BRITISH MEDICAL JOURNAL

LONDON SATURDAY OCTOBER 15 1949

# MALARIA

## WITH SPECIAL REFERENCE TO CERTAIN EXPERIMENTAL, CLINICAL, AND CHEMOTHERAPEUTIC INVESTIGATIONS*

BY

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## LECTURE I: THE LIFE CYCLE

Under the stimulus of war in the Tropics, more scientific effort and medical man-power were directed to intensive study and research on malaria than to any other disease in history. In such circumstances it is hardly surprising that great advances in our knowledge of this disease and of its control have resulted during the past decade.

The discovery of insecticides like D.D.T. and "gammexane" is now leading to the control of mosquito-breeding and the destruction of adult mosquitoes on a scale which is revolutionary. Similarly, the synthesis of new antimalarial drugs in the U.K. and the U.S.A. proceeded on a scale that far surpassed all previous achievements and brought the control of malaria by chemotherapeutic means within the range of practical possibility. In connexion with the discovery of proguanil (paludrine) by Curd, Davey, and Rose (1945) over 1,000 new compounds were produced and tested against bird malaria. In the United States over 14,000 new compounds were screened for their therapeutic action in bird malaria, and over 100 of these were subsequently tested on man. The most promising of the antimalarial drugs synthesized in U.S.A. and U.K., such as certain sulphonamides, mepacrine, "sontochin," "chloroquine," and proguanil, were tested for suppressive and prophylactic action on experimentally infected volunteers living under tropical conditions by Australian medical research units established near Cairns, in Northern Queensland.

The discovery made by Shortt and his colleagues last year of the pre-erythrocytic and exo-erythrocytic cycle in monkey malaria (*Plasmodium cynomolgi*) and of pre-erythrocytic schizonts in benign tertian malaria in man was long overdue, establishing as it did the tissue phase in mammalian malaria some fifteen years after its demonstration in avian malaria. It was a discovery of outstanding importance not only from the parasitological point of view but also because of its clinical and chemotherapeutic implications. This first lecture will be devoted to this subject.

*First of the Croonian Lectures delivered at the Royal College of Physicians of London on May 3 and 5, 1949.

### Erythrocytic and Sporogonous Cycle

At the beginning of the present century the life cycle of the malaria parasite appeared to be completely known. A schizogonous cycle in the red cells which accounted for clinical attacks and a sporogonous cycle in the mosquito had been firmly established. It is true Grassi (1900) had considered there might be another developmental cycle in man during the incubation period, but after Schaudinn (1902) vividly described just how the sporozoites actively penetrated the red cells the chain of events in the life cycle appeared complete. The whole question was reopened some thirty years later mainly because it was not possible to explain the behaviour of antimalarial drugs like quinine and "plasmoquine" (pamaquin) on this basis.

Sergent and Sergent (1922) had noted that though quinine destroyed malaria parasites once they appeared in the blood it exerted no therapeutic action when given during the incubation period. Yorke and Macfie (1924) next reported that quinine given during the incubation period resulted in radical cure of benign tertian malaria which had been transmitted by blood inoculation (trophozoite-transmission), whereas it merely prolonged the incubation period slightly when the disease was transmitted by infective mosquitoes (sporozoite-transmission).

In a paper read before the Royal Society of Tropical Medicine and Hygiene in January, 1931, James (1931a) confirmed these observations and discussed several views which had been put forward to explain the different therapeutic response induced by quinine in patients infected by blood inoculation and those infected by mosquitoes. One theory was that some of the sporozoites, instead of entering the red blood corpuscles, entered connective-tissue cells or the cells lining the capillary blood vessels; another theory was that the sporozoites actually penetrated into the interior of the red cell, whereas merozoites merely attached themselves to the outside of the corpuscle. James favoured the latter view and considered that Schaudinn's account of the penetration of the corpuscle by the sporozoite was "so clear-cut and detailed as to admit

of no question." In the discussion which followed, Warrington Yorke (1931) was frankly sceptical, stating that he had spent some weeks trying to repeat Schaudinn's observations without success.

