CHAPTER III

RESULTS

1. ATP contents in red blood cell.

Since the method for determination of ATP contents in RBC using the luciferase enzyme and the liquid scintillation counter was very sensitive, therefore the reproducibility, and the recovery of this method and the effect of temperature and storage on the ATP level in the red blood cell were studied.

1.1 Reproducibility. A blood sample was simultaneously determined 30 times for the ATP levels and the result is shown in Table 1. A mean value of ± one standard deviation of red blood cell ATP level was found to be 153.16 ± 3.30 μM/100 ml RBC with a range of 148.26 μM/100 ml RBC to 158.87 μM/100 ml RBC and a standard error of 0.60 μM/100 ml RBC.

Table 1.

The reproducibility study of the same sample.

No.	ATP Content (µM/100 ml RBC)	No.	ATP Content (µM/100 ml RBC)
1	151.72	16	151.72
2	155.30	17	151.72
3	148.26	18	158.87
4	151.72	19	148.26
5	158.87	20	151.72
6	158.87	21	151.72
7	151.72	22	155.30
8	151.72	23	151.72
9	155.30	24	148.26
10	151.72	25	155.30
11	155.30	26	158.87
12	151.72	27	158.87
13	151.72	28	155.30
14	151.72	29	151.72
15	151.72	30	151.72

1.2 Recovery of added ATP. Blood with known amount of red blood cell ATP concentration and the potassium phosphate buffer were mixed with the known amount of standard ATP solution. The ATP contents in these samples were determined and the percentage of recovery was calculated from a formula:-

$$\text{Percentage recovery} = \frac{\text{Determined value}}{\text{Theoretical value}} \times 100$$

The results of the recovery experiments are shown in Table 2.

Table 2.

Recovery experiments after adding the known amount of standard ATP solution.

	Theoretical value (μM/100 ml)	Duplicated determined value (μM/100 ml)	Percentage recovery
Total volume of 8 ml of buffer with:-			
2.0 ml 200 μM/100 ml Std.	50	50, 48	100, 96
3.0 ml	75	64, 64	85, 85
4.0 ml	100	92, 91	92, 91
5.0 ml	125	103, 104	82, 83
6.0 ml	150	158, 162	105, 108
7.0 ml	175	170, 182	97, 104
8.0 ml	200	195, 199	98, 100

Table 2. (Cont.)

	Theoretical value (µM/100 ml)	Duplicated determined value (µM/100 ml)	Percentage recovery
Total volume of 10 ml of buffer with 1 ml blood pool and:-			
1.0 ml 500 µM/100 ml Std. added	57	55, 55	97, 97
1.5 ml	82	69, 69	84, 84
2.0 ml	107	105, 102	98, 96
2.5 ml	132	115, 112	87, 85
3.0 ml	157	145, 138	92, 88
3.5 ml	182	152, 142	83, 78
4.0 ml	207	200, 209	97, 101
4.5 ml	232	235, 225	102, 97
5.0 ml	257	303, 297	118, 115
5.5 ml	282	318, 325	113, 115
6.0 ml	307	303, 303	99, 99

1.3 Effect of temperature and time on red blood cell ATP levels. Samples of blood were collected in the heparinized test tubes and were divided into 2 portions. The first portion was extracted immediately with the perchloric acid and divided into 3 tubes. The first tube was assayed for the red blood cell ATP level and served as the control at the zero time. The other two tubes were kept at room temperature and in the

refrigerator. The second portion of blood was divided into two tubes and kept also in the refrigerator and at room temperature. All these samples were assayed for ATP level at 1, 2, 3, 4 and 27 hours. The percentages of initial value were calculated as follow:-

$$\text{Percentage of initial value} = \frac{\text{ATP level at time t.}}{\text{ATP level at zero time}} \times 100$$

The results of this study are shown in Table 3. and Fig. 1. It was quite apparent that the temperature and the process of extraction had a considerable effect on the ATP levels in the red blood cell.

Table 3.

