1. Title: The Physiology of the Malarial Parasite.

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OBJECTIVE

It is the purpose of this study to investigate, in detail, the anabolic and catabolic metabolism of rodent, simian, and human malaria parasites, as well as the same pathways in the host cell. These data will permit quantitative and qualitative comparison of the metabolism of the parasite and host cell with the objective of clarifying the biochemical interactions of the host–parasite relationship.

METHODS

The metabolic activity of the parasite and host cells is being investigated utilizing a combination of isotopic, spectrophotometric and respirometric methodologies.

PROGRESS

Table I presents a compilation of the various enzyme systems of cell–free homogenates of normal and infected mouse erythrocytes and host cell–free *Plasmodium berghei* studied during the reporting period.

Lactic acid dehydrogenase (LDH). The electrophoretic mobility, substrate stereospecificity, and apparent Michaelis constant (K_m) values for lactic acid, pyruvic acid, and nicotinamide adenine dinucleotide (oxidized and reduced forms) were examined. The parasite enzyme was electrophoretically distinct from that of the host cell. Both enzymes were stereospecific for L (+) lactic acid. No significant differences existed between the host cell and the parasitic LDH's in the K_m values for lactic acid and nicotinamide adenine dinucleotide (oxidized and reduced forms). However, the value for pyruvic acid with the parasitic LDH was 1/36th that of the host cell enzyme showing an increased affinity of the former system for pyruvate. The parasitic LDH had higher specific activities with both lactic acid and pyruvic acid than did the host cell enzyme.

L and D Amino Acid Oxidases–The presence of a flavin mononucleotide dependent L–amino acid oxidase has been demonstrated in host cell–free extracts of *P. berghei*. There was no evidence suggesting the presence of a D–amino acid oxidase, in fact, the addition of D–alanine inhibited oxygen consumption by the extracts.

Glutamic acid dehydrogenase–The presence of a NADP–dependent glutamic acid dehydrogenase was established in cell–free extracts of host cell–free *P. berghei*. The activity of the enzyme was not affected by the presence of L–thyroxine; 3,3, 5–triiodo–L–thyronine; ADP; or ATP. The pH optimum was found to lie between 8.0 and 8.5.

Methyl group metabolism–The proteolytic release of methionine by extracts of *P. berghei* has been demonstrated. The data support the conclusion that the parasite cannot store or retrieve labile methyl groups in or from phospholipid choline. However, the parasite does have the ability to synthesize methionine methyl groups *de novo*. The inability of the parasite to recycle methyl groups through methionine and the requirement for methionine in protein synthesis explains, at least in part, the need of the parasite for a continuing source of this amino acid.

TABLE 1 The net specific activities of the enzymes of *Plasmodium berghei* studied during the reporting period.

Enzyme	NET SPECIFIC ACTIVITY		
	Source of enzyme		
	P. berghei	Infected RBC	Normal RBC
Lactic acid dehydrogenase			
Lactic acid as substrate	0.38[a]	—	0.06[a]
Pyruvic acid as substrate	2.8[b]	—	0.18[b]
L—Amino acid oxidase	1.1×10^{-3}[c]	—	0.13×10^{-3}[c]
D—Amino acid oxidase	0[c]	—	0[c]
Glutamic acid dehydrogenase			
NAD as cofactor	0.05×10^{-3}[a]	0.14×10^{-3}[a]	0
NADP as cofactor	15.5×10^{-3}[d]	0.4×10^{-3}[d]	0
Methionine methyl group synthesis system	51.9×10^{-3}[e]	—	0
Methyl transferases			
Choline—homocysteine	0[e]	—	0
Betaine—homocysteine	3.3×10^{-3}[e]	—	0
Methionine—phospholipid (cephaline)	0[f]	—	0

Specific activities

a. = μmoles NAD reduced/mg protein/minute
b. = μmoles NADH oxidized/mg protein/minute
c. = μmoles O_2 consumed/mg protein/minute
d. = μmoles NADP reduced/mg protein/minute
e. = mμmoles methionine synthesized/mg protein/minute
f. = mμmoles lecithin formed/mg protein/minute

This work has resulted in the preparation of the following manuscripts:

1. Malarial parasite metabolism: The lactic acid dehydrogenase of Plasmodium berghei. P. Phisphumvidhi and B.W, Langer, Jr., Exptl. Parasitol., 24:37 (1969).
2. Malarial parasite metabolism: The amino acid oxidase of Plasmodium berghei. B.W. Langer, Jr., and P. Phisphumvidhi. Submitted to Nature.
3. Malarial Parasite metabolism: The glutamic acid dehydrogenase of Plasmodium berghei. B.W. Langer, Jr., P. Phisphumvidhi, and D. Jiampermpoon. Submitted for clearance.
4. Malarial parasite metabolism: The metabolism of methionine by Plasmodium berghei. B.W. Langer, Jr., P. Phisphumvidhi, D. Jiampermpoon, and R.P. Weidhorn. To be published as part of a supplement of Military Medicine presenting the Proceedings of the Malaria Workshop held in Washington, D.C., May 1969.