993. *Self-decomposition of* [^{14}C]*Glucose.*

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D-[^{14}C]Glucose undergoes appreciable self-decomposition when stored *in vacuo* as a freeze-dried sample. Some of the products have been identified by chromatography, electrophoresis, and carrier-dilution analysis. A comparison of these products with the products of the oxidation of D-glucose with Fenton's reagent suggests a similarity of the two reactions, and the participation of hydroxyl radicals in the self-decomposition.

In the application of [^{14}C]-tracer techniques to chemical or biochemical reactions, it is normally important that the [^{14}C]-compounds employed should be chemically pure, and it is frequently assumed that such compounds are virtually stable during storage before their use. In 1953, Tolbert *et al.*[1] and Lemmon [2] reported that considerable radiation decomposition had occurred to [^{14}C]-labelled amino-acids, amino-alcohols, purine derivatives, calcium glycollate, cholesterol, thyroxine, and succinic acid during storage. The products of self-decomposition of [^{14}C]methanol were examined by Scraba *et al.*,[3] who identified some of them as methane, hydrogen, ethylene glycol, glycerol, and erythritol. Wagner and Guinn [4] have studied the self-decomposition of [^{14}C]methyl iodide. From the magnitudes of the decompositions of these compounds it appears that various groups of [^{14}C]-labelled compounds are differently affected by radiation self-decomposition.

Bayly and Weigel [5] reported the self-decomposition of [^{14}C]carbohydrates, stored under various conditions, and noted that this decomposition could be due to one or more of four effects, *i.e.*, primary (internal) radiation, primary (external) radiation, secondary radiation effect, and chemical decomposition. They concluded that the self-decomposition of [^{14}C]sucrose when stored as a freeze-dried sample *in vacuo* at room temperature was due mainly to the primary (external) radiation effect, and that the secondary radiation effect was prevalent in the decomposition of D-[^{14}C]glucose stored under the same conditions. A further illustration of the importance of chemical structure in determining the susceptibility of a [^{14}C]-compound to self-decomposition is the rapid destruction of [^{14}C]-dextran sulphate (*ca.* 100% in 3 weeks) which was attributed to a secondary radiation effect involving the liberation of sulphuric acid.

The products of self-decomposition are of interest as their presence can lead to erroneous interpretations of tracer experiments, and a knowledge of their nature can give a guidance to the purification of labelled compounds before use. We now report the analysis of the products of self-decomposition of freeze-dried D-[^{14}C]glucose and evidence regarding the reaction mechanism involved.

A sample of D-[^{14}C]glucose (*ca.* 6 mg., generally labelled), with a specific radioactivity of *ca.* 14·44 mC per mmole, which had been purified by paper chromatography and crystallisation, was stored in the freeze-dried state in a vacuum-sealed tube in the dark. Analysis after 26 months revealed that appreciable decomposition had occurred. Radiochromatograms with a butanol solvent showed the presence of at least 11 new components (Table 2) and the disappearance of 14·5% of the glucose. The $R_{glucose}$ values of the products suggested that acids and neutral compounds with the same or a smaller number of carbon atoms than glucose had been produced, together with polymeric material. These included compounds such as gluconic, ketogluconic, arabonic, and smaller acids, and arabinose, erythrose, and glycerose.

[1] Tolbert, Adams, Bennet, Hughes, Kirk, Lemmon, Noller, Ostwald, and Calvin, *J. Amer. Chem. Soc.*, 1953, **75**, 1867.
[2] Lemmon, *Nucleonics*, 1953, **11**, No. 10, 44.
[3] Scraba, Burr, and Hess, *J. Chem. Phys.*, 1953, **21**, 1296.
[4] Wagner and Guinn, *J. Amer. Chem. Soc.*, 1953, **75**, 4861.
[5] Bayly and Weigel, *Nature*, in the press.

Electrophoresis of the self-decomposition product in phosphate buffer (pH 7·2) revealed the presence of a large quantity of acidic compounds (8·2%). Although at least three fractions, in addition to neutral compounds, could be distinguished, considerable streaking prevented their quantitative estimation. Similarly, electrophoresis in borate buffer [6] did not yield sufficient separation for the fractions to be determined quantitatively, although at least 9 components were evident.

