

Normal Mouse Erythropoiesis

I. The Role of the Spleen in Mouse Erythropoiesis¹

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Summary

Erythropoiesis in normal Swiss Webster female mice was studied by routine hematologic parameters, ferrokinetics (⁵⁹Fe), the uptake into and release of ⁵⁹Fe from the spleen, and RBC survival (⁵¹Cr). The mean radioiron clearance (⁵⁹Fe T_{1/2})² is 61.7 min; plasma iron turnover is 2.4 mg/24 hr/100 ml of whole blood; and the reappearance of ⁵⁹Fe is 62.4% and 82.0% at 24 and 48 hr, respectively. The RBC survival (⁵¹Cr T_{1/2}), with the use of whole-body counting, is 19.0 days. With the use of whole blood technics, the ⁵¹Cr T_{1/2} is 12-15 days. The uptake into and release of ⁵⁹Fe from the spleen parallels the ⁵⁹Fe T_{1/2} and ⁵⁹Fe reappearance in the RBC. The data in this study indicate that, from the standpoint of erythropoiesis, the mouse spleen is analogous to bone marrow.

Introduction

A knowledge of normal mouse erythropoiesis is important with regard to comparative physiology, erythropoietic-stimulating factor assays (13, 14), and a proper evaluation of abnormal erythropoiesis in Friend leukemia (7). Early in infection with the Friend leukemia virus, histopathologic studies have demonstrated increased erythropoiesis in the spleen with erythroblastosis in the peripheral blood (10, 11). Ferrokinetic studies with iron (⁵⁹Fe) are important in defining the pathophysiology of the human anemias in various disease states, including the leukemias (1). However, normal mouse ferrokinetics have not been systematically studied with technics commonly applied to human erythropoiesis. The normal mouse plasma iron clearance (⁵⁹Fe T_{1/2}) and plasma iron turnover rate (PITR) have not been reported. In this study the following aspects of normal mouse erythropoiesis will be presented: hemoglobin concentration; packed cell volume; RBC count; ferrokinetics, including ⁵⁹Fe T_{1/2}, PITR, and percent of ⁵⁹Fe reappearance in RBC; and normal mouse RBC survival, with the use of chromium (⁵¹Cr). Ferrokinetics were used to evaluate splenic function in normal mouse erythropoiesis. The normal mouse spleen, in contrast to the human spleen, demonstrates active erythropoietic, myeloid and megakaryocytic activity and is a major site of activity of the Friend leukemia virus (9, 10, 11). In this report ferrokinetics indicate that mouse splenic erythropoiesis mirrors that of the bone marrow. Thus the spleen can be used as an easily accessible and convenient experimental model for the study of erythropoiesis. The establishment of these normal

parameters is essential for a systematic evaluation of the altered erythropoiesis in Friend leukemia.

Materials and Methods

MICE. Weanling female (16-18 gm) mice of the "general purpose" noninbred Swiss Webster mouse strain from the Huntingdon Farms production colony were used in this work. They were maintained in plastic cages 10/cage and fed Purina laboratory chow small with water *ad libitum*.

ROUTINE HEMATOLOGIC PARAMETERS. RBC count/cu mm; hemoglobin concentration (gm %); and packed cell volume (PCV %) were determined in individual mice by puncture of the retroorbital venous plexus of the eye with the use of heparinized capillary tubes. RBC were diluted 1:200 in Gower's solution and counted in a Newbauer counting chamber. Hemoglobin concentration was determined by the cyanmethemoglobin method and the packed cell volume by the microhematocrit technic. Platelet counts were determined by the Brecher-Cronkite technic (4). Reticulocyte counts were determined with the use of new methylene blue (3).

IRON DETERMINATION. Serum iron was determined in individual mice by the micro technic of Natelson (12).

FERROKINETICS. Individual mice received i.v. in the tail vein 1.0 μc of radioiron (⁵⁹Fe Ferrutope Squibb) in a volume of 0.25 ml of physiologic saline (PSS). At 15, 30, and 60 min after injection, with the use of a double oxalated capillary tube, 36 μl of blood were obtained from the retroorbital venous plexus of each mouse. The radioactivity in the capillary tube was then determined in a well-type scintillation counter. The cpm in each specimen were plotted against time on semilogarithmic paper, and the ⁵⁹Fe T_{1/2} was determined.

