9. Title: THE PHYSIOLOGY OF THE MALARIAL PARASITE

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Objective -

It is the purpose of this project to investigate in detail the degradative and biosynthetic metabolism of rodent, simian, and human malarial parasites. In addition, concurrent investigations are planned in which the mechanism of action of anti-malarial drugs, in particular chloroquine, will be studied. Certain aspects of host metabolism, particularly those which might bear directly on the host-parasite relationship, are also being examined.

Description -

Both the degradative and biosynthetic metabolism of malarial parasites will be investigated using tracer techniques with C^{14} labelled substrates: Respiratory function is being investigated using a combination of isotopic and respirometry methodologies.

The development of chloroquine-resistant Plasmodium berghei is being conducted according to the method of Peters (W. Peters; Exp. Parasitol. 17:80 (1965).

Progress -

Pertinent host metabolism -

A series of preliminary investigations on the respiratory metabolism of mouse red blood cell preparations were conducted utilizing the Warburg respirometry technique. Heparinized blood was obtained from normal, uninfected mice. The cells were separated from the plasma by centrifugation at 10,000 g for 10 min. The white cells, buffy coat, and plasma were removed and the RBC were resuspended in 0.9% saline. The centrifugation and washing were repeated 2 more times. The cells (approx. 1.5 cc packed cells) were resuspended in 0.05M Tris buffer (pH7.6). This resuspension in buffer resulted in approximately 75% hemolysis in 30 min. No increase in hemolysis was noted over a 90 min. period. This preparation was used as the source of metabolic activity.

Two different substrates, glucose and glucose-6-phosphate, (10^{U} moles/reaction vessel) were tested in the presence and absence of 0.1^{U} mole of methylene blue, a well-established RBC respiratory stimulator.

The experimental design is shown in Table I.

In Figure 1 are shown the results of a typical study. Each curve is the result of the average of duplicates.

The use of glucose and methylene blue, or glucose-6-phosphate does not result in respiratory activity different from that of the boiled control. The only significant respiratory activity occurred in those vessels in which the substrate was glucose-6-phosphate and methylene blue was added as a respiratory stimulant. These results are in agreement with previous work which has shown that hexose phosphate is metabolized by RBC hemolysates in the presence of methylene blue. It is surprising, however,

