MALARIA AND HUMAN POLYMORPHISMS 3020

Frank B. Livingstone

Department of Anthropology, University of Michigan Ann Arbor, Michigan

Malaria has probably killed more human beings than any other single disease (Garnham 1). In 1952 Russell (2) estimated that there were about 350 million cases of malaria per year with an estimated 1 percent mortality. More recently Bruce-Chwatt (3) has estimated that 1741 million people now live in areas that were once malarious; of that number 381 million are still subjected to endemic malaria, and 710 million inhabit areas where malaria is only partially controlled. As a leading cause of human morbidity and mortality, malaria has undoubtedly been a major agent of natural selection and consequently a determinant of man's genetic evolution. Haldane (4–6) was primarily responsible for directing the attention of geneticists to the importance of infectious disease including malaria in man's recent evolution. Although man undoubtedly has had many parasites during his long history as a hunter and collector, the domestication of animals and plants about 9000 years ago increased the number and severity of parasitic infections, since it enormously increased the population density of the human species and made it one of the most available hosts for parasites. The effects of either epidemic or endemic malaria are also so disruptive to human affairs that malaria has often been considered a major cause of historical events from the declines of Greece and Rome to the failure of the Crusades and the Gallipoli campaign of World War I (Jones 7, Hackett 8, Garnham 1). Shortt (9) has discussed the economic importance of malaria and given examples of its disastrous effects. He also points out that this disease may have prevented the settling of some areas of India.

Because of its distinctive symptoms, malaria can be traced more precisely than most infectious diseases in the writings of antiquity. References on papyri of the 16th century B. C. to splenomegaly, and hieroglyphic inscriptions referring to intermittent fever seem to indicate the presence of malaria in ancient Egypt (Hoeppli 10, Garnham 1). But the characteristic symptoms of malaria, tertian or quartan fever and an enlarged spleen, were specifically recorded in Greece by Hippocrates in the 4th century B. C. and were also known in Roman times in Italy (Boyd 11, Russell 12). Pre-Christian written records from China and India also refer to the same symptoms (Gwei-Djen & Needham 13, Hoeppli 14, Russell 10); so we know that malaria was present throughout the civilizations of antiquity.

Epidemiology of malaria.—Although malaria has been present for millenia in most human populations of the tropical and sub-tropical regions of the Old World, its importance as a selective factor in these populations is a more complicated question. Selection results from either differential fertility or mortality among the genotypes of a particular locus and is dependent, obviously, upon the total effect of the selective factor. In a population with endemic malaria, the effects of the disease fall primarily on the infants and children. With the most intense transmission of malaria, on the average a person is bitten by an infected mosquito every 10 days or even more frequently; so that everyone is infected in the first year of life, and close to 100 percent of the young children will have detectable parasites and an enlarged spleen. Although the adults in the population will be continuously re-infected and occasionally show parasites, there will be little adult morbidity or mortality due to the disease. With less transmission, the child parasite rate will drop to 50 percent or less, the adult parasite rate will increase, and there may be some adult mortality. However, malaria is still continuously present, and everyone is infected. Malaria becomes epidemic when transmission is interrupted for considerable lengths of time. In an epidemic all ages are almost equally affected, and the mortality rate can exceed that for endemic malaria for the same period of time.

As for any disease, the amount of transmission of malaria can vary in any population. In arid or temperate climates such as Northern Europe, there can be pronounced seasonal variation in transmission, but the disease will still remain endemic. MacDonald (15) developed an index to measure the stability of the transmission of malaria which depends only on the life expectancy of the mosquito species transmitting malaria and the probability that it will bite man. Stability and endemicity are closely correlated, and with greater stability the demographic and selective effects of malaria would be more constant and operate continuously generation after generation.

On the other hand, since epidemic malaria is not constantly present, its selective and demographic effects are not continous and consequently not as great. A disastrous epidemic of malaria or any other disease, since it is only a single occurrence, is comparable to a single generation of selection in its effect on gene frequencies. For example, if an epidemic caused a mortality of 10 percent and the difference in fitness was 10 percent, then for the usual models of gene frequency change this would result in a selection coefficient of 1 percent, and it takes many generations of epidemics to effect appreciable gene change with this amount of selection. There are few data on the selective advantage of heterozygotes at loci that are considered to be balanced by infectious disease. The presumed higher equilibrium frequency of the sickle cell gene (hemoglobin S) and the very low fitness of homozygotes for this gene would seem to indicate that its selective advantage is the highest of any, and with an average equilibrium gene frequency of about .13, the selective advantage would be 15 percent. But even for this gene a malaria epidemic with 25 percent mortality would increase the heterozygotes by 2–4 percent, depending on their frequency. Similar epidemics would have to

occur continuously over a considerable length of time to change the gene frequency significantly, and most epidemic diseases do not have such a consistent pattern. The absence of high frequencies of abnormal hemoglobins and the glucose-6-phosphate dehydrogenase deficiency in many populations of India, Spain, and Portugal may well be due to the fact that malaria is more epidemic than endemic in these areas.

For several years there has been a controversy as to how the mortality and morbidity due to malaria vary with the endemicity of the disease. With the great advances in the program of world-wide eradication of malaria, this debate has tended to lose its significance for public health policy, but the significance remains for the evolutionary effects of malaria. Wilson, Garnham & Swellengrebel (16) in an extensive review of hyperendemic malaria [now called holoendemic (Covell, Russell & Swellengrebel 17)] expressed the view that there was less mortality and morbidity under conditions of greatest transmission. They cite the parasite rates and spleen rates of populations from many different parts of the world to support this position and infer from these data that a solid immunity to malaria is acquired in childhood with little effect on the health of the population. Despite the diversity of the populations involved, the authors considered that the ability to acquire this immunity has an innate or racial component. Garnham (18) has presented the most extensive data to support this view, from the Kavirondo Gulf region of Kenya, which in addition seem to show that malaria has little effect on the outcome of pregnancy. Shortt (9) and Macdonald (19) disagreed with this view, and Macdonald pointed out the deficiencies of the data supporting it. The controversy did result in the publication of some data on the age-specific mortality rates for malaria (Colbourne & Edington 20, Bruce-Chwatt 21, Duren 22); but since these data referred to urban or cosmopolitan populations, the debate was not resolved. In the intervening years there has still been no study of the age-specific mortality rates for malaria in a holoendemic population; and since such populations are now almost nonexistent, the crucial data may never be collected. Hence the controversy is moot.

Nevertheless, if small isolated human populations could evolve a commensal relationship with malaria that is based on a very high transmission rate, this would imply that any human polymorphism that is maintained by malaria would occur in higher frequencies in populations with less intense malaria. By considering various models for the age-specific mortality from malaria, Lehmann & Raper (23) attempted to determine the malaria mortality necessary to balance the sickle cell polymorphism among the Bwamba tribe of Uganda, who have one of the highest sickle cell frequencies in the world (.195). They found that if the malaria mortality occurred later in life, as would be the case with less transmission, then a lower mortality would be necessary to sustain this high frequency. The parasite rates for the Bwamba, which they cite, seem to reflect a lower endemicity than the hyperendemic rates in Wilson, Garnham & Swellengrebel (16). However, Wilson (24) and Brass (25) disputed Lehmann & Raper's conclusions, and Wilson also at-

tempted to show that the malaria mortality known in Africa was not sufficient to maintain the sickle cell polymorphism. Many others (e.g. Neel 26) have speculated on this problem because the mortality does seem to be less than is necessary. More recently, Raper (27) has constructed the hypothetical age-specific mortalities that would be associated with different endemicities of malaria. These malaria death rates are constructed to show that malaria could both balance the sickle cell polymorphism and result in higher frequencies of this gene at lower endemicities. However, he concludes by suggesting that criticisms by malariologists would be helpful to resolve the problem, but I am unaware of any further contributions to the issue.

To balance the sickle cell polymorphism when it is the only abnormal allele present, we can use the formula, $\hat{q} = s/(s+t)$, where s and t are the selections against the AA and SS homozygotes, respectively, and $\hat{q}$ is the equilibrium frequency of the S gene. If we assume that $t = 1.0$, which seems reasonable for most primitive populations in Africa (Lehmann & Raper 23), then for $\hat{q} = .2$, $s = .25$, and for $\hat{q} = .1$, $s = .11$. In these cases s is the proportion of AA homozygotes who would have to die of malaria if mortality from malaria was the only force balancing the polymorphism and no heterozygotes died from malaria. For the total population, these would be approximately .16 and .09 dying from malaria.

The calculation of the amount of selection due to malaria is complicated by the fact that only about 50 percent of the individuals born survive to the age of reproduction in Africa and in most other regions with highly endemic malaria. If the other deaths are assumed to be random with respect to the sickle cell genotypes, then the total population to which the selection would apply is the number (N) surviving to reproductive age plus the number (M) dying of malaria. Thus, with our assumptions, $s = M/(M+N)$. The total and malaria age-specific mortalities do not seem to be approximated by any general equation, but for any number of individuals born into a population we can compute from numerical estimates of these mortalities the number who survive to reproduction and the number who die of malaria. Colbourne & Edington's (20) data from Accra, Ghana, yield estimates of s ranging from .08 to .11, which would seem to be adequate to maintain the S gene frequency of about .1 found there. The presence of hemoglobin C, however, complicates the population dynamics in Ghana. For example, in 1948 Wards A and B on the outskirts of Accra had an infant mortality rate of 297/1000 and an infant mortality from malaria of 22/1000. The death rates of the older age classes were much lower, so that of 1000 born 583 survived to reproductive age and 57 died of malaria. Thus, $s = 57/(583+57) = .09$. For Ward D in the center of Accra, the infant mortality is 86, but the malaria mortality is somewhat higher so that $s = .088$.

The age-specific mortalities of many African populations are now known (Caldwell & Okonjo 28, Brass et al 29), and many tend to be higher for ages 2–5 than Colbourne & Edington's figures indicate. Malaria mortality also seems to be higher in other areas (Janssens et al 30, McGregor et al 31). McGregor et al followed two groups of children for the first three years of

life in a holoendemic population in the Gambia. One of the groups was given antimalarial drugs, the other was not. The groups were small, 26 in each at the start. While only 3.8 percent of the protected group died, 19.2 percent of the unprotected group died. This difference seems to indicate that malaria may be responsible for as much as 15 percent of the childhood deaths in a holoendemic population. If the age-specific malaria mortality rate for young children is estimated as 15–20/1000 instead of 11/1000, the figure of Colbourne & Edington, then estimates of s from .13 to .18 can be generated with many reasonable age-specific mortality estimates. With the assumptions of the model it is also easy to show that malaria mortality among older children makes a greater proportional contribution to s. This analysis thus supports Raper's (27) conclusions: malaria can maintain the sickle cell polymorphism and lower frequencies of the sickle cell gene may be expected with holoendemic malaria.

There are many populations in West Africa, Cameroons, and the Congo with very low sickle cell frequencies and holoendemic malaria. For those in West Africa, I have postulated that they were until comparatively recently hunters and collectors and would not have had endemic malaria in their unbroken tropical rain forest habitat (Livingstone 32). However, these populations may have had holoendemic malaria but a commensal relationship with the parasite, although at present there is considerable mortality due to malaria. In West Africa malaria is endemic almost everywhere, but there is a correlation between the frequencies of the sickle cell gene and population density (Livingstone 33). The high death rate from infectious diseases, particularly among infants and children, is one of the major factors controlling population size among primitive agriculturalists. The correlation between sickling and population density seems to implicate malaria as one of the major diseases controlling population in West Africa. Haldane (5, 6) has emphasized that causes of mortality that act homeostatically to control the size of a population, or what he called "negative density-dependent factors," have the most effect as agents of natural selection. Thus, malaria may have increased in importance as a selective factor as it increasingly became a factor controlling population size.

The effect of malaria on population growth.—Just as natural selection results from differential mortality and fertility, the growth rate of any population, which can be approximated by the difference between the crude birth rate and the crude death rate, is also determined by the forces controlling mortality and fertility. In addition to the data on the effect of malaria on mortality and fertility, indirect evidence for its effect can be inferred from the changes in the birth and death rates that have resulted from the eradication of malaria. Populations with endemic malaria have among the highest birth and death rates found in the world today, and these are comparable to the rates found in most human populations prior to the control of infectious disease. The average birth rate is perhaps 40/1000; and since these populations were comparatively stable until recently, the death

rate averaged perhaps 39/1000. As Coale (34) has pointed out, the growth rate of the whole human population has averaged about 1/1000 from the time of Julius Caesar to the 19th century. Malaria will have a maximum effect on the growth rate since most malaria mortality occurs before reproductive age. Precise estimates of this effect require age-specific mortalities, but in the absence of such data a comparison of crude death rates from malaria can provide a rough indication. Henceforth, as in previous discussion, all birth, death, and growth rates will be expressed per 1000 per year.

