

Title : Etiology of Anemia in Chronic Malaria in Rhesus Monkeys

Principal Investigator: Vithune Yuthasastr—Kosol, M.D.

Associate Investigators: Robert S. Desowitz, Ph.D., D.Sc.  
Tan Chongsuphajaisiddhi, M.D., Ph.D.

Assistant Investigator : Barnyen Permpanich

Objective To study the etiology of anemia in chronic P. coatneyi and P. inui malaria.

Description Anaemia is an important factor in the pathogenesis of malaria. This may be caused in part, either to impaired erythropoiesis or to increased red cell destruction by the parasite or reticuloendothelial elements. In the primary phase, when the parasitemia is high, it is due to erythrocyte destruction by the multiplying parasites. In chronic malaria, however, the anemia often persists and is inappropriate to the very scanty parasitemia present at this stage. One explanation given is that the anemia of chronic malaria is caused by an autoimmune reaction. This theory has not been completely accepted and some workers have maintained that an anemia is not present, since in experimental systems its existence has been based on complicated enumerative procedures.

Our earlier studies on the primate malarias, Plasmodium coatneyi and Plasmodium inui, have now provided a number of rhesus with long term chronic infections. These animals seemed highly suitable subjects to study the mechanism of anemia in chronic malarias

A proper assessment of the relative importance of these factors can only be made if both erythropoiesis and red-cell destruction can be measured simultaneously. In this study  $Fe^{59}$  was used to study iron utilization and erythropoiesis and  $Cr^{51}$  labelled red cells to follow red-cell survival in vivo.

Three groups of experimental animals were studied, each group consisted of four animals: group 1 of normal, uninfected Macaca mulatta; a second group of four M. mulatta with chronic infections of P. coatneyi; and a third group of four rhesus with chronic infection of P. inui. Hematologic data on these animals at the time of the experiment are summarized in Tables 1 and 2. Red cell survival was studied by the method of Weinstein and LeRoy (J. Lab. Clin. Med., 1955, 3) and ferrokinetics by the technique of Huff et al. (J. Clin. Invest., 1950, 29).

#### Progress

Results of the ferrokinetic and red-cell survival time studies are summarized in Tables 3 & 4, and figures 1, 2 and 3. The experimental data are presented in Table 5. All infected animals studied suffered from long term malarial infections. At the time of study there was a low parasitemia of not more than 1 %, accompanied by incommensurate anemia to different degrees.

Plasma Iron half-disappearance time Although the plasma half-disappearance time varied between 27.5 to 62.2 minutes, in P. coatneyi, and 32-68 minutes in P. inui respectively, it was markedly reduced as compared to the normal value of 110 minutes (Table 3 and Figure 1). Each animal had approximately the same range of normal serum-iron levels, thus excluding possibility of iron-deficiency anemia in the experimental animals.

Plasma-iron Turnover Plasma-iron turnover as calculated as milligrams per kilogram bodyweight per day, were also increased to a greater extent in all animals: 3.65 to 5.2 times of normal in P. coatneyi.

TABLE 1. Hematologic observations on normal, *P. coatneyi*- and *P. inui*-infected rhesus monkeys.

Animal number	Hematocrit	Hgb gm%	bl. type ABO	grouping Rh	Hgb. electrophoresis*	% Fetal Hgb.**	Spot test for G <sub>6</sub> PD deficiency	Red cell morphology	% Nucleated red cell
Group I-Normal (Mean values)									
	36.95-39.3	11.46-12.9	0	neg	A	1.45	neg	normal	0
Group II- <i>P. coatneyi</i>									
MS 2	28-38.3	8.6-11.1	0	neg	A	1.2	neg	normal	0
KL 1	21-27.4	6-8.6	0	neg	A	0.8	neg	mild hypochromic	1-4
KL 3	22.8-27.4	6.9-9.5	0	neg	A	1.4	neg	mild hypochromic	1-4
KL 9	21.2-28.3	6-7.5	0	neg	A	1.4	neg	mild hypochromic	1-2
Group III- <i>P. inui</i>									
SP 2	27.2-32	8.9-9.5	0	neg	A	1.6	neg	normal	0
SP 4	31-35.5	10.6-11.3	0	neg	A	1.4	neg	normal	0
SP 5	29.5-32.9	9.3-10.4	0	neg	A	1.6	neg	normal	0
KL 14	24.8-29.4	7.4-10.4	0	neg	A	1.82	neg	mild hypochromic	1-2

