

Title: Hypovolaemia in Plasmodium Coatneyi malaria

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Introduction There is a growing body of evidence that hypovolaemia may occur during acute infection in many malarias. This has been documented by a sudden rise in haematocrit (haemoconcentration) and by actual determination of the plasma volume. Haemoconcentration associated with an acute hypotensive episode was described in a patient with acute falciparum malaria by Kean and Taylor (1964). Feldman and Murphy (1945) observed a contracted plasma volume and anaemia accompanied by the sudden onset of shock in a patient with induced falciparum malaria. A decreased plasma protein concentration led them to postulate an increased capillary leakage of protein. During the terminal phase of P. knowlesi malaria in the rhesus, haemolytic anaemia and a fall in plasma volume and plasma protein concentration has also been demonstrated (Overman and Fellman, 1964). Occasionally during the progressive parasitaemia early in knowlesi malaria, the animals had a sudden rise in haematocrit (haemoconcentration) and developed hypotension (Skirrow, 1962 and Chongsuphajaissiddhi, 1966).

Recently we have shown that in P. coatneyi malaria haemoconcentration was associated with hepatic and renal dysfunction and overt signs of illness in the animals (Desowitz *et al.*, 1967). The present study was undertaken to measure the changes in blood volume in P. coatneyi malaria and to relate these to events in the schizogonic cycle. The importance of hypovolemia was estimated by the magnitude of the reduction in blood volume, changes in arterial pressure, and chemical evidence of alterations in kidney and liver function.

Methods Six North Indian (KL) and 8 West Pakistani (PK) rhesus monkeys (Macaca mullatta) were studied. Tuberculin skin tests performed on each animal were negative and repeated blood smears showed all to be free of natural malaria. With two exceptions the animals were splenectomized at least 3 weeks before any studies. These two (KL 16 and KL 20) were studied during the control period both before and after splenectomy.

The monkeys were infected intravenously with approximately 10^8 parasites of P. coatneyi. The infective dose in PK 8 and PK 10 was unknown. Thick and thin malaria smears, haematocrit, and rectal temperature were obtained daily at 8:00, 11:30, and 16:30 and more frequently at certain critical periods. Early in the infection urine was collected continuously for haemoglobin. The determination of the plasma volume, red cell mass, arterial pressure, and plasma chemistries were performed prior to and at varying intervals after the infection. Four monkeys (2 PK and 2 KL) had 4-7 repeated studies prior to infection.

Procedure for Determination of Blood volume All experiments were performed on fasting animals. Water intake was ad libitum. Three ml of venous blood were delivered to a sterile tube containing 0.9 ml of an ACD solution (sodium citrate, glucose and citric acid) for tagging of the red cells with Cr^{51} to measure the red cell mass. The blood was centrifuged at 1800 r.p.m. and the plasma discarded. Ten to 15 μC of Cr^{51} were added to the packed cells and incubated for 30 minutes at room temperature. For the initial study, 10 μc of Cr^{51} were employed. During repeat studies more isotope was used. The red blood cells were washed three times and resuspended in isotonic saline. After gentle mixing 2 ml were drawn into a disposable plastic syringe for reinjection into the monkey and 0.5 ml into two other syringes as the standards. Each syringe was attached to a 3-way stopcock and weighed on an analytical balance.

The animal was anaesthetized by an intravenous injection of a 3:1 mixture of thiopental and pentobarbital. The experiment was begun after the animal had been asleep for 30 minutes.

To correct for any circulating isotope from previous experiments (whole blood background) 1.4 ml of blood were obtained from the femoral vein and delivered to a test tube containing .5 mg of dried heparin. Two microhaematocrits were obtained directly from the residual blood in the syringe and two from that in the test tube. After thorough mixing, 0.5 ml of whole blood were pipetted in duplicate into counting tubes. Saponin power was added to haemolyze the red blood cells. The haemolyzed blood was set aside for later counting.

The Cr⁵¹ tagged red blood cells were injected through a venous catheter. The stopcock valve was again closed to the injection syringe. The residual tagged red blood cells in the stopcock and catheter were washed into the animal with 3 ml of isotonic saline. The stopcock was disconnected from the catheter and thoroughly dried. The stopcock together with the empty syringe was reweighed in order to calculate the radioactivity administered.

At 15, 25, and 35 minutes after the injection, 1.4 ml of blood were obtained from the femoral vein opposite to the injection site. These samples were then treated in the same manner as that described above for the whole blood background.

Upon completion of the red cell mass determination, plasma volume was estimated by the following technique. Three ml of blood were drawn into a heparinized test tube for plasma radioactive background. Two haematocrits were determined from the test tube and 2 from the syringe. The blood was spun at 1800 rpm for 5 minutes in a refrigerated centrifuge and 0.5 ml of plasma pipetted in duplicate into counting tubes.

Human serum albumin (RISA)*, labelled with I¹³¹ was drawn into three syringe (one for injection into the animal and two for standards), and each was connected to a 3-way stopcock. In the initial study 1.75 μ C of RISA were given while in subsequent experiments the activity injected was increased. The RISA was then injected through the catheter and the residual in the catheter flushed with saline. Three ml blood samples were obtained at 15, 25, and 35 minutes. Two 0.5 ml aliquots were pipetted from each sample and set aside for later counting.

The two red blood cell standards were injected into 50 ml volumetric flasks containing saline, and the two RISA standards were injected into 100 ml flasks containing isotonic saline and 40 mg of untagged albumin. The delivery and flushing of standards were the same as for the injection. Duplicate 0.5 ml samples were pipetted into counting tubes from each of the duplicate standards.

The whole blood, plasma and standards were counted in a well scintillation counter with a NaI crystal. The counts were recorded on a Nuclear-Chicago scaler, Model 181 B. The I¹³¹ and Cr⁵¹ were counted at the same base and voltage setting. All counts were at least 10,000 above background.

The calculations for plasma volume and red cell mass were as follows:

1) Plasma Volume. The counts per minute (activity) for the plasma background was subtracted from the 15, 25, and 35 minute sample. These three activities were back-extrapolated to the time of injection (0 time) on semilogarithmic paper.

$$\text{Plasma Volume (ml)} = \frac{\frac{\text{weight of injection}}{\text{weight of standard}} \times \text{activity of standard} \times 100}{\text{activity of plasma (0 time)}}$$

* Abbott Laboratories, North Chicago, Illinois.

