531. *Effects of γ-Radiation. Part I. Polymer Formation * from Sugars, Hydroxy-acids, and Amino-acids.*

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Acidic polymers have been formed by γ-irradiation of aqueous solutions of maltose, glucose, 1 : 4-gluconolactone, lactic acid, glycollic acid, and amino-acid solutions *in vacuo*. The isolation and initial structural investigations of some of these unusual polymers are described.

Prolonged irradiation of aqueous glucose solutions (0·1%) *in vacuo* from a 200 c ^{60}Co source has been found to lead to an acidic polymer.[1] Irradiated solutions were freeze-dried since the ultraviolet absorption spectrum of the aqueous polymer solution changed slowly at 20° and rapidly at 100° (Fig. 1). This change was not reversed on subsequent freeze-drying, and did not appear to be associated with an appreciable change in molecular size. Dialysis was, in some ways, not an ideal technique for the isolation of the polymer since the pore-size of the membrane varied, and some material was not of a sufficiently high molecular weight to be retained. Cetavlon [2] (cetyltrimethylammonium bromide) precipitated more polymer from the dialysate, presumably of a lower molecular weight. M-Sodium chloride, added to an ethanolic solution of the polymer–Cetavlon complex, precipitated a material having the same infrared and ultraviolet absorption spectra and identical chromatographic and ionophoretic properties as the polymer initially obtained by dialysis.

The yields of polymeric material recovered by dialysis of a number of γ-irradiated solutions of glucose, maltose, 1 : 4-gluconolactone, lactic acid, and glycollic acid are shown in Table 1. Carbon dioxide was evolved in each case during the irradiations. In the case of glycollic acid, the low yields were increased by further irradiation.

Table 1. *Yields of polymers.*

			Yields (%)	
Ref. No.	Substrate	Dose ($\times 10^{20}$ ev ml.$^{-1}$)	Non-volatile	Polymer (non-diffusible)
1	Maltose	4·44	83	1·4
2	Glucose	1·46	75	1·3
3	Glucose	4·44	62	45
4	1 : 4-Gluconolactone	4·54	68	51
5	Lactic acid	4·54	40	4·8
6	Glycollic acid	4·54	39	4·5
7	,,	0·91	85	0
8	,,	4·3	42	2·2
9	,, *	9·2	36	6·4
10	Mandelic acid	4·3	68	61 †

* 0·15%, otherwise 0·1%. † Yield of precipitate; no dialysis in this case.

The polymers show considerable similarity, apart from the special case of mandelic acid (see below). Elemental analyses showed, *e.g.*, the polymer no. 4 to be approximately

* Patent Appln., 698/59.

[1] Barker, Grant, Stacey, and Ward, *Nature*, 1959, **183**, 376.

[2] Jones, *Biochim. Biophys. Acta*, 1953, **10**, 607; Bera, Foster, and Stacey, *J.*, 1955, 3788.

TABLE 2. *Properties of polymers.*

Ref. no.	Polymer Parent substance	Found (%) C	H	Ionophoresis M_G	$M_{Gluconic}$	"Apparent CHO" (%)	Acid-labile CO_2 (%)	Equiv. at pH 7	pH 9
1	Maltose	—	—	0·7—1·0	0·2—1·0	—	—	—	—
2	Glucose	43·99	4·61	—	—	—	—	—	—
3	Glucose	51·91	5·07	1·1	1·25	16·0	4·2	450	328
4	1 : 4-Gluconolactone	50·37	5·40	1·1	1·25	16·8	2·2	408	322
5	Lactic acid	52·21	6·32	1·1	1·22	7·8	—	—	—
8	Glycollic acid	50·46	5·25	1·1	1·32	12·0	7·7	196	165
9	Glycollic acid	52·14	4·75	1·1	1·32	10·4	—	249	196
10	Mandelic acid (ppt.)	70·04	6·10	—	—	—	—	—	—

