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Population studies of *Plasmodium vivax*. 1. The theory of polymorphism of sporozoites and epidemiological phenomena of tertian malaria

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The understanding of clinico-epidemiological phenomena of tertian malaria has been the subject of controversy. The authors suggest a system of postulates which give a non-contradictory explanation of the phenomena of relapses and long incubation. The main idea is that the duration of exoerythrocytic development of Plasmodium vivax *is a polymorphic characteristic controlled by a set of genes. According to these postulates sporozoites may be subdivided into two groups designated as tachysporozoites and bradysporozoites, responsible for early and late manifestations, respectively. The logical analysis of the system suggests that it does not contradict the experimental facts. Moreover, the theory of polymorphic sporozoites permits an explanation and quantification of interrelations between different phenomena. The authors stress that the variation of genes is much greater in natural populations of parasites than in individual isolates and strains and therefore that the features of strains do not fully reflect the features of populations. Classical laboratory experiments should be combined with epidemiological experiments which allow a study of the population as a whole. The methodology of experiments to be undertaken in further investigations of the long latency period is discussed.*

Tertian malaria has two particular features that are probably interrelated: firstly, the incubation period may be short (12-20 days) or long (6 months or more); and secondly, periods of parasitaemia alternate with periods when parasites are absent from the blood (periods of latency). Relapses that take place after latency are designated as " true relapses ". With the exception of vivax malaria, true relapses occur only in a few primate malarias, namely in *Plasmodium cynomolgi*, *P. fieldi*, *P. ovale*, *P. simiovale*, and *P. schwetzi* infections. From the type of incubation period and the type of latency period, three different strains of *P. vivax* have been distinguished (Fig. 1).

It is evident that the ability of *P. vivax* to become active again after several months, or even several years, of latency hinders eradication of this species

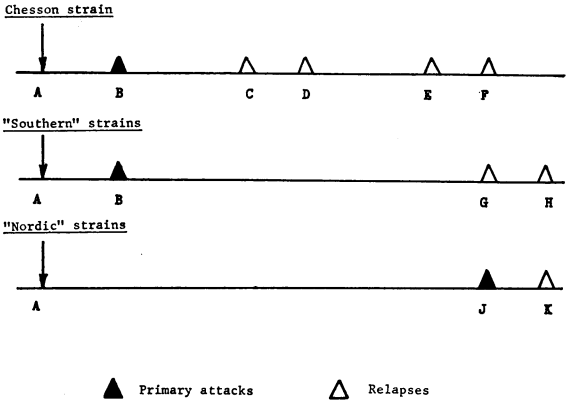

Fig. 1. The phases of tertian malaria in different strains of *Plasmodium vivax*. A = the moment of the infection; B = primary manifestations after short incubation period; C, D, E, F, G, H, & K = relapses; J = primary manifestations after long incubation period.
Periods of latency: B-C, B-G, & J-K = pre-relapse periods; C-D, D-E, E-F, & G-H = inter-relapse periods.
Incubation periods: A-B = short incubation period; A-J = primary long latency (long incubation period).

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from the foci; therefore, an explanation of the mechanism of latency may have great practical importance. Several major theoretical works on this subject have recently been published (*11*, *7*, *21*, *39*, *17*) but there is still no theory that fully explains all the phenomena related to relapses and long incubation periods. In a recent article, Coatney (*6*) discussed relapses in tertian malaria as being an enigma. It seems, however, that the facts so far collected can be explained from the point of view of population genetics.

THE MAIN POSTULATES

We have made an attempt to build a non-contradictory logical system based on a few postulates. It was considered essential that these postulates did not contain any statement that had already been disproved; however, if a statement had not yet been confirmed it could be incorporated into the system.

As the basis of our system we chose four postulates; some of them are not new but, as a whole, the theory that we propose is formulated for the first time.

