Radiation Sensitivity of Amino Acids in Solution and in Protein to Gamma Rays+

Hiroyuki HATANO*, Shigetake GANNO* and Akira OHARA**

(*Department of Chemistry, Faculty of Science, Kyoto University and
**Department of Biochemistry, Kyoto College of Pharmacy)

Received December 25, 1962

Radiation sensitivity of amino acids in solution and in protein to gamma rays was investigated by determining a rate of destruction of the amino acids in aqueous solutions and in proteinase proteins in solution after exposure to various doses of γ-radiation.

In aqueous solution, methionine, arginine and histidine were proved to be radiosensitive but tyrosine, proline and alanine relatively radioresistant. In protein in solution, the constituent tyrosine of the proteinase was found to be most destructive contrary to free tyrosine in solution. The constituent phenylalanine and arginine of the protein were relatively more radiosensitive whereas the constituent proline, histidine and threonine were less destroyed in the protein.

Reaction yields and relative doses of 37 per cent destruction of amino acids were presented. It was remarkable that even after exposure to about 100 kiloroentgen dose of γ-rays, which cause destruction of almost the constituent tyrosine of the proteinase, about 50 per cent remaining activity of the enzyme could be detected on the damaged enzyme protein.

INTRODUCTION

Investigating the specificity of deamination of amino acids by X-radiation, Dale *et al.*[1] observed various ionic yields of the deamination, though the yield was highly dependent on the conditions of the irradiation. Similar results of γ-radiation were reported on amino acids and peptides in aqueous solution by Hatano[2~4]. Radiation-induced decomposition of amino acids in solution was studied by Ohara[5] and a rank in terms of γ-radiation lability of free amino acids was determined. The radiation sensitivity of the constituent amino acids of proteins to X- or γ-rays was attempted to determine on insulin by Drake *et al.*[6], on some proteins by Barron *et al.*[7], on desoxyribonuclease by Okada *et al.*[8], on ATP-creatine phosphotransferase by Friedberg *et al.*[9], and on bacterial proteinase by Ganno[10]. It appears from these studies that the radiation sensitivity of amino acids is characteristic in solution, in constituent of protein, and in a dry or an anerobic condition.

One of major reactions of amino acids in aqueous solution was oxidative deamination to produce the corresponding keto acids accompanying by liberation

+ The material in this paper was presented at the Fifth Symposium on Radiation Chemistry of the Chemical Society of Japan, Tokyo, Oct. 1962; The Proceedings, p. 33.

* 波多野博行, 鳰野 重威

** 小原 晃

of ammonia under the presence of oxygen. Oxidative radiolysis of peptides and proteins caused appearance of the keto acids which were derived from the constituent amino acids of the parent peptides and proteins in γ-irradiated aqueous solutions though the keto acids were rendered to further destruction to produce aldehyde and carbon dioxide[2~4,11~14]. The yield of the keto acid formation, however, seems likely to be not enough to show the radiation sensitivity of amino acids because the keto acid was extraordinarily unstable in aerobic aqueous solutions. An accurate yield of radiation-induced destruction of amino acid should be given by measuring directly the amino acids remaining intact in the irradiated solutions.

This study deals with the γ-radiation sensitivity of amino acids in aqueous solution and in constituent of protein in aqueous solutions.

EXPERIMENTAL

Materials. Amino acid, which were all paperchromatographic pure grade, were available in commercial from Ajinomoto Co. Ltd., Tokyo. Ten mM amino acid solutions were used throughout this study. Crystalline bacterial proteinase of Daiwa Kasi Co. Ltd., Osaka, was used as a protein specimen. This enzyme has been observed to contain no sulfhydryl amino acids and characterized to be not sulfhydryl enzyme by Ganno[10]. The proteinase showed a single peak on ultracentrifugation[10]. For irradiation and other measurements 125 mg of the crystalline enzyme was dissolved in 100 ml of water.

Methods. Irradiation of γ-rays: The two-kilocurie cobalt-60 gamma ray irradiation facility[15] was used for γ-irradiation. The dose rate was approximately 140 kiloroentgens per hour. Aliquots were pipetted into 7×70 mm glass tubes and the tubes were sealed. The irradiation was performed at room temperature.