TABLE 3. *Infrared absorption bands (cm.$^{-1}$).*

Polymers					Acid-hydrolysis of glycollic acid polymer (no. 9)		Mandelic acid ppt.
Maltose 1	Glucose 3	1 : 4-Gluconolactone 4	Lactic acid 5	Glycollic acid 8, 9	Ppt.	Sol.	(no. 10)
3350s	3400s	3350s	3350s	3400s	3400s	3400s	3400s
2900w	2910w	2950w	2950w	2950w	2950w	2950w	2940m
					2600w	2600m	
2350w	2350w	2350w		2350w			
2170w	2170w	2130w	2100w	2100w		2050w	
						1925m	
1710m	1710w	1710w	1710w	1780m	1725s		
			1650s			1650s	1680s
1600m	1590s	1600s			1635m		1620w
					1525w		1510m
			1460w				1470m
1405w	1415s	1410s	1410m	1410m	1400w	1360s	
					1200m		1250b
			1130w			1125w	
1020s	1090m	1090m	1110w		1100m	1050w	
			1050w			1035w	1040mb
						1000m	930w
						835s	770m
						700s	710s

$(C_6H_8O_4)_n$ (Table 2). The polymers were immobile during chromatography in butan-1-ol–ethanol–water (4 : 1 : 5 v/v) or butan-1-ol–propionic acid–water (6 : 3 : 4 v/v) and were detected by alkaline silver nitrate [3] or Chlorophenol Red.[4] On ionophoresis in 0·2M-borate buffer (pH 10), the polymers had M_G ~1·1 and in 0·2M-acetate buffer (pH 5) had $M_{Gluconic\ acid}$ 1·2—1·3.

All the polymers were precipitated from solution by Cetavlon,[2] the precipitate being soluble in M-sodium chloride. The polymers were also precipitated by cold dilute acid, the precipitates having infrared spectra identical with those of the original polymers (Table 3). These precipitates (contrast the hot acid-hydrolysis below) were readily soluble in cold dilute ammonia solution.

The infrared absorption spectra of these polymers were similar (Table 3). The ultraviolet absorption spectra show peaks in the region 260—270 mμ which were less pronounced in the spectra of the polymers from lactic and glycollic acid (Fig. 1).

Treatment of the polymers from glucose (no. 3 *) and glycollic acid (no. 9) with hot dilute acid formed precipitates (in 61% and 41% yield respectively) having infrared absorption spectra similar to those of the original polymers. The precipitates were insoluble in aqueous ammonia and sodium hydroxide. The soluble fractions from the acid-treatments were examined by paper chromatography. The soluble fraction from the glucose polymer contained no detectable mobile components, but the soluble fraction from the glycollic acid polymer contained at least two unidentified non-reducing components.

* Such numbers refer to Table 1.

[3] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.

[4] Block, Durrum, and Zweig, "A Manual of Paper Chromatography and Paper Electrophoresis," Academic Press, Inc., New York, 1955, p. 169.

The polymers are oxidised by alkaline hypoiodite. The method of Hirst, Hough, and Jones [5] was used to measure the " apparent aldehyde " group content (Table 2).

The polymers from glucose (no. 3) and glycollic acid (nos. 8 and 9) were oxidised with periodate.[6] All showed a rapid uptake followed by a slower reaction; increased dosage of radiation yielded a polymer which was attacked more slowly (Fig. 2). At the end of the periodate oxidation, the reaction mixtures yielded non-diffusible materials. The periodate-oxidised polymers thus obtained had infrared absorption spectra very similar to those of the parent polymers. Alkaline hypoiodite oxidation [5] of the periodate-oxidised glycollic acid polymer (no. 9) showed 5·8% of " apparent aldehyde " group, approximately half the value obtained before periodate oxidation.

Similarly alkaline hypoiodite oxidation [5] of the polymer from glycollic acid (no. 9) yielded non-diffusible materials (84%), and the periodate-oxidised glycollic acid polymer

FIG. 1.

