THE MECHANISM OF CARBOHYDRATE OXIDATION[1]

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The carbohydrates are a group of chemical compounds which have always been of great theoretical interest to the student of organic chemistry. The splendid contributions from this area of our science have become milestones in general chemical progress. The literature of chemistry has been constantly enriched throughout the years by the solution of many difficult problems concerning the molecular architecture of various kinds of sugars. As a present moment example, may I remind you of the masterly contributions now being made with reference to the structure of the disaccharides. Recall, if you please, the recent startling announcement that the synthesis of sucrose had been finally achieved. An enumeration of the workers who have labored in the field of carbohydrate chemistry would constitute a brilliant array of names.

As rapidly as the structural and synthetic questions have been gradually cleared up the demand has been insistent that we place an increasing emphasis on a better understanding of the reactions of the carbohydrates more especially that of oxidation. By reason of both its theoretical and biological significance this latter chemical change needs our most earnest attention. In attempting work in this direction it was hoped that the oxidation mechanism of these complex substances could be traced more surely by using the data which we had obtained in some earlier experiments with compounds of the more simple type. The necessity for scientific work of this character seems to find a complete justification in the recent significant statement of one of your own distinguished members, Dr. P. A. Levene (1), who

[1] The William H. Nichols Medal Address for 1929, delivered before the New York Section of the American Chemical Society, March 1, 1929.

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said: "Glucose is one of the most important substances in the economy of the world."

In placing before you the results of the researches upon which the Jury of Award of the William H. Nichols Medal rested its decision for 1929, may I briefly recall at least two of the important characteristics of the period in which our earlier work was undertaken nearly a quarter of a century ago. One of these was the rapid expansion which was beginning to take place in the realms of physical chemistry. A number of causes contributed to this increasing activity. In the eighties Arrhenius announced his views with reference to the nature of electrolytes. In the nineties a group of important discoveries were made which were destined to exercise a marked influence on the views then held concerning the fundamental nature of material things. As you are aware, reference is here made to the radioactive elements, the rare gases of the atmosphere, the Roentgen ray, and the electron. These were memorable events. They did much towards focusing the interest of investigators on the physical aspects of chemistry. Many of us recall the profound impressions which these far reaching discoveries made on the scientific world at that time.

The influence of this growing interest in physical chemistry immediately began to bear fruit in the field of reaction mechanism. The application of the Law of Guldberg and Waage to some of the postulates of the newly enunciated solution theory gave a satisfying starting point for a better understanding of inorganic chemical reactions. The importance of this contribution to our knowledge of reaction mechanism and the incentive which it proved to be for many workers can hardly be over-emphasized. The present efforts which are being made to successfully utilize the electronic conceptions of matter in formulating a sound theory of chemical behavior should receive the most sympathetic encouragement.

On this occasion it is of much interest to note that it was in these years that Dr. Claude S. Hudson began his work on the use of physical chemical methods in the solution of carbohydrate problems. His contributions in both that field and also in that of the more strictly organic character have won for him a place

of distinguished leadership, which has received the highest recognition of his fellow scientists.

The second of these movements which characterized the opening years of this century was the continued vigor with which the classical organic synthesis was extending its conquest in every direction as it had been doing for many years previously. It is well known that this great activity produced a constantly increasing number of compounds of the element carbon.

Many new questions began to arise from this constant multiplication of new compounds. Prominent among these were the phenomena which are now grouped under the familiar name of *tautomerism*, a concept to which we shall have occasion to refer at several important places in our discussion. Another problem which was constantly demanding more attention was the great need of a satisfying theory of organic chemical behavior. The triumphs of the ionization theory in the field of inorganic chemistry only served to accentuate this need. Three theories of chemical behavior were claiming the attention of the organic chemists at this time. One of these was the fruitful partial valence theory of Thiele. The other two were those fostered by the Americans, Arthur Michael and the late John Ulric Nef. The problem still confronts us. Out of the continued efforts of these many years and with a continued zeal in chemical research aided by modern methods of attack, it is hoped that a simple and helpful theory of organic chemical behavior may finally be evolved.

Such, in brief, was the spirit of chemical science when we undertook in the Chemical Laboratory of The Ohio State University a study of the oxidation of organic compounds. The principal objective in these experiments was the determination of the molecular stages through which organic compounds passed when they were subjected to oxidizing conditions. In pursuing this line of investigation it was desired to test Nef's (2) theory of organic chemical behavior from the standpoint of its usefulness in coördinating facts already known as well as to test its power of predicting unknown chemical behavior. It was hoped that this brilliant investigator's views might thus serve as a means of

establishing some useful generalizations concerning the oxidation of organic compounds.

To accomplish these ends it became necessary to conduct all our experimental work under very carefully chosen conditions. The accurate methods of quantitative analysis were used in gathering the experimental facts. In our latest work with the carbohydrates we have been confronted with the problem of quantitatively separating certain reaction products which were very closely related to one another. Obviously, this challenge is a difficult one in some cases yet withal it is one full of interest.
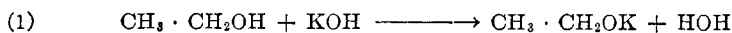
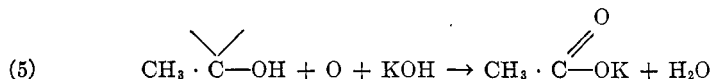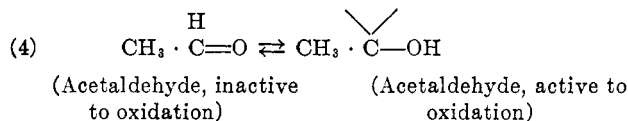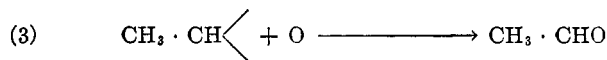### ETHYL ALCOHOL AND ACETALDEHYDE

The first substance to be studied in this general manner was ethyl alcohol. This compound was oxidized with aqueous solutions of potassium permanganate in the presence and in the absence of added potassium hydroxide. An examination of the literature of this particular reaction disclosed the fact that previous investigators had found the following compounds among the oxidation products: acetaldehyde, formaldehyde, oxalic and acetic acids and carbon dioxide. The point of interest in all this work was the notable fact that the presence of added alkali seemed to be necessary for oxalic acid formation, although Chapman and Smith (3) reported the presence of this acid even in the absence of any added alkali. Morawsky and Stingl (4) believed that temperature merely influenced the rate of the reaction, while Pean de Saint Gilles (5) was of the opinion that the temperature was also an important factor in determining both the character and the amounts of the products formed. No complete systematic study had been previously made of the permanganate reaction for the purpose of ascertaining the effect of temperature and the alkali concentration on the character and the amounts of the oxidation products. We found that carbon dioxide, acetic and oxalic acids were the only reaction products when the alcohol was oxidized under the experimental conditions which we adopted (6). Acetic acid was the sole product in the absence of any added alkali. Our data accounted for a quantitative return of the carbon introduced as alcohol. A consideration of our quanti-

tative results showed that the yields of these oxidation products were functions of the initial concentration of the alkali used and of the temperature employed.

In order to gain an insight into the exact reaction mechanism which produced these results, it was found necessary to subject the various oxidation products of ethyl alcohol which were theoretically possible to exactly the same experimental conditions as those used in these oxidations. As is well known chemical theory indicates that the formation of acetaldehyde is the *first step* in this oxidation mechanism, while the formation of acetic acid is regarded as the *second step*. An accurate experimental study of acetaldehyde (7) showed that this first intermediate behaved in the same general manner as did ethyl alcohol, i.e., the amounts of the reaction products were found to be functions of the same experimental conditions as those surrounding the oxidation of the alcohol. Hence it may be safely concluded that acetaldehyde is the first oxidation product of ethyl alcohol in neutral and alkaline potassium permanganate, and that the acetic acid is derived from the acetaldehyde.

From the general character of our quantitative data it is clear that the alkali must play an important rôle in the oxidation mechanism of ethyl alcohol. As the concentration of the potassium hydroxide is increased the alcohol molecules seem to become much more sensitive to attack. According to Nef's views (8) the alcohol and the alkali react to form an alcoholate which in turn is dissociated into ethylidene with much greater ease than is the ethyl alcohol itself. Hence, the mass action effect of the alkali would increase the speed of the ethylidene formation. The unsaturated compound thus formed would be oxidized to acetaldehyde. The acetaldehyde thus formed is undoubtedly in equilibrium with its enolic form which may be regarded as the *active form* of this molecule (9). This enol is easily oxidized to acetic acid. It is clear that the tendency of the alkali would be to increase the velocity of this change. These changes are shown in the following equations.

