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COMPARATIVE STUDIES ON THE OXIDATION OF POLYHYDRIC ALCOHOLS BY BIOLOGICAL AND NON-BIOLOGICAL MEANS

by

JOSEPH EVERETT HUNTER, JR.

A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject Biophysical Chemistry

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INTRODUCTION

During the past quarter of a century the competition between chemical synthesis and micro-biological methods for producing numerous organic compounds has become ever increasingly keen. A number of so-called heavy chemicals have been prepared at one time or another by both methods. Notable among these are acetone, acetic acid, glycerol, ethyl alcohol, higher alcohols, and gluconic acid. This last is usually obtained as the calcium salt.

Of considerable interest at present is the oxidation of glucose in the presence of calcium carbonate to obtain calcium gluconate. This oxidation has been carried out by Wells, Moyer, Stubbs, Herrick, and May (1937) using *Aspergillus niger* in nutrient medium and by Isball and Frush (1931) using carbon electrodes in a glucose solution containing sodium bromide as the electrolyte. Since both methods give excellent yields under almost identical conditions of temperature and concentration, it would seem that there is some relationship between micro-biological oxidation and oxidation at an electrode.

Fink and Summers (1938) show in their investigation that both sodium chloride and sodium iodide may be used instead of sodium bromide but the current yield is decidedly less. The explanation offered is that sodium bromide acts as a catalyst for the oxidation of glucose to gluconic acid. If this is so, and the evidence is all in its favor, why then cannot the conditions such as current density, electrode material, electrolytes, and catalysts or carriers be so arranged as to carry out a large number of reactions previously considered to be essentially biochemical?

In the case of the Acetobacter it is well known that different species will oxidize compounds to different end products depending on the intensity of their oxidation.

There is also a variation of oxidation ability of various electrodes. This phenomenon may well be associated with the "oxygen overvoltage." It should be at once stated that the term "oxygen overvoltage," since the oxygen electrode is not thermodynamically reversible, may not be strictly analogous to true "overvoltage." Nevertheless, it will be shown that the concept has a practical utility in describing the phenomena to be discussed. Certain species of Acetobacter show a specific oxidation toward polyhydric alcohols, that is, they show a preferential oxidation of the secondary alcohol group next to the terminal primary alcohol group. The question at once arises as to whether the above phenomenon is associated with a definite oxidation potential, with the ability of the organism to activate the molecule in a particular manner, or both. Marek and Hahn (1932) in speaking of catalytic dehydrogenation of alcohols state, "The higher primary alcohols require relatively higher temperatures for decomposition than the corresponding secondary alcohols." This statement leads one to believe that there is an oxidation potential which will just oxidize a secondary alcohol group but will not affect a primary alcohol group.

It is the purpose of this thesis to compare pure chemical and electrolytic oxidations of hydroxyl-containing compounds with the biochemical oxidation of similar compounds in the hope that material will be compiled which, though it may not fully answer the questions previously raised, will serve as part of the foundation for further work. It is doubtful that oxidations in vitro can be controlled to such an extent that yields as high as those obtained in biochemical oxidation will be realized, but data should be obtained which may be helpful in correlating these oxidation mechanisms.

HISTORICAL

When living organisms grow and multiply in some nutrient medium the carbon source serves two purposes: a source of energy for the organism and a source of cell constituent building material. Most reactions used by organisms to obtain energy are oxidation reactions, since, as a rule they are exothermic, that is, show a decrease in free energy. The above are spoken of as oxidation-reduction reactions because one never observes the oxidation of a substrate without a corresponding reduction of some substance, either atmospheric oxygen or another hydrogen acceptor. During the process of cell metabolism the materials which undergo chemical conversion by the enzymic system of the cell are produced in various degrees of oxidation depending on the organism. For example, Acetobacter suboxydans will oxidize glucose to gluconic acid, while Clostridium butylicum changes it to butyl alcohol and acetone; yeast forms ethyl alcohol and carbon dioxide by breaking down the glucose molecule.

Ever since Pasteur proved that micro-organisms are the cause of the seeming spontaneous chemical changes which take place in sugar containing medium, man has been trying to explain how these changes are brought about. As a result of the great volume of work done it has been shown that enzymes or organic catalysts are responsible for the activation of the molecules which undergo change. This activation is necessary; for even though a chemical transformation serves as a source of energy by showing a decrease in free energy, the reaction does not necessarily go spontaneously until the reacting molecules have been put in an activated state. For example, let us consider any compound *A* with a certain energy content which reacts in such a way that the resulting compound *B* has a smaller energy content. In this hypothetical case the molecules of compound *A* must have their energy content raised to *C* before they can react to give *B*. Figure I shows that the system must go over an "energy hill" of height E_1 before the chemical change can occur. As the change occurs energy E_2 is evolved, and the net decrease in free energy for the reaction is $E_2 - E_1$. It is evident, therefore, that synthetic processes by bacteria, E_1 is larger than E_2, must be coupled with reactions corresponding to a diminu-

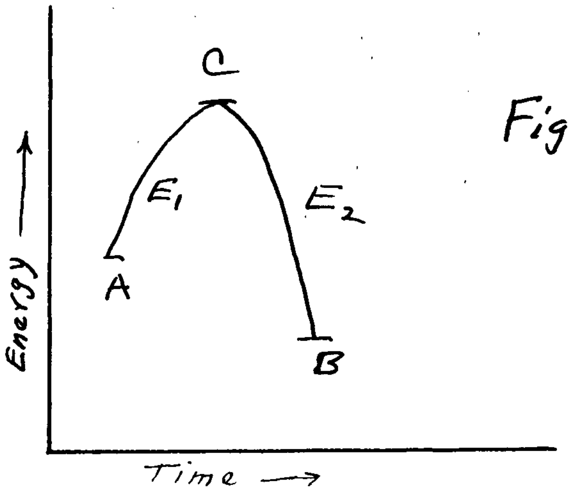


Figure I

tion of free energy, that is, the reaction is a biochemical oxidation-reduction.

By using a catalyst it is possible to go from A to B at a greater velocity than without a catalyst. Concerning the action of the catalyst, Schwab (1937) states:

> "It is evident that the velocity is unequivocally fixed by the constants and interrelationships of the separate elementary processes. However it is not necessary to assume that the 'catalytic force' is a mysterious action at a distance of which a particular substance brings about an alteration in the constants. We have to suppose, rather, that the catalyst enters directly into the mechanism of the reaction. Because of the definiteness of the velocity which we have just mentioned, the appearance of new velocities simply means the operation of fresh elementary reactions or of new arrangements between them, with the participation of the catalyst. Thus, it is not so much that the velocity of the old reaction is raised, but that the catalyst creates a new path, which makes possible a greater velocity and this new path is dependent upon the presence of the catalyst, because the latter takes part in the reaction. The requirement that the catalyst, K, should 'not appear in the end products of the reaction' is fulfilled by its final regeneration in a manner such as the following:
>
> 1. $A + K = AK$
>
> 2. $AK + B = AB + K$
>
> **Total:** $A + B = AB$ catalyzed by K
>
> This is an example of catalysis by an intermediate compound. Most positive catalyses can be explained in this way."

In respect to activation by the catalyst Schwab states: "Activation leading to a spontaneous reaction can always be interpreted as a non-spontaneous endothermic initial step and its acceleration by a reaction involving the catalyst can be conceived as an induction of this kind. The distinction is only that a successive exothermic reaction releases enough energy to return the catalyst partly or completely to its original state. Such processes would be considered as catalytic, since here the amount of catalyst consumed depends on the conditions and, from the standpoint energetic, has zero as a limit." These ideas are very much in agreement with the ideas of enzymic action given below.

Kluyver and Donker (1925) postulate a theory of microbiological oxidation which combines with the oxygen activation theory of Warburg (1924) with that of Wieland (1923) which explains biochemical oxidation as a catalytic transfer of hydrogen. By combining these theories, a respiration becomes a process whereby cells can derive energy necessary for growth either by aerobic or anaerobic oxidation of the substrate.

Aerobic respiration, termed oxidative dissimilation, is the transformation of the substrate in the presence of free oxygen into compounds which are excreted. Anaerobic respiration, termed fermentative dissimilation, is the transformation of the substrate in which free oxygen takes no part; instead other hydrogen acceptors are present.

The oxidation of glucose to gluconic acid or of sorbitol to sorbose by Acetobacter suboxydans is a very good example of an aerobic oxidation, whereas the transformation of glucose into butyl alcohol and other products of *Clostridium acetobutylicum* is anaerobic respiration or fermentative dissimilation.

The respiration processes, according to Kluyver and Donker (1925), are fundamentally the same, and the observed differences are due to varying affinity of the protoplasm of the cells for hydrogen. According to this concept it is no longer necessary to assume a different enzyme system for each single biochemical reaction. However they admit that different hydrolytic enzymes are necessary for complex carbohydrates, fats, and proteins. The differences which have been accredited to such enzymes as carboxylase, alcoholoxidase, aldehydomutase, catalase, etc., are supposed to be due to different affinity for hydrogen shown by the protoplasm. In view of the ideas presented here a classification has been presented which groups the species of bacteria of similar respiratory and morphological actions together. In all there are seven groups which are placed in the order of the decreasing affinity of the protoplasm for hydrogen and increasing affinity for oxygen.

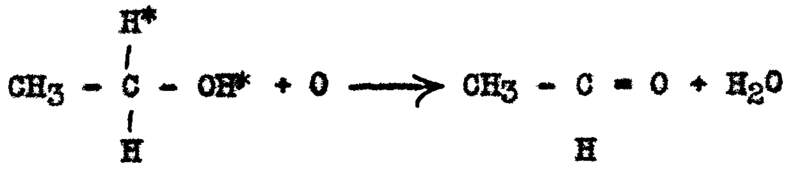
Keilin (1929) has formulated a scheme of micro-biological respiration which also combines the theories of Warburg and Wieland and seems to give a very good picture of the mechanisms of respiration. This theory of aerobic respiration mechanism introduces the pigment cytochrome. The scheme then becomes dehydrogenase-substrate-cytrochrome-oxidase-oxygen. The dehydrogenase in Keilin's system, as in Wieland's, has the power to activate the substrate molecules, while the cytochrome-oxidase portion is identical with the substance which Warburg regards as essential for biological oxidation by molecular oxygen. In the presence of a suitable dehydrogenase the cytochrome-oxydase-oxygen system may be replaced by some other hydrogen acceptor such as methylene blue, thus becoming anaerobic. The occurrence of the pigment cytochrome appears to be very widespread; it has been isolated from numerous bacteria. A great deal of experimental evidence has been found which tends to show that it is quite important in oxidation-reduction systems. On consideration of what has been said concerning the proposed respiration schemes, it is clear that the actual mechanism of cell respiration probably is a rather complicated setup.

Stephenson (1935) states that the cytochrome system in Acetobacter is responsible for practically all the respiration. This is rather interesting because their respiratory metabolism is characterized by the incompleteness of the oxidations which they bring about. Bertrand (1898) was the first to publish an extensive study of the Acetobacter. Later (1904) he presented a literature review of the biochemistry of the organism.

It was the characteristic action of the Acetobacter, the transformation of ethyl alcohol into acetic acid, which led Weiland to extend his dehydrogenation theory of oxidation to biological processes. The controversy which arose over the mechanism of the acetaldehyde to acetic acid reaction has not been completely settled, but the evidence is very strong that under usual conditions the transformation is a direct dehydrogenation of the hydrated acetaldehyde and not a dismutation (Cannizzaro reaction).

The generally accepted mechanism of the conversion of ethyl alcohol to acetic acid may be visualized as:

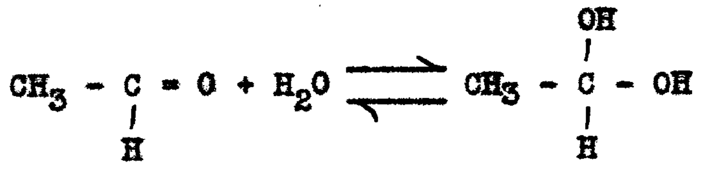
1. A catalytic dehydrogenation of ethyl alcohol to acetaldehyde.



$$CH_3 - \overset{H^*}{\underset{H}{C}} - OH^* + O \longrightarrow CH_3 - \underset{H}{C} = O + H_2O$$

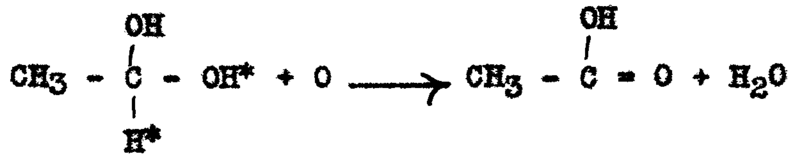
Neuberg and Nord (1919) proved acetaldehyde was an intermediate product by fixing it with neutral calcium sulfite.

2. Hydration of acetaldehyde molecule.



$$CH_3 - \underset{H}{C} = O + H_2O \rightleftharpoons CH_3 - \overset{OH}{\underset{H}{C}} - OH$$

3. Activation and dehydrogenation of the hydrated acetaldehyde.



$$CH_3 - \overset{OH}{\underset{H^*}{C}} - OH^* + O \longrightarrow CH_3 - \overset{OH}{C} = O + H_2O$$

*Denotes activated atom.

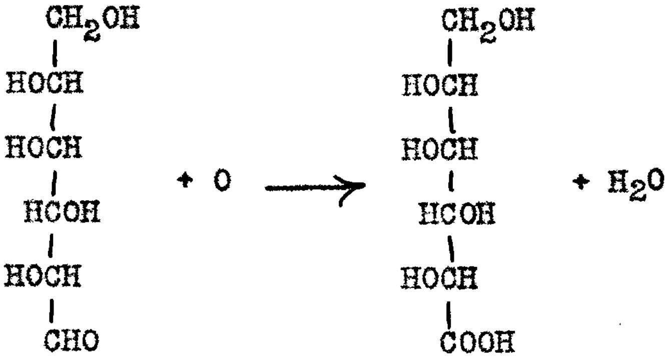
In defense of the last two points Wieland and Bertho (1928) showed that atmospheric oxygen could be replaced by other hydrogen acceptors such as methylene blue and benzoquinone. When both oxygen and quinone were present the oxygen was used only after practically all the quinone was reduced. They also showed that, although a Cannizzaro reaction does take place, it is so slow that it can account for only a very small fraction of the acetic acid formed. Tammiya and Tanaka (1930) have shown that the dehydrogenation of both alcohol and acetaldehyde hydrate is strongly inhibited by carbon monoxide in the dark. This would seem to show that the cytochrome-oxidase system is the one responsible for the oxidation.

Butlin (1936) groups the various chemical transformations of the acetic acid bacteria under six heads as follows:

(a) The oxidative (aerobic) dissimilation of sugars.
(b) Fermentative (anaerobic) dissimilation of the acetic acid bacteria.
(c) The oxidative dissimilation of alcohols.
(d) The oxidative dissimilation of acids.
(e) Polysaccharide synthesis by the acetic acid bacteria.
(f) The oxidative dissimilation of amino-acids.

Only two, (a) and (c), of these are relative to this research and are therefore the only parts which will be discussed.

