A strain of *Plasmodium vivax* characterized by prolonged incubation: the effect of numbers of sporozoites on the length of the prepatent period*

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**Summary**

The North Korean strain of *Plasmodium vivax* was characterized by its extraordinarily prolonged incubation period in certain circumstances. It was clearly demonstrated by quantitative observations that the phenomenon may be elicited by the inoculation of small numbers of sporozoites. After the intradermal inoculation of 10 or 100 sporozoites, the incubation period was delayed for periods varying between 262 and 628 days; after 1,000 sporozoites, one exception, the same delay occurred; after 100,000 sporozoites, the incubation period was always of normal duration (13-16 days).

Two laboratory-acquired infections in workers who had taken a prophylactic drug showed incubation periods of 315 and 329 days, respectively.

Various theories for the phenomenon of prolonged prepatent periods are examined, and the most satisfactory one is based on the presumed existence of two populations of sporozoites in *P. vivax*. In temperate strains, sporozoites requiring long prepatent periods (LPF) for development are present in great excess over a much smaller proportion of sporozoites characterized by short prepatent periods (SPP); thus small doses will elicit the phenomenon, though doses of over 1,000 sporozoites will mask the effect as the few SPP sporozoites will produce an infection with a normal (i.e. short) prepatent period. In tropical strains, the relative proportions are different, perhaps in equal numbers, and even in small doses some SPP sporozoites will be present and normal prepatent periods should ensue whatever the dosage.

**Introduction**

The phenomenon of a prolonged prepatent period of certain strains of *Plasmodium vivax* malaria is well known, though its explanation remains an enigma (COATNEY, 1976). Many infections acquired in the autumn do not become patent until the following spring. Such strains occur especially in temperate zones, but occasionally in the tropics (e.g. in El Salvador as described by MASON (1975)).

For at least 40 years, the idea has been advanced that prolonged delay in the incubation period might be due to the small number of sporozoites in an infective bite, and some support for this explanation has been provided by the results of infecting volunteers by single rather than multiple mosquito bites. It could be expected that after a single bite, the dosage of sporozoites would be proportionately lower than after multiple bites.

We have investigated the delay in the incubation of a strain characterized by predominantly prolonged prepatent periods by the administration of graded doses of sporozoites to psychiatric patients thought likely to benefit from malaria therapy. The results of these observations, given in the present paper, clearly show that the number of sporozoites of the North Korean strain of *P. vivax* profoundly influences the length of the prepatent period.

**Materials and Methods**

**Strain.** The North Korean strain was isolated in Moscow in 1953, and has subsequently been maintained there in patients by blood infection and mosquito bites. When transferred to England the strain was kept in splenectomized chimpanzees, and infected blood and suspensions of sporozoites were preserved at −70°C. A summary of the extensive observations of Russian workers on this strain has been made by BRUCE-CHWATT (1977) and a detailed account of its morphological and biological characters are given by GARNHAM et al. (1975).

**Production of sporozoites.** Sporozoites were harvested from the salivary glands of an English strain of laboratory-bred *Anopheles atroparvus* which had been infected by permitting them to engorge on chimpanzees with gametocytes of the North Korean strain in their blood. In this way there was no passage of blood from the chimpanzee to the patients. From May, 1969 to October, 1972 three chimpanzees were used. They were first splenectomized, and their blood was then observed for several weeks to ensure that they were not naturally infected with malaria parasites.

These animals were:

(i) "Justine": inoculated with preserved blood and sporozoites derived from chimpanzee Bonnie (see GARNHAM et al., 1975) on 5th and 19th May, 1969.

(ii) "Abigail": inoculated with blood of Justine and preserved sporozoites on 18th May, 1971.

(iii) "George": inoculated with blood of Abigail and preserved sporozoites on 6th October, 1972.
Inoculations of patients with sporozoites. Infected mosquitoes were taken to Romania where suspensions of sporozoites were prepared and the patients inoculated. A summary of the procedures is given in Table I.

