Effect of Gamma Irradiation upon Cellulose

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The purified cotton linter and purified wood pulp were irradiated by gamma radiation at various doses, and the number-average DP of each sample was determined according to the method worked out by Immergut *et al.* by the measurements of intrinsic viscosity. The log-log plots of the difference between the reciprocal of DP of the irradiated cellulose and that of the original cellulose against the radiation dose ranging from $5.2 \cdot 10^5$ to $1.1 \cdot 10^8$ roentgens gave a straight line, which indicated that the chain cleavage took place in proportion to $R^{0.85}$, R being the radiation dose. The rapid decrease in the leveling off DP with increasing dose suggested that the cleavage took place equally likely both in amorphous domains and in crystalline domains. The reduction in size of crystallites was shown by electron microscopic observation. The analysis by a mass-spectrometer of the gas evolved from deuterated cellulose during the irradiation revealed that it was composed mostly of H_2 and HD, being accompanied by only a small amount of CO, CO_2 and CH_4. The primary reactions induced by gamma radiation was discussed. The carbonyl group and the carboxyl group were identified in the irradiated samples, which, however, showed enormously large copper numbers in comparison with the carbonyl content. Therefore, the measurements of copper number results in greatly overestimated values of carbonyl content. The high ratio of oxidant to carbonyl content would be due to the formation of various alkali-sensitive groups by irradiation. The formation of carbonyl group and carboxyl group were estimated to be proportional to $R^{0.85}$ similar to the case of chain rapture. A considerable amount of formic acid ester was found in the irradiated samples, which also would be an origin of producing very high copper numbers. The paper chromatographic analysis showed the hydrolyzate of strongly irradiated cellulose to contain an appreciable amount of xylose, arabinose and glucuronic acid besides glucose, which suggested the transformation of carbohydrate components by irradiation. Hypothetic formulae were given.

INTRODUCTION

There has been a series of researches published in recent years on the effect of high energy radiation upon cellulose, to some of which reference will be made shortly.

Saeman, Millet and Lawton[1] studied the effect of high energy electron beam upon wood, wood pulp and cotton linter, and found that the effect of irradiation is not appreciable up to a dose of 10^5 equivalent roentgens, while the rapid decrease in degree of polymerization happened at the greater dosages. The three types of cellulose treated by them depolymerized at about the same rate, from which they concluded that the degradation would not be affected by the crystallinity. Charlesby[2] treated the viscosity data of Saeman *et al.* to verify the

theoretical relation deduced by him between intrinsic viscosity and dosage, and calculated the probability of main chain fracture per million roentgens. According to him a glucose unit was destroyed per one scission, and this accounted for the loss in weight at irradiation. Glegg and Kertesz[3] found an after-effect with the cellulose samples irradiated under sufficient dryness. As to the chemical modification of cellulose by irradiation Gilfillan and Linden[4] concluded from their experiments that an oxycellulose of acidic character containing cellulose peroxide is produced by gamma-irradiation. Blouin and Arthur[5] found the generation of carbonyl and carboxyl groups at the irradiation of cotton with gamma radiation. Florin[6] investigated the electron spin resonance of irradiated cellulose by gamma radiation and found the formation of free radicals.

The literature referred to above covers the important items concerning the effects of high energy radiation upon cellulose, but it is to be noticed that the studies such as to allow to postulate the chemical reactions have thus far been only little published. The main purpose of the present work is, therefore, to enhance the knowledge on the radiation chemistry of cellulose by supplying the materials which help to clarify the chemical changes induced by high energy radiation. However, the kinetics of the chain fracture will also be dealt with first because of its being of fundamental interest.

KINETICS OF MAIN-CHAIN FRACTURE

Reference must be made first to the method proposed by Charlesby[2] to analyze the kinetics of degradation of cellulose by high energy radiation. He presented an equation representing the relation between intrinsic viscosity of irradiated cellulose and radiation dose by introducing the concept of "virtual" radiation dose, which, however, prevents to solve the equation directly, because it cannot be determined independently. He, therefore, made use of the data in the region of very high doses where it was assumed that the virtual radiation dose could be ignored against the dosage applied, and the relations among the molecular weight, intrinsic viscosity and radiation dose were deduced in an indirect manner. This analysis is indirect further in the sense that the number-average molecular weight was not directly determined. Another approach was proposed by Arthur[7], who showed that the logarithm of viscosity-average degree of polymerization has a linear relation with the logarithm of radiation dose.

In the present study, attention has been drawn to the systematic study on the molecular weight of cellulose elaborated by Immergut, Rånby and Mark[8], who made it possible to find the number-average molecular weight directly from the intrinsic viscosity of cellulose in cupriethylenediamine. The application of this method to the present case is very useful, because the number of main-chain fracture can be directly deduced from the number-average molecular weight before and after irradiation, whatever the distribution of molecular weight may be. In this manner a very simple relation between molecular weight and radiation dose can be deduced, which empirical as it may be in the present state of things, is applicable to the experimental data covering a very wide range of

doses employed in this study.

If P_n and P'_n denote respectively the degree of polymerization corresponding to the number-average molecular weight before and after irradiation, the following relation applies, whatever the distribution of molecular weight may be :

$$\frac{1}{P'_n} - \frac{1}{P_n} = \frac{S}{N} \tag{1},$$

where S is the number of fractured bonds between monomer units, N the total number of monomer units. Since N is nearly equal to the total number of bonds between monomer units, S/N gives the number-fraction of fractured bonds.*

Now, the most simple relation to be thought of between the number of fracture S and radiation dose R would be the direct proportion, as can be written by :

$$S = NpR \tag{2},$$

where p gives the probability of bond fracture occuring at a given monomer unit, per unit radiation dose.

It has been shown, however, that the expression generalized as below is more widely applicable to the experimental data :

$$S = NpR^m \tag{3}.$$

Combination of (1) with (3) results in :

$$\frac{1}{P'_n} - \frac{1}{P_n} = pR^m \tag{4}.$$

The constant m in (4) is generally smaller than unity, but we are not in the position to find the theoretical interpretation of this exponential relation, although the relation similar to (3) can be found in photochemical reactions.**

The relation derived by Immergut et al. by the studies on the osmotic molecular weight and intrinsic viscosity of cellulose in cupriethylenediamine permits to determine the number-average molecular weight M_n directly by the measurement of intrinsic viscosity $[\eta]_{OED}$. The determining equation is :

$$[\eta]_{OED} = 1.33 \cdot 10^{-4} \ M_n^{0.905} \tag{5}.$$

According to Conrad[9] the intrinsic viscosity of cellulose in cuprammonium $[\eta]_{OAM}$ can be expressed in terms of $[\eta]_{OED}$ by :

$$[\eta]_{OAM} = \frac{1}{1.365} [\eta]_{OED} \tag{6}.$$

From (5) and (6) we have :

$$[\eta]_{OAM} = 9.74 \cdot 10^{-5} \ M_n^{0.905} \tag{7}$$

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 * In this calculation the loss in weight due to the decomposition of monomer units by irradiation with high energy radiation is not taken into account.
** The most typical example is the blackning of photographic emulsion, which is proportional to It^m, where I and t denote respectively light intensity and time of exposure, and m a constant called the Schwarzschild's constant. m ranges from 0.8 to 1. Since the dose rate is constant in the present case, the chain fracture S is proportional to t^m as in the case of blackning of photographic emulsion.

or
$$[\eta]_{OAM}=9.74\cdot10^{-3}\ P_n^{0.905} \qquad (7').$$

In Table 1 the radiation dose, $[\eta]_{OAM}$, P_n and P'_n calculated by (7') and

Table 1. Intrinsic viscosity in cuprammonium $[\eta]_{OAM}$, number-average DP P'_n, and $1/P'_n-1/P_n$ as a function of radiation dose.