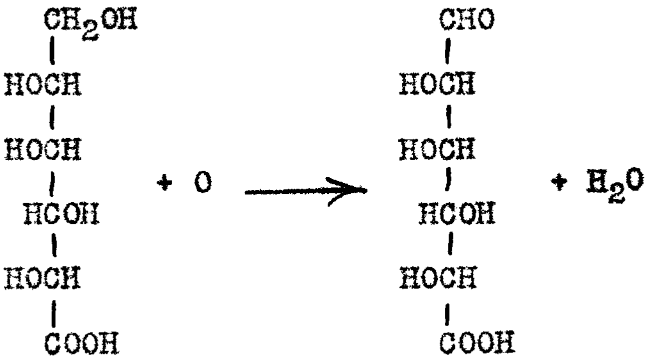
The acetic acid bacteria have been classified by Kluyver and Donker (1924) as "aerobic organisms with strong oxidative (dehydrogenating) powers, characterized by the direct dehydrogenation of glucose to gluconic acid in the presence of powerful hydrogen acceptors such as oxygen and methylene blue." The reaction expected would be:



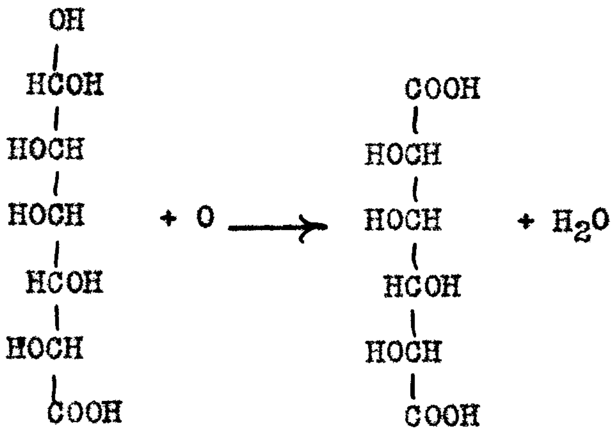
$$\begin{array}{c} CH_2OH \\ | \\ HOCH \\ | \\ HOCH \\ | \\ HCOH \\ | \\ HOCH \\ | \\ CHO \end{array} + O \longrightarrow \begin{array}{c} CH_2OH \\ | \\ HOCH \\ | \\ HOCH \\ | \\ HCOH \\ | \\ HOCH \\ | \\ COOH \end{array} + H_2O$$

Under the right conditions, given by Currie and Carter (1933), gluconic acid is formed and can be isolated as the calcium salt.

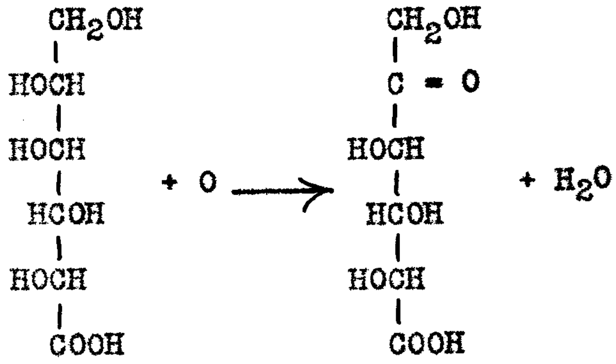
If, after the formation of gluconic acid, a further dehydrogenation takes place one would expect the primary alcohol group to be converted into 6-aldehydo-gluconic acid and then further dehydrogenated to saccharic acid according to the equations:



$$\begin{array}{c} CH_2OH \\ | \\ HOCH \\ | \\ HOCH \\ | \\ HCOH \\ | \\ HOCH \\ | \\ COOH \end{array} + O \longrightarrow \begin{array}{c} CHO \\ | \\ HOCH \\ | \\ HOCH \\ | \\ HCOH \\ | \\ HOCH \\ | \\ COOH \end{array} + H_2O$$



Visser't Hooft (1925) reports that 5-keto-gluconic acid is formed by the second dehydrogenation and no saccharic acid is found, showing that due to steric configuration or some other cause the secondary alcohol group is attacked before the primary alcohol group. Therefore instead of the above reactions the actual path taken by the dehydrogenation is:



Very little can be found in the literature concerning the aerobic dissimilation of other simple sugars, but it is to be expected that dehydrogenations similar to that of glucose would take place in the case of the aldehydo-trioses, tetroses, pentoses, and hexoses. Visser't Hooft (1925) reports acid formation in rhamnose, galactose, and maltose solutions while Kluyver and de Leeuw (1924) find that 5-ketogluconic acid is produced from maltose solutions. This would suggest that the maltose is first hydrolyzed to its components.

Due largely to Bertrand's (1898) classical research with the sorbose bacteria their dehydrogenation action on polyhydric alcohols has been much more thoroughly investigated. Although more recent research has rather confused the problem of configuration in regard to dissimilation by <u>Acetobacter</u>, Bertrand's ideas on this subject are still generally accepted. He tested a number of polyalcohols in 2 per cent solutions containing 5 per cent yeast water and concluded that when compounds made up of primary and secondary alcohol groups, as are the polyhydric alcohols, are oxidized by the sorbose bacteria, only the secondary alcohol grouping is attacked and converted into the ketone group in the beta position, and the dehydrogenation depends on the configuration of the molecule. The favorable configuration is that in which the beta and gamma hydroxyls are in the cis position. To support these conclusions Bertrand (1898) reported that glycerol, erythritol, l-arabinose, d-sorbitol, mannitol, persitol, and volimitol were dehydrogenated to beta-ketonic compounds, while l-xylitol and dulcitol were not attacked.

The mechanism of the dehydrogenation is explained by assuming that the sorbose bacteria activate the beta hydrogen atoms in such a way that they are easily stripped off by oxygen or other hydrogen acceptors.

The rules concerning favorable configuration of the alcohol molecule which Bertrand gave have been questioned by Votocek, Valentin, and Rac (1930) who were unable to oxidize rhodeitol, l-rhamnose, or alpha- and beta-rhamnohexitol, and more recently by Hann, Tilden, and Hudson (1938) who carried out experiments using sixteen sugar alcohols and related compounds. They reported that d-arabitol and l-fucitol were oxidized whereas l-arabitol and d-lactositol were not attacked.

The action of <u>Acetobacter suboxydans</u> which was first reported by Kluyver and de Leeuw (1924) differs from that of the acetic acid bacteria as regards intensity of oxidation. Their action is so mild that glucose is dehydrogenated to gluconic acid or 5-keto-gluconic acid and no further even when the culture is artificially aerated. This characteristic suggests a solution to the question of the feasibility of greater aeration of culture to increase the rate of oxidation, which was brought up when Bertho (1929) found that the velocity of dehydrogenation of isopropyl alcohol to acetone using oxygen as a hydrogen acceptor is directly proportional to the concentration of oxygen.

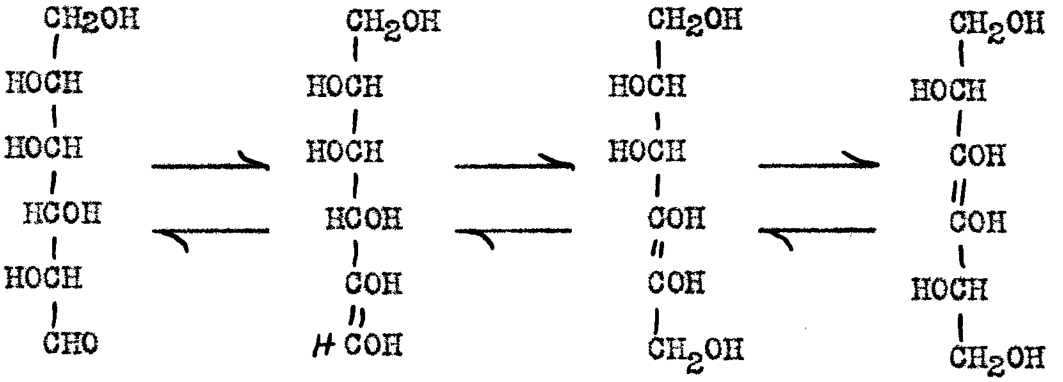
Fulmer, Dunning, Guymon, and Underkofler (1936) have studied the effect of concentration of sorbitol upon the production of sorbose by the action of Acetobacter suboxydans. They found that at a concentration of 35 per cent sorbitol or below approximately 80 per cent can be recovered as sorbose. The use of high concentrations of sorbitol affords an easy method for laboratory production of sorbose and therefore has stimulated interest in the production of sorbose for the synthesis of vitamin C. Wells, Stubbs, Lockwood, and Roe (1937) have adapted the process to use submerged growth of Acetobacter suboxydans in a rotating drum developed for the production of gluconic acid by molds. By using pressure aeration of the culture a 93 per cent yield of sorbose from 15 per cent sorbitol solution can be obtained in 24 hours.

The above brief discussion has given an insight into the biological oxidation action, especially aerobic dissimilation of certain carbohydrates and polyhydric alcohols. While a great deal of information is available concerning the pure chemical oxidation of sugars, very few data are available on the oxidation of polyhydric alcohols.

In strong acid and basic solutions the carbohydrates undergo rather complicated isomerization and degradation reactions giving many and varied products. When they are subjected to long treatment, at high temperature, with powerful oxidizing agents such as potassium permanganate, potassium dichromate, and ammonium persulfate the products range from the mono- and di-carboxylic acids to carbon dioxide and water. Of more interest to this study, however, are the products of oxidation using mild conditions which cause the minimum of degradation of the molecule.

In general, chemical oxidation of the sugars can be divided into two classes: (1) those carried out in alkaline medium and (2) those accomplished in acid solution. The first class has been studied especially by Nef (1904) and Evans (1929). The second has been investigated by Everett and Sheppard (1936) using bromine and dilute nitric acid and by Ridgeway (1931) employing potassium permanganate at a pH corresponding to that used with bromine.

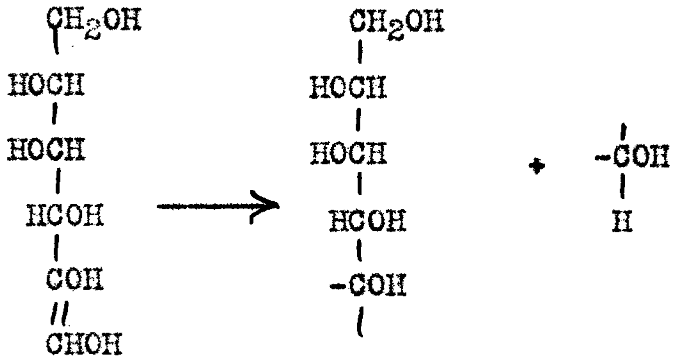
In alkaline solutions sugars show a marked tendency to isomerize. At low temperatures and very dilute solutions this isomerization may be negligible but as a rule degradation of the molecule is predominant. Evans (1929) has reviewed the studies made in his laboratory on the reactions of sugars in alkaline solutions both in the presence of and absence of an oxidizing agent. The products of the oxidation are explained on the basis of the formation of "enediol" and subsequent oxidation and splitting of the sugar at the double bond thus formed.



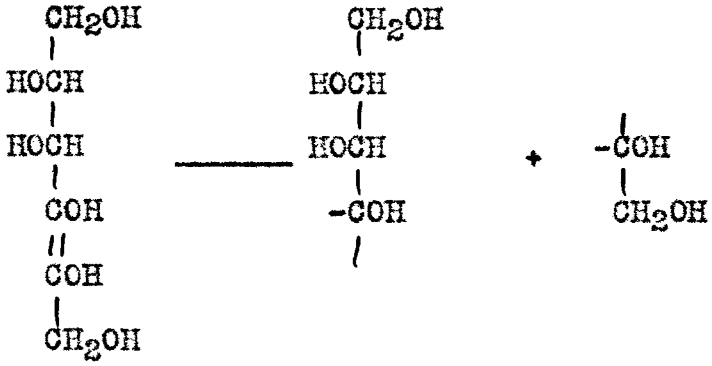
d-glucose glucose 1-2 enediol glucose 2-3 enediol glucose 3-4 enediol

When the double bonds are ruptured the following reactions take place:

(1)



(2)



To support the theory set forth by the equations given above, a study of the reactions of sugars in alkaline solution but in the absence of an oxidizing agent was made. By comparing the formation of the several products to the concentration of the base Evans (1929) found that the amounts of lactic acid, pyruvic aldehyde, acetic acid, and formic acid formed on oxidation were definite functions of the alkali concentration.

Everett and Sheppard (1936) have made extensive quantitative studies of the oxidation of more than fifty carbohydrates by bromine and less thorough studies using dilute nitric acid. In many respects, they found the oxidation by dilute nitric to resemble very closely the oxidation by bromine. All oxidations at 25° C. were allowed to run for forty-two days, and in the course of the oxidation by bromine several stages could be recognized.

Aldoses as a rule were oxidized in three stages: first, monocarboxylic acids were formed; the second step resulted in keturonic acid and some dicarboxylic acid formation; and finally, the keturonic acids themselves were oxidized. When ketoses were subjected to the same conditions of oxidation as the aldoses it was found that they were only partially changed to keturonic acids. The sugar alcohols were found to be oxidized first to ketones and then the oxidation characteristic of ketones took place.

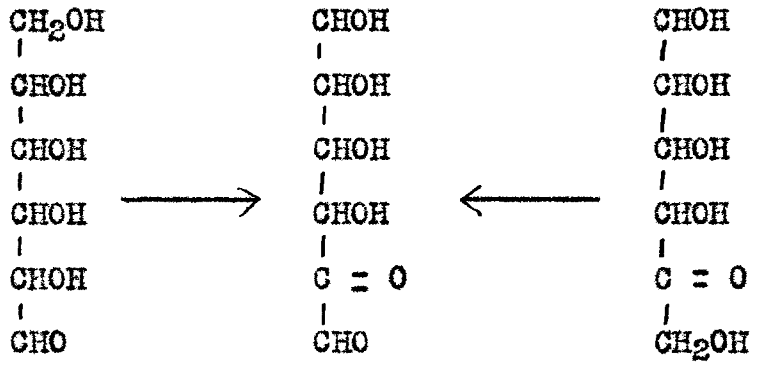
The formation of aldonic acids followed by the formation of keturonic acids from aldoses and the formation of ketones from sugar alcohols is very interesting, since it would seem to indicate that in some way the hydrogens of the secondary alcohol groups have been more highly activated than the hydrogens of the primary alcohol groups, suggesting a close connection between the oxidation of these compounds by bromine and by Acetobacter suboxydans. At this point the statement, "This reagent (bromine) and nitric acid produce oxidation products which are similar to those formed by living bacteria and fungi, as shown by Bertrand and subsequent investigators," by Everett and Sheppard (1936) becomes quite significant. A discussion of the similarities of the products produced by the pure chemical oxidation and by bacteria will be given later.

Studies of the effect of concentration, temperature, acidity, and buffers indicate that, (1) the liberated bromide slows down the oxidation to keturonic acids, so more of this acid is formed in dilute solutions, (2) buffers accelerate all stages of the oxidation and seem to materially increase the keturonic acid formation, and (3) increased temperature increases the speed of the reactions as would be expected.

Everett and Sheppard (1936) concluded that: "Oxidation paths are determined by three general structure influences: (a) substitution upon the upper, or reducing, carbon of the ring; (b) cis-trans isomerism of intermediate cyclic carbons; (c) substitution on the lower carbon. The first is manifested in the great differences between alpha- and beta-glycosides; the second determines the relations of isomeric sugars; the third becomes important for methyl pentoses and dicarboxylic acids."

Ridgeway (1931) attempted to oxidize glucose using potassium permanganate in sulfuric acid at the same pH at which the sugar is oxidized by bromine to gluconic acid; however no gluconic acid was isolated. It was found that the oxidation proceeds very rapidly to a certain point and then goes on at a much slower rate. The decrease in velocity is attributed to formation of a more stable oxidation product and a more stable oxidant.

