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The Effect of Gamma Radiation on Collagen[1]

INTRODUCTION

Investigations on the effect of ionizing radiations on proteins have mainly been concerned with soluble proteins. Irradiation of these, either in solution or in the dry state, leads to aggregation and decreased solubility, and at the same time to fragmentation of the protein molecule (*1–4*). Little detailed information has been published regarding the insoluble fibrous proteins, but in the few investigations carried out irradiation has been found to increase solubility (*5–9*).

The strength of wool fibers decreases and their solubility in alkali has been found to increase on irradiation with soft X-rays (*5*), neutrons (*6*), or γ-rays (*5*, *7*, *8*) after doses of 5 Mrad and above. Perron and Wright (*10*) report that the electron irradiation of rat-tail tendon with doses of about 1 Mrad causes decrease in intensity of the X-ray pattern, shrinkage, and increased solubility in water. Little (*11*) found that the crystalline areas of collagen become disordered at relatively low doses compared with silk, keratin, and other proteins.

The present investigation has been undertaken with the object of obtaining some information on the effect of γ-radiation on collagen. Such information, besides being of general theoretical interest, is of practical importance with respect to the increasing use of ionizing radiation for the sterilization of sutures, bone grafts, etc. Its use has also been suggested in connection with the preservation of skins and the elimination of anthrax infection.

Only a few detailed examinations of the chemical effects of irradiation have been made (see, for example, Cassell, *9*; Drake *et al.*, *12*; Caputo and Dose, *13*; Alexander and Hamilton, *14*). The present work was, therefore, concentrated on this aspect, and the doses were high so that the changes taking place could be more readily detected.

[1] The work reported in this paper forms part of a research program sponsored by the U. S. Department of Agriculture under the authority of U. S. Public Law 480.

EXPERIMENTAL PROCEDURE

Raw Material

A piece of oxhide was obtained immediately after slaughtering and washed in several changes of 5% sodium chloride solution to remove serum proteins. It was then treated in a suspension of calcium hydroxide for 6 days to remove hair and mucoproteins, neutralized, washed free of salts, and acetone-dehydrated. The grain and flesh layers were removed, and the middle layer was cut into pieces about 0.5 cm^2. These pieces were degreased in petroleum ether, b.p. 40° to 60°C, and air-dried. Some of this material was ground in a Wiley mill (laboratory model) to pass 20 mesh.

Irradiation

The conditions of irradiation were as follows: (1) in oxygen, "wet"; (2) in oxygen, "dry"; (3) in nitrogen, "wet"; (4) in nitrogen, "dry". Five-gram samples of the ground collagen together with a few pieces were placed in 1-inch-diameter boiling tubes. Water was added to two of these samples until they were obviously wet, and a further two were dried under reduced pressure in a freeze-dryer (Edwards Model 10). The tubes were filled with oxygen or nitrogen and sealed. The final moisture contents determined after irradiation were 80% (wet) and 5% (dry).

Spent fuel elements discharged from the Harwell reactor 22 days previously were the source of γ-radiation. The samples were placed in aluminum cans and exposed to radiation of mean energy 1 Mev in a homogeneous γ-ray field at a dose rate of 1 Mrad per hour. The total dose given was 50 Mrad ($\pm 2\%$) as measured by means of the ferric–ferrous system, a G value of 15.5 being assumed (see U.K. Atomic Energy Authority Publication AERE-R3665).

Further samples of oxhide (full thickness) containing 18% moisture were enclosed in polythene bags and irradiated with 5 and 50 Mrad.

Water Uptake

One-gram samples were exposed for 4 days to atmospheres of relative humidity from 40% to 90% obtained by the use of saturated solutions of suitable salts.

Swelling in water was determined by the porous plate procedure described by Preston and Nimkar (*15*) for cellulose fibers. About 3 gm of the powdered collagen was soaked in water overnight, and samples of approximately 1 gm were filtered off on sintered-glass crucibles (porosity 5) by using a maintained suction of 30 cm mercury. One to two hours was allowed for equilibrium to be attained. Then the fibrous mat was removed, weighed, dried at 105°C for 16 hours, and reweighed. The water uptake was calculated as a percentage of the final dried weight. Duplicate determinations differed by less than 1%.

Shrinkage Temperature

Measurements were made in water on samples of collagen 1 × 5 cm. The lower end of the sample was fixed, and the upper end was attached to a chain passing over a pulley and operating a lever, thus magnifying changes in length. The water was heated by 1° to 2°C per minute, and the point at which shrinkage commenced was recorded. Determinations were made in duplicate or triplicate. Replicates did not differ from the mean by more than 1°C.

