HETEROLOGOUS IMMUNITY IN HUMAN MALARIA

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Human hosts exposed to infection are model systems for studying the interactions of parasites with each other and with their environments. This paper uses published epidemiological data to demonstrate an interaction among the species of human malaria that is expected from ecological and evolutionary theory.

Under certain circumstances, there are fewer mixed malarial infections in human beings than would be expected if infection with one species of malaria were independent of infection with each other species. This reduction in the number of mixed infections is strongly associated with enlargement of the spleen of the human hosts, and less strongly with situations that stimulate immune responses.

Such heterologous resistance is probably best explained as a partial heterologous immunity to malaria in man, since experiments in other mammals have shown that immune mechanisms can eliminate or reduce the level of mixed infections. Though competition among mixed malaria species for nutrients in limited supply in hosts with splenomegaly is possible, most hypotheses which explain the reduction in mixed infections as an artifact or as a result of nonimmune mechanisms are either not consistent with all the observed data or are not now known to suffice to account for them.

If heterologous immunity can indeed greatly reduce the prevalence of mixed infections, as is claimed, then a malaria vaccine need not be specific to each of the species, strains, or antigenic variants of Plasmodium *in order to be effective.*

INTRODUCTION

TO HIS parasites, each man is an island, John Donne notwithstanding. Islands have been classic laboratories in which to study the ecology and evolution of species since Darwin reflected on the finches of the Galapagos. As model systems for studying the interactions of parasites with each other and with their environments, human hosts offer at least three advantages over the traditional islands of the field ecologist.

Human hosts exposed to infection with parasites are often far more numerous than the islands exposed to colonization in a natural archipelago. Hence, human hosts offer many more natural replications of the interactions among a set of parasite species and more opportunity to study the range of natural variation in these interactions.

Human hosts, as environments for their parasites, have been far more extensively and subtly characterized than many island habitats. Current knowledge of human physiology often compares favorably with the information in hand when the field ecologist first approaches his islands.

Human hosts matter, as people. Many observations of human parasites have been recorded for practical medical and administrative purposes and published without further analysis. These data offer the ecologist a base of information comparable to that which business statistics, assembled for commerce and government, offer the economist. Cheap data make it much easier to confirm good ideas and to discard bad ones. In addition, the importance of human

hosts as people assures the ecologist that some truly good ideas may matter beyond the confines of his craft.

These advantages of human hosts for studying the interactions of species do not end the need for studying islands or other ecological models. These advantages simply suggest that investigating the distribution of parasites in populations of human hosts may lead to valuable complementary information.

Among the most widespread pathogenic parasites of man today are four species of blood sporozoans that cause the human malarias. The purpose of this paper is to use published epidemiological data on the human malarias to demonstrate an interaction among these parasitic species in this population of hosts that can be expected from ecological and evolutionary theory (Cohen, 1970). From the point of view of the hosts (human beings), this phenomenon is called "heterologous resistance." This means that a host previously infected (one or more times) with one species of malaria will, under circumstances to be reviewed below, resist a second, different species of malaria better than a host which has never been infected with the first species.

When the mechanism underlying this heterologous resistance is the immune response of the host, then the phenomenon deserves the name of "heterologous immunity." The review below of relevant evidence and alternative possible explanations suggests that the heterologous resistance which will be demonstrated in human beings may indeed be heterologous immunity. Some possible consequences of the reality of heterologous immunity will be suggested.

This heterologous resistance is not a classical sterile immunity, which would absolutely stop infection with the second species of malaria. Rather, this heterologous resistance may be revealed by decreases in the fraction of red blood cells that are parasitized (lowered parasitemias), increases in the interval between inoculation with the challenge infection and observable infection (an increased prepatent period), reduction in the number and severity of relapses with the challenge species, or some combination of these and other characteristics.

THE EVOLUTIONARY ARGUMENT

Heterologous resistance is predicted by the following simple evolutionary argument. This argument provides an evolutionary explanation of the phenomenon, though not an explanation of the mechanisms of the phenomenon.

If an obligatory parasite restricted to a single species of host destroys the members of that species which it infects faster than it propagates from infected individuals to other individuals, then the parasite will quickly go extinct. Hence, tautologously, among parasites restricted to a single host, only those sufficiently adapted to their respective hosts persist in evolutionary time. (Parasites adapted to at least one reservoir species of host can, like rabies, be arbitrarily virulent in other hosts which they infect incidentally.)

In a host to which it is evolutionarily adapted, a parasite species may be caricatured as if natural selection drove it to maximize its prevalence, at least during the reproductive or dispersal stages of its life cycle. (The prevalence of a parasite is the number of host individuals who carry the parasite at any time. The incidence of a parasite is the number of host individuals who become infected with the parasite per unit of time. Neither prevalence nor incidence, measured in host individuals, should be confused with parasitemia, measured in parasite individuals per host.) Natural selection similarly favors increased contributions to succeeding generations on the part of host species, subject to the requirements on the hosts for nondestructive adaptation to their environment.

Now suppose a host species is liable to infection with each of two species of parasites, species 1 and species 2. One theoretically possible relation between the two parasites is complete neutrality: the presence of species 1 in a host in no way affects the likelihood of the presence or absence of species 2, and vice versa. The prevalences of the two parasites are then independent. Species 1 does not gain any selective advantage by influencing the prevalence of species 2, because the prevalence of species 2 in no way affects the prevalence of species 1.

But following any slight change in the interaction of the two parasites away from complete neutrality, natural selection will drive the two

parasites further and further in one of two directions.

If, for any reason, the presence of parasite 1 in a host makes it more difficult for parasite 2 to be present in that host than if parasite 1 were absent, those genotypes of parasite 2 which inhibit the presence of parasite 1 will be selected over those genotypes of parasite 2 which do not. Carried to its extreme in evolutionary time, this *mutual inhibition* leads either to no hosts being infected with both parasites, or possibly to an alternation of parasites, in which infection by parasite 2 of a host already occupied by parasite 1 results in the elimination of parasite 1. (Some hosts may not be infected at all.)

On the other hand, *mutual facilitation* may lead, in evolutionary time, to both species of parasites being present in all hosts that either one can infect.

If infection with either or both species of parasites hurts the host's chances of surviving to maturity and contributing offspring to the next generation, then those host-parasite systems in which the presence of one species of parasite lessens the chance that the other species will also be present will be selected over those systems in which the parasite species interact neutrally or symbiotically. On the contrary, if infection with either or both species is advantageous to the host, natural selection will favor those host-parasite systems in which the parasites facilitate each other's presence.

This argument may be formalized (Cohen, 1970). Analysis confirms that neutrality between parasite species is an unstable equilibrium (saddle point), while both mutual inhibition and mutual facilitation lead to stable equilibria. Moreover, the fraction of hosts infected, whether singly or doubly, is largest in the extreme of mutual facilitation and is smallest in the extreme of mutual inhibition.

APPLICATION TO HETEROLOGOUS IMMUNITY IN MAMMALIAN MALARIA

According to the argument just outlined, two (or more) different species or strains of malaria should inhibit each other's presence in mammalian hosts if

(1) the presence of one species in a host can somehow influence the presence of another; that is, if there is some mechanism provided by the parasites or the host through which the parasites can interact; and if

(2) the species or strains of malaria or their direct evolutionary antecedents have infected the mammalian hosts or their direct evolutionary antecedents over a period of time long enough for evolutionary adaptation to occur; and if

(3) the presence of either or both parasites is bad for the host; that is, there is a selective advantage to the hosts in whom the mechanism assumed in (1) can be brought into play. Evidence supports all three of these assumptions.

First, antigenically similar parasites can interact through the immune mechanism of the host. Antigenic similarities among the strains and species of the genus *Plasmodium* could be presumed on the basis of their similarities in morphology and life cycle. Common antigens have been demonstrated directly for both blood and sporozoite stages of the human malarias (Voller, 1971). These common antigens make it possible for the immune response stimulated by one species to affect another.

Second, species of malaria have infected men long enough to cause genetic adaptations (Cavalli-Sforza and Bodmer, 1971). Comparisons of the malarial parasites of contemporary non-human mammals, birds, and reptiles support persuasive arguments that malarial parasites have evolved with the vertebrates since well before the rise of the mammals (Bruce-Chwatt, 1965; Manwell, 1955; Fiennes, 1967:70–75).

Third, human malarias contribute in varying degrees to childhood mortality and to spontaneous abortions in women who do survive to pregnancy. Various of the fulminating rodent and primate malarias are equally disadvantageous.

The above argument that under certain circumstances it is evolutionarily advantageous for a host to use the presence of one deleterious parasite to protect itself against the presence of another does not require that the mechanism through which it does so be the immune responses. The following experiments point more specifically to immunity as a mechanism of heterologous resistance.

HETEROLOGOUS MALARIAL IMMUNITY IN MAMMALS

In experiments with more than one variant (or strain or species) of malaria, it is essential to

distinguish among susceptibility, partial resistance, and sterile immunity. Suppose a host has been exposed to a single variant of malaria, whether by means of sporozoites (the form in which infection is transmitted from mosquitoes) or by means of blood stages. Then suppose the host is challenged with a second variant of parasite. If no subsequent trace of this second variant can ever be found in the host, the animal displays sterile immunity. A control not previously exposed to the first variant is required to demonstrate that the host species is in fact susceptible to infection with the second variant, and that the particular inoculum of the second variant used was not impotent for some reason unrelated to immunity.

If the host previously infected with the first variant does show infection with the second, then susceptibility to the second has been established, even in the absence of a control. But this does not establish that the previous infection has stimulated *no* resistance. To establish the absence of resistance requires showing in detail that the time-courses of infection in the previously infected and the control animals do not differ.

