Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. 81, Suppl. II :149-152, 1986. *Intern. Symp. on Malaria*

OVERVIEW: CARBOHYDRATE METABOLISM OF MALARIAL PARASITES

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The major pathway for carbohydrate metabolism in plasmodia is the glycolisis. The pentose phosphate pathway participates on a minor proportion and in some species like Plasmodium knowlesi *isolated from red cell no activity was found. The whole part of the citric acid cycle is admitted in avian plasmodia but not in rodent or mammalian plasmodia. Some inhibitory effect of the antimalarials on carbohydrate metabolism of* Plasmodium *is shown. A quick method to obtain a selective index of inhibition on lactate dehydrogenase isoenzymes in crude extracts of* P. falciparum *and human erythrocyte is discussed.*

Bass & John (1912) observed that Plasmodia infected erythrocytes consume more glucose than uninfected erythrocytes. The glucose consumption increase rate for infected and uninfected erythrocyte varies from 2.6-fold for *Plasmodium lophurae*-duckling red blood cell (Sherman, Ruble & Ting, 1969), up to 79-fold for *P. vivax* human red blood cell (McKee, 1951), passing through the intermediate values for different plasmodia species and their erythrocyte hosts (Sherman, 1984). Although most of the time blood cell components other than erythrocyte have been neglected, the observed high proportion of glucose consumption in infected *versus* uninfected erythrocyte suggest that most of the excess glucose consumed in the infected erythrocyte should be due to the plasmodia.

Additionally to parasite consume itself another fact that may explain the high glucose consumption in *Plasmodium*-infected erythrocyte is the alteration in the erythrocyte membrane permeability caused by the presence of the parasites intracellularly. Using the non-metabolisable sugars 3-0-methyl glucose and L-glucose (Sherman & Tanigoshi, 1974 and Neame & Homewood, 1975) showed that the presence of *P. lophurae* and *P. berghei* intraerythrocytically accelerated the entry of the sugars by changing the membrane permeability of the host cell.

Lactate has been found to be the main product of glucose metabolism in almost all *Plasmodium* species. For the avian plasmodia, *P. lophurae* and *P. gallinaceum* in addition to lactate it has also been found a high percentage of CO_2, pyruvate, citrate, succinate, aminoacids and acetate (Bovarnick, Lindsay & Hellerman, 1946; Sherman, Ruble & Ting, 1969; Speck, Moulder & Evans, 1946 and Marshall, 1948). Furthermore, for isolated P. *knowlesi* free from erythrocyte cells, lactate, CO_2 and at a great proportion volatile acids were found after glucose consumption (Christophers & Fulton, 1939 and Scheibel & Miller, 1969). Among the different *Plasmodium* species the substance that stimulates more oxygen consumption is glucose, followed by mannose, frutose, maltose, glycerol and lactate (Fulton, 1939; Maier & Coggeshall, 1941; Marshall, 1948; McKee, 1951 and Nagarajan, 1968). For *P. lophurae* and *P. gallinaceum* oxygen consumption level as high as that found for glucose was also found when using lactate as substrate (Bovarnick, Lindsay & Hellerman, 1946).

The main pathway for glucose catabolism in mammal and rodent plasmodia seems to be the glycolisis whereas in the avian plasmodia it seems to be the glycolisis added to the citric acid cycle. Homewood & Neame (1983) showed that the glycolisis pathway in *P. berghei*-infected erythrocyte although high, has been understimated in many reports. After correcting an interference on the lactate assay, they observed that the glucose consummed was completely converted into lactate. Conversely to the glycolisis, the pentose phosphate pathway is responsible for a very small proportion of the carbohydrate catabolysed by *P. gallinaceum* (Herman, Ward & Herman, 1966), *P. lophurae* (Sherman, Ting & Tanigoshi, 1970), *P. berghei* (Bryant, Voller & Smith, 1964), *P. knowlesi* (Shakespeare, Trigg & Tappenden, 1979). Sheibel & Pflaum (1970) found no activity for the pentose phosphate pathway in purified *P. knowlesi*. In such a metabolically active parasites it seems paradoxal the low efficiency observed for such an important pathway, since it has the ability to regenerate NADPH for almost all synthetic purposes and provide pentoses for the nucleic acid synthesis. It is believed that most of the NADPH come from a plasmodial specific glutamate dehydrogenase (Walter, Nordmeyer & Königk, 1974) and the pentoses from ATP catabolites or direct uptake from the host.

