729. *Radiation Chemistry of Carbohydrates. Part VII.*[1] *Action of γ-Radiation on Aqueous Solutions of* D-*Sorbitol in Oxygen.*

By G. O. Phillips and W. J. Criddle.

Paper-chromatographic and radioactive-tracer methods reveal that when D-sorbitol solutions are irradiated in oxygen with ^{60}Co γ-radiation, the primary alcohol groups are preferentially oxidised. The primary products are D-glucose and L-gulose, each formed with initial G 1·2. D-Sorbitol is degraded with initial $-G$ 3·5. D-Arabinose, L-xylose, formaldehyde, formic acid, and two- and three-carbon aldehydic fragments are formed by secondary processes. It is probable that acid is directly formed, with initial G 0·3. Changes in absorption spectra and the formation of hydrogen peroxide have also been measured.

There is evidence from the irradiation of aqueous hexose and D-mannitol solutions that the CH_2·OH group is more susceptible to attack by free radicals formed during primary radiolysis of water than are the normal secondary alcohol groups in the molecule.[2] In a preliminary survey,[3] it was reported that when D-sorbitol is irradiated in oxygenated solution, D-glucose and L-gulose are the primary products. Similar results have since been reported by Wolfram *et al.*[4] who irradiated concentrated aqueous solutions of D-sorbitol. In this paper, the action of γ-radiation on aqueous oxygenated solutions of D-sorbitol is described in detail, with particular attention given to the nature of the primary degradation processes.

Results and Experimental

Details of the ^{60}Co source used for all irradiations, dosimetric and analytical techniques have been given in previous papers in this series. The dose rates employed were $1·45 \times 10^{17}$ ev min.$^{-1}$ ml.$^{-1}$ in the large cell (100 ml.) and $1·0 \times 10^{17}$ ev min.$^{-1}$ ml.$^{-1}$ in the small cell (30 ml.).

Chromatographic Analysis of Irradiated Solutions.—A solution (100 ml.) of D-sorbitol (5·50 millimoles) was irradiated to a total energy input of $7·0 \times 10^{22}$ ev, and chromatographed on paper with two irrigants. The organic constituents detected by sprays of *p*-anisidine are shown in Table 1.

Table 1. *Constituents in irradiated solutions of* D-*sorbitol.*

	(*a*) Butan-1-ol–acetic acid–water (4 : 1 : 5)			(*b*) Ethyl methyl ketone–acetic acid–saturated boric acid (9 : 1 : 1)		
Autoradiograph	Colour	R_F	Product	Colour	R_G	Product
IV	Brown	0·18	Glucose and D-sorbitol	Brown	1·0	Glucose
V	Brown	0·22	Gulose	Brown	1·3	Gulose
VI	Pink	0·24	Arabinose	Pink	1·3	Arabinose
VII	Pink	0·27	Xylose	Pink	1·9	Xylose and D-sorbitol

A solution (100 ml.) of D-[^{14}C]sorbitol (5·50 millimoles; *ca.* 25 μc) was irradiated to a total energy input of $6·1 \times 10^{22}$ ev. After chromatography in butan-1-ol–acetic acid–water (4 : 1 : 5), an autoradiograph was prepared and scanned with a Hilger photoelectric densitometer. Fig. 1 shows the intensities of the spots, giving a measure of the ^{14}C concentration along the paper chromatogram. The constituents I, II, and III were not detected with *p*-anisidine at low energy inputs. At high energy inputs, constituent III gave a pink colour with *p*-anisidine.

[1] Part VI, *J.*, 1960, 3404. In that paper the following errors are to be noted: p. 3405, line 8, *for* [1-^{14}C]mannose *read* [^{14}C]mannose; p. 3408, line 2, *for* D-[1-^{14}C]mannose *read* D-[^{14}C]mannose; p. 3411, lines 3 and 6, *for* ^{14}C *read* 1-^{14}C; p. 3406, Fig. 8, the scale for rate of formation of carbon dioxide is 10^{20} molecules.

[2] Phillips, *Nature*, 1954, **173**, 1044; Phillips, Moody, and Mattok, *J.*, 1958, 3522.

