A REVIEW

Innate Resistance in Malaria

LOUIS H. MILLER AND RICHARD CARTER

Laboratory of Parasitic Diseases,
National Institute of Allergy and Infectious Diseases,
National Institutes of Health, Bethesda, Maryland 20014, U.S.A.

The ability of the host to overcome a malarial infection is determined not only by immunologic mechanisms but also by certain innate characteristics of the host. Innate resistance characteristics are inherited, often as simple Mendelian factors, e.g., the sickle cell trait and the Duffy blood group determinants. They are not elaborated as the direct result of an individual's exposure to infection. In evolutionary terms, however, the selective pressures of malarial infection on human populations profoundly affect the distribution and frequency of traits conferring resistance to the disease.

Mechanisms of innate resistance to malaria have only been described against the blood stages. As yet nothing is known of the influence of the host upon gametocytogenesis or the establishment of the sporozoite in the liver of the mammalian host in the initial stages of infection. Innate resistance to the asexual stages in the blood may operate by creating conditions at the erythrocyte membrane, within the erythrocyte, or in the plasma, which limit the ability of the parasite to multiply. The demonstration that a surface receptor on the erythrocyte controls the parasite's ability to invade the erythrocyte suggests that such a mechanism may underlie the host specificity in other Sporozoa.

INDEX DESCRIPTORS: Malaria; Resistance innate; Genetics; Plasmodium falciparum; P. vivax; P. ovale; P. knowlesi; P. coatneyi; P. cynomolgi; P. berghei; P. vinckei; P. lophurae; Asexual erythrocytic cycle; Gametocytogenesis; Gametogenesis; Sporozoite; Exoerythrocytic forms; Merozoites; Erythrocytes; Reticulocytes; Surface receptors; Erythrocyte glycoproteins; Chymotrypsin; Pronase; Trypsin; Neuraminidase; Blood group determinants; Duffy blood group determinants; Population genetics; Electrolytes erythrocytic; Sickle trait; Thalassaemia; Glucose-6-phosphate dehydrogenase (G6PD) deficiency; Ovulating ducks; Histocompatibility antigens; Parasite modification; Man; Monkeys; Mice; Rats; Birds; Immunity; Parasitological Reviews.

I. Introduction
II. Asexual erythrocytic cycle
   A. Susceptibility of erythrocytes to merozoite invasion
      1. Erythrocyte maturation and susceptibility to malaria
      2. Erythrocyte surface receptors and host-specificity
      3. Chemical analysis of the receptor
      4. The erythrocyte receptor for P. knowlesi merozoites and the Duffy blood group determinants
      5. Summary
   B. Erythrocyte cytoplasmic factors in parasite survival
   C. Plasma factors in innate resistance
   D. Genetic control of resistance to malaria
   E. Parasite modifications under the genetic control of the host
III. Gametocytogenesis, gametogenesis and infectivity to mosquitoes
IV. Sporozoite infectivity and exoerythrocytic development
I. INTRODUCTION

Because of host factors that reduce parasite viability, malaria parasites rarely multiply at their maximum rate. Mechanisms of resistance include nonimmunologic factors (innate or natural resistance) and acquired immunity. The relative importance of the two varies throughout the course of infection. Each may change independent of the other, so that reduced parasite survival may result from either innate resistance or acquired immunity. In addition, the host itself may induce the parasite to undergo biological modifications that may affect the course of infection (Greenberg and Kendrick 1957a, b).

Innate resistance is expressed regardless of the environment or previous exposure to the parasite and has no immunologic specificity. It does, however, have specificity and may relate to a requirement of the parasite (e.g., an erythrocyte receptor or a nutritional requirement) or a deleterious substance within the host (e.g., SA hemoglobin). Where a specific requirement of the parasite is reduced or lacking, the parasite must adapt to its new environment if it is to survive.

The majority of studies on innate resistance in the recent literature have focused on the erythrocytic cycle. Factors that influence gametocytogenesis, mosquito infectivity, and sporozoite development within the vertebrate host are largely unknown and deserve equal emphasis. Stages in the life cycle at which innate factors might influence parasite survival are as follows:

1. Asexual erythrocytic cycle.
2. Gametocytogenesis, gametogenesis and infectivity to mosquitoes.
3. Sporozoite infectivity andexoerythrocytic development.