In August of the same year, James, Nicol, and Shute (1931) reported the prevention of mosquito-transmitted benign tertian malaria in volunteers by the use of pamaquin: 20 mg. was given on the day before infection, and 60 mg. for the next six days. James (1931b) considered that these findings regarding pamaquin favoured the conception that the malaria parasites passed through a tissue stage and, in a communication read by Schüffner at Amsterdam in December, suggested that the time had come "to consider whether knowledge concerning the life cycle of the malaria parasite was complete or not." He pointed out that Schaudinn's observations had never been confirmed, that sporozoites were essentially parasites of tissue cells, and suggested that possibly, on entering the blood, they were carried to reticulo-endothelial cells of the lungs and other organs, where they underwent "a cycle of growth or sporulation similar to the cycle of the allied bird parasite, *Halteridium*."

## Tissue-phase Parasites in Avian Malaria

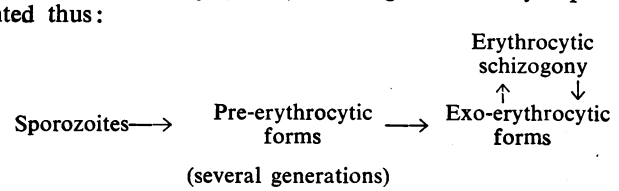
The hypothesis that there was a tissue phase of the parasite soon received support from the discoveries made in bird malaria, but it took another ten years' intensive experimental work to complete the story.

Garnham (1948), in his excellent review on this subject, regards Raffaele (1934, 1936) as probably being the first investigator to recognize the significance of exo-erythrocytic parasites in bird malaria, though undoubtedly many observers had seen them much earlier. The important publications of Huff and Bloom (1935), Brumpt (1937), and James and Tate (1937, 1938) followed shortly after those of Raffaele.

Two distinct schizogonous cycles have been recognized in the tissues: (1) the pre-erythrocytic, which occurs during the incubation period before asexual parasites appear in red blood corpuscles; and (2) the exo-erythrocytic schizogony, which is found after parasites have gained access to the corpuscles and persists in the late stages of the infection.

In seven species of plasmodia producing malaria in birds, Porter (1942) differentiated between two types of exo-erythrocytic schizogony—one occurring in *P. gallinaceum* and the other in *P. elongatum* infections. Schizogony of gallinaceum type occurred mainly in the reticulo-endothelial system, that of elongatum type was confined to the wandering cells of the blood and haemopoietic organs.

The salient features in the tissue cycle in avian malaria, modified from Davey (1944), are diagrammatically represented thus:




1. *Pre-erythrocytic Schizogony.*—The pre-erythrocytic forms in *P. gallinaceum* were first discovered by Mudrow (1940) and Shortt, Menyon, and Iyer (1940). A few years later Huff and Coulston (1944) injected enormous numbers of sporozoites into localized skin areas in chicks, demonstrating that sporozoites penetrated the reticulo-endothelial cells of the skin in from half an hour to six hours and underwent a schizogonous cycle lasting 42 hours. Primary forms also developed in the spleen and other organs intercurrently. When the schizont ruptured, the first-generation merozoites (cryptozoites) penetrated adjacent reticulo-endothelial cells, where they underwent one or more schizogonous cycles before liberating second- or third-generation merozoites (metacryptozoites) into the blood. By the fifth day Reichenow and Mudrow (1943) distinguished two types of schizonts—the macroschizonts containing 100–200 macromerozoites which are destined to invade fresh reticulo-endothelial cells, and the microschizonts containing up to 1,000 micromerozoites which invade the red blood corpuscles. Subinoculations with saline suspension of organs containing pre-erythrocytic parasites consistently yield positive results in avian malaria.

2. *Exo-erythrocytic Schizogony.*—Little difference has been observed between the pre-erythrocytic and the later exo-erythrocytic schizonts; pigment is absent from both, since they develop in cells containing no haemoglobin. The distribution of exo-erythrocytic schizonts in the organs has been described by several different workers. James and Tate (1937, 1938), who independently described the schizogonous cycle in the reticulo-endothelial cells and monocytes of fowls infected with *P. gallinaceum*, first suggested that the non-committal title "exo-erythrocytic" be used to differentiate it from erythrocytic schizogony. They found that the brain, liver, lungs, and spleen were important sites for the tissue stages, and that, even after the erythrocytic parasites had disappeared from the blood with quinine treatment, birds died from blockage of the vessels of the brain resulting from schizonts developing in the reticulo-endothelial lining.

