INFECTION AND IMMUNITY IN BIRD MALARIA [1]

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Infections with bird malaria offer certain unique possibilities for the study of the biological nature and immunity of the infection: the life-cycle of the parasite takes place in the peripheral blood stream so that representative samples for study can be obtained throughout the infection; the comparatively large size of the parasite permits conclusions in regard to the effects of resistance which are probably impossible with the smaller bacterial invaders; the characteristic periodic method of asexual reproduction permits the differentiation between those factors in the host which inhibit the rate of reproduction *per se* and those factors which are lethal to the parasites; and, finally, as the infection can be studied in the ordinary canary, a source of naturally uninfected laboratory animals is always at hand. The bearing of these various advantages on the study of the infection will be brought out in the following pages.

There are many reasons for supposing that the type of avian parasite considered in this review is very similar to infections of both *Plasmodium vivax* and *P. malariae* of man, but just how far the results obtained with bird malaria can be applied to the human infections cannot be definitely known until much more work is done on human malaria.

The accumulation of such data is much handicapped because humanitarian reasons make it unjustifiable to study uninfluenced, *i.e.*, untreated, human cases and no suitable laboratory animals tolerate the infection. Recently however, the use of malaria in the treatment of paresis offers a new method of studying the human disease which has already yielded many interesting results.

THE PARASITE AND THE INFECTION PRODUCED

The bird malaria parasites [2] undergo an alteration of generation, with the English sparrow (and possibly certain other birds) as their vertebrate host and certain *culicine* mosquitoes as their invertebrate host. In the bird the parasites occur within the red cells [3] where they exhibit two types of development, asexual and sexual.

The characteristically vertebrate part of the development is the asexual cycle which is indicated in figure 1. The small parasite

which enters the cell to start the asexual cycle is termed a merozoite. As it grows it is termed a trophozoite and eventually a schizont. The schizont by a process of multiple division (schizogony) gives rise to a number of merozoites which are liberated into the plasma by the rupture of the infected cells and which may infect new red cells, thus completing the cycle.

The sexual cycle cannot be completed in the vertebrate host. Some of the merozoites after penetrating new cells grow into large forms termed gametocytes, which do not divide and which perish unless taken up by a suitable culicine mosquito. In the mosquito they produce gametes which undergo sexual development and eventually give rise to sporozoites some of which localize in the salivary glands ready to infect new birds.

The laboratory infections, considered in the present review, have been transmitted from bird to bird, not by mosquitoes, but by the subcutaneous, intramuscular or intraperitoneal injection of saline suspensions of infected blood. In geneal, the course of such infections in the canary is as follows (Fig. 2): After a parasitological incubation period (1) during which no organisms can be found, the parasites rapidly increase in the blood (2, acute period) until sometimes every other cell is parasitized; then if the bird does not die, there is a crisis (3), when most of the parasites are killed, but some may remain for a week or more (4, chronic peiod, or as this term is used to signify a different thing in human malaria, it is also called the period of developed infection), and in time all the parasites apparently disappear from the blood, (5, latent period). Thereafter, if the bird's resistance is lowered in any way, they may reappear for a week or more in secondary acute rises and crises (6, relapse).

The exact enumerative studies made by the Sergents (see especially 1918),[4] Ben Harel (1923),[5] L. G. Taliaferro (1925),[6] Boyd (1925),[7] and Hartman (1927)[8] all indicate that the outline just given is the general type of infection encountered in birds. There may be, however, great variations in the lengths of the different periods and in the severity of infection during those periods in which the parasites can be demonstrated in the blood. Ben Harel also described "extended irregular infections", but these are probably cases in which a series of relapses were superimposed on the usual acute rise and "chronic" portion of the infection, and possibly are due to the daily removal of blood samples for erythrocyte counts which she carried out.