\* Hemoglobin electrophoresis done by Microzone technic \*\* Fetal Hemoglobin determination done by method of Singer et al.

TABLE 2. Results of differential counts of bone-marrow in normal, chronic *P. coatneyi* and *P. inui* infected rhesus monkeys.

Type of cell	Normal (Mean values)	<i>P. coatneyi</i> —infected					<i>P. inui</i> —infected							
		MS <sub>2</sub>	KL <sub>1</sub>	KL <sub>3</sub> (Per cent)	KL <sub>9</sub>	Av	SP <sub>2</sub>	SP <sub>4</sub>	SP <sub>5</sub> (Per cent)	KL <sub>14</sub>	Av			
Blasts, unclassified	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Myeloblasts	2.6	.5	1	1.5	.5	.9	.5	1	2	2	1.1	—	2	1.1
Progranulocytes	2.7	1.5	1	1.5	1.5	1.4	1	1	1	1	1.1	—	1	1.1
Myelocytes neutrophilic	4.1	2	2	3	1.5	2.1	2	2	3	3	3.5	—	7	3.5
Eosinophilic	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Basophilic	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Metamyelocytes neutrophilic	7.9	3.5	4	3.5	4	3.8	3.5	3	3	3	3.1	—	3	3.1
Eosinophilic	1	1	1.5	.5	.5	.9	1	.5	.5	.5	.5	—	—	.5
Basophilic	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Band cells neutrophilic	11.7	8	9	4.5	7.5	7.3	9.5	7	6	6	7.4	—	7	7.4
Eosinophilic	1	1.5	1	2	1	1.4	2	1	1.5	1	1.4	—	1	1.4
Basophilic	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Segmented neutrophilic	20.9	10	10.5	7	9.5	9.3	10	14	12	14	12.5	—	14	12.5
Eosinophilic	1.7	1.5	2	1	1	1.4	1	4.5	1.5	1	2	—	1	2
Basophilic	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lymphoblasts	1.2	1	1.5	2.5	1.5	1.5	1.5	1	2.5	2	1.8	—	2	1.8
Prolymphocytes	2	1	2	1.5	2.5	1.8	1	1	1.5	0	.9	—	0	.9
Lymphocytes	8.4	4.5	10	9	9	5.9	5.5	6	7	5	5.9	—	5	5.9
Monoblasts	.9	.5	.1	1	.5	.9	.5	—	.5	1	.5	—	1	.5
Promonocytes	.4	.5	1.5	1.0	.5	.9	1	—	.5	—	.4	—	—	.4
Monocytes	1.6	.5	1.5	2	1	1.3	2	1	1	1	1.3	—	1	1.3
Plasmocytes	.9	1	1	1.5	—	.9	.5	1	1	1	1.5	—	1	1.5
Megakaryocytes	3.8	2	3.5	3.5	3.5	3.1	3.5	3.5	3	3	3.1	—	3	3.1
Pronormoblasts	5.2	9	10	10.5	10.5	10	9.5	7.5	9	4	7.5	—	4	7.5
Normoblasts	2.4	50	32	43	43.5	42.1	43	40	43	44	42.5	—	44	42.5
Seticulum cells	.4	.5	.5	1	.5	.6	.5	—	.5	.5	.4	—	.5	.4
M:E	1.84:1	1:2	1:1.4	1:2	1:2	1:1.9	1:1	721:1.2	1:1.7	1:1.3	1:1.5	—	1:1.3	1:1.5