(1) $CH_3 \cdot CH_2OH + KOH \longrightarrow CH_3 \cdot CH_2OK + HOH$

(2) $CH_3 \cdot CH_2OK \longrightarrow CH_3 \cdot CH< + KOH$

(3) $\quad$ $CH_3 \cdot CH{\Big\langle} + O \longrightarrow CH_3 \cdot CHO$

(4) $\quad$ $CH_3 \cdot \overset{H}{C}{=}O \rightleftarrows CH_3 \cdot \overset{\diagdown\diagup}{C}{-}OH$

(Acetaldehyde, inactive   (Acetaldehyde, active to
to oxidation)   oxidation)

(5) $\quad$ $CH_3 \cdot \overset{\diagdown\diagup}{C}{-}OH + O + KOH \rightarrow CH_3 \cdot \overset{O}{\overset{\diagup\diagup}{C}}{-}OK + H_2O$

Our next logical step was to find the sources of the other two products, oxalic acid and carbon dioxide. A consideration of quantitative data obtained from a study of the oxidation of potassium acetate (10) showed conclusively that this salt could not be seriously regarded as a source of these two oxidation products. Since acetaldehyde had been oxidized with potassium permanganate in the absence of alkalies to acetic acid exclusively it was thought best to make an accurate quantitative study of the conditions which give rise to vinyl alcohol formation when acetaldehyde is treated with aqueous solutions of potassium hydroxide, i.e., study the behavior of acetaldehyde towards potassium hydroxide itself in the absence of potassium permanganate. A knowledge of these data became the more desirable since it had long been known that vinyl alcohol possessed a structure which was most easily attacked with alkaline potassium permanganate. Much to our satisfaction we found that vinyl alcohol production from acetaldehyde in the presence of an aqueous solution of potassium hydroxide was a linear function of the alkali concentration (11). Therefore, it must be concluded that in the oxidation of ethyl alcohol there is an equilibrium existing between acetaldehyde (active form), acetaldehyde (inactive to oxidation), and vinyl alcohol which may be shifted in the direction of vinyl alcohol by an increased concentration of alkali. It is

(6) $\quad$ $CH_3 \cdot \overset{\diagdown\diagup}{C}{-}OH \leftrightarrows CH_3 \cdot CHO \leftrightarrows CH_2{=}CHOH$

(vinyl alcohol)
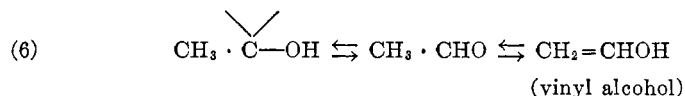
clear that such a change would result in diminished yields of acetic acid and increased yields of oxalic acid and carbon dioxide,

a point of view which is in harmony with our results. It is interesting to point out that a definite concentration of alkali was found to be necessary before oxalic acid formation began. On the basis of the relationship existing between the concentration of the potassium hydroxide used and the amounts of acetic acid formed we were able to calculate these values (12). By experimentation we found that the limiting upper value for the alkali concentration which would still yield the maximum amount of acetic acid was between 0.415–0.461 gram per liter. Our calculation gave 0.46 gram. Hence, it was concluded that vinyl alcohol must be the next intermediate after acetaldehyde in the series of reactions producing oxalic acid and carbon dioxide when ethyl alcohol is oxidized with potassium permanganate in the presence of alkalies. Since vinyl alcohol has a momentary existence only, it also became necessary to study its theoretically possible oxidation products for the purpose of establishing that this alcohol is an intermediate in these reactions producing oxalic acid and carbon dioxide. These were glycolic aldehyde, glyoxal, glycolic acid and glyoxylic acid. All of these yielded oxalic acid and carbon dioxide in amounts which showed that complete oxidation had taken place. Furthermore, it was found that the yields of these products were related to the alkali concentration in the same general manner as had been observed with ethyl alcohol and acetaldehyde (7). The neutralization of the acids formed in these changes is obviously an important factor which will influence any equilibrium conditions that may arise in such systems as those studied.

ISOPROPYL ALCOHOL AND ACETONE

We next investigated the behavior of isopropyl alcohol (13) and acetone (14). Precisely the same procedures and general experimental conditions were used as had been employed with ethyl alcohol and acetaldehyde. Carbon dioxide, acetic and oxalic acids were found to be the oxidation products both in the presence and in the absence of added alkali. A small amount of acetone was also found at 25°. As had been the case with ethyl alcohol and acetaldehyde, temperature and alkali concentration were the important factors controlling the character and

the amounts of oxidation products formed from isopropyl alcohol and acetone. One notable difference was found in the products obtained from these two pairs of compounds. With neutral potassium permanganate, oxalic acid was always found as an oxidation product of isopropyl alcohol and acetone, while as shown above, this acid was absent in the oxidation products of ethyl alcohol and acetaldehyde with the same reagent.

A study of the intermediates which might conceivably be formed in the oxidation of isopropyl alcohol showed that acetone must be the first oxidation product formed in this reaction. This arises from the fact that none of the possible intermediates save acetone will yield all the products which isopropyl alcohol will give. Therefore, it is clear that the source of the oxidation pr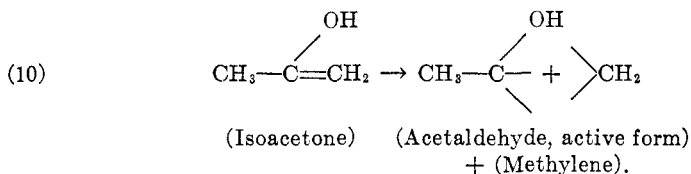oducts must be sought for in those chemical changes through which acetone passes when it is oxidized under the same conditions. The reaction mechanism of acetone formation from isopropyl alcohol is analogous in every way to the formation of acetaldehyde described above; namely, isopropylidene is the unsaturated intermediate.

These results are best understood on the ground that the added alkali reacts on acetone in the same general way as it does on acetaldehyde, i.e., it shifts the equilibrium existing between acetone and isoacetone molecules in the direction of the isocompound. In order to test this point of view we studied its behavior in the same general manner as we did that of acetaldehyde; i.e., its action towards aqueous solutions of potassium hydroxide in the absence of potassium permanganate. Again, as in the case of the acetaldehyde we found a definite relationship existing between the production of isoacetone (15) and the concentration of the potassium hydroxide used. This analogous enolization of acetaldehyde and isoacetone is shown in the following equations.

(8)
$$CH_3 \cdot CO \cdot CH_3 \leftrightarrows CH_3 \cdot \underset{\underset{CH_2}{\|}}{C}-OH$$

(Acetone)   (isoacetone)

(9)
$$CH_3 \cdot \overset{O}{\underset{}{C}}-H \leftrightarrows CH_2{=}CHOH$$

(acetaldehyde)   (vinyl alcohol)
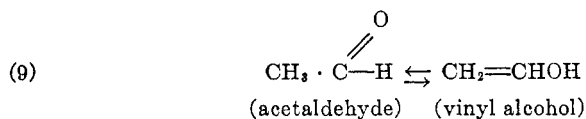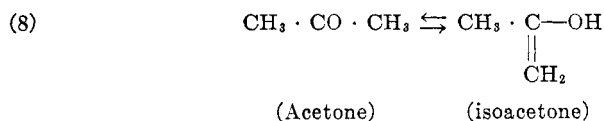
The isoacetone molecule should then react in accordance with the following equation.

(10)

$$CH_3-\overset{\displaystyle OH}{\overset{\diagup}{C}}{=}CH_2 \rightarrow CH_3-\overset{\displaystyle OH}{\overset{\diagup}{\underset{\diagdown}{C}}}- + \overset{\diagdown}{\diagup}CH_2$$

(Isoacetone)   (Acetaldehyde, active form)
+ (Methylene).

In the presence of an oxidizing agent, acetic acid and formaldehyde would be the products. The formaldehyde would subsequently be converted to carbon dioxide. Dumas and Stas (16), Gottlieb (17), and Herz (18) obtained acetic acid from acetone by using chromic acid as the oxidizing agents. Others have obtained formic and acetic acids by means of silver oxide. Since acetaldehyde is a possible dissociation product of isoacetone, it is clear that vinyl alcohol will form provided the concentration of the base is great enough.

A study of the data obtained in the oxidation of acetone with neutral potassium permanganate will show that there is a sufficient concentration of potassium hydroxide arising from the following reaction

(11) $3\,CH_3 \cdot CHOH \cdot CH_3 + 2KMnO_4 \rightarrow 3CH_3 \cdot CO \cdot CH_3 + 2KOH + 2H_2O + 2MnO_2$
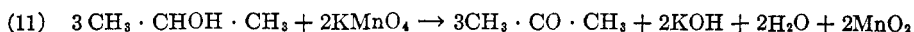
to bring about this vinyl alcohol formation. As shown above this will give rise to oxalic acid and carbon dioxide.

Since Fournier (19) had shown that acetone may be oxidized in part to pyruvic acid with alkaline potassium permanganate, it is obvious that isoacetone, as pointed out by Witzemann (20), must be oxidized to acetol in accordance with the following equation.

(12) $CH_3 \cdot CO \cdot CH_3 \rightleftarrows CH_3 \cdot C(OH){=}CH_2 + 2OH \rightarrow CH_3 \cdot CO \cdot CH_2OH + H_2O$
    (Acetone)   (Isoacetone)   (Acetol)
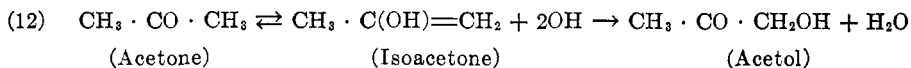
That acetone is not oxidized exclusively in alkaline solutions through the intermediate formation of acetol is shown by the fact that acetol differs from acetone in that the amounts of oxalic

and acetic acids obtained from it show certain maxima and minima which acetone does not (21). Finally, it is to be observed that the presence of the alkali exerts a marked effect on the oxidation of isopropyl alcohol as it did in the case of ethyl alcohol i.e., the sensitivity of these compounds to chemical attack is greatly increased by the mass action effect of the added alkali.
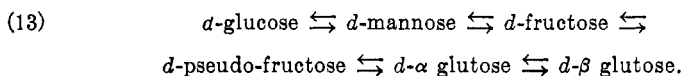
THE OXIDATION OF CARBOHYDRATES IN ALKALINE SOLUTION

The work described thus far is fairly typical of the experiments which might well be considered as the forerunners of our subsequent studies on the carbohydrates. One of the most outstanding observations which we made in this study of the oxidation of the simpler organic compounds was the marked influence which the alkali normality seemed to have on the general course of a given reaction. In our desire to obtain more data with reference to this effect we turned our attention to the oxidation of the carbohydrates in alkaline solutions.