II. ASEXUAL ERYTHROCYTIC CYCLE

Every 24 to 72 hr (depending on the species), merozoites are released from one erythrocyte and invade others. From the length of the asexual cycle and the number of merozoites per schizont, the reproductive potential for any species of malaria is determined. Host factors that influence this potential such as species, age, sex, and nutritional and physiologic state ultimately affect the ability of merozoites to invade erythrocytes and to develop normally within erythrocytes.

A. Susceptibility of Erythrocytes to Merozoite Invasion

In order to evaluate relative susceptibility of different populations of erythrocytes to invasion by a particular malaria parasite, the erythrocytes must be studied under standard conditions; the quality of the merozoites and the conditions of interaction with different erythrocytes must be identical. Preferential invasion of one erythrocyte population over another in a single host or in defined culture fulfills these criteria.

1. Erythrocyte maturation and susceptibility to malaria. The preferential infection of a subpopulation of erythrocytes (reticulocytes vs mature erythrocytes) within one host (Craik 1920; Kitchen 1939) was the first evidence that the erythrocyte determined specificity in malarial infection. It could be argued that erythrocytes of different ages are invaded with equal frequency and that the parasite within certain aged erythrocytes either fails to mature or is rapidly removed from the circulation and destroyed. Since differences in reticulocyte preference have been demonstrated for very young parasites, this preference is most likely based on susceptibility to invasion, but as yet no direct evidence is available.

Erythrocytes can be divided into young erythrocytes (reticulocytes or polychromatophilic erythrocytes) that are a few days old and mature erythrocytes that include the rest of the life span of the erythrocyte. Erythrocytes can also be separated into multiple subpopulations of different aged erythrocytes by density gradient (Corash et al. 1974), or osmotic fragility (Marks et al. 1958). Erythrocyte density increases
progressively as the cells age. Since malaria infection itself affects erythrocyte density, the different aged erythrocytes must be fractionated before infection, and each subpopulation tested for susceptibility \textit{in vitro} or in another host. These age-dependent erythrocyte separation methods could identify subpopulations of mature erythrocytes that have varying susceptibility to infection by a particular malaria parasite.

The restriction of a parasite to a particular subpopulation of erythrocytes or its ability to invade all erythrocytes determines the maximum parasitemia in a host, and this, in turn, influences morbidity and mortality. \textit{P. falciparum}, because it infects erythrocytes of all ages (Bruce-Chwatt 1948), causes infections with high parasitemia, morbidity, and mortality. Vivax and ovale malarias are largely limited to young erythrocytes (Craik 1920; Kitchen 1939; Garnham 1966), and consequently the parasitemia rarely exceeds 1%.

Young rats infected with \textit{P. berghei} have a higher parasitemia and mortality than old rats (Zuckerman 1957). This age-determined difference results in part from the fact that \textit{P. berghei} preferentially invades reticulocytes, and the mean percentage of reticulocytes in young and old rats is 18.6 and 3.9, respectively. When reticulocytosis was induced in old rats, they also developed high parasitemia. Since the onset of crisis (acquired immunity) in old rats with and without reticulocytosis was similar, it was evident that induced reticulocytosis lowered innate resistance without an effect on acquired immunity. The onset of crisis in young rats was later than in old rats. It would be of interest to know if suppression of the reticulocyte concentration in young rats would change the time of crisis. In experiments with mice, reduction in the reticulocyte count by transfusion suppressed parasitemia (Ladda and Lalli 1966). Interpretation of this experiment is complicated by the possible effect of transfusion on host defense (Hejna \textit{et al.} 1974).

The ratio of the percentage of parasitemia in reticulocytes and mature erythrocytes may not remain constant during the course of infection. Greenberg (1956) observed that \textit{P. berghei} in Swiss mice preferred immature erythrocytes but also could infect mature erythrocytes. During the first few days of infection, parasites predominantly invaded immature erythrocytes. By Day 4, most of the immature erythrocytes were destroyed, and the parasite began to infect mature erythrocytes. Later as reticulocytes began to appear in large numbers in the peripheral blood, the infection in mature cells decreased.

