711. *Radiation Chemistry of Carbohydrates. Part II.*[1] *Irradiation of Aqueous Solutions of Dextran with Gamma Radiation.*

By G. O. Phillips and G. J. Moody.

The degradation of dextran in aqueous solution by ^{60}Co γ-radiation is examined by measuring the amount of acid formed and the changes in optical rotation and reducing power. Chromatographic and isotope dilution methods reveal that the main products are glucose, isomaltose, isomaltotriose, gluconic acid, glucuronic acid, glyoxal, erythrose, and glyceraldehyde.

These products are considered to arise from at least two independent degradation processes: (*a*) hydrolysis to glucose, isomaltose, and isomaltotriose, while secondary reactions involving glucose give rise to erythrose, glyoxal and glyceraldehyde; (*b*) the formation of gluconic acid and glucuronic acid.

The irradiated solution shows an absorption at 265 mμ due to dihydroxyacetone, which is also formed during the irradiation of glucose solutions.

The chemical effects of ionising radiations on aqueous dextran are interesting because it is used as a blood-plasma expander,[2] and its study follows our work on glucose.[1] Degradation of dextran is normally accomplished by acid, but yields are poor, and other methods, *e.g.*, use of heat, ultrasonic waves, and alternating electric fields, have been examined.[3] Although it is known that dextran is degraded on irradiation with fast electrons [4] or gamma radiation,[5] there is no information on the nature of the products. In the present work a straight-chain dextran, which can be synthesised by the bacterium leucenostoc, was used.[6] Dilute aqueous solutions of the polysaccharide under evacuated and fully oxygenated conditions have been irradiated with ^{60}Co γ-radiation, and the products have been identified and estimated by chromatographic, spectroscopic, and isotope dilution methods for the purpose of ascertaining the main steps of the degradation process.

Results and Experimental

The ^{60}Co source, irradiation vessels, dosimetric techniques, and the chromatographic, spectroscopic, and isotope dilution methods were similar to those used for glucose.[1] Reducing

[1] Part I, Phillips, Moody, and Mattok, preceding paper.
[2] (*a*) Lockwood, *Chem. and Ind.*, 1951, 46; (*b*) Joseph, *ibid.*, p. 312.
[3] Watson and Wolff, *J. Amer. Chem. Soc.*, 1955, **77**, 196.
[4] Price, Bellamy, and Lawton, *J. Phys. Chem.*, 1954, **58**, 821.
[5] Ricketts and Rowe, *Chem. and Ind.*, 1954, 189.
[6] Barker, Bourne, James, Neely, and Stacey, *J.*, 1955, 2096.

power was measured by Somogyi's method.[7] Jeanes and Wilham's procedure [8] was used for the determination of the periodate consumed, and formic acid formed was estimated as described by Halsall, Hirst, and Jones.[9]

Identification of Products.—A solution of dextran (1·5 g.) in water (150 ml.) was irradiated in oxygen to a total energy input of $4{\cdot}6 \times 10^{22}$ ev. Successive 50 ml. portions were dialysed against distilled water (500 ml.) for 24 hr. in " Visking " tubing, and the solutions were then concentrated under reduced pressure at <35°. The ultraviolet spectrum of the dialysate showed a maximum at 265 mμ. The dialysate was chromatographed with butan-1-ol–acetic acid–water (4 : 1 : 5), with silver nitrate and *p*-anisidine as spray reagents. This showed that the main products were isomaltotriose R_F 0·05, isomaltose R_F 0·09, glucose R_F 0·18, and erythrose R_F 0·49. Butan-1-ol–ethanol–water–ammonia (40 : 10 : 49 : 1) disclosed glucose R_F 0·08 and erythrose only, since the di- and tri-saccharides moved so slowly in this solvent. The sprayed