It is well known that the more common carbohydrates such as glucose, fructose, galactose and sucrose have been oxidized under a great variety of experimental conditions. Many valuable results have been obtained in such work. We were anxious to make a study from the standpoint above noted. In our experiments, certain carbohydrates were oxidized by means of aqueous solutions of potassium permanganate both in the absence and in the presence of varying concentrations of potassium hydroxide. Under these conditions, the carbohydrates chosen were converted exclusively into carbon dioxide, oxalic acid and traces of acetic acid. The general procedures were exactly the same as those used with the compounds of the simpler type. Our average total carbon return was within a few tenths of 100 per cent. From our data we have made the following general observations. (a) *Effect of varying the alkali concentration.* (1) When each of the individual sugars (glucose, mannose, fructose and galactose) which were chosen for our study was oxidized at 25°, 50° and 75°, it was found the amounts of oxalic acid and carbon dioxide formed at a given alkali normality were different. This was especially true at the lower alkalinities. As the concentration of the potassium hydroxide was increased
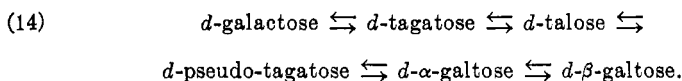
there was a general tendency for the oxalic acid—carbon dioxide ratios to become more nearly the same for all the various temperatures used. (2) At lower normalities of alkali and at a temperature of 50°, the amounts of oxidation products from glucose, mannose and fructose were found to be different for each individual carbohydrate. As the alkali normality was increased, there was a tendency for the amounts of oxalic acid and carbon dioxide from these three hexose sugars to become more nearly the same. At first it was thought that these rather small differences were negligible but our later work showed that they were really differences of some significance. (b) *Effect of temperature.* As the temperature was increased from 50° to 75°, this tendency towards identical values in the amounts of the oxidation products became more marked. At 75° the amounts of oxalic acid and carbon dioxide obtained from glucose and fructose were almost the same. Although we did not study mannose at 75°, yet it will be shown later that it may be predicted with a fair degree of certainty that the amounts of oxalic acid and carbon dioxide obtained from it will be practically the same as that derived from glucose and fructose. Although galactose belongs to another series in the hexose group, namely, galactose, tagatose and talose, yet it was observed that this carbohydrate showed a marked tendency at higher alkali normalities and at higher temperatures to yield oxalic acid and carbon dioxide in amounts which were practically the same as those in our experiments with glucose, fructose and mannose. So much was this the case that we found the data at 75° to be practically identical for glucose and galactose.

These differences and these tendencies toward identical yields of the products obtained in the oxidation of these well known hexose sugars with neutral and alkaline permanganate at different temperatures are very important matters in the chemistry of these compounds. Hence, we turned our attention to the task of finding a reaction mechanism that would account for the facts which we had observed. More especially were we anxious to know whether our data would harmonize with the classical work in the literature of this field. As is well known, the experiments of Lobry de Bruyn and Alberda van Ekenstein (22) concerning

the action of alkalies on aqueous solutions of glucose, mannose and fructose showed that these carbohydrates undergo reciprocal transformations into each other. The equilibrated system formed by the use of either glucose, mannose or fructose is represented by the following equation,

(13)   *d*-glucose $\leftrightarrows$ *d*-mannose $\leftrightarrows$ *d*-fructose $\leftrightarrows$

d-pseudo-fructose $\leftrightarrows$ *d*-α glutose $\leftrightarrows$ *d*-β glutose.

The similar behavior of galactose is shown in the following equation:

(14)   *d*-galactose $\leftrightarrows$ *d*-tagatose $\leftrightarrows$ *d*-talose $\leftrightarrows$

d-pseudo-tagatose $\leftrightarrows$ *d*-α-galtose $\leftrightarrows$ *d*-β-galtose.

These workers found that glucose, mannose, and fructose did not give rise to equilibrated systems which were quantitatively identical. The same relationship is probably true with reference to the galactose series. This general fact is of extreme importance in understanding the differences and the similarities in the behavior of these hexose sugars under the experimental conditions which we used.

In their study of the behavior of alkalies towards the sugars Nef (23) and his students elaborated the suggestion of Wohl and Neuberg (24) with reference to the existence of a 1-2 enediolic form of glucose. They postulated the presence of two other enediols, namely, the 2-3 and the 3-4 isomers. From the following equation it is clear that mannose and fructose would give rise to exactly the same enediols as glucose.

(15) CH_2OH CH_2OH CH_2OH CH_2OH
              | | | |
            HOCH   HO—C—H   HO—C—H   HO—C—H
              | | | |
            HOCH   HO—C—H   HO—C—H   C—OH
              | | | ||
            H—COH $\leftrightarrows$ H—C—OH $\leftrightarrows$ C—OH $\leftrightarrows$ C—OH
              | | || |
            HOCH   C—OH   C—OH   HO—C—H
              | || | |
             CHO   CHOH CH_2OH CH_2OH

      (d-Glucose)   (Glucose 1-2   (Glucose 2-3   (Glucose 3-4
                          Enediol)   Enediol)   Enediol)
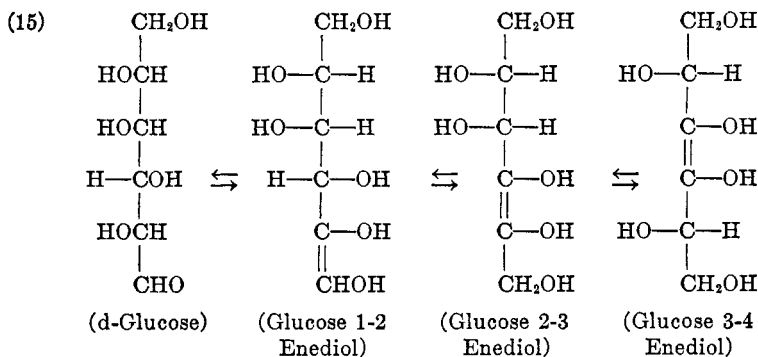
Furthermore, it is equally clear that the enediols of the galactose-tagatose-talose series must be structurally different than the (1-2) and (2-3) isomers from the corresponding ones in the glucose series.

(16)

```
      CH₂OH              CH₂OH              CH₂OH              CH₂OH
        |                  |                  |                  |
  HO—C—H             HO—C—H             HO—C—H             HO—C—H
        |                  |                  |                  |
   H—C—OH             H—C—OH             H—C—OH              C—OH
        |                  |                  |                 ‖
   H—C—OH  ⇄  H—C—OH  ⇄    C—OH   ⇄         C—OH
        |                  |                 ‖                  |
  HO—C—H              C—OH              C—OH             HO—C—H
        |                 ‖                  |                  |
      CHO               CHOH              CH₂OH              CH₂OH

  (d-Galactose)     (Galactose 1-2     (Galactose 2-3     (Galactose 3-4
                      Enediol)           Enediol)           Enediol)
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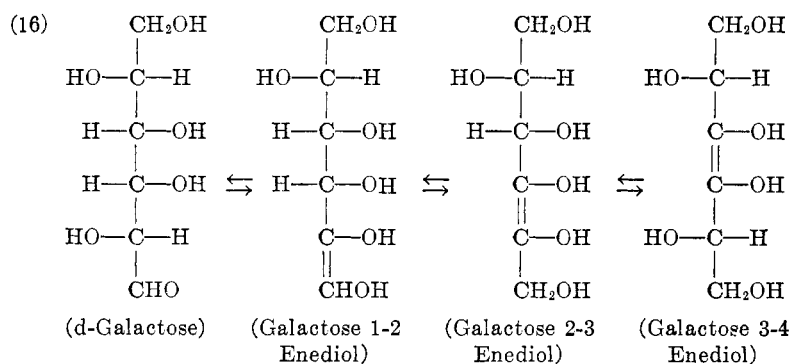
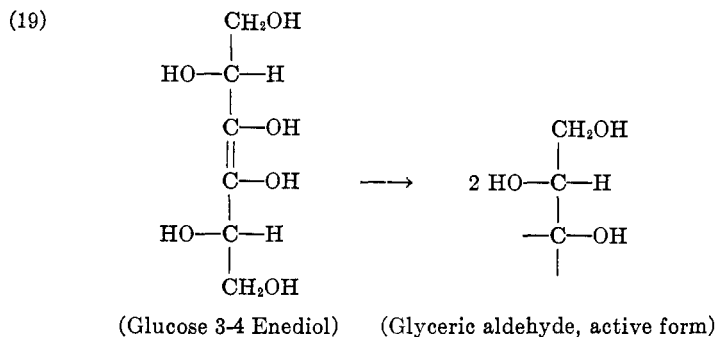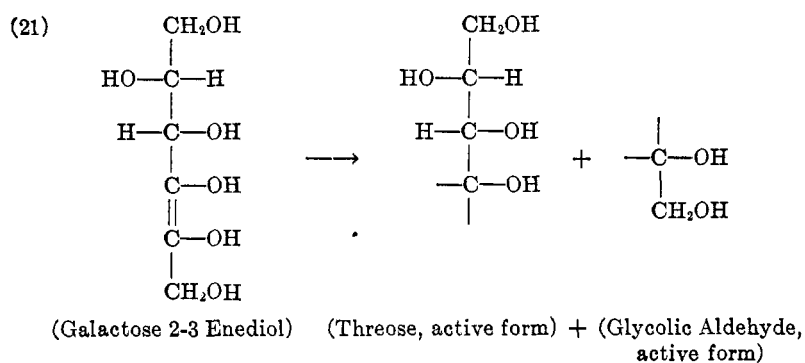When the double bonds of the several glucose enediols are ruptured the following reactions take place.

(17)

```
      CH₂OH                    CH₂OH
        |                        |
  HO—C—H                   HO—C—H
        |                        |                    OH
  HO—C—H                   HO—C—H             ⁄
        |          ⟶            |        +   C
   H—C—OH                   H—C—OH             ⎝
        |                        |                    H
      C—OH                  —C—OH
       ‖                        |
     CHOH

  (Glucose 1-2 Enediol)    (Arabinose, active form) + (Hydroxymethylene)
```

(18)

```
      CH₂OH                    CH₂OH
        |                        |
  HO—C—H                   HO—C—H
        |                        |                    |
  HO—C—H          ⟶       HO—C—H        +     —C—OH
        |                        |                    |
      C—OH                  —C—OH               CH₂OH
       ‖                        |
      C—OH                                   (Glycol Aldehyde,
        |                                        active form)
     CH₂OH

