CXC. THE EFFECT OF ULTRA-VIOLET, RADIUM AND X-RAY RADIATION ON GLUTATHIONE IN PURE SOLUTION.

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GLUTATHIONE has recently been shown to influence the activity of many enzyme systems [Pringsheim *et al.*, 1931; Lohmann, 1932; Waldschmidt-Leitz *et al.*, 1933] and has received considerable attention as a possible regulator of growth in the cancer cell. In view of the retarding action of radium and X-rays on cancerous growth and of the lethal effect of ultra-violet radiation of shorter wave-length than 2900 Å., the question arises as to the effect of these rays on the glutathione molecule. Accordingly the effect of such radiation on pure glutathione in aqueous or buffered solution has been investigated with regard to the fate of the sulphur in the molecule.

ULTRA-VIOLET RADIATION.

Glutathione solutions were exposed to radiation from the full quartz mercury arc¹ at a distance of 25 cm. The solutions were contained in flat dishes, giving thin layers of solution, and covered with transparent quartz plates. The dishes were surrounded by an ice-bath during the radiation to remove any possibility of destruction by heat. Samples were removed at intervals, made to 2 % sulphosalicylic acid and titrated by the iodate procedure [Woodward and Fry, 1932] for the reduced glutathione remaining. The total glutathione remaining was also determined after zinc reduction in the acid solution. By analogy with known sulphur compounds, glutathione which has been oxidised as far as its disulphoxide form (G.SO.SO.G) would be included in this figure. Table I shows the results obtained.

Table I. *Decomposition of glutathione by ultra-violet radiation.*

p_H	Original glutathione concentration mg. per 100 cc.	Glutathione remaining after radiation					
		4 hours		6 hours		8 hours	
		Reduced %	Total %	Reduced %	Total %	Reduced %	Total %
3.5	50	51	75	—	—	—	—
3.8	33	50	77	—	—	—	—
7.0	33	16	63	6	—	4	40
7.3	33	0	35	—	—	—	—
6.8	200	35	62	—	—	—	—
7.5	200	14	44	12	44	—	—
8.0	200	13	52	9	48	—	—

¹ A Uviarc Laboratory Arc, 3.5–4.5 amperes, 110 volts D.C.

The solution radiated at p_H 3.5 was unbuffered, being at the p_H of pure aqueous glutathione. The other solutions were buffered in 0.02 *M* phosphate. It will be observed that there was a very rapid loss in total glutathione, that is, it was changed in such a way that it could not be reduced back to a sulphydryl compound by nascent hydrogen. Glutathione not determined by this analysis will be considered "destroyed" since it can no longer act as a member of the biologically active oxidation-reduction system, $2G.SH \rightleftharpoons G.S.S.G.$

The amount of destruction of glutathione as well as oxidation of sulphydryl is shown to be dependent upon the concentration, p_H and time of radiation. In the higher concentrations, a smaller percentage loss of glutathione was observed. The effect of p_H is shown in Table I. At a concentration of 33 mg. per 100 cc., slight changes in p_H around 7.0 produced much greater increases in both the oxidative and destructive effects than at more acid p_H values. Increasing the p_H from 7.5 to 8.0, however, in the higher concentration did not cause any greater effect. At constant p_H (7.0) oxidation of sulphydryl to disulphide glutathione proceeded much more rapidly than destruction and is, therefore, probably a primary step in the destructive effect of the radiation.

Using the closely related sulphur compounds, cysteine and cystine, Lieben and Molnar [1931] have shown a similar destructive effect of ultra-violet light.

The fate of the sulphur in the glutathione which was destroyed was investigated in the solutions of higher concentration. Free sulphate could be detected in all these by means of barium sulphate precipitation. Analytical fractionation of the forms of sulphur present was therefore made by a procedure adapted from the macro-method of Denis and Reed [1927] for sulphur compounds in blood.

Total sulphur and inorganic sulphide. No sulphur was lost as gaseous products, since 100 % was recovered as barium sulphate after oxidation by Benedict's [1909] copper nitrate oxidising reagent. Therefore no inorganic sulphide was formed. This was confirmed with lead acetate paper after acidifying and boiling the solution.

Sulphates. Both free and hydrolysable sulphate were found. There was some barium sulphate precipitation even if the solution was not boiled with acid, representing the free sulphate in the solution. This was removed after standing 20 hours, and a further precipitate of barium sulphate was obtained on gentle boiling. This was classified as the hydrolysable sulphate and is probably of the sulphonie ester type.