2) Red Cell Mass. The red cell mass was calculated for 15, 25, and 35 minutes and an average obtained.

$$\text{Red Cell Mass (ml)} = \frac{\text{weight of injection}}{\text{weight of standard}} \times \frac{\text{activity of standard} \times \text{haematocrit} \times 50}{\text{activity of whole blood}}$$

The cpm of whole blood for any sample was corrected for plasma and red blood cell background. For example, the corrected activity of whole blood at 15 minutes = activity of whole blood at 15 minutes - activity of plasma background \times (1 - haematocrit at 15 minutes) - activity of red blood cell background \times haematocrit at 15 minutes.

$$\frac{\text{The activity of the red blood cell background} = \text{activity of whole blood background} - \text{activity of plasma background} \times (1 - \text{whole blood background haematocrit})}{\text{haematocrit of the whole blood background}}$$

All haematocrits employed in these and subsequent calculations were corrected for trapped plasma from a table derived by Albert (1964).

3) Total blood volume = plasma volume (RISA) + red cell mass (Cr^{51})

4) Total body haematocrit = $\frac{\text{red cell mass}}{\text{total blood volume}}$

5) F_{cells} = $\frac{\text{total body haematocrit}}{\text{peripheral haematocrit}}$ The peripheral haematocrit was the average of 7 femoral venous specimens obtained during the blood volume study.

Certain theoretical objections may be raised to the methods employed. The use of Cr^{51} tagged red blood cells to estimate the red cell mass presented the potential problem of *in vitro* red cell lysis during the tagging with Cr^{51} . Red blood cells infected with Plasmodia have increased mechanical fragility. In an attempt to minimize lysis, low speed centrifugation and gentle mixing were employed. The close relationship between the per cent change in haematocrit and red cell mass during infection (with the exception of the hypovolaemic period) validated this method (Table 1).

The plasma volume was measured by a heterologous protein, ^{131}I labelled human serum albumin. There was no evidence of hypersensitivity such as bronchospasm or hives following its injection. Repeated determinations in uninfected animals for up to 3 1/2 months showed little variation (Table 2). The average blood volume (± 1 standard deviation) in 14 uninfected rhesus (57.6 ± 5.2 ml/kg) in this study was not significantly different than that obtained by Gregerson *et al.*, (54.1 ± 4.72 ml/kg) utilizing Evans blue and P^{32} tagged red blood cells in 18 unanaesthetized rhesus monkeys.

Arterial Pressure At the end of the plasma volume study, arterial pressure was measured through a number 19 Courmand needle. Two per cent procaine was infiltrated around the femoral artery. The Courmand needle was passed through the artery and then slowly withdrawn until arterial blood pumped through the needle. The blood pressure was transmitted to a mercury column by isotonic saline and was recorded on a kymograph.

Aseptic technique was maintained throughout the study. The red blood cells remaining in any sample were re injected into the animal. The estimated blood loss from an experiment was 8-10 ml.

Blood chemistries From the samples obtained during the blood volume studies approximately 2 ml of plasma were retained for the determination of total protein, protein electrophoresis, urea nitrogen, creatinine, glutamic-oxaloacetic transaminase (GOT), and glutamic-pyruvic transaminase (GPT). The GOT and GPT determinations were performed with the Sigma Test Kit (Sigma Chemical Co., St. Louis, Mo.). Urea nitrogen was determined by the Hyland UN Test Kit method (Hyland Laboratory, Los Angeles, California.)

Table 1. The Relation between Mean Per Cent Change in Red Cell Mass and Haematocrit during the Course of *P. coatneyi* Malaria in KL and PK Rhesus

Monkey	Time After Major Sporulation	Number Studied	Red Cell Mass Mean % Change From Control	Haematocrit Mean % Change From Control	Significance of Difference Between Means
KL	1 day	3	- 32.3	- 5.4	P < 0.01
	All other times	5	- 29.9	- 29.9	N.S.
PK	2 hours	2	- 15.6	- 12.0	-
	1 day	2	- 11.2	- 3.8	-
	2-3 days	5	- 43.0	- 43.0	N.S.
	4-6 days	6	- 57.6	- 56.5	N.S.
	7-13 days	4	- 30.2	- 28.9	N.S.
	14-20 days	4	- 30.2	- 33.1	N.S.
	21-27 days	3	- 17.1	- 19.4	N.S.
	27-34 days	4	- 13.2	- 17.4	N.S.
	42-55 days	4	- 5.5	- 10.0	N.S.

Table 2. Repeated Determinations of Total Blood Volume, Ratio of Total Body Haematocrit: Peripheral Haematocrit (Fcells), and Intravascular Albumin in Four Uninfected Rhesus

Monkey	Date	Weight kg	Total Blood Volume ml/kg	Venous Haemat- ocrit %	Fcells	Serum Albumin g/100 ml	Intravas- cular Albumin g/kg
PK 8	12 Apr 67	3.47	51.4	32.3	.876	3.2	1.18
	18 Apr 67	3.46	50.5	31.2	.891	3.1	1.13
	21 Apr 67	3.34	51.6	30.6	.876	2.5	.94
	26 Apr 67	3.38	51.6	30.5	.849	3.0	1.14
	Average S.D.**	3.41	51.3 0.5	31.2	.873 .055	2.95 .31	1.10 .10
PK 10	2 May 67	4.31	61.0	32.5	.865	3.6	1.58
	8 May 67	4.11	64.5	30.8	.854	3.5	1.66
	10 May 67	3.94	62.9	29.2	.894	3.4	1.58
	15 May 67	4.23	62.4	27.8	.863	3.7	1.76
	18 May 67	4.09	59.0	27.3	.886	3.5	1.57
Average S.D.**	4.14	62.0 2.07	29.5	.872 .017	3.54 .11	1.63 .16	
KL 16	20 Feb 67*	5.3	59.2	32.3	.833	—	—
	22 Feb 67*	5.4	56.8	29.5	.871	—	—
	28 Feb 67*	5.2	57.4	28.6	.909	—	—
	29 May 67	5.6	53.2	35.2	.832	3.3	1.24
	1 June 67	5.43	59.7	31.3	.859	3.5	1.53
Average S.D.**	5.39	57.3 2.6	31.4	.861 .032	—	—	
KL 20	24 Feb 67*	6.1	57.1	33.5	.878	—	—
	27 Feb 67*	6.1	57.5	33.6	.917	—	—
	1 Mar 67*	6.0	58.6	32.5	.975	—	—
	14 Mar 67*	6.3	50.0	35.5	.882	—	—
	17 Mar 67*	6.3	56.9	31.4	.837	—	—
	22 Mar 67*	6.0	56.2	31.5	.867	—	—
	15 May 67	6.33	55.5	35.8	.891	3.5	1.32
Average S.D.**	6.16	56.0 2.8	33.5	.892 .044	—	—	

* Non splenectomized Rhesus at the time of the study.