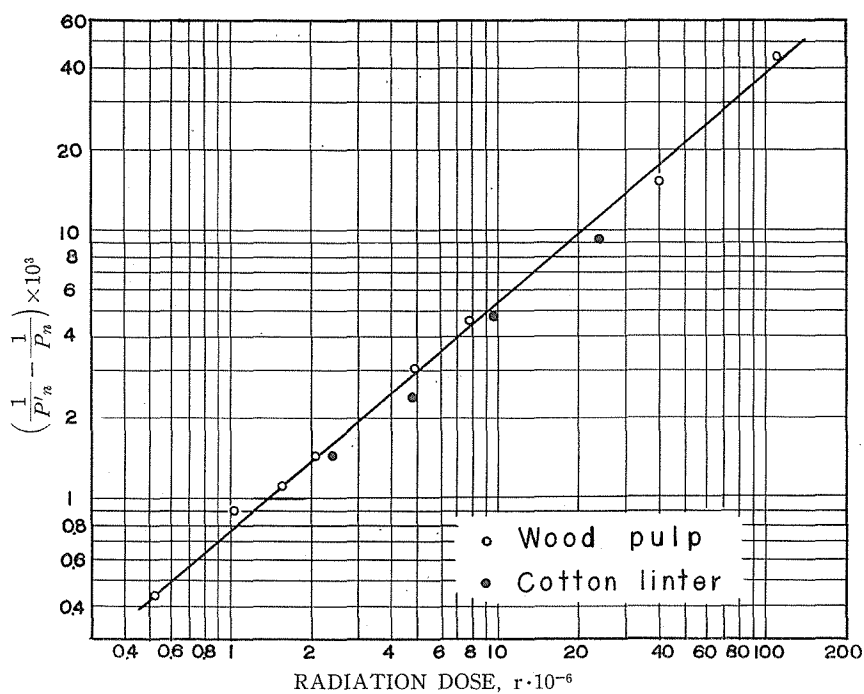| Radiation dose in million roentgens	$[\eta]_{OAM}$	P'_n	$1/P'_n-1/P_n$
Wood pulp			
0	3.88	747 (P_n)	—
0.56	3.00	562	0.44
1.04	2.43	445	0.91
1.56	2.25	409	1.11
2.08	2.00	360	1.44
4.98	1.33	229	3.03
7.80	1.00	167	4.65
40.00	0.40	61	15.06
110.00	0.16	22	44.11
Cotton linter			
0	8.88	1864 (P_n)	—
2.4	2.59	474	1.45
4.8	1.91	341	2.39
9.6	1.13	191	4.80
24.0	0.58	92	9.33

Fig. 1. Log-log plots of $\left(\dfrac{1}{P'_n}-\dfrac{1}{P_n}\right)$ vs. radiation dose.

$1/P'_n - 1/P_n$ for the samples of wood pulp and cotton linter are given. The logarithmic plots of $(1/P'_n - 1/P_n)$ vs. radiation dose are shown in Fig. 1, which gives a straight line in the very wide range of doses covering from $5.2 \cdot 10^5$ to $1.1 \cdot 10^8$ roentgens, and verifies the relation given by Equation (4). The constant m is determined to 0.85, and therefore Equation (4) becomes:

$$\frac{1}{P'_n} - \frac{1}{P_n} = pR^{0.85} \qquad (4')$$

From Fig. 1 the probability of bond fracture p results in $7.6 \cdot 10^{-4}$, that is — 0.076% at the dosage of million roentgens.

A radiation dose of one million roentgens liberates approximately $0.58 \cdot 10^{20}$ e.V. per gram of cellulose, which contains $3.7 \cdot 10^{21}$ glucose units. Since 0.076% of the bonds between these units are fractured at the radiation dose of million roentgens, the G-value for main chain fracture is determined to 4.8. This is the value reasonable for the reactions of this kind.

It is to be noticed that the probability of bond fracture p and G-value obtained here are about half of the values deduced by Charlesby from the data of Saeman *et al*. In this regards, he is also of opinion that the values obtained by him is larger than those of degradation reactions of other polymers, and a more reliable value of the constant K of the intrinsic viscosity-molecular weight formula is desirable to make his results more certain.

CHANGE IN SIZE OF CRYSTALLITES

It is generally assumed that the bond fracture is effected by high energy radiation randomly along every molecular chain regardless of crystallinity, that is — the bonds both in the amorphous and crystalline domains are expected to be equally sensitive to the radiation. Although the random fracture is always the premise of the discussion of the main chain fracture by high energy radiation, the direct evidences have little been presented.* It in anticipated as a result of random fracture that the size of crystallites is reduced as the degradation progresses, which may be demonstrated by measuring the "leveling-off" degree of polymerization, or by direct observation of crystallites by an electron microscope.

The wood pulp was irradiated with various doses of gamma radiation, and subjected to the measurement of degree of polymerization. On the other hand, each irradiated sample was hydrolyzed with $2.5N$ hydrochloric acid at 90°C for 2 hours up to the "leveling-off" point, and the residue also was subjected to the measurement of degree of polymerization. The leveling-off degree of polymerization thus obtained represents on the whole the size of crystallites. In Table 2 the result of measurements is given together with the yield of residue is also given in the table.

It can be seen from Table 2 that the leveling-off degree of polymerization

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* That the crystalline domains are also affected by irradiation with high energy radiation is evidenced by the fact that the cellulose irradiated with a very high dose of high velocity electrons becomes completely soluble in water[1].

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Table 2. Comparison of DP of irradiated wood pulp with leveling-off DP.

Radiation dose in roentgens	0	1.56×10^6	7.8×10^6	4.0×10^7	1.1×10^8
DP of irradiated samples	775	450	200	80	32
Leveling-off DP	178	150	112	72	38
Yield at leveling-off point	95.9	94.9	93.1	87.1	65.3

DP is calculated by Staudinger's equation, $DP = 250 \; [\eta]$.

decreases as the radiation dose is increased, and the value at the dosage of $1.1 \cdot 10^8$ roentgens is about one fifth of the value of unirradiated sample. This suggests that the main chain fracture takes place also in the crystalline domains.

It is natural that the leveling-off degree of polymerization is smaller than the degree of polymerization of unhydrolyzed sample at the region of small radiation doses, but it is very interesting to note that both the values are almost the same at a higher dose such as $1.1 \cdot 10^8$ roentgens. This would be one of the most convincing evidences to demonstrate the random fracture of main chains.

The reduction of size of crystallites can be demonstrated more visually by electron microscopic observation. Fig. 2 shows the electron photomicrographs

(a) Unirradiated pulp   (b) Irradiated pulp
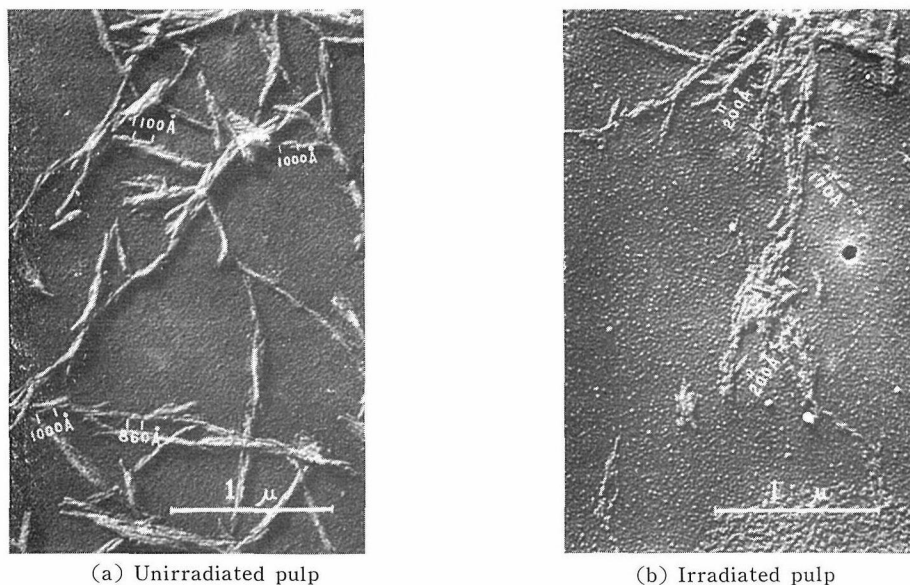
Fig. 2. Electron photomicrographs of residues of hydrolysis at leveling-off point.
Many small separated particles which are seen in the photomicrograph (b) of the irradiated pulp would also be the degraded crystallites.

of residues of hydrolysis at leveling-off point. The average length of crystallites of the original sample is determined to be about 750Å from Fig. 2 (a), which is the length that is computed from the degree of polymerization shown in Table 2. The length of crystallites is reduced to about one fifth of the original length by irradiating with a dose of $1.1 \cdot 10^8$ roentgens, as can be deduced from Fig. 2 (b). This also is consistent with the result shown in Table 2.