According to Raymond (1938) glucose is oxidized by hot dilute nitric acid principally to gluconic acid but boiling concentrated nitric acid oxidizes both terminal carbon atoms forming saccharic acid. The oxidation of sugars using hydrogen peroxide is catalyzed by both ferrous salts and colloidal ferric hydroxide. In the presence of ferrous salts both aldoses and 2-ketoses are converted into osones accompanied by some degradation of the molecule.



Colloidal ferric hydroxide catalyses the decarboxylation of sugar acids to give aldoses of one less carbon than the parent acid.

Oohi (1931) investigated the action of bleaching powder on glucose, fructose, and sucrose at different temperatures. Using a 0.05 molar solution of sugar and hypochlorite, he obtained gluconic acid with a small amount of oxalic and saccharic acid. On doubling the concentration of hypochlorite a 94 per cent yield of gluconic acid was obtained, together with approximately 3 per cent of oxalic and some saccharic acid. Fructose treated in the same way and under the same conditions as glucose was only 37 per cent oxidized. These results suggested a possible separation of fructose from glucose by oxidation of sucrose with bleaching powder. Experimental verification was obtained when the solutions resulting from this oxidation of sucrose showed the presence of glucose amounting to only 21 per cent of the amount of fructose remaining.

Fang (1932) reports the formation of xylonic acid when xylose is oxidized with chlorine in ammoniacal solution.

As would be expected, the aldehyde group of aldose sugars can be oxidized to the acid group provided a relatively mild oxidizing agent is used. Kiliani (1880) was among the first to prepare gluconic acid by oxidizing glucose with bromine. While investigating this method Bunzel and Mathews (1909) found that the hydrobromic acid produced retarded the speed of the reaction and if the aldose is not a monosaccharide hydrolysis may cause poor yields of the desired acid. Fischer and Meyers (1889) obtained only about 30 per cent yields of aldobionic acids from the oxidation of lactose and maltose by bromine water. Kunz and Hudson (1926) were rewarded with similar yields from neolactose. In both cases the presence of gluconic and galactonic acids, probably resulting from the hydrolysis of the disaccharide and then oxidation of the simple sugars, rendered crystallization of the aldobionic acids difficult.

Recently several investigators attempting to eliminate the inhibition of the hydrobromic acid have obtained very good yields of the aldonic acids of the monosaccharides by electrolytic oxidation of the aldoses in the presence of various inorganic bromides. Isbell and Frush (1931) and Fink and Summers (1938) have both prepared gluconic acid in the form of calcium gluconate by very similar methods. Essentially both processes are about the same, oxidizing an aldose at a carbon electrode using a bromide or bromide-containing electrolyte solution. Although both authors have operated their processes on a semi-commercial scale and have applied for patents, the conditions of the processes are quite fully given and discussed in the articles cited.

Isbell and Frush (1931) have also applied their method to the oxidation of d-galactose, d-mannose, d-xylose, l-arabinose, l-rhamnose, d-lactose, and d-maltose with good yields of the acid obtained in all cases.

Cook and Major (1935), using the method described by Isbell and Frush (1931), report that 5-keto gluconic acid as well as gluconic acid is formed. This compound is the second dehydrogenation product produced by Acetobacter suboxydans so its appearance here illustrates the possible parallelism of electrochemical and biochemical oxidations.

Strictly speaking, any process involving the transfer of electrons to or from a substance is a reduction or oxidation, respectively. On this basis the electrodeposition or dissolution of metals, and likewise the liberation of oxygen or hydrogen, could be referred to as reduction or oxidation. For our purpose we shall define an electrochemical reduction as the transformation of the depolarizer taking place at the cathode when a current passes through the electrolytic cell. Likewise we shall define an electrochemical oxidation as the transformation of the depolarizer taking place at the anode when a current passes through the electrolytic cell. The depolarizer may be defined as any material in a solution which takes up the hydrogen or oxygen liberated at an electrode.

In general, the successful electrochemical oxidation of one organic compound to form some other definite compound is much more difficult to carry out and control than electrochemical reduction because of the innumerable oxidation products possible, ranging from the simplest oxidation products to the ultimate formation of carbon dioxide and water. In the case of electrochemical reduction, the end product is usually the material sought, and there is not the tendency at the cathode to cause such deep-seated changes in a compound but rather to saturate its structure with hydrogen. Since electrochemical reduction takes place at the cathode which is negative and therefore only attracts positive (metallic) ions, almost any metal which will not react with water can be used as an electrode for electrochemical reduction. The opposite is true for the anode and great difficulty is experienced when one tries to use the majority of metals as electrodes for electrochemical oxidation.

Electrochemical methods in general appear to have at least one advantage over ordinary chemical methods. The final product should contain no considerable amount of impurities resulting from the use of a chemical oxidizing or reducing agent. A very good example is the reduction of nitrobenzene to aniline which, if carried out using iron and acid as the reducing agent, necessitates the separation of iron salts from the aniline. On the other hand, electrochemical reduction of nitrobenzene in acid solution using a lead cathode yields almost pure aniline as the salt. It is also possible, by altering the conditions of nitrobenzene reduction, to stop at any of the intermediate reduction products shown in Haber's (1898) proposed scheme of electrochemical reduction of nitrobenzene which gives all the possible intermediate products and conditions necessary for their formation as the final product.

One should not get the idea that electrochemical preparation of organic compounds has no disadvantages for several seem to stand out very obviously. Usually electrochemical reactions are very slow and hence require a great deal of time and space for application on a commercial scale; as a rule an aqueous solution is necessary so the insolubility of many organic compounds in water offers quite a problem. Moreover, the conditions under which the electrolysis is conducted have a marked influence on the nature of the final products, making careful control and handling absolutely necessary.

Electrochemical reduction of organic compounds has been more thoroughly investigated than electrochemical oxidation due largely to the greater choice of electrode materials and the absence of degradation reactions at the cathode. The factors which appear to influence the products formed by electrochemical reductions are (a) nature and condition of the electrode, (b) electrode potential, (c) concentration of the depolarizer, (d) temperature of the electrode and electrolyte, and (e) catalysts or carriers. Due to the great number of combinations of conditions which are possible, only a general discussion of them can be made in this thesis.

The property of the cathode material which appears to have as much influence as any other on the nature of the electrochemical reduction process is that known as "overvoltage." To understand this property it is necessary to summarize a few of the principles of electrochemistry.

If a strip of a metal is placed in a solution of the salt of that metal, the metal shows a definite tendency to go into solution and form ions. Because of this tendency there is a potential setup between the metal and the solution the value of which depends on the active mass or concentration of the metal ions already in solution. If this strip of metal is made the cathode of an electrolytic cell containing a solution of a salt of the metal and a current is passed through the cell, it will be found that the potential or E. M. F. required to force the current through the cell and cause the metal to plate out will be of opposite sign and just slightly greater than the potential set up by the tendency of the metal to go into solution. This potential is known as the reversible potential of the electrode.

As an example let us consider the silver electrode whose reversible potential in a one molar solution of silver nitrate is 0.52 volts. If this electrode is made the cathode of an electrolytic cell containing one molar nitric acid instead of silver nitrate and a current is passed through the cell, it will be found that a potential approximately 0.15 volts greater is required to make a current flow than was required when silver nitrate was the electrolyte. It will also be noted that gas bubbles form on the electrode. This difference between the reversible potential of the electrode and the potential required to cause evolution of gas is called overvoltage. Each metal has an overvoltage of its own which varies from 0.005 volts for platinized platinum to 0.78 volts for mercury. Table I given below is from Glasstone and Hickling (1935).

TABLE I. Minimum Overvoltages in 2 N Sulfuric Acid.

Metals	Volts
Platinized platinum	0.005
Gold	0.02
Iron (in alkali)	0.08
Smooth platinum	0.09
Silver	0.15
Nickel	0.21
Copper	0.23
Cadmium	0.48
Tin	0.53
Lead	0.64
Zinc	0.70
Mercury	0.78

It should be understood, however, that the overvoltage of a metal is not a property which can be measured with any high degree of accuracy, so most of the values given are only approximate.

Allman and Ellingham (1924) prefer the concept that the reaction of the hydrogen ion at the cathode takes place in three steps: the discharge, the formation of atomic hydrogen followed by the production of molecular hydrogen. If this latter reaction is catalyzed by the metal of the electrode, molecular hydrogen will form readily, but if the process does not occur rapidly there should be an accumulation of atomic hydrogen on the electrode. Assuming that a certain gas pressure is necessary for bubble formation, it is obvious that there probably will be an appreciable accumulation of hydrogen atoms on an electrode which does not catalyse the formation of hydrogen molecules. Such metals will assume a high cathode potential before bubble evolution begins, and so will show a high overvoltage. Conversely if the metal catalyses the formation of hydrogen molecules, there will be no considerable concentration of atomic hydrogen present when bubbles begin to form, and so the overvoltage is low.

Now it is possible to obtain some idea of the reaction at the cathode during an electrochemical reduction. As the reducible depolarizer comes near or in contact with the cathode, it will react readily with the atomic hydrogen and yield a reduction product. If the overvoltage of the electrode is high there will be a large concentration of active hydrogen present and a vigorous reduction will ensue, but if the overvoltage of the cathode is not so high, the concentration of hydrogen atoms will be smaller, in some cases little more than in ordinary hydrogen gas at atmospheric pressure, and the reduction is much milder. The above explanation agrees very well with the findings of Tafel (1900) who reduced the difficultly reduced carbonyl compounds and pyridine electrochemically at mercury, lead, and zinc electrodes.

Elbs and Silbermann (1901) found that nitrobenzene can be reduced quantitatively to aniline at zinc, lead, tin, and mercury cathodes, but at nickel, carbon, and platinum cathodes benzidine and phenylhydroxylamine are products as well.

Electrochemical oxidation has been applied to the manufacture of a number of inorganic substances for some time, but its use in the realm of organic chemistry has found little application although in general, according to Fichter and Stocker (1914), the products of electrochemical oxidation are similar to those formed by the action of hydrogen peroxide. In the first place the mechanism of the evolution of oxygen at the anode and consequently the mechanism of oxidation is obscure, and in the second place every anode, as a rule, at first tends to dissolve in the electrolyte, limiting the choice of anode materials to those metals which can be made passive in the particular electrolyte used. Herein lies one of the main drawbacks to electrochemical oxidation for there appears to be no series of stable potentials between the high potential of oxygen evolution and the low potential at which anode solution occurs, therefore as good control of the intensity of the anode is not possible, as in the case of the cathode, where a range of overvoltage potentials and a wide choice of electrode materials are available.

The difference in overvoltage range may be seen by comparing Table II below, taken from Glasstone and Hickling (1935), with Table I.

TABLE II. Oxygen Overvoltages in 1 N Potassium Hydroxide.

Metal	Volts
Nickel	0.13
Cobalt	0.14
Platinized platinum	0.25
Iron	0.25
Lead	0.31
Silver	0.41
Palladium	0.43
Smooth platinum	0.45
Gold	0.53

Garrison and Lilly (1934) who have measured oxygen overvoltages in N/10 sodium hydroxide give the following values:

TABLE III.

Metal	Volts
Nickel	0.245
Cobalt	0.300
Iron	0.345
Iridium	0.367
Platinum (smooth)	0.402
Gold	0.580

As seen from Table III above most usable electrodes show rather high overvoltage tending to high oxidizing power; according to Fichter (1929) this should be compared only with the strongest oxidizing agents.

Perkins (1905) claims that electrochemical oxidation is due to the action of the discharged hydroxyl groups and not to the discharged oxygen, basing his contention on the fact that toluene and related compounds yield similar products when oxidized with chromyl chloride and electrochemically.

Glasstone and Hickling (1932) have proposed a hydrogen peroxide theory of anodic oxidation. When the hydroxyl ions are discharged at the anode they immediately combine to form hydrogen peroxide which becomes concentrated in a layer at the electrode and can be removed either by reaction with an oxidizable substance, or by decomposition into oxygen and water.

It would be most convenient to think of oxygen overvoltage as being due to a phenomenon similar to that of hydrogen overvoltage. If one visualizes the reactions which take place at the anode as being,

1. $2OH \longrightarrow 2OH + 2e$
2. $OH + OH \longrightarrow H_2O + O$
3. $O + O \longrightarrow O_2$

a similarity between oxygen liberation at an anode and hydrogen liberation at a cathode is noted. As has already been stated, hydrogen discharge takes place as follows:

1. $H^+ + e \longrightarrow H$
2. $H + H \longrightarrow H_2$

The irreversible reaction of two nascent hydroxyl radicals at the anode to form water and oxygen would be cause enough for the irreversibility of the oxygen electrode.

The oxidation of primary and secondary alcohols to carbonyl compounds can be more easily understood if one thinks of electrochemical oxidation as being brought about by activated or atomic oxygen adsorbed on the anode in much the same way that hydrogen is held on the cathode. Even though the oxygen overvoltage is not a direct measure of the oxidizing power of an anode as the hydrogen overvoltage is a measure of the reducing power of the cathode, the active oxygen theory can still be used to explain a large number of oxidations.

Due to the large number of isolated experiments, information concerning the electrolytic oxidation of organic compounds is rather fragmentary. Knobel and Brockman (1926) have compiled a very complete bibliography of the work previous to 1926.

When comparing the oxidation reactions brought about by Acetobacter suboxydans with those caused by other agents, it is most logical to begin with the oxidation of ethyl alcohol and the products formed. It is rather difficult to compare the products of electrochemical oxidation of ethyl alcohol obtained by different investigators because of the wide variation in conditions and current densities used. However it seems to be the consensus of opinion that ethyl alcohol is oxidized to acetic acid at high current density and is oxidized only to acetaldehyde at low current density. Current density may be expressed either in amperes per square decimeter or in amperes per square centimeter, but unless otherwise stated it is usually assumed that current density is given in amperes per square decimeter of electrode surface exposed to the solution.

Using low current density and a one per cent sulfuric acid solution Jaillard (1864) oxidized ethanol to acetaldehyde. Elbs and Brunner (1900), who have investigated alcohol oxidation rather thoroughly, reported that with high current density acetic acid was the main product with only about five per cent of acetaldehyde being found.

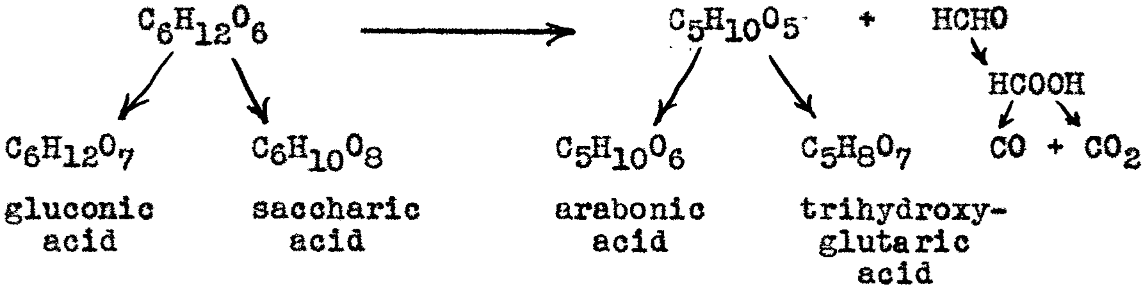
Dony-Henault (1900) suggested that whether or not a reaction stopped at a certain point depended on the depolarizing action of the first product formed. At a potential of 1.66 volts acetaldehyde is an excellent depolarizer, but at 1.3 volts and a small current density acetaldehyde is formed almost quantitatively from ethyl alcohol. It might be that low current density and therefore low rate of oxygen discharge gives the acetaldehyde time to diffuse away from the electrode, whereas high current density and its corresponding rapid oxygen discharge does not. This idea is substantiated by Slaboszewiez (1903) who found that acetaldehyde is a better depolarizer than is ethyl alcohol in equal concentrations. Acetaldehyde has been proved to be the first oxidation produce of ethyl alcohol by Schlotter (1902) who obtained eighty per cent current yields by working at high temperatures so that the acetaldehyde distilled out and was removed from the sphere of oxidation.