The salivary glands of the mosquitoes were dissected in cold physiological fluids and a suspension of sporozoites prepared by gently grinding the glands with a Teflon grinder in a glass tube. The quantity of fluid was measured and estimates of the total numbers of sporozoites were made from counts in a Neubauer counting chamber. Tenfold dilutions were then prepared so that the required doses were obtained in volumes of 0.1 ml (KILLICK-KENDRICK, 1973). Throughout the manipulations the sporozoites were kept at approximately +4°C. The time from the beginning of the dissection until the inoculation of the last patient of a series never exceeded two hours. The lowest doses were given first and the highest last. All inoculations except when otherwise stated were intradermal. In each of the three investigations (see Table I) different fluids were used to prepare the suspensions of sporozoites: in the first it was "199" tissue culture medium; in the second, Grace's fluid; and in the third, 20% human serum in Locke's fluid.

Observations on patients. Thick and thin blood films were taken from the patients three times a week for the first two months after the inoculation of sporozoites. From two months until a maximum of just over two years, films were taken twice weekly. A countercheck on negative results was made by testing sera from some of the patients by the IFA test at suitable intervals; no antibodies were detected in these samples. The temperatures of the patients were recorded daily until the administration of radical treatment.

Results

The work was limited to observations on the interval (prepatent period) elapsing between the intradermal inoculation of graded doses of sporozoites and the appearance of malaria parasites in the blood of the recipients and also on two infections acquired in the laboratory. No attempt was made to follow in detail the subsequent course of infections of North Korean P. vivax, which has already been described by TIBURSKAYA (1962).

Therapeutic infections. Fig. 1 illustrates the combined results of the first and third trials. The second investigation was a complete failure, with no evidence of infection in any of the patients. Possibly something was wrong with the batch of Grace's fluid in which the sporozoites were suspended. In the two successful investigations, the prepatent periods were as follows:

(i) After the injection of 10 sporozoites. Six patients were inoculated and three showed parasites for the first time after 329, 386 and 628 days respectively (the last two infections being asymptomatic). The other three patients remained free from parasites for 306, 448 and 475 days when they received radical treatment. It is impossible to be sure if these patients were truly uninfected, or if they would subsequently have shown parasites.

(ii) After the injection of 100 sporozoites. Seven patients were inoculated and three showed parasites (two with fever and one without) after 262, 355 and 387 days respectively. Three other patients received radical cure on days 369, 479 and 734 respectively and a fourth died 229 days after inoculation without showing parasites. His death was due to circulatory failure.

(iii) After the injection of 1,000 sporozoites. All five patients developed parasitaemia, four after prolonged prepatent periods of 257, 345, 365 and 386 days (the last being asymptomatic) respectively, and one 16 days after inoculation.

(iv) After the injection of 100,000 sporozoites. In the first trial, only two patients were inoculated; one died after 225 days without showing parasites, the other had a normal attack starting 13 days after inoculation. In the third trial, four of the five patients inoculated intradermally showed fever and parasites (three on the 15th

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<th>Table I – Showing the production of sporozoites of the NK strain of P. vivax and the doses given to 36 patients; all inoculations were intradermal unless otherwise stated.</th>
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<td><strong>Group and place</strong></td>
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<tr>
<td>I (Bucarest)</td>
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<td>III (Iasi)</td>
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1 Two patients inoculated intravenously.
2 One patient inoculated intravenously.
3 One patient inoculated intravenously with an uncounted number of sporozoites estimated to be > 100,000.
Fig. 1. Prepatent periods of patients inoculated with estimated doses of 10, 100, 1,000 and 100,000 sporozoites of the North Korean strain of \textit{P. vivax}

day, and one on the 16th day). One patient remained negative throughout the observation period. The blood of a patient inoculated intravenously with an unknown (but large) number of sporozoites, first showed parasites on day 31, and fever began the next day.

