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CLINICAL STUDIES ON IRON KINETICS

I. IRON KINETICS STUDIES IN BLOOD DISORDERS

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ABSTRACT

Ferrokinetics data on patients with blood disorders of diverse etiology were presented and the clinical implication of this test was discussed. Ferrokinetics data on normal adults as well as on the aged were also presented.

Ferrokinetic patterns of ineffective erythropoiesis were obtained on patients with pernicious anemia, erythroleukemia, myelofibrosis, paroxysmal nocturnal hemoglobinuria and refractory anemia as well. Ferrokinetic studies on patients with portal hypertension with congestive splenomegaly have disclosed that the erythropoietic activity in this condition is rather increased and the liver damage does not necessarily exert inhibitory effect on erythropoiesis in these patients.

The studies on patients with acute leukemia did not reveal the patterns typical of each cell type of leukemia, and the results indicate that the myelophthisic effect due to the encloachment of leukemic cells is a major factor in the development of anemia in acute leukemia.

Finally, it is stressed that the ferrokinetic measurements with body surface counting are of great use in diagnosis and treatment of patients with myeloproliferative syndrome.

I. INTRODUCTION

Since the pioneering work of Huff and associates, ferrokinetic studies have had much application in clinical medicine as well as experimental works. In this paper, the author presents ferrokinetics data on patients with blood disorders of diverse etiology and discusses the clinical significance of ferrokinetic studies as well as on some basic problems of iron kinetics.

II. MATERIALS AND METHODS

Materials

Normal controls:

Seven normal adults (from 26 to 56 years old) and seven aged subjects (from 63 to 75 years old) who were all in good health, with normal hematolo-

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gical values and without history of chronic diseases or blood loss were studied for controls.

Patients with blood disorders of diverse etiology:

Eighty four patients with blood disorders of diverse etiology on whom the diagnosis was confirmed by clinical and hematological examinations and no treatment were done before and during examinations, were studied for ferrokinetic measurements.

The numbers of patients with blood disorders were as follows: 8 of iron deficiency anemia, 18 of portal hypertension with splenomegaly, 13 of hemolytic anemia, 3 of paroxysmal nocturnal hemoglobinuria, 2 of pernicious anemia, 11 of aplastic anemia, 5 of polycythemia vera, 3 of Di Guglielmo's syndrome, 3 of myelofibrosis, 14 of acute leukemia and 4 of chronic leukemia patients.

Body surface counting studies were simultaneously performed on some of these cases.

Methods

Ferrokinetic studies: 5 to 10 μc of ^{59}Fe in the form of ferric citrate was diluted in 10 ml of isotonic saline and injected intravenously without incubation with plasma.

Plasma iron disappearance rate (PID, $T\frac{1}{2}$), plasma iron turnover rate (PIT), percent red cell utilization of ^{59}Fe (%RCU), red cell iron turnover rate (RCIT) and mean red cell life span (MRCLS) were calculated according to the method of Huff and associates[1] with minor modification.

Plasma volume was measured by ^{59}Fe dilution method. PIT was expressed in mg/kg/day. And, to exclude the error inherent to the measurement of plasma volume, PIT was also expressed in mg/100 ml plasma/day by using the formula as follows:

Plasma iron turnover rate (PIT) (mg/100 ml plasma/day)

$$= \frac{\text{Serum iron } (\mu g/100 \text{ ml})}{T\frac{1}{2} \text{ (min)}}$$

The % RCU was calculated by the formula as follows:

Percentage of red cell utilization of ^{59}Fe (%RCU)

$$= \frac{\text{Counts/min/ml hemolyzed whole blood}}{\text{Counts/min/ml plasma at 0 time}} \times \frac{100}{100-\text{Ht}} \times 100$$

(Ht=Hematocrit %)

This formula made calculation simpler and excluded the error inherent to the counting of the injected dose.

Serum iron was measured by the method of Matsubara using *o*-phenanthroline as a colour reagent and hydroxylamine hydrochloride as a reducing agent[2]. The unsaturated iron-binding capacity was measured by a modification of the radioiron method of Tauxe[3],

Body surface counting was performed over the precordium, liver, spleen and the bone marrow (sacrum) hourly during the first day and with 2 to 3 days interval for 2 weeks until the completion of % RCU studies. The directional scintillation counter was placed on the skin and appropriate oblique placements were chosen to minimize the influence from the vertebrae of the sternum. As to the activities, gross body surface counting was used without respects to body background counting. The results were plotted with activities (count/min/μc) on a linear ordinate scale against time or day on a linear absicca scale.

III. RESULTS

A. Ferrokinetic data (Turnover indice)

I) Normal subjects

Ferrokinetic data in 7 healthy adults were as follows: PID ($T\frac{1}{2}$) being 96.1±19.3 minutes, PIT 0.45±0.07 mg/kg/day, % RCU 92.1±8.7%, and RCIT being 0.41±0.06 mg/kg/day. On the other hand, ferrokinetic data in 7 normal aged subjects were as follows: the mean values of PID ($T\frac{1}{2}$), PIT, % RCU and RCIT were 89.9±19.5 minutes, 0.49±0.07 mg/kg/day, 84.6±6.5% and 0.42 ±0.06 mg/kg/day respectively. The mean values of PID ($T\frac{1}{2}$), PIT, % RCU and RCIT show no statistically significant differences between normal adults and aged.

II) Blood disorders

1) Iron deficiency anemia

Table 1 depicts the hematological and ferrokinetic data in 8 patients with

TABLE 1. Hematological and Ferrokinetics Data on Patients with Iron Deficiency Anemia

Case No.	Age	Sex	Hemoglobin (%)	Reticulocyte (‰)	Serum Iron (μg/dl)	PID $T\frac{1}{2}$ (min)	PIT mg/kg/day	PIT mg/100 ml plasma/day	RCU (%)	Remarks
1	25	M	31	28	32	14	1.58	2.28	100	gastrectomy
2	14	F	50	3	55	20	2.02	2.75	109	idiopathic
3	15	F	49	21	35	16	1.30	2.19	100	〃
4	20	M	58	14	20	18	0.68	1.11	105	〃
5	18	M	18	8	14	14	0.66	1.00	110	〃
6	47	M	55	19	42	14	1.45	3.00	100	gastrointestinal bleeding
7	23	F	45	21	20	13	1.21	1.54	100	idiopathic
8	34	F	34	8	21	13	1.32	1.61	99	hypermenorrhea
mean			42.5	15.3	29.9	15.3	1.28	1.94	102.9	
S.D.						2.4	0.42	0.69	4.2	

PID = Plasma Iron Disappearance.
PIT = Plasma Iron Turnover.
RCU = Red Cell Utilization of ^{59}Fe.

iron deficiency anemia. All patients had remarkable hyposideremia. PID was very rapid (mean PID ($T\frac{1}{2}$) = 15.3±2.4 min) and % RCU was complete. The mean value was 1.28±0.42 mg/kg/day and 1.94±0.69 mg/100 ml plasma/day. These values were 2.8 and 2.0 times that of normal respectively. The elevation of PIT values in patients with iron deficiency anemia is a queer and important finding.