GAS EVOLVED DURING IRRADIATION

Saeman *et al.*[1] report that considerable loss in weight is found at irradiation, which amounts to about 14% for cotton and 17% for wood pulp at hundred million equivalent roentgens, and attributes this to the decomposition of carbohydrate residues. Charlesby's calculation[2] results in that approximately one glucose unit is decomposed per chain fracture. The loss in weight takes place in consequence of the liberation of gaseous products generated by irradiation, and therefore it is very interesting to investigate the constitution of evolved gas with a view to supply materials to interpret the reactions induced by high energy radiation.

The samples of thin cellulose films prepared from cellulose acetate by hydrolysis were treated with heavy water to substitute the hydrogen atoms of "accessible" hydroxyl groups in the amorphous regions by deuterium, and subjected to gamma-irradiation in a tube sealed off under a high vacuum, respectively with the dose of $2.1 \cdot 10^7$ and $1.0 \cdot 10^8$ roentgens. The gas evolved was analyzed by a mass spectrometer. Fig. 3 shows the spectra thus obtained.
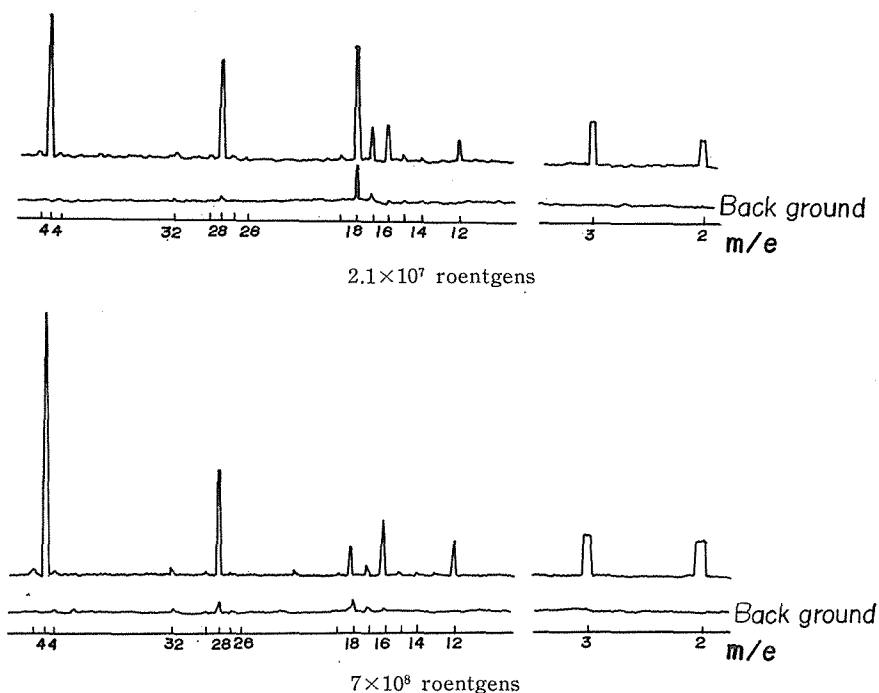

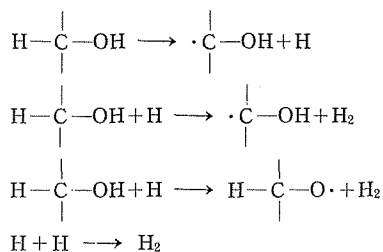Fig. 3. Mass spectra of the gas evoluted from irradiated cellulose.

The peaks and the corresponding values of m/e prove the existence of H_2, HD, CH_4, H_2O, HDO, CO and CO_2. The relative quantity of each constituent is shown in Table 3 in molar percent, from which it is concluded that gas evolved during irradiation is composed mostly of H_2 including HD. The content of H_2O

Table 3. Mas spectrometric analysis of gas (Molar ratio in %).

dosage	2.1×10^7 roentgens	1.0×10^8 roentgens
$H_2 + HD$	97.3%	95.5%
CH_2	0.2	0.1
CO	1.0	0.6
CO_2	1.5	3.8

is not shown in the table, since the accurate determination of water by this method is very difficult, but it is certain that the amount of water generated would be very small, if any.

The hydrogen evolution is found in many cases of radiation-induced reactions of a number of organic compounds, and this results from the abstraction of hydrogen atoms from molecules. In the case of cellulose also the hydrogen abstraction from glucose residues can happen in various manners. However, considering the precedents that the hydrogen atoms attached to the carbon atoms bonding with a hydroxyl group or other oxygen atom are assumed to be splitted more readily, a postulated schema of probable reactions is presented as follows :

$$H-\overset{|}{\underset{|}{C}}-OH \longrightarrow \cdot\overset{|}{\underset{|}{C}}-OH+H$$

$$H-\overset{|}{\underset{|}{C}}-OH+H \longrightarrow \cdot\overset{|}{\underset{|}{C}}-OH+H_2$$

$$H-\overset{|}{\underset{|}{C}}-OH+H \longrightarrow H-\overset{|}{\underset{|}{C}}-O\cdot+H_2$$

$$H+H \longrightarrow H_2$$

The formation of carbonyl groups which will be treated in the next section is readily inferred from this schema as follows :

$$\cdot\overset{|}{\underset{|}{C}}-OH \longrightarrow H-\overset{|}{C}=O$$

$$H-\overset{|}{\underset{|}{C}}-O\cdot \longrightarrow H-\overset{|}{C}=O$$
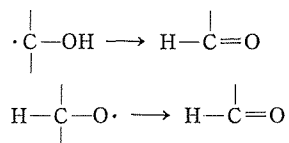
The generation of CO, CO_2 and CH_4 suggests the splitting of the bonds C—C and C—O by irradiation. The production of CO_2 is interesting with regard to the fact that xylose units are produced from glucuronic acid units during the irradiation, as will be dealt with later.

The atomic ratio of H : D in the gas is 69 : 31 in the case of irradiation at $2.1 \cdot 10^7$ roentgens, and 73 : 27 at $1.0 \cdot 10^8$ roentgens, and both the values are very close to each other, while the ratio in the original deuterated cellulose sample is determined by infra-red spectrum to about 90 : 10. It follows, therefore, that the gas evolved from the deuterated cellulose is richer in deuterium than the original substrate. The interpretation of this fact is not so simple. If the hydrogen atoms attaching directly to carbon atoms are preferably abstracted by

(24)

the primary action of high energy radiation as postulated before, the product should be purely hydrogen, because only the hydroxyl hydrogen is substituted by deuterium. Then the presence of deuterium as HD would be due to the exchange reaction given by:

$$H-\overset{|}{\underset{|}{C}}-OD+H \longrightarrow H-\overset{|}{\underset{|}{C}}-O\cdot+HD$$
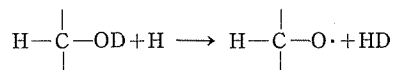
The content of deuterium depends upon the readiness of the reaction given above. The primary dissociation of deuterium from OD:

$$H-\overset{|}{\underset{|}{C}}-OD \longrightarrow H-\overset{|}{\underset{|}{C}}-O\cdot+D$$
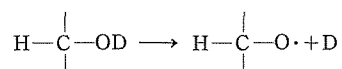
would be very rare, since D_2 is not identified in the gas mixture.