Coulston and Manwell (1941) actually produced experimental infections with *P. circumflexum* by the injection of a single parasitized corpuscle, and after several passages exo-erythrocytic forms appeared. After intravenous inoculation of blood containing *P. gallinaceum*, Tullis (1947) found that small numbers of exo-erythrocytic schizonts appeared on the fourth day in reticulo-endothelial cells of the spleen and on the sixth day in the liver, heart, lungs, and intestine. Thus in bird malaria tissue schizonts may originate directly from pre-erythrocytic parasites or indirectly from the blood parasites.

## Tissue-phase Parasites in Monkey Malaria

In monkeys, malaria which is caused by *P. cynomolgi* is readily transmitted experimentally by *Anopheles maculipennis atroparvus*. Other well-known simian parasites, like *P. knowlesi* and *P. brazilianum*, are unfortunately unsuitable for studying tissue forms, since the mosquito vector is unknown.

In *P. cynomolgi* infections the erythrocytic parasites closely resemble vivax parasites morphologically and appear in the blood about the ninth day. Relapses are common up to six to eight months, when spontaneous cure generally occurs. Coatney and Cooper (1948) report that pentaquine and pamaquin with or without quinine bring about radical cure. The disease closely resembles benign tertian malaria in man, especially the New Guinea and Chisson strains characterized by short-term relapses. Much intensive research work has been undertaken in America, India, England, and other parts of the world during the past decade in an effort to demonstrate tissue forms in monkeys infected with this species of plasmodium. It was not until last year, however, that Shortt, Garnham, and Malamos (1948), at the London School of Hygiene and Tropical Medicine, discovered pre-erythrocytic forms in the liver of a monkey (*Macaca mulatta*) intensely infected with sporozoites seven days previously.

1. *Pre-erythrocytic Parasites.*—In this experiment over 500 hyperinfected mosquitoes (*A. maculipennis atroparvus*) were

first fed on this monkey; then the entire batch was ground up with heparinized plasma and injected parenterally. The pre-erythrocytic forms were confined to the parenchyma cells of the liver, sections of which showed ovoid blue areas, averaging about 26 $\mu$ in diameter, composed of plasmodial masses containing chromatin particles. Later work showed that mature schizonts on the eighth or ninth day contained nearly 1,000 merozoites; each merozoite measured a little over 1 $\mu$ in diameter and was composed of a mass of cytoplasm with a small fragment of chromatin on one side. When the schizont ruptured, the merozoites escaped into the circulation via the hepatic sinusoids, and invasion of the residual mass by lympho-macrophage cells, plasma cells, and polymorphonuclear cells occurred. Pre-erythrocytic schizogony in *P. cynomolgi* was later confirmed by Hawking, Perry, and Thurston (1948).

2. *Exo-erythrocytic Forms.*—Some months later Shortt and Garnham (1948) demonstrated the late tissue stages (exo-erythrocytic schizonts) in the parenchyma cells of the liver of a monkey which had been hyperinfected 103 days previously; by this time the disease was latent and parasites had temporarily disappeared from the peripheral blood. The late exo-erythrocytic forms resembled the pre-erythrocytic forms in most respects, a possible difference being the presence of a limiting membrane surrounding the younger schizonts. These exo-erythrocytic forms undoubtedly represent the relapse forms. Shortt and Garnham (1948) consider that exo-erythrocytic schizogony is maintained throughout the whole course of the disease, and that the cellulo-humoral antiparasitic defence mechanism of the host is operative only against the erythrocytic parasites, resulting in the destruction of merozoites entering the circulating blood. This immunity mechanism, which comes into play exclusively against the erythrocytic parasite, is not active against the exo-erythrocytic forms themselves, possibly because of their intracellular habitat. Those merozoites destined to invade other liver cells are similarly protected, so that the exo-erythrocytic cycle can go on indefinitely quite independently of blood infection.