Another intriguing aspect of the pentose phosphate pathway that remained unsolved for years was the facility the second enzyme of the sequence, 6-phosphogluconate dehydrogenase

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(6PGD), was found whereas glucose 6 phosphate dehydrogenase (G6PD), the first enzyme, was never found. Only very recently, Usanga & Luzzato (1985) showed that *P. falciparum* possesses the G6PD gene which, however, is only expressed under stressing conditions, for instance, when the parasites are cultivated serially on human erythrocytes G6PD deficient. Although only malate dehydrogenase (MDH), succinate dehydrogenase (SD) and isocytrate dehydrogenase (ICD) has been reported in avian plasmodia (Sherman, 1984), the existence of the whole or part of the citric acid cycle in those plasmodia should be considered. On the other hand, the only putative citric acid cycle enzyme found in mammal and rodent plasmodia is the malate dehydrogenase (Vander Jagt et al., 1982 and Momem, 1979), therefore ruling out the possibility of existence of the citric acid cycle on mammal and rodent plasmodia.

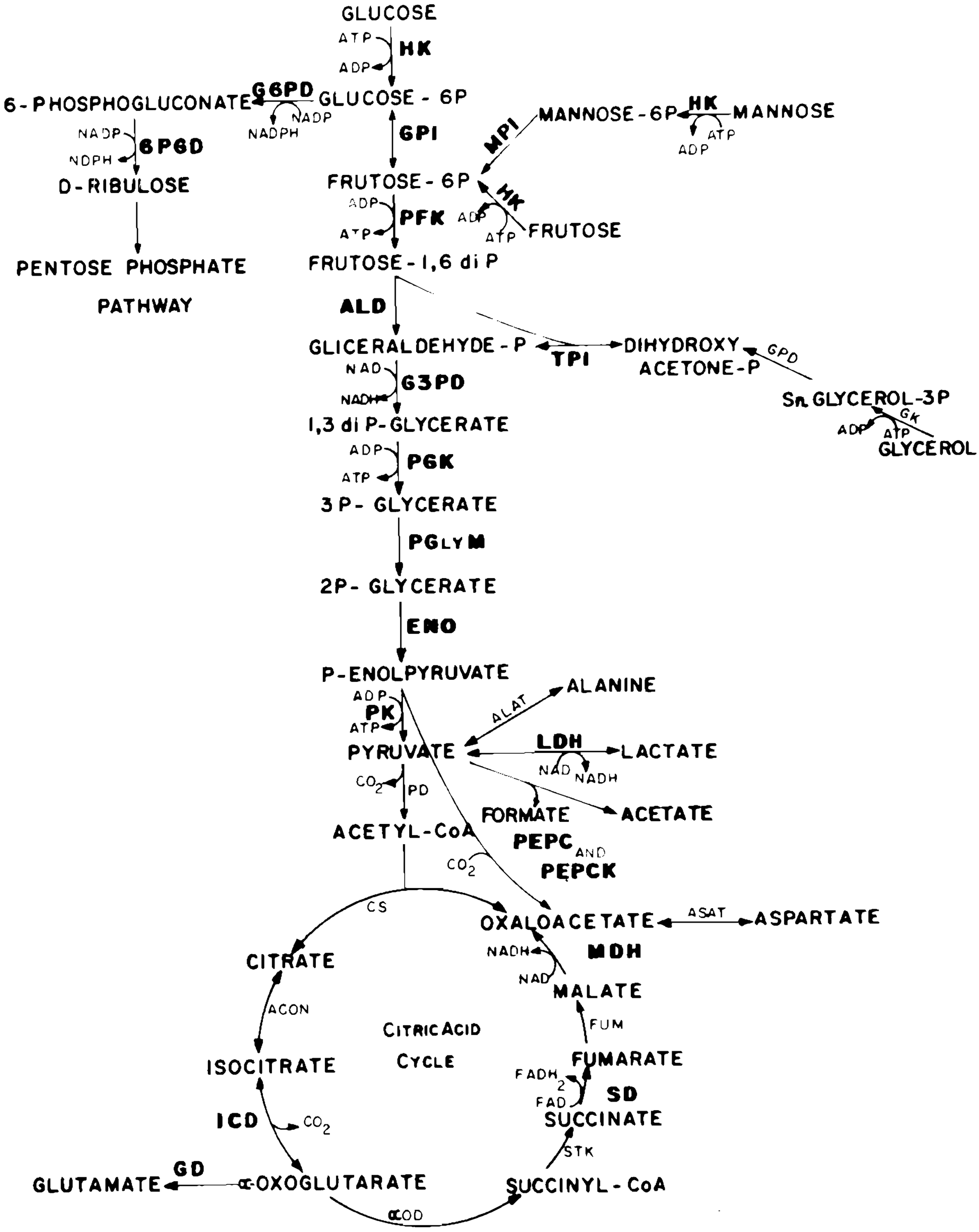

Fig. 1: Probable carbohydrate metabolism in malarial parasites. The enzymes already described in *Plasmodium* are presented in dark. Enzyme abbreviations: **HK** = hexokinase; **GPI** = glucose phosphate isomerase; **G6PD** = glucose-6-phosphate dehydrogenase; **6PGD** = 6-phosphogluconate dehydrogenase; **MPI** = mannose phosphate isomerase; **PFK** = phosphofrutokinase; **Ald** = aldolase; **TPI** = triosephosphate isomerase; **GPD** = glycerol phosphate dehydrogenase; **GK** = glycerol kinase; **G3PD** = glyceraldehyde 3-phosphate dehydrogenase; **PGK** = phosphoglycerate kinase; **PGlyM** = phosphoglyceromutase; **Eno** = enolase; **PK** = pyruvate kinase; **ALAT** = alanine aminotransferase; **LDH** = lactate dehydrogenase; **PD** = pyruvate dehydrogenase; **PEPC** = phosphoenol pyruvic carboxylase; **PEPCK** = phosphoenol pyruvic carboxy kinase; **CS** = citrate synthase; **Acon** = aconitase; **ICD** = isocitrate dehydrogenase; **GD** = Glutamate dehydrogenase; **αOD** = α-oxoglutarate dehydrogenase; **STK** = succynil thiokinase; **SD** = succinate dehydrogenase; **Fum** = Fumarase; **MDH** = malate dehydrogenase; **ASAT** = aspartate aminotransferase.