A high resolution of the products was achieved by two-dimensional paper chromatography–paper electrophoresis, which revealed the presence of 37 components (Fig. and Table 3). The fractions were numbered according to their $R_{glucose}$ values as shown in Table 2; the letters refer to their sequence in electrophoresis. Fractions 5-C, 7-B, 9-B, and 12-A had $R_{glucose}$ and $M_{glucose}$ values identical with those of glucose (86·4%), arabinose (0·44%), erythrose (0·59%), and glycerose (0·12%). Other fractions corresponded to aldohexonic acids, aldopentonic acids, their keto-derivatives, and the lactones of these acids.

In order to make a more accurate determination of some of the products of the self-decomposition, the mixture was analysed for specific compounds by the carrier-dilution technique. With those compounds which establish equilibria in aqueous solution, such as α- and β-sugars, acids and lactones, it was necessary to allow sufficient time for the carrier to equilibrate with the [^{14}C]-product of the self-decomposition (cf. 2-keto-D-gluconic acid results). Neglect of this step could have given a determination of only one of the components of the equilibrium. The results are shown in Table 1. It will be seen that

TABLE 1. *Products of self-decomposition of* D-[^{14}C]*glucose and oxidation of* D-*glucose by Fenton's reagent.*

Compound	Yield (%) on self-decompn. for 26 months	Yield (%) on oxidn. by Fenton's reagent	Compound	Yield (%) on self-decompn. for 26 months	Yield (%) on oxidn. by Fenton's reagent
D-Glucose	79·95	40·12	D-Glucurone	<0·10	
D-Arabinose	0·43	0·49	D-Arabonic acid ...	0·07	1·11
D-Erythrose	*		Oxalic acid	<0·0005	1·02
Glycerose	*		D-Glucosone		12·6 *
D-Gluconic acid	0·62	11·68	Formaldehyde		0·02
2-Keto-D-gluconic acid	0·38	2·32	Carbon dioxide		0·49

* Identified by chromatography. † Total osones calculated as D-glucosone.

these measurements indicated that the [^{14}C]glucose sample had decomposed to the extent of 20% in 26 months, giving *inter alia* D-arabinose (0·43%), D-gluconic acid (0·62%), and 2-keto-D-gluconic acid (0·38%). No attempt was made to determine all the many products because the pattern was already evident and because of the small amount (6 mg.) of the stored parent compound at our disposal.

The $G(-M)$ values of freeze-dried samples of [^{14}C]sucrose and D-[^{14}C]glucose, when stored in vacuum-sealed tubes at room temperature, have been found to be 4 and 53 respectively.[5]

Sucrose can, with efficient freeze-drying techniques, be obtained anhydrous. A freeze-dried sample of D-glucose, prepared in the same way as the radioactive sample, was shown, by the presence of a broad absorption band at 1640 cm.$^{-1}$ in its infrared spectrum, to contain an appreciable quantity of non-bonded water. It is therefore reasonable to assume that the freeze-dried D-[^{14}C]glucose sample also contained non-bonded water. The interaction of the β-particles from ^{14}C with the water could thus produce hydroxyl radicals, which on reaction with D-[^{14}C]glucose would enhance the degree of decomposition.

[6] Foster, *J.*, 1953, 982.

To test the validity of this theory a comparison was made with the oxidation of D-glucose with Fenton's reagent,[7] which is known [8] to generate hydroxyl radicals in solution, according to the process $H_2O_2 + Fe^{2+} \longrightarrow Fe^{3+} + OH^- + OH\cdot$. A mixture of the products formed by Fenton's reagent and by self-decomposition was analysed by paper chromatography. A radiochromatogram showed seven components, six of which had $R_{glucose}$ values identical with six of the eight coloured spots which appeared when the chromatogram was sprayed with alkaline silver nitrate [9] and which arose from the chemical oxidation (Table 4).

