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Monosaccharide Autoxidation in Health and Disease

by Paul J. Thornalley*

The reduction of oxygen by the ene-diol tautomer of simple monosaccharides produces hydrogen peroxide and α-oxoaldehydes. This process, termed monosaccharide autoxidation, occurs at physiological pH and temperature and may contribute to the development of several pathological processes.

Enolization of the monosaccharide to an ene-diol tautomer is a prerequisite for the reaction of the monosaccharides with oxygen. The reaction kinetics suggest a two step process: the enolization of the monosaccharide to the ene-diol followed by the reaction of the ene-diol with oxygen. Free-radical reactive intermediates are formed by the reaction of the ene-diol with oxygen: superoxide, semidione, and 1-hydroxyalkyl radicals are formed under physiological conditions (hydroxyl radicals are also detected at high pH).

The autoxidation of monosaccharides stimulates the oxidation of oxyhemoglobin in erythrocytes, producing methemoglobin and hydrogen peroxide, and the oxidation of reduced pyridine nucleotides NAD(P)H to the oxidized congener $NAD(P)^+$ and enzymatically inactive nucleotide. This stimulates oxidative metabolism (via the hexose monophosphate shunt) and α-oxoaldehyde metabolism (via the glyoxalase system) in erythrocytes *in vitro*. The oxidative challenge is relatively mild even with very high concentrations (50 mM) of monosaccharide. However, crosslinking of membrane proteins by α-oxoaldehydes is enhanced; this effect may exacerbate ageing and decrease the lifetime of erythrocytes in circulation.

***In vivo*, the autoxidation of monosaccharides is expected to be a chronic oxidative process occurring in biological tissue which utilises simple monosaccharides, e.g., in glycolysis and gluconeogenesis. Monosaccharide autoxidation is suggested to be a determinant in the control of cellular mitosis and ageing, providing physiological substrates for the glyoxalase system, and may contribute to the chronic disease processes associated with diabetes mellitus and the smoking of tobacco.**

Introduction

Susceptibility of Monosaccharides to Oxidative Degradation

The spontaneous reaction of monosaccharides with oxygen in aqueous solution under physiological conditions (pH 7.4, 37°C) is, perhaps, one aspect of chemistry of monosaccharides which does not normally give the toxicologist cause for concern. Yet this process has been known for many years (*1*) and produces potentially noxious α-oxoaldehydes, otherwise known as osones and glycosuloses. Over the short term (hours), sterile aqueous solutions of monomeric and oligomeric hexoses can be stored without significant deterioration by nonenzymic oxidative processes. Over longer periods (days, months), or much shorter periods (minutes) for simple aldoses and ketoses existing predominantly as acyclic α-hydroxyaldehydes and ketones (and their hydrates) in aqueous solution (glycolaldehyde, glyceraldehyde, dihydroxyacetone, erythrose), the nonenzymic oxidative degradation of monosaccharides is pronounced and is not without deleterious effect on biological systems.

Monosaccharide Autoxidation: A Definition

Monosaccharide autoxidation is defined as the nonenzymatic reaction of the α-hydroxycarbonyl group of monosaccharides with oxygen. The predominant products are hydrogen peroxide and α-oxoaldehydes (*2*).

$$RCH(OH)COR' + O_2 \rightarrow RCOCOR' + H_2O_2 \qquad (1)$$

Other products are: α-oxoacids, decarboxylation products, and water. The reaction is free radical-mediated, although the enolization (and dehydration of hydrates) of the monosaccharide to an ene-diol is an obligatory first step.

$$RCH(OH)COR' \rightarrow RC(OH)=C(OH)R'$$

It is axiomatic that enolization of the monosaccharide is a prerequisite to autoxidation of the monosaccharide.

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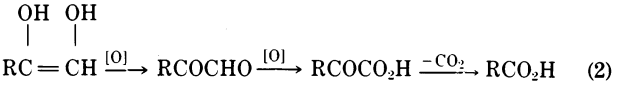
Historical

Reduction of Oxygen by Monosaccharides. Studies on the reactions of monosaccharides with oxygen have historically centered on the reaction of monosaccharides under alkaline conditions (monosaccharide autoxidation is usually enhanced at high pH, but complex aldol condensation and retro-aldol reactions also occur).

The first systematic investigation of the reactions of sugars in aqueous alkaline solution was reported by Nef in 1907 (*3*). He found the D-glucose, in alkaline solution in the presence of air, yields principally formic and D-arabinonic acids, small amounts of CO_2 and saccharinic, D-ribonic, D-erythronic, DL-glyceric, glycollic, and oxalic acids. He suggested that these products (except for saccharinic acids) are formed by oxidative cleavage of 1,2-, 1,3-, and 1,4- ene-diols. Later workers have followed the general thesis of Nef but there is still dispute over the mechanisms of oxidative cleavage of the ene-diol.

In 1934, Spoehr and Milner investigated the attack of oxygen on D-glucose, D-glyceraldehyde, glycerol, and related polyhydric alcohols in neutral aqueous solution at 37.5°C (*1*). One mole of carbon dioxide was formed per mole of D-glucose consumed. Sodium ferropyrophosphate was used as a catalyst. D-Fructose was much more sensitive to oxidative degradation than D-glucose in the presence of phosphate or arsenate. The rate depends on the concentration of phosphate or arsenate present but not so on pH (*4*). The major products found were aldonic acids. (The author notes the results with polyhydric alcohols are difficult to reproduce and may reflect some impurity in the polyhydric alcohols of the day).

In 1963, Stanek et al. (*5*) proposed a mechanism for the autoxidation of hexoses which is close to our present understanding of the mechanism of monosaccharide autoxidation under physical conditions. It was proposed that the 1,2-ene-diol tautomer of the monosaccharide reduces oxygen forming an α-oxoaldehyde, followed by oxidation to a 2-oxoaldonic acid, which in turn gives the next lower aldonic acid by decarboxylation.



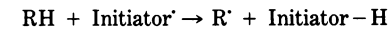
(2)

This process has now been observed *in vitro* for many simple biologically active monosaccharides under physiological conditions and may afford a chronic mild oxidative stimulus and source of α-oxoaldehydes in biological tissue (*2,6*).

