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Life Cycle and the Phenomenon of Relapse

A. LIFE CYCLE

IN the Primate Host. The cycle of malaria in the primate host is initiated by the inoculation of sporozoites by the female mosquito when she punctures the skin to obtain blood (Fig. 3). Instead of setting-up a cycle in the blood, as maintained by Schaudinn in 1902, and not disproved until 46 years later by Shortt and Garnham (1948), the sporozoites soon leave the blood stream and enter the parenchyma cells of the liver, initiating the cycle of exoerythrocytic (EE) schizogony. As the parasite grows, it assumes different shapes (round, oval, or lobulate). It may contain vacuoles, and from several to many flocculi. Normally, the EE body develops within a single parenchyma cell of the liver, displacing its nucleus. The nuclei of the parasite multiply by division, eventually increasing in number up to as many as 40,000 per EE body; pigment is absent. When the schizont is mature, in 5 to 15 days, depending on the species, the merozoites are released into the blood where they invade the host's erythrocytes, and thereby initiate the schizogonic cycle in the blood.

The merozoites of some species display a marked predilection to invade reticulocytes, others prefer mature red cells and some are non-selective. The young asexual parasite, or trophozoite, appears ring-shaped, the "stone" being the nucleus. As the trophozoite grows, it may assume various shapes, amoeboid as in *Plasmodium vivax*, compact as in *P. inui*, or it

may be decidedly vacuolated, as in *P. hylobati*. The growing trophozoite feeds on the hemoglobin of the host red cell by phagotrophy. However, the hemoglobin is incompletely metabolized by the parasite and what remains, principally hematin, is malaria pigment. The shape and color of these pigment particles vary from one species of malaria to another and, consequently, often serve as an aid in species diagnosis. Just prior to nuclear division, the vacuole disappears and the cytoplasm appears more compact. The parasite nucleus now undergoes a series of mitotic divisions which ultimately produces a mature schizont. The nuclei surrounded by a small amount of cytoplasm are now termed merozoites. The number of merozoites in a schizont varies according to the species of *Plasmodium* although there is considerable variation even within a given species. *Plasmodium eylesi* produces 20 to 34 merozoites, the average is 25, whereas *P. malariae* may produce 4 to 12 merozoites with an average number of 8. When the schizont is mature, the merozoites are released into the blood and, unless destroyed by the host's immune mechanism, invade other erythrocytes which initiates another asexual cycle.

The length of time required for completion of the erythrocytic cycle ranges from approximately 24 hours (quotidian periodicity) to approximately 72 hours (quartan periodicity). Only one species of primate malaria, *P. knowlesi*, has a 24-hour cycle. Most of the primate malarias have a 48-hour