  (Glucose 2-3 Enediol)    (Erythrose, active form)
```

(19)

$$\begin{array}{c}
CH_2OH \\
| \\
HO-C-H \\
| \\
C-OH \\
\| \\
C-OH \\
| \\
HO-C-H \\
| \\
CH_2OH
\end{array}
\longrightarrow
2\;
\begin{array}{c}
CH_2OH \\
| \\
HO-C-H \\
| \\
-C-OH \\
|
\end{array}$$

(Glucose 3-4 Enediol)   (Glyceric aldehyde, active form)
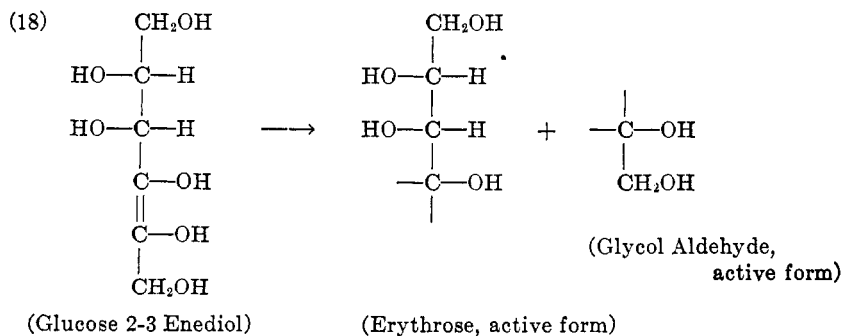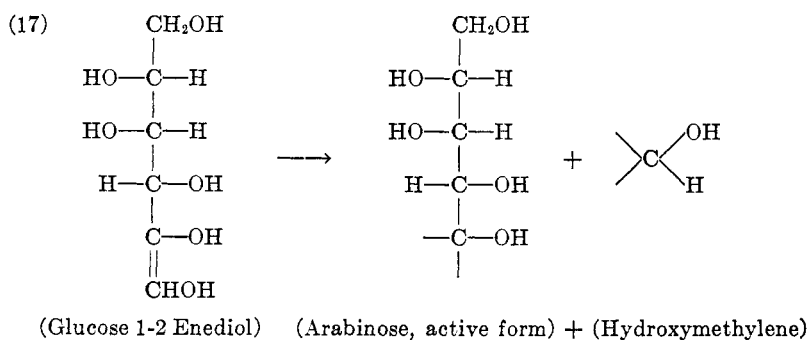
The following reactions take place when the above galactose enediols are ruptured.

(20)

$$\begin{array}{c}
CH_2OH \\
| \\
HO-C-H \\
| \\
H-C-OH \\
| \\
H-C-OH \\
| \\
C-OH \\
\| \\
HCOH
\end{array}
\longrightarrow
\begin{array}{c}
CH_2OH \\
| \\
HO-C-H \\
| \\
H-C-OH \\
| \\
H-C-OH \\
| \\
-C-OH \\
|
\end{array}
+
\begin{array}{c}
\diagdown \\
\diagup C \diagdown \overset{OH}{\underset{H}{}}
\end{array}$$

(Galactose 1-2 Enediol)   (Lyxose, active form)   (Hydroxymethylene)

(21)

$$\begin{array}{c}
CH_2OH \\
| \\
HO-C-H \\
| \\
H-C-OH \\
| \\
C-OH \\
\| \\
C-OH \\
| \\
CH_2OH
\end{array}
\longrightarrow
\begin{array}{c}
CH_2OH \\
| \\
HO-C-H \\
| \\
H-C-OH \\
| \\
-C-OH \\
|
\end{array}
+
\begin{array}{c}
| \\
-C-OH \\
| \\
CH_2OH
\end{array}$$

(Galactose 2-3 Enediol)   (Threose, active form) + (Glycolic Aldehyde, active form)

(22)

$$CH_2OH$$
$$|$$
$$HO—C—H$$
$$|$$
$$C—OH$$
$$||$$
$$C—OH$$
$$|$$
$$HO—C—H$$
$$|$$
$$CH_2OH$$

$$\longrightarrow$$

$$CH_2OH$$
$$|$$
$$2\ HO—C—H$$
$$|$$
$$—C—OH$$
$$|$$

(Galactose 3-4 Enediol)　(Glyceric Aldehyde, active form)

Since mannose and fructose give rise to the same enediols as glucose then it is clear that the qualitative character of the solutions produced through a rupture of these hexose enediols will be the same in each case. The amounts of the fission products should be different in the alkaline solution of these three sugars because of the fact observed by de Bruyn and van Ekenstein namely, that glucose, mannose and fructose did not give equilibrated systems that were quantitatively identical. A rupture of the galactose enediols would result in a system that is chemically different from that formed by the glucose enediols except in the 3-4 enediol. In this case as in all the hexose sugars glyceric aldehyde would be a common component.

It occurred to us (25) that should the equilibrium consisting of the three enediols of a given hexose together with their respective fission products be an integral part of the one observed by de Bruyn and van Ekenstein then there might be certain experimental factors which ought to produce a shifting in such a system. If the alkaline solutions of these carbohydrates give rise to an irreversible equilibrium, then it is probable that the speed of the slowest reaction in the series of changes involved must be influenced by the concentration of the alkali used and the temperature employed. Nef (26) believed that there were no less than 116 substances in these equilibrated mixtures produced by the action of alkalies on the hexose sugars. From the differences and similarities observed in the oxidation products obtained in the glucose-mannose-fructose series, and the relation of the results obtained in the galactose series to those in the

glucose series, it is clear that these experimental factors must undoubtedly be the temperature and the alkali normality.

Sources of the oxidation products. The sources of the reaction products found in these permanganate oxidations and the effect of the variable experimental factors on their amounts may be understood on the basis of these views with reference to the equilibrated condition which is thought to exist in alkaline solutions of these sugars. *Carbon dioxide.* (*a*) The carbon dioxide obtained in the oxidation of glucose, fructose, mannose, and galactose with potassium permanganate in the absence of any added alkali must come from the ultimate oxidation of hydroxymethylene molecules obtained when the hexose 1-2 enediol undergoes splitting. It is evident that various aldehydes arising from the successive degradation of the hexose through a loss of hydroxymethylene should react in the same manner. We found that arabinose, lyxose and glyceric aldehyde may be oxidized completely to carbon dioxide in the absence of alkali. We had no erythrose or threose available. Our results show a maximum yield of carbon dioxide in those regions of alkalinity where the 1-2 enediol formation is favored. (*b*) Carbon dioxide may arise from the glycolic aldehyde obtained from the splitting of the *hexose 2-3 enediol.* Nef has shown that the tetroses tend to react as 2-3 enediol and these on splitting would eventually yield more glycolic aldehyde. (*c*) It has been also shown that glyceric aldehyde in alkaline solution is oxidized in part to carbon dioxide. Hence, the *hexose 3-4 enediol* thus becomes a source of carbon dioxide by reason of its rupture into glyceric aldehyde. *Oxalic acid.* As the alkali concentration is increased the enediolic equilibrium would tend to shift to the region in which the 2-3 hexose enediol predominates. This would tend towards the production of glycol aldehyde, a compound which is oxidized exclusively to oxalic acid and carbon dioxide under our experimental conditions. It was found that the oxalic acid reaches a maximum, after which the yield begins to diminish. This has been thought to be due to the oxalic acid—carbon dioxide ratio in glyceric aldehyde, a fission product of the hexose 3-4 enediol, being of less value than in glycol aldehyde at given alkali normality.

This would mean a tendency in the oxalic acid to approach a minimum. *Acetic acid*. This acid undoubtedly arises from the oxidation of pyruvic aldehyde, pyruvic acid and lactic acid. The sources of these will be discussed below.

Amounts of the oxidation products. The differences in the amounts of the oxidation products in the glucose-mannose-fructose series at the lower alkalinities must be due to a lack of identity in the equilibrium system formed by each individual sugar. As the alkali concentration is increased it is clear that these equilibrated mixtures must shift in each case towards the glucose 3-4 enediol. These alkaline solutions tend therefore to become more nearly identical quantitatively. Hence the amounts of their oxidation products should become more nearly the same. The effect produced by an increase in temperature seems to bring about the same end result. As noted above, the splitting products of the galactose (1-2) and (2-3) enediols are different from those of the glucose series. Hence, quantitative differences in the amounts of oxalic acid and carbon dioxide are rather to be expected. However, the splitting products of the galactose (3-4) enediol are like those of the glucose series. If the increased alkali concentration changes the first splitting products of all hexose enediols into other enediols which in themselves undergo the same fission reaction also, then it becomes clear that the alkaline solutions of all hexose sugars will tend towards identical composition at the higher alkalinities. An added emphasis is given to this point of view when one reflects that all the hexose sugars form a common 3-4 enediol, which, as has been repeatedly stated, will rupture into two molecules of glyceric aldehyde. Whether the saccharines play a part in these reactions has not been established. It is highly probable that the speed of oxidation of the fission products is much greater than that of saccharine formation under these conditions. Hence this factor would have little influence on our results.

ACTION OF POTASSIUM HYDROXIDE ON THE HEXOSES

It now became increasingly important that the alkaline solutions of these carbohydrates should be studied in the absence of