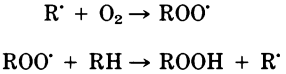
The potential ability of oxygen radicals to mediate oxidative damage, and α-oxoaldehydes to induce cross-linking, in protein and membrane lipid of biological tissue (*7–9*) suggests the time is ripe for a reappraisal of our understanding of the biocidal, biostatic and degenerative (aging) effects of abnormal and senile carbohydrate metabolism (*10,11*). Central to the theme of this review is the reactivity and role of free radical intermediates in these processes.

Free Radical Involvement in Monosaccharide Autoxidation. Historically, the involvement of free radicals in the oxidative degradation of sugars has been more suspected than proven. For example, Isbell (*12*), in reviewing enolization and oxidation of sugars in solution, could find little relevant literature on free radical involvement. Moreover, when Kasimura et al. (*13*) reported the production of superoxide by autoxidizing sugar and sugar phosphates, they were unable to cite a precedent and mechanism for the mode of action of autoxidizing sugars. Entwistle et al. (*14*) in 1949 suggested the reaction of oxygen with cellulose under alkalinic conditions is free-radical-mediated and postulated the formation of monosaccharides from trace quantities of initiator.

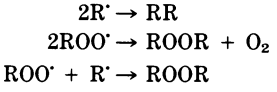
Initiation step:



Propagation steps:

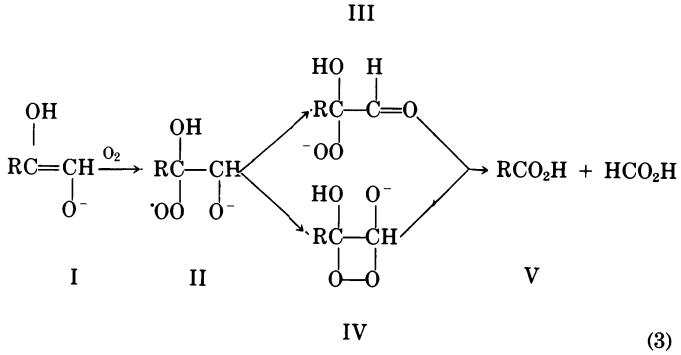


Termination steps:



RH is the monosaccharide substrate.

In 1976, Isbell proposed a diradical mechanism for the degradation of reducing sugars by oxygen (*15*). He suggested that the ene-diolate anions (I) reacts with oxygen to form a peroxy diradical (II). The diradical (II) is proposed to undergo an internal disproportionation to an α-hydroperoxyaldehyde III) or undergo spin inversion and form a dioxetane IV). Both (III) and (IV) are suggested to decay to formic and aldonic acids (V).



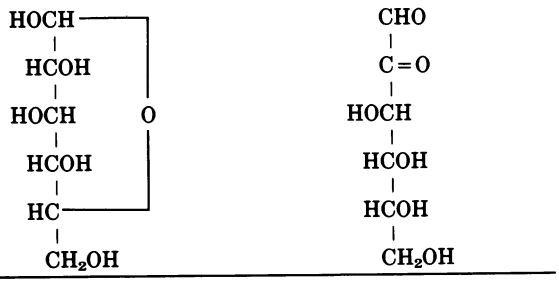
(3)

Yet there is no experimental evidence for monosaccharide hydroperoxides, alkyperoxyl radicals or diradicals in these autoxidative processes.

In this review, data are collected from radiolysis stud-

Table 1. α-Oxoaldehyde products from the autoxidation of simple monosaccharides.

Monosaccharide	α-Oxoaldehyde
Glycolaldehyde $HOCH_2CHO$	Glyoxal $(CHO)_2$
Glyceraldehyde $HOCH_2CH(OH)CHO$	Hydroxypyruvaldehyde $HOCH_2COCHO$
Dihydroxyacetone $(HOCH_2)_2CO$	
Glyceraldehyde-3-phosphate $Na+^-OPO_3CH_2CH(OH)CHO$	Hydroxypyruvaldehyde phosphate $Na+^-OPO_3CH_2COCHO$
Dihydroxyacetone phosphate $Na+^-OPO_3CH_2COCH_2OH$	
D-Glucose	D-Arabino-hexos-2-ulose (D-glucosone)



ies of the reactions of oxygen radicals with sugars, direct election paramagnetic resonance (EPR) studies of semidiones generated by oxidative degradation of sugars, and spin-trapping studies of oxygen and carbon-centered free radical formation in monosaccharide autoxidation under physiological conditions, finally to present a unified free-radical mechanism for the autoxidation of monosaccharides. The result is a novel insight into the nonenzymatic reactions of monosaccharides and an exciting prospect for further development in the future into biomedical implications of monosaccharide autoxidation.

Autoxidation of Monosaccharides

Products of Autoxidation

Aqueous solutions of monosaccharides, buffered at pH 7.4 and incubated at 37°C, react with oxygen. Oxygen and monosaccharide are consumed; α-oxoaldehyde and hydrogen peroxide are the initial products (*2,8*).

$$RCH(OH)CHO + O_2 \rightarrow RCOCHO + H_2O_2 \quad (4)$$

Some of the α-oxoaldehyde products formed from the autoxidation of common biological monosaccharides are indicated in Table 1.

An early report (*16*) has indicated that methylglyoxal is produced from glyceraldehyde and dihydroxyacetone in phosphate buffer at pH 7.4. Recent reports have

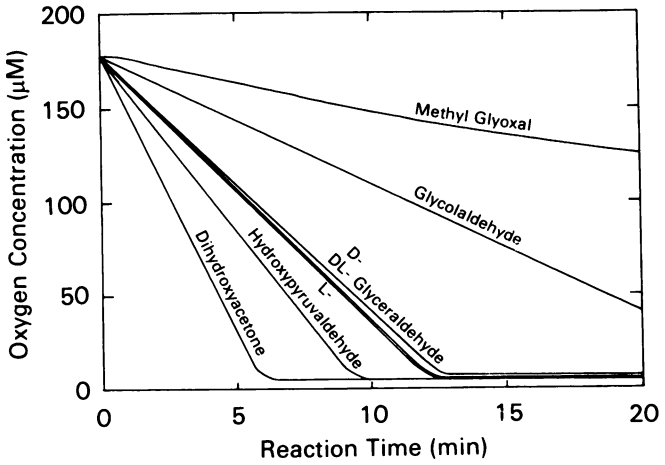


FIGURE 1. Oxygen consumption during the autoxidation of monosaccharides. Reaction mixtures contain 50 mM monosaccharide (as indicated) in 100 mM sodium phosphate, pH 7.4 at 37°C (*6*).

