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Publication Date
1972
Submitted to Int. Journal of Applied Radiation and Isotopes

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January 1972

AEC Contract No. W-7405-eng-48

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University of California.
A method is described which uses L X-ray counting, rather than $\alpha$-counting, for studies of the metabolism of certain actinide elements in mice. To demonstrate the effectiveness of this method, mice were injected with citrated $^{243}$Am and two separate metabolic patterns were determined based on data obtained from two different photopeaks: One was from the L X-rays and the other from the 74 keV $\gamma$-ray. The results were comparable, and indicate L X-ray counting will be useful in studies of actinide elements that do not emit abundant $\gamma$-rays.

*This work was done under the auspices of the U. S. Atomic Energy Commission.*
INTRODUCTION

Until recently only a few of the actinide elements were used in industry or laboratories. However, newfound practical applications and increasing studies of the actinides have brought these elements into more common usage. As a result, a greater potential exists for accidental human exposure to these nuclides. Because the α-emitting transuranic elements are the most toxic radionuclides available to man, it becomes essential that we predict their metabolic behavior in man.

Several programs have been designed to study the metabolism of the actinides in selected animal species from which, hopefully, we can extrapolate the results to man. The study of actinide metabolism in mice (1) is an integral part of our comparative investigation of the metabolism of these elements.

Some of the more important actinides (2) do not emit abundant γ-rays, (3) and so study of their metabolic patterns requires laborious, time-consuming, α-counting procedures. (4) In such instances it is desirable to seek methods which will save time and still produce accurate results. Measurement of the L X-rays is such an alternate method that we have used successfully to study the metabolism of $^{253}$Es and $^{243}$Am in mice. For rapid assessment of the results of various therapeutic treatments, this counting method could be particularly useful. This paper describes the method in detail in a study of the metabolism of $^{243}$Am in mice.

MATERIALS AND METHODS

Detection System

The detection system consisted of two NaI crystals (12-cm dia. X 3-mm thick) mounted inside a special, small animal, whole-body
counter. The detectors were horizontally opposed, and their separation could be varied from 1 to 430 mm. The signals from the detectors went through a summing amplifier and then into a 400-channel pulse-height analyzer. The digital readout of the spectrum was by standard teletype and punch paper tape, which was processed by a CDC 6600 computer. The computer program was designed to plot each spectrum and integrate any photopeak that was designated as a region of interest by the investigator.

In this study all counts were accumulated with the crystals separated a distance of 180 mm and each sample placed at the midpoint of the separation. In this position the counting efficiency is the same for a source of activity whether distributed in a 0.1-ml or a 30-ml volume.

**Animal Study**

Seven syringes were prepared, each with a 100 nCi dose of $^{243}$Am sodium citrate in a 0.1-ml volume. The syringes were counted before and after the injections were made. Five 20-g female mice were each injected intramuscularly in the left hind leg. To be certain that all animals were counted at the same postinjection time, single animals were injected at intervals of 15 min, placed in separate plastic vials, and whole-body counted 5 min after they were injected. Then they were housed in metabolism cages designed for separate collection of urine and feces. The remaining two doses were used to make counting standards: One dose was emptied into a small plastic vial and the volume left unchanged; the final dose was injected into a mouse that was allowed to live for 5 min to permit some circulation of the nuclide. Thereafter, the dead animal was sealed in a plastic vial and maintained
as the whole-body reference standard for this specific experiment.

The animals were counted again at 2-hr postinjection, and then counted on Days 1, 2, 3, 4, 7, 9, 11, and 14.

At various times throughout the experiment each animal was also counted with the semiconductor detector to determine any change in the ratios of the discrete L X-rays. (5)

One animal was sacrificed on Day 1, two on Day 4, and two on Day 14 postinjection. The spleen, liver, lungs, kidneys, and hind legs were removed from the animal; the remaining tissues were left together as the "carcass." The activity in each tissue and excreta sample was determined by the whole-body counting system with the same crystal separation and calibration settings used for whole-body counting of the mice. The tissue distribution and whole-body retention were calculated from the integrated photopeak counts from the L X-rays, and the results were compared to those based on the photopeak from the 74 keV γ-ray.

A question arose as to whether there was Compton scattering into the L X-ray photopeak from the higher energy photons. It was convenient to establish this empirically using 195Au and some tissue-equivalent absorbers. Gold-195 has a photon energy spectrum similar to that of 243Am with the exception of the L X-ray region where 195Au has no significant emissions. We placed 1 mm each of bone-equivalent and tissue-equivalent material over a thin source of 195Au and determined that the higher-energy areas did not significantly contribute counts to the L X-ray photopeak (Fig. 1).
RESULTS

Whole-body Retention

The retention curves were plotted as true net counts per min (Fig. 2a) and then as a per cent of the initial dose in the "mousewhole-body standard" (Fig. 2b).