potassium permanganate in order to ascertain whether they would react as though an equilibrated condition was present. This seemed to be the logical procedure because of the obviously important part played by the alkali in our experiments with the simpler types of compounds. We applied the same experimental treatment in this case that we had used in studying the effect of the alkali concentration on the formation of vinyl alcohol from acetaldehyde, and the effect of the same factor on the formation of isoacetone from acetone. The general chemical change taking place in alkaline solutions of these sugars is undoubtedly the same as that in the alkaline solutions of the simpler compounds; i.e., a keto-enol tautomerism, a phenomenon now being studied by W. Lee Lewis and his students in the case of the methylated sugars. We felt that data obtained from experiments involving changing temperature and variable alkalinity ought to shed much light on the validity of our explanation. The brilliant experiments of Nef and his students in this difficult field are well known and in my opinion will stand unchallenged as masterpieces of chemical workmanship. Our procedure consisted in subjecting the carbohydrate under investigation to the influence of variable temperature and changing alkali normality for a definite length of time. The presence of certain reaction products were regarded as evidence for the previous existence of hexose enediolic forms in the alkaline solutions. A consideration of the properties of the various substances which might form in the reaction between potassium hydroxide and these hexose sugars showed that a study of the relationship between the lactic acid yields and the alkali normality would be the most practical method of establishing the presence of an equilibrated system in these carbohydrate solutions. The following will illustrate our plan: *Lactic Acid*: If a hexose (3-4) enediol is ruptured at the double bonds, it should give two molecules of the active form of glyceric aldehyde. This fission product is then converted to pyruvic aldehyde which in turn is changed to lactic acid in alkaline solutions. Hence, *lactic acid* was regarded as an index for the presence of a hexose (3-4) enediol. If the normality of the alkali and temperature are the two factors which influence the status of the general

equilibrated condition in these solutions, then the yields of lactic acid should be functions of these experimental conditions. A comparison of the amounts of lactic acid obtained from glucose, mannose and fructose at 25°, 50° and 75° bears out this point of view (27). It should be emphasized in this connection that the amounts of lactic acid derived from these three well known sugars are practically identical at 75°. These facts are in harmony with the tendency of the oxalic acid-carbon dioxide ratios in the oxidation of fructose and glucose to become more nearly the same at 75° and at the higher alkalinities. Since the amount of lactic acid from alkaline solutions of mannose is practically the same as that from the other two sugars, it is a fair prediction that the oxalic acid carbon-dioxide ratio will also be the same. *Pyruvic aldehyde.* If pyruvic aldehyde is a forerunner of lactic acid then the amounts of this compound should also bear a definite relationship to the variable experimental factors employed. This keto-aldehyde was quantitatively determined through its osazone. Our production curves rose to a maximum (28) with an increase in alkalinity and then they receded. These maxima were thought to be points of alkalinity at which the rate of osazone formation was just balanced by that of lactic acid formation. These reactions show a definite temperature coefficient, the maximum point occurring at lower alkalinities with an increase in temperature. *Acetic and formic acids.* Acetic acid and a portion of the formic acid may come from the dissociation of pyruvic aldehyde into acetaldehyde and carbon monoxide. These aldehydes are then converted into acetic and formic acids. If a definite hydroxyl ion concentration is necessary to change pyruvic aldehyde into lactic acid, it is obvious that the amounts of acetic and formic acids will tend to diminish in an amount which will be inversely proportional to the amount of lactic acid formed. These maxima in the amounts of acetic and formic acid were found to be present in the alkaline solutions of glucose, mannose, fructose and galactose. Furthermore, we began to find significant yields of lactic acid at approximately those alkali normalities which marked the maxima for acetic and formic acids. This intimate relationship between pyruvic aldehyde, acetic, formic
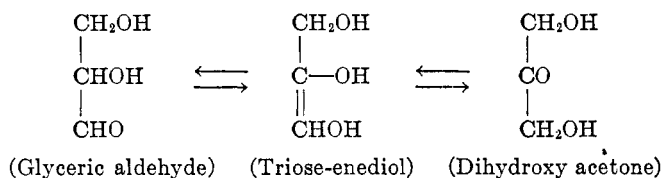
and lactic acid production has been observed in all the hexoses thus far studied. If glyceric aldehyde is the intermediate producing these changes then it follows that all the hexose sugars should react in the same way by reason of the presence of the common hexose (3-4) enediol formation. Formic acid is also regarded as being derived from the splitting of a 1-2 hexose enediol, and also as stated above from a succession of such reactions arising from the residual pentose, tetrose, triose and diose, resulting in each case. The recent paper of Gustus and Lewis (29) on the alkaline oxidation of 2, 3, 4, 6, tetramethyl glucose is an exceedingly important contribution in this field. Glycolic acid would be thought of as an index for the presence of a hexose 2-3 enediol. A precise quantitative method for its determination was not devised since in the glucose-mannose-series the glucose 3-4 enediol seemed to be the favored structure. However, in the galactose series we found that the amounts of galacto-meta-saccharinic acid lactone showed a definite relationship to the alkali normality used. If the increased concentration of the alkali changes the first splitting products of the hexose enediols into other enediols of smaller carbon atom content which will in themselves undergo a further fission reaction then it becomes clear that the alkaline solutions of all the hexose sugars will tend towards an identical composition at the higher alkalinities in the presence of oxidizing agents. These facts account for the greater reducing power observed in carbohydrate solutions as the concentration of the alkali is increased. In his recent important contribution on the copper number of glucose (30) Amick has shown that "the amount of the oxidation is dependent on the alkalinity of the solution." Stieglitz (31) has previously emphasized the fact that "the oxidation of glucose is enormously accelerated in the presence of alkali and it is retarded by the presence of neutralizing acids."

Finally, the experimental results obtained from a study of the behavior of the hexose sugars towards the variable factors of temperature and alkali concentration point strongly to the correctness of the view that a general equilibrated condition is present in these systems and that it may be disturbed by these same factors.

ACTION OF POTASSIUM HYDROXIDE ON THE TRIOSES

It now became necessary to test the validity of the assumption made above with reference to glyceric aldehyde being the forerunner of the lactic acid which was being derived from the alkaline solutions of these hexose sugars. If this assumption is valid then the same relationships which were found to exist between pyruvic aldehyde, lactic, acetic and formic acids in the alkaline solutions of glucose, mannose, fructose and galactose should also be found in alkaline solutions of glyceric aldehyde. Aqueous solutions of equivalent amounts of pure glyceric aldehyde crystals were treated with potassium hydroxide under exactly the same experimental conditions as those used with the sugars themselves (32). Much to our satisfaction *these same general relationships were found to exist in the reaction products of the triose as were found present in the hexoses.*

It should be noted that an equilibrium undoubtedly exists in the alkaline solutions of glyceric aldehyde similar to that observed by de Bruyn and van Ekenstein in the cases of the hexoses. This may be represented by the following equation:

$$
\begin{array}{ccccc}
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH} & & \text{CH}_2\text{OH} \\
| & & | & & | \\
\text{CHOH} & \rightleftharpoons & \text{C—OH} & \rightleftharpoons & \text{CO} \\
| & & \| & & | \\
\text{CHO} & & \text{CHOH} & & \text{CH}_2\text{OH} \\
\text{(Glyceric aldehyde)} & & \text{(Triose-enediol)} & & \text{(Dihydroxy acetone)}
\end{array}
$$