Prior to 1966, many experiments in animals, including humans, established that hosts previously infected with one variant of malaria were susceptible to infection with another. Most reports of these experiments concluded that the first infection had had no effect on the course of the second, without an adequately detailed comparison with control individuals. For example, the experiments of Yorke and Macfie (1924), Ciuca, Ballif, and Vieru (1928, 1930), Ciuca, Ballif, and Chelarescu-Vieru (1934), James (1931), and Boyd, Kitchen, and Matthews (1939) are frequently cited to support the claim that "a man immune to one species of *Plasmodium* exhibits no heterologous immunity to another species" (Taliaferro, 1949: 941, who also provides an extremely useful guide to the earlier experiments in birds and rodents). Not one of these experiments compared controls infected with only the second species with people who were infected with two species of malaria, nor did any report prepatent periods and daily parasitemias in sufficient detail to make the comparison possible. The same experimental weakness, and unjustified conclusion, appears in ethically less questionable studies of monkeys — e.g., that of Garnham and Bray (1955), whose single control rhesus died on the eleventh day after inoculation.

Boyd and Kitchen (1945) and Jeffery (1966) confirmed that under laboratory conditions people previously infected with one strain of malaria were universally susceptible to infection with another strain or species. But by keeping records of the daily course of the infections (fevers, parasitemias) and the frequency of chemotherapy required to preserve the lives of the patients, they clearly demonstrated heterologous resistance, as well as a difference between homologous and heterologous resistance.

Cleaner experiments are possible with nonhuman animals because infections can run their course without chemotherapy and because there are fewer constraints on the conditions of inoculation and observation. These experiments have demonstrated heterologous resistance at three levels: between antigenic variants within the same geographical strain; between geographical strains within the same species; and between different species of *Plasmodium*.

The recent experiments in rats, mice, rhesus macaques, gibbons, and chimpanzees (Cox and Voller, 1966; Yoeli, Nussenzweig, Upmanis, and Most, 1966; Sadun, Hickman, Wellde, Moon, and Udeozo, 1966; Cadigan and Chaicumpa, 1969; Cox, 1970; Cox and Turner, 1970; Cox, 1972a,b,c; Nussenzweig, Vanderberg, Spitalny, Rivera, Orton, and Most, 1972; and most elegant of all, Voller and Rossan, 1969a,b,c,d) justify several generalizations, as follow: (1) Heterologous resistance between two variants (or strains or species) need not be symmetrical; one variant may be superior as an "immunizing" agent in a particular host to another variant. (2) The more frequently an individual is inoculated with one variant, the greater its protection against challenge with another variant. (3) The longer the interval since the last infection, the less the protection it affords against challenge. (4) Heterologous resistance between two variants of malaria is somehow specific to some characteristics of those two variants and is not a product of a generalized arousal of the host which could be produced by an arbitrary infection. Indeed, the absence of resistance between *Trypanosoma musculi* and intraerythrocytic protozoa in mice (Cox, 1972b) suggests that in

analyzing the interactions of malaria species in humans, the possible concurrent presence of trypanosomiasis can be neglected.

By sampling and comparing successive peak parasitemias of a rhesus monkey infected with *Plasmodium knowlesi*, Voller and Rossan (1969c) demonstrated antigenic differences, not only between species of malaria, or between localities within species, or between individuals within localities, but also over time within the same individual. In other rhesus monkeys challenged repeatedly with the parasites from a single one of these peaks, the proportion of animals increased which completely resisted infection by successive inoculations. Among those which were infected at each challenge, the mean prepatent period increased, and the average number of parasites per 10,000 red blood cells declined.

Subsequent challenges of these monkeys with antigenic variants from different peak parasitemias of the original donor monkey led to parasitemias sooner than did the homologous challenge, but much later than did infections in monkeys which had not been previously "immunized" at all. These experiments point to a specifically immunological mechanism for heterologous resistance.

When these and other monkeys chronically infected with *P. knowlesi* were challenged with *P. cynomolgi* and *P. coatneyi*, the "immunized" and control groups displayed no differences in the course of parasitemia (Voller and Rossan 1969d). But the parasitemia in "immunized" animals resulting from challenge with *P. inui* was reduced, and developed more slowly than in controls.

Voller and Rossan (1969a,b,c,d) have emphasized repeatedly that the detailed mechanism of protection (e.g., humoral versus cellular immunity) is unknown and that serological methods can overestimate or underestimate the actual protective immunity (Voller, 1971).

The experiments of Voller and Rossan (1969a,b,c,d) involved transferring blood stages of malaria. From a review of experiments on sporozoite-induced immunity in mammalian (primarily rodent) malarias, Nussenzweig et al. (1972) reached the same conclusions. The sporozoite stage induces substantial resistance, both within and across strains and species of malaria, and this resistance is not entirely due to nonspecific reticuloendothelial arousal; the actual amount of protection can be overestimated or underestimated by serological methods; its actual mechanism is inadequately known. Nussenzweig et al. (1972) reached one additional conclusion. Protection is stage-specific, in spite of common antigens among the different stages of the malaria life cycle. Neither did a sporozoite-induced protection stop infection with blood forms, nor vice versa.

ANALYTICAL EPIDEMIOLOGY OF HUMAN MALARIA

Experiments with humans would have difficulty imitating, under laboratory conditions, the pattern and duration of infections with the diverse malarial strains found in the field. Such experiments could not reproduce the nutritional conditions and presence of other infections that may affect heterologous resistance under real circumstances. To assess the extent of heterologous resistance to malaria in humans in the field, the only recourse is to data from the field on humans.

A search of field studies of malaria has yielded 14 usable reports of the following data: N, the total sample size; N_0, the number of uninfected individuals; N_1, the number of individuals infected with *Plasmodium falciparum* only; N_2, the number of individuals infected with *P. vivax* only; N_3, the number of individuals infected with *P. malariae* only; and N_4, the number of individuals with mixed infections (any two or all three of these species). The fourth species of human malaria, *P. ovale*, was always missing. In areas with only two species of malaria, one of N_1, N_2, and N_3 is equal to zero, and N_4 counts just double infections. In advance of submitting these data to the analysis that follows, I decided not to use several dozen other malaria surveys because of their various internal inadequacies of procedure or reporting.

The useful reports also classify the individuals by one or more other characteristics of possible epidemiological importance. Among these are: (1) geographic locale, (2) ecological setting (wet or dry, sprayed with DDT or not sprayed, altitude, and season), (3) age, and (4) spleen size.

The results of analyzing the data recovered from these reports will be presented in tables and graphs. Each table contains the data, which are often based on recalculation from the original publication; χ^2-tests of homogeneity, not reported here, were performed before pooling some categories (of locale, setting, age, or spleen size) in which the distributions of infections did not differ. Bishop (1971) has analyzed some possible effects of this procedure.

There are two reasons for presenting these data in detail. The first is so that others may compare the figures in the original publications with mine and thereby confirm or challenge mine. The second is that others who wish to use the data in ways which have not occurred to me, or to confirm or challenge my calculations, may do so without repeating the labor of extracting the numbers from the original publications.

Each table also contains the maximum likelihood estimates (Cohen, 1971) of α_i; $i=1, 2, 3$, where α_i is the fraction of the population infected with species i, assuming that the prevalences of the three species are independent, that is, that the presence of one species in a host is independent of the presence of any of the other species. From these values of α_i were calculated the expected numbers (not shown in the tables) corresponding to each kind of infection, assuming no interaction between species. The tables show two measures of the goodness of fit of these expected frequencies to the corresponding observed frequencies N_i; X^2 is Pearson's estimate of χ^2 with one degree of freedom, and LLR is -2 times the log likelihood ratio with one degree of freedom.

For each set of data in a table, a graph (with a matching number) plots an estimate of a statistic called s and the 99 per cent confidence interval of this estimate. The statistic s (which might be thought of as "susceptibility") is the ratio of the observed number N_4 to the expected number of mixed infections upon the assumption of no interaction between species. The 99 per cent confidence intervals around the estimated values of s presented below were calculated following the idea of Mantel and Patwary (1961) as described in Cohen (1971: 382). The numerical procedure used to find the critical values of s was the method of false position (Ralston, 1965:323). If the estimated value of s falls below 1, and the 99 per cent confidence interval around that estimate does not include 1, then there are significantly fewer mixed infections than are to be expected from the model of no interaction.

The idea of studying the ratio s of the number of observed joint occurrences of species to the number expected if species were independent, though proposed independently for this study, dates back in ecology at least to Forbes (1907).

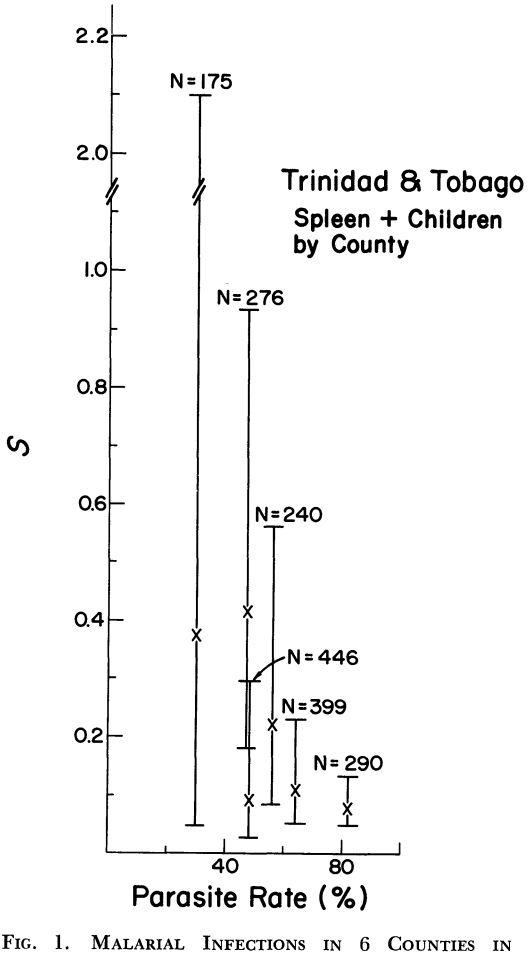

FIG. 1. MALARIAL INFECTIONS IN 6 COUNTIES IN TRINIDAD AND TOBAGO

Estimates (x) and 99% confidence intervals (vertical bars) of s as a function of parasite rate (fraction of the population with malarial infection). See Table 1, based on Downs, Gillette, and Shannon (1943). In this and all of the following figures, s is the ratio of the number of mixed infections observed to the number that would have been expected if there were no interactions among the parasite species.