Strength

Measurements were made on strips of oxhide 2 × 4 inches as described in the *Official Methods of Analysis* of the Society of Leather Trades' Chemists (*16*). A steel pin, ⅛-inch in diameter, is inserted through the strip half an inch from one end, and a pull is exerted on this until tearing occurs. The results are expressed as tearing load per unit thickness.

Such measurements were made on three samples at each level (5 and 50 Mrad), both before and after irradiation, and the mean percentage loss in strength was calculated.

Analysis

Total nitrogen was determined by a standard Kjeldahl procedure (*17*). Amide nitrogen was determined by hydrolyzing with 2 *N* HCl for 3 hours at 100°C and distilling aliquots from borate buffer, pH 11.0, in a Markham still.

Free ammonia was determined by taking a sample of the irradiated collagen immediately after opening the tubes and extracting with 5% NaCl. Samples were then distilled from borate buffer as for amide nitrogen.

For determination of amino acids the protein was hydrolyzed with 12 *N* HCl in a sealed tube for 18 hours at 105°C. The amino acids were separated by the procedure of Moore *et al.* (*18*) and determined colorimetrically with ninhydrin (*19*). Hydroxyproline was determined by a modification of the Neuman and Logan method (*20*), and tyrosine by the method of Udenfriend and Cooper (*21*). For the majority of amino acids the estimated error was ±3%, or less. With glycine + alanine, valine, methionine, threonine, and hydroxylysine the error was slightly greater, ±5%.

Terminal Amino Groups

One-gram samples of the powdered collagen were treated with 1 ml of 1-fluoro-2,4-dinitrobenzene in 25 ml of saturated sodium bicarbonate solution for 48 hours at room temperature. The α-dinitrophenyl (DNP) derivatives were separated on buffered Celite columns and determined as described by Courts (*22*). Determinations were made in duplicate or triplicate, the estimated error being ±10%.

Carbonyl Compounds

Samples of the protein were heated in sealed tubes for 3 hours with 12 *N* HCl; the solutions were cooled and made to a known volume. Aliquots were treated with a saturated solution of 2,4-dinitrophenylhydrazine in 2 *N* HCl and heated to 50°C for 30 minutes. After cooling, the 2,4-dinitrophenylhydrazones were determined by the method of Lappin and Clarke (*23*). Methanol and potassium hydroxide were added, the solutions were centrifuged to clarify them, and the optical density of the solutions was determined by a Uvispek spectrophotometer. The solutions were read against a blank prepared from the reagents alone, and acetophenone 2,4-dinitrophenylhydrazone was used as a standard.

The absorption maximum of the unknowns was at a slightly lower wavelength (440 mμ) compared with that of the acetophenone derivative (465 mμ). It was assumed that the molecular absorptions of the DNP-hydrazones were similar, and the optical density of each was measured at its maximum. The estimations were repeated on aliquots of the hydrolyzed protein after evaporation to dryness, the difference in values obtained giving a measure of volatile compounds.

RESULTS

Properties of Irradiated Collagen

Except for a slight yellowing, the samples of collagen irradiated at low moisture contents (5% and 18%) were not visibly affected, and examination under the microscope indicated that the fiber structure was unchanged. The yellow color tended to fade on heating or long storage. When immersed in water, however, the collagen irradiated at 5% moisture content swelled to a soft gel which slowly dissolved.

Irradiation in the "wet" condition (80% moisture) led to loss of fibrous structure. The collagen swelled to a firm gel which did not obviously shrink or dissolve in boiling water. On drying, a tough, slightly plastic mass was formed which could not readily be subdivided by cutting or grinding.

Electron-microscope examination[2] showed that changes in structure had occurred with doses of 50 Mrad. With irradiation in the "dry" state there were changes in the amorphous ground substance which was no longer able to hold the bundles of fibrils together. Little change in the fibrils themselves was observed. The samples irradiated "wet," however, showed no individual fibrils; bundles of fibrils could not be distinguished, and the whole had a homogeneous appearance.

The wide-angle X-ray diffraction pattern of the irradiated samples showed much diffuse and amorphous scatter. Loss of crystallinity was noticeable in the "dry" irradiated samples and very marked in the "wet" irradiated samples.

[2] We are indebted to Dr. K. Little of the Radcliffe Infirmary, Oxford, for both electron-microscope and X-ray examinations.

