47. *Radiation Chemistry of Carbohydrates. Part XII.** *Action of Ionising Radiations on Aqueous Solutions of* D-*Mannitol.*

By G. O. PHILLIPS.

When oxygenated D-mannitol solutions are exposed to ionising radiations, the initial product is D-mannose. Initial G(D-mannose) is the same for fast electrons, Co^{60} γ-radiation, and 190 kv radiation (G 2·25); initial $G(H_2O_2)$ is 3·0. A radical mechanism is proposed which is in fair agreement with the overall stoicheiometry.

The results indicate that oxygenated D-mannitol solutions may be a convenient dosimeter for ionising radiations up to a dose rate of *ca.* $1{\cdot}7 \times 10^{18}$ ev min.$^{-1}$ ml.$^{-1}$.

WHEN hexitols are irradiated in aqueous solution in oxygen, the primary alcohol groups are attacked preferentially.[1] Irradiation of D-sorbitol solutions gives D-glucose and L-gulose as the initial products, with D-arabinose and L-xylose produced by secondary processes. The corresponding reactions with D-mannitol should give D-mannose from both extremities of the molecule, and would therefore provide a simpler system for investigation. Mannitol in solution is non-reducing and, therefore, formation of D-mannose may be accurately determined by measurement of reducing power during the initial reaction when the amount of secondary reducing products will be negligible. In the present investigation the effects of electrons ^{60}Co γ-radiation and 190 kv X-rays on oxygenated D-mannitol solutions have been examined. The results indicate that D-mannitol solutions may provide a suitable dosimeter for these radiations. The nature of the peroxide formed during radiation is examined, and from the over-all stoicheiometry of the reaction indications may be obtained about the mechanism of the process.

EXPERIMENTAL AND RESULTS

Electron Beam.—The irradiations were carried out with a beam of electrons from the 2 Mev van de Graaf generator of the Chemistry Division, A.E.R.E., Harwell, and the 4Mev linear accelerator at the Wantage Radiation Laboratories, U.K.A.E.A. Details of cells and irradiations for the van de Graaf generator have been described.[2] Energy-input rates were measured directly from the beam voltage and the current within the cell. The current was collected on a platinum wire and led along a shielded lead to earth through a D.C. amplifier and current integrator which would take into account variations in cell current. The generating voltmeter measured the beam voltage directly and a correction was applied for energy losses in the cell window.

Irradiations with 4 Mv electrons were carried out in thin-walled glass tubes (vol., 10 ml.), cooled during irradiation with a blast of cold air. The dose was measured calorimetrically by a copper absorber linked to a water-chamber by a thin nickel tube. The beam impinges on the absorber, and the heat leaks across the nickel into the water where it is measured at equilibrium by means of a thermocouple. The absolute dose values were confirmed by using the ferrous sulphate dosimeter on the basis of $G(Fe^{3+})$ 15·5.

Dose rates from the van de Graaf generator were $3{\cdot}54 \times 10^{17}$ to $3{\cdot}75 \times 10^{18}$ and from the linear accelerator were 8×10^{17} ev min.$^{-1}$ ml.$^{-1}$. For electrons, the dose rate is a nominal value, calculated on the basis of complete absorption of the energy into *ca.* 10 ml. solution.

200 *k*v *X-Radiation.*—The irradiations were from a Phillips Metalix 200 kv X-ray tube at the Medical Research Council's Radiotherapeutic Unit at Hammersmith Hospital. The tube and experimental arrangement have been described by Ebert and Boag.[3] In our irradiations

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* Part XI, *J.*, 1962, 4216.

[1] Phillips, *Nature*, 1954, **173**, 1044; Phillips, Mattok, and Moody, Proc. Internat. Conf. Peaceful Uses Atomic Energy, Geneva, 1958, Vol. XXIX, p. 92; Phillips and Criddle, *J.*, 1961, 3756, 3763.

[2] Phillips, Moody, and Mattok, *J.*, 1958, 3522.