Forbes proposed a "coefficient of association" which is exactly equal to s in the case of two species, for his investigation of a thousand museum collections of the subfamily Etheostominae (darters). He had no measure of the sampling variability of s. Mozley described Forbes's method as "forgotten" in 1936. Since then it seems to have been forgotten again.

In interpreting any of the measures of prevalence of infection which follow, three cautions need be observed. First, the absence of parasites in single or repeated blood smears does not prove the absence of infection, since stages in the life cycle of the malaria parasite may be present in other tissues of the body. Nonetheless, the frequency with which parasites appear in peripheral blood is an accepted indicator of prevalence. (The technical problem of diagnosing an individual as having only the first parasite species seen in a blood smear will be discussed later.) Second, just as some nonhuman primates may be infected while showing no clinical signs of disease, humans may attain substantial resistance to their parasites. Malarial infection, particularly a chronic, low parasitemia, does not necessarily imply disease. Third, a difference between two populations in prevalence of infection may reflect a difference in either duration of infection or in incidence. Only if duration does not vary greatly from one area to another is prevalence an indicator of the incidence.

Parasite Rate

A general indicator of prevalence, the parasite rate calculated as $100(1-N_0/N)$, is the percentage of the population sampled who displayed malarial infection of any kind.

Fig. 1 plots the estimates of s and the 99 per cent confidence intervals for children with any observable enlargement of the spleen ("spleen-positive children") in six counties of Trinidad and Tobago, as a function of the parasite rates in those counties. Only counties with well over 100 such children are analyzed.

For the lowest parasite rate observed (Caroni, 30%), the confidence interval around s includes the value 1. Hence the number of mixed infections observed there does not differ significantly from the number expected in the absence of interaction between the species. For the other counties, the higher the prevalence, the lower the number of mixed infections relative to the number expected if the species of parasites were not interacting.

TABLE 1

Malarial infections in six counties in Trinidad and Tobago, in order of increasing parasite rate

In this and the following tables, N = total sample size, N_0 = number of uninfected individuals, N_1 = number of individuals infected with *Plasmodium falciparum* only, N_2 = number of individuals infected with *P. vivax* only, N_3 = number of individuals infected with *P. malariae* only, N_4 = number of individuals with mixed infections; α_i = maximum likelihood estimate of the fraction of the population infected with species i, assuming that the prevalences of the three species are independent; X^2 = Pearson's estimate of χ^2 with one degree of freedom; $LLR = -2 \times$ log likelihood ratio, which also has asymptotic distribution of χ^2 with one degree of freedom.

COUNTY	PARASITE RATE (%)	N	N_0	N_1	N_2	N_3	N_4	$\hat{\alpha}_1$	$\hat{\alpha}_2$	$\hat{\alpha}_3$	X^2	LLR
Caroni	30.3	175	122	17	32	2	2	.108	.194	.013	1.003	1.999
St. George (excluding Port of Spain)	46.7	276	147	74	30	14	11	.304	.138	.067	4.763	6.055
St. Andrew	47.8	446	223	129	51	28	5	.299	.122	.068	32.814	42.321
St. Patrick	55.8	240	106	89	24	13	8	.402	.123	.068	12.664	15.346
Tobago	63.4	399	146	154	22	65	12	.414	.065	.187	53.323	60.773
St. David	82.1	290	52	63	88	56	31	.291	.389	.261	81.438	85.135

Based on data from Downs, Gillette, and Shannon (1943).

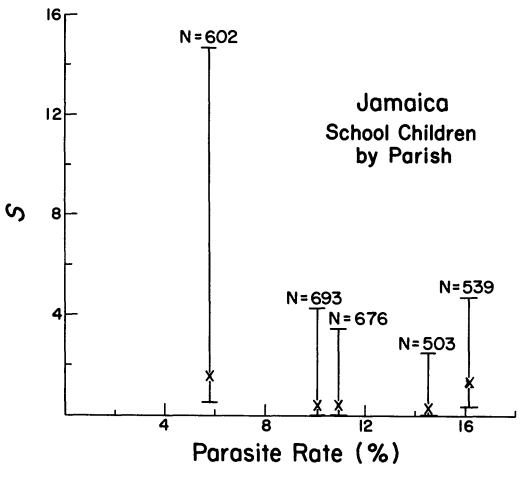

FIG. 2. ESTIMATES AND CONFIDENCE INTERVALS OF s IN FIVE PARISHES OF JAMAICA AS A FUNCTION OF PARASITE RATE

See Table 2, based on Boyd and Aris (1929).

In Fig. 2, the parasite rates among school children in the five parishes of Jamaica included range only from just under 6 to just over 16 per cent. If s declines as the parasite rate increases, the trend is very weak.

A possible bias due to diagnostic technique may mask a stronger negative association between the parasite rate and s, but at least assuredly does not contribute to the slight negative association observed. The bias is that the lower the density of parasites in the blood, the less likely it is that parasites of more than one species are to be found. In many (though not all) instances, lower parasite rates are associated with lower parasitemias. Hence one might expect the above bias to produce higher, rather than lower, values of s in areas with higher parasite rates.

Ecology

In Qatif Oasis, Saudi Arabia, among children aged 2 to 14 years, there were significantly fewer mixed infections than expected in the absence of interaction, in the three nongarden towns as well as in the five garden towns of the oasis (Fig. 3). In the garden towns, which offer a more favorable habitat for mosquitoes and which have a higher parasite rate, as shown in Table 3, the estimated value of s was about half that estimated for the three nongarden towns. Although the confidence intervals for the two estimates overlap, the interval for the garden towns falls below the estimate of s for the nongarden towns.

In Pattukkottai Taluk, Madras, India, statistics on hospital outpatients and on sizes of spleens suggested that the period from July to January constituted a "malaria season," and the rest of the year an "off season." Infants (children less than one year old) were surveyed from March to May, during the "off season," and from November to December, during the "malaria season." The original report does not specify to what extent the same children appear in both surveys. The only mixed infections found were *P. falciparum* and *P. vivax*. During the "off season," when the parasite rate was low, there were not significantly fewer mixed infections than expected from the model of no interaction. During the malaria season, there were significantly fewer mixed infections than expected. The estimate of s for the "off season"

TABLE 2

Malarial infections in five parishes of Jamaica, in order of increasing parasite rate

PARISH	PARASITE RATE (%)	N	N_0	N_1	N_2	N_3	N_4	$\hat{\alpha}_1$	$\hat{\alpha}_2$	$\hat{\alpha}_3$	X^2	LLR
St. Mary	5.8	602	567	16	6	12	1	.028	.011	.021	.069	.159
St. Catherine	10.1	693	623	40	11	18	1	.059	.017	.027	.155	.739
St. Elizabeth	10.9	676	602	37	23	13	1	.056	.035	.020	.436	1.310
St. Thomas	14.5	503	430	33	11	28	1	.067	.023	.057	1.104	2.492
Portland	16.1	539	452	8	12	63	4	.018	.027	.124	.030	.226

Based on data from Boyd and Aris (1929).

