

## STUDIES ON THE MECHANISM OF ACTION OF IONIZING RADIATIONS

### VI. THE OXIDATION OF THIOLS BY IONIZING RADIATIONS

BY E. S. GUZMAN BARRON AND VERONICA FLOOD

*(From the Argonne National Laboratory and the Chemical Division,  
Department of Medicine of the University of Chicago, Chicago)*

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The importance of thiols in general physiology is now generally recognized. The -SH groups that exist in the cell both in peptides or free amino acids and as an integral part of proteins play a very important rôle in the processes of cell division and cell growth. This has been shown by the investigations of Hammett (1), Voegtlin and Chalkley (2), and Rapkine (3) among many others. The -SH groups in the proteins of a large number of enzymes have also been found to be essential for enzyme activity. It is known that during mitosis and during the phase of growth, cells are more easily injured by the action of ionizing radiations, and a relation between these two processes, destruction of -SH groups and inhibition of cell growth, has been frequently sought. Woodward (4) studied the effect of ultraviolet light, radium, and x-irradiation on aqueous solutions of glutathione; and although he found that ultraviolet light produced oxidation of the -SH groups more easily than destruction of the molecule, he was unable to observe measurable oxidation on irradiation with both beta and gamma rays from radium, and with x-rays. Kinsey (5) studied the effects on glutathione. However, he avoided a study of the oxidizing action of the ionizing radiations and confined himself to measuring the destruction of the molecule. In previous communications (6, 7) it was shown that enzymes requiring -SH groups for activity were readily inhibited by ionizing radiations; furthermore, the reversibility of this inhibition was an indication of the oxidation of the -SH groups of the protein. It was, for these reasons, necessary to reinvestigate the problem of the oxidation of thiols by ionizing radiations under carefully controlled conditions. We present in this paper the results of such studies which show that these substances are easily oxidized by x-rays and by beta and gamma rays.

#### EXPERIMENTAL

It is known that glutathione and other thiols are not autoxidizable, but are rapidly oxidized by catalytic amounts of copper (8, 9). Heavy metal contamination has to be avoided carefully in experiments where oxidation of easily oxidizable systems is to be studied. The distilled water used in these experiments was prepared from the triple distilled water used in the laboratory. It was distilled again twice in a pyrex

glass distillation apparatus used for the preparation of conductivity water. The first distillation was over acid permanganate; the second, over barium hydroxide. At first, the distilled water thus obtained was kept in well paraffined pyrex bottles. It was found, however, that when this water was kept for some days it gave non-reproducible results. The experiments were then started again with freshly distilled water, the distillation being conducted at a moderately slow rate. All the chemicals used were recrystallized from the five times distilled water and were free of copper and iron, as tested by sensitive colorimetric qualitative tests. Glutathione was obtained from Schwartz and Co., New York; the other thiols were samples from this laboratory. The salts, acids, and bases used in the preparation of buffers were Merck's reagent grade. X-irradiation was performed with a 250 kv., 15 ma. machine with a 0.5 mm. Cu filter at a focal distance of 48.3. cm. The tubes containing the solutions were kept on a lucite holder over cracked ice. Radiations with gamma rays were performed according to the technique of Barron and Dickman (7). Radiations with beta rays were performed by the addition of increasing quantities of carrier-free  $P^{32}$  as the Na salt, buffered to pH 7.0. The sulfhydryl groups of glutathione and of the thiols were determined by the colorimetric method of Anson (10), namely oxidation with ferricyanide and formation of Prussian blue with the ferrocyanide thus formed. The method was adapted for use in the Beckman spectrophotometer, the total volume of the solution being 5 cc. Readings were made at 7100 Å. Under those conditions, from 0.2 to 3  $\mu$ g. glutathione can be estimated; *i.e.*, from 0.04 to 0.6  $\mu$ g. per cc.

*1. Oxidation of Thiols by X-Irradiation.*—The first thiol compound to be studied was propane-1,3-dithiol, which was dissolved in ice cold phosphate buffer, pH 6.8 (0.01 M), immediately before irradiation. Vials containing 1 cc. of the dithiol solution ( $9.2 \times 10^{-8}$  M) were irradiated with increasing dosages of x-rays from 500 r to 5000 r. As can be seen in Fig. 1, the per cent oxidation was directly proportional to the dosage of irradiation. For the calculation of the ionic yield (the number of molecules,  $M$ , oxidized divided by  $N$ , number of ion pairs produced on ionization of water) the value of  $1.601 \times 10^{12}$  was taken as the number of ions produced on ionization of 1 cc. of water by 1 roentgen unit of x-rays. The average ionic yield was 3.5 (Table I).