2) Portal hypertension with congestive splenomegaly (Banti's syndrome)

Patients with congestive splenomegaly due to portal hypertension, on whom the diagnosis were established by diagnostic laporatomy, measurement of portal pressure as well as liver biopsy, were studied before the treatment. These patients were divided into 3 major groups according to Imanaga's classification of portal hypertension[4]. Group I has extra-hepatic portal vein obstruction without liver damage. Group II is those with intra-hepatic portal vein obstruction associated with fibrosis but no parenchymal damage of the liver. Patients in group III have intra-hepatic obstruction of hepatic vein and

TABLE 2. Ferrokinetics Data on Patients with Portal Hypertension

Case No.	Age	Sex	Hematocrit (%)	Serum Iron (γ/dl)	PID $T\frac{1}{2}$ (min)	PIT mg/100 ml plasma/day	RCU (%)	Classification
9	18	M	46	93	33	2.83	100	group I
10	15	M	15	52	60	0.87	100	group II
11	42	F	42	72	69	1.04	99	
12	26	F	26	30	20	1.50	100	
13	45	F	45	52	47	1.10	98	
14	43	F	43	55	45	1.22	99	
15	13	F	13	60	35	1.72	96	
16	57	F	36	60	68	0.88	90	
17	37	F	37	70	45	1.56	99	
Mean			32.1	64.4	55.6	1.24	97.6	
S.D.					17.1	0.30	3.1	
18	65	F	35	102	74	1.38	92	group III type 1
19	45	F	35	120	108	1.11	90	
Mean			35	111	91	1.25	91	
20	18	F	34	120	80	1.50	85	group III type 2 Hematemesis (−)
21	27	M	41	82	141	0.58	95	
22	41	M	34	80	82	0.98	100	
23	35	M	38	92	83	1.11	99	
Mean			36.8	93.5	96.5	1.04	94.8	
S.D.					24.7	0.33	5.93	
24	41	M	24	82	20	4.10	99	group III type 2 Hematemesis (+)
25	68	M	26	82	38	2.16	97	
26	21	M	30	86	55	1.56	100	
Mean			27.7	83.3	37.7	2.61	98.7	
S.D.					14.3	1.08	1.25	

portal vein with liver cirrhosis. Group III was subdivided further into groups with hematemesis and without hematemesis.

To exclude an error inherent to the measurement of plasma volume in these patients with massive splenomegaly, PIT was expressed in mg/100 ml plasma/day. The results were shown in Table 2. One patient in group I (No. 9) showed rapid PID ($T\frac{1}{2}$) with complete % RCU and increased PIT. However, this finding does not necessarily represent ferrokinetic changes of this group because of the paucity of the cases studied. Patients in group II (No. 10–17) showed remarkable hyposideremia and rapid PID ($T\frac{1}{2}$) (mean value: 55.6 ± 17.1 minutes). Group II and group III (No. 18–23) differ significantly in the values of serum iron and PID ($T\frac{1}{2}$). The mean value of PIT on patients in group II was 1.24 mg/100 ml plasma which was significantly higher than normal. Percent RCU in this group was rapid and full (97.6%). On the other hand, patients in group III showed normal range of serum iron and PID ($T\frac{1}{2}$) and increased rates of PIT (mean value: 1.11 mg/100 plasma). In group III, patients with hematemesis or melena (No. 24–26) showed accelerated PID ($T\frac{1}{2}$) and remarkably increased PIT values (mean value: 2.61 mg/100 ml plasma).

3) Hemolytic anemias

i) Congenital hemolytic anemia

In hereditary spherocytosis, PID ($T\frac{1}{2}$) was accelerated (less than 30 minutes) and % RCU has varied between 11 and 84. The PIT has been found to be between 1.72 and 7.64 mg/kg/day (mean value: 3.50 mg/kg/day) (Table 3). The patient No. 34 was the case of congenital hemolytic anemia which was different hematologically from the other cases and simulated the thalassemia. However, in this case alkaline resistant hemoglobin was 2.6% and abnormal hemoglobin was not demonstrated. This patient showed the pattern of high degree of ineffective erythropoiesis.

ii) Autoimmune hemolytic anemia

Ferrokinetic data in patients with autoimmune hemolytic anemia were shown in Table 3. All 5 patients were cases of idiopathic autoimmune hemolytic anemia, having positive Coombs test and no underlying disease. Two cases (No. 35 and No. 36) were examined before treatment and the others (No. 37–39) during treatment. Three patients under treatment were in remission from the anemia by steroid hormone. Non-treated patients showed rapid PID ($T\frac{1}{2}$) and increased rates of PIT. After treatment, PIT tends to improve rapidly but remain at relatively high levels.

iii) Paroxysmal nocturnal hemoglobinuria

The results of ferrokinetic study in 3 patients with paroxysmal nocturnal hemoglobinuria were shown in Table 3. The case No. 40 was studied at the early stage of non-treatment and the others were studied after prolonged hemoglobinuria and varieties of treatments. The case No. 41 was studied

TABLE 3. Hematological and Ferrokinetics Data on Patients with Hemolytic Anemia and Patients with Pernicious Anemia

Case No.	Age	Sex	Diagnosis	Hemoglobin (%)	Reticulocyte (%)	Serum Iron (μg/dl)	PID $T\frac{1}{2}$ (min)	PIT mg/kg/day	PIT mg/100 ml plasma/dal	RCU (%)	RCIT (mg/kg/day)	RBC Life Span ^{51}Cr $T\frac{1}{2}$ (days)
27	20	M	Hereditary spherocytosis	77	79	120	22	2.53	5.45	62	1.57	
28	18	M	〃	61	142	110	15	3.97	7.33	50	1.99	
29	27	M	〃	83	26	158	40	1.72	3.95	50	0.86	
30	40	M	〃	69	26	120	24	3.95	5.00	70	2.77	11
31	19	F	〃	67	186	95	23	2.26	4.13	74	1.68	6.5
32	17	F	〃	68	144	76	15	2.45	5.07	84	2.05	7
33	11	F	〃	45	101	118	14	7.64	8.43	50	3.82	11
Mean				67.1	100.6	113.9	21.9	3.50	5.62	62.9	2.11	
S.D.							8.4	1.57	1.54	12.6	0.88	
34	16	M	Congenital hemolytic anemia	28	132	150	15	8.59	10.00	11	0.91	
27*	20	M	Hereditary spherocytosis	114	6	90	84	0.49	1.07	69	0.34	
35	17	F	IAHA	40	118	90	27	1.66	3.33	99	1.65	
36	39	F	〃	46	245	71	62	1.65	1.15	70	1.16	
Mean				43	181.5	80.5	44.5	1.655	2.24	84.5	1.41	
S.D.							17.5	0.005	1.09	14.5	0.24	
37*	28	F	IAHA	75	20	50	30	0.83	1.67	73	0.61	
38*	14	F	〃	95	10	66	47	0.64	1.40	96	0.62	
39*	16	F	〃	84	18	220	90	0.87	2.44	49	0.43	
40	34	M	PNH	35	81	187	47	2.00	3.98	47	0.78	
41	26	M	〃	33	317	65	36	0.97	1.81	86	0.83	11
42	32	F	〃	35	30	60	48	0.85	1.25	37	0.31	12
Mean				34.3	142.7	104.0	43.7	1.27	2.35	56.7	0.64	
S.D.							5.44	0.52	1.18	21.1	0.23	
43	50	F	Pernicious anemia	62	7	167	30	3.11	5.57	50	1.56	
44	15	M	〃	41	22	153	32	2.54	4.78	35	0.89	
Mean				51.5	14.5	160.0	31.0	2.83	5.18	42.5	1.23	
S.D.							1.0	0.28	0.39	7.5	0.32	

IAHA=Idiopathic autoimmune hemolytic anemia.

PNH=Paroxysmal nocturnal hemoglobinuria.

Case No. 27*: Post splenectomy.

Case No. 37*, 38* and 39*: During treatment of steroid hormone or 6 MP.

while under the steroid hormone maintenance therapy. This patient had blood transfusions and sodium bicarbonate therapy several months prior. Ferrokinetic study in case No. 42 was carried out during the non-treatment period 6 months after the onset of the disease. Previous therapies were the washed erythrocytes transfusion and administration of steroid hormone. In general, these patients showed an accelerated PID, increased PIT and depressed % RCU. Case No. 41, studied after prolonged massive hemoglobinuria, exhibited the ferrokinetic pattern similar to that in patients with iron deficiency anemia.

4) Pernicious anemia

As shown in Table 3, the studies on two patients with pernicious anemia had the rapid PID in spite of hypersideremia and depressed % RCU, averaging 43%. Values of PIT were 3.11 mg/kg/day and 2.54 mg/kg/day (mean value: 2.83 mg/kg/day). These were 6.9 and 5.6 times normal.