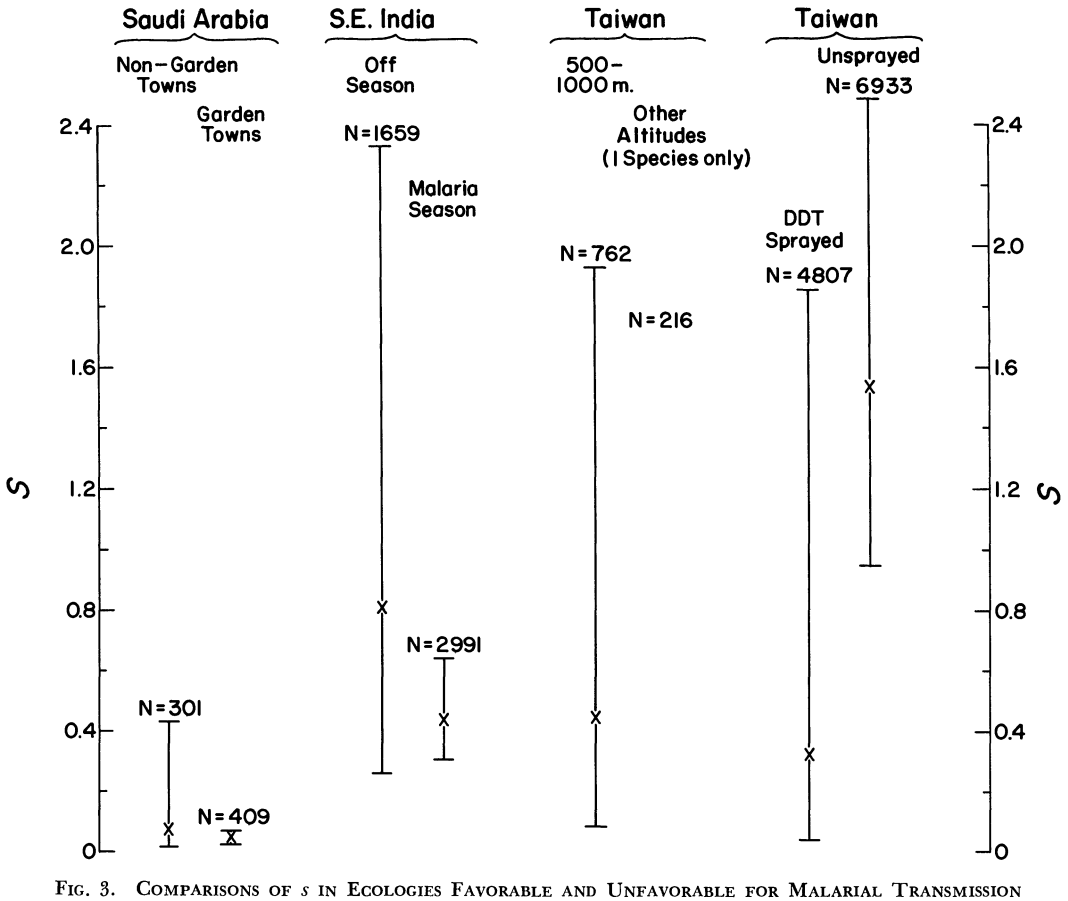

FIG. 3. COMPARISONS OF *s* IN ECOLOGIES FAVORABLE AND UNFAVORABLE FOR MALARIAL TRANSMISSION

See Table 3, based on Daggy (1959) for Saudi Arabia; Russell, Menon, and Rao (1938) for India; Wu (1956) for altitude data from Taiwan; Wu and Chuang (1956) for DDT data from Taiwan.

fell above the confidence interval for the "malaria season," although the confidence intervals for the two seasons overlap (see Fig. 3).

The great majority of towns and villages of central Taiwan are located at altitudes between 500 and 1000 m. In these towns, the prevalences of single and mixed infections did not differ significantly from those expected in the absence of interaction (Fig. 3). In the three towns outside of this range of altitude, only *P. vivax* was observed in the few infected people. Since no other species was found, no mixed infections were possible.

During the middle two years of a four-year malaria control program in Taiwan, a survey of school children between 6 and 14 years of age compared prevalences in a region that had originally been selected because of its high malaria endemicity for residual house spraying of DDT, with another area that had not been sprayed. In neither region did the number of mixed infections differ significantly from that expected. However, in the formerly highly malarious area that had been sprayed, the estimate of *s* fell below the confidence interval of the estimate for the unsprayed area (Fig. 3). Presumably, children in the unsprayed area never suffered from intense transmission of malaria, while children in the sprayed area were still affected, at least to some extent, by conditions prior to spraying.

These ecological comparisons suggest that where the setting favors transmission of the parasites, *s* tends to be lower than where the setting does not. This trend is contrary to the

TABLE 3

Ecological comparisons of malarial infections in Saudi Arabia, India, and Taiwan

UNIT	N	N_0	N_1	N_2	N_3	N_4	$\hat{\alpha}_1$	$\hat{\alpha}_2$	$\hat{\alpha}_3$	X^2	LLR
3 nongarden towns, Qatif Oasis, Saudi Arabia, ages 2–14	301	178	51	55	15	2	.175	.188	.052	17.141	24.717
5 garden towns, Qatif Oasis, Saudi Arabia, ages 2–14	409	67	145	112	58	27	.410	.325	.174	166.381	175.841
Off season, Pattukkottai, India, infants	1659	1370	243	30	10	6	.150	.021	.007	.060	.219
Malaria season, Pattukkottai, India, infants	2991	1936	702	291	12	50	.251	.113	.005	26.142	30.057
500–1000 m towns, central Taiwan	762	635	66	52	6	3	.090	.072	.009	1.162	2.063
Other altitudes, central Taiwan	216	213	0	3	0	0					
DDT sprayed areas, Taiwan, school children	4807	4494	123	167	21	2	.026	.035	.004	1.873	3.335
Unsprayed areas, Taiwan, school children	6933	6226	362	292	23	30	.056	.046	.004	4.265	4.276

Based on data from Daggy, 1959 (Saudi Arabia); Russell, Menon, and Rao, 1938 (India); Wu, 1956 (altitude data from Taiwan); Wu and Chuang, 1956 (DDT data from Taiwan).

TABLE 4

Malarial infections by age groups in Saudi Arabia, Sarawak, Borneo, Uganda, India, and Taiwan

UNIT	AGE RANGE	N	N_0	N_1	N_2	N_3	N_4	$\hat{\alpha}_1$	$\hat{\alpha}_2$	$\hat{\alpha}_3$	X^2	LLR
garden towns, Qatif Oasis, Saudi Arabia	0–9	249	28	95	65	37	24	.464	.334	.197	109.817	116.278
garden towns, Qatif Oasis, Saudi Arabia	10–14	192	40	68	51	25	8	.390	.298	.150	71.156	79.260
Sarawak and Brunei Dayak and Murut	0–9	375	259	46	18	49	3	.129	.051	.137	7.773	11.312
Sarawak and Brunei Dayak and Murut	10–	282	222	25	6	26	3	.098	.025	.101	.089	.379
Borneo, Baram DDT Test Area	1–9	231	148	33	34	14	2	.150	.154	.065	7.794	11.683
Borneo, Baram DDT Test Area	10–	139	107	14	7	9	2	.112	.059	.074	.009	.169
Kigeza, Uganda-Bunyoni & Bufuka	0–9	5466	5322	83	0	55	6	.016	0	.011	21.022	12.294
Kigeza, Uganda-Bunyoni & Bufuka	10–	157	132	14	0	11	0	.089	0	.070	.043	2.134
India, NE Frontier Agency	infants	124	70	34	12	6	2	.289	.108	.055	3.733	6.006
India, NE Frontier Agency	children	877	723	102	24	24	4	.120	.030	.030	1.007	1.758
Taiwan towns	infants	517	498	10	8	0	1	.021	.017	0	.517	1.829
Taiwan Hu-Chu	primary school	1430	1190	132	85	7	16	.103	.070	.006	1.470	1.770

Based on data from Daggy, 1959 (Saudi Arabia); DeZulueta, 1956a (Sarawak); DeZulueta, 1956b (Borneo); DeZulueta et al., 1964 (Uganda); Misra, 1956 (North East Frontier Agency, India); Wu, 1956 (Taiwan).

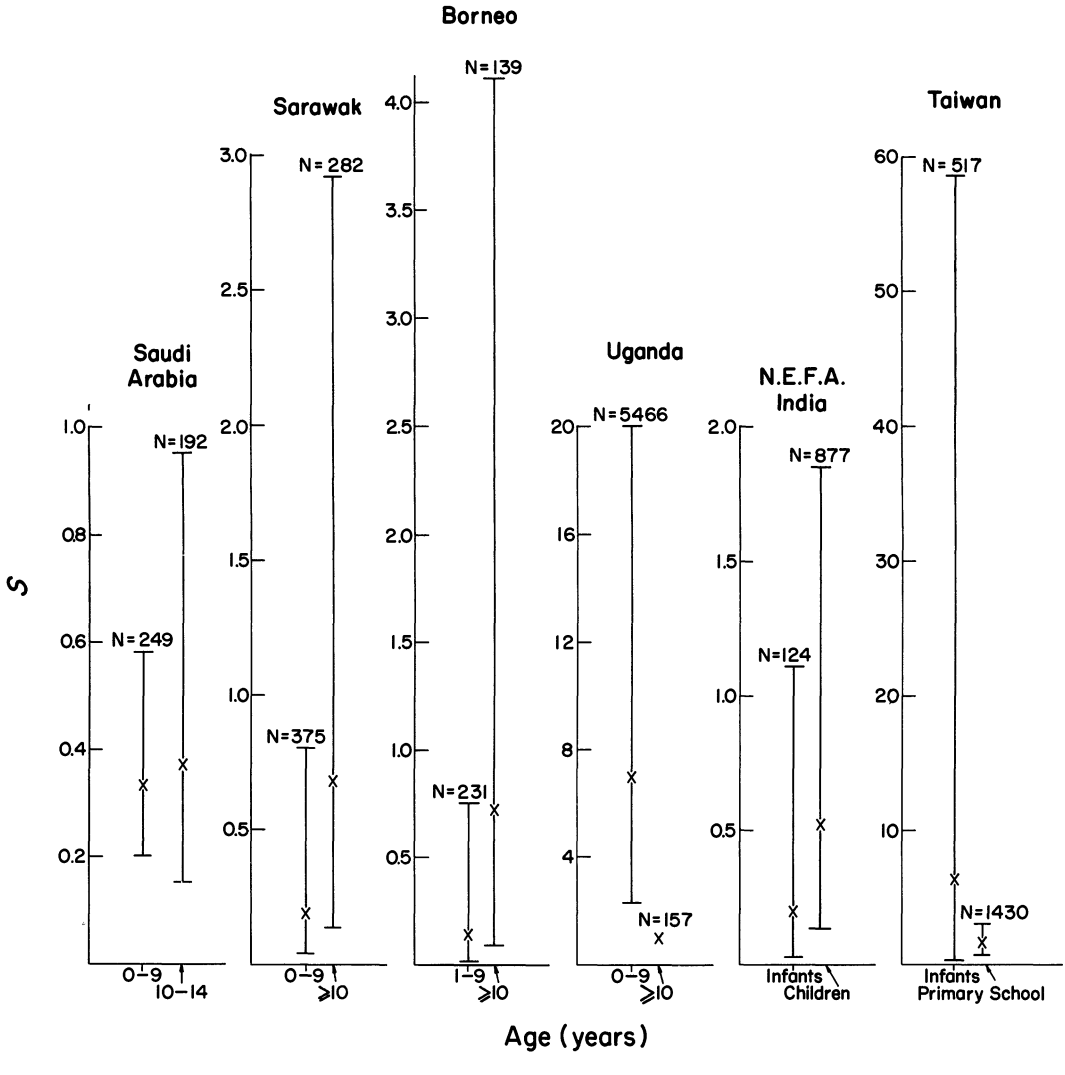

FIG. 4. COMPARISONS OF s BETWEEN GROUPED AGE CATEGORIES

See Table 4, based on Daggy (1959) for Saudi Arabia; DeZulueta (1956a) for Sarawak; DeZulueta (1956b) for Borneo; DeZulueta et al. (1964) for Uganda; Misra (1956) for North East Frontier Agency, India; and Wu (1956) for Taiwan.

bias noted in the discussion of parasite rates above.