*Comment.*—The success attained by Shortt and his colleagues, where so many others had failed, was no doubt due to the intense hyperinfection adopted and the improved technique devised for staining tissue parasites. In the monkey it involved the introduction of hundreds of thousands of sporozoites by mosquito-bite and by inoculation either of a whole mosquito intraperitoneally or of the salivary glands intravenously (Garnham, 1948). Investigators had also been misled to some extent because the tissue phase in mammalian malaria differed in certain unexpected ways from avian malaria. Thus in monkey malaria schizogonous development takes place in the parenchymatous cells of the liver, not in reticulo-endothelium, while subinoculation with saline suspensions of the liver into clean monkeys invariably yields negative results, probably because there is only one generation of pre-erythrocytic schizonts whereas in avian malaria there are several.

## TISSUE-PHASE PARASITES IN HUMAN MALARIA

### A. Benign Tertian Malaria

*Pre-erythrocytic Phase.*—Shortt, Garnham, Covell, and Shute (1948) also reported the pre-erythrocytic stage of *P. vivax* in a volunteer patient who had been exposed to heavy biting by infected mosquitoes (*A. maculipennis atroparvus*) and had in addition received an intravenous injection of sporozoites derived from their salivary glands. Laparatomy and liver biopsy were performed seven days later under local analgesia. Sections revealed plasmodial masses in the parenchyma cells, measuring up to 42 $\mu$ in diameter, which were studded with chromatin particles and some of which contained vacuoles. In appearance they closely resembled the pre-erythrocytic schizonts found in *P. cynomolgi* in monkeys.

From subinoculation experiments undertaken by Fairley (1945) and Fairley *et al.* (1947) it is evident that vivax sporozoites after being inoculated by mosquitoes rapidly gain access to the blood but soon again leave the circulation, invading the parenchyma cells of the liver, as shown by Shortt and his colleagues. Here they pass through a prolonged developmental cycle lasting at least eight days. The mature schizonts rupture and merozoites enter the circulation with great regularity on the ninth day of infection, producing a subpatent parasitaemia which may last another one to seven days. In thick films scanty parasites —i.e., to the order of 1 per c.mm.—appear on an average 12 days after exposure (range 10–15 days). This at least is the chain of events so far as the New Guinea strain of *P. vivax* is concerned.

*Exo-erythrocytic Forms.*—There is so much indirect clinical evidence supporting the presence of persistent exo-erythrocytic forms in benign tertian malaria, and vivax infection with short-term relapses is so similar to cynomolgi malaria, that for all practical purposes their existence can be accepted. Latency and the tendency to multiple relapses can best be explained on the basis of persisting exo-erythrocytic forms, while the different therapeutic response to various schizonticidal drugs like mepacrine, chloroquine, and quinine observed in blood-inoculated and mosquito-transmitted malaria is otherwise inexplicable. The schizonticidal drugs rapidly produce radical cure in trophozoite-transmitted vivax malaria. In sporozoite-transmitted malaria, though they rapidly produce clinical cure and clear the blood completely of parasites as revealed by negative subinoculations and negative blood smears, parasites later reappear and overt relapses follow. To establish radical cure during the overt attack one of the 8-amino-quinoline compounds like pamaquin or pentaquine is given in full dosage in combination with quinine. During the long latency characteristic of the St. Elizabeth strain of *P. vivax* radical cure has been reported by White *et al.* (1948) at a time when erythrocytic parasites are entirely absent from the blood: such a result can be due only to exclusive action on exo-erythrocytic forms.

*Exo-erythrocytic Forms and Immunity Response.*—According to Shortt and Garnham (1948) exo-erythrocytic schizogony is maintained throughout the whole course of the disease, and the antiparasitic defence mechanism of the host is directed essentially against the erythrocytic parasites, not against pre-erythrocytic or exo-erythrocytic forms. They suggest that latency and relapse in vivax malaria are governed by this immunity defence mechanism. Acquired antiparasitic immunity and tolerance result from a specific cellulo-humoral response to the invasion of the blood by erythrocytic parasites—provided that the parasitaemia is of sufficient duration and intensity to elicit an adequate response. The experimental studies of Taliaferro and Mulligan (1937) indicate that the spleen, liver, and bone marrow undergo considerable lymphoid hyperplasia with cytogenesis of macrophages, parasites being mainly destroyed locally in these organs. Immunity is paid for in terms of blood destruction, overworked sweat glands, and fever, but it is more easily acquired with some vivax strains than with others. Whether it is complete or partial will be determined by the trophozoite experience of the individual (total antigenic stimulus) and the strain of *P. vivax* implicated. With New Guinea strains solid immunity results in untreated patients only after prolonged primary fever, or if this be cut short by antimalarial

therapy it may arise later during febrile relapses in which treatment is withheld or markedly delayed.

