Biochemical and immunochemical investigation of some South African strains of the human malaria parasite, *Plasmodium falciparum*

by

Anton Carel Stoltz

Submitted in partial fulfilment of the requirements for the degree of

*Master of Science*

in the Faculty of Science
Department of Biochemistry
University of Pretoria

*February 1992*
ACKNOWLEDGEMENTS

I acknowledge, with gratitude, the following persons:

Professors A.I. Louw and J.A. Verschoor for their academic input, support and guidance during my studies and assistance with the completion of the thesis.

Professor L. Visser for his unfailing support and encouragement.

Mrs. J. Freese and Dr. Schutte from NIDTE for sharing their knowledge of malaria cultures with us and teaching me the technique at their laboratories in Durban.

Professor Fripp and Drs. Hansford and Sieling for making the anti-malaria drug resistance survey possible by providing the necessary equipment and funds.

Professor Coetzee and his colleagues of the Electron Microscopy Unit, University of Pretoria for expert technical assistance and support.

Marietjie Meyer and all my friends and colleagues who supported me during difficult periods.

Last but not least, my parents for their moral support and encouragement.
## TABLE OF CONTENTS

### CHAPTER 1

**GENERAL LITERATURE REVIEW**

1.1 Diagnosis of malaria  1  
1.2 Epidemiology  2  
1.3 Life cycle of the malaria parasites  4  
1.4 The different malaria species  6  
1.5 Pathology of malaria  12  
1.6 Drug resistance  15  
1.7 Immunology of malaria  16  
1.8 Metabolism of malaria  20  
1.9 Continuous culture of *P. falciparum*.  22  
1.10 Objectives  23  
1.11 REFERENCES  25  

### CHAPTER 2

**DRUG SUSCEPTIBILITY AND SHORT-TERM CULTURE OF Plasmodium falciparum.**

2.1 INTRODUCTION  31  
2.1.1 Antimalarial drug classification and mechanism of action  33  
2.1.2 Cloroquine resistance  37  
2.1.3 New drugs  38  
2.1.4 Objectives  39  
2.2 MATERIALS AND METHODS  39  

2.2.1 Microtest
2.2.2 Samples
2.2.3 Blood smears
2.2.4 Giemsa staining
2.2.5 Dill Glazko's reagent
2.2.6 Preparation of growth medium
2.2.7 Performance of the microtest

2.3 RESULTS
2.4 DISCUSSION
2.5 REFERENCES

CHAPTER 3
CONTINUOUS CULTURING AND BIOCHEMISTRY OF THE ERYTHROCYTIC STAGES OF Plasmodium falciparum

3.1 INTRODUCTION
3.1.1 Factors affecting the continuous cultivation of P. falciparum.

3.1.2 Nutritional requirements of P. falciparum. during in vitro culturing

3.1.3 Objectives

3.2 MATERIALS AND METHODS
3.2.1 Serum
3.2.2 Erythrocytes
3.2.3 Mediums
3.2.4 Gassing of cultures
3.2.5 Continuous cultivation of *P. falciparum*.

3.2.6 Gassing experiments

3.2.7 Cryopreservation of *P. falciparum*-infected blood or cultures

3.2.8 Thawing of cryopreserved infected erythrocytes

3.2.9 Medium supplements and parasite growth

3.2.10 Metabolite concentrations in *P. falciparum*-infected cultures

3.2.11 RESULTS

3.3.1 Time required to displace the air above thin-layered cultures with the special gas mixture

3.3.2 Effect of gassing method on parasite growth

3.3.3 Gas profile of medium under culture conditions

3.3.4 Parasite growth in mediums with different supplements

3.3.5 Metabolite concentrations in *P. falciparum*-infected cultures

3.4 DISCUSSION

3.5 REFERENCES

CHAPTER 4

A MICRO-ENZYME-IMMUNOASSAY FOR THE DETERMINATION OF THROMBOCYTE-ASSOCIATED IMMUNOGLOBULINS IN MALARIA PATIENTS

4.1 INTRODUCTION

4.2 MATERIALS AND METHODS

4.2.1 Preparation of partially purified immunoglobulin
4.2.2 Comparison between coating buffers and pin coating samples

4.2.3 Thrombocyte preparation

4.2.4 Immunoglobulin coated TSP

4.2.5 Determination of thrombocyte-associated IgG and IgM

4.3 RESULTS

4.3.1 Preparation of partially purified immunoglobulin

4.3.2 Coating buffers and immunoglobulin source for coating pins

4.3.3 Optimization of the coupling of immunoglobulins onto TSP-pins

4.3.4 Standard curves

4.3.5 Measurement of thrombocyte-associated IgG and IgM

4.4 DISCUSSION

4.5 REFERENCES

CHAPTER 5
ULTRASTRUCTURAL STUDIES OF ERYTHROCYTES INFECTED WITH *P. falciparum*.

