demonstrably more effective in producing passive transfer reactions, or in shocking sensitized guinea pigs.

The obvious fact that biological tests on castor bean allergenic preparations are more sensitive than chemical tests for a given constituent provides justification for having employed the involved procedures used to isolate CB-65A. That CB-65A was obtained by such prolonged, varied, and drastic fractionation must be regarded as substantiating evidence that its activity is inherent and not owing to the presence of a difficultly removable contaminant. Osborne, Mendel and Harris[22] used similar reasoning in deciding that the powerful toxic action of ricin, from castor beans, was inherent in the albumin fraction exhibiting maximum toxicity and not owing to a difficultly removable contaminant.

Summary

An essentially carbohydrate-free allergenic protein, CB-65A, has been isolated from fraction CB-1A from castor beans by a prolonged series of procedures involving chromatographic adsorption of the picrate, electrophoretic recovery of the protein from its picrate, electrophoresis of the protein, and solvent fractionation. Elementary analyses and properties of CB-65A are compared with those of an analogous fraction CS-60C from cottonseed.

Positive passive transfer reactions were produced by 1 × 10^{-10} g. of CB-65A. Fraction CB-65A was antigenic as shown by tests using guinea pigs. CB-65A was as potent as precursor fraction CB-1A in producing passive transfer reactions and in shocking guinea pigs, but its sensitizing capacity was approximately one-eleventh that of CB-1A. Available evidence indicates that the principal allergenic and anaphylactogenic specificities of both CB-1A from castor beans and CS-1A from cottonseed are inherent in the essentially carbohydrate-free protein fractions CB-65A and CS-60C obtained from them.

WASHINGTON, D. C. RECEIVED FEBRUARY 17, 1944

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(22) Osborne, Mendel and Harris, *Am. J. Physiol.*, **14**, 259 (1905).

[CONTRIBUTION FROM THE INSTITUTE OF PAPER CHEMISTRY]

The Action of Ultraviolet Light upon Cellulose. I. Irradiation Effects. II. Post-Irradiation Effects[1]

BY ROBERT A. STILLINGS[2a,b] AND ROBERT J. VAN NOSTRAND[2a,3]

Irradiation Effects[2a,b]

Although a number of studies of the effect of ultraviolet light on cellulose have been reported, the results are in many ways contradictory and incomplete, especially with respect to the role of oxygen and the mechanism of the reaction. The objectives of this investigation were to clear up existing discrepancies and to gain information as to the reaction mechanism.

Apparatus, Materials and Methods

Irradiation Apparatus.—The apparatus used in this investigation (Fig. 1) consisted of a gas purification train, an exposure chamber with a magnetic stirrer and capable of being evacuated, a mercury vapor lamp, and a gas analysis train with provision for the quantitative estimation of water, carbon dioxide, formaldehyde, hydrogen, carbon monoxide and organic combustibles.

The gas used as the atmosphere surrounding the cellulose sample during the experiment was passed first through a calibrated flowmeter of the orifice type. The gas line leading from the flowmeter had a side connection leading to a bubbler, in which the water level was maintained about two inches above the gas outlet. This side tube served as a safety valve, never permitting the gas pressure in the exposure chamber to rise above two inches of water. From the flowmeter, the gas passed through a calcium chloride tower to remove water vapor, a copper gauze furnace to remove oxygen, an Ascarite–Anhydrone bottle to remove carbon dioxide and the last traces of water vapor and, finally, through a three-way stopcock into the exposure chamber. The copper gauze furnace was of the type described by Savage and Ordal[4]; it consisted of 40-mesh copper wire gauze wound on a Calrod heating element (General Electric Company), the assembly being encased in a tight-fitting Pyrex tube. When oxygen was used as the gaseous atmosphere, the furnace was turned off; otherwise, the gas received the same purification treatment.

The outer shell of the exposure chamber consisted of an open-top brass cylinder 2.25 inches high by 10.25 inches in diameter (inside dimensions). Inside of this was a concentric, rotatable, open-top brass cylinder 1.5 × 10 inches (outside dimensions) supported on ball bearings. Two pieces of soft iron were fastened to the bottom of the inner cylinder, so that it might be turned by a motor-driven electromagnet rotating in a horizontal plane below the outer shell. Direct current was fed to the coils of the magnet by means of slip rings, about 50 watts being required for satisfactory operation of the agitator. The cellulose was placed in the inner cylinder. When the latter rotated, the material was mixed by stationary plows attached to the upper rim of the outer shell. The plow holder was removable, allowing the inner cylinder to be taken out. A mercury-in-glass thermometer, shielded from direct radiation, was attached to the plow holder. To keep the temperature down during irradiation, a fan blew air over the exposure chamber.

The cover consisted of four pieces (each 5.625 inches square) of Vycor high-silica ultraviolet-transmitting glass (Corning Glass Works), sealed in place with beeswax-

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(1) Original manuscript received December 14, 1942.

