SOME PRELIMINARY OBSERVATIONS ON THE EFFECTS OF
ULTRAVIOLET LIGHT, ALPHA RAYS AND X RAYS
ON 2,3,5-TRIPHENYL-TETRAZOLIUM CHLORIDE SOLUTIONS*

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ABSTRACT

SOME PRELIMINARY OBSERVATIONS ON THE EFFECTS OF ULTRAVIOLET LIGHT, ALPHA RAYS AND X RAYS ON 2,3,5-TRIPHENYL TETRAZOLIUM CHLORIDE SOLUTIONS*

OBJECT

Noticing that ultraviolet light produced color changes in 2,3,5-triphenyltetrazolium chloride solutions, it was believed important to explore this phenomenon in relation to different types of radiant energy. The experiments here reported are to be regarded as exploratory in nature.

RESULTS AND CONCLUSIONS

Ultraviolet light (3650 Å to 2536 Å) reduces 2,3,5-triphenyltetrazolium chloride in solution to its red formazan. The amount of reduction depends upon the intensity of the light source and/or the time of exposure. The rate of formation of the red formazan increases with an increase in concentration of the salt solution. The reduction is influenced by pH and temperature.

Alpha radiation and x radiation also reduce 2,3,5-triphenyltetrazolium chloride to its red formazan.

It is believed that this study is an experimental contribution to the theory of Weiss on radiochemistry of aqueous solutions.

RECOMMENDATIONS

The importance of the application of this phenomenon in ultraviolet dosimetry, in medical therapy and in climatological problems is obvious.

More extensive studies on types and amounts of radiation, especially as to exact ionic yield should be undertaken. The radiochemical behavior of other tetrazolium compounds such as "Neotetrazolium" and 2,3-diphenyl-5-methyltetrazolium should be investigated.

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I. INTRODUCTION

2,3,5-Triphenyltetrazolium chloride (TTC) was first synthesized in 1894 by Echtmann and Range (1). Kuhn and coworkers (2,3) took up the study in 1941 and reported, among other properties, its oxidation reduction potential, namely, -0.03 volt in the pH range 5.7 to 6.8. Attention to this substance in America was brought about by the work of Dutcher (4) who reviewed the work of Lakon (5) on topographical staining and analysis of grain seed. Porter, Durrell and Romm (6), also reported on its use as an indicator of seed viability. Cottrell (7) confirmed Lakon's work in 1947 as did also Shuel (8). Mattson, Jensen and Dutcher (9) used it as a test reagent for checking vitality of tissues other than seeds. Waugh (10) then used this salt as a stain for living stem tissue and leaves. Pratt, Dufrenoy and Pickering proposed it as a reagent in cellular physiology (11). Straus, Cheronis and Straus (12) were the first to explore its uses in animal tissue, both normal and malignant.

According to the above published works, the formula for TTC and its reduced state is:

\[
\begin{align*}
R - C & \quad H \\
\mid & \quad I \\
N & \quad N - R \\
\mid & \quad I \\
N & \quad N - R \\
\mid & \quad I \\
CL & \quad - \\
\end{align*}
\]

Because of its low reduction potential, this colorless water soluble tetrazol is easily reduced by chemical as well as phytochemical means to its red, water-insoluble, nondiffusible formazan.

Noticing that ultraviolet light influenced solutions of this salt, it was believed important to explore this phenomenon further, not only studying light, but also other radiations. The experiments to be reported are to be regarded as purely exploratory in nature.

II. EXPERIMENTAL

A. Methods and Procedures

1. Photochemical Investigations. The first investigations into the effect of ultraviolet light on TTC were started with fluorescence equipment assembled for work with fluorescent vital stains. It consisted of an ordinary microscope, ultraviolet light source and eye protective filter. Illumination sources were a Leitz carbon arc lamp with a Beckman B-2 and B-4 filter combination or the Spencer 353 fluorescence lamp. The eye protective filter was a Wratten K-3, located in the eyepiece. To obtain better efficiency, the standard microscope mirror was replaced by an aluminized front surface mirror.
Doing vital staining work with Allium Cepa epidermis and modern fluorochromes, TTC was also included in the investigations. It was noted that a section of living onion epidermis subjected to TTC solution became pink. This was expected according to the published work. However, while observing this preparation in the fluorescence equipment, it developed in addition to the normal pink color a bright red spot. To be certain that this was not due to a possible stimulation of cellular activity, a clear drop of TTC solution was placed on a glass slide and observed under identical conditions. Here also, the red precipitate was formed. With the formation of the red spot, fluorescence appeared in the fluid and increased in intensity to saturation during the observation period.