The first postulate: exoerythrocytic (EE) schizogony is a direct (non-cyclic) process

The well known fact that merozoites do not emerge in the blood simultaneously after a single inoculation of sporozoites may be explained in two ways:

1. the development of EE schizonts is cyclic (EE schizont → merozoite → EE schizont, etc.) and relapses are provoked by some merozoites that deviate from this cyclic development and appear in the blood;

2. the development of the progeny of a set of sporozoites is asynchronous, the development of some EE schizonts being retarded in the liver while the progeny of other schizonts is already developing in the red blood cells.

The first explanation was given by Shortt & Garnham (*29*) and for a long time it remained the prevailing point of view. It was adopted by the authors of the *Terminology of malaria and of malaria eradication* (*31*) and is still prevalent in the literature, though it has not yet been confirmed experimentally. Moreover, several facts disproving this point of view have been collected; these were excellently presented by Coatney et al. (*7*). The existence of a paraerythrocytic cycle was subsequently denied by a WHO Scientific Group on Developments in Malaria Immunology (*37*).

The second explanation seems to be simpler and more realistic. It was first suggested by Shute (*30*) and was later supported by Lysenko (*14*, *15*, *17*), Yoeli (*38*), Moškovskij (*21*), Coatney et al. (*7*), and others. The asynchronous character of the passage of merozoites into the blood may be explained by the slow maturation of some EE schizonts or, more probably, by an interruption in the development of sporozoites, EE schizonts, or some hypothetical transitional stage of the parasite that remain dormant for different periods of time. During dormancy, the metabolic rate of the parasite is low, thus drugs inhibiting cell multiplication (pyrimethamine, etc.) are not effective.

Lysenko (*17*) designated sporozoites that develop into EE schizonts immediately as tachysporozoites (TS) and those sporozoites that develop only after a period of dormancy, bradysporozoites (BS).

As the slow-maturing EE schizonts seem not to be descendants of the immediately-developing forms and as EE development seems not to be cyclic, the terms " primary " and " secondary " EE schizonts and " paraerythrocytic cycle " should be avoided. The terms " paraerythrocytic development " or " paraerythrocytic forms " are, howewer, quite correct and indicate that erythrocytic and EE forms coexist in the same host.

The second postulate: sporozoites are polymorphic, and the duration of EE development of the progeny of an individual sporozoite is a polymorphic characteristic

Though the genetics of plasmodia has not yet been intensively studied, several important findings have been made during the past decade. It has been demonstrated that *P. falciparum*, *P. vivax*, and rodent plasmodia possess several polymorphic characteristics: isoenzyme types, drug resistance, and antigenic composition (*36*, *24*). Natural populations of *P. falciparum* have also been found to be polymorphic (*4*, *5*).

It is possible that the duration of EE development is also a polymorphic characteristic, which may be " any phenotypic character, be it morphological, physiological, or behavioural, provided it is genetically controlled and more or less discontinuous " (*19*).

The third postulate: the duration of EE development is controlled by several loci

This postulate is proposed in order to explain the following phenomenon: the bite of a mosquito fed

on a patient with a late manifestation of malaria can provoke malaria after a short incubation period and *vice versa* (*1*). As plasmodia are haploid, the hidden transmission of a character cannot be explained by the recessive nature of the corresponding allele as in diploid organisms.

This apparent contradiction made Bray (*2*), and later Coatney (*6*), doubtful about the validity of the theory proposed by Moškovskij (*21*). They did not take into account that Moškovskij had postulated the hidden genetic transmission of the character, though he had not studied the mechanism of such a transmission. If the duration of EE development is controlled by several loci, haploid organisms of the same phenotype may possess different genetic compositions. Reproduction of such organisms may lead to the formation of a new phenotype, not identical to the parental one.

The fourth postulate: the progenies of sporozoites belonging to different phenotypes form independent lines of erythrocytic schizonts

We do not consider cases where patients have a high level of immunity; such cases are common in hyperendemic conditions. But even if the level of immunity is low, an observer is not always able to distinguish separate waves of parasitaemia resulting from separate waves of EE schizogony. If successive broods of tissue merozoites appear shortly one after another, their manifestations are superimposed and can be distinguished only if a blood schizonticide is given after each emergence of parasitaemia. When such treatment was applied to *P. cynomolgi* infection, the number of distinct manifestations (relapses) considerably increased (*7*).