For the Western Hemisphere, the estimates of the crude death rate from malaria are higher than those for Africa despite the fact that endemicity is generally lower. For Central America Russell (12) has estimated the crude mortality rate from malaria to be 4.32, and Faust (35) gives figures of over 10 for parts of Guatemala and Honduras. In South America, the malarious districts of Guyana had malaria death rates up to 3–4, and when deaths from nephritis are added, the total due to malaria would be 5–7 (Giglioli 36, 37, Newman 38). In Trinidad the crude malaria mortality rates ranged up to 2 for the most malarious districts (Downs et al 39), and even in Arkansas in 1933 the malaria mortality rate was .5, which was the highest rate in the United States at that time (Faust 35). Since less than half of the population of Arkansas was subjected to malaria, the amount of selection there was comparable to many areas of endemic malaria in Africa. Colbourne & Edington's (20) data show a crude malaria mortality rate of 2.0 for Wards A and B on the outskirts of Accra, Ghana, and 2.5 for Ward D in the center of the city, which has a lower endemicity. In Lagos, Nigeria, Bruce-Chwatt (21) recorded a crude mortality rate from malaria of 1.4, and Duren (22) in a summary of the data from the Congo found an average death rate from malaria of 1.7. More recent data from endemic areas of the Sudan show the extremely low malaria death rates of .04 in the south and .03 for the Central Sudan (Wernsdorfer & Wernsdorfer 40).

Given an average mortality rate of 30–40, these data from Africa imply that malaria has only a minimal effect on the growth rate of populations with endemic malaria. However, the recorded declines in the crude mortality rates that follow the control of malaria in endemic populations seem to contradict this conclusion. In one of the pioneer studies of human ecology, Hackett (8) states that the mortality rate in Sermoneta, a small village on the Pontine Marshes of Central Italy, fell from 40 to less than 20 after the eradication of malaria. The Montagnard populations of the highlands of South Vietnam were subjected to some of the most severe holoendemic malaria in the world, and with malaria control the crude death rate fell by 15–20 (Farinaud et al 41, Farinaud & Choumara 42). In East Africa Pringle (43) found that the death rate decreased by 10 after residual insecticide spraying to control malaria; but since the death rate stayed at its lower level, after the resumption of malaria transmission, other factors seem to be involved. In any case, these studies all indicate a crude death rate due to malaria in holoendemic populations of perhaps 10–15, which is much larger than any recorded malaria death rates.

Malaria may contribute indirectly to death from other causes, nephritis being an obvious case, and these indirect effects may be the reason for such drastic declines in the death rate after malaria control. The most extensive data are from Guyana and Ceylon, the drastic decline in the death rate in the latter after malaria control is very frequently cited to show the demographic effects of malaria (Coale & Hoover 44). Newman (38) has made an extensive analysis of these two declines in the death rate. He attributes a major role to malaria and considers its effect on the death rate to be 4–5 times higher than recorded malaria death rates. However, there is still some controversy over the role of malaria in the decline of mortality rates in Ceylon (Frederiksen 45, Meegama 46), since this decline shortly after World War II was comparable in both malarious and nonmalarious districts. In Guyana there are records of the malaria mortality rates (Newman 38); and although these range up to 4 in hyperendemic districts, the decline in the death rate is still much greater. Thus, although it would appear plausible that malaria has been one of the major diseases controlling the size of human populations, conclusive evidence is still lacking. In reviewing the controversy on Ceylon, Bruce-Chwatt & Meade (47) point out that there has been a recent epidemic of malaria on Ceylon and its demographic effects may contribute further evidence.

Evidence for the influence of malaria on the birth rate of human populations is even more equivocal than for the death rate. The eradication of malaria does not have a constant effect on the birth rate (Pampana 48, Gill 49), although Khan & Zia-ud-Din (50) found a significant negative correlalation between epidemics and the next year's birth rate in the Punjab. On the other hand, there is considerable evidence that malaria infection during pregnancy causes prematurity (Blacklock & Gordon, 51, Bruce-Chwatt 52, Jelliffe 53, Gilles et al 54), which surely would result in increased risk of neonatal death. The pronounced decrease in the infant mortality rate that follows malaria control could thus be primarily a result of the decline in malaria infections of the placenta. Such an effect would obviously result in a very marked population control.

Evolution of human malaria parasites.—Heretofore malaria has been considered as a single entity, but, as is well known, human malaria can be caused by several different organisms all of which have generally been classified in the genus, *Plasmodia.* Man is the major host for four species, *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale.* The falciparum parasite is so different from the others in its morphology and life cycle that it has been classified in a different genus, *Lavarania*, by Bray (55), and this view seems to be gaining acceptance. The great majority of the obvious deaths from malaria are caused by cerebral complications, and most of these are due to falciparum malaria. But all four parasites cause the typical malaria symptoms of chills and fever and cause considerable morbidity and perhaps mortality. Mortality from malaria is closely correlated with the density of parasitemia, but chronic malaria, especially that due to *P. malariae*, has been

implicated in many diseases. It is one of the major causes of nephritis or nephrosis, which are common in many tropical countries with endemic malaria and cause a significant mortality (Giglioli 37, van der Kuyp 56, Gilles 57, Kibukamusoke 58).

There are malaria parasites of other primates that can on occasion infect humans. Most of the *Plasmodia* of the higher primates were considered to be quite species-specific until recent experimental transmissions between man and other primates of many of their malaria parasites (Bruce-Chwatt 59, Contacos et al 60). It seems that the major reasons for the absence of infections being transferred so infrequently between mammalian hosts are ecological. For example, the mosquitos that infect monkeys in South America live and breed in treetops and so have little contact with man, but they do occasionally infect humans with malaria (Deane et al 61). On the other hand, in Southeast Asia the same anopheline mosquitos bite man and other primates (Eyles et al 62, Warren et al 63), and there are more malaria zoonoses in that region.

The two malaria parasites of the South American monkeys, *P. brasilianum* and *P. simium*, are morphologically identical to the human parasites, *P. malariae* and *P. vivax* respectively; and since *P. brasilianum* has been experimentally transmitted by mosquito bite to man from the spider monkey and back again (Contacos et al 64), it would appear to be the same parasite as *P. malariae*. The lack of variation in the *Plasmodia* of South American monkeys and their identity with human malaria parasites leads to the conclusion that these monkeys were recently infected with what were originally human parasites (Dunn 65). Since the American Indians populated the New World via the Bering Straits, it would have been impossible for them to have brought malaria parasites to the New World. Thus, malaria seems to be comparable to yellow fever in having been brought here by European expansion and to be post-Columbian, although Bruce-Chwatt (66) has postulated an earlier transatlantic crossing during the Bronze Age. This implies that the American Indians have been subjected to natural selection by malaria for only the last few hundred years, and their populations seem to have no gene frequencies at the loci that have presumably been selected by malaria to contradict this hypothesis (Arends 67).

In the Old World, malaria would also seem to be relatively recent as a cause of natural selection in most human populations. During the course of man's evolution from an anthropoid ape, it is now obvious that he spent millions of years as a hunter on the tropical savannas of the Old World. During this stage of his evolution, population densities averaged at most about 1 per 10 square miles, and the species was confined to the relatively mosquito-free grasslands. Such a species would not be an adequate host for the current human malaria parasites, and this raises the question as to how these parasites evolved to their present form.

The development of drug resistance in the malaria parasites in the last few years indicates that these parasites can evolve much faster than their

hosts, so that the present malaria parasites may have been quite different in the past and adapted to a sparse hunting host. But it seems more likely that other primates in the tropical forest, which have population densities of about 10 per square mile, were the principal hosts in the past. When man became more populous and began to change drastically the flora and fauna in his environment, he became infected with the parasites of other mammals. At first these parasites would be zoonoses, but with the elimination of the other host the parasite would become totally adapted to man. Assuming host transfer to be the mode of evolution of human malaria parasites, one can then attempt to find the nearest malaria ancestor among the similar parasites of other primates from which possible alternate hosts can be determined (Bruce-Chwatt 59, Livingstone 68). It should be pointed out, however, that there is a strong tendency among parasitologists to assume that parasites co-evolve with their host (Bruce-Chwatt 66). This phenomenon has been given a name, vicariance (Garnham 1), and would obviously minimize the importance of host transfers.

Man's closest relative, the chimpanzee, has two malaria parasites, *P. reichenowi* and *P. schwetzi*, which are very similar to the human parasites, *P. falciparum* and *P. vivax*, respectively, and a third parasite, *P. malariae*, is found in both man and the chimpanzee. It is thus a zoonosis, although there seems to be little opportunity for transfer between hosts (Bruce-Chwatt 59). *P. vivax* is also extremely similar to *P. cynomolgi* of the macaque and other Old World monkeys. The other human malaria parasite, *P. ovale*, is not very much like any other parasite, but it does share some similarities with *P. gonderi* of the African monkeys, with *P. fieldi* of Malayan macaques, and with *P. simiovale* of Ceylonese macaques (Garnham 1).

All the human malaria parasites are found throughout most of the range of human malaria, but the more localized distributions of the alternate hosts or similar simian parasites, or both, make it possible to speculate as to the possible places and times of evolution of the human parasites from zoonoses. *P. vivax* is more adapted than the others to northern temperate climates where there is a marked interruption in transmission during one season. Since it is almost identical to *P. cynomolgi* of the macaques who were once extremely widespread from Europe through the Middle East and India to Japan, the elimination of the macaque in many of these areas by man's agricultural activities created the circumstances for *P. cynomolgi* to evolve into *P. vivax*. Thus, it would seem that *P. vivax* was the first major human malaria parasite and the primary one in the civilizations of antiquity.

The close relatives of *P. ovale* are widespread, so that its origins are obscure. However, it is most frequent in West Africa, where most of the monkeys are infected with *P. gonderi*, which could be an ancestor. On the other hand, *P. falciparum* and *P. malariae* would both seem to have been acquired from the chimpanzee. Since *P. malariae* is a low density, chronic infection, which is adapted to a low rate of transmission, it has survived relatively unchanged in its new niche. On the other hand, *P. falciparum* has evolved

into an extremely virulent parasite with many of the same pathological symptoms as *P. knowlesi* when it infects macaques other than its normal host. It would thus appear that man acquired falciparum malaria in Africa, and since the tropical forest and thereby one of its inhabitants, the chimpanzee, have been greatly reduced in geographic range in the last 2000 years due to man's activity, the circumstances for host transfer of parasites have surely arisen many times. In Africa the major anopheline carriers of human malaria, *A. gambiae* and *A. funestus*, are adapted to water sources that arise with human disruption of the forest. There is thus little opportunity for zoonoses with apes, and hunters would not be expected to have endemic malaria. In Southeast Asia, however, many zoonoses are possible in the tropical forest (Eyles et al 62, Warren et al 63), so that hunters may be expected to have had endemic malaria.

Despite the speculation involved in these attempts to derive human malaria parasites from those of other primates, many of the broad conclusions can aid in solving the problems concerning the distributions of abnormal hemoglobin and other human genes related to malaria. After the evidence for the effect of malaria on human genes is reviewed, their distributions will be considered in more detail. Some of the conclusions based on parasite evolution seem to be in accord with expectations from genetic distributions. The absence of abnormal hemoglobins and the recency of malaria in the New World has already been mentioned as one of the most striking of these concordances.

Racial immunity to malaria.—With the expansion of Western European culture in the 16th century, modern ideas on the nature and extent of human genetic variability began to develop. In the 18th century Linnaeus, Blumenbach, and others constructed racial classifications of mankind, and their major outlines have persisted to this day. In addition to genetic traits many other biological and even cultural characteristics were associated with the different races. The great differences in the intensity of infectious diseases among the world's populations, and the obvious differences in susceptibility to various diseases including malaria, were thus easily considered to have a racial basis. The fact that malaria contributed more than any other factor to making West Africa the "White Man's Grave" seemed to be convincing evidence that races differed in their immunity to malaria. More recently Wilson, Garnham & Swellengrebel (16) have suggested that the solid immunity found in hyperendemic regions has a genetic basis, but it is not confined to any one race. However, for malaria and many other diseases the importance of the specific epidemiological circumstances are being increasingly recognized as the major determinant of differences in the susceptibility of the individual to the disease and of its severity.

