[CONTRIBUTION NO. 32 FROM THE RETINA FOUNDATION, DEPARTMENT OF OPHTHALMOLOGY, MASSACHUSETTS EYE AND EAR INFIRMARY, AND HARVARD MEDICAL SCHOOL]

Effect of Ultraviolet Light on Alkaline Solutions of Glucose and Certain Other Sugars[1,2]

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RECEIVED JULY 22, 1955

In the course of ultraviolet irradiation of alkaline solutions of different monosaccharides, compounds are formed which exhibit an absorption band between 200–300 mμ. The absorption maximum of irradiated glucose at 265 mμ shifts to 245 mμ after acidification below *p*H 3. In nitrogen atmosphere the formation of these compounds is accelerated by heat. The calculated *pK* of the ionizable chromogen group is 4.5. The possible nature of the ultraviolet absorbing substances is discussed.

It was reported in an earlier paper[3] that in the course of ultraviolet irradiation of alkaline solutions of certain carbohydrates an absorption band was found, with a maximum at 265 mμ. Similar observations were described earlier by Holtz[4,5] who irradiated monosaccharides at *p*H 7.6 with ultraviolet light and X-rays. He found that in an oxygen atmosphere compounds were formed which had strong negative oxidation–reduction potentials and which showed absorption maxima between 265 and 290 mμ. The experimental conditions used by Holtz were quite different from the conditions described here, and a different absorption maximum was obtained for irradiated glucose (290 instead of 267 mμ). Berl and Feazel[6] studied a substance formed in alkaline glucose solutions after these had been allowed to stand at room temperature for several days. The maximal optical density was at 295 mμ in alkaline solution, and at 265 mμ in acid solution. Its *pK* was 7.5. This differs markedly from our substance. In order to avoid the reaction described by Berl and Feazel, only fresh sugar solutions were used in our experiments. Recently, Phillips[7] studied the effect of ionizing radiation on aqueous solutions of carbohydrates and found that uronic acids were formed.

The ultraviolet spectrum of a fresh alkaline glucose solution (*p*H > 9) shows only end absorption at wave lengths below 230 mμ. After ultraviolet irradiation (wave length 200–400 mμ) a strong maximum appears at 267 mμ. When the irradiated solution is acidified, the optical density decreases and the maximum is shifted to 245 mμ (Fig. 1). Within the range of our determinations (optical density <4.0) the ultraviolet absorption follows Beer's law both in neutral and in acid solutions at 267 and 245 mμ.

It was demonstrated earlier[3] that the yield of the absorbing material increases with increasing *p*H or glucose concentration. Furthermore, the yield of the absorbing material is higher in a nitrogen and lower in an oxygen atmosphere (Fig. 2). After a certain time of irradiation the optical density of the solution reaches a maximum, and then the amount of ultraviolet absorbing material decreases slowly with further irradiation. The optical density of the maximum varies with the glucose concentration, the *p*H and the temperature during the irradiation. Further irradiation when the maximum level is obtained gives yellow solutions and a spectrum quite different from the one described above. The influence of temperature is demonstrated in Fig. 3. There is a marked increase in the yield of this material with increasing temperature.

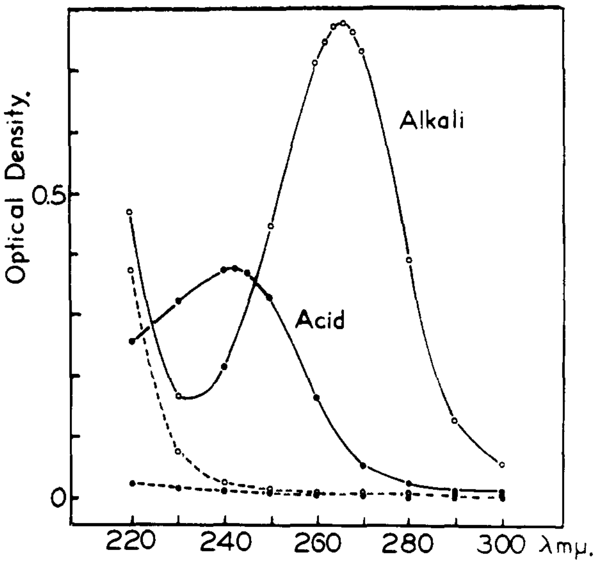

Fig. 1.—The absorption spectrum of 0.01 *M* *d*-glucose in 0.01 *N* sodium hydroxide before (------) and after (——) ultraviolet irradiation for 40 min. at 20° in nitrogen atmosphere. The absorption is measured at once at *p*H 11.5 (○) and after acidification at *p*H 2.0 (●).