Figure 2a shows that a consistent relationship exists between counts in the L X-ray photopeak and the counts from the 74 keV γ-ray.

At the time of injection, the counts in the 74 keV photopeaks from each animal and each standard agreed within ±1 percent of each other. This was expected due to the counting geometry of the samples. While on the other hand, the L X-ray photopeaks from these same spectra indicated a 30 per cent decrease in counts a few minutes after the isotope was injected into the animal thus showing that the greatest percentage of the attenuation by the tissues occurs almost immediately. By one day after injection the attenuation loss amounted to approximately 40 per cent. Thereafter, the attenuation did not change a significant amount, as can be seen from the parallel relationship of the retention curves.

Tissue Distribution

The tissue distribution is more difficult to relate to the whole-body retention due to the differences in attenuation that result from the variation in sample size. To determine the correction factors for losses due to self-absorption, we diluted 0.1-ml samples of activity serially by 2-ml increments to a maximum volume of 25-ml, and plotted the change in count rate as a function of volume. All tissue samples were normalized using this curve and tabulated as a per cent of the initial dose as contained in a 0.1-ml volume. Table 1 shows the tissue distribution pattern based on the 74 keV γ-ray and L X-ray data.
Excreta

The excreta were normalized in the same manner as the tissues and the amount of activity present in each sample correlated with the losses determined by whole-body counting of the animals. The urine-to-fecal ratio on Day 1 was about 5:1, but thereafter the radioactive material was predominantly excreted in the feces.

DISCUSSION

This experiment required a nuclide which had not only L X-ray emissions similar to that of the more widely used actinides, but also a higher-energy γ-ray emission that could be used to compare with the L X-ray data. Therefore, it was not acceptable to use $^{241}$Am in our study because counts from the 26-keV γ-ray interfered with the counts from the L X-ray component. We chose $^{243}$Am because it has a 74 keV γ-ray emission and no significant photons which would contribute counts to the L X-ray photopeak. By obtaining two groups of data, one based on the L X-rays and one based on the higher-energy γ-ray, it is possible to define the error due to attenuation in the lower-energy region. These values agree quite closely with those obtained from the self-absorption curve described in the tissue section. This curve indicates that 25 ml of water absorbs 36 per cent of the photons from the L X-rays, which is slightly lower than the 40 per cent absorption obtained from the difference in the two whole-body curves based on the separate photopeaks.

If the whole-body counts are plotted as a per cent of the mouse whole-body standard, the initial losses from self-absorption are corrected, as can be observed in Fig. 2. A further correction is necessary at Day 1.
postinjection; this additional 10 per cent loss is probably due to absorption of the nuclide into the bone. This last correction factor should apply to other actinides, provided the retention curves are plotted as a function of a mouse standard.

Although the main purpose of this study was to compare data obtained from the two different photopeaks, it is of interest to note that the whole-body retention and tissue distribution data based on the 74 keV photopeak were significantly different from the results of an earlier mouse study (1) using 241 Am. To elucidate these differences, it would be necessary to use larger groups of animals in order to obtain statistically significant numbers. The L X-ray technique is not concerned with the 74 keV photopeak and could not contribute to this variance.

Semiconductors, whose value is not fully appreciated by many investigators, can be used in animal studies to provide a general idea about the extent of photon attenuation in any sample. The L X-ray spectrum from a sample is easily resolved into 3 or 4 distinct photopeaks, the relative heights of which are a function of the magnitude of the photon attenuation. (5) The semiconductor spectra in Fig. 3 show the effects of soft tissue and bone attenuation on the individual photopeaks from the L X-rays. In earlier studies (7) we observed that 1 mm of compact bone removes approximately 85 per cent of the 14 keV X-ray. The spectra from our 243 Am mice indicated only about one-half this magnitude of attenuation.

We have very little evidence to indicate that attenuation by mouse bone cannot be treated as a soft tissue attenuation, when studied under experimental conditions as used by the author. To the contrary, it has
been shown that the mean diameter of mouse bones ranges from 0.2 mm for the scapula to only 1.5 mm for the femur. Other evidence indicates that the actinides are bone-surface rather than bone-volume seekers. These two factors can explain the minimal degree of attenuation observed in this study.