On the other hand, the evidence for the existence of differences among human populations in their resistance to some species of malaria is quite concrete. Many studies have shown that American Negroes are refractory

to infection with *P. vivax* (Boyd & Stratman-Thomas 69, Young et al 70), and Liberians have a similar resistance (Bray 71). In addition, Negroes in the South had proportionally less vivax malaria than Whites (Rice & Watson 72), and recent experimental transmissions to man of simian parasites similar to *P. vivax* have shown that Negroes are less susceptible to these parasites (Beye et al 73, Contacos et al 60).

Despite these apparent differences among human populations in their ability to resist vivax infections, the differences cannot at present be associated with any specific genetic or biochemical difference. For some time human populations have been known to vary in their levels of γ globulin, with those in the tropics usually having marked elevations. Recent advances have shown that these elevations in West Africans (McFarlane 74, Rowe et al 75), in populations of West African origin (Low-Beer et al 76), and in New Guinea (Wells 77) are due principally to IgG, which also contains most malaria antibodies (Zuckerman 78, McFarlane & Voller 79). Curtain & Baumgarten (80) did not find malaria antibody to be associated with the Gm factors of immunoglobulin. Cohen & Butcher (81) have found that the bivalent portion had to be present to have an antimalarial effect. Targett (82) has found that the increase in IgG is correlated with the malaria antibody titer, and in addition the IgM fraction is higher in those with malaria antibodies. In any case the current rapid advances in the genetics of the immunoglobulins will surely contribute to the solution of many problems of malaria antibody formation and its genetic basis.

Another kind of recent evidence for racial differences in susceptibility to malaria is the varying amounts of adenosine triphosphate (ATP) in the red cells of different populations. Brewer & Powell (83) found a relationship between ATP content of the red cells and the course of falciparum malaria infection, and Eaton & Brewer (84) found the same inverse relationship between ATP and *P. cynomolgi* infection in the rhesus monkey. Brewer (85) provided some evidence for the inheritance of the level of ATP in red cells, and Brewer & Coan (86) showed that hyperoxia leads to a lowering of the red cell ATP, which could alleviate the severity of a malaria infection. There is also some evidence (Brewer 87) that heterozygotes for β thalassemia have a lower level of red cell ATP, which could contribute to their presumed resistance to malaria.

Malaria and the blood groups.—The ABO blood groups were the first genetic polymorphism found to vary significantly among human populations, and in the early part of the century many studies were done on the association of the ABO blood groups with a great variety of diseases. Malaria was one of the diseases tested, and, as for most of the other diseases, the results were equivocal and subsequently forgotten. The largest study was done in Russia (Rubaschkin & Leisermann 88), and a χ^2 test of the results indicates a very significant association between malaria and the ABO blood groups, with AB having a greater frequency in malaria patients. Other

studies (Parr, see 88) were not significant. Still others claimed significance in another direction; but since the blood group frequencies diverge considerably from the Hardy-Weinberg expectations, doubt is cast on these associations.

In the past few years with the recognition of the problems raised by the distributions of the ABO blood group genes, there has been renewed interest in the association of the blood groups with infectious disease. Malaria, like many other infectious diseases, shares common antigens with the ABO blood groups (Oliver-Gonzalez & Torregrosa 89), which has led Athreya & Coriell (90) to postulate that blood group B may have an advantage in malarious regions. However, in what must be the most extensive controlled experiment with malaria, 3500 patients have been therapeutically treated with malaria in Rumania, and the blood groups of the patients have been recorded. Raper's (91) analysis of the data shows no evidence of an association of the ABO blood groups with malaria.

To my knowledge there has been little work on the relationship between the other blood groups and malaria. Butts (92) postulated an association between the Rh- blood group and blackwater fever. Gorman (93) has also postulated that the Rh- gene would be selected against in the tropics by malaria and other infectious diseases that raise antibody production. These diseases would select for good antibody producers that would select against the Rh- gene by increasing the probability of erythroblastosis fetalis. However, there seems to be little evidence for this hypothesis. Winston (94) in Nigeria and Livingstone et al (95) in Liberia found no cases of anti-D in pregnant women, although in the latter study the frequency of Rh- was increased above expectation in pregnant women. More recently, a larger study by Worlledge et al (96) in Nigeria has found a lower frequency of immunization in multiparous females than expected.

Malaria and hemoglobin S.—In the last decade the association between the sickle cell gene (Hemoglobin S) and falciparum malaria has become the classic example of natural selection in man and the textbook illustration of the concept of balanced polymorphism. Despite the uncertainty of much of the evidence, malaria seems to be the only factor that could balance the sickle cell polymorphism throughout the range of its high frequencies. As Bowman (97), one of the more cautious investigators of the malaria hypothesis, has said, "Although the author has presented reservations, he cannot reject the thesis; for one who does so must provide a substitute; this has not been done. The lack of another reasonable explanation for the high frequencies of certain genetic abnormalities of the erythrocyte is, perhaps, the best evidence for the malarial selective effect." If the evidence for the association of malaria with the sickle cell gene is not all one would hope for, the evidence for the other abnormal hemoglobins and red cell defects is practically nonexistent.

When Allison first produced some evidence for the malaria hypothesis,

he attempted to show three different kinds of associations between the sickle cell gene and malaria. First, he showed that children with the sickle cell trait (heterozygotes) had a lower frequency of malaria parasitemia; second, that adult sicklers inoculated with falciparum malaria did not show infections as frequently or as severely; and third, that the frequency of the sickle cell gene in East Africa was correlated with the endemicity of malaria (for recent reviews, see Rucknagel & Neel 98, Allison 99, Motulsky 100, Jonxis 101, Livingstone 102). Immediately after Allison's work, a spate of investigations attempted to duplicate his results. Most were concerned with the different frequencies of parasitemia in sicklers and nonsicklers, and these were almost equally divided between those finding significant differences and those that did not. Allison's inoculation experiment was repeated on nonimmunes in the United States, and no significant differences were found (Beutler et al 103). Others also quickly pointed out that the correlation between sickling and malaria endemicity was not very pronounced in many tropical areas and even in East Africa. If sicklers have a higher fitness than nonsicklers in a malarious area, then it should be possible to detect a significant age trend in the frequency of sickling. The studies cited in Rucknagel & Neel (98) and Allison (104) tend to support the malaria hypothesis, since adults have a higher sickling frequency than children. But earlier studies (Neel 26) were more equivocal, and in the most extensive study to date (Burke et al 105) in which over 33,000 individuals were examined, no significant trend was detected except that the frequency of sickling was somewhat higher in individuals over 45 years old.

Despite the uncertainty of much of the preceding evidence, the most conclusive data to support a selective effect would be differential mortality rates from falciparum malaria for sicklers and nonsicklers, and a significant difference in these rates has been found in every study. Raper (106) in Uganda, Vandepitte & Delaisse (107) and the Lambotte-Legrands (108) in the Congo, and Edington & Watson-Williams (109) in Ghana and Nigeria have all found that mortality from cerebral malaria is practically nonexistent in sicklers. Rey et al (110) in the Senegal found less cerebral malaria in sicklers, and Gilles et al (111) in Nigeria found fewer severe falciparum infections in sicklers. In Ibadan, Nigeria, Brew & Edington (112) found 15.7 percent sicklers in post-mortems of children from 1 to 4 years old, and in Lagos Smith (113) found only 4.8 percent sicklers in child post-mortems. Since the frequency of sickling is about 25 percent in this part of Nigeria, these data seem to be further evidence of a selective advantage. Recent studies on the frequency of parasitemia in sicklers and nonsicklers have continued to be less conclusive but do tend to show lower rates for sicklers (Rey et al 110, Santos David & Trincao 114, Motulsky et al 115).

In addition to the differences in mortality from malaria, it is also possible that the selective advantage of sicklers is due to differential fertility. Since malaria has serious effects on the outcome of pregnancy (51–54), female sicklers may have an increased ability to cope with malaria infections during

pregnancy (Livingstone 116). Bodmer (117) has given the solutions for various mathematical models of differential fertility, and Firschein's (118) data for the Black Carib show an increased fertility of female sicklers sufficient to balance the polymorphism. There seems to be less evidence for differential fertility in Africa (99), but significant differences have been found (Delbrouck 119). Allison (99) has remarked that the absence of differences in Africa in contrast to the New World may indicate that fertility differences are important only with less malaria transmission. Ashcroft et al (120) have found an increased fertility of female sicklers in a Jamaican population with very little malaria, but the difference is not significant. Recent studies in Africa (Gilles et al 54, Jilly 121) on the effect of malaria in pregnancy are inconclusive with regard to sickling, but in Jilly's study there were no sicklers with greater than 100,000 parasites per cu mm which is usually considered to be the level associated with severe effects. Fleming et al (122) found a lower frequency of sicklers with anemia in pregnancy, which is highly significant, and the ten stillbirths in Fletcher's (123) study all occurred in normals in a population with 30 percent sickling.

Some studies (Allard 124) have shown male sicklers to have a higher fertility. Eaton & Mucha (125) have suggested that this may be the result of malaria, since high fever is known to reduce fertility by decreasing spermatogenesis. Miller (126) found that resistant adult African males suffered from 1.5 malaria attacks per year on the average and these were always accompanied by fever. If sicklers have a resistance to malaria, they would presumably also have less fever from the disease, which makes this a plausible hypothesis, but its quantitative effect may be small. Since sicklers apparently survive with a greater frequency to old age, the fertility effects may be the result of this greater survival.

Although the sickle cell gene has been correlated with almost every known disease, recent advances in medical knowledge have led to new associations of sickling with diseases or syndromes related to malaria. Beet (127) first noted an inverse correlation between sickling and the spleen rate, which is the percentage of individuals with a palpable spleen and is a good indication of the endemicity of malaria. Monekosso & Ibiama (128) found a similar association in Nigeria, and further studies (Fleming et al 123, Hamilton et al 129) have shown an almost total absence of the tropical splenomegaly syndrome in sicklers. This syndrome now seems to be primarily due to an abnormal immune response to malaria (Wells 77, Ziegler et al 130) and can be cured by antimalarial drugs (Watson-Williams & Allan 131). Malarial nephrosis also seems to be due to a hyper-immune response to malaria (Allison et al 132, Kibukamusoke & Voller 133), but there seems to be no association with hemoglobins S or C (Gilles et al 111). However, malarial nephrosis is due primarily to *P. malariae;* and, although Garlick (134) found an association between sickling and *P. malariae*, most other surveys have not.

The stress on the immune mechanism by malaria causes two other com-

plications that could affect the selective advantage of sicklers. Shaper et al (135) found high levels of malaria antibodies, particularly of IgM, to be associated with high levels of autoantibodies to heart and other cells. This may increase susceptibility to endomyocardial fibrosis, but to my knowledge no studies have been done on sickling and this disease. The geographical distribution of Burkitt's lymphoma within the malaria belt of Africa has led to considerable speculation about their association (136), and there does seem to be a strong correlation between the two diseases in Uganda (Kafuko et al 137). Olufemi Williams (138) and Pike et al (139) have reported a lower frequency of sicklers with Burkitt's lymphoma, although only the former was statistically significant. Since malaria causes similar lymphomas in mice (Jerusalem 140, Wedderburn 141), the lower frequency in sicklers would seem to be further indirect evidence of their increased ability to resist malaria infections. Furthermore, these recently discovered complications of malaria may be the factor causing the greater survival of sicklers to old age in Africa.

Finally, there have been some laboratory experiments that have attempted to elucidate the physiological mechanism that is the basis of the resistance of sicklers to malaria. At first, this resistance was generally thought to be due to some biochemical cause such as the inability of the parasite to metabolize hemoglobin S. However, Raper (142) found that falciparum malaria seemed to develop as well in red cells with hemoglobin S as in normal cells. He was able to grow the parasites for only one generation; but more recent advances in the cultivation of malaria parasites (Siddiqui et al 143) may permit more conclusive experiments in the future—not only for hemoglobin S but for all the red cell defects that seem to be associated with malaria. Since sickling seems to confer a resistance to falciparum malaria but not to the other human malaria species, it seems more probable that the basis of the sickler's resistance lies in the fact that later stages of the falciparum parasite are removed from circulation and lodge in the capillaries where they mature. The lower oxygen tension in this environment would increase the likelihood of sickling and thus cause an interruption of parasite multiplication (Miller et al 144). The reduced blood flow in the spleen in malaria infection (Sheagren et al 145) would also increased the tendency of the cells to sickle. Recently Luzzatto et al (146) have shown that parasitized red cells have a greater tendency to sickle in vitro. This tendency should also occur in vivo and would presumably increase as the parasite matures. As Luzzatto et al say, this amounts to a "suicidal" infection and would probably result in most parasiticized cells being phagocytized. This evidence seems to show conclusively that it is the sickling process that results in the resistance of hemoglobin S to malaria.