5) Aplastic anemia

Table 4 depicted the hematological and ferrokinetic data on 7 patients with panmyelophthisis, 3 patients with erythroblastophthisis and one patient with refractory anemia. In patients with panmyelophthisis, PID ($T\frac{1}{2}$) was markedly retarded and % RCU was depressed (mean value: 31.3%).

PIT values have been normal or subnormal except high values in case No. 45 and No. 51. Percent RCU was moderately depressed in patients with

TABLE 4. Hematological and Ferrokinetics Data on Patients with Aplastic Anemia

	Case	Age	Sex	Hemoglobin (%)	Reticulocyte (‰)	Serum Iron (μg/dl)	PID $T\frac{1}{2}$ (min)	PIT mg/kg/day	PIT mg/100 ml plasma/day	RCU (%)	RIT (mg/kg/day)	E/M Bone Marrow
A. Panmyelophthisis	45	42	M	35	18	270	198	0.88	1.36	64	0.56	1,000
	46	21	M	69	10	195	150	0.58	1.30	54	0.56	1,030
	47	30	F	25	65	238	520	0.32	0.46	34	0.11	665
	48	65	M	55	2	120	164	0.25	0.73	42	0.13	740
	49	37	F	58	0	168	175	0.57	0.96	10	0.06	(1,520)'
	50	41	F	38	0.5	134	346	0.27	0.39	2	0.01	(581)'
	51	55	M	30	3	199	154	0.81	1.29	13	0.11	156
	Mean			44.3	14.1	189.1	243.9	0.53	0.93	31.3	0.22	
	S.D.						129.2	0.24	0.38	21.9	0.22	
B. Erythroblastphthisis	52	47	M	45	0	200	440	0.34	0.45	3	0.01	0
	53	53	F	24	0.5	182	348	0.46	0.52	3	0.01	0
	54	74	M	35	14	145	160	0.54	0.91	47	0.25	154
	Mean			34.7	4.8	175.7	316	0.45	0.63	17.7	0.09	
C	55	62	M	44	54	82	22	2.76	3.72	64	1.76	1,500

Case No. 51: Hypoplastic anemia associated with secondary hemochromatosis.
Case No. 52 and No. 53: Pure red cell anemia associated with thymoma (mediastinal).
C=Refractory anemia with erythroid hyperplasia in bone marrow.
( )'=Scarce cell count of bone marrow aspirate.

erythroid normo- or hypercellular marrow (No. 45–48), but was markedly decreased in patients with severely hypocellular bone marrow (No. 49–51). Case No. 51 was the patient of hypoplastic anemia who had secondary hemochromatosis due to parenteral iron therapy and blood transfusions. This case showed relatively fast PID ($T\frac{1}{2}$) in spite of markedly depressed % RCU. PIT was 0.81 mg/kg/day which is significantly higher than normal.

Two of 3 cases with erythroblastphthisis were pure red cell anemia patients associated with thymoma. On ferrokinetic studies, both patients showed almost 0 in reticulocyte count and complete erythroid aplasia in the bone marrow. Case No. 52 was studied after extirpation of thymoma and case No. 53 before thymoma extirpation. These two patients showed conspicuously retarded PID ($T\frac{1}{2}$) and almost 0 in % RCU. PIT rates were 0.34 and 0.46 mg/kg/day respectively. Case No. 54 had presented cellular bone marrow with erythroid hypoplasia until death and thymoma was not found by autopsy. Erythroid cells in bone marrow remained at a relatively high percentage at the time of examination, so % RCU was relatively high.

Case No. 55 in Table 4 was diagnosed as refractory normoblastic anemia, based on the following findings, that is, persistent erythroid hyperplasia in bone marrow, the presence of many ringed sideroblasts, absence of hemolytic factors in serum and refractoriness to treatments with iron, B_6, B_{12}, and folic acid. In this case, PIT was 2.76 mg/kg/day with RCU of 64%.

6) Myeloproliferative disorders

a) Polycythemia vera

The results in 5 patients with polycythemia vera were shown in Table 5. No treatment were given to case No. 56, 58 and 60 before and during ferrokinetic studies. Case No. 57 was diagnosed polycythemia vera 9 years ago, and treated with venesections, ^{32}P and Thio TEPA for the last 7 years except the last two years. Case No. 59 had venesection of up to 2 litre 3 months prior to examination. On case No. 60 the diagnosis was made 20 years ago but no treatment was done what so ever. At the time of examination, this patient (No. 60) showed a moderate degree of myelofibrosis of the bone marrow and massive splenomegaly in which on body surface counting and spleen biopsy the presence of extramedullary erythropoiesis was revealed. The average half-time of PID in patients with polycythemia vera was 26 minutes and % RCU was rapid and complete (mean value: 99%). The mean value of PIT rates was 1.29 mg/kg/day, ranging from 0.68 to 2.22 mg/kg/day.

b) Myelofibrosis

All 3 patients with myelofibrosis showed dry bone marrow tap and massive splenomegaly. On all of 3 cases extramedullary erythropoiesis was demonstrated by ^{59}Fe *in vivo* counting. The bone marrow biopsies revealed the myelofibrosis in all cases. Two of them were primary myelofibrosis and the other (No. 63)

TABLE 5. Hematological and Ferrokinetics Data on Patients with Myeloproliferative Syndrome

Case No.	Age	Sex	Diagnosis	Hemoglobin (%)	Reticulocyte (%)	Serum Iron (μg/dl)	PID $T\frac{1}{2}$ (min)	PIT		RCU (%)	RCIT (mg/kg/day)
								mg/kg/day	mg/100 ml plasma/day		
56	43	M	Polycythemia vera	114	2	60	40	0.83	1.50	100	0.83
57	54	F	〃	96	12	85	31	1.60	2.74	100	1.60
58	48	M	〃	132	4	106	24	2.22	4.41	98	2.21
59	48	F	〃	70	6	38	16	1.12	2.37	99	1.12
60	44	F	〃	117	12	30	17	0.68	1.77	98	0 67
Mean				105.8	7	64	26	1.29	2.56	99	1.29
S.D.							9.0	0.18	1.02	0.9	0.56
61	63	F	Primary myelofibrosis	52	45	50	36	0.76	1.32	63	0.46
62	29	F	〃	73	31	50	22	2.16	2.27	48	1.04
63	34	F	Secondary myelofibrosis	43	82	62	30	1.10	2.07	75	0.82
Mean				56.0	52.7	54.0	29.3	1.34	1.89	62.0	0.77
S.D.							5.7	0.60	0.41	11.1	0.24
64	54	M	CML	74	15	87	66	0.69	1.32	68	0.47
65	34	F	〃	65	5	35	30	0.77	1.17	100	0.77
66	24	F	〃	82	8	80	79	0.46	1.01	95	0.44
Mean				73.7	9.3	67.3	58.3	0.64	1.17	87.7	0.56
S.D.							20.7	0.13	0.13	14.1	0.15
67	48	M	CLL	92	2	138	83	0.85	1.66	92	0.78
68	43	F	Erythroleukemia	21	12	202	84	2.08	2.40	9.6	0.21
69	64	F	〃	32	19	177	50	2.37	3.54	9.0	0.21
70	31	M	〃	33	8	113	50	1.46	2.26	2.8	0.13
Mean				28.7	13.0	164.0	61.3	1.97	2.73	7.1	0.18
S.D.							16.0	0.38	0.57	3.1	0.04

CML = Chronic myelogenous leukemia.
CLL = Chronic lymphatic leukemia.

was secondary to stomach cancer. Scores of alkaline phosphatase in neutrophiles were high in the primary ones and normal in the secondary one. As a common finding in this disease, the PID ($T\frac{1}{2}$) was rapid and % RCU was moderately depressed (mean value: 62%). The PIT was high in two cases (No. 62 and 63) and moderately raised in one (No. 61), with an average of 1.34 mg/kg/day (Table 5).

c) Chronic leukemia

In the studies of 3 patients with chronic myelogenous leukemia, PIT was raised in two (No. 64 and 65) and normal in one (No. 66), with an average of 0.64 mg/kg/day. Percent RCU varied between 68 and 100% (Table 5).