The plasma iron turnover rate was calculated by the method of Bothwell *et al.* (2):

PITR (mg/24 hr/100 ml of whole blood)

$$= \frac{\text{Serum Fe } \mu\text{g } \%}{^{59}\text{Fe T}_{1/2}} \times \frac{100 - \text{PCV}}{100}$$

The ⁵⁹Fe clearance curve was extrapolated back to zero time, and the total counts at time zero (cpm₀) in 36 μl of whole blood were determined. The radioactivity of 36 μl of whole blood obtained 24 and 48 hr after injection of ⁵⁹Fe was divided by the cpm₀ and the % of ⁵⁹Fe reappearance determined.

In 2 experiments animals were injected with ⁵⁹Fe as above, but were sacrificed and exsanguinated by decapitation at 15, 30, and 60 min; the blood was pooled at these times. The RBC were separated from the plasma by centrifugation, and the radioactivity in the plasma and RBC was determined. The ⁵⁹Fe T_{1/2} was calculated as above. Ten mice were used to establish each point.

¹ This investigation was supported by Grant E 305 from the American Cancer Society, and Grant CA 06832 from the USPHS, National Cancer Institute.

² T_{1/2}, half life.

Received for publication May 24, 1965.

ORGAN COUNTING. Groups of individual mice used in the ^{59}Fe clearance experiments were exsanguinated *via* the retroorbital venous plexus and sacrificed by cervical fracture at 1, 6, 24, 48, and 168 hr after ^{59}Fe injection. The livers and spleens were removed and weighed on a shadowgraph tumor-weighing scale. The radioactivity in the individual livers and spleen was determined in a well-type scintillation counter. The % of ^{59}Fe uptake was determined by dividing the cpm in either the liver or the spleen by the cpm of a ^{59}Fe standard prepared at the time of radioiron injection. The % of uptake was also expressed per gm of spleen or liver weight.

In addition to removal of the spleen and liver, certain animals were eviscerated and the % of uptake of ^{59}Fe in the carcass was determined in a Tobor small-animal whole-body counter. The assumption was made that the radioactivity in the carcass represented that in the skeletal system and bone marrow (5).

RBC SURVIVAL. The RBC survival was determined in individual mice by injecting i.v. into the tail vein 0.25 ml of homologous mouse whole blood incubated for 30 min at 20°C with 1.25 μc of ^{51}Cr (Chromitope, Squibb). At the end of the period of incubation prior to injection, 100 mg of ascorbic acid was added. The radioactivity of the whole animal was determined immediately after injection and daily thereafter in a Tobor whole-body small-animal counter. The data were plotted on semilogarithmic paper, and the ^{51}Cr $T_{1/2}$ was determined. In 3 experiments 10 mice each received i.v. 0.25 ml of homologous mouse whole blood, incubated as above with 5.0 μc of ^{51}Cr . Thirty-six μl of blood were obtained from the retroorbital venous plexus of each mouse on Days 1, 2, 5, 12, and 15 after injection. Twenty-four hr was taken as 100%, and the radioactivity of the samples on the following days was expressed as % of the 24-hr sample counted on a particular day. The data were plotted on semilogarithmic paper, and the ^{51}Cr $T_{1/2}$ was determined.

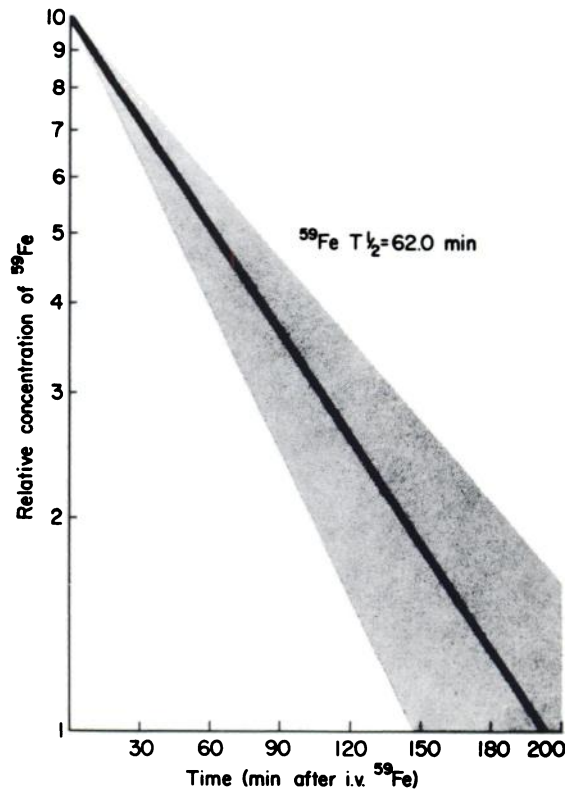


CHART 1. The clearance of radioactive iron from the plasma. The stippled area represents 1 S.D. from the mean.