As the evidence for an association between hemoglobin S and falciparum malaria began to accumulate, the implications for the explanation of other genetic variation quickly began to be explored. It was perhaps the primary evidence that has led to increased awareness of the importance of balanced

polymorphism as a mechanism maintaining genetic variability. In particular, the sickling-malaria association seemed to have two broad implications: (*a*) other deleterious genes found in appreciable frequencies in some human populations may be explained by selection against normal homozygotes by some disease, and (*b*) malaria may be responsible for balancing many other genetic traits of the red cell that vary in human populations. This review will explore only the second of these implications, but as examples of the first, recent studies have suggested that such diverse genetic conditions as Tay-Sachs disease (Myrianthopoulos & Aronson 147), cystic fibrosis (Knudson et al 148), and schizophrenia (Huxley et al 149) may be balanced polymorphisms.

Obviously thalassemia and the other abnormal hemoglobins found in high frequencies were quickly assumed to be balanced by malaria, but many other genetic polymorphisms that were being discovered in rapid succession were also considered for a possible relationship to malaria. There was especially much speculation about the haptoglobin polymorphism. Blumberg et al (150) tested the effect of malaria infection on haptoglobin level, and Curtain et al (151) examined the spleen rate and parasitemia in relation to haptoglobin type. They found no associations, but populations in malarious areas did have higher frequencies of anhaptoglobinemia and the Hpl allele, which has been considered to have a selective advantage to malaria since it can bind hemoglobin more efficiently. However, there seems to be no conclusive evidence for an association of malaria with any of the serum polymorphisms (Buettner-Janusch 152).

Malaria and the glucose-6-phosphate dehydrogenase deficiency.—Of the red cell traits that have been investigated following the sickling-malaria developments, the deficiency of the enzyme, glucose-6-phosphate dehydrogenase, at present has the most evidence supporting its association with malaria. Earlier studies on the level of parasitemia in G6PD deficients and normals provided some evidence for an association (Motulsky 153), and some later studies have been claimed to support it (Santos David & Trincao 114, Motulsky et al 115), while others have not (Edington & Watson-Williams 109, Pene et al 154). Similarly, in some studies severe malaria infections were found to be less frequent in G6PD deficients (Gilles et al 111, Kruatrachue et al 155), but no difference was found in another (Rey et al 110). Although Krautrachue et al (155) found very high parasite densities to be significantly less frequent in G6PD deficients, they found no differences in mortality rates from malaria. Inoculation experiments on nonimmunes in the United States have also proved inconclusive both for *P. falciparum* infections (Powell & Brewer 156) and for those due to *P. vivax* (Powell et al 157), but Devakul et al (158) found no very heavy infections in G6PD deficients in a study of induced falciparum malaria. As in the case of hemoglobin S, the experimental data on the parasitization of individual red cells have shown G6PD deficient red cells to be much less frequently

parasitized in both hemizygous males and homozygous and heterozygous females (Kruatrachue et al 159, Luzzatto et al 160). This would seem to be the best evidence for malaria balancing the G6PD polymorphism, but the correlation of the G6PD deficiency frequencies with those of hemoglobin S in Africa, with thalassemia in the Mediterranean area, and with hemoglobin E in Southeast Asia (Motulsky 153) is usually cited as the major evidence. However, the distribution of the G6PD deficiency frequencies in human populations raises many questions since it is not always associated with malaria, and these will be discussed later in this review.

Since the first indications that the G6PD deficiency may be related to malaria, the lower level of reduced glutathione (GSH) in G6PD deficient red cells has been considered to be a possible deterrent to malaria parasite growth and hence the mechanism by which G6PD deficients are resistant to malaria (153). Pollack et al (161) simulated the effects of G6PD deficiency with chemicals in mice and found that in some cases it conferred a resistance to *P. berghei* malaria. However, the chemicals that did prolong survival did not seem to have much effect on the level of GSH. Beaconsfield et al (162) discussed the importance of the pentose phosphate shunt to rapid cell growth and immunological response, and Peters (163) pointed out that it would have the same increased significance for a rapidly growing malaria parasite. Recently Kosower & Kosower (164) have proposed that since oxidized glutathione (GSSG) inhibits protein synthesis, higher levels of GSSG in the red cells of G6PD deficients would inhibit parasite growth. Despite earlier contradictory results, the G6PD deficient red cells do have increased levels of GSSG (Srivastava & Beutler 165). Although the data seem to support the Kosower's hypothesis, the reluctance to accept not only their hypothesis but any association of the G6PD deficiency with malaria is illustrated by the editorial comment (166) on their paper. On the other hand, the fact that there are many other red cell enzymes that would also change GSH/GSSG levels and for which inherited variants are now known (Beutler 167, Bloom & Zarkowsky 168) raises the question as to why these enzyme variants are not increased by malaria selection.

Any association between the G6PD deficiency and malaria is complicated by the fact that this locus is sex-linked. The fitnesses of the hemizygotes and homozygotes for the G6PD deficiency are certainly diminished by favism, allergic drug reactions, neonatal jaundice (Valaes et al 169), and by their susceptibility to viral infections (Kattamis & Tjortjatou 170, Nagaratnam et al 171). Petrakis et al (172) have found that in the United States there is a significant decrease of G6PD deficiency with age in American Negroes, and Wiesenfeld et al (173) found an association of the deficiency with elevated blood pressure and other physiological variables. The question then arises that if these individuals have a resistance to malaria, how much does this resistance increase their fitness and counteract the deleterious effects of the gene? According to the models of fitness values proposed by Siniscalco et al (174) it would increase their fitnesses to a value greater than those of the

normal hemizygotes and homozygotes. This would make the equilibrium gene frequency for the G6PD deficiency greater than .5, and if the female heterozygotes are intermediate in fitness, then the G6PD deficiency should become fixed in populations with malaria. Siniscalco et al (174) consider that this is the most likely possibility, but since there are only a few populations of Jews and Arabs that have frequencies of G6PD deficiency greater than .5 (Livingstone 102) and most human populations average about .2, it does not seem to me to be the most probable. Of course, the fitness values will vary from one population to another, but since it takes only about 100–200 generations to attain equilibrium, surely we would encounter more frequencies close to equilibrium. With an equilibrium gene frequency of about .2-.3 for the G6PD deficiency in a malarious region and a maximum fitness for the heterozygote, there is perhaps a 10 percent decrease in fitness of the hemizygotes and homozygotes for the G6PD deficiency compared to normal hemizygotes and homozygotes. However, this decrease depends on the decrease in fitness of the normal homozygotes and hemizygotes relative to the heterozygote, and if this is very small, the difference in fitness between the two sets of hemizygotes and homozygotes could be as little as 1 percent. In this case, the G6PD deficient alleles would take much longer to increase in frequency, and one would not expect to find any age trends in the frequency of the G6PD deficiency, which in fact have not been found.

Advances in electrophoretic techniques have led to the discovery of many different alleles that can result in the deficiency of G6PD (Motulsky & Yoshida 175), and these alleles vary considerably in their biochemical properties and the amount by which they decrease the activity of G6PD. Throughout tropical Africa, the G6PD deficiency is due to a fast electrophoretic variant that is only moderately deficient, and among Chinese, it is due to a comparable although different fast variant. In the Mediterranean area most of the deficiency is due to a variant with no electrophoretic difference from normal G6PD but with a very severe decrease in enzyme activity. However, there are several variants in this area, and one with moderate deficiency is rather common (Stamatoyannopoulos et al 176). The populations of New Guinea also have a severe and a moderately deficient variant in polymorphic frequencies (Kirkman et al 177). The presence of this great number of G6PD variants raises problems as to the equilibrium frequencies. Variants with all degrees of G6PD deficiency are found in most populations, so that it would seem possible for any degree of deficiency to increase in any population. It seems paradoxical that a moderate deficiency is most frequent in tropical Africa, which has the most severely endemic malaria, and that this variant occurs in a lower average frequency than the severely deficient variant in malarious areas of the Mediterranean. Perhaps part of the answer lies in the relationship of G6PD deficiency to the various species of human malaria. In the Mediteranean area the severely deficient allele also affects the parenchyma cells of the liver, which are the host cells for the pre-erythrocytic and exoerythrocytic phases of malaria (Brunetti et al 178, Garn-

ham 1). *P. falciparum* has only one cycle in the liver, but the three other human malaria species continue to re-infect the liver cells, which leads to malaria relapses after several years. Since *P. vivax* assumes more importance as a selective agent in the Mediteranean area, this could account for some of the variation in G6PD deficient allelic frequencies in human populations. However, other factors seem to be necessary to explain the Chinese-Africa and Mediterranean-New Guinea similarities. Carson & Frischer (179) suggested that plague may have played a selective role on the G6PD deficiency, although many populations with high frequencies have not been subjected to this disease for many generations. More recently, Carson (180) has suggested nutritional factors, but in any case malaria seems to have played a major role in determining the frequencies of the G6PD deficiency.

Malaria and other abnormal hemoglobins.—In addition to hemoglobin S, two other abnormal hemoglobins, C and E, attain high frequencies in a great number of human populations. There are also appreciable frequencies, which would seem to be polymorphic, of hemoglobin D in India, hemoglobin K in North and West Africa, and hemoglobin O in Indonesia; and perhaps others yet to be discovered in more isolated populations. Since homozygosity for any of these abnormal hemoglobins would seem to result in reduced fitness, there seems to be no evidence from the human hemoglobin variants for non-Darwinian evolution. In fact, the most frequent hemoglobin variants seem to have the most selection against them and thus are the most likely examples of balanced polymorphism. However, King & Jukes (180a) have stated that the evolution of hemoglobin differences among animal species is non-Darwinian, or due to the constant random replacement of one neutral variant by another, and Arnheim & Taylor (180b) have suggested that 50 percent of the human hemoglobin alleles are neutral or not associated with any difference in fitness. Nevertheless, within the human species there seems to be no known high frequency of a neutral variant that would be in the process of replacing the normal allele. It would appear instead that some selective factor is balancing the human hemoglobin polymorphisms.

All of the elevated frequencies of abnormal hemoglobins are found in populations that are or have been subjected to endemic malaria, which seems to implicate malaria as the selective factor. Nevertheless, no conclusive evidence has been produced to show the resistance to malaria of the carriers of any abnormal hemoglobin other than hemoglobin S. Thompson (181) reported a lower frequency of parasitemia in hemoglobin C carriers in Accra, Ghana, but an earlier study (Edington & Laing 182) and later studies (109–111) have failed to reveal any differences. Among deaths from cerebral malaria and cases of cerebral malaria, hemoglobin C carriers were found in the same frequency as normals (109, 110). Gilles et al (111) also state that hemoglobin C is equally frequent among children with the nephrotic syndrome due to *P. malariae.* More work has been done on hemoglobin E in Southeast Asia, and the results are also rather inconclusive. Kruatrachue et

al (183) found no differences in parasitemia or very high density infections, and Kruatrachue et al (184) found similar frequencies of cerebral complications in hemoglobin E carriers and normals. On the other hand, they also found the mortality rate from falciparum malaria for hemoglobin E carriers was about one half that of normals, but the numbers are too small for this difference to be significant. In Cambodia So Satta et al (185) have shown that hemoglobin E carriers with malaria have a higher hemoglobin level than normals, which seems to indicate a better ability to handle this infection. They also did not find any hemoglobin E carriers with cerebral malaria but 16 cases in normals, which is significant for a population with 32 percent carriers of hemoglobin E. Goueffon & du Saussay (186) found a slight decrease in the frequency of parasitemia among hemoglobin E carriers, and their four cases of cerebral malaria all had normal hemoglobin.

Despite the equivocal nature of these results, they are suggestive of a relationship of these hemoglobins to malaria, particularly when the small numbers in these studies and the expected magnitude of the association are taken into consideration. In contrast to hemoglobin S, homozygosity for either hemoglobin C or E does not result in a severe anemia and consequently in a greatly reduced fitness. Estimates of the fitness of hemoglobin E homozygotes range from about .6 to .9 that of normals (Flatz 187); and since the consequences of homozygosity for hemoglobin C are comparable, their fitness should be similar. For this range of homozygote fitness values, the heterozygote for either hemoglobin C or E would have a selective advantage of at most 10 percent over normal homozygotes, which is about one third the advantage of the hemoglobin S heterozygote. For other abnormal hemoglobins found in lower frequencies, the selective advantage would be even less, assuming homozygosity results in a moderate depression of fitness. Given the difficulties associated with demonstrating the selective advantage of hemoglobin S heterozygotes, it seems likely that with the increasing eradication of malaria a selective advantage for any other abnormal hemoglobin will never be "proven" for a natural human population.

