710. *Radiation Chemistry of Carbohydrates. Part I. Action of Ionising Radiation on Aqueous Solutions of* D-*Glucose.*

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When irradiated in dilute aqueous solution, D-glucose is degraded to D-glucuronic acid, D-gluconic acid, glyoxal, D-arabinose, D-erythrose, formaldehyde, saccharic acid, and 1 : 3-dihydroxyacetone, which have been estimated quantitatively. Hydrogen peroxide is also formed.

Under fully oxygenated and evacuated conditions, variation in glucose concentration and dose rate does not influence the yield of acid; the extent and pattern of degradation are the same for 1 Mv electrons and cobalt gamma-radiation.

The liberation of gas indicates a post-irradiation process, also shown by changes in ultraviolet absorption spectra and hydrogen peroxide concentration.

The absorption spectrum of the irradiated solutions shows a characteristic absorption maximum at 265 mμ, sensitive to alkali; it is related to 1 : 3-dihydroxyacetone.

By following the yield–dose curves for the main products, the primary and secondary products are distinguished. The main types of degradation are thus identified.

ALTHOUGH the chemical action of ionising radiations on organic compounds has been widely studied, information regarding the behaviour of carbohydrates is meagre. Attention has been focused mainly on some of the physical changes observable during irradiation, such as pH, optical rotation, reducing power, viscosity and ultraviolet absorption spectra.[1,2] In a preliminary study we found that an aqueous solution of D-glucose after irradiation with fast electrons shows an absorption band at 200—300 mμ, and that glucuronic acid is formed.[3] Our subsequent investigations, however, revealed several other products. Bothner-By and Balazs [2] recently reported that solutions of the sugar irradiated with *X*-rays at doses ranging from 1 to 10^7 rads show an absorption band in this range; although no products were identified they state that glucuronic acid was not formed. In the present work we have examined the behaviour of aqueous solutions

[1] Holtz and Becker, *Arch. Expt. Pathol. Pharmakol.*, 1936, **182**, 160; Holtz, *ibid.*, 1936, **182**, 141; Slosse, *Compt. rend. Soc. Biol.*, 1923, **89**, 96; Kertesz, Bruce, Tuttle, and Lavin, *Radiation Res.*, 1956, **5**, 372.

[2] Bothner-By and Balazs, *Radiation Res.*, 1957, **6**, 302.

[3] Phillips, *Nature*, 1954, **173**, 1044.

of D-glucose under evacuated and fully oxygenated conditions when irradiated with 1 Mv electrons and with gamma radiation.

Experimental

Electron Beam.—The irradiations were carried out with a beam of high-energy electrons from the 2 Mv van de Graaf Generator of the Chemistry Division, A.E.R.E., Harwell. The vertical electron beam accelerated to 1·25 Mv was turned through 90° by means of a magnet, and directed into the cell through a graphite collimator. This arrangement reduced stray radiation effects and ensured that the main electron beam entered the cell without impinging on the glass sides. The cell was placed 3 cm. from the aluminium window of the generator. The cells [4] were provided with windows of thin Pyrex glass to minimise energy losses. One type of window weighed 100 mg./cm.,[2] corresponding to a threshold voltage of the order of 0·24 Mv. The other type was much thinner (30 mg./cm.^{2}), and the energy loss was only 0·07 Mv.[5] A well containing mercury permitted connection between the charge-collecting lead (through a platinum–tungsten lead sealed in the cell wall) and the current-collecting lead from the measuring instrument. The volumes of the cells ranged from 10 to 11 ml. Irradiation times could be reduced to 10 sec. by means of a beam interrupter,[6] and cell currents ranging from 0·01 to 1·0 μA were employed, corresponding respectively to energy-input rates of $3{\cdot}7 \times 10^{18}$—$3{\cdot}7 \times 10^{20}$ ev/min. Energy-input rates were calculated directly from the beam voltage and current within the cell and refer to a total volume of 10 ml. The current in the platinum wire within the cell was led along a shielded lead to earth through a D.C. amplifier and current integrator which would take into account any variation in cell current. Since the currents measured were small, it was necessary to ensure that the leakage resistance of the current-carrying lead to earth was high. The generating voltmeter measured the beam voltage directly, and a correction for energy losses in the cell window was applied as described.

Electron-beam Irradiation of Glucose Solutions.—A de-oxygenated solution (10 ml.) of D-glucose (5·5 millimoles) was irradiated with electrons (1 Mv) at 1 μA for 1 hr. (energy-input rate $3{\cdot}75 \times 10^{20}$ ev/min.); the pH fell to 3·25, and $[\alpha]_D$ from +51·6° to +0·14°. At this high dose rate similar behaviour was shown by the air-equilibrated solution, because the depletion of oxygen in the vicinity of the beam is faster than its diffusion from the body of the solution. The distillate obtained by heating the solution below 40° under reduced pressure contained a trace of acid, but did not form a complex with dimedone or *p*-bromoformanilide. Paper chromatography with butan-1-ol–pyridine–water, followed by spraying with aniline hydrogen phthalate and silver nitrate, gave highly streaked chromatograms, but with practice it was possible to identify three spots corresponding to glucose (R_F 0·37), glucuronic acid (R_F 0·07), and glucurone (R_F 0·66).

In another experiment, [^{14}C]glucose (generally labelled) (100 μc) was added to the original solution, and the mixture irradiated. The autoradiograph (24 hr.) was identical in pattern with the chromatogram obtained with the spray reagent.

Rate of Acid Formation in the Absence of Oxygen.—The concentration of acid increased linearly with energy input (Fig. 1). The yield was independent of the initial glucose concentration in the range $5{\cdot}5 \times 10^{-2}$ to $5{\cdot}5 \times 10^{-3}$M, and of the energy absorption in the range $3{\cdot}75 \times 10^{20}$ to $3{\cdot}75 \times 10^{19}$ ev. By assuming that the acid is monobasic, it was found that G(acid) = 0·41.

Evolution of Gas.—The cell employed for investigating the evolution of gas on irradiation [4] was provided with a mercury bellows-manometer [7] and a break-seal for use when gas is pumped off. The cell was charged with the glucose solution, and the system freed from air by the freeze-pump technique before irradiation. During irradiation the cell was maintained at the selected temperature within ±0·05°. After irradiation of the solution, the gases were removed at −196° and the residual volatile material at −90°. The gases were measured in a calibrated Töpler pump before analysis.

