EXPERIMENTAL STUDIES ON THE MALARIA OF MONKEYS.*

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Some of the fundamental problems of immunology can be more easily studied in the blood protozoa than in the smaller bacterial invaders. Thus, I have previously shown (See reviews in Taliaferro, 1929 and 1932) that the trypanosomes and plasmodia in particular, because of their easily accessible location, their even distribution throughout the blood and their large size, permit the direct correlation of the serological and cellular reactions of the host with the course of primary infections and the acquisition of acquired immunity. My subject this afternoon involves such a study of the quartan malaria parasite, *Plasmodium brasilianum* of Panamanian monkeys.

My chief emphasis will be centered on an analysis of acquired resistance in the monkey as exemplified by the course of the initial infection, the high degree of immunity to superinfection following the initial acute infection and the cellular bases for this acquired immunity. This presentation will necessarily involve a consideration of the morphology of the parasite and its normal course of infection. These results, I hope, will be of intrinsic interest to you as an attack on the question of infection and resistance in a protozoan infection. But, in addition, they are of particular interest as they advance our knowledge of infection and resistance in a form more closely related to man than has heretofore been accomplished.

In spite of the enormous amount of work done on human malaria it has been almost impossible to subject the infection to an experimental

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analysis because of the unsuitability of man as an experimental animal. For this reason we have analyzed in detail during the past several years the infection and immunity in an avian malarial organism, *P. cathemerium* in the canary. Our results on infections in the monkey so closely parallel those on infections in the bird that I shall use the latter constantly throughout my talk as a comparative standard.

So far we have made observations on 342 monkeys, including uninfected animals, monkeys shot in various expeditions by Dr. H. C. Clark and his associates (see Clark, 1930 and 1931) and monkeys experimentally infected in the laboratory. All of these specimens belong to the Cebidae and include representatives of the red spider monkey, *Ateles geoffroyi* Kuhl, the Darien black spider, *A. dariensis* Goldman, two varieties of the white throated or white face monkey, *Cebus capucinus* Linnaeus, (*C. c. capucinus* and *C. c. imatator*), and two varieties of the Panama howling monkey, *Alouatta palliata inconsonans* Goldman and probably *A. p. palliata*. The present account is a progress and preliminary report.

Distribution of malaria among the primates.

The higher anthropoid apes, the chimpanzee and gorilla, harbor three species of malarial parasites morphologically indistinguishable from *P. vivax*, *P. malariae* and *P. falciparum* of man. Although Reichenow (1917 and 1920) believes that these are identical and Mesnil and Roubaud (1917) have apparently successfully infected one chimpanzee with *P. vivax* from man, the question of their identity is still unsettled. Of particular interest is the fact that several investigators have entirely failed to infect apes successfully with human species or to infect man with the malaria of the chimpanzee (See Blacklock and Adler, 1922 and 1924).

Among the lower primates, malarial parasites have been found in both the Old World Cercopithecidae and the New World Cebidae. There seems, however, to be a sharp delineation in type of parasite harbored by the two groups of monkeys. Thus, all of the parasites from the Old World monkeys are tertian-like in morphology and similar to *P. vivax*, although described under several specific names (see review in Wenyon, 1926) and those from the New World monkeys are quartan-like in morphology and similar to *P. malariae* according to the evidence I shall present shortly. In spite of their similarity in morphology to the human species, these parasites of the lower primates are apparently distinct. All cross-infection experiments have failed with the exception of a possible evanescent infection of man with a

parasite from the Panamanian monkey described by Clark and Dunn (1931).

The parasite.

In 1908 Gonder and Berenberg-Gossler first discovered a malarial parasite in a New World monkey. This parasite they found in a specimen of *Brachyurus calvus* imported to Hamburg from the Amazon district and named it *P. brasilianum*. They gave an excellent description and noted the quartan periodicity and similarity to *P. malariae*. Their work was amplified by Berenberg-Gossler (1909) with the same material from the same monkey. With the exception of the finding of unpigmented rings in a specimen of *Ateles* of Yucatan by Seidelin (1912) no further work was published on the parasites of the New World monkeys until the extensive investigations of Clark who examined a large number of spider, white throated and howler monkeys of Panama.