Age

In studies that classified usable data on infections by the ages of the individuals examined, exact determinations of age were not to be hoped for. In recognition of the uncertainty about age, age categories that did not have distributions of infections that differed significantly according to a χ^2-test of homogeneity were pooled. In 6 studies, two age classes remained after this pooling (Table 4; Fig. 4).

This gross categorization lumps together individuals with very different immunological capacities. For example, infants protected by maternal antibodies differ from those in the vulnerable 2- to 4-year age group, who again differ from children aged 5 to 9 years, who can begin to protect themselves against malaria. Nevertheless, the data reported here either did

not make such a fine division by age possible or did not justify it.

In four of these studies, individuals 9 years of age and younger are divided from the individuals aged 10 years and over (though the Borneo study leaves out infants). In three of these four studies, there are significantly fewer mixed infections than expected in the younger age group; the estimate of s is larger, though never significantly so, in the older age group.

In the other two of the six studies, "infants" are divided from "children." In these, the number of mixed infections does not differ significantly from the number expected in the absence of interaction between parasite species.

In a survey of Sepik District in New Guinea, there were significantly fewer mixed infections than expected in the group aged 5 to 9 (Table 5; Fig. 5). This is consistent with the findings for Saudi Arabia, Sarawak, and Borneo seen in Fig. 4. In the youngest age group there appeared to be more mixed infections than expected. At the other ages there were about as many as expected. (The overlap in ages between the two youngest groups appears in the original publication. Probably the youngest age group includes those individuals estimated as not having reached their second birthday.)

One study (Schüffner, 1938) reported data for Mandailing, Sumatra, September 1917, from which it is possible to reconstruct the exact number of individuals in eight age groups who were observed with each possible combination of three species of parasite.

Underneath each observed frequency in Table 6 are two expected frequencies obtained by fitting the data to two models (Fienberg, 1970). The models were fitted by a slightly modified version of an iterative proportional

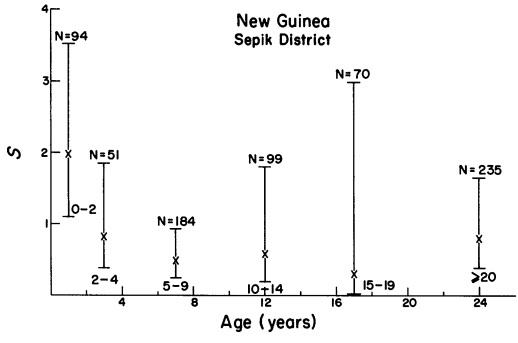

FIG. 5. ESTIMATES OF s AS A FUNCTION OF AGE IN NEW GUINEA

See Table 5, based on Peters and Standfast (1957).

fitting program for multidimensional contingency tables written by Dr. Y. M. M. Bishop.

The first expected frequency results from fitting a model which assumes that the prevalence of each species (the α_i, $i = 1, 2, 3$ described above) changes with age, but that within each age class the species do not interact. For this model, which has 32 degrees of freedom, the value of Pearson's X^2 is 40.1 and of the log likelihood ratio is 39.1, neither of which is significant at the 1 per cent level. Hence these data do not provide statistical evidence for interaction between species within any age class.

This model is a minimal model in the sense that if any fewer interactions among the four variables (age and the three prevalances) are assumed, the frequencies expected do not match the observed at any reasonable level of significance. For example, if the prevalence of *P. falciparum* were assumed to be independent of age, while the other two prevalences were assumed to change with age, then even if all three parasite species were assumed to interact

TABLE 5

Malarial infections by age groups in Sepik District in New Guinea

AGE RANGE	N	N_0	N_1	N_2	N_3	N_4	$\hat{\alpha}_1$	$\hat{\alpha}_2$	$\hat{\alpha}_3$	X^2	LLR
0–2	94	16	28	8	2	40	.697	.431	.182	.952	1.732
2–4	51	4	10	2	7	28	.689	.296	.606	.088	.066
5–9	184	77	38	32	19	18	.284	.247	.157	3.509	4.377
10–14	99	52	23	10	8	6	.284	.141	.115	.422	.962
15–19	70	46	14	4	5	1	.213	.065	.080	.420	1.477
20–	235	136	20	22	43	14	.121	.132	.232	.168	.391

Based on data from Peters and Standfast (1957).

TABLE 6

Exact distribution of infections by age groups in Mandailing, Sumatra

+, species is present; −, species is absent. Thus, the column under the heading "*P. falciparum* −, *P. vivax* +, *m* +" gives the number of individuals of each age infected with *P. vivax* and *P. malariae* but not *P. falciparum*. *m*, the species *Plasmodium malariae;* Obs., number of individuals observed; E1, number expected, assuming no interactions among the species at each age and taking the marginal distribution by age of each species as given; E2, number expected assuming, in addition to the marginal distributions by age, a constant pairwise interaction between *P. falciparum* and *P. malariae* at every age.. Both models fit the data acceptably.

		P. falciparum +				*P. falciparum* −			
		P. vivax +		*P. vivax* −		*P. vivax* +		*P. vivax* −	
AGE		*m* +	*m* −	*m* +	*m* −	*m* +	*m* −	*m* +	*m* −
0–1	Obs.	3	8	11	70	6	30	12	203
	E1	1.2	11.4	7.4	72.0	3.2	31.2	20.2	196.4
	E2	0.9	11.7	5.8	73.6	3.5	30.9	21.8	194.8
2–3	Obs.	4	17	12	99	4	25	32	169
	E1	2.6	15.6	16.3	97.4	4.6	27.2	28.5	169.8
	E2	2.1	16.1	13.4	100.4	5.0	26.7	31.4	166.8
4–5	Obs.	1	2	13	86	2	6	43	178
	E1	0.6	2.8	17.6	81.0	1.4	6.3	39.5	181.9
	E2	0.5	2.9	14.3	84.3	1.5	6.1	42.8	178.6
6–7	Obs.	0	1	3	74	1	12	34	211
	E1	0.4	2.9	8.5	66.3	1.2	9.5	28.0	219.3
	E2	0.3	3.0	6.6	68.1	1.3	9.5	29.8	217.4
8–9	Obs.	0	1	2	45	1	3	15	176
	E1	0.1	0.9	3.5	43.5	0.3	3.7	14.1	176.8
	E2	0.1	0.9	2.7	44.3	0.3	3.7	15.0	176.0
10–11	Obs.	0	0	1	30	1	1	8	95
	E1	0.0	0.4	2.2	28.3	0.1	1.4	7.6	95.8
	E2	0.0	0.4	1.7	28.8	0.1	1.4	8.1	95.3
12–15	Obs.	0	0	0	17	0	1	8	106
	E1	0.0	0.1	1.0	15.8	0.1	0.8	6.9	107.2
	E2	0.0	0.1	0.8	16.1	0.1	0.8	7.2	107.0
16–	Obs.	0	1	0	93	0	5	17	1267
	E1	0.0	0.4	1.2	92.4	0.1	5.5	15.8	1267.6
	E2	0.0	0.4	0.8	92.8	0.1	5.5	16.1	1267.3

Based on data from Schüffner (1938).

in pairs, the value of Pearson's X^2 is 291.9 and the value of the log likelihood ratio is 300.9, with 36 degrees of freedom. Hence the prevalences of all species must be assumed to interact with age.

The second expected frequency results from assuming, in addition to the interaction between each species' prevalence and age, that *P. malariae* and *P. falciparum* interact. The model with this added interaction gives Pearson's X^2 of 38.4 and a *LLR* of 34.8, with 31 degrees of freedom. Since the models are fitted to the data in such a way as to minimize the *LLR*, the difference in goodness of fit between the first fitted model and this second one corresponds to a change in the log likelihood ratio of $39.1 - 34.8 = 4.3$ with $32 - 31 = 1$ degree of freedom (Fienberg, 1970). This change is not significant at the 1 per cent level which has been adopted throughout this paper.

Adding the assumption of a pairwise interaction between *P. falciparum* and *P. malariae* causes the largest reduction in the log likelihood ratio of any additional pairwise interaction that could be added to the minimal model. So none of the other pairwise interactions is statistically

significant, either. Thus, the data in Table 6 show that prevalences change with age, but that within any age class prevalences of different species do not interact.

A confirmation of this conclusion is offered by Fig. 6, which shows the estimates and confidence intervals of s that result when the various mixed infections within each age class are combined to yield data of the form in Tables 4 and 5. There were about as many mixed infections as expected, except among children of ages 6 and 7 years, who had significantly fewer than expected. Although no confidence interval is shown from the group 12 to 15 years of age (because no mixed infections were observed), the log likelihood ratio and the χ^2-test of goodness of fit did not reject the model of no interaction within that age group.

The data relating prevalence of infection to age accordingly indicate neither a striking interaction among species at all ages nor systematic changes in interaction with age. They do repeatedly suggest that there are fewer mixed infections than expected among children in the age range from 5 to 9 years.