suggested that methylglyoxal is not formed in such systems at physiological pH but rather the acidified 2,4-dinitrophenylhydrazine used to assay methylglyoxal, as the dihydrazone, may dehydrate glyceraldehyde and dihydroxyacetone to methyl glyoxal (*2,8*) under non-physiological pH. This observation, and the earlier report that 2,4-dinitrophenylhydrazine reacts with glyceraldehyde and dihydroxyacetone to form the dihydrazone of hydroxypyruvaldehyde (*17*), suggest that the use of 2,4-dinitrophenylhydrazine to detect α-oxoaldehydes is substantially artifactual. Better methods are to use more specific agents to produce chromophoric α-oxoaldehyde adducts e.g. Girard T reagent, and to separate monosaccharide autoxidation products by chromatography before locating/assaying agents (*8*).

Kinetics of Autoxidation

The rate of monosaccharide autoxidation, r_{autox}, is defined by the rate of oxygen consumption by the monosaccharide,

$$r_{autox} = -d[O_2]/dt$$

Experimental curves for oxygen concentration in aqueous solutions of simple monosaccharides with 100 mM sodium phosphate, pH 7.4, at 37°C are shown in Figure 1. The oxidative instability of DL-glyceraldehyde, for example, is demonstrated by the observation that a solution of 50 mM DL-glyceraldehyde in 100 mM sodium phosphate, pH 7.4 and 37°C, in a closed vessel becomes anaerobic after ca. 10 min.

One other striking feature of these oxygen concentration curves is their linearity to very low concentrations of oxygen. This indicates that the rate of monosaccharide autoxidation is independent of oxygen concentration where oxygen is not limiting and suggests a reaction step prior to the oxygen consumption step is rate-determining.

Superoxide dismutase does not inhibit monosacchar-

Table 2. Relative rates of oxygen consumption and iodine uptake by some simple monosaccharides in aqueous phosphate buffer, pH 7.4 and 37°C.[a]

Monosaccharide	Iodine uptake, $-d[I_2]_o/dt$	Oxygen consumption, $-d[I_2]_o/dt$
Dihydroxyacetone	120	120
Glyceraldehyde	100	100
Glycolaldehyde	80	75
Erythrose	38	30
Ribose	13	4
Glucose	2	1

[a] Reaction mixtures contained 50 mM monosaccharide in 100 mM sodium phosphate, pH 7.4 and 37°C. For measurement of $-d[I_2]/dt$, 40 μM I_2 with 50 mM KI was included in the incubation and iodine uptake followed by loss of I_3^-, using the absorption band at 351 nm where E_{351} is 26500 $M^{-1}cm^{-1}$ (*19*). For the measurement of $-d[O_2]/dt$, the reaction mixture was incubated isn the reaction chamber of a Clark-type oxygen monitor (YSI Model 53), and the oxygen concentration was followed potentiometrically with the Clark-type oxygen electrode (*19*).

Table 3. The effect of buffering systems on the rate of oxygen consumption by glyceraldehyde[a]

Buffer system	$-d[O_2]_o/dt \times 10^{-9}$, M^{-1} sec^{-1}
Sodium phosphate	
125 mM	300
100 mM	230
50 mM	120
10 mM	34
HEPES	
50 mM	37
Tris/HCl	
50mM	2.7

[a] DL-Glyceraldehyde in aqueous solution at pH 7.4, 37°C. Initial rate measurements of oxygen consumption using a Clark-type oxygen electrode with 50 mM DL-glyceraldehyde.

ide autoxidation, indicating that although superoxide is formed in the reaction, it is not a chain carrier in the free-radical mechanism. Catalase suppresses oxygen consumption by a factor of 2 which is consistent with the production of hydrogen peroxide in the autoxidation of monosaccharides and detoxification by catalase (*8*). Metal ion chelating agents, such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DETAPAC), and desferrioxamine, suppress but do not totally inhibit monosaccharide autoxidation. Moreover, addition of pro-oxidant metal ions, Fe^{3+}, Cu^{2+}, does not enhance the rate of monosaccharide autoxidation. It appears that trace metal ions may catalyze the autoxidation of monosaccharides up to a maximum limiting rate, but the availability of trace metal ion catalysts is not an obligatory feature for monosaccharide autoxidation to proceed.

The kinetics of the autoxidation of monosaccharides (measured by the rate of oxygen consumption, $-d[O_2]/dt$), closely resembles the rate of enolisation of monosaccharides to an ene-diol (measured by the rate of uptake of added molecular iodine, $-d[I_2]/dt$).

The rates of autoxidation and enolization of glyceraldehyde, dihydroxyacetone, erythrose, ribose and glucose are similar (Table 2). The autoxidation of monosaccharides under these conditions appears to be enolization rate-controlled. However, both the rates of enolization and autoxidation of these monosaccharides are influenced by buffer ions (Table 3). This may be due to the effects of buffer ions on the rates of dehydration and/or enolization, or scavenging of the ene-diol by the conjugate base of the buffer; amines and phenolate anions are known to form adducts with ene-diols (*18*). In any event, it is clear that the buffer greatly influences the autoxidation kinetics. This is a critical factor when assessing the rate of monosaccharide autoxidation in biological systems, as will be discussed below.

Although physiological pH and temperature are important incubation conditions for considering the biomedical implications for monosaccharide autoxidation, the autoxidation reaction occurs over a wide range of pH and temperatures. Figures 2 and 3 show the dependence of the rates of enolization and autoxidation of DL-glyceraldehyde on pH and temperature. The pH profile of the rates of enolization and autoxidation of DL-glyceraldehyde shows that enolization and autoxidation increase together from pH 6 to pH 7, consistent with an enolization rate-controlled autoxidation process. From pH 7 to pH 8 the rate of autoxidation markedly exceeds the rate of enolisation. The relatively high rates of hydrogen peroxide produced in this pH range may catalyze further autoxidation of the monosaccharide substrate (autocatalysis). Above pH 8.5, the rate of autoxidation of DL-glyceraldehyde decreases, whereas the rate of enolization continues to increase with pH. This may indicate that, at high pH, there are fates for the ene-diol of DL-glyceraldehyde other than reaction with oxygen (autoxidation), typical of early studies on monosaccharide autoxidation, e.g., oligomerization of the ene-diol.