In his published papers (1930 and 1931) he concluded that there were basically two types of malaria: First, a species in the white throated monkey which was fundamentally quartan-like in structure causing no hypertrophy of the red cell and producing 8 to 10 merozoites, and second, a species in the red spider monkey which was fundamentally tertian in structure, producing some hypertrophy of the red cells and from 12 to 14 merozoites. He noted, however, that the number of merozoites in this tertian species was still only about half the number generally found in human tertian and in his later experimental work which is unpublished he began to question whether this differentiation of species was valid.

After a detailed study of the morphology and asexual cycle in 139 monkeys of Panama, including natural infections in red spider, white throated and black howlers, and cross-inoculations among these species and black spiders, we have verified all of Clark's findings except that we have shown conclusively that there is only a single species of malaria in the Panamanian monkeys, which is fundamentally quartan in periodicity, but varies somewhat in morphology depending upon the species in which it is grown. Two examples will emphasize the great importance of studying infections with the same parasite when grown in different species of monkeys. The natural infection encountered in the black howler monkey showed a large number of the band-like schizonts so characteristic of human quartan malaria, whereas natural infections of the white throated and red spider monkeys showed comparatively few. Subsequent experimental work demonstrated that when malaria from black howlers was grown in the

other two species it did not form bands to as large an extent and that, vice versa, when malaria from white throated and red spider monkeys was grown in black howlers it exhibited a very high percentage of bands. Furthermore, the number of merozoites produced by the same strain varied in individual monkeys, but in general was higher in the red spider than in the white throated and howler monkey. These findings together with morphological and cross-inoculations have convinced us that all of the parasites found in Panamanian monkeys belong to a single species which is identical with *Plasmodium brasilianum* described by Gonder and Berenberg-Grossler from their Brazilian monkey.

The general morphology of *P. brasilianum* is very similar to that of *P. malariae* of man. In fact, in those animals such as the black howler where band stages occur in quantity it is indistinguishable in our experience. In other species where bands are not common, the young trophozoites are similar to those of *P. vivax* except that the red cell does not become enlarged except occasionally during the final stages of segmentation and even then never as much so as in *P. vivax*. Gametocytes appeared comparatively seldom in all of the infections whether naturally or experimentally acquired and whether initial infections or relapses. When they were found, however, they agreed in every respect to the morphology of *P. malariae*.

The asexual cycle.

The development of the asexual forms is of interest from two angles. In the first place, as will be discussed in the next section, the fact that these forms develop synchronously permits a direct analysis of certain factors in the resistance of the host to the parasite. In the second place, the synchronous asexual cycle is so characteristic of malarial infections in general that its description and causation is of fundamental interest.

The study of the asexual cycle was carried out in connection with the morphological study of the parasite and involved the making of blood smears at 4, 8, or 12-hour intervals. Two methods were adopted to ascertain the presence, nature and synchronicity of the asexual cycle. In some cases the mean-size-curve was used, as described by Mrs. Taliaferro (1925) in her work on *P. cathemerium*. From 25 to 50 parasites were drawn from each blood film at 12-hour (or less) intervals and then measured in square microns. When the mean size of these measurements is plotted, it is low when only merozoites and young trophozoites are in the blood, gradually rises as the parasites

grow and reaches its peak at the time of sporulation. In other cases the percentage of sporulators was ascertained, as used by Boyd (1929*a*). In the present work the number of asexual forms having 5 or more nuclei in a sample of 50 (25, occasionally) was ascertained at 12-hour (or less) intervals. These numbers, when plotted in percentages, show a series of peaks just before each sporulation, as is represented in fig. 1. The second method is much less laborious than the first and has the further advantage of being applicable to low grade infections where parasites can be found only in thick films.

The asexual cycle has been studied throughout the major portions of 19 infections and during lesser portions of 57 infections. Briefly stated, these studies indicated that the asexual cycle is quartan in type, that each parasite takes 3 days to grow, divide into approximately 8 to 10 merozoites and sporulate, that sporulation normally takes place from 10 A.M. to 2 P.M. every third day, that most infections are composed of a single brood, i.e., sporulation occurs regularly every third day around noon, that some consist of double broods, i.e., sporulation occurs at noon on two successive days followed by a day when no sporulation occurs and that at least one infection was made up of three broods, i.e., sporulation occurs regularly every day around noon. That the latter was not a quotidian type was clear since at each sporulation time there were also present large schizonts whose nuclei had not begun to divide, representing the brood that would sporulate the day following, and immature schizonts, representing the brood that would sporulate 2 days hence.