If a selective advantage is to be demonstrated for other abnormal hemoglobins, it will probably associate the hematological characteristics of these hemoglobin carriers with an inhibition of the growth of the malaria parasites. No other polymorphic abnormal hemoglobin results in the sickling phenomenon, but the red cells of hemoglobin C or E heterozygotes are significantly different from normal red cells despite the fact that their hematological indices fall within the normal range. Hemoglobin C heterozygotes have a decreased red cell survival time (Prindle & McCurdy 188), an excess of target cells, which are flatter with an increased surface area and normal volume, and an increased red cell osmotic resistance (Wintrobe 189). Hemoglobin E heterozygotes have a slightly lower mean cell volume (Chernoff et al 190, Lie-Injo 191), which leads to an increased osmotic resistance; but to my knowledge no survival studies have been done on them. Although from a standpoint of pathology these differences from normal are minimal, they

could very easily result in a fitness advantage of 5 percent by altering the growth rate of the malaria parasite by a similar amount.

Since individuals who are simultaneously heterozygous for two abnormal β hemoglobin alleles have a markedly reduced fitness, these alleles are in competition with one another and hence tend to eliminate each other from any population (Kirkman 192). Homozygosity for either hemoglobin C or E is less deleterious than for hemoglobin S or β thalassemia, so it seems most likely that the former alleles would replace the latter. Hemoglobin E does seem to be replacing β thalassemia in Southeast Asia (Flatz 187), but with the most plausible set of fitness values for the six genotypes hemoglobin S seems to be replacing hemoglobin C in West Africa (Livingstone 193). The almost exclusive distributions of hemoglobins S and E do not give any clues as to their interrelationships or possible replacement of one by the other. However, hemoglobin E is found among the Eti-Turks of Southeast Turkey and sporadically elsewhere in the Middle East and tropical Africa; so that it seems to have had the "opportunity" to replace hemoglobin S. On the other hand, the distribution of hemoglobin S is much more contiguous and apparently is due to the diffusion of a very few or perhaps only a single mutant. It seems to have replaced most other abnormal β alleles in East Africa and Saudi Arabia, which may indicate its origin in this area. The high frequencies of other abnormal hemoglobins occur in populations that are usually characterized by an absence of the major abnormal hemoglobins and by an absence of gene flow from populations with these variants. They are different responses to malaria selection but lose out in competition with the major variants.

Malaria in relation to thalassemia and other red cell defects.—Prior to the discovery of the sickling-malaria association, Haldane (4) suggested that the high frequencies of thalassemia could be due to selection by malaria. Earlier work (Vezzoso 194) indicated that the distribution of thalassemia in Italy was correlated with that of malaria, but despite the strong possibility of an association between malaria and thalassemia, no studies of parasitemia were done until recent work in Thailand (Kruatrachue et al 183,184), in which the numbers are quite small and the results inconclusive.

The overall correlation of thalassemia with malaria has continued to be the only evidence in favor of malaria balancing this polymorphism. In Sardinia and other regions of Italy there is a strong correlation, but in Greece (Stamatoyannopoulos & Fessas 195), Cyprus (Plato et al 196), Malta, the Sudan, and New Guinea (102) this association is less striking or even nonexistent. Thalassemia has not been correlated with any other disease, but because of its inconsistent relationship to malaria and its altered iron metabolism, a resistance to iron deficiency anemia and particularly that caused by hookworms has been suggested (Sijpesteijn 197).

Thalassemia is now known to be due to alterations of both α and β chain synthesis. For the most part the high frequencies of thalassemia are due to β

chain mutants, and thus all of the high frequency hemoglobin polymorphisms are β chain variants. But α thalassemia is polymorphic in many diverse populations. Within the human species there now seems to be evidence for a polymorphism in the number of α chain loci, since two loci are present in European populations (Kattamis & Lehmann 198) and most likely one locus in some Melanesian populations (Abramson et al 199). Since hemoglobin H disease seems to be due to three α thalassemia alleles and one normal allele (Lehmann 200), the absence of this disease in most of tropical Africa may indicate that one α locus is most frequent in these populations. There seems to be no obvious association between this new discovery of hemoglobin synthesis and malaria, but if the α thalassemia polymorphism is balanced by malaria, this variation should be of significance.

No work has been done on the individual red cells of thalassemics in relation to malaria infection. But since their red cells differ from normal ones in many of the same measurements as those of hemoglobin C and E heterozygotes (174), the same possible reductions in the growth of the malaria parasites would be expected. In fact thalassemic red cells are even more abnormal and in particular are much smaller. Since malaria parasites fill the entire cell, the growth of the later stages may be inhibited in the smaller cells. *P. vivax* enlarges the red cell, which would seem to increase even more the resistance of thalassemics to this parasite. Perhaps for the same physiological reasons polycythemic mice have a resistance to *P. berghei* (Ladda & Lalli 201), although erythropoesis is reduced in polycythemic mice in contrast to its increase in thalassemia due to their decreased red cell survival time. The alterations of GSH, ATP, and G6PD levels in thalassemia seem comparable to other traits that have also been postulated to have a resistance to malaria (Chatterjea et al 202).

Thalassemia is more widespread than the other specific abnormal hemoglobins, and this would be expected since it encompasses more than one specific change from normal hemoglobin synthesis. When malaria becomes an important selective factor, it is more likely first to encounter one of the thalassemia mutants and begin to increase its frequency. However, the low fitness of the thalassemia homozygotes results in the replacement of these alleles by hemoglobin E in Thailand (187) and by hemoglobin S in Greece (Barnicot et al 203), Saudi Arabia, and East Africa (102).

The restricted distributions of the abnormal hemoglobins point out the recency of the importance of malaria as a selective factor, and they also show that when it becomes a selective factor it will act on any genetic variability that is present. Any abnormality that in some way decreases the ability of the malaria parasite to grow will be selected for. Thus increased frequencies of other red cell abnormalities in small tropical isolates may be more common than elsewhere and be explained by malaria. For example, elliptocytosis is found in high frequencies in Malaya and in a small area of Sulawesi (Lie Injo 204). It also seems to be slightly more frequent in Negroes in United States and in Southern Italy (Torlontano et al 205). The clinical consequences of

this trait vary, but homozygosity does lead to a hemolytic anemia (Nielsen & Strunk 206, Geerdink et al 207). Chastel & Thomas (208) have suggested that this trait may protect against malaria, and thus these isolated frequencies may be the result of such selection. On the other hand, the absence of high frequencies of other red cell defects in large populations may be due to their deleterious interaction with the widespread abnormal hemoglobins or thalassemia.

Problems of the distributions of the red cell defects.—Although the major evidence for malaria balancing the hemoglobin and G6PD polymorphisms is the correlation of the distributions of the high frequencies of the abnormal alleles with endemic malaria, there are nevertheless many populations whose frequencies do not appear to be explained by the amount of malaria to which the population has been exposed. In addition, there are many populations for which the frequencies of the hemoglobinopathies and the G6PD deficiency are not correlated. However, as Allison (99) and Motulsky (100) have pointed out, these problematic frequencies do not by themselves "disprove" the malaria hypothesis. This hypothesis states that malaria selection maintains the high frequencies of the abnormal alleles, so that to disprove it, a high frequency would have to be found in a population with no history of malaria. Some have been cited, but it is first of all necessary to show that these contradictions cannot be explained by other evolutionary forces. Obviously, these frequencies are not close to the deterministic equilibrium if malaria is the major selective factor, hence they necessitate first an examination of the world's frequencies with regard to the presence of genetic equilibrium. Haldane (209) gave several reasons for the absence of genetic equilibrium in modern human populations, and these apply especially to the hemoglobinopathies and the G6PD deficiency.

All American Indian populations with endemic malaria have an almost complete absence of the abnormal alleles at these loci and thus are far from equilibrium. Since malaria is post-Columbian in America (65), these populations have obviously not had enough time to attain equilibrium. Some Indian populations in the Guianas have intermediate frequencies of thalassemia and the G6PD deficiency, which would be the most likely to be initially present and thus first to be acted on by selection. Malaria also seems to have spread in recent years in the Southwest Pacific (Lambert 210), and thus the absence of high frequencies of abnormal alleles in many populations in this area with endemic malaria seems to be due to the same lack of time to attain equilibrium. It should also be noted that thalassemia and the G6PD deficiency are the abnormal alleles present here.

From the standpoint of the ultimate equilibrium frequencies with endemic malaria, most populations in the Old World would also appear to be far from equilibrium. The distributions of hemoglobin S in the Mediterranean, Middle East, India, and Africa, and of hemoglobin E in Southeast Asia, would seem to be due to recent diffusions and hence indicate that

malaria has become a selective factor only since these populations have been in their present habitats. The border of the two hemoglobin distributions in India near Calcutta is also the approximate border of the expansions into the Indian sub-continent from the west by Middle Eastern peoples and from the east by Oriental groups. This border thus seems to be determined to a greater extent by "ethnic" factors or migration than by selection.

There are many problematic frequencies in the Mediterranean area, the Middle East, and Africa, and these seem to be more difficult to explain. In the Po River Valley of Italy, which was highly malarious, there are high frequencies of thalassemia but very low frequencies of the G6PD deficiency (Gandini et al 211). This may indicate that it takes more time for the G6PD deficiency to attain equilibrium or perhaps that it is not as effective against falciparum malaria. On the other hand, in the Middle East the G6PD deficiency seems to be more correlated with malaria (Beaconsfield et al 212). But in the oasis villages of Saudi Arabia there seems to be no correlation between sickling and the G6PD deficiency, and neither is correlated with malaria endemicity (Gelpi 213). In the Nile Delta of Egypt there are high frequencies of the G6PD deficiency (102) and endemic malaria but apparently very low frequencies of abnormal hemoglobin alleles. In the Sudan higher sickling frequencies are found in the central region than in the south, but there seems to be more malaria in the south (40). There are also tribes in the adjacent areas of northern Uganda with endemic malaria but very low sickling frequencies. On the island of Zanzibar there is great variation in the frequency of sickling, which does not seem to be correlated with differences in malaria endemicity (Chopra & Mbaye 214). Finally, in South Africa and Mozambique the Bantu tribes have high frequencies of the G6PD deficiency (Reys et al 215) but low frequencies of sickling (102), while on Madagascar, which has been settled for only the last 2000 years, there seems to be a close correlation of these abnormal alleles.

Despite all these contradications to the malaria hypothesis, it is possible to develop explanations for them. Differences in time to attain equilibrium is one possibility. Differences in the endemicity of malaria and in the interactions between the different malaria parasites and abnormal alleles are others. Other factors in addition to malaria will have to be examined, and, as was previously suggested, the demographic or ecological circumstances of the population through their influence on the total birth and death rates can be a major determinant of genetic change. In any case, there is considerable evidence both circumstantial and factual to indicate that malaria has played a major role in determining the frequencies of these abnormal red cell alleles in human populations. By examining in more detail the apparent contradictions between the general malaria hypothesis and the gene frequencies in human populations, further advances in our knowledge of the importance and variability of selective forces and of the effects of other genetic and demographic factors on evolution will surely be forthcoming.