At high concentrations of alkali and sugar, and at high temperatures, there are several side reactions, and very complex absorption spectra are obtained. It was therefore decided to perform the experiments at 20° in a nitrogen atmosphere, using solutions which were 0.01 *M* with respect to both sugar and sodium hydroxide. Under these conditions irradiation times up to two hours gave the absorption spectrum shown in Fig. 1.

In order to find the most active band of radiation, a glass filter was used. It transmitted 26% of the total energy; at 300 mμ it transmitted 65%, at 280 mμ 25%. For different times of irradiation (up to 40 minutes exposure at 74°) with this filter, the rise in the absorption of the sugar solutions was less than 0.5% of the rise without filter. This means

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(1) This paper was presented before the Division of Carbohydrate Chemistry at the 124th Meeting of the American Chemical Society, Chicago, Ill., September, 1953.

(2) This investigation was supported in part by funds provided under the Contract AF 18(600)627 with the USAF School of Aviation Medicine, Randolph Field, Texas, and by the Kresge Foundation.

(3) T. C. Laurent and E. M. Wertheim, *Acta Chem. Scand.*, **6**, 678 (1952).

(4) P. Holtz, *Arch. exp. Path. Pharmakol.*, **182**, 141 (1936).

(5) P. Holtz and J. P. Becker, *ibid.*, **182**, 160 (1936).

(6) W. G. Berl and C. E. Feazel, *Abstr. Am. Chem. Soc. Meeting* (September, 1952).

(7) G. O. Phillips, *Nature*, **173**, 1044 (1954).

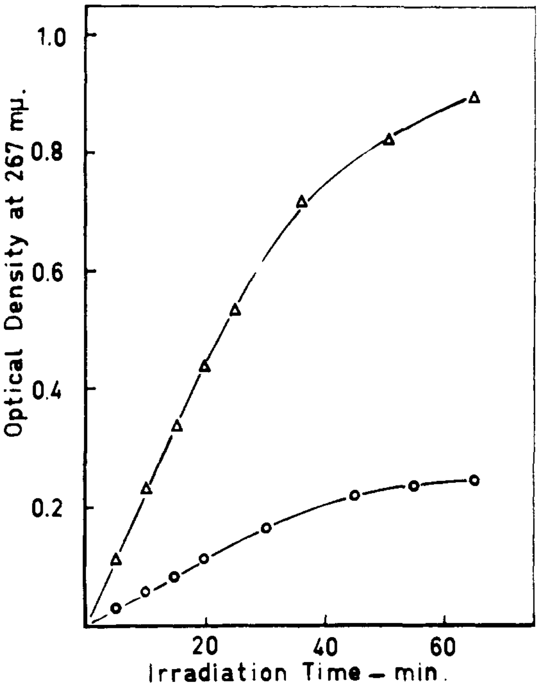


Fig. 2.—Increase of the optical density of glucose solutions (0.01 *M* *d*-glucose in 0.01 *N* sodium hydroxide) at 267 mμ following ultraviolet irradiation at 20° in nitrogen (△) and oxygen (○) atmosphere.

that the most active part of the spectrum is below 280 mμ.

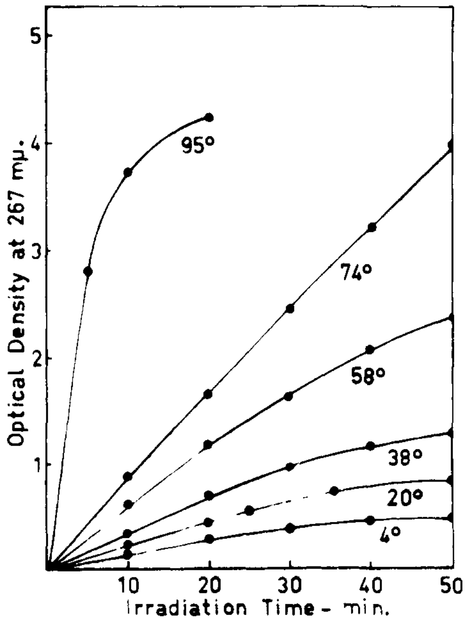


Fig. 3.—Increase of the optical density of glucose solutions (0.01 *M* *d*-glucose in 0.01 *N* sodium hydroxide) at 267 mμ following ultraviolet irradiation at different temperatures in nitrogen atmosphere.

