

CHAPTER III

RESULTS

Since the determination of ATP in red cells by the firefly luciferase enzyme and liquid scintillation counter is a very sensitive method, therefore the reproducibility and recovery of the ATP level in the red blood cells were performed in the present study.

Reproducibility

A blood sample pool of Thai blood donors was simultaneously determined 20 times for ATP levels. A mean value \pm one standard deviation of red cell ATP levels was found to be $111.84 \pm 10.33 \mu\text{M}/100 \text{ ml RBC}$ (range $101.51 - 122.17 \mu\text{M}/100 \text{ ml RBC}$) with a standard error of $2.30 \mu\text{M}/100 \text{ ml RBC}$. The individual values of this study are shown in Table 2.

Recovery of added ATP

A standard ATP solution was mixed with the potassium phosphate buffer to the final concentrations of 40 to $200 \mu\text{M}/100 \text{ ml}$ solutions. A blood sample with the known amount of erythrocyte ATP level was mixed with the different amount of a standard ATP solution to the final concentrations of 35 to $215 \mu\text{M}/100 \text{ ml RBC}$. ATP contents in these samples were estimated and compared with the theoretical

TABLE 2

The reproducibility of ATP values in a same
pooled blood sample

| No. | ATP content ($\mu\text{M}/100$ ml RBC) | No. | ATP content ($\mu\text{M}/100$ ml RBC) |
|-----|--|-----|--|
| 1 | 107.35 | 11 | 115.03 |
| 2 | 97.91 | 12 | 111.13 |
| 3 | 112.41 | 13 | 90.33 |
| 4 | 127.59 | 14 | 96.79 |
| 5 | 115.03 | 15 | 104.91 |
| 6 | 120.45 | 16 | 117.71 |
| 7 | 115.03 | 17 | 103.71 |
| 8 | 126.13 | 18 | 107.35 |
| 9 | 130.56 | 19 | 117.71 |
| 10 | 108.60 | 20 | 111.23 |

values. 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 3.

TABLE 3

Percentage recovery after adding the known amount of the standard ATP solution into the buffer solution and a blood sample

| | Theoretical value ($\mu\text{M}/100$ ml) | Duplicate determined values ($\mu\text{M}/100$ ml) | Percentage recovery |
|---|--|--|---------------------|
| Total volume of 5 ml of buffer with: | | | |
| 1.0 ml 200 $\mu\text{M}/100$ ml standard | 40 | 43, 43 | 106, 106 |
| 1.5 ml 200 $\mu\text{M}/100$ ml standard | 60 | 59, 57 | 98, 96 |
| 2.0 ml 200 $\mu\text{M}/100$ ml standard | 80 | 76, 77 | 95, 97 |
| 2.5 ml 200 $\mu\text{M}/100$ ml standard | 100 | 89, 85 | 89, 85 |
| 3.0 ml 200 $\mu\text{M}/100$ ml standard | 120 | 120, 120 | 100, 100 |
| 3.5 ml 200 $\mu\text{M}/100$ ml standard | 140 | 148, 147 | 106, 105 |
| 4.0 ml 200 $\mu\text{M}/100$ ml standard | 160 | 159, 170 | 99, 106 |
| 4.5 ml 200 $\mu\text{M}/100$ ml standard | 180 | 169, 169 | 94, 94 |
| 5.0 ml 200 $\mu\text{M}/100$ ml standard | 200 | 188, 191 | 94, 95 |
| Total volume of 5 ml of buffer with 1 ml blood pool and: | | | |
| 0.25 ml 400 $\mu\text{M}/100$ ml standard | 35 | 38, 40 | 109, 114 |
| 0.50 ml 400 $\mu\text{M}/100$ ml standard | 55 | 53, 47 | 96, 85 |
| 0.75 ml 400 $\mu\text{M}/100$ ml standard | 75 | 71, 72 | 95, 96 |
| 1.00 ml 400 $\mu\text{M}/100$ ml standard | 95 | 98, 105 | 103, 111 |
| 1.25 ml 400 $\mu\text{M}/100$ ml standard | 115 | 128, 109 | 111, 95 |
| 1.50 ml 400 $\mu\text{M}/100$ ml standard | 135 | 123, 126 | 91, 94 |
| 1.75 ml 400 $\mu\text{M}/100$ ml standard | 155 | 145, 154 | 94, 99 |
| 2.00 ml 400 $\mu\text{M}/100$ ml standard | 175 | 198, 189 | 113, 108 |
| 2.25 ml 400 $\mu\text{M}/100$ ml standard | 195 | 212, 224 | 109, 115 |
| 2.50 ml 400 $\mu\text{M}/100$ ml standard | 215 | 214, 200 | 107, 98 |

Calibration for the optimal amount of luciferase enzymes

The effect of different concentrations of the enzyme on the standard curves of ATP was studied, 0.01 to 0.1 ml of the enzyme were used and the result of the standard curve are illustrated in Fig. 1.

The optimal amount of 0.05 ml of the enzyme was chosen in the present study because most of the samples studied have the counts on the range of this standard curve.

Studies on the effect of the temperature on erythrocyte ATP levels in the stored blood

A blood sample pool of Thai blood donors was divided into 2 portions. The first portion was extracted immediately with perchloric acid and divided into 3 tubes. One tube was assayed for the red cell ATP level and served as the control at the zero time. The other 2 tubes were kept at room temperature and in a refrigerator, respectively. The second portion of blood was divided into 2 tubes and also kept in the refrigerator and at room temperature. All of these samples were assayed for ATP content at 1, 2, 3 and 4 hours after collection and the values were expressed as the percentage of the initial value by the following formula:

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

The result of these studies are shown in Table 4 and Fig. 2.

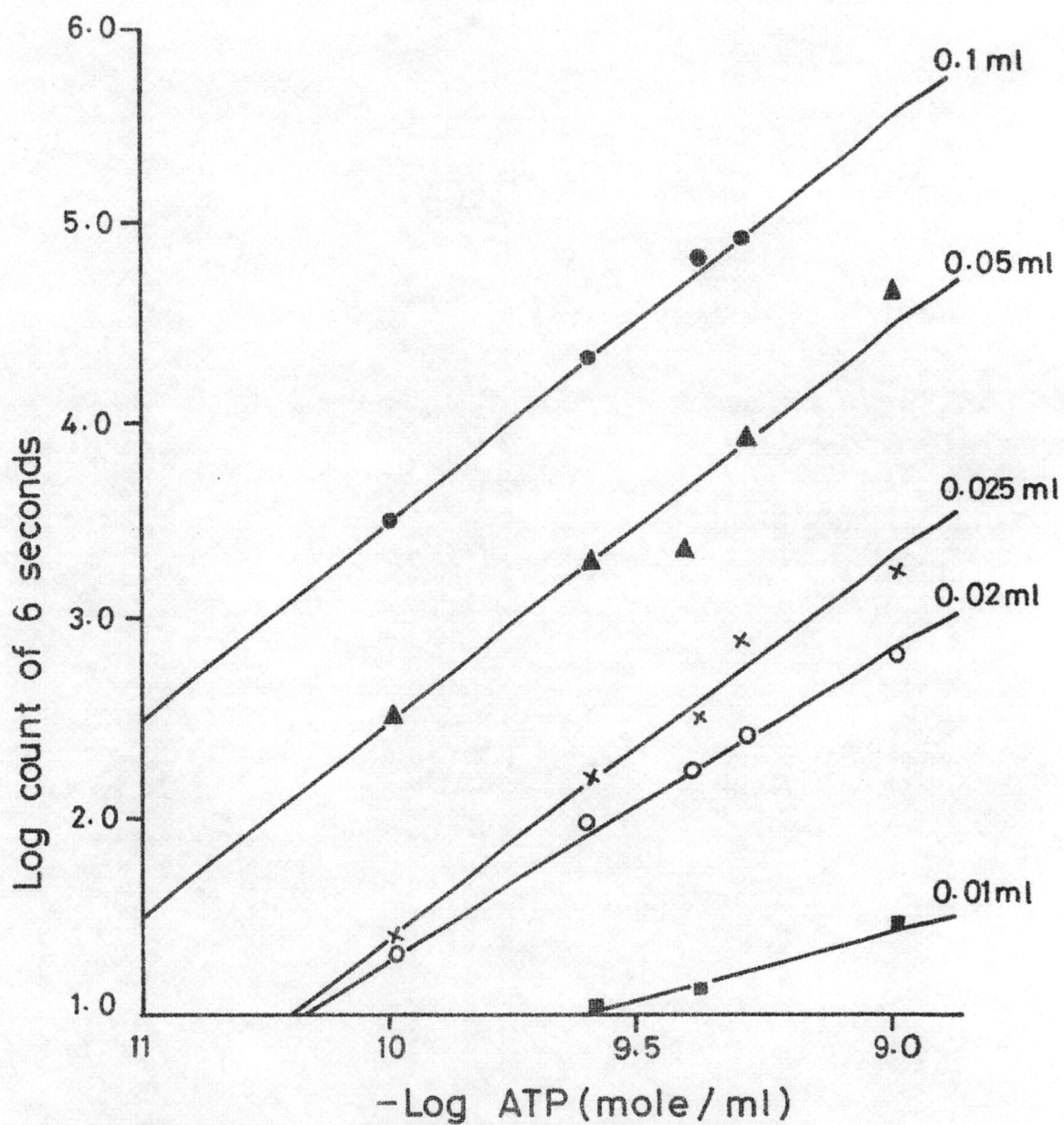


Fig.1 Calibration for the optimal amount of enzymes .