[3] Phillips, Moody, and Mattok, Proc. 2nd Conf. Peaceful Uses of Atomic Energy, 1958, Vol. XXIX, p. 92.

[4] Wolfram, Binkley, Shen Han, and Michelakis, *Radiation Res.*, 1959, **10**. 37.

Treatment of the irradiated D-sorbitol with phenylhydrazine and glacial acetic acid gave a mixture of osazones which were separated by circular paper chromatography.[5] The following constituents were detected: R_F 0·40, glucosazone; R_F 0·42, possibly due to gulosazone, but no control sample was available for confirmation; R_F 0·45, arabinosazone; and R_F 0·48, xylosazone.

Formic acid was detected in the irradiated solution as follows. The irradiated solution was distilled *in vacuo*. The distillate, collected in a receiver cooled in liquid air, was treated with an excess of aqueous ammonia and concentrated. The resultant solid was chromatographed in 95% ethanol–concentrated aqueous ammonia [6] (100 : 1); and spraying with Bromocresol Green in absolute alcohol [7] revealed a blue spot on a yellow background, with R_F 0·31, due to ammonium formate.

Acid Formation.—Volatile acid. After distillation of the irradiated solution *in vacuo*, formic acid was estimated in the distillate by direct titration with 0·01N-barium hydroxide. The rate of formation of volatile acid is shown in Fig. 2.

FIG. 1. *Density of spots on autoradiograph, giving a measure of* ^{14}C *concentration along the paper chromatogram.*

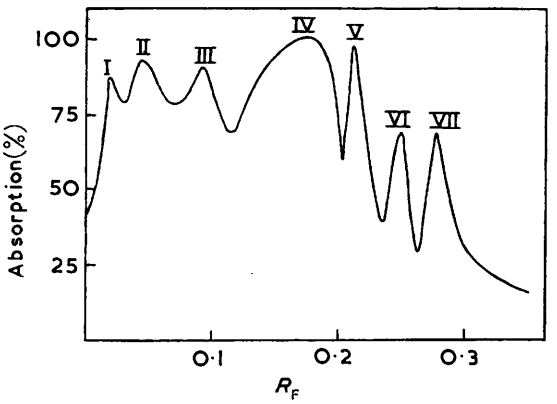
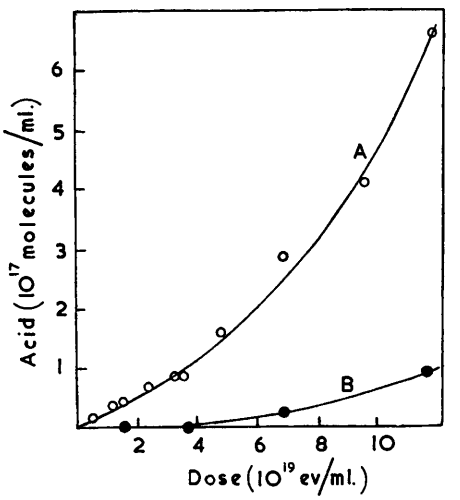


FIG. 2. *Formation, during irradiation of* D-*sorbitol solutions in oxygen, of* (A) *total and* (B) *volatile acid.*



Total acid. The rate of formation of acid in the irradiated solution was measured by direct titration with barium hydroxide (phenolphthalein) and potentiometrically. Both methods indicate initial G 0·3 for acid, if the acid is assumed to be monobasic. The results are shown in Fig. 2.

Formation of Hydrogen Peroxide.—The rate of production of hydrogen peroxide was measured with titanium sulphate reagent [8] and is shown in Table 2. Initial $G(H_2O_2)$ is 2·9; after the irradiation there was a decrease in hydrogen peroxide concentration at the rate of 4×10^{13} molecules ml.$^{-1}$ min.$^{-1}$.