A quantitative determination of some of the products from the chemical oxidation was made by treating freshly purified D-[^{14}C]glucose with Fenton's reagent (1·014 mol. of peroxide) and applying carrier-dilution techniques for D-glucose, D-arabinose, D-gluconic acid, 2-keto-D-gluconic acid, D-glucurone, D-arabonic acid, and oxalic acid. D-Glucosone,[7,10] formaldehyde,[11] and carbon dioxide were also determined. The results are shown in Table 1. The production of D-glucosone was confirmed when paper chromatography in phenol–water (4 : 1; to give a clear separation from glucose) revealed a component with R_F 0·25, identical with that previously reported.[12] A radiochromatogram of the products of the self-decomposition of D-[^{14}C]glucose did not reveal D-glucosone.

Immobile components were present on paper chromatograms of the products from both the self-irradiation and the chemical oxidation of D-glucose. That from the former was treated with sulphuric acid and then with barium carbonate, but no radioactivity was found in the filtrate. It is suggested that this material was a non-glycosidic acidic polymer, which was either precipitated by the sulphuric acid or formed an insoluble barium salt; this is supported by its streaking towards the anode during electrophoresis. It is possible that it arose by synthesis of carbon–carbon bonds from radicals, since it is known [3] that [^{14}C]methanol gives ethylene glycol, glycerol, and erythritol. The immobile product from the chemical oxidation of D-glucose was precipitated from aqueous solution by acetone and contained 17·9% of iron. It may have been similar to an iron complex found by Küchlin,[13] and was not necessarily related to the material formed by self-irradiation.

Clearly, there is a marked similarity between the products arising from self-decomposition of D-[^{14}C]glucose and those formed when glucose is oxidised by Fenton's reagent. The only significant difference was the absence of D-glucosone from the products of irradiation. This may have been due to the fact that the irradiation decomposition proceeded in the absence of air, whereas no precautions were taken to exclude air during the chemical oxidation. Moreover, glucosone is a very reactive compound and may well have undergone considerable chemical change during the long storage period, but not during the rapid chemical oxidation.

The similarity between the products of the chemical and radiation-induced reactions suggests that they arise by similar routes, presumably involving the participation of hydroxyl radicals. Abstraction of hydrogen by these radicals would yield polymers, keto-groups, and carboxyl groups, thus producing gluconic acid and keto-gluconic acids. In conjunction with C–C bond fission, lower aldoses and their acids and keto-derivatives would result. This is also supported by the work of Phillips *et al.*[14] and Grant *et al.*,[15] who studied the action of ionising radiation on aqueous solutions of D-glucose in the presence of oxygen and *in vacuo*, respectively. The similarity between some of their products and those produced by self-decomposition suggests a similarity of the reaction mechanisms.

[7] Morrel and Crofts, *J.*, 1899, **75**, 786.
[8] Waters, *Ann. Reports*, 1945, **42**, 130.
[9] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[10] Cross, Bevan, and Smith, *J.*, 1898, **73**, 468.
[11] Gibbons and O'Dea, *Biochem. J.*, 1953, **55**, 580.
[12] Bayne and Fewster, *Adv. Carbohydrate Chem.*, 1956, **11**, 67.
[13] Küchlin, *Rec. Trav. chim.*, 1932, **51**, 892.
[14] Phillips, Moody, and Mattok, *J.*, 1958, 3522.
[15] Grant and Ward, *J.*, 1959, 2871.

Experimental

Material.—D-[^{14}C]Glucose, generally labelled, was obtained from the Radiochemical Centre, Amersham. It had been prepared by photosynthesis and had been purified by chromatography and crystallisation, after which no impurities could be detected.

Determination of Radioactivity.—Radioactivity was determined after conversion of the compound into carbon dioxide, and thence into barium carbonate.[16] The amount used was sufficient to give a thickness greater than 20 mg. per cm.^{2}. The β-emission was measured by using a Geiger–Müller end-window tube with an EKCO scaler (type N 529A) and for times sufficient to give a standard error of better than ±2%, except for samples of specific radioactivity lower than 1·6 μc per g.-atom of carbon. All figures quoted are corrected for background and paralysis time. A sample of poly([^{14}C]methyl methacrylate), supplied by the Radiochemical Centre, Amersham, was used as a standard source of barium [^{14}C]carbonate.