TABLE 4

The effect of time and temperature on ATP levels in red blood cells. The mean values of ATP levels in red blood cells in 4 samples are expressed as the percentage of the "Zero time".

| Samples | Time (hours) | | | | |
|-------------------------------------|--------------|-------|-------|-------|-------|
| | 0 | 1 | 2 | 3 | 4 |
| Extracted blood in refrigerator | 100 | 97.71 | 91.49 | 85.88 | 84.56 |
| Extracted blood at room temperature | 100 | 89.96 | 82.54 | 77.14 | 72.55 |
| Whole blood in refrigerator | 100 | 94.50 | 82.91 | 73.30 | 66.01 |
| Whole blood at room temperature | 100 | 84.64 | 79.51 | 68.01 | 60.40 |

The post-collection storage and the temperature had a profound effect on the red cell ATP levels. The ratio of activity of extracted blood after storage for 4 hours at 4°C and at room temperature to the initial activity were 0.84 and 0.72 respectively. The corresponding figures for the whole blood kept at the same condition were 0.66 and 0.60 respectively. It is apparent, therefore that approximately 16% of red cell ATP activity was lost in the extracted blood kept at 4°C for 4 hours; and that accurate values can be

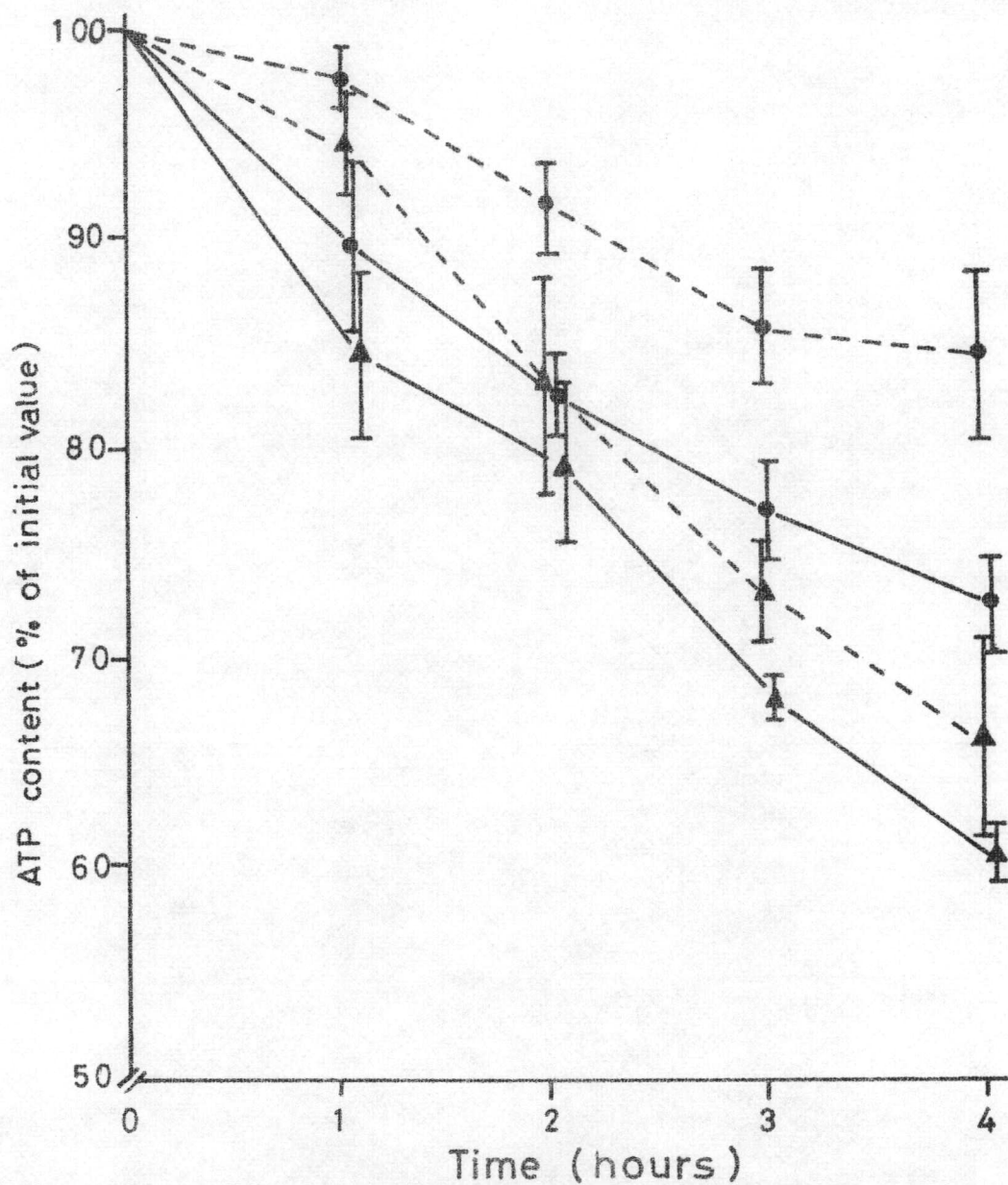


Fig.2 Effect of time and temperature on ATP levels.
 ●---● Extracted blood in refrigerator.
 ●—● Extracted blood at room temperature.
 ▲---▲ Whole blood in refrigerator.
 ▲—▲ Whole blood at room temperature.
 Each value represents the mean \pm S.E. of 4 blood samples.

obtained from blood sample only when the samples are extracted and assayed immediately.

ATP content in red blood cells of Thai blood donors

Red cell ATP levels were determined in 151 Thai blood donors, 94 males and 57 females. The ages ranged between 18 and 53 years of age. An average value \pm standard deviation of red cell ATP levels in 151 Thai blood donors was found to be $101.86 \pm 19.96 \mu\text{M}/100 \text{ ml RBC}$ (range $79.87 - 124.21 \mu\text{M}/100 \text{ ml RBC}$).

There is no significant difference ($P > 0.1$) between the mean values of red cell ATP levels in 94 males and 57 females, i.e., $103.74 \pm 20.47 \mu\text{M}/100 \text{ ml RBC}$ (range $83.27 - 124.27 \mu\text{M}/100 \text{ ml RBC}$) and $98.72 \pm 18.85 \mu\text{M}/100 \text{ ml RBC}$ (range $79.87 - 117.57 \mu\text{M}/100 \text{ ml RBC}$), respectively. The comparison of the red cell ATP levels from the present studies with the previous results obtained by various methods is shown in Table 5.

The frequency distributions of red cell ATP levels in 151 Thai blood donors 94 males and 57 females are shown in Fig. 3, 4 and 5 respectively.

The effect of temperature and post-collection storage of blood on the red cell ATP levels of patients with P. falciparum malaria are shown in Table 6. It is apparent

TABLE 5

Red cell ATP levels obtained by various methods*

| Method of extraction | Method of ATP assay | Normal | | References |
|---|--|---|----------------------------------|-------------------------------|
| | | As given | Calculated as $\mu\text{M/g Hb}$ | |
| Whole blood TCA extract | GAPD back reaction | $1.13 \pm 0.27 \mu\text{M/ml RBC}$ | 3.32 ± 0.08 | Minikami <u>et al.</u> , 1965 |
| Whole blood perchloric acid extract | Hexokinase | $3.86 \mu\text{M/g Hb S.E. 0.13}$ | 3.86 ± 0.13 | Gross <u>et al.</u> , 1963 |
| Perchloric acid extract of washed cells | Paper chromatography | $106 \mu\text{M}/100 \text{ ml RBC}$ | 3.12 | Greenwalt and Ayers, 1960 |
| TCA extract of washed cells | Hexokinase | $2.75 \mu\text{M/g Hb}$ | 2.75 | Brewer and Powell, 1966 |
| PCA extract of washed cells | Column chromatography | $82.4 \mu\text{M}/100 \text{ ml RBC}$ | 2.42 | Mandel <u>et al.</u> , 1962 |
| TCA extract of washed cells | Paper chromatography | $0.68 \mu\text{M/g RBC}$ | 2.19 | Gerlach <u>et al.</u> , 1958 |
| PCA extract of washed cells | Column chromatography | $69.2 \mu\text{M}/100 \text{ ml RBC}$ | 2.04 | De Luca <u>et al.</u> , 1962 |
| TCA extract of washed cells | Column chromatography | $2.7 - 3.7 \mu\text{mole/ml RBC}$ | $2.03 - 3.63$ | Bartlett, 1959 |
| Whole blood | Firefly | $5.2 \mu\text{M/g Hb}$ | 5.2 | Beutler and Baluda, 1964 |
| Whole blood | Firefly | $5.45 \pm 1.36 \mu\text{M/g Hb}$ | 5.45 ± 1.36 | |
| Whole blood | Firefly | $138 \mu\text{M}/100 \text{ ml RBC}$ | 4.05 ± 0.11 | Beutler and Mathai, 1967 |
| Whole blood PCA extract | Firefly and liquid scintillation counter | $101.86 \pm 19.96 \mu\text{M}/100 \text{ ml RBC}$ | 3.02 ± 0.59 | Present study |

* This table was adapted from Beutler and Mathai (1967)