[3] Ebert and Boag, *Discuss. Faraday Soc.*, 1952, **12**, 189.

the tube was run at 190 kv and at currents up to 6 mA with no filtration except by the tube window. In each experiment mannitol solution (5 ml.) was irradiated in open dishes, and variation of the dose rate was obtained by varying the distance of the dishes from the target. Dosimetry was carried out with ferrous sulphate, by using $G(\mathrm{Fe}^{3+})$ 15·5. Dose rates used were 2·27, 1·34, and 0·78 × 10^{17} ev min.$^{-1}$ ml.$^{-1}$. During irradiations there was a rise in temperature of the solution which caused premature oxygen-depletion. To minimise this effect, all solutions were initially pre-oxygenated. D-Mannitol solutions were initially 5 × 10^{-3} M.

γ-Radiation.—Two ^{60}Co sources of γ-radiation were used. At Harwell the source was similar to that described by Hochanadel and Ghormley [4] (850 c), and at Cardiff of the type described by Gibson and Pearce.[5] Dose rates were varied by moving cells away from the source. Dose rates of 1·5—5·9 × 10^{17} ev min.$^{-1}$ ml.$^{-1}$ were employed.

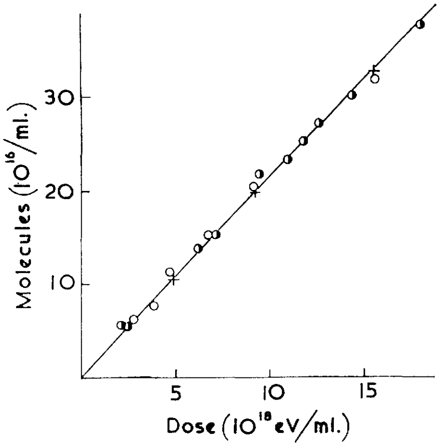

FIG. 1.

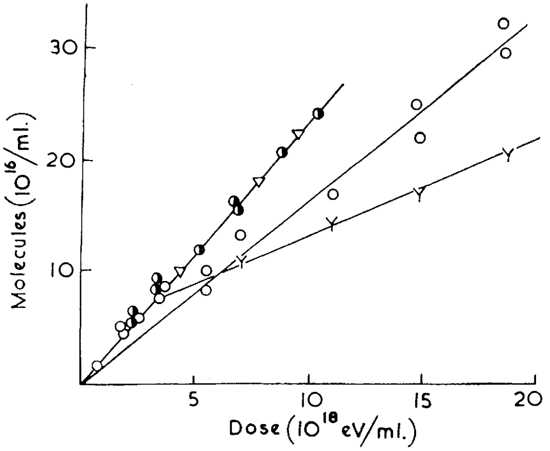

FIG. 2.

FIG. 1. Production of D-mannose during the irradiation of aqueous D-mannitol solutions (5·5 × 10^{-3}M) with γ-radiation. Dose rate = (◑) 5·9, (○) 4·8, and (+) 1·5 × 10^{17} ev min.$^{-1}$ ml.$^{-1}$.

FIG. 2. Production of D-mannose during the irradiation of aqueous D-mannitol solution (5 × 10^{-3}M) with fast electrons.

(◑) Beam current *ca.* 0·01 μA; dose rate 3·54 × 10^{17} ev min.$^{-1}$ ml.$^{-1}$; (○) beam current *ca.* 0·1 μA; dose rate of 3·75 × 10^{18} ev min.$^{-1}$ ml.$^{-1}$; oxygen passing through cell. (Y) beam current *ca.* 0·1 μA; dose rate 3·75 × 10^{18} ev min.$^{-1}$ ml.$^{-1}$; air-equilibrated solution. (△) 4 Mv electrons; dose rate 8 × 10^{17} ev min.$^{-1}$ ml.$^{-1}$.

Characterisation of D-*Mannose.*—Solutions (40 ml.) of D-mannitol (5 × 10^{-2} M) were irradiated with ^{60}Co γ-radiation in oxygen to a total energy input of 7 × 10^{22} ev and chromatographed with butan-1-ol–acetic acid–water (4 : 1 : 5) and butan-1-ol–pyridine–water (10 : 3 : 3). The presence of mannose and arabinose in the irradiated solution was revealed by spraying with *p*-anisidine. The solution was freeze-dried to 5 ml. and treated with phenylhydrazine (1 ml.) and glacial acetic acid (1 ml.) at 10° for 8 hr. The solid which separated was recrystallised until a pure D-mannose phenylhydrazone was obtained (m. p. 199°, not depressed on admixture with an authentic sample).