Chromatography.—(i) *Solvents.* The solvents used in paper chromatography were: (*a*) butanol–ethanol–water (4 : 1 : 5) (organic phase); (*b*) acetone–water (4 : 1); (*c*) phenol–water (4 : 1).

(ii) *Radiochromatograms.* Radiochromatograms were obtained by exposure of the paper chromatograms to Ilford *X*-ray films (Industrial G) for an appropriate length of time, or by scanning the paper chromatograms with a Geiger–Müller end-window counter.

Chromatography and Electrophoresis of Self-decomposition Products from D-[^{14}C]*Glucose.*—D-[^{14}C]Glucose (481 μc contained in *ca.* 6 mg.; spec. radioactivity *ca.* 2400 mc per g.-atom of carbon) was stored in the freeze-dried state in a vacuum-sealed tube in the dark for 26 months. A radiochromatogram in solvent (*a*) revealed the presence of 11 components in addition to glucose, as shown in Table 2. Fractions No. 2, 3, 5, 6, 8, 9, and 11 had $R_{glucose}$ values similar to those of reference samples of D-gluconic acid and 2-keto-D-gluconic acid, D-arabonic acid, D-glucose and D-glucosone, D-arabinose, D-gluconolactone, D-arabonolactone and D-erythrose, and glycerose, respectively.

TABLE 2. *Radiochromatogram of* D-[^{14}C]*glucose stored for* 26 *months.*

Fraction no.	1	2	3	4A	4B*	5	6	7	8	9	10	11	12	13 †
$R_{glucose}$	0	0·1	0·2	0·3		1·0	1·3	1·5	2·1	2·6	2·9	3·1	3·5	
Radioactivity (% of total)	2·9	2·6	1·6	1·2	1·1	85·5	1·4	1·3	0·7	1·0	0·3	0·2	0·1	0·1

* Trail between fractions 4A and 5. † Trail between fraction 12 and bottom of chromatogram.

Electrophoresis (15 v per cm.) of the self-decomposition product in 0·2M-phosphate buffer (pH 7·2) and estimation of the distribution of the radioactivity showed that 8·2% of the material moved towards the anode and was thus acidic. In addition to the neutral material (91·8%), three fractions having $M_{gluconic\ acid}$ values of 1·00, 1·13, and 2·43 could be distinguished, but the considerable streaking did not allow their independent quantitative determination.

TABLE 3. *Self-decomposition products from* D-[^{14}C]*glucose after* 26 *months' storage.*

Fraction	1-A	1-B	2,3-A	2,3-B	2,3-C	2,3-D	2,3-E	Trailing of 4-A	4-A
Radioactivity *	0·64	0·11	2·14	1·46	0·07	0·04	0·02	0·44	0·89
Fraction	4-B	4-C	5-A	5-B	5-C	5-D	6-A	6-B	7-A	7-B
Radioactivity *	0·01	0·03	0·30	0·30	86·4	0·03	0·09	0·47	0·06	0·44
Fraction	7-C	7-D	8-A	8-B	8-C	8-D	8-E	9-A	9-B	9-C
Ratioactivity *	3·19	0·04	0·04	0·06	0·32	0·70	0·08	0·11	0·59	0·25
Fraction	9-D	10-A	11-A	11-B	11-C	11-D	12-A	12-B	12-C	
Radioactivity *	0·11	0·06	0·05	0·05	0·15	0·04	0·12	0·08	0·02	

* *I.e.*, radioactivity as % of total.

Paper electrophoresis in 0·2M-borate buffer [6] (pH 10), with subsequent exposure to *X*-ray film, revealed nine components with M_G values of 0·12, 0·25, 0·37, 0·60, 0·70, 0·80, 1·00, 1·15, and 1·87. Streaking prevented their quantitative determination.

[16] Skipper, Bryan, White, and Hutchinson, *J. Biol. Chem.*, 1948, **173**, 371; Calvin, Heidelberger, Reid, Tolbert, and Yankwich, "Isotopic Carbon," Wiley, New York, 1949; Henriques, Kistiakowsky, Margnetti, and Schneider, *Ind. Eng. Chem., Analyt.*, 1946, **18**, 349.