Amino acid analysis: A new analyser, KLA-2 Hitachi Amino acid Analyzer, with three new pumps, a three wavelength photometer and a programing drum, was used for automatic amino acid analysis. The analyzer was designed by Hatano and his coworkers and manufactured by Hitachi Ltd., Tokyo[16]. The analytical procedure was identical with the method of Moore *et al.*[17].

Preparation of samples for the analysis: Twenty 10 mM amino acid solutions were combined with together after individual γ-irradiation. Resulting mixture, contained 1.0μ mole of each amino acid in 2 ml of the aliquout was analyzed in the Hitachi amino acid analyzer. To the γ-irradiated protein solution, equal volume of a 50% trichloroacetic acid solution was added to cause precipitation of residual protein. Resulted precipitate of the protein was collected by centrifugation and hydrolyzed with a few volumes of a 6 N hydrochloric acid solution in an evacuated tube for 34 hr. at 100°C. The resulting hydrolyzate was taken to dryness repeatedly, dissolved in sodium citrate buffer pH 2.2, and analyzed in the Hitachi amino acid analyzer.

Determination of enzyme activity: Proteolytic activity of the bacterial proteinase was assayed by the usual method using a Folin's reagent and a casein

substrate[18].

RESULTS

(1) Radiation sensitivity of free amino acids in solution. After exposure to γ-ray doses in a range from 100 kr. to 10 Mr., twenty 10 mM amino acid solutions were combined with together and the resulted mixtures in which were contained the amino acids remaining intact in each irradiated solution were analyzed automatically by the analyzer. The analytical results were presented in Table 1. Typical chromatograms of amino acid irradiated in solution were shown in Fig. 1 (dose : 420 kr.), Fig. 2 (dose : 3.4 Mr.) and Fig. 3 (dose : 6.7 Mr.). Whereas little disappearance of every amino acid was observed on the analytical result of 140 kiloroentgen dose irradiation, little amino acids remaining intact were found on that of 10 megaroentgen dose irradiation, accompanying by several uncharacterized products of x_1 to x_8 of the chromatogram.

Table 1. Amino acids remaining intact in a γ-irradiated 10mM solution.

Amino Acid	Irradiation Dose, Mr.						
	0.14	0.42	0.70	1.12	3.40	6.70	10.0
$CySO_3H$	1.02	0.99	0.97	0.90	0.68	0.47	0.35
Asp	0.74	0.71	0.68	0.58	0.52	0.44	0.32
Hypro	0.98	0.75	0.60	0.56	0.42	0.39	0.28
Thr	0.88	0.87	0.66	0.64	0.31	0.10	0.03
Ser	0.96	0.84	0.73	0.67	0.43	0.18	0.07
Glu	0.92	0.78	0.65	0.62	0.42	0.16	0.09
Pro	0.93	0.90	0.88	0.84	0.69	0.54	0.48
Gly	0.90	0.77	0.72	0.69	0.59	0.40	0.28
Ala	0.86	0.80	0.72	0.66	0.58	0.41	0.37
CyS	0.83	0.73	0.61	0.59	0.58	0.37	0.14
Val	0.85	0.84	0.75	0.58	0.53	0.29	0.12
Met	0.80	0.67	0.55	0.54	0.26	0.02	0.00
Ileu	0.90	0.86	0.72	0.71	0.44	0.21	0.07
Leu	0.89	0.68	0.63	0.53	0.36	0.10	0.08
Tyr	0.97	0.90	0.88	0.81	0.73	0.71	0.51
Phe	0.89	0.88	0.84	0.70	0.35	0.12	0.03
Try	0.98	0.71	0.66	(0.64)	0.56	0.38	0.26
Lys	1.10	0.89	0.81	0.73	0.49	0.19	0.07
His	0.99	0.86	0.78	0.72	0.37	0.13	0.04
Arg	1.09	0.77	0.66	(0.62)	(0.38)	0.16	0.06
NH_3	0.38	0.99	1.19	1.55	1.80	1.91	2.35
Total Amino Acid	18.48	15.20	14.50	12.07 13.33*	9.31 9.70**	5.77	3.65

* contained x_1 and x_2 compounds; ** contained x compound. ( ) : calculated

Reaction yields, G-values, at 37 per cent amino acid destruction were given in Table 2. Higher radiation sensitivity of methionine, threonine, phenylalanine,

leucine, histidine, arginine and glutamic acid was observed contrary to relatively more radioresistant cystine, tyrosine, glycine, alanine, proline, tryptophan and aspartic acid.