A number of investigators have been interested in ascertaining whether this cycle is determined by the genetical constitution of the parasite or by the physiological activities of the host. For many years most investigators leaned toward the first explanation. Thus, where double and triple broods of parasites existed it was assumed that these separate broods represented the progeny of separate mosquito inoculations, each brood maintaining the synchronicity which was determined by the moment the sporozoites were introduced into the body. A few investigators have begun to doubt this, however, and some of the recent work on induced malaria in man, particularly that of S. P. James and Shute (1926), indicated that a single brood of parasites might vary in its synchronicity and even split up into separate broods.

Experimental work on the quotidian *P. cathemerium* of canary birds has paved the way for an experimental analysis of this problem. In 1928 Mrs. Taliaferro showed that the time of sporulation in *P. cathemerium* could be delayed by placing the parasites at 0.5° C., and that

the delay was commensurate with the interval of refrigeration when the parasites were subsequently injected into birds. Quite to her surprise, however, each succeeding sporulation occurred a few minutes earlier until the parasites gradually returned to their accustomed schedule. Later, Boyd (1929*a*) performed the ingenious experiment of reversing the normal periods of light and dark and found that the parasites reversed their periodicity, viz., instead of sporulating between 5 and 8 in the afternoon, they sporulated between 3 and 8 A.M. He also (1929*b*) was able to lengthen the cycle to 28 hours by subjecting the birds to a 14-hour day and a 14-hour night. These experiments indicate clearly that the time at which sporulation occurs is determined by some physiological reaction within the host.

We have repeated one of the experiments of Boyd in one specimen of white throated monkey undergoing an intense relapse of malaria. In this monkey, sporulation was very synchronous and the peak in the number of segmenters with 5 or more nuclei occurred at 8 A.M. every third day. On reversing the normal periods of light and dark for 48 days, sporulation became less and less synchronous so that in twelve days sporulation instead of being limited to a period of about 4 hours was spread over an entire day. Thereafter, a tendency was observed for the parasites to sporulate in two broods; one, 12 hours prior to the original time, the other 12 hours later than the original time, until eventually a typical double quartan infection ensued with the exception that the peaks in the number of segmenters occurred at 8 P.M. instead of 8 A.M. This result is exactly what would be expected from the avian work, remembering that a quartan and not a quotidian infection is under consideration. We hope to continue this work and I cannot resist pointing out that theoretically after an infection changed in this manner is again subjected to normal light and day, we would expect to find 3 separate broods with sporulation each day.

The general observations on human malaria and the experimental observations on avian and simian malaria indicate strongly that the time of sporulation and the synchronicity of sporulation is largely determined by the host. On the other hand, the length of the asexual cycle must be largely determined by the genetical constitution of the parasite as otherwise all malarial infections would be quotidian. It seems probable that light and dark indirectly affect the activity of the host, but just what mechanism or mechanisms mediate the effect is still a complete mystery.

No study of periodicity in malaria is complete without mention of the fever curve. The normal temperature curve varies in type and

degree in the different species of monkey which we studied, but in general averages around 101° F. and in the white throated monkey tends to be around 99° in the morning and to rise gradually during the day until it reaches about 102°–103° in the evening after which it gradually recedes. Ordinarily no demonstrable rise in temperature occurs when there are 50 or less parasites per 10,000 red cells. When the infection rises above this level, there is generally a pronounced peak of 104°–106° just at the time of sporulation which persists for approximately 4 hours (fig. 1).