The equilibrium moisture content of the collagen irradiated with 5% moisture ;ended to be lower than that of the original collagen at all relative humidities ɔetween 40% and 90% (see Table I). A similar observation was made by Cas-ɜel (*9*).

Measurements of strength, swelling, shrinkage temperature, and solubility made on some of the irradiated samples are recorded in Table II. Strength was

TABLE I

WATER UPTAKE OF IRRADIATED COLLAGEN AT VARIOUS RELATIVE HUMIDITIES
(Grams of water per 100 gm dry weight)

Relative humidity (%)	*Control*	*Collagen irradiated with 5% moisture Dose—50 Mrad*	
		In oxygen	*In nitrogen*
52	23.3	21.8	21.4
58	23.4	20.7	21.4
70	24.9	21.7	22.7
80	31.5	26.8	28.4
90	40.9	38.0	37.5

TABLE II

PROPERTIES OF IRRADIATED COLLAGENS

Property	*Control (not irradiated)*	*Irradiated at 18% moisture in air*		*Irradiated (50 Mrad)*			
				At 5% moisture		*At 80% moisture*	
		5 Mrad	*50 Mrad*	*Oxygen*	*Nitrogen*	*Oxygen*	*Nitrogen*
Strength (% retained)		68[a]	35[b]	—	—	—	—
Shrinkage temperature (°C)	61	43	25	<25	<25	>100	>100
Swelling (gm water uptake per 100 gm)	175	254	370	—	—	—	—
	Nitrogen dissolved as % total protein nitrogen (Mean of duplicate determinations)						
Solubility							
Water (1 gm in 50 ml for 24 hours at 25°C)	0.1	9	36	ca90	ca90	—	—
0.1 *M* acetic acid (1 gm in 50 ml for 24 hours at 25°C)	0.4	18	53	97	96	14	6
5 *M* acetic acid (1 gm in 10 ml for 5 days at 20°C)	4	90	92	—	—	—	—

[a] Mean of three values: 61, 71, and 73.
[b] Mean of three values: 23, 40, and 41.

decreased by irradiation, even 5 Mrad causing a 30% reduction. The shrinkage temperature of the collagen irradiated "dry" could not be measured as it was very weak and began to disintegrate almost at once. On irradiation in air with 18% moisture the shrinkage temperature was decreased nearly 20° by a dose of 5 Mrad, and after a dose of 50 Mrad the collagen disintegrated and dissolved at 25°C. As already noted, the collagen irradiated "wet" did not appear to shrink even in boiling water.

Increases in solubility were greatly affected by the moisture content at the time of irradiation. Irradiation "wet" caused relatively little increase in solubility even in 0.1 *M* acetic acid, but irradiation at low moisture contents caused the collagen to become almost completely soluble in water at 20°C. The solutions were only slightly viscous and showed little tendency to gel, though in some instances the protein content was as much as 2%. On dialysis of the 0.1 *M* acetic acid extracts of the collagen irradiated in air, no precipitate or gel was formed, and only about one-third of the nitrogen was retained in the dialysis sac (Visking tubing).

An attempt was made to obtain information on the molecular weight of the dissolved protein by light-scattering measurements (*24*) on a solution of the collagen irradiated "dry" in nitrogen.[3] Certain difficulties were encountered; only about 90% of the collagen dissolved in the pH 8 phosphate buffer used for this determination, and there was interference from fine suspended matter. Indications were that the average molecular weight was of the order of 60,000. The low limiting viscosity number (10 to 11) obtained on the same solution was also in agreement with this low molecular weight. There were indications that the molecule was more rigid than a gelatin molecule of the same molecular weight.

Composition

The composition of the samples irradiated in oxygen and nitrogen with doses of 50 Mrad was examined in detail (see Table III).

There were small losses in total nitrogen during irradiation, especially with the collagen exposed "wet," but less than 0.1% free ammonia was detected in samples taken immediately after the tubes were opened. Irradiation under all conditions led to an apparent increase in amide nitrogen of about 0.4 gm of nitrogen per 100 gm of protein or to 2% of the total nitrogen. In the irradiated collagens only 83% to 89% of the total nitrogen was recovered as amino acids and ammonia, compared with 95% in the control. The sum of the amino acid residues was also low, particularly with the samples irradiated "wet." Three unidentified peaks were observed on the Moore and Stein columns. The positions in which these were eluted are indicated in Table III. No attempt was made to identify these, but it

[3] We are indebted to Dr. J. Wootton of the British Gelatin and Glue Research Association, London, for these measurements.

