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Enhanced *Plasmodium falciparum* Merozoite Phagocytosis by Monocytes from Immune Individuals

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Phagocytosis of merozoites and schizont-infected erythrocytes prepared from continuous cultures by peripheral blood monocytes from patients with falciparum malaria was investigated with an in vitro assay. Monocytes do ingest merozoites of *Plasmodium falciparum*, but rarely phagocytose parasitized or nonparasitized erythrocytes in the absence of immune serum. The monocytes from hyperimmune subjects were significantly more efficient in the ingestion of merozoites than were those obtained from sensitized or noninfected subjects. These data indicate, first, that the merozoite rather than the parasitized erythrocyte is the specific target for blood phagocytic cells in human falciparum malaria and, second, that the phagocytosis of merozoites by peripheral blood monocytes increases depending on the level of specific immunity.

Phagocytosis has long been recognized to play a crucial role in the defense of the host during malarial infections. In experimental infection of animals with *Plasmodium gallinaceum*, *P. brasilianum*, *P. cynomolgi*, and *P. knowlesi*, it has been shown that the number of macrophages increases especially in the spleen and liver (1, 7, 20, 21). The clearance of carbon particles injected intravenously is accelerated in *P. berghei*-infected animals (8). In in vitro studies, it has been shown that splenic macrophages obtained from infected animals can phagocytose parasitized and nonparasitized erythrocytes (6, 19, 24).

In human malaria, the involvement of reticuloendothelial cells in the resistance to infection has been suggested by researchers who observed an increase in the number of phagocytic cells as a result of infection, particularly in the spleen, liver, and bone marrow; by histopathologists, who observed malarial pigment as well as red cell debris in the vacuoles of these cells (22); and by researchers who have made occasional observations of phagocytic images in the peripheral blood of infected patients. Trubowitz and Masek (26) observed the ingestion of a merozoite by a polymorphonuclear leukocyte. On stained blood smears, Vernes (27) reported phagocytosis of parasitized erythrocytes by monocytes.

This study was undertaken to investigate in vitro, in controlled conditions, the ability of purified human monocytes from malarial subjects to ingest *P. falciparum* merozoites and schizonts, normal erythrocytes, and nonrelated particles (latex and yeast particles).

MATERIALS AND METHODS

Patients. Peripheral monocytes were obtained from 11 malarial subjects and 12 normal controls. Since no laboratory test is as yet able to reflect the resistance of a person to the parasite, we have defined four classes of subjects, depending on their past history of malaria.

(i) Hyperimmune individuals were born and had been living all their lives in areas of hyperendemic *P. falciparum* infection. In the absence of prophylaxis, they had been repeatedly infected at least once a year and had reached a state of "premunition" (protection of infected individuals against severe clinical manifestations). We selected only those who had arrived in France within the preceding 6 months. (ii) Immune individuals were repeatedly infected but might have lost their state of premunition because they had been away from endemic areas for more than 1 year. (iii) Primary attack individuals were infected only once with acute *P. falciparum* malaria. They had a high level of specific antibodies, but presumably had no protection against the disease. (iv) Normal individuals were normal healthy controls with no history of malaria.

We tested the monocytes from six African hyperimmune subjects whose ages were between 18 and 70 years, coming from West Africa; two African immune subjects, between 30 and 40 years of age; three primary attack subjects, between 22 and 42 years of age (two French, one African); and 12 normal controls (11 French, 1 Thai).

All except the normal controls had high levels of specific malarial antibodies as measured by the fluorescent-antibody test and a precipitation test. There was no significant difference among the antibody levels of the three groups.

Although the patients from groups i and ii had been repeatedly infected, we did not observe parasites in their blood. The presence of *P. falciparum* on blood smears was recorded only in those from group iii. In

these patients, monocytes were obtained a few days (2 to 8 days) after treatment was initiated.

Malaria parasites. *P. falciparum* was obtained from in vitro continuous cultures of strain FCR3. The culture was performed by the technique of Trager and Jensen (25) as modified by Druilhe et al. (12). The extracellular merozoites were obtained by natural release into the medium from schizont-infected erythrocytes, and the merozoites were separated by centrifugation. Schizont-enriched populations of *P. falciparum*-infected erythrocytes extracted on a 2% solution of Plasmagel and from day 4 cultures were used in all studies.