By reason of this possibility dihydroxyacetone (33) was studied in exactly the same manner as glyceric aldehyde had been. Here also we found the same facts with reference to pyruvic aldehyde, lactic, acetic, and formic acids as had been observed in alkaline solutions of glyceric aldehyde. It should be pointed out that lactic acid is the saccharinic acid of the trioses. In connection with the facts represented by the above equation it is to be noted that Fischer, Taube and Baer (34) recently converted glyceric aldehyde to dihydroxyacetone to the extent of 49 per cent.

From the relationships here described it seems to follow that the splitting product of a 3-4 hexose enediol, namely, glyceric

aldehyde is responsible for the production of lactic acid in alkaline solutions of glucose, fructose, mannose and galactose.

OXIDATION OF THE POLYSACCHARIDES. ACTION OF POTASSIUM HYDROXIDE ON MALTOSE

By reason of the results obtained with the trioses and the hexoses it became of much interest to examine the behavior of certain polysaccharides towards the oxidizing conditions employed throughout this work. We have studied the behavior of lactose, sucrose, maltose, cellose and raffinose towards neutral and alkaline potassium permanganate. Maltose is the only one of these compounds whose action towards aqueous solutions of potassium hydroxide has thus far been examined (35). These solutions were of such a strength that a comparative study could easily be made of the data obtained with our previous work on glucose.

The most recent work in the literature (36) shows that maltose is a 4-glucosido-glucose.
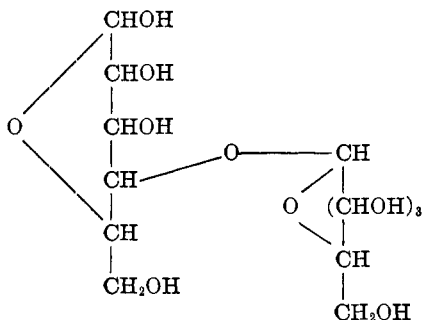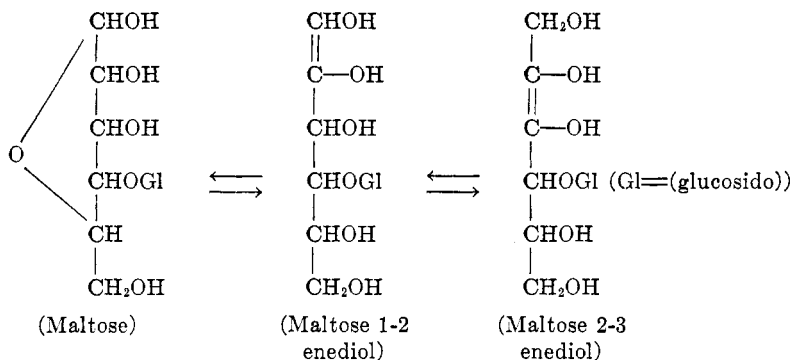

The oxidation products of maltose and of cellose were the same as those obtained with glucose under the same experimental conditions. Our experimental data show two important facts: (a) that the alkali concentration and temperature are the two factors which influence the amount and character of the reaction products; (b) that the first step in the alkaline oxidation of the maltose molecule is not one of a complete hydrolysis of the disaccharide into two molecules of glucose. If such an hydrolysis

had taken place it would naturally follow that the yields of oxalic acid and carbon dioxide would be identical with those of glucose. When maltose and cellose are oxidized at 50° with neutral and alkaline potassium permanganate, the reducing character of the solutions of maltose and cellose are not so great as those of an equivalent solution of glucose at the lower alkalinities and temperature. As the alkali normality is increased the reducing power of the disaccharides is greatly increased. These facts are shown in table 1.
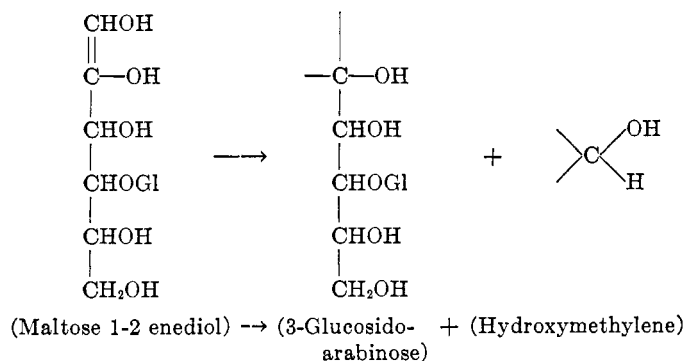
TABLE 1

DISACCHARIDE	NORMALITY OF KOH	WEIGHT OF DI-SACCHARIDE USED	DISACCHA-RIDE RECOVERED (GLUCOSE)	DISACCHA-RIDE OXIDIZED (GRAMS)	KMnO₄ USED BY DI-SACCHARIDE ACTUALLY OXIDIZED	KMnO₄ USED BY GLUCOSE EQUIVALENT TO 1.98 GRAMS OF DISAC-CHARIDE
Maltose	0.00	1.98	0.42	1.56	3.84	6.80
	0.05	1.98	0.34	1.64	5.34	8.53
	0.10	1.98	0.34	1.64	6.43	8.53
	0.25	1.98	0.31	1.67	6.80	8.53
	0.50	1.98	0.28	1.70	7.16	8.52
	1.00	1.98	0.25	1.73	7.35	8.53
Cellose	0.10	1.98	0.31	1.67	6.43	8.53
	0.25	1.98	0.29	1.69	6.80	8.53
	0.50	1.98	0.26	1.72	7.16	8.52

By reason of these facts a study was next made of the behavior of potassium hydroxide solutions towards maltose in the absence of potassium permanganate to see if the disaccharide would react in response to changes in alkali concentration and also to changes in temperature. This was found to be the case. Therefore *alkaline solutions of maltose must undoubtedly contain an equilibrated condition similar to that observed with the hexose sugars.* The complex components of such a system would be the 4-glucosido-hexose analogues of those compounds observed by de Bruyn and van Ekenstein in the glucose-mannose-fructose series. In addition to these there should be present certain 4-glucosido-glucose enediols (37) and their fission products.

$$
\begin{array}{ccc}
\text{CHOH} & \text{CHOH} & \text{CH}_2\text{OH} \\
| & \| & | \\
\text{CHOH} & \text{C—OH} & \text{C—OH} \\
| & | & \| \\
\text{CHOH} & \text{CHOH} & \text{C—OH} \\
\text{O} \quad | & | & | \\
\text{CHOGl} \rightleftharpoons & \text{CHOGl} \rightleftharpoons & \text{CHOGl (Gl=(glucosido))} \\
| & | & | \\
\text{CH} & \text{CHOH} & \text{CHOH} \\
| & | & | \\
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & \text{CH}_2\text{OH}
\end{array}
$$

(Maltose)   (Maltose 1-2   (Maltose 2-3
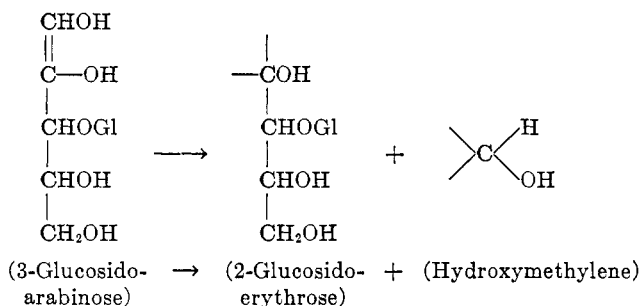   enediol)   enediol)

It is obvious that such a system should react to the changing alkali concentration and temperature in quite the same general way as the hexose sugars did.

Maltose 1-2 enediol (4-glucosido-glucose-1-2 enediol). A rupture of maltose 1-2 enediol would result in the formation of hydroxymethylene and 3-glucosido-arabinose according to the following equation.

$$
\begin{array}{cccc}
\text{CHOH} & & & \\
\| & & | & \\
\text{C—OH} & & \text{—C—OH} & \\
| & & | & \\
\text{CHOH} & \longrightarrow & \text{CHOH} & \quad + \quad \text{OH} \\
| & & | & \text{C} \\
\text{CHOGl} & & \text{CHOGl} & \quad \quad \text{H} \\
| & & | & \\
\text{CHOH} & & \text{CHOH} & \\
| & & | & \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH} &
\end{array}
$$

(Maltose 1-2 enediol) --→ (3-Glucosido- + (Hydroxymethylene)
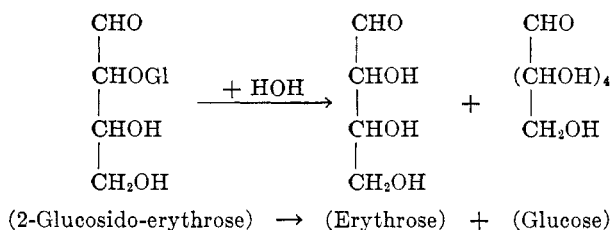   arabinose)

If the CHOGl group does not take part in the enolic changes then it is clear that the 3-glucosido-arabinose may lose an hydroxymethylene group and give 2-glucosido-erythrose as shown in the following equation.

$$
\begin{array}{ccc}
\text{CHOH} & & \\
\|\| & & | \\
\text{C—OH} & & \text{—COH} \\
| & & | \\
\text{CHOGl} & \longrightarrow & \text{CHOGl} \\
| & & | \\
\text{CHOH} & & \text{CHOH} \\
| & & | \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH}
\end{array}
\; + \; \;>\!\!C\!\!<^{\displaystyle H}_{\displaystyle OH}
$$

| (3-Glucosido- | → | (2-Glucosido- | + | (Hydroxymethylene) |
| arabinose) | | erythrose) | | |

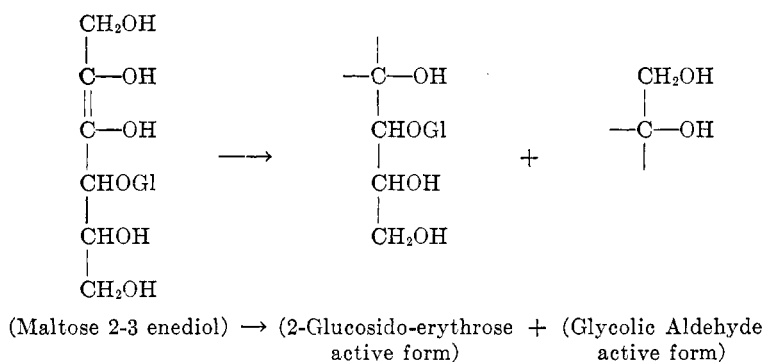The hydroxymethylene molecules are then converted to formic acid.

The 2-glucosido-erythrose must now hydrolyze to erythrose and glucose, as shown by the following equation, because it can not further enolize if the CHOGl group remains intact.

$$
\begin{array}{ccccc}
\text{CHO} & & \text{CHO} & & \text{CHO} \\
| & & | & & | \\
\text{CHOGl} & \xrightarrow{\;+\;\text{HOH}\;} & \text{CHOH} & + & \text{(CHOH)}_4 \\
| & & | & & | \\
\text{CHOH} & & \text{CHOH} & & \text{CH}_2\text{OH} \\
| & & | & & \\
\text{CH}_2\text{OH} & & \text{CH}_2\text{OH} &
\end{array}
$$

(2-Glucosido-erythrose) → (Erythrose) + (Glucose)

Nef (38) believed that tetroses in alkaline solutions reacted as 2-3 enediols, $CH_2OH \cdot C(OH) = C(OH) \cdot CH_2OH$, rather than as tetrose 1-2 enediols, $CH_2OH \cdot CHOH \cdot C(OH) = CHOH$. Under these circumstances erythrose could not yield lactic acids because glyceric aldehyde, the forerunner of lactic acid, would not be a fission product of the general reaction. Nef reached this conclusion because he was unable to find the least trace of lactic acid in (8.N) alkaline solutions of glycol aldehyde. However, he did obtain significant amounts of a four carbon atom saccharinic acid (1,3 dihydroxybutyric acid), thus showing that a tetrose had formed from glycolic aldehyde. We confirmed Nef's observation with reference to the presence of lactic acid. We used solutions of less normality. On the other hand Hutchman did succeed in obtaining from alkaline solutions of glycol aldehyde very minute amounts of 1-2 dihydroxybutyric acid and

the 1-3 dihydroxybutyric acid. These were identified through a synthesis of them and through a comparison with specimens kindly furnished to us by Dr. J. W. E. Glattfeld of the University of Chicago. The formation of these two acids seemed to be in harmony with the suggestion made by Miss Marjorie Pickard Benoy and the author with reference to the enediolic forms of the carbohydrates being forerunners of the saccharines. Although our proposed mechanism rests on no experimental basis yet it has aided us in tracing out the compounds which should form in these alkaline solutions.

