

9. Title: Mechanisms of Anemia in Chronic malaria; Plasmodium coatneyi and Plasmodium inui in rhesus monkeys.

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Period of Report: 1 April 1968 — 31 March 1969

OBJECTIVE

The objective of this study is to elucidate the mechanisms and etiology of anemia developed in chronic malaria caused by Plasmodium coatneyi and Plasmodium inui in rhesus monkeys.

DESCRIPTION

Anemia is undoubtedly the most common pathologic consequence of malaria. In the primary phase, when parasitemia is high, it is possibly due to erythrocyte destruction by multiplying parasites. In chronic malaria, however, the anemia often persists and is inappropriate to the very scanty parasitemia present at this stage.

Zuckerman in a series of papers suggests that the anemia of chronic malaria is caused by an autoimmune reaction. Other workers might not accept this theory, or do not even believe that inappropriate anemia is present, since its detection has been based on complicated enumerative procedures, the precision of which can be questioned.

Our earlier studies on the chronic malarias due to Plasmodium coatneyi and Plasmodium inui, involving the simultaneous use of Radioactive Cr⁵¹ and Fe⁵⁹ show definite shortening of Cr⁵¹ half survival time combined with hyperactive erythropoiesis and an increase of body iron utilization. The results of blood and bone marrow examinations further indicate erythroid hyperplasia and reticulocytosis.

Apparently there is no impairment of the bone marrow function, since the bone marrow is hyperactive, probably to compensate for the anemia developed due to hemolysis in the circulation of these chronically infected monkeys. These animals had an almost negligible parasitemia for more than 180 days prior to the studies, a circumstance unlikely to result in destruction of a sufficient number of red cells to cause the degree of anemia encountered.

Our further investigations, presented here, are the results of a comparison of the red cell survival times of normal red cells in chronically infected animals, and the red cells of chronically infected animals transfused into normal monkeys.

Three groups of animals were studied; one group of five normal animals, two chronic P. coatneyi, and two chronic P. inui infected animals. These four chronic infected animals were subjects in simultaneous studies of Cr⁵¹ and Fe⁵⁹ as previously reported in the SMRL Annual Report, 1968.

All the techniques in radioisotopes studies, blood tests, and bone marrow examinations are the same as mentioned in the previous Annual Report.

All five normal animals were studied for red cell survival by using Cr⁵¹ tagged autologous red cells, and were given one course of Chloroquine 25 mg (base)/kg, body weight i. m. for three days before running the survival tests reported here.

Each normal was paired with one infected animal, and a reciprocal exchange of labelled erythrocytes was performed. One normal animal was given normal red cells as a control. The pairs employed are shown in Table 1.

Table 1 Experimental pairing of monkeys.

First Member		Second Member (Normal animals)
<u>P. coatneyi</u>	KL ₁	PK ₂₂
	KL ₃	PK ₂₄
<u>P. inui</u>	SP ₂	MS ₆₃
	SP ₄	MS ₆₀
Normal	SP ₆	MS ₆₀

Routine human blood cross matching was employed. As previously reported for other monkeys in the colony, all animals are type O, Rh negative. An addition cross matching in each pair between serum and cells revealed no incompatibility. Each animal received 8 ml of Cr⁵¹ labelled red cells tagged with 30 microcuries of Cr⁵¹.

PROGRESS

Results of the studies are summarized in Table 2A and 2B, Figure 1, 2 and 3 respectively.

All infected animals suffered from long term chronic malarial infections with hematocrits between 61.7—79% of normal values, and hemoglobins of 47—81% of normal values. Each of these animals had a low grade parasitemia (negative, thick film to 1%) so the anemia developed here is incommensurate with the amount of parasitized red cells. In the normal animals with injections of Chloroquine, parasites are absent throughout the tests.

The survival of normal red cells in the chronically infected animals are very interesting showing definitely shortened $T_{\frac{1}{2}}$ values of 7.3, 7.0, 7.0 and 3.5 days respectively in KL₁, KL₃, SP₂ and SP₄, values similar in most animals to those of autologous cells. Thus the clearance rates were 2.2 times that of normal survival in KL₁, KL₃, and SP₂, and 4.6 times normal in SP₄. However, the survival time control in the normal monkey SP₆ is 15.5 days, 2 days shorter than with autologous cells, as shown in Table 2—A and Figure II a—b.