Yoeli (*39*) demonstrated two waves of EE schizogony in *P. berghei* by a direct observation. The development of one brood of EE schizonts took 48 hours. The second brood of EE schizonts completed its development only 96 hours after inoculation.

It is possible that the same phenomenon also occurs in falciparum malaria. There is an obvious contradiction between this theory and practical experience. The theory suggests that EE schizogony is short in this species (not exceeding one week) and that true relapses are nonexistent. On the other hand, nonimmune subjects who have lived in an endemic area are advised to continue chemoprophylaxis for four or even eight weeks after arriving in a nonendemic area. Vachon (*35*) mentioned a case of cerebral malaria occurring on the 42nd day after the return from an endemic area, in spite of chemoprophylaxis of 3 weeks duration. This contradiction may easily be explained by assuming that, in falciparum malaria, some EE schizonts are also retarded in their development, but that the period of latency lasts for days or weeks, not for months as in *P. vivax*.

POLYMORPHISM OF NATURAL POPULATIONS AND CLINICO-EPIDEMIOLOGICAL PHENOMENA

The development of *P. vivax* in the vertebrate host is described in terms of four interrelated processes (Fig. 2): a mosquito inoculates a heterogeneous

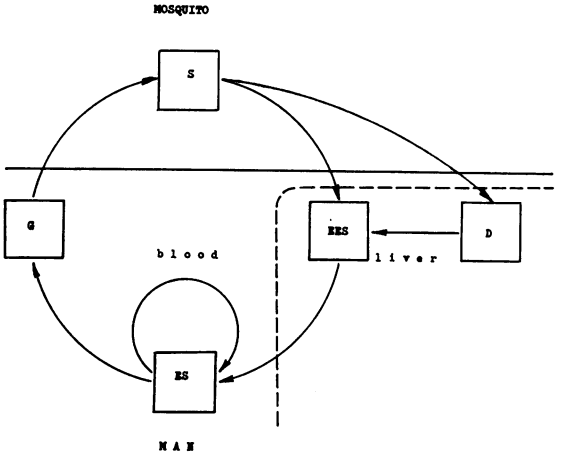

Fig. 2. Life cycle of *Plasmodium vivax*. EES = exo-erythrocytic schizogony; D = dormancy; ES = erythrocytic schizogony; G = gametogony; S = sporogony.

mixture of sporozoites; tachysporozoites (TS) begin developing immediately; bradysporozoites (BS) develop after a more or less protracted period of dormancy; and the duration of dormancy is predetermined genetically. If this were true, the course of infection would be determined by the composition of the inoculum.

Type of sporozoite	*Designation of the infection*	*Clinical interpretation*
TS	pure TS infection	short incubation without late relapses
BS	pure BS infection	long incubation
TS + BS	mixed infection	short incubation with late relapses

The development of parasitaemia with time is shown in Fig. 3. The same curve of parasitaemia

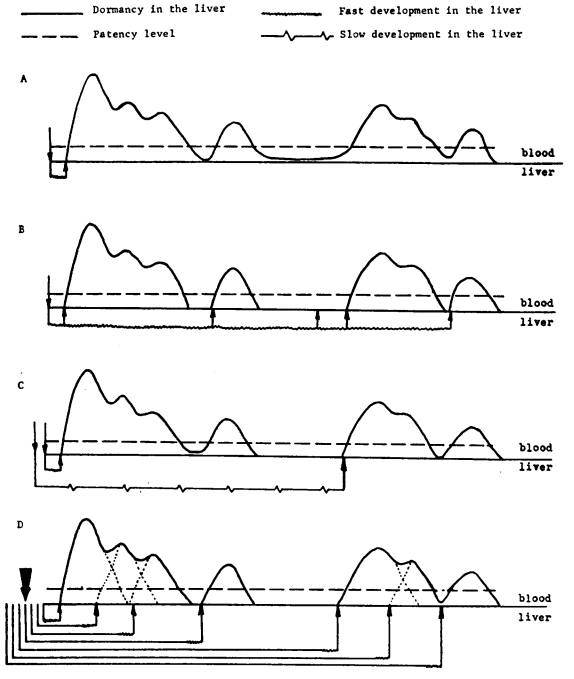

Fig. 3. The course of development of *Plasmodium vivax*, after different theories.