FIGURE 1. PLASMA IRON DISAPPEARANCE CURVES IN 3 DIFFERENT GROUPS OF RHESUS MONKEYS

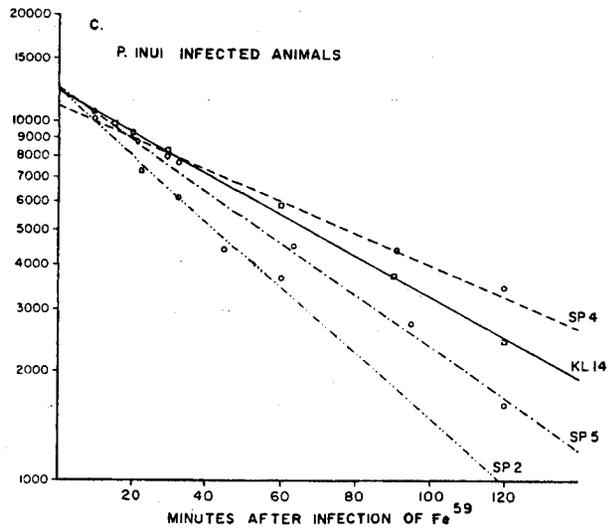
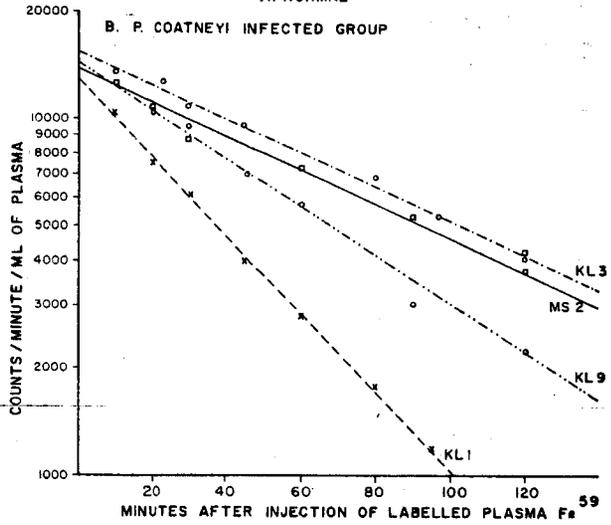
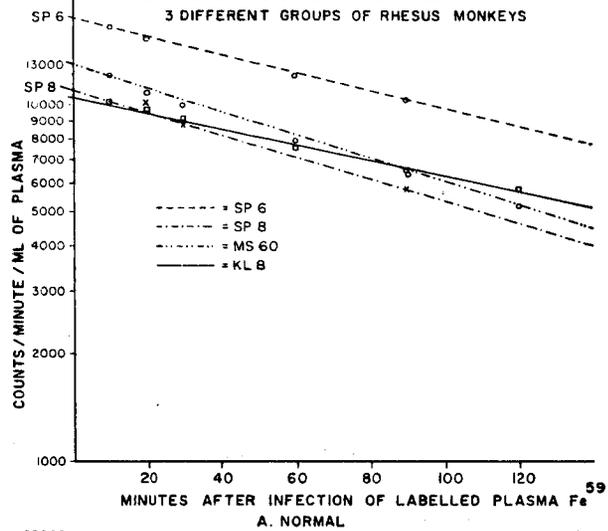


TABLE 3. Results of ferrokinetic studies of normal *P. coatneyi* and *P. inui*-infected rhesus monkeys.

