

ACTIVE GLUCOSE*

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Glucose is one of the most important substances in the economy of the world. In the living organism it is the source of animal heat. In every-day life it is important as the material from which alcohol, acetone, lactic and tartaric acids are made; furthermore, it is the substance from which are built the molecules of important products such as starches, gums, cellulose.

It is easy then to understand the reasons behind the search for an explanation of the mechanism by which glucose is transformed into these important substances. As a result of the research directed toward this goal, there have been advanced novel theories concerning the forces which serve to combine the smaller molecules into the larger and concerning those forces which lead to the disintegration of the small molecules into still smaller. One of the new conceptions is that of "active" glucose. A concrete, final, undisputed theory of the nature or of the structure of this form of glucose has not yet been furnished. The concept arose as a matter of expediency in order to explain some puzzling observations on the conduct not only of glucose but also of a much simpler sugar, namely, of a triose, a substance which can be derived from glucose and which may condense into glucose.

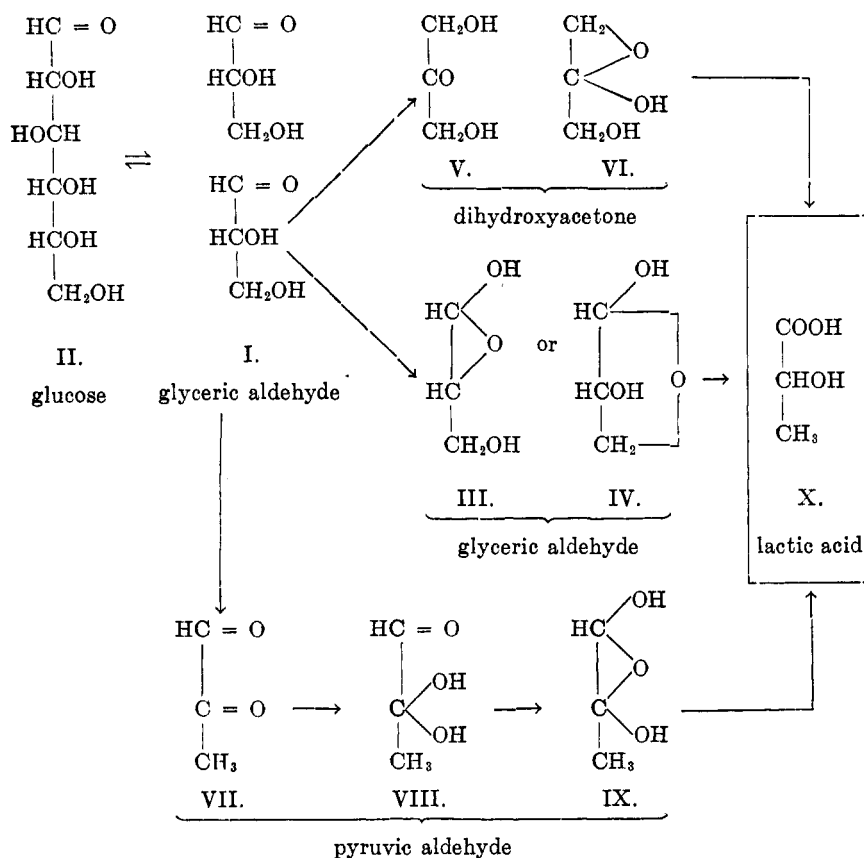
This is a very fortunate occurrence for the reason that the ideas concerning the "active" form of triose are easier to test experimentally than those regarding the "active" form of glucose.

The gradual evolution of the idea regarding triose, therefore, will be presented first by way of introduction to the more involved topic of "active" glucose.

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ACTIVE TRIOSE

From the following formulae



it is readily seen that glyceric aldehyde may, on one hand, condense into glucose and, on the other, through intramolecular rearrangements, assume a variety of forms of which pyruvic aldehyde and lactic acid are of special interest.

Theoretically, both pyruvic aldehyde and lactic acid can readily be transformed into acetaldehyde and a substance readily convertible into carbon dioxide. Further, acetaldehyde can easily be either reduced to alcohol or oxidized to carbon dioxide and water. Thus, the chemical theory permits us to outline the

individual phases or steps through which a molecule of glucose has to pass on its way to carbon dioxide and water or on its way to carbon dioxide and alcohol and permits us to formulate a satisfactory theory of fermentation and of the biological oxidation of sugar. Indeed, Nef and Wohl have done so (1).