may be interpreted in different ways. Fig. 3 (A) reflects the point of view of Moškovskij (*20*), Corradetti & Verolini (*10*), and Corradetti (*9*): the erythrocytic schizogony is constant but sometimes subpatent. Fig. 3 (B) reflects the theory of the paraerythrocytic cycle proposed by Shortt & Garnham (*29*), adopted later by WHO (*31*). Fig. 3 (C) reflects the idea of dimorphism of schizonts that was supported by Shute (*30*), Lysenko (*16*, *17*), Yoeli (*38*), and Coatney et al. (*7*).

The latter point of view seems to be the most likely, but it is probably over-simplified. It is difficult to explain the nature of a serial pattern of late relapses on the basis of this view and the relapse pattern of the Chesson strain, in which there is no marked delimitation between early and late manifestations, also remains inexplicable. It therefore seems necessary to proceed to the more complicated explanation shown in Fig. 3 (D). There are probably not two, but several phenotypes which differ as regards the duration of dormancy, and TS and BS are likely to be more or less complex groups of phenotypes, rather than two homogeneous entities.

The existing terminology of manifestations of malaria distinguishes between the following categories of manifestations of the disease: firstly, primary manifestations and relapses; secondly, early and late relapses; and thirdly, manifestations after a short incubation period and manifestations after a long incubation period. The first consideration is important: however it seems that the place of a given manifestation in a sequence of manifestations does not depend on any substantial feature of the parasite but rather on a random combination of different types of sporozoites. A manifestation caused by BS can be secondary if the subject was infected with a mixture of BS and TS, or primary if he contracted only BS. The third consideration is the most important because the time required for EE development is an essential feature of the parasite; this leads us to distinguish *early* and *late* manifestations (both may be subdivided into primary attacks and relapses). It may also be useful to distinguish *intermediate* and *ultra-late* manifestations. The former occur 2–5 months after inoculation. Primary manifestations of this kind were observed by Nikolaev (*23*) and Tiburskaja et al. (*34*). The ultra-late manifestations occur one year or more after inoculation; they also may be primary (*1*, *33*).

Each case of infection by *P. vivax* may be thought of as the result of several acts of infection. The number of these acts corresponds to the number of phenotypes that have been inoculated. These multiple acts of infection may take place at one moment when large amounts of sporozoites are inoculated, as happens in artificial infections, or they may be distributed over a period of several weeks, as usually happens under natural conditions.

Let us suppose that there is a heterogeneous set of sporozoites. The development of the infection will depend on the number of sporozoites that have grown to the stage of mature EE schizonts (n); it will also depend on the proportions of the TS and BS in the sample (T and B, respectively; $T+B = 1$). The larger the number of sporozoites, the greater is the probability of the presence of both types of sporozoites in the sample. The distribution of probabilities will fit the expansion of a binomial $(T+B)^n$. The probability of pure TS infections is T^n; of pure BS infections, B^n; and of mixed infections, $1-T^n-B^n$.

The probability of pure infections sharply decreases with an increase in the infecting dose, and when n is large this probability is low, despite an overwhelming prevalence of the corresponding type