Table 2. Reaction yields, G-values, at 63 per cent destruction.

Amino Acid	G moles/100eV*
Met	3.79
Thr	3.03
Phe	2.69
Leu	2.69
His	2.49
Arg	2.33
Glu	2.33
Ser	2.24
Ileu	2.13
Lys	2.09
Val	2.02
Hypro	1.35
CyS	1.26
Try	1.21
Gly	1.13
Asp	1.00
$CySO_3H$	0.90
Ala	0.84
Pro	(0.62)
Tyr	(0.55)

* 1 r.=93 erg/solution. ( ) : calculated.

(2) **Radiation sensitivity of the constituent amino acids of the protein.** Results of automatic amino acid analysis on the hydrolyzate of the irradiated proteinase, which was precipitated in the irradiated protein solution, were presented in Table 3. The chromatograms of the analyses were typically shown in Fig. 4 (non-irradiated), Fig. 5 (dose : 1.1 Mr.) and Fig. 6 (dose : 13 Mr.).

Here was found that tyrosine first disappeared from the irradiated protein

Table 3. Inactivation and destroyed amino residues in γ-irradiated 0.125 per cent proteinase solutions.

Irradiation Dose, Mr.	Inactivation, %	Disappeared Amino Acid Residues
1.0	32.7	—
1.5	39.3	—
1.8	(44.0)*	Tyr
3.0	58.1	—
11.0	100.0	Arg, Phe
13.0	—	His, Thr, Pro, Ileu, Leu

* Calculated

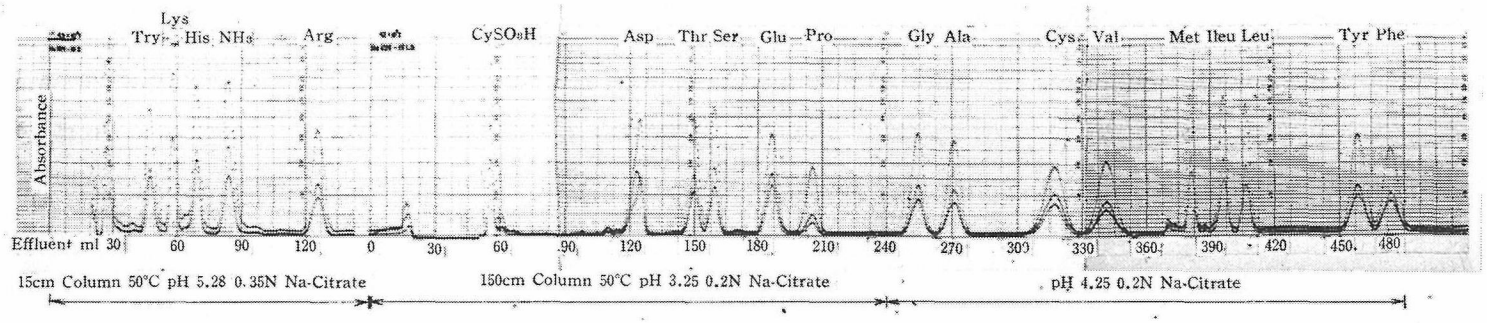

Fig. 1. Chromatogram of amino acid irradiated in the individual solution. Dose : 420 kiloroentgens.

