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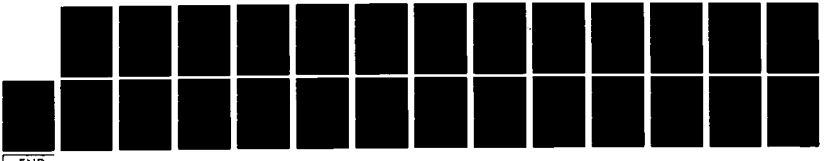

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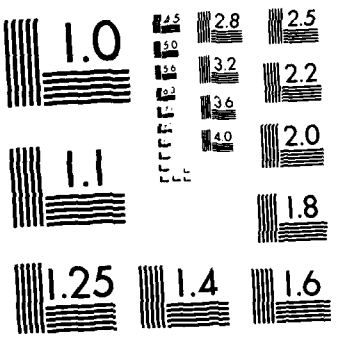

Isolation and Characterization of Erythrocyte and Parasite Membranes from Rhesus Red Cells Infected with P. Knowlesi

III

Annual Summary Report

June 1, 1978 - May 31, 1979

Donald F.H. Wallach

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

Intraerythrocytic maturation of P. knowlesi was achieved by cultivating infected cells in FEP-teflon containers, at controlled pO_2, and pH, allowing labeling of parasite components and parasite-induced glycoproteins in host-cell membranes with ^{14}C-amino acids and ^{14}C-sugars. Aminoacid and glucosamine labeling were localized primarily in the parasite whereas ^{14}C-galactose

(Continued on reverse)

Block 20 (continued). and ^{14}C-fucose associated mostly with host cell membranes. Among host-cell membrane proteins preferential incorporation of ^{14}C-amino acids and ^{14}C-glucosamine occurred at pI 4.5-5.2 upon isoelectric focusing and these proteins/glycoproteins migrated between 90,000 and 45,000D upon SDS-PAGE.

Monkey anti-schizont antisera and the serum of a monkey rendered naturally immune after infection with P.knowlesi showed the following immunoelectrophoretic results: (a) twenty-four immune precipitates identified in purified schizonts. Seven components were common to schizonts and erythrocyte membranes, four unique to the infected membrane and the thirteen were exclusively found in the parasite. (b) Absorption of sera with schizont-infected erythrocytes eliminated at least three schizont immunoprecipitates and at least five host-cell membrane components. (c) CIE of ^{125}I-labeled membrane proteins followed by autoradiography revealed that antigens 13 and 19 are exposed on the surfaces of infected cells. (d) Parasite-induced membrane components, 1 and 13, were immunogenic in vivo. Schizonts and host-cell membranes from Rhesus erythrocytes infected with the Philippine strain of P. knowlesi gave the same immune precipitates as equivalent material from a Malaysian strain of P. knowlesi.

1. Introduction

High resolution protein fractionation techniques have been applied to investigate proteins/membrane proteins of purified P. knowlesi schizonts and membranes of normal and parasitized erythrocytes. Immunochemical analyses using a rhesus monkey hyperimmune serum against P. knowlesi schizonts and bidimensional IEF-DS-PAGE allowed us to identify several parasite-induced proteins/glycoproteins of which at least three are unique for the membrane of infected erythrocytes; the apparent molecular weights and isoelectric points of these components are 55,000D/pI 4.5, 65,000D/pI 4.6, and 90,000D/pI 5.2 (1, 2).

Based on these results, our research was continued to answer the following questions:

(a) The nature of the proteins/glycoproteins; their partial or complete origin of the intracellular parasite.

(b) Subfractionation of schizonts (6-10 nuclei) after disruption by nitrogen decompression to assign membrane proteins of the schizont and components of the vacuolar membrane.

(c) The isolation of plasma membrane vesicles derived from parasitized erythrocytes enriched in parasite-specific antigens.

(d) Immunogenicity of parasite-induced proteins/glycoproteins in vivo and its correlation with a protective immunity. Variation of antigenicity in different strains and variants of P. knowlesi.

2. Procedures; Results and Discussion

2.1 Metabolic labeling of parasitized erythrocytes in vitro.

Metabolic labeling of erythrocyte membrane proteins/glycoproteins must be

Abbreviations: CIE- crossed immune electrophoresis; DS PAGE-dodecyl sulfate polyacrylamide gel electrophoresis; IEF-isoelectric focusing; pI - isoelectric point.

mediated by the intracellular parasite because erythrocytes lack any active metabolism of their protein components. Although the technique of metabolic labeling we employed can by no means be considered optimal, we have obtained reproducible data using short term cultures in a large scale.

