Effects of ^{59}Fe on Blood and Bone Marrow of Rabbits

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Abstract □ Effects of ^{59}Fe on blood and bone marrow of rabbits, *Oryctolagus*, were studied. Erythrocyte counts and leukocyte differential counts at three- and eight-day intervals and bone marrow differential counts after the 19th, 28th, and 40th day were performed for each rabbit. Increased ^{59}Fe produced increased destruction of all blood cells except lymphocytes. The ratio of lymphocytes in bone marrow increased.

Keyphrases □ ^{59}Fe effect—blood, bone marrow, rabbits □ Erythrocytes—^{59}Fe effect □ Leukocytes—^{59}Fe effect □ Bone marrow produced cells—^{59}Fe depletion

"Sufficient knowledge has been gained through an intensive research program to indicate that radioactive isotopes have therapeutic value, are excellent diagnostic agents, and are worthwhile research tools" (1). A number of compounds of importance in pharmaceutical as well as medical research have been developed.

When the radioisotopes of iron (^{55}Fe, ^{59}Fe) became available, it gave impetus to research on iron metabolism (2). Lawrence found that studies with a very small dose of ^{59}Fe could be carried out safely (3). ^{59}Fe is a β- and γ-emitter with a half-life of forty-six days. Less than 10 μc. are required for a determination in the clinical study of blood disorders (4).

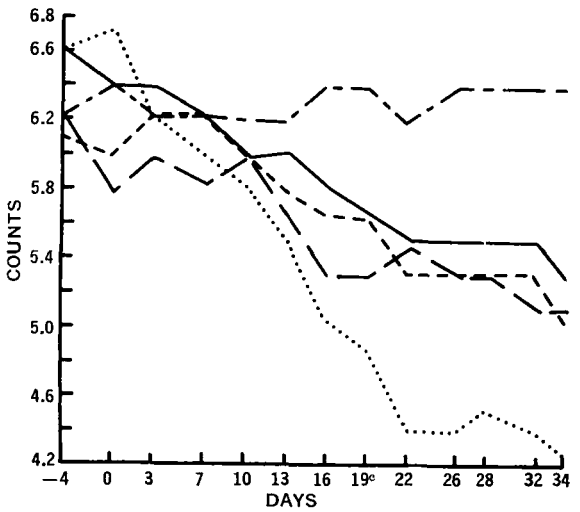

Figure 1—*Red blood cell counts of Groups I through V. Each rabbit in Groups I, II, III, and IV received total doses of 5, 10, 15, and 20 μc. of ^{59}Fe, respectively; Group V was not dosed and served as the control. The counts represent the average for the 10 rabbits of each group and are given in millions of red blood cells per cu. mm. c denotes dates of administration of one-half total dose of ^{59}Fe. Key: ——, Group I; — —, Group II; - - -, Group III; ..., Group IV; — - —, Group V.*

Anemia may result from large doses of ionizing radiation on the bone marrow since the damage reduces the ability to produce red cells in sufficient numbers. A much more common effect is the depression of white cell production, resulting in a leukopenia which may be transient or protracted. The ultimate result of serious damage to the blood-forming organs may be the development of some form of leukemia (5).

In the metabolism of iron, most of dietary iron is in complex organic combination, usually in the form of ferric or trivalent iron. Therapeutic iron is available in many chemical forms both bivalent and trivalent. These are usually administered orally and sometimes parenterally. From 70 to 100% of newly ingested iron can be found in the circulating red cells in 7 to 14 days after absorption (6). When orally administered, it was found that the absorption of this important element was increased as the iron stores were depleted (7). However, once absorbed, the iron was rapidly picked up by the bone marrow and converted into blood hemoglobin (8). On the other hand, following intravenous injection, up to 40% of the dose was stored temporarily in the liver as ferritin for ready availability to the bone marrow (9). In regard to total storage iron, 41.7 to 83.3% has been reported to be in the liver with the remainder in the spleen, bone marrow, and the reticuloendothelial system (10).