The temperature profiles for the rates of enolization and autoxidation of DL-glyceraldehyde show an acceleration of both the rates of enolization and autoxidation approaching physiological temperature (37°C). An estimate of the activation energy of enolization for dl-glyceraldehyde was found to be ~1 kJ/mole (*8*).

Overall, the kinetics of monosaccharide autoxidation can be described as a two-step process: an initial rate-determining enolization of the α-oxoaldehyde to an ene-diol, followed by an autoxidation step with reduction of oxygen by the ene-diol.

$$\text{monosaccharide} \xrightarrow{r_{enol}} \text{ene-diol} \xrightarrow{r_{autox.}} \alpha\text{-oxoaldehyde} + H_2O \quad (5)$$

Under normal circumstances (nonlimiting oxygen), $r_{enol} < r_{autox}$, and the reaction kinetics of monosaccharide autoxidation are expected to be first-order with respect to glyceraldehyde and zeroth-order with respect to oxygen. However, the rate of monosaccharide autoxidation is first order with respect to buffer concentration. So,

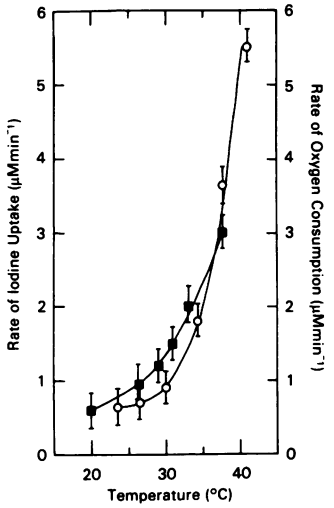


FIGURE 2. Temperature dependence of the initial rates of (■) iodine uptake and (○) oxygen consumption by autoxidizing DL-glyceraldehyde. Reaction mixtures contain 12.5 mM DL-glyceraldehyde in 10 mM sodium phosphate, pH 7.4. No other additions were necessary for oxygen consumption measurements. For iodine uptake measurements, the reaction mixtures also contained 50 mM KI and 25 μM iodine (*6*).

the kinetics of autoxidation of glyceraldehyde in phosphate buffer (P), for example, can be defined by

$$r_{autox} = -d[O_2]/dt = k^P_{autox.}\,[\text{glyceraldehyde}][P]$$

where $k^P_{autox.} = (5 \pm 1) \times 10^{-5}\ M^{-1}\ sec^{-1}$ at pH 7.4 and 37°C. Estimates of similar rate constants for the autoxidation of pentoses and hexoses are ca. 100 times slower than for DL-glyceraldehyde (*8*).

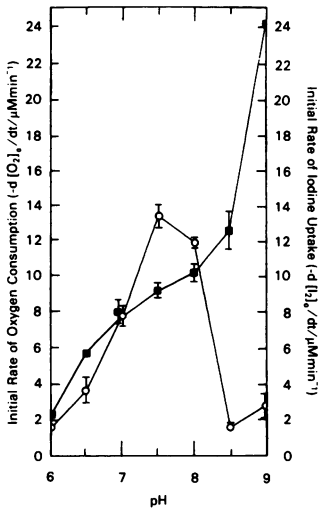


FIGURE 3. pH Dependence of the initial rates of (■) iodine uptake and (○) oxygen consumption by autoxidizing DL-glyceraldehyde. Reaction mixtures contain 10 mM DL-glyceraldehyde at 37°C. 100 mM sodium phosphate was used as buffer from pH 6.0 to pH 8.0. 100 mM sodium pyrophosphate was used from pH 8.5 to 9.0 Oxygen consumption and iodine uptake measurements were made as described previously (*19*).

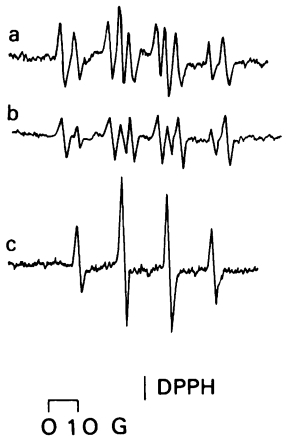


FIGURE 4. Free-radical involvement in the autoxidation of monosaccharides at pH 8.5, of reaction mixtures containing (*a*) 50 mM DL-glyceraldehyde, (*b*) 50 mM glycolaldehyde, and (*c*) 50 mM dihydroxyacetone, in 100 mM sodium pyrophosphate, pH 8.5 at 37°C. Reaction mixtures were incubated for 10 min prior to recording of the EPR spectrum.

Free-Radical Involvement

The production of free radicals during the autoxidation of simple monosaccharides at 37°C has been studied by the EPR technique of spin trapping (*2,8,19*). In the presence of the spin trap, 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), monosaccharides undergoing autoxidation form spin adducts derived from hydroxyl and 1-hydroxyalkyl free radicals. Spin-adduct formation was dependent on the monosaccharide, oxygen, buffer ions and pH, as is the rate of the autoxidation reaction.

At pH 8.5, 10 mM monosaccharide incubated in 100 mM sodium pyrophosphate for 10 min at 37°C gave the EPR spectra shown in Figure 4. The EPR spectra observed for incubation with glyceraldehyde and glycolaldehyde can be assigned to two spin adducts: the hydroxyl radical spin adduct of DMPO, 5,5-dimethyl-2-hydroxypyrrolidino-1-oxyl (DMPO-OH), and a carbon-centered free-radical-derived spin adduct,

$$\text{DMPO-R}_{\text{monosaccharide}}$$

which we will refer to as DMPO-R_{ms}. For the incubation with dihydroxyacetone only DMPO-OH was observed. Similar spin-trapping studies with chemically generated hydroxyl radicals [Fe(II) + H_2O_2] and studying the ratio of DMPO-OH to DMPO-R_{ms} formation with a range of monosaccharide concentrations suggested that the carbon-centered free radical, R_{ms}, is formed by reaction of hydroxyl radicals (generated by the autoxidation of monosaccharides) with the parent monosaccharide. Overall, spin adduct production (both DMPO-OH and DMPO-R_{ms}) is stimulated by superoxide dis-