2,3-Dimercaptopropanol (BAL) was x-irradiated at a concentration of  $2.008 \times 10^{-7}$  M per cc. It was dissolved in phosphate buffer, pH 6.82 (0.01 M) shortly before irradiation. The extent of oxidation was directly proportional to the dosage of irradiation (Fig. 2). The ionic yield, 3.72, was a little higher than that found in the oxidation of propane-1, 3-dithiol (Table II).

The oxidation of glutathione by x-irradiation was also directly proportional to the dosage (Fig. 3). In these experiments the amount of glutathione was  $2 \times 10^{-7}$  M per cc. dissolved in phosphate buffer, pH 7.3 (0.01 M). The ionic yield was 3.35, a value somewhat lower than that of the other two thiol compounds (Table III). The ionic yield did not change on increasing the glutathione content ten times, to  $2 \times 10^{-6}$  M per cc.

Great difficulty was encountered on measuring the rate of oxidation of

*N*-phenylaminopropanedithiol-HCl because of the low solubility of this compound. As can be seen in Fig. 4, there appeared to be two different rates; the

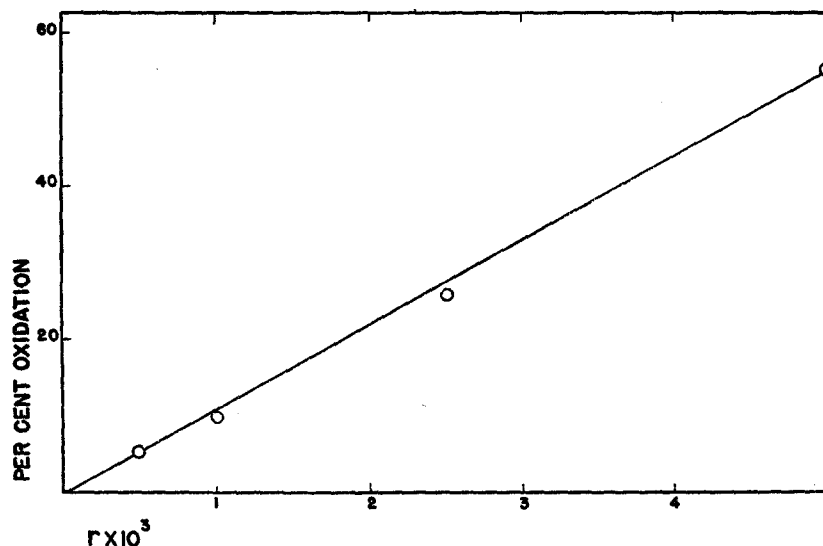


FIG. 1. Oxidation of propane-1,3-dithiol by x-rays. 1 cc. thiol,  $9.2 \times 10^{-3}$  M pH, 6.82.

TABLE I

*Oxidation of Propane-1,3-Dithiol by X-Irradiation*  
*Effect of X-Ray Dosage*

1 cc. dithiol =  $9.2 \times 10^{-3}$  M irradiated at  $0^\circ$ . pH, 6.82. Phosphate buffer, 0.01 M.  $M$  = number of molecules oxidized;  $N$  = number of ion pairs produced on irradiation ( $1.601 \times 10^{18}$  per dose in r).  $\frac{M}{N}$  = ionic yield.

| X-ray dosage | $M$              | Ionic yield |
|--------------|------------------|-------------|
| $r$          | $\times 10^{18}$ |             |
| 500          | 2.88             | 3.6         |
| 1000         | 5.21             | 3.2         |
| 2500         | 14.1             | 3.5         |
| 5000         | 30.1             | 3.7         |

first which was fast went up to 40 per cent oxidation; the second, was slow. No attempts were made to recrystallize the compound which is very slowly oxidized by copper, when the latter is used as the oxidizing catalyst (9).

