243. *The Action of γ-Radiation on Aqueous Solutions of Cysteine.*

By A. J. Swallow.

It is shown that dilute aqueous solutions of cysteine hydrochloride are oxidised by the action of γ-radiation, and that for air-saturated solutions the ionic yield increases with concentration. For a 0·051M-solution of cysteine hydrochloride the ionic yield is 24, which is too great to be explained without postulating a chain reaction. In the absence of dissolved oxygen the ionic yield for a 0·051M-solution is 3. These results are explained by equations which may have implications for enzyme chemistry.

The deactivation of enzymes in solution by ionizing radiations can be inhibited by the addition of various protective substances (Dale, *Biochem. J.*, 1942, **36**, 80; *Brit. J. Radiol.*, 1947, Suppl. No. 1, 46). It is generally considered that these substances act by competing with active groups of the enzyme for the radicals produced by irradiation. Amongst other substances, those containing thiol groups have been used in studying the radiation chemistry of enzymes. For example, glutathione is a protective substance for ribonuclease (Collinson, Dainton, and Holmes, *Nature*, 1950, **165**, 266). The role of thiol groups in the enzymes themselves has been investigated by Barron, Dickman, Muntz, and Singer (*J. Gen. Physiol.*, 1948—49, **32**, 537; Barron and Dickman, *ibid.*, p. 595). The interesting result that cysteine caused *increased* deactivation of catalase while cystine protected it has been reported by Forssberg (*Nature*, 1947, **159**, 308). In the biological field, cysteine was one of the first

substances to be injected into rats or mice to protect them from the lethal effect of *X*-radiation (Patt, Tyree, Straube, and Smith, *Science*, 1949, **110**, 213).

It was thought of interest to study the effect of radiation on cysteine itself, partly with regard to its action as a protective substance, but more particularly for its own radiation chemistry. Recent work by Dale and Davies (*Biochem. J.*, 1951, **48**, 129) has been concerned with the production of hydrogen sulphide from cysteine, and Rotheram, Todd, and Whitcher (AECD, UCLA-119, 1951) have studied the oxidation of the thiol group and the formation of hydrogen peroxide and hydrogen sulphide in aqueous solutions of cysteine. Dale and Davies (*loc. cit.*) have also shown that deamination, which is important with other amino-acids, does not proceed appreciably with cysteine.

FIG. 1. *Irradiation of* 0·00051M-*cysteine* (*air-saturated*).

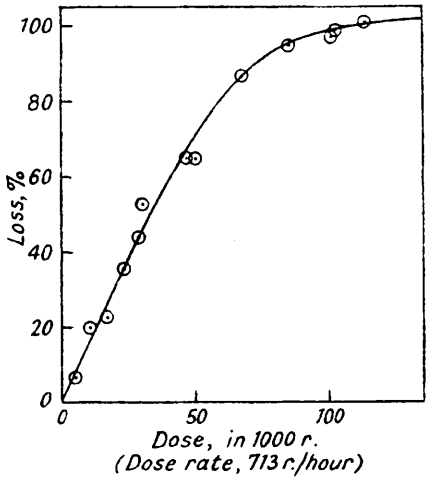


FIG. 2. *Irradiation of* 0·0051M-*cysteine* (*air-saturated*).

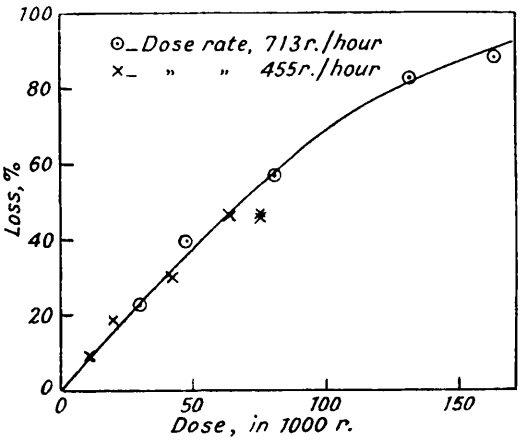
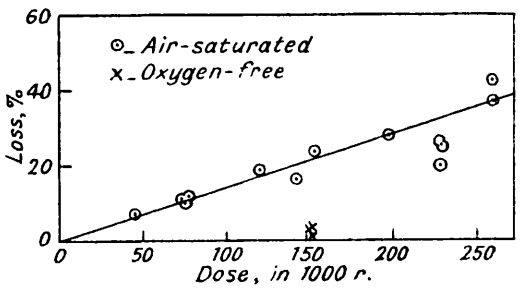


FIG. 3. *Irradiation of* 0·051M *cysteine* (*dose rate*, 455 *r./hour*).