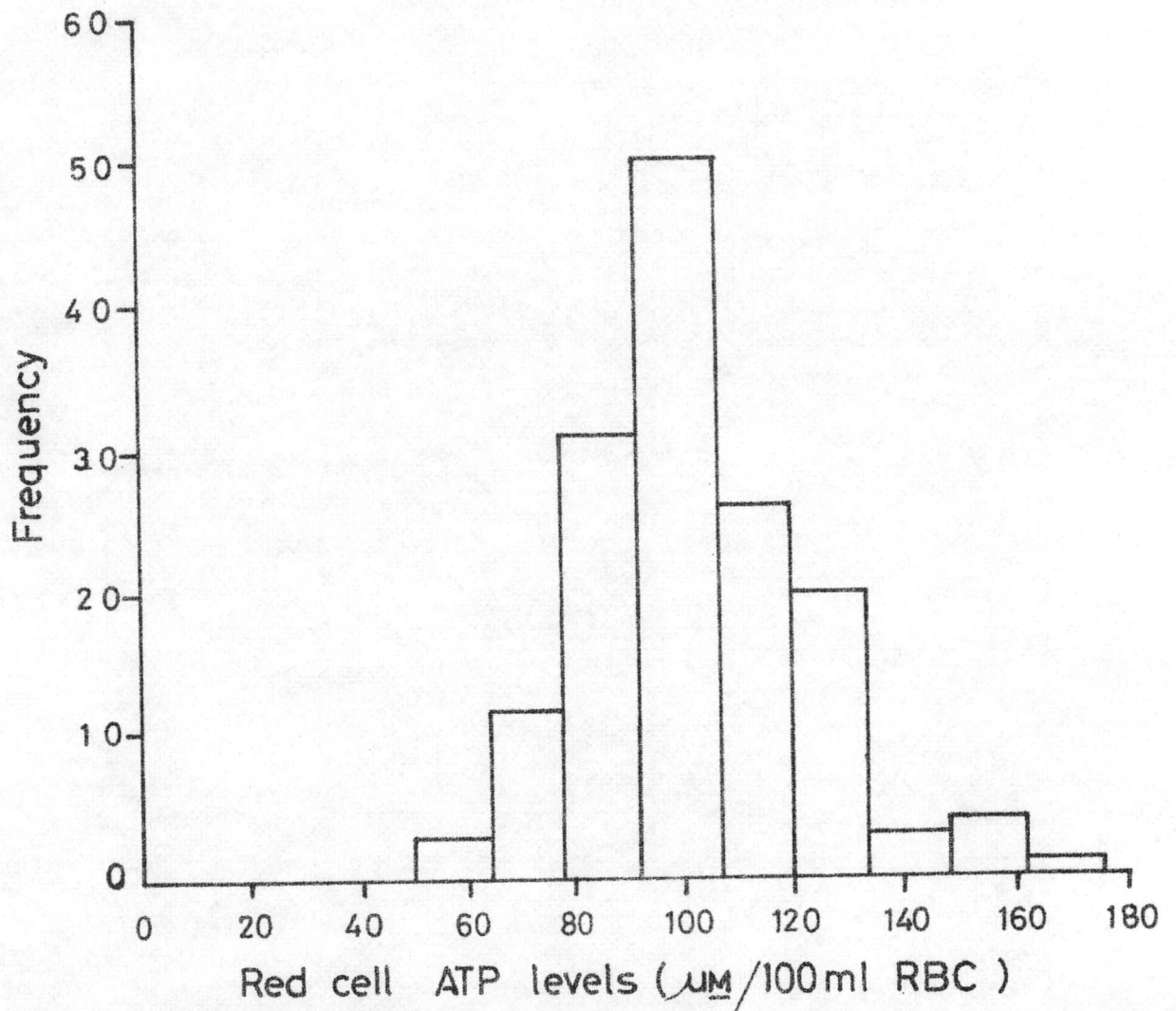


Fig.3 The frequency distribution of red cell ATP levels in 151 Thai blood donors.

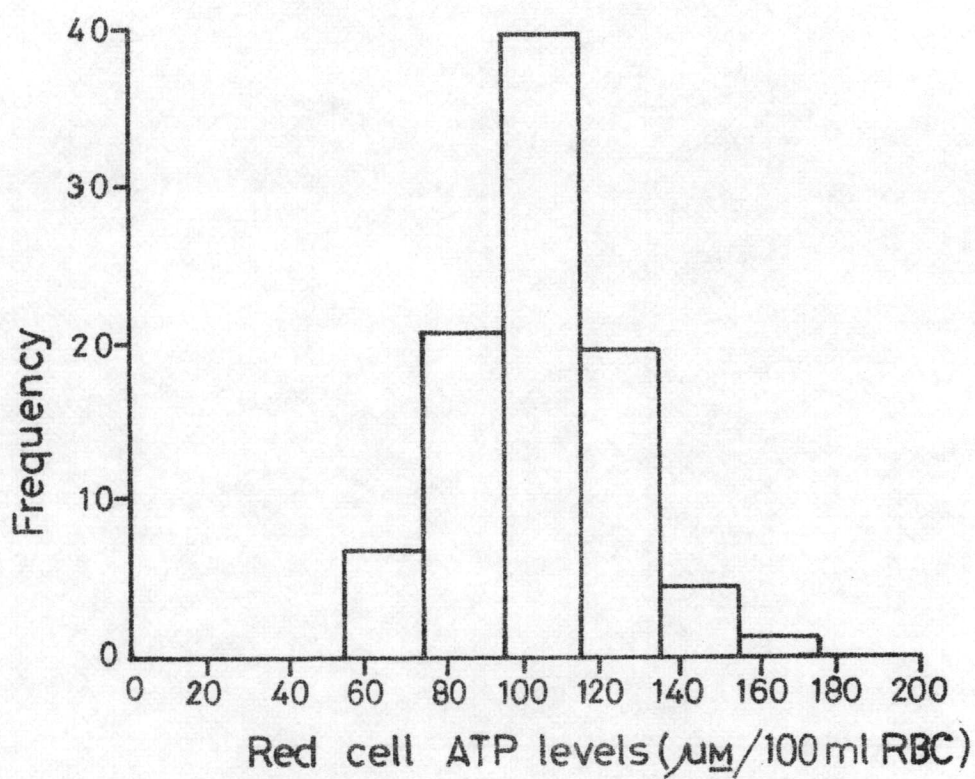


Fig. 4 The frequency distribution of red cell ATP levels in 94 males.

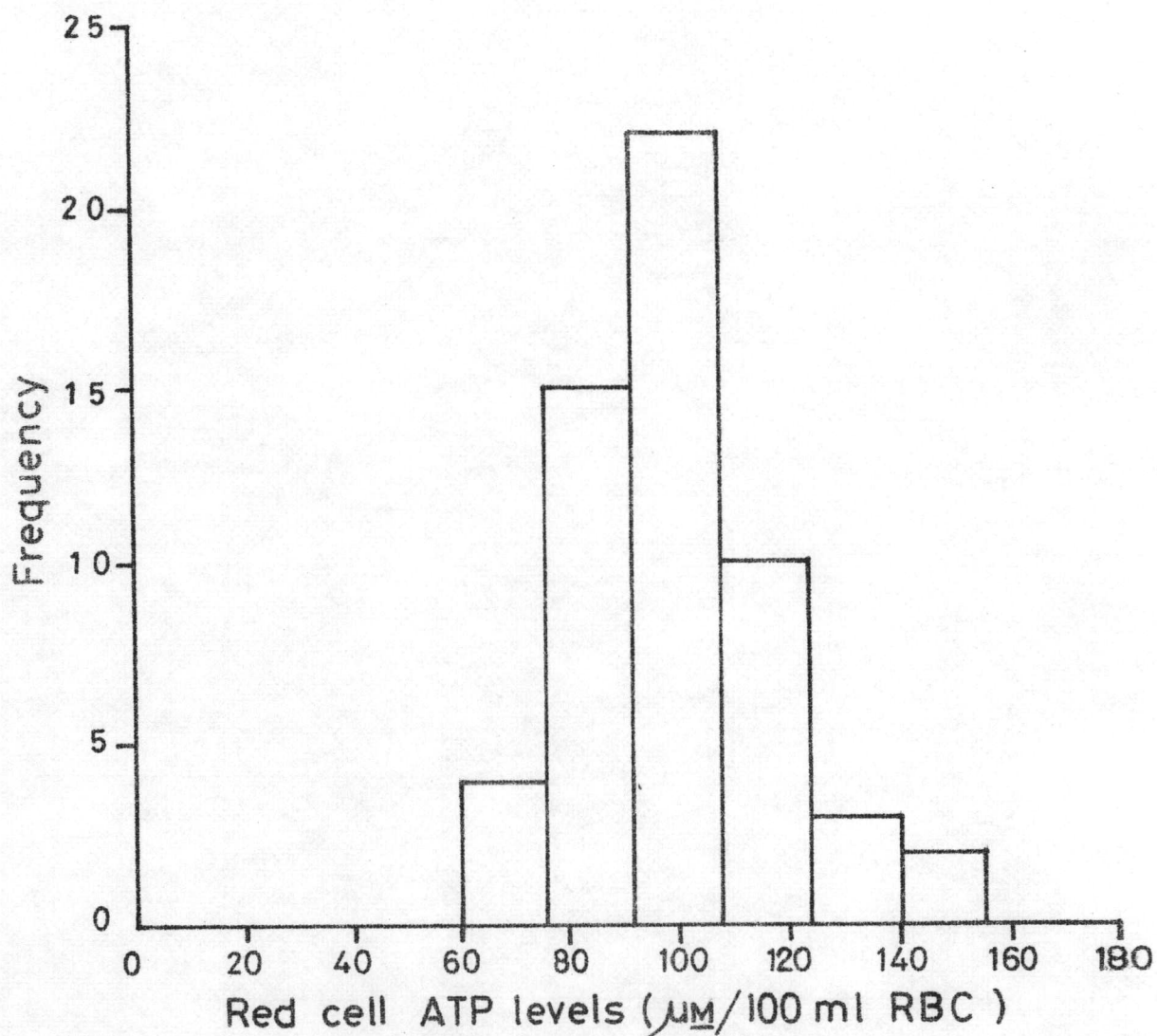


Fig.5 The frequency distribution of red cell ATP levels in 57 females.

that the ATP levels in the extracted blood and the whole blood of malarial subjects decrease faster than those of the normal subjects both at the room temperature and at 4°C as illustrated in Fig. 6.