(2) (a) A portion of a thesis submitted in partial fulfillment of the requirements of The Institute of Paper Chemistry for the degree of Doctor of Philosophy from Lawrence College, Appleton, Wis. This work was carried out under the direction of Emil Heuser. Presented before the Division of Cellulose Chemistry at the 106th Meeting of the American Chemical Society, Pittsburgh, Pa., Sept., 1943.

(2) (b) Present address, Kimberly-Clark Corporation, Neenah, Wis.

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(4) Savage and Ordal, *Science*, **91**, 222 (1940).

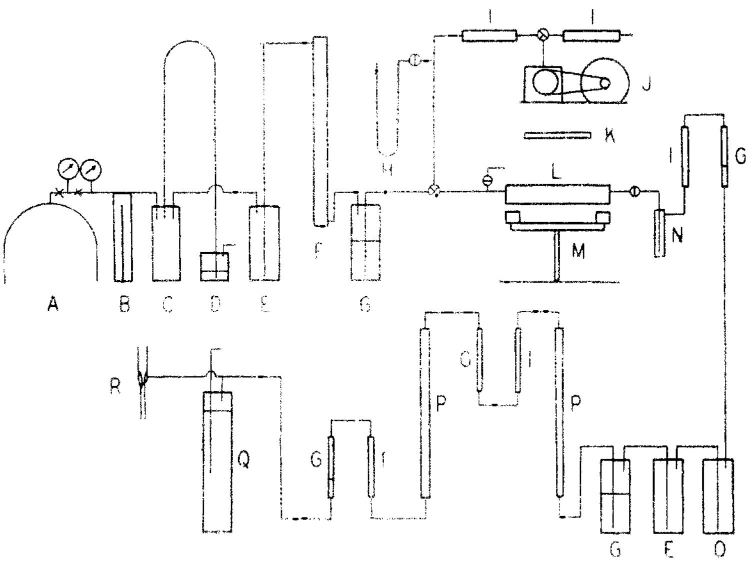

Fig. 1.—Schematic diagram of irradiation apparatus: A, nitrogen tank; B, flowmeter; C, trap; D, bubbler; E, $CaCl_2$ tube; F, copper gauze furnace; G, ascarite–anhydrone tube; H, manometer; I, anhydrone tube; J, Cenco pump; K, Uviarc lamp; L, exposure chamber; M, electromagnet; N, trap; O, NaOI tube; P, combustion tube; Q, pressure regulator (H_2O); R, aspirator.

rosin mixture at the start of each experiment. An auxiliary cover (consisting of a vacuum desiccator lid) was placed on top of the Vycor glass during evacuation, which was carried out with a Cenco Hyvac pump; the pressure was indicated by a closed-tube mercury manometer opening into the evacuation line, which also contained an Anhydrone tube to absorb water vapor.

A General Electric Portable Uviarc outfit, consisting of a UA-26A2 quartz tubular, A. C. Uviarc in a mat aluminum reflector, was used as the source of radiation. Technical data for this 360-watt discharge tube have been given by Johnson and Webster.[5] In all the experiments the distance from the discharge tube to the glass cover of the chamber was eight inches.

The gas emerging from the exposure chanber was passed through a glass-wool trap to remove any entrained particles of cellulose, through an Anhydrone tube for the estimation of water, an Ascarite–Anhydrone tube for the estimation of carbon dioxide, and an alkaline solution of standard iodine for the estimation of formaldehyde or other easily oxidizable substances. The mixture of gases then passed through a calcium chloride bottle to remove water, an Ascarite–Anhydrone bottle to remove any carbon dioxide and the last traces of water, and into the first cupric oxide-filled combustion tube. This tube was maintained at 260–270° for the combustion of carbon monoxide to carbon dioxide and hydrogen to water, which were estimated by absorption in Anhydrone and Ascarite–Anhydrone tubes. The gas was then passed into the second cupric oxide-filled tube, which was maintained at about 800° for the combustion of organic matter to carbon dioxide and water (estimated as before). The pressure drop through the gas analysis train was compensated by a pressure regulator connected to the end of the train, thus maintaining the pressure in the exposure chamber at the desired value.

The procedure followed in making a run on cellulose was as follows: The sample, which had been dried at room temperature in a vacuum desiccator over Anhydrone, was introduced into the exposure chamber. The latter was closed and the system evacuated. The cellulose sample was dried either by means of a small electric hotplate beneath the exposure chamber (35–40°) or by radiation from a 250-watt General Electric infrared drying lamp in a gold-plated reflector (60°), while passing through the chamber a current of air or nitrogen dried over Anhydrone. The first method permitted drying of the cellulose to 0.2% and the latter to less than 0.02% moisture (as determined by oven drying at 105°).

When the exposure was to be made in the absence of oxygen, the drying period was followed by repeated evacuation to a pressure less than 0.1 mm. of mercury and admission of purified nitrogen to a pressure of about 200 mm. of mercury. Calculation showed that seven of these cycles were sufficient to eliminate all oxygen which can be removed by the procedure. The entire system, including the gas analysis train, was then swept out by passing purified nitrogen through it for about five hours at a rate of 50 ml. per minute. The infrared lamp was then replaced by the mercury arc and the gas flow was continued at 50 ml. per minute throughout the exposure, except when the absorption tubes were being weighed.