2. *Oxidation of Propane-1,3-Dithiol by Beta Irradiation.*—The first experiments on the effect of beta rays were carried out with  $\text{Sr}^{89}$  as  $\text{Sr}^{89}\text{Cl}_2$ . The

results were inconsistent, probably because of the large amount of impurities contained in the samples, as shown by the ash content. More reproducible

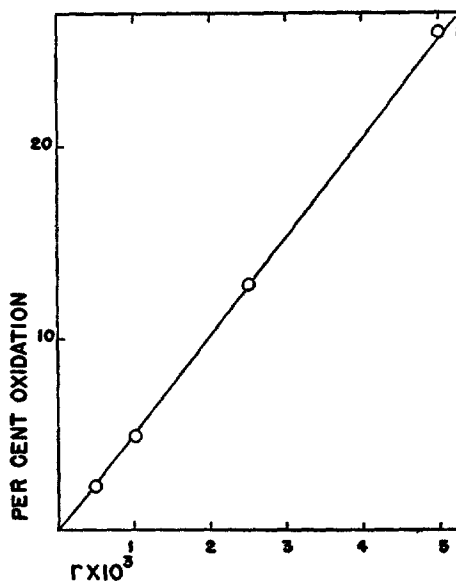


FIG. 2

FIG. 2. Oxidation of 2,3-dimercaptopropanol by x-rays. 1 cc. thiol,  $2 \times 10^{-7}$  M, pH, 6.82.

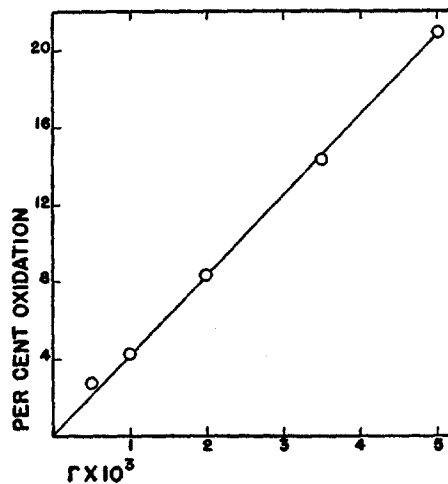


FIG. 3

Fig. 3. Oxidation of glutathione by x-rays. 1 cc.,  $2 \times 10^{-7}$  M, pH, 7.3.

TABLE II

*Oxidation of 2,3-Dimercaptopropanol (BAL) by X-Irradiation*  
*Effect of X-Ray Dosage*

1 cc. BAL,  $2.008 \times 10^{-7}$  M irradiated at 0°. pH, 6.82. Phosphate buffer, 0.01 M.

| X-ray dosage | M                | Ionic yield |
|--------------|------------------|-------------|
| $r$          | $\times 10^{15}$ |             |
| 500          | 2.78             | 3.44        |
| 1000         | 6.045            | 3.73        |
| 2500         | 15.475           | 3.83        |
| 5000         | 31.434           | 3.88        |

results were obtained when carrier-free  $P^{32}$  was used as the source of beta radiation. The experiments were performed by adding to the solution of propane-1,3-dithiol increasing amounts of  $P^{32}$  (as the Na salt buffered to pH 7.0) so as to give in 2.5 hours doses of radiation varying from 281 to 3375 r. Spherical vessels were used in these experiments. The per cent oxidation was directly

proportional to the intensity of irradiation (Fig. 5). For the calculation of the ionic yield, the value of  $1.83 \times 10^{12}$  ion pairs was taken as that produced on ionization of 1 cc. water by 1 r of beta rays. The average ionic yield was 3.32 (Table IV). Since about 10 per cent of irradiation was lost at the surface of the spheres, the ionic yield is in close agreement with that obtained on irradiation by x-rays.

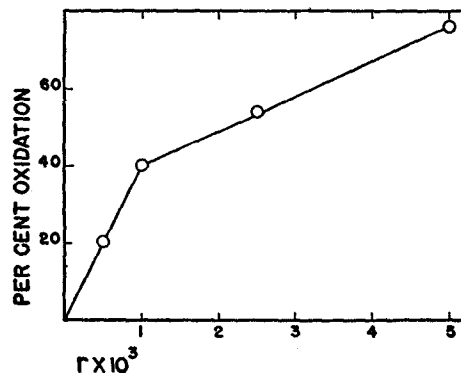


FIG. 4. Oxidation of *N*-phenylaminopropanedithiol-HCl by x-rays. 1 cc.,  $1 \times 10^{-7}$  M, pH, 6.97.