Monocytes. A 50-ml portion of heparinized blood (20 IU/ml) (Liquemine; Roche) was obtained from each subject. The mononuclear cells were separated by centrifugation on a high-density Ficoll-Hypaque solution (density, 1.078) at 20°C (Ficoll 400; Pharmacia Fine Chemicals, Uppsala, Sweden) according to the technique of Boyüm (2). The cell preparations were then suspended in a mixture of RPMI 1640 supplemented with 10% fetal calf serum (GIBCO Europe). The monocytes were further isolated by a simple method based on their ability to adhere to serum-coated plastic dishes (14). This technique allowed the recovery of highly purified monocytes (90 to 95% on average); the remaining cells were lymphocytes and a few neutrophils. Viability based on dye exclusion (0.2% trypan blue in Hanks balanced salt solution) ranged from 85 to 95%.

Phagocytic assay. Aliquots, 0.5 ml, of approximately 10^6 monocytes in RPMI 1640 containing either fetal calf serum or human umbilical cord serum (collected through the kind cooperation of obstetricians at the Hôpital de la Pitié-Salpétrière) were placed in a siliconized glass tube (100 by 15 mm) and mixed with 0.5-ml aliquots of approximately 10^7 target cells: merozoites, schizont-infected erythrocytes, normal erythrocytes, latex particles (latex 0.81; Difco Laboratories, Detroit, Mich.), or yeast particles (*Candida albicans* obtained from our laboratory) (ratio, monocytes/target cells = 1:10). This ratio was chosen after preliminary experiments with ratios of 1:5, 1:10, 1:15, and 1:20. The cultures were incubated at 37°C on a rotator platform (10 cycles/min; CENCO Instrument, Breda, The Netherlands) for 45 min. Duplicate tubes were used for each particle tested. The mixtures were then centrifuged for 10 min at 400 × *g*, and the pellets were suspended in RPMI-10% fetal calf serum or human umbilical cord serum. Wet preparations and smears were prepared on 75- by 25-mm slides and stained with Giemsa; then the slides were examined. For each test, three slides were done; 500 cells were counted on each slide. Phagocytosis was expressed as the percentage (mean and standard deviation) of monocytes that had ingested one or more particles.

RESULTS

Phagocytosis by monocytes obtained from normal and malarial subjects against various targets is shown in Fig. 1 and 2. The functional activity of the tested monocytes was demonstrated by their ability to ingest latex or yeast particles. Normal monocytes actively phagocytosed latex and yeast particles (46.29 ± 4.21 and 43.21 ± 5.42%, respectively). The monocytes obtained from all groups of malarial subjects were able to ingest both particles at approximately the same rate (42.83 ± 3.77 and 42.62 ± 6.45%). No significant difference between phagocytosis of latex and yeast particles by monocytes from normal and malarial subjects was found ($P > 0.90$).

The level of ingestion of noninfected homologous erythrocytes was very low. No difference

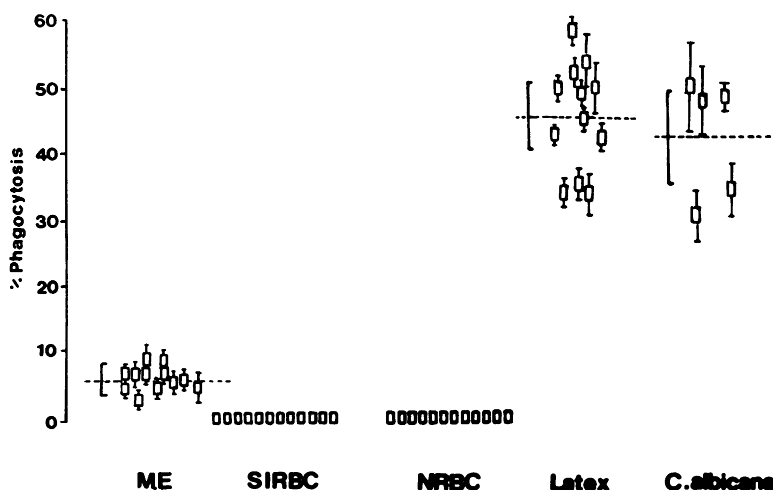

FIG. 1. Phagocytosis of various targets by monocytes from normal subjects. ME, Merozoites; SIRBC, schizont-infected erythrocytes; NRBC, normal erythrocytes. Each value is the mean ± standard deviation from duplicate tubes of each subject tested (□). The dotted line indicates the mean percent phagocytosis of all subjects tested against each target, and the bracket indicates the standard deviation.