The experimental evidence in support of the theory had a slow development. The formation of lactic acid from glucose was observed by Reichard in 1870, more recently by Kiliani, still more recently by Meisenheimer, and many times since (2).

The discovery of pyruvic aldehyde among the products of dissociation of glucose in alkaline aqueous solution is of more recent date. It was made first by Pinkus in 1898, later by W. Löb, by Dakin and Janney, by Neuberg and Oertel and by others (3). Finally, the conversion of pyruvic aldehyde into lactic acid through the action of alkali has been demonstrated by Evans and his coworkers (4).

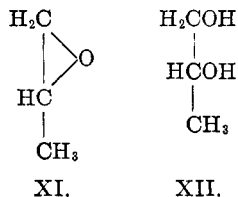
Gradually evidence accumulated to show that biological agents were capable of transforming glucose into lactic acid and to show that its precursor is pyruvic aldehyde. Many investigators, on the basis of animal experimentation, had accepted the view that lactic acid is an intermediary substance in the process of biological utilization of sugar (5). By a more direct and rigorous method the biological conversion of glucose into lactic acid was shown simultaneously and independently by Slosser, by Levene and Meyer, and by Embden and coworkers (6). Levene and Meyer have shown also on experimental grounds that glucose in its conversion into lactic acid passes through the stage of pyruvic aldehyde (7). Dakin and Dudley, Neuberg, and Levene and Meyer have shown independently and practically simultaneously that methyl glyoxal is converted into lactic acid by animal cells or tissues (8). Still later methylglyoxal was isolated from among the products of bacterial action.

Thus it seemed that the mechanism of alcoholic fermentation outlined on purely theoretical grounds had been well supported by experimental evidence. There remained to test the fermentability of pyruvic aldehyde and of lactic acid. It was expected that, as intermediary products, they should ferment at a higher

rate than glucose. The test of the fermentability of pyruvic aldehyde and of lactic acid and the test of their biological oxidation led to very disappointing results. It was found that not one of the several possible forms of pyruvic aldehyde or the substances formed from it through intramolecular rearrangement fermented with the same velocity as glucose (9). In order, therefore, to reconcile the older theory with the newer facts, an assumption was made of the transitory existence of an "active" form of the triose. A concrete formulation, however, of the structure of the "active" form had at that time not been advanced.

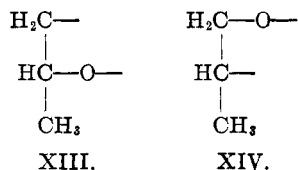
The key for further development of the idea into a more concrete formula was found in the chemical theory.

Scrutinizing all the isomeric forms of a triose, our attention centered on the cyclic structures. The existence of the cyclic forms was demonstrated by the preparation of the corresponding glucosides by Bergmann (10). The free cyclic trioses have not yet been prepared. However, analogous cyclic structures are known for certain organic oxides. Of these, one, propylene oxide, has recently been studied by Levene and Walti (11).

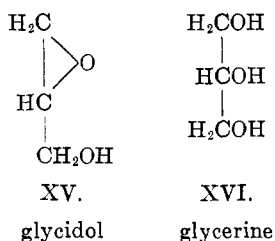


Propylene oxide (XI) is characterized by much greater reactivity than propylene glycol (XII) from which it is readily formed and into which it is transformed still more readily.

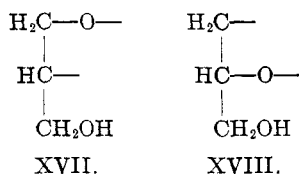
Propylene oxide is easily converted into propionic aldehyde which is formed by an intramolecular rearrangement analogous to that observed in trioses and expressed in formulae from (I) to (X). It had been assumed by Nef that in solution propylene oxide may exist in one of the following two forms.



More definite experimental evidence in favor of the transitory existence of such forms is furnished by recent work of Levene and Walti (11). It has been shown also that oxides have a great tendency towards condensation in a manner similar to that by which simple sugars condense to di- and polysaccharides (12). The conduct of the cyclic substance glycidol and of glycerine is very similar to that of propylene oxide and propylene glycol.