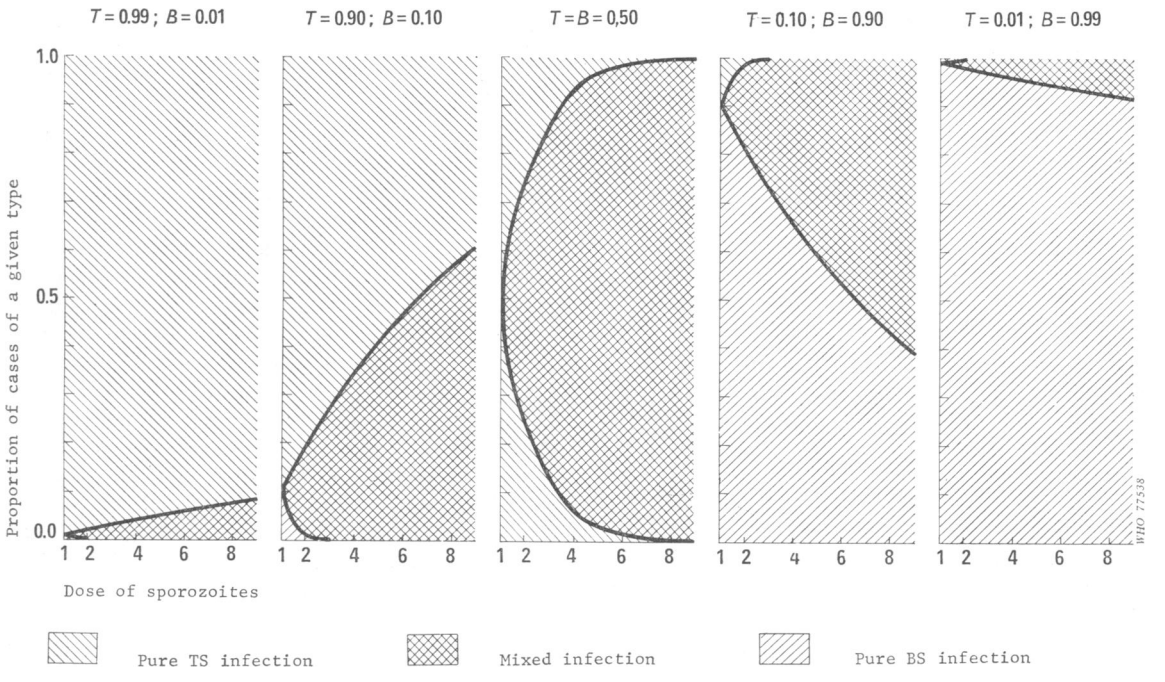

Fig. 4. Frequency distribution of different types of infection according to the dose of sporozoites and the proportions of TS and BS in the inoculum. T = proportion of TS; B = proportion of BS.

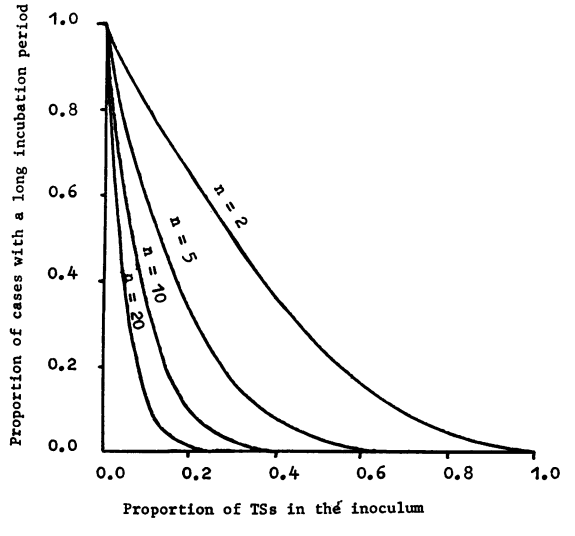

Fig. 5. The frequencies of cases with long incubation periods according to the dose and the proportion of TS in the inoculum. n = dose of sporozoites.

of sporozoite; the majority of cases in fact are of mixed infections (Fig. 4). Usually, only pure BS infections are taken into consideration in the analysis of long incubation periods. As shown in Fig. 5, the probability of a pure BS infection is considerable only when T and n are both small. The participation of BS in mixed infections is not taken into account and the frequency of BS is strongly underestimated. For this reason, the idea still prevails that there is a very low incidence or even of an absence of parasites with delayed activity in the subtropics and tropics.