Contrarily, the survival times of infected cells in normal animals indicate approximately normal life spans as shown in Figure 1 a—b. Table 2—B and Figure 3. Thus, there is definite prolongation of survival of erythrocytes from infected animals after transfusion to normal animals. Judging by all these findings, it seems quite clear that red cell destruction in these animals is not due to a defect of the erythrocytes per se. It would appear, then, that there is destruction of both normal and parasitized red cells in the circulation of these chronically infected animals. Since the patterns of destruction of red cell from infected animals in normal animals all shows an essentially normal red cell life span, with a shortened life span of normal red cells in infected animals, the phenomenon can be categorized as an extrinsic defect resulting in acquired hemolytic anemia. However, in a preliminary experiment, transfusion of serum from a chronically infected animal into a normal animal showed no change in the red cell survival time. Thus, if there is a humorally mediated hemolytic process, it has thus far evaded detection. The possibility of cell destruction by a hyperplastic reticuloendothelial system must also be entertained. This has been previously stressed by George and other workers. However, the splenectomized animals in the present study showed no difference in red—cell life span as compared to animals with intact spleens.

TABLE 2 A

Survival of Cr-51 labelled normal Erythrocytes in Monkeys with Chronic Malaria

Animal Number	Clinical condition	% parasitemia during test period	Using autologous labelled red cells		Using normal labelled red cells		Bone-marrow myeloid: erythroid	%Reticulo-cytes
			Cr-51 $T_{1/2}$, days	rate of rbc destruction (per day)	Cr-51 $T_{1/2}$, days	rate of rbc destruction (per day)		
<u>P. coatneyi</u> KL ₁ female 3.83 kg	spleen intact anemia 1+—2+	thick to neg	8.0	8.6	7.3	9.6	1:1.4	1.2—3.4
KL ₃ female 4.85 kg	splenectomized anemia 2+	thick to neg	10.3	6.7	7.0	9.9	1:2	2.2—6.2
<u>P. inui</u> SP ₂ female 4.05 kg	spleen intact anemia 1+—2+	.02—1 to thick	6.8	10.2	7.0	9.9	1.7:1	2.8—3.6
SP ₄ female 4.8 kg	spleen intact anemia 1+	thick to neg	12.8	5.4	3.5	19.8	1:1.2	.7—4.0
Normal SP ₆	spleen intact	—	17.5	4.0	15.5	4.5	2.2:1	1—1.2
Average of 6 normal monkeys	—	—	17.4 (SD ± 2.1)	4.0 ± (SD ± 0.4)	—	—	1.9:1 1.7:1	0.2—1.2

TABLE 2 B

Survival of Cr-51 labelled erythrocytes from monkeys with chronic malaria in normal monkeys.

Animal Number	Clinical condition	% parasitemia during test period	Using autologous labelled red cells		Using labelled red cells from chronic infected		Bone-marrow myeloid-erythroid ratio	% Reti-culocytes
			Cr-51 $T_{1/2}$, days	rate of rbc destruction (per day)	Cr-51 $T_{1/2}$, days	rate of rbc destruction (per day)		
MS 60 Male 5.4 kg	normal spleen intact	0	16.0	4.3	15.8	4.4	1.7:1	.2-1.2
MS 63 Male 2.7 kg	normal spleen intact	0	15.0	4.6	14.5	4.8	2:1	.4-2
PK 22 Male 3.7 kg	normal splenectomized	0	16.0	4.3	15.85	4.4	1.7:1	.2-1.8
PK 24 Male 3.8 kg	normal splenectomized	0	14.5	4.8	13	5.3	1.8:1	.2-2.2
Average	—	0	15.4	4.3	14.5	4.8	—	—