Animal number	Day of infection	%Parasitemia during period of study	Red cell mass (ml/kg)	Serum iron ( $\mu$ g/100 ml)	Plasma Fe <sup>59</sup> half-clearance Time (min)	Plasma iron Turnover (mg/kg/day)	Plasma iron clearance rate/hr	% iron utilization
<b>I. Normal group</b>								
(Mean values) of 4 (wt 4.0-5.43 kg)	—	0	16.24 $\pm$ 1.05	178 $\pm$ 13	102.9 $\pm$ 10.6	.663 $\pm$ .069	.416 $\pm$ .006	79.14 $\pm$ 4.94
<b>II. <i>P. coatneyi</i> group</b>								
KL <sub>1</sub> female 3.63 kg	355 th	thick to neg	12.4	178	27.5	3.48	1.51	97.35
KL <sub>3</sub> female 4.85 kg	233 rd	thick to neg	14.7	162	62.2	2.43	.67	88.2
KL <sub>9</sub> female 4.85 kg	194 th	.18-.86	12.28	175	43	1.8	.97	88.2
MS <sub>2</sub> male 7.53 kg	346 th	0.4-thick	16.3	165	60	2.42	.77	86.4
<b>III. <i>P. inui</i> group</b>								
SP <sub>2</sub> female 4.05 kg	461 th	.24-1.04	16.04	158	32	2.6	1.3	91.8
SP <sub>4</sub> female 4.77 kg	469 th	thick to neg	14.2	175	68	1.64	.87	80.5
SP <sub>5</sub> female 4.15 kg	461 th	.14-.52	13.46	172	41.8	1.27	.99	91.18
KL <sub>14</sub> female 4.65 kg	326 th	.32-.94	13.55	160	54	1.8	.77	78