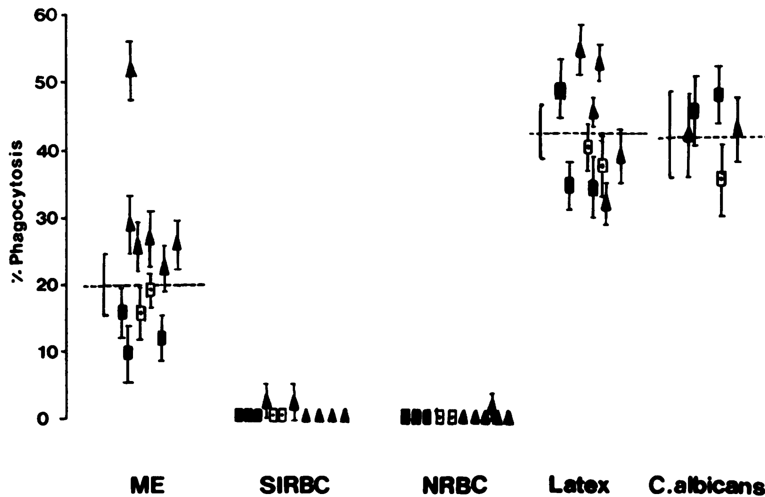

FIG. 2. Phagocytosis of various targets by monocytes from malarial subjects. See legend to Fig. 1 for abbreviations. Symbols: (▲) hyperimmune; (⊡) immune; (■) primary attack; (· · ·), mean percent phagocytosis of all subjects tested against each target; ([), standard deviation: Each value is the mean ± standard deviation from duplicate tubes of each subject tested.

between malarial (0.17 ± 0.14%) and normal (0%) subjects was found.

Monocytes from most groups of malarial subjects did not ingest more schizont-infected erythrocytes (0.33 ± 0.05%) than did those from normal groups (0%) (Fig. 3). The same results were obtained when we used only schizonts or mixtures of trophozoites and young and mature schizonts. In the group of hyperimmune subjects, phagocytosis of intact schizonts was rarely found. Phagocytosis of intact schizonts was only slightly enhanced in two of the six subjects (2.00 ± 0.5%). Nevertheless, merozoites released from schizonts ruptured during the experiment were phagocytosed. The release of merozoites, as well as their phagocytosis, was inconsistent since the percentage of intact mature schizonts was variable. The total number of released merozoites was lower in these experiments than in the experiment in which previously released merozoites were added to the cultures. Nevertheless, the same pattern of ingestion was observed, but at a lower degree.

When merozoites were used as the targets (Fig. 4), it was found that the peripheral blood monocytes from malarial subjects were able to ingest merozoites to a higher degree (22.75 ± 3.70%) than were monocytes from individuals

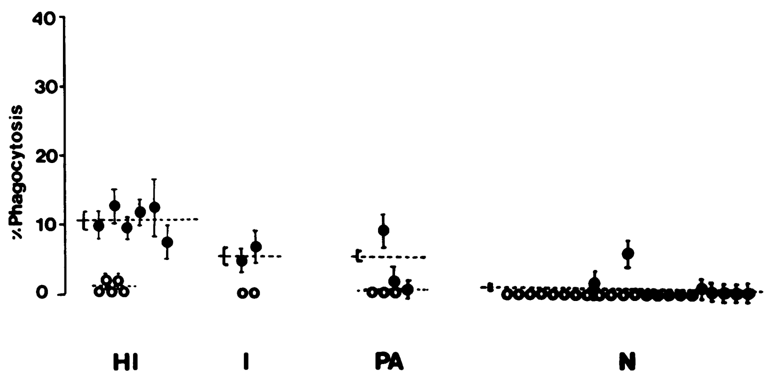

FIG. 3. Phagocytosis of *P. falciparum* schizont-infected erythrocytes (SIRBC) and merozoites released by monocytes from malarial subjects: HI, hyperimmune; I, immune; PA, primary attack; N, normal. Symbols: (○) SIRBC; (●) merozoites released from SIRBC ruptured during the test; (· · ·) mean percent phagocytosis of subjects from each group; ([) standard deviation. Each value is the mean ± standard deviation from duplicate tubes of each subject tested.