2.1.1 Procedures

At a parasitemia of 30-50%, erythrocytes were harvested at the early schizont stage. The erythrocytes were washed three times in Dulbecco's PBS and then freed of leukocytes and thrombocytes using a Ficoll-Hypaque gradient (density 1.08) as in (1, 3). The erythrocytes have been suspended at a concentration $2 \cdot 10^8$ cells/ml in RPMI 1640 medium (4), 10% fetal calf serum adjusted to 3.0 g glucose, 5 mg adenosine, 1.25 g glycylglycine, 15 mg ascorbic acid, and D-Ca pantothenate and biotin/1000 ml medium (5). The cells were cultured in Teflon bags (Instrumentation Laboratory, Lexington, Mass) at 37°C in a humidified incubator perfused with 10% CO_2, 8% O_2 and 82% N_2. [^{14}C]amino acids, [^{14}C]glucosamine, [^{14}C]galactose and [^{14}C]fucose were added to a final concentration of 50 Ci/100 ml of medium and labeling was for 12-16 hrs. After cultivation, the cells were washed three times in PBS. Parasitized cells were isolated and disrupted by nitrogen decompression as described in (1, 3).

2.1.2 Results

During in vitro cultivation, the intracellular parasites exhibited a normal but delayed development to more mature schizonts (4-10 nuclei). Counts of parasitized erythrocytes prior to and after 16 hr of culture never yielded lysis of more than 20% of the infected cells. Because we observed a very low degree of reinfection the most probable reason for lysis was maturation of the schizonts to yield merozoites.

Of the total ^{14}C amino acids added to the medium 5-15% (depending upon the parasitemia) of the label was incorporated into protein. Less than 20% of the radioactivity was recovered in the membranes of infected cells, 75% and more was associated with purified schizonts (Table 1). A substantially lower proportion

tion of radioactive precursor was incorporated when the erythrocytes were cultured in presence of [^{14}C]glucosamine (Table 1), [^{14}C]galactose and [^{14}C]fucose. Despite their different relative distribution in isolated infected membrane and schizonts (Table 2) 0.5-2.0% of each of the ^{14}C-monosaccharides was covalently incorporated into glycoproteins. Data presented in Table 2 indicated that glucosamine was incorporated into the parasite to a larger extent than into the erythrocyte membrane whereas galactose and fucose was predominantly associated with the plasma membrane of infected erythrocytes.

Membrane proteins/glycoproteins of parasitized erythrocytes metabolically labelled with ^{14}C-amino acids and ^{14}C-glucosamine are identified in Figures 1 and 2. Selective labelling of both, the polypeptide and the carbohydrate moiety of proteins focusing at pH 4.5, pH 4.8, pH 5.0 and pH 5.2 is observed (Fig. 1). The apparent molecular weights of the proteins/glycoproteins range from 90,000D to 45,000D. In contrast, all proteins of the schizont are labelled with ^{14}C-amino acids; equally ^{14}C-glucosamine is incorporated into many components with isoelectric points between pH 6.5 and pH 4.0 and apparent molecular weights of 120,000 to 20,000D. In addition, our results suggest that metabolic labelling in combined use with subcellular fractionation can yield valuable information about the interaction between host cell and parasite. This is indicated by a different extent of label incorporation into identical components of the two cellular compartments: the pI 5.2 component was labeled in both the infected membrane and in the parasite. However, the degree of relative glycosylation (as determined by the ratio of [^{14}C]-glucosamine/^{14}C-amino acids) is substantially higher in the schizont. In contrast, the pI 4.5 component was labeled with [^{14}C]glucosamine in the host-cell membranes but not in the parasite.

Both, IEF and DS-PAGE reveal ^{14}C-glucosamine in the lipid region, i.e. metabolic labeling of glycolipids (peak at pI 3.8 in Fig. 1; label at the electrophoresis front in Fig. 2).

2.1.3 Discussion

Cultivation of P. knowlesi-infected erythrocytes in a large scale (10^{10} cells and more) initiated at the early schizont stage revealed a sufficiently synchronized maturation to schizonts with 4-10 nuclei. As a result, we obtained a highly reproducible qualitative and quantitative labeling pattern in purified parasites and isolated infected membranes using ^{14}C-amino acids and ^{14}C-monosaccharides as precursors. In contrast, we had observed a rapid desynchronization when the cultures of infected erythrocytes were started at the trophozoite stage (2).

The initiation of culture at the early schizont stage clearly reveals the advantages of synchronized maturation and reproducibility in metabolic labeling. On the other hand, we have to accept 10-20% lysis of mature parasitized cells. Another disadvantage may be that the radioactive precursors are offered at a time at which the parasite may not exhibit its maximum metabolic activity (6).

DS-PAGE and IEF of metabolically labeled membrane proteins from infected erythrocytes revealed label incorporation of both ^{14}C-amino acids and ^{14}C-monosaccharides at molecular weights and isoelectric points similar to those described for parasite-induced components, unique for the host-cell membrane (1). However, during 16 hr of labeling the infected cells did not incorporate sufficient quantities of ^{14}C-metabolites into both parasite and erythrocyte proteins to yield satisfactory autoradiograms of bidimensional IEF-DS-PAGE electropherograms.

Our data indicate that parasite protein/glycoproteins are transported to and incorporated into the host cell membrane. However, for a more quantitative analysis of these interesting host-parasite relationships the cultivation conditions for large scale cultures need to be studied in more detail. In this, most important is the maintenance of synchronization for at least one cycle allowing effective metabolic pulse labeling at different maturation stages of the intraerythrocytic parasites.