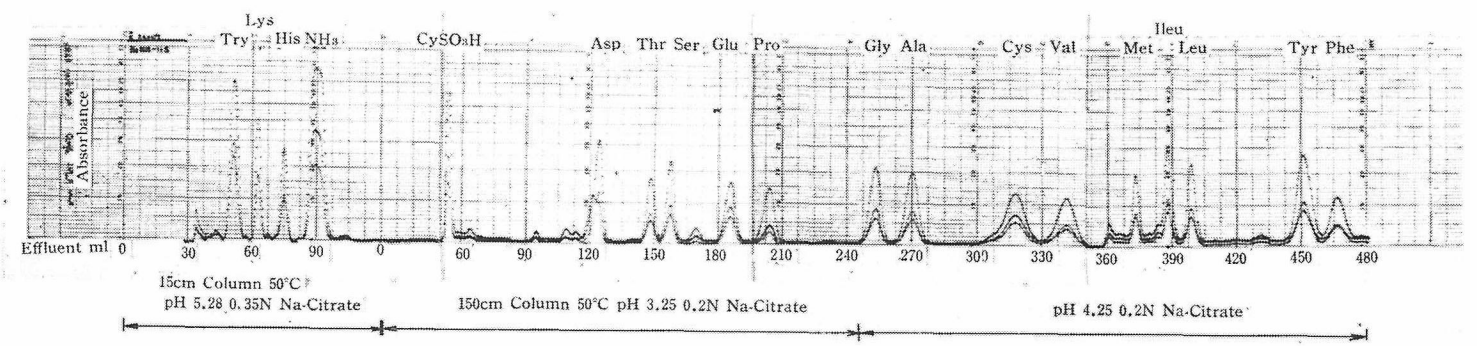

Fig. 2. Chromatogram of amino acid irradiated in the individual solution. Dose : 3.4 megaroentgens.

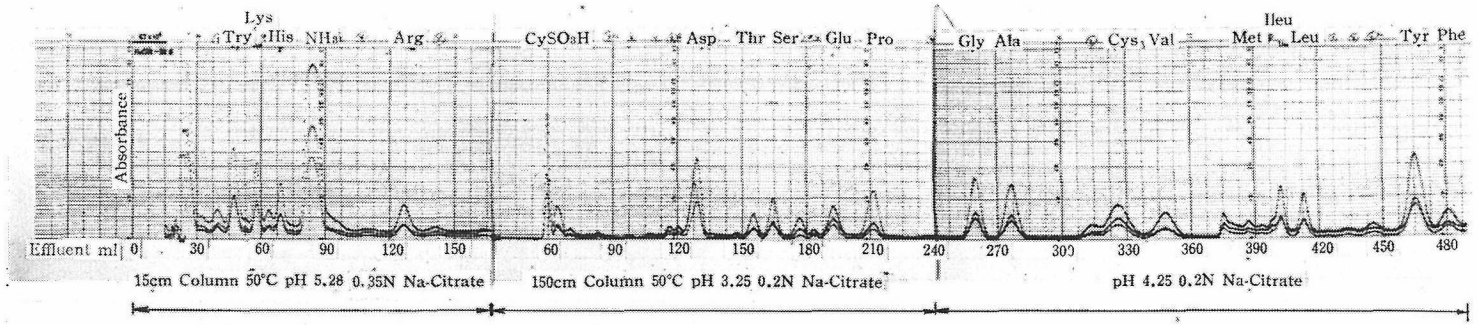

Fig. 3. Chromatogram of amino acid irradiated in the individual solution. Dose : 6.7 megaroentgens.