In systematic investigations, the nature of the red precipitate was determined, also the effective reduction wave length, the effect of temperature, pH, concentration, intensity of light and exposure time. For these purposes, aqueous TTC solutions and TTC gelatin emulsions of known concentration were prepared. The chemical was dissolved in distilled water with a pH normally adjusted to 6 with phosphate buffer; for special investigations, buffered solutions with pH ranges from 1.3 to 11 were prepared. The TTC emulsions contained in general 10% gelatin and 1% TTC.*

A series of varying exposures under standard irradiation conditions was made. Samples were exposed in the appropriate cuvettes and also as emulsions on 4 x 5 glass plates. Density measurements of the cuvettes were at first made in a Coleman junior spectrophotometer and later the results were confirmed on a Beckman quartz spectrophotometer. The density measurements of the plates were made with a Weston densitometer and later with the self-recording General Electric spectrophotometer. This instrument was also used to identify the photochemically produced red substance with chemically reduced TTC (zinc-reduction) by comparing the absorption spectra. Irradiation sources were the sun, the Hanovia U-burner and the Hanovia Alpine lamp. The General Electric H-4 lamp was also satisfactory. For studying the effective wave lengths, the above sources were filtered by Corning or Wratten filters. Filters with sharp cutoffs or monochromats were especially useful. The results obtained were later verified by exposure of gelatin emulsion plates in the large Littrow quartz spectrograph using, for slit illumination, either National Super-Tan carbons or the Hanovia Alpine mercury lamp.

2. Radiochemical Investigations. This work was extended from the photochemical into the radiochemical regions. Both alpha rays and x rays were investigated.

* TTC from 2 sources was used: (1) The Arapahoe Chemical Co., Inc., Boulder, Colorado; and (2) The Pannone Chemical Company, Farmington, Connecticut.
For the alpha ray work, three polonium preparations were available.* (The polonium was deposited on nickel foil 5 x 5 sq. mm. in size.) Their strength as of August 23, 1948 was: Preparation #1 - 2 millicuries; Preparation #2 - 0.1 millicurie; Preparation #3 - 0.0001 millicurie.

The same solutions and emulsions as used in the photochemical work were also taken in this study. The alpha ray bombardment was made in such a way as to capture the maximum energy of the alpha particles in the preparation.

Details are shown in figure 1.

The density of the alpha particle produced formazan in the plates was measured with the Weston densitometer.

The x ray exposures were carried out with aqueous TTC solutions and TTC emulsions that were sealed in small vials of 3 cc. each. These samples were exposed at a rate of 250 r/min/air and up to 130 minutes total irradiation time. The irradiation was done with 550 KV x rays through a composite filter equivalent to 9 mm. of copper.

* Obtained from Canadian Radium & Uranium Corporation, 630 Fifth Ave., New York 20, N. Y.
B. Results

The following results were observed:

1. By irradiating aqueous solutions of the clear, water-soluble 2,3,5-triphenyltetrazolium chloride with ultraviolet light, this chemical was reduced to its red water-insoluble formazan.

2. The reduction depends on the radiation wave lengths. Wave lengths longer than 3650 Å do not reduce, whereas wave lengths between 3650 Å and 2536 Å reduce effectively. The 2536 Å region was the lowest at our disposal.

3. The amount of formed precipitate is determined by the intensity of the light source, and/or the time of exposure. Figure 2 shows that graduated exposures give an increasing density.

4. The rate of formation of the red precipitate increases with increase of concentration. The radiation effects are more pronounced in the early time intervals as shown in figure 3.
5. The pH influence, noticed early by Jerchel and Möhle (3), also exists in irradiation reduction. The reduction rate is the same in pH range 5.5 to 6.6 where also the reduction potential is constant. See figure 4.
6. Higher temperatures accelerate the reduction process. For example, with a temperature difference of 100°C (0°C - 100°C) and at a two-minute irradiation under the chosen standard conditions, there is 15% difference in transmission. See figure 5.

7. Alpha rays from polonium samples behave in a similar manner as ultraviolet light. Also here, tetrazolium in aqueous solution or in a gelatin emulsion is reduced to red formazan. The millicurie strength, the exposure time and the concentration of the tetrazolium are significant. See figure 6.
8. X rays also reduce TTC solutions. The amounts necessary are relatively high but essentially of the same order of magnitude as polonium alpha rays. The earliest visual effect was produced by 32,000 r/air.