It was decided to measure the decrease of cysteine concentration with irradiation, rather than to measure the amount of product formed. This method was adopted because one of the main products of irradiation was expected to be cystine, and this cannot be measured accurately in the presence of large amounts of cysteine.

Fig. 1 shows the results obtained for a 0·00051M-solution of cysteine hydrochloride, Fig. 2 those for 0·0051M, and Fig. 3 for 0·051M. All three curves are for air-saturated solutions. Stein and Weiss have found that after a certain dose the rate of reaction sometimes drops owing to the exhaustion of oxygen originally present in the solution (*J.*, 1949, 3245; Loebl, Stein, and Weiss, *J.*, 1950, 2704). This was not the case in these experiments because the rate of irradiation was so slow (455—713 r./hour), and the volume of solution so small, that air was able to dissolve continuously and keep the solutions nearly saturated.

During the irradiation of all these solutions the smell of hydrogen sulphide became noticeable, and colourless hexagonal crystals, presumably of cystine, separated from the two more concentrated solutions.

In the absence of dissolved oxygen the loss of cysteine from the 0·051M-solution was much less pronounced, and after a dose of 150 000 r. the cysteine concentration was still more than 0·049M. Results for oxygen-free cysteine are also shown on Fig. 3.

Experiments were conducted on the effect of γ-radiation on cystine. 0·025M-Solutions of cystine in 0·12M-hydrochloric acid were freed from oxygen and saturated with hydrogen. A dose of 52 300 r. produced 0·0003M-cysteine.

The ionic yields for all these reactions are presented in the following table.

0·00051M-Cysteine, air saturated	$2{\cdot}5 \pm 0{\cdot}3$	Molecules of cysteine lost (formed) per 32·5 ev absorbed
0·0051M- ,, ,,	$13 \pm 1{\cdot}5$	,, ,, ,, ,,
0·051M- ,, ,,	24 ± 3	,, ,, ,, ,,
0·051M-Cysteine, oxygen-free	$3 \pm 1{\cdot}5$	,, ,, ,, ,,
0·025M-Cystine, hydrogen saturated	2 ± 1	,, ,, ,, ,,

It has been usual to assume that the energy required to create an ion-pair in water is the same as that for air, *viz.*, 32·5 ev in the case of γ-radiation (see Lea, "Actions of Radiation on Living Cells," Cambridge, 1946). It is now known that this assumption is not justified and that the value 32·5 ev has no significance for aqueous systems. It was decided however to express ionic yields in this paper as molecules lost per 32·5 ev absorbed so that comparison may be made with other published results. The choice of the value 32·5 ev should be regarded as purely conventional, and the conclusions presented are not dependent on its having any physical significance.

The ionic yield of 24 is exceptionally high for radiation chemistry, and is of the same order as the yield reported by Rotheram, Todd, and Whitcher (*loc. cit.*) for the oxidation of cysteine by *X*-radiation at pH 8.

Discussion.—It is now well established that the primary step in the action of ionising radiations on aqueous solutions is the formation of hydrogen atoms and hydroxyl radicals (Weiss, *Nature*, 1944, **153**, 748; Stein and Weiss, *J.*, 1949, 3245):

$$H_2O \rightsquigarrow H + OH \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

In the presence of dissolved oxygen the following reaction is also to be expected to occur to a considerable extent:

$$H + O_2 \longrightarrow HO_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

so that strong oxidising conditions are predominant, even though both OH radicals and H atoms are initially produced by radiation.

The ionic yields for the loss of cysteine are much greater than could be accounted for solely by the production of hydrogen sulphide in the yields reported by Dale and Davies, and by Rotherham, Todd, and Whitcher (*locc. cit.*). The most readily formed oxidation product of cysteine is cystine, and it is assumed that that is the main product of the reactions studied here. Rotheram, Todd, and Whitcher also consider that cystine is the main product of irradiation.

