THE QUANTITATIVE EFFECT OF X-RAYS ON ASCORBIC ACID IN SIMPLE SOLUTION AND IN MIXTURES OF NATURALLY OCCURRING COMPOUNDS*‡

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Among the many studies that have been made on biological radiation effects, relatively few (1) have given attention to the question of what compounds in tissue may react when it is irradiated. This report is concerned with a part of an approach to this question through the study of competitive reactions in aqueous solutions.

Several facts suggest the desirability of such an approach. First, in soft tissue irradiated by high voltage x-rays the bulk of the energy absorbed will be absorbed in the water and the next largest amount in proteins as a class.[1] Most of the energy available for causing immediate reaction is therefore in many tissues initially in the water, yet even there the primary chemical reactions produced are quantitatively very small. As is plausible from purely physical data, Fricke (2) and others (3–5) working in dilute aqueous solutions have usually found that 1000 r produced measurable changes in concentration of at most a few micromoles per liter. This amount of radiation may produce profound biological effects. Finally, a great variety of inorganic and organic compounds will react in dilute aqueous solutions under the influence of x-rays.[2] While these reactions with ionized water[3] are, of course, not identical they are certainly not all independent of each other and many must occur with the same x-ray products. For instance, it is now believed, following the original suggestion by Fricke (6), that a variety of proteins, including enzymes, papilloma virus (7–8), and bacteriophage (9), compete in part with other proteins and other compounds (10) for these products. In a mixture such as non-bony

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[1] This characteristic is, of course, in marked contrast to that of the usual ultraviolet, 2500Å or more.

[2] An extensive list of references to studies on irradiated water solutions is given by Fricke (2).

[3] The phrase "ionized water" is intended to include any reactive entity which results immediately from energy absorption in the water and which involves only water and oxygen.

tissue, therefore, the many compounds there which are capable of reacting will, to an unknown but surely large extent, compete[4] for the limited amount of ionized water.

In the past, the mere fact of reaction in dilute aqueous solution has at times been adduced as evidence for the reaction of a particular compound in tissue. It is clear that while an ability to react in pure solution may be necessary, it alone gives no clue regarding the result in a complicated system. However, from a study of relatively simple mixtures, it should be possible to learn whether compounds exist which are capable of competing successfully for far more than their proportionate share of the total available ionized water.

In the initial phase of this work (11) a study was made of the x-ray inactivation of luciferin in aqueous solutions containing one of a series of compounds, including ascorbic acid. The loss of luciferin was less when the irradiated solution contained ascorbic acid than when it did not. This suggested the desirability of studying the x-ray induced reaction of ascorbic acid itself to see whether or not this reaction continued in the presence of other compounds, particularly the proteins. The results of these experiments are given below.

Methods

Stock solutions of ascorbic acid were prepared by dissolving 0.1 gm. of Merck's crystalline material in 100 ml. of 5 per cent metaphosphoric acid. Such solutions were used as the standard. Solutions to be irradiated were prepared by diluting the stock solution and neutralizing it slowly with the proper amount of sodium hydroxide in the presence of phosphate buffer of pH 6.8. The final concentration of buffer was 0.025 M. Determinations of the ascorbic acid content of control solutions were made at the beginning and end of the experiments, since spontaneous oxidation occurred in some cases. However, by chilling the solutions in an ice bath and keeping the time as short as possible the loss was not usually greater than the errors in the determination.

The titrations of ascorbic acid were made with Eastman's sodium 2,6-dichlorobenzenone indophenol (12–13) in solutions acidified with ½ or ⅓ the volume of 5 per cent metaphosphoric acid. Where proteins were present, enough 5 per cent metaphosphoric acid was added to insure complete precipitation, usually 1½ volumes, and the precipitate removed by centrifuging before titration.

Serum albumin was prepared from dried human plasma by the method of Svedberg and Sjögren (14) except that it was not reprecipitated.

Irradiation was carried out with unfiltered x-rays produced at 185 kv. The intensity was about 5000 r per minute measured in air. Exposure of solutions was in pyrex test tubes, the walls of which did not reduce the intensity of radiation by more than 10 per cent. The amounts of radiation quoted are therefore all based on the intensity in air.