In a study of \textit{P. vinckei} infected rats, the parasites were equally distributed in young and old erythrocytes (Zuckerman 1958). However, in one animal, the parasites that were equally distributed in reticulocytes and mature erythrocytes on Day 3 did not infect any mature erythrocytes on Day 7, the day of peak parasitemia (Table I). There may have been a subpopulation of mature erythrocytes with increased susceptibility that was destroyed early in the infection, or, alternatively, the parasite may

<table>
<thead>
<tr>
<th>Host</th>
<th>Infection rate in reticulocytes (^a)</th>
<th>Infection rate in mature RBC (^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult rats</td>
<td>1.2</td>
<td>0.98</td>
</tr>
<tr>
<td>Young rats (surviving)</td>
<td>0.74</td>
<td>1.15</td>
</tr>
<tr>
<td>Young rats (dying)</td>
<td>1.18</td>
<td>0.88</td>
</tr>
<tr>
<td>Young rat 32 (Day 3 of infection)</td>
<td>0.98</td>
<td>1.01</td>
</tr>
<tr>
<td>Young rat 32 (Day 7 of infection)</td>
<td>1.36</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) Data derived from paper by A. Zuckerman, 1958.
\(^b\) Infection rate in reticulocytes = \% merozoites in reticulocytes/\% reticulocytes.
\(^c\) Infection rate in mature RBC = \% merozoites in mature RBC/\% mature RBC.
have changed its host cell preference during the course of infection. Unfortunately, this single observation was not repeated. There was an apparent change in reticulocyte preference in \textit{P. knowlesi} and \textit{P. coatney} at different levels of parasitemia, but, again, the number of observations was limited (Miller and Chien 1971).

Development of parasites in abnormal hosts also demonstrated selectivity in red cell populations infected. \textit{P. berghei} inoculated into duck embryos had an abnormal development in orthochromic erythrocytes as compared to erythroblasts (McGhee 1954). In a similar experiment, erythrocytes from young and old rats were inoculated into chick embryos infected with \textit{P. lophurae} (McGhee 1953b). The erythrocytes from young rats were susceptible and those from old rats remained uninfected. It would have been of interest to know the susceptibility of erythrocytes of old rats to \textit{P. lophurae} after induced reticulocytosis. Was the difference in susceptibility between young and old rats caused by the difference in reticulocyte concentration or inherent host age determined differences in the erythrocyte?

2. 	extit{Erythrocyte} surface receptors and host specificity. Experimental evidence that host specificity is determined, in some cases, by the outer surface of the erythrocyte membrane was first presented by McGhee (1953a, c). The relative erythrocyte susceptibility of different species was studied in chick embryos. He found that merozoites of \textit{P. lophurae}, an avian malaria that infects both chick embryo and duck erythrocytes, had a much higher affinity for duck erythrocytes. Recently, it was demonstrated that in vivo infectivity of \textit{P. knowlesi} for various host species was related to the penetration of erythrocytes of these species in vitro (Butcher \textit{et al.} 1973; Miller \textit{et al.} 1973). The erythrocytes of resistant species (e.g., rodents and birds) were not invaded in vitro. The differences in virulence of \textit{P. knowlesi} between man and rhesus monkeys may be explained, in part, by the lower infection rate in human erythrocytes (25\% of the rate in rhesus erythrocytes under identical conditions). However, the invasion rate in \textit{Macaca fascicularis} and \textit{M. mulatta} were similar (Butcher \textit{et al.} 1973), although the infection is mild in the former host and fulminant and usually fatal in the latter. Therefore, the difference in virulence in these hosts is unrelated to erythrocyte susceptibility.

Sometimes susceptibility varies for hosts of the same species. West Africans are resistant to infection by \textit{P. vivax} (Bray 1958; Young \textit{et al.} 1955), although they are susceptible to the other three species of malaria. Unlike sickle trait that modulated the severity of infection with \textit{P. falciparum} (Gilles \textit{et al.} 1967) but has no influence on infectivity (Edington and Watson-Williams 1965), the vivax resistance factor completely blocks infection. Since resistance was present after inoculation of infected blood (Young \textit{et al.} 1955), the resistance factor must interfere with the merozoite’s ability to invade erythrocytes or to develop once within the erythrocyte.