2.2 Subfractionation of purified P. knowlesi schizonts

Intact schizonts are surrounded by two membrane systems; an outer layer, the vacuolar membrane, originating at least in part from the host-cell membrane and, inside of this, the parasite plasma membrane (7). Our approach to this heterogeneous system is to convert both membranes into small vesicles which could then be subfractionated into constituents of the two cellular compartments (2).

2.2.1 Procedures

Isolated schizonts (6-10 nuclei) were surface-labeled using lactoperoxidase-catalyzed radioiodination as employed for labeling of plasma membranes from parasitized erythrocytes (3). After appropriate washing, the schizonts were disrupte by nitrogen decompression (incubation at 600 psi N_2 for 15 min).

The cell homogenate was concentrated three-fold and laden onto a dextran gradient with density steps of 1.05, 1.10, 1.12 and 1.16. Ultracentrifugation was for $1.6 \cdot 10^7$ g · min. Four bands (B1-B4) were collected from the gradient, at the interphase of each density step, material that remained at the top of the tube (soluble protein) and particles that pelleted through the 1.16 density barrier. The total and specific [^{125}I]radioactivity of each fraction was determined; after two washing steps in 5 mM PO_4, pH 8.0, material from each band was analyzed for its protein composition. For this we employed isoelectric focusing in polyacrylamide (2) and crossed immune electrophoresis (see section 2.4.3).

2.2.2 Results

As documented in Table 3, only 10% of the label was associated with soluble cytoplasmic protein, indicating that most of the schizonts are intact, i.e. not permeable to molecules of the size of lactoperoxidase. We found that, Band 1 (lowest density) exhibited a five-fold higher specific [^{125}I]radioactivity than the intact schizonts and material that concentrated at high density ($\rho \sim 1.16$) most probably constituting undisrupted parasites. B2 ($\rho \sim 1.10$) and B3 ($\rho \sim 1.12$) have intermediate specific activities. B3, B4 and the pellet ($\rho > 1.16$) contain in-

creasing quantities of iron granules indicated by their intense brown color. We suspect that these granules act as natural density perturbants and therefore, cause about 50% of [^{125}I]activity to non-specifically accumulate at high density. The high specific [^{125}I]activity in B1 and B2, at densities where small membrane vesicles tend to concentrate suggests that lactoperoxidase predominantly mediates radioiodination of the intact schizonts' surface membranes. Band 3 to Band 4 of the dextran gradient exhibit a different protein composition very similar to undisrupted schizonts (1, 2). In contrast, material of B1 and B2 resembles the protein pattern of membranes from parasitized erythrocytes as obtained by IEF (Fig. 3). This conclusion is further supported by immunochemical analyses (see section 2.4.3, Fig. 9A-E).

2.2.3 Comments

The membranes under investigation resemble a natural mosaic of parasite and host cell components. Therefore, we can only make relative assignments concerning the origin of proteins using most discriminating fractionation techniques in combined use with selective labeling and absorption approaches. Beyond some helpful results obtained by IEF, analysis of the gradient fractions using CIE yields more discriminating findings (See section 2.4.3.). According to these data Band 2 exhibits the highest concentration of immune component 13, a parasite antigen exposed on the surface of infected erythrocytes. B2 may, therefore, resemble material of the vacuolar membrane because from experiments using schizonts of ^{125}I surface-labeled erythrocytes we can exclude that B2 is contaminated by erythrocyte membranes.

2.3 Fractionation of membrane vesicles using affinity density perturbation

The rationale of this approach is to purify functionally intact plasma membrane vesicles of infected erythrocytes enriched in parasite-specific components.

2.3.1 Procedures

Unshocked membrane vesicles derived from normal or parasitized erythrocytes were used. As density perturbant we employed latex spheres (600 Å diameter; 8) with a density of 1.32, derivatized with about 60 fluorescein-labeled BSA molecules and 25-30 immunoglobulin molecules per bead. (The technique was in principle as described in refs. 2 and 8; the number of BSA and IgG molecules/bead was determined by initially using ^{131}I-BSA and ^{125}IgG (Table 4). The immunoglobulin (IgG) is purified from hyperimmune sera raised in rabbits against normal monkey erythrocyte membranes and in monkeys against purified schizonts (1, 2). Latex spheres derivatized with rabbit or monkey hyperimmune IgG were reacted with membrane vesicles of normal monkey erythrocytes and erythrocytes parasitized by P. knowlesi, respectively. The specificity of each reaction was established by comparing the reactivity of beads derivatized with hyperimmune IgG vs. the respective non-hyperimmune IgG.

For density perturbation, $\sim 7 \cdot 10^{13}$ beads were reacted with 1 mg of membrane protein (unshocked and labeled with ^{125}I, using lactoperoxidase-catalyzed radioiodination) for 16 hrs at 4°C with constant slow stirring. The reaction mixture was then fractionated into latex beads, membranes and membrane-bead complexes using step gradients of CsCl (20, 25, 30, 35 and 40%, w/w; pH 7.5). The membranes do not penetrate the 20% CsCl step whereas the beads concentrate at 35% CsCl. After Ultracentrifugation at $8 \cdot 10^6$ g · min, the latex spheres were localized by their fluorescence and the membranes by determining the ^{125}I-distribution (for more experimental details see legends of Table 4 and 5).