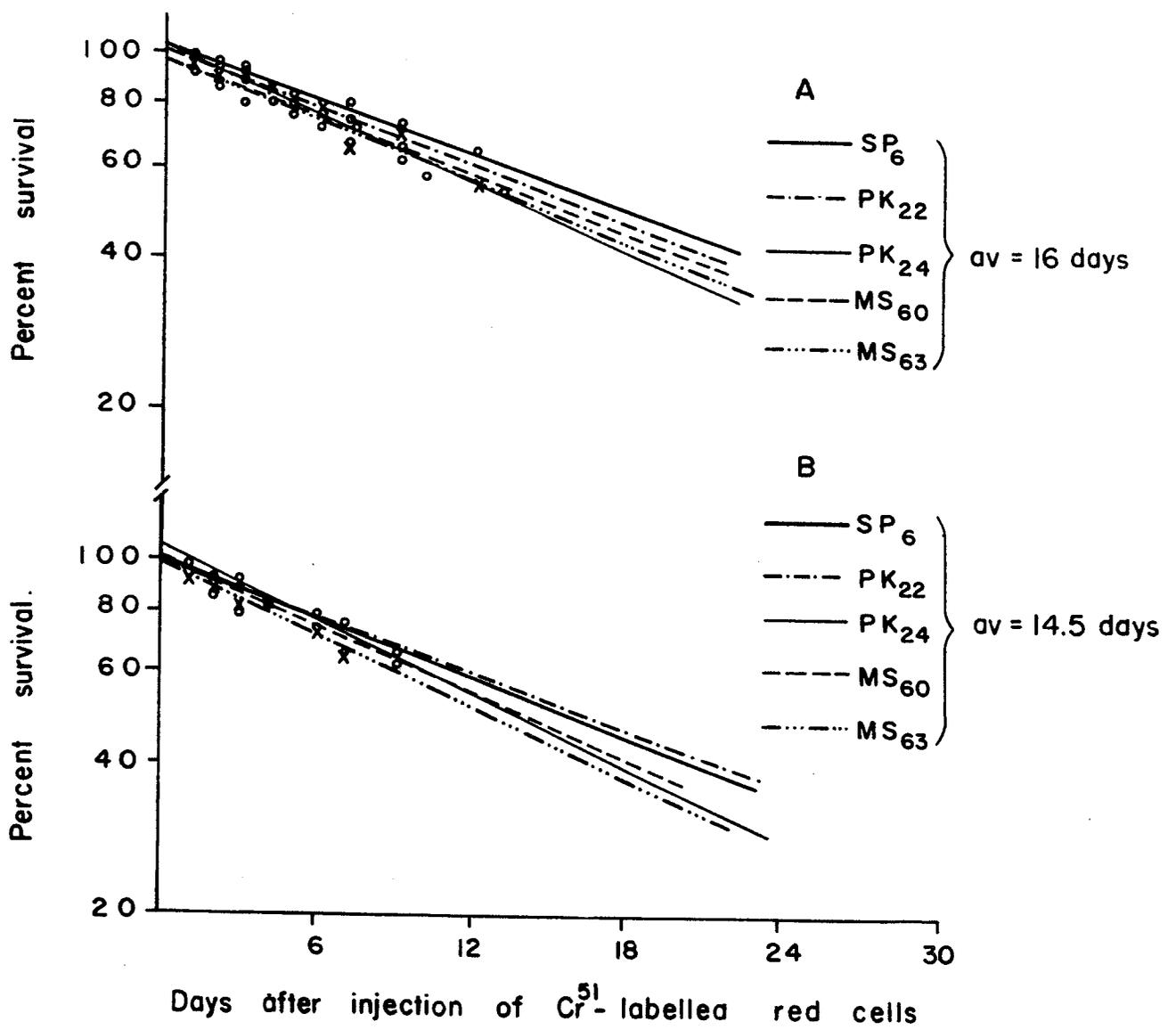


Figure 1A: Cr⁵¹ survival of normal animals using autologous red cells.

B: Cr⁵¹ survival of erythrocytes from chronic infected monkeys into normal monkeys.