3. 	extit{Chemical analysis of the receptor}. Treatment of human erythrocytes with proteolytic enzymes (chymotrypsin and pronase) blocked invasion by \textit{P. knowlesi} merozoites (Miller \textit{et al.} 1973). The fact that these enzymes only affect the cell surface supported the hypothesis that invasion is initiated by specific receptors and that these receptors are probably proteins or glycoproteins. Since trypsin and neuraminidase treatment did not inhibit penetration, receptors on human erythrocytes are not a component of the major sialoglycoprotein, and invasion is uninfluenced by surface charge. Chymotrypsin treatment that produced similar digestion of surface proteins in human and rhesus erythrocytes failed to block invasion of rhesus erythrocytes (Miller \textit{et al.} 1975b) With the discovery that Duffy blood group determinants are erythrocyte receptors for \textit{P. knowlesi}, we tested Rhesus erythrocytes for Duffy blood group determinant (Miller, F. H., and Mc-
Rhesus erythrocytes were found to have Duffy blood group determinants. Whereas chymotrypsin destroyed the Duffy determinants on human erythrocytes, these determinants on Rhesus erythrocytes were unaffected by chymotrypsin. The data are consistent with the hypothesis that the Duffy determinant is a glycolipid on rhesus erythrocytes and possibly a glycoprotein on human erythrocytes.

4. The erythrocyte receptor for P. knowlesi merozoites and the Duffy blood group determinants. Recently we have demonstrated that P. knowlesi in culture invades poorly human erythrocytes that are blood group Duffy negative (FyFy) (Miller et al. 1975a). The resistance to invasion does not appear to be caused by other associated membrane defects in Duffy negative erythrocytes, since removal of Duffy blood group determinants (Fya or Fyb) by chymotrypsin and specifically blocking of Fya with anti-Fya greatly reduce invasion.

The P. knowlesi resistance factor, Duffy negative erythrocytes, occurs in high frequency in West Africa (Sanger et al. 1955) where the people are resistant to P. vivax; it is extremely rare in most other areas of the world where P. vivax occurs (Mourant 1974; Lewis et al. 1972; Ward et al. 1975). This striking association strongly suggests that the Duffy negative blood group (FyFy) may be the resistance factor to P. vivax in West Africans and that normal invasion by P. vivax may require a Duffy positive erythrocyte (Fya or Fyb).

5. Summary. It is evident that more is known about the erythrocyte surface and its influence on innate resistance than any other aspect of the host–parasite interactions. Evidence exists that reticulocyte preference, host specificity (in some cases), and strain specificity are determined by the surface receptors on erythrocytes. The analysis of chemical structure of this receptor on human erythrocytes is now possible since the discovery of an association between Duffy blood group determinants and susceptibility to P. knowlesi, and between Duffy negative erythrocytes and the P. vivax resistance factor. Of equal importance is the question of whether the Duffy blood group determinants are the receptors for other human malarials. It should be possible to isolate the chemical groups on merozoites that attach to the erythrocyte receptors and to attempt immunization against these structures on merozoites.

B. Erythrocyte Cytoplasmic Factors in Parasite Survival

The interior of the erythrocyte may interfere with normal parasite development. Malaria parasites are not found in any animals with low K⁺, high Na⁺ erythrocytes (e.g., cats, dogs, and cattle) (Kerr 1937; McGhee 1953c). A single species of bovine (the Indian domestic buffalo, Bubalus bubalis) is infected with a malaria parasite, P. bubalis. It would be interesting to know the Na⁺ and K⁺ concentration in erythrocytes of this animal.

The association between high incidence of sickle cell hemoglobin and hyperendemic falciparum malaria suggested a causal relationship (Allison 1954, 1961). The prevalence of infection with P. falciparum in villages in West Africa is the same in children with sickle trait and normal hemoglobin (Edington and Watson-Williams 1965). On the other hand, in children hospitalized for severe malaria and in those dying from malaria, there was a significant reduction in sickle trait as compared to the expected rate in the population (Gilles et al. 1967). Therefore sickle trait does not prevent the infection but modifies the severity of the disease.

