Reports

Enzymatic Oxidation of Glucose to Glucosone in a Red Alga

The biological oxidation of gluconic acid to 2-ketogluconic acid is a well-known reaction (*1*). However, an analogous reaction, in which the second carbon of glucose is oxidized to a carbonyl group, producing an osone, has received little attention. The work of Walker *et al.* (*2*) indicated that such a reaction possibly occurs on fungi, and Berkeley (*3*) demonstrated the formation of a glucosone from glucose in the crystalline styles of Mollusca. The possible function of glucosone in the formation of glucosamine has been explored by Becker and Day (*4*) and Dorfman *et al.* (*5*).

We have shown previously that the marine red alga, *Iridophycus flaccidum,* oxidizes glucose and galactose to the corresponding aldonic acids (*6, 7*). In this report, evidence is presented that glucose and galactose are also oxidized to glucosone and galactosone, respectively, in the tissue and extracts of this alga (*8*).

In a preliminary experiment, disks cut from the thallus with a cork borer were bathed in a solution of radioactive sugar as previously described (*6*). At intervals of from 1 minute to 24 hours, the disks were removed and the tissue was inactivated by boiling in ethanol. The products present in the alcoholic extracts were then identified by means of two-dimensional paper chromatography, using solutions of phenol and water and of butanol, acetic acid, and water as solvents, followed by autoradiography of the chromatograms to locate the radioactive spots (*7*). The results showed that the major portion of the activity from the infiltrated glucose initially entered into a compound with an R_g (chromatographic movement relative to glucose) of 1.25 in the phenol-and-water solvent and 0.79 in the solvent of butanol, acetic acid, and water. This compound disappeared in the later stages of the reaction, leaving gluconic acid as the only significant product of the glucose oxidation (Fig. 1). Paper chromatographic data showed that this compound was neither a gluconolactone nor a substituted form of glucose. Similar experiments showed that the same type of reaction occurred when galactose was introduced into the plant.

For identification of this unknown compound produced from glucose, additional material was obtained by incubating radioactive glucose with sections and extracts of *Iridophycus* for short periods of time, extracting with alcohol, chromatographing the extracts, and eluting the radioactive spots from the chromatograms. The fractions representing the unknown product were pooled and this material was subjected to a number of chemical tests.

Oxidation of the unknown compound with bromine (*9*) produced a compound which migrated to the same position as an aldonic acid when it was chromatographed on paper in the afore-mentioned solvents. Reduction with sodium amalgam yielded a mixture of products, but reduction with zinc and acetic acid produced chiefly a compound having chromatographic characteristics identical with those of fructose.

For purposes of comparison radioactive glucosone was prepared synthetically (*9*) and purified chromatographically. The biologically produced material was found to cochromatograph with the major product of the chemical synthesis. When a sample of the unknown product was mixed with phenylhydrazine and sodium acetate in a capillary tube, an immediate turbidity and a color change (to bright yellow) were observed. The precipitate formed was initially amorphous, but after it had stood overnight in the cold in the sealed tube, clusters of needles that were identical in appearance with those of glucose phenylosazone (*10*) were formed. A similar reaction was observed under the same conditions with the chemically synthesized osone. This reaction did not occur with glucose alone or with glucose and an eluate from a blank paper. These tests show that glucosone is the transient compound formed as an oxidation product of glucose. The product formed from galactose was subjected to similar tests, and it was concluded that galactosone is also formed in the plant through oxidation.

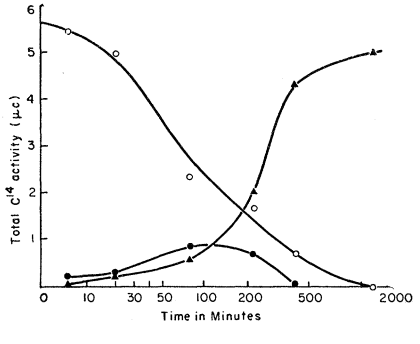

Fig. 1. Oxidation of glucose *in vivo* by *I. flaccidum*: ○, glucose; ●, glucosone; ▲, gluconic acid. For each point in the chart, 0.14 mg of C^{14}-labeled glucose (42 μc/mg) in 0.2 ml of water was incubated with two disks of 90 mg each of thallus for a given period. The disks were inactivated and extracted with hot alcohol. Aliquots of the extracts were analyzed by paper chromatography, and the radioactivity in each component was counted directly on paper.

Crude extracts of *Iridophycus* were also capable of forming glucosone and galactosone from the corresponding sugars. However, the osones did not accumulate to the same extent in the extracts as in the living tissues. Dialyzed extracts and purified sugar oxidase (*6*) from this alga did not oxidize the aldoses to osones. Thus it appears that formation of the osones is not directly related to the formation of the aldonic acids in this alga and that the two compounds may be formed by different enzymes. However, the osones can be converted to the aldonic acids through an undetermined mechanism. This is indicated by the experiments *in vivo*. Initially, comparatively high activity was observed in the osones, but, after a prolonged period of time, all the activity appeared in the aldonic acids. When isolated C^{14}-glucosone was incubated with the crude extracts of *Iridophycus,* gluconic acid was found as the only endproduct. This reaction has not yet been carried out under conditions which would permit determining whether glucose is formed as an intermediate product of the isomerization of glucosone to gluconic acid. Inasmuch as the purified oxidase from *Iridophycus* does not appear to form glucosone, it seems probable that the reaction involving the formation of osone is readily reversible to produce the original sugar, which can then be oxidized through another route directly to the aldonic acid. Further work is now in progress on the isolation and characterization of the enzyme system responsible for this oxidation reaction.