LITERATURE CITED

1. Garnham, P. C. C. 1966. *Malaria Parasites and Other Haemosporidia.* Oxford: Blackwell. 1114 pp.
2. Russell, P. F. 1952. *Malaria; Basic Principles Briefly Stated.* Oxford: Blackwell. 210 pp.
3. Bruce-Chwatt, L. J. 1970. Imported malaria—a growing world problem. *Trans. Roy. Soc. Trop. Med. Hyg.* 64:201–9
4. Haldane, J. B. S. 1949. Disease and evolution. *La Ricerca Sci.* 19:Suppl. 2–11
5. Haldane, J. B. S. 1953. Animal populations and their regulation. *New Biol.* 15:9–25
6. Haldane, J. B. S. 1956. The relation between density regulation and natural selection. *Proc. Roy. Soc. Ser. B* 145:306–8
7. Jones, W. H. S. 1967. *Diseases in Antiquity,* ed. D. Brothwell, A. T. Sandison, 170–76. Springfield: Thomas. 766 pp.
8. Hackett, L. W. 1937. *Malaria in Europe; an Ecological Study.* London: Oxford. 336 pp.
9. Shortt, H. E. 1951. The impact of Protozoa on world economic problems. *Brit. Med. J.* 2:1103–10
10. Hoeppli, R. 1969. *Parasitic Diseases in Africa and the Western Hemisphere, Acta Trop.* Suppl. 10. 240 pp.
11. Boyd, M. F. 1949. *Malariology,* ed. M. F. Boyd, 3–28. Philadelphia: Saunders. 2 vols. 1643 pp.
12. Russell, P. F. 1955. *Man's Mastery of Malaria.* London: Oxford. 308 pp.
13. Gwei-Djen, L., Needham, J. 1967. *Diseases in Antiquity,* ed. D. Brothwell, A. T. Sandison, 222–37. Springfield: Thomas. 766 pp.
14. Hoeppli, R. 1959. *Parasites and Parasitic Infections in Early Medicine and Science.* Singapore: Univ. Malaya. 526 pp.
15. Macdonald, G. 1957. *The Epidemiology and Control of Malaria.* London: Oxford. 201 pp.
16. Wilson, D. B., Garnham, P. C. C., Swellengrebel, N. H. 1950. A review of hyperendemic malaria. *Trop. Dis. Bull.* 47:677–98
17. Covell, G., Russell, P. F., Swellengrebel, N. H. 1953. *Malaria Terminology. World Health Organ. Monogr. Ser. No. 13.* 82 pp.
18. Garnham, P. C. C. 1949. Malarial immunity in Africans: effects in infancy and early childhood. *Ann. Trop. Med. Parasitol.* 43:47–61
19. Macdonald, G. 1951. Community aspects of immunity to malaria. *Brit. Med. Bull.* 8:33–6
20. Colbourne, M. J., Edington, G. M. 1954. Mortality from malaria in Accra. *J. Trop. Med. Hyg.* 57:203–10
21. Bruce-Chwatt, L. J. 1952. Malaria in African infants and children in Southern Nigeria. *Ann. Trop. Med. Parasitol.* 46:173–200
22. Duren, A. N. 1951. Essai d'étude sur l'importance du paludisme dans la mortalité au Congo Belge. *Ann. Soc. Belge Méd. Trop.* 31:129–47
23. Lehmann, H., Raper, A. B. 1956. Maintenance of high sickling rate in an African community. *Brit. Med. J.* 2:333–36
24. Wilson, W. A. 1956. Sickling in an African community. *Brit. Med. J.* 2:481
25. Brass, W. 1956. Sickling in an African community. *Brit. Med. J.* 2: 1117–18
26. Neel, J. V. 1956. The genetics of human haemoglobin differences: problems and perspectives. *Ann. Hum. Genet.* 21:1–30
27. Raper, A. B. 1960. Sickling and malaria. *Trans. Roy. Soc. Trop. Med. Hyg.* 54:503–4
28. Caldwell, J. C., Okonjo, C., eds. 1968. *The Population of Tropical Africa.* New York: Columbia Univ. Press. 457 pp.
29. Brass, W., et al. 1968. *The Demography of Tropical Africa.* Princeton: Princeton Univ. Press. 539 pp.
30. Janssens, P. G., Vincke, I. H., Bafort, J. 1966. Le paludisme d'Afrique centrale; son influence sur la morbidité et la mortalité des enfants. *Bull. Soc. Pathol. Exot.* 59:665
31. McGregor, I. A., Gilles, H. M., Walters, J. H., Davies, A. H., Pearson, F. A. 1956. Effects of heavy and repeated malarial infections on Gambian infants and children. *Brit. Med. J.* 2:686–92
32. Livingstone, F. B. 1958. Anthropological implications of sickle cell gene distribution in West Africa. *Am. Anthropol.* 60:533–62
33. Livingstone, F. B. 1962. Population genetics and population ecology, *Am. Anthropol.* 64:44–53

34. Coale, A. J. 1964. *Population: The Vital Revolution*, ed. R. Freedman, 47–58. Garden City: Doubleday. 274 pp.
35. Faust, E. C. 1941. *Symposium on Human Malaria, Publ. Am. Assoc. Advan. Sci.*, No. 15, ed. F. R. Moulton, 8–18. Washington: Am. Assoc. Advan. Sci. 398 pp.
36. Giglioli, G. 1930. *Malarial Nephritis.* London: Churchill. 160 pp.
37. Giglioli, G. 1962. Malaria and renal disease, with special reference to British Guiana. I.-Introduction. *Ann. Trop. Med. Parasitol.* 56: 101–9
38. Newman, P. 1965. *Malaria Eradication and Population Growth with Special Reference to Ceylon and British Guiana. Bur. Public Health Econ., Res. Ser. NO. 10.* Ann Arbor: Sch. Public Health, Univ. Michigan
39. Downs, W. G., Gillette, H. P. S., Shannon, R. C. 1943. *A Malaria Survey of Trinidad and Tobago British West Indies, J. Nat. Malaria Soc.* 2:Suppl. 44 pp.
40. Wernsdorfer, G., Wernsdorfer, W. 1967. Malaria im mittleren Nilbecken und dessen Randgebieten. *Z. Tropenmed. Parasitol.* 18:17–44
41. Farinaud, M. E., Choumara, R., Royer, J. 1950. *Infestation palustre et demographiê dans les populations Montagnards du Sud-Indochinois (P.M.S.I.), Bull. Écon. Indochine*, No. 22, Suppl. 48 pp.
42. Farinaud, M. E., Choumara, R. 1954. La prophylaxie du paludisme dans les pays Montagnards du Sud Viet-Nam. *Bull. WHO* 11:793–837
43. Pringle, G. 1969. *Proc. Joint Meet., London, 15 May, 1969. Malaria, Trans. Roy. Soc. Trop. Med. Hyg.* 63:No. 4 Suppl. 56 pp.
44. Coale, A. J., Hoover, E. M. 1958. *Population Growth and Economic Development in Low-Income Countries.* Princeton: Princeton Univ. Press. 389 pp.
45. Frederiksen, H. 1970. Malaria eradication and the fall of mortality. A note. *Pop. Stud.* 24:111–13
46. Meegama, S. A. 1969. The decline of maternal and infant mortality and its relation to malaria eradication. *Pop. Stud.* 23:289–302
47. Bruce-Chwatt, L. J., Meade, T. W. 1968. Malaria and the population explosion. *Lancet* 2:568
48. Pampana, E. J. 1955. Effect of malaria control on birth and death rates. *Proc. 1st World Pop. Conf. Rome* 1:497–509
49. Gill, C. A. 1940. The influence of malaria on natality with special reference to Ceylon. *J. Malaria Inst. India* 3:201–52
50. Khan, M. K. H., Zia-ud-Din, M. 1955. Effect of malaria on the birth rate in the Punjab Province (Pakistan). *Proc. 1st World Pop. Conf. Rome* 1:917–19
51. Blacklock, B., Gordon, R. M. 1925. Malaria parasites in the placental blood. *Ann. Trop. Med. Parasitol.* 19:37–46
52. Bruce-Chwatt, L. J. 1959. Birth weight and prematurity, Tanganyika. *Trans. Roy. Soc. Trop. Med. Hyg.* 53:363–64
53. Jelliffe, E. F. P. 1968. Low birth-weight and malarial infection of the placenta. *Bull. WHO* 38:69–78
54. Gilles, H. M., et al. 1969. Malaria, anaemia and pregnancy. *Ann. Trop. Med. Parasitol.* 63:245–63
55. Bray, R. S. 1958. Studies on malaria in Chimpanzees. VI. *Laverania falciparum. Am. J. Trop. Med. Hyg.* 7:20–24
56. van der Kuyp, E. 1950. Malarial nephritis in Surinam 1930–40. *Doc. Neer. Indones. Morbis Trop.* 2:363–73
57. Gilles, H. M. J. 1968. *Tropical Medicine Conference, 1967*, ed. J. H. Walters, 29–36. London: Pitman. 163 pp.
58. Kibukamusoke, J. W. 1969. The riddle of malarial nephrosis. *East Afr. Med. J.* 46:13–17
59. Bruce-Chwatt, L. J. 1968. Malaria zoonosis in relation to malaria eradication. *Trop. Geogr. Med.* 20: 50–87
60. Contacos, P. G., et al. 1970. Transmission of *Plasmodium schwetzi* from the chimpanzee to man by mosquito bite. *Am. J. Trop. Med. Hyg.* 19:190–95
61. Deane, L. M., Deane, M. P., Neto, J. F. 1966. Studies on transmission of simian malaria and on a natural infection of man with *Plasmodium simium* in Brazil. *Bull. WHO* 35: 805–8
62. Eyles, D. E., Wharton, R. H., Cheong, W. H., Warren, McW. 1964. Studies on malaria and *Anopheles balabacensis* in Cambodia. *Bull. WHO* 30:7–21
63. Warren, McW., Cheong, W. H., Fredericks, H. K., Coatney, G. R.

1970. Cycles of jungle malaria in West Malaysia. *Am. J. Trop. Med. Hyg.* 19:383–93

64. Contacos, P. G., Lunn, J. S., Coatney, G. R., Kilpatrick, J. W., Jones, J. E. 1963. Quartan type malaria parasite of New World monkeys transmissible to man. *Science* 142: 676–78
65. Dunn, F. L. 1965. On the antiquity of malaria in the Western Hemisphere. *Hum. Biol.* 37:385–93
66. Bruce-Chwatt, L. J. 1965. Paleogenesis and paleo-epidemiology of primate malaria. *Bull. WHO* 32: 363–87
67. Arends, T. 1964. Search for abnormal hemoglobins in South American Indians living in malarious zones. *Proc. 7th Int. Congr. Trop. Med. Malaria* 4:179–82
68. Livingstone, F. B. 1967. *Reconstructing African Culture History*, ed. C. Gabel, N. R. Bennett, 141–66. Boston: Boston Univ. Press. 246 pp.
69. Boyd, M. F., Stratman-Thomas, W. K. 1933. Studies on benign tertian malaria. 4. On the refractoriness of Negroes to inoculation with *Plasmodium vivax*. *Am. J. Hyg.* 18:485–89
70. Young, M. D., Eyles, D. E., Burgess, R. W., Jeffery, G. M. 1955. Experimental testing of the immunity of Negroes to *Plasmodium vivax*. *J. Parasitol.* 41:315–18
71. Bray, R. S. 1958. The susceptibility of Liberians to the Madagascar strain of *Plasmodium vivax*. *J. Parasitol.* 44:371–73
72. Rice, M. E., Watson, R. B. 1943. The relationship between malaria morbidity and parasitemia. *Am. J. Hyg.* 37:164–72
73. Beye, H. K., Getz, M. E., Coatney, G. R., Elder, H. A., Eyles, D. E. 1961. Simian malaria in man. *Am. J. Trop. Med. Hyg.* 10:311–16
74. McFarlane, H. 1968. Comparative quantitation of immunoglobulins in Nigerians. *West Afr. Med. J.* 17: 149–52
75. Rowe, D. S., McGregor, I. A., Smith, S. J., Hall, P., Williams, K. 1968. Plasma immunoglobin concentrations in a West African (Gambian) community and in a group of healthy British adults. *Clin. Exp. Immunol.* 3:63–79
76. Low-Beer, T. S., McCarthy, C. F., Austad, W. I., Brzechwa-Ajdukiewicz, A., Read, A. E. 1968. Serum vitamin B_{12} levels and vitamin B_{12} binding capacity in pregnant and non-pregnant Europeans and West Indians. *Brit. Med. J.* 4:160–61
77. Wells, J. V. 1968. Serum immunoglobulin levels in tropical splenomegaly syndrome in New Guinea. *Clin. Exp. Immunol.* 3:943–52
78. Zuckerman, A. 1969. Current status of the immunology of malaria and of the antigenic analysis of *Plasmodia*. *Bull. WHO* 40:55–66
79. McFarlane, H., Voller, A. 1966. Studies on immunoglobulins of Nigerians. II. Immunoglobins and malarial infection in Nigerians. *J. Trop. Med. Hyg.* 69:104–7
80. Curtain, C. C., Baumgarten, A. 1965. The distribution of genetic factors in malaria antibodies as determined by a fluorescent antibody test. *Aust. J. Exp. Biol. Med. Sci.* 43: 351–58
81. Cohen, S., Butcher, G. A. 1970. Properties of protective malarial antibody. *Nature* 225:732–34
82. Targett, G. A. T. 1970. Antibody response to *Plasmodium falciparum* malaria. *Clin. Exp. Immunol.* 7: 501–17
83. Brewer, G. J., Powell, R. D. 1965. A study of the relationship between the content of adenosine triphosphate in human red cells and the course of falciparum malaria: a new system that may confer protection against malaria. *Proc. Nat. Acad. Sci. USA* 54:741–45
84. Eaton, J. W., Brewer, G. J. 1969. Red cell ATP and malaria infection. *Nature* 222:389–90
85. Brewer, G. J. 1967. Genetic and population studies of quantitative levels of adenosine triphosphate in human erythrocytes. *Biochem. Genet.* 1:25–34
86. Brewer, G. J., Coan, C. C. 1969. Interaction of red cell ATP levels and malaria, and the treatment of malaria with hyperoxia. *Mil. Med.* 134:1056–67
87. Brewer, G. J. 1969. *Biochemical Methods in Red Cell Genetics, ed.* J. J. Yunis, 201–30. New York: Academic. 530 pp.
88. Rubaschkin, W. J., Leisermann, L. I. 1929. Blutgruppen und Krankheiten. *Ukrainisches Zentrabl. Blutgruppenforsch.* 3:303–11, Data taken from Parr, L. W. 1930. On isohemagglutination, the hemolytic