TABLE III

AMINO ACID COMPOSITION OF IRRADIATED COLLAGEN

Component	*Control*	*Irradiated in:*				*Control*	*Irradiated in:*			
		Oxygen		*Nitrogen*			*Oxygen*		*Nitrogen*	
		Dry	*Wet*	*Dry*	*Wet*		*Dry*	*Wet*	*Dry*	*Wet*
Total N (gm/100 gm)	18.2	17.9	17.0	17.9	17.3	—	—	—	—	—
Amino acids	*Residues (gm/100 gm)*					*Amino acid N (% total N)*				
Unknown 1[a]	—	—	—	—	—	—	—	—	0.3	0.4
Hydroxyproline[b]	12.2	10.5	9.6	11.4	10.7	8.3	7.3	7.3	7.9	7.7
Aspartic acid	5.3	5.0	4.2	4.3	3.3	3.6	3.4	3.0	3.0	2.3
Threonine	1.6	1.5	1.4	1.5	1.6	1.2	1.2	1.1	1.2	1.3
Serine	2.6	2.2	2.6	2.7	2.8	2.3	2.0	2.4	2.4	2.6
Glutamic acid	9.3	8.4	7.5	9.2	7.1	5.5	5.1	4.8	5.6	4.5
Proline	9.9	9.0	7.8	8.5	6.1	7.8	7.3	7.6	6.8	5.1
Glycine	20.6	17.4	19.1	18.2	16.8	27.8	23.9	27.5	24.9	23.9
Alanine	8.1	7.4	6.4	6.1	7.5	8.7	8.1	7.5	6.8	8.5
Unknown 2[a]	—	—	—	—	—	—	0.2	0.3	—	—
Valine	2.2	2.5	2.0	2.3	1.8	1.7	1.9	1.7	1.8	1.5
Methionine	0.7	0.7	0.5	0.6	0.5	0.4	0.4	0.3	0.4	0.3
Isoleucine	1.4	1.3	1.3	1.4	1.1	1.0	0.9	0.9	1.0	0.8
Leucine	2.7	2.4	2.3	2.5	2.4	1.9	1.7	1.7	1.8	1.7
Unknown 3[a]	—	—	—	—	—	—	—	0.2	0.1	0.3
Tyrosine[b]	0.4	0.1	0.2	0.4	0.2	0.2	0.1	0.1	0.2	0.1
Phenylalanine	2.0	1.2	1.2	1.8	1.2	1.1	0.9	0.7	1.0	0.7
Unknown 4[a]	—	—	—	—	—	—	0.1	0.2	0.1	—
Hydroxylysine	0.9	0.8	0.7	0.8	0.8	0.9	0.8	0.8	0.9	0.9
Lysine	3.5	3.1	2.8	3.1	2.7	4.2	3.8	3.5	3.8	3.4
Histidine[c]	1.0	0.8	0.4	0.5	0.5	1.7	1.3	0.8	0.9	0.8
Arginine	7.6	5.9	6.0	6.4	6.0	15.0	11.8	12.7	12.8	12.4
Amide N[b]	(0.4)	(0.8)	(0.8)	(0.7)	(0.7)	2.0	4.1	3.9	3.6	3.6
Total:	92.0	80.2	76.0	81.7	73.1	95.3	86.3	89.0	87.3	82.8

[a] Calculated on the assumption that it contains one amino group.

[b] Direct determination.

[c] High value in control indicates presence of some amino compound in addition to histidine.

seems unlikely that they could account for the missing nitrogen. The peak emerging before valine on the 150-cm column was probably ε-hydroxyaminocaproic acid resulting from the deamination of lysine. Cassel (*9*) also reports a low recovery of amino acids and the presence of several unidentified spots on paper chromatograms.

The results as a whole indicate the presence of about 10% of unidentified compounds in the "dry" irradiated collagen and up to 20% in the "wet" irradiated.

Losses of nitrogen also indicate that "wet" irradiation causes more deamination than "dry" irradiation.

Individual losses of amino acids varied with the conditions of irradiation, but in general the basic and acidic amino acids and those with a ring structure, particularly phenylalanine, tyrosine, and histidine, were the most affected, whereas leucine, isoleucine, valine, and rather surprisingly serine and threonine were almost completely unattacked. Losses were, in most instances, greater with the "wet" irradiated samples, particularly of proline and glutamic acid. Losses of tyrosine and hydroxyproline were greater in oxygen; those of aspartic acid and proline were greater in nitrogen.