Prior to DS-PAGE, the bead-membrane complexes were washed in 5 mM PO_4, pH 8.0. The membranes were dissolved in 1% DS/40 mM DTT and the beads removed by ultracentrifugation at 10^7 g · min. Using DS-PAGE the protein composition of membranes specifically reacted with latex beads was compared with unreacted membranes and the whole vesicles population.

2.3.2 Results and Discussion

Preliminary experiments using normal monkey erythrocyte membranes showed that these membrane vesicles specifically bound to beads derivatized with hyperimmune IgG (Table 4). At high membrane concentrations, about 50% of the beads (hyperimmune IgG) concentrate at the density of the membranes; this was not the case when the membranes were reacted with beads containing normal IgG. At an increased ratio of bea mg membrane protein (Table 4, B) about 30% of the membrane vesicles bound a sufficien number of beads to concentrate at the density of beads themselves. Qualitatively similar results have been obtained when membrane vesicles of infected erythrocytes were reacted with beads derivatized with monkey anti-schizont IgG (T e 5). Whereas in the control experiments (Table 5, column 2 and 3) only about 11% the ^{125}I-membranes were recovered at the density of the latex beads (35% CsCl ity 1.29) 35% of membranes reacted with "hyperimmune beads" (Table 5, column 1).

Despite the specific density perturbation of ∿ 25% of the erythrocyte membrane vesicles to a high density of 1.29 their membrane protein composition in comparison to unreacted membranes revealed only some minor differences in the quantitative protein composition. One of the reasons for this finding may be that proteins are extracted from the membrane when centrifuged into high concentrations of CsCl. We currently investigate this possibility by centrifuging the sphere-membrane complexes in sucrose or sucrose/D_2O density gradients.

2.4 Immunochemical analyses of schizonts and membrane components of parasitized erythrocytes.

Important candidate antigens that could mediate an effective immune defense against P. knowlesi merozoites and intracellular forms of this parasite are those being parasite specific and expressed on the surface of infected erythrocytes. We have employed immunochemical techniques to investigate whether the parasite specific antigens in the plasma membrane of infected cells (1, 2) are exposed on the cell surface and whether they are immunogenic in vivo.

2.4.1 Antisera

Ammonium sulfate precipitated immunoglobulin of two types of antisera was employed; one serum was raised in a rhesus monkey immunized and repeatedly boosted with purified P. knowlesi schizonts in Freund's complete adjuvant, the other serum was obtained from a rhesus monkey rendered naturally immune against P. knowlesi during the first infection. Since then, this monkey has been challenged twice with the same strain of P. knowlesi until precipitating antibodies of sufficiently high titer (required for immunochemical reactions) developed.

2.4.2 Procedures

2.4.2.1 Absorption of antisera

To identify parasite-specific components of the surface of infected erythrocytes 0.1 ml of antiserum was absorbed three times with $2 \cdot 10^8$ infected cells (schizont stage, 4-10 nuclei). For each absorption the incubation was at 37° and 4°C for 20 min each. There was no cell lysis during the absorptions.

2.4.2.2 Membrane proteins

As an alternative approach to identify antigens on the cell surface, membrane proteins/glycoproteins exposed on the surface of parasitized erythrocytes were selectively labelled using the lactoperoxidase-catalyzed radioiodination (2, 3). Proteins of purified schizonts and isolated infected membranes were solubilized in 1% Triton X-100 as described in (1, 2).

2.4.2.3 Crossed immune electrophoresis

CIE was performed as described earlier (1). The identity of antibodies in different sera was established by electrophoresing the antigens separately into each antibody and in addition, into a mixture of both immunoglobulins (9). In this system, a selective decrease in the height of a precipitation arc represents presence of IgG against this antigen in both the antisera (9).

2.4.3 Results

Immunization of immune competent monkeys (previous data were obtained with sera of a splenectomized animal; 1) with purified schizonts yielded a very complex immune electrophoretic pattern of 24 well defined, but partially heterogeneous components. The number of precipitation arcs and their relative height is highly reproducible when tested against different preparations of schizonts and membranes of parasitized erythrocytes. None of the sera yielded an immunochemical reaction with membranes of normal erythrocytes.

Immunoplates of both schizonts and infected membrane reveal numerous precipitation arcs, which, as before (1) are numbered according to their relative mobility in the first dimension. Although some of the components are found in both parasite and infected membrane, this cannot be a simple contamination because many components predominant in the parasite (e.g. components 2 A, B, 6, 12, 14, 16, 21 and 23) are absent in the infected membrane. On the other hand, as described before (1), there are components exclusively found in the infected membrane (components 13i, 10, 22 and 24). The other antigens can be identified in both schizonts and host cell membranes but in different proportions (e.g. 1, 3, 4, 5, 16, etc., ref. 1). However, not all of these components are accessible on the surface of parasitized erythrocytes (see below).