Laboratory infections. Laboratory infections of the North Korean strain of \textit{P. vivax} occurred in two technicians who handled the infected mosquitoes in the insectaries in London, probably while collecting the mosquitoes for dissection. Precautions included the wearing of rubber gloves and the prophylactic administration of proguanil. It is impossible to pinpoint the precise days when these two men (M. W. Guy and G. S. Gill) received the infective bites (see Table II) but, dating the event from the earliest exposure (7th June) to the onset of symptoms, the incubation periods were 315 days in one and 329 days in the other. These periods are comparable to the prolonged prepatent periods observed in the patients. We cannot draw any conclusions on the effect of dosage in these two cases, though it is likely that a small number of sporozoites was introduced because the technicians were well aware of the danger. Also drug prophylaxis is known often to cause a delay in the incubation period.

Table II. – Details of two laboratory acquired infections of the North Korean strain of \textit{P. vivax}

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<th>Guy</th>
<th>Gill</th>
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<tr>
<td>Symptoms began</td>
<td>18th April, 1969</td>
<td>2nd May, 1969</td>
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<tr>
<td>Parasites first detected</td>
<td>24th April, 1969</td>
<td>4th May, 1969</td>
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<tr>
<td>Spleen</td>
<td>Not palpable</td>
<td></td>
</tr>
<tr>
<td>Radical treatment and cure</td>
<td>26th April, 1969</td>
<td>4th May, 1969</td>
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Discussion

Earlier work (e.g. Tiburskaya, 1961), based on bites of infected mosquitoes, has sometimes suggested that the number of bites had no influence on the length of
the incubation period of malaria. However, the dosage of sporozoites can only be very roughly estimated by this technique, as indicated by the following calculation based on a typical experiment: from the salivary glands of 300 mosquitoes we harvested $24 \times 10^6$ sporozoites, i.e. a mean of 80,000 per mosquito. Assuming that as few as 10% are actually deposited during biting, the rough number from the bite of one mosquito would be 8,000. This dose lies between the number (1,000) which usually gives rise to prolonged prepatency of the North Korean strain, and the dosage (100,000) which invariably results in a short incubation.

It is important to note carefully the precise strain which is being investigated. Thus, NICOLAIEV (1951) emphasizes that his short incubation period strain IX, always gave rise to a short incubation, however few mosquitoes were used, and however few sporozoites were present in their salivary glands. His long incubation period strain XIX on the contrary was found to give rise invariably to a long incubation, irrespective of the dosage, though NICOLAIEV himself stated (1957) that this strain produced few gametocytes and presumably yielded few sporozoites. It is difficult to assess the exact significance of work in which the number of sporozoites infecting the patient was not actually counted.

A clear cut answer was elicited as the result of the present enquiry. Long incubation periods followed induction by 10 or 100 sporozoites, and occurred in four out of five patients inoculated with 1,000 sporozoites, while short prepatency was exhibited by one patient given 1,000 sporozoites and five patients who received 100,000 sporozoites.

The incubation periods were either prolonged (after small doses of sporozoites) or short (after large numbers) —and no intermediate figures were obtained. Fig. 2 shows that the prolonged periods form a cluster around 360 days with a single exception of nearly double this number (628 days). Perhaps this numerical uniformity is on a par with the constant figures relating to periodicity at other stages of the life cycle of P. vivax (48 hours erythrocytic schizogony, eight days sporogony at the optimum temperature of 26°C and eight days normal pre-erythrocytic schizogony).