TABLE 6

The effect of time on the red cell ATP levels of P. falciparum-infected blood stored in the refrigerator. Results are expressed as the percentage of the initial value.

| Sub- jects No. | Whole blood | | | | | Extraction blood | | | | |
|----------------------|--------------|-------|-------|-------|-------|------------------|-------|-------|-------|-------|
| | Time (hours) | | | | | Time (hours) | | | | |
| | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| 1 | 100 | 79.43 | 79.43 | 50.70 | 28.50 | 100 | 94.42 | - | - | 66.83 |
| 2 | 100 | 90.16 | 60.95 | 24.83 | 25.93 | 100 | - | 92.26 | 86.10 | 82.22 |
| 3 | 100 | 93.33 | 78.53 | 92.26 | 60.96 | 100 | 91.21 | 96.61 | 96.61 | - |
| 4 | 100 | 95.28 | 56.11 | 65.92 | 90.98 | 100 | 84.92 | 61.51 | 52.36 | 82.98 |
| 5 | 100 | 73.96 | 53.27 | 38.81 | 33.04 | 100 | 93.11 | - | 81.09 | 67.45 |
| 6 | 100 | 85.11 | 51.16 | 32.28 | 20.37 | 100 | - | 44.57 | 67.45 | 53.58 |
| 7 | 100 | 72.44 | 46.77 | 33.11 | 29.51 | 100 | 95.50 | 85.12 | 56.24 | 44.38 |
| Mean value | 100 | 85.24 | 60.89 | 48.27 | 41.33 | 100 | 91.83 | 76.01 | 73.31 | 66.24 |

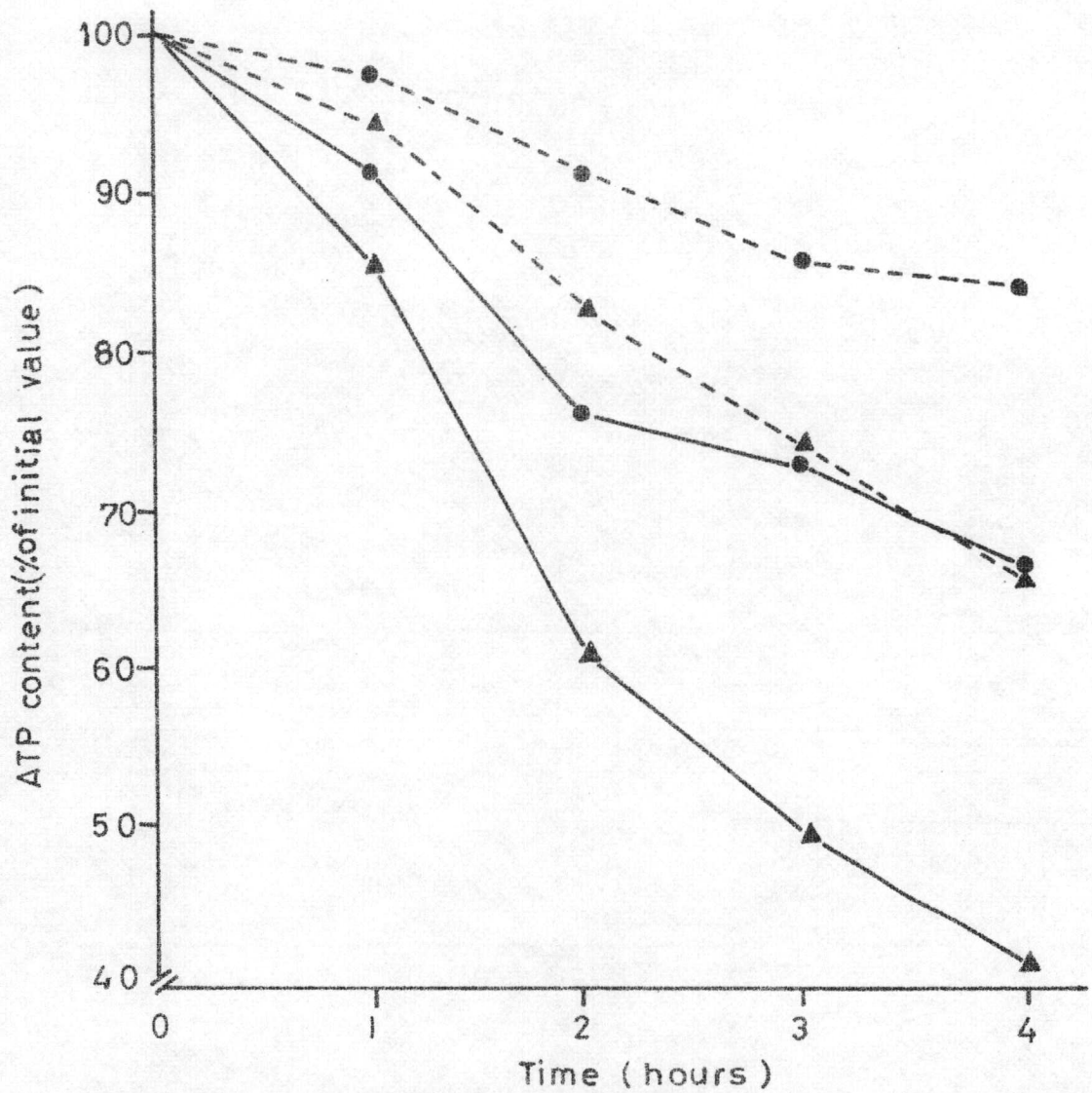


Fig.6 Effect of time and temperature on ATP levels in normal blood donors and blood infected with *P.falciparum* malaria.

- Extracted infected blood in refrigerator.
- Extracted normal blood in refrigerator.
- ▲—▲ Infected blood at room temperature.
- ▲---▲ Normal blood at room temperature.

ATP content in red blood cells of patients with *P. falciparum* malaria

Results of red cell ATP levels of 27 patients with *P. falciparum* malaria with parasitaemia ranging from 1 to 105 per 1,000 red cells are shown in Table 7. A mean value \pm standard deviation of the erythrocyte ATP levels was found to be $119.05 \pm 30.75 \mu\text{M}/100 \text{ ml RBC}$ (range $58.8 - 189.7 \mu\text{M}/100 \text{ ml RBC}$). This figure was not significantly different ($P > 0.05$) from a mean value obtained from 151 blood donors.

There was a significantly reversed relationship ($P < 0.05$) between the parasitaemia and the measured red cell ATP content as illustrated in Fig. 7.

A mean value \pm standard deviation of $105.2 \pm 40.8 \mu\text{M}/100 \text{ ml red cells}$ was found in 26 samples of blood which was separated into 104 layers using 0.7 and 0.8 M sucrose in Krebs glucose-saline solutions. This figure was not significantly different ($P > 0.05$) from a mean value of normal subjects. The calculated ATP content of parasitized red cells was found to be much higher than that of the non-parasitized red cells. There was a significantly reversed relationship ($P < 0.05$) between the parasitaemia and the ATP content of parasitized red cells. The higher the parasitaemia, the lower the ATP level of the parasitized erythrocytes (Fig. 8).

TABLE 7

ATP content in red cells of patients with
P. falciparum malaria

| No. | Sex | Age (year) | Hb (g %) | Hct (%) | Parasitaemia (1000 ⁻¹ RBC) | ATP level (μ M/100 ml RBC) |
|-----|-----|---------------|-------------|------------|--|------------------------------------|
| 1 | M* | 13 | 9.6 | 30 | 5 | 145.8 |
| 2 | M | 23 | 10.8 | 34 | 10 | 89.5 |
| 3 | M | 12 | 5.8 | 25 | 18 | 89.5 |
| 4 | M | 56 | 9.6 | 29 | 12 | 122.9 |
| 5 | M | 24 | 14.2 | 43 | 3 | 117.4 |
| 6 | M | 20 | 10.5 | 33 | 2 | 171.6 |
| 7 | M | 18 | 38.0 | 35 | 4 | 128.5 |
| 8 | M | 15 | 9.8 | 26 | 5 | 189.7 |
| 9 | M | 21 | 5.8 | 26 | 30 | 154.2 |
| 10 | M | 43 | 6.8 | 35 | 14 | 111.9 |
| 11 | F* | 21 | 6.9 | 27 | 9 | 105.1 |
| 12 | M | 22 | 14.1 | 42 | 20 | 151.3 |
| 13 | M | 17 | 13.3 | 41 | 5 | 114.9 |
| 14 | M | 23 | 12.6 | 39 | 10 | 126.5 |
| 15 | M | 58 | 11.2 | 34 | 8 | 100.4 |
| 16 | M | 23 | 7.0 | 28 | 24 | 116.4 |
| 17 | M | 32 | 12.2 | 38 | 17 | 105.5 |
| 18 | M | 24 | 10.8 | 33 | 8 | 130.1 |
| 19 | M | 20 | 12.1 | 35 | 16 | 125.3 |
| 20 | M | 29 | 11.8 | 39 | 11 | 155.2 |
| 21 | M | 24 | 11.2 | 33 | 18 | 82.9 |
| 22 | M | 17 | 10.2 | 37 | 1 | 100.9 |
| 23 | M | 29 | 12.1 | 44 | 33 | 137.9 |
| 24 | M | 38 | 12.1 | 36 | 30 | 72.6 |
| 25 | F | 38 | 9.8 | 29 | 62 | 58.8 |
| 26 | M | 43 | 11.2 | 28 | 12 | 131.8 |
| 27 | M | 41 | 5.4 | 13 | 105 | 77.3 |