Reaction with fluorodinitrobenzene indicated the liberation of small amounts of terminal amino groups (Table IV). No new amino acids were detected as terminal residues, but all those present in the original collagen were increased. More α-amino groups were liberated in oxygen than in nitrogen. The number average chain length calculated from these values is of the same order as that of an average gelatin (*22, 25*).

TABLE IV

TERMINAL AMINO, CARBONYL, AND AMIDE GROUPS IN IRRADIATED COLLAGENS

(Millimoles per 100 gm)

Component	*Control*	*Irradiated in oxygen*		*Irradiated in nitrogen*	
		Dry	*Wet*	*Dry*	*Wet*
Terminal amino groups					
Glycine	0.10	0.37	0.31	0.36	0.21
Glutamic acid	0.01		0.11	0.03	0.01
Aspartic acid	0.02		0.08	0.06	0.03
Serine	0.02	0.20		0.06	
Threonine	0.04		0.14	0.08	0.09
Alanine	0.02	0.21	0.32	0.07	0.07
Valine	0.03	0.15	0.23	0.11	0.18
Phenylalanine	0.10	0.15	0.17	0.07	0.14
Total:	0.34	1.08	1.36	0.84	0.73
Carbonyl groups					
Total:	<0.2	7.4	4.3	4.0	3.2
Nonvolatile	<0.2	4.0	2.2	1.3	1.0
Amide N	30	60	54	53	51
Average chain weights					
From terminal amino + carbonyl groups	300,000	12,000	18,000	21,000	25,000
From terminal amino and increase in "amide" groups	300,000	3,200	4,900	4,200	4,600

The results of determinations of carbonyl groups in the irradiated samples are given in Table IV. The absorption spectrum of the compounds was displaced to rather lower wavelengths compared with that of the standard, acetophenone. The results, however, clearly indicate appreciable increases in carbonyl groups. Of these about half were volatile and were lost on evaporation of the hydrolyzate. No evidence of the presence of dicarbonyl compounds was obtained (cf. Jayko and Garrison, *26*). An attempt was made to identify the carbonyl compounds by paper chromatography, but the amounts of material available were too low for this to be done satisfactorily.

DISCUSSION

Changes in physical properties of the collagen were apparent after irradiation with 5 Mrad and became very extensive after 50 Mrad. The changes in the X-ray diffraction pattern, the fall in shrinkage temperature, and the increased solubility indicate that there has been an extensive loss of molecular structure.

The low viscosity and gelling power of the extracts suggest that there has also been considerable breakdown of the polypeptide chains to smaller units. This is also indicated by the results of the light-scattering measurements on the material irradiated "dry." The fact that the molecule still appears to be more rigid than that of most gelatins may indicate that some fraction still retains the three-stranded helical configuration of collagen (*27*). The inability to precipitate any fibrous protein from the extracts and the observation of Cassel (*9*) regarding the optical rotation of such solutions are, however, contrary to this view. Although damage appeared to be greater when the collagen was irradiated "wet," increases in solubility were much less. Chemical degradation was at least as great, if not greater, than with "dry" irradiation, which suggests that the relatively low solubility must be due to the formation of new interchain stabilizing bonds leading to a branched-chain structure. The rubbery feel of the "wet" irradiated material also supports this view.[4] It is possible that similar bonds are formed to a smaller degree during "dry" irradiation and may be the cause of the apparent rigidity.

The increase in terminal amino groups is relatively small and insufficient to account for the low molecular size indicated by the properties of the solutions. The increase in amide nitrogen and the detection of carbonyl compounds in the irradiated collagens suggests that the greater part of the decreased molecular size is due to the breaking of —N—C— bonds as suggested by Garrison and co-workers (*26, 29, 30*), i.e.:

$$\underset{\displaystyle R'}{\underset{|}{-CH}}-CO-NH-\underset{\displaystyle R''}{\underset{|}{CH}}-CO- \xrightarrow[\displaystyle +\ O_2]{H_2O} \underset{\displaystyle R'}{\underset{|}{-CH}}-CONH_2 + O{=}\underset{\displaystyle R''}{\underset{|}{C}}-CO-$$

It would be expected that this reaction would be favored by the presence of oxygen or by water from which this may be derived during irradiation. In the present work, however, increases in amide nitrogen were not greatly affected by the conditions, and, although carbonyl groups were slightly higher after irradiation in oxygen than in nitrogen, they were less after "wet" than after "dry" irradiation. The irradiation of dry serum albumin (5% moisture) *in vacuo* (*14*) has also been found to lead to increases in amide and carbonyl groups. Presumably the 5% moisture in the "dry" proteins was sufficient to supply the necessary radicals.