Intermediate sulphur. This classification includes the sulphur which was not determined in the analyses for total glutathione and total sulphate. It was

Table II. *State of sulphur after ultra-violet radiation of glutathione solutions.*

Type of sulphur compound	p_H and time of radiation		
	p_H 6.8, 4 hours %	p_H 7.5, 5 hours %	p_H 8.0, 6 hours %
Total sulphur recovered	101.0	101.5	99.5
Total glutathione { GSH GSSG Sulphoxide	62	44	48
Free sulphate	8.4		16.4
Hydrolysable sulphate (sulphonate)	4.2		5.5
Total sulphate	12.6	21.6	21.9
Inorganic sulphide	0	0	0
Intermediate sulphur { Sulphone R ₂ S	25.4	34.4	30.1

calculated by subtracting the sum of these two determinations from 100 %, and probably includes sulphur of the organic sulphide (R_2S) and sulphone ($R-SO_2-SO_2-R$) types. No free sulphur could be detected, although all solutions were slightly yellowish after radiation.

Table II summarises the analytical results thus obtained. The destruction produced seems to be due to a progressive oxidising effect of the ultra-violet radiation rather than to liberation of inorganic sulphide or free sulphur from the molecule.

RADIUM.

Radon in both glass and gold seeds was used in studying its effect on glutathione. The seeds were placed directly in 10 cc. of glutathione solution, in the first case (Table III), at p_H 3.5, unbuffered and containing 50 mg. per 100 cc.; at the higher p_H values in 0.02 *M* phosphate buffer containing 33 mg. per 100 cc. The flasks were stoppered and left to stand at room temperature. Control solutions without radium were placed so as to receive no radiation from the experimental solutions (Table III).

Table III. *Effect of radium on pure glutathione.*

p_H	Duration of treatment hours	Glass seeds ($\beta + \gamma$ rays)			Gold seeds (γ rays only)			Control Glutathione remaining	
		Amount of radiation m.c.h.	Glutathione present after treatment		Amount of radiation m.c.h.	Glutathione present after treatment		Reduced %	Total %
			Reduced %	Total %		Reduced %	Total %		
3.5	25	212	75	97	—	—	—	87	100
	68	469	31	86	—	—	—	78	100
7.0	4	38	77	99	34	79	99	88	100
	24	213	0	84	191	2	92	4	100
7.3	4	32	67	95	29	70	95	65	99
	25	180	0	85	160	0	86	0	99

The results show that glutathione is extremely resistant to both the β - and γ -rays of radium. At the acid p_H , after 212 millicurie hours of treatment, there was some oxidation, but only very slight destruction. After longer treatment, 469 millicurie hours, both effects were more pronounced. At the more alkaline p_H values the autoxidation of glutathione was so rapid that the oxidative effect of the rays was not as definite. However, the destructive effect was still quite evident.

The oxidative effect observed by Hammett [1932] as expressed by a decreased nitroprusside test is thus quantitatively demonstrated.

X-RAYS.

Glutathione in solutions similar to those used for the radium treatment was exposed for 4 hours to unfiltered X-rays at 45 cm. distance from a machine running at 120 kv. The glutathione solution was contained in a stoppered pyrex glass flask and surrounded by ice-water for cooling during the radiation. At the more alkaline p_H values some autoxidation naturally occurs during the course of 4 hours. To allow for this a control solution was allowed to stand in ice-water for the same length of time. The results (Table IV) showed that there was no effect on the glutathione which could be attributed to X-rays. The

Table IV. *Effect of X-rays on pure glutathione.*

p_H	Duration of treatment hours	Radiated solution				Control	
		Amount of radiation R units	Glutathione present after radiation			Glutathione present	
			Reduced %	Total %		Reduced %	Total %
3.5	4	15,600	95	99		100	100
3.8	4	15,600	93	104		100	100
7.0	4½	10,800	80	103		72	100
7.3	4	15,600	56	102		65	99

stability of compounds of this type toward X-rays has also been reported by Stenström and Lohmann [1928] who found no destruction of cystine in acid solution when exposed for as long as 95 hours.

SUMMARY.

Ultra-violet radiation was found to produce a very destructive effect on glutathione, radium a slight destructive effect, both of these agents causing at the same time partial oxidation of the sulphydryl to the disulphide form. X-rays had no effect on the glutathione.

The effect of ultra-violet radiation appeared to be of the nature of an oxidative destruction. Free sulphate was formed to the extent of 42 % of the glutathione which was decomposed.

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