The oxidation of higher primary alcohols follows much the same course as the oxidation of ethyl alcohol. Elbs and Brunner (1900) found that n-propyl alcohol in dilute sulphuric acid is oxidized at platinum and lead dioxide anodes to propionic acid. Ninety per cent yields of propionic acid were obtained with very little aldehyde, except at low current density. Shunzo Koizumi (1921) reports that isoamyl alcohol is transformed into isovaleric aldehyde at lead dioxide and nickel electrodes in sulfuric acid solution when the current density was less than one ampere, but under the same conditions and a current density of two amperes isovaleric acid was the main product.

Isopropyl alcohol has been oxidized electrochemically by Elbs and Brunner (1900) yielding seventy per cent of acetone along with some acetic acid, formic acid, and carbon dioxide. The oxidation of other simple secondary alcohols has not been reported. However several of the more complex secondary alcohols have been oxidized to the corresponding ketones.

Glycol in acid solution yields carbon monoxide, carbon dioxide, trioxymethylene, glycolic acid, formic acid, oxygen, hydrogen, and a sugar which Renard (1875) claimed was not a pentose but formed an osazone melting at 184 to 185° C. Glycerol yields similar products including a sugar which in this case appears to be a pentose.

Until about the turn of the century most investigations of electrochemical oxidation reactions had been carried out using compounds selected entirely at random so long as they were soluble in water. As a rule current densities were rather high and the platinum and lead dioxide electrodes employed usually caused extremely vigorous oxidation. Under the conditions of high current density and high overvoltage electrodes it is only natural that the carbohydrates which were oxidized yielded a large variety of products and underwent considerable degradation. Renard (1879) oxidized glucose electrochemically and was able to identify formic acid, trioxymethylene, and saccharic acid. Lob (1909) made the first systematic study of the electrochemical oxidation of glucose using a lead electrode in dilute sulphuric acid, and reports formaldehyde, formic acid, d-arabinose, d-arabonic acid, trihydroxyglutaric acid, gluconic acid, and saccharic acid are formed. The scheme of oxidation suggested by Lob is:



In recent years the tendency has been to subject the carbohydrates to milder electrochemical oxidation. To obtain a milder oxidation some investigators have turned from metal electrodes and electrolytes which cause oxygen to be liberated, to halogen electrolytes and carbon electrodes. The work of Isbell and Frush (1931) mentioned above has been more extensive than that of other investigators.

The information on electrochemical oxidation of polyhydric alcohols may best be summed up by the words of Glasstone and Hickling (1935), "Mannitol, erythritol, and cane sugar have been electrolytically oxidized but the results are of no special interest."

METHODS

Analytical Methods

During an electrochemical oxidation, even when very carefully controlled, it is to be expected that small amounts of compounds other than the major product will be formed. Because these are usually present in rather insignificant amounts, analyses were made only for the major product or products. By making use of various color reactions used by biochemists when analyzing body fluids, it is possible to determine very accurately what type of compound is present. When the complexity of the mixture resulting from the oxidation was not too great the final products were isolated and identified, where possible, by derivatives. In some cases it was necessary to develop analytical methods.

1. Determination of acidity

The amount of acid formed during the electrochemical oxidation was determined by titrating 2 ml. of the medium with approximately N/10 base using phenolphthalein as the indicator, and expressed as acidity in terms of mls. of N/10 base or acid required per ml. of medium.

The weight of acid formed as a result of the oxidation of a primary alcohol was calculated from the acidity by means of simple molecular relationships between the acid and the base used. When tests show that there is no appreciable aldehyde present, it is reasonable to assume that only one acid has been formed from the primary alcohol. At the end of the oxidation the resulting solution was distilled, the distillate neutralized with sodium carbonate, and then redistilled. Qualitative analyses of the second distillate and residue were carried out to determine what products were present.

2. Determination of ketone

Acetone was determined by the Goodwin (1920) modification of the Messinger method and is tabulated in milligrams per ml. of medium. One ml. of N/10 iodine solution is equivalent to 0.00096747 gm. or 0.96747 mgm. of acetone.

Methyl ethyl ketone was determined by the same method and under the same conditions but a different iodine equivalent had to be used. This value was determined by standarization of the method against solutions of varying concentrations of methyl ethyl ketone which had been purified by isolating it as the sodium bisulfite addition compound. One ml. of N/10 iodine solution is equivalent to 0.001218 gms. or 1.218 mgm. of methyl ethyl ketone.

3. Determination of reducing compounds

Polyhydric alcohols should yield either reducing compounds or acids, or both when they are oxidized. The reducing power of the medium at different stages of the oxidation was determined by the Shaffer-Hartmann (1920) method. In most cases we cannot be absolutely sure what compound causes this reducing power so all values are expressed in terms of mgm. of glucose per ml. of medium.

4. Identification of final products

Volatile acids were recovered as the sodium salt and identified as the p-toluidides.

Volatile ketones were identified as the 2,4-dinitrophenylhydrazones.

The presence of saturated aliphatic aldehydes was tested for with Schiff's reagent.

The compounds resulting from the oxidation of polyhydric alcohols could not always be conveniently separated from the original polyhydric alcohol so various specific tests were used. Phenylhydrazine aids in detecting aldehydic and ketonic polyhydric compounds, but usually forms osazones instead of the single addition hydrazones making it impossible to differentiate between isomers which differ only in the configuration of the two affected carbon atoms. Glucose, mannose, and fructose all give the same phenylosazone. There are various color tests which are specific for certain groups of compounds or groupings of atoms in a compound. Use has been made of some of these tests to determine what types of compounds are present. If the final product is known it is often possible to determine the course of the reaction. Description of the tests used are given below.

Molisch test.--A test for compounds having the carbohydrate grouping. The reagent used is a 15 per cent alcoholic solution of alpha-naphthol. Place approximately 5 ml. of sugar solution in a test tube and add several drops of Molicsh reagent. Pour 5 ml. of concentrated sulfuric acid down the side of the test tube so that the acid forms a layer beneath the carbohydrate solution. A reddish-violet zone is produced at the point of contact of the solutions. Instead of the usual solution of 15 per cent alpha-naphthol several drops of a 5 per cent alcoholic thymol solution may be used.

Seliwanoff test.--A test used primarily for fructose but also gives a positive reaction with other ketoses. Does not give positive reaction with any aldoses. The reagent was prepared by dissolving 0.10 gms. of resorcinol in 200 ml. of 1:2 (12 per cent) hydrochloric acid. To approximately 5 ml. of Seliwanoff's reagent in a test tube add a few drops of the solution to be tested and heat the mixture to boiling. A positive reaction is indicated by the production of a red color and the separation of a brown-red precipitate. Heat the solution only to boiling because excess heating may cause the sugar to isomerize.

Bial's orcinol test.--A test which is specific for pentoses. The reagent was made by dissolving 1.5 gm. of orcinol (5-methyl resorcinol) in 500 ml. of concentrated hydrochloric acid and then adding 25 to 30 drops of 10 per cent ferric chloride solution. To 5 ml. of Bial's reagent in a test tube add 2 to 3 ml. of sugar solution and heat the mixture gently until the free bubbles rise to the surface. Immediately or on cooling the solution becomes green and a flocculent precipitate of the same color may form.

Phenylhydrazine reaction.--The reagent is made by dissolving 0.5 gm. of phenylhydrazine hydrochloride and 0.75 gm. of sodium acetate (fused) in 10 ml. of water. If necessary the solution may be heated to 60° C. Use 0.5 ml. of approximately 0.2 molar sugar solution and add 1 ml. of phenylhydrazine reagent. Heat on water bath for 10 to 20 minutes and set aside to cool. After crystallization has taken place the crystals may be examined under a microscope and compared with known osazones or photomicrographs.

EXPERIMENTAL

Apparatus

It was decided that oxidation of alcohols at low current density would be most applicable in this case because a direct current of low amperage is easier to produce and control, and too, the heating effects are not nearly so great. The direct current source may be a storage battery or series of storage batteries, a motor-generator combination, or some type of rectifier. Each source has its own advantages and disadvantages which were rather thoroughly investigated before selecting a vacuum tube rectifier as the direct current source for these investigations.

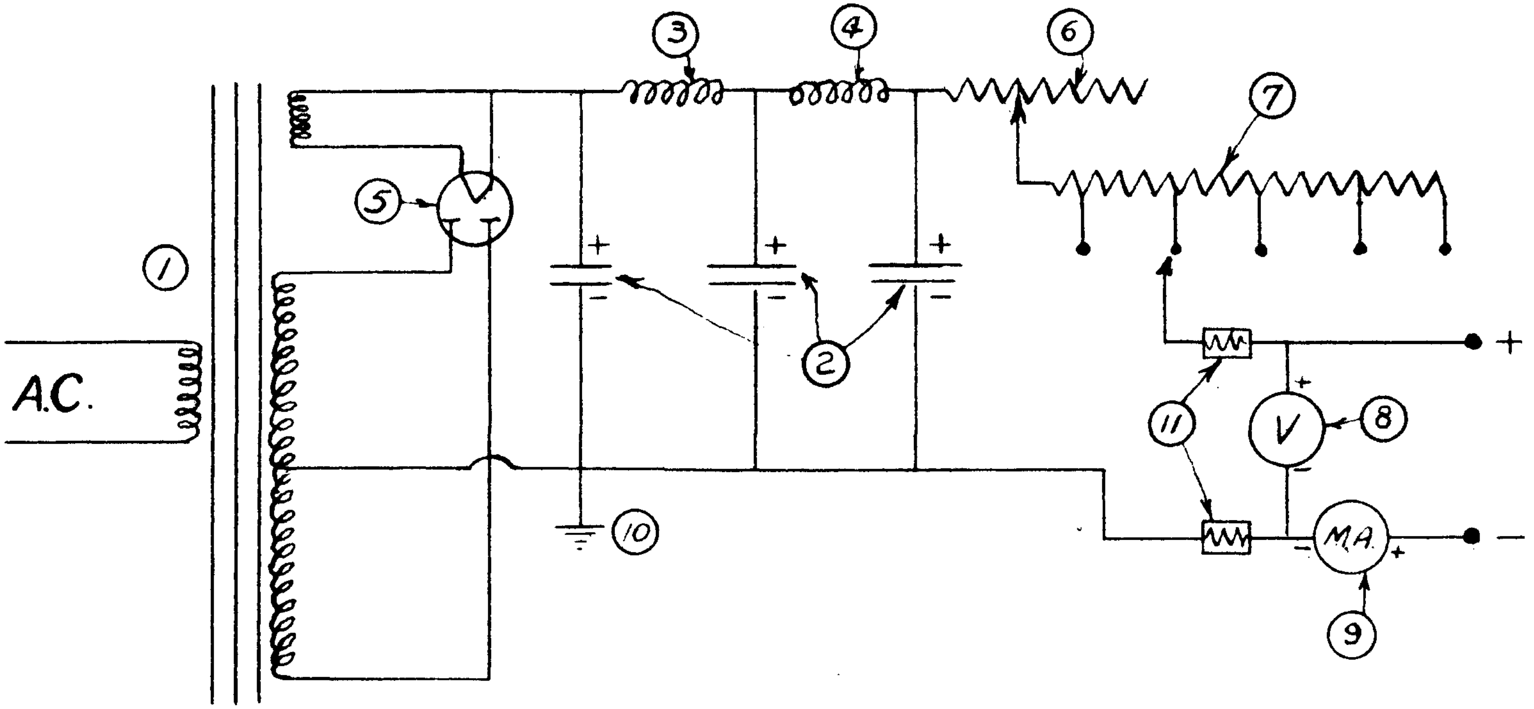
Rectifiers or power packs which change alternating current into direct are of several types: wet, dry, and vacuum tube. For our purpose a vacuum tube outfit seemed to be most satisfactory because it is comparatively foolproof and fairly inexpensive. With the large choice of rectifier tubes available it is possible to make power packs with current outputs up to 250 milliamperes at a maximum voltage of 500 volts. When a variable resistance is inserted in the circuit, the current output can be varied from 250 milliamperes downward without entirely losing the advantage of the large driving force available.

The power pack used as a source of direct current in this investigation was built so that several different tubes could be used. The only requirements are that the filament rating is 5 volts and peak plate voltage is at least 250 volts. A wiring diagram of the power pack circuit used in this investigation is shown in Figure II.

During an electrolysis there is always danger that the anode will corrode to such an extent that the external circuit will be broken, and very disastrous results may ensue if the alternating current through the primary of the transformer is not turned off within a short time. Fuses will protect the power pack from short circuits in the external circuit but some sort of relay or circuit breaker is necessary to protect the power pack against burnouts when the external circuit is broken.

The relay used in this investigation consisted primarily of an electromagnet and an iron-tipped crossbar fitted with a mercury circuit breaker. The magnet is connected in series with the electrolytic cells and as long as a current is flowing the crossbar remains horizontal. The circuit breaker is made by sealing an electrode in each end of a tube half filled with mercury. The mercury makes contact with both electrodes as long as the tube is horizontal and thus keeps the primary circuit of the transformer closed. If the external circuit is broken, the magnet ceases to function

Figure II



POWER PACK
WIRING DIAGRAM

List of Parts of Power Pack

1. Power transformer

    rated at 200-500 volts and 200-250 milliamperes

2. Electrolytic condensers

    450 volts, maximum 550 volts

3. Swinging choke

    8-30 henries, 200-250 milliamperes

4. Smoothing choke

    20 henries, 200-250 milliamperes

5. Tube

    #80, #83, or #85 volts

6. Power rheostat

    rating 75 watts and 500 ohms

7. Voltage divider

    rating 1500 ohms and 100 watts

8. Voltmeter

    300 watts

9. Milliammeter

    250 milliamperes

10. Chassis

11. Fuses

and the crossbar tilts thereby cutting off the alternating current.

It is advisable to know when the current goes off so that the length of oxidation of the compounds will be known. A self starting electric clock makes a very convenient indicator if it is wired in parallel with the primary of the power transformer.

The electrolytic cells which have been used in previous studies are of various shapes and descriptions. Several types of cells including beakers, large test tubes with sealed on side arms, 500 ml. wide-mouthed bottles, and 250 ml. wide-mouthed bottles were tried before the 250 ml. wide-mouthed bottles were finally decided upon. The bottles were fitted with number nine rubber stoppers having four holes bored in them for electrode leads, stirrer, and sampling opening.

Stirring or agitation of the solution while the current is passing is very necessary, for the rate of diffusion to and away from the electrode is seldom equal to the rate of reaction. Although any form of agitation may be used, some type of mechanical stirrer is usually most convenient. Frequently one of the electrodes is rotated so that it keeps the solution moving, but this arrangement is usually rather difficult to adjust. Among its more important purposes stirring serves to bring new depolarizer in contact with the electrode surface, remove the products of the reaction from the vicinity of the electrodes so that further oxidation is held at a minimum, and decrease the concentration of positive and negative ions around the respective electrodes. For this investigation variable speed, electric motor-driven stirrers were used. The blades of the stirring paddles drove the solution downward against the bottom of the cell where it moved upward over the electrode surfaces.