An evacuated glucose solution (5×10^{-2}M) on irradiation at 30° with 1 Mv electrons at an energy input of $3{\cdot}75 \times 10^{20}$ ev/min. evolved gas at the rate of 0·114 ml./min. The pressure in

[4] Amphlett and Williams, *J. Sci. Instr.*, 1956, **33**, 64.
[5] Williams and Wilkinson, *J. Chim. phys.*, 1955, **52**, 600.
[6] Miller, A.E.R.E. C/R. 1068.
[7] Spence, *Trans. Faraday Soc.*, 1940, **36**, 417.

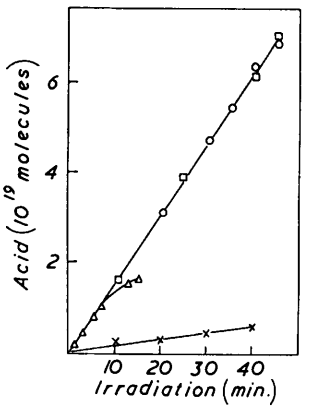
the cell increased linearly with energy input (Fig. 2). After irradiation, the evolution continued for 30 hr. Of the total gas (8·5 ml.), the part gaseous at −196° consisted of hydrogen (98%) and carbon monoxide (2%), while the part gaseous at −90° (0·9 ml.) contained about 90% of carbon dioxide. Experiments at 30° and 40° are reported in Table 1.

TABLE 1. *Irradiation of glucose solutions with* 1 Mv *electrons (energy input* $3{\cdot}25 \times 10^{20}$ ev/*min.*).

Temp.	Time (min.)	G (total gas)	Post-irradiation evolution (%)
30°	66	1·1	24
40	60	1·1	20

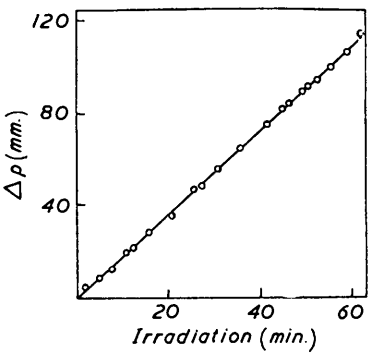
Acid Yield under Oxygenated Conditions.—Owing to the depletion of oxygen in the vicinity of the electron beam (1 μA and energy input of $3{\cdot}75 \times 10^{20}$ ev/min.), the passage of oxygen through the irradiated glucose solution did not affect the yield of acid. For measurements of acid yield under fully oxygenated conditions, we employed lower dose rates with beam current

FIG. 1. *Acid production during irradiation of aqueous* D-*glucose with* 1 Mv *electrons.*



□ $5{\cdot}5 \times 10^{-3}$M, ○ $5{\cdot}5 \times 10^{-2}$M, Δ $5{\cdot}5 \times 10^{-3}$M (*energy input* $3{\cdot}75 \times 10^{20}$ ev/min.); × $5{\cdot}5 \times 10^{-3}$M (*energy input* $3{\cdot}75 \times 10^{19}$ ev/*min.*)

FIG. 2. *Gas evolution during irradiation of evacuated* D-*glucose solutions with* 1 Mv *electrons.*



Glucose $5{\cdot}5 \times 10^{-2}$M. *Energy input* $3{\cdot}75 \times 10^{20}$ ev/*min. Temp.* 30°.

varying from 0·01 to 0·05 μA. The results for glucose solutions (10^{-2}—10^{-3}M) are shown in Fig. 3. The rate of formation of acid increases gradually with dose, and the initial rate corresponds to G(acid) 1·2—1·3, on the assumption that the acid formed is monobasic.

Absorption Spectrum of Irradiated Solutions.—A typical absorption spectrum of an irradiated glucose solution is shown in Fig. 4. Further evidence of a slow post-irradiation reaction was furnished by the change in the ultraviolet spectrum of the irradiated solution. The absorption at 265 mμ (Fig. 4) increased steadily and attained a maximum 20—30 hr. after irradiation had ceased. The addition of potassium hydrogen carbonate led to a sharp increase in the absorption in this region.

Action of Gamma Radiation.—Gamma radiation was provided by a cobalt-60 source (200 curies) similar in design to that developed at A.E.R.E., Harwell, and described by Gibson and Pearce,[8] and varying dose rates were obtained by arranging the irradiation vessels at different distances from the source.

The chemical method employed for dosimetry was based on the oxidation of ferrous sulphate in highly purified water,[9] the solution being 10^{-3}M in ferrous ion and 0·8N in sulphuric acid. The concentration of ferric ion was determined by means of a Unicam S.P. 500 spectrophotometer: max. 301 mμ, ε 2050, and $G(Fe^{3+})$ was taken as 15·5. Addition of inorganic ions (KCl 10^{-3}M) had no effect on the yield.

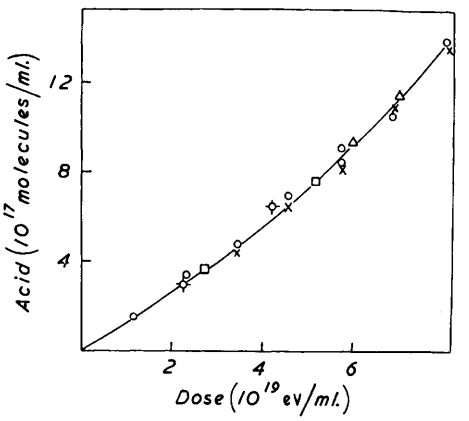
Where maximum energy deposition into the solution was required, glass cells with a central

[8] Gibson and Pearce, *Chem. and Ind.*, 1957, 613.
[9] Amphlett, *Discuss. Faraday Soc.*, 1952, **12**, 145.

annulus, into which the source could be inserted, were used. In this type of cell volumes of 100—150 ml. were irradiated. For lower dose rates, glass cells (*ca.* 40 ml.) fitted with a sintered disc for oxygenation were placed around the source on a turn-table (4 revs./3 min.) to ensure even and reproducible dose rates. During the irradiations, the system was either de-aerated on a high-vacuum line or saturated with oxygen by passing the gas through a sintered disc into the solution. The dose rates into the annular cells were $2{\cdot}13 \times 10^{17}$ ev min.$^{-1}$ ml.$^{-1}$ and into the smaller cells $9{\cdot}65 \times 10^{16}$ ev min.$^{-1}$ ml.$^{-1}$.

