent than so far observed in other sugar acids.

Although the amino acid, aspartic acid, is known to form an insoluble compound with calcium, its function as a catalyst seems to indicate that it is not completely precipitated under the conditions of our experiment. This compound might enolize as an enol and an amine-enol at the same time:



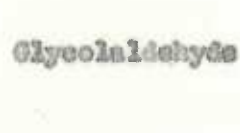
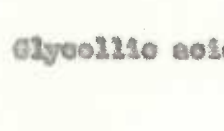
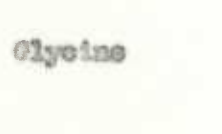
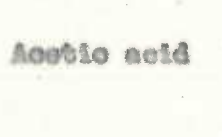
The third division of table X includes a number of structures, none of which exert any appreciable catalytic effect. As has been shown previously, certain of these molecules (hydroxy acids) are theoretically able to furnish endiol groups. Their inability to appreciably catalyze the condensation may possibly be a measure of the extent of their enolization. Other compounds in this group have potential enol groups, these also are quite inert. Certain of these molecules form only partially soluble salts with calcium — a fact which might seriously interfere with the compound functioning as a catalyst.

A more definite relationship between structure and catalytic activity is shown when related molecular structures are compared. Such a comparison is given in tables XI - XIII, in which certain of the

catalysts are grouped together to contrast related configurations:

Table XI

CATALYSTS WITH TWO CARBON ATOMS

Catalyst	Structure	Milli-moles Catalyst 5.55	1.11
Glycolaldehyde		24 minutes	36 minutes
Glyoxal		40 minutes	69 minutes
Glycollic acid		94 minutes	115 minutes
Glycine		120 minutes	----------
Acetic acid		150 minutes	156 minutes

Compounds having four carbon atoms are shown in table XII. Aside from the fact that practically all these compounds are only slightly active, the ten variations in structure enable us to draw certain conclusions relative to the activity of these catalysts.

Table XII

CATALYSTS WITH FOUR CARBONS PER MOLECULE

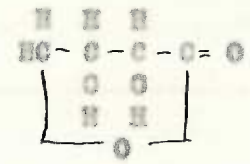
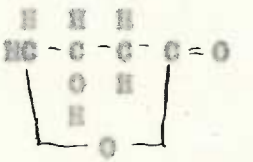
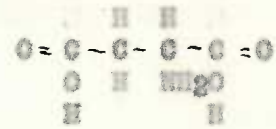
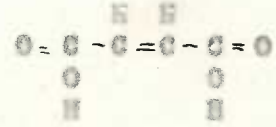
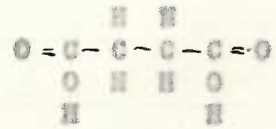
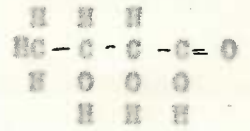
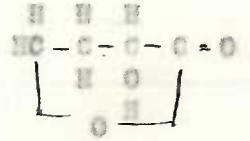
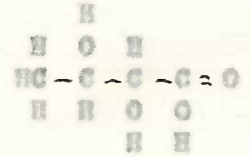
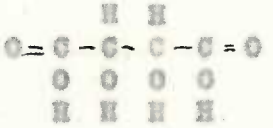
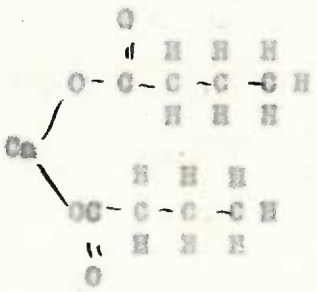
Catalyst	Structure	Milli-moles catalyst 8.66	1.11
Erythronic acid lactone		72 minutes	118 minutes
2,3 di OH butyric acid lactone		76 minutes	110 minutes
Aspartic acid		80 minutes	98 minutes
Maleic acid		88 minutes	116 minutes
Succinic acid		93 minutes	112 minutes
Erythro 1,2 di OH butyric acid		93 minutes	126 minutes
1,3 di OH butyric acid lactone		96 minutes	120 minutes
Threo 1,2 di OH butyric acid		103 minutes	121 minutes