In a patient with chronic lymphatic leukemia having no anemia (No. 67), PIT was 0.85 mg/kg/day, with % RCU of 92.

d) Erythroleukemia (Di Guglielmo's syndrome)

Table 5 also depicts the results in 3 patients with Di Guglielmo's syndrome. All of the data were obtained from the patients in the initial eryhtremic stage prior to medication. An average half-time of PID was 61.3 minutes and an average of % RCU was 7.1. PIT was 4.4 times normal, with an average of 1.97 mg/kg/day. The patterns showed the intense degree of ineffective erythropoiesis in this condition. Case No. 68 and 69 died after transformation to acute myelogenous leukemia, while case No. 68 showed an interesting chronic course of 3 years and 3 months which was divided into 3 stage, that is, erythremic stage, erythroid hypoplastic stage and myeloid leukemic stage. Ferrokinetics measurements obtained in each stage of this case were shown in Table 6. At the stage of erythroid hypoplasia in bone marrow, the patient showed the ferrokinetic pattern of aplastic anemia. And in the terminal stage complicating secondary hemochromatosis after c.a. 60,000 ml blood transfusion, patient showed relatively fast PID and high value of PIT which is believed to be due to the increase of non-erythroid tissue iron turnover.

7) Acute leukemia

Table 7 depicted the ferrokinetic results in patients with acute leukemia of lymphatic and myelogenous type. In patients with acute myelogenous leukemia, PID retarded generally and PIT rates were slightly elevated in 4

TABLE 6. Transitional Changes of Ferrokinetic Data in a Patient with Erythroleukemia (Case No. 82)

Data	Serum Iron (μg/dl)	UIBC (μg/dl)	PID $T\frac{1}{2}$ (min)	PIT		RCU (%)	RCIT (mg/kg/day)	E/M in Bone Marrow
				mg/kg/day	mg/100 ml plasma/day			
Aug. '63 (onset)	202	32	84	2.08	2.40	10	0.21	2,178
Dec. '63	185	24	156	0.67	1.19	17	0.11	796
June '66	200	13	140	1.13	1.43	1	0.01	29

UIBC = Unsaturated Iron-binding Capacity.

TABLE 7. Hematological and Ferrokinetics Data on Patients with Acute Leukemia

Case	Age	Sex	Diagnosis	Hemoglobin (%)	Hematocrit (%)	Reticulocyte (‰)	Serum Iron (μg/dl)	PID $T_{\frac{1}{2}}$ (min)	PIT mg/kg/day	PIT mg/100 ml plasma/day	RCU (%)	RCIT (mg/kg/day)
71	50	M	AML	34	16	1	195	166	0.78	1.17	27	0.21
72	17	M	〃	71	33	2	180	165	0.61	1.09	52	0.32
73	50	M	〃	20	12	15	159	172	0.73	0.92	63	0.46
74	50	M	〃	38	16	19	146	250	0.40	0.58	34	0.14
75	22	M	〃	85	42	3	180	177	0.51	1.02	65	0.33
76	17	M	〃	43	18	—	190	190	0.60	1.00	39	0.23
Mean				48.5		8.0	175.0	186.7	0.61	0.96	46.7	0.28
S.D.								29.5	0.13	0.19	14.4	0.10
77	22	M	AML*	91	39	12	115	90	0.78	1.28	100	0.78
78	32	M	AML*	116	42	10	295	180	0.82	1.64	68	0.56
79	12	M	AML*	83	36	11	201	60	0.52	3.35	85	0.44
80	49	M	AML°	53	25	3	240	720	0.20	0.33	3.1	0.01
81	34	F	CML→AML′	63	29	2	128	155	0.44	0.86	43	0.19
82	22	F	ALL	73	31	2	135	154	0.50	0.88	12	0.11
83	17	M	〃	75	31	5	178	162	0.58	1.10	43	0.47
84	26	M	〃	43	22	9	295	368	0.53	0.80	16	0.13
Mean				63.7	28.0	5.3	202.7	228	0.54	0.93	23.7	0.24
S.D.								99.0	0.03	0.13	13.8	0.17

AML = Acute myelogenous leukemia.
ALL = Acute lymphatic leukemia.
* = Remission after treatment.
° = End stage of AML.
′ = Acute transformation of CML.

cases (No. 71, 72, 73 and 76) and close to normal in 2 cases (No. 74 and 75). Percent RCU in these cases varied between 27 and 65%, averaging 46.7%. Three patients with acute lymphatic leukemia (No. 82-84) showed also retarded PID ($T\frac{1}{2}$), depressed % RCU and almost normal levels of PIT. Three patients with acute myelogenous leukemia (No. 77-79) who were under complete hematological remission by chemotherapy, showed a ferrokinetic data close to normal.

B. Body surface counting by ^{59}Fe

Body surface counting for tissue localization of radioiron were performed on 33 patients with various hematological diseases.

1) Fig. 1 shows the normal distribution of ^{59}Fe over the bone marrow (sacrum), liver, spleen and heart with daily counting. The maximum uptake over the marrow was reached in 12 to 24 hours and then showed progressive diminution after the release of ^{59}Fe incorporated red blood cell into circulation. The count over the liver usually remained constant and was slightly higher than that over the spleen. In Fig. 1 is also shown the curves of a patient with polycythemia vera (case No. 56).

2) Fig. 2 shows body surface counting curves in patients with portal hypertension. The countings were preformed in 2 patients of group II (No. 11 and 13) and 2 patients of group III (No. 19 and 22). These 4 patients showed a similar pattern of the curve on each organ. The counting curves over the liver and bone marrow showed normal patterns. The counts over the spleen showed a progressive increase 2 or 3 days after ^{59}Fe injection, paralleling to the increase of the counts over the heart (secondary spleen curve). This secondary spleen curves were obtained on all the patients of both group II

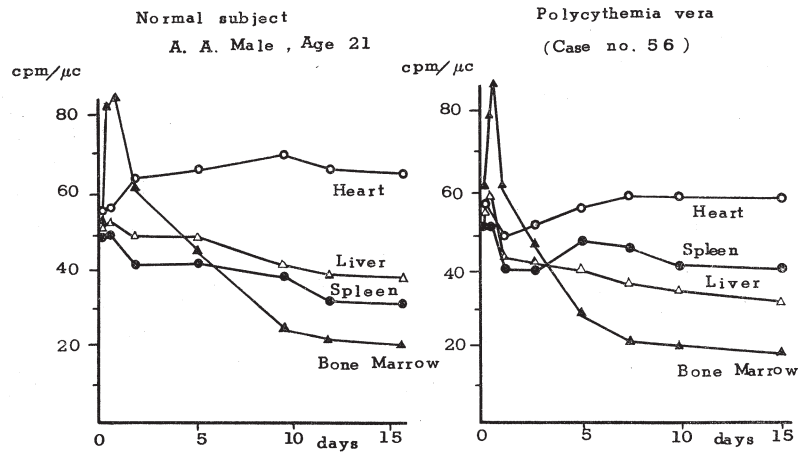

FIG. 1. Body surface patterns on a normal subject and a patient with polycythemia vera.