FORMATION OF CARBONYL GROUPS AND CARBOXYL GROUPS

That cellulose is chemically modified upon irradiation with high energy radiation has been pointed out by several authors, who attributed this to the formation of a type of oxycellulose characterized by a greater affinity to the basic dyes. However, the conspicuous changes chemically identified are the carbonyl group formation and carboxyl group formation by irradiation. According to Blouin and Arthur[5] the gamma-irradiation of a series of samples of purified cotton resulted in carbonyl group formation and carboxyl group formation and cleavage of main chain in approximate ratio of 20 : 1 : 1. They measured also the infrared spectra of the irradiated samples, and found the relatively unchanged spectra: the only difference from the unirradiated sample was the appearance of a small band at 5.75μ, which is designated to the C=O bonding of both carbonyl and carboxyl groups. The intensity of this absorption band was by far smaller than the value resulting from the content of carbonyl groups, and this was attributed to the hemiacetal formation and hydration of carbonyl groups. Therefore, even this weak band could not definitely be designated to carbonyl groups, since the presence of carboxyl groups had been detected.
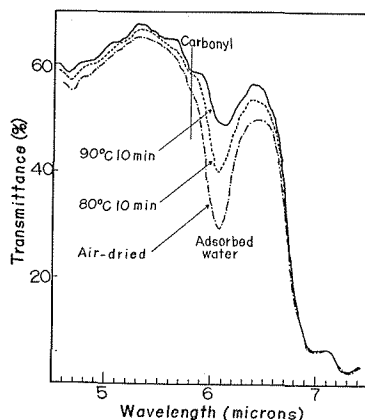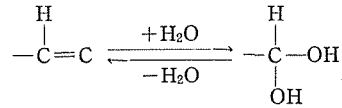

Fig. 4. Infrared spectra of irradiated cellulose.

Fig. 4 shows the infrared spectra of the cellulose film irradiated with gamma radiation at the dosage of $2.66 \cdot 10^6$ roentgens. The film was prepared in the same manner as described in the previous section. The band due to carbonyl groups is scarecely identified with the sample subjected to the measurement of infrared spectrum immediately after irradiation, but, when the same sample is heated respectively at 80° and 90°C for ten minutes to remove the adsorbed water, a sign of weak band becomes visible at the wave-length of 5.82μ, which is attributed to $C=O$ bondings. The indication becomes more distinct with decreasing water content, which is estimated by the decrease in the intensity of the adsorbed water band at the wave-lengths around 6.00μ. In this case also the intensity of carbonyl band is by far smaller than that which would result from the content of carbonyl groups, but the dependence of the intensity upon the water content suggests that the band at the wave-length of 5.82μ as observed here would be mostly ascribed to carbonyl groups, which are hydrated and dehydrated reversibly as can be expressed by the following schema :

$$\begin{array}{ccc} \text{H} & & \text{H} \\ | & +\text{H}_2\text{O} & | \\ -\text{C}=\text{C} \underset{-\text{H}_2\text{O}}{\overset{}{\rightleftharpoons}} & -\text{C}-\text{OH} \\ & & | \\ & & \text{OH} \end{array}$$

The $C=O$ bonding can be detected more clearly by the measurements of ultraviolet absorption spectra, as shown in Fig. 5. The spectra were measured with the solutions of cellulose samples in 80% phosphoric acid. It is well known
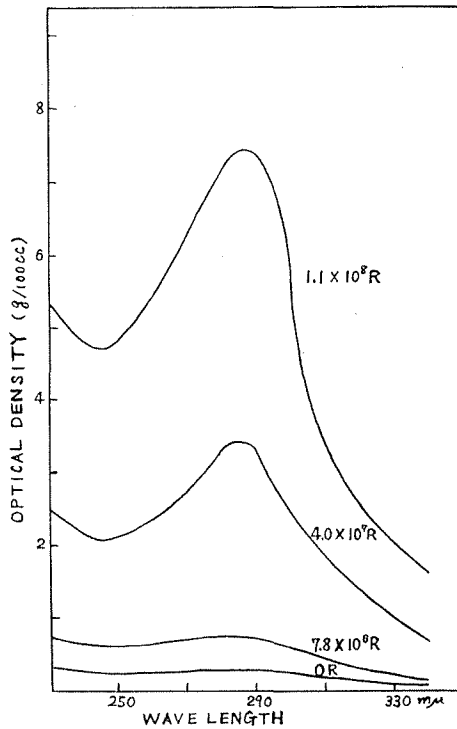

Fig. 5. Ultraviolet absorption spectra of irradiated cellulose samples.

that the broad band at the wave-lengths around $285m\mu$ is due to $C{=}O$ bonding. It is interesting to notice that the optical density of this band increases as the radiation dose is increased, as can be seen in Fig. 5.

Blouin and Arthur estimated the content of carbonyl groups by the determination of copper number, which is the most common method of carbonyl determination of carbohydrates. As is well known, however, the reaction of Fehling's solution with carbohydrates is not stoichiometric, but different substances give different factors for calculation of carbonyl content. Recently Lindberg et al.[10] pointed out that the oxidized cellulose, such as chromate oxycellulose, shows anomalously high copper number compared to the other samples of modified cellulose. They opened up a new stoichiometric method for the determination of carbonyl groups, based upon the reduction with sodium borohydride, and determined the ratio of equivalents of copper to moles of carbonyl groups to 11.3 for the cotton oxidized by potassium dichromate, while the ratio is 4.3 for the cotton oxidized by hypochlorite at pH 9, and 2.9 for the dry-milled cellulose. The stoichiometric ratio based upon the formation of cuprous oxide is 2. Thus the ratio of equivalents of copper to moles of carbonyl groups differ diversely according to the type of modified cellulose. Therefore, the results obtained by the copper number measurements cannot be interpreted directly in terms of carbonyl content. The stoichiometric method of determination of carbonyl content other than the sodium borohydride method was presented by Nevell[11], who made use of the stoichiometric oxidation of aldehyde groups to
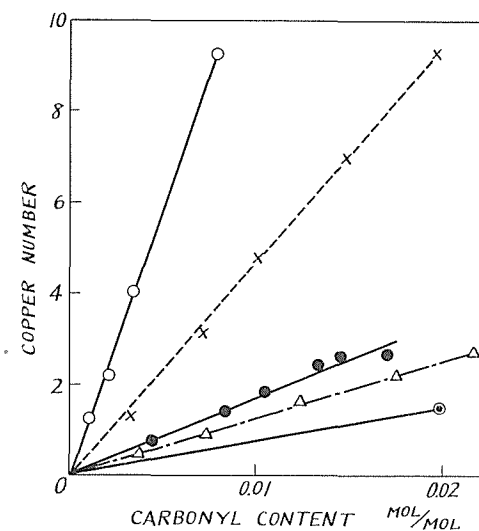

Fig. 6. Plots of copper number vs. carbonyl content.
—○—○— Gamma-irradiated cotton cellulose
—×—×— Cotton cellulose oxidized by potassium dichromate
—●—●— Cotton cellulose oxidized by hypochlorite at pH 9.
—△—△— Hydrate cellulose
——◉—— Theoretically expectable value
The data other than those of irradiated cotton are the duplication of the data of S. Lidman-Safwat and O. Theander[10].

carboxyl groups by sodium chlorite. The carboxyl groups produced can be determined by the usual method using calcium acetate.[12] The comparison of these two methods resulted in conclusion that both the methods give almost the same value of carbonyl content. In the present study the sodium chlorite method was employed for determination of carbonyl content.

The determination of carbonyl groups by the stoichiometric method as described above resulted in a very astonishing effect, as can be seen in Fig. 6, which shows the plots of copper number of irradiated cellulose together with those of other modified celluloses against the "true" carbonyl content estimated by the sodium chlorite method and the borohydride method. The copper number of irradiated cellulose shows the linear relation with carbonyl content like in the cases of other cellulose samples, but the point to be noticed is the fact that the ratio of copper number to carbonyl content of the gamma-irradiated cotton is by far greater than those of other cellulose samples, and indeed greater than even that of dichromate oxycellulose which has thus far been characterized by the greatest ratio of copper number to carbonyl content.

In Table 4 the carbonyl content, determined by sodium chlorite method and

Table 4. Carboxyl formation and carbonyl formation as a function of radiation dose.