The shift of the absorption with increasing hydrogen ion concentration suggests the presence of an ionizable chromophore group. The degree of ionization at different *p*H's can be determined from the optical density values. We assume that above *p*H 7 the substance is fully ionized and below *p*H 2 the ionization is negligible because the absorption spectra are constant within these ranges. The optical density (*D*) at two different wave lengths is measured for the same solution at *p*H 8.5, *p*H 1.0 and intermediate *p*H's (*p*H x), and the degree of ionization (α) for each *p*H is calculated as

$$\alpha = \frac{D_{pH\,x} - D_{pH\,1}}{D_{pH\,8.5} - D_{pH\,1}}$$

α was calculated at 267 and 245 mμ. The values obtained for the two wave lengths did not differ from each other by more than 0.02. Figure 4 shows the calculated dissociation curve which corresponds to a *pK* value of 4.5.

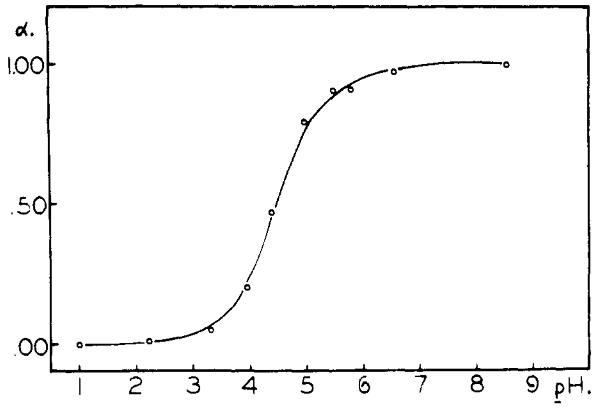
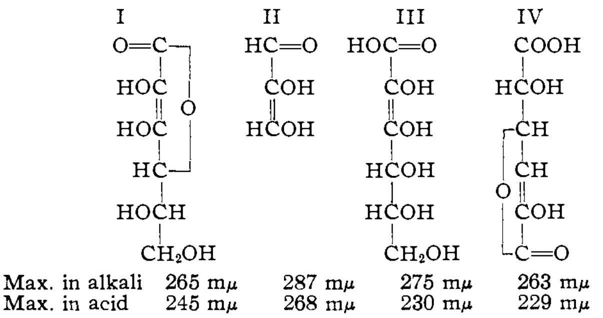


Fig. 4.—Dissociation curve of the ultraviolet absorbing material calculated from the optical density values (*pK* 4.5); points calculated from the experimental data; full line: theoretical dissociation curve for a monovalent acid, *pK* 4.5.

There are four substances described in the literature with absorption spectra similar to the one described above, *i.e.*, ascorbic acid (I),[8] reductone (II),[9] 2-keto-gluconic acid (dienol form) (III)[10] and 4-desoxy-5-keto-3,6-mannosaccharolactone (IV).[11] The four substances have in common a carbonyl group on the first (or sixth) carbon atom and an enolized keto group adjacent to it.



	I	II	III	IV
Max. in alkali	265 mμ	287 mμ	275 mμ	263 mμ
Max. in acid	245 mμ	268 mμ	230 mμ	229 mμ

It is highly probable that the latter group is formed during irradiation, either by the oxidation

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[8] R. W. Herbert, E. L. Hirst, E. G. V. Percival, R. J. W. Reynolds and F. Smith, *J. Chem. Soc.*, 1270 (1933).

[9] H. von Euler and C. Martius, *Ann.*, **505**, 73 (1933).

[10] P. Niederhoff, *Z. physiol. Chem.*, **181**, 83 (1929).

[11] W. N. Haworth, D. Heslop, E. Salt and F. Smith, *J. Chem. Soc.*, 217 (1944).

of an α-hydroxyl group and subsequent enolization, or by dehydration between C_2 and C_3, resulting in the direct formation of the enol.

The irradiation of a glucose solution in a nitrogen atmosphere is accompanied by a decrease in both *p*H and reducing power. The decrease of hydroxyl ion concentration is equivalent to the decrease of reducing power of the solution (Fig. 5). When irradiation was performed in an oxygen atmosphere, the decrease in reducing power was not accompanied by an equivalent decrease in hydroxyl ion concentration.

When an alkaline solution of gluconic acid was irradiated in a nitrogen atmosphere for the same length of time as glucose, no change in the *p*H of the solution could be observed.