THE ANTI-PARASITE RESISTANCE OF THE HOST

In the present review I am considering only those factors in the resistance of the host which will affect the numbers of the parasites in the blood of the bird. In other words, can the peculiar course of the infection just described be explained by the conditions imposed on the parasite by the host? The methods for analyzing the factors of host resistance, which may affect the numbers of parasites, have been repeatedly pointed out in recent papers by the author. Briefly, they may be summarized as follows: If a protozoon, such as the malarial parasite, successfully invades the host and finds conditions suitable, it reproduces by schizogony, viz. during each asexual generation the small merozoite grows large, undergoes division and finally produces about 15 small merozoites which enter new cells and repeat the process (Fig. 1). Thus, if the rate of reproduction is unhampered and if all the progeny survive, the organisms in the blood would be multiplied by 15 at each asexual generation. Such an increase of parasite population would describe a geometrical progression series. Host resistance can, however, effect this steady increase of population in one or both of two ways: the rate of reproduction of the parasites may be inhibited, in which case not as many progeny would be produced, or the parasites may be killed, in which case fewer progeny would survive. Briefly, this may be expressed by the following equation:

(1)	(2)	(3)
No. of parasites per cmm. of blood.	No. produced by reproduction.	No. destroyed.

To differentiate the two mechanisms fairly accurate conclusions have been made possible by determining the first and second terms and evaluating the third (L. G. Taliaferro 1925)[6]. The first term is obtained by making frequent parasite counts during the course of the infection. The second term is obtained indirectly, since to be valid it must be independent of both the first and third terms, and hence can in no way depend on number counts. Since the asexual forms grow up and sporulate nearly synchronously, only merozoites will be found in the blood at one time, schizonts at another, etc. (Fig. 1). The length of time required for these cycles of growth and reproduction, *i.e.* the time it takes for one merozoite to become 15, is actually a measure of the rate of reproduction of the parasites, and should it vary, the rate of reproduction may be said to vary. Accordingly, blood smears were made at two- or four-hour intervals during as much of the infection as parasites were

found and from them fifty parasites were drawn and their mean size obtained. As can be seen from figure 3, the data obtained showed a series of cycles, each cycle consisting of a gradual rise in the mean size of the parasites and an abrupt fall. Comparing the time it takes this cycle to be completed during the various stages of the infection (acute, chronic, relapse) shows whether the rate of reproduction is constant or varying. In one strain of bird malaria, for example, the cycle took twenty-four hours throughout the infection, and therefore the same number of parasites were being produced throughout. This measure of the rate of reproduction presuposes that the average number of young produced by each fullgrown parasite does not vary at any time and has been shown to be valid by L. G. Taliaferro.

HOST RESISTANCE THROUGH THE INFECTION

All evidence is consistent in indicating that the host acquires no resistance during the incubation period and the acute rise of the infection, but that the parasites reproduce at a uniform rate and that a constant number of them survive. Evidence for this is clearer in the acute rise of the infection which will, therefore, be considered first.

During the acute rise of the infection L. G. Taliaferro (1925)[6] found that the rate of reproduction was constant (the length of the asexual cycle was 24 hours throughout[9] and that the infection increased in the blood according to a geometrical progression which would be expected if the host acquired no resistance. She found, however, that out of an average of 15.5 merozoites produced by each mature shizont approximately 10 die. The constant rate of increase during the acute period has been verified by Hartman (1927),[8] who finds, however, that the actual rate may vary considerably among different birds. Furthermore, he has made a careful study of the rate of death of the parasites which perish between each asexual generation and finds that the rate of death is a constant for twenty-one hours of the twenty-four-hour period.[10] As pointed out by Taliaferro, this non-viability of the majority of merozoites produced by each sporulation probably indicates the suitability of the bird as a culture medium for the parasites and is not in any sense an acquired resistance.

