THE EFFECT OF X-RAYS ON GLUTATHIONE

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Hammett (1), using the qualitative nitroprusside reaction, demonstrated the destruction of glutathione when water solutions were irradiated with $\beta$ and $\gamma$ rays from radium. Woodward (2) confirmed Hammett's observations quantitatively with radon, but failed to find any destruction with as high as 15,600 $r$ units of Roentgen rays. She used the accurate titration method of Woodward and Fry (3) for making her analyses. Hueper et al. (4) could find no effect with Roentgen rays when the same and twice the dose cited above were used, although they noted a drop from 37 mg. per cent to 32 mg. per cent of total glutathione with 255 millicurie hours of radium.

Materials and Technique

A water-cooled Seeman x-ray tube having a molybdenum target and a thin aluminum portal was employed throughout. The tube was run for varying lengths of time at 60 kilovolts, 15 milliamperes, 6 cm. target distance. Dosage was determined by the use of a Victoreen dosimeter. The average wave-length reaching the solutions was found to be 0.56 Å.

Glutathione, obtained from the Eastman Kodak Company, was irradiated in 2 cc. samples contained in Pyrex test-tubes. The solution was 1.5 cm. deep and 1.4 cm. in diameter. Stoppered and open tubes gave identical effects in all cases. The walls of the test-tubes had some filtering effect because of the comparatively low voltage used; a correction which took this into account was made in calculating the average wave-length. Heat effects are extremely unlikely when a Seeman water-cooled tube is used. Nevertheless they were checked by immersing a thermometer in the solution to be x-rayed; they were found to be nil. Controls
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were run in all of the experiments reported below under identical conditions except for a thick sheet of lead which was placed between the sample and the x-ray tube.

2 drops of 5 per cent NaCN were added to each cc. of glutathione before the analyses in order to reduce to the G—SH form any glutathione which might have been oxidized to the G—S—S—G state. Oxidation beyond the G—S—S—G form will not be reduced to G—SH by NaCN and consequently will not show up in either the qualitative or quantitative tests used. Glutathione is said, in this paper, to be destroyed when such is the case. No attempt has been made to determine the oxidation products of glutathione in this investigation. See Woodward (2).

Qualitative Study

Freshly prepared solutions of 0.01 per cent glutathione were exposed to 20,000, 50,000, and 100,000 r units of x-rays. Immediately following the irradiation the nitroprusside reaction\(^1\) was applied. As shown in the controls a positive test for glutathione at this concentration gives a deep reddish color; the red fades after 5 to 10 minutes to a straw color. The x-rayed solutions gave a graduated series: Solution 1 was slightly less red than the control, Solution 2 pinkish, and Solution 3 almost a straw color. All of the irradiated solutions after testing faded faster than the controls.

Quantitative Study

Variation with Dosage—It now appeared desirable to make a quantitative study of the effects of x-rays. For this purpose the titration method of Woodward and Fry\(^2\) (3) was adopted. Glu-

\(^{1}\) To 5 drops of glutathione solution are added 4 drops of (NH\(_4\))\(_2\)SO\(_4\), 2 drops of NaCN, 4 drops of nitroprusside, and 2 drops of NH\(_2\)OH (concentrated). A red coloration shows a positive test.

\(^{2}\) To 10 cc. samples are added 2.5 cc. of 4 per cent sulfosalicylic acid, 2.5 cc. of 5 per cent KI (free of I), and 2 drops of 1 per cent starch. This mixture is titrated with 0.001 N KIO\(_3\) containing 2 per cent sulfosalicylic acid. Persistence of blue color marks the end-point. The temperature for titration is maintained at 19-20°. The calculation is as follows:

\[
\frac{\text{cc. 0.001 N KIO}_3 \text{ (for 10 cc. of filtrate)}}{3.26 \text{ (theoretical titer for 1 mg.)}} \times 100 = \text{mg. of G—SH per 100 cc. of sample.}
\]
tathione dissolved in distilled water to 0.01 per cent concentration, pH 3.5, was irradiated for 15, 30, 45, and 60 minutes; this corresponds to 11,500 \( r \) units for each 15 minutes. The average destruction for five tests was 11.5, 21.0, 29.5, and 37.5 per cent respectively, with a maximum variation from the average of \( \pm 1.5 \) per cent. The per cent decrease plotted against dosage shows a linear relationship within the range studied (see Fig. 1).