M* = Male, F* = Female

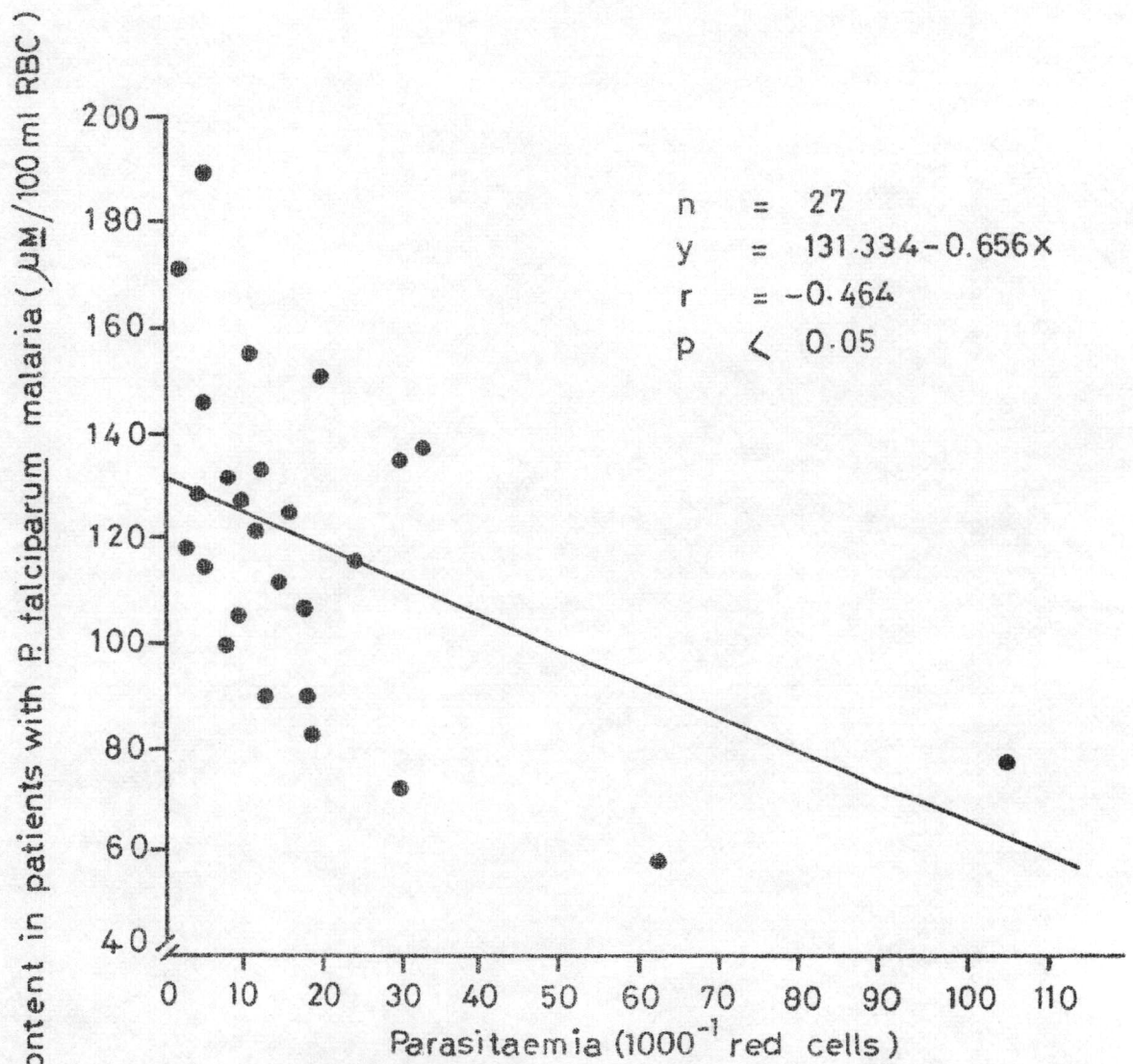


Fig.7 Relationship between the parasitaemia and the measured red cell ATP content of 27 patients with *P. falciparum* malaria.

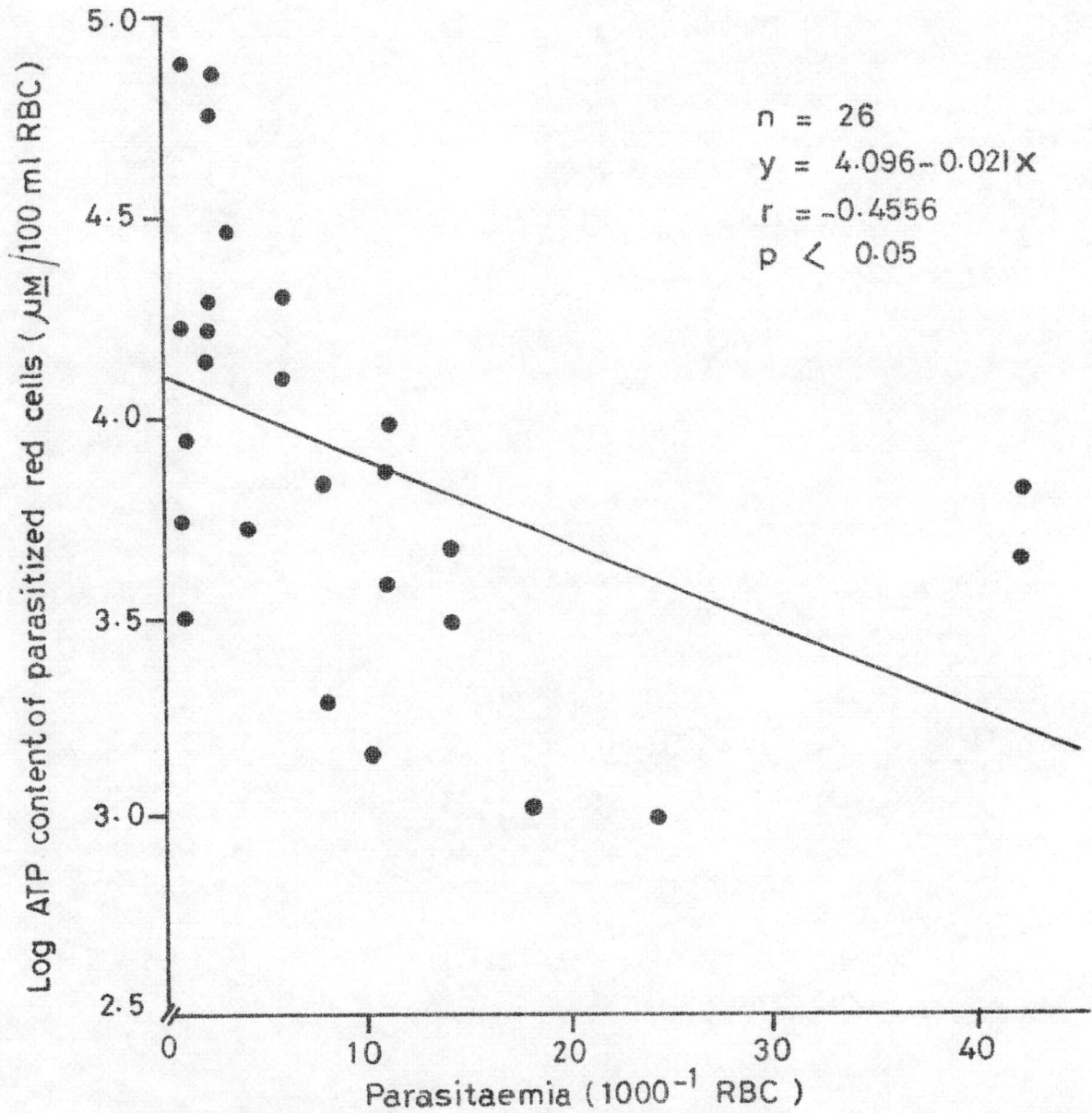


Fig.8 Relationship between the parasitaemia and the log ATP content of parasitized red cells with *P. falciparum* malaria.

STUDIES ON ERYTHROCYTES ATP LEVELS IN MONKEYS

1. The ATP contents in red blood cells of normal monkeys

A mean value \pm standard deviation of red cell ATP levels in 20 normal monkeys was found to be $99.02 \pm 27.75 \mu\text{M}/100 \text{ ml RBC}$ (range $51.5 - 149.9 \mu\text{M}/100 \text{ ml RBC}$) with a standard error of $6.20 \mu\text{M}/100 \text{ ml RBC}$. The individual values are shown in Table 8.

2. The ATP contents in red blood cells of *P. knowlesi*-infected monkeys

Red cells ATP levels were also determined in 21 samples of 8 monkeys infected with *P. knowlesi* at different stages of parasitaemia ranging from 1 to 103 per 1,000 red blood cells. A mean value \pm standard deviation of this results are found to be $105.3 \pm 40.1 \mu\text{M}/100 \text{ ml RBC}$ as shown in Table 9.

3. The ATP contents in the red blood cells of monkeys at the convalescent stage

The erythrocyte ATP levels of 9 monkeys at the convalescent stage was $116.5 \pm 37.2 \mu\text{M}/100 \text{ ml RBC}$ (Table 10).

There was no significant difference ($P > 0.05$) between the mean values of the normal monkeys, and the malaria infected monkeys or the monkeys at the convalescent stage. The individual values are illustrated in Fig. 9.