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[4] Examples are known (5) where some enhancement of the radiation effect on a given compound occurs in the presence of other compounds but these are small in comparison to the inhibitions, indicative of competition, that have been observed.

Distilled water was either from a tin still or redistilled from pyrex glass. For the comparative purposes of this investigation, it did not seem necessary to take the additional precautions used by Fricke (2).

RESULTS

The data in Table I show the extent to which ascorbic acid in a solution containing only inorganic constituents, 0.025 M phosphate buffer and about 0.5 M sodium metaphosphate, reacts under the influence of x-rays. Very low concentrations of ascorbic acid were used in order to increase the relative size of the x-ray effect with moderate amounts of radiation. About one-half of the ascorbic acid reacted upon exposure to 5500 r. The data show a reasonable reproducibility in a series of comparable experiments. They also show that under

TABLE I

Concentration of unirradiated ascorbic acid solutions	Loss of concentration after		Loss per 1000 r	
	5,500 r	11,000 r		
mg./100 ml.	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*μ moles/1000 ml.*
0.36		0.32	0.029	1.7
0.43		0.35	0.032	1.8
0.45	0.23		0.042	2.4
0.43	0.22		0.040	2.3
0.42	0.21		0.038	2.2

The concentration of each unirradiated ascorbic acid solution is the average of the concentrations found at the beginning and end of the experiment. Each value given as a loss in concentration is the average value found in two to four solutions irradiated at the same time.

these conditions 1000 r produced changes in concentration of about 2 micromoles per liter. This is definitely not an absolute value since it apparently is dependent on the extent of the reaction and depends on other variables such as the absolute concentration and the pH. That the bulk of the reaction occurs during the irradiation, or at most a very few minutes thereafter, was shown by titrating at intervals a series of solutions irradiated at the same time. The average values for the remaining ascorbic found in the titrations done first, that is within a few minutes after the end of the irradiation, and the last, done perhaps half an hour later, were 0.23 and 0.20 mg./100 ml. The difference is within the error.

The extent to which ascorbic acid reacts under a particular set of conditions in relatively simple solutions having been shown, the next point to be studied was what part, if any, of this reaction would occur in the presence of proteins. To test this, the data given in Table II were obtained. It is evident that 0.2 per cent serum albumin, about 5×10^{-5} M, does not reduce the radiation effect

on 3.5×10^{-5} M ascorbic acid by more than the variation between the individual control experiments.

Since a single experiment with 2.5 per cent albumin still showed the usual loss of the ascorbic acid, the behavior of the reaction in a much more complicated system, blood plasma, was tested. Dried plasma, to which ascorbic acid had been added, was first used, followed by a few confirmatory experiments with fresh plasma. These results are given in Table III. For 11,000 r, the average

TABLE II

Loss of Ascorbic Acid

Concentrations of the unirradiated solutions are given at the head of each column.

Amount of irradiation					0.2 per cent serum albumin		
0	0.57	0.53	0.51	Average	0.60	0.62	Average
	mg./100 ml.	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*
2750r	0.11	0.12	0.14	0.12	0.08	0.11	0.10
5500r	0.22	0.20	0.22	0.21	0.19	0.20	0.20
11,000r	0.36	0.33	0.37	0.35	0.35	0.38	0.37
16,500r	0.46	0.42	0.45	0.44	0.50	0.51	0.51

TABLE III

Ascorbic acid irradiated in	Amount of irradiation	Concentration of ascorbic acid				Average loss
	r	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*
Buffer solution	0	0.41	0.40	0.43	0.43	
	11,000	0.10	0.07	0.06	0.08	0.34
7 per cent dried plasma	0	0.47	0.46	0.47	0.42	
	11,000	0.17	0.17	0.18	0.16	0.29
Fresh plasma	0	0.57	0.83	0.86		
	5,500	0.39				0.18
	11,000	0.25				0.32
	22,000		0.16	0.19		0.67

loss from the controls is 0.34 mg./100 ml., while from the dried plasma it is 0.29 mg./100 ml. The data show that the ascorbic acid reaction continues even in fresh plasma.