TABLE III  
*Oxidation of Glutathione by X-Irradiation*  
*Effect of X-Ray Dosage*

1 cc. glutathione,  $2 \times 10^{-7}$  M irradiated at 0°. Phosphate buffer, 0.01 M, pH, 7.3.

| X-ray dosage | <i>M</i>         | Ionic yield |
|--------------|------------------|-------------|
| <i>r</i>     | $\times 10^{15}$ |             |
| 500          | 3.39             | 4.13        |
| 1000         | 5.08             | 3.14        |
| 2000         | 10.16            | 3.14        |
| 3500         | 17.3             | 3.06        |
| 5000         | 26.62            | 3.29        |

3. *Oxidation of Glutathione by Gamma Irradiation.*—To study the oxidation of glutathione by gamma irradiation, glutathione was dissolved in phosphate buffer, pH 6.85 (0.01 M) so that 1 cc. contained  $4 \times 10^{-8}$  M. The solutions, prepared immediately before irradiation, were irradiated with radium according to the technique of Barron and Dickman (7). Here, too, the extent of oxidation was directly proportional to the dosage of radiation (Fig. 6). For the calculation of the ionic yield, the value of  $1.71 \times 10^{12}$  ion pairs was taken as that produced on ionization of 1 cc. of water by 1 r of gamma rays. By discarding the low

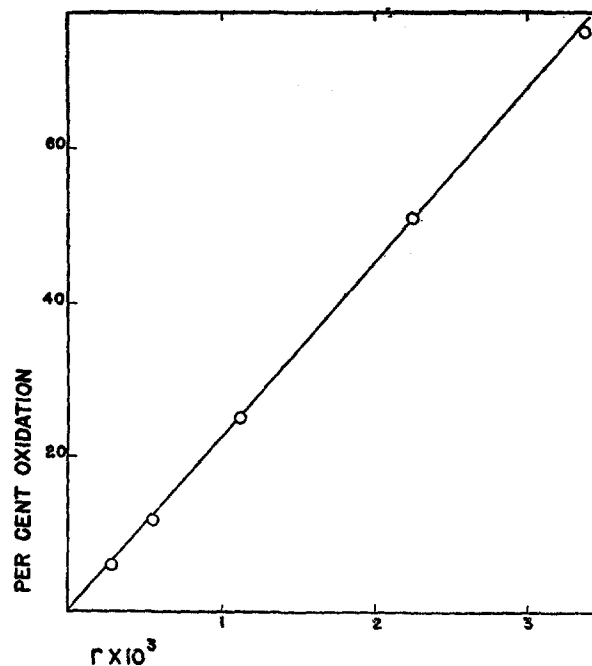


FIG. 5. Oxidation of propane-1,3-dithiol by beta rays. (carrier-free  $P^{32}$ ). 1 cc. thiol  $4.6 \times 10^{-8}$  M. pH, 7.0.

TABLE IV

*Oxidation of Propane-1,3-Dithiol by Beta Irradiation (Carrier-Free  $P^{32}$ )*  
*Effect of Beta-Ray Dosage*

1 cc. of propane-1,3-dithiol.  $4.6 \times 10^{-8}$  M irradiated at  $0^\circ$ . Buffer, phosphate, 0.01 M, pH, 7.0.  $P^{32}$  as the Na salt brought to pH 7.0 added in increasing quantities to increase the irradiation dosage. Time of exposure, 2.5 hours. 1 beta r taken as giving  $1.83 \times 10^{18}$  ion pairs on ionization of 1 cc.  $H_2O$ .

| Beta-ray dosage | $M$              | Ionic yield |
|-----------------|------------------|-------------|
| $r$             | $\times 10^{18}$ |             |
| 281             | 1.66             | 3.23        |
| 562             | 3.32             | 3.23        |
| 1125            | 6.92             | 3.34        |
| 2250            | 14.13            | 3.43        |
| 3375            | 20.77            | 3.37        |

value obtained on irradiation with 270 r, the average ionic yield was 2.16 (Table V), a value which is 77 per cent the ionic yield obtained on irradiation of glutathione by x-rays at this pH (2.80).