The increase in amide nitrogen found in this and other investigations (*14, 31*) is nearly four times the increase in carbonyl groups. Either amide groups are formed by an alternative mechanism not yielding carbonyl groups, or else ammonia is derived from some other source, for example, arginine or lysine, both of which are decreased by irradiation. If all the increase in "amide nitrogen" arises from the breaking of —C—N— bonds in the polypeptide chain, the average chain weight would be of the order of 3000 to 5000. If, however, only increases in terminal amino and carbonyl groups indicate such breaks, the average chain weight would be of the order of 12,000 for the collagen irradiated "dry" in oxygen, and 18,000 to 25,000 for the other three samples. If new crosslinks are formed between polypeptide chains, the molecular weight may be several times that of the average chain weight, but, even so, the value of 21,000 for the collagen irradiated "dry" in nitrogen seems to be more in accord with the light-scattering measurements than the lower value of 4200.

Analysis indicates the over-all loss of about 10% of amino acids in the samples irradiated "dry" and up to 20% in those irradiated "wet." The amounts of carbonyl compounds detected are relatively small and, if an average molecular weight of 100 is assumed, would account for less than 1% of the weight. Hence, appreciable amounts of other breakdown products remain to be identified. Losses of nitrogen on irradiation indicate that deamination products of amino acids will amount to about 1.5% of the weight in the case of the "dry" irradiation and nearly 7% in the case of the "wet" irradiation. The mechanisms by which the other unidentified products are formed is not clear. The low nitrogen recovery after hydrolysis suggests that some of these contain nitrogen.

Cassel (*9*) also reports a low recovery of amino acids, but the losses of individual amino acids found by him are not the same as those reported here. The conditions of irradiation in the two investigations are, however, rather different. As found by most other workers, the acidic and basic amino acids and those having a ring structure were the most radiosensitive. Relative losses varied with the conditions of irradiation and were not the same as those reported for other proteins. It seems that the susceptibility of the amino acids varies to some extent with their local environment. The acidic and basic amino acids, proline and hydroxyproline, account for over 50% of the amino acids in collagen (*32*), and it is possible that this

high content of the more radiosensitive amino acids is one of the reasons for the readiness with which the X-ray pattern is disrupted compared with that of silk fibroin, keratin, and other polyamides. The triple helix structure of collagen, which is maintained by interchain hydrogen bonds, may also be more susceptible than the folded structures of other proteins.

It is interesting to compare the chemical changes occurring as a result of irradiation with those arising from other types of degradation. The α-amino groups liberated are similar to those produced on prolonged alkaline (*33, 34*) or other treatments (*33*). Only under relatively drastic hydrolytic conditions do other amino acids appear as terminal residues, so that it may be concluded that certain bonds are more labile than the rest. The exposure of collagen to warm moist conditions (60°C over water) presents a rather similar picture of degradation.[5] There is a small loss in total nitrogen, a low recovery of amino acid residues, and the increase in α-amino groups is of the same order. The solubility in 0.1 *M* acetic acid is, also, similar to that of the "wet" irradiated collagen. It is possible that similar mechanisms are involved in each case.

SUMMARY

Collagen has been irradiated with 5-Mrad and 50-Mrad doses of γ-rays under various conditions. Such doses lead to loss of crystallinity, as indicated by the X-ray diffraction pattern, increase in solubility, and other changes in physical properties indicative of extensive loss of molecular structure and breakdown to smaller units. Determination of N-terminal residues using fluorodinitrobenzene indicates that there has been relatively little hydrolytic scission of peptide bonds. Increase in amide nitrogen and in carbonyl groups indicates that —N—C— bonds have been broken by the mechanism suggested by Garrison and co-workers. On irradiation with doses of 50 Mrad there was some loss of nitrogen and an over-all loss of some 10 to 20% of amino acids. The formation of carbonyl compounds accounts for only a small proportion of this loss, and large amounts of breakdown products remain unidentified.

Relative losses of amino acids varied with the conditions of irradiation, but in general the acidic and basic amino acids and those having a ring structure were the most radiosensitive.

The chemical changes occurring as a result of γ-radiation are compared with those caused by other forms of degradation.

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