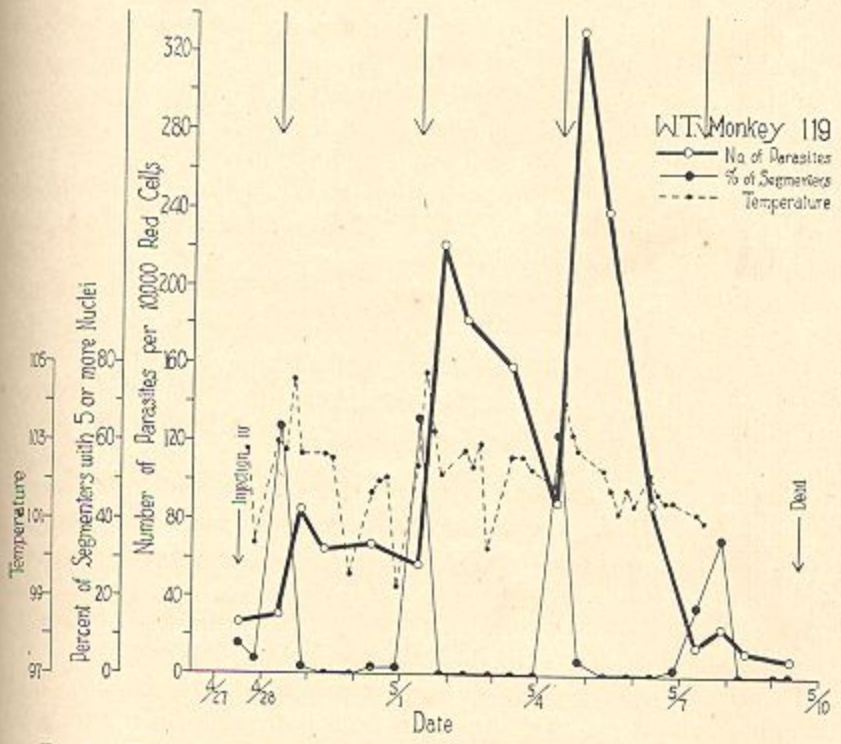

FIG. 1. Temperature, percentage of segmenters and number of parasites during the acute rise and crisis of the infection with *P. brasilianum* in white throated monkey number 119.

Analysis of the resistance of the host.

The consideration of the malarial cycle brings us directly to a description of the normal course of an infection and an analysis of the factors involved in the resistance of the host to the parasite. The course of infections was studied by ascertaining the number of parasites per 10,000 red cells at 12 or 24-hour intervals. In some animals

erythrocyte counts were made so that the ratio of parasitized to normal red cells could be reduced to the number of malarial organisms per cmm. of blood.

Although the type of initial infection observed in monkeys presents certain constant characteristics, the time at which these appear depends largely upon the number of organisms inoculated and possibly the route of inoculation. When an enormous number of parasites are introduced intravenously so that they can be found in the blood immediately after inoculation, the infection usually progresses quite rapidly (fig. 1). There is an initial acute rise of the infection often reaching as many as 250 parasites per 10,000 red cells by the 9th to 12th day. This peak of the infection is in turn followed by a sharp number crisis in which the majority of the parasites disappear from the blood and then a developed infection ensues during which there are varying but comparatively few parasites found in the peripheral blood. Finally, after a few weeks or months of this low grade infection a period of latency or semilatency sets in during which no parasites are observable in thick films for weeks at a time, but generally slight relapses occur at irregular intervals.

When a few parasites are introduced subcutaneously, the same general type of infection follows except that the number of parasites increases very gradually and may never reach a high point. Thus, following infection, no parasites can be found for several weeks, then they increase gradually to a somewhat low peak, and eventually, most of them are swept from the peripheral blood. The number crisis may be postponed for as long as two months. When it occurs, however, the same type of developed infection with latency and semi-latency ensues. The initial infection may present almost any gradation between the two extremes which I have just described depending upon the dose of malarial organisms or the particular monkey involved. It is interesting to note that in *P. cathemerium* of the bird exactly the same general characteristics of the initial infection are seen. In our own experimental work on birds, however, we used what might be termed a fixed virus and large infecting doses so that the bird infections presented the first type of infection described for monkeys, that is, a sharp acute increase with a number crisis about the 10th day followed by a developed infection and latency. It seems probable, then, that were monkeys as available as canary birds their infection could also be standardized.

Throughout our analysis of the effect of acquired resistance in initial infections of various protozoan species we (see W. H. and L. G.

Taliaferro, 1922, and W. H. Taliaferro, 1929 and 1932) have emphasized that where blood protozoa reproduce unhampered and the progeny survive, the infection progresses steadily at an uninterrupted rate until the host succumbs. Any modification of this uninterrupted increase can be brought about by one or both of two entirely different factors. In the first place, the basic rate of reproduction of the parasites may be inhibited or the organisms may succumb after they are formed. In *T. lewisi* both of these effects are operative and are mediated by two different antibodies (see review in Taliaferro, 1932).