It was therefore of interest to know what happens to the reaction in a still more complicated system such as muscle. A large number of experiments were carried out in which muscle tissue was irradiated after removal from the animal. Table IV shows experiments on muscle taken from the hind legs of rats. The muscles were cut into pieces with scissors, either immediately or 24 hours after removal from the animal, the pieces mixed thoroughly, and then a number of samples were taken as controls and for irradiation. The data shown

TABLE IV

Ascorbic Acid in Milligrams per 100 Gm. of Rat Muscle

Experiment	Controls	After 22,000 r	Differences
	mg./100 gm.	*mg./100 gm.*	*mg./100 gm.*
108	2.73	2.67	
	2.47	2.17	−0.18
112	1.15	0.87	
	1.29	0.73	−0.42
116	2.53	2.12	
	2.30	1.96	
	2.25		−0.32
117	1.70	1.80	
	1.74	1.91	+0.14
118	1.30	1.26	
	1.10	1.12	−0.01
119	1.76	1.76	
	1.82	1.78	−0.02
121	1.82	1.42	
	1.64	1.48	
	1.69	1.51	−0.25
122	1.92	0.99	
	1.66	1.29	
	1.65	1.38	−0.52
123	1.76	1.33	
	1.75	1.47	
	1.66	1.44	−0.31
127	1.41	1.41	
	1.42	1.42	−0.00
128	1.60	1.31	
	1.57	1.54	−0.16
Average			−0.19

are from only those experiments in which the controls showed reasonable agreement. The average difference between the controls and the samples given 22,000 r of irradiation is only 0.2 mg./100 gm., compared with nearly 0.7 mg./100 ml., in blood plasma. If two out of the 25 pairs of samples are

omitted, the average difference between control and irradiated muscle becomes only 0.15 mg./100 gm. The questions of whether or not this average apparent effect is real and whether the variability is due to random error or to real differences in the tissue need not be considered now. The important point shown in this series of experiments is that in most of the excised rat muscle tissue, the observed effect of x-rays on ascorbic acid is relatively small.

DISCUSSION

The change produced in ascorbic acid per 1000 r is within the usual range of 1 to 4 micromoles per liter reported for a number of organic compounds (2, 4, 15) under various conditions. It is reasonable to assume, therefore, that the ascorbic acid reaction represents a substantial fraction of the total ionized or activated water available. Hence it is of considerable interest that the change occurs without great interference in the presence of all of the compounds in oxalated blood plasma at the concentrations in which they are there found. This is particularly striking in the case of the albumins which, even on a molar basis, were 15 or 20 times as concentrated as the ascorbic acid. This is in contrast to the behavior of the x-ray induced reactions of ferrous sulfate and of thiamin. In unpublished work[5] it has been found that both reactions are hindered by the presence of a few tenths per cent of serum proteins. Whether or not this property of ascorbic acid is due solely to an exceptional speed of reaction with ionized water is, of course, not known. Possibly it reacts secondarily with other affected compounds since it is such a good reducing agent. In any case, it seems unlikely that ascorbic acid is the only substance capable of reacting to an unusual extent in the presence of other naturally occurring compounds. Not all of the reactive entities formed in water by radiation would be expected to react with ascorbic acid. Also, the difference in the behavior of the reaction in plasma and in muscle suggests that in the latter, compounds are present which do interfere with the reaction of ascorbic acid. This may be due to compounds absent from plasma or present there in much lower concentration. Work is now in progress on this phase of the problem.

There is no suggestion from this work that small losses of ascorbic acid which may occur are, as such, factors in the destructive biological effects of x-rays. However, in view of its very wide distribution, the possibility that ascorbic acid exerts significant protective action on important compounds such as the proteins, or in biological effects, is being investigated. It would also be of interest to know the competitive behavior in simple solutions of ascorbic acid, and other compounds, with auxin because of Skoog's report (1) on the relation of auxin to some radiation effects on plants.

[5] These experiments were carried out by Mr. Bieber.

SUMMARY

Data on the x-ray induced reaction of ascorbic acid in simple inorganic solution, in solutions containing serum albumin, in plasma, and in muscle have been presented. The reaction occurred in the presence of serum albumin and in human plasma but was relatively small in excised rat muscle.

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