Two-dimensional paper chromatography [solvent (*a*)]–paper electrophoresis (0·2M-borate buffer, pH 10), with subsequent exposure to *X*-ray film and determination of the distribution of the radioactivity, revealed the presence of 37 components (Fig. and Table 3).

Fractions 2,3-A, 2,3-B, 4-A, 5-C, 7-B, 8-D, 9-B, 9-D, and 12-A had $R_{glucose}$ and $M_{glucose}$ values similar to those of aldohexonic acids and their keto-derivatives, aldopentonic acids and their keto-derivatives, D-glucuronic acid, D-glucose, D-arabinose, lactones of fraction 2,3-A, D-erythrose, D-arabonolactone, and D-glycerose, respectively.

Paper chromatography of fraction 5 (Table 2) in solvent (*c*) revealed only one component with R_F value identical with that of D-glucose. D-Glucosone was thus not present.

Carrier-dilution Analysis of Self-decomposition Products from D-[^{14}C]*Glucose.*—(i) α-D-Glucose (3·998 g.) was dissolved in an aliquot part of a solution of the self-decomposition product (20·83 μc) in water (15 ml.). The pH of the solution was adjusted with ammonia solution to 7·5. The solution was stored until the optical rotation had reached equilibrium value, and then freeze-dried. The solid was crystallised by dissolving it in boiling 96% methanol (15 ml.) and adding propan-2-ol (10 ml.), and was recrystallised until three consecutive samples possessed constant specific radioactivity (125·07 μc per g.-atom of carbon; 79·95%; m. p. 147°).

(ii) D-Glucono-δ-lactone (1·019 g.) was dissolved in an aliquot part of a solution of the self-decomposition product (9·98 μc) in water (10 ml.). The solution was allowed to equilibrate for 12 hr., as it was found by measurement of the optical rotation that maximum concentration of free acid was obtained after 7 hr. The solution was neutralised with aqueous potassium hydroxide. Potassium D-gluconate was obtained by evaporation to *ca.* 5 ml., addition of ethanol (15 ml.) to incipient cloudiness, and crystallisation at 0°. The material was recrystallised from aqueous ethanol until three consecutive samples possessed constant specific radioactivity (1·81 μc per g.-atom of carbon; 0·62%; m. p. 176°).

(iii) A solution of 2-keto-D-gluconic acid in water (10 ml.), obtained by treatment of calcium 2-keto-D-gluconate (0·634 g.) with Amberlite IR-120 [H^+], was added to an aliquot part of the self-decomposition product (13·87 μc) in water (15 ml.). The solution was allowed to equilibrate for 18 hr., then treated with De-Acidite F.F. (carbonate form) (15 g.). The acid was desorbed by treatment with 3% ammonium carbonate solution (50 ml.). The solution was freed from cations by treatment with Amberlite IR-120 [H^+] (10 g.) and neutralised with aqueous potassium hydroxide. Potassium 2-keto-D-gluconate (2·98 μc per g.-atom of carbon; 0·38%; m. p. 152°) was obtained as described for potassium D-gluconate.

(iv) Potassium 2-keto-D-gluconate (0·471 g.) was dissolved in an aliquot part of the self-decomposition product (19·04 μc) in water (25 ml.), and the solution stored for 1 hr. Potassium 2-keto-D-gluconate (1·43 μc per g.-atom of carbon; 0·09%; m. p. 152°) was obtained as described for potassium D-gluconate.

(v) D-Glucurone (0·994 g.) was dissolved in an aliquot part of the self-decomposition product (15·09 μc) in water (10 ml.). The solution was set aside for 20 hr., evaporated to a syrup, and seeded with a trace of D-glucurone. The crystalline product did not show constant specific radioactivity after 11 recrystallisations (0·45 μc per g.-atom of carbon; maximum 0·10%).

(vi) A solution of D-arabonic acid in water (10 ml.), obtained by treatment of potassium D-arabonate (0·517 g.) with Amberlite IR-120 [H^+], was added to a solution of the self-decomposition product (8·33 μc) in water (10 ml.). The solution was allowed to equilibrate for 24 hr. D-Arabonolactone was separated by paper chromatography in solvent (*a*). Potassium D-arabonate was obtained after neutralisation with aqueous potassium hydroxide and crystallisation from aqueous ethanol. It was recrystallised from aqueous ethanol until three consecutive samples possessed constant specific radioactivity (0·45 μc per g.-atom of carbon; 0·07%; m. p. 219°, decomp.).