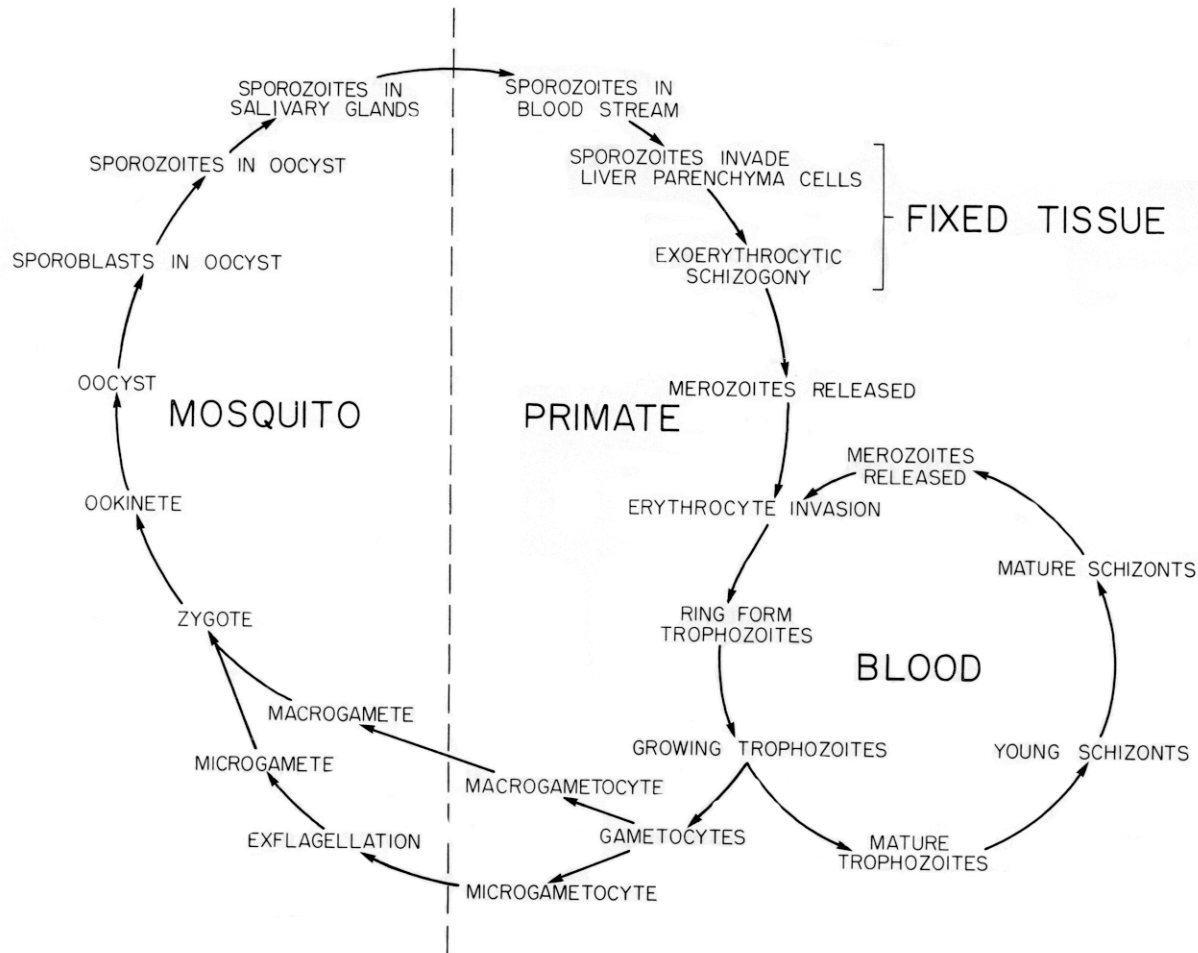


FIGURE 3.—Diagrammatic presentation of the life cycle of the primate malaria parasite.

asexual cycle (tertian periodicity). The exact interval varies; for example, different strains of *P. vivax* require from 41.5 to 45.8 hours (Young, 1944), whereas *P. ovale* requires 49 hours (Jeffery *et al.*, 1954). The primate malaras known to have a 72-hour cycle are *P. malariae*, *P. brasilianum*, and the various strains of *P. inui*. The parasites in subsequent chapters will be referred to as having developmental cycles of 24, 48, and 72 hours.

During the growth of the parasite, the host erythrocyte often exhibits secondary changes; the most prominent are enlargement (as with *P. vivax* and *P. cynomolgi*) and the presence of stippling. The latter is made visible by staining the air-dried blood film with Romanowsky stains. These important diagnostic features will be readily seen in the illustrations of the different species. After one or more generations

of erythrocytic schizogony, some parasites develop into gametocytes. It is impossible in the very early stages of development to differentiate the young gametocytes from the developing trophozoites. However, as the gametocytes approach maturity, they are easily distinguished from the mature trophozoites (see individual chapters for specific differences). The exact origin of these forms is unknown. They must arise as a product of schizogony, but whether certain schizonts are predestined to produce asexual parasites and others sexual forms is one of several mysteries in the life-cycle of malaria. Their tendency to be produced in waves would suggest that the host's immune response may be associated with gametocyte production.

However, certain strains have become gametocyteless, such as the Santee Cooper strain of *P. falciparum* (Jeffery, 1951), which suggests that the host may have little, if any, influence on the initiation of gametocyte production. Two forms are produced: micro- and macro-gametocytes, commonly referred to as male and female gametocytes. In general, the latter predominate. In some cases, notably *P. falciparum*, gametocytes will continue to circulate in the peripheral blood long after their initial production. However, their infectivity appears to be short-lived and there is some evidence to suggest that their infectivity may be associated with a certain period of the day (Hawking *et al.*, 1968).

In the Mosquito. The cycle in the peripheral blood exists only, in terms of species survival, for the production of sexual forms, the gametocytes. The gametocytes of the primate malarias can only complete their destiny in an anopheline mosquito.

The female anopheline seeks a primate host because she is hungry, a fact clarified by the late Dr. Robert W. Burgess in the early 1940's. Her hunger is satisfied by biting and in the process she takes blood containing gametocytes and other blood-stages of the parasite into her digestive tract. In the mid-gut, or mesenteron, the mature gametocytes shed their red cell envelopes and transform into gametes, which we now know to be the mature sexual forms. Laveran (1880) and many other workers had seen the 'strange' forms in the blood, but it was a 3rd year American medical student, William George MacCallum, who discovered their sexual nature. MacCallum (1897), during his summer vacation in Canada, studied the malaria parasites in crow blood and deduced that the non-segmenting forms were male and female parasites. Upon his return to Johns Hopkins Medical School in the fall, he confirmed his suspicions while studying blood from a woman infected with *P. falciparum*. He watched the process of exflagellation whereby the male (micro-) gametocyte throws out thread-like processes which lash about and soon separate from the parent body. He observed one of these thread-like bodies, saw it penetrate and thus fertilize the rounded-up female (macro-) gamete.

