Studies on the relationship between the function of reticuloendothelial system and the hematopoiesis. II. Experimental studies on 59Fe ferrokinetics in the induced hematological disorders of mice

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Abstract

The following conclusions were drawn from the ferrokinetic studies using 59Fe in mice, whose hematological disorders were induced by various treatments. 1. The ferrokinetics in the normal mice were studied. 2. Chloramphenicol (CP) administration in mice first induced ferrokinetics disturbances and then suppressed erythropoiesis. 3. Splenectomy induced hyper-erythropoiesis in the bone marrow, and CP administration after splenectomy suppressed this hyper-erythropoiesis. 4. Human gamma-globulin (H.G.G.) caused hypersplenism and a marked suppression of erythropoiesis in the bone marrow, and Chlorabulin administration suppresed erythropoiesis. Finally, the author has summarized the relationship of the RES function and hematopoiesis in mice as follows. 1. The spleen and liver reacted in the same manner with respect to the RES function to sequestrate 51Cr-labelled heat-damaged erythrocytes when hematological failures were induced. 2. The spleen and bone marrow reacted reversely with regard to the RES function. 3. When the RES function, especially that of the spleen was accentuated, the suppression of hematopoiesis was observed. 4. Chloramphenicol administration was followed by the suppressed hematopoiesis and the accentuated RES function. 5. Splenectomy accentuated the RES function in the bone marrow and liver, and also increased hematopoiesis in the bone marrow. 6. Human γ-globulin hypersensitization induced hyperfunction of the RES, especially of the spleen and suppression of the hematopoiesis.

STUDIES ON THE RELATIONSHIP BETWEEN THE FUNCTION OF RETICULOENDOTHELIAL SYSTEM AND THE HEMATOPOIESIS
II. EXPERIMENTAL STUDIES ON ⁵⁹FE FERROKINETICS IN THE INDUCED HEMATOLOGICAL DISORDERS OF MICE

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It is assumed that there is a close relationship between the spleen and the other hematopoietic organs. Many attempts have been made to demonstrate the inhibitory effects of splenic extracts on hematopoiesis, with equivocal results (1, 2). The existence of a humoral factor is suggested by observations of the effects of splenectomy and reimplantation of splenic tissue (3, 4). This possibility has intrigued investigators for many years without conclusive evidence. The author already described the relationship between RES function and hematopoietic organs in the preceding paper (5). This paper is concerned with the ferrokinetics in mice whose hematological disorders were induced by various methods.

MATERIALS AND METHODS

1. *Mice:* ICR female mice weighing 20 to 24 g were used in this experiment.
2. *Peripheral blood pictures:* Erythrocyte count/cmm and leucocyte count/cmm were determined with blood from the tail vein of an individual mouse. Reticulocyte counts were determined by the Pappenheim method.
3. *Ferrokinetics:* Plasma iron levels were measured by the Bathophenanthroline method with pooled plasma of several mice. Each mouse received $1.0\ \mu c$ of ⁵⁹Fe-ferrous citrate in 0.25 ml of physiologic saline from the tail vein. Twenty-five μl of blood was obtained from the retroorbital venous plexus after ⁵⁹Fe injection. The radioactivity in the capillary tube was then determined in a well-type scintillation counter. The cpm in each specimen was plotted against time on semilogarithmic paper, and plasma iron disappearance time (P. I. D. T.) was determined. The ⁵⁹Fe disappearance curve was extrapolated on the Y-axis at zero time, and the total cpm at zero time (cpm.) in 25 μl of the whole blood was calculated. The radioactivity of 25 μl of the whole blood obtained at 24 and 48 hours after injection of ⁵⁹Fe was divided by cpm_0 so that the percentage of ⁵⁹Fe reappearance was determined. All the mice in this experiment were sacrificed by decapitation at 1, 6, 24 and 48 hours after ⁵⁹Fe injection. The liver, spleen, femur, tibia and fibula were excised and weighed. The radioactivity of the liver

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and spleen was determined in a well-type scintillation counter. The percentage of ^{59}Fe uptake was calculated by dividing the cpm of either the liver or spleen by the cpm of the ^{59}Fe standard prepared at the time of radioiron injection. The percent uptake of the bone marrow was assumed to be 13 times the total cpm of femur, tibia and fibula.