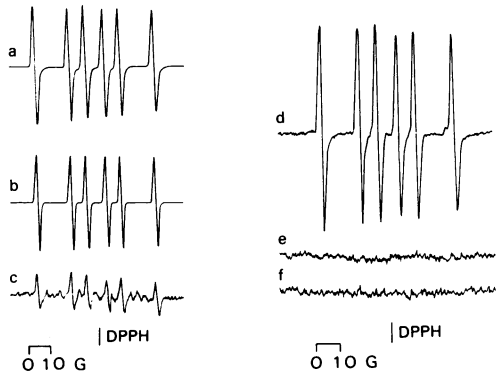


FIGURE 5. Free-radical involvement in the autoxidation of monosaccharides at pH 7.4 of reaction mixtures containing 100 mM sodium phosphate, pH 7.4, and (*a*) 50 mM DL-glyceraldehyde, (*b*) 50 mM glycolaldehyde, (*c*) 50 mM dihydroxyacetone, and (*d*) 50 mM erythrose. Incubation conditions as for Figure 4.

mutase and inhibited by catalase. Metal ion chelators, ethylenediaminetetraacetic acid, diethylenetriaminepentaacetic acid and desferrioxamine, suppress free radical production by 40-60%.

At pH 7.4, incubations with glycolaldehyde, glyceraldehyde, dihydroxyacetone and erythrose, show only DMPO-R_{ms} in the EPR spectrum (Fig. 5). Again, superoxide dismutase stimulates, and catalase inhibits, free radical formation. Incubation with ribose and glucose gave no detectable free radical production.

The identification of R_{ms}, formed by the reaction of hydroxyl radicals with the parent monosaccharide, can be deduced from considering data from radiolysis studies (*20,21*). Hydroxyl radicals abstract hydrogen atoms from monosaccharides. For glycolaldehyde, glyceraldehyde and erythrose, the hydrogen atoms most susceptible to abstraction are those of the acetal bond in the aldehyde hydrates.

$$\mathrm{RCH(OH)C(OH)(OH)-H + {}^{\cdot}OH \rightarrow RCH(OH)\dot{C}(OH)(OH) + H_2O} \qquad (6)$$

For glycolaldehyde, glyceraldehyde and erythrose, $R_{monosaccharide}$ therefore can be assigned to VI, VII and VIII where

$\mathrm{HOCH_2C(OH)(OH)}$ VI $\quad \mathrm{HOCH_2CH(OH)C(OH)(OH)}$ VII $\quad \mathrm{HOCH_2(CH(OH))_2C(OH)(OH)}$ VIII

For dihydroxyacetone hydrate, however, there is no acetal C–H group and an α-hydroxy hydrogen atom (one of four) is abstracted.

$$\mathrm{C(OH)(CH_2OH)_2 + {}^{\cdot}OH \rightarrow \dot{C}(OH)(CHOH)(CH_2OH)_2} \qquad (7)$$

IX

This analysis is supported by direct EPR observation of the radicals VI–VIII in irradiated aqueous solutions of monosaccharides (*20*).

Reaction time courses, concentration dependence (pH 7.4) and pH profile of free-radical production from autoxidizing glyceraldehyde at 37°C has been reported (*19*). Spin adduct formation is proportional to the concentration of the monosaccharide but shows an anomalous pH profile. Formation of the product,

$$\mathrm{DMPO\text{-}R_{glyceraldehyde}}$$

referred to as DMPO-R_{gly}, increases with pH from pH 6 to pH 7.5. At pH 7.4, the DMPO-R_{gly} formation peaks and falls to low levels at higher pH. DMPO-OH formation is detectable only at, and above, pH 8.5 (Fig. 5). This may indicate a change in mechanism for the autoxidative process at pH 8.0–8.5.

The effect of pH on the formation of monosaccharide radicals from the reaction of hydroxyl radical with monosaccharides, has not been fully investigated. Kuwabara et al. (*22*) used a nitroso spin trap to observe the free radicals produced from hexoses with radiolytically generated hydroxyl radicals but were restricted to acid and neutral pH by the instability of the spin trap. The work of Gilbert et al. (*23*) on free radicals produced by the reaction of hexoses with hydroxyl radicals relates only to pH ≤ 4. The monosaccharide free radicals formed are a mixture of all possible hydroxyalkyl radicals. Steeken and Schulte-Frohlinde (*20*) suggested that decarbonylation and decarboxylation reactions are involved in the decay of monosaccharide free radicals:

$$\mathrm{I} \xrightarrow[-H_2O\ -CO]{-H_2O} \begin{matrix} \mathrm{HOCH_2\dot{C}CHO_2H} \\ \mathrm{HO\dot{C}HCH_2OH} \end{matrix} \qquad (8)$$

$$\mathrm{III} \xrightarrow[-H_2O]{-H_2O} \begin{matrix} \mathrm{\dot{C}H_2CO_2H + H_2O} \\ \mathrm{HOCH_2\dot{C}O \rightarrow CO + \dot{C}H_2OH} \end{matrix} \qquad (9)$$

Dehydration processes to $\mathrm{RCH\dot{C}O_2H}$ are expected to be favored where 1> pH > 10. The decarbonylation/dehydration reaction would be favored by an increase in temperature.

Semidione free radicals from monosaccharides, despite their simplicity and ease of generation, have been little investigated (cf. the very similar semiquinone free radicals). The work of West el al. (*24*) demonstrates semidione formation from monosaccharides at pH 8. The formation of semidione radicals was proposed to occur by rearrangement of the hydroxyalkyl radicals

$$\mathrm{I\ or\ IV} \xrightarrow{-H_2O} \underset{\text{Semidione}}{\mathrm{HOCH_2\dot{C}(O^{\cdot})=CH(OH)}} \qquad (10)$$

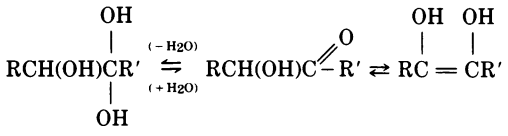
It is interesting to note that, if semidiones can re-

versibly hydrate/dehydrate, then at least part of the DMPO-R_{ms} EPR signal may represent effectively spin-trapped semidione intermediates.