More information about the exposure of these schizont-associated antigens on the cell surface has been obtained by absorption of anti-schizont sera with purified infected cells. When parasite antigens are electrophoresed against the absorbed serum components 1, 23 and one sub-component of antigen 16 (16B) are eliminated. An unchanged pattern of major components 2, 3, 4, 6, 14, 16A, and 18 suggest that these antigens are associated with the intracellular parasites. When membranes of infected membranes are run, components 1, 10, 11, 19 (in part) and 22 are eliminated indicating that they are parasite-induced surface antigens on the parasitized cells.

The data obtained with absorbed sera are compatible with immunochemical analyses of membranes isolated from surface-labeled cells. Autoradiograms were

obtained from immunoplates in which proteins of infected membranes were electrophoresed against an anti-schizont immunoglobulin. Only two components, antigen 13 and 19, are labeled at a comparably high specific activity. One of these components, antigen 13, is precipitated by sera of monkeys naturally immune against P. knowlesi. Another component identified by some such sera is component 1, present in both parasite and infected membrane.

Immunochemical analyses did not yield any major differences when schizonts or infected membranes of two different P. knowlesi strains (Malayan and Philippine, both kindly provided by Drs. Miller and Gwadz) and two apparently different variants of P. knowlesi (this was assumed when the identical monkey was infected in sequence with the identical batch of inoculum) were tested against an identical serum. However, these preliminary data suggest that component 13 in the parasite may carry strain and variant specific antigens.

Subfractions of disrupted schizonts isolated in dextran density gradients (see section 2.2) were analyzed by CIE using an anti-schizont Ig (Fig. 9A-E). The precipitation patterns of B1 and B3 (not shown) resemble those documented for B2 and B4. B2 is highly enriched in components 13 and 19 known to be associated with the erythrocyte membrane (see section 2.2.3).

2.4.4 Discussion

Anti-schizont antisera raised in immune competent rhesus monkeys have proven to be highly discriminating reagents comparable to the resolution obtained by bidimensional IEF-DS-PAGE. Unfortunately, bidimensional IEF-IE yields only a few precipitation arcs probably due to denaturation of antigen or antibody by 8M urea.

CIE reveals qualitatively similar results to those previously obtained by bidimensional IEF-DS-PAGE (1). Seven schizont-induced antigens are found in membranes of infected cells, four of which are exclusively found in the infected membrane (1). Only two of the antigens, component 1 and 13, are inducing a humoral immune response in the natural host. Component 13 and component 19 are intensely labeled in intact cells using lactoperoxidase catalyzed radioiodination (this is in

contrast to data by Deans et al (10) who report on four parasite-induced components exposed on the surface of parasitized erythrocytes). Therefore, component 13 fulfills three features highly relevant to a specific immune response in vivo: (a) parasite-specificity, (b) exposure on the surface of infected erythrocytes and (c) immunogenicity in vivo [and (d) possibly expressing strain and/or variant specificity].

References

1. R. Schmidt-Ullrich and D. F. H. Wallach (1978) Proc. Natl. Acad. Sci. USA 75, 4949-4953.

2. R. Schmidt-Ullrich, J. Lightholder and D. F. H. Wallach (1978) Proceedings of the NMRI/USAID/WHO Symposium on the Immunology of Malaria, Bethesda, Md.

3. D. F. H. Wallach and M. Conley (1977) J. Mol. Med. 2, 119-132.

4. W. Trager and J. B. Jensen (1976) Science 193, 673-675.

5. P. I. Trigg and W. E. Gutteridge (1971) Parasitology 62, 113-123.

6. I. W. Sherman (1977) Bulletin of the World Health Organization 55, 211-225.

7. M. Aikawa (1977) Bulletin of the World Health Organization 55, 139-156.

8. D. F. H. Wallach and R. Schmidt-Ullrich (1977) Methods Cell Biol. 15, 235-276.

9. N. H. Axelsen, E. Bock and J. Krøll, in Quantitative Immunoelectrophoresis (N. H. Axelsen, J. Krøll and B. Wecko, eds.) Universitetforlaget Oslo, 1973; pp. 101-103.

10. J. Deans, G. H. Mitchell and S. Cohen (1978) Proceedings of the NMRI/USAID/WHO Symposium on the Immunology of Malaria, Bethesda, Md.

Table I: Metabolic labelling of P. knowlesi-infected monkey erythrocytes

Fraction	Isotope incorporation (cpm)			
	^{14}C-aa[2]		^{14}C-glc-NH$_2$[2]	
	TA[3]	%[3]	TA[3]	%[3]
E[1]	$4.8 \cdot 10^6$	100	$8.0 \cdot 10^5$	100
IM[1]	$7.0 \cdot 10^5$	15	$5.6 \cdot 10^4$	7
P[1]	$3.5 \cdot 10^6$	7.3	$6 \cdot 10^5$	7.5

1) E = infected and normal erythrocytes; IM = membranes of parasitized erythrocytes; P = purified schizonts.