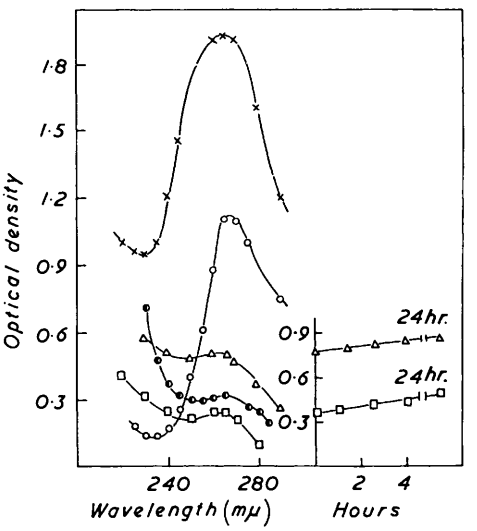
Chromatography.—The constituents in the irradiated glucose solution were separated chromatographically on Whatman paper No. 1. The free sugars were irrigated with butan-1-ol–acetic acid–water (4 : 1 : 5), and detected with *p*-anisidine hydrochloride, aniline hydrogen phthalate, or silver nitrate.[10] Osazones were separated by the circular paper method [11] with toluene–ethanol–water (9 : 1 : 1) and detected with ammoniacal silver nitrate. Anilides were separated with benzene–acetone–water (2 : 9 : 1) containing 0·01% of rhodanine and were

FIG. 3. *Acid production during irradiation of* D-*glucose solutions in oxygen.*



	Electron beam (μA)	Energy input (10^{19} ev/min.)	Glucose concn. (10^{-3}M)
⊕	0·02	0·74	50
△	0·05	1·85	50
□	0·05	1·85	5
	^{60}Co γ radn.		
○	—	0·38	50
×	—	0·38	5

FIG. 4. *Ultraviolet absorption spectra of irradiated* D-*glucose solutions.*



1 Mv *electrons, energy input* (△) $2{\cdot}4 \times 10^{22}$ ev, (×) $2{\cdot}4 \times 10^{22}$ ev (*aq.* $KHCO_3$ *added after irradiation*), (□) 1×10^{22} ev, in vacuo, *vol.* 10 *ml., optical density* ÷ 4.
^{60}Co γ *radiation, energy input* (◐○) 8×10^{22} ev, (○ only) *aq.* $KHCO_3$ *added after irradiation*), *in* O_2 *vol.* 40 ml.
Inset: post-irradiation increase in optical density at 265 mμ.

detected by their fluorescence in ultraviolet light.[12] Lactones of the sugar acids were separated with butan-1-ol–ethanol–water (4 : 1 : 5) and detected by spraying with hydroxylamine in potassium hydroxide solution and then with dilute ferric chloride.[13] For the autoradiographic examination, known volumes of irradiated solutions of [^{14}C]glucose were applied to the paper and irrigated as described for 24—30 hr. The dried paper was placed in contact with Ilford "B" *X*-ray film in lead-backed holders, and satisfactory autoradiographs were obtained after 2—4 months.

Chromatographic Separation of Irradiation Products.—A solution (150 ml.) of D-glucose (5×10^{-2}M) was irradiated to total energy input $4{\cdot}6 \times 10^{22}$ ev and then chromatographed with butan-1-ol–acetic acid–water. Excessive streaking precluded the use of silver nitrate as a spray

[10] Hough, Jones, and Wadman, *J.*, 1950, 1702.
[11] Barry and Mitchell, *J.*, 1954, 4020.
[12] Green, *J. Amer. Chem. Soc.*, 1956, **78**, 1894.
[13] Abdel-Akher and Smith, *ibid.*, 1951, **73**, 3859.

reagent. Streaking was less marked with *p*-anisidine which revealed five spots: brown, R_F 0·18, glucose; pink, R_F 0·11, glucuronic acid; pink, R_F 0·34, glucuronolactone; pink, R_F 0·22; faint yellow, R_F 0·48. The relative sizes of the spots indicated that glucuronic acid was present mainly as the lactone.

A solution (150 ml.) of [^{14}C]glucose (5×10^{-2}M; 50 μc) was irradiated with energy input varying from $1{\cdot}9 \times 10^{21}$ to $4{\cdot}19 \times 10^{23}$ ev. After chromatography, the autoradiographs showed a spot (R_F 0·75) which was not revealed by the spray reagent.

The irradiated solution was treated with phenylhydrazine in acetic acid, and the solid phenylhydrazones were separated by circular paper chromatography.[11] This method indicated the presence of erythrosazone (R_F 0·52) and glyoxal bisphenylhydrazone (R_F 0·97) in addition to glucosazone (R_F 0·42).

The distillate from the irradiated solution contained a small amount of volatile acid, but gave no complex with dimedone. Gluconic and glucuronic acid in the irradiated solution were converted into anilides, which where chromatographed and identified in ultraviolet light: anilide of gluconic (R_F 0·52), and of glucuronic acid (R_F 0·36) We distinguished between the substance in the irradiated solution corresponding to glucuronic (pink, R_F 0·11), 5-oxogluconic acid (brown, R_F 0·28), and 2-oxogluconic acid (pink, R_F 0·14) by chromatography with butan-1-ol–acetic acid–water In view of the similarity between the first and the third acid, we applied a further distinguishing test The irradiated solution (0·5 ml.) was treated with iodine solution (4·0 ml., 3×10^{-2}M in I_2 and 0·1M in potassium iodide made alkaline with 0·4 ml. of N-sodium hydroxide). After 15 min. the solution was neutralised with solid carbon dioxide. Control solutions containing glucose, glucuronic acid, and the oxogluconic acids were similarly treated. Paper electrophoresis (550 v in 0·2M-acetate buffer of pH 5) for 6—8 hr., followed by spraying with alkaline silver nitrate, revealed a fast-running spot (brown) corresponding to saccharic acid in both the glucuronic acid and the irradiated solution. This was not shown by the untreated irradiated solution or the other control solutions.

The irradiated solution of D-glucose was also chromatographed with butan-1-ol–ethanol–water, and the purple spot (R_F 0·33) produced by successive spraying with hydroxylamine and ferric chloride [13] corresponded to D-glucurone.