The mechanism by which sickle trait hemoglobin reduced parasitemia is unknown. P. falciparum infected erythrocytes sickle more easily than uninfected erythrocytes (Luzzatto et al. 1970). Since P. falciparum infected erythrocytes adhere to venous endothelium, the anoxic environ-
ment might induce sickling that, in turn, reduced parasite survival (Miller et al. 1956). There does not appear to be an obstacle to infection of AS erythrocytes (Luzzatto 1974). P. falciparum merozoites invaded in vitro AS erythrocytes as frequently as AA erythrocytes.

The influence of glucose-6-phosphate dehydrogenase (G6PD) deficiency on severity of infection with P. falciparum is controversial (Devakul et al. 1960; Powell and Brewer 1965; Gilles et al. 1967; Bienzle et al. 1972). This controversy has been reviewed by Livingstone (1971). It should be remembered that small differences in severity of infection and thus survival between genetically different groups may be difficult to demonstrate but could have a marked effect on selection over many generations. Recently, Bienzle et al. (1972) claimed that "amongst non-deficient male subjects, those with the electrophoretic variant A of glucose-6-phosphate dehydrogenase have significantly lower parasite counts than those with B variant" and in females, "subjects heterozygous for glucose-6-phosphate-dehydrogenase deficiency (specifically, those with the GdA/B genotype)" and demonstrated that the difference was significant by the chi-square test. However, chi-square test of the entire male and female population failed to demonstrate a significant difference in the number of individuals above and below the median parasitemia (P = 0.25 and 0.11, respectively). Luzzato et al. (1969) demonstrated that the parasite rate was higher in normal than G6PD deficient erythrocytes. This observation may have had nothing to do with the parasite's ability to invade or survive within deficient erythrocytes. The elution test that was used to differentiate normal and deficient erythrocytes may be invalid during malarial infection. If normal erythrocytes were indeed more frequently infected, then multiple invasion should occur more often in normal than deficient erythrocytes.

C. Plasma Factors in Innate Resistance

Certain nutritional deficiencies such as PABA deficiency have marked effect on parasite development (review by Geiman 1964). Toxic factors from the parasite or a sick host may adversely affect parasite development, especially at high parasitemia. In addition, the physiologic state of the host may influence parasite development. Trager (1948) demonstrated that some ovulating ducks have a suppressed peak parasitemia; the infection had an invariable fulminant course in ducklings. Trager and McChee (1950) showed that a factor in plasma of these ducks was partially responsible for the reduced parasitemia. It would have been interesting to observe the susceptibility of the erythrocytes from susceptible and partially resistant adult ducks in the P. lophurae infected chick embryo in order to exclude an erythrocyte difference.

In some cases, the effect of plasma on the course of parasitemia may be an artifact of the test system and unrelated to the malarial parasite. Becker et al. (1951) noted that P. lophurae infected ducks produced a poor infection in chickens unless accompanied by duck plasma (the sparing phenomenon). They later found that chicken serum agglutinated duck erythrocytes (infected or uninfected) and that duck serum diminished the agglutination of duck erythrocytes by chicken serum. Antibodies in chickens against duck erythrocytes probably caused rapid clearance of duck erythrocytes, and duck plasma protected the duck erythrocytes from the chicken serum. Therefore the parasitized duck erythrocyte in duck plasma could survive in the circulation of chickens; the parasite could undergo full maturation and invade chicken erythrocytes.

It is theoretically possible that plasma factors could protect the parasite from the immune mechanisms of the host. Although these have not been observed in malaria, evidence for their presence in trypanosomiasis has been presented by Desowitz
FIG. 1. The course of infection with *Plasmodium berghei* (K173) in Swiss mice (a virulent infection) and in STR × C57BL mice (a mild infection). All mice were infected with parasites adapted to Swiss mice. Daily mortality rates \[\text{Erythrocytes/mm}^3 \times 10^9\]. Percentage of immature erythrocytes \[\bullet \bullet \bullet \bullet \bullet \]. Percentage of immature erythrocytes parasitized \[\Delta \Delta \Delta \Delta \]. Percentage of mature erythrocytes parasitized \[\bigcirc \bigcirc \bigcirc \bigcirc \]. Percentage of parasitemia (in all erythrocytes) \[\triangle \triangle \]. Data derived from Greenberg (1956) and Greenberg and Kendrick (1957a).