Variation with Concentration—The concentration of glutathione was then varied from 0.001 to 0.06 per cent, while the dose (23,000 \( r \) units) and pH (3.5) were kept constant. The average destruction found for three tests, with a maximum variation of \( \pm 2.0 \) per cent was 73, 30, 20.0, 11.0, 6.5, and 5.0 per cent for concentrations of 0.001, 0.005, 0.01, 0.02, 0.04, and 0.06 per cent respectively. The per cent concentration has been reduced to \( M \) concentration in Fig. 2 and is plotted against the moles destroyed (see also Table II). It will be seen that 0.01 per cent or \( 3.4 \times 10^{-4} \) \( M \) concentration represents a critical point in the destruction of glutathione. Evidently a different mechanism operates above and below this concentration. No explanation of this observation is offered at this time.
Variation with $pH$—Solutions of glutathione (0.02 per cent concentration) were maintained at $pH$ values varying from 3.5 to 9.00 by using appropriate buffers. These were then irradiated with 23,000 $r$ units. In the higher $pH$ range there was considerable autoxidation; e.g., at $pH$ 9.0 a 50 per cent decrease in glutathione in the course of 90 minutes was noted. The reason for the autoxidation is not known; it may be due to the presence of heavy metals such as Cu or Fe which have been shown to increase autoxidation for some substances (e.g. vitamin C) in the higher $pH$ range. Since control solutions at identical $pH$ values were employed, the destructions noted in Table I represent the action of the x-rays alone.

Variation with Media—It was thought possible that the glutathione was destroyed only on contact with the surface of the test-tubes in which it was contained. Accordingly, analyses were made in control solutions kept for some hours in Pyrex test-tubes, and in Pyrex test-tubes containing several hundred fine Pyrex
capillary tubes. Calculation showed that no appreciable destruc-
tion had occurred. A test-tube was then prepared with areas
and volumes corresponding with previous experiments, but in
such manner that capillaries could be introduced into the solution
and the whole irradiated. The x-rays entered the base of this
test-tube and passed longitudinally through the capillaries. The
glutathione received the same dosage as in the previous cases,
but the glass presented a surface several hundred times greater
for an equal volume. The results were identical with those of
the corresponding earlier experiments.

The test-tube containing capillaries was then turned 90°, so
that the x-rays passed through the walls of the capillaries. At
0.01 per cent concentration and 23,000 r units of x-rays the gluta-

<table>
<thead>
<tr>
<th>Buffer used</th>
<th>pH</th>
<th>Concentration</th>
<th>Average decrease</th>
<th>Range of decrease</th>
<th>No. of determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3.5</td>
<td>0.02</td>
<td>11</td>
<td>10-12</td>
<td>6</td>
</tr>
<tr>
<td>Acetate</td>
<td>4.63</td>
<td>0.02</td>
<td>11</td>
<td>10-13</td>
<td>4</td>
</tr>
<tr>
<td>Veronal-HCl</td>
<td>7.0</td>
<td>0.02</td>
<td>12</td>
<td>11-14</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>0.02</td>
<td>33</td>
<td>30-37</td>
<td>3</td>
</tr>
</tbody>
</table>

thione destroyed was found to have increased from 21 per cent
to 27.5 per cent. This increased effect is probably due to the
increased number of secondary electrons emitted by the capil-
laries, rather than to any adsorption of glutathione on the surface
of the glass.

Energy Efficiency of Glutathione Destruction

In order to determine the stability of glutathione relative to
other compounds affected by x-rays, it is necessary to know how
much energy is required to destroy 1 mole of this substance.
Such a calculation may be made for the various concentrations
irradiated by using the value obtained from the formula proposed
by Rump (5). In this formula, von Kulenkampff’s (6) experi-
mental value of 35 electron volts is taken as the work required to
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form one ion pair in air. (This value may be used approximately for water.)

\[
\frac{E}{i} = \frac{\epsilon}{0.36} \left( \frac{\rho}{\bar{\mu} + \sigma_v} \right)
\]  

(1)

\(\epsilon = 35\) electron volts, \(E = \) energy, \(i = \) dosage in \(r\) units, \(\rho = \) density, \(\bar{\mu} + \sigma_v = \) part of the absorption coefficient due to photoelectrons and recoil electrons.