Rate of Formation of Acid.—The rate of formation of acid in oxygenated solutions (40 ml.) was similar to that found with the electron beam. The yield–dose curve (Fig. 3) shows a gradual rise with energy input, and the yield is independent of concentration within a ten-fold range. On the assumption that the acid is monobasic, the initial slope corresponds to G(acid) 1·2—1·3.

Absorption Spectra of Irradiated Solutions.—A typical absorption spectrum of an irradiated solution of D-glucose is shown in Fig. 4. On addition of potassium hydrogen carbonate to the solution the peak at 265 mμ increases in intensity, and the minimum moves to 230 mμ. The compound responsible for the peak was formed throughout the irradiation and more rapidly in a vacuum than in oxygen (Fig. 5). Of the spectra represented in Fig. 6, that of dihydroxyacetone alone has a peak at 265 mμ. A mixture of dihydroxyacetone (0·115M) and glyoxal (0·24M) gave a spectrum similar to that of the irradiated glucose solution. Moreover, the ultraviolet spectrum of a solution of D-glyceraldehyde had no peak but, on the addition of a small amount of alkali to the solution, the spectrum became identical with that of dihydroxyacetone. This behaviour was thus similar to that of the irradiated solution of glucose on treatment with alkali (Fig. 4). In alkali 2-oxogluconic acid enolised and gave a spectrum with a peak of 275 and minimum at 250 mμ (Fig. 6).

Formation of Hydrogen Peroxide during Irradiation.—The results obtained by colorimetric estimation of hydrogen peroxide with titanium sulphate [14] are represented in Fig. 7. In the initial stages, the rate of formation of the peroxide was constant until 4×10^{17} molecules per ml. had been formed, but then fell as equilibrium was established.

When irradiation ceased, the peroxide decreased at a rate of 3×10^{13} molecules ml.$^{-1}$ min.$^{-1}$, which is comparable with the decrease (4×10^{13}) observed when glucuronic acid and hydrogen peroxide are mixed under similar conditions. After 7 days this solution did not exhibit absorption at 265 mμ.

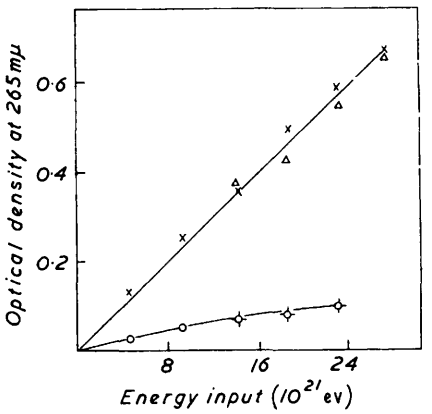
Separation of Neutral and Acidic Products.—Before the application of the isotope dilution method, attempts were made to separate the neutral and the acidic constituents by the use of ion-exchange resins. Inasmuch as Phillips and Pollard [15] observed considerable degradation

[14] Eisenberg, *Ind. Eng. Chem., Analyt. Ed.*, 1943, **15**, 327.
[15] Phillips and Pollard, *Nature*, 1953, **171**, 41.

of glucose and almost complete retention on Amberlite, and Machell [16] found difficulty in removing sugar lactones from Deacidite FF, we employed less basic exchangers. The best separations were obtained with Deacidite E and Zeo-Karb 225. By using the former charged with N-sodium hydroxide, quantitative recovery of neutral sugars was possible.

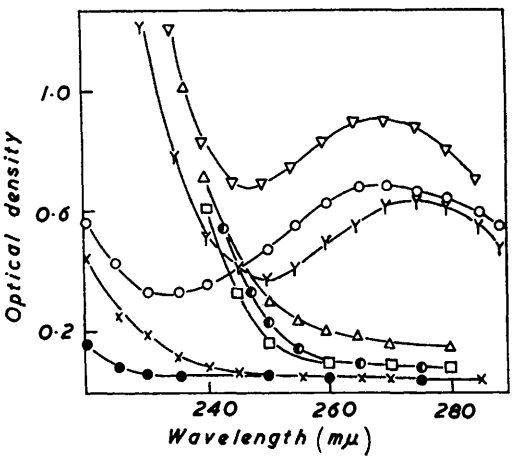
In a typical experiment, an aqueous solution (150 ml.) of glucose (7·8 millimoles) of specific activity 10·2 μc/millimole was irradiated at a dose rate of $2{\cdot}13 \times 10^{17}$ ev min.$^{-1}$ ml.$^{-1}$ in a stream of oxygen for 24 hr. The solution was passed through a column of Deacidite E which was washed with water to produce 250 ml. of eluant (neutral fraction). The acids were removed

FIG. 5. *Increase in ultraviolet absorption at 265 mμ with energy input during irradiation of aqueous* D-*glucose.*



Dose rate $9{\cdot}65 \times 10^{16}$ ev *min.*$^{-1}$ *ml.*$^{-1}$. *Glucose concn.* (Δ) 5×10^{-2}M (*vac.*), (×) 5×10^{-3}M (*vac.*), (○) 5×10^{-2}M (O_2), (-◇-) 5×10^{-3}M (O_2).

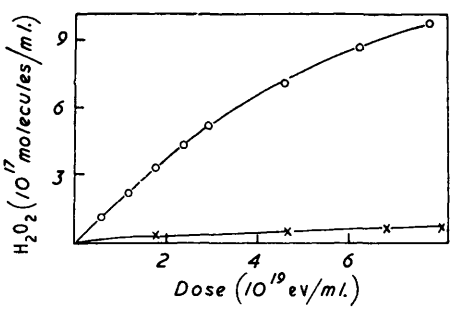
FIG. 6. *Ultraviolet absorption spectra of aqueous solutions.*



● Ca 2-*oxogluconate.* × Ca 5-*oxogluconate.* Y Ca 2-*oxogluconate in aq.* NaOH. ○ 1 : 3-*Dihydroxyacetone.* △ *Glyoxal.* ▽ *Mixture of the previous two.* □ D-*Glucuronolactone.* ◐ D-*Gluconolactone.*

FIG. 7. *Formation of hydrogen peroxide during irradiation of aqueous* D-*glucose with* ^{60}Co γ *radiation.*

Glucose concn. 5×10^{-2}M. *Dose rate* $9{\cdot}65 \times 10^{16}$ ev. *min.*$^{-1}$ *ml.*$^{-1}$. ○ O_2. × *Vacuum.*



from the column with 2N-ammonia, and the free acids liberated by passage through a column of Zeo-Karb 225. The eluted solution was made up to 100 ml. (acid fraction).