(1963). Serum derived from host normally susceptible to *Trypanosoma vivax* facilitated the infection in rats. Desowitz proposed that the serum supplement protects the trypanosome from the action of antibodies in immune rats.

**D. Genetic Control of Resistance to Malaria**

Reference has already been made to the inherited red blood cell traits in man, the sickle cell hemoglobin trait, G6PD deficiency, and the Duffy negative blood group determinants, each of which is associated with increased innate resistance to malaria. In addition to these, evidence has been produced that the \(\beta\)-thalassaemia trait and certain HL-A types may be involved in conferring resistance to malaria in man. Sinsiscalco et al. (1966) found that the frequency of the \(\beta\)-thalassaemia trait in the native populations in 47 villages in Sardinia showed a positive correlation with the previous incidence of malaria. Piazza et al. (1972) observed that the HL-A frequencies in four Sardinian villages appeared to be related to the previous incidence of malaria in these areas. They speculated that the HL-A variation might be an adaptation to malaria. Since the immune response genes (Ir genes) are closely linked to the major histocompatibility locus (McDevitt and Benacerrat 1969), they suggested that this variation may be related to the immune response to the malaria parasite. Further studies into the HL-A frequencies in malaria should be pursued.

Genetic studies on differences in response to infection with *P. berghei* (K173) in inbred strains of mice were carried out by Greenberg and co-workers. Their results are summarized in the following discussion. In all strains of mice studied, an invariant characteristic was the marked preference of *P. berghei* for reticulocytes rather than mature erythrocytes. On the other hand, the most obvious differences between
mouse strains were related to the degree of virulence of the infections (parasitemia in mature erythrocytes on Day 6 (6DP) and mortality) (Greenberg 1956).

In a virulent infection (Fig. 1, Swiss mice), the rapid increase in parasitized immature erythrocytes early in the infection quickly destroyed these erythrocytes. For a period after this, the rise in overall parasitemia was almost entirely due to the slower multiplication of parasites in the less preferred mature erythrocytes. In response to the destruction of erythrocytes, reticulocytosis developed and the circulation became flooded with immature erythrocytes in increasing numbers. As the reticulocytosis increased, the infection in the mature cells began to fall as the parasites preferentially invaded the young erythrocytes.

In a mild infection (Fig. 1, STR × C57BL mice) the earliest events developed more slowly. Due to the slow rise in parasitemia, the original population of immature erythrocytes was not entirely destroyed by the parasites before the new generation of reticulocytes appeared in the blood. The peak of infection in mature cells was very low as compared to that in virulent infections and occurred somewhat later in the infection.

The mortality rates in the early infection appeared to be directly associated with the rate of destruction of erythrocytes determined by the rate of rise of parasitemia in mature erythrocytes (Fig. 1). Thus during the early period of infection there was a strong correlation between mortality rates and the level of parasitemia in mature erythrocytes [measured as parasitemia in mature erythrocytes on the sixth day of infection (6DP)] (Fig. 2). When sufficient numbers of mice of different strains were compared, large differences in mortality rates and 6DP values could be demonstrated (Fig. 2). In addition, within each strain of inbred mice infected with *P. berghei*, wide variations (among individual mice of the same age and sex) occurred in the time of death and to a lesser extent in 6DP. Since such individuals are presumed to be genetically identical, or nearly so, there can be little doubt that nongenetic factors also played a role in influencing the response of mice during infection.