Although a definite result was obtained regarding the effect of dosage of sporozoites, many questions remain unanswered, particularly in relation to the mechanism of the phenomenon as seen in experimental conditions. Three theories can be advanced and these are discussed below in reverse order of that which we consider to be the most acceptable:

(i) Immunity. One possibility is that the inoculation of sporozoites may have temporarily immunized the patients. This theory is untenable because (a) immunofluorescent antibody tests were negative during the prolonged prepatent period and (b) if the host is inoculated with blood stages of the parasite during this period, an infection immediately follows (SHUTE & MARYON, unpublished observations). Moreover, there is good evidence that immunological response to the exoerythrocytic stages (GARNHAM & BRAY, 1956) is entirely absent, and only starts after the parasite begins to multiply in the blood.

(ii) Persistence of some sporozoites or latent forms in the skin or tissues other than liver. HUFF (1947) and colleagues spent much time in examining the skin and other tissues of man and monkeys for evidence of primary tissue forms of P. vivax and P. cynomolgi respectively, and found no signs of cryptozoites. Their successful experiments with avian malaria parasites (COULSTON & HUFF, 1947) show that they were well equipped to find similar stages in mammalian malaria if such indeed existed, and their negative results must be
regarded as highly significant. Dormancy elsewhere (other than in the liver) of the sporozoite is a possibility, but rather begs the question for the same problems are presented in both cases. Garnham et al. (1975) reported the occurrence of late tissue stages of the North Korean strain in the liver of an experimentally infected chimpanzee, suggesting that this organ is capable of harbouring such stages for many months. This observation makes it unlikely that some special cell "X" elsewhere may harbour either the stages or the sporozoite itself, during the prolonged prepatent period. No certain figures are available for the length of life of the parenchymal cell of the liver, but estimates of about a year have been given, which approximate to the "critical" period observed in the present work. However, we cannot entirely exclude the possibility that the parasite initially lies dormant in a tissue other than the liver, and then moves to that organ where it completes its development.

(iii) The presence of two populations of sporozoites. Saengthay & Tiburskaya (1967) formulated the hypothesis that certain strains of P. vivax give rise to long or short incubation periods, the result of infection with at least one of two types of sporozoites. This hypothesis is confirmed by the result of the present work, which suggests that in the North Korean strain of P. vivax and other temperate strains, a double population of sporozoites exists, one of which gives rise to a short prepatent period and the other to a long prepatent period. We refer to these as SPP and LPP sporozoites respectively. Their relative proportions in the North Korean strain are assumed to be very different, and our figures (see Fig. I) suggest that no more than one in a thousand of the sporozoites were SPP and the remainder LPP. Thus, when successful, an inoculation of 100 or fewer sporozoites would almost always be followed by prolonged prepatency (as in six cases in the present work), 1,000 would usually produce it (as in four out of five cases), and 100,000 would be most unlikely to produce it (in none of five cases).

This hypothesis has received a mixed reception from several experienced workers, both malarialogists and geneticists. It was supported independently by Moshkovski (1973) (p. 27), but R. S. Bray (personal communication) suggested that if the theory were valid, the LPP population should eventually breed true and a pure strain would be produced; he has since rejected the theory, while freely acknowledging the lack of an alternative working hypothesis (1975). We know that the inoculation of sporozoites derived from long term relapses, or after prolonged prepatency of the Madagascar strain, can give rise to normal infections of short term type (Shute & Maryon, unpublished observations). G. Beale (personal communication) agrees that, genetically, two populations (arising respectively from SPP and LPP sporozoites) could co-exist, and suggested that attempts should be made to select pure strains with fixed long or short prepatent periods. To some extent the objections of Bray and the suggestion of Beale are answered by the observations of Nicolaiev (1951) on P. vivax hibernans, which continued to breed true. Thus, in a long series of volunteers, infections by this strain always exhibited delayed patency, irrespective of the number of bites of infected mosquitoes. While it might be feasible experimentally to select a line with a constant short prepatent period, it is hardly practicable to attempt to select a hibernans-type line; such work would take many years, and require many volunteers under constant observation for long periods. Splenectomized chimpanzees or Aotus monkeys would be unsuitable because they are such abnormal hosts that the results would be difficult to interpret.