The effect of time and temperature on ATP levels in red blood cell, expressed as the percentage of the zero time.

	Time (hours)				
	1	2	3	4	27
Ext. + Refrig.	94.9	90.1	89.0	72.7	65.5
Ext. + Room T.	90.1	84.9	86.4	65.6	15.5
Blood + Refrig.	75.8	75.1	65.5	52.3	11.1
Blood + Room T.	82.2	69.2	56.4	42.4	16.3

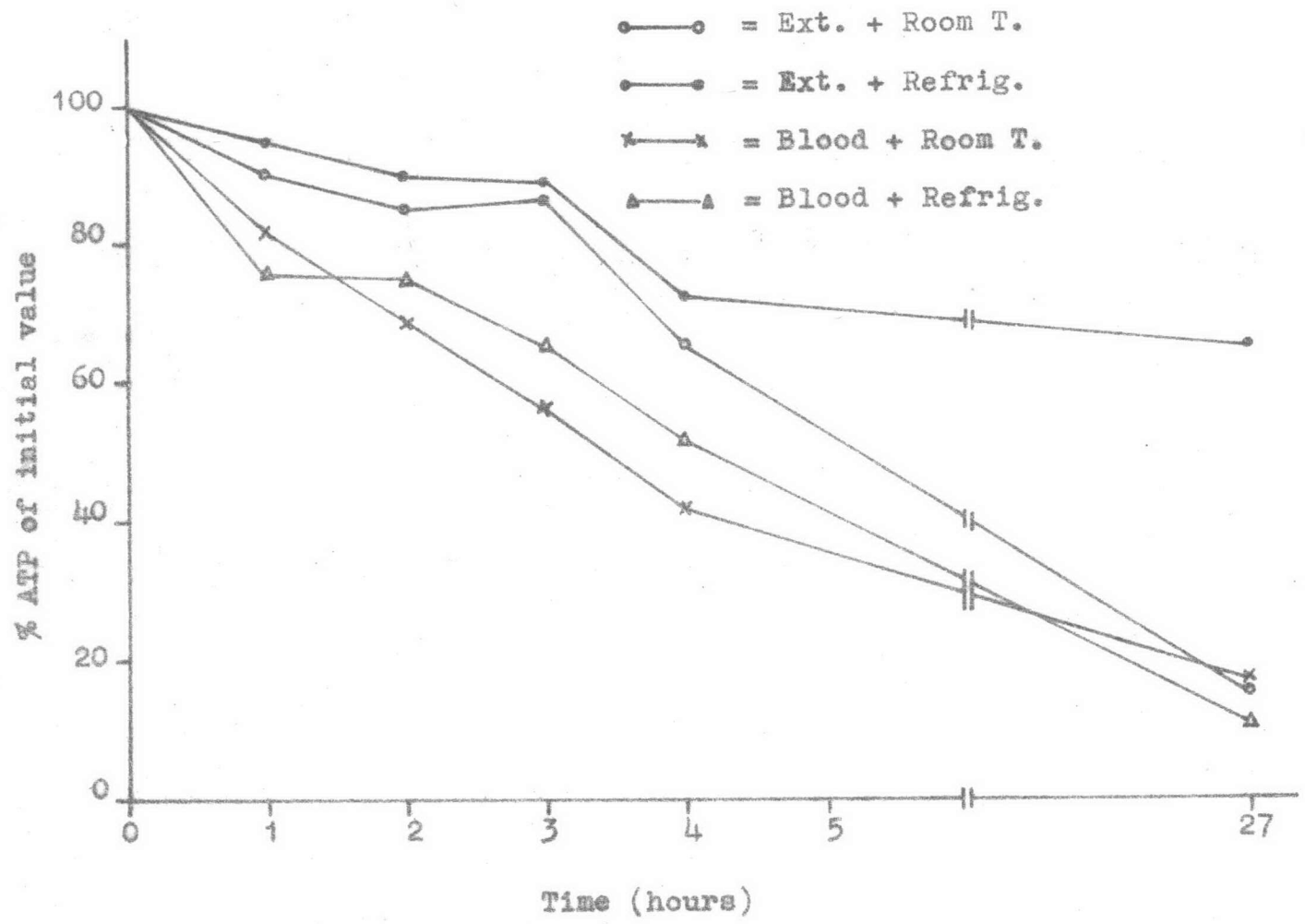

Fig. 1- Effect of time and temperature on ATP levels.