Radiation dose	0	2.4×10^6	4.8×10^6	9.6×10^6	2.4×10^7
COOH group (m. moles/162 g)	3.2	3.5	3.9	4.5	5.9
△COOH		0.3	0.7	1.3	2.7
CHO group, I* (m. moles/162 g)	0.2	1.1	2.5	3.6	8.0
△CHO		0.9	2.3	3.4	7.8
CHO group, II* (m. moles/162 g)	0.3	15.2	27.7	50.9	116.5
△CHO		14.9	27.4	50.6	116.2

* CHO group, I denotes the value determined by the sodium chlorite method, while CHO group, II the value estimated by copper number.

copper number method, and the carboxyl content of irradiated cotton are tabulated as a function of dose. It is interesting to note that the carbonyl formation calculated from copper number is about fifteen times as great as the value determined by the sodium chlorite method. The ratio of carbonyl formation resulting from copper number to carboxyl content is about 40 : 1, while the ratio is about 3 : 1, when the "true" carbonyl formation determined by the sodium chlorite method is reckoned. Blouin and Arthur gave the ratio of 20 : 1, but, as explained above, it is very probable that the carbonyl content given by them by the copper number measurements is greatly overestimated.

It is interesting to notice here that the cellulose irradiated by gamma radiation is sensitive to alkalis like chromate oxycellulose and periodate oxycellulose, whose degree of polymerization determined from cuprammonium viscosity is apparently smaller than that evaluated from the viscosity of solution of nitrate. The alkali-sensitive linkages would be one of the origins giving rise to a higher ratio of oxidant to carbonyl content. In Table 5 the values of degree of polymerization determined by two methods are shown in comparison with each

Table 5. Comparison of cuprammonium DP and nitrate DP of gamma-irradiated cellulose.

Radiation dose in million roentgens	Cuprammonium DP	Nitrate DP	Nitrate DP / Cuprammonium DP
0	863	775	1.114
0.8	628	522	1.204
1.2	583	469	1.243
1.6	552	423	1.305
2.0	528	397	1.330
4.0	405	306	1.325
20.0	176	122	1.442
40.0	112	82	1.366

other as a function of radiation dose. It can be seen that cellulose becomes more alkali-sensitive by irradiation.

If the carbonyl group formation and carboxyl group formation take place in proportion to chain rapture, the log-log plots of both the quantities against the radiation dose should give straight lines having the slope of 0.85, since chain rapture takes place in proportion to $R^{0.85}$, as shown in the previous section. The range of dosage covered by the present experiment is not wide enough to make a confident discussion on the relation, but, as can be seen from Fig. 7,
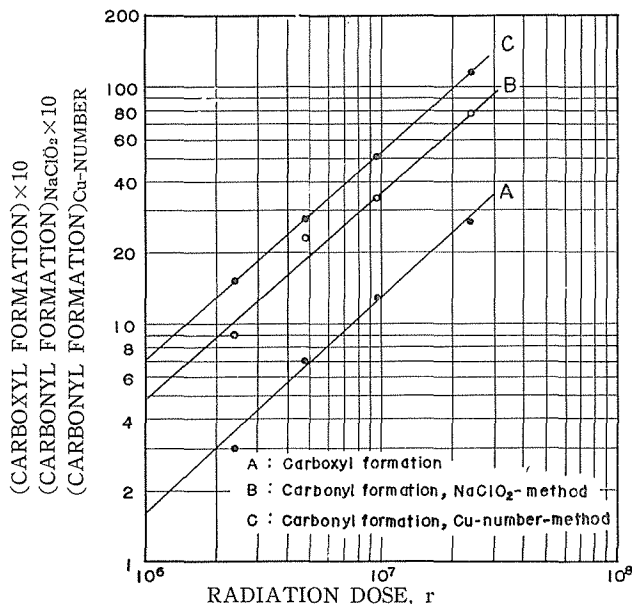

Fig. 7. Log-log plots of carboxyl formation and carbonyl formation vs. Radiation dose.

the log-log plots of carbonyl groups and carboxyl groups generated by irradiation against radiation dose give straight lines whose slopes are about 0.85. Therefore, both carbonyl groups and carboxyl groups are assumed to be generated in proportion to $R^{0.85}$ just like the case of chain scission.

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It is interesting to refer to the analysis presented by Arthur[7] who also showed that the log-log plots of carbonyl groups and carboxyl groups against dosage prove the linear relation. The slopes of the straight lines in his case also are determined to 0.80~0.85, and are coincident with ours.

From these relations it is inferred that the formation of carbonyl groups and carboxyl groups would probably take place in proportion to chain cleavages. However, since a series of manifold reactions develops in succession induced by the excitation with high energy radiation, any simple stoichiometric relations between reaction products and chain cleavage are not to be expected. The calculation in the present case results in the carbonyl formation, carboxyl formation, and chain cleavage in the approximate ratio of 0.63 : 0.21 : 1, or more roughly in the ratio of round numbers of 3 : 1 : 5. Further, the amount of carbonyl formation and that of carboxyl formation at one million roentgens are deduced from Fig. 7 to be approximately 0.48 and 0.16 m. moles/mole glucose.

FORMATION OF LOWER FATTY ACID GROUPS

It has been shown in the previous section that the cellulose irradiated by high energy radiation shows an enormously large copper number considering its carbonyl content. Although it has been assumed that the alkali-liable linkages resulting from the formation of dialdehyde groups as is the case in dichromate oxycellulose and periodate oxycellulose are responsible for this, the extremely high ratio of oxidant to carbonyl content is not completely accounted for only by these linkages. Another origin thought of as a reason would be the production of other types of reducing groups, which are intact in acid media but are labile in alkaline media as is used in the copper number determination and generate reducing groups by hydrolysis. Of these groups the esters of formic acid come first into consideration, since the determination of lower fatty acid groups including formic acid groups and acetic acid groups is one of the common items in the practice of chemical analysis of wood and cellulose.

The formyl groups and acetyl groups were determined by the method originated by Klason and modified by Klingstedt[13] with a sample of cotton irradiated with a gamma radiation dose of $1.5 \cdot 10^8$ roentgens. The analysis results in a considerable amount of formyl groups generated by irradiation, as shown in Table 6. It is important to notice that most of formyl groups are assumed to

Table 6. Formation of formyl groups and acetyl groups by irradiation of cotton with the dose of $1.5 \cdot 10^8$ roentgens in m moles/mole glucose.

	Unirradiated cotton	Irradiated cotton
Formyl group + acetyl group	1.0	49.2
Formyl group	—	45.3
Acetyl group	—	3.9
Free acids*	—	3.5

* Free acid was determined by the titration of ether extract of the irradiated cotton before subjecting to hydrolysis.

be found as formate ester, and the amount of free acid is very small as can be deduced from Table 6.

It is possible that the formic acid ester formed by irradiation is saponified in the alkaline medium as is employed in copper number measurement, and generates free formic acid, which, in turn reduces cupric copper to cuprous copper, giving a higher copper number than that resulting only from free carbonyl groups existed from the first. On the other hand, these alkali-senstive linkages are resistant to the hydrolysis in acid media as are used in the boro-hydride method and the sodium chlorite method of carbonyl determination, which, therefore, give the original content of carbonyl groups. So far as the copper nummber method is concerned, the effects of the secondarily generated products should not be ignored, and a number of reactions other than those described above would also take place in the case of modified cellulose. This would be the reason for resulting in an abnormally high copper number of the cotton cellulose irradiated by high energy radiation.