It should be possible to find evidence in support of the theory of two populations of sporozoites by comparing the density of exoerythrocytic schizonts in the liver of animals which have been inoculated with sporozoites of temperate and tropical strains of P. vivax. On the assumption that the LPP sporozoites remain latent and that only SPP sporozoites grow immediately into exoerythrocytic schizonts, the density of the two types should be markedly different—presumably only 0-1% of the LPP would be demonstrable compared with about 99-9% of the SPP.

Fortunately, we have some data on this point, although it relates only to the chimpanzee which is not the best model for these experiments. We compared the density of exoerythrocytic schizonts eight days after the inoculation of sporozoites of the North Korean and Madagascar strains of P. vivax into splenectomized chimpanzees and the results were as follows:

P. vivax, North Korean strain. Twenty-four million sporozoites from 300 mosquitoes (Garnham et al., 1975) were harvested and inoculated intravenously into a chimpanzee ("Sally", five years old) which was also bitten by 500 mosquitoes. The latter probably introduced a further six million sporozoites, and it may be assumed that the animal received a total of at least 30 million sporozoites. About one exoerythrocytic schizont per standard sized section was subsequently found in the liver.

P. vivax, Madagascar strain. Eleven million sporozoites from 140 mosquitoes (Bray, 1957) were harvested and were inoculated intravenously into a splenectomized chimpanzee aged two years. About two pre-erythrocytic schizonts were counted per section in its liver.

As a five-year-old chimpanzee is likely to have a liver two-and-a-half times the size of an animal only two years old, it is necessary to correct the comparative figures by a factor of 2.5. The dosage of sporozoites also differed: 30 million were given to the former animal and 11 million to the latter, and an inverse factor of about three must be introduced into the comparison. Thus the comparative density of exoerythrocytic forms is estimated as follows:

North Korean strain: \(1 \times 2.5 \div 3 = 0.8\) per section

Madagascar strain: \(1 \times 2.5 \div 3 = 0.8\) per section

From this extremely crude estimation and bearing in mind the wide fluctuations in density always seen in such experiments, the animal given the North Korean strain produced about half the number of schizonts compared to the other animal. If there had been 99-9% of LPP sporozoites in the former we should have expected nothing like this number: in fact primary exoerythrocytic schizogony might well have been undetectable. We might go as far as to suggest that this comparison indicates no real difference between the sporozoites of the two strains, and fail to support the theory of the existence of two different populations. However, the above considerations are based on an unnatural model in a state profoundly altered by splenectomy, and have insufficient weight to persuade us to abandon the theory which still seems to fit the facts best.

Besides low dosage of sporozoites, other experimental factors are known to induce prolongation of the prepatent period. The administration of meperidine, chloroquine or other drugs (but not quinine) concurrently with the inoculation of sporozoites suffices to delay multiplication of parasites in the blood for about the same
period (200-365 days) as in the laboratory infections reported above. The nature of this form of dormancy remains unknown.

Another suppressive factor is the presence of a second species of Plasmodium, namely, P. falciparum in the inoculum containing the sporozoites of P. vivax and this phenomenon has been reported by Shute (1946) both after bites of mosquitoes doubly infected and after the inoculation of sporozoites of the two species. P. falciparum dominates the picture and P. vivax is suppressed for a period of long duration. The exoerythrocytic cycle of P. falciparum is two days shorter than that of P. vivax, but it is unknown what happens in the liver during the incubation of the latter. Unfortunately the key experiment has never been carried out on chimpanzees and vital information would undoubtedly be obtained if sporozoites of the two species were mixed and inoculated into a splenectomized chimpanzee (to watch the ensuing parasitaemia)—and into an intact animal. Biopsies of the liver of both animals would be taken on the fifth and eighth days and sections examined. It does not seem likely that immunity plays any part in this phenomenon, as little if any cross immunity exists between the two species.