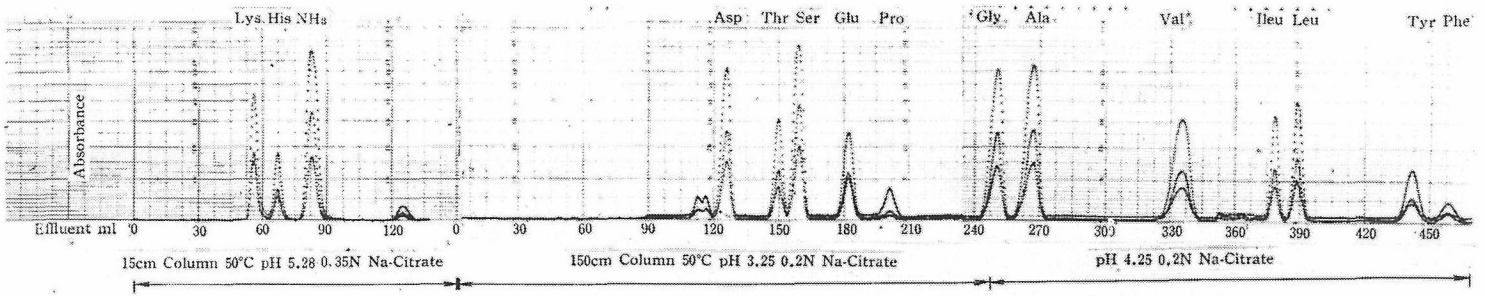

Fig. 4. Chromatogram of amino acid in the hydrolyzate of the non-irradiated proteinase in solution.

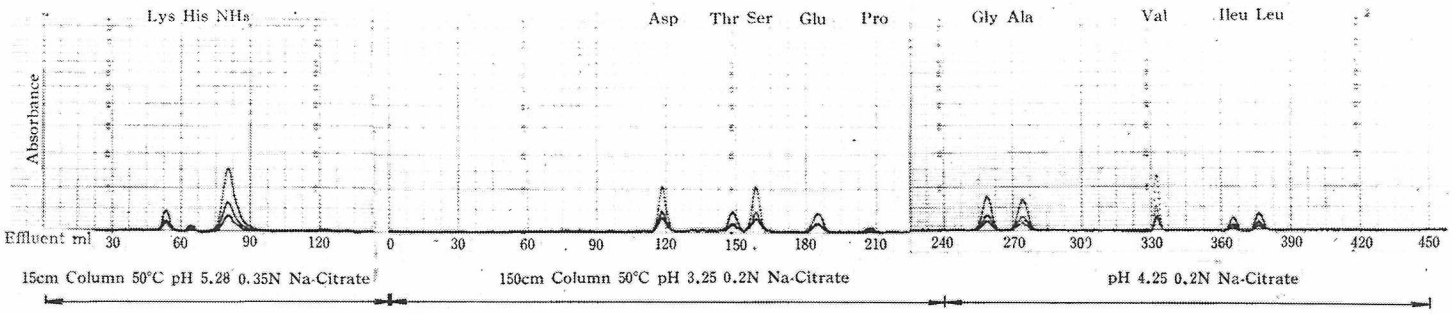

Fig. 5. Chromatogram of amino acid in the hydrolyzate of the γ-irradiated proteinase in solution.
Dose : 1.1 megaroentgens.

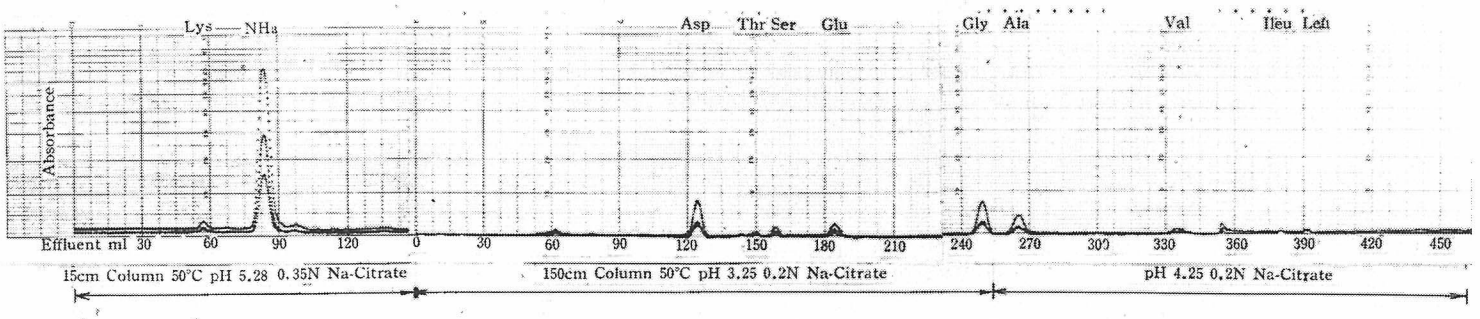

Fig. 6. Chromatogram of amino acid in the hydrolyzate of the γ-irradiated proteinase in solution.
Dose : 13 megaroentgens.