Also, of these two substances, the first is the more reactive. Glycidol, upon opening of the ring, is capable of a transitory existence in the unsaturated forms (XVII) or (XVIII).



These unsaturated forms, as free radicals, possess much greater molecular asymmetry and because of it much greater reactivity than the saturated forms and to them one may justly apply the term "active." Thus, each cyclic triose may exist in its own "active" form, namely, in that which has a transitory existence upon the opening of the ring.

"ACTIVE" GLUCOSE

The evolution of the concept of "active" glucose passed through the same phases as that of "active" triose.

The biological observations which led to the belief in the existence of an "active" form of glucose are the following.

First. The different sugars ferment at different velocities and yet the intermediate and final products of their fermentations are identical. Thus, it was found that when a mixture of fermentable hexoses is exposed to the action of yeast, a marked difference is observed in the rate of fermentation of individual isomers (13).

It is possible that in this respect individual yeasts show different behavior for the reason that the results reported by individual workers are not always in agreement with each other. Thus, according to Fernbach (14), all yeasts ferment fructose with greater velocity in the early phases of fermentation, but the velocity lags behind that of glucose in the later stages of fermentation. However, by a suitable adjustment of the hydroxyl ion concentration, the more rapid fermentability of fructose may be maintained until the very end. If a mixture of glucose and fructose is employed, then according to Willstätter, the rate of fermentation of fructose to glucose is 3:1 (15).

If instead of living yeast, zymon or yeast juice is employed, then, according to Harden (16), fructose has the highest rate of fermentation in the early stages, the second place belongs to mannose, but in the later stages the rate of fermentation of mannose falls behind that of glucose.

Also animal cells and tissues seem to discriminate between the individual hexoses. Thus, Levene and Meyer have observed that the yield of lactic acid from mannose is lower than that from glucose and fructose when living leucocytes or aseptic kidney tissue (17) is allowed to act on them, and according to Laquer (18) the rates of glycolysis in muscle tissue of the three hexoses—fructose, mannose and glucose—are the following: 0.484, 0.395, 0.327. According to Warburg, the cells of various normal and pathological tissues oxidize the three hexoses in the following order, glucose, mannose and fructose, the ratios being 24, 22, 3 (19). Galactose is the least fermentable hexose and in fact, yeast has to undergo special training in order to ferment this sugar.

All the above quoted observations are interpreted to mean that the various sugars are transformed into a form common to all

which then undergoes further decomposition, and that this form is the so-called "active" glucose.

Second. Euler and his coworkers have found that glycogen ferments at a higher rate than free glucose (20). More striking is the high rate of dissociation of glycogen by animal tissues. Thus Laquer found that the formation of lactic acid from glycogen proceeds at a higher speed than that from any other sugar. Embden (21) found that after addition of sodium fluoride, glycogen gives a larger yield of lactacidogen than does glucose. Neuberg and Gottschalk found that not only lactic acid, but also the intermediate products of glycolysis, are formed at a higher rate from glycogen than from other sugars (22).

The nearly unanimous conclusion drawn from these observations is that in glycogen glucose is present in a more labile state than in the common forms of glucose.

Third. A disparity was observed between the fall of the reducing power of a fermenting solution and the amount of alcohol and CO_2 formed. The earlier observations in this direction were made by Euler and his coworkers and by Lebedev (23). The interpretation of Euler was that in the early phase of the process an intermediary product was formed which subsequently underwent fermentation. Indeed, Euler sought for ways to depress fermentation without affecting the first phase of the process and by this means to isolate the intermediate substance. His efforts in this direction, however, failed. Harden and Young, Buchner and Meisenheimer have thought to explain the phenomenon by the assumption of reconversion of glucose to a polysaccharide. Their conclusions, however, were contraverted by Euler. Also Lebedev on theoretical grounds disagreed with the theory of synthesis of a polysaccharide. The observations of Lebedev were interesting also for the reason that he observed a greater lowering in the rotatory power of the fermenting solution than that expected on the basis of the amount of sugar converted into alcohol and CO_2 .