** Standard deviation.

Creatinine values were obtained by the Technicon Autoanalyzer. The total protein was measured by the method of Shank and Hoagland (1946). Albumin and globulin fractions were determined by microzone electrophoresis on cellulose acetate strips and analyzed by the Spinco Analytrol apparatus. The calculation of intravascular albumin was as follows:

$$\text{Intravascular albumin (g/kg)} = \frac{\text{plasma albumin (g/100 ml)} \times \text{plasma volume (ml/kg)}}{100}$$

Parasite enumeration

The parasitaemia was estimated by counting the number of rings, schizonts and total asexual parasites in 50 thin film oil immersion fields.

Results

Studies in uninfected animals. There was no significant difference in total blood volume (ml/kg), Fcells (total body haematocrit/peripheral haematocrit) and intravascular albumin (g/kg) between the 6 KL and 8 PK monkeys (Table 3). For the 14 uninfected rhesus the mean (± 1 standard deviation) total blood volume was 57.6 ± 5.2 ml/kg and the mean Fcells was 0.869 ± 0.021 . The weight, haematocrit, and plasma albumin were higher in the KL than in the PK monkeys (Table 3).

Table 3. Average Data in 8 PK and 6 KL Uninfected Splenectomized Rhesus During the Initial Study

	Weight kg	Red Cell Mass ml/kg	Plasma Volume ml/kg	Total Blood Volume ml/kg	Venous* Haemat- ocrit %	Fcells	Serum Albumin g/100 ml	Intravascu- lar Albumin g/kg
Average 8 PK	3.47	16.0	42.7	58.7	31.2	.871	2.75	1.16
S.D.**	.57	1.7	4.2	5.4	2.3	.016	.55	.21
Average of 6 KL	5.57	17.1	39.1	56.2	35.2	.867	3.38	1.33
S.D.**	.90	1.2	4.9	5.2	2.6	.028	.26	.26
Significance of Difference Between PK & KL	< .01	N.S.	N.S.	N.S.	< .02	N.S.	< .02	N.S.

* The haematocrits are corrected for trapped plasma.

** Standard deviation.

Repeated determinations of the blood volume, Fcells, plasma albumin, and intravascular albumin in two PK and two KL monkeys over a period ranging from 14 days to 3 1/2 months showed relatively little variation and no progressive change (Table 2). As a result of blood loss during the experiment, there was a slight decrease in the haematocrit (Table 2) and red cell mass. The compensatory rise in plasma volume maintained a constant total blood volume during the repeated experiments.

The peripheral venous haematocrit fell following anaesthesia in all animals and stabilized at the lower level after the animal was asleep for 15–30 minutes. The mean (± 1 standard deviation) decrease in haematocrit expressed as per cent change from the pre-anaesthetic value was 13.9 ± 4.8 in 66 experiments. This decrease in haematocrit in the control animals was not significantly different than in infected animals. In addition, there was no correlation between per cent decrease in haematocrit during anaesthesia and the F cells.

Course of parasitaemia The parasitaemia exhibited a high degree of tertian synchronicity early in the infection. Sporulation usually occurred around 13:00. There was a logarithmic progression of peak peripheral parasitaemia over the first three cycles (Fig. 1). The parasitaemia was usually highest following the third sporulation (between 700 and 6500 parasites per 50 thin film fields), and this period will be referred to as the "major" sporulation. The synchrony was usually evident for the succeeding cycle, and then was difficult to discern. There was no difference in the pattern or level of parasitaemia between KL and PK monkeys (Fig. 1). The number of schizonts in the peripheral blood increased logarithmically from the second to the fourth cycle and then decreased in number. The highest count occurred just prior to sporulation. The number of schizonts in the peripheral blood accounted for only a fraction of the total schizonts in the body. For example, on the morning before the major sporulation the average schizont count was 9 per 50 thin film fields. Following sporulation there were 3400 young rings per 50 thin film fields. In one animal, KL 16, there was evidence of infection with a double brood (Fig 2). In those that survived, the parasitaemia was again synchronized approximately 2 weeks after major sporulation with a maximum parasitaemia of about 100 parasites per 50 thin film fields. During this period the sporulation occasionally occurred as late as 18:00.

Anaemia The anaemia was first observed after the major sporulation and in those that survived, the red cell mass was lowest 4–6 days later. Haemoglobinuria accompanied the rapidly falling haematocrit during the four days following major sporulation.

Mortality All six KL monkeys died during the acute infection whereas only 2 out of 8 PK monkeys died. The PK and KL monkeys were infected with equal inocula of *P. coatneyi* and were studied during the same period (March to June, 1967). Since no other obvious difference in handling of the animals was evident, the difference in mortality reflected a basic difference in host response. Because of this the data will be analyzed separately for the PK and KL rhesus.

Blood Volume, Arterial, Pressure and Plasma Chemistries in the Infected KL Rhesus

The experimental data for 5 of the KL monkeys are given in Table 4 and summarized in Table 5. The three animals studied at the time of major sporulation failed to show any change in blood volume, blood pressure, intravascular albumin, or plasma chemistries.

Three monkeys (KL 5, 15, and 20) studied 24 hours after major sporulation showed a marked fall in blood volume (11–27 per cent) which was accompanied by hypotension. There was a relatively minor fall in haematocrit (5 per cent) in spite of a 32 per cent decrease in the red cell mass (Table 1). The plasma volume remained unchanged in one and decreased in the other two. The three monkeys had a fall in intravascular albumin, especially marked in KL 5 and KL 15 (36 and 30 per cent, respectively). There was an elevation of plasma creatinine, urea nitrogen, and transaminases in all three animals (Tables 4 and 5). KL 5 and 15 were lethargic on the morning of the experiment and died a few hours after the study. KL 20 died the following night. A fourth monkey, KL 11, died 18 hours after major sporulation before any study could be performed.