III. DISCUSSION

The observed results raise the question as to the mechanism of these effects.

TTC is reduced, according to chemical knowledge, by taking up hydrogen atoms. Consequently, hydrogen atoms must be produced by irradiation. This could happen by an indirect effect of the radiation on the solvent (water in this case), or by direct effect on the solute (TTC), raising it to an activated state.

That ionizing radiation converts water molecules into free hydrogen atoms and hydroxyl radicals has been postulated by the theory of Weiss (13). According to Weiss, in the radiation of water, the following electron transfers occur:

\[ \text{H}^+ \quad \text{OH}^- \quad \xrightarrow{\text{Radiation}} \quad \text{H} \quad + \quad \text{OH} \]

\[ \text{H}_2\text{O} \quad \rightarrow \quad \text{H}_2\text{O}^+ \quad + \quad \text{Radiation} \quad \xrightarrow{\text{Radiation}} \quad \text{H}_2\text{O}^- \quad \rightarrow \quad \text{H}_2\text{O}^+ \]

followed by:

\[ \text{H}_2\text{O}^- \quad \rightarrow \quad \text{H} \quad + \quad \text{OH}^- \]

\[ \text{H}_2\text{O}^+ \quad \rightarrow \quad \text{H}^+ \quad + \quad \text{OH} \]

All of these processes are energetically possible and have been confirmed independently by photochemical evidence.

The produced hydrogen atoms and hydroxyl radicals are chemically very reactive (14, 15) and if there are in the solution appropriate acceptors, they will react with these radiation products. Since biological systems are primarily solutions, the radiobiological implications of the above statements become apparent. So, for instance, dissolved oxygen in water is such an acceptor and irradiation leads to the production of \( \text{H}_2\text{O}_2 \). Such an acceptor, favored by its low reduction potential, must be also tetrazolium chloride.

The production of the effect by ultraviolet light with wave lengths up to 3650 A requires further investigation. It may be explained by a direct effect on TTC, so that similar to photosynthetic processes (Rabinowitch (16)) excited intermediate states are formed. The observed fluorescence, which increases with the irradiation time, favors this hypothesis.

There may also be an explanation on the basis of the data given by Lea. According to Lea (17), 5 electron volts (eV.) are required to convert water into H and OH radicals. The wave length 2536 A has a quantum energy of 4.39 eV, an amount close to the number given by Lea, but very dissimilar to the known water dissociation energy of about 13 eV (18). Further inves-
tigations, especially into the ionic yield, will give additional data and also new insight as to the parallelism between photochemical and radiochemical effects.

In a certain degree, these results and this discussion of photochemical and radiochemical behavior of TTC solutions are a further continuation of the theory of Weiss on the effects of radiation on water and aqueous solutions as it has been discussed and used by Lea (17) and at the conference in London on certain aspects of the action of radiation on living cells (19), and by Barron (20) at the forty-ninth meeting of the American Roentgen Ray Society, 1943.

IV. CONCLUSIONS

Previous investigations have shown that TTC solution can be reduced chemically. These experiments show that this substance can also be reduced by photochemical and radiochemical means.

Factors influencing this reduction, such as tetrazolium concentration, intensity of radiation, time of exposure, pH and temperature, were investigated. It is believed that this study is an experimental contribution to the theory of Weiss on the radiochemistry of aqueous solutions.

V. RECOMMENDATIONS

The importance of the application of this phenomenon is ultraviolet dosimetry, in medical therapeutics and in climatological problems is obvious. For instance, one can easily show in a pilot experiment how the ultraviolet intensity of the sun varies during the day. For this purpose a series of small vials containing 2-3 cc. of gelatin tetrazolium emulsion was exposed for two minutes on the hour every hour to the sun. The result is plotted in figure 7.

![SUN ULTRAVIOLET CURVE EXPOSED FOR 2 MIN.](image-url)
Following this exploratory work, more extensive studies should be undertaken as to different types of radiation, the amounts of each necessary to reduce tetrazolium, and the exact ionic yield.

Other substituted tetrazolium salts such as Neotetrazolium (21) and 2,3 diphenyl-5-methyl TTC should be studied as to their radiochemical behavior.

VI. ACKNOWLEDGMENTS

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VII. BIBLIOGRAPHY


4. Dutcher, R. A. See reference (9).