When metal electrodes were used, they were cut long enough to extend approximately 5 cm. below the surface of the liquid and were suspended by means of copper wire passed up through glass tubes in the rubber stopper. The cathodes in all cases were surrounded by alundum cups; extraction thimbles measuring 19 by 90 mm. with flat bottoms and in three grades of porosity were used. These porous cups almost entirely eliminated the reduction of the oxidation products at the cathode by decreasing diffusion of the depolarizer while offering very little hindrance to the ions of the electrolyte.

Investigation of Electrode Materials and Electrolytes

In investigations of the electrochemical oxidation of alcohols, it is necessary to determine the manner in which the different electrodes react when used in a variety of electrolytes. The electrodes tested in connection with this investigation were lead, nickel, copper, tin, zinc, carbon, and aluminum. The behavior of each of these will be discussed under the electrolyte employed.

Sulfuric acid as electrolyte

When 2 per cent sulfuric acid was used as the electrolyte, a covering of black lead dioxide formed on the lead anode and made it almost immediately passive. Oxygen was liberated quite rapidly after the electrode surface had been changed to the dioxide. A nickel anode was gradually dissolved in the electrolyte with very little gas evolution. Copper anodes dissolved rather rapidly in the sulfuric acid electrolyte, while tin first became covered with the black oxide followed by the dissolving of the electrode; some gas was evolved. Zinc and aluminum electrodes, as would be expected, dissolved quite rapidly in acid electrolyte. The carbon anode was oxidized by the oxygen liberated at the electrode so is not very useful when sulfuric acid is the electrolyte.

Sodium hydroxide as electrolyte

Lead electrodes dissolve very rapidly without gas evolution or apparent oxide formation when 2 per cent sodium hydroxide was used as the electrolyte. A nickel anode became covered with an oxide film which made it passive in basic solution, and oxygen was liberated quite rapidly. The inertness of nickel in alkaline solution should make it a very good oxidation electrode. Copper also became passive in basic solution and showed promise of being quite useful as an anode. Both copper and nickel anodes, however, dissolved very rapidly when a polyhydric compound such as glycerol or sorbitol was present in the solution. Apparently a complex was formed which is similar to the one which copper forms with tartrates. The findings of Lieser and Ebert (1937) that cupric hydroxide formed complexes with polyalcohols such as glycerol, adonitol, and inositol support the above statement. Tin, zinc, and aluminum anodes dissolved quite rapidly in 2 per cent sodium hydroxide since they are amphoteric metals. Carbon anodes disintegrated in alkaline solution as a result of oxidation by nascent oxygen which was liberated.

Sodium sulfate as electrolyte

A neutral solution such as sodium sulfate has been successfully used as the electrolyte in electrochemical reductions. Therefore it probably would have certain advantages for electrochemical oxidation. Anodes of lead, when employed in 2 per cent sodium sulfate solution, were covered with lead dioxide just as they were in sulfuric acid, and quickly became passive so that oxygen was liberated fairly rapidly. Nickel, copper, zinc, and aluminum dissolved very much the same as they did in sulfuric acid solutions with little or no gas evolution. Tin electrodes seemed to be slightly more passive than other electrodes tested but they gradually corroded with very little gas evolution. Carbon electrodes were attacked very severely by the oxygen liberated at the anode. In general, when sodium sulfate was used the electrodes behaved much the same as they did in sulfuric acid. Therefore since a neutral solution is much more easily handled than an acid electrolyte, sodium sulfate appears to have certain advantages over sulfuric acid.

Halides as electrolytes

The halide compounds, either acid or neutral, have essentially the same effect on all metal electrodes tested. They dissolved without any apparent liberation of gas. Lead and tin were corroded more slowly than the other metals. A carbon anode was not attacked by the halogens liberated from the halide electrolytes and so seemed to be the only anode material which could be used under the above conditions. After long use, however, the carbon anode showed some wear but not enough to cause trouble.

Phosphate electrolytes

The experiments already carried out indicate that a protective coating or film must be formed on the electrodes if they are to be passive enough to use as anodes in prolonged electrochemical oxidations. To meet this requirement electrodes are necessary which will form insoluble compounds with the metal used as the anode. It is also necessary that these insoluble compounds adhere very strongly to the electrode. With these two requirements in mind numerous electrodes and solutions were tested in hopes that combinations would be found which fulfilled them.

Nickel, zinc, and copper phosphates are all insoluble but when these electrodes were tested using 2 per cent primary or secondary phosphate as electrolyte, the insoluble compound was formed as a finely divided precipitate instead of the desired film on the electrode. Aluminum on the other hand formed a film under the above conditions but it had such high resistance that a potential of 125 volts was required to make current flow. Tin and lead both formed black oxides in phosphate solutions which protected them against excessive corrosion. When 2 per cent phosphoric acid was used, results were obtained which were quite similar to those reported for primary and secondary phosphates.

Miscellaneous electrolytes

The chromium ion has been reported to be a good oxygen carrier. A 2 per cent solution of potassium chromate was tried as the electrolyte using zinc, copper, nickel, aluminum, tin, and lead electrodes. In most cases a flocculent yellow precipitate was noted after several hours with no apparent film formation on the electrode. Nickel anodes did not seem to be attacked while again a film formed on the aluminum electrode which had a very high resistance.

When potassium permanganate was used as the electrolyte, manganese dioxide was precipitated as a flocculent precipitate leaving the solution basic. An alkaline solution probably would cause excessive degradation of the sugars and therefore is to be avoided.

Discussion

In view of the results of the above experiments it became obvious that there are only a limited number of materials which are suitable for use as anodes for the type of investigation in hand. Disregarding the noble metals, which were not tested, only four metals and one nonmetal seemed usable, and these can be employed only under certain specific conditions. Lead anodes may be used in sulfate or phosphate solutions either acid or neutral. However they are more resistant to corrosion in sulfate than in phosphate electrolytes. Carbon anodes are most effective when used in halide solutions both acid and neutral. Nickel and copper anodes are passive only in alkaline solution and are quite resistant to corrosion except when polyhydric compounds of the glycerol type are present. Hence it is doubtful whether nickel and copper anodes can be used to oxidize sugar or sugar alcohol solutions. Then too alkaline solutions tend toward excessive sugar degradation, so are to be avoided.

The electrodes and electrolytes selected were: (1) lead in sulfate solutions both acid and neutral, (2) carbon in neutral halide solutions, (3) nickel in sodium hydroxide solution, and (4) copper in sodium hydroxide solution. The electrolyte solutions used were all at 2 per cent concentration.

Oxidation of compounds containing only one functional group

Most polyhydric alcohols contain both primary and secondary alcohol groups, so before attempting the oxidation of sugar alcohols preliminary experiments were made using compounds which contain only a secondary alcohol group and only a primary alcohol group. By using compounds with only one functional group it is possible to determine how each group reacts at the various electrodes and in the presence of the different electrolytes. The course of the reactions was followed by analyzing samples taken from each electrolytic cell at 8 to 12 hour intervals. The 5 ml. samples of medium were diluted to 50 ml. and appropriate aliquots were analyzed for acidity and ketone formation by methods already given.

Approximately 10 per cent solutions of two primary alcohols, n-propyl and isobutyl, were oxidized under the following conditions. The electrodes used were: (1) sheet lead anode measuring 2 by 5 cm. with 2 per cent sulfuric acid as electrolyte, (2) sheet lead anode measuring 2 by 5 cm. with sodium sulfate as electrolyte, (3) cylindrical carbon anode approximately 0.75 cm. in diameter by 5 cm. long with 2 per cent sodium bromide as electrolyte, (4) sheet nickel anode measuring 2 by 5 cm. with 2 per cent sodium hydroxide as electrolyte, and (5) sheet copper anode measuring 2 by 5 cm. with 2 per cent sodium hydroxide as electrolyte. The current was adjusted so that 150 m.a. were flowing through the cells under sufficient potential to keep a constant flow. All solutions were vigorously stirred during the course of the reaction, and the level of the liquid was kept constant by addition of water or appropriate aqueous solution to replace that decomposed by electrolysis and removed as samples for analysis.

When a primary alcohol is oxidized, it is first changed into an aldehyde. Whether the aldehyde formed will be further oxidized to an acid depends on the relative depolarizing action of the original alcohol and of the aldehyde which is formed by the initial oxidation. In most cases an aldehyde is at least as good if not better depolarizer than the corresponding alcohol. The results of these experiments support this last generality since no test for aldehyde was obtained with Schiff's reagent and acid was present in considerable quantities. Saturated aliphatic acids are, as a rule, quite resistant to oxidation so there is little danger that appreciable quantities of the acid will be degraded.

The data summarized in Table IV give the amounts of acid formed at different stages of the oxidation calculated from the acidity of the solution. Assuming that the oxidation of a primary alcohol follows the equation,

$$C_2H_5CH_2OH \xrightarrow{O} C_2H_5CHO \xrightarrow{O} C_2H_5COOH$$

it is possible to calculate the amount of acid which could be expected if the process were 100 per cent efficient. Let us take for an example an electrochemical oxidation of n-propyl alcohol which has proceeded for 40 hours with 150 m.a. passing. On titrating one ml. of the solution from the cell which contains 200 ml. an acidity of 2.0 ml. of N/10 base was found. There would be

$$2 \times 0.0074 \times 200 = 2.96 \text{ gm. of acid in the cell.}$$

The Faradays which have passed are

$$\frac{40 \times 3600 \times 0.150}{96500} = 21600/96500 = 0.2238$$

From the equation given above one sees that two atoms of oxygen, equivalent to four Faradays per mole, are required to change the alcohol to the acid. To form one gm. of acid 4/74 is required and so 0.2238 Faraday will form

$$\frac{0.2238 \times 74}{4} = 4.14 \text{ gms. of acid.}$$

TABLE IV

Effect of different electrolytes and anodes on the electrochemical oxidation of typical primary alcohols.

Time, hours	Acidity[1]	Yield[2]	Current efficiency[3]
I. 10 per cent n-propyl alcohol as depolarizer			
	lead anode in 2 per cent sulfuric acid current 150 m.a.		
10	0.65	4.95	95.6
21	1.15	8.00	73.6
32	1.70	12.55	75.8
44	2.30	17.50	76.8
57	2.95	22.40	75.8
64	3.27	24.80	74.8
	lead anode in 2 per cent sodium sulfate current 150 m.a.		
12	0.11	0.84	36.05
21	0.54	4.10	37.70
28	0.82	6.22	42.90
35	1.18	8.97	49.50
45	1.72	13.07	56.10
68	2.76	20.95	59.40

[1] Acidity is expressed in ml. of N/10 base equivalent to acid in one ml. of medium.

[2] Yield is expressed in gm. of acid formed per 100 gm. of alcohol in original medium, and since a 10 per cent alcohol solution was used it also shows mgm. of acid per ml. of medium.

[3] Current efficiency is ratio of acid formed to that which should have been formed on basis of current consumption.

TABLE IV. (Continued).

Time, hours	Acidity[1]	Yield[2]	Current efficiency[3]
carbon anode in 2 per cent sodium bromide current 150 m.a.			
8	0.025	0.19	4.6
19	0.23	1.75	17.7
31	0.45	3.40	21.2
43	0.65	4.95	22.2
55	0.85	6.45	22.6
68	1.05	8.00	22.8
nickel anode in 2 per cent sodium hydroxide current 170 m.a.			
13	0.48	3.65	54.3
20	0.81	6.16	59.5
26	1.15	8.75	65.0
37	1.51	11.48	59.9
44	1.73	13.15	57.6
50	1.95	14.70	56.8
61	2.15	16.30	51.6
copper anode in 2 per cent sodium hydroxide current 150 m.a.			
8	0.92	7.0	169.0
19	1.50	11.4	
31	2.15	16.3	101.5
43	2.90	22.0	
55	3.45	29.8	104.8

TABLE IV. (Concluded).

Time, hours	Acidity[1]	Yield[2]	Current efficiency[3]
II. 10 per cent isobutyl alcohol as depolarizer			
lead anode in 2 per cent sulfuric acid current 150 m.a.			
10	0.55	4.96	80.8
21	0.95	8.58	66.5
32	1.30	11.73	59.7
44	1.65	14.90	55.0
57	2.02	18.22	52.0
lead anode in 2 per cent sodium sulfate current 150 m.a.			
8	0.40	3.52	71.6
19	0.80	7.23	61.9
31	1.20	10.86	57.0
43	1.82	16.46	62.3
55	2.20	19.90	58.8
68	2.65	24.00	57.4
carbon anode in 2 per cent sodium bromide current 150 m.a.			
10	0.025	0.22	3.6
21	0.15	1.35	9.6
32	0.30	2.70	13.7
44	0.45	4.06	15.0
57	0.60	5.42	15.2

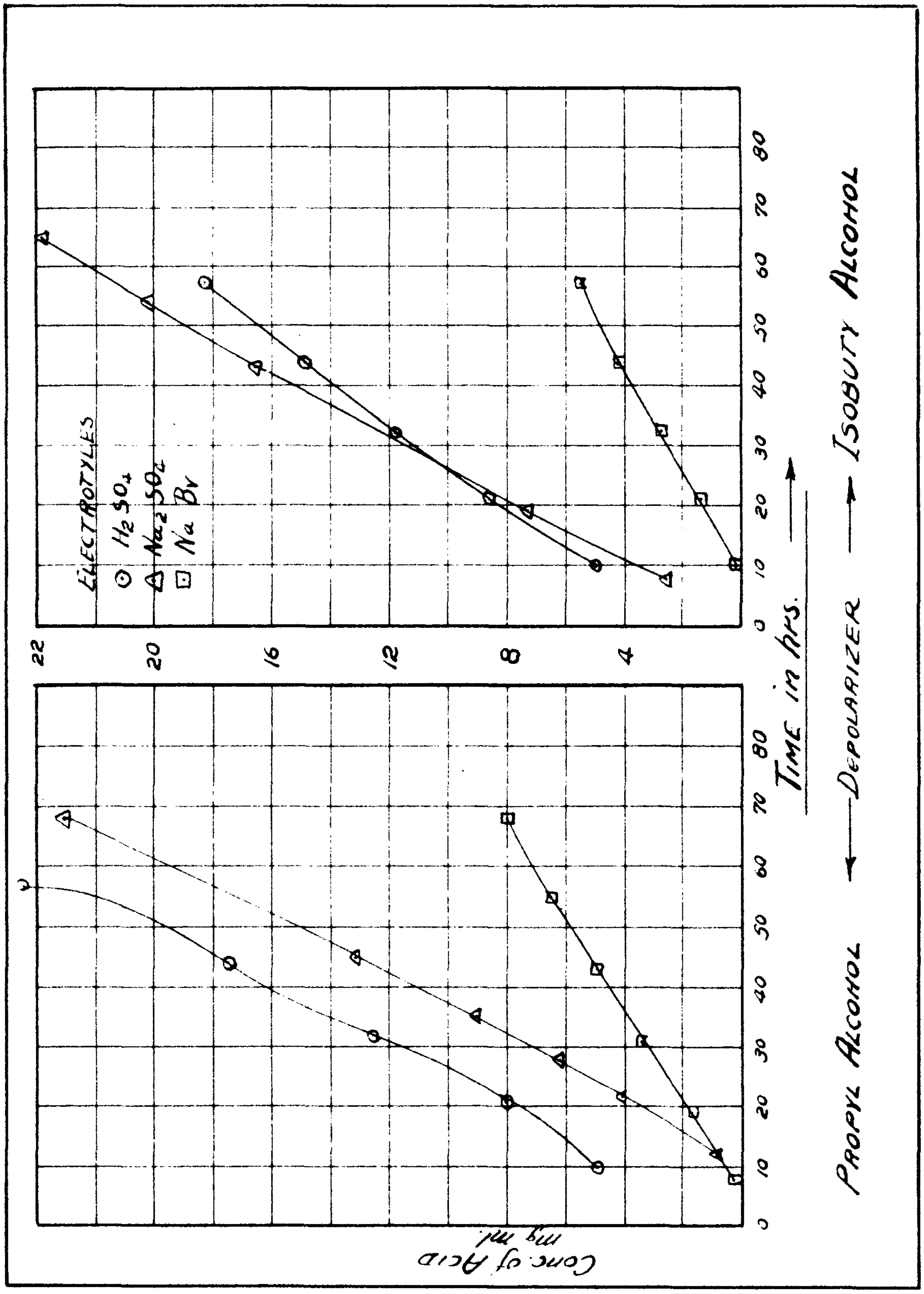

ELECTROTYLES
⊙ H_2SO_4
△ Na_2SO_4
⊡ Na Br
Conc. of Acid mg/ml
22
20
16
12
8
4
0
10
20
30
40
50
60
70
80
TIME in hrs.
PROPYL ALCOHOL ← DEPOLARIZER → ISOBUTY ALCOHOL

Figure III

The current efficiency is 2.96/4.14 = 0.715 or 71.5 per cent.