The findings of Lebedev bring us close to the observations made on the blood sugar after administration of insulin. In several publications from the most reputed laboratories the claim was made that after administration of insulin the blood sugar acquired

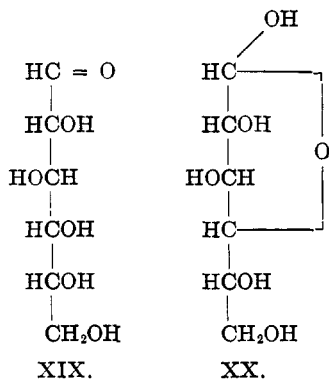
special properties which distinguished it from ordinary glucose, namely, that it had a lower rotatory power than the common forms of glucose and that on standing the rotatory power rose to that of the equilibrium form of common glucose (24).

These claims have been challenged and, to say the least, need corroboration, but whatever the value of the observations may be, they stimulated the belief in the existence of the unstable form of glucose.

Fourth. In the course of fermentation with zymin, several hours pass before the evolution of carbon dioxide begins. It seems as if a period of incubation were required during which the sugar undergoes certain changes necessary for its fermentability. The period of inactivity may be shortened by the addition of some chemical substances, particularly by a substance itself a product of fermentation, namely, a hexosediphosphoric ester. The details of the chemical changes taking place during the incubation period are as yet not known.

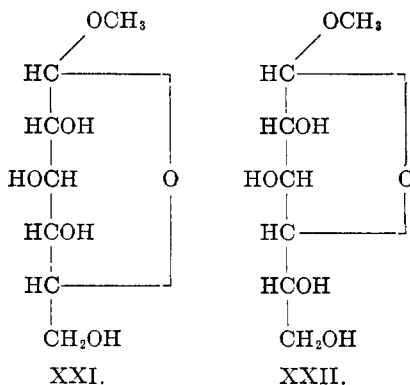
Thus far the problem had been of a purely biological nature. The conclusions had been reached by purely biological methods and lacked concreteness.

For more precise and more accurate conclusions, a deeper insight into the chemistry of sugars is needed. Indeed, the further development of the idea of "active" glucose goes hand in hand with the progress of pure chemistry. In the early days of sugar chemistry it was known that glucose is capable of existence in two forms, one having the structure of an ordinary aldehyde and the other, a cyclic form.



If we had at that period of our knowledge speculated on the nature of an "active" glucose, the choice would have been limited to the two forms only. Indeed, even today, authorities in sugar chemistry are inclined to regard the aldehydic form as the "active" form of glucose.

In recent years, however, heretofore unknown isomers of derivatives of glucose have been discovered. These isomerisms were explained by the assumption of differences in the ring structures. The greatest mass of work was done on the glucosides. Through the work done on these substances by Pryde, Levene and Meyer, Haworth and coworkers and by Levene and Simms, the characters of the different ring structures are now definitely established (25). The common forms of glucosides have the oxygen bridge between the first and the fifth carbon atoms (XXI) and the newly-discovered forms have the oxygen bridge between the first and the fourth carbon atoms (XXII).



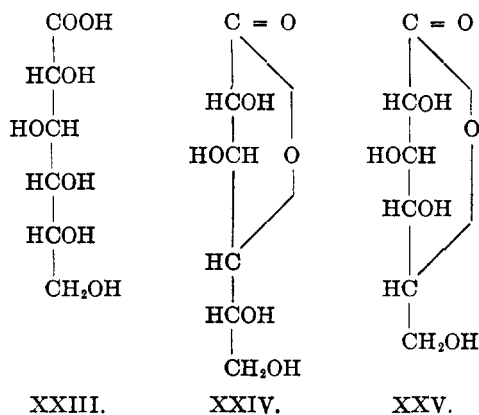
It was proposed to refer to the cyclic forms as lactal forms, naming the first $\langle 1,5 \rangle$ lactal and the second $\langle 1,4 \rangle$ lactal.

From the facts established for glucosides, it was surmised that free glucose also exists in $\langle 1,5 \rangle$ and in $\langle 1,4 \rangle$ lactal forms and that the $\langle 1,4 \rangle$ lactal form is the more unstable and hence the form to be regarded as the "active" form of glucose.