(vii) Oxalic acid (0·5 g.) was dissolved in an aliquot part of the self-decomposition product (4·75 μc) in boiling water (0·5 ml.), and crystallised by cooling. After 6 recrystallisations its specific radioactivity (0·002 μc per g.-atom of carbon; maximum 0·0005%; m. p. 99°) was below the limit of accurate determination.

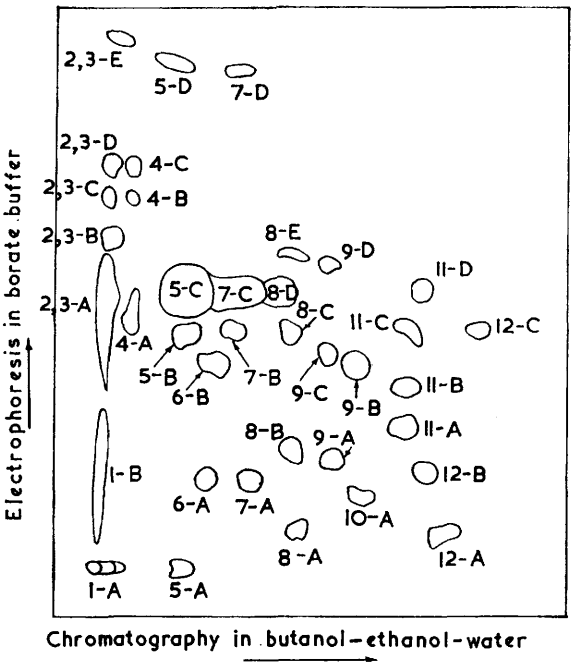
(viii) D-Arabinose (0·544 g.) was dissolved in an aliquot part of the self-decomposition product (19·16 μc) in water (10 ml.) and isolated by chromatography on Whatman paper No. 3 in solvent (*a*). The eluate was freeze-dried, and the solid crystallised by dissolving it in boiling methanol (4 ml.) and adding propan-2-ol (10 ml.). It was recrystallised until three consecutive samples possessed constant specific radioactivity (4·58 μc per g.-atom of carbon; 0·43%; m. p. 159°).

(ix) D-Mannitol (1·974 g.) was dissolved in an aliquot part of the self-decomposition product (4·75 μc) in boiling 90% methanol (40 ml.), and crystallised by cooling. Six recrystallisations yielded non-radioactive D-mannitol (m. p. 166°).

Examination of Polymeric Component of the Self-decomposition Products from D-[^{14}C]*Glucose.*—This component, remaining immobile during paper chromatography (Fraction 1, Table 2), was eluted with boiling water. Sulphuric acid was added to portions of the eluate to obtain 0·05N- and 0·5N-sulphuric acid, severally. The solutions were kept at 100° for 2·5 hr., neutralised with barium carbonate, and concentrated. No radioactivity could be detected in the concentrates.

Analysis of Products of Oxidation of D-*Glucose by Fenton's Reagent.*—(i) *Chromatography.* Hydrogen peroxide (20-vol.) (10 × 0·32 ml.) was added to a solution of ferrous sulphate heptahydrate (30 mg.) and D-glucose (1 g.) in water (10 ml.). After each addition, time was allowed

Self-decomposition products of D-[^{14}C]*glucose.*



for the deep yellow colour to disappear or fade to a light yellow. Paper chromatography [solvent (*b*)] of the solution in admixture with the self-decomposition products from D-[^{14}C]-glucose, exposure to *X*-ray film, and spraying with acetone–silver nitrate–alcoholic sodium hydroxide revealed 8 coloured spots, 6 of which had $R_{glucose}$ values similar to those of 6 of the 7 components present in the self-decomposition product, as shown in Table 4.