Results

Normal peripheral blood studies in female Swiss Webster mice are presented in Table 1. Normal ferrokinetics in female Swiss Webster mice are presented in Table 2.

The normal ^{59}Fe $T_{1/2}$, plasma iron, and the reappearance of ^{59}Fe in the peripheral blood at 24 and 48 hr are presented. The calculated PITR was 2.4 mg/24 hr/100 ml of whole blood. In 2 experiments the ^{59}Fe $T_{1/2}$ was calculated from pooled plasma with 10 mice used per point. The mean ^{59}Fe $T_{1/2}$ with this method was 64.5 min. No reappearance of ^{59}Fe in the RBC was noted at 1 hr. The normal ^{59}Fe $T_{1/2}$ is presented graphically in Chart 1.

The uptake of ^{59}Fe in the spleen, liver, and carcass is presented in Table 3. The data are presented at 1, 6, 24, 48, and 168 hr after injection of ^{59}Fe . The peak uptake of ^{59}Fe into spleen and carcass occurs 6 hr after injection. On the basis of the ^{59}Fe $T_{1/2}$, 6 hr corresponds to 6 half lives, implying complete clearance of the ^{59}Fe from the plasma. The highest percentage of ^{59}Fe at all times is in the carcass. The % of uptake of ^{59}Fe /gm of carcass is not presented because the major portion of carcass is muscle, skin, and bone, rather than bone marrow. The radioactivity drops in both the spleen and the carcass at 24 and 48 hr. The pattern of ^{59}Fe uptake into the spleen and release of ^{59}Fe from the spleen coincide more closely than the carcass with the ^{59}Fe $T_{1/2}$ and ^{59}Fe reappearance in the RBC. In contrast to the spleen and carcass, the ^{59}Fe uptake in the liver remains relatively constant during the period of observation. These data are presented graphically in Chart 2.

RBC SURVIVAL WITH ^{52}Cr . The RBC survival in normal female

TABLE 1

HEMATOLOGIC PARAMETERS IN NORMAL SWISS WEBSTER FEMALE MICE

	Hb ^a (gm %)	PCV (%)	RBC (X 10 ³ /cu mm)	WBC cu mm	Platelets (X 10 ³ /cu mm)	Reticulo- cytes (%)
Mean	13.0	43.4	7688.2	7099.0	786.8	2.1
S.D.	1.5	4.0	1606.9	3621.0	238.4	1.5
No. of mice	38	69	11	49	50	29

^a Hb, hemoglobin concentration; PCV, packed cell volume.

TABLE 2

FERROKINETICS IN NORMAL SWISS WEBSTER FEMALE MICE

	^{59}Fe $T_{1/2}$ (min)	REAPPEARANCE (%)		PLASMA FE (μg %)	PCV ^a (%)	PIT
		24 hr	48 hr			
Mean	61.7	62.4	82.0	247.4	43.4	2.4
S.D.	17.7	18.1	17.0	107.2	4.0	1.3
No. of mice	166	90	57	55	69	55

^a PCV, packed cell volume; PIT, plasma iron turnover, mg/24 hr/100 ml of whole blood.