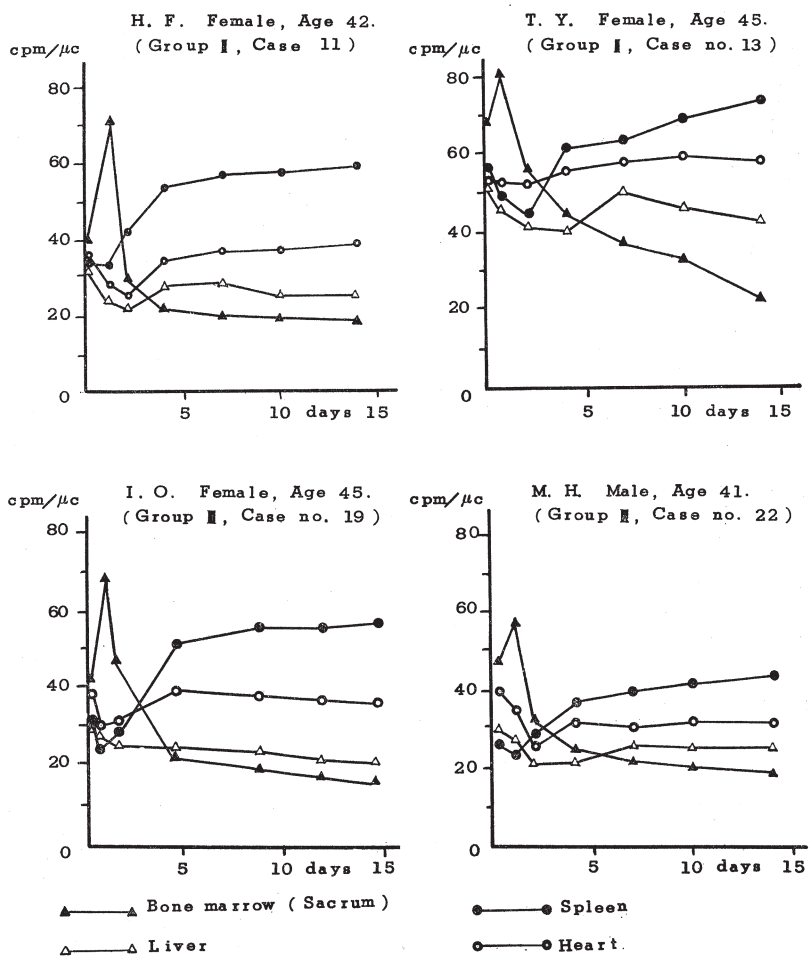

FIG. 2. Body surface patterns on patients with portal hypertension having congestive splenomegaly.

and III examined. Any different pattern in body surface counting was not observed between group II and group III.

3) The body surface countings were done on 4 patients with congenital hemolytic anemia (case No. 27, 29, 30 and 34) and a patient with autoimmune hemolytic anemia (case No. 37*). The characteristic picture is the progressive increase of counts over the spleen which was also observed on patients with portal hypertension. This secondary spleen curve was obtained on all of the 4 patients with hereditary spherocytosis (Fig. 3). The secondary spleen curve in case No. 27 disappeared after splenectomy. The uptake of ^{59}Fe by the bone

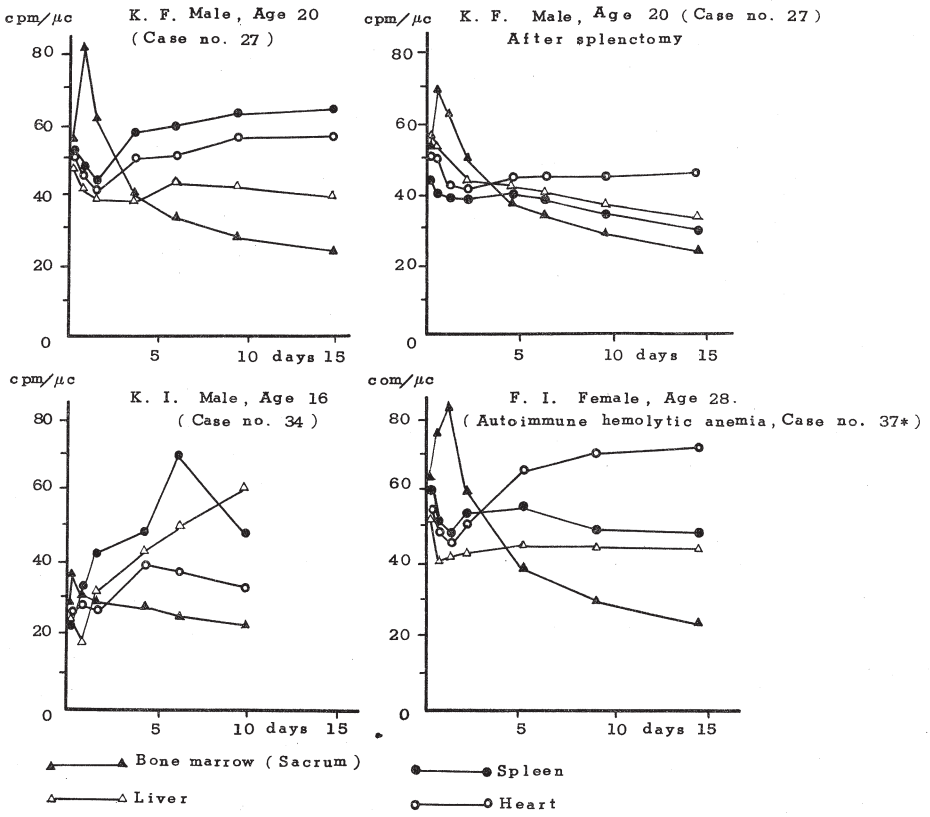

FIG. 3. Body surface patterns on patients with hemolytic anemia.

marrow and liver were normal in this disorder. However, patient No. 34 showed an unusual picture such as depressed rise in radioactivity on the bone marrow and the progressive increase of counts on the liver which was supposed to reflect aplastic crisis.

4) Fig. 4 shows the body surface counting curves in 3 patients with aplastic anemia (No. 49, 51 and 52) and a patient with acute leukemia (No. 81). These patients all having pronounced erythroid hypoplasia in bone marrow presented a typical aplastic pattern which shows a maximum uptake on the liver, but little or none on the bone marrow or on the spleen.

5) Body surface countings in all 3 patients with myelofibrosis (No. 61-63) showed the evidence of extramedullary erythropoiesis in the spleen (Fig. 5). These patients showed a marked initial rise and fall over the spleen (primary spleen curve) and low or no marrow uptake. These splenic curves resemble the normal marrow curves but do not necessarily fall at the same rate as marrow, as seen in case No. 63. In this studies, the extramedullary patterns

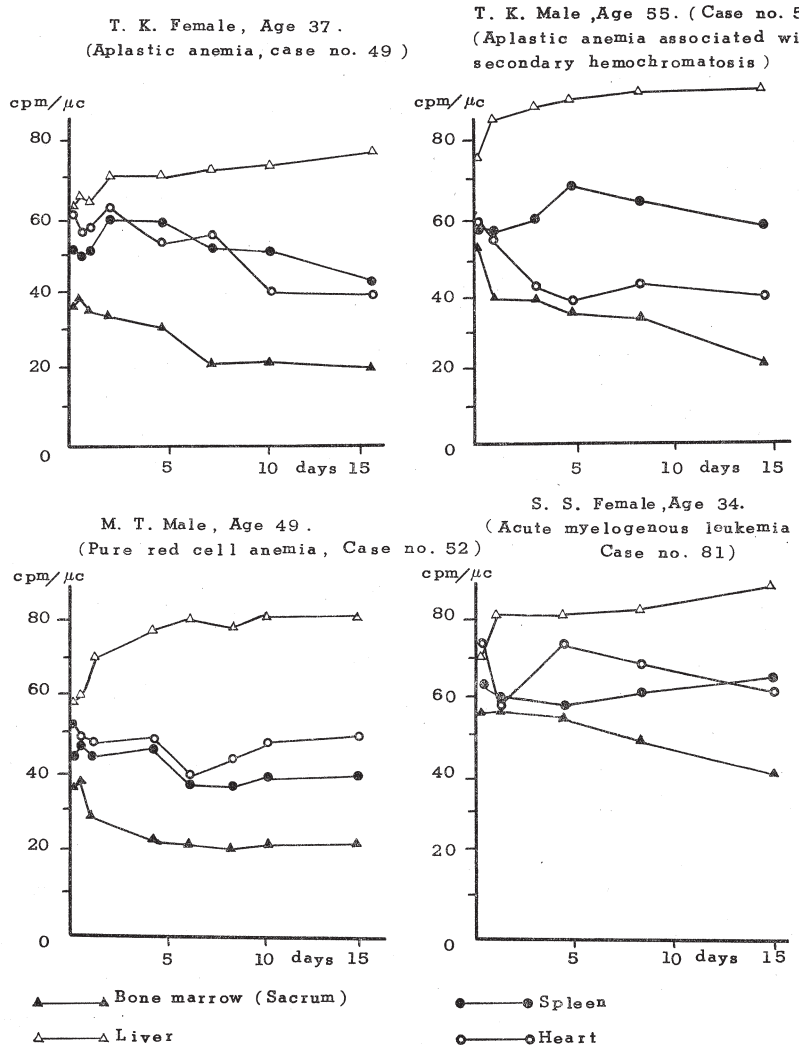

FIG. 4. Body surface patterns on patients with erythroid hypoplasia.

were also obtained in a patient with burnt out polycyhthemia vera (No. 60) and a patient with chronic myelogenous leukemia (No. 64).