The microgametes are produced by a process termed exflagellation. Soon after ingestion, the nucleus of the microgametocyte divides, giving rise to 8 nuclei. These migrate to the periphery and enter long cytoplasmic processes which project from the surface of the parasite. The microgametes lash about vigorously and soon separate from the parent body. The microgamete enters the macrogamete to form the zygote. Shortly thereafter, the ookinete is formed. This vermiculate body moves actively and forces its way into an epithelial cell of the host's mid-gut. A cyst wall is formed and this stage, the oocyst, now commences its growth lying between the basement membrane of the gut wall and the cells, and projecting into the haemocoel of the mosquito. Oocysts are only found on the mid-gut and are usually more concentrated toward the posterior end. Meiotic division occurs within the young oocyst, usually commencing about 48 hours after the blood meal (Bano, 1959). Succeeding mitotic divisions produce nuclei with the particular haploid number of chromosomes for that species. As the oocyst grows (sometimes reaching a diameter of 100 μ) the number of nuclei increases; vacuoles appear as the cytoplasm divides into sporoblasts. From these arise the sporozoites. Oocysts in which the sporoblasts and developing sporozoites are visible under light microscopy will be referred to, in subsequent chapters, as differentiated. Each sporozoite contains a single nucleus and the number of sporozoites produced per oocyst has been estimated as about 10,000 (Pringle, 1965). Upon maturity, the time depending on the temperature and humidity conditions, the oocyst ruptures and the sporozoites are released into the haemocoel of the mosquito. The sporozoites migrate to all parts of the body but some enter the acinar cells of the salivary glands. When the infected mosquito begins to feed, the sporozoites enter the salivary duct and are thereby introduced into the blood stream of the primate host, initiating the infection in a new environment.

B. RELAPSE

The phenomenon of relapse has intrigued malariologists for decades. As early as 1893,

Golgi suggested that parasites of human malaria may develop in endothelial cells not affected by antimalarial drugs and that the protected parasites could be the source of relapses.

In 1897, almost at the time that Ronald Ross was completing his basic studies on the transmission of malaria, Thayer published a series of lectures with several references to relapse in vivax malaria. In his speculations to explain latency, which must obtain preceding a relapse, he postulated that there must be an undiscovered form of the parasite. He wrote "the organism may remain perhaps within the cell body of certain phagocytes for long periods of time, only to be set free again as a result of some insult, the nature of which is not as yet appreciable to us." More than 70 years later, we still do not know the exact nature of these phenomena, but it is generally accepted that: 1) fixed-tissue forms of the true relapsing malarials (i.e., *Plasmodium vivax*, *P. ovale*, *P. cynomolgi*, *P. fieldi*, and *P. simiovale*) release young forms periodically which parasitize the erythrocytes and thereby initiate the relapse (Fig. 4) and 2) along with the above type of reactivation of the infection is the one arising from renewed activity, after a period of quiescence, in the conventional red cell cycle. This renewed activity, which we call a recrudescence, is due, possibly, to the development of new antigenic variants of the parasite as was shown by Brown

et al (1968) for *P. knowlesi*, Voller and Rossan (1969) for *P. cynomolgi*, and by ourselves for *P. brasilianum*.

The first experimental evidence of rhythm in relapse activity came in 1900 when Sir Patrick Manson allowed infected mosquitoes to bite his son, P. Thurburn Manson, then a young medical student. The younger Manson came down with vivax malaria and the infection was treated with quinine. Following treatment, he continued in good health until 9 months later when he had a typical relapse which he himself reported in detail in 1901. Another volunteer, in the same time period, was Major C. F. Fearnside who had a relapse experience similar to that of young Manson which he reported in 1903. In retrospect, these early reports set a pattern which was later recognized as a common phenomenon in many strains of *P. vivax*.

After Schaudinn's description of the direct invasion of a red blood cell by the sporozoite of *P. vivax* (1902), attention was concentrated, almost entirely, on this stage of the parasite. However, as information accumulated, especially the data derived from the study of induced malaria in the treatment of general paresis, it became apparent that all human malarials did not have the same relapse potential, and that there was a difference in the response to therapy in blood-induced and sporozoite-induced infections (Yorke, 1925;

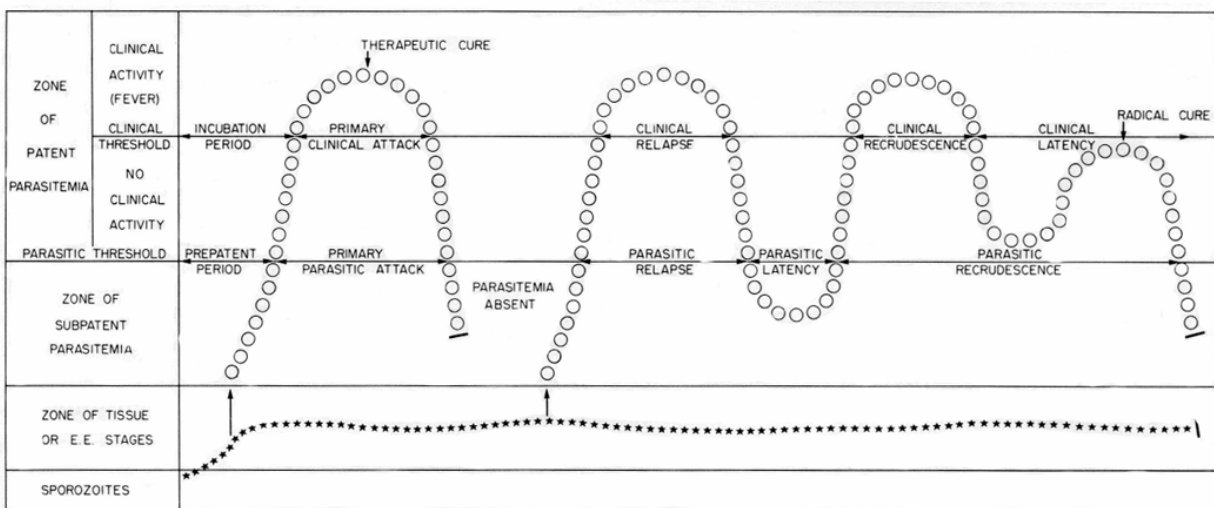


FIGURE 4.—Diagrammatic presentation of the parasitologic and clinical cycle in the vertebrate host of those malarials which have a true relapse mechanism.