4. *Procedures to produce the hematological disorders:* The hematological disorders were induced with the same treatment as described in the preceding paper (5), (1) CP (chloramphenicol) 3-day group, (2) CP 30-day group, (3) Spx. (splenectomy) 10-day group, (4) Spx. 20-day group, (5) Spx. 10-day plus CP group, (6) Spx. 20-day plus CP group, (7) H. G. G. (human gamma globulin) group, (8) H. G. G. & F. C. Adj. (Freund's complete adjuvant) group and (9) Chlorabulin group. The study of ferrokinetics was started at 12 hours after the last administration of CP, and four days after H. G. G. or Chlorabulin injection.

RESULTS

1. *Normal mice :* The normal value was determined in 20 normal mice;

Table 1

		Normal	CP 3-day group	CP 30-day group
Erythrocyte ($\times 10^4$/cmm)		1081±85†	1053±119	757±72*
Reticulocyte (‰)		28±15	16±11	54±23
Plasma Fe (γ/dl)		242	298	350
P. I. D. T. (min.)		70±10	100±30**	100±20*
^{59}Fe Reappearance rate (%)	24 h	83±13	33±13*	67±6
	48 h	97±12	55±20*	82±3
Liver	1 h	8.2±0.9	6.3±2.6	6.3±0.4
	6 h	12.3±1.0	15.4±1.7	8.4±0.5*
	24 h	13.5±1.2	17.3±2.0	9.3±0.2*
	48 h	13.0±1.6	22.7±4.4	9.4±0.8*
Spleen	1 h	8.5±2.3	2.3±1.8*	5.1±2.4
	6 h	11.7±2.6	2.7±1.9*	12.9±3.0
	24 h	5.9±0.8	2.1±0.5*	3.5±1.3**
	48 h	1.4±0.1	0.8±0.2*	1.9±0.5
Bone Marrow	1 h	22.1±2.6	23.4±9.1	20.8±2.6
	6 h	31.2±3.9	33.8±10.4	24.7±5.2**
	24 h	18.2±2.6	19.5±3.9	16.9±2.6
	48 h	9.1±1.3	14.3±3.9	7.8±1.3

(left vertical label: Percent Organ Uptake (%))

† : Mean value±standard deviation.
  * : P value showed significant difference less than 1 percent error (p < 0.01),
 ** : less than 5 percent (p < 0.05) comparing normal group.

Plasma iron levels: $242\gamma/dl$, erythrocyte counts: $1081 \pm 85 \times 10^4/cmm$ (M$\pm$SD), reticulocyte counts: $28 \pm 15 \text{‰}$, P. I. D. T.: 70 ± 10 min, ^{59}Fe reappearance rate: $83 \pm 13 \%$ after 24 hours, and $97 \pm 12 \%$ after 48 hours. The uptake of ^{59}Fe in the spleen, liver and bone marrow is also presented in Table 1. The maximum uptake of ^{59}Fe into the spleen and bone marrow occurred at 6 hours after ^{59}Fe injection. The uptake of ^{59}Fe in the liver increased until 6 hours after injection and then remained relatively constant during 48 hours. The radioactivity of the spleen dropped in parallel with that of bone marrow after 24 and 48 hours.

2. *CP 3-day group*: As presented in Table 1, this group showed elevated plasma iron levels, prolonged P. I. D. T. ($p<0.01$), decreased reticulocytes, and decreased ^{56}Fe reappearance rate ($p<0.01$). The uptake of the bone marrow was normal but that of the spleen was significantly low ($p<0.01$). The uptake of the liver was significantly high ($p<0.01$) and increased with time during the observation. These facts indicated that CP administration suppressed the erythropoietic function in the spleen but not in the bone marrow.