Table XII
(Continued)

Catalyst	Structure	Milli-moles catalyst 8.56	Milli-moles catalyst 1.11
Tartaric acid		133 minutes	137 minutes
Calcium betyrate		136 minutes	139 minutes

Part one of table XIII compares various glucose derivatives while part two compares galactose compounds and the last part, mannose derivatives:

Table XIII

CATALYSTS WITH SIX CARBONS PER MOLECULE

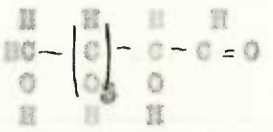
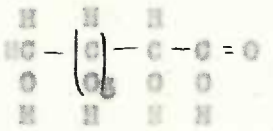
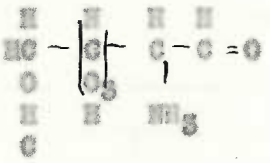
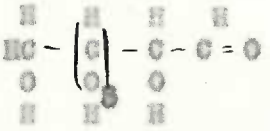
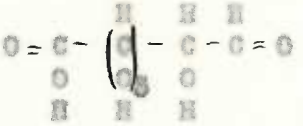
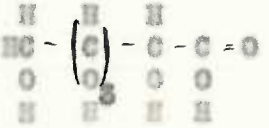
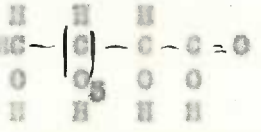
Catalyst	Structure	Milli-moles catalyst 8.56	Milli-moles catalyst 2.22
Glucose		32 minutes	34 minutes
Gluconic acid		43 minutes	47 minutes

Table XIII
(Continued)

Catalyst	Structure	Milli-moles catalyst 5.55	Milli-moles catalyst 2.22
Glucosamine		45 minutes	58 minutes
N-Tetra methyl glucose		99 minutes	----------
Alpha methyl glucose		100 minutes	----------
Galactose		52 minutes	54 minutes
Galacturonic acid		45 minutes	46 minutes
Galactonic acid		74 minutes	73 minutes
Mucic acid		84 minutes	84 minutes
Mannose		36 minutes	44 minutes
Mannonic acid		56 minutes	68 minutes

It should be noticed that of the three aldohexose sugars listed above, two are almost identical in activity.

The Effect of Temperature Upon the Reaction. The effect of temperature on the condensation reactions is hown by the following results obtained in determinations carried out at different temperatures.

Temperature	Time of blank reaction
30° C.	600 minutes
40° C.	170 minutes
50° C.	55 minutes
60° C.	25 minutes

This striking relationship between time of condensation and temperature is also shown by catalysed reactions.

Millimoles of catalyst per 100 ml.		5.55	4.44	3.32	2.22	1.11	0.56	0.27	0.11	0.05	0.02	0.01	0.001
		Time in minutes											
Glucose	30°	167											
	40°	55	38	39	42	47	65	63	78	88		116	
	50°	11	11	11	13	17		21	28	34	38	41	44
	60°	3	4	4	4	5		8	10	11	14	14	16
Fructose	30°	132											
	40°	22	26	26	31	37		40	40	60	69	81	89
	50°	7	7	8	9	10		14	17	20	24	28	32
	60°	2	2	2	2	3		5	6	7	9	11	13
l-Arabinose	40°	20											
	60°	3											
Calcium Butyrate	40°	133											
	50°	48											

It is evident that with the use of an active catalyst such as fructose, and a reaction temperature of 80 degrees Centigrade, the condensation is extremely rapid. Because of a relationship between the time of condensation and the amount of reducing substances formed that has already been mentioned, sugar determinations were made on a blank reaction at 60 degrees Centigrade.

Temperature	Maximum reduction of blank
40° C.	1350 mg.
60° C.	1400 mg.

The small difference shown above is well within the limits of experimental error.