Chromatography of the fractions gave no clear-cut separations. The radioactivity of the spots was measured, and the results are shown in Table 2, where the units refer to counts per 5 min.

TABLE 2. *Relative amounts of products.*

Product	Acidic fraction	Neutral fraction	Unseparated mixture
Glucose	342	2366	2610
Glucuronic acid (lactone + free acid)	36	230	262
Compound (R_F 0·22)	40	128	170

[16] Machell, *J.*, 1957, 3389.

Unchanged glucose and the main products in the acidic and the neutral fractions were quantitatively estimated by the isotope dilution method (Table 3*a*), and where possible the data in Table 2 were used to estimate the products in the whole irradiated mixture.

Estimation of Products by Isotope Dilution Method.—The isotope dilution method applied directly to the irradiated mixture confirmed the results obtained by paper chromatography. In a typical estimation an aqueous solution (100 ml.) of glucose (11·07 millimoles) of specific activity 2·8 μc/millimole was irradiated at a dose rate of $2{\cdot}13 \times 10^{17}$ ev min.$^{-1}$ ml.$^{-1}$ in oxygen for 46·5 hr. The isotope dilution method was applied directly to the untreated irradiated solution.

Glucose. The irradiated solution (10 ml.) was freeze-dried and any trace of water removed *in vacuo*. Carrier D-glucose (1·37 millimoles), acetic anhydride (1 ml.), and fused sodium acetate (200 mg.) were added. The mixture was kept at 100° for 2·5 hr., and the penta-*O*-acetate was then precipitated by water (5 ml.). After 9 recrystallisations from ethanol, the penta-*O*-acetylglucose had m. p. 135° and a constant specific activity 0·952 μc/millimole.

Glucuronic acid. Carrier D-glucuronolactone (0·42 millimole) was added to the irradiated solution (5 ml.), and the solution reduced to small bulk by distillation. The deposited D-glucurone after five recrystallisations melted at 175° and had constant specific activity 0·35 μc/millimole.

Gluconic acid. The irradiated solution (10 ml.) was treated with carrier D-gluconolactone (0·52 millimole) and excess of calcium carbonate. The solution was filtered after 2 days, and the gluconate precipitated with ethanol. Ten recrystallisations gave material of constant specific activity 0·44 μc/millimole.

Saccharic acid. Carrier saccharic acid (0·35 millimole) was added to a portion of the irradiated solution (5 ml.). The solution was treated in the usual way, and the deposited acid after six recrystallisations had m. p. 125° and constant specific activity $6{\cdot}8 \times 10^{-3}$ μc/millimole.

Formaldehyde. The distillate obtained during the estimation of glucuronic acid was treated with carrier formaldehyde (0·113 millimole) and 10% dimedone solution (5 ml.). After 48 hr. the dimedone complex separated and was recrystallised until it had m. p. 189° and constant specific activity 0·02 μc/millimole.

Glyoxal. The irradiated solution (10 ml.) was treated with phenylhydrazine (1·5 ml.) and glacial acetic acid (1·0 ml.). After seven recrystallisations from benzene, glyoxal bisphenylhydrazone was obtained with m. p. 170° and constant specific activity 0·066 μc/millimole.

Dihydroxyacetone. Carrier dihydroxyacetone (1·19 mmoles), acetic acid (1 ml.), and phenylhydrazine (1·2 ml.) were added to the irradiated solution (20 ml.) and the mixture was boiled for 5 min. Seven recrystallisations from benzene gave pure D-glycerosazone, m. p. 129°, constant specific activity 0·068 μc/millimole.

D-*Arabinose.* Attempts to estimate D-arabinose as the tetra-*O*-acetate were unsuccessful. Accordingly isotope dilution was applied by using the osazone. Carrier D-arabinose (1·04 mmoles), acetic acid (1·5 ml.), and phenylhydrazine were added to the irradiated solution (10 ml.), and the mixture was boiled for 15 min. The product required ten recrystallisations to give pure D-arabinosazone, m. p. 159°, of constant specific activity 0·099 μc/millimole.

D-*Xylose.* The irradiated solution (10 ml.) after freeze-drying was treated with carrier D-xylose (1·88 millimoles), sodium acetate (160 mg.), and acetic anhydride (1 ml.) and refluxed for 2 hr. Eight recrystallisations from ethanol gave tetra-*O*-acetyl-β-D-xylose, m. p. 124°, of constant specific activity $7{\cdot}6 \times 10^{-3}$ μc/millimole.

D-*Erythrose.* A glucose solution (specific activity 4·00 μc/millimole) (150 ml.) was irradiated as above and 10 ml. were treated with carrier D-erythrose (0·68 mmole) and phenylhydrazine (2 ml.) in glacial acetic acid (1·5 ml.). The solid which separated after eight recrystallisations from benzene gave D-erythrosazone, m. p. 157°, specific activity 0·024 μc/millimole.

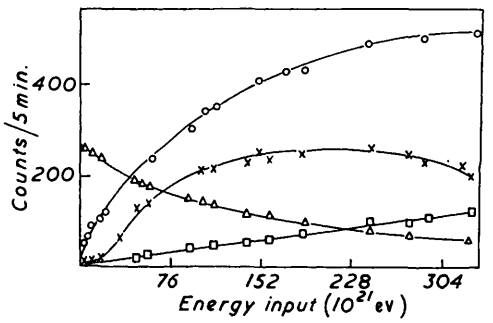
Table 3 shows the yields of the main products at different energy inputs under fully oxygenated conditions. In some cases where it was difficult to purify the osazones to constant specific activity by recrystallisation, a preliminary separation was carried out on an alumina column.

Rate of Formation of Products.—Accurately known amounts of irradiated [^{14}C]glucose solution which had received progressively increasing doses of radiation were chromatographed and the radioactivity of the spots was measured. The rate of formation with energy input was measured for glucuronic acid (sum of the amounts of the free acid and the lactone), the substances showing the pink spot, R_F 0·22, and the yellow spot, R_F 0·48. The rate of

decomposition of glucose with energy input was also measured. The results are shown in Fig. 8.