index and heterohemagglutination. *J. Infec. Dis.* 46:173–85

89. Oliver-Gonzalez, J., Torregrosa, M. V. 1944. A substance in animal parasites related to the human isoagglutinogens. *J. Infect. Dis.* 74:173–77
90. Athreya, B. H., Coriell, L. L. 1967. Relation of blood groups to infection. I. A survey and review of data suggesting possible relationship between malaria and blood groups. *Am. J. Epidemiol.* 86:292–304
91. Raper, A. B. 1968. ABO blood group and malaria. *Trans. Roy. Soc. Trop. Med. Hyg.* 62:158–59
92. Butts, D. C. A. 1945. The Rh factor and blackwater fever. *Am. J. Trop. Med.* 25:417–20
93. Gorman, J. G. 1964. Selection against the Rh negative gene by malaria. *Nature* 202:676–77
94. Winston, R. M. 1954. The Rh factor in Lagos Nigeria. *West Afr. Med. J.* 3:17–21
95. Livingstone, F. B., Gershowitz, H., Bacon, E., Keller, F. J., Robinson, A. R. 1960. The blood groups, abnormal hemoglobins, and hemoglobin values of pregnant women in Liberia. *Am. J. Phys. Anthropol.* 18:1–4
96. Worlledge, S., Luzzatto, L., Ogiemudia, S. E., Luzzatto, P., Edington, G. M. 1968. Rhesus immunization in Nigeria. *Vox Sang.* 14:202–10
97. Bowman, J. 1964. Comment on hereditary red cell traits and malaria. *Am. J. Trop. Med. Hyg.* 13:159–61
98. Rucknagel, D. L., Neel, J. V. 1961. The hemoglobinopathies. *Progr. Med. Genet.* 1:158–260
99. Allison, A. C. 1964. Polymorphism and natural selection in human populations. *Cold Spring Harbor Symp. Quant. Biol.* 29:137–49
100. Motulsky, A. G. 1964. Hereditary red cell traits and malaria. *Am. J. Trop. Med. Hyg.* 13:147–58
101. Jonxis, J. H. P., ed. 1965. *Abnormal Haemoglobins in Africa.* Philadelphia: F. A. Davis. 477 pp.
102. Livingstone, F. B. 1967. *Abnormal Hemoglobins in Human Populations.* Chicago: Aldine. 470 pp.
103. Beutler, E., Dern, R. J., Flanagan, C. L. 1955. Effect of sickle-cell trait on resistance to malaria. *Brit. Med. J.* 1:1189–91
104. Allison, A. C. See Ref. 101, 365–91
105. Burke, J., deBock, G., deWulf, O. 1958. La drépanocytémie simple et l'anémie drépanocytaire au Kwango (Congo belge). *Mémoires in-8°, Acad. Roy. Sci. Coloniales, Bruxelles, Nouvelle Série,* Tome VII, fasc. 3. 126 pp.
106. Raper, A. B. 1956. Sickling in relation to morbidity from malaria and other diseases. *Brit. Med. J.* 1:965–66
107. Vandepitte, J., Delaisse, J. 1957. Sicklémie et paludisme. *Ann. Soc. Belge Méd. Trop.* 37:703–35
108. Lambotte-Legrand, J., Lambotte-Legrand, C. 1958. Notes complémentaires sur la drépanocytose. I. Sicklémie et malaria. *Ann. Soc. Belge. Méd. Trop.* 38:45–53
109. Edington, G. M., Watson-Williams, E. J. See Ref. 101, 393–401
110. Rey, M., Oudart, J. L., Diop Mar, I., Nouhouayi, A., Camerlynck, P. 1967. Erythrocytopathies héréditaires et paludisme. *Bull. Soc. Méd. Afr. Noire Langue Fr.* 14:253–55
111. Gilles, H. M., Fletcher, K. A., Hendrickse, R. G., Lindner, R., Reddy, S., Allan, N. 1967. Glucose-6-phosphate dehydrogenase deficiency, sickling, and malaria in African children in South Western Nigeria. *Lancet* 1:138–40
112. Brew, D. StJ., Edington, G. M. See Ref. 101, 213–31
113. Smith, E. C. 1943. Child mortality in Lagos, Nigeria. *Trans. Roy. Soc. Trop. Med. Hyg.* 36:287–303
114. Santos David, J. H., Trincao, C. 1963. Drepanocitemia, deficiência da desidrogenase da glucose-6-fosfato (G-6-PD) eritrocitária e paludismo no posto do Cuango (Lunda-Angola). *An. Inst. Med. Trop. Lisbon* 20:5–15
115. Motulsky, A. G., Vandepitte, J., Fraser, G. R. 1966. Population genetic studies in the Congo. I. Glucose-6-phosphate dehydrogenase deficiency, hemoglobin S, and malaria. *Am. J. Hum. Genet.* 18:514–37
116. Livingstone, F. B. 1957. Sickling and malaria. *Brit. Med. J.* 1:762–63
117. Bodmer, W. F. 1965. Differential fertility in population genetics models. *Genetics* 51:411–24
118. Firschein, I. L. 1961. Population dynamics of the sickle-cell trait in the Black Caribs of British Honduras, Central America. *Am. J. Hum. Genet.* 13:233–54
119. Delbrouck, J. 1958. Contribution à la génétique de la sicklémie. Maintein de la fréquence élevée de

sicklémie a u Congo belge. *Ann. Soc. Belge Méd. Trop.* 38:103–33

120. Ashcroft, M. T., Miall, W. E., Milner, P. F. 1969. A comparison between the characteristics of Jamaican adults with normal hemoglobin and those with sickle cell trait. *Am. J. Epidemiol.* 90:236–43
121. Jilly, P. 1969. Anaemia in parturient women with special reference to malaria infection of the placenta. *Ann. Trop. Med. Parasitol.* 63:109–16
122. Fleming, A. F., Allan, N. C., Stenhouse, N. S. 1968. Splenomegaly and sickle-cell trait. *Lancet* 2:574–75
123. Fletcher, T. E. 1966. The continuing study of the effect of malaria on selection for the sickle-cell gene in the Wa-Taveta tribe. *East Afr. Inst. Malaria Vector-borne Dis. Ann. Rep. 1965*, 32–35
124. Allard, R. 1955. A propos de la conservation génétique du sickle cell trait. *Ann. Soc. Belge Méd. Trop.* 35:649–60
125. Eaton, J. W., Mucha, J. I. In press
126. Miller, M. J. 1958. Observation on the natural history of malaria in the semi-resistant West African. *Trans. Roy. Soc. Trop. Med. Hyg.* 52:152–68
127. Beet, E. A. 1947. Sickle cell disease in Northern Rhodesia. *East Afr. Med. J.* 24:212–22
128. Monekosso, G. L., Ibiama, A. A. 1966. Splenomegaly and sickle-cell trait in a malaria-endemic village. *Lancet* 1:1347–48
129. Hamilton, P. J. S., Morrow, R. H., Ziegler, J. L., Pike, M. C., Wood, J. B., Banyikidde, S. K., Hutt, M. S. R. 1969. Absence of sickle trait in patients with tropical splenomegaly syndrome. *Lancet* 2: 109
130. Ziegler, J. L., Cohen, M. H., Hutt, M. S. R. 1969. Immunological studies in tropical splenomegaly syndrome in Uganda. *Brit. Med. J.* 4:15–17
131. Watson-Williams, E. J., Allan, N. C. 1968. Idiopathic tropical splenomegaly syndrome in Ibadan. *Brit. Med. J.* 4:793–96
132. Allison, A. C., Houba, V., Hendrickse, R. G., de Petris, S., Edington, G. M., Adeniyi, A. 1969. Immune complexes in the nephrotic syndrome of African children. *Lancet* 1:1232–38
133. Kibukamusoke, J. W., Voller, A. 1970. Serological studies on nephrotic syndrome of quartan malaria in Uganda. *Brit. Med. J.* 1:406–7
134. Garlick, J. P. 1960. Sickling and malaria in South-west Nigeria. *Trans. Roy. Soc. Trop. Med. Hyg.* 54:146–54
135. Shaper, A. G., Kaplan, M. H., Mody, N. J., McIntyre, P. A. 1968. Malarial antibodies and autoantibodies to heart and other tissues in the immigrant and indigenous peoples of Uganda. *Lancet* 1:1342–47
136. Editorial. 1970. Malaria and lymphoma. *Lancet* 2:1121–22
137. Kafuko, G. W., Baingana, N., Knight, E. M., Tibemanya, J. 1969. Association of Burkitt's tumour and holoendemic malaria in West Nile District, Uganda: malaria as a possible aetiologic factor. *East Afr. Med. J.* 46:414–36
138. Williams, A. O. 1966. Haemoglobin genotypes, ABO blood groups and Burkitt's tumour. *J. Med. Genet.* 3:177–79
139. Pike, M. C., Morrow, R. H., Kisuule, A., Mafigiri, J. 1970. Burkitt's lymphoma and sickle cell trait. *Brit. J. Prev. Soc. Med.* 24:39–41
140. Jerusalem, C. 1968. Relationship between malaria infection (*Plasmodium berghei*) and malignant lymphoma in mice. *Z. Tropenmed. Parasitol.* 19:94–108
141. Wedderburn, N. 1970. Effect of concurrent malarial infection on development of virus-induced lymphoma in BALB/c mice. *Lancet* 2:1114–16
142. Raper, A. B. 1959. Further observations on sickling and malaria. *Trans. Roy. Soc. Trop. Med. Hyg.* 53:110–17
143. Siddiqui, W. A., Schnell, J. V., Geiman, Q. M. 1970. In vitro cultivation of *Plasmodium falciparum*. *Am. J. Trop. Med. Hyg.* 19:586–91
144. Miller, M. J., Neel, J. V., Livingstone, F. B. 1956. Distribution of parasites in the red cells of sickle-cell trait carriers infected with *Plasmodium falciparum*. *Trans. Roy. Soc. Trop. Med. Hyg.* 50:294–96
145. Sheagren, J. N., Tobie, J. E., Fox, L. M., Wolff, S. M. 1970. Reticuloendothelial system phagocytic function in naturally acquired human malaria. *J. Lab. Clin. Med.* 75:481–87
146. Luzzatto, L., Nwachuku-Jarrett, E. S.,

Reddy, S. 1970. Increased sickling of parasitised erythrocytes as mechanism of resistance against malaria in the sickle-cell trait. *Lancet* 1: 319–22