Yorke and Macfie, 1924). In 1931, James suggested that sporozoites, after being injected by the mosquito, are carried to the internal organs where they enter reticulo-endothelial cells and go through a cycle of development with the eventual production of merozoites which parasitize red blood cells. This proposal was based primarily on the fact that therapeutic regimens known to be effective against malaria could not cure an infection when administered during the incubation period. It was reasoned, that if sporozoites entered directly into the red blood cells and became trophozoites and schizonts, they would have been destroyed by the drugs and no active infection could have resulted. Meanwhile, research in the avian malarias had been developing rapidly, and in 1935, Huff and Bloom clearly demonstrated exoerythrocytic stages to be a fundamental part of the life cycle of *P. elongatum*.

As data about fixed tissue parasites from birds accumulated, mainly the work of James and Tate (1937) and the brilliantly executed studies of Huff and his co-workers (1943 to 1948), it became abundantly clear that such a cycle must also occur in primate malarias. In 1946, Saper, in his Craig Lecture (Saper, 1947) presented presumptive evidence for the link between fixed tissue stages and relapse in such lucid language that their actual demonstration seemed an easy task.

In 1945, Fairley *et al* had shown that sporozoites, following their introduction, disappeared from the circulating blood during the first hour and no evidence of infection was to be found until 6 days later in falciparum infections and 8 days later in vivax infections, when blood transfusions from the infected individuals produced infection in the recipients.

The evidence in support of the existence of preerythrocytic stages in primate malarias was now so strong that it seemed only a matter of time until they were demonstrated. Coatney and Cooper (1948) summarized the information available on drugs with apparent action against preerythrocytic stages of avian and primate malarias. These investigators reported that 8-aminoquinolines and certain biguanides were active against the presumed exoerythrocytic forms of human and simian malarias, the presence of which had been deduced from

indirect evidence. The direct evidence came in the same year, with the description by Shortt and Garnham (1948) and Shortt, Garnham, and Malamos (1948) of the exoerythrocytic stages of *P. cynomolgi* in the liver of an experimentally infected rhesus monkey. Soon, similar preerythrocytic forms were demonstrated in the livers of human volunteers infected with *P. vivax* (Shortt *et al*, 1948) and *P. falciparum* (Shortt *et al*, 1949; Jeffery *et al*, 1952).

With the demonstration of the exoerythrocytic stages of avian and primate malarias, a whole new developmental phase of the parasites could be examined. Various proposals were advanced to explain the phenomenon of relapse in malaria. The hiding place of the parasite, during long periods when the patients were clinically and parasitologically negative, had been debated for many years. The discovery of the exoerythrocytic stages of primate malarias seemed to have revealed their hiding place. With the report of an EE schizont in the liver of a monkey 3½ months after sporozoite inoculation (Shortt and Garnham, 1948), most workers in the field came to believe that a direct relationship existed between these fixed tissue stages and true relapses.

The relationship of relapse of primate malarias to an exoerythrocytic stage of the parasite was not new. Various workers, involved in experimental therapy of malaria, had predicted that an exoerythrocytic stage of the parasite was responsible for long term relapses in *P. vivax*. The absence of a persistent tissue phase in *P. falciparum* was also proposed (Shannon and Earle, 1945; Fairley *et al*, 1947) prior to the actual demonstration of EE schizonts of *P. cynomolgi* by Shortt and Garnham (*loc. cit.*). The persistent tissue phase as the source of the parasites in typical *P. vivax* relapses was firmly supported by Shannon and Earle (1945) and Fairley *et al* (1947), and at least tentatively, by Huff (1947). The evidence to support this hypothesis was primarily based on the extensive work on EE stages in avian malarias by a variety of workers in the 30's and 40's and the striking differences in response to therapy in blood-induced and sporozoite-induced infections with *P. vivax*. Coatney and Cooper (1948a), based on data from studies with the St. Elizabeth strain of *P. vivax*, not only supported the EE stage theory

as the mechanism of relapse, but proposed a concept of latency which we will reintroduce later in this discussion.

It is clear that a large number of workers in the field of malaria were convinced, not only of the existence of EE stages in primate malaras, but of their association with relapses. Therefore, the results of the remarkable series of experiments by Shortt and his group which conclusively demonstrated the EE schizonts of *P. cynomolgi*, *P. vivax*, and *P. falciparum* were generally sympathetically received. When the work of Coatney and Cooper (1948a), showing that massive blood transfusions during latency, following treatment of the initial attack, failed to produce infections in recipients, although the donors' infections relapsed later, was combined with the demonstration of an EE schizont of *P. cynomolgi* 3½ months after sporozoite inoculation, most workers considered the relapse story to be complete (Shortt and Garnham, 1948a). The specific mechanism proposed was that merozoites from mature EE schizonts enter red blood cells, producing the familiar clinical and parasitological features of malaria. Other merozoites, possibly from the same schizont, enter normal liver cells and continue the cycle of exoerythrocytic schizogony. This latter process would continue indefinitely, and, when the active immunity of the host was, for any reason, reduced, some of the merozoites would invade the red blood cells to produce a relapse. This concept of Shortt and Garnham (loc. cit.) was widely but not universally accepted as the most likely explanation for the production of relapses in certain species of both human and simian malaria.