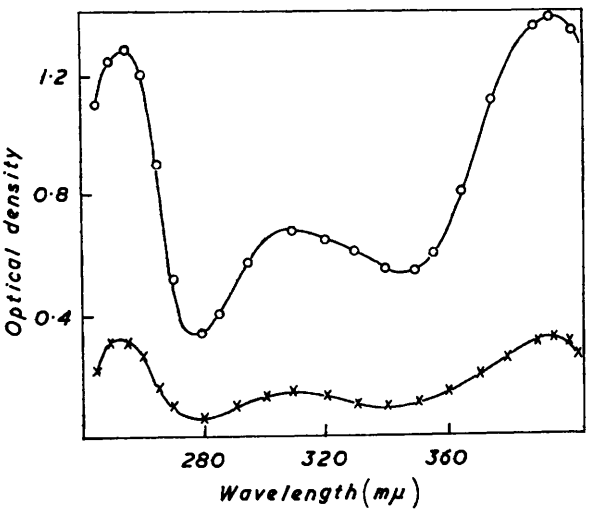


FIG. 1. *Ultraviolet absorption spectra, in methanol, of glucosazone* (○) *and a sample isolated from irradiated dextran solution* (×).

chromatograms showed the characteristic streaking found with irradiated solutions of glucose (Part I).

The solid mixture of osazones obtained by treatment of the dialysate with phenylhydrazine was separated by circular paper chromatography,[10] and the presence of glyoxal bisphenylhydrazone R_F 0·97, erythrosazone R_F 0·58, and glucosazone R_F 0·41 was established. *N*-Acetylphenylhydrazine produced from the reagents alone was also detected. The presence of erythrose, glucose, isomaltose, and isomaltotriose was confirmed by paper electrophoresis. The osazones of glyoxal, erythrose, and glucose were readily separated on a column of alumina.[10] Elution with benzene gave glyoxal bisphenylhydrazone; further treatment with ether gave erythrosazone, and ethanol–water (9 : 1) yielded glucosazone. The identity of the osazones separated in this way was confirmed by comparing the infrared spectra with those of authentic specimens. Further confirmation was furnished by the identity of the ultraviolet spectra of methanol solutions (Fig. 1) with those of authentic samples.

According to Khenokh [11] formaldehyde is one of the major products formed during the irradiation of carbohydrates since the solutions give a precipitate with phenylhydrazine. We found, however, that the distillate obtained by concentrating the irradiated solution gave a negative test for formaldehyde on treatment with dimedone or phenylhydrazine. There is evidence [12] that glyoxal bisphenylhydrazone may be formed from formaldehyde. To ascertain whether it is formed in this way, formaldehyde was subjected to the same treatment as the reaction mixture. The phenylhydrazone obtained, however, had a different m. p. (182—184°) and a different ultraviolet spectrum ($\lambda_{max.}$ 280 mμ; ε 857 in MeOH). We conclude

[7] Somogyi, *J. Biol. Chem.*, 1952, **22**, 195.
[8] Jeanes and Wilham, *J. Amer. Chem. Soc.*, 1950, **72**, 2655.
[9] Halsall, Hirst, and Jones, *J.*, 1947, 1427.
[10] Barry and Mitchell, *J.*, 1954, 4020.
[11] Khenokh, *Doklady Akad. Nauk S.S.S.R.*, 1955, **104**, 746.

that glyoxal bisphenylhydrazone is not formed from formaldehyde, and that the latter is not present as a major product. By the more sensitive test described below, it was possible to detect a small amount of formaldehyde, but its concentration was considerably lower than that of glyoxal. The presence of glyoxal in the irradiated solution was readily shown by Bamberger's test.[13] and it appears that this compound is responsible for the extensive streaking observed on the paper chromatograms.

Isotope Dilution Method.—The products were also identified and estimated by the isotope dilution method (Part I). [^{14}C]Dextran diluted with the straight-chain dextran used in the above experiments was dissolved in water (100 ml.), and the solution (0·15%; specific activity 2·7 μc/millimole of carbon) was irradiated at $2{\cdot}13 \times 10^{17}$ ev min.$^{-1}$ ml.$^{-1}$ for 20·5 hr. After dialysis against two 500 ml. portions of water of the irradiated solution (89 ml.) to constant rotation the individual products were estimated in the concentrated dialysate (50 ml.).

