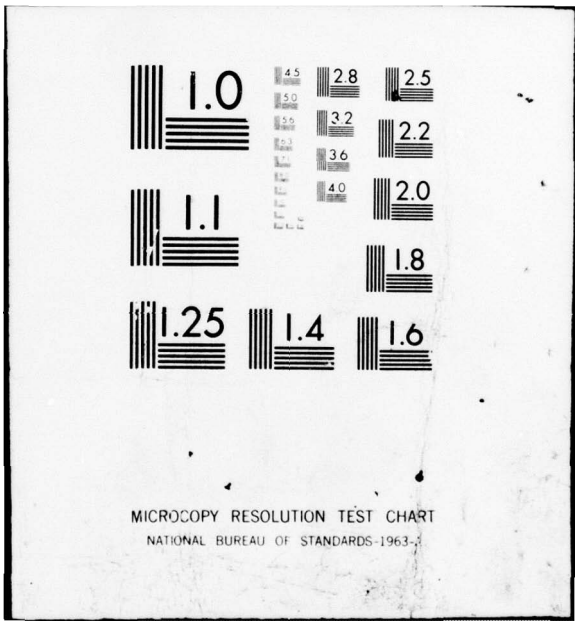
AD-A068 262 TUFTS-NEW ENGLAND MEDICAL CENTER BOSTON MA F/G 6/5
ISOLATION AND CHARACTERIZATION OF ERYTHROCYTE AND PARASITE MEMB--ETC(U)
APR 78 D F WALLACH DAMD17-74-C-4118

UNCLASSIFIED NL

1 OF 1
AD
A068262

END
DATE FILMED
6 -79
DDC


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
6.3
7.1
8.0
9.0
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

LEVEL II

12

Isolation and Characterization of Erythrocyte and Parasite Membranes from Rhesus Red Cells Infected with P. Knowlesi

I

Annual Summary Report

June 1, 1974 - May 31, 1975

Donald F. H. Wallach

April 1978

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD 17-74-C-4118

Tufts-New England Medical Center
171 Harrison Avenue
Boston, MA 02111

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents

Approved for public release; distribution unlimited.

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER	(9) Summary
4. TITLE *(and Subtitle)* (6) Isolation and Characterization of Erythrocyte and Parasite Membranes from Rhesus Red Cells Infected with P. Knowlesi. I.	5. TYPE OF REPORT & PERIOD COVERED Annual Report 1 Jun 1974 - 31 May 1975
	6. PERFORMING ORG. REPORT NUMBER I
7. AUTHOR(s) (10) Donald F. H. Wallach, M. D. (17) 00	8. CONTRACT OR GRANT NUMBER(s) (15) DAMD 17-74-C-4118
9. PERFORMING ORGANIZATION NAME AND ADDRESS Tufts-New England Medical Center Boston, Massachusetts 02111	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62759A (16) 3A762759A829 00.228
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick, Frederick, Maryland 21701	12. REPORT DATE (11) Apr 1978
	13. NUMBER OF PAGES 15
14. MONITORING AGENCY NAME & ADDRESS *(if different from Controlling Office)* (12) 6p.	15. SECURITY CLASS. *(of this report)* Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

P. knowlesi, membrane fractionation techniques.

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The first six months of the contract period covered by this report have been devoted to training of personnel in the handling of rhesus monkeys, transmission of P. knowlesi malaria and membrane fractionation techniques. In addition, monkeys have been purchased and are currently in quarentine.

410941

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

79 05 03 018

a. Organization

The first six months of the contract period covered by this report have been largely devoted to "setting up".

First, Dr. J. Alroy has spent time at the Laboratory of Parasitic Diseases, NIAID, under the supervision of Dr. L. Miller, Head of the Malaria Section, for thorough training in the handling of P. knowlesi.

Second, a laboratory assistant, Miss Margaret Conley, hired in December, 1974, has been rigorously trained in methods for surface iodination of intact erythrocytes for erythrocyte disruption, erythrocyte membrane fractionation, and separation of erythrocyte membrane proteins by sodium dodecyl sulfate polyacrylamide electrophoresis, isoelectric focusing as well as crossed immune electrophoresis. Rigorous application of these methods is required to distinguish between the properties of the membranes from normal and parasitized erythrocytes.