In addition to the main problem posed by these investigations, a number of other questions remain unanswered or are only partly solved. They all bear on the phenomenon of prolonged prepatency, and they are considered below:

(i) Why are there no intermediate periods between long and short prepatency? The simplest answer is that in temperate climates the phenomenon of prolonged prepatency is a device of the parasite to increase its chances of survival (Hackett, 1937). Parasitaemia is useless if it arises at the end of the summer when transmission ceases and mosquitoes hibernate; it may therefore become delayed until the late spring or summer when mosquitoes become abundant and the ambient temperature is suitable for sporogony (Swelengrebel & De Buck, 1938). There would thus be no selective forces leading to the production of intermediate forms.

(ii) Why are a large proportion of strains of P. vivax occasionally characterized by long prepatent periods? The strains of P. vivax isolated from Central and South America often show a 'temperate climate' pattern particularly in regard to relapses (e.g. Mason, 1975). P. vivax is thought to have been introduced into the New World either before Columbus from Asia (Bruce-Chwatt, 1965) or by the Conquistadores from Europe in the 16th and 17th centuries; in the latter instance, the parasite would presumably have been of the temperate zone type. Clyde (1972) even reported some delay (36 and 68 days) in patency of volunteers infected with very small doses of the typically tropical Chossen strain, but such minimal delay appears to be of a different nature from that of the greatly prolonged prepatency of P. vivax hibernans. Jeffery (1956) studied the date of onset of relapses after treatment of the primary attack with chloroquine and no evidence of 'clustering' was obtained, although the interval in one case was over a year; in many instances the parasitaemia was afebrile. A distinction must be made between the true P. vivax hibernans of Nicolaiev and allied strains as found in Northern Europe and parts of Asia, with highly characteristic prolonged prepatency, and strains like the so-called temperate P. vivax St. Elizabeth strain from North America, which on occasions show prolonged prepatency, e.g. in 78 inductions with this strain there was a short incubation in 75 and a long (about 300 days) in only three (Coatney et al., 1950); the relapse pattern however was of the temperate zone type. Some prolongation of the prepatent period may have been the result of undisclosed suppression by drugs.

(iii) Why is the interval between the primary attack (after a short prepatent period) and its first relapse of the same duration as the prolonged prepatent periods of temperate zone P. vivax? The simplest answer to this question is that given by Moshkovski (1973) who states that amongst the sporozoites of certain strains of P. vivax two types will be present: one type gives rise to a primary attack seven or eight days after infection (followed by a long-term relapse) and the other remains latent for a similar interval as this relapse. It is therefore probable that the long-term relapse and prolonged prepatency represent one and the same phenomenon, i.e. dormancy for many months of a certain type of sporozoite.

This paper does not present any data on relapses of the North Korean strain as the work was done on patients who were radically cured after the primary attack and information on splenectomized chimpanzees is valueless in this respect. Tiburskaya et al. (1968) gave details of the relapse patterns in various strains (including the North Korean).

One can only conclude that in relapses some sporozoites (or their immediate successors) persist in a dormant condition and resume activity in about a year. The demonstration of later exoerythrocytic schizonts in the liver of the chimpanzee 'Bonnie' (Garnham et al., 1975) is evidence for this conclusion. The preceding short prepatent period is the result of the introduction of some SPP sporozoites in a heterogeneous infection.

(iv) Is the phenomenon of delayed patency the result of the introduction of aged sporozoites? This theory was considered by Swelengrebel & De Buck (1938) and by earlier Dutch workers in order to explain the extraordinarily long incubation period of P. vivax malaria in the Netherlands. Although their mosquitoes were found to be capable of surviving from autumn to spring, they discovered that the sporozoites were not as long-lived and that their infectivity appeared to be "non-existent" after a few months: the aged sporozoites were degenerate.