TABLE 2. *Formation of hydrogen peroxide during the irradiation of oxygenated* D-*sorbitol solutions.*

Dose (10^{19} ev ml.$^{-1}$)	0·42	0·84	1·27	2·11	3·37	4·0	5·0
H_2O_2 (10^{17} molecules ml.$^{-1}$)	1·23	2·46	3·33	4·7	7·0	9·7	12·0

Ultraviolet Absorption Spectra.—The ultraviolet absorption spectrum of a solution (100 ml.) of D-sorbitol (5·5 millimoles) which had absorbed an energy input of $8{\cdot}1 \times 10^{22}$ ev is shown in

[5] Barry and Mitchell, *J.*, 1954, 4020.
[6] Kennedy and Barker, *Analyt. Chem.*, 1951, **23**, 1033.
[7] Block, Durrum, and Zweig, "Paper Chromatography and Paper Electrophoresis," Academic Press, New York, 1955, p. 160.
[8] Eisenberg, *Ind. Eng. Chem., Analyt.*, 1943, **15**, 327.

Fig. 3. The absorption maximum at 275 mμ is not affected by the addition of potassium hydrogen carbonate. The rate of increase in ultraviolet absorption at 275 mμ with energy input is given in Table 3. At energy inputs of ~5 × 10^{22} ev, no characteristic absorption maximum was observed.

TABLE 3. *Increase in ultraviolet absorption at* 275 mμ *during irradiation of aqueous* D-*sorbitol solutions.*

Optical density (275 mμ)	0·15	0·16	0·21	0·38	0·85	1·80
Dose (10^{20} ev ml.$^{-1}$)	0	1·5	5·7	8·1	12·0	19·6

Formation of Carbon Dioxide.—Carbon dioxide liberated during irradiation was trapped in barium hydroxide solution and estimated gravimetrically. The rate of formation with energy input is shown in Table 4.

TABLE 4. *Formation of carbon dioxide during irradiation of* D-*sorbitol solutions* (100 ml.).

Energy input (10^{22} ev)	1·7	5·3	9·7	13·7	17·5	20·0
Carbon dioxide (10^{20} molecules)	0·2	0·3	0·5	1·5	5·5	8·0

Polymer Formation.—Since we have observed the formation of a polymer during irradiation of D-sorbitol solutions *in vacuo*, a rigorous search was made to detect any polymer which may have been formed under oxygenated conditions. The irradiated solution (total energy input

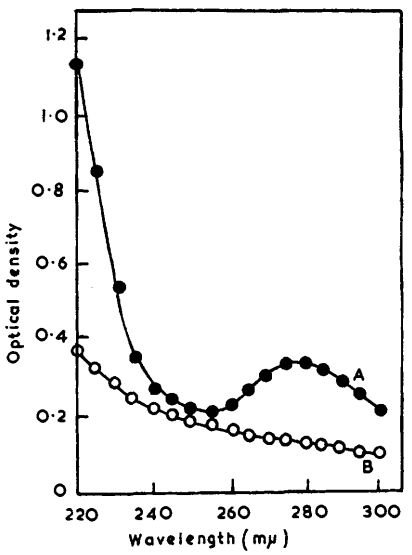


FIG. 3. *Ultraviolet spectra of irradiated* D-*sorbitol solutions* (100 ml.). (A) D-*Sorbitol after energy input of* 8·1 × 10^{22} ev. (B) *Unirradiated* D-*sorbitol.*

12 × 10^{22} ev) was dialysed against running water, and the solution remaining was freeze-dried. No residue was obtained. Additional evidence for the absence of polymer came from the observation that for paper chromatograms of D-[^{14}C]sorbitol solutions irradiated in oxygen, no radioactivity was retained on the starting line. *In vacuo*, polymer was detected by both these methods, as described in the following paper. Therefore, it is concluded that no polymer is formed in oxygen.

Estimation of Products by Isotope Dilution Analysis.—A solution (100 ml.) of D-sorbitol (5·50 millimoles) containing sufficient D-[^{14}C]sorbitol to give a specific activity of 3·8 μc/millimole was irradiated to a total energy input of 6·1 × 10^{20} ev. Isotope dilution analysis was used to estimate unchanged D-sorbitol and the products of irradiation. A typical estimation is described below.