Swiss Webster mice was determined with whole-body counting in 2 experiments with 22 mice used per experiment. The normal $^{51}\text{Cr } T_{1/2}$ is 9.0 days calculated from Day 0 and 19 days calculated from Day 1. Generally, RBC survival is calculated from Day 1

TABLE 3
UPTAKE OF ^{59}Fe IN SPLEEN, LIVER, AND CARCASS IN NORMAL SWISS WEBSTER FEMALE MICE

	% in carcass	% in spleen	% in liver	% /gm spleen ^a	% /gm liver ^b	Spleen/liver ratio
1 hr						
Mean	41.0	8.1	13.1	75.6	9.9	8.1
S.D.	4.8	4.8	3.1	39.5	2.7	4.2
No. of mice	11	43	43	43	43	43
6 hr						
Mean	45.0	13.7	10.4	140.2	9.0	18.5
S.D.	5.2	5.7	4.1	61.3	4.2	9.7
No. of mice	33	33	33	33	33	33
24 hr						
Mean	26.4	4.0	9.9	27.3	6.3	4.7
S.D.	7.8	2.4	1.8	10.9	1.6	2.4
No. of mice	33	33	33	33	33	33
48 hr						
Mean	22.6	0.5	11.6	4.4	8.2	0.6
S.D.	3.8	0.2	3.2	1.9	2.8	0.3
No. of mice	11	20	20	20	20	20
168 hr						
Mean		0.5	7.2	3.2	5.6	0.6
S.D.		0.5	2.2	1.9	1.8	0.3
No. of mice		20	20	20	20	20

^a Spleen weight: mean, 0.122 gm; S.D., 0.046 gm; No. of mice, 83.

^b Liver weight: mean, 1.369 gm; S.D., 0.218 gm; No. of mice, 85.

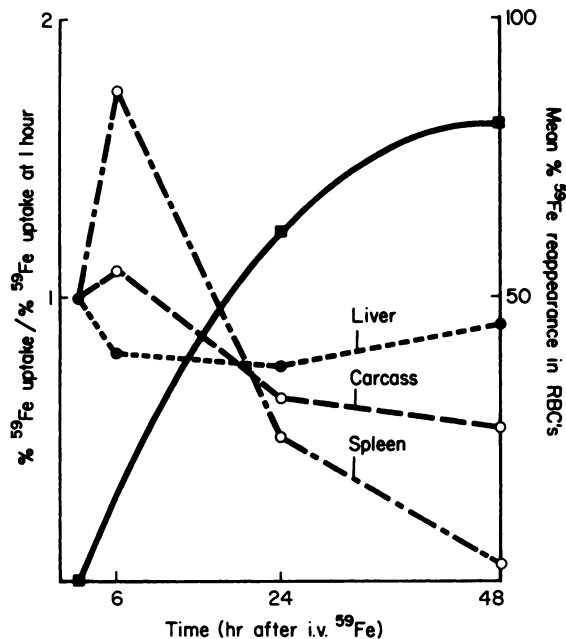


CHART 2. The uptake of radioactive iron into, and release from, the spleen, liver, and carcass, compared with the reappearence of radioactive iron in the peripheral blood.

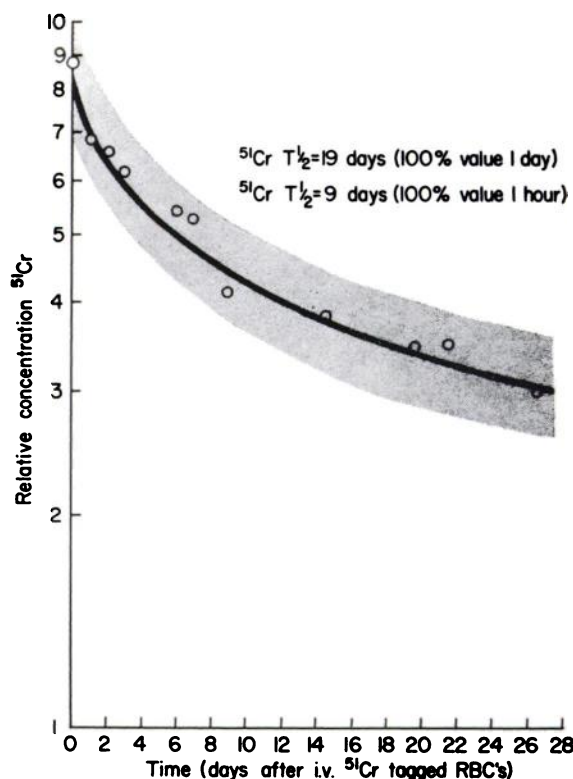


CHART 3. The RBC survival of homologous mouse erythrocytes determined by ^{51}Cr tagging and whole-body counting. The *stippled area* represents 1 S.D. from the mean.

because of the rapid clearance of unlabeled ^{51}Cr from the blood in the 1st 24 hr. With the use of a formula for the elution of ^{51}Cr from human RBC, the mean RBC survival calculated from Day 1 is 46 days (6). The ^{51}Cr survival is plotted graphically in Chart 3.