1.4 Red blood cell ATP level in normal monkeys. The result of ATP content in red blood cell of 20 normal monkeys is shown in Table 4.

Table 4.

The ATP content in the red blood cell of the normal monkeys.

	Hb. (gm %)	Hct. (%)	ATP content (μM/100 ml RBC)
M-4	12.5	33.0	116
M-5	11.8	36.0	117
M-15	11.9	27.0	142
M-18	12.5	27.0	52
M-22	11.5	33.0	111
M-23	11.9	27.0	118
M-24	9.1	33.0	111
M-25	13.1	32.0	109
M-26	12.2	40.0	73
M-29	9.3	44.5	94
M-31	13.2	42.0	91
M-35	11.2	34.0	123
M-36	13.4	44.0	64
M-37	13.4	49.0	68
M-38	11.9	34.0	55
M-39	12.9	37.0	99
M-41	13.0	39.0	150
M-42	10.0	33.5	120
M-43	12.9	41.5	82
M-44	12.2	45.0	115

An average value ± one standard deviation was found to be 99.02 ± 27.75 µM/100 ml RBC with a range of 52 µM/100 ml RBC to 150 µM/100 ml RBC with a standard error of 6.20 µM/100 ml RBC.

1.5 Red blood cell ATP level in monkeys infected with P. knowlesi. The study was performed in 5 monkeys infected with P. knowlesi. Red blood cell ATP level was determined before and during the infection. The results are shown in Table 5. and Fig. 2.

These values in comparison with the normal values are illustrated in Fig. 3.

Table 5.

The ATP content in the red blood cell of monkeys with *P. knowlesi* infection.

	Days after infection	Hb. (gm %)	Hct. (%)	Parasite count (1000^{-1} RBC)	ATP content (μM/100 ml RBC)
M-38	0	11.9	34.0	0	55
	3	9.8	35.0	1	64
	6	11.9	35.0	36	71
	8	7.6	25.5	8	72
	10	5.5	23.5	3	83
M-41	0	13.0	39.0	0	150
	5	9.1	35.0	6	136
	10	3.5	14.0	89	175
M-42	0	10.0	33.5	0	120
	5	10.6	32.5	5	115
	7	6.4	27.0	103	172
M-43	0	12.9	41.5	0	82
	5	11.9	39.0	11	128
	7	9.4	31.5	30	124
	9	5.3	23.0	12	132
M-44	0	12.2	46.0	0	75
	4	14.7	45.0	2	83
	6	12.0	39.0	10	85
	8	7.6	22.5	15	88