D-*Sorbitol.* The irradiated solution (5 ml.) was freeze-dried and rigorously dried *in vacuo* over phosphorus pentoxide. Carrier D-sorbitol (0·48 millimoles), anhydrous sodium acetate (0·2 g.), and freshly distilled acetic anhydride (1 ml.) were added and the mixture was refluxed for 30 min. The solid which separated on addition of ice-cold water (15 ml.) was recrystallised

8 times from ethanol to give pure hexa-*O*-acetyl-D-sorbitol, m. p. 99°, constant spec. activity 0·52 μc/millimole.

D-*Glucose*. (*a*). As penta-*O*-acetate. The irradiated solution (5·0 ml.) was freeze-dried, further dried as above, and treated with carrier D-glucose (0·54 mmole), anhydrous sodium acetate (0·2 g.), and acetic anhydride (1 ml.). The mixture was heated at 100° for 1 hr. and poured into ice-cold water (15 ml.). The solid which separated was recrystallised seven times from ethanol, to give pure penta-*O*-acetyl-β-D-glucose, m. p. 131°, constant spec. activity 0·27 μc/millimole.

(*b*) As D-glucosazone. The irradiated solution (5·0 ml.) was heated with carrier D-glucose (1·0 millimole), glacial acetic acid (1 ml.), and phenylhydrazine (1·5 ml.) at 100° for 20 min. The solid which separated was recrystallised seven times from ethanol, to give pure D-glucosazone, m. p. 199°, constant spec. activity 0·14 μc/millimole.

L-*Xylose*. The irradiated solution (5·0 ml.) was freeze-dried, dried as above, and heated

TABLE 5. *Products when aqueous* D-*sorbitol is irradiated with γ-radiation in oxygen.*

(a) *Initial* D-*sorbitol*, 5·5 millimoles. *Energy input* 6·1 × 10^{22} ev (vol. 100 ml.).

		D-Glucose				
Product	D-Sorbitol	*a*	*b*	D-Arabinose	L-Xylose	D-Xylose
Carrier (millimoles)	0·48	0·54	1·00	1·00	0·36	0·96
Spec. activity (μc/millimole)	0·52	0·27	0·14	0·09	0·23	—
Yield (millimoles) ...	1·58	0·82	0·84	0·50	0·51	—

Product	Gluconic acid	Glucuronic acid	Two-carbon fragments	Three-carbon fragments	Oxalic acid	Formaldehyde
Carrier (millimoles)	1·02	0·54	1·05	1·00	2·00	0·13
Spec. activity (μc/millimole)	0·04	0·03	0·006	0·002	0·002	0·16
Yield (millimoles) ...	0·11	0·08	0·09	0·024	0·66	0·70

Formic acid determined as volatile acid by titration, 0·06 millimole. Carbon dioxide determined gravimetrically, 0·02 millimole.

a, As penta-*O*-acetate. *b*, As glucosazone.

(b) *Initial* D-*sorbitol* 5·53 millimoles. *Energy input* 12·0 × 10^{22} ev (vol. 100 ml.).

Product	D-Sorbitol	D-Glucose	D-Arabinose	L-Xylose	D-Xylose	Gluconic acid
Carrier (millimoles)	1·10	1·00 [a]	1·03	1·01	1·05	1·01
Spec. activity (μc/millimole)	0·16	0·12	0·065	0·065	—	0·08
Yield (millimoles) ...	0·96	0·62	0·42	0·41	—	0·21

Product	Glucuronic acid	Two-carbon fragments	Three-carbon fragments	Oxalic acid	Formaldehyde
Carrier (millimoles)	1·00	1·13	0·97	1·93	0·133
Spec. activity (μc/millimole)	0·033	0·006	0·003	0·003	0·20
Yield (millimoles) ...	0·18	0·10	0·028	0·098	1·04

Formic acid determined as volatile acid by titration, 0·15 millimole. Carbon dioxide determined gravimetrically, 0·04 millimole.

(c) *Initial* D-*sorbitol* 5·56 millimoles. *Energy input* 20·0 × 10^{22} ev (vol. 100 ml.).