The $^{51}\text{Cr } T_{1/2}$ determined by direct counting of whole blood varied between 12 and 15 days, with a mean RBC survival of 23-32 days.

Discussion

The hemoglobin concentration and packed cell volume in mice are essentially in the same range as the values for humans, but the RBC count and the platelet count are higher. On the basis of a slightly higher reticulocyte count and the increased RBC count, the absolute reticulocyte count (% of reticulocytes \times RBC/cu mm) is approximately 3 to 4 times greater in the mouse than in the human. In humans the average $^{59}\text{Fe } T_{1/2}$ is 90 min; the P.I.T.R. is 0.6 mg/24 hr/100 ml of whole blood, with an average ^{59}Fe reappearence of 80-100% in 7-10 days (1) and average serum iron of 125 $\mu\text{g } \%$ (12). The normal human $^{51}\text{Cr } T_{1/2}$ is 24-28 days, with an RBC life-span of 120 days (6).

With the use of whole blood technics, the $^{51}\text{Cr } T_{1/2}$ is 12-15 days. Ultmann and Gordon (15) obtained a $^{51}\text{Cr } T_{1/2}$ of 20.2 days in DBA/2 brown male and female mice, and Goodman and Smith (8), in several inbred strains of mice, found a variation in the $^{51}\text{Cr } T_{1/2}$ from 15-20 days, depending on the strain. Our results indicate that the RBC survival is shorter in Swiss Webster mice compared with the inbred strains (8, 15). This may be related to

genetic differences. Blood incompatibility in noninbred strains may influence the results. However, naturally occurring antibodies and blood groups in mice have not been reported.

With total-body counting, ^{51}Cr $T_{\frac{1}{2}}$ of RBC in Swiss Webster female mice is 19 days. The overestimation of the ^{51}Cr $T_{\frac{1}{2}}$ is probably due to some retention of ^{51}Cr in the animal's body. Schiffer and Cronkite³ demonstrated in 3 human beings that the ^{51}Cr $T_{\frac{1}{2}}$ is longer when determined by whole-body counting than by whole blood technics. Whole-body counting is a relative method for determining the RBC survival and was used to establish normal controls for similar studies to be done on the RBC of mice infected with Friend leukemia virus. The method is reproducible, and its major advantage is speed and convenience.

The ^{51}Cr $T_{\frac{1}{2}}$ in mice is short compared with human beings (^{51}Cr $T_{\frac{1}{2}}$, 24–26 days) (6). Since the hemoglobin concentration and pack cell volume in mice and human beings are similar, the accelerated mouse erythropoiesis is probably a physiologic compensation for the mouse's shortened RBC survival.

In addition to the increased rate of erythropoiesis, a major qualitative difference between mouse and human erythropoiesis is the persistence of normal erythropoietic activity in the spleens of adult mice. The ferrokinetic studies indicate that the uptake of ^{59}Fe into and release from the spleen parallels the ^{59}Fe $T_{\frac{1}{2}}$ and ^{59}Fe reappearance in the RBC (Chart 2). The apparent discrepancy between spleen and carcass may be due to the fact that uptake of ^{59}Fe was determined indirectly, since the bone marrow was shielded by skin, muscle, and bone. The geometry of the counting was more efficient for spleen and liver than for carcass. However, the pattern of ^{59}Fe uptake and release is essentially similar for both spleen and carcass (Chart 2). External counting of ^{59}Fe in normal humans demonstrates that the bone marrow, and not the spleen, is the major site of erythropoiesis (1).

With a knowledge of normal mouse ferrokinetics and erythropoiesis it will be possible to evaluate such factors as increased RBC destruction and the effectiveness of erythropoiesis in the altered RBC production associated with Friend virus leukemia. Since the mouse spleen is analogous to bone marrow, it will be further possible to evaluate the early effects of the Friend virus on mouse RBC production. This work will be the subject of subsequent reports.

³ L. Schiffer and E. P. Cronkite, personal communication.

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Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

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Cancer Res 1966;26:198-201.

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