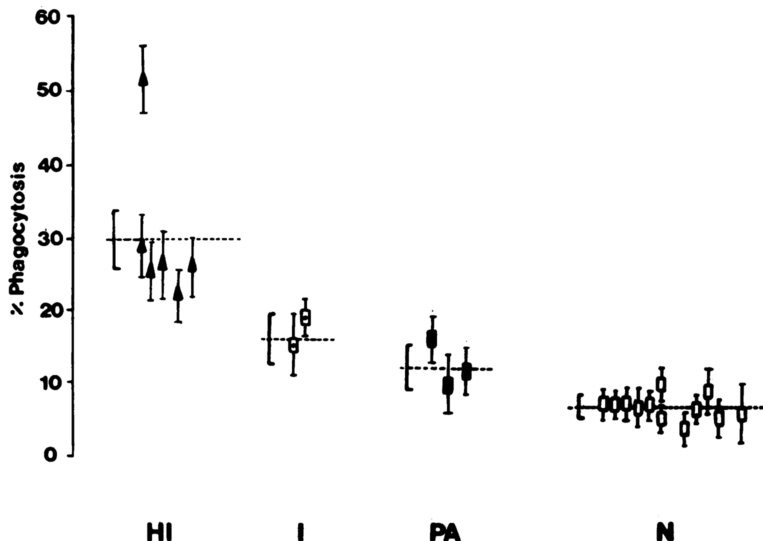

FIG. 4. Phagocytosis of *P. falciparum* merozoites by monocytes from normal and malarial subjects. Symbols: (▲) hyperimmune (HI); (⊡) immune (I); (■) primary attack (PA); (□) normal control (N); (. . .) mean percent phagocytosis of subjects from each group; ([) standard deviation. Each value is the mean ± standard deviation from duplicate tubes of each individual.

without any past experience of malaria (6.68 ± 1.71%). The difference between these two groups was statistically significant ($P < 0.001$).

The level of merozoite ingestion was highest by monocytes from the group of hyperimmune subjects (30.09 ± 4.07%) whose individual rates of phagocytosis were above the rates of the other subjects in every case. One of these showed an extremely high rate of phagocytosis (51.69 ± 4.18%), twice the average rate of the others (25.78 ± 4.05%). The difference between the phagocytosis of hyperimmune and immune (16.59 ± 3.38%) subjects was statistically significant ($P < 0.05$), as was the difference between hyperimmune and primary attack groups (12.19 ± 3.19%) ($P < 0.05$). However, the difference between immune and primary attack groups was not significant ($P > 0.1$).

DISCUSSION

With an in vitro assay, phagocytosis by peripheral blood monocytes from patients with falciparum malaria was investigated against parasites contained in erythrocytes and free parasites prepared from continuous cultures. Our report demonstrates that, in culture, phagocytic cells from malarial subjects are not able to ingest schizont-infected erythrocytes, normal erythrocytes, and unrelated particles at higher rates than are phagocytes from normal subjects. The main target of the monocyte in culture is the merozoite.

No increase of phagocytosis by monocytes from malarial subjects against inert particles was found. These monocytes, like those from normal controls, showed a rate of latex and yeast particle ingestion similar to that reported in previous studies (14, 23, 28). The accelerated clearance of carbon particles injected intravenously during malarial infection (8) is then more likely related to an increase in the number of phagocytic cells rather than to an increase of their individual activity.

No ingestion of normal human erythrocytes by monocytes from both normal and malarial subjects was observed. This result is in accordance with the findings obtained in animal malaria (9, 16, 17). Shear et al. (19) demonstrated that splenic macrophages from normal and malaria-infected mice showed little ingestion of normal erythrocytes or reticulocytes, and phagocytosis increased only against heterologous sheep erythrocytes, probably because of Forssman antibodies. Zuckerman (29) described an increase of erythrophagocytosis by chicken blood monocytes only in the presence of immune serum to infected erythrocytes. This increase was probably due to blood group antibodies.