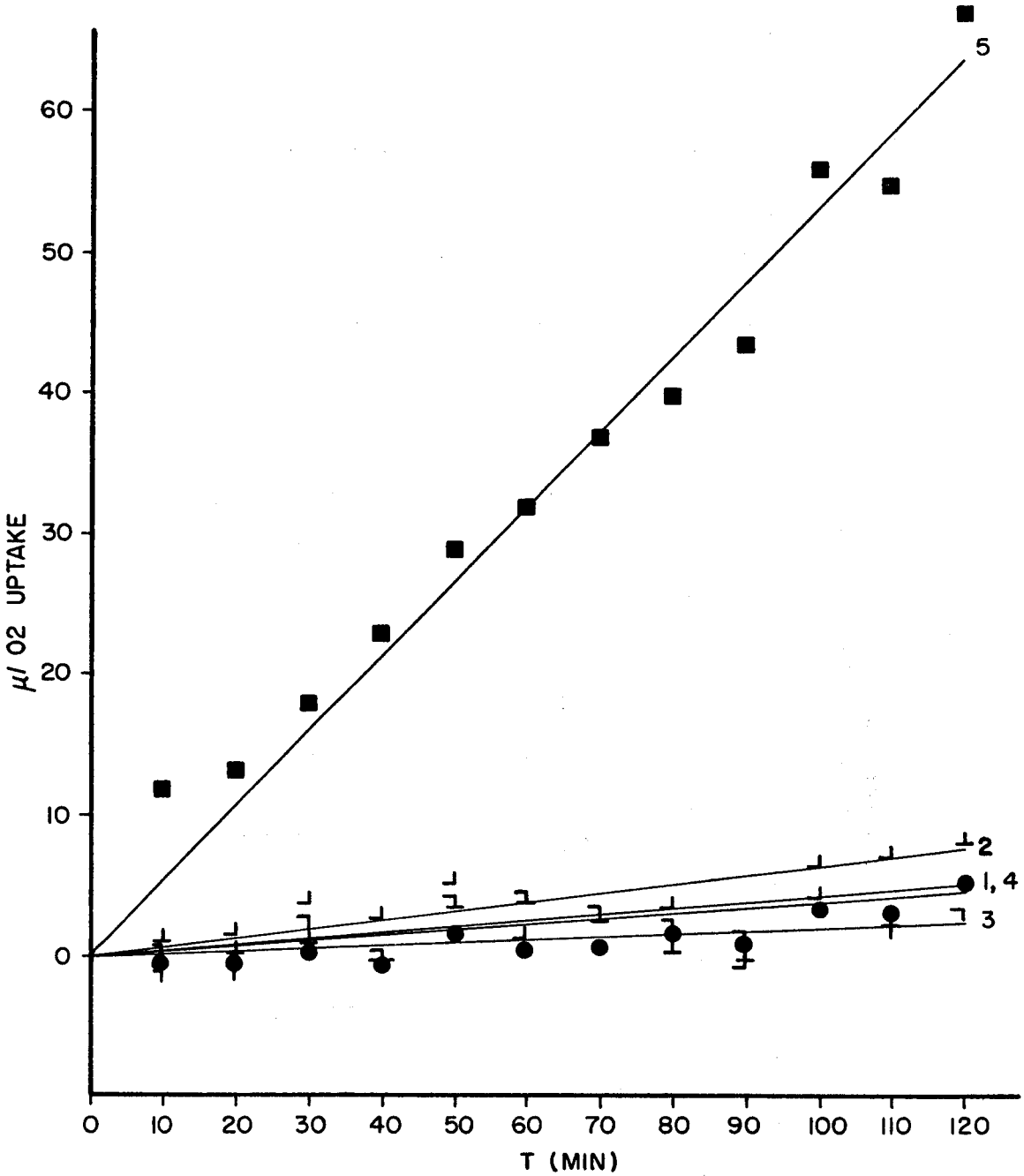
TABLE 1

Experimental design for the study of the respiration of mouse red blood cell preparations

Flask No.	Main Vessel			Side Arm		Center Well	Remarks
	ml. cell-buffer suspension	ml. Methylene blue (10. um/ml)	ml. water	ml. glucose (100 um/ml)	ml. glucose-6-phosphate (100 um/ml)	ml. 20% KOH	
1	—	—	1.1	—	—	—	Thermo-Barometer Boiled Controls
2.3	0.8	—	0.1	0.1	—	0.1	
4.5	0.8	—	0.1	0.1	—	0.1	
6.7	0.8	0.1	—	0.1	—	0.1	
9.10	0.8	—	0.1	—	0.1	0.1	
11.12	0.8	0.1	—	—	0.1	0.1	

FIGURE I.

THE RESPIRATION OF MOUSE RED BLOOD CELLS



SUBSTRATE:

CURVE 1 — BOILED CONTROL (●); 2 — GLUCOSE (⌋); 3 — GLUCOSE + METHYLENE BLUE (¬); 4 — GLUCOSE-6-PHOSPHATE (⊥), 5 — GLUCOSE-6-PHOSPHATE + METHYLENE BLUE (■)

(GLUCOSE & GLUCOSE-6-PHOSPHATE-10 μ MOLES; METHYLENE BLUE-0.1 μ MOLE)

that no respiration was observed with glucose, and methylene blue, and glucose-6-phosphate because a low unstimulated respiration is believed to exist. At present we have no explanation for these results.

Further work in this area is required to more clearly define and quantitate the respiratory system of mouse red blood cells. Studies on the general metabolism of other rodent, simian, and human red blood cells are planned. The study of normal cells will provide data to which the metabolic activity of uninfected RBC's from infected animals can be compared. This comparison will aid in determining what effect malaria infection has on the uninfected cells of the body, an important consideration in the host-parasite relationship.

Parasite metabolism.

Since the initiation of this project, the major part of our effort has been expended in setting up and standardizing methodologies.

The method for protein determination will be that of Lowry et al. (O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall; J. Biol. Chem. 193:265 (1951) which provides a sensitive measurement of protein concentration over the range from 50 to 300 ug/ml. Hemoglobin determination will be done by the cyanomethemoglubin method.

Recently it was discovered that the mice possessed an infection other than malaria. The organism is rod shaped and almost strictly intracellular (within the RBC). Blood has been taken for culture and antibiotic treatment has begun. This infection has delayed the initiation of studies on the respiratory activity of P. berghei parasites.

5. Preparation of antisera.

Antiserum for use in the disruption of RBC has been prepared for mouse and rat RBC. Antiserum for use against simian and human RBC's will be prepared.

The antiserum is made by inoculating normal rabbits with 0.5 ml. of packed normal RBC at 5 day intervals for a total of 5 injections. Booster shots are given every two weeks to maintain high lysin titer.

Figure 2 shows that essentially complete lysis of mouse RBC's occurs in about 30 minutes using rabbit anti-mouse RBC serum. The fact that over 100% hemolysis is observed is due to low hemoglobin values, because of dilution, with the spectrophotometer readings falling in the low, unreliable region. However, several repeat runs have indicated that hemolysis is consistently in excess of 90%

6. Preparation of parasites freed from the red cell.

The procedure of Targett and Fulton (G.A.T. Targett and J.D. Fulton; Exp. Parasitol. 17:180 (1965) is being used to free the parasite from the host cell. The procedure consists of lysing the RBC's with anti-serum and isolation of the freed parasites by differential centrifugation.