C. Integrative analysis of ferrokinetic data

In ferrokinetic studies of blood disorders of diverse etiology, some diseases present a consistent pattern, but others are highly heterogeneous in ferrokinetic

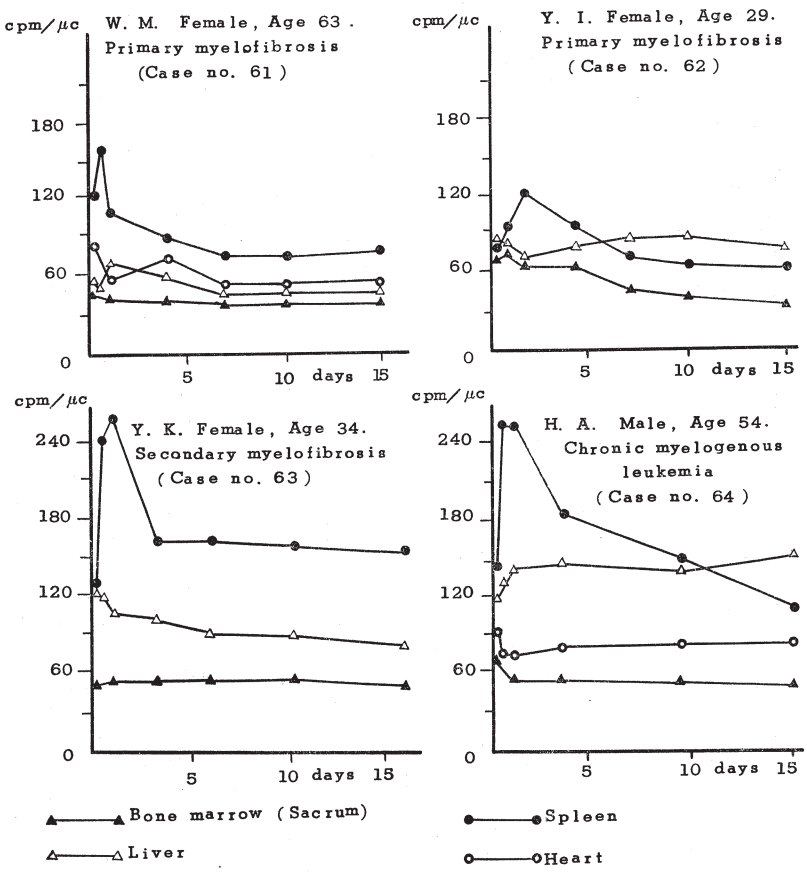

FIG. 5. Body surface patterns on patients with extramedullary erythropoiesis.

measurements. Perspective review and integrative analysis on ferrokinetic data descrived above are beneficial for the correct interpretations of these data and for an application of this technique in clinical practice. Fig. 6 presented the average of PIT with their standard deviations in various blood disorders. PIT and RCIT rates in these hematopoietic disorders were visualized in Fig. 7. Between hematological and ferrokinetic parameters the correlation between serum iron and PID ($T\frac{1}{2}$) is the highest. Correlation coefficient in whole subjects (normal subjects and patients with blood disorders) was +0.687. A significantly high correlations between PID ($T\frac{1}{2}$) and serum iron value were observed in normal subjects as well as patients with portal hypertension and patients with acute leukemia (Fig. 8). Correlation coefficients were +0.785 in normal subjects, +0.678 in patients with portal hypertension and +0.782 in

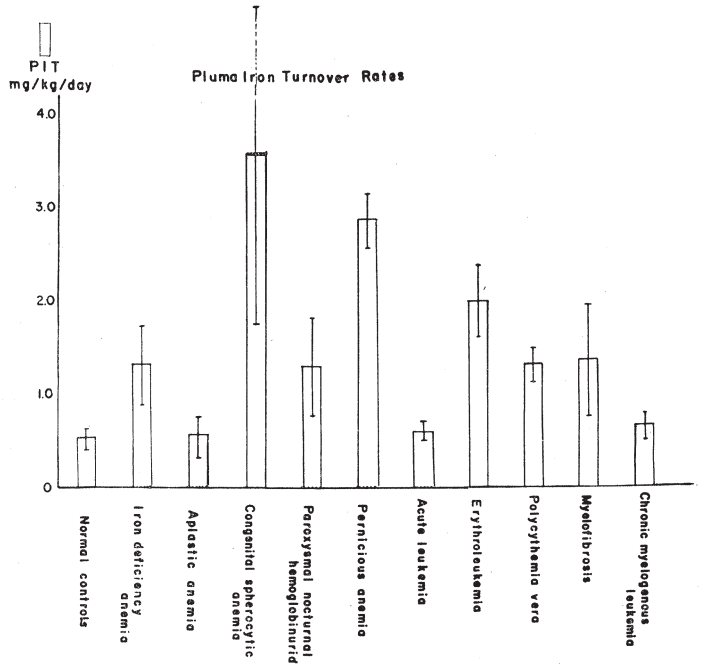

FIG. 6. Plasma iron turnover rates in blood disorders.

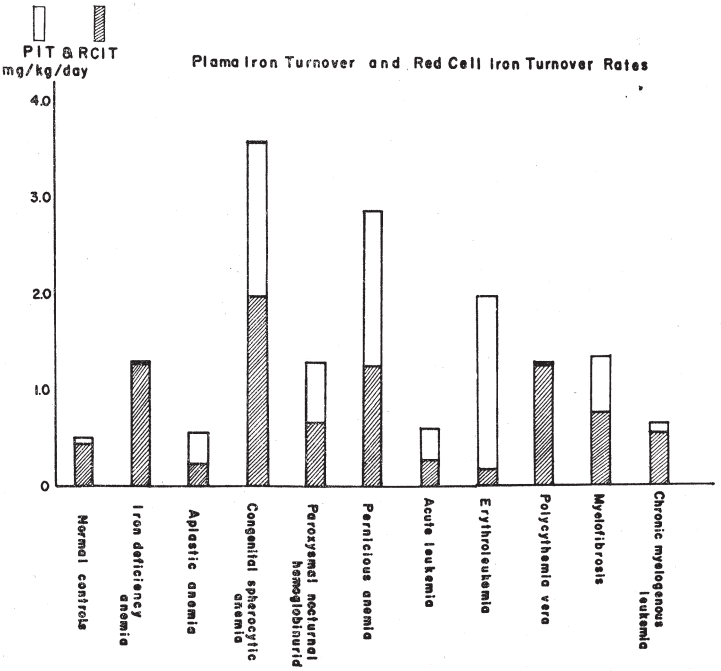

FIG. 7. Plasma iron turnover and red cell iron turnover rates in blood disorders.