The ionic yield of 24 for a 0·051M-solution is exceptionally high. It is difficult to explain it by any simple reaction of the radicals formed by the primary act, because even in water vapour the maximum possible number of radicals is 6 hydrogen and 6 hydroxyl from each 32·5 ev of energy absorbed (Dainton, *Ann. Reports*, 1948, **45**, 5). In liquid water the number is likely to be less (*idem*, *J. Phys. Coll. Chem.*, 1948, **52**, 490). A much more likely explanation is that a chain reaction is taking place. The system is very complex but the following equations are put forward tentatively to show how a chain reaction might take place:

$$RSH + OH \longrightarrow RS + H_2O \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

$$RS + RSH \longrightarrow RS{\cdot}SR + H \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

where RSH is cysteine and RS·SR cystine. A reaction like that of equation (4) has been suggested previously for the photolysis of ethanethiol (Meissner and Thompson, *Trans. Faraday Soc.*, 1938, **34**, 1238). The hydrogen atom reacts with oxygen according to equation (2), and the HO_2 radical so formed reacts with cysteine :

$$HO_2 + R{\cdot}SH \longrightarrow R{\cdot}S{\cdot} + H_2O_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

The hydrogen peroxide so formed may also react slowly with cysteine to increase the ionic yield, while the R·S· radical reacts according to equation (4).

Equations (4), (2), and (5) constitute a chain reaction. The chain can be terminated by many reactions, amongst which are the combination of H, OH, HO_2, and RS radicals. In the absence of oxygen, of course, the chain reaction would not proceed, and this explains the low ionic yield in the absence of dissolved air. In the more dilute solutions, the ionic yield is less, since the termination reactions compete more favourably with the reactions whose equations are given above.

The reduction of cystine to cysteine in the presence of hydrogen is not a chain reaction, and may be due to the reverse of reaction (4).

The existence of free thiol radicals has often been postulated and may be responsible for the action of certain enzymes (Waters, "Chemistry of Free Radicals," 2nd edn., Oxford, 1948, 73, 188, 257, 282). In fact, the reactions described above have many implications for enzyme chemistry, and it is hoped elsewhere to discuss the possibility that by radiation-chemical methods one may imitate more or less exactly the action of certain enzymes. The work of Weiss and his collaborators has provided valuable evidence in this field by showing that the products of biological reactions and of radiation-induced reactions are often the same.

Experimental

Cysteine.—The DL-cysteine hydrochloride used in these experiments was obtained from two sources, L. Light and Co. Ltd. and British Drug Houses Ltd. Results obtained with the two kinds were identical. Cysteine hydrochloride was always dried before use, as it is slightly deliquescent. Cysteine solutions were always fairly stable, and even for the longest irradiations with the most dilute solutions the decrease in cysteine concentration of a control due to normal air oxidation was not more than 12%. Freshly made solutions were always used for irradiations of 0·00051M- and 0·0051M-cysteine. For 0·051M-cysteine this was not necessary.

L-Cystine was obtained from British Drug Houses Ltd.

Water.—Water was specially purified by distillation from alkaline permanganate followed by redistillation and a second redistillation directly into the flask in which the solution was to be made. Pyrex apparatus was used throughout.

De-aeration.—The apparatus used for de-aeration is shown in Fig. 4. The tubes at the lower end of each limb were removed, and 1 ml. of purified water was introduced into each, followed by 1 ml. of the solution to be irradiated. The tubes were then replaced on the ground-glass joints which had been greased round the top with Apiezon L grease. Air was removed by alternate evacuation through a liquid-air trap and saturation with oxygen-free nitrogen (British Oxygen Company Ltd., Wembley) until the volume of the solutions was reduced to 1 ml. by evaporation. The whole apparatus was shaken to avoid bumping. The evacuation–saturation cycle had to be repeated about 12 times. Finally, the tap *A* was closed while the solutions were under a pressure of nitrogen a few cm. above atmospheric, and the lower part of the apparatus was detached at *B*.

A similar procedure, but with use of oxygen-free hydrogen, was adopted when preparing cystine for irradiation.

pH *of Solutions.*—No attempt was made to adjust all solutions of cysteine to the same pH or chloride concentration. The pH of 0·00051M-cysteine is 3·3, of 0·0051M, 2·6, and of 0·051M, 1·9. After irradiation with a dose of 100 000 r., the pH of the 0·00051M-solution had been reduced to 3·15. The decrease of pH did not cease when all the cysteine had been oxidised, presumably owing to the formation from cystine of further products such as cysteic acid.

Irradiation Arrangements.—0·00051M-Solutions and some 0·0051M-solutions were irradiated in the apparatus shown in Fig. 5. A 100-mc metallic source of ^{60}Co (from A.E.R.E., Harwell) was lowered into the central tube with a bar magnet. Other solutions were irradiated in flat-bottomed tubes, 1 cm. in diameter, fixed with elastic bands round a central tube con-

taining the ^{60}Co. Air-free and hydrogen-saturated solutions were arranged round the ^{60}Co in this way, and 2" lead blocks placed between the two limbs of the apparatus to protect the control solution from radiation. All solutions were irradiated at room temperature (5—15°) and no attempt was made to control this since it was not expected that the results would be sharply temperature-dependent. It may be, however, that some of the errors in the experiments are due to fluctuation in temperature.