Glucose. The dialysate (10 ml.) was freeze-dried and treated with carrier glucose (0·59 millimole), acetic anhydride (0·75 ml.), and fused sodium acetate (0·06 g.). The mixture was kept at 100° for 2·5 hr. The resulting penta-*O*-acetylglucose after six recrystallisations had m. p. 135° and constant specific activity $3{\cdot}5 \times 10^{-2}$ μc/mmole.

Glucose, erythrose, and glyoxal. Carrier glucose (0·21 millimole), glyoxal (0·5 millimole), and erythrosazone (0·004 millimole) were added to the dialysate (20 ml.). The mixture was treated with phenylhydrazine (1·8 ml.) and acetic acid (1·5 ml.) at 100° for 15 min. The solid mixture of osazones which separated was fractionated on alumina. Fraction 1, eluted with benzene (95 ml.), gave glyoxal bisphenylhydrazone which after 7 recrystallisations from benzene had m. p. 169°. Fraction 2, eluted with ether (60 ml.), gave erythrosazone, m. p. 157° after three recrystallisations. Fractions 3, eluted with methanol–water (9 : 1), gave glucosazone, m. p. 201° after seven recrystallisations.

Glucuronic acid. The dialysate (5 ml.) was treated with glucuronic acid (0·41 millimole), and the solid remaining after freeze-drying recrystallised seven times from hot water to give pure D-glucurone, m. p. 175°.

Gluconic acid. The dialysate (5 ml.) was treated with carrier D-gluconolactone (0·52 millimole) and excess of calcium carbonate, and filtered after 3 days. The calcium gluconate was precipitated with ethanol, and the solid was separated. Ten successive precipitations from solution gave the pure gluconate of constant specific activity.

Formaldehyde. The distillate obtained by concentrating the irradiated solution (300 ml.) was treated with carrier formaldehyde (0·113 millimole) and 5% ethanol–dimedone (10 ml.). Four recrystallisations of the solid which separated gave the pure complex, m. p. 189°.

Dihydroxyacetone. The dialysate (10 ml.) was treated with carrier dihydroxyacetone (1·0 millimole), phenylhydrazine (1 ml.), and glacial acetic acid (0·5 ml.). The mixture was heated at 100° for 5 min. and the osazone separated. Seven recrystallisations from benzene gave pure glycerosazone, m. p. 128°, and constant specific activity. The results for the isotope dilution estimations are shown in Table 1.

TABLE 1. *Irradiation of dextran in aqueous solution* (0·15%).

Compound	Specific activity of sample (μc/mmole of carbon)	Carrier (mmole)	Amount (mg.)
Glucose			
(*a*) penta-*O*-acetate	$3{\cdot}5 \times 10^{-2}$	0·59	7·9
(*b*) osazone	$1{\cdot}7 \times 10^{-1}$	0·21	7·1
Erythrose	$7{\cdot}9 \times 10^{-1}$	0·004	0·45
Glyoxal	$2{\cdot}7 \times 10^{-2}$	0·50	0·82
Gluconic acid	$1{\cdot}2 \times 10^{-2}$	0·52	5·10
Formaldehyde	$8{\cdot}3 \times 10^{-3}$	0·113	0·03
Glucuronic acid	$1{\cdot}3 \times 10^{-2}$	0·41	4·5
Dihydroxyacetone	$1{\cdot}08 \times 10^{-2}$	1·0	2·0

Changes Produced by Irradiation.—Irradiation of straight-chain dextran in aqueous solution (1%) under evacuated conditions to a total energy input of $2{\cdot}9 \times 10^{21}$ ev led to a marked fall in pH. The amount of acid formed increased linearly with dose, and the yield was slightly higher under oxygenated conditions (Fig. 2). If the acid is assumed to be monobasic, G (in oxygen) = 1·5, and G (in vacuum) = 1·1. In both cases the yield did not depend on the dose