It has been noted that acceptance of the exoerythrocytic stage of malaria as the source of relapses was not universal. Huff (1948) and Huff and Coulston (1948) were convinced of the existence of the exoerythrocytic stages in primate malaras, but Huff (1950) expressed serious reservations concerning the parasitic nature of the bodies described by Shortt. Fairley (1949) did not entirely agree with the continuing exoerythrocytic cycle, as described by Shortt and Garnham, either. In 1950, Corradetti expressed categorical disagreement with the idea that relapses in mammalian malaria were in any

way related to the exoerythrocytic stages of the parasite. This investigator insisted that "not a single fact" went against the initial opinion of Bignami (1910) that relapses were related to the persistence of endoerythrocytic parasites.

It is appropriate, at this point, to discuss these two theories concerning the origin of relapses in malaria inasmuch as the controversy has remained essentially unchanged since the early 50's. Corradetti accepts the existence of EE stages of primate malaras, but still (1966), believes that relapses are due entirely to the persistence of erythrocytic stages of the parasite; Corradetti's position has not changed appreciably since 1950 (Corradetti and Verolini, 1950). Bray (1967) produced an excellent review of his position on the relationships between EE stages and relapse mechanisms in which he considered the individual points in Corradetti's thesis. Bray's arguments generally reflect the contemporary opinion of most malariologists. There are several aspects of this controversy which warrant a more detailed consideration.

Corradetti (1965) presented a number of well reasoned points and his strongest argument rests with the fact that blood-induced infections, of quite long duration, have been observed with *P. cynomolgi*, *P. malariae*, and *P. falciparum*. It is also agreed, that the blood may be negative for malaria parasites by routine microscopic examination for periods of varying duration during any of these extended infections. Actually, work from our laboratory indicates that even stronger support for his thesis would be found with blood-induced infections of *P. inui* which are known to persist in monkeys for years. This we consider to be a well reasoned argument and could well support Corradetti's case if it were taken no further. However, all of the evidence must be considered. It is true that blood-induced infections with *P. cynomolgi* persist for long periods, but such infections can be quickly and totally eradicated with schizonticidal drugs, such as chloroquine or quinine. Sporozoite-induced infections, with the same parasite, can be cleared of parasites in the peripheral circulation with the same drugs, but the infection returns with a frequency which is dependent upon the strain of parasite used.

This evidence is, of course, not new. Differences in the response of sporozoite- and blood-induced infections of *P. vivax* to schizonticidal drugs have been recognized for many years (Yorke and Macfie, 1924). Corradetti (1966) did not include data from chemotherapeutic studies of *P. vivax* and *P. cynomolgi* in his discussion of relapse mechanisms.

The extreme duration of infections with *P. malariae* naturally occupies an important position in any discussion of relapse mechanisms in malaria. There are reports of persistence of infection for 30 to 40 years and recrudescences are generally expected through a period of from 5 to 8 years. Ciuca *et al* (1964) followed the course of a blood-induced infection and found erythrocytic parasites 525 days after inoculation. The same workers reported that schizonticides were radically curative in sporozoite-induced *P. malariae* infections, and, they concluded, that this parasite possessed no secondary exoerythrocytic stages. Corradetti called attention to this finding, a point which was well taken since radical cures with schizonticidal drugs had been interpreted by others to be associated either with blood-induced infections or with sporozoite-induced infections of malarials not possessing a relapse potential. More recent data from our laboratory suggest that *P. malariae* has no true relapse mechanism. Furthermore, schizonticidal drugs have been found to produce radical cures of sporozoite-induced *P. inui* infections, the quartan parasite of Asian monkeys.

One of the strongest pieces of evidence to support the exoerythrocytic source of malaria parasites in relapse situations is the total absence of these parasites from the peripheral blood during negative intervals. These negative intervals may occur naturally or may be induced by the use of schizonticidal drugs. The absence of parasites during these periods was established by the inoculation of massive amounts of whole blood from infected to malaria-free volunteers without producing infections (Cooper *et al*, 1949). Cooper *et al* (1947) demonstrated the susceptibility of volunteers during the long negative interval in St. Elizabeth strain *P. vivax* infections by successfully superimposing a blood-induced infection, with the homologous

strain parasite, in the same person. This experiment clearly indicated that the absence of parasites from the peripheral circulation was real and not a suppression to very low levels by immunologic activities. We consider these experiments to be conclusive, but Corradetti (1966) felt that it would be necessary to transfer every red blood cell without producing an infection, before one could know that no parasites were present. In the same paper, it is noted that Corradetti and Verolini (1950) subinoculated blood during negative periods from monkeys with blood-induced *P. cynomolgi* infections with negative results. We have carefully reviewed this reference and cannot find the account of a subinoculation, with negative results, of whole fresh blood from an infected, but negative donor, that at a later time demonstrated a patent parasitemia. Even a single result of this nature would be interesting; if any parasite could consistently produce this response, it would be quite important.