In duplicate experiments the irradiated solutions (40 ml.) were freeze-dried until all the solvent was removed. The solid, after thorough drying over P_2O_5, was treated with pyridine (10 ml.) and acetic anhydride (7 ml.) at 0° for 20 hr.; the mixture was poured into water and the gum which separated crystallised from ethanol. After recrystallisation, pure hexa-*O*-acetyl-D-mannitol was obtained with m. p. and mixed m. p. 124°. From the filtrate, after removal of hexa-*O*-acetyl-D-mannitol, a sample of penta-*O*-acetyl-β-D-mannopyranose was

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[4] Hochanadel and Ghormley, *Nucleonics*, 1955, **13**, No. 10, 74; *Rev. Sci. Instr.*, 1951, **22**, 473.
[5] Gibson and Pearce, *Chem. and Ind.*, 1957, 613.

separated by a procedure similar to that of Wolfrom *et al.*[6] After removal of the solvent, the solid was chromatographed on alumina and eluted with alcohol. The product was detected with a fresh 1% solution of permanganate in 2·5N-sodium hydroxide. Elution with acetone gave a syrup, which slowly crystallised on addition of ethanol. After recrystallisation pure penta-*O*-acetyl-β-D-mannopyranose was obtained with m. p. 117° and $[\alpha]_D$ −25° in $CHCl_3$.

Rate of Mannose Production.—Somogyi's [7] reducing-power method was used to estimate the formation of mannose. Fig. 1 shows the rate of formation during irradiation of 5×10^{-3}M-mannitol in oxygen with ^{60}Co γ-radiation to a total dose of 2×10^{19} ev ml.$^{-1}$. The rate is linear with dose and independent of dose rate within the range 1·57—5·9 $\times 10^{17}$ ev min.$^{-1}$ ml.$^{-1}$. The results give initial *G*(mannose) 2·25.

With 1 Mv electrons at beam current 0·01 μA, giving a dose rate of $3{\cdot}54 \times 10^{17}$ ev min.$^{-1}$ ml.$^{-1}$, mannose formation is linear with dose and gives initial *G*(mannose) 2·25. A linear relation of reducing power to dose is also shown at a beam current of 0·1 μA (dose rate $3{\cdot}75 \times 10^{18}$ ev min.$^{-1}$ ml.$^{-1}$) although the results are more erratic. Here, initial *G*(mannose) is 1·6. The importance of maintaining continuous oxygenation is demonstrated when initially air-equilibrated solutions are irradiated. The oxygenated rate is maintained only to a dose of *ca.* 6×10^{18} ev ml.$^{-1}$. Then, after oxygen depletion, the rate of mannose formation decreases. The electron beam results are shown in Fig. 2.

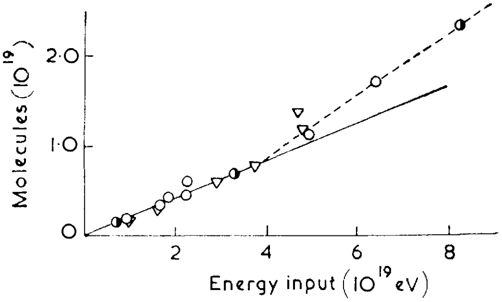

FIG. 3.

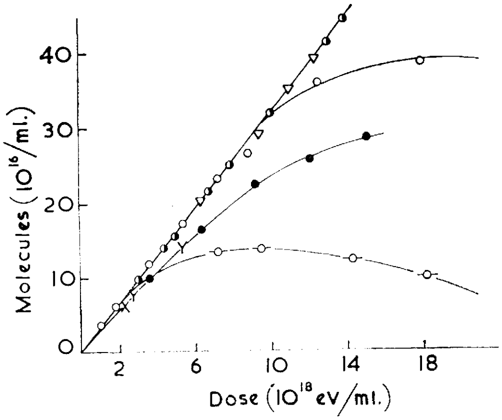

FIG. 4.