In KL 16 there was early sporulation because of a double brood infection (Fig. 2). At the time of the study the parasitaemia was 2400 per 50 thin film fields. The plasma creatinine was 3.9 mg per cent. There was no change in total blood volume, arterial pressure, or intravascular albumin, and the red cell mass was only mildly reduced (from 15.7 to 12.2 ml/kg) (Tables 4 and 5). A few hours following the

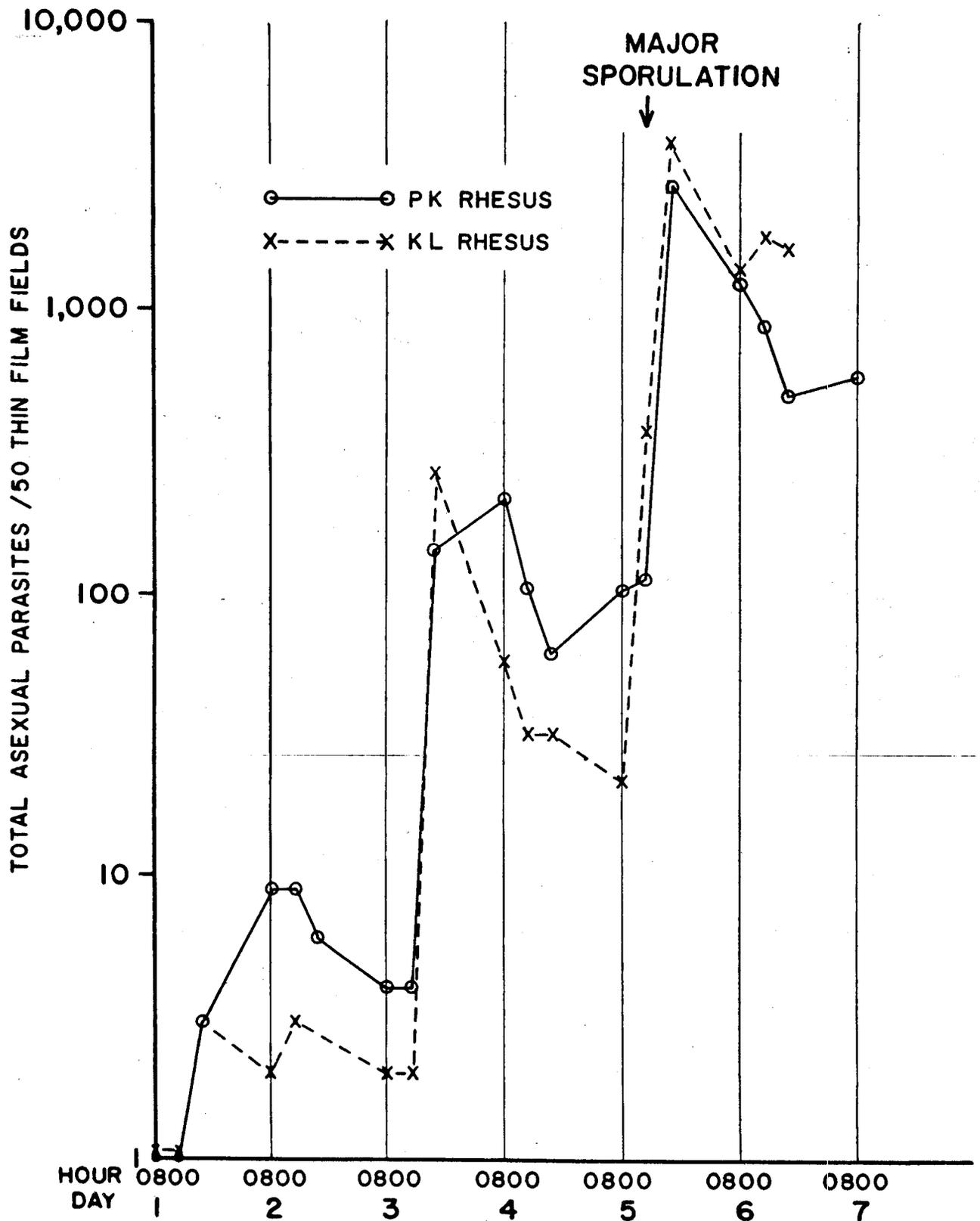


Figure 1. The course of parasitaemia in PK and KL monkeys infected with *P. coatneyi*. (Note the synchronized tertian periodicity.)