Product	D-Sorbitol	D-Glucose	D-Arabinose	L-Xylose	D-Xylose	Gluconic acid
Carrier (millimoles)	1·00	1·03 [a]	0·95	0·99	1·13	1·00
Spec. activity (μc/millimole)	0·06	0·06	0·036	0·039	—	0·11
Yield (millimoles) ...	0·37	0·38	0·23	0·26	—	0·28

Product	Glucuronic acid	Two-carbon fragments	Three-carbon fragments	Oxalic acid	Formaldehyde
Carrier (millimoles)	1·02	0·97	1·00	2·00	0·133
Spec. activity (μc/millimole)	0·08	0·008	0·003	0·009	0·13
Yield (millimoles) ...	0·22	0·14	0·034	0·26	0·62

Formic acid determined as volatile acid by titration, 0·23 millimole. Carbon dioxide determined gravimetrically, 0·50 millimole.

with carrier L-xylose (0·36 millimole), anhydrous sodium acetate (0·2 g.), and acetic anhydride (1 ml.) at 100° for 1 hr. It was necessary to recrystallise the solid which separated on addition of crushed ice (15 g.) eight times from ethanol to give pure tetra-*O*-acetyl-β-L-xylose, m. p. 126°, constant spec. activity 0·23 μc/millimole.

D-*Xylose*. By the procedure described for L-xylose, inactive tetra-*O*-acetyl-β-D-xylose was obtained after addition of D-xylose as carrier.

D-Arabinose, D-glucuronic acid, D-gluconic acid, two- and three-carbon aldehydic fragments, oxalic acid, and formaldehyde were estimated as described previously. The results for the above estimation, and for two further isotope dilution estimations after energy inputs of $12{\cdot}0 \times 10^{22}$ and $20{\cdot}0 \times 10^{22}$ ev, are shown in Table 5.

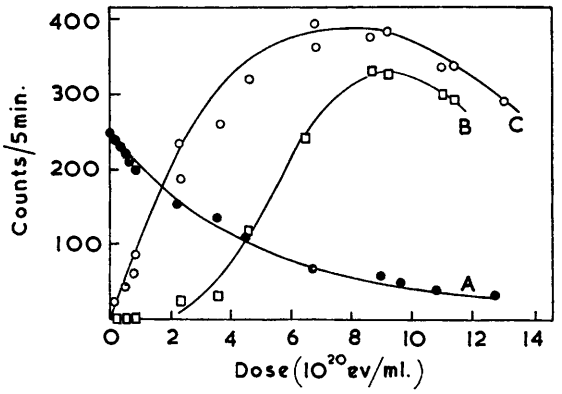


FIG. 4. *Rate of formation of* (B) *pentose and* (C) *hexose during irradiation of oxygenated* D-*sorbitol solutions* (100 ml.), *determined by paper chromatography*. A = *Sorbitol* ($\times 10^{-1}$).

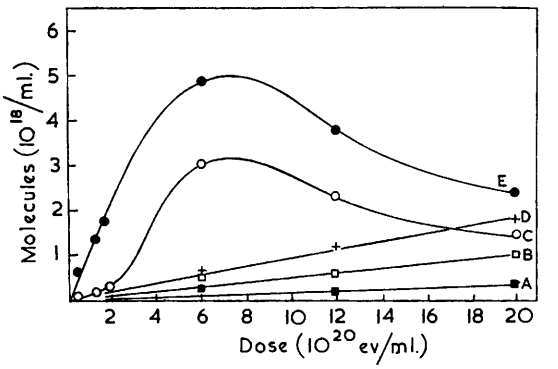


FIG. 5. *Rate of formation of products during irradiation of oxygenated* D-*sorbitol solutions, determined by isotope dilution.*