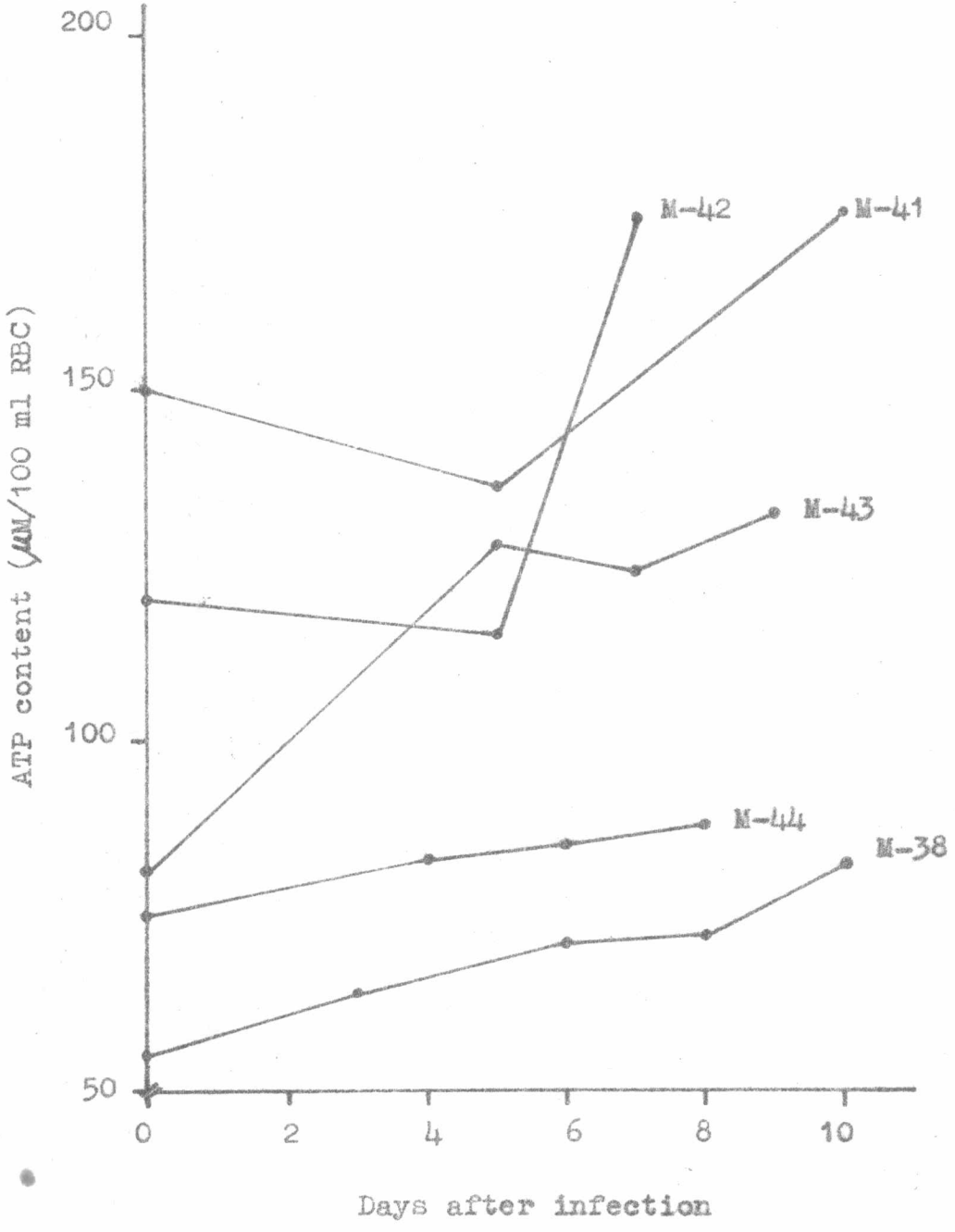

Fig. 2- The ATP content in red cell of monkeys infected with P. knowlesi.