Materials.—Hercules cotton linters, cut to an average fiber length of 0.4 mm. by the Clairemont Waste Manufacturing Co., had the characteristics given in Table I. Purified *β-d*-glucose[6] and *β*-cellobiose gave specific rotations of $[\alpha]^{0}\text{D}$ 20.0° (water, $C = 5.02$) and $[\alpha]^{20}\text{D}$ 35.8° (water, $C = 3.82$), respectively.

Tank nitrogen, after passage through the copper gauze furnace, contained about 0.002% by volume of oxygen as determined by the starch-iodine complex.[7]

Analytical Methods.—Copper number (according to Schwalbe), *α*-cellulose, ash and furfural were determined according to generally accepted methods. The reducing power of the glucose and cellobiose was determined by the method of Auerbach and Bodländer.[8] Staudinger's viscosity method was used to determine the average chain length of the cellulose, employing the apparatus and procedure described by Heuser and Green.[9] In calculating the degree of polymerization, (D. P.), 5×10^{-4} was used as the value of Staudinger's m constant.

Blank Tests.—To ascertain any increase in weight of the gas analysis absorption tubes when nitrogen or oxygen was passed through the system, blank tests were run before and after each exposure to ultraviolet light, and the small increases observed were taken into consideration. In addition, a gas mixture of known composition (consisting of carbon dioxide, hydrogen, carbon monoxide and methane) was allowed to pass through the apparatus while the chamber was being exposed to ultraviolet light and was analyzed as described above. The accuracy was quite satisfactory, and there was no indication that any appreciable interaction of these gases took place under the action of the ultraviolet light.

Standardization.—In all experiments with cellulose, the weight of sample used was 38.87 g. (oven-dry basis). The temperature of the cellulose during exposure was 45±5°. In all experiments except 8, the rate of gas flow through the apparatus was maintained at 50 ml. per minute. A positive pressure of about one inch of water was maintained in the sample chamber during exposure. The time of irradiation varied between 24 and 336 hours.

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(5) Johnson and Webster, *Rev. Sci. Instruments*, **9**, 325 (1938).

(6) Hudson and Dale, THIS JOURNAL, **39**, 320 (1917).

(7) Snell and Snell, "Colorimetric Methods of Analysis," 2nd edition, Vol. 1, p. 133, D. Van Nostrand Co., New York, N. Y., 1936.

(8) Auerbach and Bodländer, *Angew. Chem.*, **36**, 602 (1933).

(9) Heuser and Green, *Ind. Eng. Chem.*, **33**, 868 (1941).

TABLE I

IRRADIATION IN NITROGEN AND EFFECT OF OXYGEN

Expt.	Atmosphere	Length of exp., hr.	Final H_2O, %	Copper no.	Time,[a] days	α-Cellulose, %	Time,[a] days	D. P.[b]	Time,[a] days	Gases evolved millimoles	
										CO_2	CO
Unexposed			2.82	0.28		96.8		1400			
5	N_2	24	0.22	.42	4	95.1	4	1290	4	0.06	0.08
				.44	103			1070	93		
10	10% O_2	24	.00	.58	4-5	92.2	4-5	950	50	.18	.17
9	20% O_2	24	.02	.78	4-5	90.0	4-5	870	57	.22	.22
6	O_2	24	.24	1.49	4-5	82.9	4-5	740	85	.39	.37
Blank	O_2	22	.28	0.29	..	..	..	..	..	..	..
4	N_2	96	.00	.65	7	92.6	7	1040	16		
				.80	97	88.6	108	930	35	.24	.39
								870	84		
								860	113		
8	N_2	96	.30	.70	11	91.4	11	1050	12		
								840	63	.34	.44
								860	88		
2	N_2	48	.19	.50	8	93.8	8	1020	64	.12	.15
								970	146		
3	N_2	48	.38	.53	4	93.8	4	1000	71	.21	.22
				.66	131			960	106		
7	H_2O, N_2	48	4.34	.40	17	95.8	17	1260	11		
				.40	95			1210	17	.16	.11
								1230	86		
12	N_2	336	0.09	1.20	20	88.8	20	860	19		
								780	26	.33	.88
								700	39		

[a] The values in the column headed "Elapsed Time" indicate the number of days elapsed between the beginning of the exposure and the determination. The ash content of the unexposed material was 0.086%; the copper content of the ash amounted to not more than 0.4 p. p. m. and its iron content was not more than 16 p. p. m. Where the atmosphere was part oxygen, the rest was nitrogen. [b] D. P. is used to signify degree of polymerization.

Experimental Results

Experiments 4, 5, 6, 8–10, as well as the blank test, in Table I, summarize the results of a series designed to discover the effect of oxygen during the irradiation of cellulose. As will be discussed later in more detail, the specific viscosities as well as the copper numbers and the α-cellulose contents, continued to change for several months after the samples had been removed from the exposure chamber and while they were being stored in stoppered glass bottles. These changes were quite unexpected and were observed incidentally only toward the end of this investigation. Nevertheless, the values quoted in Table I would seem to be fairly comparable, either because the analyses (copper number and α-cellulose) were made after approximately the same elapsed time or the elapsed time was sufficiently long to insure approach to the ultimate values (D. P.).