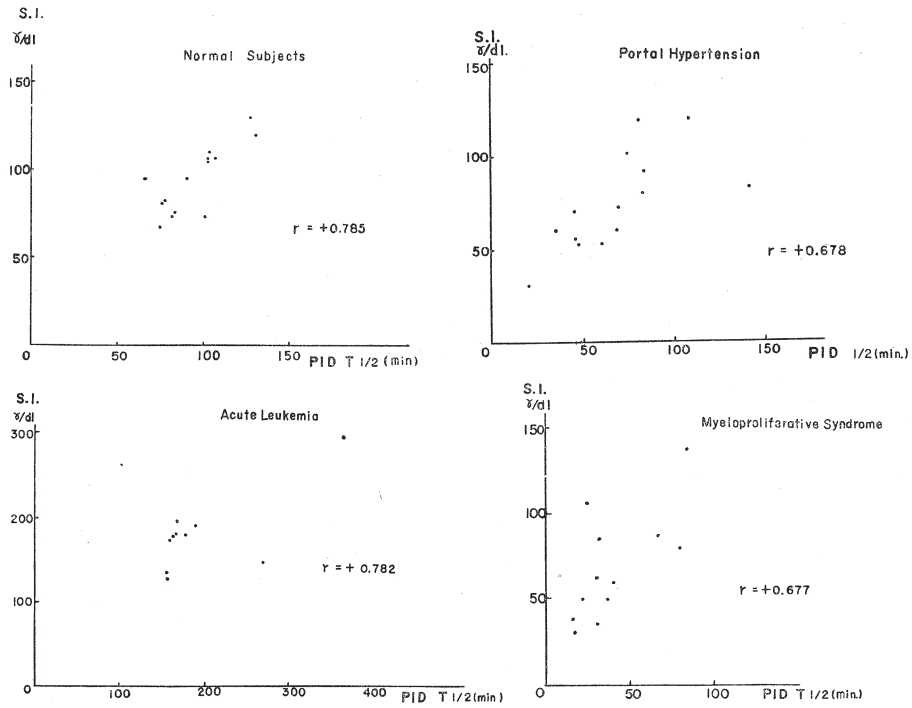

FIG. 8. Correlations between serum iron and PID ($T\frac{1}{2}$).

patients with acute leukemia respectively. Significant correlations between them were also observed in iron deficiency anemia ($r=+0.569$) and myeloproliferative disease ($r=+0.677$), but absent in hemolytic anemia ($r=-0.238$). The significant correlation was not observed in patients with aplastic anemia ($r=+0.334$).

The relationship between PID ($T\frac{1}{2}$) and erythroid/myeloid ratio in bone marrow in 48 patients with blood disorders was signifcant. The correlation coefficient was -0.435, while it was -0.484 in 29 patients excluding patients with pernicious anemia, paroxysmal nocturnal hemoglobinuria, erythroleukemia, polycythemia vera and patients with aplastic anemia. Significant relationship between erythroid/myeloid ratio in bone marrow and PIT was also noted in 47 patients with blood disorders. The correlation coefficient was $+0.378$. But, when the patients with hemolytic anemia, pernicious anemia, erythroleukemia and polycythemia vera were excluded from them, the correlation coefficient was $+0\,524$. Otherwise, cerrelation between hemoglobin concentration and PID ($T\frac{1}{2}$), that of hemoglobin concentration and % RCU and that of PID ($T\frac{1}{2}$) and % RCU were not significant or low in patients with blood disorders described.

IV. DISCUSSION

Normal values of ferrokinetic measurement in normal adults were reported by Wakisaka[5] and Sekiya[6]. The mean values of PIT in normal adults presented by author were slightly less than those reported by Wakisaka and Sekiya but close to the data of these two workers using ^{59}Fe-globulinate. The ferrokinetic data of present study in the aged had fallen within the range which had been obtained in normal adults. PIT and RCIT rates in the aged showed no statistically significant difference to the results in normal adults. Similar results on the aged subjects were reported by Takaku[7] and Maekawa[8]. Thus, it will be said that so far as ferrokinetic studies are concerned, the erythropoietic activity of the aged is within normal range. Results in 8 patients with iron deficiency anemia gave values for PIT higher than normal, though normal or decreased values might be anticipated in this condition. The mean value of PIT was 1.28 mg/kg/day and 1.94 mg/100 ml plasma/day. Several investigators[9][10][11] have also reported high values of PIT in this condition, while PIT values between 20 and 60 percent of normal were reported in the study of Finch[12]. Bothwell and Finch[13] speculate two possible reasons for elevated PIT values in iron deficiency anemia. The one is the inaccuracy in measurement of serum iron at low levels found in iron deficiency anemia. The other relates to the finding that the line of clearance of iron from the plasma tends eventually to deviate from a straight line. Pollycove[14] disclosed by means of complete iron kinetics including *in vivo* counting that the increased PIT in iron deficiency anemia is related to the intramedullary hemolysis of maturing erythrons. However, it is doubtful whether this could be a significant feature and reach to a considerable amount, since the % RCU is complete. Moreover, it should be considered that the elevated values of PIT can be related to the hemorrhage occurring occasionally in this condition. In the present study, the remarkable elevation of PIT were observed in patients with portal hypertension associated with acute massive blood loss. This effect of hemorrhage on PIT value was shown in the experiment using rats by Girvin and associates[15].

In the present study, patients of portal hypertension with congestive splenomegaly were grossly divided into two groups, that is, the non-cirrhotic group (group II) and the cirrhotic group (group III) according to the hemodynamic findings of intrahepatic and portal blood flow. The ferrokinetic studies disclosed that the erythropoietic activity on patients in both group is increased rather than depressed, and the liver damage does not necessarily show inhibitory effect on the red cell production in this disorder. However, it is evident that the bone marrow in this condition is still unable to compensate for the decrease of red blood cells in spite of an increase of erythropoiesis.

In body surface counting, the spleens in patients of both groups showed

the progressive uptake of ^{59}Fe which reflects the splenic sequestration of labeled young red cells. However, it may be that sequestrated red cells are not necessarilly damaged cells and are exchanging with circulating blood, since the % RCU is rapid and full. On the other hand, it was confirmed by several workers[16)17)] that a large quantity of blood was sequestrated in the enlarged spleen of the patients with portal hypertension. And it may be that this finding has to do with the pathogenesis of the anemia in this condition. Summing up these results, it is possible to speculate that red cells trapped within the spleen are actually still within circulating blood and therefore the stimulus to increase red cell production may not reach the marrow.

In congenital hemolytic anemia, there are a marked increase of PIT values and moderate degree of depression in % RCU. PID curve is not purely exponential in this condition, therefore PIT calculated according to the method of Huff and associates is over-estimated. Interpretation of the decreased % RCU is difficult because of the random destruction of newly formed red cells and the possible effect of increased iron stores on the utilization of radioiron.

In hereditary spherocytosis, the ineffective erythropoiesis was not demonstrated except rare cases[18)19)]. Meanwhile, it has been reported that there is a marked degree of ineffective erythropoiesis in thalassemia[20)].

Ferrokinetic studies on patients with autoimmune hemolytic anemia has been reported by several investigators[21)22)]. Kuroyanagi[22)] demonstrated the presence of ineffective erythropoiesis in some of patients with autoimmune hemolytic anemia.

The present data in ferrokinetic study on patients with pernicious anemia have also shown a marked increase of PIT and a marked depression of % RCU. The PID curve is not purely exponential and PIT is also over-estimated in this disease. The ferrokinetic results similar to those presented have been reported in literatures[23)24)]. These ferrokinetic patterns are one of ineffective erythropoiesis. In recent study of Myhre[24)] were presented the erythrokinetic findings suggesting that great destruction of young erythroid cells occurs either within the bone marrow or shortly after delivery of the red cells into the peripheral blood in this condition.

Only a few ferrokinetic studies[25)26)] in patients with paroxysmal nocturnal hemoglobinuria have known to the author. The results presented by the author suggest the presence of ineffective erythropoiesis in this disorder. However, it is uncertain, as Kummer[26)] stated, that a marked increase of PIT is due to the presence of second red cell population with very short survival time, as postulated by many investigators.