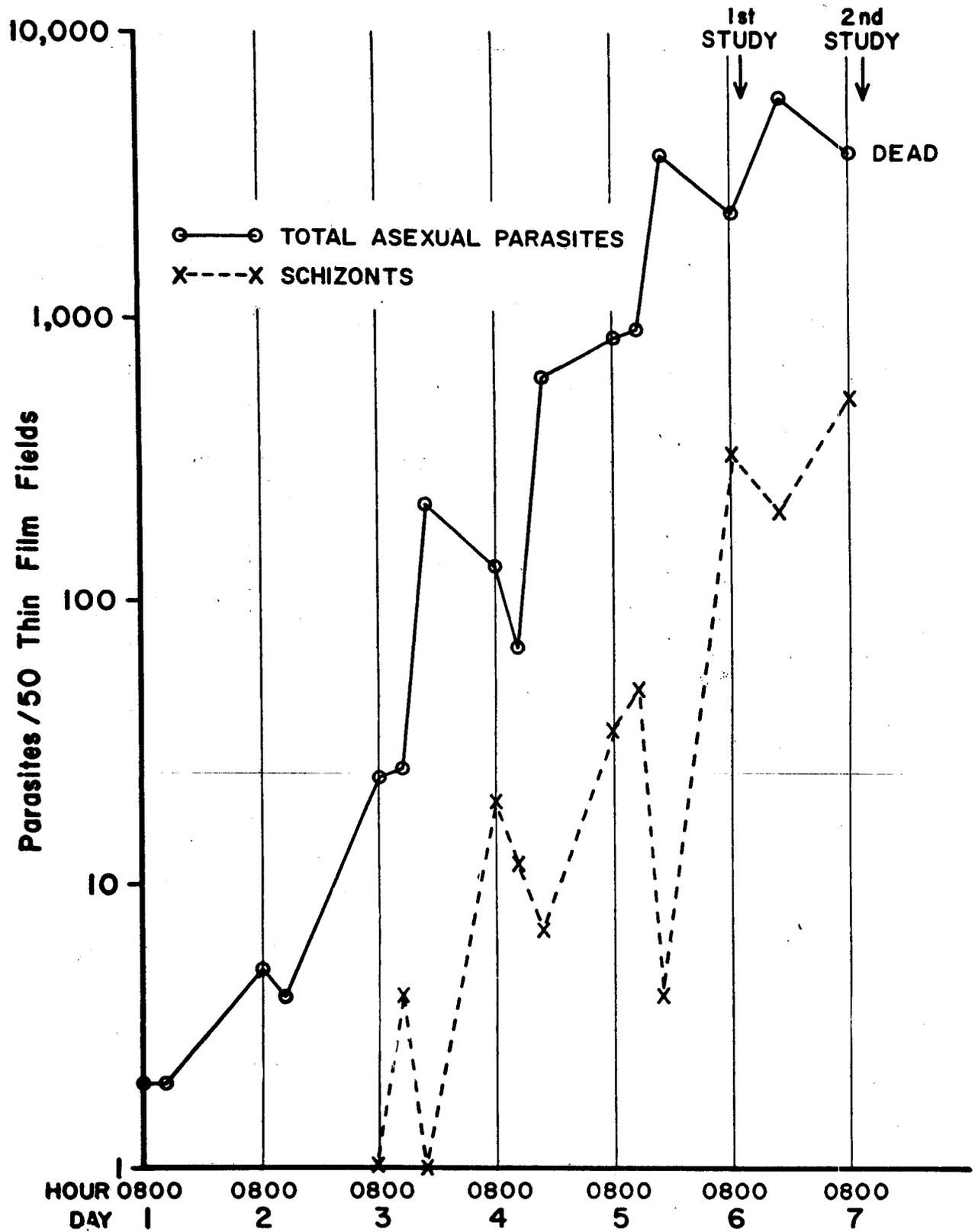


Figure 2. A double brood infection with *P. coatneyi* in rhesus KL 16. Each day there is a rise in total parasitaemia and a rise and fall in schizonts.

Table 4. The Blood Volumes, Arterial Pressures, and Plasma Chemistries in the KL Rhesus Before and After Infection with *P. coatneyi*

Monkey	Stage of Infection	Weight kg	Red Cell Mass ml/kg	Plasma Volume ml/kg	Total Blood Volume ml/kg	Venous Haemat- ocrit %	Fcells	Intra- vascular Albumin g/kg	Mean Arterial pressure mm Hg	Plasma Creati- nine mg/100ml	Plasma GOT SF units
KL 5	Control	6.92	15.9	34.1	50.0	35.2	.903	1.16	112	1.4	26
	M.S.*	7.06	—	34.3	—	36.0	—	1.30	137	1.6	28
	24 hr. after M.S.	6.96	11.0	25.4	36.4	34.0	.888	.74	66	6.0	119
KL 15	Control	4.83	17.4	40.9	58.3	34.6	.864	1.35	137	0.9	22
	Control	4.71	16.1	42.2	58.3	31.1	.887	1.35	107	1.0	25
	M.S.	4.63	16.0	47.4	63.4	32.0	.788	1.56	111	1.1	26
	24 hr. after M.S.	4.66	10.8	41.2	52.0	29.7	.700	0.95	84	1.8	54
KL 20	Control	6.33	17.7	37.8	55.5	35.8	.891	1.32	134	1.1	24
	24 hr. after M.S.	6.42	12.2	31.2	43.4	34.7	.810	1.12	84	5.1	69
KL 16	Control	5.6	15.6	37.6	53.2	35.2	.832	1.24	116	1.1	27
	Control	5.43	15.9	43.7	59.6	31.3	.853	1.53	—	1.0	26
	Before 6 th Spor.	5.87	12.2	43.1	55.3	24.4	.906	1.42	102	3.9	26
	Before 7 th Spor.	5.67	8.2	41.4	49.6	17.4	.948	1.37	died at end of exp.	12.0	194
KL 17	Control	5.13	17.2	48.1	65.3	31.2	.843	1.83	126	1.0	19
	Control	5.33	15.3	38.9	64.1	27.8	.856	1.62	—	1.0	18
	3 days after M.S.	5.25	6.6	53.4	60.0	12.7	.853	1.71	92	1.4	82
	4 days after M.S.	5.40	3.1 (†)	55.6	58.7	6.4	—	1.67	died at end of exp.	2.6	130
KL 11	Control	4.63	18.8	36.2	55.0	39.3	.870	1.08	112	1.2	18
	1 hour after M.S.	4.41	15.9	42.4	58.3	33.2	.819	1.44	109	1.1	26

* M.S. = major sporulation

** The haematocrits are corrected for trapped plasma

Table 5. Changes in Blood Volume, Arterial Pressure and Chemistries Between the Control Period and Acute Infection in 5 KL Rhesus

Monkey	Stage of Infection	Red Cell Mass	Plasma Volume	Total Blood Volume	Intra-vascular Albumin	Arterial Pressure	Plasma Creatinine	Plasma Transaminases
KL 5	24 hours after major sporulation	↓*	↓	↓↓	↓↓	↓	↑↑	↑↑
KL 15	24 hours after major sporulation	↓	no change	↓↓	↓↓	↓	↑	↑
KL 20	24 hours after major sporulation	↓	↓	↓↓	↓	↓	↑↑	↑
KL 16	Before 6th sporulation	↓	↑	no change	no change	no change	↑↑	no change
	Before 7th sporulation	↓↓	↓	↓	no change	died at end of exp.	↑↑↑	↑↑
KL 17	3 days after major sporulation	↓↓	↑	↓	no change	↓	↑	↑
	4 days after major sporulation	↓↓↓	↑	↓	no change	died at end of exp.	↑↑	↑↑

* ↑ or ↓ = mild change
 ↑↑ or ↓↓ = moderate change
 ↑↑↑ or ↓↓↓ = severe change.

study, the parasitaemia was 6100 per 50 thin fields. By the following day the parasitaemia was 4000 per 50 thin film fields. The animal was lying in the cage. The creatinine had risen to 12.9 mg per cent, the anaemia was more severe and the blood volume was below the control value. The animal died soon after the study.

The final study in KL 17 was performed 4 days after the major sporulation. It was the most severely anaemic of any animal studied (haematocrit of 6 per cent and red cell mass of 3 ml/kg) and was unable to sit up. The blood volume was mildly reduced in the absence of any change in intravascular albumin (Tables 4 and 5). The plasma creatinine and transaminases were elevated and the animal died immediately following the experiment.