TABLE 8

The ATP content in the red blood cells of the normal monkeys

| No. of monkey | Hb (g %) | Hct (%) | ATP content (μ M/100 ml RBC) |
|---------------|-------------|------------|--------------------------------------|
| M - 4 | 12.5 | 33 | 116.03 |
| M - 5 | 11.8 | 36 | 116.62 |
| M - 15 | 11.9 | 27 | 141.81 |
| M - 18 | 12.5 | 27 | 51.50 |
| M - 22 | 11.5 | 33 | 110.80 |
| M - 23 | 11.9 | 27 | 117.95 |
| M - 24 | 9.1 | 33 | 111.00 |
| M - 25 | 13.1 | 32 | 109.13 |
| M - 29 | 9.3 | 45 | 94.34 |
| M - 31 | 13.2 | 42 | 90.59 |
| M - 35 | 11.2 | 34 | 123.00 |
| M - 36 | 13.4 | 44 | 64.15 |
| M - 37 | 13.4 | 49 | 68.03 |
| M - 39 | 12.2 | 37 | 98.50 |
| M - 38 | 11.9 | 34 | 55.20 |
| M - 41 | 13.0 | 39 | 149.90 |
| M - 42 | 10.0 | 34 | 119.90 |
| M - 43 | 12.9 | 42 | 82.20 |
| M - 44 | 12.2 | 45 | 115.00 |
| M - 26 | 12.2 | 40 | 73.00 |
| Mean | 11.9 | 41 | 99.02 |

TABLE 9

The ATP content in the red blood cells of monkeys with
P. knowlesi malaria

| No. of monkey | Days after infection | Hb (g %) | Hct (%) | Parasitaemia (1000 ⁻¹ RBC) | ATP content (μ M/100 ml RBC) |
|---------------|----------------------|----------|---------|---------------------------------------|-----------------------------------|
| M - 23 | 7 | - | 28 | 8 | 108.2 |
| M - 24 | 3 | 8.6 | 32 | 1 | 106.1 |
| | 4 | 10.6 | 38 | 3 | 116.0 |
| | 6 | 7.8 | 27 | 8 | 63.0 |
| | 7 | 5.3 | 19 | 9 | 105.3 |
| | 11 | 4.1 | 16 | 7 | 184.9 |
| M - 36 | 1 | - | 57 | 1 | 64.2 |
| M - 37 | 8 | 11.5 | 39 | 20 | 49.4 |
| | 12 | - | 28 | 6 | 57.3 |
| | 51 | 11.5 | 41 | 9 | 93.4 |
| M - 38 | 3 | 9.8 | 35 | 1 | 64.9 |
| | 6 | 11.9 | 35 | 36 | 61.9 |
| | 8 | 7.6 | 26 | 8 | 71.9 |
| | 10 | 5.5 | 24 | 3 | 85.5 |
| M - 41 | 5 | 11.5 | 35 | 6 | 136.2 |
| | 10 | 3.5 | 13 | 89 | 169.5 |
| M - 42 | 5 | 10.6 | 33 | 5 | 115.2 |
| | 7 | 6.4 | 27 | 103 | 174.5 |
| M - 43 | 7 | 11.9 | 39 | 11 | 129.1 |
| | 9 | 9.4 | 32 | 30 | 124.1 |
| | 11 | 5.3 | 23 | 12 | 131.9 |
| Mean | 9 | 8.4 | 31 | 18 | 105.3 |

TABLE 10

ATP levels in the red blood cells of convalescent monkeys

| No. of monkey | Haematocrit (%) | ATP content ($\mu\text{M}/100 \text{ ml RBC}$) |
|---------------|-----------------|--|
| M - 15 | 35 | 181.7 |
| M - 22 | 33 | 110.8 |
| M - 22 | 33 | 166.9 |
| M - 23 | 27 | 117.9 |
| M - 29 | 45 | 94.3 |
| M - 31 | 52 | 90.6 |
| M - 34 | 37 | 98.8 |
| M - 35 | 34 | 123.5 |
| M - 36 | 57 | 64.2 |
| Mean | 39 | 116.5 |

The result also show that there was no relationship ($P > 0.05$) between the erythrocyte ATP levels and the parasitaemia in the present study. Alteration of the erythrocyte ATP levels during malarial infection is depicted in Fig. 10.

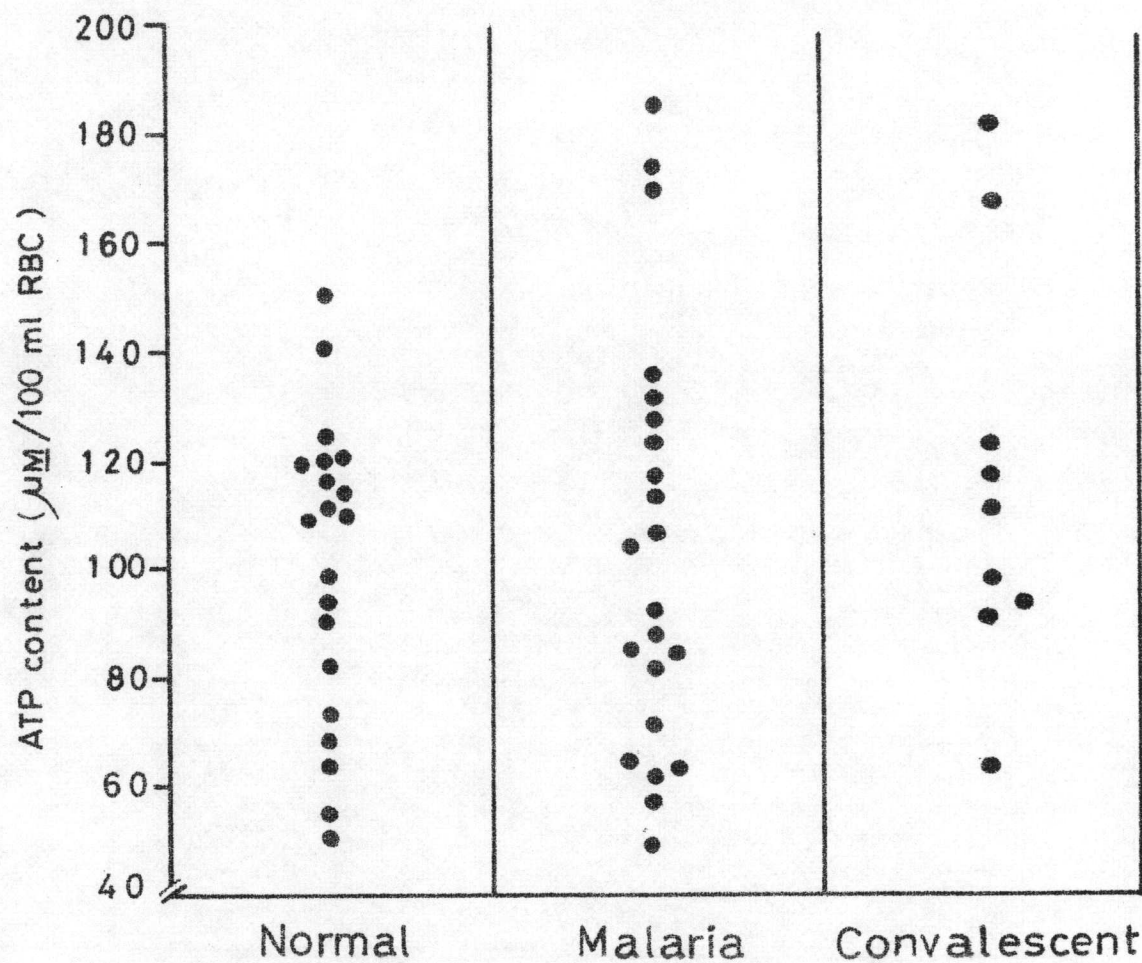


Fig.9 The individual values of red cell ATP levels in normal, malaria infected and convalescent monkeys.

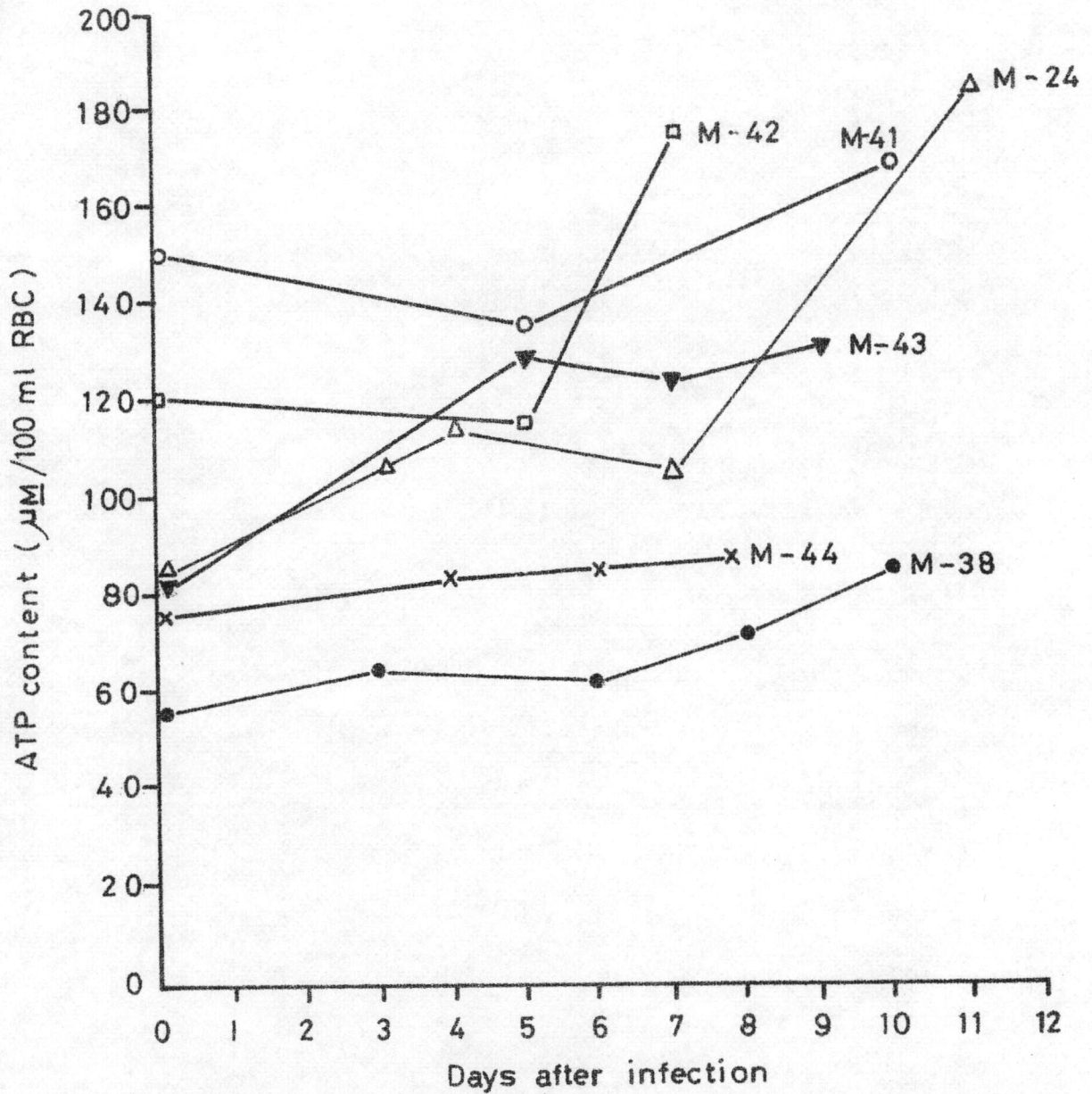


Fig.10 The erythrocyte ATP levels of infected monkeys with *P. knowlesi* malaria at different stage of infection.