To our knowledge, no other in vitro experiment has been done with *P. falciparum* and human phagocytes. However, phagocytosis by tissue macrophages has been commonly observed in tissue sections and has been occasionally reported to occur in infected peripheral blood. On stained blood smears, Vernes (27) found that monocytes were able to display en-

gulfment or digestion of *P. falciparum*-parasitized erythrocytes. However, in five patients such ingestion was found mainly in one and rarely in two other patients. Trubowitz and Masek (26) observed wet preparations of *P. falciparum*-infected blood by phase microscopy and reported the absence of phagocytosis of parasitized erythrocytes, whereas polymorphonuclear leukocytes were found to move toward, attach, and ingest free merozoites.

Our results obtained from in vitro studies, carried out on a large number of patients in various states of immunity, confirm the initial observation of Trubowitz and Masek concerning merozoites and show that phagocytosis of parasitized erythrocytes by blood monocytes is infrequent. However, our results are in agreement with both observations since both targets, parasitized erythrocytes and merozoites, can be phagocytosed, but at different rates. The very high rate of merozoite ingestion by monocytes from some patients probably was not observed before since the occurrence of free merozoites in peripheral blood is infrequent.

From our results, it is interesting to note that in its phagocytic process, the monocyte appears to exhibit some specificity since, first, this process occurred in the absence of any immune serum in the medium used (human umbilical cord serum and fetal calf serum were negative for malarial antibodies in the fluorescent-antibody test and precipitation performed with *P. falciparum* antigen) and, second, the phagocytic activity was directed to the merozoite stage, not to other asexual blood stages of the same parasite. Third, there was a marked and significant difference in merozoite ingestion ability between the cells of malarial and nonmalarial subjects, and a higher percentage of merozoite ingestion was observed in hyperimmune subjects. Finally, there was no significant difference in the phagocytosis of latex and yeast particles between the monocytes from normal and malarial subjects.

That there is only rare ingestion of parasitized erythrocytes is in contrast to some previous studies in animal malaria models. In *P. knowlesi*-infected rhesus monkeys (4–6, 10, 18) and *P. berghei*-infected rodents (11, 19, 24), it was shown that splenic macrophages from malaria-infected animals were able to phagocytose parasitized erythrocytes in vitro at a high rate. This ingestion appeared to be mediated by disease-associated immunoglobulin, which was previously found to be able to bind to the surface of parasitized cells (15).

In our experiments the difference may result from the use of peripheral monocytes instead of splenic macrophages, from the absence of specific antibody on schizont-infected erythrocytes obtained from continuous cultures, or from modifications of the erythrocyte membranes after long-term cultivation.

However, blood monocytes from malaria-sensitized individuals behave as if they were able both to distinguish one target from another and to exhibit a higher level of specific target ingestion depending on the degree of sensitization. The mechanism of such recognition cannot be precisely formulated based on the results of this study, but the role of cytophilic antibodies can be highly suspected since in the *P. berghei* rat model (3, 13) it has been shown that cytophilic and opsonizing antibodies are involved in in vitro attachment and phagocytosis of free parasites by peritoneal macrophages.

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LITERATURE CITED

1. **Ben Harel, S.** 1923. Studies of bird malaria in relation to the mechanism of relapse. Am. J. Hyg. **3**:652–685.
2. **Boyüm, A.** 1968. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scand. J. Clin. Lab. Invest. **21**(Suppl. 97):77–89.
3. **Brooks, C., and J. P. Kreier.** 1978. Role of the surface coat in *in vitro* attachment and phagocytosis of *Plasmodium berghei* by peritoneal macrophages. Infect. Immun. **20**:827–835.
4. **Brown, I. N.** 1969. Immunological aspects of malarial infection. Adv. Immunol. **11**:267–349.
5. **Brown, K. N., I. N. Brown, P. I. Trigg, R. S. Phillips, and L. A. Hills.** 1970. Immunity to malaria. II. Serological response of monkeys sensitized by drug-suppressed infection or by dead parasitized cells in Freund's complete adjuvant. Exp. Parasitol. **28**:318–338.
6. **Brown, K. N., and L. A. Hills.** 1971. Macrophage activation and the action of opsonizing antibodies in malaria. Trans. R. Soc. Trop. Med. Hyg. **65**:6.
7. **Cannon, P. R., and W. H. Taliaferro.** 1931. Acquired immunity in avian malaria. III. Cellular reactions in infection and superinfection. J. Prev. Med. **5**:37–64.
8. **Cantrell, W., and E. E. Elko.** 1976. *Plasmodium berghei*: phagocytic activity in two strains of rats. Exp. Parasitol. **40**:281–285.
9. **Chow, J. S., and J. P. Kreier.** 1972. *Plasmodium berghei*: adherence and phagocytosis by rat macrophages *in vitro*. Exp. Parasitol. **31**:13–18.
10. **Cohen, S., I. A. McGregor, and S. C. Carrington.** 1961. Gamma globulin and acquired immunity to human malaria. Nature (London) **192**:733–737.
11. **Criswell, B. S., W. T. Butler, R. D. Rossen, and V. Knight.** 1971. Murine malaria: the role of humoral factors and macrophages in destruction of parasitized erythrocytes. J. Immunol. **107**:212–221.
12. **Druilhe, P., P. Jacquier, D. Lambert, and M. Gentilini.** 1980. *P. falciparum in vitro* cultures: improvements using umbilical cord serum and medium modification. Tropenmed. Parasitol. **31**:409–413.
13. **Green T. J., and J. P. Kreier.** 1978. Demonstration of the role of cytophilic antibody in resistance to malarial para-