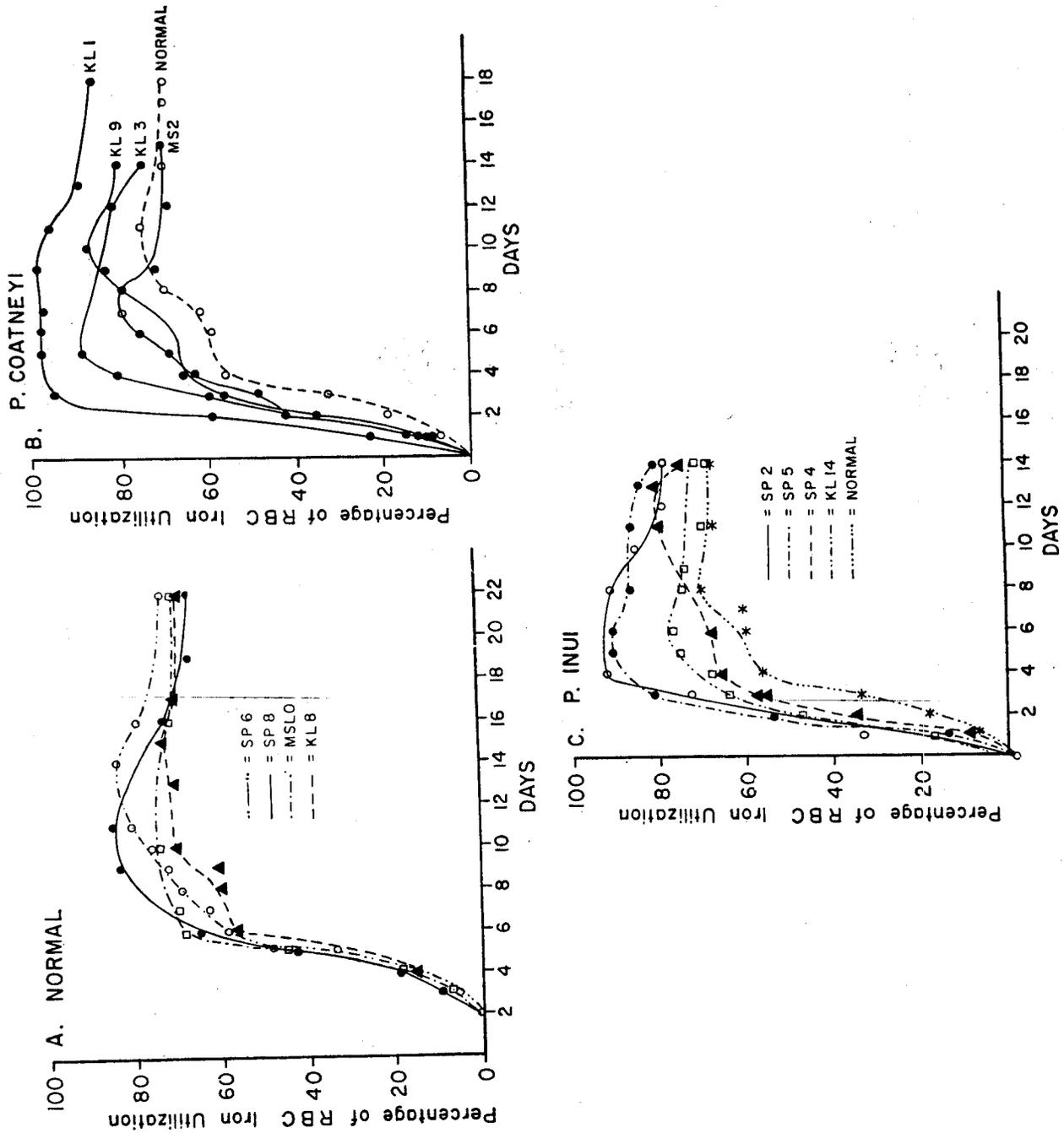


Figure 2 DIAGRAM SHOWS PER CENTAGE OF IRON UTILIZATION IN DIFFERENT GROUPS OF ANIMALS.

infected animals, and 1.91 to 3.92 times in P. inui-infected animals. Concurrently, the plasma-iron clearance rate increased, as shown in Tables 5-a and 5-b.

Red cell utilization of Iron As illustrated in Figure 2, the percentage of iron-utilization in the P. coatneyi-infected group was 23.2% to 8.86% higher than normal; and in P. inui-infected animals 16.13% to slighter higher than normal. Moreover, the radioactivity appeared to reach the peak more rapidly than normal. The uptake curves of all infected animals are some what steeper with an ensuing longer plateau than normal.

Red cell survival data As shown in Table 4 and Figure 3, the values of the  $Cr^{51}$  half-survival time in the P. coatneyi group was markedly reduced to 1.7 to 3.02 times that of normal and 1.4 to 2.6 times of normal in the P. inui-infected animals. The rate of red-cell destruction increased 1.75 to 3.05 times normal, and 1.4 to 2.6 times in P. coatneyi and p. inui-infected groups, respectively.

In the P. inui group, parasitemias were higher during chronicity than in the P. coatneyi group. It is interesting to note that the survival of red cells in P. inui group was somewhat longer than in P. coatneyi, although the percentage of iron utilization was not higher than in P. coatneyi. The ferrokinetic data indicated that there was a hyperactive erythropoiesis in these infected animals. Collateral results of blood and bone marrow examinations further indicate an erythroid hyperplasia and reticulocytosis. Obviously there is no impairment of bone-marrow function. We are investigating the cause of the abnormal shortening of the red-cell life span.

#### Summary

A definite shortening of erythrocyte survival in M. mulatta with chronic infections of P. coatneyi and P. inui was observed. Active erythropoiesis balanced any anemia that might caused by this phenomenon.

TABLE 4. Results of red-cell survival studies in normal, P. coatneyi and P. inui-infected rhesus monkeys.