### Latency and Relapse

1. *Latency with Immunity.*—Blackburn (1948) was able to show that volunteers infected with the New Guinea strain of *P. vivax* who developed solid tolerance and antiparasitic immunity to their infections constantly had vivax trophozoites in the peripheral circulation, though their density rarely exceeded 1 per c.mm. over several months of observation. Gametocytes were never seen during the period of immunity, and mosquitoes fed on these subjects failed to become infected. The low densities of trophozoites caused no constitutional disturbances whatsoever. These volunteers had normal haemoglobin concentrations and red blood cell counts, their spleens were not palpable, and they were perfectly fit. Attempts to induce relapses by reinfection with the same strain of *P. vivax* (superinfection) were unsuccessful.

2. *Latency and Suppression with Mepacrine.*—In experimentally infected volunteers taking 0.1 g. of mepacrine daily for many months, Fairley and his colleagues (1945, 1947) found that vivax parasites were not demonstrable in thick smears, that subinoculations invariably became positive on the 9th day and were generally negative after the 14th day of infection. In one patient receiving 50 infective vivax bites on zero day, subinoculations with 20 ml. of blood were made at four-day intervals between the 9th and 142nd days while receiving suppressive mepacrine. Positive results were recorded on the 9th, 13th, and 42nd days after infection; the remaining 32 subinoculations were negative. Parasites were absent in blood films on the 42nd day, when the positive subinoculation occurred. Overt vivax malaria occurred on the 177th day, 35 days after mepacrine ceased. It is noteworthy that in heavy experimental infections the 42nd day is about the time the first relapse appears in patients whose primary fever has been treated with schizonticidal drugs. After stopping suppressive mepacrine overt vivax malaria occurred within three to ten weeks in 97% of cases. No evidence of immunity was observed in these patients: this is hardly surprising, as merozoites are destroyed shortly after reaching the blood with the New Guinea strain of *P. vivax*, provided the plasma mepacrine concentration is satisfactory.

When suppressive drug medication is stopped overt attacks occur in a few weeks with the ordinary New Guinea and the Chisson strain of *P. vivax*, which have a close relapse pattern, but with the St. Elizabeth, Madagascar, and local Dutch strains, which have had suppressive treatment during the first four weeks after infection, overt malaria does not appear until approximately nine months after the original infection.

3. *Latency and Long-term Relapse without Immunity.*—True latency with long-term relapse is encountered with certain strains of *P. vivax* both in natural infections and where the primary fever has been suppressed by antimalarial drugs or treated early in the febrile attack.

(*a*) *Latency Following Suppression of Primary Fever.*—Important experimental investigations in volunteer prisoners in the U.S.A. have recently been carried out with the St. Elizabeth strain of *P. vivax*. This strain produces an infection with a long latent period of eight to nine months between the early treated primary attack and the first relapse; thereafter a series of short-term attacks begin. White *et al.* (1948) report the complete absence of circulating parasites during this long latent phase; thick blood smears were always negative, and massive subinoculations with 250–300 ml. of blood to non-immune recipients failed to produce malaria. In addition Cooper *et al.* (1947) report that during this period the individual harbouring a latent infection is susceptible to superinfection with parasites of the same strain, indicating that no immunity has developed. Coatney and Cooper (1948) conclude that it is impossible to account for this long latent interval on the basis of immunity to erythrocytic parasites, and suggest that it may represent a period when the fixed-tissue reservoir is not casting erythrocytic invading forms into the circulation.

(*b*) *Natural Latency with Prolonged Incubation Period.*—This condition is well recognized in natural infections in Europe, where in the early spring primary attacks may be caused by infections contracted in the previous summer and autumn. James (1931a) reported that 12 patients at Horton did not develop overt vivax malaria until six to ten months after being bitten by infective mosquitoes (Madagascar strain). Swellengrebel and de Buck (1938) found that many of their patients infected with the local Dutch strain fell into this category, not developing overt malaria for some nine months after exposure to infective mosquitoes biting at any time of the year. There is a normal average incubation period of 21 days with this Dutch strain, and eight to nine months elapse between primary fever and the first relapse.