4. *The Effect of pH on the Oxidation of Glutathione by X-Rays.*—It was shown by Lyman and Barron (8) that the oxidation of glutathione with  $\text{Cu}^{++}$  as the oxidizing catalyst depended, among other factors, on the degree of dissociation

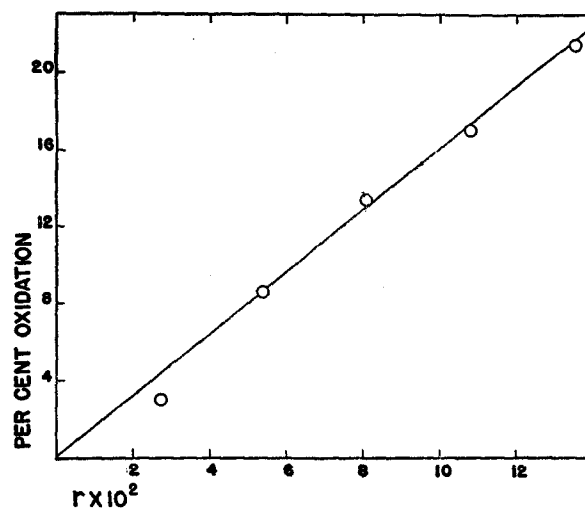


FIG. 6. Oxidation of glutathione by gamma rays (radium). 1 cc.,  $4 \times 10^{-8}$  M. pH, 6.85

TABLE V

*Oxidation of Glutathione by Gamma Irradiation*  
*Effect of Gamma-Ray Dosage*

1 cc. glutathione,  $4 \times 10^{-8}$  M irradiated at  $0^\circ$ . Phosphate buffer, pH, 6.85, 0.01 M.  $M$ , number of molecules oxidized;  $N$ , number of ion pairs formed on ionization of 1 cc.  $\text{H}_2\text{O}$ , 1 gamma r taken as giving  $1.71 \times 10^{22}$  ion pairs.

| Gamma-ray dosage | $M$              | Ionic yield |
|------------------|------------------|-------------|
| $r$              | $\times 10^{14}$ |             |
| 270              | 7.23             | 1.49        |
| 540              | 20.73            | 2.15        |
| 810              | 32.29            | 2.23        |
| 1080             | 40.97            | 2.12        |
| 1350             | 51.81            | 2.15        |

of the -SH group. There was a direct relation between the logarithm of half-oxidation time and the pH value of the solution. On studying the effect of the hydrogen ion concentration on the oxidation of glutathione by x-irradiation, the Sørensen and Clark buffers were used at first. It was found that although the per cent oxidation was greater at higher pH values with the same intensity of radiation (5000 r), there was an added effect, that of the nature of the

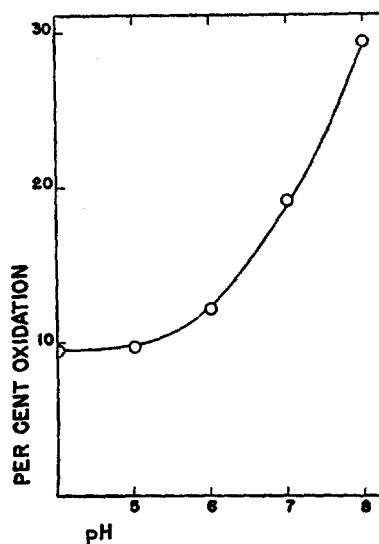


FIG. 7. Effect of pH on the oxidation of glutathione by x-rays. 1 cc. of glutathione  $2 \times 10^{-7}$  M.

TABLE VI  
*Oxidation of Glutathione by X-Irradiation*  
*Effect of pH*

1 cc. of glutathione,  $2 \times 10^{-7}$  M. Theorell's universal buffer. X-ray dosage, 5000 r.

| Buffer     | pH   | M                | Ionic yield |
|------------|------|------------------|-------------|
|            |      | $\times 10^{18}$ |             |
| Theorell's | 4.01 | 1.08             | 1.33        |
| "          | 5.02 | 1.15             | 1.42        |
| "          | 6.01 | 1.46             | 1.61        |
| "          | 7.02 | 2.25             | 2.78        |
| "          | 8.02 | 3.53             | 4.37        |
| Phthalate  | 5.25 | 1.75             | 2.16        |
| Phosphate  | 6.31 | 1.88             | 2.32        |
| "          | 7.44 | 2.49             | 3.08        |
| "          | 8.28 | 2.55             | 3.15        |
| Veronal    | 8.23 | 3.10             | 3.83        |
| Borate     | 8.37 | 3.22             | 3.98        |

buffer. Thus, at the same pH value, there was more oxidation in the presence of borate buffer than in the presence of phosphate buffer (Table VI). The experiments were repeated with the universal buffer of Theorell and Stenhagen (11). The oxidation by x-irradiation of glutathione dissolved in this buffer, at pH values 4 and 5 was the same. At these pH values the degree of dissociation of



the -SH group (pK 9.6) is practically nil. From pH 5 to pH 8 there was a direct relation between the per cent oxidation and the pH value of the solution (Fig. 7). As a consequence, the ionic yield increased as the pH value increased (Table VI).