Animal number	Day of infection	% Parasitemia during period of study	Red cell mass ml/kg	$Cr^{51}$ half survival Time (day)	Rate of red destruction	Bone-marrow myeloid erythroid ratio	% Reticulocytes
I. Normal group (Mean values) from 8 (wt 4.63-9 kg)	—	0	16.42 $\pm 1.89$	17.4 $\pm .2$	3.95 $\pm .001$	1.9:1	0.2-1.2
II. <u>P. coatneyi</u> group							
KL <sub>1</sub> female 3.83 kg	355 th	thick to neg.	12.4	8	8.6	1:1.4	1.2-3.4
KL <sub>3</sub> female 4.85 kg	233 th	thick to neg.	14.7	10.20	6.7	1:2	2.2-6.2
KL <sub>9</sub> female 4.85 kg MS <sub>2</sub> male 7.53 kg	194 th 346 th	.18-.86 thick-.4	12.7 16.3	7 5.75	9.9 12.05	1:2 1:2	3.9-6.9 1.6-6.9
III. <u>P. inui</u> group							
SP <sub>2</sub> female 4.05 kg	461 th	.24-1.04	16.04	6.75	10.2	1:1.7	2.8-3.6
SP <sub>4</sub> female 4.77 kg	489 th	thick to neg.	14.2	12.75	5.4	1:1.2	.7-4.0
SP <sub>5</sub> female 4.15 kg	461 th	.14-.52	13.46	9	7.7	1:1.7	3.0-3.1
KL <sub>14</sub> female 4.85 kg	326 th	.32-.94	13.55	7.5	4.3	1:1.3	2.8-3.6

