^{59}Fe LABELING IN BONE

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Autoradiographs of the tibia of rabbits revealed labeling of osteocytes and/or associated lacunae at 24 hr after the intravenous injection of ^{59}Fe-labeled ferrous citrate. Following x-irradiation of the tibia in eight rabbits, labeling was increased in six instances and decreased in two. Evidence of ^{59}Fe labeling in sites other than marrow in bone is presented.

The use of ^{59}Fe for studies of erythropoietic activity is well known. Autoradiographic methods that utilize ^{59}Fe relate mainly to labeling of the erythropoietic cells of the bone marrow (*1*). Iron uptake in some bones reflects little change following increasing doses of x-irradiation and suggests no significant involvement with hematopoiesis (*2*). Marrow extracted from the shaft of normal and x-irradiated bones contained 60% (*3*) to 99% (*4*) of the total activity in the bone. Apparently ^{59}Fe labeling in sites other than marrow can occur in bone.

Findings of ^{59}Fe labeling in bone and differences in labeling between untreated and x-irradiated bones are presented.

MATERIALS AND METHODS

The right tibiofibula of young adult, white, female New Zealand rabbits was x-irradiated while the rabbit was under pentobarbital anesthesia and the remainder of the body shielded with 3-mm thick lead. X-radiation was from a 280-kVp Picker Vanguard therapy unit at 16 mA, filtration 0.2 mm Cu and 1.0 mm Al, HVL 1.0 mm Cu. Daily doses of 200 R were given 5 days a week at a rate of 260 R/min until a total dose of 2,000 R, 3,000 R, 4,000 R, or 5,000 R was administered. Sham exposures for periods of time equivalent to the total x-irradiation schedule were done. The rabbits were studied at various times after x-irradiation (Table 1). Fifty microcuries ^{59}Fe-labeled ferrous citrate was injected intravenously into the marginal ear vein and 24 hr later the rabbit was euthanized. The tibias were dissected, cleaned of all surrounding tissue, and counted "in toto" for radioactivity with a Picker Magnascaler using a 3-in. NaI(Tl) crystal. Residual ^{59}Fe activity in bone after the removal of marrow was determined in one rabbit. In this case, the tibia was sectioned in two pieces longitudinally and the marrow was washed out with saline and removed with a semi-micro spatula.

One to 3 months after autopsy, paraffin sections of tibia were decerated and coated in a darkroom with Kodak NTB2 emulsion which was diluted 1:1 with distilled water, then stored in light-tight slide boxes. Lead strips, minimal total thickness of 6 mm, were inserted between each slide and between slide boxes to reduce the radiation between slides. After an exposure period of 24–34 days, the coated slides were developed at 20°C in Kodak D-19 for 4 min, washed in a running water bath for 3 min, fixed in Kodak acid fixer for 5 min, and rewashed for 30 min. Routine hematoxylin and eosin staining were done using Harris' hematoxylin and a 0.5% aqueous solution of Eosin Y. The grains in the autoradiographs were noted using an oil immersion objective and a Howard micrometer reticule which permitted areas 100 microns square divided into 36 subunit squares to be observed. Random distribution of the grains per subunit with limits between 0 and 10 close to the site to be studied averaged less than 4 grains per subunit with limits between 0 and 10 grains. The length of the lacunae was usually within the limit of the dimensions of a subunit square. Where osteocytes and/or lacunae had aggregations of ten or more grains, ^{59}Fe labeling was considered definite. A total of 400 osteocytes and/or associated

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TABLE 1. LABELING OF OSTEOCYTES AND/OR ASSOCIATED LACUNAE WITH ^{59}Fe

Rabbit number	Time after exposure	Percent labeling		Ratio	Percent ^{59}Fe incorporation
		Untreated tibia	Treated tibia		Treated tibia / Untreated tibia
144	2 week $\bar{p}$ sham (OR × 25)	29	26	1:0.9	90
155	3 month $\bar{p}$ sham (OR × 10)	56	59	1:1.0	94
111	6 month $\bar{p}$ sham (OR × 10)	43	40	1:0.9	103
156	3 month $\bar{p}$ 2,000 R*	55	63	1:1.1	64
134	6 month $\bar{p}$ 2,000 R	48	58	1:1.2	45
65	2 week $\bar{p}$ 3,000 R	6	20	1:3.3	86
117	6 month $\bar{p}$ 3,000 R	47	60	1:1.3	39
113	2 week $\bar{p}$ 5,000 R	27	51	1:1.9	32
152	3½ month $\bar{p}$ 5,000 R	20	50	1:2.5	54
158	3 month $\bar{p}$ 4,000 R	38	27	1:0.7	35
119	6 month $\bar{p}$ 4,000 R	52	41	1:0.8	31

* 200 R/day, 5 days/week.

lacunae, 200 each per opposing side for each section of bone with marrow from the distal third portion of the tibia, were examined.