The chances of obtaining a pure BS infection are particularly low in artificially induced infections because the recipient usually receives very large and uncontrolled amounts of sporozoites. On the other hand, if the number of sporozoites is small, the variability of phenotypes decreases, the number of manifestations per person drops (*8*, *41*), the probability of a pure BS infection increases, and therefore a strain that has not previously resulted in long incubation may begin to do so (Garnham, quoted by Bray (*2*)).

ADVANTAGES AND DISADVANTAGES OF LABORATORY AND FIELD EXPERIMENTS

Laboratory experiments are thought by many investigators to be crucial, even if the results are negative. On the other hand, laboratory methods have several limitations, as demonstrated above. It is high time to weigh up the advantages and disadvantages of laboratory experiments in studying the problem of relapses and long latency.

The advantages of laboratory experiments are that: the moment of infection is known; it is possible to observe a patient continuously; and there is a negligible risk of re- and superinfection. The disadvantages are as follows: a large and often an uncontrollable dose of infection; underestimation of the number of late manifestations (because it is often impossible to wait long enough for the beginning of symptoms in the case of a long incubation period in a psychiatric patient); the impossibility of early and systematic administration of blood schizonticides to record the number of waves of EE schizogony; the moderate size of samples; the impossibility of studying the population of parasites as a whole and the restriction of the study to a very limited part of the population, an isolate. It should be noted that some of these disadvantages can be overcome.

The results of laboratory experiments have very often been interpreted in such a way that the properties of individual isolates have been ascribed to the totality of parasites circulating in a given area. Such interpretations remain prevalent, as demonstrated by a wide use of phrases such as: " strains with short incubation circulate in this area ". Statements of this kind are incorrect, not only terminologically, but also semantically. Strains are artificial sets of parasites.[a] In nature, there are neither strains nor combinations of strains, but only populations. The gene pool of a population is much richer than the gene pools of strains. The latter are limited from the outset and become less variable during their maintenance in the laboratory, due to inevitable inbreeding. Of course, a strain represents the population to some extent, but to judge the properties of a population by the properties of a strain is like judging the sea by a drop of water. We stress that the study of the phenomenon of latency should be based both on laboratory and field experiments.

The advantages of field experiments are: the large number of possible observations and the possibility of studying the population as a whole. The disadvantages are: the lack of information about the exact moment of infection of each patient; the impossibility of measuring directly the dose of infection; and the uncertainties connected with migrations. In the past, the possibilities of field experiments were limited by the uneven response of various population groups to the infection. Now, in the countries where antimalaria campaigns are in progress, even where residual transmission still occurs, the level of immunity has dropped dramatically because of the early detection and treatment of cases (*3*, *18*) and this limitation is no longer important. The possibilities of epidemiological experiments have also increased because new effective drugs have become available. For example, in areas where there is effective surveillance, the use of chloroquine makes it possible to record separately multiple infections occurring during one epidemiological season which, under other circumstances, would have been recorded as the result of a single act of transmission. Drugs acting selectively at particular stages of parasite development have become an important tool of epidemiological research.

We have attempted to investigate the distribution of manifestations in foci of malaria, bearing in mind the theory of polymorphism of sporozoites. The results of this investigation will be published as the second part of this paper (*26*). In general, they are in compliance with the theory.

SOME CONSIDERATIONS ON THE EVOLUTION OF *PLASMODIUM VIVAX*

The area of speciation of *P. vivax* was somewhere in the tropics, most probably in South-East Asia (*28*). We suppose that the prototype of *P. vivax* was the richest in genes controlling the duration of latency. The South-East Asian populations follow the relapse pattern of the Chesson strain (multiple true relapses occurring throughout the course of infection, without any concentration in a specific period).

In moving to temperate zones, *P. vivax* lost some of the phenotypes responsible for the intermediate manifestations, and a bimodal pattern of activity appeared. It is possible that these intermediate phenotypes are not completely lost and that they are responsible for true relapses occurring early after the infective bite and for the rare cases with an intermediate incubation period not exceeding six months.

[a] According to a recent definition, a " strain is a group of organisms isolated from a wild population on one occasion and maintained in the laboratory " (*13*).