Blood Volume, Arterial Pressure, and Plasma Chemistries in the Infected PK Rhesus

The studies in PK monkeys were grouped according to the temporal relationship to major sporulation (Fig. 3). Immediately following the major sporulation there was a mild anaemia in the absence of abnormalities in blood volume, arterial pressure or plasma chemistries. Twenty-four hours after the major sporulation two PK monkeys died, but unfortunately these animals were not studied on this day. These were the only fatal infections in the PK series. The results of two other animals studied during this period were unremarkable.

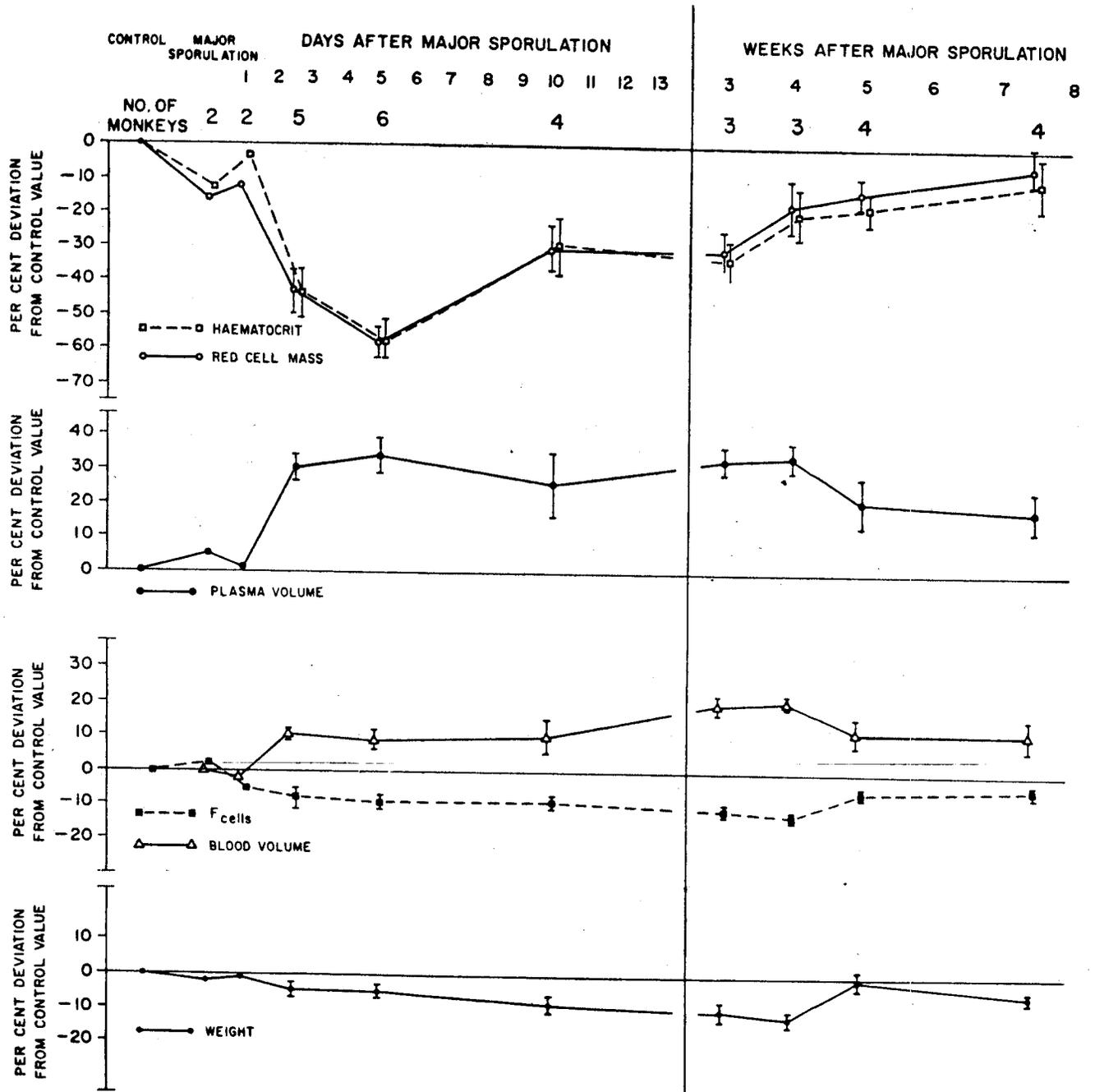


Figure 3. The per cent change from control in blood volumes and F cells (ratio of total body haematocrit to peripheral haematocrit) in the PK rhesus during the first 8 weeks of infection.

By the second to third days after major sporulation there was a 43 per cent average reduction in red cell mass. This was associated with an expansion of the plasma volume, which resulted in a 10 per cent increase in blood volume (expressed per kg of body weight). The ratio of total body haematocrit to peripheral haematocrit (F_{cells}) had decreased. The mean arterial pressure was low in 3 out of 5 monkeys (infected/control: 92/110, 54/126, and 88/113 mm Hg). The plasma urea nitrogen was elevated in 3 of 5 monkeys (34, 46, and 126 mg/100 ml), the creatinine in 2 (2.6 and 1.3 mg/100 ml), and the GOT in 1 (120 S.F. units). During the 4th to 6th days after major sporulation the maximum reduction in haematocrit and red cell mass occurred (average, 59 per cent reduction of red cell mass). The increased plasma volume and total blood volume and decreased F_{cells} persisted. Hypotension was still present in 3 out of 6 animals (infected/control: 54/126, 93/113, and 60/100 mm Hg). The plasma urea nitrogen and creatinine had returned towards normal.

The red cell mass slowly increased towards the control value during the succeeding weeks. The elevation in total blood volume accompanied by a low F_{cells} persisted for at least 8 weeks.

In 4 chronically infected rhesus monkeys (80-286 days), whose infections were described in an earlier paper (Desowitz *et al.*, 1967), the mean F_{cells} was significantly lower than in uninfected animals (Table 6). It is of interest to note that in one animal, PK 3, in which only an occasional parasite could be detected after the 40th day, the F_{cells} and blood volume had returned to preinfection levels in repeated studies.