Determination of Cysteine.—The method used was that of Kuhn, Birkofer, and Quackenbush (*Ber.*, 1939, **72**, 407), based on the reaction of cysteine with excess of iodine in glacial acetic acid. The exact procedure depended on the concentration of cysteine. The following was used for cysteine $\leqslant$ 0·00051M.

Cysteine solution (1 ml.) in a weighing bottle was evaporated to dryness on a steam-bath, and, when cool, water (two drops) was added, followed by distilled " AnalaR " glacial acetic

FIG. 4. *Apparatus for removing oxygen from solutions.*

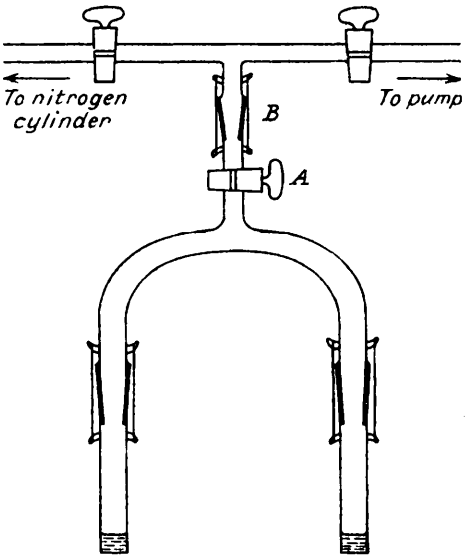
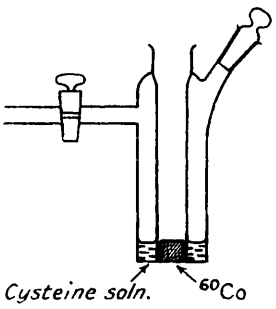


FIG. 5. *Irradiation apparatus.*



acid (1 ml.). A glacial acetic acid solution (0·5 ml.) of iodine was then added rapidly, followed after 1 minute by water (about 1·5 ml.). This solution was then titrated with 0·001N-sodium thiosulphate, starch being used as indicator. Since the reaction of cysteine with iodine did not proceed stoicheiometrically, it was necessary to calibrate with a freshly made standard solution of cysteine for each set of titrations. Typical results are shown in the following table.

Results of irradiating 0·00051M-*cysteine hydrochloride.*

Solution titrated	0·001N-$Na_2S_2O_3$ required, ml.	Concn. (M) of solution titrated	Decrease of concn. (M) during irradiation
0·00051M-Cysteine standard ...	0·18, 0·20, 0·20, 0·19, 0·20 Average = 0·194	0·00051	—
Irradiated solution (101 000 r.)	0·575, 0·56, 0·57, 0·59, 0·58 Average = 0·575	0·000025	0·000485
Control solution	0·245, 0·235, 0·23, 0·24, 0·24, 0·25 Average = 0·24	0·00045	0·00006
Blank (no cysteine)	0·57, 0·60, 0·605, 0·60, 0·57, 0·59, 0·605 Average = 0·595	0	—

The titration of 0·00051—0·0051M-cysteine was carried out in a similar manner except that only 0·1 ml. of cysteine was used.

In order to titrate 0·0051—0·051M-cysteine, 1 ml. of the solution was diluted to 10 ml., and 0·1-ml. quantities were used. No calibration was necessary in this case as the control solution could within the experimental limits be assumed to be exactly 0·051M.

The process of evaporation to dryness, mentioned above, was necessary to remove hydrogen sulphide. Solutions evaporated at room temperature in a vacuum desiccator gave the same result, but this procedure was slower.

The following substances were shown not to interfere with the determination : hydrogen sulphide, hydrogen peroxide, pyruvic acid, and glyceraldehyde.

Dose Measurement.—Dose measurement was by Day and Stein's chemical method (*Nucleonics*, 1951, **8**, No. 2, 34). Aqueous solutions of benzene were irradiated to produce phenol, which was measured colorimetrically. The dose rate produced in the apparatus shown in Fig. 5 was 713 r./hour, and in the flat-bottomed tubes 455 r./hour.

This work was carried out while the author was first a Nuffield Research Fellow, and later an I.C.I. Fellow of the University of Birmingham.

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