In malaria of monkeys, which we have just described, there are obviously several deviations from an acute progressive steady rise in numbers. As there are no tissue localizations of the parasites (except for a temporary concentration of the parasites in the spleen at the time of the crisis), the number curve itself permits certain conclusions in reference to acquired resistance. Thus, at the time of the crisis there must be a large mortality of the parasites, because even if the rate of reproduction were reduced to zero, the parasites would simply not increase, viz., no decrease could be accounted for. The number curve alone, however, cannot demonstrate whether or not there is any effect on the basic rate of reproduction. For this there must be some measure of the rate of reproduction which is independent of the number of parasites destroyed. As was first pointed out in avian malaria by Mrs. Taliaferro (1925), such a measure is found in the asexual cycle. In simian malaria the asexual cycle is the time it takes one parasite to become on the average 9 merozoites and is, therefore, a direct measure of the rate of reproduction which is independent of the number of organisms that may die as long as there is no change in the number of progeny formed. Thin and thick film studies of the asexual cycle of *P. brasilianum*, during the acute rise of the infection, the developed infection, and minor and major relapses, all indicate that whenever the parasites can be found in the peripheral blood the asexual cycle, and hence, the basic rate of reproduction, is constant. The only exception is an occasional delay of about one day during the crisis. Once delayed, however, the parasites thereafter continue their usual 3-day periodicity. From this we can conclude that there is no pronounced inhibition of reproduction throughout the infection.

In addition to the enormous mortality of parasites at the crisis, there is, even during the acute rise of the infection, a comparatively high death rate of the progeny from each schizont. Thus, if all the progeny survived, the infection should increase at each sporulation by

a factor of about 9. An examination of the three sporulation periods in figure 1 shows clearly that this is not the case. The sharp increase in parasites at the first sporulation is less than 2 times and at the second and third is a little over three times. This indicates that of the brood of 9 merozoites coming from each schizont only about 3 parasitize new red cells. This, however, is not the whole story. Another examination of fig. 1 shows that of the 3 which do infect new cells many die so that the number curve decreases during each intersporulation period and the net gain is between 1.5 and 2 parasites. Thus, it is evident that of each brood of nine many do not parasitize new cells and of the few which do get into new cells only a few complete their full developmental cycle. The net result is that at least 7 of each 9 progeny perish. This mortality of asexual parasites is similar to that first described by Mrs. Taliaferro (1925) in *P. cathemerium* and studied in detail by Hartman (1927). It is also in line with the results of Knowles and Das Gupta (1930) on *P. malariae*. The present results differ from those on *P. cathemerium* in the high death rate between the time of segmentation and the infection of new red cells.

Immunity to superinfection.

The parasiticidal mechanism which the host acquires at the time of the crisis and which holds the infection down during the developed infection and latency can be directly demonstrated by the high grade immunity to superinfection present during latency. Thus, if large numbers of washed parasitized red cells are injected intravenously into a monkey after it has apparently recovered from an initial infection, the parasites steadily decrease so that within 10 to 19 days none can be demonstrated in the blood by thick film examination. In marked contrast, when such parasitized cells are injected into previously uninfected monkeys, the parasites increase rapidly and go through a typical initial infection.

Cellular basis for the initial infection.

Professor P. R. Cannon and the speaker have now examined, in detail, the histological picture in 89 monkeys, of which 17 were normal and the remainder were killed or died during various stages of an infection. In some of the animals a small portion of the spleen was removed before infection and served as a direct control of the cellular changes observed in the spleen of the same animal after infection. In addition, we have studied, more summarily, the tissues from the animals obtained by Dr. Clark (1930 and 1931) in his various

expeditions. The tissues for the detailed study were fixed in Zenker-formol, embedded in celloidin and stained by Maximow's (1909) method with hematoxylin-eosin-azur II. This stain not only permitted the differentiation of the types of host cell, but clearly demonstrated the malarial parasites within the tissues.

The most marked histological changes occur in the spleen and liver. The normal spleen of these monkeys shows in general compact follicles of basophilic lymphoid tissue around the follicular arteries and comparatively little lymphoid tissue in the red pulp, while the macrophages (splenocytes) of the reticulum of the splenic pulp are compressed and oat-like in shape. The normal liver shows the macrophages (Kupffer cells) for the most part flattened and inactive.

The cellular responses to malaria consist of two closely related but independent processes—the increase in number and phagocytic activity of the differentiated macrophages and the activation of the lymphoid tissues, the cells of which are not themselves phagocytic, but which, according to Maximow (1927), may develop into macrophages. These two processes can best be considered separately.