The final evidence in support of the direct involvement of exoerythrocytic stages in malarial relapses which has been challenged is the demonstration of the parasites themselves. Corradetti was skeptical because of the low number of late EE schizonts that have been seen. Actually, as Bray (1967) pointed out, the number of liver stages seen 30 days or more after sporozoite inoculation is considerably greater than that noted by Corradetti. However, the questions posed by the latter investigator concerning: 1) how such stages are known to be secondary and not the result of some form of latency and 2) why 'nests' of parasites are not found in the area where an earlier schizont matured, have not been answered satisfactorily.

The accumulation of evidence relating long term relapses to a persistent tissue stage of the parasite seems to us to be absolutely conclusive. There remain a number of gaps in our knowledge and a number of areas in which generally accepted concepts do not provide all of the answers. We consider the theory of a continuing cycle of exoerythrocytic schizogony to be an inadequate explanation from two points of view. It seems inescapable that the mechanism responsible for the long term relapse

in the St. Elizabeth strain of *P. vivax* is also involved in the delayed patency phenomenon seen with other strains of *P. vivax* (Tiburskaya, 1964). The delayed primary attacks of *P. ovale* reported by Chin and Contacos (1966) and Trager and Most (1963) were apparently due to the use of suppressive drugs at the time of infection. There seems to be no doubt that each of these infected individuals was susceptible during the long period between infection and the primary clinical attack. Therefore, there is no immediately available explanation why an earlier erythrocytic multiplication would not have occurred if parasites were continually being released into the blood from maturing liver schizonts. The same question can be asked concerning the delayed patency phenomenon in certain strains of *P. vivax* (Tiburskaya, loc. cit.). This weakness in the Shortt and Garnham (1948b) theory of continuing exoerythrocytic schizogony was considered much earlier by Fairley (1949) and by Coatney *et al* (1950).

The second area in which the cyclic maturation of fixed tissue schizonts might be challenged is in the relationship between declining immune levels and the eventual reappearance of parasitological and clinical relapse. The original concept, as introduced by Shortt and Garnham (1948a), was that merozoites continually entering the blood from maturing tissue schizonts would be destroyed by the immune mechanisms of the host. Eventually, when the level of immune activity was sufficiently low, the parasites would be able to multiply and thereby produce a relapse. Cooper *et al* (1947) were able to superinfect volunteers with sporozoite-induced infections of the St. Elizabeth strain of *P. vivax* by inoculating whole blood infected with the homologous strain during the long interval between primary attack and initial relapse. It is therefore clear, that the subject was susceptible to blood-stage parasites from an extraneous source--why not, then, from tissue schizonts? It is difficult to understand how the immune response in sporozoite-induced infections would ever drop to the level postulated by Shortt and Garnham (1948b) with the constant antigenic stimulus that would be present with merozoites entering the blood continually from exoerythrocytic schizonts. It is

generally believed that the immune mechanisms of the malaria-infected vertebrate do not reach the liver stages but are only effective against the erythrocytic stages of the parasite. If exoerythrocytic schizogony were a continuing process, it would follow that, once established, the infection would be terminated only by the death of the host.

Garnham (1967) has reviewed this subject and agrees that the process of continuous secondary exoerythrocytic schizogony has not been established. In this publication, he reintroduced the concept of latency or a dormant stage of the parasite as a possible explanation for the relapses seen in certain species of primate malaria and noted that "if such an idea could be proved, then many puzzling features of relapses and latency would be explained." Shute (1946) pointed out that sporozoites could survive in the salivary glands of mosquitoes for considerable periods of time and suggested that they might also be able to do the same thing in the human host. It is interesting that Corradetti (1966) suggested that the late tissue forms described by Bray (1957) might have been derived directly from sporozoites whose development had been delayed by some "unfavorable condition." However, the same author apparently never entertained the possibility that the same delayed development could be responsible for relapses.

There is recent experimental evidence to support the concept of a latent stage as an essential part of the mechanism of relapse in malaria. Warren *et al* (1970) have found that *P. cynomolgi* sporozoites from mosquitoes exposed to X-irradiation immediately prior to dissection and inoculated into malaria-free monkeys produced infections which appeared to be normal, although profound damage was observed in EE schizonts from the same animals. However, monkeys infected with irradiated sporozoites demonstrated fewer relapses than seen in the controls which had received comparable numbers of sporozoites. The authors concluded, that their findings were the result of a random destruction of sporozoites with the concomitant elimination of specific, predetermined relapses.

Experiments, more specifically designed to

demonstrate this point, were carried out by Warren *et al* (manuscript) in which a series of monkeys were inoculated with varying numbers of sporozoites of *P. cynomolgi*. Prepatent periods were only slightly delayed in animals which received low numbers of sporozoites. Infections followed the expected course in all animals and no differences were noted until after treatment with chloroquine. Once relapses began to occur, there was a clear-cut reduction in their number directly related to the number of sporozoites received. These authors concluded that the results strongly challenged the concept of a continuing cycle of histiotrophic merozoites in the liver being responsible for relapses in malaria, since, by this theory, the number of relapses should not be influenced by the number of sporozoites in the original infecting inoculum; one sporozoite should be capable of initiating the complete sequence. In Figure 5 we have presented the hypothetical course of infection for malaras with different potentials for relapse and/or recrudescence.