This assumption, plausible as it seemed, at the time when it was announced had little experimental evidence in its support. It is the outstanding problem of the day in sugar chemistry to

unravel the ring structure of the free sugars and to correlate chemical structure with biological conduct.

The approach towards the solution of the problem was the following. Sugar acids resemble sugars in their ability to acquire cyclic structures, as shown in the following figures (26).



The advantage of choosing lactones is that, unlike the lactals, they can be prepared easily in crystalline form. It was shown by Levene and Simms that individual lactone forms have different degrees of stability and that peculiarities arising from the ring structure in the case of unsubstituted and in that of substituted acids are practically identical. Thus the conclusion is warranted that also in the case of sugars, there exists an analogy between the properties of the substituted and of the unsubstituted forms.

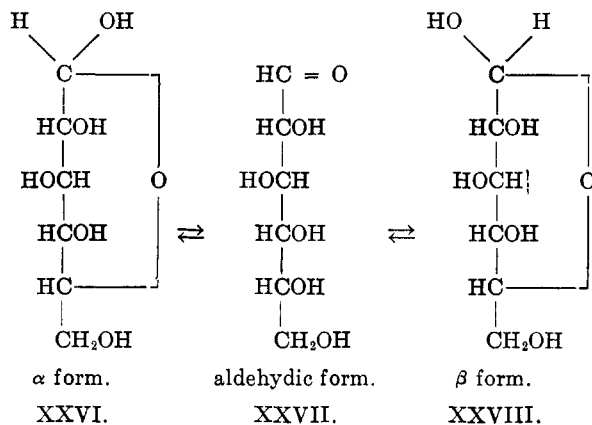
From the observations on substituted and unsubstituted sugar acids the conclusion was formulated that in a solution of a sugar acid, all theoretically possible lactones are formed, but only the more stable are present in measurable quantities and that the proportions of the different lactones are a function of time. In the fresh solutions the unstable forms predominate.

Inasmuch as lactone and lactal formation are analogous processes, the thought was expressed that in a solution of sugar also, all theoretically possible lactals are formed and that at the point of equilibrium the more stable forms predominate. This view is supported by the fact that from the same common glucose,

depending upon the conditions of experiment, three different forms are obtainable. Thus, in methyl alcoholic solution, methyl glucosides are obtained having the $\langle 1,5 \rangle$ and the $\langle 1,4 \rangle$ lactal structures. In acetone solution, only the $\langle 1,4 \rangle$ lactal derivative is formed, whereas in aqueous hydrochloric acid glucose condenses with mercaptans into the noncyclic aldehydic derivatives.

On the basis of these considerations, the assumption of the simultaneous existence in solution of the aldehydic and of several cyclic forms gains in probability.

At the time when this assumption was made by Levene and Simms, there existed no experimental evidence for the view that individual lactals possess different degrees of stability. In sugars and in certain of their derivatives, the stability of a ring may be measured by the phenomenon of mutarotation. In sugars the mutarotation is explained by the change of one isomeric form into another—(α) into (β).



The intermediate is the noncyclic form. Hence the sugars or their derivatives which show a higher velocity of mutarotation are also those in which the opening of the cyclic structures proceeds with higher velocity.

Up to last year there was lacking material of the $\langle 1,5 \rangle$ lactal structure on which the rate of mutarotation could be studied. In course of last year such material was furnished through the

work of Levene and Meyer (27), and today it is quite certain that in free sugars also, the stability of the cyclic structures depends upon the ring structure, and in addition that in free sugars different ring isomers actually exist.

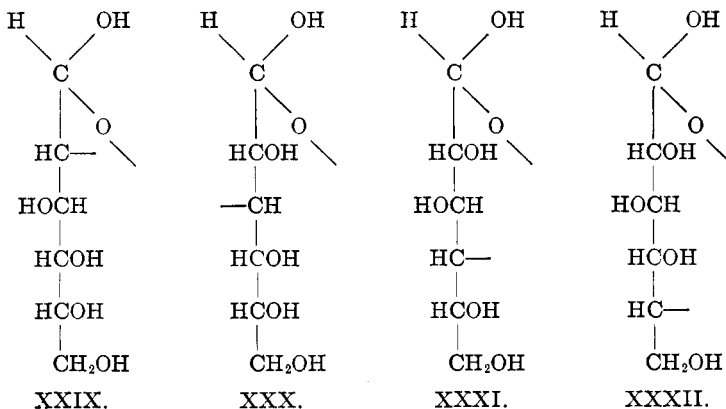
In order to correlate the chemical findings of the stabilities of the different lactal structures with the concept of "active" glucose demanded by the observations on fermentation, it was now necessary to prove that the velocity of fermentation also is influenced by the stability of the lactal structures. Indeed, Willstätter and Sobotka found (28) that α -glucose, (having a more unstable ring structure) ferments with a higher velocity than the β -form.