147. Myrianthopoulos, N. C., Aronson, S. M. 1966. Population dynamics of Tay-Sachs disease. I. Reproductive fitness and selection. *Am. J. Hum. Genet.* 18:313–27
148. Knudson, A. G., Wayne, L., Hallett, W. Y. 1967. On the selective advantage of cystic fibrosis heterozygotes. *Am. J. Hum. Genet.* 19: 388–92
149. Huxley, J., Mayr, E., Osmond, H., Hoffer, A. 1964. Schizophrenia as a genetic morphism. *Nature* 204: 220–21
150. Blumberg, B. S., Kuvin, S. F., Robinson, J. C., Teitelbaum, J. M., Contacos, P. G. 1963. Alterations in haptoglobin levels. *J. Am. Med. Assoc.* 184:1021–23
151. Curtain, C. C., Gajdusek, D. C., Kidson, C., Gorman, J. G., Champness, L., Rodrigue, R. 1965. Haptoglobins and transferrins in Melanesia: relation to hemoglobin, serum haptoglobin and serum iron levels in population groups in Papua-New Guinea. *Am. J. Phys. Anthropol.* 23:363–80
152. Buettner-Janusch, J. 1970. Evolution of serum protein polymorphisms. *Ann. Rev. Genet.* 4:47–68
153. Motulsky, A. G. See Ref. 101, 143–96
154. Pene, P., Sankale, M., Linhard, J., Bernou, J. C., Diebolt, G., Gueye, I. 1967. Etude de l'évolution du paludisme rural Africain en fonction des glucose 6 phosphate deshydrogenase. *Bull. Soc. Méd. Afr. Noire Langue Fr.* 14:257–59
155. Kruatrachue, M., Sadudee, N., Sriripanich, B. 1970. Glucose-6-phosphate-dehydrogenase-deficiency and malaria in Thailand: the comparison of parasite densities and mortality rates. *Ann. Trop. Med. Parasitol.* 64:11–14
156. Powell, R. D., Brewer, G. J. 1965. Glucose-6-phosphate dehydrogenase deficiency and falciparum malaria. *Am. J. Trop. Med. Hyg.* 14:358–62
157. Powell, R. D., Brewer, G. J., De Gowin, R. L., Carson, P. E. 1966. Effects of glucose-6-phosphate dehydrogenase deficiency upon the host and upon host-drug-malaria parasite interactions. *Mil. Med.* 131:1039–56
158. Devakul, K., Harinasuta, T., Kanakakorn, K. 1969. Erythrocyte destruction in *Plasmodium falciparum* malaria: an investigation of intravascular haemolysis. *Ann. Trop. Med. Parasitol.* 63:317–25
159. Kruatrachue, M., Klongkumnuanhara, K., Harinasuta, C. 1966. Infection-rates of malarial parasites in red blood-cells with normal and deficient glucose-6-phosphate dehydrogenase. *Lancet* 1:404–6
160. Luzzatto, L., Usanga, E. A., Reddy, S. 1969. Glucose-6-phosphate dehydrogenase deficient red cells: resistance to infection by malarial parasites. *Science* 164:839–42
161. Pollack, S., George, J. N., Crosby, W. H. 1966. Effect of agents simulating the abnormalities of the glucose-6-phosphate dehydrogenase-deficient red cell on *Plasmodium berghei* malaria. *Nature* 210:33–35
162. Beaconsfield, P., Ginsburg, J., Kosinski, Z. 1965. Glucose metabolism via the pentose phosphate pathway relative to cell replication and immunological response. *Nature* 205: 50–51
163. Peters, W. 1969. Recent advances in the physiology and biochemistry of *Plasmodia. Trop. Dis. Bull.* 66:1–29
164. Kosower, N. S., Kosower, E. M. 1970. Molecular basis for selective advantage of glucose-6-phosphate-dehydrogenase-deficient individuals exposed to malaria. *Lancet* 2:1343–45
165. Srivastava, S. K., Beutler, E. 1968. Oxidized glutathione levels in erythrocytes of glucose-6-phosphate-dehydrogenase-deficient subjects. *Lancet* 2:23–24
166. Editorial. 1970. Glucose-6-phosphate-dehydrogenase deficiency and malaria. *Lancet* 2:1347–48
167. Beutler, E., ed. 1968. *Hereditary Disorders of Erythrocyte Metabolism.* New York: Grune & Stratton. 343 pp.
168. Bloom, G. E., Zarkowsky, H. S. 1969. Heterogeneity of the enzymatic defect in congenital methemoglobinemia. *N. Engl. J. Med.* 281:919–22
169. Valaes, T., Karaklis, A., Stavrakakis, D. 1969. Incidence and mechanism of neonatal jaundice related to glucose-6-phosphate dehydrogenase deficiency. *Pediat. Res.* 3:448–58
170. Kattamis, C. A., Tjortjatou, F. 1970. The hemolytic process of viral

hepatitis in children with normal or deficient glucose-6-phosphate dehydrogenase activity. *J. Pediat.* 77: 422–30

171. Nagaratnam, N., Subawickrama, C. S., Kariyawasam, T. 1970. Viral infections in G-6-PD deficiency. *Trop. Geogr. Med.* 22:179–82
172. Petrakis, N. L., Wiesenfeld, S. L., Sams, B. J., Collen, M. F., Cutler, J. L., Siegelaub, A. B. 1970. Prevalence of sickle-cell trait and glucose-6-phosphate dehydrogenase deficiency. *N. Engl. J. Med.* 282: 767–70
173. Wiesenfeld, S. L., Petrakis, N. L., Sams, B. J., Collen, M. F., Cutler, J. L. 1970. Elevated blood pressure, pulse rate and serum creatinine in Negro males deficient in glucose-6-phosphate dehydrogenase. *N. Engl. J. Med.* 282:1001–2
174. Siniscalco, M., Bernini, L., Filippi, G., Latte, B., Meera Khan, P., Piomelli, S., Rattazzi, M. 1966. Population genetics of haemoglobin variants, thalassaemia and glucose-6-phosphate dehydrogenase deficiency, with particular reference to the malaria hypothesis. *Bull. WHO* 34:379–93
175. Motulsky, A. G., Yoshida, A. 1969. *Biochemical Methods in Red Cell Genetics*, ed. J. J. Yunis, 51–93. New York: Academic. 530 pp.
176. Stamatoyannopoulos, G., Kotsakis, P., Voigtlander, V., Akrivakis, A., Motulsky, A. G. 1970. Electrophoretic diversity of glucose-6-phosphate dehydrogenase among Greeks. *Am. J. Hum. Genet.* 22:587–96
177. Kirkman, H. N., Kidson, C., Kennedy, M. See Ref. 167, 126–45
178. Brunetti, P., Rossetti, R., Broccia, G. 1960. Nuove acquisizioni in tema di bioenzimologia del favismo itteroemoglobinurico: Nata III, L'attivita glucosio-6-fosfato-deidrogenasica del parenchima epatico. *Rass. Fisiopat. Clin. Ter.* 32:338–50
179. Carson, P. E., Frischer, H. 1966. Glucose-6-phosphate dehydrogenase deficiency and related disorders of the pentose phosphate pathway. *Am. J. Med.* 41:744–61
180. Carson, P. E. See Ref. 167, 329–30
180a. King, J. L., Jukes, T. H. 1969. Non-Darwinian evolution. *Science* 164: 788–98
180b. Arnheim, N., Taylor, C. E. 1969. Non-Darwinian evolution: consequences for neutral allelic variation. *Nature* 233:900–3
181. Thompson, G. R. 1963. Malaria and stress in relation to haemoglobins S and C. *Brit. Med. J.* 2:976–78
182. Edington, G. M., Laing, W. N. 1957. Relationship between haemoglobins C and S and malaria in Ghana. *Brit. Med. J.* 2:143–45
183. Kruatrachue, M., Bhaibulaya, M., Klongkamnuanhara, K., Harinasuta, C. 1969. Haemoglobinopathies and malaria in Thailand. *Bull. WHO* 40:459–63
184. Kruatrachue, M., Sriripanich, B., Sadudee, N. 1970. Haemoglobinopathies and malaria in Thailand: a comparison of morbidity and mortality rates. *Bull. WHO* 43:348–49
185. So Satta, Bernard O., Long Yann 1970. Résultats préliminaires d'une étude des rapports existant entre l'hémoglobine E (Hg E) et le paludisme au Cambodge. *Nouv. Rev. Fr. Hematol.* 10:317–19
186. Goueffon, S., du Saussay, C. 1969. Enquête systématique sur l'hémoglobine E at la glucose-6-phosphate déshydrogénase au Cambodge. (Octobre 1965–Juin 1966). *Bull. Soc. Pathol. Exot.* 62:1118–32
187. Flatz, G. 1967. Hemoglobin E: distribution and population dynamics. *Humangenetik* 3:189–234
188. Prindle, K. H., McCurdy, P. R. 1970. Red cell lifespan in hemoglobin C disorders (with special reference to hemoglobin C trait). *Blood* 36:14–19
189. Wintrobe, M. M. 1967. *Clinical Hematology, 6th edition.* Philadelphia: Lea & Febiger. 1287 pp.
190. Chernoff, A. I., Minnich, V., Na-Nakorn, S., Tuchinda, S., Kashemsant, C., Chernoff, R. R. 1956. Studies on hemoglobin E. I. The clinical, hematologic and genetic characteristics of the hemoglobin E syndromes. *J. Lab. Clin. Med.* 47:455–89
191. Lie-Injo Luan Eng 1956. *Penjelidikan Hemoglobin Patologik di Indonesia.* Thesis. Univ. Indonesia di Djakarta. 154 pp.
192. Kirkman, H. N. 1967. Haemoglobin abnormalities and stability of triallelic systems. *Ann. Hum. Genet.* 31:167–71
193. Livingstone, F. B. 1969. Gene frequency clines of the β hemoglobin locus in various human populations and their simulation by models in-

volving differential selection. *Hum. Biol.* 41:223–36

194. Vezzoso, B. 1946. Influenza della malaria sulla mortalita infantile per anemia con speciale riguardo al morbo di Cooley. *Riv. Malariol.* 25:61–77
195. Stamatoyannopoulos, G., Fessas, Ph. 1964. Thalassaemia, glucose-6-phosphate dehydrogenase deficiency, sickling, and malarial endemicity in Greece: a study of five areas. *Brit. Med. J.* 1:875–79
196. Plato, C. C., Rucknagel, D. L., Gershowitz, H. 1964. Studies on the distribution of glucose-6-phosphate dehydrogenase deficiency, thalassemia, and other genetic traits in the coastal and mountain villages of Cyprus. *Am. J. Hum. Genet.* 16: 267–83
197. Sijpesteijn, J. A. K. 1959. Thalassemia minor in een Nederlandse familie. *Ned. Tijdschr. Geneesk.* 103:838–43
198. Kattamis, C., Lehmann, H. 1970. Duplication of Alpha-thalassaemia gene in three Greek families with hemoglobin H disease. *Lancet* 2:635-37
199. Abramson, R. K., Rucknagel, D. L., Schreffler, D. C., Saave, J. J. 1970. Homozygous Hb J Tongariki: evidence for only one alpha chain structural locus in Melanesians. *Science* 169:194–96
200. Lehmann, H. 1970. Different types of alpha-thalassaemia and significance of haemoglobin Bart's in neonates. *Lancet* 2:78–80
201. Ladda, R., Lalli, F. 1966. The course of *Plasmodium berghei* infection in the polycythemic mouse. *J. Parasitol.* 52:383–85
202. Chatterjea, J. B., Swarup-Mitra, S., Ghosh, S. K. 1969. Some observations on thalassemia trait with reference to the enzymatic stability of the red cells. *Ann. N. Y. Acad. Sci.* 165:42–53
203. Barnicot, N. A., Allison, A. C., Blumberg, B. S., Deliyannis, G., Krimbas, C., Ballas, A. 1963. Haemoglobin types in Greek populations. *Ann. Hum. Genet.* 26:229–36
204. Lie-Injo Luan Eng. 1965. Hereditary ovalocytosis and haemoglobin E-ovalocytosis in Malayan Aborigines. *Nature* 208:1329
205. Torlontano, G., Tonietti, G., Centurelli, G., Conti, C. 1965. Incidence, haematological, enzymological findings and haemolysis in the hereditary elliptocytosis. Study of fifty four cases. *Rass. Fisiopat. Clin. Ter.* 37:1–10
206. Nielsen, J. A., Strunk, K. W. 1968. Homozygous hereditary elliptocytosis as the cause of haemolytic anemia in infancy. *Scand. J. Haematol.* 5:486–96
207. Geerdink, R. A., Helleman, P. W., Verloop, M. C. 1966. Hereditary elliptocytosis and hyperhaemolysis. *Acta Med. Scand.* 179:715–28
208. Chastel, C., Thomas, J. 1968. Elliptocytose constitutionnelle et paludisme. *Bull. Soc. Pathol. Exot.* 61: 605–12
209. Haldane, J. B. S. 1961. Natural selection in man. *Progr. Med. Genet.* 1:27–37
210. Lambert, S. M. 1949. *Malariology*, ed. M. F. Boyd, 820–30. Philadelphia: Saunders. 2 vols. 1643 pp.
211. Gandini, E., Menini, C., De Filippis, A., Dell'Acqua, G. 1969. Deficienza di glucosio-6-fosfato deidrogenasi eritrocitaria. Studio della distribuzione nella provincia di Ferfara e dei rapporti con malaria e talassemia. *Acta Genet. Med. Gemellol.* 18:271–84
212. Beaconsfield, P., Mahboubi, E., Rainsbury, R. 1967. Epidemiologie des glukose-6-phosphat-dehydrogenasemangels. *Munchen Med. Wochenschr.* 109:1950–52
213. Gelpi, A. P. 1967. Glucose-6-phosphate dehydrogenase deficiency, the sickling trait, and malaria in Saudi Arab children. *J. Pediat.* 71: 138–46
214. Chopra, S. A., Mbaye, A. H. 1969. Sickling in Zanzibar Island—its incidence, regional variation, and relationship with splenomegaly rates. *Trans. Roy. Soc. Trop. Med. Hyg.* 63:270–74
215. Reys, L., Manso, C., Stamatoyannopoulos, G. 1970. Genetic studies on Southeastern Bantu of Mozambique. I. Variants of Glucose-6-phosphate dehydrogenase. *Am. J. Hum. Genet.* 22:203–15