Shute (1946) produced good evidence that natural latency in vivax malaria—i.e., an overt attack in patients infected eight to nine months previously—was attributable to low sporozoite dosage; he suggested that relapses might be due to sporozoites which had been lying dormant in tissue cells, pointing out that they were able to survive for many months in the insect carrier. According to Shute, natural latency occurs only when the sporozoites injected are too few to set up an immediate attack: about 2,000 sporozoites were regarded as the minimum necessary to establish infection by intravenous injection.

Serial subinoculations have not been performed on and after the ninth day following infection in these latent cases, but it seems likely that owing to a paucity of inoculated sporozoites only scanty pre-erythrocytic schizonts are produced; the resulting erythrocytic merozoites are evidently so few that non-specific phagocytosis in the liver, spleen, bone marrow, and blood—i.e., natural immunity—suffices to prevent the establishment of an effective parasitaemia. In consequence primary fever fails to develop, and with the Madagascar and Dutch strains an overt attack is not encountered for eight to nine months.

*Comment.*—Results based on infections with the New Guinea strain of *P. vivax*, which has a short-relapse pattern, support the view that latency is related to, and controlled by, the immunity response of the host as in cynomolgi malaria in monkeys.

In vivax infections with the St. Elizabeth, the Madagascar, and the local Dutch strains, which are characterized by long-term latency before relapse, it is difficult to visualize how any immunity could have been acquired. In these circumstances the most likely explanation is a failure of erythrocytic merozoites to reach the blood stream and establish a parasitaemia, as Coatney and Cooper (1948) have pointed out. The failure to establish even a subpatent parasitaemia during this long-term latency is revealed by the negative results obtained with massive subinoculations in non-immunes. This might result from (1) cessation of exo-erythrocytic schizogony with the production of resting-stage parasites; (2) a slowing down of exo-erythrocytic schizogony and/or a decrease in the ratio of erythrocytic to liver-invading merozoites, so few of the former reaching the circulating blood that natural immunity suffices to prevent an effective parasitaemia developing; and (3) a switch-over to the exclusive production of liver-invading merozoites during exo-erythrocytic schizogony, the rate of which may or may not be retarded. It is suggested that No. 3 constitutes the most feasible working hypothesis, and that the atypical exo-erythrocytic schizogony which characterizes long-term latency is determined by the innate

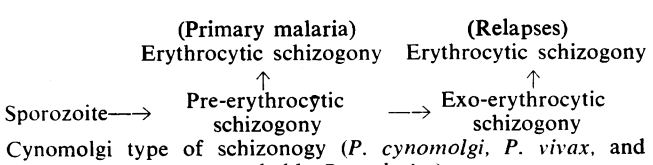
biological qualities of the vivax strains under consideration. Latency ends when there is a reversion to normal exo-erythrocytic schizogony, with the production of the usual ratio of erythrocytic to liver-invading merozoites.

Once overt malaria supervenes after this long latent period it is interesting to note that several relapses not infrequently follow at monthly periods (close relapse pattern), indicating that normal exo-erythrocytic schizogony has been established and is being maintained.

### B. Quartan Malaria (P. malariae)

In quartan malaria both sporogonous and erythrocytic schizogonous cycles proceed very slowly. Nothing is known of the tissue cycle. Unfortunately, subinoculations have not been performed during the incubation period, so no assessment can be made of the probable duration of the pre-erythrocytic cycle. The long latent periods, the presence of late relapses, and the difficulty of attaining radical cure suggest that exo-erythrocytic schizogony persists for many years. Blood-transfusion accidents, in which quartan malaria is transmitted by a donor who has been away from malarious areas for many years and been completely free from clinical attacks during this period, also support this view.

The following diagram depicts the tissue phases of the malaria parasite in monkeys infected with *P. cynomolgi*. It is suggested that the term "cynomolgi type" could usefully be employed in mammalian malaria for this type of schizogony. There is abundant indirect evidence that *P. vivax* follows the cynomolgi type of schizogony, and it seems highly probable that *P. malariae* does likewise.