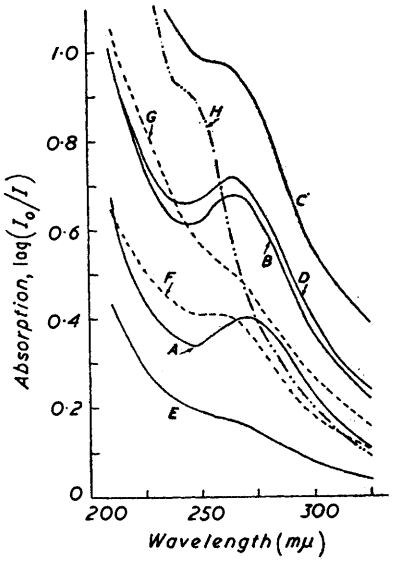

FIG. 2.

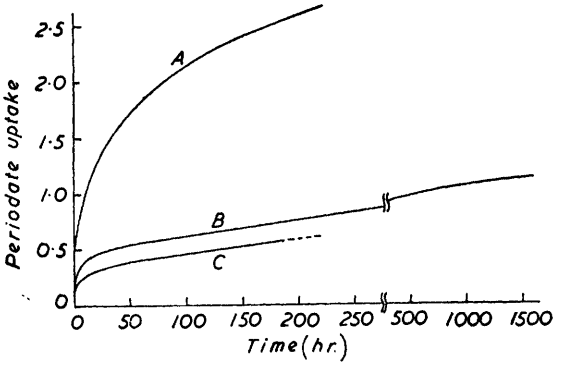

FIG. 1. *Ultraviolet absorption spectra of polymers. Polymers from (A) maltose (no. 1 in Table 1), (B) glucose (no. 3), (C) glucose (no. 3) after being heated at 100° for 10 min., (D) 1 : 4-gluconolactone (no. 4), (E) lactic acid (no. 5), (F) glycollic acid (no. 9), (G) periodate-oxidised glycollic acid polymer (no. 9), (H) mandelic acid precipitate (no. 10). A—G, 0·1% solutions in water. H, 0·05% solutions in ethanol.*

FIG. 2. *Oxidation of polymers by periodate. (A) Glucose polymer (no. 3). (B) Glycollic acid polymer (no. 8). (C) Glycollic acid polymer (no. 9). Periodate uptake is plotted in mmole per 180 mg. for (A), and in mmole per 76 mg. for (B) and (C).*

(no. 9) when oxidised with alkaline hypoiodite yielded 90% of non-diffusible material. All the hypiodite-oxidised polymers had infrared absorption spectra very similar to those of the original polymers.

The acidimetric equivalent was determined by potentiometric titrations (Table 2). The carbon dioxide evolved when the materials were heated with 19% hydrochloric acid was determined as a measure of ready decarboxylation [7] (Table 2).

1 : 4-Gluconolactone was irradiated in 99·78% deuterium oxide solution. The infrared absorption spectra of the non-volatile solute and polymer showed no indication of carbon–deuterium bond formation, indicating that virtually no carbon–hydrogen bonds underwent hydrogen exchange with the solvent or radicals derived therefrom.

[5] Hirst, Hough, and Jones, *J.*, 1949, 928.

[6] Dyer, " Methods of Biochemical Analysis," Interscience Publ., Inc., New York, 1956, Vol. III, p. 111.

[7] Barker, Foster, Siddiqui, and Stacey, *Talanta*, 1958, **1**, 216.

Glycine, alanine, and phenylalanine solutions on irradiation also yielded materials which were precipitated by Cetavlon, the complex being soluble in M-sodium chloride. The isolation of polymeric material by dialysis has not yet been attempted in these cases.

The presence of oxygen was found completely to inhibit polymer formation in all cases, but polymer formation ensued when the irradiation occurred under a stream of nitrogen.

