

USE OF RADIOIRON (^{59}Fe) AS AN INDEX TO HEMOPOIETIC DAMAGE CAUSED BY IONIZING RADIATION¹

J. T. KITCHINGS III, P. B. DUNAWAY, J. D. STORY,
AND L. E. TUCKER

Radiation Ecology Section, Health Physics Division,
Oak Ridge National Laboratory, Oak Ridge, Tennessee

ABSTRACT

Elimination rates and biological half-lives of radioiron (^{59}Fe) in seven species of wild rodents ranged from *Oryzomys palustris* ($\lambda_b = 0.18\%$, $T_b = 385$) to *Reithrodontomys humulis* ($\lambda_b = 0.50\%$, $T_b = 137$). No significant difference in retention of ^{59}Fe was noted after injections of 0.475 μCi or 0.110 μCi . The animals were exposed to ionizing radiation on the one hundred-fourteenth day of the experiment. One group (400 rad) exhibited no decrease in iron retention with the exception of radiosensitive rice rats, while in the other group (doses approaching the LD_{50-90}) all species except the house mouse showed a marked decrease in retention.

It is believed that the normal mechanism involved in the return of hemoglobin disintegration products, notably released iron, to the hemopoietic system is impaired. A discussion of renal elimination of the released iron is presented.

INTRODUCTION

The normal mammalian system is highly dependent upon iron for several vital functions. Iron is incorporated into a number of enzymes associated with metabolism, e.g., catalase, succinic dehydrogenase and cytochrome oxidase. Hemoglobin, an iron-porphyrin-protein complex, has the capacity to combine reversibly with oxygen and thus is used for oxygen transportation by the erythrocytes.

Most of the iron assimilated into the body is absorbed in the mucosal cells of the proximal duodenum and is transported by the plasma in the form of transferrin, a specific iron-binding B_1 globulin. The bulk of body iron resides in red cell hemoglobin, but smaller quantities are stored, in the form of ferritin and hemosiderin, in the liver, spleen, and bone marrow.

The body conserves and reutilizes iron tenaciously. The rate of loss of radioiron from laboratory mice (Carlton Farm Webster strain) has been investigated by Bonnet *et al* (1960) and found to be 0.39% per day with a biological half-life of 180 days. Earlier work by Chappelle (1955) and his associates with Swiss mice indicates rates of 0.29% per day to 0.73% per day. Stevens *et al* (1953) obtained a 140 day biological half-life based upon a daily excretion rate of 0.50% per day for Swiss mice.

We investigated the response of seven species of wild rodents to intraperitoneal injections of radioiron (^{59}Fe) in an effort to establish elimination rates for each species, to determine what effect varying doses of the isotope have on the elimination of iron from the body, and to test the effect of ionizing radiation on the established elimination patterns. This was done in order to learn whether iron metabolism and damage to the

erythropoietic system caused by radiation can be measured by this technique in future field experiments.

METHODS

Seven species of wild rodents indigenous to the Atomic Energy Commission installation at Oak Ridge, Tennessee, were live trapped for this study. These species were: harvest mouse (*Reithrodontomys humulis*), white-footed mouse (*Peromyscus leucopus*), golden mouse (*Peromyscus nuttalli*), rice rat (*Oryzomys palustris*), cotton rat (*Sigmodon hispidus*), house mouse (*Mus musculus*), and Norway rat (*Rattus norvegicus*). A two-week period was allowed to lapse between time of capture and the beginning of the experiment. Animals were caged singly, and food and water were available *ad libitum*. The temperature and relative humidity in the caging room were 21-22°C and 40-45%, respectively. Prior to the initial injection the animals were assigned randomly to either ^{59}Fe dose group I (0.475 μCi or II (0.110 μCi) so that two animals of each species were in each group. Each animal was given a 0.5 ml intraperitoneal injection of an iron-saline solution (the isotope was originally FeCl_2 in an HCl solution). Polyethylene bottles, similar to the geometry of each species and filled with distilled water to approximate the body weight of each species, were used as standards. Each standard was injected with amounts of ^{59}Fe corresponding to either dose group I or II. Activity measurements were taken with an Armac whole body counter on the day of injection and every three or four days thereafter until it became apparent that the iron was being eliminated at a constant rate, then every seven days and eventually every fourteen days until time of irradiation on day one hundred fourteen.