Thus, it was on one hand shown that in free sugars rings of different stability may exist and on the other, that the rate of fermentation is influenced by the stability of the ring.

Here again we see that after certain facts have been established by the chemical method and after certain conclusions have been drawn from them, it is pertinent to test the chemical theories by the biological method.

If we now return to the view that the "active" form is the one possessing the properties of a free radicle, it will at once be evident that the product of fermentation is in a way determined by the character of the ring structure.

A glance at the free radicles derived from some of the theoretically possible lactals



reveals the fact that the radicles (XXX) and (XXXI) are most likely to undergo the cleavage of the six-carbon chain into two three-carbon chains. These forms therefore may be regarded as the "active" forms of glucose in the fermentations forming alcohol, acetone and lactic acid. On the other hand, for fermentations resulting in butyric acid the $\langle 1,2 \rangle$ and the $\langle 1,3 \rangle$ lactals are the most favored forms. Thus, each form of fermentation has its own "active" form of glucose.

Granting that the views here expressed are correct, one question still remains unanswered, namely, as to the nature of the mechanism by which in a solution of glucose the concentration of the favored form increases above the normal concentration. Normally, in a fresh aqueous solution of glucose, all lactal forms coexist and after some interval of time, gradually the most stable form predominates.

How then is the concentration of the less stable form increased over that of the more stable forms? The simplest mechanism would be one by which an enzyme enters in combination with a carbon atom which normally serves for the formation of the oxygen bridge. Thus, if a methyl group is introduced in the hydroxyl of the fifth carbon atom, then the formation of the $\langle 1,5 \rangle$ lactals is made impossible. The same would happen if an enzyme had combined in place of the methyl group. Often the nature of the group entering into union with the first carbon atom influences the ring structure of the resulting derivative. Thus, acetone glucose in which the acetone is linked to the first and the second carbon atoms has the $\langle 1,4 \rangle$ lactal structure.

Indeed, the assumption of the formation of a complex glucose prior to fermentation acquired an explicit character when it was discovered that in the course of fermentation as well as in the course of the combustion of sugar in the muscle tissue, there takes place a condensation of the glucose with phosphoric acid giving rise to a hexose diphosphate. It was then concluded that in this substance the sugar is contained in a more unstable state. In other words, that either the hexose diphosphate itself or the sugar liberated from it is the unstable form. This contention, however, is not fully in accord with experimental evidence.

Phosphorylated glucoses, whether synthetic or formed on fermentation, are not fermented by living yeast or by zymine at a higher rate than free glucose. However, the failure of the phosphoric ester theory does not exclude the possibility of the formation of another complex substance. It is safer, however, to admit that as yet the mechanism by which the concentration of the unstable lactal structures is increased above the normal is unknown.

Thus, the arguments advanced in this discussion are the following.

1. Observations on the processes of fermentation and of animal combustion of glucose led to the conclusion of the existence of an "active" form of glucose.

2. Observations on organic oxides and glycols bring out the fact that cyclic structures are more active than noncyclic structures, because they are readily transformed for a short but finite interval of time into free radicals.

3. Observations on glucose derivatives show that they are capable of existence in several ring isomers differing from each other in their stability.

4. Observations on sugar acids demonstrate that in aqueous solution all theoretically possible cyclic structures can exist simultaneously.

5. The conclusion is drawn that in aqueous sugar solutions, all theoretically possible cyclic structures coexist in equilibrium with each other.

6. The agents bringing about fermentation produce an increase in concentration of that cyclic form of glucose which gives rise to that radical which is most apt to cause the required dissociation of the glucose molecule, the initial phase in the process being the formation of a free radical.

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