Cynomolgi type of schizonogy (*P. cynomolgi*, *P. vivax*, and probably *P. malariae*)

### C. Malignant Tertian Malaria (P. falciparum)

The present position in regard to the tissue phase of *P. falciparum* is much the same as that of *P. vivax* last year, before pre-erythrocytic forms had been discovered.

*Pre-erythrocytic Cycle.*—Subinoculation experiments reported by Fairley (1945) and Fairley *et al.* (1947) have shown that when donors are bitten by from 7 to 24 infective mosquitoes at a single session lasting 5 to 15 minutes sporozoites may gain access to the circulation during the time of biting or be detected at intervals up to 60 minutes after cessation of biting. Thereafter subinoculation results were uniformly negative until the seventh day, when they again became consistently positive. All of 41 subinoculations with 500 ml. of blood made from volunteers infected 6½ to 8½ days previously were positive.

The only reasonable interpretation of these findings is that, in falciparum as in vivax malaria, sporozoites inoculated into the tissues by infective anophelines rapidly gain access to the circulating blood and subsequently disappear rapidly from the circulation. Presumably they enter the parenchyma cells of the liver, where they develop into pre-erythrocytic schizonts, which liberate mature merozoites into the blood with extraordinary regularity on the seventh day of infection.

The causal prophylactic action of proguanil in malignant tertian malaria also suggests the existence of early tissue forms which are many times more sensitive to the drug than either sporozoites or merozoites. Fairley *et al.* (1946) found that a single dose of 50 mg. given between the 39th and the 131st hour following exposure to biting afforded complete protection; parasites never appeared in the blood and overt malaria failed to develop. Eight out of 10 volunteers receiving as little as one single dose of 10 mg. between 48 and 120 hours after infection were completely protected. It was found that proguanil (0.3 g.) given about the time of exposure to infection generally failed to protect; malaria could be prevented with certainty only if 1 g. of the drug was given. The conclusion reached was that proguanil had no action on the sporozoites as such, its lethal effect being due to small quantities persisting in the circulation and acting on tissue forms early in the pre-erythrocytic cycle. Later on the seventh day, by which time merozoites were entering the circulation, a single dose of 0.3 g. failed to give protection, though it was effective up to 144 hours. The most reasonable explanation of these experimental findings is that proguanil has a highly selective action on the pre-erythrocytic forms in falciparum malaria.

*Exo-erythrocytic Cycle.*—Though indirect evidence favouring a pre-erythrocytic stage is entirely convincing in malignant tertian malaria, the same cannot be said in regard to the existence of later exo-erythrocytic forms. None of the evidence advanced as indicating a persistent phase in vivax malaria is forthcoming for falciparum malaria. Thus in falciparum malaria (1) long latent periods are absent and recrudescences occur separated by only two to three weeks of apyrexia; (2) once the blood has been completely cleared of falciparum parasites by schizonticidal drugs like quinine, mepacrine, or chloroquine radical cure has been achieved; (3) it is useless to resort to the 8-amino-quinolines for radical cure as in benign tertian malaria: drugs of this group, though they are effective in preventing malignant tertian malaria when they are given in full dosage during the first six days of infection, are quite ineffective in the treatment of the acute attack and the eradication of the infection; this is because they are very poor schizonticides for *P. falciparum*; and (4) sporozoite-transmitted malaria is just as rapidly and radically cured by schizonticidal drugs as is trophozoite-transmitted malaria; this is contrary to the findings in benign tertian malaria.

To explain these therapeutic results it would be necessary to assume that, while the hypothetical exo-erythrocytic forms in falciparum malaria are just as susceptible to schizonticidal drugs as are asexual erythrocytic parasites, they are insusceptible to the 8-amino-quinolines. This would be exactly the reverse of the action of these drugs in benign tertian malaria. In toxic dosage the 8-amino-quinolines are known to have a true causal prophylactic effect in malignant tertian malaria, where their action on pre-erythrocytic forms is much more potent than in vivax malaria. Why, then, should they have no effect on exo-erythrocytic forms in malignant tertian malaria while destroying the exo-erythrocytic forms in benign tertian malaria?