STUDIES ON ERYTHROCYTES ATP LEVELS IN MICE

1. The ATP levels in red blood cells of the normal mice

An average value \pm standard deviation of ATP contents in red cells of 21 normal mice was found to be $105.94 \pm 31.27 \mu\text{M}/100 \text{ ml RBC}$ (range $74.67\text{-}137.21 \mu\text{M}/100 \text{ ml RBC}$) and a standard error of the mean $6.82 \mu\text{M}/100 \text{ ml RBC}$. (Table 11).

2. The erythrocyte ATP levels in *P. berghei*-infected mice

The erythrocyte ATP levels in 42 *P. berghei*-infected mice at various stages of infection are shown in Table 12. A mean value \pm standard deviation was found to be $147.72 \pm 74.3 \mu\text{M}/100 \text{ ml RBC}$ with a standard error of $11.4 \mu\text{M}/100 \text{ ml RBC}$. These values were found to be significantly higher than those of the normal mice ($P < 0.01$) and the individual values are illustrated in Fig. 11.

There was no relationship between the parasitaemia and the measured ATP levels in this experimental ($P > 0.05$) as illustrated Fig. 12.

3. Studies on the ATP contents of red cells in the parasitized and non-parasitized red blood cells

Twenty samples of blood from infected mice were separated into different layers by using 0.7 M and 0.8 M

TABLE 11

ATP levels in the red blood cells of the normal mice

| No. of mice | Haematocrit (%) | ATP levels ($\mu\text{M}/100$ ml RBC) |
|-------------|-----------------|--|
| 1 | 36 | 116.07 |
| 2 | 35 | 98.45 |
| 3 | 39 | 144.53 |
| 4 | 39 | 107.14 |
| 5 | 38 | 152.51 |
| 6 | 36 | 135.79 |
| 7 | 36 | 124.96 |
| 8 | 36 | 150.24 |
| 9 | 37 | 74.96 |
| 10 | 37 | 123.24 |
| 11 | 28 | 110.60 |
| 12 | 43 | 141.13 |
| 13 | 34 | 120.68 |
| 14 | 30 | 94.61 |
| 15 | 34 | 102.71 |
| 16 | 32 | 70.45 |
| 17 | 33 | 50.64 |
| 18 | 33 | 92.17 |
| 19 | 37 | 50.56 |
| 20 | 30 | 60.57 |
| 21 | 36 | 102.76 |
| Mean | 35 | 105.94 |

TABLE 12

The ATP content in the red blood cells of mice
with P. berghei malaria.

| No. | Hct (%) | Parasitaemia (1000 ⁻¹ RBC) | ATP levels $\mu\text{M}/100 \text{ ml RBC}$ | No. | Hct (%) | Parasitaemia (1000 ⁻¹ RBC) | ATP content $\mu\text{M}/100 \text{ ml REC}$ |
|-----|---------|---------------------------------------|---|-----|---------|---------------------------------------|--|
| 1 | 19.5 | 134 | 123.90 | 22 | 24.5 | 285 | 66.67 |
| 2 | 12.5 | 161 | 156.73 | 23 | 31.0 | 300 | 135.43 |
| 3 | 13.6 | 312 | 169.25 | 24 | 27.0 | 242 | 162.83 |
| 4 | 15.0 | 165 | 140.28 | 25 | 27.5 | 10 | 145.80 |
| 5 | 11.5 | 274 | 76.09 | 26 | 39.0 | 278 | 105.20 |
| 6 | 15.5 | 307 | 85.45 | 27 | 24.0 | 388 | 103.01 |
| 7 | 22.0 | 216 | 153.34 | 28 | 22.5 | 483 | 95.70 |
| 8 | 22.5 | 474 | 53.69 | 29 | 11.5 | 466 | 252.58 |
| 9 | 13.0 | 387 | 228.09 | 30 | 34.0 | 502 | 93.67 |
| 10 | 18.0 | 190 | 202.67 | 31 | 19.0 | 133 | 98.47 |
| 11 | 17.0 | 214 | 145.08 | 32 | 40.0 | 373 | 144.89 |
| 12 | 29.0 | 442 | 144.43 | 33 | 32.0 | 123 | 119.37 |
| 13 | 24.0 | 612 | 155.54 | 34 | 41.0 | 319 | 75.73 |
| 14 | 10.5 | 687 | 388.90 | 35 | 33.0 | 583 | 90.07 |
| 15 | 30.0 | 236 | 187.89 | 36 | 31.0 | 445 | 117.96 |
| 16 | 20.5 | 535 | 250.77 | 37 | 37.0 | 474 | 136.10 |
| 17 | 18.5 | 332 | 326.48 | 38 | 27.0 | 655 | 144.78 |
| 18 | 13.0 | 568 | 354.12 | 39 | 35.0 | 455 | 67.27 |
| 19 | 16.0 | 182 | 104.45 | 40 | 28.0 | 691 | 121.59 |
| 20 | 30.5 | 276 | 68.98 | 41 | 14.5 | 349 | 112.66 |
| 21 | 10.5 | 234 | 145.14 | 42 | 29.0 | 174 | 123.22 |

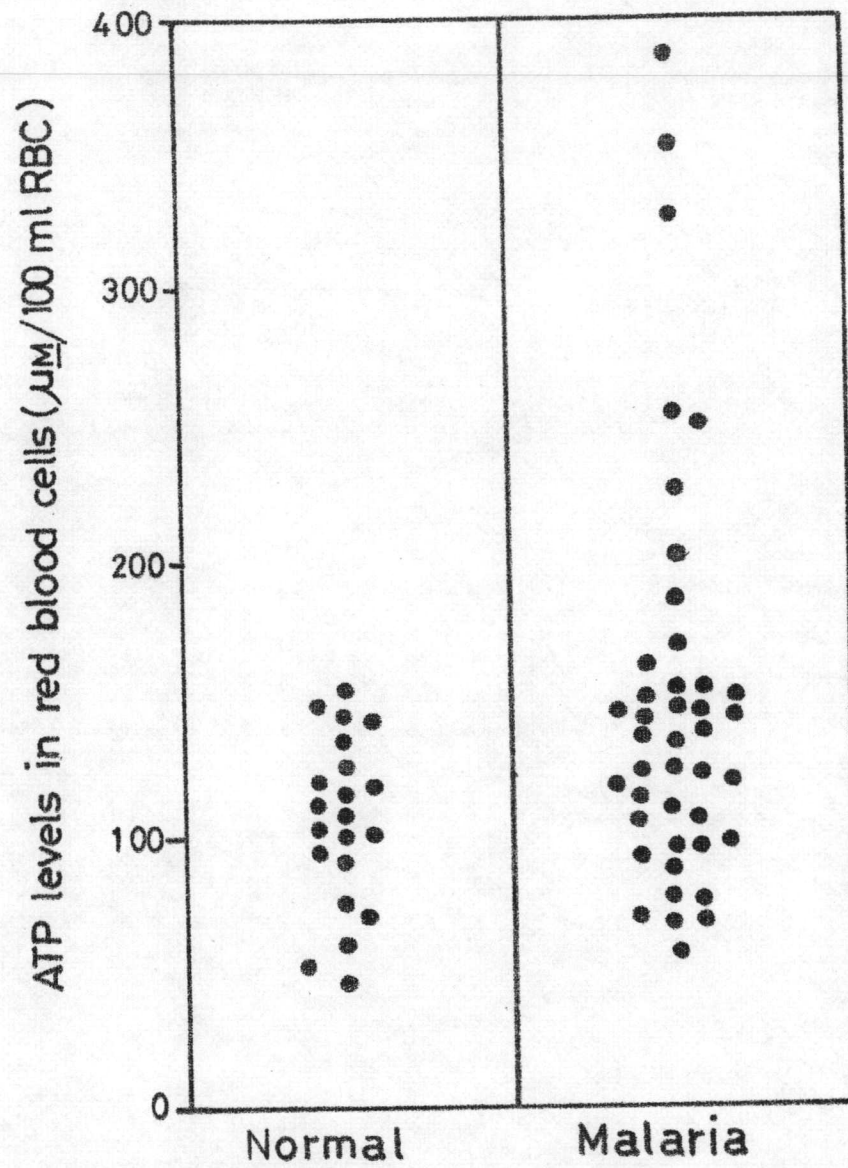


Fig.11 ATP content in red cells of normal and the P. berghei-infected mice.