The reaction mechanism of producing formate groups is not simply postulated, but the hypothesis presented by Daniel[14] for the interpretation of reaction mechanism based upon his studies on the saponifiable carbon dioxide of acid

Anhydroglucose   Dialdehyde

Carbonic acid ester

or

γ-Lacton
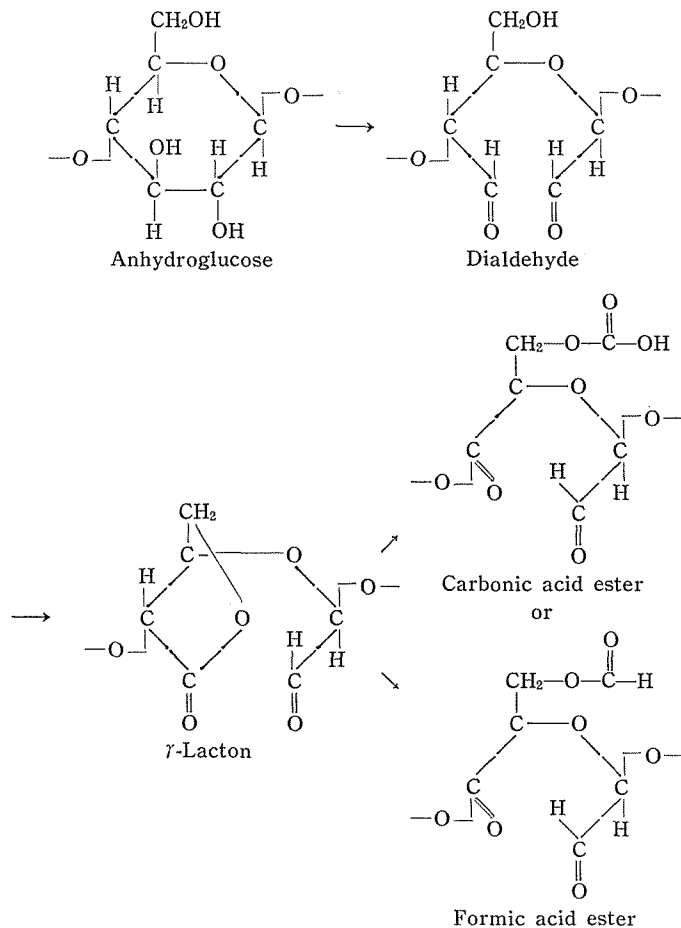
Formic acid ester

chlorine-oxidized cellulose is very interesting. Daniel's hypothesis is referred to as follows :

The schema showing the formation of formic acid ester from γ-lacton is not given by Daniel in this manner, but is hypothetically supplemented by the present authors.
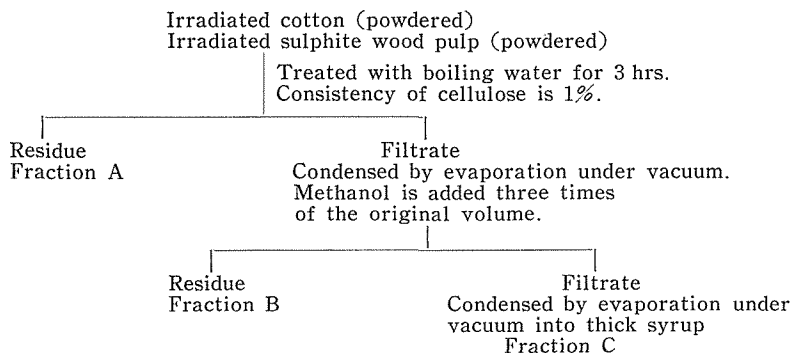
PAPER CHROMATOGRAPHIC ANALYSIS OF SUGARS

As described above, the depolymerization of cellulose by irradiation with high energy radiation is accompanied with chemical modification, whic has been evidenced by the formation of carbonyl groups, carboxyl groups, formyl groups and various alkali-sensitive linkages. It is inferred from these findings that the modified groups as identified above are, on the one hand, the results of structural changes of sugar components, on the other hand, the origin from which a number of reactions evolve into further changes in structure of carbohydrates. An introductory example is presented in Table 7, which shows the increase in the content of pentosans determined by the formation of furfural together with the increase in water solubility as a function of radiation dose.

Table 7. Solubility in boiling water and pentosan content of cotton irradiated by gamma radiation as a function of radiation dose.

Dose, roentgens	0	$4.8 \cdot 10^6$	$9.6 \cdot 10^6$	$2.4 \cdot 10^7$	$1.5 \cdot 10^8$
Solubility in boiling water, %		0.3	0.9	1.7	23.5
Pentosan, %	1.16	0.87	1.46	1.87	4.30

Since it is deduced from the data shown in Table 7 that the fundamental change in structure of carbohydrates take place by the irradiation with high energy radiation, it was attempted to study the sugar chemical changes by the aid of paper chromatography.

In order to study the change in carbohydrate components, it is recommendable to fractionate the original sample into several fractions differing in molecular weight, since the modified sugars are concentrated in the low-molecular fractions. The samples of purified cotton and sulphite wood pulp were irradiated by gamma radiation at the dose of $1.5 \cdot 10^8$ roentgens, and fractionated into three fractions as shown below :

Irradiated cotton (powdered)
Irradiated sulphite wood pulp (powdered)
  Treated with boiling water for 3 hrs.
  Consistency of cellulose is 1%.

Residue
Fraction A
              Filtrate
              Condensed by evaporation under vacuum.
              Methanol is added three times
              of the original volume.

Residue
Fraction B
                        Filtrate
                        Condensed by evaporation under
                        vacuum into thick syrup
                          Fraction C

The relative quantity of each fraction is as follows:

	Irradiated cotton	Irradiated wood pulp
Fraction A	76.5	74.8
Fraction B	8.8	9.9
Fraction C	14.7	15.3

It is to be noticed that pH of the Fraction A is 3.2 for irradiated cotton and 2.8 for irradiated wood pulp, while it is 6.0 and 6.4 respectively for unirradiated cotton and unirradiated wood pulp. This shows that acidic groups are produced by irradiation.
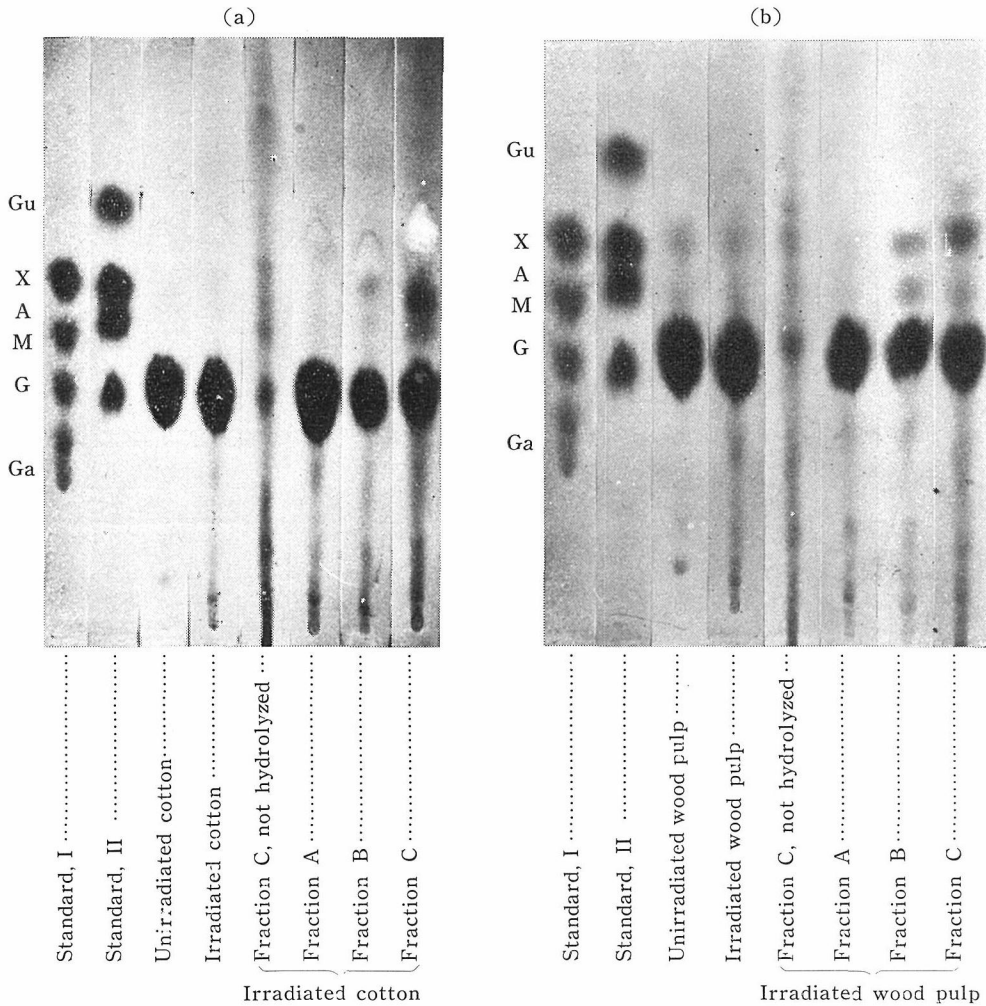

Fig. 8. Paper chromatograms of irradiated cotton linter (a), and irradiated wood pulp (b). Developing solution is 1-n-butanol-acetic acid-water 4 : 1 : 1.