All available data indicate that in malignant tertian malaria, after the pre-erythrocytic schizonts have ruptured and discharged their merozoites into the circulation on the seventh, and possibly the eighth and ninth days as well, the pre-erythrocytic schizonts disappear entirely without giving rise to later exo-erythrocytic forms. The subsequent course of the disease will thus be determined solely by erythrocytic schizogony, and if the parasitaemia be eradicated radical cure results.

It is suggested that the following diagram adequately depicts the tissue phase of schizogony as it occurs in malignant tertian malaria in man:

(Primary fever and recrudescences)
↑
Erythrocytic schizogony
↑
Sporozoite⟶Pre-erythrocytic schizogony

### Comment and Conclusions

At the beginning of the present century the life cycle of the malaria parasite in man appeared to be completely known. It is a matter of considerable interest that, nearly half a century later, hiatuses in precise knowledge of the subject still exist. Despite the brilliant work of Shortt and his colleagues, only the pre-erythrocytic stage in *P. vivax* has so far been demonstrated in man. The technical difficulties associated with the production of hyperinfection in man and the demonstration of tissue schizonts in the liver have been overcome. No suitable laboratory animal is known which can be experimentally infected with any of the human species of malaria parasite, and in these circumstances the various problems concerning tissue forms are unlikely to be solved finally without the use of experimentally infected volunteers.

In the meantime the following tentative conclusions based mainly on indirect evidence are put forward regarding the tissue phase of the three species of malaria plasmodia commonly affecting man.

1. In benign tertian and quartan malaria there are both pre-erythrocytic and exo-erythrocytic stages of schizogony similar to those now known to occur in cynomolgi malaria in monkeys.

2. In vivax malaria characterized by short-term relapses such as are produced by the New Guinea or Chisson strains, normal exo-erythrocytic schizogony is maintained throughout the whole course of the disease and erythrocytic merozoites reach the circulating blood with regularity. Relapses cease when solid immunity and tolerance have developed or radical cure has been established.

3. In vivax infections characterized by long-term latency before relapse, such as is seen in infections with the St. Elizabeth, Madagascar, and Dutch strains, parasitaemia and immunity fail to become established during this initial latent period. As a working hypothesis it is suggested that during latency the exo-erythrocytic cycle is atypical, inasmuch as schizogony is switched over to the exclusive production of liver-invading merozoites, so that no erythrocytic merozoites reach the circulation. Latency ends when there is a reversion to normal exo-erythrocytic schizogony; this is indicated by the close relapse pattern which supervenes after the long period of latency has terminated in an overt malaria attack.

4. In malignant tertian malaria, after the pre-erythrocytic schizonts have ruptured and discharged their erythrocytic merozoites into the circulation on the seventh and possibly the eighth and ninth days as well, they disappear without giving rise to late exo-erythrocytic forms. The subsequent course of the disease will be determined solely by erythrocytic schizogony, and if parasitaemia is eradicated radical cure will result.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# CLINICAL VALUE OF PREGNANEDIOL ASSAYS*


BY

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A laboratory technique such as pregnanediol assay may lay claim to clinical value if it can be shown that its results yield information about diagnosis, prognosis, or treatment which will supplement that gained by unaided clinical examination. In assessing the clinical value of a given technique various factors must be considered and given due weight. The accuracy of the method, for example, is not of primary importance, though its ability to give unequivocal answers to the clinical questions posed is clearly a first requirement. Ease and speed of performance are significant considerations, and the nature and quantity of the clinical specimens required deserve attention. If to these we add the uniqueness of the information yielded by the technique, it should be possible to form an opinion of its value to the clinician in comparison with other laboratory procedures. In this paper the attempt is made to evaluate the position of pregnanediol assay, as judged by the above criteria and using the data that I have myself obtained.

A large number of different procedures for the assay of urinary pregnanediol have been described, some undoubtedly being far more accurate than others. I shall not attempt a comparative discussion of these rival methods, but certain general remarks are warranted. In the first place, methods requiring three or four days and many man-hours to complete are of little potential clinical value, no matter what their excellence as purely research techniques may be. Secondly, since by the nature of the clinical problems concerned repeated assays may be necessary, it becomes a matter of urgency to avoid, if at all possible, urine-collection requirements which would limit the application to in-patients only.

*Paper read at the British Congress of Obstetrics and Gynaecology, London, on July 6, 1949.