Conclusions

In the section of this thesis entitled "Standardisation of Reagents and Conditions", it was established that the kind, brand and concentration of reagents used in the formaldehyde condensation are important variables. In the case of the formaldehyde solutions, it is difficult to set up any absolute standard of comparison. One brand of reagent may be more easily condensed because of an impurity that exerts a positive catalytic effect. Another brand may have an impurity that tends to slow down the condensation. Because of such an unpredictable variation in the reagent, it is suggested that future work done on this problem should be carried out with reagents that are standardized in some way before use.

The lime used in the condensation is another variable but this reagent is more easily standardised by comparison with a known pure lime. In this connection, it is recommended that spectrographic studies be made on such limes. A number of such studies have been made on the limes discussed in an earlier sections however, the results are not as yet complete enough to be of much value. We are grateful to Dr. J. Beeman for this help in the preparation of spectroscopic slides and their interpretation.

Because of the facts that have been brought out in regard to the purity of the reagents used, we conclude that in the condensation the

presence of certain impurities either organic or inorganic may markedly affect the reaction rate and any attempt to correlate contributing data must take these things into consideration. In none of the published reports of work done on the problem has this phase been studied.

The products of the condensation, studied largely by their reducing power, appear to be sugars that as yet have not been crystallised. The relationship between the maximum amount of sugars formed, and the disappearance of the formaldehyde at what we call the color end point has been pointed out. The significance of such a color end point has not been noted by other workers, due presumably to the kinds of reagents used. Orthmer and Gerish, in their quantitative studies, used a colored condensing agent ($Pb(OH)_2$). Certain studies made on a syrup isolated from the condensation mixture are discussed in the concluding section of this thesis. For a more complete understanding of the condensation reaction, a comprehensive study of the sugars formed should be carried out. The catalytic hydrogenation method of Orthmer and Gerish seems to be ideally suited to such a purpose.

The studies made on the catalysis of the condensation seem to substantiate the general observation of Kusin that the endiol group catalyses the condensation. From out experiments we have found that certain other groups may possibly catalyse the reaction, although most of the molecules concerned are theoretically able to furnish the endiol

group. For example, it seems that glucosamine probably yields an amine-enol group under the conditions of our work and that this functions as the catalytic center. Kojic acid catalyzes the reaction, but it is theoretically difficult to understand how it may produce a true endiol group. The most reactive molecular structure seems to be that of an endiol formed from the enolization of an aldose or ketose sugar, and the most reactive of such sugars are those that have from two to six carbon atoms per molecule. The molecular grouping that is second in order of activity is furnished by the sugars of the first group in which the carbon atom at the opposite end from the aldehyde group has been oxidized to an acid group, yielding a uronic acid. The acids formed when the aldehyde group of a sugar is oxidized are next in order of activity. In this connection the anomalous activity of gluconic acid as compared with mannonic and galactonic acids is unique. Gluconic acid is peculiarly more active as a catalyst than is galacturonic. As has been previously pointed out many other molecules in table X have hydroxyl groups adjacent to the carboxyl group. Their activity (usually less than the sugar acids) may be a measure of their ability to enolize. In this connection, the configuration of the hydroxy butyric acids, presents an interesting case. These compounds show varying activities which are undoubtedly related to specific configurations, yet our present knowledge is insufficient for profitable speculation. It is interesting, for example, that the lactone of erythronic acid is considerably more active than the acid. Erythro-dihydroxy butyric

acid is more active in the higher concentrations than is 1,2 three-dihydroxy butyric acid.

When one studies the catalytic rates of various substances at concentrations below 0.27 millimoles per 100 milliliters, it is observed that the order of activity may be reversed. Especially in the case of the ketoses, points of inflection in the concentration catalytic time curves are generally very noticeable. The explanation for this phenomenon is unknown. The over all catalytic effect in the formaldehyde condensation seems to be referable to a few characteristic groupings, yet there are modifying effects exerted by a variety of structures which enter into the total catalysis.

Preparation and Properties of the Condensation Syrup.