It is evident therefore that the glucose section of the maltose molecule does not give rise to lactic acid through its action on potassium hydroxide. This follows as a matter of course from the preceding discussion. Obviously the lactic acid obtained from maltose must therefore be a product of the glucosido section which arises from the hydrolysis of the 2-glucosido-erythrose. *Maltose 2-3 enediol* (4-glucosido-glucose 2-3 enediol). As the concentration of the alkali is still further increased the equilibrium will be shifted towards the formation of maltose 2-3 enediol. When the maltose 2-3 enediol undergoes a fission at the double bond, 2-glucosido-erythrose and glycolic aldehyde will be formed as shown by the following equation.

$$
\begin{array}{ccccc}
\text{CH}_2\text{OH} & & & & \\
| & & | & & \\
\text{C—OH} & & \text{—C—OH} & & \text{CH}_2\text{OH} \\
\| & & | & & | \\
\text{C—OH} & \longrightarrow & \text{CHOGl} & + & \text{—C—OH} \\
| & & | & & | \\
\text{CHOGl} & & \text{CHOH} & & \\
| & & | & & \\
\text{CHOH} & & \text{CH}_2\text{OH} & & \\
| & & & & \\
\text{CH}_2\text{OH} & & & &
\end{array}
$$

(Maltose 2-3 enediol) → (2-Glucosido-erythrose + (Glycolic Aldehyde
active form)   active form)

Then it is clear that the 2-glucosido-erythrose will become a source of lactic acid through hydrolysis of the compound into glucose and erythrose as pointed out above.

If the maltose molecule reacts as just outlined, then the yield of lactic acid obtained from the action of aqueous solutions of potassium hydroxide will never be greater than approximately one-half that obtained from glucose. Obviously, the same should also be true for pyruvic aldehyde and acetic acid. Our experimental data show that this is true. Had hydrolysis preceded enolization then our yields of pyruvic aldehyde, lactic and acetic acids should be identical with those obtained from glucose under the same conditions. Glattfeld and Hanke (39) oxidized maltose in alkaline solution with air and also with hydrogen peroxide. On hydrolysis of the glucosido-acids obtained by them as reaction products, they identified d-erythronic lactone and arabonic acid, which was recognized by means of its phenylhydrazide. Another notable fact in this connection was the formation of the C_4 saccharinic acids. All these facts are in harmony with the reactions which have been outlined above for the alkaline decomposition of maltose. A comparison of the data obtained from the oxidation of lactose (40) with those obtained from the oxidation of an equivalent mixture of glucose and galactose shows that the disaccharide is not completely hydrolyzed into its monosaccharide components at the lower alkalinities and temperatures. At the higher alkalinities and higher temperatures our data are practically identical. The same general effect was observed with sucrose (41) and equivalent solutions of fructose and glucose. Raffinose (42) was oxidized and its products compared with the following equivalent solutions; (a) fructose, glucose and galactose; (b) sucrose and galactose; (c) melibiose and fructose.
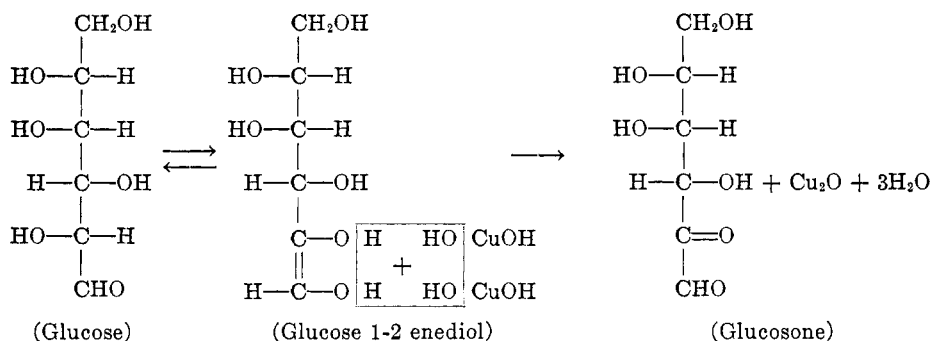
OXIDATION OF CARBOHYDRATES IN ACID SOLUTION

It is evident from the above discussion that the alkali is one of the most important factors having a definite influence in determining the character of the reaction taking place when the carbohydrates and the simpler organic compounds are oxidized in alkaline solutions. Hence, it became of great interest to know whether the reaction mechanism of carbohydrate oxidation in acid solutions could be explained on the principles given above.

In view of the results obtained with the hexose sugars, the question arose whether one or more of these enediols did not exist in solutions of pH value less than 7.

Copper acetate as an oxidizing agent. Copper acetate (43) had previously been used as an oxidizing agent in reactions of this character. It possesses the following distinct advantages; (*a*) The cupric ion is easily reduced to the cuprous condition and at times metallic copper is also found associated with the cuprous oxide. These facts furnish a ready and an accurate means of calculating the oxygen consumed. (*b*) The reaction with the hexoses was found to proceed fairly slowly, and could be stopped most easily. (*c*) Several of the theoretically possible intermediates were known to be stable towards this reagent. Obviously this fact was of extreme value in ascertaining some of the stages through which these oxidations passed. (*d*) The unused copper ion could be easily removed.
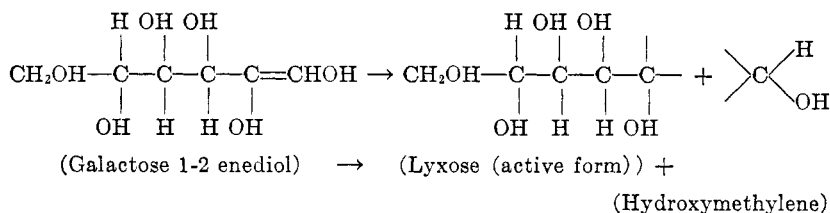
Reaction products. When glucose and fructose were oxidized with copper acetate the reaction mixtures were examined for the following products: formic, oxalic, and glyoxylic acids, carbon dioxide and glucosone. Although not measured quantitatively the presence of glycolic acid was also established. In galactose the same reaction products were obtained save the characteristic hexosone, galactosone. As the hydrogen ion concentration of the reaction mixtures increased the oxidation of glucose and fructose gradually ceased. This fact is one of the most importance when viewed in the light of the discussion above with reference to the function of the alkali (or the hydroxyl ion concentration) in the oxidation.

Reaction mechanism. Glucose and fructose. Owing to the presence of glucosone as one of the reaction products in the oxidation of glucose and fructose with copper acetate solutions, it was concluded that the formation of the hexosone was the first step in the reaction. The formation of gluconic acid from glucose is excluded as an intermediate since it was found that gluconic acid and galactonic acid lactone had no marked effect on aqueous copper acetate solutions. The presence of glucosone is easily understood from the following equations.

$$
\begin{array}{cccc}
\text{CH}_2\text{OH} & \text{CH}_2\text{OH} & & \text{CH}_2\text{OH} \\
| & | & & | \\
\text{HO--C--H} & \text{HO--C--H} & & \text{HO--C--H} \\
| & | & & | \\
\text{HO--C--H} & \text{HO--C--H} & & \text{HO--C--H} \\
| & | & & | \\
\text{H--C--OH} \;\rightleftharpoons\; & \text{H--C--OH} & \longrightarrow & \text{H--C--OH} + \text{Cu}_2\text{O} + 3\text{H}_2\text{O} \\
| & | & & | \\
\text{HO--C--H} & \text{C--O}\;|\;\text{H}\quad\text{HO}\,|\;\text{CuOH} & & \text{C=O} \\
| & \quad||\qquad\quad + & & | \\
\text{CHO} & \text{H--C--O}\;|\;\text{H}\quad\text{HO}\,|\;\text{CuOH} & & \text{CHO} \\
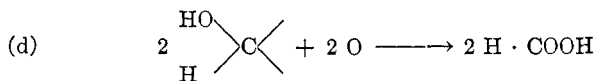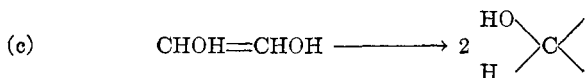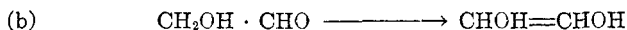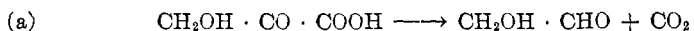\text{(Glucose)} & \text{(Glucose 1-2 enediol)} & & \text{(Glucosone)}
\end{array}
$$

This interpretation would regard the hexose enediol as an acid. Some of the evidence in favor of the acidic character of the carbohydrates is as follows: (*a*) Michaelis and Rona (44) found the dissociation constant for glucose to be 6.6×10^{-13}, for fructose 8.8×10^{-13}, and for galactose 5.3×10^{-13}. Osaka (45) computed that of glucose to be 5.9×10^{-13} at 25°. Kullgren (46) found that of invert sugar to be 7.2×10^{-13} and that of cane sugar 1.05×10^{-13} at 21°. (*b*) Powell (47) has shown both by conductivity methods and also by the change of rate in the hydrolysis of ethyl acetate in the presence of fructose and glucose that these carbohydrates form definite compounds with the base, and in the case of sodium hydroxide the reaction takes place in the ratio of one molecule of each. (*c*) Wolfrom and Lewis (48) found that the pH value of one liter of lime water changed from 12.6 to 10.6 on the addition of one mole of d-glucose.

Formic acid and hydroxymethylene. The 1-2 hexose enediols may suffer a decomposition as is shown for example in the case of galactose in the following equation.

$$
\begin{array}{c}
\quad\;\text{H}\;\;\text{OH}\;\text{OH} \qquad\qquad\qquad\quad \text{H}\;\;\text{OH}\;\text{OH} \\
\quad\;|\;\;\;|\;\;\;| \qquad\qquad\qquad\qquad |\;\;\;|\;\;\;|\;\;\;| \\
\text{CH}_2\text{OH--C--C--C--C=CHOH} \rightarrow \text{CH}_2\text{OH--C--C--C--C--} + \; \begin{array}{c} \diagup\text{H} \\ \text{C} \\ \diagdown\text{OH} \end{array} \\
\quad\;|\;\;\;|\;\;\;|\;\;\;| \qquad\qquad\qquad\qquad\; |\;\;\;|\;\;\;|\;\;\;| \\
\quad\;\text{OH}\;\text{H}\;\;\text{H}\;\text{OH} \qquad\qquad\qquad\; \text{OH}\;\text{H}\;\;\text{H}\;\text{OH}
\end{array}
$$

(Galactose 1-2 enediol) $\longrightarrow$ (Lyxose (active form)) $+$