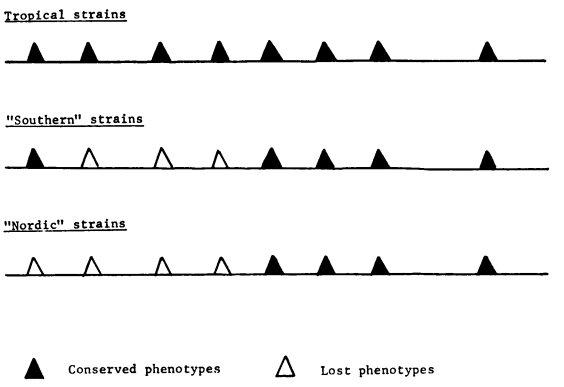

Fig. 6. The spectrum of phenotypes of different strains of *Plasmodium vivax*.

Finally, *P. vivax* lost all phenotypes without latency, and the long incubation period of the " Nordic strains " appeared (Fig. 6).

Nikolaev (*22*) suggested that the " Nordic " and " Southern " strains of *P. vivax* were subspecies, *P. v. hibernans* and *P. v. vivax*. But it is now clear that *P. v. hibernans* does not meet the current criteria for a subspecies (*19*). Subspecies are now considered as a complex of populations; Nikolaev dealt with isolates, and some of the isolates that he attributed to different subspecies might have belonged to the same populations. It is surprising that Garnham et al. (*12*) considered *P. v. hibernans* to be a valid subspecies, although this statement was not based on the facts given in their interesting paper.

It is well known that populations cannot exist without the recombination of genes and the creation of new gene combinations. According to the third postulate (above), recombination and the formation of new gene combinations may take place when a mosquito is infected by a pair of genetically different gametes that may be of different phenotypes or of the same phenotype. Recombination is facilitated if the mosquito is infected with several pairs of gametes when feeding on one or on several carriers. As a result, one mosquito may harbour a heterogeneous mixture of sporozoites. It is thus probable that a person may be inoculated with heterogeneous parasites even after the bite of a single mosquito.

A population reacts to selective pressures like a single system. Under conditions of seasonal transmission, parasites that become reactivated in periods when effective infection of vectors is impossible are eliminated. Some phenotypes therefore become uncommon or disappear. Other factors may also be involved in selection. As a rule, the action of genes is pleiotropic. If genes control the duration of EE development, then a specific duration of development might be regularly correlated with some other characteristics, such as temperature requirements or survival rate. In that case, temperature and other environmental factors might act directly by eliminating phenotypes with a certain duration of development. The results of experiments by Yoeli et al. (*40*) may be interpreted in this way. The authors infected rats with *P. berghei* and demonstrated that in rats maintained at a low temperature the infection rate was lower, the EE schizonts were of a peculiar morphology, and the beginning of parasitaemia was delayed. This might be explained by a modification of parasites under the direct influence of the lowered temperature or, more probably, by the elimination of quickly developing parasites from the mixture of EE forms. The development of the infection would then be determined by the forms with delayed activity which otherwise would not be detectable.

New factors have been added to those which have operated for centuries. Among them are the antimalaria activities, which lead, first of all, to an elimination of forms with early activity.

CONCLUSIONS

The theory described above cumulates the ideas of several authors. It seems to be in good agreement with the facts; at least, we do not know of any fact that could not be explained on the basis of this theory. Acceptance of this theory involves several new considerations. The most important of them are the following.

1. The controversy on the subject of the prevalence of long incubation periods in different geographical zones results from an incorrect discrimination of phenotypes causing long incubation periods. We suggest that these phenotypes are widespread all over the area of distribution of *P. vivax*, but that in areas closer to the equator their presence is camouflaged by the high prevalence of phenotypes that cause short incubation periods. The prevalence of phenotypes responsible for long incubation periods should be evaluated as the sum of cases with long incubation periods and those with late relapses.

2. Divergences between field observations and laboratory experiments may be readily explained by the differences in infective doses; the larger the dose, the greater the variability of genotypic and pheno-

typic makeup of the inoculum, the higher the number of manifestations per patient, and the smaller the probability of a pure infection by bradysporozoites (i.e., of cases with long incubation periods).