Evidence that there is similarly no resistance acquired by the host during the incubation period is purely inferential, since the parasites are not in the blood. It is significant, however, that Boyd

(1925)[7] found a definite correlation between the number of parasites injected into the bird, and the length of the incubation period (—.522 + .053) and the number of parasites at the peak of the infection (.340 + .074). This is just what would be expected if the rate of reproduction and the rate of accumulation of parasites were constant throughout and indicates that the incubation period is simply the time it takes the parasites to become numerous enough to be found in the blood. During the remainder of the infection, it is evident from the wholesale decrease of the parasites at the crises which terminate the acute, chronic, and relapse periods (Fig. 2), that some type of resistance is acquired which destroys large numbers of the parasites after they are formed. There is, however, no retardation of the rate of reproduction of the parasites, according to L. G. Taliaferro (1925).[6] She found that the asexual cycle, which is a measurement of the rate of reproduction, as explained previously, takes the same time (twenty-four hours in one strain) throughout the acute, chronic, and relapse periods (this is shown for the acute period and crisis in Fig. 3), and that each stage in the cycle takes place at exactly the same time in the relapse as it had in the acute and chronic periods—a fact which indirectly indicates that the cycle had continued undisturbed throughout the latent period.

Although there is no conclusive evidence as to the defense mechanism involved in the destruction of the malaria parasites to such a great degree at the crisis and to a lesser extent throughout the remainder of the infection a number of recent investigations on bird malaria have a bearing on this point. It seems very probable from the work of Ben Harel (1923),[5] and from the well-established fact that an enlarged spleen containing many parasites occurs after the crisis, that the wandering mononuclears and fixed tissue phagocytes of the spleen and other organs ingest the parasites at the crisis and during the latent infection. Furthermore, the author and L. G. Taliaferro (1927)[11] have shown that if a large number of washed parasitized cells are injected intravenously into an infected canary during the latent period, they are quickly removed from the circulation (probably by the phagocytes), whereas in a normal canary they are not removed but steadily increase in numbers until the death of the canary or until a crisis ensues (Fig. 4). One very interesting feature in this work is that the parasites are being removed from the peripheral blood continuously[12] a fact which is not in accord with Bass and John's (1912)[13] assumption that the greatest hazard is when the parasite is passing from one cell to another. Evidently,

there is some factor in the blood stream of the infected bird which affects either the parasites or the phagocytes or both.

All attempts to show that this factor is an antibody have failed. The author and L. G. Taliaferro have been unable either to sensitize infected red cells with serum from infected birds in the latent period or to obtain a passive transfer of the lethal factor. This might be taken to indicate that the parasites simply injure the red cells to such a degree that they are phagocyted, but such an assumption is not tenable because the same infected cells are not phagocyted by the normal uninfected bird. The failure to obtain evidence of an antibody together with the recent work of Hegner and MacDougall (1926)[14] and MacDougall (1927)[15] suggests that the factor which kills the parasites, or which makes the parasite-red cell combination phagocytable is connected with the simpler serum constituents. Hegner and MacDougall have found that increasing the blood sugar by feeding solutions of glucose brings about conditions favorable to the accumulation of the parasites in the blood, whereas decreasing the blood sugar by injecting insulin probably inhibits the accumulation of the parasites. Their experiments are in accord with the work of Bass and Johns (1912)[13] and others who have found sugar necessary for the cultivation of the malarial organisms outside of the body and with the results of Bass and Johns (1913)[16] who succeeded in cultivating the parasites in the blood form a case of diabetes without the addition of sugar.

The failure to find an antibody basis for the destruction of the parasites in birds suggests the same lack of antibody basis in man, but one hesitates to draw too close a parallelism because no antimalarial antibodies of any type have been found in the bird whereas they have been found in man. Thus, Maldovan (1912)[17] failed to obtain any evidence of protective or complement-fixing antibodies in the avian infection. This has been corroborated by the present author (unpublished work), who in addition found no evidence of a lytic antibody. Fisher (unpublished work) could not demonstrate precipitins. In human malaria, however, both complement-fixing antibodies and precipitins have been demonstrated. (For a review of the literature see the author, L. G. Taliaferro, and Fisher (1927).[18] Furthermore, the results on the relation of sugar to the human infection are rather inconclusive. Working with induced benign tertian malaria in general paresis, Rudolf and Marsh (1927)[19] found glycosuria in a higher percentage of cases of general paresis treated with malaria than untreated. (Also see their paper for a review of

previous work on the same subject). During the induced malaria the blood sugar varied inversely as the temperature, although it might be higher after the fall in temperature than before pyrexia. Administration of glucose gave no effects on objetive symptoms nor parasites but apparently relieved subjective symptoms. Administration of insulin produced indefinite results, but in 60 per cent of the cases the fever terminated after administration, and although relapses followed, they were of a lower degree of pyrexia than those following quinine therapy. They conclude that lowering the blood sugar does not appear to be the cause of the cessation of the fever.