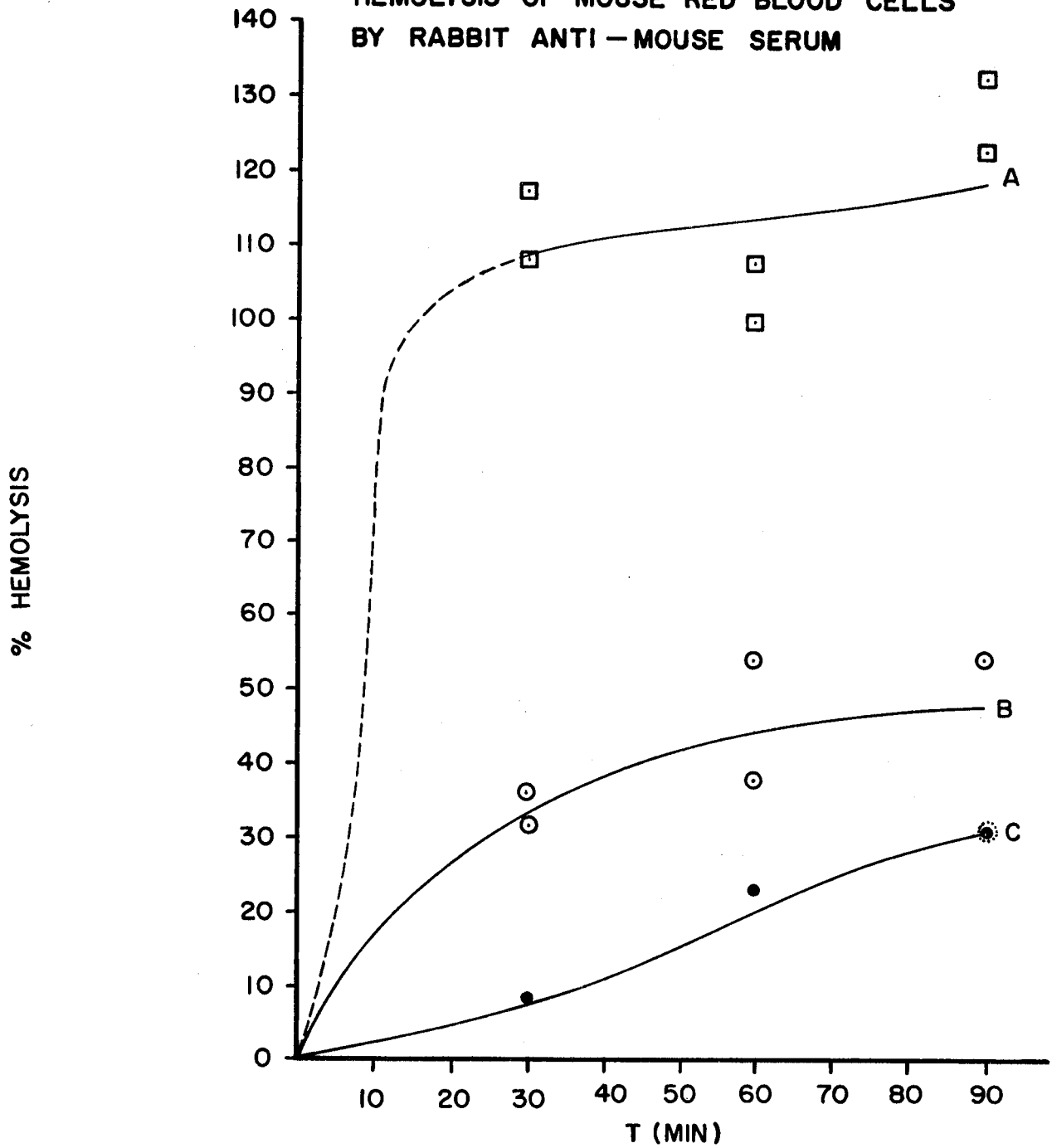
Trial runs have been conducted which have yielded preparations which microscopically correspond very closely to the pictures published by Targett and Fulton.

Summary.

Since the initiation of this project the major part of our effort has been expended in setting up and establishing the reliability of our general methodologies as well as preparing anti-sera and proper quantities of parasites. Studies on the respiration of normal mouse RBC hemolysates have yielded results which indicate that additional work is required in this area of host metabolism prior to the undertaking of studies dealing with the host-parasite relationship.

FIGURE 2.

HEMOLYSIS OF MOUSE RED BLOOD CELLS BY RABBIT ANTI-MOUSE SERUM



A — RABBIT ANTI-MOUSE SERUM
B — NORMAL RABBIT SERUM
C — SALINE CONTROL