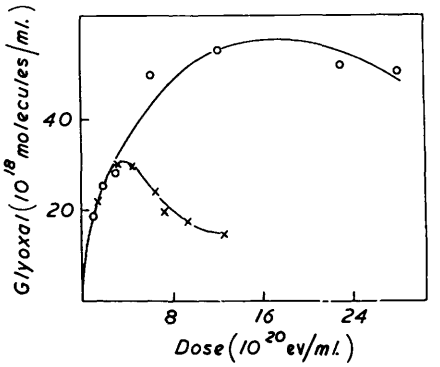
Formation of Glyoxal.—Glyoxal does not move as a discrete spot on paper chromatograms, but gives rise to extensive streaking, and in this respect resembles the behaviour of irradiated glucose solutions. To obtain the yield–dose curve for glyoxal, therefore, it was necessary to apply the more laborious isotope dilution method at each dose level. Individual experiments were carried out by using [^{14}C]glucose (generally labelled), and the glyoxal concentration measured at each dose level (Fig. 9).

FIG. 8. *Effect of energy input on rate of formation of products.*



△ D-*Glucose* ($\times 10^{-2}$). ○ D-*Glucuronic acid* + *lactone*. × *Arabinose*. □ D-*Erythrose*.

FIG. 9. *Rate of formation of glyoxal during irradiation of aqueous* D-*glucose with* ^{60}Co γ *radiation in oxygen.*



× [1-^{14}C]*Glucose*. ○ [^{14}C]*Glucose* (*general labelling*).

TABLE 3. *Products when aqueous solution of* D-*glucose is irradiated with gamma radiation in oxygen.*

(*a*) Initial glucose, 7·8 mmoles. Energy input, 4·6 × 10^{22} ev (*volume* 150 *ml.*).

Product:	D-Glucose	D-Erythrose	Glyoxal	Formaldehyde	D-Gluconic acid	D-Glucuronic acid	Saccharic acid
Carrier (mmole)	0·57	0·011	1·5	0·113	0·38	0·47	0·36
Sp. activity (μc per mmole) ...	4·1	3·44	0·60	0·012	2·8	0·71	0·03
Fraction	Neutral	Neutral	Neutral	Distillate	Acid	Neutral	Neutral
Yield (mmole)...	4·6	0·05 *	0·64	0·005	0·24	0·45	0·012

Corrected yield of glucose in whole irradiated solution ..... 5·2 mmoles
Corrected yield of glucuronic acid in whole irradiated solution ..... 0·50 mmole
D-Arabinose estimated from paper chromatography ..... 0·30 mmole

* Confirmed by estimation from paper chromatogram.

(*b*) Initial glucose, 5·5 mmoles. Energy input, 32·6 × 10^{22} ev (*volume* 115 *ml.*).

Product:	D-Glucose	D-Erythrose	Glyoxal	Formaldehyde	D-Gluconic acid	D-Glucuronic acid
Carrier (mmole)	1·7	0·40	1·26	0·113	1·26	0·47
Sp. activity (μc per mmole) ...	0·483	0·77	0·49	0·053	0·50	0·92
Yield (mmole) ...	1·13	0·22	0·96	0·147	0·63	1·01

D-Arabinose estimated from paper chromatogram, 0·6 mmole.

(*c*) Initial glucose, 11·07 mmoles. Energy input, 5·8 × 10^{22} ev (*volume* 100 *ml.*).

Product:	D-Glucose	D-Erythrose	Glyoxal	Formaldehyde	D-Gluconic acid	D-Glucuronic acid
Carrier (mmole)	1·37	0·68	1·13	0·113	0·52	1·04
Sp. activity (μc per mmole) ...	0·95	0·024	0·066	0·02	0·44	0·35
Yield (mmole) ...	7·05	0·09	0·85	0·05	0·44	0·60

	D-Arabinose	Saccharic acid	D-Xylose	Dihydroxyacetone
Carrier (mmole)	1·04	0·35	1·88	1·19
Sp. activity (μc per mmole)...	0·099	$6·8 \times 10^{-3}$	$7·69 \times 10^{-3}$	0·068
Yield (mmole)	0·45	0·016	0·06	0·31

Since glyoxal is a fragment of the glucose molecule, we have attempted to determine the part of the glucose molecule involved in its formation. The above experiment was therefore repeated with [1-^{14}C]glucose, with results shown in Fig. 9.

The rate of formation of active glyoxal is initially the same from [^{14}C]glucose and [1-^{14}C]-glucose, but after a dose of 5×10^{22} ev the rate of formation from the latter falls markedly.

Discussion

It is evident from the present work that D-glucose in aqueous solution undergoes extensive degradation under the action of ionising radiations. Solutions irradiated with 1 Mv electrons at high energy input gave chromatograms with pronounced streaking, but it was possible to distinguish spots which run identically with glucuronic acid and glucurone.

Chromatography of solutions irradiated with gamma radiation indicated the presence of a variety of constituents, and the main organic products are listed in Table 4. The behaviour of products (I) and (IV) is identical with that of glucuronic acid and glucurone,

Table 4. *Constituents in D-glucose solutions after irradiation.*

	Method	Irrigant	Colour	R_F	
I	Paper chromatography	Butan-1-ol–acetic acid–water	Pink	0·11	Glucuronic acid
II	,,	,,	Brown	0·18	Glucose
III	,,	,,	Pink	0·22	Arabinose
IV	,,	,,	Pink	0·34	Glucurone
V	,,	,,	Yellow	0·48	Tetrose
VI	Autoradiography	,,	—	0·75	
VII	Anilide method [12]	Water–benzene–acetone	Fluorescent (ultraviolet)	0·52	Gluconic acid

and these compounds could readily be distinguished from 2- and 5-oxogluconic acid. Since previous workers [2] have reported that glucuronic acid is not present in the irradiated solution, particular attention has been paid to the identification of this acid by paper chromatography. The presence of glucuronic acid was further confirmed by the methods of Green [12] and Abdel-Akher and Smith.[13] Moreover, oxidation of the irradiated solution with alkaline iodine gave saccharic acid, which could be formed from glucuronic acid but not from the keto-acids. Control mixtures of gluconic and glucuronic acid behaved identically with the irradiated glucose systems. In view of the variety of positive tests for glucuronic acid and the subsequent confirmation by the isotope dilution method, we conclude that this acid is formed during the irradiation.