3. *CP-30 day group*: This group showed higher plasma iron level, prolonged P. I. D. T., and decreased erythrocytes ($p<0.01$). ^{59}Fe reappear-

Table 2

			Spx 10-day group	Spx 20-day group
Erythrocytes ($\times 10^4$/cmm)			784 ± 103†*	866 ± 75**
Reticulocyte (‰)			30 ± 6	26 ± 5
Plasma Fe (γ/dl)			258	214
P. I. D. T. (min.)			75 ± 15	70 ± 10
^{59}Fe Reappearance rate (%)		24 h	70 ± 13	49 ± 10*
		48 h	80 ± 9	79 ± 10
Percent Organ Uptake (%)	Liver	1 h	7.6 ± 1.4	9.2 ± 0.4
		6 h	11.8 ± 1.0	7.3 ± 0.9
		24 h	12.9 ± 2.3	11.2 ± 1.9
		48 h	10.2 ± 1.3	10.6 ± 0.4
	Bone Marrow	1 h	29.6 ± 5.2	23.4 ± 3.0
		6 h	36.4 ± 0.2	35.1 ± 7.8
		24 h	16.9 ± 0.2	23.4 ± 1.3
		48 h	6.5 ± 1.3	10.4 ± 1.3

† : Mean value$\pm$standard deviation.
* : P value showed significant difference less than 1 percent error ($p<0.01$),
** : less than 5 percent ($p<0.05$) comparing normal group.

ance rate was low after both 24 and 48 hours. The uptake of the marrow and the liver was low, but that of the spleen was normal (Table 1). These facts indicated that long-term administration of CP suppressed the erythropoietic function in the bone marrow but not in the spleen.

4. *Spx. 10-day group* : Erythrocyte counts decreased but the uptake of the bone marrow was higher than that of the normal group (Table 2).

5. *Spx. 20-day group* : Erythrocyte counts returned to a normal range and ^{59}Fe reappearance rate was slightly low but the uptake of the bone marrow was within the normal range (Table 2).

6. *Spx. 10-day plus CP group* : This group showed decreased erythrocyte counts and high plasma iron levels. ^{59}Fe reappearance rate was low and the uptake of the bone marrow was decreased (Table 3).

7. *Spx. 20-day plus CP group* : Erythrocyte counts and ^{59}Fe reappearance rate were decreased and plasma iron levels were elevated in this group as observed in Spx. 10-day plus CP group. But the uptake of the bone marrow was within normal limit (Table 3).

Table 3

		Spx 10-day group + CP	Spx 20-day group + CP
Erythrocyte ($\times 10^4$/cmm)		866±75†**	878±74**
Reticulocyte (‰)		26± 5	29± 7
Plasma Fe (γ/dl)		320	403
P. I. D. T. (min.)		75±10	80±15
^{59}Fe Reappearance rate (%)	24 h	58±10	61±13
	48 h	74± 6	74±13
Percent Organ Uptake (%) — Liver	1 h	9.2±3.1	8.8±1.1
	6 h	13.1±2.2	9.5±2.5
	24 h	13.1±2.2	13.7±1.5
	48 h	12.4±1.1	15.0±1.6
Bone Marrow	1 h	26.0±6.5	23.4±3.9
	6 h	23.1±9.1*	32.4±3.9
	24 h	23.4±9.1	16.9±2.6
	48 h	10.4±1.3	9.1±1.3

† : Mean value±standard deviation.
* : P value showed significant difference less than 1 per cent error ($p < 0.01$),
** : less than 5 percent ($p < 0.05$) comparing normal group.

8. *H. G. G. group* : This group showed elevated plasma iron levels, prolonged P. I. D. T. and decreased ^{59}Fe reappearance rate. These results were analogous to those of CP 3-day group but conversely the uptake of bone marrow was significantly decreased (Table 4).

9. *H. G. G. & F. C. Adj. group* : This group showed markedly high plasma iron level, prolonged P. I. D. T. and decreased ^{59}Fe uptake of the bone marrow (Table 4).

10. *Chlorabulin group* : There were little changes in the ferrokinetics study except elevated plasma iron levels (Table 4).