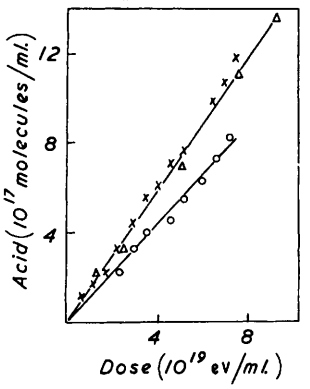
[12] von Pechmann, *Ber.*, 1897, **30**, 2459.
[13] Bamberger, *Ber.*, 1899, **32**, 1806.

rate in the range $2{\cdot}13 \times 10^{17}$ to $9{\cdot}65 \times 10^{16}$ ev min.$^{-1}$ ml.$^{-1}$. The proportion of volatile acid was estimated in the distillate obtained by concentrating the irradiated solution. After a total dose of $6{\cdot}9 \times 10^{22}$ ev, in oxygen, the irradiated dextran solution contained 17% of the total acid in the distillate.

The optical rotation of the solution decreased steadily during irradiation, and little difference was apparent between the evacuated and the oxygenated system (Fig. 3).

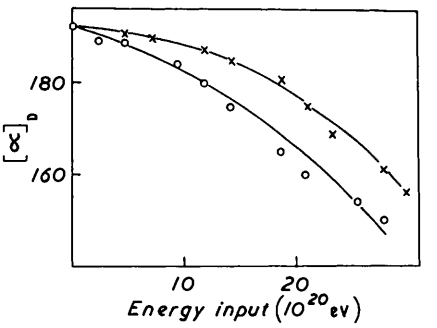
The conversion of dextran into lower saccharides would be expected to lead to an increase

FIG. 2. *Acid formation during irradiation of 0·1% aqueous dextran.*



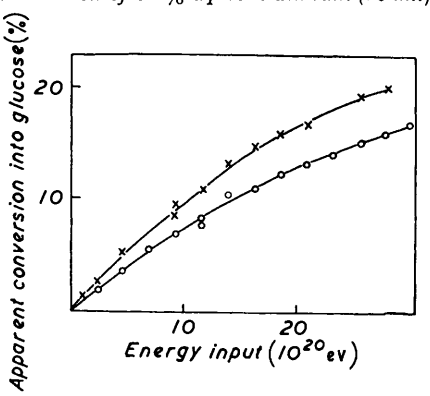
Dose rate: ×, $9{\cdot}65 \times 10^{16}$ (*in* O_2); Δ $2{\cdot}13 \times 10^{17}$ (*in* O_2); ○ $9{\cdot}65 \times 10^{16}$ (*vac.*) ev min.$^{-1}$ ml.$^{-1}$.

FIG. 3. *Change in optical rotation during irradiation of 0·1% aqueous dextran (40 ml.).*



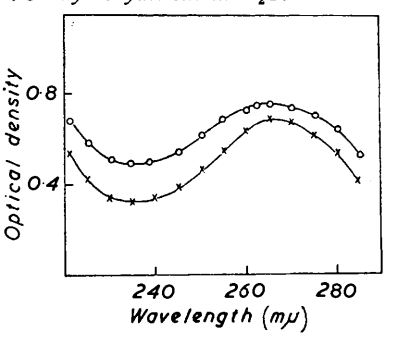
Dose rate: $9{\cdot}65 \times 10^{16}$ ev *min.*$^{-1}$ *ml.*$^{-1}$ (○) in vacuo *and* (×) *in* O_2.

FIG. 4. *Increase in reducing power during irradiation of 0·1% aqueous dextran. (40 ml.)*



Dose rate $9{\cdot}65 \times 10^{16}$ ev *min.*$^{-1}$ *ml.*$^{-1}$ (×) in vacuo *and* (○) *in* O_2.

FIG. 5. *Ultraviolet absorption spectra of* (○) *0·1% aqueous dextran after irradiation* in vacuo ($2{\cdot}78 \times 10^{21}$ ev) *and* (×) 0·19M-1 : 3-*dihydroxyacetone in* H_2O.



in the reducing power of the solution. The relation between energy input and reducing power (expressed as apparent conversion into glucose) is shown in Fig. 4. On this basis, it appears that the extent of degradation is greater under evacuated conditions.