after exposure to about 100 kiloroentgen dose which caused destruction of about 50 per cent of the total amino acid residues of the protein. Similar disappearance of the constituent arginine and phenylalanine was observed after irradiation of about one megaroentgen. Histidine, threonine, proline, isoleucine and leucine were lost from the irradiated protein by the irradiation of about 10 megaroentgens.

(3) **Radiation-induced changes of amino acid composition and biological activity.** Inactivated activity of the irradiated proteinase after exposure to various γ-ray doses was measured and tabulated in Table 3. The survival activity after exposure to about 180 kiloroentgen dose was found to be about 50 per cent whereas the constituent tyrosine had been damaged completely and about 50 per cent of total amino acids destroyed in the irradiated enzyme.

When the proteinase in solotion was irradiated with more than 10 megaroentgen doses of γ-rays, the enzyme was inactivated irreversibly completely and histidine, threonine, proline, leucine and isoleucine residues did not be detected as the constituents of the damaged enzyme.

DISCUSSION

In the previous papers[19~23] it was found that sulfhydryl amino acid and enzymes were most radiosensitive and sulfhydryl group of the compounds was characteristic to the radiation-induced effects. By electron spin resonance studies[24~26] on irradiated proteins the specific behavior of the sulphur-containing compounds was realized to show. This was a main reason to be chosed the bacterial proteinase, which had been characterized to be non-sulfhydryl enzyme, as a typical enzyme protein of which chemical composition and structure were studied after exposure to irradiation of γ-rays throughout this experiment.

It was very remarkable that whereas phenylalanine was labile both in solution and in constituent of protein, tyrosine was most radioresistant in solution contrary to be most radiosensitive in constituent of protein. Some of other amino acids showed more or less similar behaviors in solution and in constituent of protein. It was found that in free amino acids in solution methionine, threonine and leucine was more radiosensitive than histidine. From these results it was shown that incorporation of amino acids into a polypeptide chain alters the radiation sensitivity and the radiation-induced chemical changes of the amino acids. Free tyrosine in solution seems to be less destructive by γ-radiation owing to its highly resonance structure of aromatic ring. Constituent tyrosine in protein, however, appeared to be affected by any other damages caused on the protein molecule. This latter property of the radiation sensitivity was resemble to that of sulfhydryl group on protein molecules or thiol compounds.

Friedberg *et al.*[8] showed about their specimen of sulfhydryl enzyme, ATP-creatine phosphotransferase, that in the intact protein in solution histidine was most radiosensitive and Drake *et al.*[7] that in insulin proline appeared more

radiosensitive than histidine. In the author's specimen of non-sulfhydryl enzyme, bacterial proteinase, it was observed that tyrosine, arginine and phenylalanine were more radio-sensitive than histidine, even if the protein does not be hydrolyzed before radiation exposure. It seems likely that a rank of constituent amino acids in terms of radiation sensitivity varies with individual proteins. In the hydrolyzate of ATP-creatine phosphotransferase, methionine was reported to be most labile to γ-radiation and phenylalanine and arginine were also faster destroyed when the irradiation was performed after pydrolysis of the protein. The fact should be considered to be resulted each other from a protecting effect of co-existing amino acids in the hydrolyzate of the enzyme because the hydrolyzate was a mixture of the constituent amino acids of the protein. It has been established that a protecting effect of co-existing compounds in an irradiated solution was observed about amino acids and other many compounds[20,21].

In the early stage of γ-irradiation, the enzyme activity seems to disappear at a faster rate than in the loss of amino acid content of the enzyme protein. This is attributed to radiation-induced changes of the conformation of the enzyme protein. On the damaged enzyme of which the constituent tyrosine residue was destroyed completely by γ-irradiation, was found about half residual activity of the full proteolytic activity. This fact lead us to a hypothesis that tyrosine may be not essential for the proteolytic activity of the enzyme.