The purpose of this study was to determine the effects of increased doses of radioactive ^{59}Fe on the blood and bone marrow of rabbits, *Oryctolagus*. Previous research has indicated that large doses of this isotope have resulted in the depression of white cell production (5). This study was particularly concerned with a comparison of the effects of radioactive ^{59}Fe on the different types of leukocytes.

EXPERIMENTAL

Five groups of adult rabbits, each group consisting of five females and five males, were used. Each rabbit weighed approximately 2.3 kg. The rabbits[1] were normal at birth, received the same food, and as nearly as possible, were maintained under identical conditions throughout the study. Each rabbit in Groups I, II, III, and IV received an intravenous dose of 2.5, 5, 7.5, and 10 μc. of ^{59}Fe, respectively. One milliliter of Ringer's injection USP was used as the vehicle in each injection. Group V served as a control and each rabbit received 1 ml. of the official Ringer's injection. This procedure was repeated after an interval of 18 days for total doses of 5, 10, 15, and 20 μc. of ^{59}Fe, respectively. A vein in the ear was used as the site of injection.

[1] California Breed.

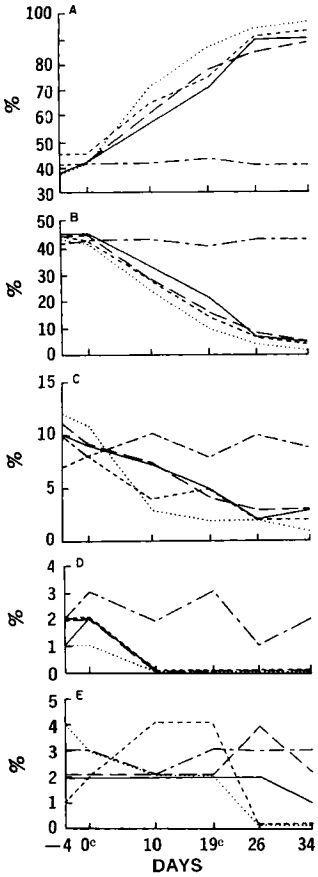

Figure 2—*White blood cell differential counts of Groups I through V. Each rabbit in Groups I, II, III, and IV received total doses of 5, 10, 15, and 20 μc. of ^{59}Fe, respectively; Group V was not dosed and served as the control. The percentage is the percentage of each type of cell of the white cell differential count and represent the average for the 10 rabbits of each group.* c *denotes dates of administration of one-half total dose of ^{59}Fe. Key: ——, Group I; — —, Group II; - - -, Group III; ..., Group IV; — - —, Group V.* A, *lymphocytes;* B, *neutrophils;* C, *monocytes;* D, *basophils;* E, *eosinophils.*

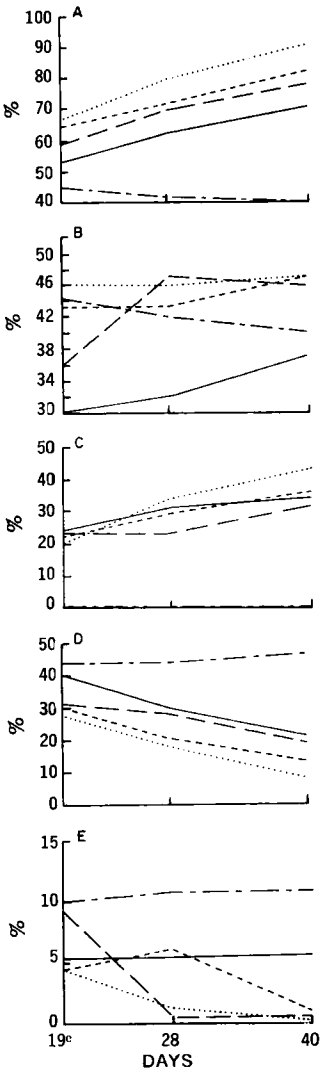

Figure 3—*Bone marrow differential counts of Groups I through V. Each rabbit in Groups I, II, III, and IV received total doses of 5, 10, 15, and 20 μc. of ^{59}Fe, respectively; Group V was not dosed and served as the control. This percentage is the percentage of each type of cell of the bone marrow differential count and represents the average for the 10 rabbits of each group.* c *denotes date of administration of one-half total dose of ^{59}Fe; the first half-dose was administered on day 0. Key: ——, Group I; — —, Group II; - - -, Group III; ..., Group IV; — - —, Group V.* A, *total lymphocytes;* B, *normal lymphocytes;* C, *atypical lymphocytes;* D, *total neutrophils;* E, *total monocytes, basophils, and eosinophils.*