On the one hundred-fourteenth day after the initial injection, Group I received a 400 rad dose from a cobalt-60 source. The 400 rad level was chosen because it was below the LD_{50-90} for the rice rat which has been shown by Dunaway *et al* (1968) to be the most radiosensitive of the seven species tested. Group II received a dose 125 to 150 rad below the LD_{50-90} for each species. Whole body counts were taken on the second and fifth day after irradiation.

RESULTS

Biological elimination constants and biological half-lives were established after an initial period of rapid

¹ Research sponsored by the U. S. Atomic Energy Commission under contract with the Union Carbide Corporation.

loss. *Mus musculus*, *Oryzomys palustris*, *Peromyscus leucopus*, and *Sigmodon hispidus* required eleven days to reach a steady loss rate. *Peromyscus nuttalli* and *Reithrodontomys humulis* eliminated at a constant rate after the fifteenth day, and *Rattus norvegicus* required twenty-two days before reaching a constant loss level. These figures do not agree with the six and two day periods needed for initial clearance as reported by Bonnet *et al* (1960) and Chappelle *et al* (1955), respectively. The total loss of radioiron from the body was not influenced significantly by the different con-

centrations of isotope administered; therefore, the results of both dose levels were averaged and plotted in Fig. 1. The elimination rates and biological half-lives ranged from $\lambda_b = 0.18\%$, $T_b = 385$ days (*Oryzomys palustris*) to $\lambda_b = 0.50\%$, $T_b = 137$ days (*Reithrodontomys humulis*).

Table I summarizes the effect of various doses of irradiation on the elimination rates. It became apparent after counting the activity of Group I on the second and fifth days after irradiation that the level of radiation was too low to elicit a response in most species and the dose was increased in Group II to approach the species LD_{50-80} . In Group I only the radiosensitive rice rat (Dunaway *et al*, 1968) displayed any response to irradiation. This was by a decrease of 1.2% from the elimination constant after five days. In Group II retention rates of all species, with the exception of the house mouse, decreased from the elimination curves, ranging from 1.9% (*O. palustris*) to 4.4% (*R. humulis*). The experiment was terminated after 121 days (seven days after irradiation) due to low amounts of radioactivity in Group II.

TABLE I.

THE EFFECTS OF IONIZING RADIATION ON THE RETENTION OF RADIOIRON (^{59}Fe)

Taxon	Injected Dose ^{59}Fe	Weight ^A (grams)	Irradiation (rad)	Percent Deviation from ^B Established λ_b
I. Cricetidae				
<i>(Reithrodontomys humulis)</i>	0.475 μc	8.9	400	+0.8
	0.110 μc		800	-4.4
<i>(Peromyscus leucopus)</i>	0.475 μc	19.3	400	+1.0
	0.110 μc		900	-2.0
<i>(Peromyscus nuttalli)</i>	0.475 μc	23.4	400	+0.2
	0.110 μc		875	-4.0
<i>(Oryzomys palustris)</i>	0.475 μc	48.0	400	-1.2
	0.110 μc		400	-1.9
<i>(Sigmodon hispidus)</i>	0.475 μc	110.0	400	+1.0
	0.110 μc		800	-2.5
II. Muridae				
<i>(Mus musculus)</i>	0.475 μc	16.5	400	+1.3
	0.110 μc		700	+0.3
<i>(Rattus norvegicus)</i>	0.475 μc	218.6	400	+0.7
	0.110 μc		750	-2.4

^A Average weight of animals in both groups from time of injection to day of irradiation.

^B Five days postirradiation.