5. *The Influence of Dissolved Oxygen.*—It is well known that the amount of  $\text{H}_2\text{O}_2$  produced on irradiation of water depends on the presence of dissolved oxygen. Furthermore, when water containing dissolved oxygen is irradiated

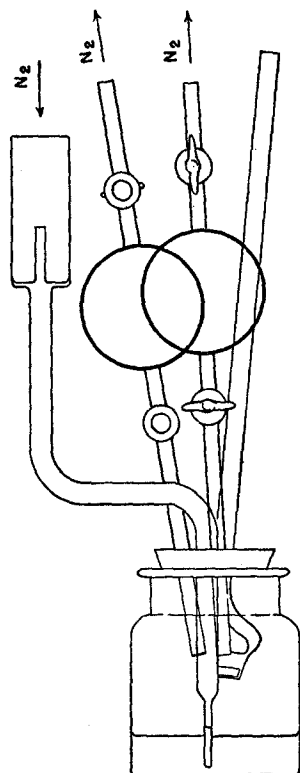


FIG. 8. Apparatus for the saturation of solutions with  $\text{N}_2$ .

with ionizing radiations, the atomic hydrogen produced on irradiation of water will reduce oxygen, thus starting a series of reactions that will give powerful oxidizing agents: the radicals  $\text{O}_2\text{H}$  and  $\text{OH}$ , and  $\text{H}_2\text{O}_2$ :  $\text{O}_2 \rightarrow \text{O}_2\text{H} \rightarrow \text{O}_2\text{H}_2 \rightarrow \text{OH} + \text{OH}_2 \rightarrow 2 \text{OH}_2$ . The univalent reduction of  $\text{O}_2$  which produces these free radicals,  $\text{OH}$  and  $\text{O}_2\text{H}$ , besides  $\text{H}_2\text{O}_2$ , contributes to the oxidations produced by ionizing radiations. For the experiments on the effect of dissolved oxygen on the oxidation of thiols by ionizing radiations, glutathione was chosen because it could be kept outside the solution without difficulties.

For the preparation of air-free glutathione solutions the apparatus in Fig. 8 was used. It consisted of a wide-mouth flat bottle of 95 cc. capacity stoppered with a rubber stopper. Through this stopper passed, (1) a tube for the bubbling of  $N_2$ , (2) a glass rod ending in a spoon inside which was kept a platinum dish containing the sample of glutathione, and (3) two vessels to hold the glutathione solution. The vessels were flat cylinders of 10 mm. height and 40 mm. diameter with stopcocks at the both ends. Commercial tank nitrogen was freed of the residual oxygen through passage of a 1 meter pyrex tube of 36 mm. inside diameter filled with pellets of copper and copper gauze. The tube was heated to  $500^\circ$  and the connections to the tanks of  $N_2$  and of  $H_2$

TABLE VII

*Oxidation of Glutathione by X-Irradiation*  
*The Influence of Dissolved Oxygen*

1 cc. of glutathione,  $2 \times 10^{-7}$  M. Buffer, 0.01 M phosphate, pH, 7.0. X-ray dosage: 10,000 r irradiated through closed vessels of plane-parallel pyrex glass of 1.2 mm. thickness.

| Gas in solution | Oxidation |
|-----------------|-----------|
|                 | per cent  |
| Air.....        | 25.9      |
| Nitrogen.....   | 8.6       |

TABLE VIII

*Effect of Catalase on the Oxidation of Propane-1,3-Dithiol by X-Irradiation*

1 cc. thiol,  $2 \times 10^{-7}$  M. Phosphate buffer, 0.01 M, pH, 7.0. Catalase, 5  $\mu$ g.; crystalline egg albumin, 40  $\mu$ g.