TABLE 4. *Comparison of self-decomposition products of* D-[^{14}C]*glucose with products from* D-*glucose and Fenton's reagent.*

	Fraction	1	2	3	4	5	6	7	8
$R_{glucose}$ in solvent (*b*)	Fenton's reagent	0	0·60	0·77	1·00	1·20	1·37	1·57	1·70
	Self-decompn.	0	0·70	0·78	1·00	1·22	1·37	1·56	—

Paper chromatography of the oxidation product in solvent (*a*), elution of the components with $R_{glucose}$ 1·00, and chromatography of the eluate in solvent (*c*) revealed the presence of a component with R_F 0·25, identical with that of D-glucosone.

In the five following experiments, the same conditions were used but the weight of salt and the volume of the portion of peroxide were varied on a scale noted in parentheses.

(ii) *Carrier-dilution analysis of oxidation products from* D-[^{14}C]*glucose.* The reagents (one-tenth scale) were used with freshly purified D-[^{14}C]glucose (*ca.* 100 mg., 1165 or 1229 μc per g.-atom of carbon) in water (1 ml.), as described above. A carrier compound was dissolved in each solution and allowed to equilibrate for 24 hr. D-Glucose, potassium D-gluconate, D-mannitol, and oxalic acid were isolated and recrystallised as described for the carrier-dilution analysis of the self-decomposition product. Potassium 2-keto-D-gluconate, potassium D-arabonate, and D-arabinose were separated by paper chromatography on Whatman paper No. 3 in solvent (*a*) and then purified as described for the carrier-dilution analysis of the self-decomposition product. The details of the analysis are shown in Table 5.

TABLE 5. *Carrier-dilution analysis of products from pure* D-[^{14}C]*glucose and Fenton's reagent.*

Carrier compound	W_G (mg.)	S_o (μc per g.-atom of C)	W_c (g.)	S_i (μc per g.-atom of C)	Yield (%)
D-Glucose	100	1229	3·988	12·24	40·12
K D-gluconate	100	1229	0·980	17·25	11·68 *
K 2-keto-D-gluconate	98·7	1165	0·503	6·29	2·32 *
D-Arabinose	96·9	1165	0·500	1·10	0·49
K D-arabonate	99·0	1165	0·502	3·13	1·11 *
Oxalic acid	100	1229	0·500	2·51	1·02
D-Mannitol	100	1229	2·000	0	0

W_G = Weight of D-[^{14}C]glucose oxidised. S_o = Specific radioactivity of D-[^{14}C]glucose. W_c = Weight of carrier added. S_i = Specific radioactivity of isolated sample. * Calc. for free acid.

(iii) *Osones.* The reagents (original scale) were used with D-glucose (1 g.) in water (10 ml.). Acids were removed by treatment with barium carbonate (2 g.). Acetone (50 ml.) was added, and the precipitate centrifuged off. The acetone was distilled off, and the solution concentrated to *ca.* 4 ml. Addition of phenylhydrazine (2 g.) in glacial acetic acid (1·5 ml.) and water (15 ml.) produced an immediate precipitate. This was filtered off after 10 min. and dried *in vacuo* over P_2O_5 (0·25 g.; 12·6%, calculated as D-glucosone). The recrystallised D-glucosazone had m. p. 204°, $[\alpha]_D^{27}$ −77°.[17] D-Glucose, treated with phenylhydrazine for 10 min. at room temperature, did not give a precipitate.

(iv) *Formaldehyde.* The reagents (10-fold scale) were used with D-glucose (10 g.) in water (100 ml.). The mixture was steam-distilled, and formaldehyde determined with chromotropic acid (2·3 mg.; 0·02%).

(v) *Carbon dioxide.* The reagents [scale as in (i)] were used with D-glucose (971 mg.) in carbon dioxide-free water (10 ml.). The solution was warmed to 40° for 30 min. The carbon dioxide evolved was isolated as barium carbonate (32 mg., 0·49%).

(vi) *Iron complex of oxidation product.* The reagents (20-fold scale) were used with D-glucose (20 g.) in water (200 ml.). Addition of acetone (1·2 l.) produced a precipitate (40 mg.) (Found: Fe, 17·9%).

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[17] Neuberg, *Ber.*, 1899, **32**, 3384.