The survival value of a long term relapse mechanism to a malaria parasite is undoubtedly great and contributes significantly to its ability

to be transmitted to new non-immune hosts. We believe that the need for making gametocytes available in the peripheral blood of appropriate hosts is of such biological urgency that two mechanisms for such an activity have evolved and have been maintained by the primate plasmodia.

We would surmise that a dormant or latent tissue phase of the parasite was the first to evolve and was probably brought forward from an ancient coccidian ancestor. Sufficient information is not available at the present time to establish the exact developmental stage of the parasite which becomes dormant, but one must certainly suspect that either the sporozoite or an intermediary stage, between the sporozoite and the exoerythrocytic schizont, possesses the capacity to settle down and remain quiescent for long periods of time. The extent of this period would seem to be dependent upon the species and even the strain of parasite in question.

It is clearly evident that there are many lacunae in our knowledge of the relapse mechanism. Eventually these gaps will be filled in but until then, the complete picture of the relapse phenomenon is denied to us.

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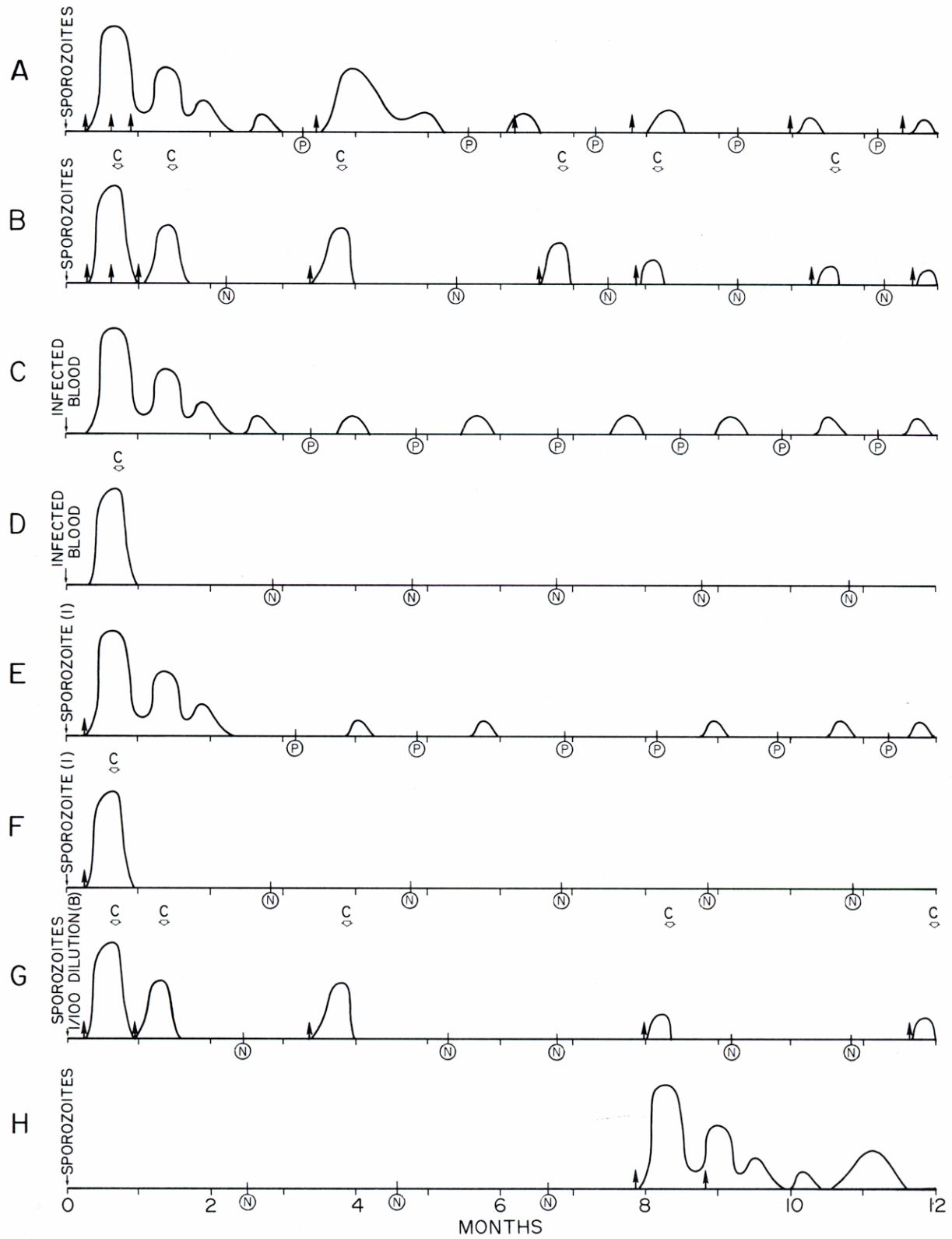
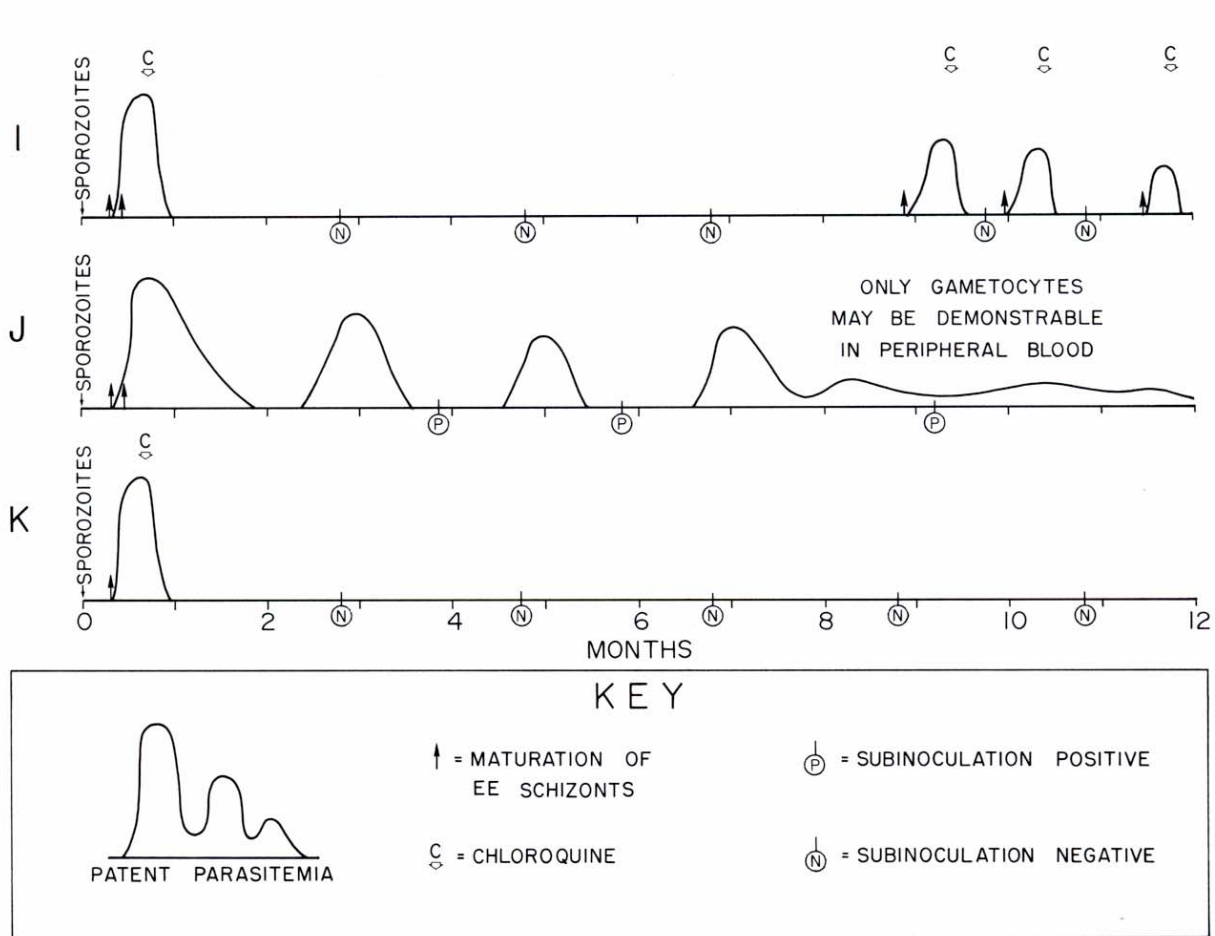