| SH compound              | X-ray dosage | Oxidation        |               | With albumin |
|--------------------------|--------------|------------------|---------------|--------------|
|                          |              | Without catalase | With catalase |              |
|                          |              | per cent         | per cent      |              |
| Propane-1,3-dithiol..... | 2500         | 26               | 25.6          | 27           |
|                          | 5000         | 58.7             | 45.4          | 59           |
| Glutathione.....         | 2500         | 9.2              | 6.75          | —            |
|                          | 5000         | 20.0             | 15.7          | —            |

and to the flask were made with copper tubing. After a preliminary bubbling of  $H_2$ ,  $N_2$  was bubbled through the buffer solution (50 cc.) for 30 minutes; the gas outlets were the two flat cylindrical vessels. After this time the spoon containing the platinum dish with glutathione was lowered, and  $N_2$  was continued bubbling for 10 minutes more, at the end of which the vessels were lowered and the glutathione solution was pushed inside by the pressure of the  $N_2$ . As soon as the vessels were filled, the stopcocks were closed. Two other vessels of identical dimensions contained glutathione dissolved in the usual buffer solution not freed of air. The solutions were then irradiated with 10,000 r. A portion of this radiation was absorbed by the glass which was of 1.2 mm. thickness. On irradiation of the non-deaerated solution of glutathione ( $2 \times 10^{-7}$  M

per cc.) there was 25.9 per cent oxidation, while irradiation of the oxygen-free solution produced only 8.6 per cent oxidation (Table VII). In the absence of oxygen, the oxidizing power of x-irradiation was reduced to 33 per cent of that found in the non-deaerated solution.

6. *The Rôle of H<sub>2</sub>O<sub>2</sub> in the Oxidation of Thiols by Ionizing Radiations.*—The H<sub>2</sub>O<sub>2</sub> produced on irradiation of water contributes somewhat to the oxidations produced by ionizing radiations, as was shown by Barron and Dickman (7) in their study of the action of ionizing radiations on the activity of enzymes. To study the rôle of H<sub>2</sub>O<sub>2</sub>, crystalline catalase (5 µg.) was added to the thiol solution previous to irradiation. On x-irradiation of propane-1,3-dithiol with 2500 r addition of catalase had almost no effect. On irradiation with 5000 r, catalase protected oxidation of this thiol by 23 per cent. With glutathione, catalase protected 27 per cent on irradiation with 2500 r, and 22 per cent on irradiation with 5000 r (Table VIII). On irradiation of propane-1,3-dithiol

TABLE IX

*Effect of Catalase on the Oxidation of Propane-1,3-Dithiol by Beta Irradiation (Carrier-Free P<sup>32</sup>)*

1 cc. thiol,  $4.6 \times 10^{-8}$  M irradiated at 0°. Buffer, Phosphate buffer, 0.01 M, pH, 7.0. Catalase, 5 µg.

| Beta-ray dosage | Oxidation        |               |
|-----------------|------------------|---------------|
|                 | Without catalase | With catalase |
| r               | per cent         | per cent      |
| 3375            | 75               | 62            |

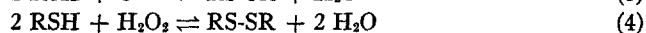
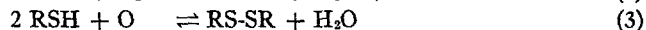
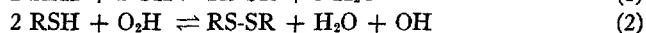
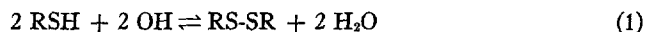
with beta rays from carrier-free P<sup>32</sup>, catalase protected only 17 per cent when the solutions were irradiated with 3375 r (Table IX). This protection must be due to the destruction of the H<sub>2</sub>O<sub>2</sub> by the enzyme. Addition of crystalline egg albumin (40 µg.) in an amount 200 times the molar concentration of catalase had no effect at all (Table VIII).

7. *On the Reduction of Disulfides by X-irradiation.*—It is known that ionizing radiations produce both oxidation and reduction reactions, although no studies have been made to determine which one of these reactions occurs to a greater extent. The thiol compounds here studied are sluggish oxidation-reduction systems; *i.e.*, they are thermodynamically reversible with very low reaction rates in the absence of a catalyst. In order to study the possible reduction of the -S-S- form of these thiols, glutathione was titrated with iodine in the presence of KI. A solution of 0.01 M glutathione was prepared, to which was added the required amount of iodine, and it was then left at 3° for 24 hours. At the end of this time no reduced glutathione was left in the solution. Vials containing  $2 \times 10^{-7}$  M of oxidized glutathione per 1 cc. in phosphate buffer, 0.01 M, pH 7.0 were then irradiated with 5000 r of x-rays and 1350 r of gamma rays from a

radium source. At the end of irradiation no reduced -SH groups, as tested by the ferricyanide reaction, were found either on irradiation with x-rays or with gamma rays. It must be concluded from these experiments that while oxidation of the -SH groups proceeded efficiently, the reverse process, reduction of the -S-S- form, did not take place under the conditions above stated.