DISCUSSION

It is apparent that each species had its own iron requirement, as indicated by the relatively wide variation of elimination rates. The elimination rate of all

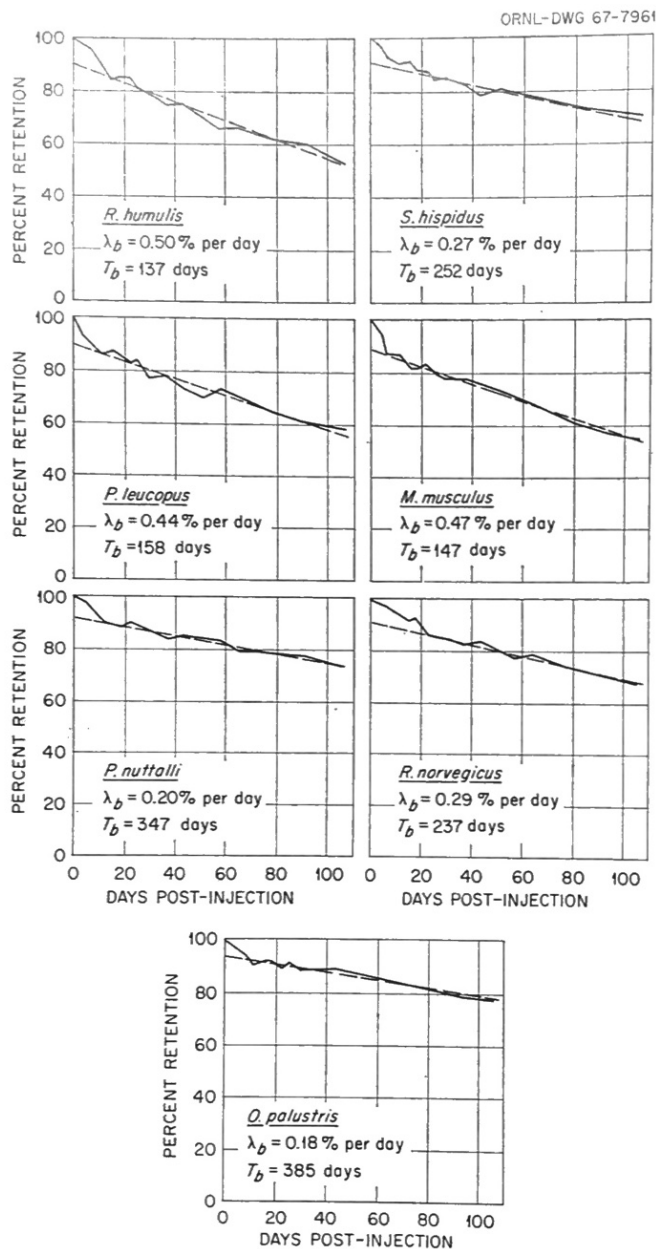


Fig. 1. The retention of radioiron (^{59}Fe) by seven species of wild rodents. Solid line represents experimental results; broken line denotes biological elimination constant.

λ_b = biological elimination constant: computed by standard linear regression analysis, with y values transformed to natural log equivalents.

T_b = biological half-life = $\frac{0.693}{\lambda_b}$

where λ_b represents biological elimination constant.

species tested except *Oryzomys palustris* and *Peromyscus nuttalli* tended to decrease as the body weight increased. The elimination rate of *Oryzomys palustris* was the lowest of all species tested. Further study may relate its aquatic habits with its tenacious retention of iron stores.

For each species the effect of ionizing radiation on the biological elimination of iron was rapid as the dose approached the LD_{50-30} . A decrease in retention was noted as early as the second day. There was no apparent decrease in iron retention in Group I after 400 rad except in the rice rat. It has been observed, however, that radiation levels as low as 200-400 rad, cause disturbances in the peripheral blood, especially to the white blood cells (Gambino and Lindberg 1964, Dunaway *et al* 1967, Langham *et al* 1956, and Krayevskii 1965). The radiation level of Group I was apparently of such a magnitude that the components of the hemopoietic system which are involved in the return of disintegration products, notably iron, were not damaged and were capable of returning iron to the system.

It is generally accepted that the tissues of the hemopoietic system are among the most radiosensitive (Hornykiewytch and Syedl 1955, Krayevskii 1965, Barbieve *et al* 1958, and Mathé as in Szirmai 1965). A dose approaching the LD_{50-30} , as in Group II and the rice rat in Group I, may damage the hemopoietic organs (e.g., liver, spleen, bone marrow) thus impairing the utilization of iron released from the destroyed hemoglobin.