Since the condensation of formaldehyde is brought about by the presence of an alkaline condensing agent, in our work calcium hydroxide, it should be possible to interrupt the condensation by removing the lime from the solution. Oxalic acid makes an ideal reagent for this purpose. By adding oxalic acid in slight excess, the calcium is quantitatively removed and the solution is made slightly acid, a condition which tends to protect the sugars from destruction. To prepare a maximum amount of condensation product the reaction should be interrupted just as the reducing values reach their peak, at the end point. In such a preparation, if the oxalate is added too soon, the product will be contaminated with formaldehyde. On the other hand, if the oxalate is added after the end point when the solution has definitely changed color the product is contaminated by breakdown products. Although there may be a complex mixture of such products, we know of only one that would have deleterious physiological effects. -This product gives several tests characteristic of formate, namely: 1., a silver mirror is produced when an impure syrup is heated with $AgNO_3$; 2., mercuric chloride, when heated with formate, is reduced to the insoluble mercurous salt.

To obtain aproduct suitable for physiological work, the condensation reaction should be interrupted just as the first faint yellow color

appears. In order to remove formate, the solution must be made acid and the formic acid removed by evaporation and concentration.

It has been noted in an earlier section of this thesis that formaldehyde added to the condensation reaction a few minutes before the end point is quickly used up. Since it appeared probable that at this stage of the reaction there is a large number of active catalytic molecules present, a method of sugar preparation was worked out that took advantage of these catalytic molecules.

Although it is possible to get a syrup from the condensation of four per cent formaldehyde, the volume of solution and the time required for a number of such condensations, make it more desirable to proceed according to the following procedure. Two hundred milliliters of four per cent formaldehyde (Merck), eight grams lime (Mallinckrodt) and two hundred milligrams of glucose are placed in a liter round bottom flask. The flask is immersed in a water bath at 40 degrees Centigrade and the mixture stirred with a glass stirrer attached to an electric motor. Such a mixture is comparable to other reactions listed in this paper in which one hundred milliliters of formaldehyde are condensed by four grams lime and 0.27 millimoles of glucose, and should show an end point in 64 minutes. After 64 minutes, or just as the color appears, 100 milliliters of 38 - 40 per cent Merck formaldehyde is added. Four to five grams of lime is also added to maintain the proper proportions

of reagents. The appearance of the final end point or the disappearance of formaldehyde, as followed with dimedon, depends upon the exact moment the concentrated formaldehyde has been added. This end point, however, should appear within 30-45 minutes. Several times in preparing this syrup, the 40 per cent formaldehyde was added supposidly at just the proper moment, but condensation occured in from three to five minutes instead of 40 minutes. There is evidently a point in the condensation of the four per cent formaldehyde at which the reaction is very fast. When this point happens to be taken advantage of, the condensation is fast, but the syrup is unusable for the reaction turns a dark brown within a minute or two. Just as this color returns, an excess (22 grams) of oxalic acid, in solution, is quickly added. The mixture is now filtered and tested for formate. If the solution gives a positive formate test, it should be made definitely acid with oxalic or phosphoric acid and the volume of the solution reduced considerable by evaporation or vacuum distillation. With any such procedure the temperature of the solution must not be raised to more than 40 degrees Centigrade. If the formate is not removed by this first treatment, a liter or so of water should be added and the solution again evaporated -- preferably by blowing a strong blast of warmed air across the surface of the solution which is contained in a large flat dish. When the solution is free from formate, according to the mercuric chloride test, it is neutralised with $CaCO_3$, filtered and concentrated to a thick syrup. The syrup is now

taken up in 95 per cent alcohol to precipitate salts in solution. The alcohol solution is concentrated at a temperature not above 40 degrees Centigrade, preferably in a closed system free from dust particles. During the various steps of the procedure, care must be taken so that appreciable amounts of the syrup is not lost because of improper washing of precipitates, etc. A syrup prepared as above and thoroughly dried weighed 37.6 grams. A sample of this syrup was further dried in an Abderhalden dryer. By this treatment, it was found that there was still 1.7 per cent water in the large batch. Correction for this water gives 37 grams of dry syrup. Several analytical determinations were made on the syrup and the results are tabulated below.