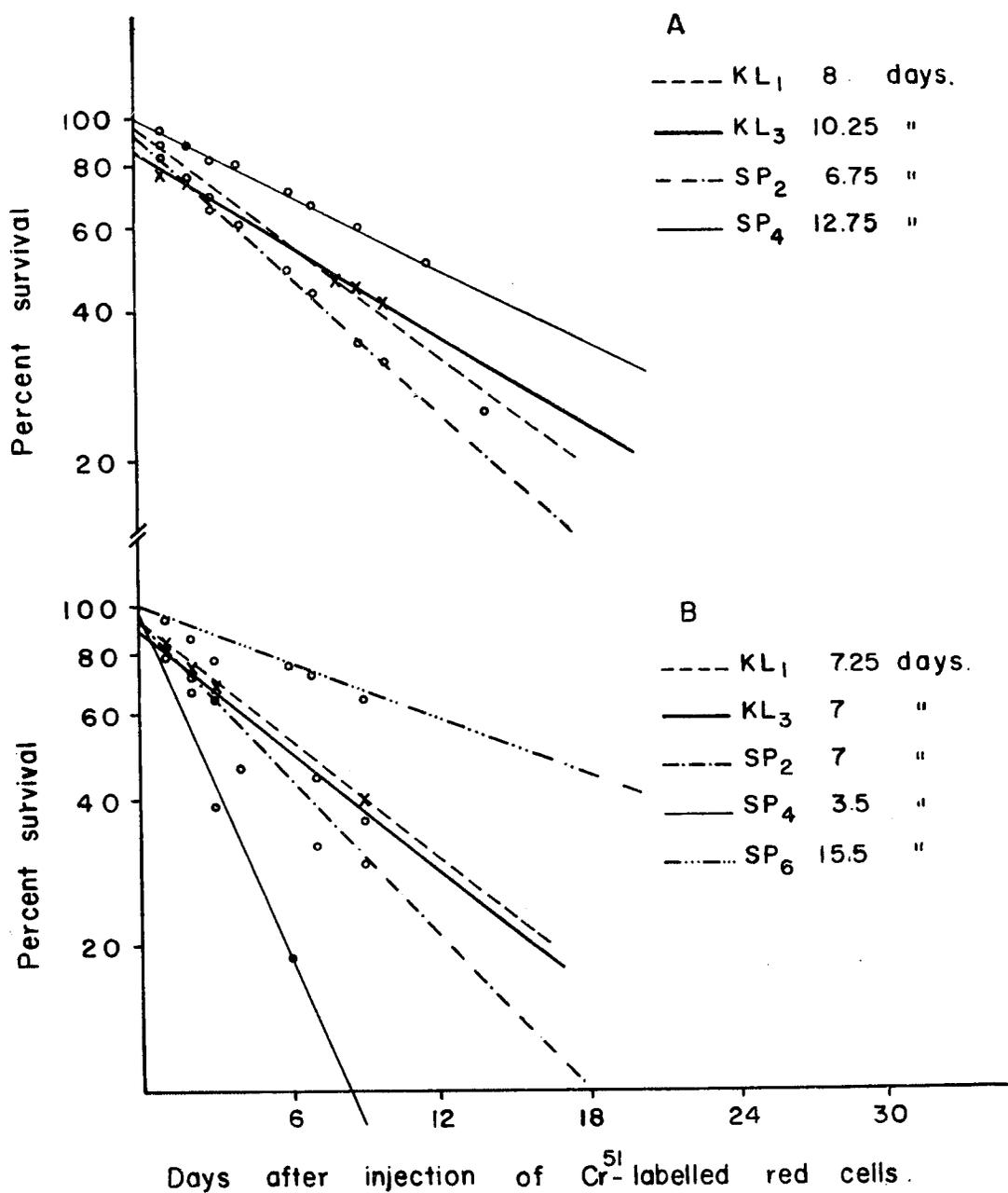


Figure II A: Cr⁵¹ of chronic infected monkeys using autologous red cells.

B. Cr⁵¹ survival of normal - labelled erythrocytes transfused into chronic infected monkeys, with comparison of SP₆ normal animal.