Irradiation of other hexoses (galactose, mannose, fructose, rhamnose and fucose), pentoses (ribose, arabinose and xylose), sucrose and gluconic acid gave the maxima and optical densities shown in Table I. Neither *d*-glyceraldehyde nor glycerol showed any increase in ultraviolet absorption when irradiated under the same conditions as the other sugars. At higher temperatures and concentrations, however, a maximum was formed at 267 mμ in the glyceraldehyde solution, but this formation was about 1/20 as fast as in glucose solution. This might be due to hexose formation during the irradiation.[12]

TABLE I

ABSORPTION MAXIMA AND OPTICAL DENSITIES OF DIFFERENT SUGARS

20 ml. of 0.01 *M* sugar solutions in 0.01 *M* NaOH irradiated for 20 min. in N_2 atmosphere.

Compounds	Max. at mμ	Op. den.
d-Glucose	267	0.44
d-Galactose	267	.60
d-Mannose	264	.31
d-Fructose	266	.47
l-Rhamnose	265	.28
l-Fucose	266	.29
d-Ribose	259	.22
l-Arabinose	256	.17
d-Xylose	252	.25
Sucrose	266	.45
Gluconic acid (and its γ- and ϑ-lactones)	265	.70

Experimental

Irradiation.—Twenty ml. of 0.01 *M* sugar solution in 0.01 *N* sodium hydroxide was placed in an open glass beaker with a surface area of 10 $cm.^2$ and irradiated from above for 20 minutes by a General Electric Uviarc (UA-3) lamp. The lamp had strong emission lines, with about the same order of magnitude of energy, at 254, 265, 297, 302, 313 and 365 mμ. The distance between the bulb and the surface of the solution was 25 cm. No fluctuation in the lamp output was observed when measured by a uranyl oxalate actinometer. A constant stream of nitrogen or oxygen was passed through the solution by a capillary. The solution was kept at a constant temperature in a water-bath. The effect of an increase in concentration of the solutions as a result of evaporation at 20° was negligible (<2%), but at higher temperatures the solutions had to be diluted to the original volume after irradiation. A Corning ultraviolet transmitting glass filter (color specification 9-53, thickness, 1.9–2.1 mm.) was used to filter out the shorter wave lengths.

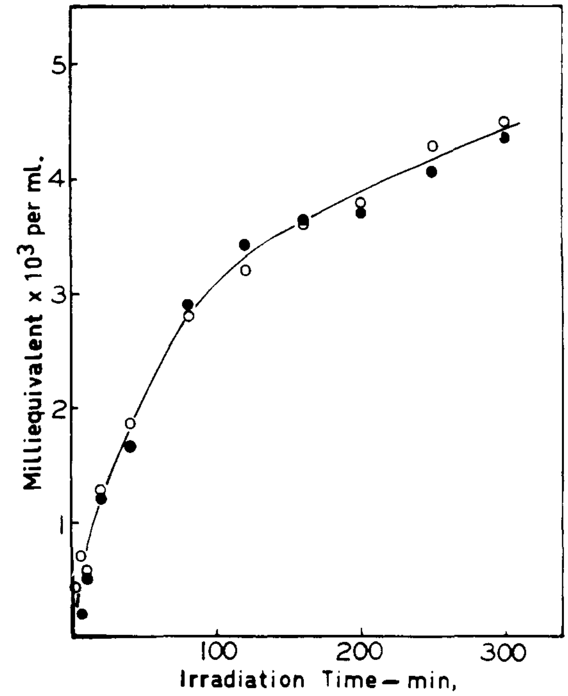

Fig. 5.—Decrease of hydroxyl ion content (●) and reducing substance (○) during 5 hours of irradiation of 20 ml. of 0.01 *M* *d*-glucose solution in 0.01 *N* sodium hydroxide at 20° in nitrogen atmosphere.

Analysis.—The optical density was measured in quartz cells with a Beckman model DU spectrophotometer tested according to Haupt.[13] The *p*H was measured with a Beckman model G *p*H meter and a glass electrode. The *p*H was adjusted with 0.1 *N* HCl or 0.1 *N* NaOH. Reducing substance was determined by Park-Johnson's ferricyanide reduction method.[14] The hydroxyl ion concentration was measured by titration with 0.01 *N* HCl with brom thymol blue as indicator.

Acknowledgments.—The author is greatly indebted to Dr. Endre A. Balazs for his invaluable help and advice and to Drs. Christine Bothner-By and John Gergely for their helpful criticism of the manuscript.

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[12] W. G. Berl and C. E. Feazel, THIS JOURNAL, **73**, 2054 (1951).

[13] G. W. Haupt, *J. Research Nat. Bureau Standards*, **48**, 414 (1952).

[14] J. T. Park and M. J. Johnson, *J. Biol. Chem.*, **181**, 149 (1949).