Approximately 10 per cent solutions of two secondary alcohols, isopropyl and secondary butyl, were oxidized under the same conditions given for the primary alcohols. Secondary alcohols yield ketones as oxidation products in one step. To undergo further oxidation it is necessary to split the molecule at the carbonyl group, a reaction which requires rather drastic conditions, so it is not surprising that only slight increase in acidity was noted during these oxidations.

Assuming that the oxidation of a secondary alcohol conforms to the equation,

$$CH_3CHOHCH_3 \xrightarrow{O} CH_3COCH_3$$

it is possible to calculate the amount of ketone which could be expected if the process were 100 per cent efficient. Since the amount actually present is known, the current efficiency can be determined as it was for the acid formation.

The data summarized in Table V give the amounts of ketone formed at different stages of the oxidation determined as previously described. The acidity of each solution was also determined because any splitting of the ketones initially formed would become evident as increased acidity due to the two acids formed b the splitting.

Acids or ketones were formed at the lead electrodes used in sulfate solutions in practically equivalent amounts

TABLE V

Effect of different electrolytes and anodes on the electrochemical oxidation of typical secondary alcohols.

Time, hours	Acidity[1]	Yield[2]	Current efficiency[3]
I. 10 per cent isopropyl alcohol as depolarizer			
lead anode in 2 per cent sulfuric acid current 150 m.a.			
12	0.15	10.0	93.9
20	0.30	14.4	92.0
27	0.30	17.6	84.3
36	0.27	23.9	84.8
47	0.25	30.0	81.5
62	0.27	34.6	71.3
74	0.35	43.0	74.3
lead anode in 2 per cent sodium sulfate current 150 m.a.			
12	0.47	6.3	67.1
21	0.67	10.1	61.5
28	0.67	14.6	66.6
35	0.70	19.2	70.1
45	0.72	26.9	76.5
56	0.72	33.8	77.2
68	0.81	39.3	73.8

---

[1] Acidity is expressed in ml. of N/10 base equivalent to acid in one ml. of medium.

[2] Yield is expressed in gm. of ketone formed per 100 gm. of alcohol in original medium, and since a 10 per cent alcohol solution was used it also shows mgm. of ketone per ml. of medium.

[3] Current efficiency is ratio of ketone formed to that which should have been formed on basis of current consumption.

TABLE V (Continued).

Time, hours	Acidity[1]	Yield[2]	Current efficiency[3]
carbon anode in 2 per cent sodium bromide current 150 m.a.			
8	0.025	6.8	108.5
19	0.05	11.5	77.4
31	0.05	20.7	85.5
43	0.05	28.0	83.2
55	0.05	35.2	81.8
68	0.05	43.5	81.8
nickel anode in 2 per cent sodium hydroxide current 150 m.a.			
13	0.53	9.55	93.8
20	0.54	12.0	76.6
26	0.54	13.5	66.3
37	0.59	15.6	54.0
44	0.63	16.6	48.2
50	0.68	17.5	44.7
61	0.73	18.4	38.5
copper anode in 2 per cent sodium hydroxide current 150 m.a.			
8	0.55	3.26	52.1
19	0.72		
31	0.75	7.6	31.4
43	1.05		
55	1.25	9.56	22.2
68	1.55	11.6	21.8

TABLE V (Concluded).

Time, hours	Acidity[1]	Yield[2]	Current efficiency[3]
II. Secondary butyl alcohol as depolarizer			
	lead in 2 per cent sulfuric acid		
12	0.40	6.38	52.7
24	0.65	8.82	36.3
36	0.90	14.50	39.9
48	1.10	17.32	35.8
60	1.37	20.10	33.3
72	1.75	20.85	28.7
	lead in 2 per cent sodium sulfate		
12	0.70	3.07	25.4
24	0.90	5.57	23.0
36	1.10	10.45	28.8
48	1.37	14.05	29.0
60	1.75	17.30	28.6
72	2.10	18.70	25.7
	carbon in 2 per cent sodium bromide		
12	0.025	5.57	46.0
24	0.025	6.10	25.2
36	0.025	8.70	23.9
48	0.025	9.64	19.8
60	0.025	10.45	17.2
72	0.025	11.10	15.3

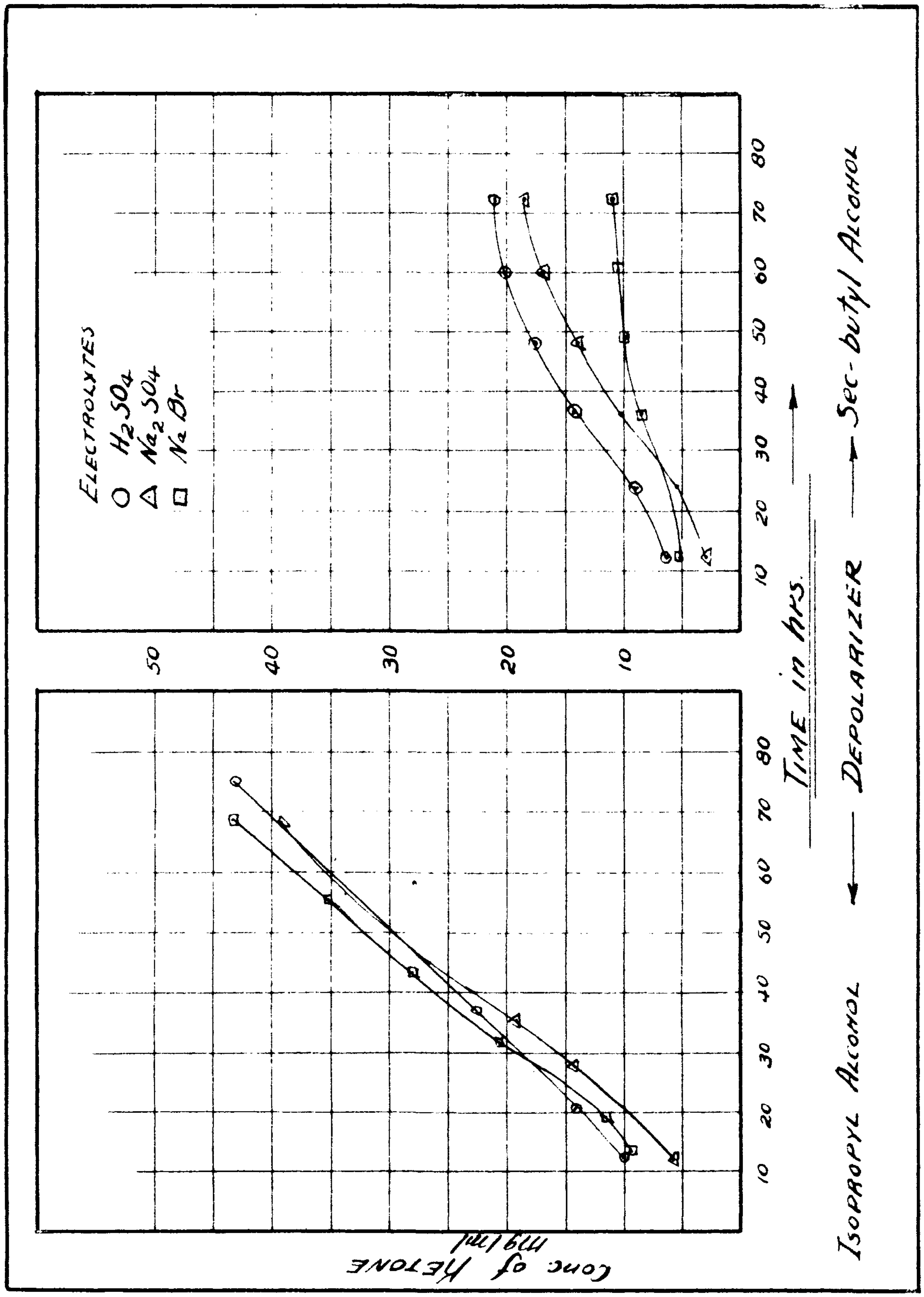

ELECTROLYTES
○ H_2SO_4
△ Na_2SO_4
□ NaBr
Conc. of Ketone mg/ml
50
40
30
20
10
10
20
30
40
50
60
70
80
Time in hrs.
Isopropyl Alcohol
Depolarizer
Sec-butyl Alcohol

Figure IV

calculated on the basis of the equations given. The carbon anode, when used with sodium bromide as electrolyte, oxidized a secondary alcohol to the ketone just as efficiently as did the lead anode in sulfate solutions; however its effect on primary alcohols was considerably less. Acid was formed at the carbon electrode as the result of the oxidation of primary alcohols in quantities only about 40 per cent of the amount formed at the lead electrodes. No aldehyde was indicated in the final solution by the Schiff test.

Nickel electrodes tended to catalyse acid formation more than ketone formation. The decrease in rate of acid formation shown toward the end of the electrolysis is probably due to the decomposition of sodium proprionate according to the Kolbe (1849) syntheses.

The results obtained using a copper anode in sodium hydroxide solution is obviously in error for it is very unlikely that a yield of over 100 per cent is possible. The acidity of the solution was determined by titrating the base which remained at any particular time, so any reaction which used up sodium hydroxide would appear as acid formed, and thereby cause the error.

When the results of the above experiments were considered in their entirety, they indicated that the carbon electrode used in sodium bromide solution probably would oxidize the secondary alcohol group of a compound in preference to the primary alcohol group. Further experiments will be made in an attempt to verify this possibility.

To prove the assumption that the expected acids were actually the oxidation products of the primary alcohols, p-toluidides were made and the melting points determined. Approximately 100 ml. of the final solution was fractionated and the acid portion neutralized with sodium carbonate. The sodium salt solution was evaporated to dryness and a portion used to prepare the p-toluidide as described by Mullekin (1904). The derivative of the acid formed during the oxidation of n-propyl alcohol melted at 124° C. and did not lower the melting point of propion-p-toluidide. The derivative of the acid formed during the oxidation of isobutyl alcohol melted at 105° C. and did not lower the melting point of isobutyl-p-toluidide.

The ketones formed by electrochemical oxidation of the secondary alcohols were identified by preparing the 2,4-dinitrophenyl hydrazones and comparing the melting points with those of known compounds. Approximately 100 ml. of the final solution was fractionated and a derivative made from the lower boiling fraction using the method described by Allen (1930). In all instances the solution gave materials whose boiling points corresponded to the unknown, the initial alcohol, and water. The derivative of the ketone formed during the oxidation of isopropyl alcohol melted at 127° C.

and did not lower the melting point of acetone-2,4-dinitrophenyl hydrazone. The derivative of the ketone formed during the oxidation of secondary butyl alcohol melted at 115° C. and did not lower the melting point of methyl ethyl ketone-2,4-dinitrophenylhydrazone.

During the determination of acetone by the Goodwin (1920) modification of the Messinger method, it was found that under the conditions of the analysis isopropyl alcohol caused a small error when present in fairly large amounts. Goodwin (1920) states that ethyl alcohol does not interfere with the analysis even when the concentration is ten times that of the acetone present, but makes no mention of isopropyl alcohol.

A series of solutions was made which contained varying amounts of acetone and isopropyl alcohol and aliquot portions were analyzed for acetone. The data obtained are given in Table VI, and show that an appreciable error in the acetone value is caused by isopropyl alcohol when present in concentrations greater than one-half the amount of acetone present.

When the molar ratio of acetone to isopropyl alcohol is two or greater, the error in the analysis is within the limits of experimental error. If the analysis is made using 15 ml. of 0.2 N iodine solution and 0.1 N sodium thiosulfate solution, best results can be obtained when the concen-

TABLE VI

Error in acetone value caused by isopropyl alcohol.

Molar ratio acetone/ alcohol	Acetone in mgm./ml.		Actual error in mgm.
	Present	Found	
0.1	5.60	6.525	+0.93
0.2	5.60	5.995	+0.39
0.25	5.60	5.89	+0.29
0.33	5.60	5.83	+0.23
0.50	5.60	5.72	+0.12
1.00	5.60	5.72	+0.12
2.00	5.60	5.65	+0.05
3.00	8.40	8.43	+0.03
4.00	11.11	11.1	-0.01
5.00	14.00	13.92	-0.08
6.00	16.67	16.72	+0.05
7.00	19.60	19.55	-0.05
8.00	22.22	22.14	-0.08
9.00	25.20	25.16	-0.04
10.00	27.78	27.82	+0.04
12.00	33.33	33.38	+0.05
16.00	44.44	44.36	-0.08
20.00	55.56	55.48	-0.08

tration of acetone in the sample used is between 5 and 20 mgm.; however a maximum of 25 mgm. can be determined with fair accuracy. Since solutions of greater concentration of acetone than the maximum given above must be diluted and aliquots used for the analysis, any error in titration will be multiplied by an integer whose value depends on the amount of dilution of the original solution. The predominance of negative errors in the acetone values is probably due to the tendency to slightly over titrate the excess iodine liberated by the acid.

The above discrepancy in the acetone value, when considerable isopropyl was present, led to experiments which were made to determine whether alkaline iodine solution such as that used in the acetone determination showed any difference in its oxidizing action on primary and secondary alcohol groups. Ethyl alcohol and isopropyl alcohol were selected for the comparison because both yield oxidation products which form iodoform quite readily. The remaining iodine may be easily determined by titrating the acidified solution with sodium thiosulfate. The oxidation of both alcohols to their respective initial oxidation compounds and the subsequent formation of iodoform, as shown by equations below, require equivalent amounts of iodine, thus allowing a direct comparison on the titration values to be made.