3. The gene pool of strains maintained in the laboratory is far poorer than the gene pool of natural populations; therefore, strains do not fully represent populations. Moreover, a strain may possess, as the result of a random selection, a set of characteristics that are not typical of the population as a whole.

4. The differences between strains are of a quantitative nature. The classification of strains should be based not on the length of the incubation period but on a measurement of the prevalence of different phenotypes. The existence of strains that have both types of incubation period (*32*) cannot be explained by Nikolaev's theory of two subspecies, but it is not surprising from the point of view of the theory of polymorphic sporozoites.

5. The forms of the waves of early and late manifestations resemble each other in natural conditions because both waves are provoked by the same parasite transmitted over the same period of time and from the same source of infection. Empirical methods for prognosis of incidence proposed by Sergiev et al. (*27*) are theoretically well grounded.

6. It is well known that post-eradication epidemics of malaria are provoked almost exclusively by *P. vivax*. One of the reasons for this is the fact that transmission may be re-established from subjects with ultra-late manifestations. Such subjects may be effective sources of infection because genetic recombination in mosquitos may again produce phenotypes with early activity.

7. Persons who have been under an equal risk of infection are under an equal risk of late manifestations (relapses or long incubation periods), irrespective of whether they have had manifestations during the epidemic season or not. Primaquine treatment of individual cases cannot exhaust the reservoir of infection; therefore, mass primaquine treatment, first tested in the Tadzik SSR in 1955 (*16*) and then in the Azerbajdžan SSR and Afghanistan (*25*), is justified.

Suggestions for further research

The fact that the theory discussed above explains many phenomena suggests that it might be valid but is not sufficient to prove that it is absolutely true. It is therefore desirable to continue collecting experimental evidence for and against this theory. Experiments should be planned using modern techniques, for although classic experiments have provided much information, they now seem too unsophisticated. The following items are of particular interest:

1. Classic laboratory experiments performed under better defined and more rigid conditions (infection by a small and known number of sporozoites, early application of schizonticides, etc.);

2. Experiments on clones (originating from a single sporozoite or schizont, etc.);

3. Genetic analysis of the duration of EE development;

4. Epidemiological experiments in foci using different operational techniques;

5. The search for evidence of the possible pleiotropic action of genes controlling the duration of EE development;

6. The study of analogous phenomena in other plasmodia, especially in *P. ovale*, the species of human plasmodia that is the closest to *P. vivax*. There is some evidence that sporozoites of other plasmodia that do not produce true relapses (e.g., of *P. falciparum*) are also polymorphic, though the range of variation is much narrower in them than in *P. vivax*.

RÉSUMÉ

ÉTUDES DES POPULATIONS DE *PLASMODIUM VIVAX*. 1. LA THÉORIE DU POLYMORPHISME DES SPOROZOÏTES ET LES PHÉNOMÈNES ÉPIDÉMIOLOGIQUES DE LA FIÈVRE TIERCE

Les auteurs proposent un système de quatre postulats pour expliquer les phénomènes d'incubation prolongée et de rechutes tardives qui sont propres à *P. vivax*. Ils admettent que la durée du développement exoérythrocytaire est un trait polymorphe contrôlé par plusieurs gènes. Les sporozoïtes sont subdivisés en deux groupes: les tachysporozoïtes qui sont responsables des manifestations précoces, et les bradysporozoïtes responsables des manifestations tardives. L'analyse logique de ce système montre que cette théorie est compatible avec tous les faits expérimentaux connus; et elle permet d'expliquer et de mesurer les relations entre les phénomènes cliniques

et épidémiologiques. Les auteurs insistent sur le fait que la variation des gènes est beaucoup plus accusée au sein des populations naturelles que dans des souches individuelles, et que les propriétés des souches ne reflètent pas fidèlement celles des populations entières. C'est pourquoi les expériences de laboratoire classiques doivent être combinées avec des expériences épidémiologiques. La méthodologie de recherches ultérieures est discutée.

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