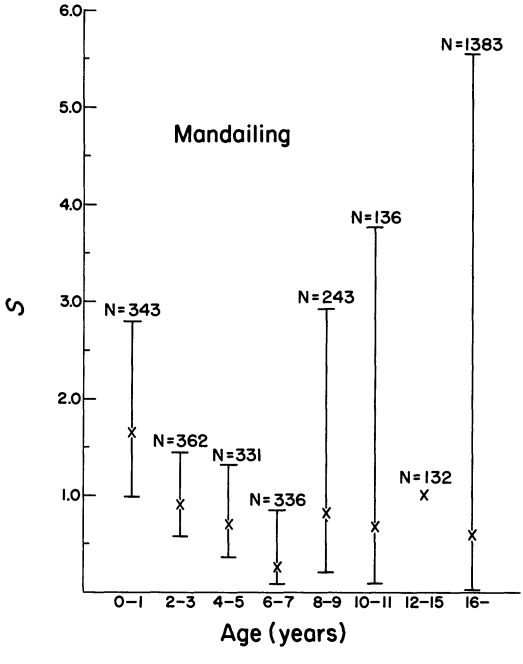

FIG. 6. ESTIMATES OF s AS A FUNCTION OF AGE IN MANDAILING, SUMATRA

See Table 6, based on Schüffner (1938).

Size of Spleen

Humphrey and White (1970: 263) cite experiments which "stress the role of the spleen in forming antibody to antigens which are particulate and distributed *via* the bloodstream." This role of the spleen is consistent with its enlargement, that is, splenomegaly, which very often accompanies sustained malarial infection (Brown, 1969). In areas like Trinidad and Tobago, Costa Rica, and southeast India, where other causes of splenomegaly such as schistosomiasis are absent, the finding of an enlarged spleen upon palpation has served as a first indicator of malarial infection prior to a definitive diagnosis from a blood smear.

Three observations limit the universality of splenomegaly as an indicator of sustained malarial infection, without disqualifying it as an indicator in the situations to be described below. First, concurrent infections such as schistosomiasis and others may cause splenomegaly. Hence their presence must be attended to and ruled out. Second, the significance of splenomegaly depends on whether the malarial ecology is hyperendemic or holoendemic (Russell, West, Manwell, and MacDonald, 1963: 444). In hyperendemic areas, more than 50 per cent of the children aged 2 to 9 years have enlarged spleens, and this splenomegaly continues into adulthood, giving a high fraction of adults with enlarged spleens. In holoendemic areas, more than 75 per cent of the children aged 2 to 9 years have enlarged spleens, but only a small fraction of adults have enlarged spleens and adult tolerance of malaria is high. In these areas, spleens may reach a maximum size around age 10 years, then shrink progressively after adolescence to become "pancake spleens" as a result of continuous malarial assault (Richard H. Morrow, pers. commun.). Confusion about the level of malarial transmission by reason of this possible decrease in spleen size is averted by surveying school children or children in the age range of 2 to 9 years. Third, at least in rodents, the spleen is not necessary for marked resistance to sporozoite-induced infection (Nussenzweig, et al., 1972). In these animals, an absence of enlargement does not demonstrate an absence of infection or resistance.

Malaria surveys usually measure the splenomegaly in a population by a "spleen rate" or

TABLE 7

Malarial infections by spleen size in Egypt

SPLEEN INDEX	N	N_0	N_1	N_2	N_3	N_4	$\hat{\alpha}_1$	$\hat{\alpha}_2$	$\hat{\alpha}_3$	X^2	LLR
0	1543	1306	50	183	1	3	.034	.121	.001	1.846	2.742
PDI, P, 1	309	123	103	71	3	9	.362	.258	.012	31.082	37.633
2, 3, 4	110	25	62	20	1	2	.582	.199	.010	26.433	32.003

Based on data from Barber and Rice (1937).

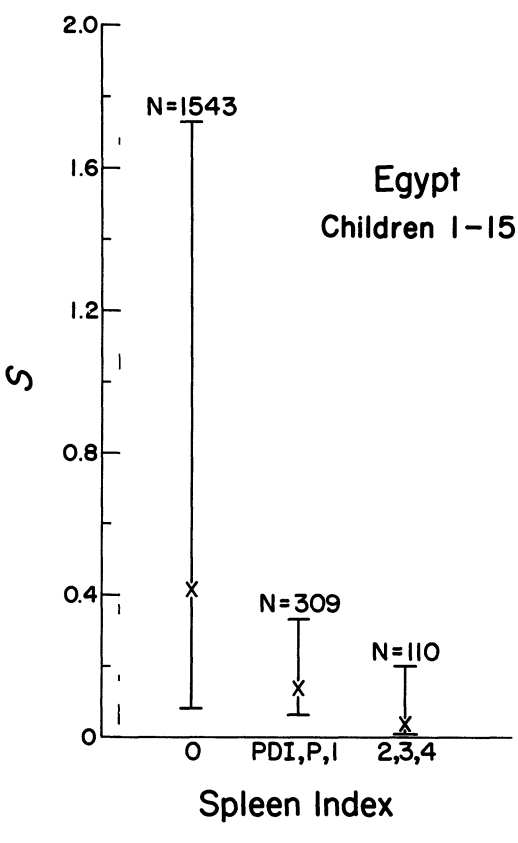

FIG. 7. ESTIMATES OF s AS A FUNCTION OF SPLEEN SIZE IN EGYPT

See Table 7, based on Barber and Rice (1937).

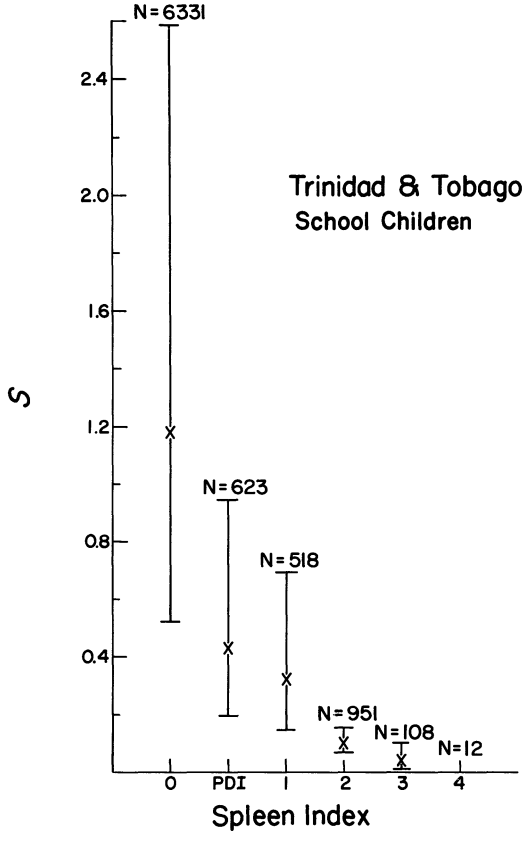

FIG. 8. ESTIMATES OF s AS A FUNCTION OF SPLEEN SIZE IN TRINIDAD AND TOBAGO

See Table 8, based on Downs, Gillette, and Shannon (1943).

TABLE 8

Malarial infections by spleen size in Trinidad and Tobago

SPLEEN INDEX	N	N_0	N_1	N_2	N_3	N_4	$\hat{\alpha}_1$	$\hat{\alpha}_2$	$\hat{\alpha}_3$	X^2	LLR
0	6331	5856	298	127	39	11	.049	.021	.007	.116	.253
PDI	623	425	97	60	30	11	.171	.109	.056	5.673	7.226
1	518	311	112	47	36	12	.236	.106	.082	11.435	13.973
2	951	294	338	159	114	46	.398	.200	.145	177.853	189.252
3	108	16	43	18	23	8	.462	.209	.263	42.252	46.783
4	12	6	2	2	0	2	.333	.333	0	.047	.734

Based on data from Downs, Gillette, and Shannon (1943).

by the distribution of a "spleen index." The spleen rate is the percentage of individuals examined who show any enlargement of the spleen. The spleen index is a scale of categories originally based on clinical criteria proposed by Hackett (Russell, West, Manwell, and MacDonald, 1963: 482–484). In order of increasing spleen size, the categories are 0 (no enlargement), PDI (palpable on deep inspiration), P (palpable), 1, 2, 3, 4, and 5. Different surveys omit some of these categories.

In a survey of Egyptian children aged 1 to 15 years (Fig. 7), children with no splenomegaly did not have significantly fewer mixed infections than expected in the absence of interaction between species of parasites. But children with spleens rated PDI, P, or 1 (among whom the distribution of infections was statistically homogeneous) had significantly fewer mixed infections than expected. Children with larger spleens (sizes 2, 3, or 4) had still fewer mixed infections. In this survey all of the mixed infections were double infections of *P. falciparum* and *P. vivax*.

In the malaria survey of Trinidad and Tobago, when the school children from all counties are combined and categorized by spleen size (Fig. 8), the children with no splenomegaly again had about as many mixed infections as expected from the model of no interaction, but the children with enlarged spleens had significantly fewer. As spleen size increased, the estimate of s decreased monotonically. The estimate of s for children with spleen index 4 is omitted, since there were only 12 children in that class.