RESULTS

An example of ^{59}Fe labeling in bone is shown in Fig. 1. Varying degrees of aggregations of the grains in the autoradiographs were associated with osteocytes and/or lacunae. A marked increase in distribution of the grains, but no aggregations, was observed along the endosteum marrow interface and in proximity to the endothelium of some blood vessels.

Labeling of osteocytes with ^{59}Fe and/or lacunae in the tibia following sham x-irradiation varied from 26% to 59% (Table 1). Comparable values, 29%–56%, were observed in the untreated contralateral tibia. In the untreated contralateral tibia of the x-ray treated rabbits, ^{59}Fe labeling ranged from 6% to 55%. There were greater differences between the ^{59}Fe labeling in the treated and untreated tibia in the x-irradiated group than in the sham treated group. Six of the eight tibias that were x-irradiated had increased ^{59}Fe labeling of 1.1–3.3 times the labeling found in the untreated contralateral tibia; in the remaining two tibias it was approximately three-fourths. Incorporation of ^{59}Fe at 24 hr after intravenous administration as determined by external counting was comparable in both the treated and untreated tibia in the sham exposure group and decreased in tibias exposed to x-radiation.

The x-irradiated tibia of Rabbit 152 (Table 2) had approximately half as much ^{59}Fe, 54.6%, as was incorporated in the untreated contralateral tibia. Without marrow, the tibia had a residual activity of ^{59}Fe that was 41% of the total amount incorporated while in the untreated tibia only 17.5% was present. Twenty-seven percent more ^{59}Fe activity was found in the x-irradiated tibia than in the untreated tibia after the marrow was removed.

DISCUSSION

Evidence is presented to show that following the intravenous administration of ^{59}Fe-labeled ferrous citrate, labeling of bone with iron is not exclusive to the erythropoietic constituents of the marrow. Deposition of ^{59}Fe in osteocytes and/or associated lacunae is evident by the localization and aggregations of grains in autoradiographs (Fig. 1). The labeling appears to be random and mostly extranuclear. These observations raise questions as to the mechanisms of deposition of ^{59}Fe in the lacunae, its pathway there, and the physicochemical changes involved. Answers to these questions were not being sought in this study. The evidence presented here was found incidental to the work of developing an autoradiographic procedure for study of ^{59}Fe labeling of bone

TABLE 2. ^{59}Fe INCORPORATION IN TIBIA

Rabbit 152—3½ month $\bar{p}$ 5,000 R (200 R × 25)

$$\frac{\text{X-irradiated}}{\text{Untreated}} = 54\%$$

$$\frac{\text{X-irradiated (marrow removed)}}{\text{Untreated (marrow removed)}} = 127\%$$

$$\frac{\text{Untreated (marrow removed)}}{\text{Untreated}} = 17.5\%$$

$$\frac{\text{X-irradiated (marrow removed)}}{\text{X-irradiated}} = 41\%$$

FIG. 1. Autoradiograph of ^{59}Fe labeling in bone (original magnification approximately × 1,200).

marrow in rabbits available from a nearly completed experiment being done for other purposes.

Because of the apparent radiation effect on labeling in bone with ^{59}Fe (Tables 1 and 2) the mere determination of ^{59}Fe activity in whole bones may not truly reflect the status of hematopoietic activity in the marrow. This could be an explanation for the finding that iron uptake ratios in x-irradiated bones changed little with increasing x-ray dose and suggested non-involvement with hematopoiesis (*2*). Reticuloendothelial (RE) cell and erythron function in bone marrow observed at least 2 weeks after x-irradiation were found to be similar (*4*). Technetium-99m-sulfur colloid was employed to determine RE cell activity, ^{59}Fe to determine erythron activity, and parallel responses were found. It has been suggested that radiocolloid photoscans of the spatial distribution of colloids in the marrow will reflect the pattern of erythropoietic marrow activity if such studies are performed several weeks after irradiation. A similar suggestion was made following another study (*5*) in which 6 weeks after irradiation marrow uptake of ^{59}Fc-labeled ferrous sulfate and ^{99m}Tc-sulfur colloid was measured, marrow cellularity counts taken, and correlations made. Marked variations in marrow cellularity were evident when comparison was made with the uptake of ^{99m}Tc. If the uptake of iron is not exclusive to the marrow of bone as our study indicates, then the possibility exists that only a fraction of the total uptake of the ^{59}Fe represents erythron activity in the marrow. This may be an explanation for the variations observed in the relationship between marrow cellularity and ^{99m}Tc activity. In addition to the evidence presented here, there is recent evidence of bone concentration of iron in rabbits indicating that 60% of the ^{59}Fe was in the extracted marrow, the remainder in the bone (*3*). Our autoradiographic studies reveal ^{59}Fe labeling in bone. Caution should therefore be exercised in the interpolation of ^{59}Fe uptake values with the uptake values for other radioisotopes in bone when used as substitutes for ^{59}Fe to indicate erythron activity in the marrow.

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