Red blood cell counts (Fig. 1) and white cell differential counts (Fig. 2) were performed for each rabbit at 3- and 8-day intervals, respectively. Bone marrow samples were withdrawn from the scapula of each rabbit on the 19th, 28th, and 40th day after the initial dose of ^{59}Fe. Bone marrow differential counts (Fig. 3) were obtained from each sample. The final average red blood cell counts, the white cell differential counts, and the bone marrow differential counts are shown in Tables I, II, and III, respectively.

DISCUSSION

The decrease of erythrocytes in blood (Table I) and the decreased ratio of the other leukocytes to the lymphocytes of the differential counts (Table II) indicate that increased dosage of ^{59}Fe is related to the increased destruction of all of the blood cells except the lymphocytes. One may assume that these effects could be due to the increased damage to the bone marrow according to Quimby *et al.* (4).

The effects of increased doses of ^{59}Fe on the bone marrow (Table III) also was related to decreased ratio of all the other leukocytes to the lymphocytes in the differential counts. The immature neutrophils (segmented, stabs, juveniles, myelocytes, and promyelocytes) were decreased in approximately the same ratio. Increased ratio of the lymphocytes to the other leukocytes occurred as it did in the blood. However, a large percentage of atypical lymphocytes were found in the bone marrow of the dosed rabbits. These effects of ^{59}Fe on the bone marrow further support Quimby's statement that ^{59}Fe damages the bone marrow.

The red bone marrow, which consists of myeloid tissue, has been established as producing the erythrocytes and the granulocytes (neutrophils, basophils, and eosinophils) (11). Many hematologists believe that the monocytes are also formed in the myeloid tissue (12). The lymphocytes originate in the lymphatic tissue, primarily in the nodular, but also to some extent in the diffuse and loose lymphatic tissue (13).

Since the granulocytes and the monocytes originate in the red bone marrow, it is logical to conclude that their decreased appearance in the bone marrow and blood was the result of the ^{59}Fe that was administered. Increased ratio of lymphocytes to the other leukocytes in the differential counts may be explained by the fact that they are not comparably affected by the ^{59}Fe. This lesser effect of ^{59}Fe on the lymphocytes is possibly due to the fact that the lymph nodes, which do not store iron (10), are the principal site of origin for the lymphocytes. Thus their exposure to ^{59}Fe is considerably less than that of the other leukocytes. It is possible that the presence of atypical lymphocytes in the bone marrow of the dosed rabbits could be due to their origin in the lymphoid tissue of

Table I—Comparison of Final Red Blood Cell Counts of Groups I–V[a]

Group V (Control)		Group I			Group II			Group III			Group IV		
Count[b]	±*SD*	Count	±*SD*	*p*[c]	Count	±*SD*	*p*	Count	±*SD*	*p*	Count	±*SD*	*p*
6.40	0.048	5.35	0.056	<0.01	5.17	0.047	<0.01	5.01	0.055	<0.01	4.24	0.052	<0.01

[a] Each rabbit in Groups I, II, III, and IV received total doses of 5, 10, 15, and 20 μc. of ^{59}Fe, respectively. Group V was not dosed and served as the control. [b] The counts represent the average for the 10 rabbits of each group and are given in millions of red blood cells per cu. mm. [c] *p* = significance determined by subjecting data to Student *t* test.