The fact that the blank experiment in the presence of oxygen without irradiation caused no significant increase in the copper number of the cellulose, eliminated heating (43°) during drying and exposure and also the presence of oxygen in itself as the cause of the effects observed in the other experiments. These effects, measured by changes in degree of polymerization, copper number, α-cellulose, and by the evolution of gases must, therefore, have been photochemical, and experiments 4, 5 and 8 show them to be considerable, even in samples exposed in nitrogen with all possible precautions to exclude and remove oxygen. When oxygen-containing atmospheres were used (Experiments 10, 9 and 6), the rate of degradation of the cellulose increased over the whole range of oxygen concentration.

In spite of the precautions to remove and exclude oxygen during the exposures in nitrogen, significant amounts of oxygen could have been present in the nitrogen atmosphere or the cellulose. A comparison of experiments 4 and 8 eliminates the first possibility. The two experiments were identical, except that in experiment 4 nitrogen was passed continuously through the system, whereas in experiment 8 the nitrogen-filled chamber was sealed off. If the oxygen in the nitrogen were responsible for the degradation, the effect should have been greater in experiment 4, but this was not the case. In experiment 8, the calculated amount of oxygen introduced with the nitrogen (0.002% by volume of oxygen) was 0.024 millimole or 1.8×10^{-4}%, based on the cellulose. To prepare an oxycellulose of the same copper number as exposed sample 8 by other methods would require at least 0.05% oxygen, or 280 times the amount which was present in the nitrogen in experiment 8.

If oxygen retained by the cellulose had been the sole cause of the degradation, the rate of reaction would be expected to be maximum at the beginning and fall off to zero as the oxygen was consumed. This was not the case. During experiment 12, the rates of evolution of water, carbon dioxide, and carbon monoxide actually *increased* continuously during the course of the irradiation.

Kinetics and Mechanism of the Reaction in Nitrogen.—A study of the kinetics of the reaction was based upon experiments 2–8 and 12 of Table I.

Decrease in Chain Length.—For the early stages of a chain-splitting reaction, Sakurada and Okamura[10] developed the following equation relating the average number of scissions per molecule to the change in average degree of polymerization as determined by the viscosity method.

$$P^1/P = (2/S^2)(S - 1 + e^{-S})$$

where P = original D. P.
P^1 = D. P. after the reaction, and
S = average number of scissions per molecule.

Although the application of this equation to the present case would appear to be somewhat hampered by the instability in which the samples are left after irradiation, an attempt was made to calculate S by taking P^1 as the ultimate D. P.—*i. e.*, that reached after the post-irradiation effect had practically come to an end.

If the photolysis proceeded as a first order reaction, the rate of cleavage (rate of change of S) should have been essentially constant over the limited range studied. Figure 2 is a comparison of the actual rate of decrease of chain length with a theoretical, first-order rate chosen to make the curves intersect at the two-day mark. It is apparent that the reaction cannot be regarded as first-order, as the actual rate was too fast near the start and became too slow as the degradation progressed.

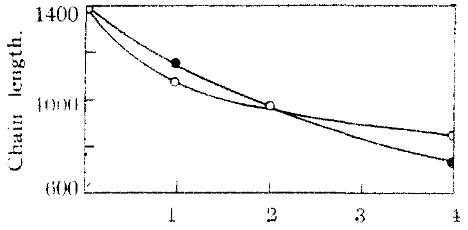

Fig. 2.—Actual (○) and theoretical (●) decrease in degree of polymerization during irradiation in nitrogen.

Possible explanations of this difference are the presence of oxygen in the system at the start of each exposure, and the formation of products of reaction having a higher absorption of ultraviolet light than the original material.

If some oxygen were present at the start of each experiment, the rate of cleavage would be higher at the start and would come to a constant rate only after all the oxygen was consumed. A correction for such an effect might be applied by subtracting a constant from the calculated S values. When 0.45 is used as the constant, the corrected curve is first-order for the first forty-eight hours, but the ninety-six-hour value is still significantly off. Thus, the deviation from the first-order rate cannot be completely explained by assuming the presence of oxygen, although oxygen as a contributing factor is not ruled out. With regard to the second possibility, the irradiated material did indeed show a decreased reflectance in the blue end of the visible spectrum, and indications were that the difference was even greater in the ultraviolet.

A third possibility is that the difference is the result of the presence in cellulose of cross linkages which, according to Steurer,[11] could have undergone cleavage at a faster rate than did the predominating 1,4-glycosidic linkages.

Increase in Copper Number.—The above considerations are equally applicable in explaining the fact that the increase in copper number (increase in reducing groups) proceeded at a decreasing rather than at a constant rate.

