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MASTER

TECHNICAL PROGRESS REPORT

AEC Contract No. AT(11-1)1728 "Chemical Consequences of the Auger Effect in Thyroxine and its Analogues"

1. Personnel:

a. Principal Investigator: Dr. Bodo Diehn, Assistant Professor.

Time devoted to project: 1/2 time September 1967 to February 1968,
1/4 time since March 1968, when an NSF contract entitled "Biochemistry and Biophysics of Phototaxis" was awarded to the Principal Investigator.

b. Postdoctoral Associate: Dr. El-Hussieny Diefallah, devoting full time to the project since joining it in December, 1967.

c. Graduate Student: Mr. Lothar Stelter. As a first year graduate student, Mr. Stelter has to fulfill a one year teaching assignment which is part of the Ph.D. requirements at Toledo. He is not being paid by the project this academic year, but will be employed as a full time research assistant in the summer.

2. Work done on the project as of May 1, 1968:

For the X-irradiations, a General Electric X-ray generator with copper target was made available by the Physics Department of the University of Toledo. In preliminary experiments it was found that during irradiations directly in front of the beam window, the temperature of the sample rose to nearly 100°C, at which temperature significant thermal decomposition of thyroxine can occur. Air cooling did not relieve this

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problem sufficiently. A water-cooled copper irradiation block with tight-fitting thin-walled lucite sample cells was therefore designed and constructed and proved satisfactory.

It was decided to irradiate the compounds under investigation in the solid state. In solution, chemical effects are generally determined by the interaction of radicals from radiolysis of solvents. This would unnecessarily complicate the initial studies which must be designed to show whether there is any chemical effect due to resonance absorption of x-rays in the iodine. A marked release of iodine was observed in the first irradiations of thyroxine. The same situation was found in subsequent irradiations of 3,5-diiodothyronine, 3,5-diiodotyrosine, and 3-iodotyrosine.

Figure (1) shows a plot of the weighed atomic cross-sections of the elements constituting diiodothyronine as a function of x-ray energy. It can be seen that the total x-ray absorption cross-sections in diiodothyronine approximates that of the weighed absorption cross section in iodine for x-ray energies above about 5 keV and that contributions due to other elements present in the molecule are practically negligible.

Figure (2) shows the calculated x-ray resonance absorption in the systems under consideration in this study.

The existence of a resonance effect would require that the observed radiation damage effect vs. x-ray energy exhibit a break in the curve at the iodine absorption edges. The spectrum of damage is usually similar to that of absorption of the radiation.

Since an x-ray monochromator is not available at present, and would only offer very low beam intensities, it was decided to determine whether a chemical effect of resonance absorption can be shown by measuring the yield of I_2 as a function of accelerating voltage and radiation dose.

The total number of I_2 molecules found after chemical analysis of the x-irradiated sample (I_2 - yield) may be calculated using the equation,

$$Y_{I_2} = G_{I_2} \cdot 10^{-2} \int_{E_0}^{E_{max}} (2\nu^2 (\nu_0 - \nu) F(\nu) (1 - \frac{I}{I_0}) dE$$

where G_{I_2} is the number of I_2 molecules produced for each 100 ev. of energy absorbed. $F(\nu)$ is a correction factor which corrects for the reduction in x-ray intensity due to absorption by the Be-window, air and the polyacetate filter. Other symbols in the equation have their common significance. The integral over E has been determined graphically.

The experimental I_2 - yield found for 3,5-diiodothyronine and thyroxine are shown in Figs. (3) and (4) respectively. The corresponding calculated values of (Y_{I_2}/G_{I_2}) are shown on the same figures. It can be seen that the 3,5-diiodothyronine is much more sensitive to radiation than thyroxine. Moreover, the value of G_{I_2} seems to depend on E_{max} in both cases. This may be due to a dose effect and/or differences in the Auger yields associated with the K- and L- shell electron vacancies. This point is now under investigation and no details can be given at this time. Figures (6) and (7) shows radiation dose effects in thyroxine and diiodothyronine.

The experimental excitation functions for the appropriate reaction can be calculated from the experimental yield curve by a differential method. This requires a yield curve with better accuracy and work is still in progress. However, it can be seen [Figs. (3) and (4)] that the data exhibit a resonance behavior.

The determination of the spectrum of irradiation products other than iodine was also initiated. Using paper chromatography, D,L triiodothyronine as well as another, as yet unidentified compound were found as the result of irradiation with 40 kV X-rays. Since gas chromatography is a much faster and more flexible analytical technique than paper chromatography, and has recently been applied to thyroid hormones^{1,2}, a Varian 90 P 3 gas chromatograph was purchased. Since ultimately, irradiation with monochromatic X-rays will be attempted and low yields of products are expected in this case, the 90 P 3 is now being converted to operation as radio gas chromatograph³. If radioactively labeled substances are used as starting materials, even minor products should be detectable with this apparatus.

References:

- ¹ Stouffer et al., Biochim. Biophys. Acta 127, 261(1966)
- ² N.M. Alexander and R. Scheig, Anal. Biochem. 22, 187 (1968)
- ³ Bodo Diehn et al., Z. Anal. Chemie 204, 112 (1964)

FIG. 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6