Note : Gu glucuron, X xylose, A arabinose, M mannose, G glucose, Ga glucuronic acid.
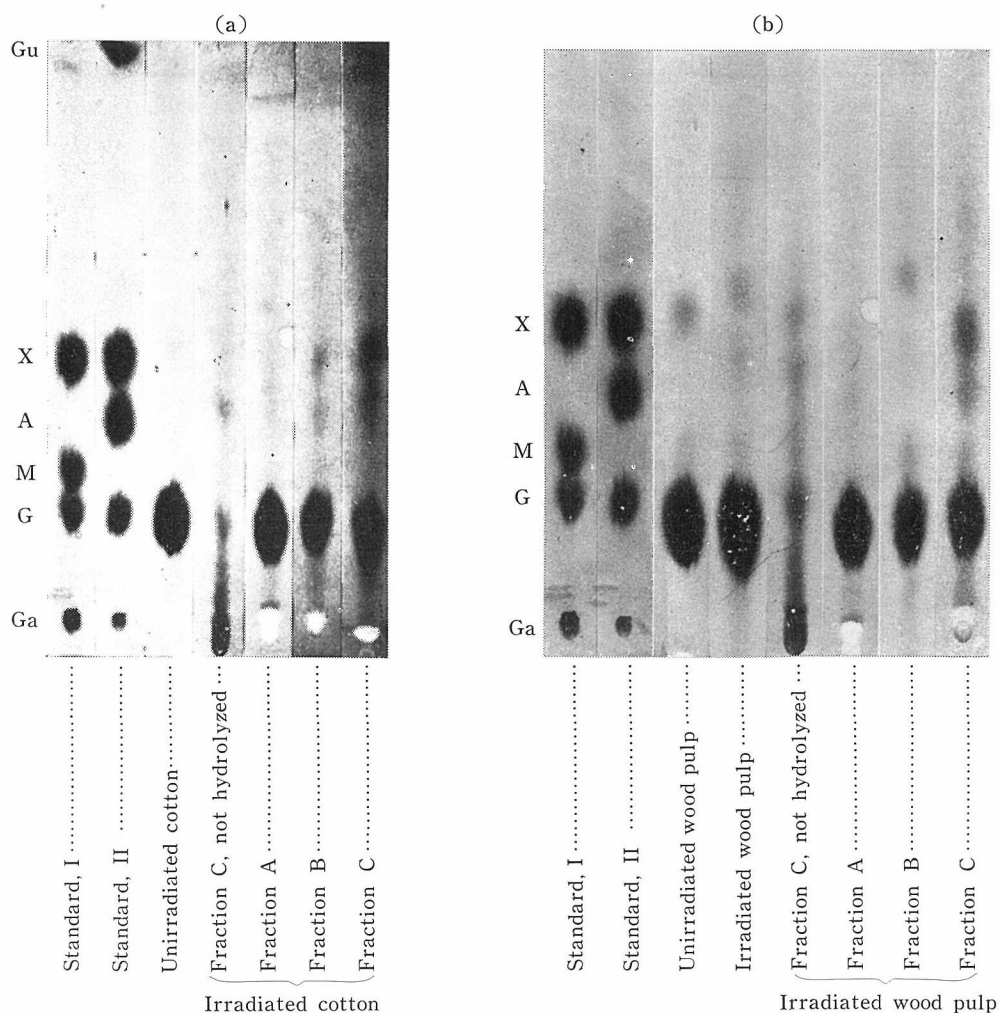
Fig. 9. Paper chromatograms of irradiated cotton linter (a), and irradiated wood pulp (b). Developing solution is pyridine-ethyl acetate-water 8 : 2 : 1.
Note : Gu glucuron, X xylose, A arabinose, M mannose, G glucose, Ga glucuronic acid.

Fraction A, B and C were hydrolyzed by 72% sulphuric acid prior to subjecting to paper chromatography. In the case of Fraction C, however, the unhydrolyzed syrup also was directly employed for chromatography. The multiple development technique worked out by Jeanes et al.[15] was used to obtain better separation of sugar components. Two kinds of sovents were used, that is -- 1-n-butanol-acetic acid-water 4 : 1 : 1 and pyridine-ethyl acetate-water 8 : 2 : 1. While the former is acidic, the latter is reckoned among neutral developing solutions. The number of developments is three for the former solvent and five for the latter. As the reagent for detecting the sugar spots the aniline phthalate solution of Partrige[16] was used.

The chromatograms are shown in Figs. 8 and 9. It is noticed that the spots of monomeric sugars are apparently underlain by continuous coloring on

the chromatograms of the unhydrolyzed sample of Fraction C. This indicates that the radiation has effected the chain rapture to such an extent as to produce monosaccharides. The *continuous coloring would probably be due to oligosac-charides* and various other modified sugars. The spots of monosaccharides become more apparent when the hydrolyzed sample of Fraction C is used.

Now, it is very important to notice that arabinose which is not found in the unirradiated sample is clearly perceptible on the chromatograms of the irradiated samples. Another important point to be noticed is that the spots of glucuronic acid is very apparent on the chromatograms of irradiated samples. The spots of these modified sugars are recognized more apparently in the chromatograms of low-molecular fractions, especially Fraction C, which manifests also the spot of xylose. From these observations it is inferred that arabinose
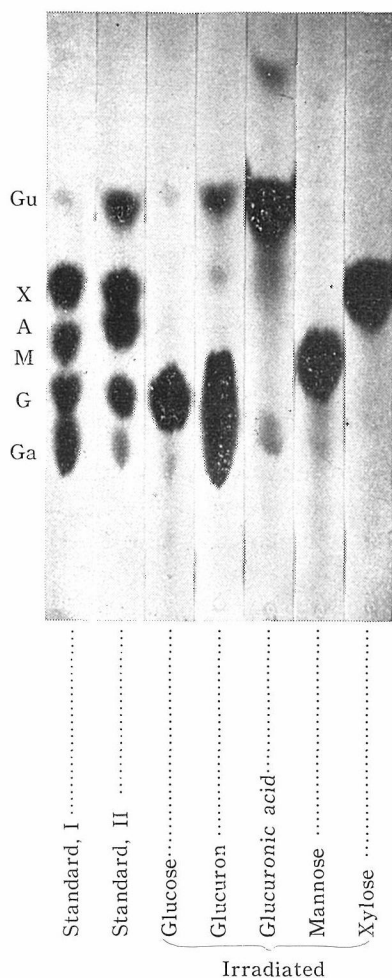

Fig. 10. Paper chromatograms of the irradiated monosaccharides.
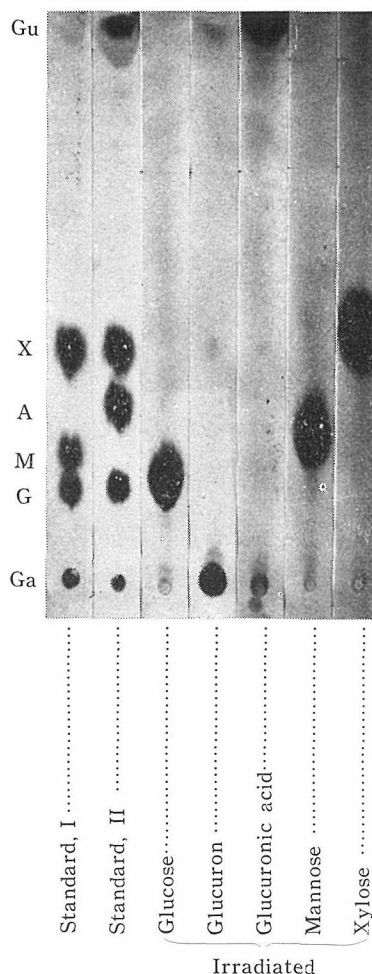Developing solvent is 1-n-butanol-acetic acid-water 4 : 1 : 1.