In a series of papers Greenberg and coworkers (Greenberg et al. 1953; Nadel et al. 1955; Greenberg and Kendrick 1955; Greenberg and Kendrick 1958; Greenberg and Kendrick 1959) reported the results of crosses made between inbred mouse strains differing in mortality rates and 6DP during infection with *P. berghei*. In the F1 hybrids, the mortality rates and 6DP in the earlier period of infection (up to about the end of the first week) generally approached or were lower than those of the lowest parent. These results indicate that hybrid mice tend to acquire characteristics enabling them to contain and survive the infection to a degree which approached or even exceeded that of the more resistant parent. The F1 generations, therefore, tend to exhibit a form of hybrid vigor or heterosis.
Fig. 3. 6DP and mortality during *Plasmodium berghei* infection in the parental lines, F₁, F₂, and backcrosses from a cross between Swiss and STR mice. In the F₂ and backcrosses the observed values are compared with those predicted on the assumption of inheritance of the parental differences in 6DP and mortality rates at single gene locus. In principle the prediction for the F₂ is made by taking the mean daily percent mortality of the Swiss parent, the F₁ and the STR parent in the proportion 1:2:1, respectively. Likewise the predictions for each backcross are made by taking the mean values of the appropriate parent and the F₁ in the proportions 1:1. (The theoretical daily change in the number of each parental type was included in the calculation of theoretical daily mortality rate.) There is a marked divergence between observed and predicted values for mortality in the early period of infections. The observed mortality was always lower than predicted. This suggests multiple gene involvement in the mortality characteristics in this cross. All mice were infected with Swiss adapted parasites. Data derived from the results of Greenberg and Kendrick (1958).

Although, in general, heterosis is believed to indicate multigene control, it may be controlled at a single locus. For example, the mortality caused by *P. falciparum* is less for Hb SA children than for Hb SS or Hb AA.

In experiments using two different pairs of mouse strains as parents (Nadel *et al.* 1955; Greenberg and Kendrick 1958), the F₁ generations were analyzed in the F₂ and in backcrosses to either parent. The results of these crosses indicate whether one or
more gene loci are involved in the inheritance of the characters studied. The results of Greenberg and Kendrick (1958) in which the parental strains were outbred Swiss mice and inbred STR mice (Fig. 3) strongly suggested that several genes governed mortality in the early stages of the infection.

In another experiment, Nadel et al. (1955) used DBA/2 and C57BL as parental strains. The F2 and backcross data for the inheritance of mortality rates are close to those values predicted on the assumption of inheritance at a single locus or closely linked set of genes (Fig. 4).

These results suggest that innate inherited differences between mice in their response during the early stages of infection with *P. berghei* may be controlled at many loci or by a single gene, depending on the strains studied. In view of the increasing complexity of events in the later stages of infection, it is felt that the genetic analysis of characters such as daily mor-
Mortality may become prohibitively complex. The results of the analysis of the two crosses referred to above are hard to interpret for late infection characteristics.

**E. Parasite Modification under the Genetic Control of the Host**

Protozoa are capable of active transformations in response to factors presented by the environment (Beale 1954). There is good evidence that such transformations occur in malaria. This type of phenomenon has been illustrated by antigenic changes induced in *P. knowlesi* by circulating antibodies in the Rhesus monkey (Brown 1973). As each new antigenic variant arose in the parasites, new antibodies were synthesized against this variant. These antibodies then initiated a further change of antigen type in the parasite. When a *P. knowlesi* infection was subinoculated into a nonimmune host, its antigenic type remained unchanged until antibodies in the second host initiated a further antigenic change. The parasite was thus capable of undergoing active cellular transformations in response to the changing environment presented to it by its host.

In *P. berghei* infected mice, Greenberg and Kendrick (1957a, b) presented evidence that the parasites could undergo behavioral changes in response to factors determined by the genetic constitution of the host. Parasites that had been adapted to Swiss mice were inoculated into C3H mice (Fig. 5). In the first passage, the early mortality and 6DP were similar to that seen in the Swiss mice. After several passages in C3H mice, however, the infection stabilized to a pattern characteristic of a C3H adapted line of parasites (low mortality and low 6DP). The reverse operation was also performed by subinoculating C3H adapted parasites into Swiss mice. In these experiments the infections in Swiss mice during the first one or two passages resembled the infection in C3H mice (Fig. 5). As before, however, after several passages the infections stabilized to a pattern characteristic of parasites adapted to Swiss mice.

Similar, although less pronounced, effects were found during transfer of parasites between other pairs of mouse strains. In some pairs of strains, however, (e.g., C57BL and Swiss) the effect was not apparent in spite of major differences in the patterns of mortality during *P. berghei* infection in these mouse strains.