Plasma albumin The relationship between the change in plasma albumin concentration and intravascular albumin is shown in fig. 4. In spite of the decrease in plasma albumin in 5 out of 6 animals, the intravascular albumin (expressed as g/kg body weight) remained unchanged or actually increased. Late in the infection a few animals demonstrated a rise in both plasma albumin concentration and intravascular albumin.

Discussion This study confirmed the presence of hypovolaemia during the primary parasitaemia of rhesus infected with *P. coatneyi*. Previously in this infection Desowitz *et al.*, (1967) observed haemoconcentration which suggested a contracted plasma volume. The hypovolaemia in *P. coatneyi* has been demonstrated during only one schizogonic cycle and at one specific period during that cycle. The description of the relationship of the physiologic events with the biology of *P. coatneyi* was possible because of certain characteristics of the parasite originally described by Eyles *et al.*, (1962) and confirmed in the present study. The infection had a high degree of synchronicity. The tertian periodicity separated the events of the cycle over a 48 hour period instead of the 24 hour cycle in *P. knowlesi*. The sporulation which resulted in the highest parasitaemia ("major" sporulation) was an easily defined time in each infection and physiologic events could be related to it.

Immediately following the "major" sporulation, there were no abnormalities in blood volume, arterial pressure, or serum chemistries. The critical period for 4 out of the 6 KL monkeys and 2 out of 8 PK monkeys occurred 18-36 hours after the major sporulation. Unfortunately the two PK and one KL monkey died before a study could be performed. The other three KL monkeys had a reduced blood volume and hypotension. In the presence of haemolysis the failure of expansion of the plasma volume in one, and actual contraction in two, produced the hypovolaemia. This was reflected in the disproportion between the 30 per cent fall in red cell mass and the 5 per cent decrease in venous haematocrit (Table IV). The drop in blood volume of 11-27 per cent was undoubtedly a factor in the pathogenesis of disease. The resultant altered function and organ pathology were reflected in elevations of plasma urea, creatinine, and transaminases.

The hypovolaemia can be explained by salt and water depletion of fluid shifts from the intravascular to the extravascular space. The absence of any change in weight during the study period would tend to exclude salt and water depletion. Fluid shifts within the body itself may be composed of only salt and water or a protein rich fluid. The fall in the total quantity of intravascular albumin would make the latter more likely. The extravasation of albumin might result from an increased vascular permeability.

Table 6. Blood Volumes in 4 Rhesus with Chronic coatneyi Malaria Compared to Values in 14 Uninfected Rhesus

Animal	Duration of Infection days	Weight kg	Red Cell Mass ml/kg	Plasma Volume ml/kg	Total Blood Volume ml/kg	Venous+ Haematocrit %	F cells*
KL 1	286	3.19	13.1	55.2	68.3	22.7	.846
KL 3	156	4.89	15.3	42.2	57.5	32.0	.828
KL 9	80	4.40	12.1	44.8	56.9	26.2	.812
MS 2	273	6.70	17.7	38.0	55.7	37.4	.848
Average	199	4.80	14.6	45.1	59.6	29.6	.834
S.D.**			2.7	7.3	5.9	6.6	.017
<hr/>							
Average of 14 Uninfected 4.37			16.5	41.2	57.6	32.9	.869
S.D.**			1.6	4.7	2.5	3.1	.021
Significance of Difference P Between Infected and Uninfected Rhesus			N.S.	N.S.	N.S.	N.S.	<.01

+ The haematocrits are corrected for trapped plasma.

* F cells is the ratio of total body haematocrit to peripheral haematocrit.