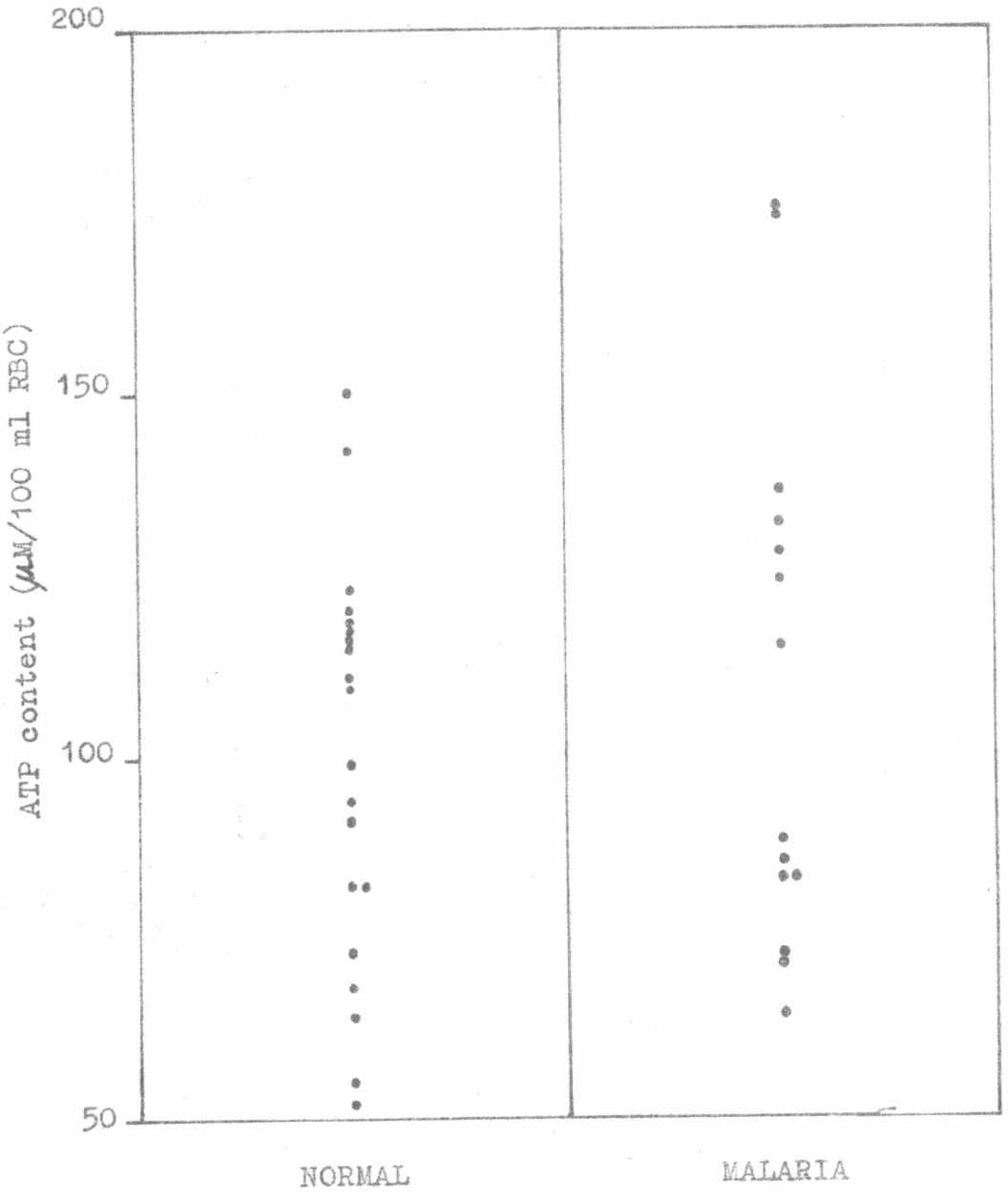

Fig. 3- ATP in red cell of normal and the P. knowlesi-infected monkeys.

2. Trapped red blood cell in the brain of normal and infected monkeys.

The studies were performed in 16 experiments of 5 monkeys infected with P. knowlesi with the infection rates of 4.7, 19.3, 3.2, 1.6 and 13.9 % and in 3 experiments in a normal monkey. The typical tracer dilution curves were shown in Fig. 4. and 5.

It was evident that the outflow pattern of ^{51}Cr-labelled red blood cells infected with P. knowlesi malaria was lower than that of ^{59}Fe-labelled normal red blood cells in both normal and infected monkeys. This indicated that the infected red blood cells travel with the same rate as the the normal red blood cells but some of the infected red blood cells were trapped in the capillaries of the brain of these monkeys.