When large doses of parasites are given intravenously to produce an acute infection, such as is shown in fig. 1, the following changes occur in the liver and spleen. For the first 48 hours the parasites occur in the sinusoids of the spleen and liver with practically no evidence of phagocytosis or stimulation of the lymphoid tissue. Thereafter, the phagocytic and lymphoid activities progressively increase and are accompanied by an increase in the macrophages of the splenic pulp and the lymphoid cells of the follicles and by an increase in phagocytosed parasites and phagocytosed pigment. The latter represents not only the remains of digested parasites, but also pigment taken up directly as it is liberated at the time of sporulation. It is interesting to note that the entire red-cell-parasite combination in all stages of growth and division of the parasite is ingested.

Just at the time of the crisis the parasites become markedly concentrated in the spleen and liver. In fact, every oil immersion field of the splenic pulp may contain numerous free parasites, while the peripheral blood may show only a few in thick film. Previously the spleen contained practically no more parasites than the peripheral blood. The liver may show a slight concentration. After a day or two of this concentrating process the macrophages suddenly become very active and quickly clear the spleen and liver of their accumulated organisms so that not infrequently no parasites can be found in the spleen, although a few are still present in the blood. Both the reticular

macrophages (splenocytes) of the splenic pulp and the Kupffer cells are greatly swollen and contain large masses of coalesced pigment and parasites in all stages of digestion.

When only a few parasites are injected, phagocytic activity develops more slowly and if the parasites never reach a high number, the cellular changes, while following the same course, are never as pronounced.

In the preceding account attention has been concentrated on the spleen and liver. Practically no phagocytosis or accumulation of malaria pigment has been observed in various sections of the alimentary tract, in the mycardium or in the kidney. The bone marrow shows some parasitized red blood cells and some phagocytosis by large mononuclears which seem to vary with the extent of the infection in the peripheral blood and with the amount of phagocytosis in the spleen and liver. There is, however, comparatively little actual phagocytosis, due, apparently, to the comparatively small number of differentiated macrophages. Similarly, the lung shows comparatively little active phagocytosis. This cannot be ascribed to the lack of phagocytic cells because the "septal" cells are highly phagocytic. It seems rather to depend upon the portal of entry. Thus, the septal cells actively phagocytose dust or bacteria which enter through the bronchi and get into the air sacs, but do not phagocytose infected red cells which occur in rapid circulation in the capillaries.

Associated with the increased physiological activity of the differentiated macrophages is an activation of the lymphoid cells which progressively increases as the infection ensues and is more intense at the end of a long initial acute rise than at the end of a short, even if more intense, acute rise of the infection. Where very heavy doses are given to produce an infection, such as shown in fig. 1, the number of mitotic figures of the lymphoid cells in the follicles has increased in 48 hours and the germinal centers are well marked. If the intense stimulation is continued, the splenic follicles swell to about 4 or 5 times their normal size in about a week, the lymphoid cells infiltrate into the red pulp and numerous active macrophages (probably a swelling of preëxisting macrophages) appear in the center of the follicles. In such infections where a crisis occurs within the second week, the general lymphoid activation is often limited to the spleen. If, however, the crisis is postponed for a month or more, or if after the crisis there is a long continued stimulation by a long continued medium grade infection, the lymphoid activation may be observable in other organs. The bone marrow may show definite hyperplasia and in time well marked islands of closely packed basophilic lymphoid cells may appear. The

liver also may show a mantling of lymphoid cells around the blood vessels similar to the ones emphasized by Epstein (1929) in his study of the cellular changes in the rabbit following the injection of non-infectious antigens. Occasionally, the kidney, which ordinarily manifests no cellular reactions, contains cortical accumulations of lymphoid cells and even differentiated macrophages containing phagocytosed pigment and parasites.

If the infection is started with a few organisms and is slow in developing, the changes in the spleen may not be observable until after 12 or 14 days.

The foregoing cellular changes have been considered through the crisis. After the intense activity of the macrophages at the time of the crisis and the concomitant stimulation of the lymphoid system, there is, as latency ensues, a gradual lessening of the histological appearances of activation and a disappearance of phagocytosed pigment. Nevertheless, activation remains. In fact, we have never found animals once infected with malaria in which the spleen has regained completely its normal histology.

The foregoing findings agree almost exactly with those reported by Cannon and the speaker (1931) for avian malaria except that in the latter case evidences of mesenchymal activation slowly subside during latency and are not evident histologically unless the animal is superinfected. This difference may be accounted for by the fact that once latency is initiated relapses in the monkey are more frequent than in the bird and the constant pouring of malarial material into the spleen and other organs maintains the lymphoid system at a high level of activation. Moreover, as monkeys grow older, they are subject to frequent infections with parasites other than malaria which may maintain the stimulation.