Alexander *et al.*[27] found a product, α-amino-n-butyric acid, which appeared to be produced from glutamic acid residue according to decarboxylation in an irradiated protein solution. The new products appeared on the chromatograms of Ohara, have not yet characterized. To solve these interconversion of amino acids during radiation exposure the procedure used in this experiment by the author may be not always a most suitable method because this procedure could not differenciate intact amino acid from converted amino acid. So far as using this procedure, therfore, it is noteworthy that the radiosensitivity of amino acids in this experiment is apparent sensitivity. It would like to be necessary to perform further experimentation to determine a true radiation sensitivity of amino acids.

ACKNOWLEDGMENT

The authors wish to thank Prof. Shozo Tanaka for his encouragement throughout this study, and are indebted to Prof. Sakae Shimizu for γ-irradiation in his laboratory and to the Daiwa Kasei Company Ltd. Osaka, for the kind supply of the specimen of bacterial proteinase.

REFERENCES

(1) W. H. Dale, J. V. Davies and C. W. Gilbert, *Biochem. J.* 45, 93 (1949).
(2) H. Hatano, *J. Rad. Res.*, **1**, 28 (1962).
(3) H. Hatano, *J. Rad. Res.*, **1**, 38 (1962).
(4) H. Hatano, *This Bulletin*, **39**, 120 (1961).
(5) A. Ohara, to be published (1963).

(6) M. P. Drake, J. W. Giffee, D. A. Johnson and V. L. Koenig, *J. Am. Chem. Soc.*, 79, 1395 (1957).
(7) E. S. G. Barron, J. Ambrose and P. Johnson, *Rad. Res.*, 2, 145 (1955).
(8) S. Okada and G. Gehrmann, *Biochim. Biophys. Acta*, 25, 179 (1957).
(9) F. Friedberg and G. A. Hayden, *Arch. Biochem. Biophys.*, 98, 485 (1962).
(10) S. Ganno, *J. Biochem.*, to be published (1963).
(11) W. M. Garrison, M. E. Jayko and W. Bennett, *Rad. Res.*, 16, 483 (1962).
(12) B. M. Weeks and W. M. Garrison, *Rad. Res.*, 9, 291 (1958).
(13) M. E. Jayko and W. M. Garrison, *Nature*, 181, 413 (1958).
(14) W. Bernnett and W. M. Garrison, *Nature*, 183, 889 (1959).
(15) S. Shimizu, S. Tanaka and Y. Nakayama, *This Bulletin*, 37, 306 (1959).
(16) H. Hatano, S. Egashira, K. Ozawa, S. Ganno, I. Kuroha and K. Miyauchi, *The Hitachi Hyoron*, 44, No. 12, (1962).
(17) S. Moore, D. H. Spackman and W. H. Stein, *Anal. Chem.*, 30, 1185 (1958).
(18) "Kosokenkyuho", Ed. by S. Akabori, (Asakura Shoten Book Co., Tokyo, 1956) Vol. 2, p. 242.
(19) H. Hatano, *J. Rad. Res.*, 1, 23 (1962).
(20) S. Tanaka, H. Hatano and S. Ganno, *J. Biochem.*, 46, 485 (1959).
(21) S. Tanaka, H. Hatano and S. Ganno, *J. Biochem.*, 46, 925 (1959).
(22) S. Tanaka, H. Hatano and S. Ganno, *J. Biochem.*, 47, 361 (1960).
(23) S. Tanaka, H. Hatano, S. Ganno and T. Okamoto, *This Bulletin*, 37, 374 (1959).
(24) W. Gordy, W. B. Ard and H. Shields, *Proc. Nat. Acad. Sci.*, 41, 983 (1955).
(25) W. Gordy and H. Shields, *Rad. Res.*, 9, 611 (1958).
(26) W. Gordy and I. Miyagawa, *Rad. Res.*, 12, 211 (1960).
(27) P. Alaxander and L. D. G. Hamilton, *Rad. Res.*, 13, 214 (1960).