Patients with aplastic anemia form a heterogeneous group in ferrokinetic patterns due to a variety of the marrow cellularity and different pathological conditions but one point in common is the decrease of production of viable red cells. It is obvious that PIT and % RCU, as they are, do not reflect the

rate of erythropoiesis in this condition, because a large quantity of iron shifts into iron stores. Bothwell and associates[27] reported PIT values twice normal with 39% RCU in patients with hemochromatosis. Thus, the increased iron stores showed a considerable effect on PIT in spite of the presence of normal erythropoiesis. In the present study, the tendency to increased PIT due to iron overload was demonstrated in 2 patients with secondary hemochromatosis (case No. 51 and case No. 68 at the end stage). Erythroid cells in the bone marrow being absent in two patients with erythroblastphthisis at the time of study, the PIT of these patients represent purely the tissue iron exchange other than erythroid marrow. The value for PIT in these cases is twice normal, for non-erythroid iron turnover rate in normal subject is about 0.2 mg/kg/day[13].

Several results[1)28)] have been reported in patients with the condition labeled "refractory anemia with hyperplastic marrow". According to these reports, PIT rates are usually considerably elevated and % RCU are depressed to less than 50%. These ferrokinetic patterns are also the type of ineffective erythropoiesis and are the same as seen in patients with untreated pernicious anemia and Di Guglielmo's syndrome.

The elevation of PIT in polycythemia vera has been recognized by Bothwell[9], Huff[1], Wasserman[11] and Kiely[29]. From a practical standpoint, it has been reported that the ferrokinetic measurements are useful in the differential diagnosis of the polycythemias[30] and in assessing the effect of therapy in patients with polycythemia vera[31]. However, it is difficult to differentiate, by ferrokinetic measurements polycythemia vera and secondary polycythemia due to associated tumor. The reason why the high values of PIT were obtained in polycythemia vera and polycythemia associated with a variety of tumours has not been solved, although a few explanations have been presented by Huff[1] and Sharney.[32] Clinically, it should be kept in mind that the stage of the disease and the complications of iron deficiency anemia and myeloid metaplasia can influence the ferrokinetic measurements.

The results in 3 patients with myelofibrosis gave values of PIT 3 times normal and diminished % RCU. It is apparent that quite various patterns can be obtained depending on the stage of the disease. Therefore, ferrokinetic measurements are useful in defining the different patterns present in individual patients. The erythrokinetic studies of our cases[33] have demonstrated the presence of ineffective erythropoiesis in this disorder.

In the present study of Di Guglielmo's syndrome, the mean value of PIT was 4.4 times normal and % RCU was 7%. Similar results to those were reported by Baldini[34] and other investigator[35]. These results show the relatively uniform pattern as one of the ineffective erythropoiesis and they are quite similar to those of untreated pernicious anemia. True mechanism of the anemia in Di Guglielmo's syndrome has not been made clear but the presence

of high degree of ineffective erythropoiesis has been demonstrated by erythrokinetic studies[19)33)].

In the present study on 4 patients with chronic myelogenous leukemia, the mean value of PIT was 1.4 times normal and that of % RCU was 88%. But lower % RCU were reported by Huff[1)]. The present study on a patient with chronic lymphatic leukemia showed PIT 1.9 times normal and 92% in RCU, while some workers[10)11)] reported normal or low PIT with lower % RCU. In the present study on 6 patients with acute myelogenous leukemia and 3 patients with acute lymphatic leukemia, the PIT was normal or slightly increased and % RCU was depressed between 12 to 65%. A high correlation between serum iron and PID ($T\frac{1}{2}$) was obtained in patients with acute leukemia and the correlation coefficient was so high as seen in normal subjects and patients with non-hematological disorders. This finding may indicate that myelophthisic effect due to the encloachment of leukemic cells is a major factor in the mechanism of anemias in patients with acute leukemia studied. However, it is generally accepted that the anemia in leukemia is not only due to erythropoietic failure by the encloachment of leukemic cells but also due to hemolytic process. Wetherley-Mein and associates[36)] demonstrated the role of hemolysis in leukemia by a combined ^{59}Fe and ^{51}Cr techniques. In the present studies and several other reports[1)9)10)11)37)38)], the ferrokinetic patterns typical of each cell type of leukemia can not be obtained on both acute and chronic leukemia.

Body surface counting tests do not always show a consistent patterns in certain hematological disorders but is useful in obtaining anatomic distribution of erythropoietic activity. Based on the results presented, three main types of surface patterns have been obtained in terms of erythropoiesis. They are (1) the normal (2) the hypoplastic and (3) the extramedullary pattern. On the other hand, there are two types of surface patterns as to the spleen. That is, primary spleen curve and secondary spleen curve. Surface patterns of extramedullary erythropoiesis have been reported in patients with polycythemia vera, myelofibrosis and chronic myelogenous leukemia[38)39)40)]. However, it does not necessarily mean that the absence of extramedullary patterns in body surface counting indicates the absence of extramedullary hematopoiesis. These false negative cases have been reported in literatures[41)42)]. Thus, viewed from clinical practice, body surface counting can be useful to obtain evidence of extramedullary erythropoiesis and give confirmatory evidence of hypoplasia or iron overload.

In clinical practice, there are three main types of parameters in ferrokinetic studies. They are measurement of PIT, measurement of % RCU and body surface counting. PIT is one of the most important parameters which reflects total erythroid activity. In clinical practice this parameter is of great use as a reproducible and rapid measure of erythropoiesis. Another important

significance of PIT is to assess the ineffective erythropoiesis in conjunction with % RCU data. However, the main disadvantage of PIT measurement is that the iron overload may give an false impression of increased erythropoiesis.

Percent RCU reflects effective erythropoiesis and the measurement of % RCU is useful for clinical assessment of effective erythropoietic function. However, it is evident that if a hemolytic process is involved, interpretation of data should be done with caution. The main disadvantage of this test is that it will not detect an increase above normal in erythropoiesis.

In conclusion, main clinical use of ferrokinetic technique is to get the approximate estimation of the extent of effective and ineffective erythropoiesis and the detection of extramedullary erythropoiesis. This technique is now of practical diagnostic as well as prognostic importance, and this is of special value in diagnosing and treating patients with myeloproliferative syndrome in whom the bone marrow examinations do not exactly show the degree of erythropoietic activity.

V. SUMMARY

(1) Ferrokinetic data in normal adults and aged were presented. No significant difference of ferrokinetic parameters was obtained between adults and aged.

(2) i) Ferrokinetic measurements in patients with various blood disorders were reported and the interpretations of ferrokinetic parameters in each disorder were discussed.

ii) Ferrokinetic patterns of ineffective erythropoiesis were obtained in pernicious anemia, erythroleukemia, myelofibrosis, paroxysmal nocturnal hemoglobinuria and refractory anemia.

iii) Ferrokinetic studies in patients of portal hypertension with congestive splenomegaly have disclosed that the erythropoietic activity is rather increased and the liver damage does not necessarily exert inhibitory effect on erythropoisis in this condition.

iv) The studies on patients with acute leukemia did not present the patterns typical of each cell type of leukemia, and the results indicate that the myelophthisic effect due to the encloachment of leukemic cells is a major factor in the mechanism of anemia in this condition.

(3) Body surface patterns in blood disorders have been demonstrated and the clinical implication of this test was discussed. It is stressed that ferrokinetic measurements with body surface counting are of great use both in diagnosis and treatment of patients with myeloproliferative syndrome.

(4) Perspective review and integrative analysis have been tried on ferrokinetic data in normal subjects and patients with blood disorders. Significant correlation between serum iron level and PID ($T\frac{1}{2}$) was observed on normal subjects, as well as patients with portal hypertension, myeloproliferative

syndrome and patients with acute leukemia. Significant correlations were observed between PID ($T\frac{1}{2}$) and erythroid/myeloid ratio in bone marrow and between PIT and erythroid/myeloid ratio in bone marrow in patients with blood disorders.

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