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References and Notes

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"Clock" Controlled Activity Rhythms in the Fruit Fly

Recent studies in this laboratory on the daily rhythm of eclosion (*1*) in fruit flies of the genus *Drosophila* have shown that it is controlled by an interval timing device—or "clock"—that is temperature independent. It is well known that the locomotor activity of *Drosophila* adults in the field also exhibits a clear daily rhythm; activity is maximal in the evening just before sunset. A question of prime importance is whether the active period is determined by strictly exogenous factors such as light intensity, relative humidity, or temperature, or whether an internal biological timing device is involved.

Experiments in the field (*2*) have shown that temperature and humidity do not play a major role in the determination of active periods in *Drosophila.* However, it has been shown that the time and duration of active periods are correlated with definite ranges of light intensity. During the late afternoon, maximum activity occurred when the light intensity ranged between 100 ft-ca and 15 ft-ca.

To study the locomotor activity of *Drosophila* in the laboratory, a small cylindrical lucite chamber was devised with a grid of fine wires on the inside walls. Every other grid wire was connected to a common terminal. When a fly walks about in the chamber, it cannot help but short-circuit any two adjacent wires. This short circuit was detected with a high gain amplifier, and contacts per hour were translated into ink marks by an operations recorder. The virtue of this system lies in the ability of the instrument to record activity of flies under wide ranges of light intensity or even in total darkness.

Figure 1 shows the pattern of activity in *D. robusta* over a period of 7 days. Six male and six female flies were placed in the chamber with food, and the humidity of the chamber was maintained at a high level. The temperature was rigidly controlled at 21°C. For the first 4 days the flies were subjected to 12 hours of bright light (25 ft-ca incident reading) alternating with 12 hours of absolute darkness. At the onset of the fifth day, a very dim light (less than 1 ft-ca incident reading) was substituted for the bright lamp. This dim light was left on for the remainder of the experiment, and the alternating dark and bright light cycle was abolished. It can be seen that the active periods for the first 4 days were restricted to a few hours before the onset of darkness and that activity fell off abruptly when the lights suddenly went out. During the last 3 days, when the flies were in constant low light, the active periods spread to some degree, but the mean remained in phase with the previous 4 days.

These data strongly suggest that a biological "clock" is operative in determining the active periods for the flies. The absolute amount of activity declined during the last 3 days of the experiment; this was probably the result of the depressing effect of dim illumination. Previous experiments in which flies were subjected to several days in absolute darkness bear this out. The amount of activity in total darkness was so slight that no rhythm could be detected.

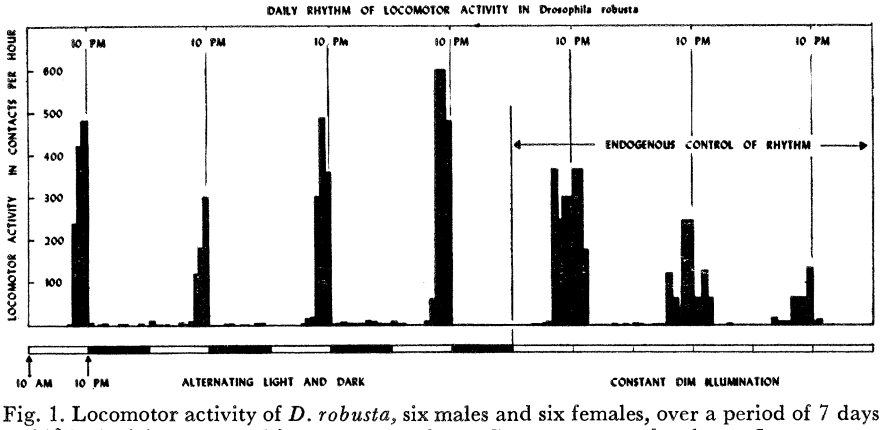

Fig. 1. Locomotor activity of *D. robusta,* six males and six females, over a period of 7 days at 21°C. Activity measured in contacts per hour. Contacts are made when a fly steps on a pair of grid wires. Under conditions of alternating light and dark, the photoperiods are 12 hours with "dawn" at 10 A.M. E.S.T.

Besides the fact that a rhythm of activity persists under constant conditions of illumination, it is also interesting to note the pattern of activity during the first 4 days of alternating light and dark. The onset of the evening peaks occurred quite regularly at about 7 P.M. without reference to any gradual fluctuation in light intensity, temperature, or relative humidity as would exist in the field. It appears then, that under alternating light conditions where the lights are turned on and off abruptly, the commencement of activity is under "clock" control.

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Hardness of Substances in the Ideal State

The relation proposed by Tabor (*1*) between Vickers indentation hardness, H_v, and ultimate tensile strength, T_u, of an ideal plastic material, $T_u = 0.33\ H_v$, may be used to calculate a hardness of the material in the ideal state since methods are known for calculating the "ideal ultimate tensile strength." The results of such calculations are interesting even though they may be considered only as approximations.

Griffith (*2*) has measured fracture strengths as large as 9×10^5 lb/in.^{2} for fine glass fibers as compared with a theoretical strength of 1.6×10^6 lb/in.^{2} and a measured strength of 2.49×10^4 lb/in.^{2} for rods of the same glass. The fine fibers have a strength of 633 kg/mm^2 and therefore a calculated Vickers hardness of 1900. This is equivalent to the hardness of corundum or a Moh's hardness of 9 as compared with about 4.5 for ordinary glass.

Herring and Galt (*3*) have measured the elastic properties of single perfect "whiskers" of tin and found a yield stress of 200,000 lb/in.^{2} The application of Tabor's equation yields a Vickers hardness of 141 for the whisker as compared with about 21 for hard drawn tin.

Zwicky (*4*) has calculated a strength of 200 kg/mm^2 for sodium chloride. The calculated Vickers hardness is 600, equivalent to a Moh's hardness of 5. The