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MODULATION OF MEMBRANE TRANSPORT: A BIOCHEMICAL APPROACH TO THE  
CHEMOTHERAPY OF MALARIA

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In this report we shall deal only with the collaborative aspect of this project. In the above mentioned period, we set out two working meetings, one in Bangkok (6 days in February 1989, by Prof. Cabantchik) and the other in Jerusalem (30 days in May 1989, by Dr. Wititsuwannakul). As a result of these meetings we have coordinated our programmes and conducted experiments in the areas of mutual interest. The area of research comprises the application of novel iron chelators as antimalarials, reported in our previous report and the delineation of the biological sources of iron in malaria parasitized cells. All of the *in vitro* work has been carried out in Jerusalem with cultures of *P. falciparum*, while all the in vivo work is being carried out with *P. Berghei*, in mice.

1. We have reported in our previous report about the IC-50 values of the

new iron chelators, some of which were 30 fold more potent than desferral. In this period we concentrated on the delineation of the compartment of the infected cell which appears to be the target for the chelators.

Plasma, uninfected cells and infected cells were treated with the chelators and used in various combinations so as to assess the compartment affected. The respective systems were treated with the chelators for up to 12 hours, washed by dialysis (plasma) or centrifugations and incubations in growth medium and tested in our bioassay system (see previous report).

It was found that while the plasma was totally unaffected by the chelators (it remained fully supportive of parasite growth), red cells lost partially the ability to support growth after invasion. Using our model of Sendai permeabilized rings (Silfen et al, 1989), we found that parasite growth was considerably less susceptible to chelator than intact rings, in agreement with the above findings.

2. Using Tb as a model metal ion for Fe, we have followed the chelating properties of the various chelators and found that they removed the metal from preformed Tb-transferrin, from preformed Tb-ferritin and from Tb loaded cells (with excess Tb/chelator in the medium). For these experiments we have used fluorescence techniques, taking advantage of the spectrofluorimetric properties of conjugated Tb. Gel filtration techniques demonstrated that the intracellular Tb was primarily sequestered by erythrocytic ferritin. Those experiments were initiated in Jerusalem by Dr. Wititsuwannakul, from Thailand.

3. In Thailand, Dr. Witsuwannakul, has defined a benzyl alcohol extractable

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fraction from parasite origin (*P. berghei*), which displayed siderophore capacity, insofar as it could remove radioactively labelled iron from ferritin. The method used to study the mobilizing properties of the putative siderophore was by gel filtration of the prelabelled ferritin.

#### Publications

- 1 . Z.I. Cabantchik (1989) Altered membrane transport of malaria infected erythrocytes: a possible pharmacological targets. Blood (in press).
- 2\*. Silfen, J. Yanai, P. and Cabantchik, Z.I. (1988). Bioflavonoideffects on *in vitro* cultures of *P. falciparum*: inhibition of permeation pathways induced in the host cell membrane by the intraerythrocytic parasite. *Biochem. Pharmacol.* 37:4269-4276.
- 3\*. Cabantchik, Z.I., Silfen, J. Krugliak, M. Firestone, J.F., Nissani, E. and Ginsburg, H. (1989) Effects of lysosomotropic detergents on the human malarial parasite *Plasmodium falciparum* in *in vitro* cultures. *Biochem. Pharmacol.* 38:1271-1277.
- 4.\* Cabantchik, Z.I., Glickstein, H. and Silfen, J. (1989). Pharmacological definition of the permeation pathways induced in *Plasmodium falciparum* infected human erythrocytes *J. Cell. Biochem* (In press).
- 5\*. Baruch, D. and Cabantchik, Z.I. (1989) Passive modulation of antigenic expressions on the surface of malarial infected red blood cells. *Mol.*

Biochem. Parasitol. 36:127-138.

6. Baruch, D., Glickstein, H. and Cabantchik, Z.I. (1989). A sensitive ELISA assay for surface antigenic properties of intact erythrocyte parasitized with *Plasmodium falciparum* (in preparation)

As we have requested a non-funded extension of this project, which has started rather belatedly, because of logistic problem, finances, etc, we are actively pursuing our research endeavours in a fully collaborative fashion, for the period approved by the AID-CDR authorities.