Discussion.—The polymers formed are acidic and are susceptible to both alkaline hypoiodite [5] and periodate [6] oxidation, but in neither oxidation is the polymeric character destroyed. The polymeric character, furthermore, survives acidic and alkaline hydrolysis, although superficial degradation occurs, *e.g.*, partial decarboxylation and slight fragmentation. This emphasises that the linkage is neither glycosidic nor that of an ester. Infrared spectroscopy reveals that the lactic acid polymer (no. 5) and mandelic acid precipitate (no. 10) retain some of the methyl and phenyl groups respectively, while the formation of small amounts of glucose on hydrolysis of the maltose polymer (no. 1) shows that some of the *O*-glucosyl substituent also survives. When the parent molecule contains phenyl groups, it might be expected that polymerisation would markedly decrease solubility. This is evident with the slightly soluble phenylalanine product and in the precipitation in the case of mandelic acid. Once precipitated, further attack will be slower, since attack by solvent radicals then becomes heterogeneous.

It has been shown [8] that the direct action of γ-radiation on polycrystalline glycollic acid *in vacuo* forms carboxyhydroxymethyl radicals. Tartaric acid has been shown to be a relatively stable primary product formed in high yield on irradiation of aqueous glycollic acid *in vacuo*,[9] and solutions both of tartaric acid and of mixtures of tartaric and glycollic acid [1] give acidic products of larger molecular size *in vacuo*. It is postulated, therefore, that the polymers are formed by such radical addition. In the case of glycollic acid, this would be expected to yield a polymeric repeating unit: $[-C(CO_2H)(OH)-]$. However, the growing molecule would be subject to further attack at alternative sites (as observed with the simpler molecules [1,9]). Comparison of the properties of the polymers (nos. 8 and 9) from glycollic acid, which have been formed after mean molecular doses of 55 and 78 ev/molecule respectively, showed lower carboxylic acid and aldehyde contents and greater resistance to attack by periodate with increasing dose. The last-mentioned effect is of particular importance since the hypothetical repeating unit above would be sensitive to periodate cleavage. It appears (Fig. 2) that further radiation is mainly affecting such structures as would be rapidly oxidised by periodate. Formation and destruction of aldehyde groups may be expected in a manner similar to the formation of glyoxylic acid from glycollic acid and its destruction.[9]

The main route for polymer formation from glucose is believed to be through gluconic acid. The evidence for this lies in the high yield of gluconic acid from glucose solutions irradiated *in vacuo*,[10] the increased yield of polymer from 1 : 4-gluconolactone, and the strong similarity between the polymers from glucose (no. 3) and from 1 : 4-gluconolactone (no. 4). Similar mechanisms are therefore believed to account for the polymer formation in each case. This is supported by the use of electron-spin resonance techniques to detect, *inter alia*, the aminocarboxymethyl radical $[\cdot CH(NH_3^+)\cdot CO_2^-]$ in irradiated crystalline glycine *in vacuo* [11] and the detection of apparent amino-acid dimers by chromatography of irradiated solutions of amino-acids.[1] Such mechanisms would produce polymer yields very dependent on the molecular size of the initial compound, and, with the exception of the polymer from maltose (no. 1), this is indicated in Table 1.

It is pertinent to notice that the presence of oxygen inhibits polymer formation and also formation of tartaric acid from glycollic acid [9] and of larger molecules from a number

[8] Grant, Ward, and Whiffen, *J.*, 1958, 4635.
[9] Grant and Ward, following papers.
[10] Grant and Ward, *J.*, in the press.
[11] Ghosh and Whiffen, *Chem. Soc. Spec. Publ.* No. 12, 1958, p. 168.

of relevant compounds in aqueous solution.[1] It is just such radical addition which would be prevented by rapid formation of peroxy-radicals in the presence of oxygen.

These investigations, which are being continued, have given evidence for carbon–carbon addition to form polymeric materials. The parts of the initial molecules not involved in addition undergo concurrent radiative degradation to yield carbonyl groups, carboxylic acid groups, and other chemical species, which are chemically labile in contrast to the inert polymeric linkage.