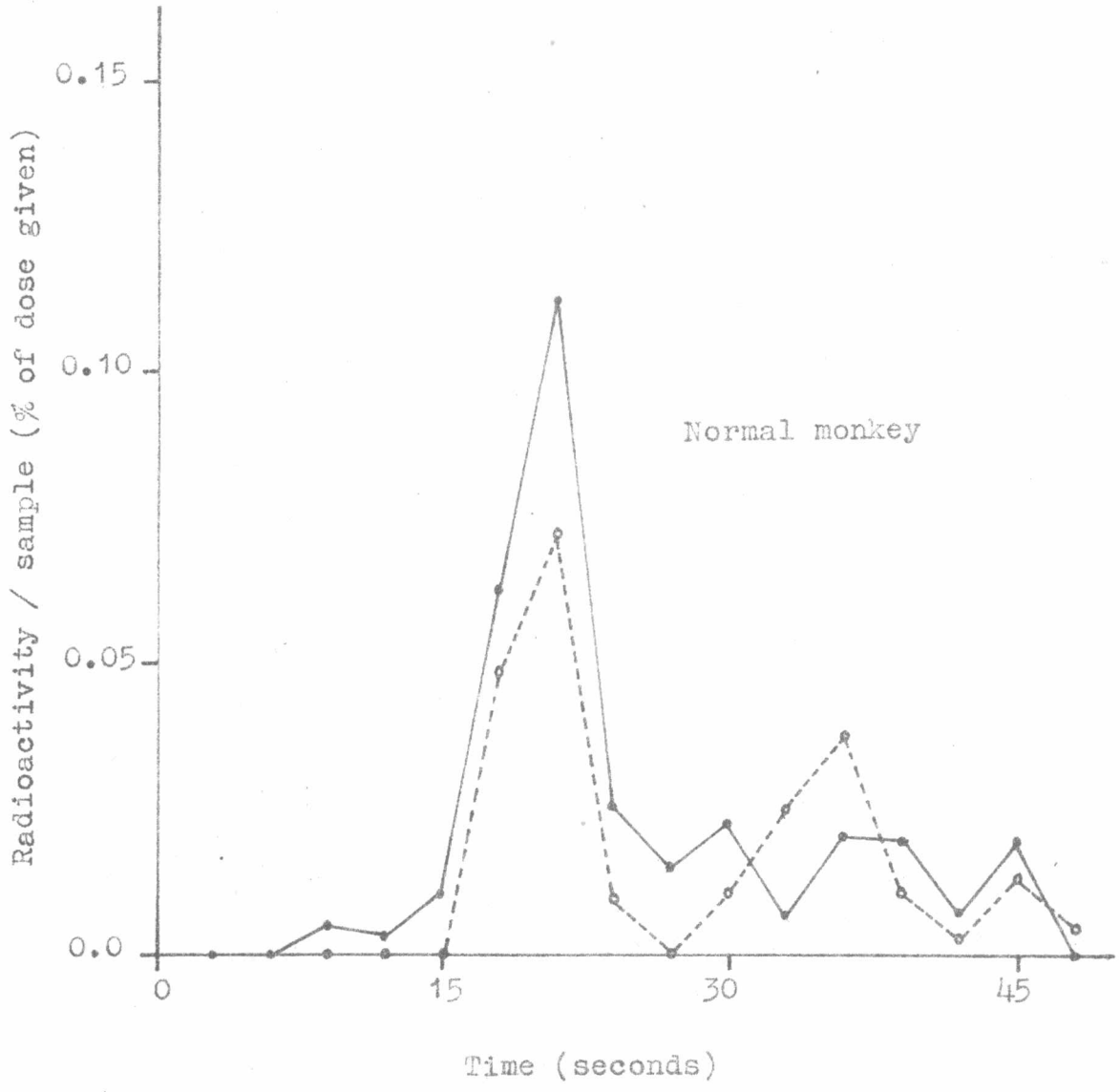

Fig. 4- A typical blood radioactivity-time pattern in normal monkey.

●——● = ^{59}Fe-labelled normal red cells.

o---o = ^{51}Cr-labelled infected red cells.