FIG. 3. Production of D-mannose during irradiation of aqueous D-mannitol solutions (5×10^{-3}M) with 190 kv *X*-rays. Dose rate = (◑) 2·27, (▽) 1·37, and (○) 0·78 × ev min.$^{-1}$ ml.$^{-1}$.

FIG. 4. Hydrogen peroxide formation during irradiation of D-mannitol solutions.

(1) γ-Radiation, dose rate $6{\cdot}44 \times 10^{17}$ ev min.$^{-1}$ ml.$^{-1}$: (▽) O_2 bubbling; (○) preoxygenated; (-○-) air-equilibrated.
(2) Fast electrons, (◑) estimated by iodide method; $2{\cdot}23 \times 10^{17}$ ev min.$^{-1}$ ml.$^{-1}$ fast electrons; energy input rate (10 ml.) $3{\cdot}75 \times 10^{18}$ ev min.$^{-1}$.
(3) *X*-Radiation, preoxygenated: dose rate = (●) 2·27, (×) 1·37, and (Y) 0·78 $\times 10^{17}$ ev min.$^{-1}$ ml.$^{-1}$.

With 190 kv *X*-rays, the curve of mannose formation with dose is initially linear (initial *G* 2·0) and independent of dose rate within the range 0·78—2·27 $\times 10^{17}$ ev min.$^{-1}$ ml.$^{-1}$ (Fig. 3). At a dose of *ca.* 8×10^{18} ev ml.$^{-1}$ the rate of mannose formation increases. All solutions were saturated with oxygen before irradiation.

Hydrogen Peroxide Formation.—Hydrogen peroxide was estimated by the titanium sulphate reagent.[8] With γ-radiation at a dose rate of $6{\cdot}44 \times 10^{17}$ ev min.$^{-1}$ ml.$^{-1}$, the yield–dose curve for hydrogen peroxide is linear to a dose of 8×10^{18} ev ml.$^{-1}$ when solutions are pre-oxygenated,

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[6] Wolfrom, Binkley, McCabe, Shen Han, and Michelakis, *Radiation Res.*, 1959, **10**, 37.
[7] Somogyi, *J. Biol. Chem.*, 1952, **195**, 19.
[8] Eisenberg, *Ind. Eng. Chem., Analyt.*, 1943, **15**, 327.

and to higher doses when oxygen is bubbled through the cell during irradiation. When air-equilibrated solutions are irradiated, the yield–dose curve of hydrogen peroxide falls from linearity at a relatively low dose (Fig. 4). Initial $G(H_2O_2)$ is 3·0. With fast electrons, initial $G(H_2O_2)$ is also 3·0, and the yield–dose curve is linear (Fig. 4). Depletion of oxygen during irradiation with X-rays interferes with accurate measurement of the initial rate of hydrogen peroxide formation. The yield–dose curve (Fig. 4) under our experimental conditions is not linear, and initial $G(H_2O_2)$, measured from the initial slope, is 2·6.

Hydroperoxide Formation.—The titanium sulphate reagent does not indicate the presence of hydroperoxides. Consequently an iodide method [9] was applied to the irradiated solutions to measure peroxide. Here, the high extinction (24,600 at 350 mμ) permits peroxide estimations with greater accuracy than the titanium method. This method estimates total hydrogen peroxide and organic hydroperoxides.

Equal amounts of two reagents are added, namely: potassium iodide (33 g.), sodium hydroxide (1 g.), ammonium molybdate (0·1 g.) in 500 ml. (reagent 1); and potassium hydrogen phthalate (10 g.) in 500 ml. (reagent 2). With hydrogen peroxide the optical density at 350 mμ develops to a maximum value immediately. With organic hydroperoxides (methyl hydroperoxide was used in control experiments), maximum optical density is attained only after several hours. On this basis, by using a first-order logarithmic extrapolation, it is possible to estimate organic hydroperoxides in the presence of hydrogen peroxide.