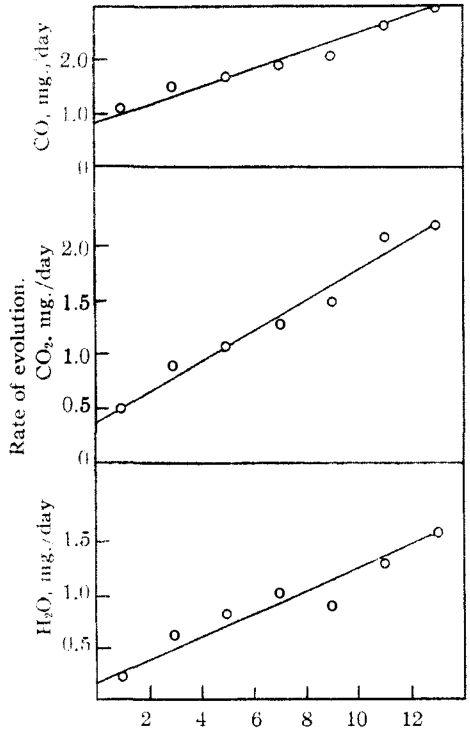

Fig. 3.—Ratio of evolution of gaseous products from cellulose during irradiation in nitrogen.

Formation of Gaseous Products.—Figure 3 shows that the rates of evolution of carbon monoxide, carbon dioxide, and water increased continuously during the course of the longest ex-

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[10] Sakurada and Okamura, *Z. physik. Chem.*, **A178**, 289 (1940).

[11] Steurer, *ibid.*, **B47**, 127 (1940); Steurer and Mertens, *Ber.*, **74**, 790 (1941).

posure made (experiment 12 of Table I). In the case of the first two, the increasing rate indicates that they were the products of secondary reactions or of both primary and secondary reactions. As the curves extrapolate to an appreciable rate at zero time, the latter alternative is more probable. The increasing rate of liberation of water may have been merely a result of the hygroscopic nature of the dried cellulose.

Control experiments were made in which glucose was irradiated for forty-eight hours and cellobiose for one hundred and eight hours. The rates of carbon monoxide and carbon dioxide evolution in millimoles per day were only 0.03 and 0.04 for glucose and 0.02 and 0.02 for cellobiose. As the rates for cellulose were 0.09 and 0.07, respectively (*i. e.*, roughly three times higher), it was concluded that these gases did not result from the photolysis of hemiacetal groups or glycosidic bonds in cellulose. The reducing power of both sugars remained constant to ± 0.2% during irradiation, but water solutions of both showed an increase in light absorption in the ultraviolet range.

Instability of Exposed Samples.—As mentioned above, degradation of the irradiated samples continued after the conclusion of the actual exposure—*i. e.*, during storage in air in screw-top glass bottles. No degradation occurred in the unexposed material stored under the same conditions during the investigation.

A portion of exposed sample 12 stored in nitrogen in the dark showed no decrease in chain of length during a two-week period, whereas the portion stored in air in the light of the laboratory underwent a drop in D. P. from 780 to 700. Two portions of another sample which had been irradiated 48 hours in the nitrogen atmosphere and had a D. P. of 1280 were stored in nitrogen and in air in the light of the laboratory. After six days of storage the sample in nitrogen showed a D. P. of 1310, whereas the sample in air had dropped to 1200. These results seem to suggest that the post-irradiation effects were due to oxidation by atmospheric oxygen. This conclusion is borne out further by the results of an experiment in which the viscosity of the last mentioned sample was determined as soon as possible after irradiation (about one hour's contact with air during the necessary manipulations). The D. P. thus determined was 1280 as compared with the original value of 1400 and with the ultimate D. P. after the post-irradiation effect of 960.

Reaction Mechanism.—If only the reducing end groups were involved in the primary reaction, no appreciable decrease in chain length would result unless the attack continued progressively along the chain, in which case the α-cellulose would show a decrease corresponding to the decrease in D. P. This was not the case. Another indication that the reducing end groups were not appreciably affected is the fact that irradiation of glucose and cellobiose resulted in no measurable change in reducing power.

Other investigators[12] have shown that, in the absence of oxygen, simple alcohols are stable to light of wave length above 200 mμ (lower wavelength limit in the present investigation was 230 mμ). This, together with the fact that no hydrogen was formed during irradiation of cellulose, indicates that photolysis of the alcohol groups did not occur, at least not in the usual manner.

If the decrease in chain length (measured immediately following exposure) was not caused by oxidation effects, it was, in all probability, the result of a photolysis of glycosidic linkages. The fact that the number 1 carbon atom is the only one linked to two oxygen atoms suggests this as the source of the carbon dioxide formed. Whether or not the photolysis of glycosidic linkages occurred as a primary reaction, there must have been some other primary reaction which did not break the chains but left them unstable to atmospheric oxygen, as evidenced by the post-irradiation decrease in chain length. The possibility that, under the action of ultra-violet light, the oxygen was converted into ozone (in which form it is known to degrade cellulose without the aid of ultraviolet light) is ruled out because no ozone could be detected inside the exposure chamber, although the surrounding atmosphere was pregnant with ozone while the lamp was operated.