Table II—Comparison of Final White Blood Cell Differential Counts of Groups I–V[a]

Type of Cell	Group V (Control) Count[b]	±SD	Group I Count	±SD	p[c]	Group II Count	±SD	p	Group III Count	±SD	p	Group IV Count	±SD	p
Lymphocytes	42	2.6	91	2.2	<0.01	89	1.8	<0.01	93	2.8	<0.01	96	2.2	<0.01
Neutrophils	44	2.7	5	2.1	<0.01	6	1.5	<0.01	5	2.7	<0.01	3	1.9	<0.01
Monocytes	9	1.3	3	1.2	<0.01	3	1.4	<0.01	2	1.2	<0.01	1	1.0	<0.01
Basophils	2	1.2	0	0.0	<0.01	0	0.0	<0.01	0	0.0	<0.01	0	0.0	<0.01
Eosinophils	3	1.3	1	1.0	<0.01	2	1.7	*NS*	0	0.0	<0.01	0	0.0	<0.01

[a] Each rabbit in Groups I, II, III, and IV received total doses of 5, 10, 15, and 20 microcuries of ^{59}Fe, respectively. Group V was not dosed and served as the control. [b] The count is the percentage of each type of cell of the white cell differential count and represents the average for the ten rabbits of each group. [c] p = significance determined by subjecting data to Student t test.

Table III—Comparison of Final Bone Marrow Differential Counts of Groups I–V[a]

Type of Cell	Group V (Control) Count[b]	±SD	Group I Count	±SD	p[c]	Group II Count	±SD	p	Group III Count	±SD	p	Group IV Count	±SD	p
Normal lymphocytes	40	2.2	37	2.8	<0.02	46	3.1	<0.01	47	4.1	<0.01	47	5.3	<0.01
Atypical lymphocytes	0	0.0	34	2.0	<0.01	32	2.9	<0.01	36	4.0	<0.01	44	3.7	<0.01
Total lymphocytes	40	2.2	71	3.2	<0.01	78	3.5	<0.01	83	4.1	<0.01	91	2.8	<0.01
Segs[d]	16	2.8	9	2.0	<0.01	8	1.8	<0.01	6	1.9	<0.01	3	2.1	<0.01
Stabs[d]	17	1.6	9	2.2	<0.01	11	2.1	<0.01	7	2.1	<0.01	4	2.4	<0.01
Juveniles[d]	5	2.7	2	1.2	<0.01	0	0.0	<0.01	1	1.1	<0.01	1	1.5	<0.01
Myelocytes[d]	6	3.1	2	1.2	<0.01	0	0.0	<0.01	0	0.0	<0.01	0	0.0	<0.01
Promyelocytes[d]	2	1.6	0	0.0	<0.01	1	1.1	*NS*	0	0.0	<0.01	0	0.0	<0.01
Total neutrophils	46	2.9	22	2.4	<0.01	20	1.8	<0.01	14	2.8	<0.01	8	2.4	<0.01
Monocytes	8	1.8	1	1.3	<0.01	1	1.5	<0.01	2	1.7	<0.01	0	0.0	<0.01
Basophils	2	1.6	0	0.0	<0.01	0	0.0	<0.01	0	0.0	<0.01	0	0.0	<0.01
Eosinophils	3	1.5	5	2.5	<0.05	1	1.3	<0.01	1	1.3	<0.01	1	1.3	<0.01
Misc.[e]	1	1.3	1	1.5	*NS*	0	0.0	<0.05	0	0.0	<0.05	0	0.0	<0.05

[a] Each rabbit in Groups I, II, III, and IV received total doses of 5, 10, 15, and 20 μc. of ^{59}Fe, respectively. Group V was not dosed and served as the control. [b] The count is the percentage of each type of cell of the bone marrow differential count and represents the average for the ten rabbits of each group. [c] p = significance determined by subjecting data to Student t test. [d] Cells in the development of the neutrophil. [e] Misc. is the total of other cells such as blast forms, plasma cells, and reticular cells.

the bone marrow where a degree of exposure of these cells to the effects of ^{59}Fe could occur. However, the possibility merits further investigation.

The data obtained in this study suggests a need for further study of the effects of ^{59}Fe on blood and bone marrow. This suggestion is particularly important when the possibility is considered, as shown in this study, that ^{59}Fe has resulted in the depletion of the granulocytes produced in the bone marrow. The granulocytes are the chief phagocytic agents in the body.

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