Mechanism of the Autoxidation of Monosaccharides

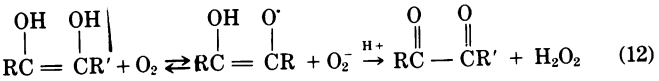
The mechanism of the autoxidation of monosaccharides must provide a route to α-oxoaldehyde and hydrogen peroxide production with the formation of superoxide, semidione, hydroxyl and 1-hydroxyalkyl free radicals. A general mechanism for the autoxidation of monosaccharides has been constructed (*19*) and is given in Eqs. (11)–(19).

Dehydration/Enolization



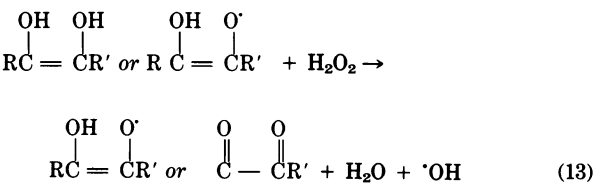
(11)

Autoxidation



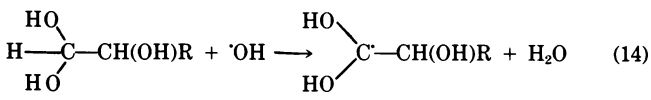
(12)

Formation of hydroxyl radicals.



(13)

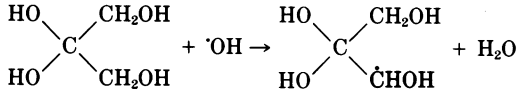
H Abstraction



(14)

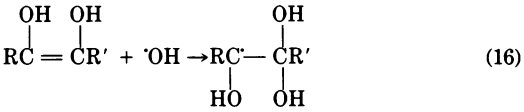
For glyceraldehyde R = CH_2OH, for glycolaldehyde R = H, and for erythrose R = $CH(OH)CH_2OH$.

For dihydroxyacetone:



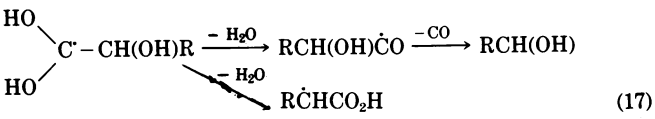
(15)

Ene-diol scavenging of hydroxyl radicals.



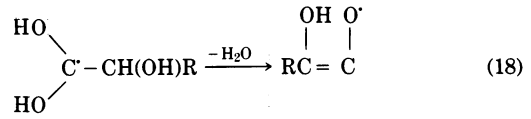
(16)

Dehydration/decarbonylation reactions of monosaccharide radicals



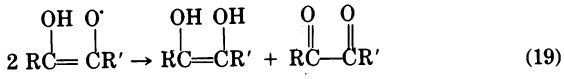
(17)

Semidione formation by dehydration of monosaccharide radicals



(18)

Semidione disproportionation



(19)

The first step in the autoxidation of monosaccharides is the formation of the ene-diol. This may involve both dehydration and enolization of the monosaccharide (hydrate). [Eq. (11)]. The ene-diol then reduces oxygen to hydrogen peroxide via superoxide intermediacy [Eq. (12)]. The initial superoxide producing step is shown as reversible to reflect the effect of superoxide dismutase on the reaction. Superoxide dismutase stimulation of free radical formation suggests that superoxide retards the autoxidation reaction, probably by reducing the semidione back to the ene-diol. α-Oxoaldehyde is produced in this reaction step.

Hydroxyl radical formation is envisaged to occur via one-electron reduction of hydrogen peroxide via the ene-diol and/or the semidione free radicals [Eq. (13)]. The course of further reactions then depends on the particular monosaccharide and pH. At high pH (pH > 8), the ene-diol may be ionized to an ene-diolate anion and, as such, is expected to be activated to electron transfer processes, rapidly reducing oxygen and hydrogen peroxide to produce (the observed) hydroxyl and hydroxyalkyl free radicals [Eq. (14)]. At lower pH (pH < 8), the ene-diol in the un-ionized form will be metastable and may scavenge hydroxyl radicals, forming hydroxyalkyl radicals by an addition reaction [Eq. (15)]. The hydroxyalkyl radicals formed in reaction (15) may decompose by dehydration and decarbonylation reactions [Eq. (17)], or, as suggested by West et al. (*24*), may rearrange to semidiones [Eq. (18)]. Semidiones may decay by disproportionation to α-oxoaldehyde and ene-diol [Eq. (19)].

Monosaccharide Autoxidation and Biochemical Metabolism

Oxidative Metabolism

The autoxidation of monosaccharides produces hydrogen peroxide and reactive free-radical intermediates. These oxidizing species may stimulate oxidative metabolism in cells (*25,26*).

Detoxication of Hydrogen Peroxide. The production of hydrogen peroxide by the autoxidation of monosaccharides normally resident in cells is expected to be relatively slow, yet chronic. Such a slow production

of hydrogen peroxide may contribute to the normally resident concentrations of hydrogen peroxide ($\sim 10^{-8}$ M) found in cells (*27*). This small amount of hydrogen peroxide is converted to water by the enzymatic action of glutathione peroxidase which is particularly suited for the detoxication of low fluxes of hydrogen peroxide (cf. catalase) (*27*).

$$2GSH + H_2O_2 \xrightarrow[\text{Peroxidase}]{\text{Glutathione}} GSSG + 2H_2O \qquad (20)$$

Oxidized glutathione is produced. This stimulates the reduction of oxidized glutathione, by glutathione reductase, and in turn, the flux of glucose oxidized through the hexose monophosphate shunt (HMS) (Fig. 6).

In DL-glyceraldehyde-treated erythrocytes, the HMS appears to be stimulated by the detoxication of hydrogen peroxide produced from the autoxidation of glyceraldehyde. However, autoxidizing glyceraldehyde also directly oxidizes NADPH to $NADP^+$ in a nonenzymatic interaction (*25*). The stimulation of the HMS in erythrocytes incubated with glyceraldehyde, dihydroxyacetone and glycolaldehyde is compared in Figure 7. The observed stimulation in the HMS is relatively small compared to that observed for classical oxidative agents, e.g., phenylhydrazine (*28*), and does not have any acute damage effect on the cell. Rather the oxidative challenge from the autoxidation of normal concentrations of cellular glycolytic monosaccharides is expected to contribute to the normal turnover of the HMS (29) and is, therefore, important only in red cell aging and senescence.