In all this work there is the possibility that a diminution of the blood sugar is a concomitant change rather than the basis for the disappearance of the parasites. Thus, in the trypanosome infections, where the death of the parasites at each number crisis in the infection is directly related to the production of antibodies, blood sugar changes have been observed quite similar to those described for bird malaria. Especially pertinent, in this connection, is the work of Schern (1925).[20] In cover slip preparations of the blood of infected rats, trypanosomes remained active for several hours if taken at the beginning of the infection, but became motionless in about ten minutes if taken a few hours before death. In fact, in this way the probable survival time of a host could be forcast. Furthermore, the trypanosomes from the terminal stages of an infection could be reanimated by the addition of dextrose, levulose, or of a thermo-stabile fermentable constituent of normal serum or liver extract. Additional evidence for these general conclusions has been given by Bruynoghe, Dubois and Bourkaert (1927).[21] In trypanosome infections there is evidence that carbohydrates, such as sugars, are necessary for motility (which is probably a delicate measure of vitality) and that these disappear from the liver and blood as the infection progresses. Just how much their disappearance plays in the destruction of the parasites in the animal body is unknown, but certainly trypanocidal antibodies play the major role. In fact, it would seem to the author that although the decrease in blood sugars during the infection would directly influence the survical time outside of the body within the body the parasites would not be actually killed by hypoglycaemia of the host until the host was also killed.

RELAPSES

The preceding discussion leads directly to a consideration of the fate of the parasites during the latent periods and the mechanism of relapse.

Infections with malaria, whether they be in man or birds, are characterized by their tendency to relapse. Many workers on human malaria divide these relapses into two types: true-relapses, which appear after short intervals, and recurrences which appear after long intervals. In spite of a long list of investigations there is no unanimity of opinion as to how the malarial parasite subsists in the body during the latent periods between relapses. The more important hypotheses may be classified under three heads:

(1) Some of the gametocytes survive the action of the quinine or the host's resistance and at the beginning of a relapse are stimulated to undergo segmentation which again initiates the asexual cycle of the parasite. This development of the gametocytes is often called "parthenogenesis" and the hypothesis is associated chiefly with Grassi, Schaudinn, Neeb, Rowley-Lawson, Biedl and Pontano, but above all with Schaudinn who has depicted in detail what he believes to be stages in the process. An able critique of this hypothesis and in particular of Schaudinn's work is given by J. D. Thomson (1917).[22] Among his criticisms the most conclusive evidence against this view is: (1) Schaudinn overlooked the fact that in all known cases of parthenogenesis the unfertilized female exhibits essentially the same general course of development pursued by the fertilized gamete. Therefore, if there were a parthenogenetic development of the female gametocyte, it should show many of the stages peculiar to the mosquito host and not give rise directly to asexual segmenting stages. (2) All of Schaudinn's figures depicting the female gametocyte undergoing segmentation can be easily interpreted as cases in which the red cell contains a female gametocyte juxtaposed to an ordinary segmenting schizont. In fact, Thomson found many such stages in his own preparations. There is, in short, very little evidence to support the so-called parthogenetic view.