Experimental

The polymers are described by reference numbers, as given in Table 1.

Polymer Preparation.—An aqueous solution (0·1% or exceptionally 0·15%) of the compound was boiled vigorously under a vacuum at room temperature for 2 hr. and sealed. The stirred solution was irradiated from a 200 c ^{60}Co γ-radiation source [12] (dose rate $14{\cdot}2 \times 10^{16}$ ev min.$^{-1}$ ml.$^{-1}$ for polymers nos. 4, 5, and 6; otherwise $3{\cdot}8 \times 10^{16}$ ev min.$^{-1}$ ml.$^{-1}$), and then freeze-dried. The residue was weighed, dissolved in water to give a 10% solution, and dialysed for 4 days against 25 vol. of distilled water which was changed daily. The non-diffusible material was recovered by freeze-drying. The parent substances were all free of non-diffusible materials before irradiation. Mandelic acid (4 g.), on irradiation as above, gave a white precipitate (no. 10) (2·43 g.), and the supernatant liquid on freeze-drying yielded 0·27 g. of a pale yellow solid.

Properties of the Polymers.—(i) *Chromatography and ionophoresis.* Chromatography with butan-1-ol–ethanol–water (4 : 1 : 5 v/v) or butan-1-ol–propionic acid–water (6 : 3 : 4 v/v) showed a single immobile component in each polymer, detectable with alkaline silver nitrate [3] and Chlorophenol Red,[4] but only faintly with aniline hydrogen phthalate.[13] Ionophoresis in 0·2M-borate buffer (pH 10) and 0·2M-acetate buffer (pH 5) showed similar mobilities for all the polymers (Table 2).

(ii) *Precipitation with Cetavlon.* Each polymer was precipitated from solution by the addition of 2% aqueous Cetavlon (cetyltrimethylammonium bromide) solution. Cetavlon also gave a slight precipitate from the concentrated dialysable fraction. The precipitates redissolved in M-sodium chloride. A portion of the Cetavlon complex was washed with water, dissolved in ethanol, and treated with M-sodium chloride, a precipitate being formed whose ultraviolet and infrared spectra and analysis were identical with those of the polymer obtained by dialysis.

(iii) *Infrared and ultraviolet absorption spectra.* Infrared spectra are shown in Table 3, and the ultraviolet spectra in Figure 1.

(iv) *Periodate oxidation.*[6] Glucose polymer (no. 3) (84·01 mg.) was oxidised with 0·15M-sodium metaperiodate (50 ml.). Glycollic acid polymers (no. 8) (15·48 mg.) and (no. 9) (30·0 g.) were oxidised with 0·05M-sodium metaperiodate (25 and 50 ml. respectively). The uptake of oxidant at room temperature in the three cases is shown in Fig. 2. The formaldehyde and acid content in the reaction mixtures was determined colorimetrically with chromotropic acid [6,14] and titrimetrically with sodium hydroxide [6] respectively after 72 hr. The values obtained for the glucose polymer (no. 3) were 0·0012 mmole of formaldehyde and 0·41 milliequiv. of acid per 180 mg. of polymer. Glycollic acid polymer (no. 8) yielded 0·019 mmole of formaldehyde and 0·316 milliequiv. of acid per 76 mg. of polymer. Glucose polymer (no. 3) (30 mg.) was oxidised at room temperature with 0·15M-sodium metaperiodate (10 ml.). After 100 hr. the excess of periodate was destroyed with ethylene glycol, and the solution dialysed for 4 days as described for the preparation of the polymers. The non-diffusible fraction yielded 10 mg. on freeze-drying. This periodate-oxidised polymer had very similar chromatographic properties and infrared absorption spectrum to its parent glucose polymer (no. 3), and gave with 2% Cetavlon solution a precipitate which was soluble in M-sodium chloride. Ionophoresis showed a single component with M_G 1·1 and $M_{\text{Gluconic acid}}$ 1·25.