The behaviour of fraction (III) was identical with that of arabinose. Evidence that it is neutral is furnished by the behaviour of the mixture on an ion-exchange resin (Table 2). D-Xylose might also be derived from glucose by complete removal of the primary alcohol group, but the isotope dilution method showed that its amount was very small, and that arabinose was the main pentose present. The R_F value of product V was close to that of erythrose (R_F 0·48) and dihydroxyacetone (R_F 0·52), but the colour of the *p*-anisidine complex indicated the former.

Glyoxal, erythrose, and dihydroxyacetone were detected and estimated as osazones, but the results require careful interpretation as the same osazone may be formed from different compounds and degradation of the irradiated solution may arise on treatment with phenylhydrazine. Glyoxal bisphenylhydrazone may be formed from glyoxal or glycollaldehyde. The experiments with [^{14}C]glucose and [1-^{14}C]glucose indicate that glyoxal is the predominating two-carbon fragment. Erythrosazone may be formed from D-threose, D-threulose, or D-erythrulose, but in the present connection the first two may be ruled out on configurational grounds. Since, moreover, D-erythrulose is necessarily formed by a secondary process from D-erythrose, the latter appears to be the product leading to D-erythrosazone. It is possible that the isotope dilution value corresponding to the concentration of erythrose contains a contribution from D-erythrulose. Chromatography, however, gave no indication of the presence of the latter in the irradiated

solutions and the low concentration of erythrose is more consistent with some form of secondary attack other than that involving formation of a keto-group at position 2. The possibility of degradation during the preparation of the osazones is, we consider, remote in view of the disparity between the observed amounts of glyoxal and erythrose, and we conclude that these compounds are formed by irradiation of the system. Estimation of dihydroxyacetone as D-glycerosazone does not differentiate it from D-glyceraldehyde, and the evidence, particularly that provided by the absorption spectrum of the irradiated solution, indicates that both are present.

A consistent feature of the irradiated glucose solutions is an absorption maximum at 265 mμ. This was observed for other carbohydrates by Khenokh.[17] Laurent [18] also pointed out that solutions of sugars irradiated with ultraviolet light show a similar absorption and suggested that ascorbic acid (*A*), reductone (*B*), 2-oxogluconic acid (dienol form) (*C*), and 4-deoxy-5-oxo-3 : 6-mannosaccharolactone (*D*) may be responsible. In this connection Bothner-By and Balazs [2] suggested that the absorption spectrum of glucose

TABLE 5.

	A	*B*	*C*	*D*
Max. in alkali (mμ)	265	287	275	263
Max. in acid (mμ)	245	268	230	229

solutions irradiated with *X*-rays was characteristic of ascorbic acid or the mannosaccharolactone, but our results (see Table 5) cannot be interpreted on this basis. Of the four substances mentioned, 2-oxogluconic acid is the only one admissible in the present case inasmuch as it could be formed by secondary attack on gluconic acid. We consider, however, that enolisation of 2-oxogluconic acid would not account for the absorption spectrum because the peak (265 mμ) is lower than that found for 2-oxogluconic acid and does not shift on treatment with alkali (Fig. 6). In fact, we find that when the irradiated solution is made alkaline the intensity of the peak at 265 mμ increases very markedly and the minimum of the curve moves to lower wavelength, so that the absorption is similar to that of dihydroxyacetone. The main observations may thus be interpreted on the basis of the formation of D-glyceraldehyde during irradiation and its subsequent isomerisation to dihydroxyacetone, a change which we have confirmed occurs readily in alkali. Solutions containing glyoxal and dihydroxyacetone in the proportion which they occur in the irradiated solution have practically the same absorption spectrum as the irriadiated solution (Fig. 6). We have observed that D-glucosone in alkali absorbs at 265 mμ. Isotope dilution work is now in progress to test this possibility further.

In view of our consistent identification of product (IV) as glucuronic acid by a variety of chromatographic procedures and the subsequent confirmation by the isotope dilution method, it is difficult to understand the statement by Bothner-By and Balazs [2] that " no evidence for the formation of glucuronic acid was found on any of the chromatograms." Indeed their results are not entirely consistent with this conclusion, and since the autoradiographs show the extensive streaking to which we have referred, the presence of a small amount of free acid in their solution cannot be discounted. In fact, the compound which they report as having R_F 0·32 in butan-1-ol–acetic acid–water corresponds fairly closely to the product which we have identified as D-glucuronolactone. The practice adopted by these workers of irradiating the solutions in the presence of sodium hydroxide is open to question inasmuch as some of the primary products are sensitive to alkali. Glyoxal, for example, easily forms a complex with sodium hydroxide.[19] Another complication which appears to have received no attention from these workers relates to the oxygen content of the solutions. No provision was made for replacing oxygen consumed during irradiation, and their observations cannot be related to either fully oxygenated or evacuated conditions.

[17] Khenokh, *Doklady Akad. Nauk S.S.S.R.*, 1955, **104**, 746.
[18] Laurent, *J. Amer. Chem. Soc.*, 1956, **78**, 1875.
[19] Taylor, *ibid.*, 1948, **70**, 455.

It follows that their quantitative results would be difficult to reproduce accurately. Moreover, their method of de-aerating the solution with nitrogen is not so satisfactory as the evacuation procedure, and Bourne, Stacey, and Vaughan [20] have shown that there are appreciable differences between sugar solutions irradiated in a vacuum and in nitrogen.

By the use of paper chromatography in conjunction with radioactive assay, we have determined the change in concentration of several of the products with dose (Fig. 8) for the purpose of distinguishing primary products. The rate of consumption of glucose in the early stages is constant and independent of concentration, but in the later stages the rate falls and depends on the amount of glucose present. This feature doubless arises from the competition of the products for the radicals formed during irradiation. As shown in Table 6, satisfactory correlation is found between the data obtained by the isotope dilution method and the radioactive measurements on the paper chromatograms.