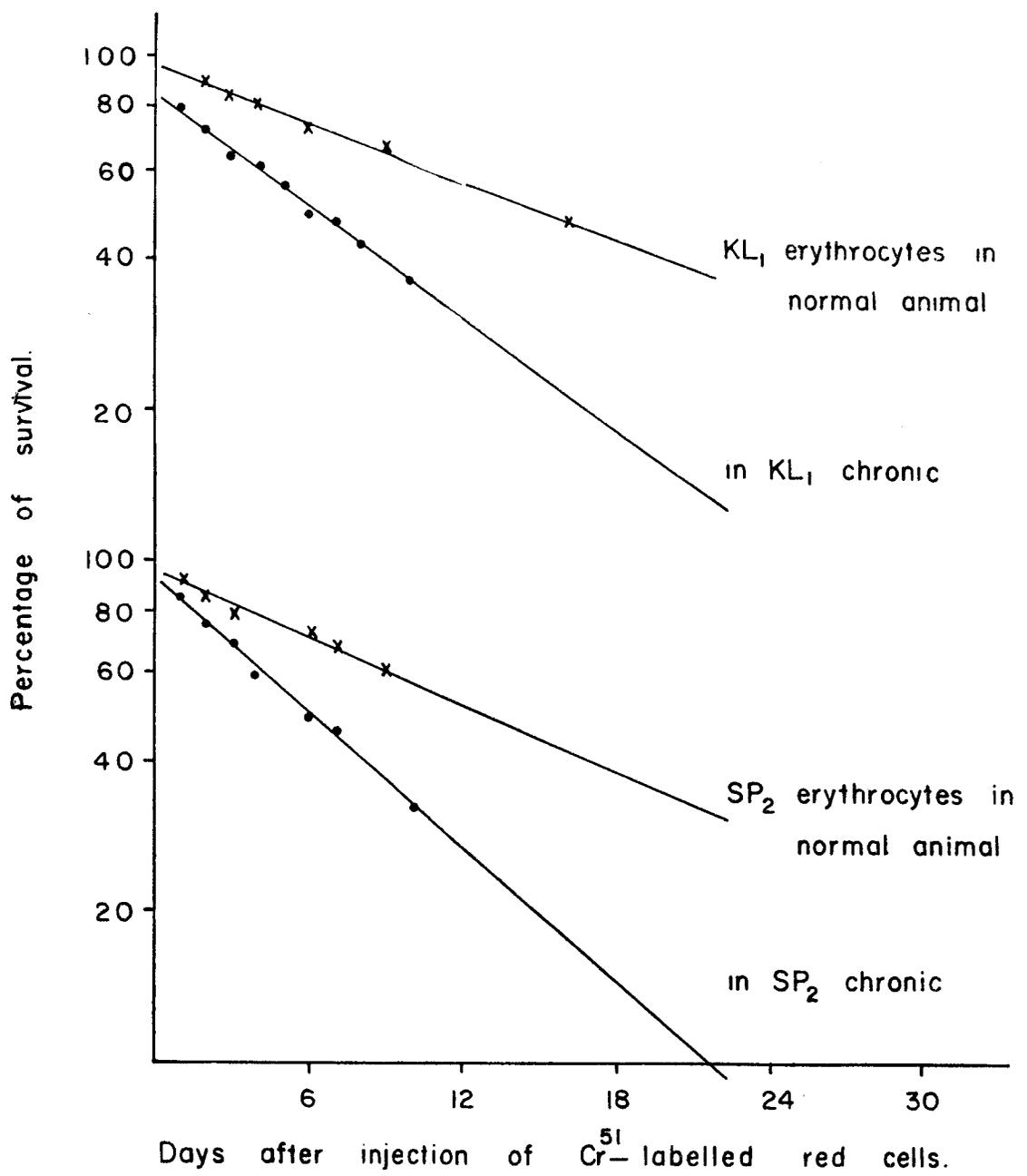


Figure III

Comparison between the results of Cr^{51} survival of erythrocytes from chronic malarial infection P. coatneyi and P. inui transfused into normal with their own red cell survival.

SUMMARY

- a) Normal erythrocytes survived for a shorter time in four monkeys with chronic malaria (two P. coatneyi and two P. inui) than in a normal control monkey.
- b) In contrast, the survival time of erythrocytes from monkeys with chronic malaria in normal animals was similar to that of normal heterologous erythrocytes.
- c) In a preliminary experiment, no change in the rate of erythrocyte destruction could be demonstrated on transfusion of a normal monkey with serum from animals with chronic malaria.
- d) These studies suggest that the inappropriate erythrocyte destruction observed in these animals with chronic malaria cannot be adequately explained by defect in the erythrocyte; however, no evidence for a humoral mediator has been obtained.