The question arises whether the other measured changes in the cellulose were concerned in the reaction which led to chain splitting. Some indication may be obtained by comparing the *S* values calculated by the Sakurada equation with the formation of gases and reducing groups expressed as millimoles per millimole of cellulose (227 g. in this case). Table II is a tabulation of such a comparison. There is a fairly good correspondence between the *S* values and the carbon monoxide and dioxide values. This is a strong indication that the formation of these gases was involved in the reaction which led to chain cleavage, one molecule of each gas being produced per scission.

TABLE II

RELATIONSHIP OF ANALYTICAL RESULTS

Expt.	D. P.	*S*	Reducing power,[a] milliequiv.	CO,[a] millimoles	CO_2,[a] millimoles
5	1070	0.87	5.0	0.47	0.35
2	970	1.22	7.9	0.88	0.70
3	960	1.26	13.6	1.29	1.23
4	860	1.70	18.6	2.28	1.40
8	860	1.70	15.0	2.57	1.99
10	950	1.30	13.5	0.99	1.05
9	870	1.65	17.8	1.29	1.29
6	740	2.31	44.3	2.16	2.28
7	1230	0.40	4.3	0.64	0.94

[a] Based on one millimole or 227 g. of cellulose.

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(12) Berthelot and Gaudechon, *Compt. rend.*, **156**, 68 (1913); Farkas and Hirschberg, THIS JOURNAL, **59**, 2450 (1937).

However, the reducing power values (calculated from the increase in copper number) are about ten times the *S* values, corresponding to the production of five aldehyde or similar groups per chain split, which seems improbable. More likely, the reducing groups were formed, at least in part, from some other reaction not ultimately resulting in chain cleavage.

The fact that carbon dioxide and monoxide were formed, necessarily means that anhydroglucose units were decomposed. No change in pentosan content could be detected, but the possibility remains that desoxypentoses or carbohydrate units of fewer carbon atoms were formed.

Post-Irradiation Effect[2a,3]

To establish more firmly the conditions under which the post-irradiation effect occurred, portions of the exposed cellulose samples were analyzed immediately after exposure, whereas the remainder were stored in the desired atmosphere by transferring them—immediately after exposure—to round-bottom flasks which were evacuated, subsequently flushed, filled with purified nitrogen, air, or oxygen and finally sealed. The analysis of the samples was confined to the degree of polymerization, the copper number, and the α-cellulose content. The time of irradiation was, except in one case, ninety-six hours, whereas the storage time varied.

The equipment used in the first part of this investigation was improved in various respects. Particularly, the construction of the irradiation chamber was such that it withstood a considerably higher vacuum than previously employed to remove the air from the system as far as possible. The vacuum amounted to 25–30 microns as read from a Pirani gage.

Evacuation and flushing with nitrogen were repeated five times, after which only $4.38 \times 10^{-17}\%$ of the original atmosphere was present.

For the purification of the tank nitrogen, the copper gauze was replaced by colloidal copper deposited upon diatomaceous earth in accordance with the suggestion made by Meyer and Ronge.[13] In this way, the oxygen content of the tank nitrogen was reduced to as low a value as 3.4×10^{-4} (against 2×10^{-3} in the first part of the investigation).

Because the Uviarc lamp had lost a good deal of its efficiency in the previous experiments, the effect of irradiation was relatively small, as was the post-irradiation effect during storage of the irradiated sample in air at room temperature (Table III, run N2). However, the latter effect was considerably increased when the temperature during storage in air was raised to 70° (run N5) or when the air at room temperature was replaced by oxygen (run N4). Only a small part of the increased post-irradiation effect at 70° was due to the temperature alone, as may be seen from the extent to which an unirradiated sample was attacked when subjected to 70° in air for about the same length of time as was used for storing the irradiated sample (run N3).

TABLE III

CHANGE OF CHARACTERISTICS OF CELLULOSE ON IRRADIATION AND SUBSEQUENT STORAGE IN VARIOUS ATMOSPHERES

Experimental runs	Description	Degree of polymerization	Copper number	α-Cellulose content, %
	Original	1250	0.31	97.5
	Irradiated in nitrogen	1155	.62	94.9
N2	Stored in air for 25 days	1072	.67	..
	Stored in air for 60 days	1079	.61	94.4
	Irradiated in nitrogen	1100	.61	94.8
N5	Stored in air at 70° for 60 days	688	1.07	92.0
N3	Original cellulose, merely heated at 70° for 55 days	1060	0.37	..
	Irradiated in nitrogen	1130	.61	95.1
	Stored in nitrogen for 20 days	1155	.60	..
N4	Stored in nitrogen for 30 days, followed by 10 days in oxygen	904	.68	..
	Same as above, followed by 20 days in oxygen	854	.75	93.2

It is of interest that, even if the effect of temperature on the unirradiated cellulose sample is taken into account, the results show that irradiation in nitrogen had sensitized the cellulose to a far greater extent than could be derived from the drop in degree of polymerization from 1250 to 1155 or from that which occurred when the irradiated sample was stored in air at room temperature (from 1155 to 1072). It is seen also from Table III that the post-irradiation effect becomes practically constant if the oxygen is given sufficient time to act (N4, last two columns).