Because the L X-ray attenuation losses are a function of energy, once they have been determined for a specific system, they should be applicable to studies using $^{238}\text{Pu}$, $^{239}\text{Pu}$, $^{244}\text{Cm}$, and $^{253}\text{Es}$. Even though these nuclides deposit in different ratios in soft tissue and bone, the attenuation should not change a significant amount.

CONCLUSIONS

The results of this study have demonstrated that measurement of the L X-rays provides not only a quantitative, but rapid means for studying the metabolism of actinide elements in mice. The rationale for using this method in mice is that the attenuation of the L X-rays by their bones is not great, and it is possible to make corrections for such losses. Although this method does not produce the high degree of accuracy needed in some instances, it does produce quite reasonable results which are adequate for many metabolism studies especially those where enhancement of radionuclide elimination is under investigation.

In a recent study of the effect of DTPA on the metabolism of $^{253}\text{Es}$ in mice, we used the routine $\alpha$-counting procedures as well as this L X-ray counting technique. The results were quite similar and we plan to use L X-ray counting as a routine procedure for more mouse studies.
ACKNOWLEDGMENTS

The author acknowledges with pleasure the constructive reviewing by Drs. H. G. Parker and Patricia W. Durbin. In addition I wish to thank Anne DeG. Low-Beer and David J. Yeager for the purification and preparation of the $^{243}$Am. I am indebted to Mary Wildensten for her valuable editorial advice.
REFERENCES


5. Parker Howard., Wright Stephen R. and Low-Beer Anne deG. Actinide element studies with a Si(Li) "wound counter". In: Semiannual Report, Biology and Medicine, Donner Laboratory, Lawrence Radiation Laboratory, Berkeley, California, UCRL-18793, Fall 1968.


7. Unpublished studies with semiconductors.

Table 1. Mouse tissue distribution and excretion of $^{243}$Am calculated as a per cent of the initial dose

<table>
<thead>
<tr>
<th>Number of mice</th>
<th>Day of experiment</th>
<th>1</th>
<th>2 (mean value)</th>
<th>14</th>
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<tr>
<td></td>
<td>Attenuation</td>
<td>UN</td>
<td>COR</td>
<td>UN</td>
</tr>
<tr>
<td></td>
<td>LX-ray</td>
<td>14</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>Urine (cumulative)</td>
<td>14</td>
<td>17</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>Feces (cumulative)</td>
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<td>2.8</td>
<td>5.4</td>
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<td>44</td>
<td>41</td>
<td>42</td>
</tr>
<tr>
<td>Injected leg</td>
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<td>4.3</td>
<td>4.4</td>
<td>2.6</td>
</tr>
<tr>
<td>Uninjected leg</td>
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<td>0.87</td>
<td>1.1</td>
<td>1.8</td>
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<tr>
<td>Liver</td>
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<td>31</td>
<td>32</td>
<td>35</td>
</tr>
<tr>
<td>Spleen</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lungs</td>
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<td>0.7</td>
<td>0.7</td>
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<tr>
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<td>1.5</td>
<td>1.9</td>
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<tr>
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<td>107.0</td>
<td>101.7</td>
<td>101.9</td>
<td>103.9</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

Fig. 1. NaI spectra of $^{243}$Am and $^{195}$Au photons after passing through 1 mm of soft tissue and 1 mm of bone. The higher energy photons do not contribute significantly to the L X-ray photopeak.

Fig. 2a. $^{243}$Am whole-body retention, plotted as the true net cpm from the L X-ray photopeak and the 74-keV gamma-ray photopeak.

Fig. 2b. $^{243}$Am whole-body retention, plotted as a per cent of the counts from the mouse whole-body standard injected with the same initial dose and sacrificed five minutes after injection.

Fig. 3. Semiconductor spectra of the $^{243}$Am photons before and after passing through soft tissue- and bone-equivalent material.
Fig. 1

Counts/min

10^3

10^2

10^1

10^0

Channel number

0

40

80

120

160

243\text{Am}

L X-ray

66\text{keV}

74\text{keV}

31\text{keV}

43\text{keV}

195\text{Au}

Higher-energy contribution into LX-ray photopeak

243\text{Am}

31\text{keV}

43\text{keV}

66\text{keV}

74\text{keV}

195\text{Au}
Counts do not change after injection into mouse.

-74 keV γ-ray photopeak
- L X-ray photopeak
- Standard in 0.1 ml volume

30% Self-absorption, 5 min after injection into mouse

Fig. 2a
Fig. 3
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