(v) Dormancy of the sporozoite is an attractive theory and perhaps this stage is capable of survival in organs other than the liver. Is it possible that some sporozoites get trapped, e.g., by cells perhaps of a particular age which hold them as prisoners for the rest of the life of the cell? Then when the cell breaks down the sporozoite finds its way to the liver where it undergoes exoerythrocytic schizogony of the normal duration (eight days). Examination of such material provides no confirmation that this process occurs, though the research has not been prolonged. However, the work of Landau (1973) and others has established that arrested development of sporozoites of, for example, intestinal coccidia and haemogregarines is common, and it would not be surprising, therefore, if the same phenomenon were to be found to occur in the closely related Haemopora.ina.

(vi) Why is prolonged prepatency confined to a few species of Plasmodium? In the human species, the phenomenon occurs only in certain strains of P. vivax and in P. ovale; it is absent in P. malariae and P. falciparum. The simian counterpart of P. vivax is P. cynomolgi in which prolonged prepatency has apparently never been observed but "relapse" bodies have been found three- and-a-half months after inoculation (Shortt & Garnham
1948). Moreover, Contacos & Collins (1973) showed that relapses occurred up to 168 days after the inoculation of 10 to 20 sporozoites of P. cynomolgi bastianelli, the primary infection of the blood having been cured with quinine; they suggested that the mechanism of dormancy is a characteristic solely of species which exhibit true relapses. Warren et al. (1974), have shown that the number of relapses in P. cynomolgi is directly related to the number of sporozoites inoculated, e.g., 19 relapses occurred in a monkey which received 1·5 million and 12 in another receiving only 300,000 sporozoites. These experiments were based on infections derived from counted sporozoites, the lowest dose of which was 700. In none was the prepatent period delayed, from which experiments were based on infections derived from that relapses occurred up to 168 days after the inoculation nor a greater degree of phagocytosis of the sporozoites relapse took place more than two years after infection to low dosage do not occur in inoculations of large numbers of sporozoites non-chloroquine.

(vii) In our patients, why were some intravenous inoculations of large numbers of sporozoites non-infective? Neither technical faults in the administration nor a greater degree of phagocytosis of the sporozoites after inoculation into a vein instead of the skin seem to provide an answer. The intravenous route was applied during which period there were many recurrences of parasitaemia; all were sterilized immediately with chloroquine. Perhaps, it may be assumed that the phenomena relating to long time that the North Korean strain of P. vivax hibernans Nikolaiev, 1949. Protozoology, 3, in press.

(viii) Another puzzle of the same type is the unusually long time that the North Korean strain of P. vivax takes to become patent after inoculation of large numbers (100,000) of sporozoites. In our observations in Romania, the prepatent period was between 13 and 17 days, while in TIBURSKAYA's series in Moscow, it was 17 to 22 days. This kind of delay is usually attributable to a low dose of sporozoites or a small number of mosquito bites (7–10), but in most of these instances 100,000 or more sporozoites were undoubtedly given. It can hardly be a peculiarity of the strain, as the number of exoerythrocytic schizonts is approximately what might be expected which indicates a normal degree of viability of the sporozoites. We must distinguish here between delayed normal (e.g. up to 22 or even 68 days) prepentrancy and the prolonged prepentrancy of hibernans and allied strains (e.g. 300 days).

(ix) What would be the effect on prepentrancy of small doses of sporozoites of other strains of P. vivax, particularly on the typically tropical Chesson strain? We have already drawn attention to the occasional prolongation of the incubation period in various strains of P. vivax but no quantitative experiments have been carried out. We feel that our work with P. vivax should be extended to the Chesson strain of the parasite, using precisely similar techniques. Such work is now in progress.

Acknowledgements

We acknowledge the help of Dr. R. S. Bray in the planning of this work and thank him for his kindness in providing material from chimpanzees infected with the Madagascar strain; we refrained from asking him to be a co-author because he is in disagreement with some of the conclusions.

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