In Taiwan, in each of two rounds of examinations in the course of a malaria control program

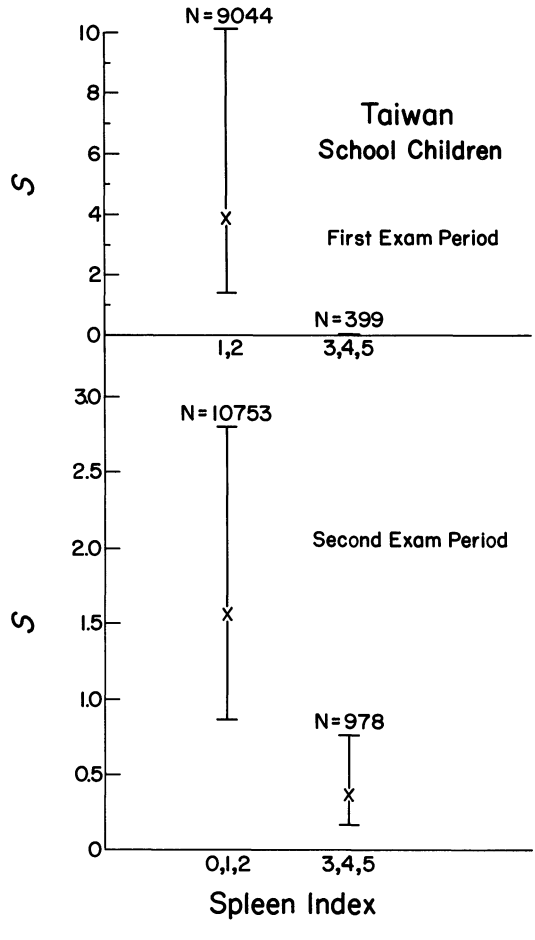

FIG. 9. ESTIMATES OF s AS A FUNCTION OF SPLEEN SIZE IN TAIWAN

See Table 9, based on Wu and Chuang (1956).

(Fig. 9), school children with spleen indexes of 3, 4, or 5 had significantly fewer mixed infections than expected with no interaction between

TABLE 9

Malarial infections by spleen size in Taiwan

SPLEEN INDEX	N	N_0	N_1	N_2	N_3	N_4	$\hat{\alpha}_1$	$\hat{\alpha}_2$	$\hat{\alpha}_3$	X^2	LLR
1,2 first exam period	9044	8767	71	189	10	7	.009	.022	.001	10.411	7.977
3,4,5 first exam period	399	325	29	44	1	0	.073	.110	.003	3.082	7.457
0,1,2 second exam period	10,753	10,032	317	366	18	20	.031	.036	.002	3.063	3.152
3,4,5 second exam period	978	680	167	93	26	12	.182	.105	.030	10.431	13.162

Based on data from Wu and Chuang (1956).

parasite species. Children with spleen indexes of 2 or smaller had as many mixed infections as expected, or more. No confidence interval is shown with the estimate of s for children in the first examination period with spleen indexes of 3, 4, and 5, but the *LLR* calculated in Table 9 demonstrates a significant deviation from the model based on no interaction.

In Pattukkottai Taluk, Madras, India, excluding infants (Fig. 10), the individuals with normal spleens had more mixed infections than expected. But those with splenic indexes of PDI, 1, 2, or 3 had significantly fewer. The estimate for people with indices of 4 is omitted, since only 46 people fell in this class.

Finally, Costa Rican children (Fig. 11) with normal spleens had just significantly more mixed infections than expected in the absence of interaction, but those with any degree of splenomegaly had significantly fewer mixed infections than expected.

In summary, every study found with usable data on the distribution of infections by degree of splenomegaly showed the same, clear pattern. Individuals with no enlargement of the spleen had as many mixed infections as would be expected in the absence of interaction between parasite species, or sometimes more. On the other hand, individuals with splenomegaly had significantly fewer mixed infections than would be expected. As the degree of splenomegaly increased, the ratio of observed to expected mixed infections never increased, and it generally declined.

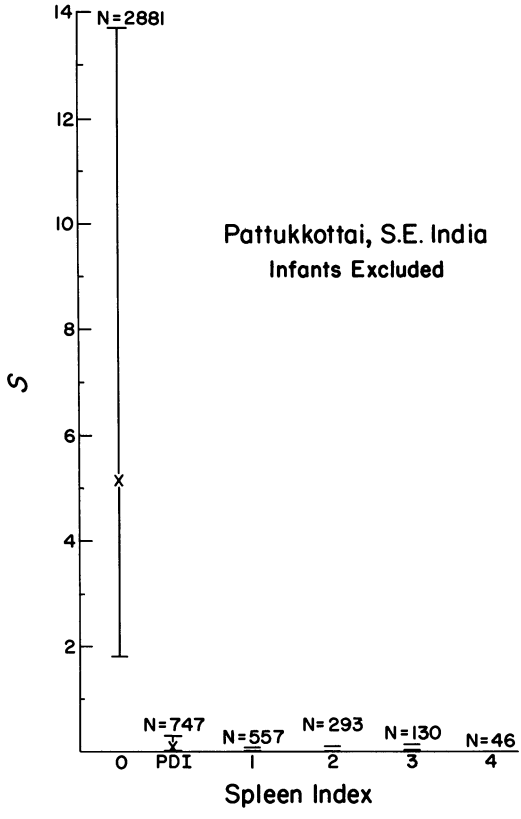

FIG. 10. ESTIMATES OF s AS A FUNCTION OF SPLEEN SIZE IN PATTUKKOTTAI TALUK, MADRAS, INDIA

See Table 10, based on Russell, Menon, and Rao (1938).

POSSIBLE EXPLANATIONS

The preceding analyses of published data on the distribution of malarial infections may be summarized briefly. (1) As the parasite rate (percentage of the whole population with one or another infection) increases from one area to another, s does not increase and may decline, where s is the ratio of the number of mixed infections observed to the number that would have been expected if the presence of one

TABLE 10

Malarial infections by spleen size in Pattukkottai, Madras, India

SPLEEN INDEX	N	N_0	N_1	N_2	N_3	N_4	$\hat{\alpha}_1$	$\hat{\alpha}_2$	$\hat{\alpha}_3$	X^2	LLR
0	2881	2746	83	45	0	7	.031	.018	0	15.383	10.639
PDI	747	374	266	91	7	9	.368	.133	.010	43.093	54.005
1	557	102	319	113	9	14	.598	.227	.018	186.337	198.666
2	293	32	186	54	4	17	.692	.239	.018	101.731	101.266
3	130	11	86	20	2	11	.746	.232	.024	36.793	37.597
4	46	27	14	3	0	2	.348	.109	0	.057	.066

Based on data from Russell, Menon, and Rao (1938).

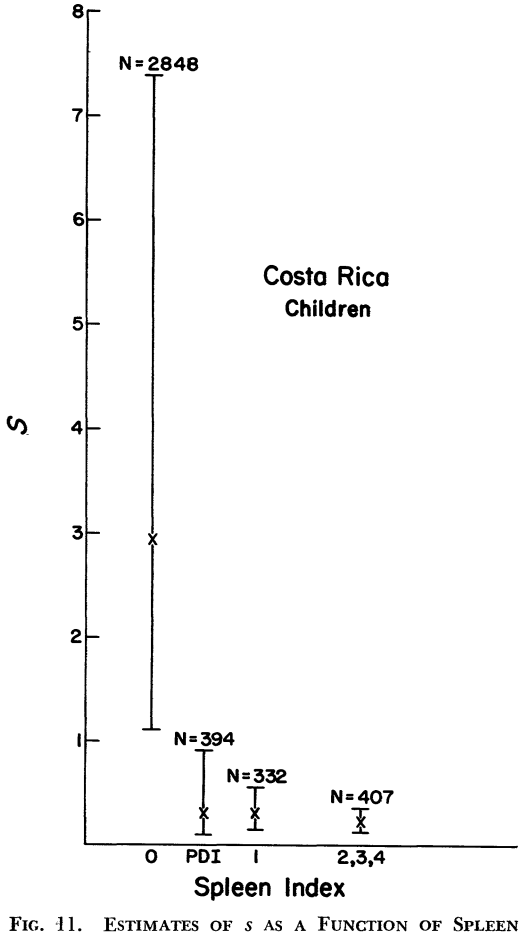

FIG. 11. ESTIMATES OF *s* AS A FUNCTION OF SPLEEN SIZE IN COSTA RICA

See Table 11, based on Kumm and Ruiz-S. (1939).

species of parasite in an individual were independent of the presence of another. (2) Where the ecology of an area favors transmission of the parasites, *s* tends to be lower than where the ecology is unfavorable. These two findings are consistent, and (1) may be a consequence of (2). (3) By itself, age bears no determinate relationship to *s*, although it is strongly related to the prevalence of each parasite separately. Several studies appeared to show fewer mixed infections than expected in children between the ages of 5 and 9 years. (4) Individuals with normal spleens had about as many mixed infections as expected, or even more. But individuals with enlarged spleens had significantly and strikingly fewer mixed infections in every study which could be located. Moreover, *s* appeared to decline with increasing splenomegaly.

The purpose of this section is to consider six possible explanations, other than genuine heterologous immunity, for the relative reduction in the number of mixed infections associated with splenomegaly.

First, the parasite species might possibly be localized in different, largely disjunct regions, allowing no opportunities for mixed infections to occur; but the sample on which the estimate of *s* is based could have been pooled over these regions. For the survey of Trinidad and Tobago (Fig. 8), this possibility is ruled out by the data given in Table 1 on the distribution of infections by county: all three species of parasites are found in all counties (although there are some variations in prevalence from county to county). A similar assurance is provided by data (not quoted here) on the distribution of parasites in Egypt (Barber and Rice, 1937: 418–419), in Pattukkottai Taluk, India (Russell, Menon, and Rao, 1938: 313–318), and in Costa Rica (Kumm and Ruiz, 1939: 435).