FIGURE 5.—Hypothetical infections of malaria with different relapse potentials. Infections A-G. Relapse and recrudescence Potential of hypothetical infections with *Plasmodium cynomolgi*.



INFECTION A.—Sporozoite-induced infection with *P. cynomolgi* (M strain). Parasitemia is present for at least one year and subinoculation during this period results in infection.

INFECTION B.—Sporozoite-induced infection as in Infection A. Following the initial attack and each subsequent relapse, chloroquinine was used to eliminate the parasitemia. Subinoculations after treatment failed to produce infection.

INFECTION C.—Infection induced by inoculation of parasitized blood. Parasitemia is present for at least one year and subinoculation during this period results in infection.

INFECTION D.—Infection induced by inoculation of parasitized blood as in Infection C. Treatment of the initial infection with chloroquine results in radical cure.

INFECTION E.—Infection induced by the inoculation of a single sporozoite. Parasitemia is present for at least one year and subinoculation during this period results in infection.

INFECTION F.—Infection induced by the inoculation of a single sporozoite. Treatment with chloroquine results in radical cure.

INFECTION G.—Infection induced by the inoculation of 1/100th of the number of sporozoites used in Infection B. The number of relapses is randomly reduced.

INFECTION H.—Infection resulting from sporozoites from a strain of *P. vivax* with extended prepatent period.

INFECTION I.—Type of infection produced by sporozoites of *P. vivax* (St. Elizabeth strain). Initial parasitemia treated with chloroquine.

INFECTION J.—Sporozoite-induced infection with a non-relapsing malaria, *P. falciparum*. Parasitemia is present for at least one year but during the last few months, only gametocytes may be demonstrable.

INFECTION K.—Sporozoite-induced infection with a non-relapsing malaria, *P. falciparum*. Treatment with chloroquine results in radical cure.

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(NS) = Not seen.