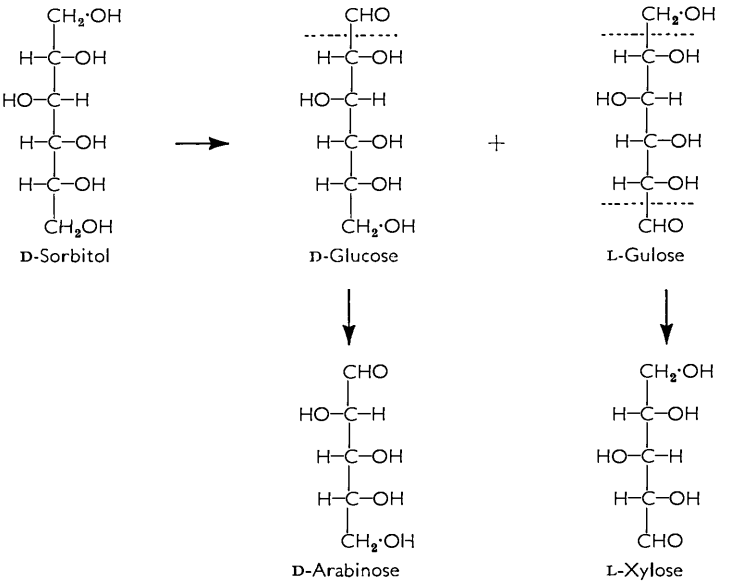
A, *Two-carbon aldehydic fragments.*
B, *Three-carbon aldehydic fragments.*
C, D-*Arabinose.*
D, D-*Gluconic acid.*
E, D-*Glucose.*

Rate of Formation of Products.—Accurately known amounts (0·05 ml.) of oxygenated D-[^{14}C]sorbitol (5·56 millimoles; *ca.* 25 μc) solution (100 ml.) which had been irradiated to progressively increasing doses were chromatographed in butan-1-ol–acetic acid–water and ethyl methyl ketone–saturated boric acid–acetic acid. In the former solvent it is possible to isolate gulose, arabinose, and xylose as discrete spots; in the latter, glucose, gulose, and arabinose. Therefore, by measuring the radioactivity of the spots at varying doses in the two solvents, the yield–dose curves for D-glucose, L-gulose, L-xylose, and D-arabinose were obtained (Fig. 4). The rate of disappearance of D-sorbitol was also measured.

In order that the accuracy of the yield–dose curves obtained from measurements on paper chromatograms could be verified and initial *G* values accurately determined, a hexose and a pentose were determined directly by isotope dilution analysis as described above at three low energy inputs. Individual samples of D-sorbitol (1·62 millimoles) in water (30 ml.) were irradiated to doses of 4·8, 12·6, and $16{\cdot}8 \times 10^{19}$ ev/ml., and D-sorbitol, D-glucose, and D-arabinose were estimated at each dose. The results are shown in Fig. 5, together with the relevant isotope dilution yields previously given in Table 5. From the yield–dose curves $-G$(D-sorbitol) is 3·5 and initial G(D-glucose) 1·2. From paper chromatography initial G(hexose) is 1·1. The yield–dose curves support the view that pentoses are formed by secondary processes.

Discussion

The results indicate that the degradation of D-sorbitol when irradiated in oxygenated solution is more specific than the degradation of hexoses under comparable conditions.[2] A similar specific attack on D-mannitol was previously noted.[2] Paper chromatography reveals four main degradation products, namely, glucose, gulose, arabinose, and xylose. Isotope dilution analysis demonstrates that the stereochemical forms present are D-glucose, D-arabinose, and L-xylose. On configurational grounds, therefore, it is probable that gulose is present as the L-isomer. Oxidation of the primary alcohol group at one extremity of the molecule leads to D-glucose, and at the opposite end to L-gulose. Similar considerations apply to pentose formation. Simultaneously, formaldehyde was detected by isotope dilution analysis. Thus the degradation occurs as illustrated.



From paper chromatographic evidence, Wolfram *et al.*[4] concluded that a similar degradation pattern occurs in a 50% aqueous D-sorbitol solution when exposed to cathode rays at doses ranging from 20 to 100 Mrep at ethanol–solid carbon dioxide temperatures.

Autoradiography indicates the presence of constituents (I, II, III) which are not detectable with *p*-anisidine at low energy inputs. These are probably non-reducing acids, but have not been identified with certainty. At high energy inputs the presence of a constituent giving a pink colour with *p*-anisidine was noted and this may be glucuronic acid, formed by secondary attack on glucose. Constituent III and glucuronic acid (pink spot) run identically. Support for the presence of glucuronic acid is provided by the slow formation of this acid indicated by isotope dilution analysis (Table 5), and the analogous formation of mannuronic acid at high doses, noted by Wolfram and his co-workers [4] during the irradiation of D-mannitol solutions.