** Standard deviation.

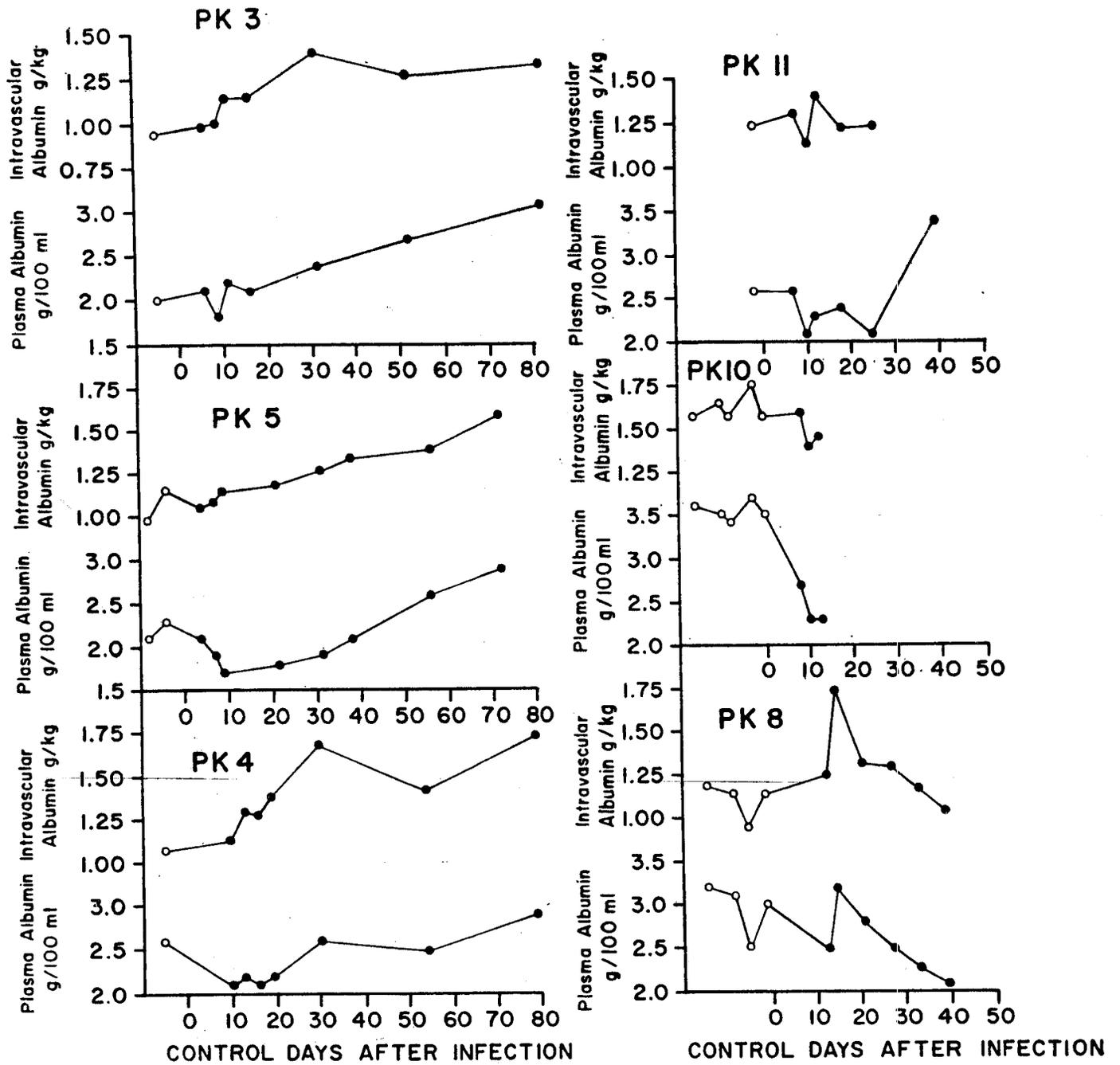


Figure 4. The relationship between the concentration of plasma albumin and the total intravascular albumin in g/kg.

Tella and Maegraith (1966) have demonstrated increased levels of bradykinin late in P. knowlesi malaria. One of the effects of this polypeptide is to increase vascular permeability. A vascular permeability increasing factor has been found in the serum of monkeys infected with P. inui and P. coatneyi (Desowitz and Pavanand, 1967). It might then be postulated that some factor in malaria causes increased vascular permeability with movement of protein rich fluid from the intravascular to the extravascular space. This is complicated by a haemolytic anemia. This vicious circle as previously described by Maegraith leads to tissue anoxia and eventual death.

The reason why hypovolaemia occurred only during this period is not completely clear at present. It is possible that changes in volume of a lesser degree could be missed because of the limitations of the methods employed.

Additional factors are of course involved in the pathogenesis of disease in malaria. Severe anaemia was present in the terminal phase in KL 17. KL 16 developed renal impairment without any change in blood volume, intravascular albumin or blood pressure. The anaemia was mild and could not explain such severe disease. The picture in this animal resembled three patients with severe falciparum malaria and renal failure described by Sitprija et al., (1967). They had no hypovolaemia, normal blood pressure and moderate anaemia. There was no biopsy evidence of tubular obstruction. The preservation of tubular function excluded tubular necrosis. They postulated an abnormality in intrarenal blood flow, although this mechanism is completely speculative.

It was possible to follow the course of P. coatneyi malaria in the PK rhesus through the acute phase into chronicity. Between the 2nd and 6th days after major sporulation there was a progressive anaemia. Hypotension developed in three in spite of a normal blood volume. Organ damage was minimal, and none of the animals died during this period.

The rapidity with which the red cell mass returned towards normal in the presence of continued parasitaemia and red cell destruction confirmed the earlier impression of an extremely active bone marrow in acute coatneyi malaria (Desowitz et al., 1967). This was contrary to the findings of Srichaikul et al., (1967) in human vivax and falciparum malaria. They observed a hypocellular bone marrow during the acute illness. Reticulocytosis and hypercellularity of the bone marrow occurred only following treatment. Possible explanations for these discrepancies were host differences and drug induced bone marrow depression.

The most consistent finding in our studies during the chronic infection was an expanded blood volume and reduced F_{cells}. It is unlikely that this was an artifact of repeated studies. A similar trend was not evident in repeated studies on uninfected animals. In addition, the F_{cells} was significantly reduced during the initial study in chronic monkey malaria (Table 6). Malloy et al., (1967) consistently observed a similar change in human acute falciparum malaria. Miller and Canfield (unpublished data) failed to confirm this finding in 18 patients with falciparum malaria. However patients studied by Miller and Canfield had been treated for approximately 12 hours prior to the initial experiment whereas Malloy et al., (1967) performed the blood volume determinations before treatment. The explanation and significance of this finding is unclear at present. The space measured by ¹³¹I labelled serum albumin (RISA) may be larger than the plasma volume in malaria. This would explain the overexpansion of the "plasma volume" and a lowered F_{cells}. Secondly, expanded blood volume (expressed in ml/kg body weight) may reflect a new relationship between blood volume and body weight as occurs in malnutrition. However a second explanation would be needed to explain the reduced F_{cells}. Chaplin et al., (1953) found the F_{cells} constant over a wide range of haematocrit (8.7 to 77.6 per cent) and variety of diseases from haemolytic anaemia to polycythemia vera. This reduction in F_{cells} in malaria, if confirmed, would be truly unique.

Hypoalbuminemia has been frequently observed in natural and experimental malaria, including P. coatneyi in the rhesus. In those PK rhesus that survived the infection, hypoalbuminaemia was not associated with a reduction in the total quantity of intravascular albumin (plasma albumin concentration ×

plasma volume in ml/kg) (fig. 4). The enlarged pool size (expanded plasma volume) diluted the plasma albumin and lowered its concentration. The plasma volume expanded secondary to a reduction in red cell mass. Overman and Feldman (1946) observed a similar rise in plasma volume and fall in plasma protein concentration in knowlesi malaria of the rhesus. However they measured only total protein and not specifically plasma albumin. The effect of malaria on synthesis and turnover of albumin must await more sophisticated studies of albumin metabolism.

Summary 1) Blood volume was determined in 6 KL and 8 PK splenectomized rhesus monkeys (*M. mulatta*) by Cr^{51} tagged red blood cells and I^{131} labelled human serum albumin before and after infection with *Plasmodium coatneyi*. Intraarterial pressure and plasma urea nitrogen, creatinine, transaminases, and albumin were also performed.

2) The blood volume in the uninfected monkeys was not significantly different from those of Gregerson utilizing Evans blue and P^{32} tagged red blood cells.

3) All of the KL monkeys died during the acute infection whereas only 2 out of 8 PK rhesus died. This could not be attributed to a difference in the parasitaemia.

4) Twenty-four hours after the "major" sporulation the KL monkeys developed hypovolaemia and hypotension. Because of a reduced intravascular albumin, increased vascular permeability seemed the most likely explanation.

5) Renal impairment one monkey (KL 16) with normal blood volume and arterial pressure and only mild anaemia indicated the presence of other factors in the pathogenesis of disease.

6) An elevation in blood volume and a reduction in Fcells (total body haematocrit/peripheral haematocrit) during the chronic infection was unexplained.

7) Hypoalbuminaemia was in part due to an expanded plasma volume.

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