In the foregoing the average \(\lambda\) reaching the solution was \(0.56\) \(\text{Å}\). Rump (5), using the above formula, has found that the energy flux \(E/i\) for this \(\lambda\) is equal to 192 ergs per sq. cm. per \(r\) unit.

It now is necessary to determine the fraction of this energy flux which is transformed into work of ionization in the water solution used. Where \(\gamma\) represents this fraction, Glockler (7) and Glockler and Risse (8) show that:

\[
\gamma = \frac{\bar{\mu} + \sigma_v}{\mu} (1 - e^{-\mu D})
\]  

(2)

where \(\mu = \) weakening coefficient, \(D = \) thickness of absorbing layer. (\(D\) averages 1.1 cm. for the test-tubes used in the experiments.)

By substitution in Equation 2 of the values obtained from the tables by Glockler and Risse (8, 9) for water and \(\lambda = 0.56\) \(\text{Å}\), \(\gamma\) is found to equal 0.34 (\(\mu = 0.65, \bar{\mu} = 0.45, \sigma_v = 0.008\), and \(D = 1.1\) cm.). Thus 34 per cent of the incident energy is absorbed and used in the formation of ions. This gives a value of 65.6

Table II

<table>
<thead>
<tr>
<th>Concentration</th>
<th>(M \times 10^{-3})</th>
<th>Destroyed</th>
<th>Energy efficiencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>per cent</td>
<td>(M \times 10^{-3})</td>
<td>per cent</td>
<td>(M) per calories</td>
</tr>
<tr>
<td>0.001</td>
<td>3.4</td>
<td>73</td>
<td>2.5</td>
</tr>
<tr>
<td>0.005</td>
<td>17</td>
<td>30</td>
<td>5.1</td>
</tr>
<tr>
<td>0.01</td>
<td>34</td>
<td>20.0</td>
<td>6.8</td>
</tr>
<tr>
<td>0.02</td>
<td>68</td>
<td>11.0</td>
<td>7.5</td>
</tr>
<tr>
<td>0.04</td>
<td>136</td>
<td>6.5</td>
<td>8.8</td>
</tr>
<tr>
<td>0.06</td>
<td>204</td>
<td>5.0</td>
<td>10.1</td>
</tr>
</tbody>
</table>
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Ergs per sq. cm. per r unit as the energy flux. In terms of calories this is equal to $1.57 \times 10^{-6}$ calories per sq. cm. per r unit, or $3.51 \times 10^{-2}$ calories per sq. cm. per standard dose (23,000 r units).

By determining the amount of glutathione destroyed in moles at various concentrations the energy efficiencies are calculated and listed in Table II.

From the foregoing it is obvious that the energy efficiency increases with the concentration. This rate throughout the range studied is rather high as compared with the energy efficiency of the photolysis of KNO₃ ($7.7 \times 10^{-6}$ mole per calorie) found by Clark and Pickett (10), and somewhat less than the extremely efficient photochemical chain reaction between HgCl₂ and K₂Cr₂O₇ (0.765 mole per calorie).

**SUMMARY**

X-Rays were found to have a destructive effect on aqueous solutions of glutathione.

A linear relation was established between the destruction noted and the dose employed.

The deleterious effects of x-rays increase linearly with the concentration from 0.001 to 0.01 per cent glutathione, and above this concentration again increase linearly, but at a lesser rate.

Augmented destruction was found with increasing pH values.

No effect was observed when glass capillaries were placed in the solutions to determine possible adsorption effects of the walls of the Pyrex glass test-tubes used for containers.

The energy efficiency of the destruction of glutathione was then determined for various concentrations, and was found to increase as the concentration.

The writer wishes to express his gratitude to those men of the Westinghouse Electric and Manufacturing Company who made this work possible by offering space and equipment in their Research Laboratory. Thanks are also extended to Mr. F. J. Hicks who assisted in this investigation.

**BIBLIOGRAPHY**

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