Ethyl alcohol

1. $CH_3CH_2OH + NaOI \longrightarrow CH_3CHO + NaI + H_2O$
2. $CH_3CHO + 3NaOI \longrightarrow CHI_3 + HCHONa + 2NaOH$

Isopropyl alcohol

3. $CH_3CHOHCH_3 + NaOI \longrightarrow CH_3COCH_3 + NaI + H_2O$
4. $CH_3COCH_3 + 3NaOI \longrightarrow CHI_3 + CH_3COONa + 2NaOH$

Ten ml. portions of two molar solutions of ethyl alcohol and isopropyl alcohol were placed in separate 300 ml. flasks together with 20 ml. of 1 N alkali. The flasks were placed in ice and after they had reached a constant temperature, 15 ml. of approximately 0.2 N iodine solution were added with vigorous shaking. The flasks were allowed to stand in the ice for varying lengths of time and the iodine liberated by acidifying with 20 ml. of 1 N acid titrated with standard sodium thiosulfate. The iodine used up during the reaction is found by subtracting the amount of sodium thiosulfate required to titrate the excess iodine from the blank. This determination is made using 10 ml. of distilled water instead of the alcohol solution.

The data summarized in Table VII indicate that the secondary alcohol group oxidizes quite readily at the potential of alkaline iodine solution while the primary alcohol group, at least the one in ethyl alcohol, is oxidized only slowly.

This difference in the action of ethyl alcohol and isopropyl alcohol in the presence of an oxidizing agent is

TABLE VII

Effect of alkaline iodine solution on ethyl alcohol and isopropyl alcohol.

Time, minutes	Weight of alcohol in sample	ml. of N/10 I_2 used by alcohol
I. Ethyl alcohol		
5	0.865	0.72
10	0.865	0.85
15	0.865	0.95
30	0.865	1.17
45	0.865	1.34
II. Isopropyl alcohol		
5	1.218	8.45
10	1.218	10.88
15	1.218	12.30
30	1.218	13.73
45	1.218	14.35

evidence in support of the idea that the action of *Acetobacter suboxydans* in oxidizing a secondary alcohol group in preference to a primary alcohol group is more a question of oxidation potential and not so much a question of specificity of the organism.

Oxidation of Compounds Containing Two Functional Groups

Effect of concentration on oxidation of sorbitol

Experiments to determine the effect of the concentration of sorbitol on the course of the oxidation gave rather inconsistent results due probably to slight variations of the conditions in different cells. It is almost impossible to secure identical agitation of the solution in different cells and equally difficult to obtain several electrodes which will all maintain like surfaces.

The course of each reaction was followed by determining the acidity of the medium at various intervals. The reducing power of the solution, using the Shaeffer-Hartmann method, was also determined because it is logical to suppose that as the result of electrochemical oxidation, a sugar alcohol will yield a product or products which show reducing power. This reducing value of the solution should be an indication of the course taken by the oxidation. Most probable products which would show reducing properties are hydroxyl ketones, aldehydes, and ketonic acids.

Since the copper number varies with the nature of the reducing agent, the reducing value is calculated as dextrose. It is evident that such values do not represent the situation quantitatively but they do give a means of determining general trends.

The data summarized in Table VIII are typical of the trend of the reaction which took place in the different concentrations of sorbitol. It is evident that there is very little difference among the reactions which took place in concentrations of sorbitol between 5 and 15 per cent at the time intervals studied. Lead electrodes, which had previously been thinly coated with lead dioxide, were used as anodes, and 2 per cent sodium sulfate served as the electrolyte. The lead electrode used in the 20 per cent sorbitol solution was prone to form a coating of insoluble lead sulfate instead of lead dioxide as was usually the case. This removal of sulfate from the solution would explain the decrease in acidity noted in this cell. In low concentrations of sorbitol it appears that oxygen was liberated at the anode faster than unaltered depolarizer could come in contact with the electrode and take up the oxygen, thus accounting for the lower reducing value shown by this solution.

Concentrations of sorbitol varying from 5 to 15 per cent seemed to be about equally suitable for electrochemical

TABLE VIII

Effect of concentration of sorbitol on the course of the oxidation by lead electrodes in sodium sulfate.

Time, hours	Acidity[1]	Reducing value, calculated as mgm. dextrose/ml.
	20 per cent sorbitol	
10	0.80	10.2
22	0.45	12.2
35	0.00	13.2
47	-0.05	14.8
58	-0.25	13.7
	15 per cent sorbitol	
10	0.62	8.8
22	0.50	10.7
35	0.78	13.3
47	0.91	14.9
58	1.12	16.7
	10 per cent sorbitol	
10	0.55	6.5
22	0.60	11.1
35	1.12	15.3
47	1.65	18.5
58	2.21	19.8

---

[1] Acidity is expressed in ml. of N/10 base equivalent to acid in one ml. of medium.

TABLE VIII. (Concluded)

Time, hours	Acidity[1]	Reducing value, calculated as mgm. dextrose/ ml.
	7.5 per cent sorbitol	
10	0.53	6.6
22	0.78	10.6
35	1.05	14.4
47	1.65	16.2
58	2.18	17.4
	2.5 per cent sorbitol	
10	0.65	5.2
22	1.25	8.2
35	1.70	9.5
47	1.95	9.9
58	2.00	9.1
	5 per cent sorbitol	
10	0.50	4.4
22	0.92	11.8
35	1.61	15.3
47	2.45	17.4
58	3.05	17.5

oxidation; however a 10 per cent solution appeared to give slightly higher reducing values. The nearly equivalent amounts of reducing substances as well as the nearly equal acidities shown by the several solutions whose concentrations are between the above limits, indicate that the depolarizer is coming in contact with the electrode in quantities large enough to take up all of the active oxygen available. Since all experiments were conducted at low current densities, only a certain amount of depolarizer can be oxidized at any one instant and any excess coming in contact with the anode is not reacted upon.

The steady increase in acidity together with the relatively small increase in reducing value of the above solutions indicate that the first oxidation products are being further oxidized to acidic compounds as the oxidation progresses.

Effect of various anodes and electrolytes on the oxidation of sorbitol

The results of the oxidations of sorbitol using lead electrodes in sulfate solutions showed that less drastic oxidizing conditions are to be preferred. On the basis of the preliminary experiments which showed that a carbon electrode in sodium bromide solution was rather mild in its oxidizing action, it appeared that the other halides would act similarly. Several 10 per cent sorbitol solutions were oxidized using the following anode-electrolyte combinations: (1) lead in sodium sulfate, (2) carbon in sodium chloride, (3) carbon in sodium bromide, (4) carbon in alkaline potassium iodide, (5) tin in primary potassium phosphate, and (6) tin in sodium carbonate. The previous experiments have shown that lead brings about essentially the same reactions when used in sulfuric acid and sodium sulfate solutions, so it was decided that sodium sulfate would be employed as reference for all succeeding experiments.

The formation of iodoform which took place at the carbon anode in the alkaline potassium iodide solution could occur only if one of the beta-secondary alcohol groups of the sugar alcohol was attacked before the terminal group was oxidized, since the structure  $R\underset{O}{C}CH_3$ is required for iodoform formation.

Tin electrodes did not give the expected passivity for the black oxide which formed did not adhere to the electrode very well. At the end of thirty hours the tin electrodes were too corroded for further use.

The data summarized in Table IX indicate that oxidation at a lead anode yields both reducing compounds and acid compounds, while oxidation at carbon anodes with sodium bromide or sodium chloride as electrolyte produced almost as large amounts of reducing compounds but very little acid. This low acid condition indicates that the alcohol molecule is being oxidized to the aldehyde or the ketone with very

TABLE IX

Effect of various anodes and electrolytes on the oxidation of sorbitol.

Time, hours	Acidity[1]	Reducing value, calculated as mgm. glucose/ml.
10 per cent sorbitol as depolarizer		
<u>lead anode in 2 per cent sodium sulfate current 150 m.a.</u>		
8	0.50	6.55
18	0.75	12.60
31	1.10	20.45
44	1.60	22.50
56	2.10	23.50
68	2.45	23.95
<u>carbon anode in 2 per cent sodium chloride current 150 m.a.</u>		
6	0.05	5.9
20	0.15	12.6
31	0.25	16.3
44	0.35	20.4
56	0.45	22.1
69	0.55	23.8
<u>carbon anode in 2 per cent sodium bromide current 150 m.a.</u>		
8	0.18	6.5
18	0.30	11.2
31	0.40	16.3
44	0.45	18.05
56	0.55	19.65
68	0.55	21.1
<u>carbon anode in 2 per cent potassium iodide and sodium hydroxide</u>		
No reducing values noted. Iodoform appeared in appreciable quantities throughout the electrolysis.		

---

[1] Acidity is expressed in ml. of N/10 base equivalent to acid in one ml. of medium.

little further oxidation to acids. It was thought rather unlikely, in view of the previous experiments, that one of the alcohol groups of the sugar would be oxidized more easily than the aldehyde group of the same molecule; hence it would appear that one of the secondary alcohol groups had been transformed into a carbonyl group, thus accounting for the reducing properties of the solution.

The sorbitol solution which had been oxidized at the carbon anode using sodium bromide as electrolyte gave a negative test for aldehyde with Schiff's reagent, while the sorbitol solutions oxidized at the carbon anode in sodium chloride and the lead anode in sodium sulfate gave positive tests with the same reagent. After distilling a portion of each of the two last mentioned solutions tests for aldehydes and acids were made on the distillate. In both cases acids and aldehydes were present, thus indicating that considerable degradation of the polyalcohol molecule had taken place.

Effect of the use of catalysts or carriers on the electrochemical oxidation of sorbitol

Previous experiments have shown that two electrodes, lead in sodium sulfate solutions and carbon in sodium bromide solutions, are typical in their action on sorbitol, so it was decided that the effect of various materials, when present in small amounts, on the course of the reaction should be determined. These minute quantities of material added to the solution have at various times been referred to as catalysts and carriers and are usually added to increase the efficiency of the oxidation. As a rule carriers are compounds containing an element which changes easily from one valence to another and therefore merely acts as host to the oxygen for a short time, hence the name carrier.

Since the lead electrode when used in sulfate solution exhibits a rather vigorous oxidizing action on sorbitol, a number of different compounds were tested to determine whether one or more of them would tend to make the lead anode milder in its action. It is assumed at this point that low acidity and high reducing value throughout the course of the oxidation is an indication of mild oxidizing action.

The data summarized in Table X wereobtained using the following compounds as catalysts or carriers: 0.25 per cent potassium ferricyanide, 0.2 per cent secondary potassium phosphate, 0.01 per cent vanadium pentoxide, 0.01 molybdenum pentoxide, 0.1 per cent ferric chloride, 0.1 per cent ceric sulfate, and 0.1 per cent mercuric sulfate.

Of the carriers tested only molybdenum pentoxide, ferric sulfate, and mercuric sulfate increased the reducing value of the solution appreciably. In the presence of molybdenum pentoxide reducing compounds were formed very rapidly during

TABLE X

Effect of catalysts or carriers on the oxidation of sorbitol by lead electrodes.

Time, hours	Acidity[1]	Reducing power mgm. glucose/ ml.
	0.25 per cent potassium ferricyanide	
8	0.52	4.0
18	0.85	11.5
31	1.35	18.1
44	1.50	19.0
56	1.65	19.8
68	1.82	20.4
	0.2 per cent secondary potassium phosphate	
11	0.42	12.6
17	1.20	16.2
25	1.68	18.3
31	1.90	20.3
37	1.95	21.1
49	2.70 1.95*	18.7
	0.01 per cent vanadium pentoxide	
11	0.42	13.1
17	1.14	15.9
25	1.41	18.7
31	1.45	20.1
37	1.55	20.95
49	2.00 1.55*	19.80

[1] Acidity is expressed in ml. of N/10 base equivalent to acid in one ml. of medium.

*Acidity shown by last figure was determined after catholyte had been mixed with anolyte.

TABLE X. (Concluded).

Time, hours	Acidity^1	Reducing power mgm. glucose/ ml.
0.01 per cent molybdenum pentoxide		
11	0.42	13.8
17	1.15	19.7
25	1.51	22.8
31	1.55	24.6
37	1.62	26.1
49	2.25	24.0
	1.55*	
0.1 per cent ferric sulfate		
6	0.70	4.2
18	0.90	12.6
30	1.35	19.65
42	1.90	22.15
53	2.28	26.1
65	2.55	27.7
0.1 per cent ceric sulfate		
6	0.50	5.1
18	0.55	8.5
30	0.75	13.8
42	0.82	15.6
53	1.45	18.3
65	1.94	20.3
0.1 per cent mercuric sulfate		
6	0.50	5.8
18	0.35	9.3
30	0.60	16.3
42	0.95	21.9
53	1.10	25.1
65	1.15	26.8

the initial stages of the oxidation, but the rate of formation fell off slowly as the reaction progressed. The increase in acidity and the decrease of reducing values at the end of the reaction indicate that further oxidation of the initial products took place. Ferric sulfate when added to the solution caused a marked increase in reducing value. This increase is especially interesting in view of the part played by iron compounds in biological oxidation-reduction processes. The higher acidity observed in the solution containing ferric sulfate is probably due partially to the acid liberated when some of the iron salt was decomposed at the cathode. Mercuric sulfate appears to have a rather desirable effect on the course of the oxidation since the combination of high reducing value and low acidity is assumed to indicate only slight degradation of the initial oxidation product.

Several of the more promising catalysts were used with carbon electrodes with sodium bromide as the electrolyte, and in addition a few miscellaneous combinations such as carbon electrodes in 2 per cent sodium chloride plus 0.1 per cent manganese chloride, nickel anode in 2 per cent sodium hydroxide plus 0.01 per cent vanadium pentoxide, and tin anode in 2 per cent sodium carbonate plus 0.1 per cent vanadium pentoxide. This last combination was tried in the hope that stannic vanadate would form and catalyse the oxidation.

Stannic vanadate is one of the most active vapor phase dehydrogenation catalysts known and has a very low temperature of activation. The tin electrode corrodes too badly to be useful for extended oxidations.

The data given in Table XI indicate that molybdenum pentoxide acts as a very good oxidation catalyst for the carbon anode, increasing the efficiency approximately 50 per cent. Manganous chloride catalyses the formation of acid by the carbon electrode when used in sodium chloride solution. Although ferric salts increased the efficiency of lead anodes, they seemed to have the opposite effect on the carbon anodes. Vanadium pentoxide has no effect on the course of the reaction caused by a carbon electrode. When nickel anodes were used in an alkaline solution, the solution became very deeply colored. Since a deep red precipitate was obtained with dimethylglyoxime, it would appear that the coloration was due to a nickel compound formed with the sorbitol which is similar to the copper complexes of polyalcohols described by Lieser and Ebert (1937).

Identification of oxidation products

The separation of the principal final products present in the solution which has been subjected to electrochemical oxidation for fifty hours would be a very lengthy and tedious procedure so some other method of identification was sought.

TABLE XI

Effect of carriers and catalysts on the oxidation of sorbitol by carbon electrodes.

Time, hours,	Acidity[1]	Reducing power mgm. glucose/ ml.
10 per cent sorbitol as depolarizer		
	carbon anode in 2 per cent sodium bromide plus 0.01 per cent vanadium pentoxide current 150 m.a.	
6	0.10	5.3
18	0.25	10.7
30	0.55	14.5
42	0.45	16.8
53	0.55	18.7
65	0.60	20.1
	carbon anode in 2 per cent sodium bromide plus 0.01 per cent molybdenum pentoxide current 150 m.a.	
6	0.15	5.65
18	0.30	14.90
30	0.40	21.15
42	0.45	24.20
53	0.55	28.20
65	0.65	30.50
72	0.65	32.20

[1] Acidity is expressed in ml. of N/10 base equivalent to acid in one ml. of medium.