Fig. 11. Paper chromatograms of the irradiated monosaccharides.
Developing solvent is pyridine-ethyl acetate-water 8 : 2 : 1.

and glucuronic acid would have been produced from glucose residue by irradiation with gamma rays. The increase in the content of xylose as deduced from chromatograms suggests also the production of xylose groups by irradiation.

The postulate presented above is supported by the experiments made with pure monosaccharides such as glucose, glucuronic acid, glucuronic lacton or glucuron, mannose and xylose. These sugars were irradiated by gamma rays with the dose of $9.6 \cdot 10^7$ roentgens and then subjected to the paper chromatography. The results are shown in Figs. 10 and 11.

The chromatograms show that the irradiation of glucose by gamma radiation results in the production of arabinose, glucuronic acid and its lacton. The latter. two yield on their turn xylose by decarboxylation. A series of reactions as mentioned above is consistent with the results obtained with cellulose. Xylose is not affected by irradiation with the dose used in this experiment, as can be seen in the chromatograms. Therefore, the content of xylose increases by the amount generated by irradiation. The chromatograms of irradiated mannose show a new spot which may be ascribed to mannuronic acid.

The production of xylose residue from glucose residue can be expressed by the schema :

Glucose reside   Glucuronic acid residue   Xylose residue
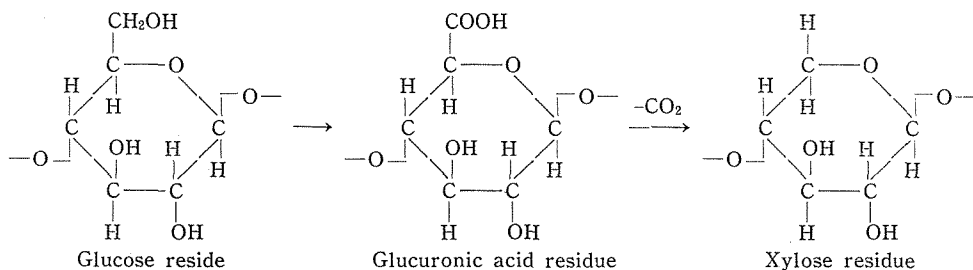
No affirmative schema for the formation of arabinose from glucose can be presented, but the hypothetic formula given by Kaverzneva $et\ al.$[17] for acid chlorine-oxidized cellulose, upon which Daniel commented is illustrated below for reference.

Glucose residue   Carbonic acid ester   Arabinose residue
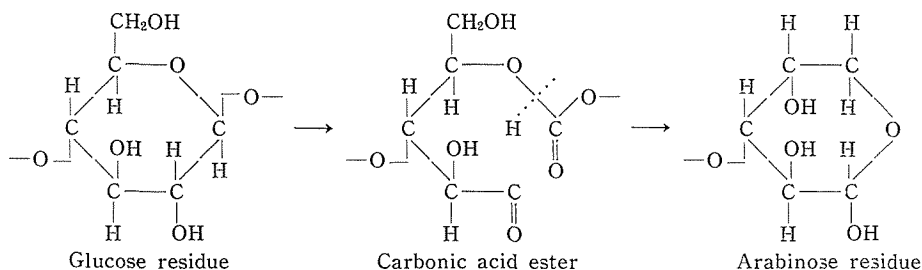
EXPERIMENTAL

Gamma-irradiation. The irradiation was performed with the two thousand Curie gamma-ray facility of the Institute for Chemical Research of Kyoto University, whose detailed descriptions about construction and performance are seen in the articles published by Shimizu and his coworkers[18]. The sample was placed

in the middle of a horizontal tubular cavity of 122 mm in diameter, which is surrounded by thirty seven pencils of Co–60 placed in horizontal holders. The pencils are arranged arround the cavity in two circles, the inner circle comprising twenty pencils, and the outer circle seventeen pencils. The radius of the inner circle, that is — the distance between the pencils in the inner circular row and the center axis of the tubular hollow is 75 mm, while the radius of the outer circle is 95 mm. The intensity of each pencil is rated by the producer (Atomic Energy Canada Limited) at 61.7 curies at the date of June, 1957. The dose rate at the middle point of cavity was normalized by Shimizu *et al.* to be $2.3 \cdot 10^5$ roentgens/hour at the date of April, 1958. The time of irradiation for desired radiation dose was found by the aid of decay curve of radioactivity of Co–60, considering the dose rate at the time when the experiment was performed.

Deuteration and mass spectrum measurement. The cellulose film of several microns in thickness prepared by hydrolysis of cellulose acetate film was placed in a tube furnished with a "breakable seal" device, and the tube was connected with a deuteration apparatus. The sample was dried under a high vacuum of 10^{-4} mmHg at 60°C for a week. After the stopcock in the pump line had been closed, the stopcock in the heavy water line was opened, and the sample was kept in equilibrium with the vapor of heavy water for twenty four hours at room temperature. This procedure was repeated three times, and the tube, after being exhausted up to the high vacuum, was separated from the deuteration apparatus, and placed in the center of radiation chamber and irradiated by gamma radiation. After irradiation the tube was attached to the mass spectrometer, and by breaking the seal of the tube by the device as mentioned above, the gas in the tube could be connected with the spectrometer. The massspectrometer used was PMU–5 Type of Hitachi Seisakusho. The principal working conditions of the spectrometry were as follows: electron emission current was 120 μA, electron accelerating voltage 90V, ion accelerating voltage 1,500V, and the temperature of ion source 130°C.

ACKNOWLEDGMENTS

REFERENCES

(1) J. F. Saeman, M. A. Millett and E. J. Lawton, *Ind. Eng. Chem.*, 44, 2848 (1952).
(2) A. Charlesby, *J. Polym. Sci.*, 15, 263 (1955).
(3) R. E. Gregg and Z. I. Kertesz, *Science*, 124, 893 (1956); J. Polym. Sci., 26, 289 (1957); *Radiation Research*, 6, 469 (1957).

(4) E. S. Gilfillan and L. Linden, *Textile Res. J.*, **27**, 87 (1957).

(5) F. A. Brouin and J. C. Arthur, Jr., *Textile Res. J.*, **28**, 198 (1958); R. J. Demint and J. C. Arthur, Jr., *Textile Res. J.*, **29**, 276 (1959).

(6) R. F. Florin, Abstract of Papers of 138th Meeting of American Chemical Society, New York, N. Y., p 16E (1960).

(7) J. C. Arthur, Jr., *Textile Res. J.*, **28**, 204 (1958).

(8) E. H. Immergut, B. G. Rånby and H. F. Mark, *Ind. Eng. Chem.*, **45**, 2483 (1953).

(9) C. M. Conrad, *J. Phys. Col. Chem.*, **55**, 1474 (1951).

(10) B. Lindberg and A. Misiorny, *Svensk Papperstidning*, **55**. 13 (1952); B. Lindberg and O. Theander, *ibid.*, **57**, 83 (1954); S. Lidman-Safwat and O. Theander, *ibid.* **61**, 42 (1958).

(11) T. P. Nevell, *J. Textile Inst*, **46**, T 91 (1951).

(12) G. F. Davidson and T. P. Nevell, *J. Textile Inst.*, **39**, T 102 (1948).

(13) R. S. Sieber, "Die Chemisch-technischen Untersuchungsmethoden der Zellstoff und Papierindustrie" (1951).

(14) J. W. Daniel, *Tappi*, **42**, 534 (1959).

(15) A. Jeanes, C. S. Wise and R. J. Dimler, *Analyt. Chem.*, **23**, 415 (1951).

(16) S. M. Partridge, *Nature*, **164**, 443 (1949).

(17) E. D. Kaverzneva, V. I. Ivanov and A. S. Salova, *Bumazh. Prom.*, **28**, No. 7, 6 (1953).

(18) S. Okamoto and Y. Nakayama, *This Bulletin*, **37**, 299 (1959); S. Shimizu, S. Tanaka and Y. Nakayama, *ibid.*, **37**, 306 (1959).