When this method was applied to irradiated D-mannitol solutions, maximum optical density was attained several minutes after the addition of reagent. This, however, was not due as first suspected to the presence of a labile hydroxy-hydroperoxide, since control hydrogen peroxide and reagents behaved identically in the presence of D-mannitol. Hydrogen peroxide in water gave maximum optical density 2 min. after the addition of the reagent. Consequently, in all estimations solutions were left for 30 min. before measurement. Typical results are shown in Table 1.

TABLE 1.

Increase in optical density at 350 mμ in peroxide estimations by the iodate method.[9]

(a) Irradiated D-mannitol (5×10^{-3}M); 10^{18} ev ml.$^{-1}$. (b) D-Mannitol (5×10^{-3}M) + H_2O_2 ($1·7 \times 10^{-5}$M). (c) H_2O_2 ($1·7 \times 10^{-5}$M).

Time (min.)		2	3	4	5	6	7	8	12	∞
0·3 × Optical density:	(a)	0·28	0·33	0·36	0·37	0·375	0·379	0·38	0·38	0·385
	(b)	0·27	0·31	0·35	0·36	0·37	0·377	0·38	0·39	0·395
	(c)	0·395	0·395	0·395						0·395

Fig. 4 shows the rate of peroxide formation in D-mannitol solutions irradiated with γ-radiation estimated by the iodide and titanium sulphate methods. Initial $G(H_2O_2)$ is 3·0.

Polarography of Irradiated Solutions.—Minkoff's polarographic method [10] was used to examine irradiated D-mannitol solutions for the presence of organic hydroperoxide. We have endeavoured in this way to eliminate the possibility that hydroperoxide originally formed was decomposing to hydrogen peroxide during addition of the reagents used for peroxide estimation.

In an alkaline medium (0·1M-lithium sulphate, 0·01M-lithium hydroxide) hydrogen peroxide in water shows two polarographic waves, an anodic step with $E_{\frac{1}{2}} = -0·1$ v (against a standard calomel electrode), and a slowly rising cathodic step, with the approximate relation $E_{\frac{1}{2}} = -0·9$ v. Organic hydroperoxides have been shown to contribute only to the second cathodic step. Quantitative estimation of peroxide is possible from the height of the waves after calibration against pure hydrogen peroxide solutions. These were prepared by the irradiation of oxygenated pure water, and estimated by the titanium and the iodide method.[8, 9] The heights of the anodic and the cathodic wave increase linearly with hydrogen peroxide concentration. A typical estimation of hydrogen peroxide in irradiated D-mannitol solutions after doses of up to $9·6 \times 10^{18}$ ev ml.$^{-1}$ (γ-radiation) is shown in Table 2. Initial $G(H_2O_2)$ is 3·0. Further, addition of lanthanum acetate (0·01M) to the solution used for polarography caused precipitation of hydrogen peroxide and the removal of both the anodic and the cathodic wave.

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[9] Allen, Hochanadel, Ghormley, and Davis, *J. Phys. Chem.*, 1952, **56**, 575.

[10] Minkoff, *Nature*, 1953, **172**, 909; Egerton, Everett, and Minkoff, *ibid.*, 1954, **173**, 399.

TABLE 2.

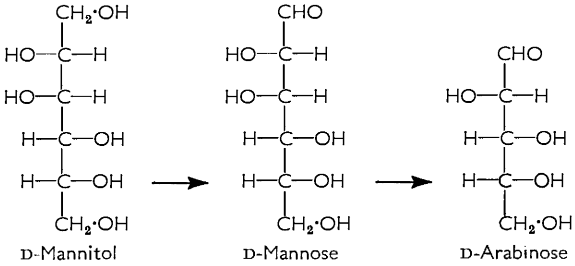
Polarographic estimation of peroxide in irradiated oxygenated D-mannitol solutions. (1 μA ≡ 2·9 × 10^{-2} mg. H_2O_2/ml.)