Nonenzymic Oxidation of Reduced Pyridine Nucleotides. The autoxidation of glyceraldehyde at pH 7.4 and 37°C, in the presence of NADH or NADPH, produces a nonenzymic oxidation of NAD(P)H to $NAD(P)^+$ and some enzymatically inactive pyridine nucleotides (*25*). This suggests that there is a co-oxidation of reduced pyridine nucleotides with autoxidizing monosaccharide. The enzymatically inactive pyridine nucleotide may result from adduct formation with glyceraldehyde (*30*). The active oxidant of the reduced pyridine nucleotide is not known. Superoxide is thought not be to a good oxidant of NAD(P)H unless the nucleotide is on an NAD(P)H enzyme-binding site. Rather, hydroxyalkyl radicals similar to those observed in monosaccharide autoxidation have been shown to oxidize selectively reduced pyridine nucleotide to its oxidized congener (*31*); the hydroxylalkyl radical (R·) oxidizes NAD(P)H to a pyridinyl radical which rapidly reduces oxygen, forming oxidized nucleotide, $NAD(P)^+$. Some formation of NAD(P) dimers from the dimerization of pyridinyl free radicals cannot be excluded as a contributory factor to the formation of enzymatically inactive nucleotide (*32*).

$$\begin{array}{l} R^{\cdot} + NAD(P)H \rightarrow RH + [NAD(P)^{\cdot}] \xrightarrow{\text{Dimerization}} [NAD(P)]_2 \\ \qquad\qquad\qquad\qquad \text{Oxidation} \downarrow O_2 \\ \qquad\qquad\qquad\qquad NAD(P)^+ + O_2^- \end{array} \qquad (21)$$

Oxidation of Oxyhemoglobin. Simple monosaccharides stimulate the oxidation of oxyhemoglobin and the reduction of methemoglobin but do not change the oxidation state of heme groups in carboxyhemoglobin (*33*).

The reaction of monosaccharides with oxyhemoglobin proceeds via the enolization of the monosaccharide to an ene-diol. Hereafter, ca. 60% of the oxyhemoglobin appears to be oxidized by hydrogen peroxide (produced from the autoxidation of the ene-diol) in a Type III hemoglobin oxidation reaction (*34*), i.e., oxidation of oxyhemoglobin through the small amount of deoxyhemoglobin present.

$$(Hb)Fe^{II} + H_2O_2 \rightarrow \rightarrow \text{methemoglobin, hemichrome} + H_2O \qquad (22)$$

The remaining ca. 40% of oxyhemoglobin is oxidized by direct reaction with the ene-diol acting as a Type II reductant (*34*).

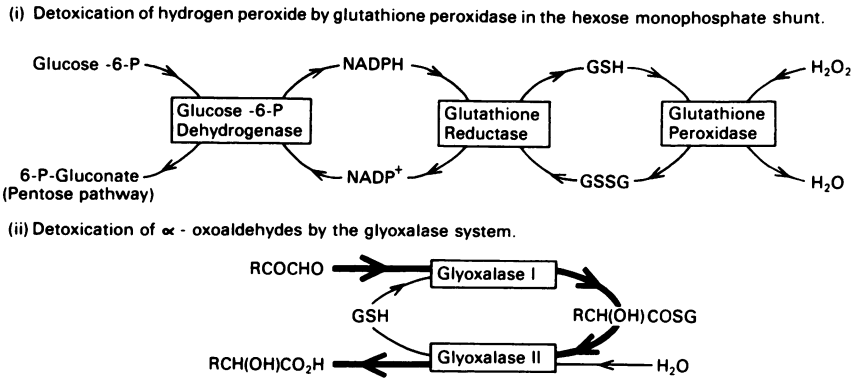


FIGURE 6. Biological metabolism of the products of monosaccharide autoxidation.

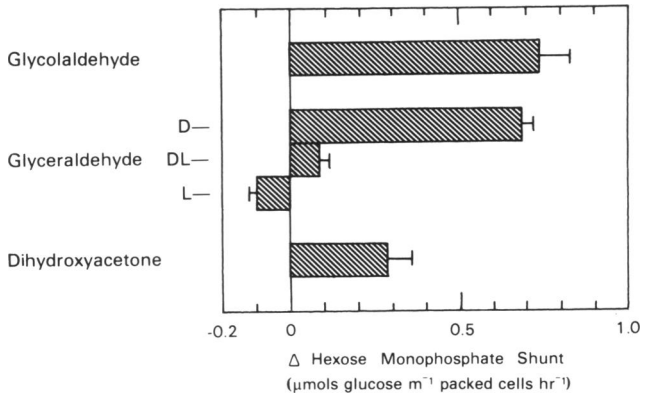
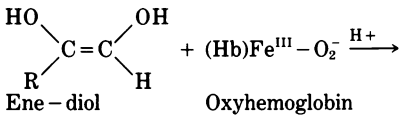
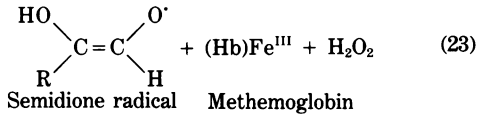


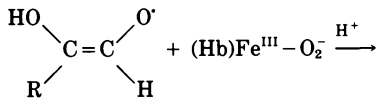
FIGURE 7. Effect of exogenous monosaccharides on the flux of glucose oxidized by the hexose monophosphate pathway in red cells. Hexose monophosphate shunt activities were measured for 25% red cell suspensions treated with 5 mM monosaccharide (*25*). Data are the mean ± standard deviation for four determinations. The hexose monophosphate shunt activity is given as μ mole glucose oxidized per 1 milliliter packed red cells per hour (calculated by appropriate corrections of the experimental data). The control hexose monophosphate shunt activity was 0.17 ± 0.02 μmole glucose/mL cells/hr.

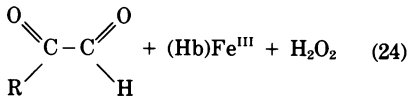




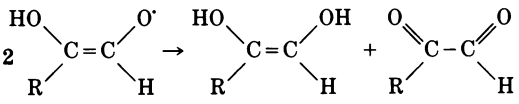
(23)

Semidione radicals decay by disproportionation and reduction of further oxyheme groups.