The irradiation effect from a new and stronger Uviarc lamp (600 as compared with 300-watt)

TABLE IV

CHANGE OF CHARACTERISTICS OF CELLULOSE ON IRRADIATION IN NITROGEN AND SUBSEQUENT STORAGE ALTERNATINGLY IN NITROGEN AND OXYGEN

Experimental Run N6

Description	Degree of Polymerization	Copper number	α-Cellulose Content, %
Irradiated	823	1.25	89.0
Stored, 10 days in nitrogen	787	1.36	89.0
Followed by 5 days in oxygen	665	..	87.3
Followed by 5 days in nitrogen	669	1.43	..
Followed by 23 days in oxygen	565	..	..
Followed by 7 days in oxygen	557	1.72	86.0

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(13) Meyer and Ronge, *Angew. Chem.*, **52**, 637 (1939).

was much greater as seen from the data in Table IV. In addition, it is shown and further illustrated in Fig. 4 that the post-exposure effect could be deferred or produced *ad libitum*, depending upon whether the sample was stored first in nitrogen and then in oxygen, then again in nitrogen, and finally again in oxygen. If sufficient time was allowed for this last phase, the post-exposure effect, as judged from the change in degree of polymerization, came to an end.

In the following series of experiments, irradiation was allowed to take place in oxygen instead of in nitrogen. As would be expected, the irradiation effect was much enhanced under these conditions. The degree of polymerization dropped from 1250 to a value as low as 450, the copper number increased from 0.31 to 5.92, and the α-cellulose content decreased from 97.5 to 56.8.

When the sample irradiated in oxygen was subsequently stored in oxygen for forty days, further drop in the degree of polymerization and increase in the copper number (6.45) occurred. However, when the storage in oxygen was lengthened by ten more days, the degree of polymerization remained practically constant, showing that the post-irradiation effect had come to an end. The data are shown in Table V.

TABLE V

CHANGE OF CHARACTERISTIC OF CELLULOSE IRRADIATED IN OXYGEN AND SUBSEQUENTLY STORED IN OXYGEN

	D.P.
Irradiated in oxygen for 4 days	450
Stored in oxygen for 40 days	320
Stored in oxygen for 10 additional days	333
Irradiated in oxygen for 10 days	272
Stored in oxygen for 10 days	240

This table contains also the data which were obtained when the cellulose was irradiated in oxygen for ten instead of four days (as in all previous experiments) and when the sample thus treated was subsequently stored in oxygen for ten days. The degree of polymerization decreased to 272 on irradiation in oxygen but remained practically unchanged on subsequent storage in oxygen.

The results reported above seem to confirm the previously advanced hypothesis—namely, that two reactions take place during the action upon cellulose of ultraviolet light in the absence of oxygen, one which results in the rupture of glycosidic linkages and another which does not rupture these linkages but leaves them in an unstable condition to oxygen. That these two reactions should take place simultaneously would imply that the glycosidic linkages are attacked to a different extent. This difference is possibly a result of the difference in their accessibility in the micellar system. Thus, the assumption may be made that those glycosidic linkages which belong to chains exposed on the surface are broken down completely, whereas those which belong to chains located in the interior of the micelles are only weakened. These weakened bonds are then completely ruptured by oxidation, either during irradiation in an oxygen atmosphere or during storage of the irradiated sample in oxygen. In other words, the activity of the oxygen may be eliminated from the irradiation stage and shifted to a subsequent and separate stage. Indeed, the two reactions—the action of oxygen during irradiation and the post-irradiation reaction—seem to be quite alike, the same changes being produced. Likewise, if a portion or all of the oxidation of weakened glycosidic linkages is allowed to take place during irradiation, only part or practically none of it occurs in the post-irradiation stage.

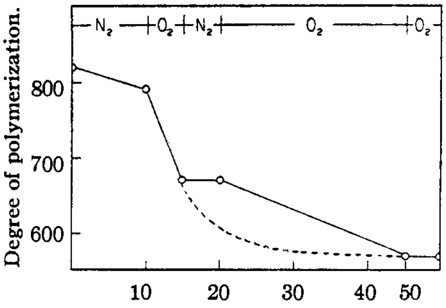


Fig. 4.—Change in degree of polymerization of cellulose previously irradiated in nitrogen and alternately stored in nitrogen and oxygen.

The mechanisms by which a portion of the glycosidic linkages is ruptured while another portion is left in an unstable condition and by which the weakened bonds are subsequently ruptured by oxidation are still obscure. Consideration also will have to be given to the possibility that the cleavage of glycosidic linkages is the result of hydrolysis caused by the small amount of water left in the cellulose and catalyzed by ultraviolet light energy.

Summary and Conclusions

An apparatus has been developed which is suitable for the ultraviolet irradiation of solids in powder form in various atmospheres and which provides for evacuation prior to exposure, continuous mixing and continuous analysis of gaseous products.

The influence of oxygen on the photolysis of cellulose has been studied, as have the kinetics of the reaction in nitrogen. In addition, samples of glucose and cellobiose have been irradiated in a nitrogen atmosphere under the same conditions.