sites (*Plasmodium berghei*) in rats. Infect. Immun. 19:138–145.

14. **Kumagi, K., K. Itoh, S. Hinuma, and M. Tada.** 1979. Pretreatment of plastic petri-dishes with fetal calf serum. A simple method for macrophage isolation. J. Immunol. Methods **29**:17–25.
15. **Lustig, H. J., V. Nussenzweig, and R. S. Nussenzweig.** 1977. Erythrocyte membrane associated immunoglobulins during malaria infection of mice. J. Immunol. **119**:210–216.
16. **McCallum, D. K.** 1969. Time sequence study on the hepatic system of macrophages in malaria-infected hamster. RES J. Reticuloendothel. Soc. **6**:232–252.
17. **McCallum, D. K.** 1969. A study of macrophage pulmonary vascular bed interactions in malaria infected hamster. RES J. Reticuloendothel. Soc. **6**:253–270.
18. **Phillips, R. S., R. A. Wolstencroft, I. N. Brown, K. N. Brown, and D. C. Dumonde.** 1970. Immunity to malaria. III. Possible occurrence of a cell-mediated immunity to *Plasmodium knowlesi* in chronically infected and Freund's complete adjuvant-sensitized monkeys. Exp. Parasitol. **28**:339–355.
19. **Shear, H. L., R. S. Nussenzweig, and C. Bianco.** 1979. Immune phagocytosis in murine malaria. J. Exp. Med. **149**:1288–1298.
20. **Taliaferro, W. H.** 1949. Immunity to the malaria infections, p. 935–959. *In* M. F. Boyd (ed.), Malariology. W. B. Saunders Co., Philadelphia.
21. **Taliaferro, W. H., and P. R. Cannon.** 1936. The cellular reactions during primary infections and superinfections of *Plasmodium brasilianum* in Panamanian monkeys. J. Infect. Dis. **59**:72–125.
22. **Taliaferro, W. H., and H. W. Mulligan.** 1937. The histopathology of malaria with special reference to the function and origin of the macrophages in defence. Indian Med. Res. Mem. **29**:1–125.
23. **Territo, M. C., and M. L. Cline.** 1977. Monocyte function in man. J. Immunol. **118**:187–191.
24. **Tosta, C. E., and N. Wedderburn.** 1980. Immune phagocytosis of *Plasmodium yoelii*-infected erythrocytes by macrophages and eosinophils. Clin. Exp. Immunol. **42**:114–120.
25. **Trager, W., and J. B. Jensen.** 1976. Human malaria parasites in continuous culture. Science **193**:673–675.
26. **Trubowitz, S., and B. Masek.** 1968. *Plasmodium falciparum*: phagocytosis by polymorphonuclear leucocytes. Science **162**:273–274.
27. **Vernes, A.** 1980. Phagocytosis of *P. falciparum* parasitized erythrocytes by peripheral monocytes. Lancet **ii**:1297–1298.
28. **Zucker-Franklin, D.** 1974. The percentage of monocytes among "mononuclear" cell fractions obtained from normal human blood. J. Immunol. **112**:234–240.
29. **Zuckerman, A.** 1945. In vitro opsonic tests with *Plasmodium gallinaceum* and *Plasmodium lophurae*. J. Infect. Dis. **77**:28–59.