5.1 INTRODUCTION

5.1.1 Parasite-induced changes to the erythrocyte membrane

5.1.2 Intraerythrocytic cytoplasmic organelles

5.1.3 Fixation of tissues for electron microscopy

5.1.4 Ultrastructural features of erythrocyte stage parasites

5.1.5 Objectives
5.2 MATERIALS AND METHODS

5.2.1 Parasite collection and in vitro culturing

5.2.2 Preparation of blood smears for light microscopy

5.2.3 Slow fixation of infected erythrocytes

5.2.4 Fast fixation of infected erythrocytes

5.2.5 Processing of glutaraldehyde-fixed, infected tissues for scanning electron microscopy

5.2.6 Processing of glutaraldehyde-fixed, infected tissues for transmission electron microscopy

5.3 RESULTS

5.3.1 Light microscopy of *P. falciparum*-infected erythrocytes

5.3.2 Scanning electron microscopy of *P. falciparum*-infected erythrocytes

5.3.3 Transmission electron microscopy of *P. falciparum*-infected erythrocytes

5.4 DISCUSSION

5.5 REFERENCES

CHAPTER 6

6.1 CONCLUDING DISCUSSION

6.2 REFERENCES

SUMMARY

OPSOMMING
LIST OF FIGURES

Figure 1.1 Malaria areas in Southern Africa 3
Figure 1.2 Malaria notifications in South Africa from 1957 to 1989 4
Figure 1.3 Life cycle of Plasmodium species 6
Figure 1.4 Comparison between the bloodstages of human malaria strains after Giemsa staining 11
Figure 1.5 Purine salvage pathway in the erythrocyte and Plasmodium 22
Figure 2.1 Structure of antimalarial compounds 37
Figure 2.2 Growth inhibition from 18 patients tested for chloroquine and mefloquine resistance in North-Eastern Transvaal during 1988 43
Figure 2.3 a) Population pie chart of chloroquine resistance in the North-Eastern Transvaal during 1988
               b) Population pie chart of mefloquine resistance in the North-Eastern Transvaal during 1988 44
Figure 3.1 Diagrammatic representation of experiments to determine metabolite concentrations in Plasmodium falciparum cultures 63
Figure 3.2 Extraction of purine nucleotides from erythrocytes 66
Figure 3.3 Time required to displace air in 250ml growth
Figure 3.4  Parasite growth in air- and gas-equilibrated culture mediums

Figure 3.5  HPLC elution pattern of erythrocyte-PCA extracts from infected and non-infected cultures from experiment A.

Figure 3.6  Comparison of various parameters during in vitro culture of *P. falciparum*-infected and non-infected erythrocytes under different conditions

Figure 3.7  HPLC elution pattern of erythrocyte-PCA extracts from infected and non-infected cultures from experiment B

Figure 3.8  Comparison of various parameters during in vitro culture of *P. falciparum* infected and non-infected erythrocytes

Figure 4.1  Diagrammatic presentation of the protocol for thrombocyte-associated immunoglobulin determination

Figure 4.2  Elution pattern of the immunoglobulin fraction isolated from human serum on a 40x1.5cm Sephacryl S-300 column

Figure 4.3  Comparison between coupling methods for IgG

Figure 4.4  Comparison between coupling methods for IgM
| Figure 4.5 | Optimization of the coupling of partially purified immunoglobulin onto the TSP-pins | 113 |
| Figure 4.6 | Optimization of the dilution of anti-IgG and -IgM-peroxidase | 114 |
| Figure 4.7 | Standard curve for the quantification of TA IgG | 114 |
| Figure 4.8 | Standard curve for the quantification of TA IgM | 115 |
| Figure 5.1 | Transmission electron micrographs of organelles seen in the cytoplasm of infected erythrocytes | 124 |
| Figure 5.2 | Light microscope photograph of the blood stages of isolate PfUP1 | 131 |
| Figure 5.3 | Scanning electron micrograph of non-infected erythrocytes after four days in continuous culture | 132 |
| Figure 5.4 | Scanning electron micrograph of PfUP1-infected erythrocytes fixed with the slow gluteraldehyde method | 133 |
| Figure 5.5 | Scanning electron micrograph of PfUP1-infected erythrocytes fixed by the fast method | 134 |
| Figure 5.6 | Scanning electron microscope photographs of PfUP1-infected erythrocytes with knobs | 135 |
| Figure 5.7 | Transmission electron micrographs of PfUP1-infected erythrocytes | 136 |
LIST OF TABLES

Table 3.1 - Supplements added to freshly prepared medium. 62
Table 3.2 - Gas analysis of culture mediums after 1 and 24
hours of incubation at 37°C 72
Table 3.3 - Growth comparison between PfUP1-infected erythrocytes
in medium supplemented with human serum 74
Table 3.4 - Precursor-product relationship and metabolic status 85
Table 3.5 - Parasite stages in relation to parasitemia 86
Table 4.1 - ELISA determination of thrombocyte-associated IgM
(TAIgM) and IgG (TAIgG) in human blood. 116
Table 5.1 - Quetol embedding resin. 130
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>AMP</td>
<td>Adenosine monophosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>CS</td>
<td>Circum-sporozoite</td>
</tr>
<tr>
<td>CSP</td>
<td>Circum-sporozoite protein</td>
</tr>
<tr>
<td>DIC</td>
<td>Disseminated intravascular coagulation</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DDT</td>
<td>Chlorophenothane</td>
</tr>
<tr>
<td>FPIX</td>
<td>Ferriprotoporphyrin IX</td>
</tr>
<tr>
<td>IMP</td>
<td>Inosine monophosphate</td>
</tr>
<tr>
<td>MDR</td>
<td>Multi-drug resistance</td>
</tr>
<tr>
<td>MSA</td>
<td>Merozoite surface antigen</td>
</tr>
<tr>
<td>NIDTE</td>
<td>National Institute for Diseases in a Tropical Environment</td>
</tr>
<tr>
<td>PABA</td>
<td>Para-aminobenzoic acid</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>PfHRP1</td>
<td><em>Plasmodium falciparum</em> histidine rich protein</td>
</tr>
<tr>
<td>PfUP1</td>
<td><em>P. falciparum</em> University of Pretoria isolate number one</td>
</tr>
<tr>
<td>PPM</td>
<td>Parasitophorous plasma membrane</td>
</tr>
<tr>
<td>PVM</td>
<td>Parasitophorous vacuolar membrane</td>
</tr>
<tr>
<td>RER</td>
<td>Rough endoplasmic reticulum</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscope</td>
</tr>
</tbody>
</table>
TSP - Transfer solid phase
WHO - World Health Organisation