A striking feature of the irradiation process was the change in the absorption spectrum of the solution, and the appearance of a peak at 263—266 mμ (Fig. 5). This behaviour was observed previously with glucose (Part I), and appears to be general for carbohydrates containing the glucose radical.[11] The concentration of the absorbing compound increased more rapidly with dose in the evacuated system (Fig. 6). Determinations of the absorption spectrum after irradiation had ceased revealed the occurrence of post-irradiation processes, which lead to an increase in absorption in vacuum but a decrease under oxygenated conditions. This

unusual behaviour was confirmed several times, and the results of a typical experiment are given in Table 2.

TABLE 2. *Change in absorption spectrum after irradiation.*

System	Energy input (ev)	Density at 265 mμ After irradiation	200 hr. later
Evacuated	$2{\cdot}78 \times 10^{21}$	0·75	1·02
Oxygenated	$2{\cdot}3 \times 10^{22}$	0·66	0·25
Oxygenated	$3{\cdot}0 \times 10^{21}$	0·20	0·10

Oxidation Measurements.—Extensive degradation was confirmed by oxidation of the polysaccharide fragment. To obtain comparable data for the original dextran and the final polysaccharide fragment, the lower saccharides were removed by dialysis. An oxygenated solution of dextran (0·25 g.) in water (50 ml.) was irradiated at $9{\cdot}6 \times 10^{16}$ ev min.$^{-1}$ ml.$^{-1}$ for

FIG. 6. *Increase in ultraviolet absorption at 265 mμ with energy input during irradiation* (○, in vacuo; × *in* O_2) *of 0·1% aqueous dextran.*

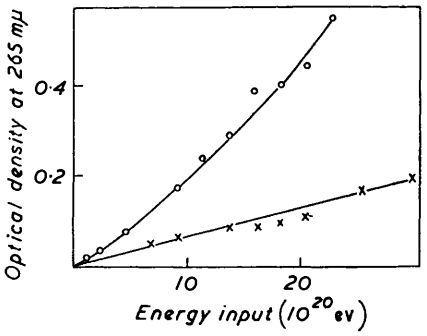
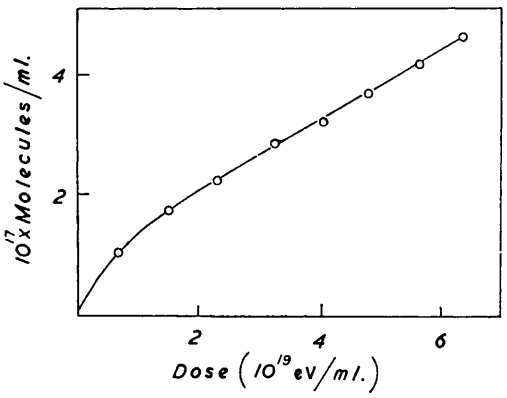


FIG. 7. *Formation of hydrogen peroxide during irradiation of 0·19% aqueous dextran in* O_2.



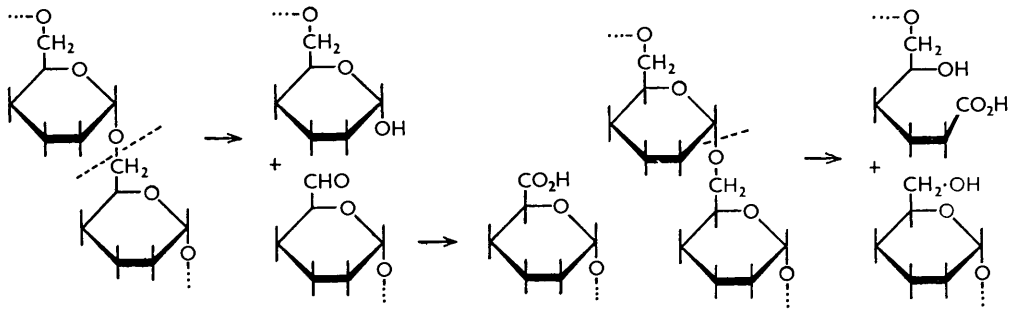
42 hr. The solution was dialysed against water (500 ml.) for 24 hr., then against running water until the rotation remained constant (0·75°). The white solid (0·11 g.) obtained on freeze-drying was shown to be homogeneous by paper chromatography and electrophoresis. This substance reduced 1·88 mols. of periodate per glucose unit forming 1·27 mols. of formic acid. Before irradiation, periodate oxidation of the original dextran gave: periodate uptake 1·96 mols.; formic acid 0·96 mol.