Table   4

		H. G. G. group	H. G. G. & F. C. Adj. group	Chlorabulin group
Erythrocyte ($\times 10^4$/cmm)		1231$\pm$189†	979$\pm$144	931$\pm$146
Reticulocyte (‰)		21$\pm$3	38$\pm$29	29$\pm$8
Plasma Fe (γ/dl)		356	465	306
P. I. D. T. (min.)		90$\pm$15**	80$\pm$20	90$\pm$25
^{59}Fe Reappearance rate (%)	24 h	68$\pm$12	79$\pm$11	66$\pm$16
	48 h	77$\pm$12	89$\pm$ 7	72$\pm$24
Percent Organ Uptake (%) — Liver	1 h	7.0$\pm$0.6	7.5$\pm$0.7	8.8$\pm$2.0
	6 h	13.9$\pm$0.8	9.8$\pm$1.8*	11.9$\pm$0.3
	24 h	12.4$\pm$1.2	9.7$\pm$2.2	12.8$\pm$1.1
	48 h	12.3$\pm$2.7	8.8$\pm$1.7	12.1$\pm$1.2
Spleen	1 h	5.4$\pm$1.5	10.6$\pm$2.3	3.2$\pm$1.6
	6 h	10.2$\pm$1.5	10.4$\pm$2.2	9.5$\pm$3.8
	24 h	5.9$\pm$1.2	8.5$\pm$1.7**	1.9$\pm$0.3
	48 h	1.8$\pm$0.3	6.5$\pm$2.6	1.7$\pm$0.2*
Bone Marrow	1 h	24.7$\pm$3.9	11.7$\pm$1.3*	15.6$\pm$3.9
	6 h	20.8$\pm$3.9*	18.2$\pm$5.2*	29.9$\pm$9.1
	24 h	16.9$\pm$5.2	14.3$\pm$5.2	13.0$\pm$2.6
	48 h	9.1$\pm$1.3	6.5$\pm$0	7.8$\pm$0

† : Mean value$\pm$standard deviation.
 * : P value showed significant difference less than 1 percent error ($p < 0.01$),
** : less than 5 percent ($p < 0.05$) comparing normal group.

DISCUSSION

The ferrokinetics study using ^{59}Fe is useful to solve the problems of erythropoiesis not only in human hematological disorders but also in

experimental animals. Few informations of normal mouse erythrokinetics using ^{59}Fe are available with regard to comparative physiology, erythro-poietic stimulating factor assays and a proper evaluation of abnormal erythropoiesis (6, 7, 8). The uptake into and release of ^{59}Fe from the spleen paralleled the ^{59}Fe P. I. D. T. and ^{59}Fe reappearance rate in the peripheral erythrocytes of normal mice. Active erythropoiesis was demonstrated in the histology and ^{55}Fe radio-autography of the spleen of normal mice.

    Reversible bone marrow depression due to chloramphenicol (CP) was first observed by KRAKOFF, KARNOFSKY and BURCHENAL (9) during the admi-nistration of large doses (6 to 12 gm per day) of the drug. RUBIN and his coworkers (10) showed that in some patients conventional doses of chloram-phenicol produced changes in iron metabolism that could precede other evidences of erythropoietic depression, whereas SAIDI, WALLERSTEIN and AGGELER (11) found that this effect of the drug was often accompanied by toxic vacuolation of the bone marrow erythroblasts, particularly when larger doses were employed or when accelerated erythropoiesis or infection were present. A number of cases of hypoplastic anemia associated with chloramphenicol therapy have been recorded in the literature (12, 13, 14, 15, 16, 17). The nature of the toxic effects of chloramphenicol on the bone marrow has been studied (10, 11, 18, 19). YUNIS (18) described two types of bone marrow reaction to chloramphenicol. The first is a reversible effect that is predictable, dose-dependent, not characterized by hypoplasia and has been produced experimentally. The second type is a later, idiosyncratic response regularly associated with hypoplasia. This usually irreversible aplastic response to chloramphenicol, as described by YUNIS, occurred later after the inception of chloramphenicol therapy and followed a larger dosage given over a longer period. Although chloramphenicol continues to be a leading cause of drug-induced hypoplastic anemia, little progress has been made in elucidating the mechanism of its toxic effect. In chloram-phenicol sensitive bacteria chloramphenicol in low concentrations causes complete inhibition of protein synthesis. There is good evidence that this action is exacted through stereospecific binding of the drug to the 50S ribosomal subunit, thereby inhibiting the formation of the peptide bond. The drug does not seem to interfere with the function of messenger RNA (mRNA) (20). Recently, WEISBERGER *et al.* (21) reported profoundin hibi-tion of mRNA-induced protein synthesis in a cell-free system from rabbit reticulocytes by a low concentration of chloramphenicol, reversed by increasing the concentration of messenger. They concluded that chloram-phenicol inhibits protein synthesis in mammalian cells by interfering with the binding of mRNA to ribosomes. Whether hematologic toxicity of

chloramphenicol is related to its inhibitory effect on protein synthesis cannot be resolved at present. Several observations in patients with chloramphenicol-induced hypoplastic anemia suggest that this drug exerts its action at the stem-cell level. BRAUER and DAMESHEK (22) suggested a possibility that acute leukemia would be seen much more frequently in patients with chloramphenicol-induced bone marrow aplasia if they survived longer. However, little can be said about this suggestion until the basic mechanisms by which chloramphenicol and other drugs injure the bone marrow are clarified.