The phenomenon of host modification of parasite infections, as illustrated by the example in C3H and Swiss mice, is open to two alternative explanations. Genetically
different hosts may select for distinct populations of parasites. Such an hypothesis involves the assumption that the strain of *P. berghei* used in this work comprised a number of genetically distinct populations and that some unusual selection conditions operated that would allow a line with low virulence to be selected in favor of one with high virulence (as, for example, in the adaptation of parasites from Swiss to C3H mice).

Alternatively, it is possible that the parasites were induced to undergo semistable cellular transformations under the influence of factors determined by the genetic constitution of the different strains of mice. This explanation is the one favored by the present reviewers. There is a resemblance between the situation proposed here and the antibody mediated induction of antigenic change in *P. knowlesi* or the temperature induced changes in surface antigens in *Paramecium aurelia* (Beale 1954).

### III. Gametocytogenesis, Gametogenesis, and Infectivity to Mosquitoes

The influence of the physiologic state of the host on gametocytogenesis and gametogenesis in Haemosporidia other than malaria has been reviewed by Bishop (1954). It should be remembered, however, that gametocytogenesis in hepatocystis and haemoproteus is probably under different controls than malaria parasites. Since exoerythrocytic parasites (in hepatocystis and haemoproteus) can only develop into gametocytes within the erythrocyte, increase in gametocyte numbers reflects increased release from the exoerythrocytic forms rather than a switch from asexual to sexual parasites as in malaria.

Abnormal hosts that support asexual development may have few or no gametocytes. Repeated subpassage of asexual parasites by syringe may, in some cases (*P. berghei, P. lophurae*), cause a loss of gametocyte production. In other situations (*P. cynomologi* and *P. knowlesi*), the repeated passage for years by syringe had no influence on gametocyte production or infectivity to mosquitoes.

In summary, it can be stated that little is known about the innate factors that influence gametocytogenesis or mosquito infectivity.

### IV. Sporozoite Infectivity and Exoerythrocytic Development

Host specificity and resistance in exoerythrocytic (EE) development probably has definable determinants as in erythrocytic infections, namely, plasma factors, receptors on cells, and intracellular environment. Resistance may reflect the fact that sporozoites cannot invade or develop within host cells. In the case of mammalian malaria, nothing is known about the sequence of events that leads to invasion of hepatic parenchymal cells. Does the sporozoite first enter the endothelial or Kupffer cells in the hepatic sinusoid or does it pass through the fenestrations into the Space of Disse and directly invade hepatic parenchymal cells?

Failure to induce an infection by sporozoite inoculation may not indicate abnormal EE development. Development within these cells may go unrecognized, especially in mammalian malaria where the forms develop within hepatic parenchymal cells; merozoites from exoerythrocytic schizonts may be unable to invade erythrocytes.

EE development after sporozoite inoculation is not an all or none phenomenon; many gradations may exist. For example, viability of *P. berghei* sporozoites varies within different hosts (Vanderberg *et al.* 1968). In the tree rat, the host in nature, approximately 50% of the sporozoites develop into EE forms within the liver. By contrast, less than 1% develop into EE forms in the mouse.

Development of EE forms from erythrocytic merozoites is unique to avian malaria. Invasion of nonerythrocytic cells after blood infection follows an ordered pattern depending on the host and parasite involved.
(Huff 1954; 1957). The reticular cells of the spleen are infected first, and, later, parasites develop within capillary and venular endothelial cells (Huff 1954). The time of endothelial infection is uninfluenced by inoculum size, onset of patent parasitemia, level of parasitemia, or splenectomy. At some point in the host-parasite site, the merozoites become able to invade endothelial cells, apparently for the first time. What leads to this enigma. Huff (1957) observed that "definite patterns of exoerythrocytic occurrence and distribution are not characteristic of host species or of parasite species, but are determined by both."

**ACKNOWLEDGMENT**

We thank Drs. F. A. Neva, I. Green, and L. Luzzatto for suggestions on the manuscript.

**REFERENCES**


McGhee, R. B. 1953b. The influence of age of the animal upon the susceptibility of mammalian erythrocytes to infection by the avian malaria parasite Plasmodium lophurae. Journal of Infectious Diseases 92, 4–9.


to compatibility Testing.” (J. Dorsett, and J. Colombani, eds.), pp. 73–84. Munksgaard, Copenhagen.