(2) Specialized resistant asexual forms, which survive the periods of latency until conditions are again favorable in the blood to renew their asexual schizogony, form an integral part of the hypotheses advanced by such investigators as Celli S. P. James and Craig. In the particular theory advanced by Craig, two young hyaline rings were supposed to conjugate within the cell and the resulting zygote to grow until it filled the red cell. It then left the red cell, was carried by the blood stream to the spleen and bone marrow where it rested until proper stimuli caused it to liberate young forms which renewed the asexual cycle. Craig (1926)[23] still holds this view for relapses occurring at long intervals, but accepts the hypothesis of Ross and

others, given later, for relapses at short intervals. In spite of Craig's description of conjugation and a similar description by Ewing (1901),[24] many biological objections can be made to the supposition that merozoites destined ordinarily to continue the asexual cycle should assume the role of gametes. Furthermore, the process is difficult to demonstrate because the close approximation of two forms within the cell can easily be mistaken for conjugation. The resistant forms described by James are asexual forms which remain quiescent until stimulated by favorable conditions to re-initiate the asexual cycle. For a discussion favoring this view, as well as illustrations, the reader is referred to J. D. Thomson and Woodcock (1922).[25]

(3) Ross advanced the hypothesis that the malarial parasites continue their normal asexual cycle throughout the infection but that during the latent periods the asexual parasites are killed off to such an extent that they can not be found (see Ross 1910).[26] Bignami, W. M. James and Whitmore hold essentially similar views. The asexual parasites during the latent period, according to Bignami, become resistant to quinine or antibodies just as trypanosomes are known to do.

In the author's opinion the studies on human and bird infections support Ross' assumptions. Not only is the asexual cycle continuous during the latent period, but continuous at the same rate (vitality) as during the acute rise, and the scarcity of the parasites is accounted for by the killing mechanism developed by the host which is removed in some way during relapse. Some of the chief evidence in favor of Ross' hypothesis may be summarized as follows:

1. Ross and D. Thomson (1910)[27] in their numerical studies of human malarial infections showed that the general trend of the number curves strongly indicated that the asexual parasites persisted during relapses of short duration but were too few to be found in blood films. Many investigators who postulate resistant forms for the production of relapses after long intervals follow Ross and Thomson's conclusions for relapses after short intervals.

2. Working with bird malaria Whitmore (1918)[28] found that throughout the latent period, although no parasites could be found, the blood was infective to other birds for as long as 29 months after infection, and as the asexual parasites are the only ones which are known to infect other hosts, he quite justifiably assumed that this is evidence that throughout the latent period there are small numbers of asexual parasites in the peripheral blood. Mazza (1924)[29] has found birds infective four years and two months after infection.

3. Ben Harel (1923)[5] after patient search was actually able to find asexual parasites in the process of asexual reproduction during latency.

4. L. G. Taliaferro (1925)[6] showed that whenever the parasites were found in the blood not only was asexual reproduction occurring, but at the same rate as during the acute rise of the infection. Furthermore, it is interesting that when they reappear during a relapse the time of each stage in the asexual cycle was exactly what would be expected if it had occurred uninterruptedly throughout the latent period. This last evidence would be conclusive except that the asexual cycle is diurnal and may be forced on the parasite by the host.

In conclusion, it seems well to quote L. G. Taliaferro's conception of the malarial infection in birds.

"After the incubation period the asexual stages of the parasites are to be found in the peripheral blood in varying numbers during the entire course of the infection and undergo their cycle of development and reproduction at the same rate throughout. From the very beginning, only a number of the merozoites are viable; this probably represents a natural resistance of the host. During the first part of the infection relatively few parasites are killed, so that they accumulate in the blood and give rise to the acute stage of the infection. Sooner or later, however, as the acquired resistance is built up, a large proportion of the parasites are killed. There may then be a temporary relapse, but eventually the number of parasites destroyed equals or exceeds the number produced by reproduction, and the chronic period with a low grade of blood infection ensues. In time, the destruction becomes so great that no parasites can be found in the blood (latent period), but their presence can be demonstrated by Whitmore's technique. This continues until some condition, such as the injection of adrenalin, temporarily stops the destruction and the parasites accumulate again, causing a relapse."