The cellular basis for acquired immunity to superinfection.

The histological picture which I have just presented gives a clear cut cellular basis for the death of parasites during the acute rise of the infection, for the great mortality of the parasites at the crisis and during the subsequent low grade infection prior to latency. We have completed a few studies on the cellular basis for the immunity to superinfection and plan to carry the study much further. Our results indicate clearly, however, that in sharp contrast to the sluggish phagocytosis of parasites by the macrophages of the normal animal, phagocytosis is rapid and more quickly effective in a previously infected animal. Just as in the case of avian malaria (Cannon and Taliaferro,

1931), immunity to superinfection rests upon a greater number of macrophages and a much greater phagocytic activity of those present.

Antibody basis for acquired immunity.

The fact that the entire picture of the initial infection in *P. brasilianum* and the immunity to superinfection is a parasiticidal mechanism without any inhibition of reproduction of the parasites and the further fact that the direct cellular basis is a phagocytosis of the organisms by the macrophages still leaves out of account the fundamental reason for the change in cellular activity. Thus, I have shown that a constant number of the parasites are phagocytosed throughout the acute rise of the infection, but that phagocytosis is greatly increased at the time of the crisis and throughout the remainder of the infection. This increase of phagocytosis is an acquired resistance developed as a result of infection. The question arises: Why do the macrophages ingest a greater number of parasites at the time of the crisis and during superinfection? Several possibilities present themselves.

In the first place, this greatly increased phagocytic activity may be the result of a non-specific stimulation of the macrophage system. In other words, at the time of the crisis due to the preceding stimulation brought about by the initial infection, the phagocytes may have simply increased in numbers and in activity so that any foreign particulate matter, such as a parasitized red cell, is taken out of the blood at a greatly heightened rate. This explanation, particularly as it applies to the greater number of phagocytes present, is partially true, but I do not believe it explains the major portion of the immune reaction. Thus, in avian malaria where the conditions seem exactly similar, the heightened phagocytic activity of the immune, that is, latently infected bird, is highly specific, since Gingrich (1930) has shown that a bird latently infected with *P. cathemerium* and highly immune to superinfection with the same species is not immune to *P. elongatum* and vice versa. Furthermore, it is possible to have two species of human malaria present and for only one to relapse. These facts indicate that the heightened phagocytic activity following the initial stimulation of the malarial infection is highly specific and dependent only to a minor extent on non-specific activation.

The most orthodox way of explaining heightened phagocytic activity following immunization is to assume the presence of an antibody which opsonizes or tropinizes the parasites so that they are readily phagocytosed. Just as in our work on avian malaria (1929*b*), however, we have been unsuccessful in demonstrating such an antibody, although

we used protective techniques which are eminently successful in demonstrating such antibodies in other protozoan infections.

Our inability to find an antibody intermediary in phagocytosis in avian malaria led Gay (1931) to describe the condition as a pure histologic immunity and to lean toward a third possible explanation, viz., that the macrophage itself has been so specifically changed in the immune animal that it will phagocytose malarial organisms to a much greater extent than in the normal. Nevertheless, I still believe that with proper technique we may eventually demonstrate some type of opsonizing antibody. It seems not unlikely that it might be produced locally in organs rich in macrophages, in amounts adequate to opsonize parasites, but not sufficient to be demonstrable in the blood serum. This would be comparable to the local production of antibodies, as described by Cannon and Sullivan (1932), who found that when an animal is immunized in the skin antibodies can be demonstrated there before they can be found in the spleen, liver or serum. Certain other work in human malaria also predicates an antibody basis for immunity. Thus, a number of investigators have demonstrated complement-fixing antibodies in malaria (cf. review in Taliaferro, 1929), and we (Taliaferro, W. H. and L. G. and Fisher, 1927 and Taliaferro, W. H. and L. G., 1928) have demonstrated precipitins. In addition, there are a few scattered investigations showing that the serum of people having recovered from an acute attack of malaria is protective, i.e., prevents infection (cf. review in Taliaferro, 1929).

The macrophage system and relapses.