TABLE 6. *Isotope dilution data and radioactive assay.*

Initial [Glucose] ≡ 2670 counts (in 5 min.)			
Energy input (10^{22} ev)	4·6	5·8	32·6
Isotope dilution			
(*a*) Initial glucose (mmole)	7·8	11·07	5·5
(*b*) Final glucose (mmole)	5·2	7·05	1·13
(*c*) Corresponding value (counts/5 min.)	1780	1700	550
Experimental value	1850	1750	600

The yield–dose curves (Fig. 8) provide valuable information as to the nature of the reactions and serve to indicate which constituents are primary products. Glucuronic acid was estimated as the sum of the free acid and lactone concentrations, and as shown in Table 7, there is close correlation between the values obtained by the chromatographic and isotope dilution methods. The yield–dose curve (Fig. 8) indicates that glucuronic acid is

TABLE 7. *Estimation of glucuronic acid.*

Energy input (10^{22} ev)	Isotope dilution	Relative value	Paper chromatogram (counts)	Relative value
4·6	0·5	**1**	210	**1**
5·8	0·6	1·2	235	1·1
32·6	1·01	2·00	525	2·16

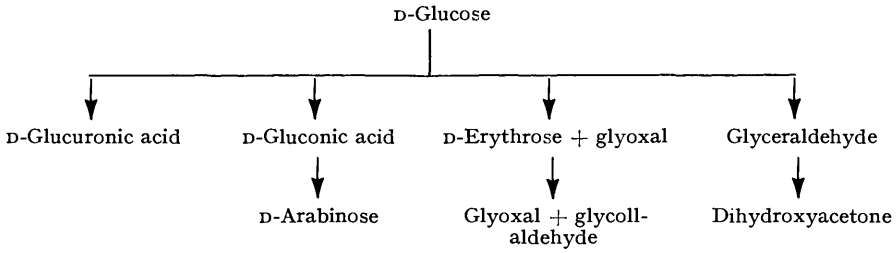
a primary product; the rate of formation is initially constant but falls at higher doses until it becomes equal to the rate of degradation. Although, the complete yield–dose curve is not available for gluconic acid, isotope dilution data indicate that this acid is also a primary product which undergoes degradation at higher doses. The rate of formation of arabinose is low but increases at the stage where the formation of the above primary products falls off. The yield–dose curve indicates that arabinose is not a primary product and that it is degraded in the subsequent stages. The constituent with R_F 0·48 is a primary product. This appears to be erythrose, and this view is supported by the good correlation between the isotope dilution measurements and radioactive assay of the spot on a chromatogram. Experiments with [^{14}C]glucose and [1-^{14}C]glucose indicate that glyoxal is a primary product. The fact that the initial rate of formation is the same in both systems suggests that the atoms $C_{(1)}$ and $C_{(2)}$ are directly involved in the formation of the molecules. Whereas the amount of glyoxal increases in the system containing [^{14}C]glucose, the concentration reaches a maximum and then falls in the solution containing [1-^{14}C]glucose. It appears, therefore, that the glyoxal formed initially from $C_{(1)}$ and $C_{(2)}$ in the latter system is effectively diluted by the contribution from the remainder of the molecule. This evidence confirms the previous conclusion that glyoxal is the main two-carbon fragment and not glycollaldehyde, although it is possible that a certain amount of this compound may be formed at high energy inputs.

[20] Bourne, Stacey, and Vaughan, *Chem. and Ind.*, 1956, 573.

In an attempt to compare the initial G values for the products with the value corresponding to the initial rate of decomposition of glucose, we find that the yield–dose curve for the consumption of glucose is linear below $4{\cdot}6 \times 10^{22}$ ev and corresponds to G 3·5 for the removal of glucose. This was calculated from a single isotope dilution value at this energy input, but it is evident from the complete yield–dose curve (Fig. 8) that this value is in error by less than 5%. The available data for the products lead to the initial G values: glyoxal 1·8, glucuronic acid 0·9, gluconic acid 0·4. The contribution of other products must be small since the above primary products together correspond to G 3·1.

There is general concordance between the yields of acid obtained by irradiation with the electron-beam and gamma-radiations under the conditions employed in the present work. The fact that the overall acid yield is independent of the glucose concentration indicates that the radiation energy is absorbed by the water and that the chemical reactions are initiated by the free radicals formed. The process is independent of dose rate in the range under investigation, and the type of degradation appears to be essentially the same for the electron- and gamma-radiations.

The occurrence of post-irradiation reactions is shown by liberation of gas for 24—30 hr., and the gradual change in the absorption spectrum of the solution after irradiation. The concentration of hydrogen peroxide also falls after irradiation, and this may be due to interaction with glucuronic acid.[21] Another observation which has not been reported in detail here is the change in the paper-chromatographic pattern observed for freshly irradiated solutions and solutions which had been kept for some weeks. Similarly, inorganic phosphate continues to be liberated after irradiation of glucose 6-phosphate. These post-irradiation reactions will be considered in more detail later.



Degradation of D-glucose in aqueous solution by ionising radiations is complex and, while a detailed mechanism cannot be advanced at this stage, it is possible to indicate the main steps of the degradation in oxygen in accordance with the annexed scheme. The formation of D-glucuronic acid involves oxidation of the $CH_2{\cdot}OH$ group while that of D-gluconic acid entails ring scission, and the former reaction appears to be favoured on the basis of the observed yields. D-Arabinose is probably formed from gluconic acid as a result of the secondary process involving OH radicals.[22] A small amount of D-xylose doubtless arises from decarboxylation of glucuronic acid, though it does not appear to be the main secondary product arising from this acid. Similarly, the low yield of saccharic acid suggests that this is not the next stage in the oxidation of the gluconic and glucuronic acid. Another important primary degradation process is the formation of glyoxal by the initial cleavage between positions 2 and 3, and subsequently further contribution from other parts of the molecule. D-Erythrose, which is probably formed simultaneously with glyoxal is degraded extensively after its formation as indicated by its low yield. This may be a complex process, and may possibly involve the formation of further two-carbon fragments or D-glyceraldehyde. The main concentration of this product, however, arises from a primary breakdown of the hexose into two triose molecules. This is substantiated by the fact that dihydroxyacetone is formed immediately at low doses owing to the ready

[21] Everett and Sheppard, Report from Univ. Oklahoma Med. School. Dept. of Biochemistry, 1944.
[22] Bourne, Stacey, and Vaughan, *Chem. and Ind.*, 1956, 1374.

isomerisation of D-glyceraldehyde. The detailed nature of some of these processes will be investigated further.

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