#### DISCUSSION

The experiments presented in this paper have shown that thiols are easily oxidized by ionizing radiations when in aqueous solution. This oxidation is undoubtedly due to the action of the different oxidizing agents formed on the decomposition of water by radiation: OH and O<sub>2</sub>H radicals, atomic oxygen, H<sub>2</sub>O<sub>2</sub>, and on the reduction of dissolved oxygen: O<sub>2</sub>H and OH radicals, and H<sub>2</sub>O<sub>2</sub>. Thiols will react with these oxidizing agents as follows:—



All these four reactions can be produced by the products of radiochemical decomposition of water, as well as by the products of the univalent reduction of molecular oxygen dissolved in water. The contribution of dissolved oxygen to the oxidation processes which occur under the action of ionizing radiations is rather large, in fact larger than hitherto assumed, since on irradiation in the absence of oxygen the oxidation of thiols was reduced by 67 per cent. There was a tendency in the past to attribute the influence of dissolved oxygen to the formation of H<sub>2</sub>O<sub>2</sub>, which has been reported to be formed only in the presence of oxygen (12, 13). However, the experiments where the effect of H<sub>2</sub>O<sub>2</sub> was stopped by catalase have shown that the contribution of H<sub>2</sub>O<sub>2</sub> to the oxidation of thiols by ionizing radiations was only 17 to 27 per cent.

The preponderant rôle of dissolved oxygen in oxidations produced by ionizing radiations has very important biologic implications, not only because biological fluids always contain dissolved oxygen, but also because there are in vertebrates and in some invertebrates circulating stores of molecular oxygen, the respiratory pigments. Diminution of these stores might increase the resistance to the damaging effect of ionizing radiations.

The great sensitivity of thiols to the oxidizing action of ionizing radiations has been shown by the large ionic yield, above 3, which was found on irradiation with x-rays, beta rays, or gamma rays. This value is higher than any reported in the literature, except that for the oxidation of FeSO<sub>4</sub>, which according to Fricke and Hart (14) would also proceed with an ionic yield of 3. In spite of the thermodynamic reversibility of the oxidation-reduction system,  $2 \text{RSH} - 2e \rightleftharpoons \text{RS-SR} + 2 \text{H}^+$ , the reverse process, reduction of the disulfide RS-SR by ionizing radiation, did not occur on irradiation with dosages of x-rays

or beta rays sufficient to produce appreciable oxidation. Therefore, from the biologic point of view it is the oxidation of the sulfhydryl compounds that is the dominant phenomenon on irradiation.

It must be emphasized that the experiments presented in this paper do not contradict those of Kinsey (5). In our experiments we were measuring *oxidation* of the -SH groups of thiols while Kinsey was measuring the unknown *destruction* of glutathione. In fact, he carefully abolished the oxidizing action of x-rays as stated in his method of glutathione determination: "2 drops of 5 per cent NaCN were added to each cc. of glutathione before the analysis in order to reduce to the G-SH form any glutathione which might have been oxidized to the G-S-S-G state." Oxidation of glutathione by x-rays occurred readily and with an ionic yield of 3 at pH values around 7, while destruction of the molecule at the same pH, according to the data of Kinsey, occurred with an ionic yield of 0.6.

#### SUMMARY

Thiol compounds, such as glutathione, 2,3-dimercaptopropanol (BAL), propane-1,3-dithiol, and *N*-phenylaminopropanedithiol, were readily oxidized by x-rays, beta rays, and gamma rays. The ionic yield for this oxidation was about the same, 3 at pH 7, on irradiation with x-rays and with beta rays; it was 23 per cent less on irradiation with gamma rays. The ionic yield varied with the hydrogen ion concentration, increasing as the pH value increased. There was no reduction of oxidized glutathione on irradiation with dosages of x-rays and gamma rays which produced oxidation of the reduced compound. In the absence of oxygen, the oxidation of thiols by ionizing radiations was only 33 per cent of that obtained in the presence of dissolved oxygen. When the thiol solutions were irradiated in the presence of dissolved oxygen, catalase protected them from oxidation by 17 to 27 per cent.

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