Dose (10^{18}/ml.)	2·2	3·3	5·1	6·5	8·8	9·6
Diffusion current (μA)	0·14	0·21	0·31	0·39	0·55	0·61

Therefore, production of significant amounts of stable hydroperoxides may safely be excluded. On the suggestion of Dr. Minkoff, we have tested also for the presence of per-acids by running the mixture into slightly acid lithium sulphate and naphthol. The maximum at +0·3 to +0·4 v characteristic of per-acids was not observed.

DISCUSSION

The chemical changes induced by ionising radiations in D-mannitol during irradiation in oxygenated solution are similar to those effected in D-sorbitol under comparable conditions. The initial process is oxidation of the primary alcohol groups to aldehyde, with secondary formation of arabinose, which may be represented:


D-Mannitol D-Mannose D-Arabinose

Similar changes were observed by Wolfrom *et al.*[6] on irradiation of 50% aqueous D-mannitol with fast electrons at doses of 50—200 megareps.

Table 3 shows the initial G values of D-mannose and hydrogen peroxide for irradiations with 190 kv X-rays, ^{60}Co γ-radiation, and fast electrons. There is excellent agreement between the values for fast electrons and γ-radiation, which is particularly satisfactory in view of the independent dosimetric techniques adopted; and the low values for X-radiation may probably be attributed to the method of dosimetry. The dosimetric solution is 0·8N in sulphuric acid, and thus gives rise to greater photoelectric absorption of 190 kv X-rays than do the thin films of D-mannitol solution used in our experiments. From the estimated mean wavelength of X-rays, it is probable that the absorption in 0·8N-sulphuric acid relative to mannitol solution is 1·07—1·12. Thus, the results for X-rays require ~10% correction if they are to be compared with those of γ-radiation and fast electrons, giving G(mannose) *ca.* 2·25 and $G(H_2O_2)$ 2·9. This effect is not significant for fast electrons and Co^{60} γ-radiation, where the energy absorption is through Compton recoil electrons having

TABLE 3.

Initial G values of D-mannose and hydrogen peroxide during irradiation of D-mannitol (5 × 10^{-2}M) in oxygen.

	X-Radiation	γ-Radiation	Fast electrons
G(mannose)	2·05	2·25	2·25
$G(H_2O_2)$	2·6	3·0	3·0

an average initial energy of 0·63 Mv. In any event, it must be remembered that hydrogen peroxide rate measurements during *X*-irradiations are less accurate because of the premature oxygen depletion encountered.

The yields of hydrogen peroxide and mannose are not significantly changed over a ten-fold variation in D-mannitol concentration. Full details of the effect of concentration on this and allied systems, however, will be presented later.

In view of the accuracy and convenience with which D-mannose may be estimated by reducing power and colorimetric methods [7] and their easy availability and preparation, oxygenated D-mannitol solutions may provide a convenient chemical dosimeter for ionising radiations, particularly in technological applications. Further, no stringent requirements of water purity are imposed by a dosimeter based on an organic solute. An upper limit of dose rate for which complete oxygenation may be maintained is indicated by experiments with fast electrons. Our experimental arrangement imposed a limit of 0·05 μA beam current (dose rate $17{\cdot}7 \times 10^{17}$ ev min.$^{-1}$ ml.$^{-1}$). This, however may result directly from the non-uniform dose rate applied to the solution, which is highest at the centre of the cell and falls off laterally in all directions. Local depletion of oxygen at the point of maximum dose rate may, therefore, result. The lower initial *G*(mannose) 1·6 at a beam current of 0·1 μA is probably due to such oxygen depletion rather than to a genuine dose-rate effect, particularly in view of the independence of dose rate shown at lower doses.

When oxygen is depleted, changes are observed in the rate of mannose production. The expected fall in rate is encountered in electron irradiations, whereas a somewhat enhanced rate is found at the point of depletion with *X*-rays. A similar short-term increase in rate of formation of pyruvic acid at the point of oxygen depletion was observed by Johnson, Scholes, and Weiss [11] during *X*-irradiations of lactic acid, and were attributed to reactions of the type: $RO_2\cdot + R\cdot \longrightarrow 2RO\cdot$. Such effects might be less marked for our electron irradiations when water radiolysis occurs within a small localised portion of the solution. Here a greater probability for reactions of the type $R\cdot + R\cdot \longrightarrow R{-}R$ would be expected. Reactions of this type were observed by Phillips and Criddle [1] during γ-irradiation of D-sorbitol solutions *in vacuo*.