Hydrogen Peroxide.—Formation of hydrogen peroxide is shown in Fig. 7. The initial rate corresponds to $G(H_2O_2) = 1{\cdot}8$. As with glucose (Part I), a slow post-irradiation decrease of concentration of hydrogen peroxide was observed corresponding to 1×10^{13} molecules ml.$^{-1}$ min.$^{-1}$. No similar post-irradiation decrease was observed in the concentration of hydrogen peroxide formed during the irradiation of pure water in oxygen.

DISCUSSION

Irradiation of aqueous solutions of dextran leads to extensive degradation with formation of a polysaccharide fragment and several products of low molecular weight which can be separated from the former by dialysis. There is a steady fall in the optical rotation during irradiation, and at the same time reducing power increases, indicating the formation of lower saccharides. The evidence provided by periodate oxidation of the remaining polysaccharide fragment after irradiation is not clear. While there is no increase in periodate consumption, the amount of formic acid increases in relation to the original dextran, and it appears accordingly that a greater number of reducing end-groups are present.

From the nature of the products formed on irradiation of dextran in aqueous solution, it appears that one of the main processes is hydrolysis to glucose, isomaltose, and isomaltotriose. Estimations of glucose on the basis of isotope dilution with glucosazone and penta-*O*-acetyl-β-D-glucose are in fair agreement, and indicate that this monosaccharide is a major product. Moreover, the yield–dose curve (Fig. 4) for reducing



substances indicates that formation of glucose and other lower saccharides is a primary process. Two other major products are gluconic acid and glucuronic acid, and the over-all yield–dose curve indicates that they also are primary products, since they comprise the major acid products.

The presence of oxygen during the irradiation of aqueous solutions generally increases the yield.[14] With dextran, the reducing power of the solution indicates that there is more extensive degradation *in vacuo*, whereas slightly more acid is produced in oxygen. Bourne, Stacey, and Vaughan [15] observed similar effects in the irradiation of aqueous amylose. These anomalies, doubtless due to the occurrence of more than one primary process, reveal that oxygen plays an important rôle in determining the reaction path. Coleby [16] observed a similar behaviour with sugar lactones.

It is evident from the present results that dextran undergoes degradation by at least two independent processes involving scission at the 1—6 link of the molecule. One leads to glucose and lower saccharides, the other to gluconic and glucuronic acid. Glyoxal and erythrose, which are present in smaller amounts, are doubtless secondary products from glucose.[1] Thus random attack along the glucose chain in dextran, as indicated in the formulæ, would lead to all the main products observed. Glucose also gives rise to D-glyceraldehyde; this isomerises to 1 : 3-dihydroxyacetone, which is responsible for the strong absorption at 265 mμ. The post-irradiation processes are also similar to those found for glucose.[1] The general similarity between irradiated solutions of glucose and dextran doubtless arises from the fact that glucose-containing polysaccharides suffer hydrolysis to glucose; this is in harmony with the observations of Bourne, Stacey, and Vaughan [15] on irradiation of aqueous amylose.

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[14] Stein and Weiss, *J.*, 1949, 3245; but see Dale, Davies, and Gilbert, *Biochem. J.*, 1949, **45**, 93, and Alexander and Charlesby, *Nature*, 1954, **173**, 578.
[15] Bourne, Stacey, and Vaughan, *Chem. and Ind.*, 1956, 573.
[16] Coleby, *Chem. and Ind.*, 1957, 111.