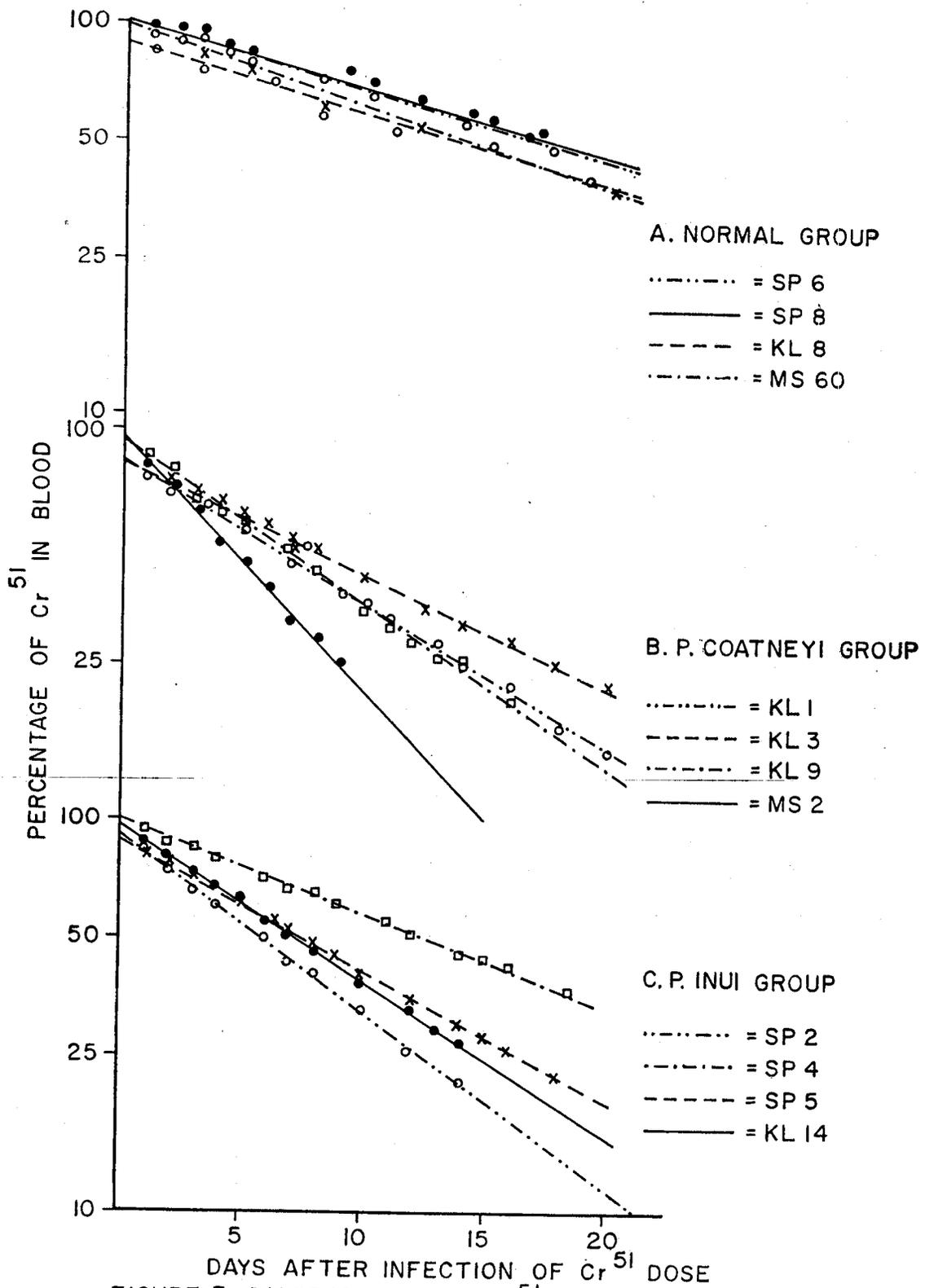


FIGURE 3 DIAGRAM SHOWS Cr<sup>51</sup> HALF - SURVIVAL TIME

TABLE 5—a. Summary of Results in simultaneous studies of Cr<sup>51</sup> and Fe<sup>59</sup>

Animal number	Clinical condition	Erythrokinetic studies				R.B.C. Survival	
		Plasma half-clearance time	Plasma iron Turnover rate	Plasma iron clearance rate	Appearance rate in erythrocytes	Cr <sup>51</sup> half-survival Time	rate of rbc destruction
Normal	Normal	110 min.	.663	.416	50% at 72 hrs, 75% peak at 5 days	17.4	3.95%
P. coatneyi KL <sub>1</sub>	Spleen intact, chronic phase of very low grade parasitemia. anemia 1+—2+	shortened 4 x normal	5.2 x increase	3.5 x increase	increase 23.2% steeper than normal with long plateau	2.2 x shortened	2 x increase
KL <sub>3</sub>	splenectomized chronic phase of every low grade parasitemia, anemia 2±	shortened 1.8 x normal	3.7 x increase	1.61 x increase	increase 11.4% with long plateau	1.7 x shortened	1.7 x increase
KL <sub>9</sub>	splenectomized with chronic phase of low grade parasitemia. Anemia 2+—3+	shortened 2.6 x normal	2.71 x increase	2.4 x increase	increase 11.4% steeper than normal with long plateau	2.5 x shortened	2.51 x increase
MS <sub>2</sub>	spleen intact, chronic phase of low grade parasitemia. Anemia 1+	shortened 1.83 x normal	3.65 x increase	1.85 x increase	increase 8.86% with steeper curve	3.02 x shortened	3.05 increase

TABLE 5—b. Summary of Results in simultaneous studies of Cr<sup>51</sup> and Fe<sup>59</sup>

Animal number	Clinical condition	Erythrokinetics studies					RBC Survival	
		Plasma Fe <sup>59</sup> half-clearance	Plasma iron Turnover rate	Plasma iron clearance rate	Appearance rate in erythrocytes	Cr <sup>51</sup> half-survival time	rate of rbc destruction	
P. inui SP <sub>2</sub>	spleen intact with chronic phase of low grade parasitemia. anemia 1+	shortened to 3.44 x normal	3.92 x increase	3.12 x increase	increase 16.13% steeper than normal	2.6 x shortened	2.6 x increase	
SP <sub>4</sub>	spleen intact with chronic phase of low grade parasitemia. no anemia.	shortened 1.62 x normal	2.5 x increase	2.1 x increase	a little higher than normal	1.4 x shortened	1.4 x increase	
SP <sub>5</sub>	spleen intact with chronic phase of low grade parasitemia. anemia 1+	shortened 2.63 x normal	1.91 x increase	2.4 x increase	increase 15.24% steeper with long plateau	1.9 x shortened	1.4 x increase	
KL <sub>14</sub>	spleen intact with chronic phase of low grade parasitemia. anemia 2+—3+	shortened 2.04 x normal	2.71 x increase	1.85 x increase	steeper curve than normal with long plateau	2.32 x shortened	2.35 x increase	