Third, affiliation has been established with the New England Primate Center and space set aside there for 20 Rhesus monkeys.

An unavoidable delay has been encountered because of the recent decline in the availability of Rhesus monkeys and their large increase in cost. Indeed, for this reason, the contract was renegotiated on Sept. 23, 1974. Twenty animals were finally delivered on January 10 and malarial infections are scheduled to begin by February 1.

This delay means that the time frame of the contract has been delayed by about six months. The only contract funds expended during the period of June 1, 1974 to December 31, 1974 have been for Dr. Alroy's salary and his visit to Dr. Miller's laboratory. Since Dr. Alroy is 50% (year) on the contract but could only spend 25% of his time in the first six

months for the reasons mentioned, he will spend 75% of his time in the second six months.

b. New Approaches to Erythrocyte Membrane Disruption

Anticipating the need for more sophisticated methods of erythrocyte disruption for the study of parasite induced alterations of erythrocyte membranes, we have explored two new alternatives to standard erythrocyte lysis techniques.

The first, involves the use of the fluorocarbon anaesthetic, halothane, to disrupt erythrocytes. This approach and its results with normal human erythrocytes is detailed in Appendix 1, and can be summarized as follows:

1. Suspension of human erythrocytes in halothane-saturated physiological media induces major cell deformation.

2. Release of halothane from erythrocyte suspensions after equilibrium with the anaesthetic produces complete hemolysis.

3. The membrane fragments isolated after halothane release are in the form of biconcave ghosts, impermeable to macromolecules (lactoperoxidase) and small molecules (ATP).

4. The protein composition of the membranes differs from that of ghosts produced by hypotonic lysis in the lack of components previously shown to be adsorbed at low ionic strength.

5. A hypothesis is presented explaining the action of halothane in terms of both its action on membranes and its capacity to modify water structure.

6. Halothane-induced hemolysis constitutes a facile method for the large-scale production of hemoglobin depleted, sealed erythrocyte ghosts, under physiologica ionic conditions.

In cooperation with Dr. L. Miller, we have tested the halothane approach on Rhesus erythrocytes infected with P. knowlesi. Not unexpectedly, the technique, as such, did not yield satisfactory release of parasites from the erythrocyte ghosts. However, such release can be achieved by subsequent vesiculation of parasite containing ghosts by liquid or mechanical shear.

The second approach, developed in cooperation with Dr. L. Miller, appears extremely promising. It employs the nitrogen-decompression method developed by us for nucleated cells (cf. Wallach, D.F.H. and Kamat, V.B., Proc. Natl. Acad. Sci., 52, 721, 1964; Wallach, D.F.H. and Lin, P.S., Biochim. Biophys. Acta, 300, 211, 1973). In this, cells are exposed to elevated pressures of nitrogen at 4°C and then returned abruptly to atmospheric pressure. Normal erythrocytes are extraordinarily resistant to disruption by this procedure, but parasitized cells disrupt readily with concurrent release of parasites.

Parasitized cells were suspended in Phosphate-buffered saline and equilibrated at various nitrogen pressures for different time intervals and erythrocyte rupture, as well as parasite release (and parasite damange) evaluated by phase-contrast microscopy.

We found that exposure to 35 atm. for 10 min. produced essentially quantitative rupture of parasitized cells with large scale release of intact parasites.

12 Copies	Director (ATTN: SGRD-UWZ-AG) Walter Reed Army Institute of Research Walter Reed Army Medical Center Washington, D. C. 20012
4 Copies	HQDA (SGRD-AJ) Fort Detrick Frederick, MD 21701
12 Copies	Defense Documentation Center ATTN: DDC-TCA Cameron Station Alexandria, Virginia 22314
1 Copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, Maryland 20014
1 Copy	Superintendent Academy of Health Sciences, US Army ATTN: AHS-COM Fort Sam Houston, Texas 78234