The Fig. 1 shows the probable carbohydrate metabolism in *Plasmodium*. This metabolic pathway was based on substrate consumption, product formation and enzyme activity assay or staining by several authors in different *Plasmodium* species. It should be noticed that a complete enzyme sequence has not been found for a single *Plasmodium* specie yet but a general *Plasmodium* metabolic picture may be drawn.

One of the problems with *Plasmodium* enzyme activity assay has been the host contaminant enzyme. This problem has been overcome by the staining on a gel of the enzymes from parasite and host separately, (Carter & Walliker, 1977; Momen, 1979; Vander Jagt, Hunsaker & Heidrich, 1981 and Sanderson, Walliker & Molez, 1981). Host and parasite enzyme are only defined after comparing the gel pattern of a non-parasitized crude host cell preparation with that of an enriched parasite-infected host cell. The quantification of the intensity of separated bands developed on gel (Romanha, Gutteridge & Colin, in preparation) enables the simultaneous inhibitory studies on lactate dehydrogenase isoenzymes in crude extracts of *P. falciparum* and human erythrocytes. They showed that kinetically *P. falciparum*-LDH resembles more human LDH-5 than human LDH-1 and that oxalate inhibited more selectively *P. falciparum*-LDH than human LDH-1 or 5. The oxalate concentration needed to inhibit fifty percent of the LDH activity on gel (I_{50}) for *P. falciparum*-LDH, human LDH-1 and 5 were 0.01, 0.38 and 0.15mM respectively. Thus giving a selective inhibitory index of 38 for *P. falciparum* LDH-human LDH-1 and 15 for *P. falciparum* LDH-human LDH-5.

Finally, to illustrate the action of some antimalarials on plasmodial carbohydrate metabolism and transport the Table I is presented below. Obviously the inhibitory effect presented by the antimalarials is neither the only nor the principal mechanism of action of these drugs. Conversely, the action described here may be irrelevant *in vivo* since the drug concentration tested *in vitro* has been rather high, in the milimolar range.

TABLE I

Inhibitory effect of some antimalarial on plasmodial carbohydrate metabolism and transport

Antimalarial	Inhibitory effect	*Plasmodium* sp.	Reference
1) Chloroquine and mepacrine	hexokinase (HK)	P. *berghei*	Fraser & Kermac (1957)
2) Chloroquine and quinine	phosphoenolpyruvate carboxykinase (PEPCK)	P. *berghei*	McDaniel & Siu (1972)
3) Mepacrine	6-phosphofrutokinase (PFK)	P. *berghei*	Bowman et al. (1961)
4) Quinine	pyruvate oxidation	P. *gallinaceum*	Moulder (1949)
5) Dapsone	glucose uptake by red cell		Cenedella & Jarrel (1970)

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