The predominant rôle played by the macrophages in acquired immunity to malaria is but one phase of much recent work indicating the great importance of these cells in general and local immunity, in antibody formation, in chemotherapy and normal metabolism (see Linton, 1929, Taliaferro, 1929, Jungeblut, 1930, and Gay, 1931). A general consideration of this field is out of the question, but one other protozoan study may serve to exemplify the kinds of data which are accumulating. In *T. lewisi* of the rat, the speaker has shown that the acquired immunity involves a reproduction-inhibiting as well as a parasiticidal process and that each is associated with a distinct antibody and several investigators have shown that the production of these antibodies is dependent upon the activity of the macrophages (see Regendanz and Kikuth, 1927, Marmorston-Gottesman, Perla and Vorzimer, 1930, Taliaferro, Cannon and Goodloe, 1930, and Taliaferro, 1932).

Assuming, as I believe we may, that what we have found true for malaria of birds and monkeys will apply in general to all plasmodial infections, we may draw some interesting inferences in regard to the mechanism of relapses. Many of you are familiar with the various hypotheses advanced to account for malarial relapse so that no extended account of them is necessary. Suffice it to say that they fall into three main categories: Those associated with Grassi (1900) and Schaudinn (1902) depend upon the resistance of the female gametocyte which ordinarily continues its existence in the mosquito, but which during latency persists and initiates the relapse by parthenogenesis. Those associated with Celli (1900), S. P. James (1917 and 1920) and Craig (1906, 1907 and 1926) depend upon some quiescent and resistant asexual stage which lies dormant in various sites, such as endothelial cells, etc. And finally, those associated with Ross (see review in Ross, 1910), Bignami (1910), W. M. James (1913) and Whitmore (1918) assume no new stage of the parasite during latency, but assume that the asexual forms continue their asexual reproductive cycle uninterruptedly, that during latency the progeny from this cycle are mostly destroyed by the defensive powers of the host and that a relapse consists essentially of a removal of the parasiticidal agent, thus allowing the parasites to accumulate in the blood. The first of these, that of parthenogenesis, is now rapidly losing ground, not only because of lack of evidence, but because the particular type of parthenogenesis predicated is unlike true parthenogenesis or other known developmental processes in other organisms. The second, that of quiescent asexual stages has not received any general acceptance, although peculiar forms have been found in the blood which authors have suggested to be resistant (see J. D. Thomson and Woodcock, 1922).

So far our work on both the avian and the simian infection all bear out the hypothesis of Ross. Thus, whenever the parasites are found in the blood, even when they are so scarce that they can only be seen in thick films, they are undergoing their usual asexual cycle which means that their basic rate of reproduction is unchanged. During latency, therefore, it seems probable that a few parasites continue to reproduce unhampered, that their progeny are never allowed to accumulate because of the phagocytic activity of the macrophages and that relapses are temporary cessations of macrophage activity which allow the progeny to accumulate in the blood until the macrophages again regain their functional level.

If this be the true picture of relapse, the various factors which are known to produce relapse should also lower the level of macrophage

activity. We are now studying this problem both in malaria and in the trypanosome infections and have obtained a few interesting preliminary results. Thus, in malaria and *T. lewisi*, removal of the spleen is often associated with relapse and is supposed to result from the removal of a large part of the macrophage system. Similarly, pregnancy is frequently associated with relapse in malaria and we (Taliaferro, Cannon and Goodloe, 1931) have recently shown that it may be associated with a lowering of the production of the *anti-lewisi* reproduction-inhibiting antibody.

Conclusions.

In concluding I wish to emphasize that the work on the asexual cycle of *Plasmodium brasilianum* and on the number curves of infections indicates that the type of infection encountered is the result of a differential mortality of the parasites which reproduce at a constant rate throughout. Thus, during the acute rise of the infection, approximately 7.5 out of each brood of about 9 merozoites die, whereas at the time of the crisis and thereafter, even more die. Before the crisis, this mortality represents a natural resistance of the host, whereas at the crisis and thereafter, it represents a true acquired immunity resulting from infection. It can be correlated with the cellular responses of the host.

The cellular responses of the host are evidenced by an increase in numbers and of phagocytic activity of the differentiated macrophages, particularly of the spleen and liver, together with a general activation of the more primitive lymphoid tissue, preëminently in the spleen, but often in other organs also. So far no intermediary antibody has been associated with this cellular activity, but there are reasons for supposing that an opsonizing antibody may be formed locally which is of too low a concentration in the peripheral blood to be demonstrated by the techniques so far employed.

One of the most interesting points in the present investigation is that the events responsible for the acquired immunity against malaria of monkeys parallel so closely those which we have already found in malaria of birds, and it seems, therefore, not unreasonable to suppose that they may also hold for malaria in man.

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