(24)



(25)

Similar reactions of ene-diol and semidione radicals may be postulated to describe the mechanism of the reduction of methaemoglobin to deoxyhemoglobin by simple monosaccharides (*33*).

α-Oxoaldehyde Metabolism

The cellular production of α-oxoaldehydes and their detoxication by the glyoxalase system have long been suspected to be a determinant of cellular ageing (*35*). Such suspicions have lost credibility over the years, probably since no major source of α-oxoaldehyde has been identified in eukaryotic cellular metabolism (bacterial production of α-oxoaldehydes has been identified) (*17,36,37*). α-Oxoaldehydes produced from the autoxidation of monosaccharides may be the physiological substrates for the glyoxalase I reaction.

Glyoxalase I (EC4.4.1.5) is a component of the glyoxalase system which catalyzes the conversion of α-oxoaldehydes to α-hydroxyacids (*38*). The glyoxalase system comprises two enzymes, glyoxalases I and II, and a catalytic amount of reduced glutathione cofactor. Glyoxalase I catalyzes the formation of S-2-hydroxyacyl glutathione esters from reduced glutathione and α-oxoaldehyde. Glyoxalase II (EC3.1.2.6) is a thiolesterase, catalyzing the hydrolysis of the S-glutathione adduct (product of the glyoxalase I reaction) to α-hydroxyacid and regeneration of reduced glutathione (Fig. 6).

The physiological role of the glyoxalase system is thought to be the enzymatic detoxication of potentially noxious α-oxoaldehydes. Typically, α-oxoaldehydes are highly toxic and lethal at relatively small doses (*8,39*). Glyoxalase activity is found in all cells. A role for glyoxalase is clearly the detoxication of monosaccharide autoxidation products and hence prolongation of the cell's useful glycolytic and metabolic life.

Biomedical Implications

Control of Mitosis and Ageing

α-Oxoaldehydes are antimitotic agents, probably by virtue of their ability to bind and crosslink DNA, RNA, and protein (*8*). The cytostatic action of abnormally high concentrations of simple monosaccharides (*8*) may also be due to the antimitotic action of the α-oxoaldehydes furnished by the autoxidation reaction.

Glyceraldehyde has been well studied for its ability to inhibit tumour growth. Riely and Pettigrew first reported a small retardation in the appearance and growth of benzopyrene-induced sarcoma when glyceraldehyde was injected subcutaneously (*40*). Twenty years later, Sartorelli et al. (*41*) reported experiments on the chemotherapy of several ascites tumors with glyceraldehyde. Screening of glyceraldehyde therapy over a wide range of tumors leads to the conclusion that glyceraldehyde was not a particularly clinically useful cytostatic agent (*42*). The very high concentrations of glyceraldehyde used in therapy and an oxygen effect on the inhibition of thymine and uracil uptake into DNA and RNA respectively by glyceraldehyde, suggest that the autoxidation of glyceraldehyde to the cytostatic α-oxoaldehyde, hydroxypyruvaldehyde (*43*), may have an important role in the manifestation of the antitumor action of glyceraldehyde. As expected for an autoxidation involvement of a general monosaccharide, a wide range of simple monosaccharides exhibit a cytostatic and antitumor effect similar to that of glyceraldehyde.

However, the very high doses of monosaccharides employed for this cytostatic effect impose such a high metabolic stress on cells (through phosphorylation and nonenzymic protein glycosylation of the monosaccharide) that such therapy appears toxic to both normal and tumor cells.

α-Oxoaldehyde-mediated protein crosslinking inactivates enzymes and decreases deformability of membrane protein networks (*8*). These effects sensitize cells to further irreversible autoxidative damage, senescence, and death. For example, the erythrocyte is thought to be sequestered by the spleen and removed from circulation when it reaches a minimum critical deformability (*44*). α-Oxoaldehydes exacerbate this aging process, whereas the glyoxalase system will protect against α-oxoaldehyde-promoted aging and senescence of erythrocytes. Physiological aging may therefore, in part, be mediated by α-oxoaldehydes produced by chronic autoxidation of glycolytic intermediates. Indeed, hydroxypyruvaldehyde phosphate—the α-oxoaldehyde formed by the autoxidation of phosphorylated triose glycolytic intermediates (*8*)—has been detected in human erythrocytes (*45*).

Abnormalities of Carbohydrate Metabolism

The effects of monosaccharide autoxidation on biological processes are expected to be enhanced when the resident cellular concentrations of monosaccharides are elevated. This is found for cells with insulin-independent glucose uptake in diabetes mellitus which become periodically hyperglycemic, e.g., erythrocytes, lens fiber cells, and endothelial cells of the microcirculation (*11*). During periods of hyperglycemia, cellular concentrations of phosphorylated glycolytic intermediates are elevated (*46*). During *in vitro* hyperglycemia, erythrocytes accumulate abnormally high levels of α-oxoaldehydes (P. J. Thornalley, unpublished observation).

The chronic pathogenesis of diabetes mellitus is thought to be stimulated by periods of hyperglycemia (*11*). During the development of diabetic cataract and microangiopathy, nonsulfhydryl protein crosslinks develop resulting in protein aggregation and basement membrane thickening (*47,48*). Red cells in the diabetic patient also show membrane changes; membrane fluidity and deformability are both decreased (*49,50*). It is postulated here that monosaccharide autoxidation and α-oxoaldehyde-mediated protein crosslinking may be important in the etiology of diabetic pathogenesis.

α-Oxoaldehydes and the Smoking of Tobacco

A recent report (*51*) has established that the smoke from the burning of dried tobacco leaves (the smoking of cigarettes) contains a number of α-oxoaldehydes which are the suspected products of the pyrolysis of cellulose, starch and other monosaccharide polymers, oligomers and monomers found in tobacco leaves.

The major α-oxoaldehyde found was methylglyoxal, although several other α-oxoaldehydes were found, e.g., diacetyl, 2,3-pentanedione, glyoxal, and 2-oxobutanal.

The role of these α-oxoaldehydes in smoking-associated diseases is not known but the smoking of tobacco is, perhaps, one of the clearest examples of an extreme form of monosaccharide autoxidation.

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