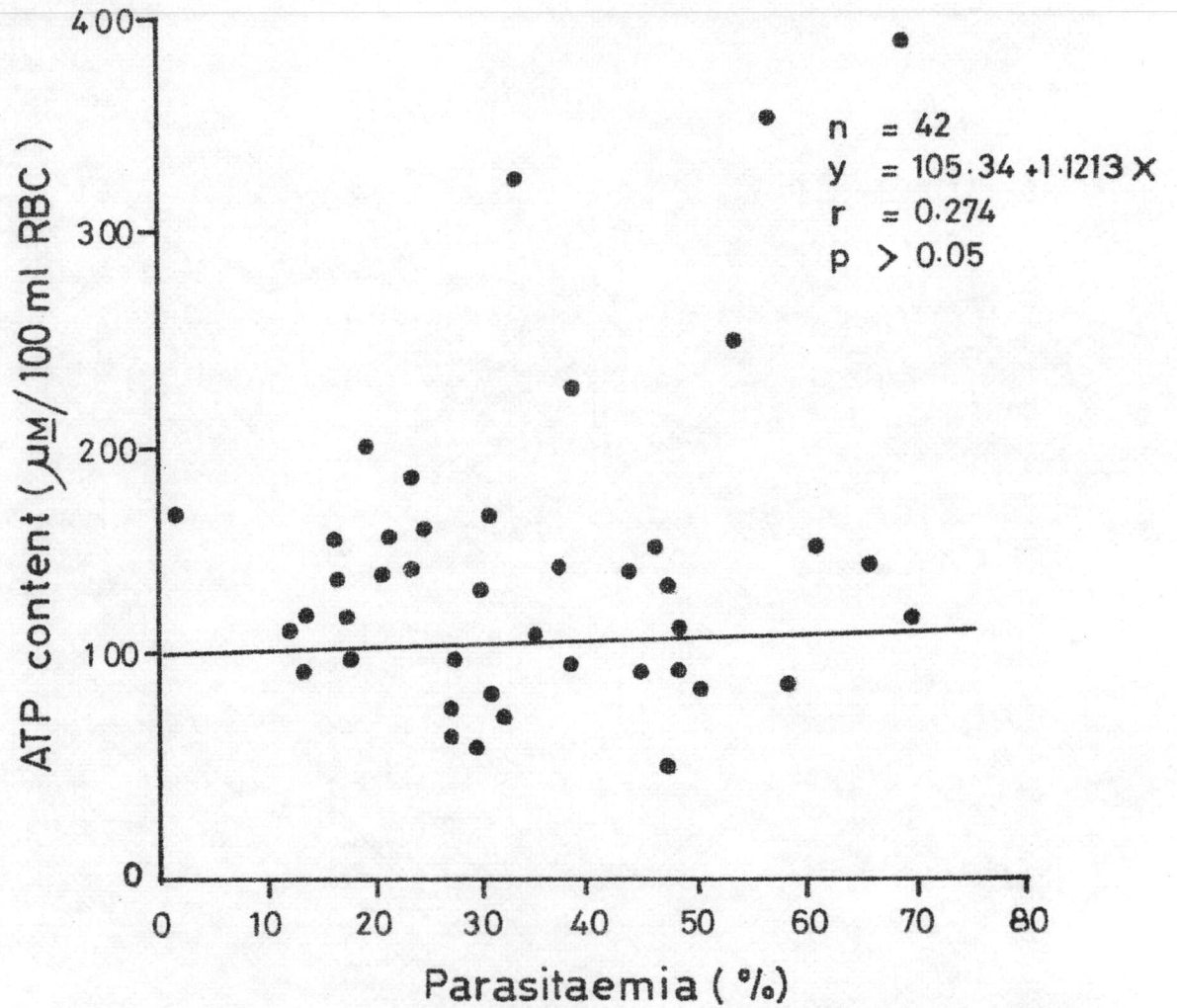


Fig. 12 Relationship between the parasitaemia(%) and the measured red cell ATP content of P. berghei-infected mice.

sucrose in Krebs glucose-saline solution. The calculated ATP content of parasitized red cells was significantly higher than that of the non-parasitized red cells ($P < 0.05$), (see Table 13).

There was a significantly reversed relationship ($P < 0.01$) between the parasitaemia and the ATP content of parasitized red cells as illustrated in Fig. 13.

The result also shows that the level of ATP in non-parasitized red cells is not greater than that in erythrocytes of the normal mice.

TABLE 13

The measured ATP content in red blood cells of mice infected with P. berghei and the calculated ATP content in the parasitized and non-parasitized red cells

| No. | Hct (%) | Parasitaemia (%) | ATP content ($\mu\text{M}/100$ ml RBC) | | |
|------|---------|------------------|---|----------------------------|--------------------------------|
| | | | Measured in RBC (a)* | Parasitized red cells (b)* | Non-parasitized red cells (c)* |
| 1 | 22 | 21.6 | 153.3 | 145.9 | 7.4 |
| 2 | 22 | 21.6 | 153.3 | 131.4 | 22.0 |
| 3 | 22 | 21.6 | 153.3 | 135.3 | 18.0 |
| 4 | 15 | 13.4 | 123.9 | 71.8 | 52.2 |
| 5 | 13 | 16.1 | 167.9 | 60.7 | 107.2 |
| 6 | 13 | 16.1 | 167.9 | 87.1 | 80.8 |
| 7 | 13 | 16.1 | 167.9 | 79.8 | 88.1 |
| 8 | 15 | 16.5 | 217.3 | 213.1 | 4.2 |
| 9 | 15 | 16.5 | 217.3 | 61.9 | 155.3 |
| 10 | 15 | 16.5 | 217.3 | 103.7 | 113.5 |
| 11 | 27 | 27.4 | 166.5 | 100.1 | 65.3 |
| 12 | 16 | 30.7 | 115.3 | 93.8 | 21.5 |
| 13 | 16 | 30.7 | 115.3 | 73.0 | 42.3 |
| 14 | 23 | 47.4 | 45.7 | 23.0 | 22.7 |
| 15 | 13 | 38.7 | 228.1 | 204.4 | 23.7 |
| 16 | 21 | 53.5 | 234.0 | 74.1 | 160.0 |
| 17 | 27 | 65.5 | 92.4 | 80.0 | 12.4 |
| 18 | 35 | 45.5 | 67.3 | 23.9 | 43.4 |
| 19 | 28 | 69.1 | 94.4 | 73.0 | 21.4 |
| 20 | 31 | 44.5 | 81.4 | 59.6 | 21.8 |
| Mean | 20 | 31.4 | 148.9 | 94.7 | 54.2 |

$$(a)^* = (b)^* + (c)^*$$

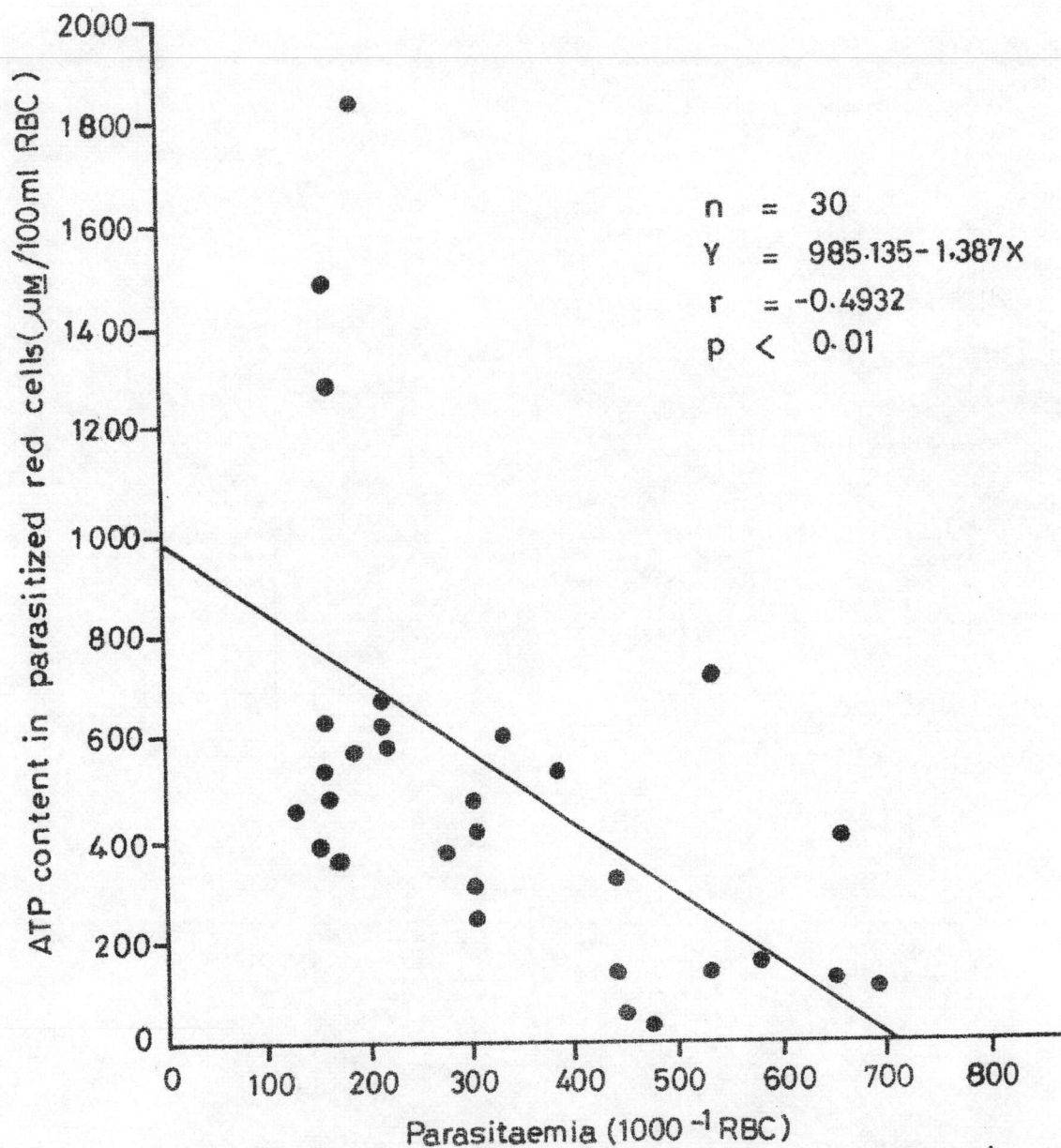


Fig. 13 Relationship between the parasitaemia (1000⁻¹ RBC) and the ATP content in the parasitized red cells.