In view of reports that hydroperoxides are formed during irradiations of certain aqueous organic compounds in oxygen,[12] and their postulation to explain certain after-effects during the irradiation of purine and pyrimidine nucleotides,[13] considerable attention was devoted to a search for such products. None was, however, detected by polarography. Further, estimations of hydrogen peroxide by this method, the iodide method, and the titanium sulphate method were identical. The overall stoicheiometry may, therefore, be considered on the basis of hydrogen peroxide yields only (initial *G* 3·0).

Product yields may be rationalised on the basis of simple radical reactions involving, first, hydrogen abstraction by hydroxyl radicals at the primary alcohol group. Such reactions have been postulated during the formation of acetaldehyde by irradiation of ethanol solutions.[14] The formation of mannose by the action of Fenton's reagent on D-mannitol solutions,[15] and the identical —*G* value for D-sorbitol on irradiation in oxygenated or evacuated solutions, indicate that hydroxyl radicals rather than the hydroperoxy-radical are the main initial reactive species produced by radiation. The following mechanism may, therefore, be operative:

$$H_2O \longrightarrow H\cdot + \cdot OH$$

$$H\cdot + O_2 \longrightarrow HO_2\cdot$$

$$R\cdot CH_2\cdot OH + \cdot OH \longrightarrow R\cdot CH\cdot OH + H_2O$$

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[11] Johnson, Scholes, and Weiss, *J.*, 1953, 3091.
[12] Scholes and Weiss, *Radiation Res.*, *Suppl.* 1, 1959, **177**, 1.
[13] Butler and Conway, *J.*, 1948, 3415.
[14] Jayson, Scholes, and Weiss, *J.*, 1957, 1358.
[15] Fenton and Jackson, *J.*, 1899, **75**, 1.

which may be followed by either

$$(a)\qquad \cdot CHR\cdot OH + O_2 \longrightarrow \cdot O_2\cdot CHR\cdot OH$$

$$\cdot O_2\cdot CHR\cdot OH + HO_2\cdot \longrightarrow R\cdot CHO + H_2O_2 + O_2$$

or

$$(b)\qquad \cdot CHR\cdot OH + O_2 \longrightarrow R\cdot CHO + HO_2\cdot$$

$$HO_2\cdot + HO_2\cdot \longrightarrow H_2O_2 + O_2$$

For both mechanisms the overall stoicheiometry is the same. Initial G(mannose) should approximate to G_{OH}, and $G(H_2O_2)$ to $G_M(H_2O_2) + \frac{1}{2}(G_H + G_{OH})$ where $G_M(H_2O_2)$, G_H, and G_{OH} are the respective yields of hydrogen peroxide, hydrogen, and hydroxyl radicals produced by irradiation of water. The most reliable estimates of these yields at the pH of the experiments are G_{OH} 2·4, G_H 2·9, and $G_M(H_2O_2)$ 0·71.[16] On this basis the above mechanism should yield initial G(mannose) 2·4 and $G(H_2O_2)$ 3·3. The experimental values are thus in fair agreement with the mechanism.

However, comparison with the corresponding D-sorbitol system indicates that another process may also operate. Here initial G(total hexose) determined by isotope dilution analysis is 2·3 and agrees with the hexose production from D-mannitol (G 2·25). $-G$(D-sorbitol), however, is 3·5 and indicates that additional mechanisms leading to degradation of D-sorbitol may occur side by side with hydroxyl-radical abstraction. Investigation of these processes is continuing.

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CARDIFF.

[*Received, June* 21*st*, 1962.]

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[16] Allen and Schwarz, Proc. Internat. Conf. Peaceful Uses Atomic Energy," 1958, Vol. XXIX, p. 30; Allen, in " Radiation Chemistry of Water and Aqueous Solutions," Van Nostrand, New York, 1961, p. 44.