TABLE XI. (Concluded)

Time, hours	Acidity[1]	Reducing power mgm. glucose/ ml.
	carbon anode in 2 per cent sodium bromide plus 0.1 per cent ferric chloride current 150 m.a.	
8	0.27	5.05
19	0.35	8.1
31	0.37	10.9
43	0.44	12.65
55	0.48	14.2
68	0.48	15.85
	carbon anode in 2 per cent sodium chloride plus 0.1 per cent manganese chloride current 150 m.a.	
8	0.58	6.5
18	1.48	12.6
31	2.45	19.8
44	3.50	22.6
56	4.62	24.5
68	6.10	25.7
	nickel anode in 2 per cent sodium hydroxide plus 0.01 per cent vanadium pentoxide current 150 m.a.	
6	Acidity is of no value because the nickel electrode formed addition-products with the polyhydric alcohol in presence of sodium hydroxide.	2.8
18		7.4
30		10.7
42		14.2
53		18.05
65		21.70
72		22.40

There are certain color tests specific for ketone groups, carbohydrate structure, and the presence of pentoses, the procedures for which have already been described, which are used by biochemists for detection of sugars in various animal fluids. These tests indicate the type of compound present and therefore given an insight into the possible mechanism of the reaction. As a control, tests previously described were made using ten compounds of known structure. The results are given in Table XII.

TABLE XII

Compound used	Tests Used		
	Bial for pentoses	Molisch for carbohydrate structure	Selivanoff for ketose grouping
Glucose	clear solution	positive	clear solution
Mannose	clear solution	positive	clear solution
Galactose	clear solution	positive	clear solution
Fructose	brown precipitate	positive	red precipitate
Sorbose	brown precipitate	positive	red precipitate
Rhamnose	brown precipitate	positive	clear solution
Arabinose	green precipitate	positive	clear solution
Xylose	green precipitate	positive	clear solution
Dihydroxyacetone	pink precipitate	positive	cloudy solution
Sorbitol	clear solution	negative	clear solution

Since a complete tabulation of the tests made in connection with the analysis for the final oxidation products present in the numerous solutions would be so long as to be impractical, unnecessary duplication will be avoided. Typical examples were selected from the reactions already discussed and their designations are given below:

1. Lead anodes with 2 per cent sodium sulfate as electrolyte
   - A. No carrier. Table IX.
   - B. Potassium ferricyanide, 0.25 per cent. Table X.
   - C. Secondary potassium phosphate, 0.2 per cent. Table X.
   - D. Vanadium pentoxide, 0.01 per cent. Table X.
   - E. Molybdenum pentoxide, 0.01 per cent. Table X.
   - F. Ferric sulfate, 0.1 per cent. Table X.
   - G. Ceric Sulfate, 0.1 per cent. Table X.
   - H. Mercuric sulfate, 0.1 per cent. Table X.
2. Carbon anodes with 2 per cent sodium bromide as electrolyte
   - I. No carrier. Table IX.
   - J. Vanadium pentoxide, 0.01 per cent. Table XI.
   - K. Molybdenum pentoxide, 0.01 per cent. Table XI.
3. Carbon anodes with 2 per cent sodium chloride as electrolyte
   - L. No carrier. Table IX.
   - M. Manganese chloride, 0.1 per cent. Table XI.
4. Nickel anode in 2 per cent sodium hydroxide as electrolyte
   - N. Vanadium pentoxide, 0.01 per cent. Table XI.

TABLE XIII

Sample	Schiff	Molisch	Seliwanoff	Bial
A	positive	positive	whitish precipitate clear solution	negative
B	positive	positive	whitish precipitate clear solution	negative
C	positive	positive	whitish precipitate clear solution	negative
D	positive	positive	whitish precipitate clear solution	decidedly positive
E	positive	positive	whitish precipitate clear solution	greenish precipitate
F	positive	positive	whitish precipitate clear solution	greenish precipitate
G	positive	positive	whitish precipitate clear solution	greenish precipitate
H	positive	positive	whitish precipitate clear solution	greenish precipitate
I	negative	positive	positive	reaction of ketose
J	slowly positive	positive	positive	reaction of ketose
K	faintly positive	positive	positive	reaction of ketose
L	positive	positive	positive	
M	positive	positive	pink precipitate	negative
N	positive	positive	positive	reaction of ketose

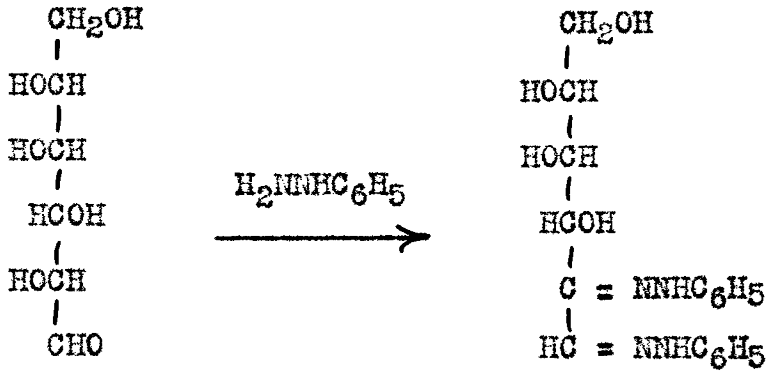
The data tabulated in Table XIII support the previous assertion that the lead anode has a degradative oxidizing action on the polyalcohol molecule while the carbon anode using sodium bromide as electrolyte does not. Using sodium chloride as the electrolyte the action of the carbon anode appears to have some characteristics of both the above materials.

A sugar even though it is an aldose does not react positively to Schiff's reagent because of the hemi-acetal linkage present. Therefore, the molecule must have been split at some point and smaller molecular weight aldehydes formed. All solutions except those oxidized at the carbon anode using sodium bromide as electrolyte gave strongly positive Schiff's tests showing that considerable amounts of aldehydes have been produced at the lead anodes.

The Seliwanoff test for ketoses indicated that they were present in five solutions, all of which had been oxidized at carbon anodes. The reactions of the other solutions tested could not be interpreted.

The Bial test for pentoses gives a bright green solution or precipitate or both when they are present. Controls have shown, however, that ketohexoses also react to Bial's reagent but give dark brown precipitates. The results of this test on the oxidized solutions showed pentoses were certainly present in one solution and probably present in four others, as well as substantiating the presence of ketoses in several more.

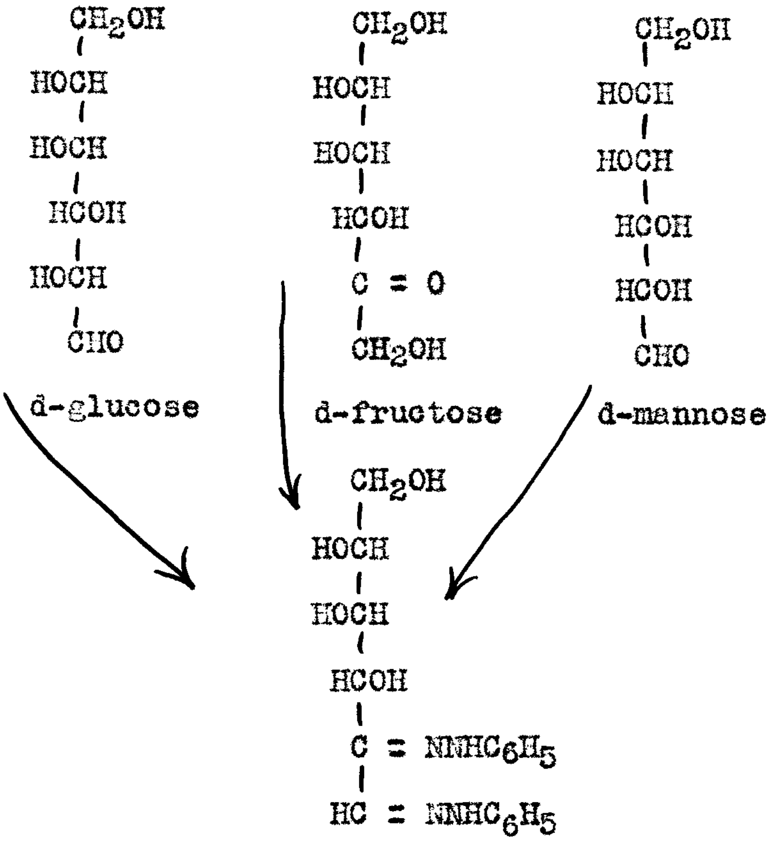
Phenylhydrazine will react with carbonyl groups to form compounds known as hydrazones. Since sugars all have carbonyl groups, they will give this characteristic reaction, but it is usually rather difficult to stop the reaction at the hydrazone. The usual procedure is to heat the mixture of sugar and phenylhydrazine on a water bath so that the osazone is formed as shown by the equation below.



$$\begin{array}{c} CH_2OH \\ | \\ HOCH \\ | \\ HOCH \\ | \\ HCOH \\ | \\ HOCH \\ | \\ CHO \end{array} \xrightarrow{H_2NNHC_6H_5} \begin{array}{l} CH_2OH \\ | \\ HOCH \\ | \\ HOCH \\ | \\ HCOH \\ | \\ C = NNHC_6H_5 \\ | \\ HC = NNHC_6H_5 \end{array}$$

d-glucose d-glucose osazone

Morrow and Sandstrom (1935) have described a method for the identification of sugars by observing the crystalline structure of the osazones under a microscope, and give a number of photomicrographs of typical osazones. The use of phenylhydrazine for qualitative identification of various sugars is not as specific as it could be for there are certain sugars which give identical osazones. The equation below shows that glucose, fructose, and mannose form identical osazones. If one is to distinguish between the several members in each of these groups, other methods must be used.



Since alpha substituted phenylhydrazines differ from phenylhydrazine in their reactions with aldose sugars, forming hydrazones instead of osazones, it is possible to identify various sugars by determining the melting points of the compounds formed. Because of the complexity of the mixture with which it was necessary to deal, it was decided that an attempt should be made to devise a method for microscopic qualitative identification based on the method given by Morrow and Sandstrom (1935) but using alpha substituted phenylhydrazines as well as phenylhydrazine. A method of this sort would have the advantage of ease of manipulation and could be used on mixtures containing many materials other than sugars without preliminary separation.

For example, consider the three sugars glucose, fructose, and mannose. When treated with phenylhydrazine hydrochloride as described on page 44, all three give the osazone shown in Figure V. Mannose, however, first forms the hydrazone which is quite insoluble and precipitates along with the osazone. On heating for a short time all the hydrazone will change to the osazone, and Figure VI shows the crystalline structure of both. If these three sugars are treated with alpha-methyl phenylhydrazine and acetic acid, glucose forms the hydrazone which is rather difficult to crystallize, while fructose and mannose form the crystals shown in Figures VII and VIII respectively.

Sorbose and its isomers, idiose and gulose, on treatment with phenylhydrazine hydrochloride form osazones whose crystals are shown in Figure IX, while sorbose, the only sugar of the three immediately available, forms the crystals shown in Figure X when allowed to react with alpha-methyl phenylhydrazine and acetic acid. The photomicrographs, Figures XI to XVII, show the appearance of the reaction products of various other sugars with phenylhydrazine and alpha-methyl phenylhydrazine.

Figure V
Glucosazone

Figure VI
Mannose hydrazone
and Glucosazone

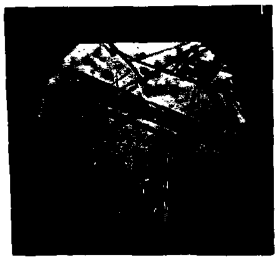


Figure VII
Sugar treated with
Fructose

Figure VIII
Methyl Phenylhydrazine
Mannose

Figure IX
Sorbose treated
with Phenylhydrazine

Figure X
Sorbose treated with
Methyl Phenylhydrazine

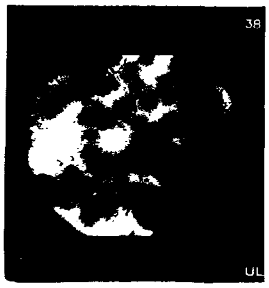


Figure XI
Arabinose

Figure XII
Xylose

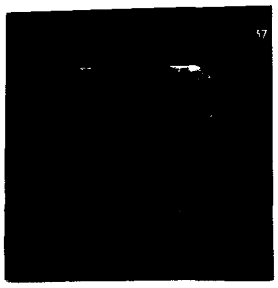


Figure XIII
Rhamnose

Figure XIV
Dihydroxy acetone

Figure XV

Treated with
Phenylhydrazine

Galactose

Figure XVI

Treated with
Methyl Phenylhydrazine

When the phenylhydrazine test was applied to the oxidation products formed under the various conditions used in this investigation, five different crystalline structures were obtained. The photomicrographs of these crystals are shown in Figures XVII to XXI inclusive and the letter designation refers to the key on page 90. The crystals in these photomicrographs do not entirely match any of the knowns and therefore were not identified, but in Figure XIX there is a small amount of glucosazone present.

Figure XVII

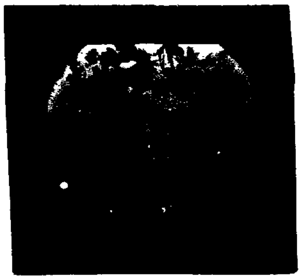


Figure XVIII

Figure XIX

Figure XX

Figure XXI

SUMMARY AND CONCLUSIONS

It has been suggested that the preferential oxidizing action which Acetobacter suboxydans shows toward secondary alcohol groups is due to the fact that secondary alcohol groups are oxidized at a lower potential than the primary alcohol groups of the same molecule. Cook and Major (1935), using the method described by Isbell and Frush (1931), reported that 5-keto-gluconic acid as well as gluconic acid was formed when glucose was oxidized at an anode in the presence of sodium bromide. This finding together with the statement that "the sugar alcohols were found to be oxidized to ketones and then the oxidation characteristic of ketones took place", made by Everett and Sheppard (1936) with reference to bromine, seems to substantiate the suggestion that the oxidizing action of the Acetobacter suboxydans is a matter of potential.

During the preparation of this thesis several experimental facts were brought to light which also substantiated the idea mentioned in the preceding paragraph.

(1) Isopropyl alcohol is oxidized quite readily to acetone at the temperature of melting ice by alkaline iodine solution while alcohol is oxidized very slowly under the same conditions.

(2) A lead electrode in the presence of a sulfate electrolyte will oxidize both primary and secondary alcohol groups equally well. However, a carbon electrode in the presence of a halide electrolyte oxidizes a secondary alcohol group very readily and a primary alcohol group almost not at all.

(3) Polyhydric alcohol solutions oxidized with lead electrodes in the presence of sulfate electrolytes reacted positively to Schiff's aldehyde test and negatively to Seliwanoff's test for ketoses. These tests would indicate that the alcohol molecule has been badly degraded.

(4) Polyhydric alcohol solutions oxidized with carbon electrodes in the presence of sodium bromide reacted negatively to Schiff's aldehyde test and positively to Seliwanoff's test for ketoses, indicating the absence of degradation.

(5) Bial's test for pentoses showed the presence of a pentose in the solution whose oxidation was catalyzed by vanadium pentoxide. This test also substantiated the Seliwanoff test in every case.

(6) The color tests used indicate that on very mild oxidation, polyhydric alcohols tend to be oxidized to ketonic compounds. However, in the presence of more vigorous oxidizing conditions the polyhydric alcohol molecule seems to be attacked at several points.

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