The most important results obtained in the investigation may be summarized as follows.

1. Cellulose irradiated in nitrogen with all possible precautions to remove and exclude oxygen showed considerable degradation (chiefly, a drop in degree of polymerization and α-cellulose content, an increase in copper number and libera-

tion of carbon monoxide and carbon dioxide), the amount of degradation increasing with lengthening time of exposure. These changes could not be explained simply by the presence of oxygen in the nitrogen or that retained in the cellulose at the start of the exposure.

2. The rate of degradation increased with increasing oxygen content in the atmosphere.

3. The rates of change of chain length and copper number during irradiation in nitrogen did not correspond to the rates calculated on the assumption that the reaction was of the first-order type.

4. Exposure of *β-d*-glucose and cellobiose likewise resulted in the production of carbon monoxide and carbon dioxide, but at a considerably slower rate than in the case of cellulose. No change could be detected in the reducing power of these sugars, but exposed samples showed an increased absorption of ultraviolet light.

5. Cellulose irradiated in the absence of oxygen was left in an unstable state at the conclusion of the exposure—*i. e.*, the changes which the cellulose underwent during irradiation continued to occur during storage when air was present, but ceased when air was absent.

6. The post-irradiation effect was increased by raising the temperature to 70° and by replacing the air in the storage bottles with oxygen. A small portion of the increased post-irradiation effect at 70° was due to the temperature alone.

7. The post-irradiation effect was deferred or produced *ad libitum* by alternating the atmospheres several times between nitrogen and oxygen. If the time of storage in oxygen was sufficiently long, the post-irradiation effect came to an end.

8. Cellulose which had been irradiated in the presence of oxygen still underwent the post-irradiation reaction. However, the effect was smaller and became almost negligible when the time during irradiation in oxygen was extended from four to ten days.

APPLETON, WISCONSIN RECEIVED FEBRUARY 12, 1944

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE ILLINOIS INSTITUTE OF TECHNOLOGY]

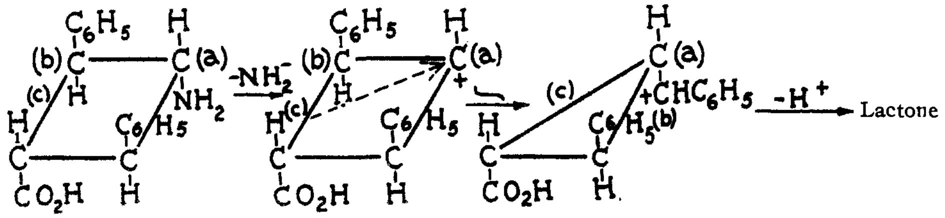
Studies in the Truxillic Acids. I. The Rearrangement of the Zeta-Truxinamic Acids and the General Theory of Molecular Rearrangements[1]

BY IRVING S. GOLDSTEIN[2] AND HERBERT I. BERNSTEIN

A general theory of molecular rearrangements was proposed by Whitmore[3] in 1932. The only essential modification since then has been that of the Walden inversion occurring on the carbon receiving the migrating group.[4]

Virtually all aliphatic rearrangements studied thus far fit into this theory. The evidence has been summarized briefly in a recent paper.[5]

It was further shown in that publication[5] that the amino acids of the truxillic and truxinic acid series lend themselves admirably for testing the stereochemical implications of the theory. Thus ε-truxillamic acid yielded the lactone of 1^c-carboxyl-2^c-benzoxyl-3^t-phenylcyclopropane upon treatment with nitrosyl bromide.[6] The theory would illustrate this as follows



The amino group is removed with its octet of electrons. Carbon a makes up for the resulting deficiency by taking a pair from the adjacent atom b, the pair constituting the bond c. The shifting electrons join carbon a opposite to the position held by the amino group. Thus carbon atom b, with only six electrons, is below the plane of the new cyclopropane ring. The missing electrons are supplied to b by a carboxyl oxygen and result from the loss of the carboxyl H, thus forming a lactone. The various steps need not be consecutive, but may all occur together, somewhat as in S_N2 substitution. Similar degradations of α-truxillaminic,[7] γ-truxillaminic,[8] β-truxinaminic,[9] and δ-truxinaminic acids[9] yielded isomeric cyclo-

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(1) Presented before the Organic Division of the American Chemical Society, Detroit, Mich., April, 1943.

(2) Submitted by Irving S. Goldstein in partial fulfillment of the requirements for the degree of M.S. Now serving in the U. S. Navy.

(3) Whitmore, THIS JOURNAL, **54**, 3274 (1932).

(4) Bartlett and Pockel, *ibid.*, **59**, 820 (1937).

(5) Bernstein and Wallis, *J. Org. Chem.*, **7** 261 (1942).

(6) Stoermer, Neumarker and Schmidt, *Ber.*, **58**, 2707 (1925).

(7) Stoermer and Keller, *ibid.*, **64**, 2783 (1931). See also ref. 8.

(8) Stoermer, Schenck and Pansegrau, *ibid.*, **60**, 2575 (1927).

(9) Stoermer and Ashrand, *ibid.*, **64**, 2793 (1931).