Similarly glycollic acid polymer (no. 9) (270 mg.) was oxidised with 0·05M-sodium metaperiodate (450 ml.). The reaction was stopped with ethylene glycol after 160 hr. and the solution dialysed as before. The non-diffusible fraction yielded 144 mg. (Found: C, 45·1; H, 4·0; Ash, 8·0%). Again the periodate-oxidised polymer was indistinguishable from its parent

[12] Gibson and Pearce, *Chem. and Ind.*, 1957, 613.
[13] Partridge, *Nature*, 1949, **164**, 443.
[14] MacFadyen, *J. Biol. Chem.*, 1945, **158**, 107.

glycollic acid polymer by means of its infrared absorption spectrum, chromatographic properties, or behaviour with 2% Cetavlon solution. Ionophoresis showed a single component with M_G 1·1—1·2 and $M_{Gluconic\ acid}$ 1·4—1·6. The ultraviolet absorption spectrum showed much less indication of a point of inflexion in the region 250—270 mμ than for the parent polymer.

(v) *Alkaline-hypoiodite oxidation.* The polymers were oxidised as described by Hirst, Hough, and Jones,[5] the reagents being calibrated with glucose (Table 2). The results are expressed as aldehyde content (%).

Additionally the periodate-oxidised polymers obtained from glucose and glycollic acid (see iv above) were oxidised with alkaline hypoiodite, and had " apparent aldehyde " contents of 1·0 and 5·8% respectively.

The polymer from glycollic acid (no. 9) (100 mg.) and periodate-oxidised glycollic acid polymer (30 mg.) were separately oxidised with alkaline hypoiodite. The solutions were neutralised with hydrochloric acid, freeze-dried, dissolved in a small volume of water, and dialysed as described for the preparation of the polymers. The non-dialysable fractions yielded 84 and 24 mg. respectively on freeze-drying. These solid oxidised polymers both gave precipitates with 2% Cetavlon solution, and the precipitates were soluble in M-sodium chloride. The infrared spectra were both very similar to that of the parent glycollic acid polymer (no. 9).

(vi) *Potentiometric titration.* The polymers were titrated with 0·0503N-sodium hydroxide in an atmosphere of nitrogen. The equivalent of the polymer was calculated on the basis of the alkali required to establish stable pH values of 7·0 and 9·0. These equivalent values are shown in Table 2.

(vii) *Acidic hydrolysis.* Maltose polymer (no. 1) (1·2 mg.) was hydrolysed for 5 hr. at 100° with 1·5N-sulphuric acid (5 ml.). The solution darkened and a brown precipitate was formed. After neutralisation of the solution with barium carbonate, glucose was detected by paper chromatography and ionophoresis as the only mobile component.

Glucose polymer (no. 3) (50·4 mg.) was dissolved in water (2 ml.), and 2N-sulphuric acid (6 ml.) was added. The immediate white precipitate dissolved at 100° in 4 hr., while the solution became darker and an insoluble material (30·7 mg.) was obtained (Found: C, 56·6; H, 5·1; ash, 0·5%). The material was insoluble in water, 2N-ammonia, acetone, or ethanol, but was slowly soluble in hot N-sodium hydroxide, from which it was precipitated by the addition of acid. The infrared spectrum of this material showed peaks at 3400s, 2950w, 1730w, 1640w, 1180s, 1070w, 1010w, and three weak peaks between 890 and 950 cm.$^{-1}$ (cf. Table 3).

The solution obtained by hydrolysis was neutralised with barium carbonate and filtered. No mobile components were detected in the filtrate on chromatographic analysis.