Presumably the kidney is capable of destroying hemoglobin if the tissues which are normally involved in this process are damaged (Crosby 1959, Hampton and Mayerson 1950, and Wintrobe 1961). Ludewig and Chanutin (1951) found an increase in the iron content of the kidney five days after irradiation. Although capable of destroying hemoglobin, the kidney is quite inefficient in returning the released iron to the body and much is spilled into the urine and excreted (Hampton and Mayerson 1950). Subsequent investigation may show that this phenomenon accounts for the decrease in iron retention in our experimental data.

Further experimentation is planned to analyze the fate of radioiron in wild populations after exposure to

ionizing irradiation. Our future work will involve: 1) increasing the number of experimental animals to exact a more precise elimination pattern for radioiron, 2) analyzing blood elements (hemoglobin, red blood cell counts, etc.) after irradiation in order to study possible correlation with iron metabolism and 3) determining radioiron distribution in several hemopoietic tissues at different time intervals after irradiation. In conjunction with the above experiments we plan to study radioiron uptake after the animal has been exposed to ionizing radiation in an effort to procure an accurate index of radiation damage which can be applied to field studies.

LITERATURE CITED

- Barbieve, R. E., R. F. Sweeton, G. P. Sakalosky and P. E. Klatt. (1958). A radiobiology guide. WADC Technical Report: 57-118 (I) 110 p.
- Bonnet, J. D., A. L. Orvis, A. B. Hagedorn and C. A. Owen, Jr. (1960). Rate loss of radioiron from mouse and man. *Am. J. Physiol.* 198(4) 784-6.
- Chappelle, E., B. W. Gabrio, A. R. Stevens, Jr., and C. A. Finch. (1955). Regulation of body iron content through excretion in the mouse. *Am. J. Physiol.* 182: 390-2.
- Crosby, W. H. (1959). Normal functions of the spleen relative to red blood cells: A review. *Blood* 14: 399-408.
- Dunaway, P. B., L. L. Lewis, J. D. Story, J. A. Payne and J. M. Inglis (1968). Radiation effects in the Soricidae, Criceidae and Muridae. In press.
- Dunaway, P. B., J. D. Story, J. T. Kitchings, III, L. E. Tucker, L. L. Lewis, C. Koh, C. M. McConnell. (1967). Radiation effects in blood of indigenous small mammals. Health Physics Division Annual Progress Report, ORNL-4168.
- Gambino, J. J. and R. G. Lindberg. (1964). Response of the pocket mouse to ionizing radiation. *Rad. Res.* 22: 586-597.
- Hampton, J. K. and H. S. Mayerson. (1950). Hemoglobin iron as a stimulus for the production of ferritin by the kidney. *Am. J. Physiol.* 160: 1-8.
- Hornykiewytch, T. and G. Syedl. (1955). Histochemical and serological studies of the effects of X-rays. *Stralenterapie* 88: 129-138.
- Krayevskii, N. A. (1965). Studies in the pathology of radiation disease. Pergamon Press, London, 219 p.
- Langham, W., K. T. Woodward, S. M. Rothermel, P. S. Harris, C. C. Lushbaugh and J. B. Storer. (1965). Studies of the effect of rapidly delivered massive doses of Gamma-rays on mammals. *Rad. Res.* 5: 404-432.
- Ludewig, S. and A. Chanutin. (1951). Effect of whole body X-irradiation on storage of iron in liver, spleen and kidney of rats. *Am. J. Physiol.* 166: 384-6.
- Stevens, A. R., P. L. White, D. M. Hegsted and C. A. Finch. (1953). Iron excretion in the mouse. *J. Biol. Chem.* 203: 161.
- Szirmai, E., ed. (1965). Nuclear Hematology. Academic Press, N. Y. 589 p.
- Wintrobe, M. M. (1961). Clinical Hematology. Lea and Febiger, Philadelphia. 1186 p.