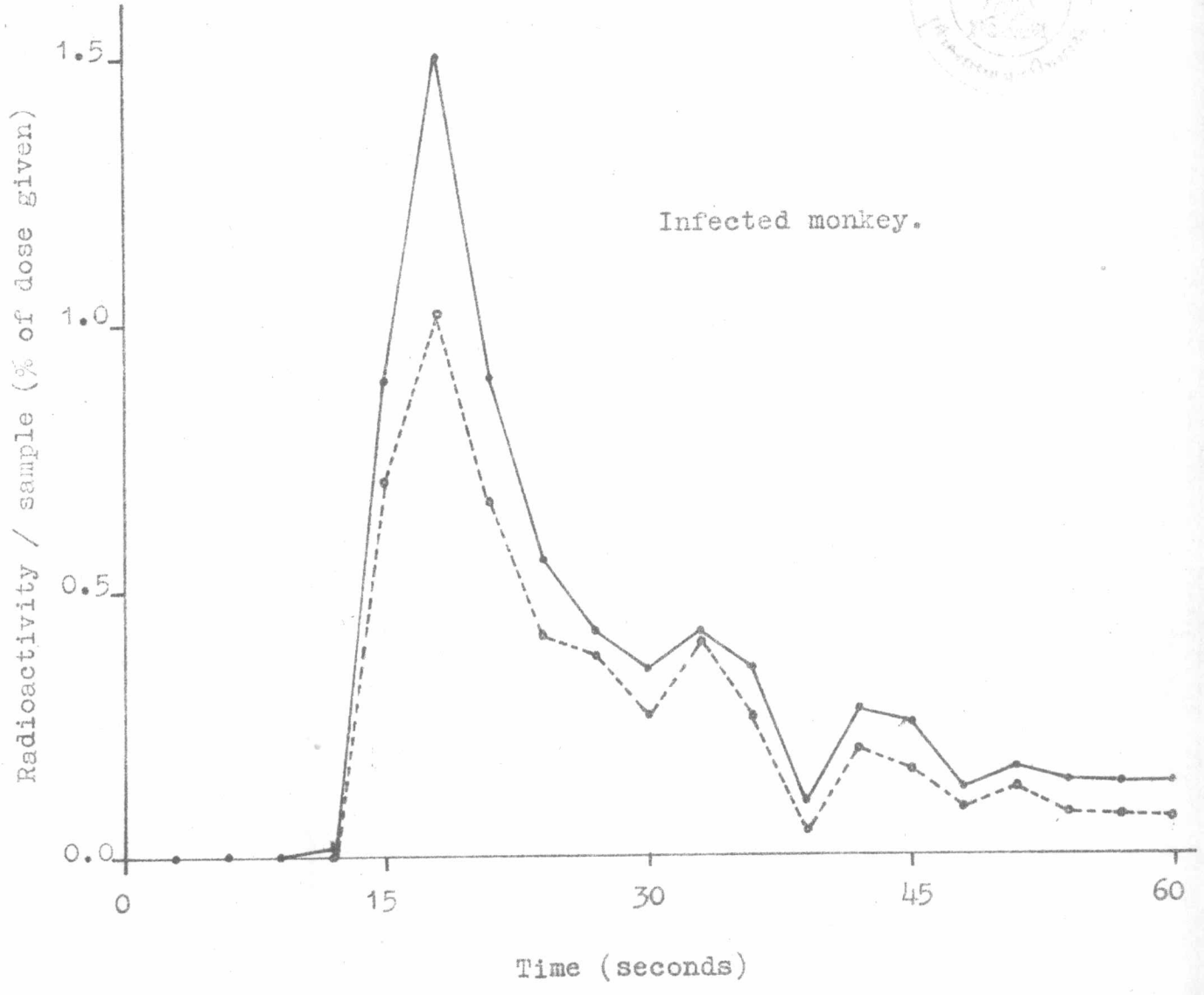

Fig. 5- A typical blood radioactivity-time pattern in P. knowlesi-infected monkey.

——• = ^{59}Fe-labelled normal red cells.

•----• = ^{51}Cr-labelled infected red cells.