2) ^{14}C-aa = ^{14}C amino acids (from protein hydrolysate); ^{14}C-glc-NH$_2$ = [^{14}C]-glucosamine.

3) TA = total activity in cpm; % distribution.

Table 2: Relative specific activities of metabolic precursors in infected membrane and purified schizonts.

	^{14}C - aa[2]	^{14}C-glc-NH_2[1]	^{14}C-gal[2]	^{14}C-fuc[2]
$\frac{SA\text{-}IM}{SA\text{-}P}$[1]	0.2	0.15	2.5	3.0

1) SA-IM = specific activity (cpm/mg protein) in membranes of parasitized erythrocytes.

SA-P - specific activity (cpm/mg protein) in purified schizonts.

2) ^{14}C-aa = ^{14}C-amino acids (from protein hydrolysate). ^{14}C-glc-NH_2 = [^{14}C]-glucosamine. [^{14}C]-gal = [^{14}C]-galactose; ^{14}C-fuc = [^{14}C]-fucose.

Table 3. Subfractionation of purified P. knowlesi schizonts[1]

Fraction	S. A.[2,4]	[^{125}I]distribution (%)[4]
Schizont (intact)	$0.91 \cdot 10^6$ (±0.05)	100.0
Cytoplasmic proteins	$0.59 \cdot 10^6$ (±0.03)	10.0
B1[3]	$4.57 \cdot 10^6$ (±0.43)	21.3
B2[3]	$2.04 \cdot 10^6$ (±0.19)	9.5
B3[3]	$1.57 \cdot 10^6$ (±0.18)	4.6
B4[3]/Pellet	$1.10 \cdot 10^6$ (±0.1)	54.3

[1] Purified intraerythrocytic parasites were [^{125}I]labeled using lactoperoxidase-catalyzed radioiodination prior to disruption by nitrogen decompression. Fractionation was on dextran density gradients.

[2] S. A.: Specific activity; ^{125}I cpm/mg protein.

[3] Band 1 (above 1.05 density step), 2(1.05/1.10 density interface), 3(1.10/1.12 density interface), and 4(1.12/1.16 density interface), and the pellet penetrating the 1.16 density step.

[4] Data from two independent fractionations (gradients run in duplicate).

Table 4. Affinity density perturbation of membrane vesicles from rhesus monkey erythrocytes in CsCl density gradients.[1]

Fraction		Immune Ig[2] ^{131}I(%)[3]	Immune Ig[2] ^{125}I(%)[3]	Control Ig[2] ^{131}I(%)[3]	Control Ig[2] ^{125}I(%)[3]
A[4]	Membranes	64	49	72	< 1
	Latex spheres	5	34	< 1	82
B[4]	Membranes	45	23	78	3
	Latex spheres	28	72	< 1	73

1 Unshocked erythrocyte membrane vesicles, [^{131}I]-labeled using lactoperoxidase-catalyzed radioiodination were reacted with latex spheres (600 Å diameter) derivatized with fluorescein-conjugated BSA and [^{125}I]-labeled IgG. Results of two independent experiments (gradients run in duplicate).

2 IgG from rabbit immune serum against normal rhesus monkey erythrocyte membranes; control IgG from non-immunized rabbits.

3 [^{131}I] and [^{125}I] distribution in percent of total membrane and bead material, respectively, laden onto the CsCl gradient.

4 $7 \cdot 10^{13}$ beads reacted with 1 mg (A) or 0.3 mg (B) of membrane proteins for 16 hrs. at 6°C with mild stirring.

Table 5

Affinity density perturbation of membrane vesicles from Pl. knowlesi-infected rhesus monkey erythrocytes in CsCl density gradients.

Density gradient. (% CsCl)	Latex spheres(HI)[1] + infected membranes[2] ^{125}I-distribution(%)	Latex spheres(N)[1] + infected membranes[2] ^{125}I-distribution(%)	Latex spheres(HI)[1] + normal membranes[2] ^{125}I-distribution (%)
20	16	20	41
25	45	66	45
30	35	11	11
35	3	3	3
40	1	1	1
	100	100	102
	(= 58.960 cpm)	(=60.260 cpm)	(=52.140 cpm)

[1] Latex spheres (600 Å) derivatized with FITC-BSA and monkey immunoglobulin from normal serum (N) hyperimmune serum against Pl. knowlesi parasites (6-10 schisont stage), respectively.

[2] Plasma membranes from normal or Pl. knowlesi-infected monkey erythrocytes. Labeling with ^{125}I by lactoperoxidase-catalysed radioiodination.

Figure Legends

Fig. 1 IEF of membrane proteins from P. knowlesi-infected monkey erythrocytes. The proteins and glycoproteins are metabolically labelled in vitro using ^{14}C-amino acids and ^{14}C-glucosamine as precursors, respectively. The abscissa gives the pH gradient and the ordinates give the activity distribution for ^{14}C-amino acids (———) and ^{14}C-glucosamine (- - -) in cpm and the absorption for Coomassie blue protein staining at 620 nm (- · -).