Glycollic acid polymer (no. 9) (100·4 mg.) was heated with 2N-sulphuric acid (20 ml.) for 3 hr. at 100°. A brown precipitate (infrared spectrum, Table 3) was formed (41 mg.) (Found: C, 51·7; H, 4·2; ash, 3·1%). The supernatant liquid was neutralised with barium carbonate, filtered, and freeze-dried to a pale brown powder (23·6 mg.; for the infrared spectrum see Table 3). Ionophoresis in 0·2M-borate buffer (pH 10) showed components with M_G values of 0·9 and 1·0; but on ionophoresis in 0·2M-acetate buffer (pH 5) no mobile components were detected. Chromatography in butanol–propionic acid–water showed components with $R_{Glyoxylic\ acid}$ 0·13 and 0·25, and a streaking, faster component; with butanol–ethanol–water components with $R_{Glyoxylic\ acid}$ 0·40 and 0·70 and a streaking, slower component were detected. The components were detected by alkaline silver nitrate but not by aniline hydrogen phthalate. Oxidation by alkaline hypoiodite showed this soluble fraction to have an " apparent aldehyde " content of only 0·8%.

(viii) *Evolution of carbon dioxide on treatment with acid.*[7] The polymers were heated in 19% hydrochloric acid. The carbon dioxide evolved was swept with nitrogen into alkali and determined titrimetrically. The carbon dioxide evolved is shown in Table 2.

(ix) *Alkaline hydrolysis.* Glycollic acid polymer (no. 8) (2 mg.) was heated at 100° for 2 hr. with 2N-barium hydroxide (2 ml.). An insoluble brown material was formed and was filtered off. The solution was neutralised with carbon dioxide, filtered, and freeze-dried twice to remove barium hydrogen carbonate. Paper chromatography and ionophoresis revealed soluble products similar to those obtained by acidic hydrolysis of the polymer.

(x) *Degradation of the glucose polymer in solution.* The ultraviolet absorption spectrum of the polymer from glucose (no. 3) changed in aqueous solution slowly at 20° and rapidly at 100°. Freeze-dried solutions had unchanged ultraviolet absorption spectra (Fig. 1).

Special Preparation of Polymers.—(i) *In deuterium oxide.* Dried 1 : 4-D-gluconolactone (20 mg.) was dissolved in deuterium oxide (20 ml.; 99·78% of D_2O) and irradiated as for polymer (no. 4) *in vacuo* in the absence of water. The solution was then freeze-dried. The non-volatile solute and the non-diffusible fraction were examined by infrared spectroscopy, but yielded no evidence of carbon–deuterium bond formation.

(ii) *Attempted preparation of glucose polymer in the presence of oxygen.* 0·1% Glucose solution (4 l.) was degassed and saturated with oxygen for 2 hr. The solution was irradiated for 8 days (dose rate $3{\cdot}8 \times 10^{16}$ ev min.$^{-1}$ ml.$^{-1}$, total $4{\cdot}4 \times 10^{20}$ ev ml.$^{-1}$) in the presence of a continuous stream of oxygen. The freeze-dried solution gave no precipitate with 2% Cetavlon solution and yielded no non-diffusible material.

(iii) *Preparation of glucose polymer in the presence of nitrogen.* The previous experiment was repeated with a nitrogen stream in place of oxygen. The freeze-dried solution gave a copious precipitate with 2% aqueous Cetavlon and yielded non-diffusible material as *in vacuo*.

Polymer Formation from Amino-acids.—0·1% Aqueous solutions (20 ml.) of glycine, alanine, and phenylalanine were irradiated *in vacuo* and in the presence of oxygen for 3 days (dose rate $14{\cdot}2 \times 10^{16}$ ev min.$^{-1}$ ml.$^{-1}$, total $6{\cdot}1 \times 10^{20}$ ev ml.$^{-1}$). No precipitation occurred when 2% Cetavlon solution was added to the oxygenated solutions, but the three amino-acid solutions irradiated *in vacuo* gave large precipitates. The freeze-dried non-volatile solutes from the evacuated systems were readily soluble in water, with the exception of the phenylalanine polymer which was soluble only to the extent of *ca.* 2 mg./ml. [cf. mandelic acid precipitate (no. 10)].

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