The form of the yield–dose curves (Figs. 4 and 5) enables primary and secondary processes to be identified. D-Glucose and L-gulose are formed at identical rates (Fig. 4), and the appreciable initial rate of formation indicates that they are primary products. There is good agreement between the initial G values obtained by paper chromatography and isotope dilution; the former method gives initial G 1·1 and the latter 1·2 for individual hexose formation. At higher doses degradation of the hexoses occurs, and this appears to be related to pentose formation (Figs. 4 and 5). Although D-arabinose and L-xylose are not formed initially, the rate of formation rises sharply at doses where considerable

degradation of the hexoses occurs. Previously it was observed that during the irradiation of D-glucose [2] and D-mannose,[9] arabinose is formed by primary and secondary processes. For hexose irradiations, after a slow initial formation of arabinose, the yield–dose curves rise sharply at higher energy input in a similar manner to Figs. 4 and 5. Thus it is probable that during the irradiation of D-sorbitol, D-arabinose and L-xylose are formed solely by secondary degradation of D-glucose and L-gulose. Similar considerations make it probable that formaldehyde, which is related to pentose formation during the irradiation of mannose,[10] is also a secondary product. Formic acid may arise subsequently. One difficulty should be noted, however, which is common to all products formed initially at very low rates. Fig. 5 shows the yield–dose curves for two- and three-carbon aldehydic fragments. At doses less than *ca.* $1{\cdot}5 \times 10^{20}$ ev/ml. it is not possible to estimate these products accurately because of their low concentration. It is difficult, therefore, to be certain on the evidence available, whether these fragments arise by primary or secondary processes. On balance, however, some primary chain scission appears probable to account for the difference between initial G(hexoses) 2·2—2·4 and the initial rate of disappearance of D-sorbitol ($-G$ 3·5). A similar difficulty exists with regard to acid formation. The rate of formation of gluconic acid is greater than that of the two- and three-carbon fragments and, if a linear rate of formation is assumed, initial G for gluconic acid formation is 0·14. To obtain more precise information about initial formation of gluconic and gulonic acid, the rate of acid formation with energy input was examined by normal titration methods and potentiometrically. The two methods indicate an initial G for total acid of 0·3, which is in agreement with isotope dilution results if D-gluconic and L-gulonic are the main acids formed initially. A fall in pH may be detected with the relatively low dose of $0{\cdot}8 \times 10^{19}$ ev/ml. and a slow primary formation of acid, therefore, appears probable.

The absorption spectra of irradiated D-sorbitol solutions show a general similarity to the spectra of irradiated hexose solutions.[2] The rate of increase in ultraviolet absorption with energy input (Table 3) demonstrates that the products responsible for the characteristic maximum at *ca.* 270 mμ are not formed in appreciable quantity until a dose of *ca.* 8×10^{20} ev/ml. is absorbed. Secondary products, therefore, are responsible for the ultraviolet absorption, and are probably formed by degradation of the initially formed hexoses. Among the last of the products to be formed in quantity is carbon dioxide (Table 4), for which the rate of formation increases sharply at *ca.* 12×10^{20} ev/ml.

The results of this investigation, therefore, confirm previous observations for D-mannitol [2,4] and hexose irradiations [2,9] that primary alcohol groups are more susceptible to attack than normal secondary alcohol groups. The degradation of D-sorbitol in aqueous solution may be summarised: $R{\cdot}CH_2{\cdot}OH \longrightarrow R{\cdot}CHO \longrightarrow$ secondary products. A similar path was observed for primary aliphatic alcohols when irradiated in solution.[10] There are also indications from the results that the process, $R{\cdot}CH_2{\cdot}OH \longrightarrow R{\cdot}CO_2H$, may occur directly without intermediate formation of the aldehyde. It is possible that a small amount of ring scission occurs by primary processes. The initial rate of disappearance of D-sorbitol (G 3·5) is in good agreement with values previously reported for the rate of degradation of sugars on irradiation in aqueous solution.[2,9,11]

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[9] Phillips and Criddle, *J.*, 1960, 3404.
[10] Jayson, Scholes, and Weiss, *J.*, 1957, 1358.
[11] Phillips and Moody, *J.*, 1960, 754.