(Hydroxymethylene)

It is clear that the pentose may undergo oxidation to lyxonic acid or it may enolize and split at the double bond in the same manner as the hexose. In our studies thus far carried out, no work has yet been undertaken along this line.
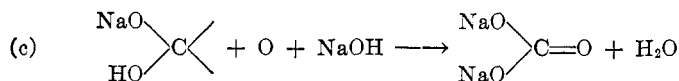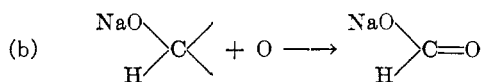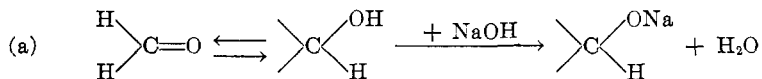
The hydroxymethylene thus formed would be oxidized to formic acid under the conditions of an experiment. In order to experimentally test the assumption that hydroxymethylene, if formed, would undergo an oxidation to formic acid, a study was made of the behavior of hydroxypyruvic acid towards aqueous solution of copper acetate. It was found that this compound was oxidized practically quantitatively to formic acid (95 per cent), and carbon dioxide. The oxygen consumed was found to be 90.5 per cent of that demanded by the use of 2.00 gram atom equivalents. This behavior is easily understood on the basis of the following equations,

(a) $$CH_2OH \cdot CO \cdot COOH \longrightarrow CH_2OH \cdot CHO + CO_2$$

(b) $$CH_2OH \cdot CHO \longrightarrow CHOH{=}CHOH$$

(c) $$CHOH{=}CHOH \longrightarrow 2 \begin{array}{c} HO \\ \\ H \end{array}\!\!>\!\!C\!\!<$$

(d) $$2 \begin{array}{c} HO \\ \\ H \end{array}\!\!>\!\!C\!\!< + 2 O \longrightarrow 2 H \cdot COOH$$

That glycol aldehyde itself will behave towards copper acetate to yield formic acid was shown to be the fact by a separate experiment. This observation is in harmony with that of Heimrod and Levene (49) on the alkaline oxidation of glycol aldehyde with hydrogen peroxide. Hence, in these studies dealing with the mechanism of carbohydrate oxidation hydroxymethylene and its derivatives are considered as active forms of these compounds. In his recent series of lectures at Rice Institute Baly (50) gave expression to the following point of view. "In view of the remarkable reactivity of activated formaldehyde it seems to me necessary to picture a different formula from that given to ordinary formaldehyde. This abnormal activity is well expressed by

the Nef formula, $C\!<^H_{OH}$, with the carbon bivalent." In 1896 Nef (51) himself stated that "Die physiologischen Wirkungen des Formaldehyds beruhen zweifellos auf einer Dissociation desselben in Oxymethylen." ("The physiological activities of formaldehyde doubtless depend upon a dissociation of it into oxymethylene.") In this connection it is interesting to observe that McLeod (52) found that formaldehyde and formic acid are unacted upon by copper acetate solutions. Again the important rôle played by alkalies in these oxidations becomes very evident when one examines the behavior of formaldehyde towards oxidizing agents. If hydroxymethylene is formed in these reactions it is clear that it will be oxidized to formic acid. It is a well known fact that formates are oxidized in alkaline solutions to carbon dioxide. The mechanism of this change is easily understood if one considers that the alkali merely causes a shift in the equilibrium expressed by the following equation.
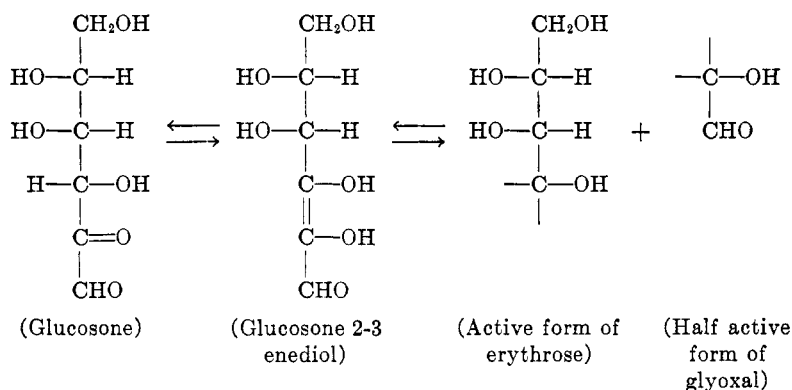
(a) $\quad {}^H_H{>}C{=}O \;\underset{\longrightarrow}{\longleftarrow}\; {>}C{<}^{OH}_{H} \;\xrightarrow{+\ NaOH}\; {>}C{<}^{ONa}_{H} + H_2O$

(b) $\quad {}^{NaO}_{H}{>}C{<} + O \longrightarrow {}^{NaO}_{H}{>}C{=}O$

(c) $\quad {}^{NaO}_{HO}{>}C{<} + O + NaOH \longrightarrow {}^{NaO}_{NaO}{>}C{=}O + H_2O$

Applying this point of view to the direct oxidation of aldehydes we have the following general reaction,

$\quad {}^R_H{>}C{=}O \quad \underset{\longrightarrow}{\longleftarrow} \quad {}^R_{HO}{>}C{<}$

(Inactive to oxidation)   (Active to oxidation)
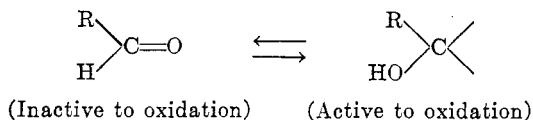
What causes the formation of the active molecules is a problem yet to be solved. *Carbon Dioxide.* The presence of carbon

dioxide in these reactions is thought to be due to the decomposition of the keto-acids. It has been shown above that hydroxy-pyruvic acid reacts in this manner. *Oxalic, Glyoxylic and Glycolic Acid.* If it is assumed that glucosone and galactosone may also form enediols which will rupture at the double bond in the same manner as in alkaline solutions, then a simple mechanism is available for the reactions producing oxalic, glyoxylic and glycolic acid. The hexosone 2-3 enediol will give rise to the corresponding tetrose and glyoxal in accordance with the following equation.

$$
\begin{array}{ccccc}
CH_2OH & CH_2OH & CH_2OH & & \\
| & | & | & & | \\
HO-C-H & HO-C-H & HO-C-H & & -C-OH \\
| & | & | & & | \\
HO-C-H & \rightleftharpoons \; HO-C-H & \rightleftharpoons \; HO-C-H & + & CHO \\
| & | & | & & \\
H-C-OH & C-OH & -C-OH & & \\
| & || & | & & \\
C{=}O & C-OH & & & \\
| & | & & & \\
CHO & CHO & & &
\end{array}
$$

(Glucosone)   (Glucosone 2-3   (Active form of   (Half active
                        enediol)   erythrose)   form of
                                                                            glyoxal)

We did not examine our reaction mixtures for the tetronic acids. However, Morrell and Crofts (53) obtained erythronic acid through the oxidation of glucosone with bromine. The glyoxal (semi-active form) will be oxidized to glyoxylic acid as shown by the following reaction.

$$
\begin{array}{ccc}
| & & \\
-C-OH & & O{=}C-OH \\
| & + O \longrightarrow & | \\
CHO & & CHO
\end{array}
$$
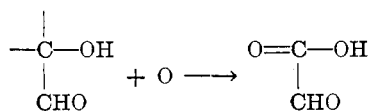
The justification for expressing the glyoxal in the active form lies in the experimental fact that specially prepared glyoxal itself does not reduce copper acetate under the conditions of our experiments (50°C). Furthermore, this would seem to be in harmony with the point of view expressed above to the effect that the activated condition of an aldehyde group is best expressed with two valences of the carbon in the unsaturated

condition. In both cases it is strongly suggestive of what has been summed up under the term *nascent*. Debus (54) has shown that glyoxylic acid will rearrange into oxalic acid and glycolic acid. Furthermore he observed that glyoxylic acid does not reduce copper acetate.

CONCLUSION

Finally, it is evident that the molecular stages through which organic compounds pass when they are oxidized in alkaline solutions are largely determined by the normality of the base used. This factor seems to have a marked influence on the status of any equilibrated condition which may be present in any of these oxidation systems. Through a knowledge of theoretically possible intermediates and this latter fact it is possible to account for the presence of a given oxidation product.

In the alkaline oxidation of ethyl alcohol the critical equilibrium is between α-hydroxyethylidene, acetaldehyde and vinyl alcohol; in isopropyl alcohol solutions it lies between isopropylidene, acetone and isoacetone; in the hexose sugars the equilibrated mixture consists of the isomeric hexoses studied by de Bruyn and van Ekenstein and the several enediolic hexose isomers postulated by Nef and their ultimate fission products. These equilibrated systems are markedly disturbed by the magnitude of the alkali concentration and the temperature used.

When these alkaline systems are studied in the absence of an oxidizing agent it is possible to prove experimentally the effect produced by the varying alkali concentration. In the α-hydroxyethylidene-acetaldehyde-vinyl alcohol system the formation of vinyl alcohol is a linear function of the alkali concentration; in the acetone-isoacetone system the concentration of the isoacetone is a logarithmic function of the base used. In alkaline solutions of the hexose sugars and in the disaccharide, maltose, the amounts of lactic acid, pyruvic aldehyde, formic and acetic acids formed are definite functions of the alkali used. The same relationship also holds for the two trioses, glyceric aldehyde and dihydroxyacetone.

In the oxidaton of glucose, fructose and galactose in copper

acetate solutions it is possible to trace the reaction mechanism in this acid medium if one assumes an equilibrated condition of certain enediolic forms of the hexose as well as of the hexosone. The tendency of the increased hydrogen ion concentration is to suppress the progress of the oxidation.

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