Concerning CP toxicity on the hematopoietic organ in the experimental animal, only one paper has been recently published by SUGA et al. in Japan (23). This paper elucidated that the suppression of hematopoiesis by CP was independent of the erythropoietic activity but dependent upon the dose of administered CP.

The data presented in this paper indicated that elevated plasma iron levels, prolonged P. I. D. T., and decreased ^{59}Fe reappearance rate were demonstrated following 3-day or 30-day administration of CP. The uptake of the spleen was suppressed in 3-day group and that of the bone marrow was suppressed in 30-day group. Therefore, short-term administration of CP suppressed the erythropoietic function in the spleen and long-term administration of CP suppressed it in the bone marrow. Consequently, the pathogenesis of CP induced anemia is presumed to be the direct damage of the hematopoietic organs by CP administration. As mentioned previously, CP administration augumented the RES function of spleen and liver, and this hyperfunction of RES in these organs will suppress still further the hematopoiesis in the bone marrows.

As already mentioned in the preface, conclusive evidence concerning the spleen and hematopoiesis is not yet available. Ferrokinetic studies were also carried in splenectomized mice to investigate the relatioship of the spleen to the hematopoiesis. Ten days after spleneotomy, erythrocyte counts were decreased but the uptake of ^{59}Fe in the bone marrow was elevated. Twenty days after splenectomy, erythrocyte counts recovered to normal and ^{59}Fe uptake in the bone marrow was increased. These data indicated that splenectomy induced hyper-erythropoiesis in the bone marrow. By CP administration in splenectomized mice the plasma iron level was elevated and the uptake of the bone marrow was suppressed. Therefore, CP administration suppressed the accentuated hematopiesis in the bone marrow which was induced by splenectomy.

The clinical use of gammaglobulin administration poses many problems, and little is known about the mechanism of its pharmacological

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effects. The author's experiments indicated that hematopoiesis in the bone marrow was suppressed by gammaglobulin administration. But concerning RES function, the bone marrow was accentuated but the spleen and liver were suppressed. Hypersensitization with gammaglobulin and Freund's complete adjuvant made the hematopoiesis in the bone marrow suppressed. Therefore, hyperfunction of the RES in the spleen will suppress the hematopoiesis in the bone marrow. The Chlorabulin administration was followed by suppressed erythropoiesis.

CONCLUSION

The following conclusions were drawn from the ferrokinetic studies using ^{59}Fe in mice, whose hematological disorders were induced by various treatments.

1. The ferrokinetics in the normal mice were studied.

2. Chloramphenicol (CP) administration in mice first induced ferrokinetics disturbances and then suppressed erythropoiesis.

3. Splenectomy induced hyper-erythropoiesis in the bone marrow, and CP administration after splenectomy suppressed this hyper-erythropoiesis.

4. Human gamma-globulin (H. G. G.) caused hypersplenism and a marked suppression of erythropoiesis in the bone marrow, and Chlorabulin administration suppresed erythropoiesis.

Finally, the author has summarized the relationship of the RES function and hematopoiesis in mice as follows.

1. The spleen and liver reacted in the same manner with respect to the RES function to sequestrate ^{51}Cr-labelled heat-damaged erythrocytes when hematological failures were induced.

2. The spleen and bone marrow reacted reversely with regard to the RES function.

3. When the RES function, especially that of the spleen was accentuated, the suppression of hematopoiesis was observed.

4. Chloramphenicol administration was followed by the suppressed hematopoiesis and the accentuated RES function.

5. Splenectomy accentuated the RES function in the bone marrow and liver, and also increased hematopoiesis in the bone marrow.

6. Human γ-globulin hypersensitization induced hyperfunction of the RES, especially of the spleen and suppression of the hematopoiesis.

ACKNOWLEDGEMENT

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