Fig. 2 DS-PAG electropherograms of membrane proteins from P. knowlesi-infected monkey erythrocytes. The proteins and glycoproteins are metabolically labelled with ^{14}C-amino acids and ^{14}C-glucosamine as precursors, respectively. The abscissas give the relative mobility and molecular size and the ordinates the distribution of ^{14}C-amino acids (———) and ^{14}C-glucosamine (- - -) and the absorbance for Coomassie blue protein staining at 620 nm (- · -).

Fig. 3 Isoelectric focusing of purified P. knowlesi schizonts, and subfractions thereof obtained from a dextran density gradient (see Table 1). Proteins were dissolved in 1% Triton X-100/0.1% dodecyl sulfate and focused in 4% acrylamide gels, containing 2% ampholytes (pH 3.5 - 10.0), 8M urea and 1% Triton X-100. The ordinate gives the pH gradient of the gels, the abscissa the absorbance (A) for Coomassie blue staining at 620 nm.

- - - Whole schizonts; 150 µg of protein.

——— Band 1 of dextran gradient (see Table 1); 100 µg of protein.

- · - Band 4 of dextran gradient; 150 µg or protein.

Figure 1

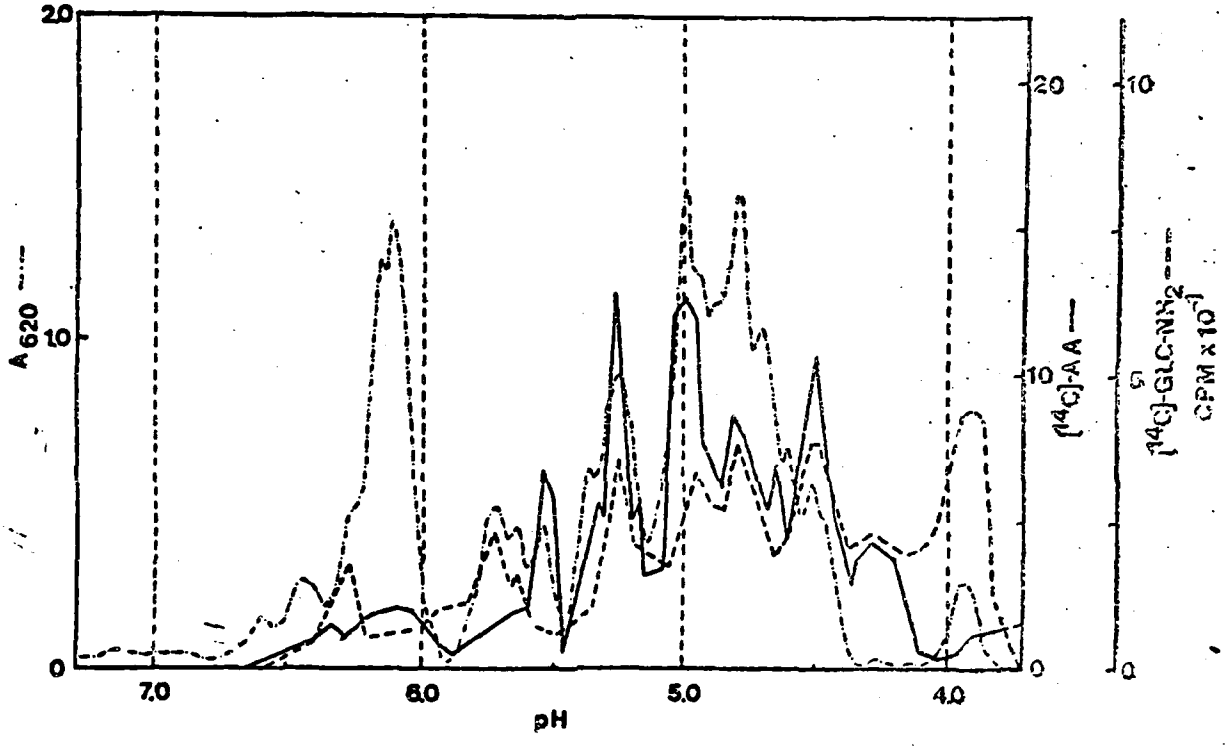


Figure 2

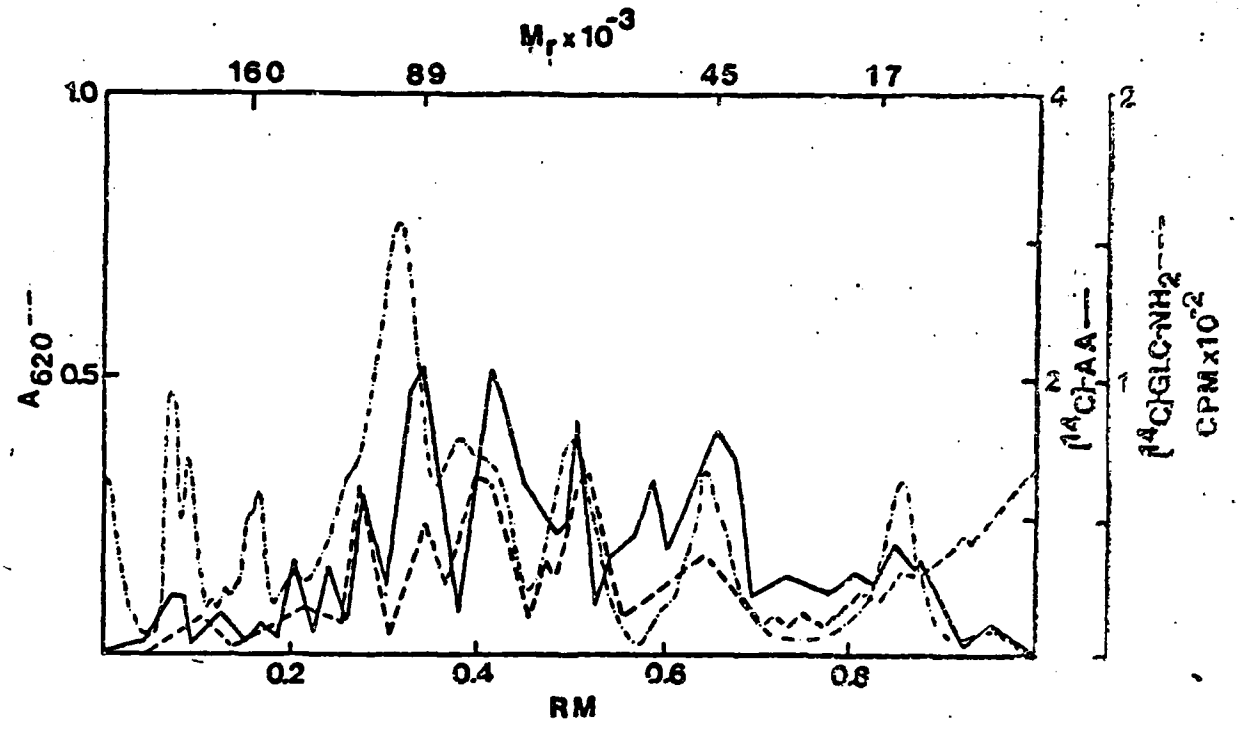
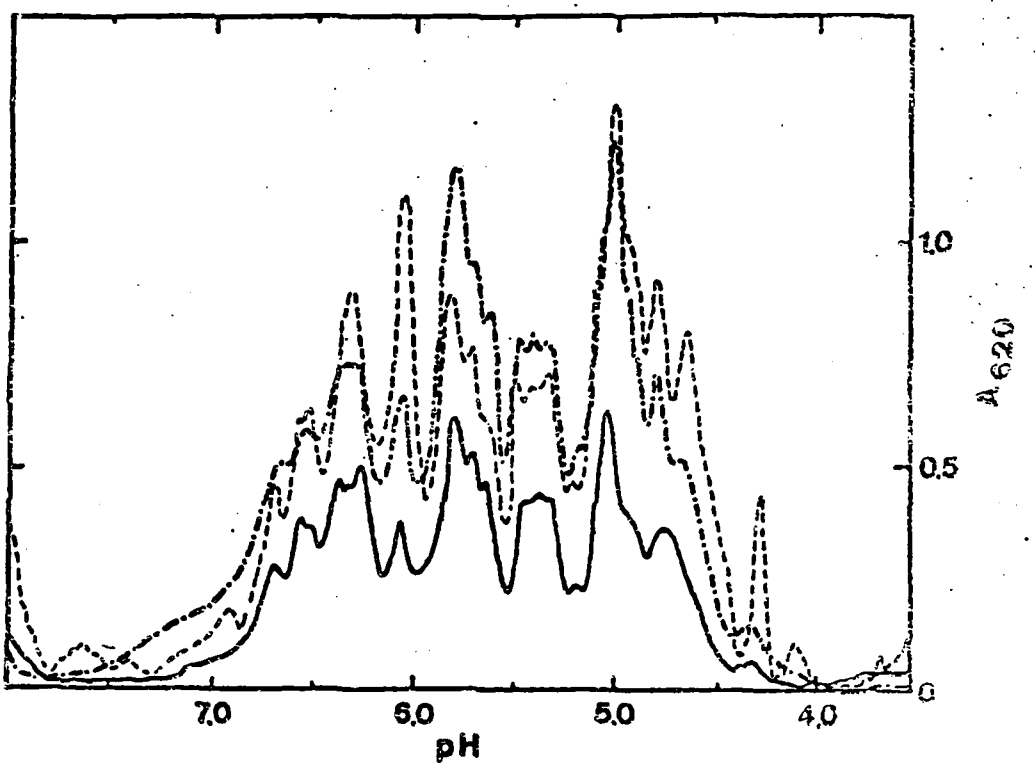

$M_r \times 10^{-3}$
160
89
45
17
1.0
0.5
A_{620}
4
2
$[^{14}C]$-AA
$[^{14}C]$GlcNH$_2$
CPM$\times 10^{-2}$
0.2
0.4
0.6
0.8
RM

Figure 3



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