Table XIV

Weight of syrup from 44 grams formaldehyde	37.0 grams
Ash	0.8 per cent
Reducing power compared to glucose	41.0 per cent
Average Molecular weight (freezing point)	180.0
Fermentation	Non-fermentable by yeast
Seliwanoff test	Ketoses
Phloroglucinol test	Pentoses

The glycogenic power of the syrup was tested on rats. Three groups of rats were used for this experiment, all of which were starved for 24 hours before use. One group was given syrup intraperitoneally, another group by stomach tube and the third group was not treated but was used as a control. The animals weregiven the syrup twice, once at 9:00 A. M.

and again at 11:45 A. M. The animals fed by stomach tube received a total of four milliliters of a 25 per cent solution of syrup. The other group received a total of eight milliliters of a 12.5 per cent solution. At 3:00 P. M., the animals were killed, the livers removed and small ground portions put into weighed centrifuge tubes containing 2 cc of 30 per cent KOH. The method was so standardized that the liver was in the tubes within one minute after the animal was killed. The tubes were now reweighed and then heated in a water bath until the solution was homogenous. Ten volumes of 95 per cent alcohol were added and the tubes reheated to boiling. The tubes were cooled and centrifuged until the glycogen was well packed in the bottom. The solution was poured off and 15 milliliters of 0.6 N HCl was added and the tubes heated in a boiling water bath for 2 hours. The solutions are neutralized and diluted to 50 milliliters, such a dilution giving a sugar concentration of proper strength with the reagent described in the section of "Methods of Analysis." The milligrams of glucose found is multiplied by 0.93 to give the glycogen equivalent. The results of the experiment are tabulated below.

Sample given by	Weight sample	Mg. glycogen (glucose x 0.93)	Glycogen per cent	Average per cent
Intraperitoneally	1.5140	4.51	0.345	0.21
	1.1767	3.05	0.261	
	1.3515	1.99	0.147	
	1.9443	4.75	0.256	
	1.6824	1.57	0.094	
Stomach tube	1.6294	1.70	0.112	0.19
	1.1314	1.26	0.110	
	1.4590	1.26	0.087	
	1.3017	2.10	0.154	
	1.4868	7.60	0.514	
Control	2.2147	1.96	0.089	0.19
	1.5124	0.95	0.150	
	1.3562	1.36	0.101	
	1.6007	0.86	0.590	
	2.6164	2.60	0.100	

The liver glycogen in normal non-fasting rats is from 2 to 5 per cent of liver weight. In fasting rats, the level may vary from 0.2 to 0.3 per cent. The figures in the above table show that the fasting rats were good controls and that the rats fed on the syrup were not able to use it for the production of liver glycogen.

BIBLIOGRAPHY

1. Butlerow, A. K., Ann. 120:295, (1861)
2. Hoffman, "Photosynthesis", Spoehr, page 265.
3. Baeyer, A., Berichte, 3:63, (1864)
4. Loew, O., J. Prakt. Chem., 33, 321 (1886)
   Loew, O., Berichte, 22: 133 (1915)
5. Fisher, E., Berichte, 20: 1093, (1887)
6. Fisher, E. and Passmore, T., Berichte, 22: 359, (1889)
7. Fisher, E. and Tafel, J., Berichte, 22:97, (1889)
8. Hof, J. U., Ann., 403; 305, (1914)
9. Ortmer, L. and Gerish, K., Biochem. Zeit. 259: 30, (1933)
10. Schumlfuss, H., Biochem. Zeit. 166: 70, (1927)
11. Husin, A., Berichte 68: 619, 1494, 2160, (1935)
12. Ronijin, J., Ann., 36: (1937)
13. Shaffer and Somogyi, M., Jr. Biol. Chem., 100: 695, (1933)
14. Euler, H., and Martius, C., Ann., 75: 505, (1933)
15. Houben, J., "Die Methodien der Organischen Chemie."
16. Fenton, G., Jr. Chem. Soc., 97; 911, (1905)
17. Isbell, H., Bureau of Standards Journal of Research, #320, 6, June 1931.
18. Quick, A. J., Jr. Biol. Chem. 74: 331, (1927)

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