Even where information on the distribution of infections by small geographical units is not available, were geographical heterogeneity solely responsible for the reduction in *s* among individuals with enlarged spleens, the same heterogeneity ought also to reduce *s* among individ-

TABLE 11

Malarial infections by spleen size in Costa Rica

SPLEEN INDEX	N	N_0	N_1	N_2	N_3	N_4	$\hat{\alpha}_1$	$\hat{\alpha}_2$	$\hat{\alpha}_3$	X^2	LLR
0	2848	2692	56	45	47	8	.022	.018	.018	6.709	6.016
PDI	394	266	30	40	52	6	.085	.112	.144	6.092	8.202
1	332	154	54	42	64	18	.201	.160	.234	15.982	18.213
2,3,4	407	156	77	75	72	27	.236	.231	.222	36.981	40.189

Based on data from Kumm and Ruiz-S. (1939).

uals without enlarged spleens or when individuals are grouped by age rather than by spleen index. Thus the failure of s to differ significantly from 1 among individuals with normal spleens provides an internal control against artifacts due to geographical heterogeneity.

Second, children with mixed infections might possibly suffer from increased rates of death or sickness, and hence be less likely to survive for or appear in a malaria survey, than children with single infections. To make this slightly vague notion precise, supose that the distribution of infections in the population is as predicted by the model of no interaction between parasite species, prior to any deaths from the disease. Suppose that all uninfected individuals appear in the malaria survey; and that for each species of parasite some fraction of individuals originally infected only with that species will be included in the malaria survey. Then, if the fraction of individuals with mixed infections who appear in the malaria survey is exactly the product of the fractions corresponding to the species with which they are infected, the estimate s of interaction between species will remain 1, the true value for the original population. Individuals with mixed infections will be considered as subject to excess mortality or morbidity only if the fraction which appears in the survey is smaller — that is, only if there is a destructive synergism between or among the infecting species.

But there is no evidence that the contributions to sickness and death of the species are not in fact independent. In Mandailing (Table 6), the change in prevalence of each species with age might be due, at least in part, to the superior survival of uninfected persons. But since the prevalences of the three species could be described as independent over all ages (except perhaps 6 and 7), and since Fig. 6 shows no monotonic trend in s away from the value 1, there is no evidence that individuals with mixed infections are subject to any excess mortality or morbidity. The similar lack of a consistent decline in s with age in the other seven studies relating age to infection also argues against this explanation of low values of s.

Third, malarial species might possibly appear alternatively in the peripheral blood which is sampled in a survey, even though both are present in the body at once; hence single-species infections may be observed whereas mixed infections actually occur (Yorke and Macfie, 1924; James, 1931).

If alternation of species in the peripheral blood caused the low values of s among individuals with enlarged spleens, then the same cause should have reduced s among individuals with normal spleens, unless splenomegaly or factors leading to it increases the alternation in hosts with mixed infections. I know of no evidence that an enlarged spleen has this effect.

A related possibility, that a parasitemia with one malarial species might depress a parasitemia with another, might also lead to reduced diagnoses of mixed infections without reducing the true prevalence of mixed infections. If such a depression were associated with splenomegaly, as it would have to be for consistency with the data, it would be evidence for a heterologous resistance within the host, rather than at the level of the population of hosts. Again, I know no evidence to substantiate this depression and its association with splenomegaly for humans.

Fourth, in poor laboratory technique, diagnosis of a blood smear might stop or tend to stop after the discovery of a single parasite species, especially if the infection with one species is much more massive than the infection with others. Zuckerman, Abzug, and Burg (1969) showed that if rats were treated with methyl cellulose long enough to develop a sterile splenomegaly, comparable to that induced in rats during the early acute rise and chronic and latent phases of malarial infection, then the anemias in the treated and infected rats were also comparable. Hence the enlargement of the spleen alone sufficed to account for the anemia of malarial infection (outside of the period of peak parasitemia). The implication of this finding for the observed association between splenomegaly in humans and the relative rarity of mixed infections depends on the stopping rule used in laboratory diagnosis.

If a fixed *volume* of blood was examined, then the anemia associated with splenomegaly could contribute artifactually to the rarity of mixed infections, because fewer potentially parasitized blood cells would be subject to examination in anemic individuals with spleno-

megaly. I do not know whether the extent of anemia, and corresponding reduction in the sample size of blood cells examined, is sufficient to account for the observed relative reduction in the number of mixed infections, but it seems unlikely.

On the other hand, if a fixed *number* of red cells was supposed to be examined, then the observations on normal individuals rule out the possibility that laboratory technique tended to stop after the discovery of a single parasite species; for these individuals had about as many mixed infections as expected.

Fifth, Benjamini and Feingold (1970: 1085–1089) have reviewed circumstantial evidence that mammalian hypersensitivity to bites by arthropod vectors may interfere with the vectors' transmission of infections. This evidence suggests that the relative reduction in mixed infections in individuals with enlarged spleens is real, but that the principal reason for it is not immune responses to the antigens of *Plasmodium* but rather hypersensitivity to mosquito bites. However, there is no direct evidence that hypersensitivity to mosquito bites interferes with the transmission of malaria to any warm-blooded host. If hypersensitivity did affect substantially the transmission of malaria, one might expect it to have an increasing effect with age, as the exposure to mosquito bites of a cohort living in an endemic area increased. Subject to the sampling error of the surveys which related infections to age, no such effect appears.

Sixth, if two or more malaria species require a common nutrient or nutrient complex in order to proliferate, and if the first species to be inoculated exhausts the supply of this nutrient, then the host could display a heterologous resistance independently of any immune responses. The ecologists calls this interaction competition for food. The shortage of a nutrient would have to be associated with splenomegaly. Moreover, the more antigenically similar the two kinds of malaria, the greater the overlap would have to be in their nutrient requirements. For lack of the relevant biochemical studies, this possibility simply cannot be evaluated at this time.

This review of six alternative explanations, other than heterologous immunity, for the decline of s with increasing spleen size leaves open the possibilities that an enlarged spleen might increase the alternation of parasites in the peripheral blood, that hypersensitivity to mosquito bites might reduce mixed infections, or that a limiting nutrient might provide the mechanism of a genuine heterologous resistance, although one not involving heterologous immunity.

The argument in favor of immunological mechanisms is supported by analogy from the nonhuman animal experiments and two further bits of evidence. MacGregor, Carrington, and Cohen (1963) treated *P. falciparum* in East Africans with IgG extracted from the sera of West Africans infected with *P. falciparum* (presumably a different strain). Protection was afforded only by "immunized" IgG. Secondly, Voller, Lelijveld, and Matola (1971) reported that, in humans, enlarged spleens are associated with high titers of IgM, which are in turn associated with high levels of malarial antibodies.

Clearly, it is also possible that immunological and other mechanisms may contribute jointly to the demonstrated rarity of mixed infections associated with enlarged spleens.

CONCLUSIONS AND IMPLICATIONS

The first thesis of this paper is that under certain circumstances there is a reduction in the number of mixed malarial infections in human beings relative to the number that would be expected if the presence of one species of malaria in a person were independent of the presence of each of the other species. This reduction is strongly associated with splenomegaly, and is weakly associated with situations that stimulate immune responses, such as an ecology favorable to malaria and high parasite rates. This reduction is significant both statistically, by the usual probabilistic criteria employed in testing data against a null model, and quantitatively, in that the reduction in the number of mixed infections is very substantial among individuals with enlarged spleens. In Taiwan, in the second examination period (Fig. 9), the ratio s of the observed number of mixed infections to the number expected in the absence of interaction was less than 0.4 among children with enlarged spleens. In Costa Rica (Fig. 11), among children with spleen indexes of 2, 3, and 4, s was estimated to be less than 0.25.

In Egypt, India, and Trinidad and Tobago (Figs. 7, 8, and 10), at the largest spleen sizes, children had less than 0.1 as many mixed infections as expected.

This conclusion is contrary to received doctrine, which holds that human resistance to malaria is highly specific (Taliaferro, 1949: 491; Russell, West, Manwell, and MacDonald, 1963: 426; Bruce-Chwatt, 1967: 355; Brown, 1969: 279–280).

The second thesis of this paper is that, at present, this resistance is best explained as a partial heterologous immunity to malaria in man. Experiments show that mammalian immunity can eliminate or reduce the level of mixed infections. Evolutionary arguments show the adaptive value to the host of such mechanisms. Other hypotheses which might explain the reduction in mixed infections as an artifact or as a result of other mechanisms are either not consistent with all the observed data or are not at present known to be sufficient to account for them.

The practical import of this conclusion, if it is true, is that a malaria vaccine need not be specific to each of the species, strains, or antigenic variants of *Plasmodium* in order to be highly effective. But since, apparently (Nussenzweig, et al., 1972), host resistance is highly specific to the stage of the malaria life cycle, it is important to know which stage contributes most importantly to heterologous resistance. All the field data on human infections that have been reviewed here are based on the blood stages of the malarial parasite. These data do not differentiate between nor reveal the relative effect of heterologous resistance at the sporozoite stage of the life cycle and heterologous resistance at later stages. Clearly, both kinds of resistance could contribute to the observed rarity of mixed infections.

Some information about the relative roles of sporozoite versus blood stages in heterologous immunity could be gathered by comparing the rate of clearance of sporozoites from blood samples taken from human volunteers with long previous histories of malarial infection, and the clearance rate of sporozoites from blood from comparable human volunteers with less, or no, previous infection. Further efforts toward the development of a vaccine could be concentrated on the stage or stages most effective in stimulating a resistance which is as heterologous as required by the local ecologies of malaria.

One problem, the difficulty of which is not to be underestimated, is to mobilize immunological defenses against the complex of malarial antigens without causing the pathology associated under natural circumstances with severe infection and splenomegaly. This problem may be easier if the attack can begin on the sporozoites, since the spleen may in that case not be involved. It cannot be argued that an enlarged spleen is good for anyone. But a nonpathogenic malaria vaccine which mobilized the immune responses that accompany the enlarged spleens of children in Egypt, India, and Trinidad and Tobago, if delivered to all of the population, could be expected to reduce the natural infections (which would then appear immunologically as mixed infections) to one-tenth or less of their former level.

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