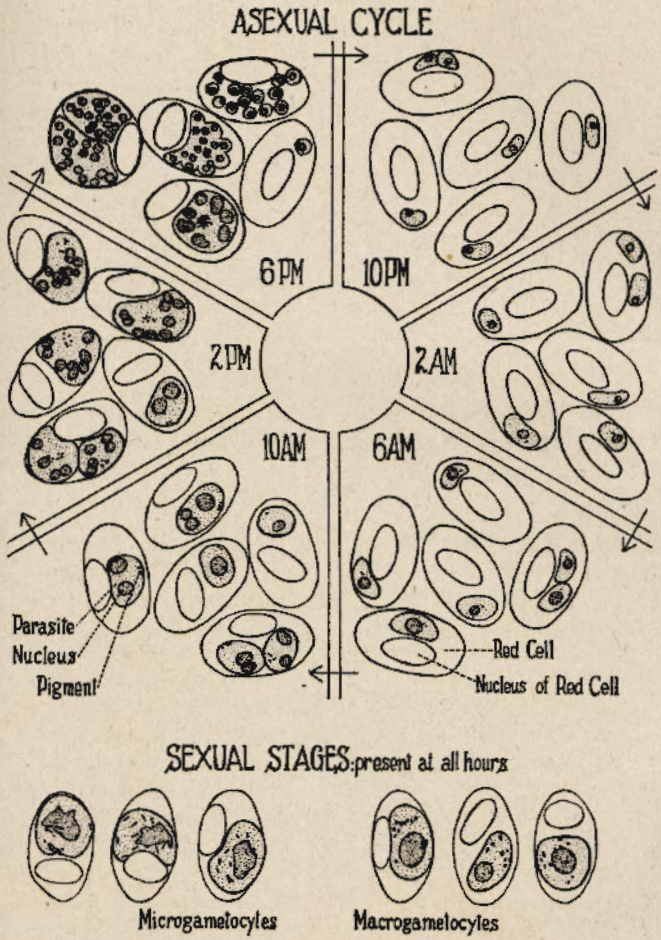

FIG. 1.—Representation of the cycle of reproduction in bird malaria showing changes in size. Outlines of the asexual stages (× 1,500) of the parasites within the nucleated red cells, showing nuclei and pigment granules, made at four-hour intervals during a consecutive period of twenty-four hours. In addition, outlines of three microgametocytes and three macrogametocytes, which occur in small numbers at all hours throughout the infection. (From drawings by L. G. Taliaferro.)

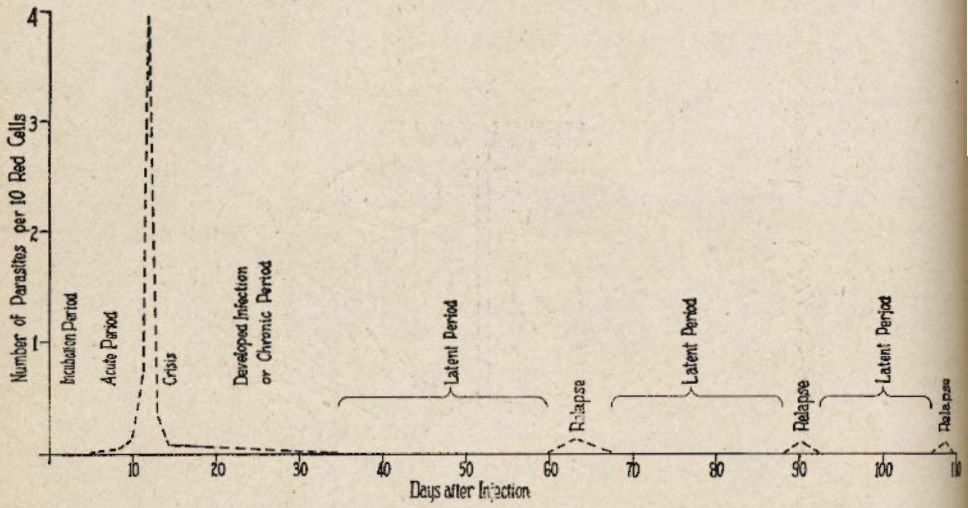

FIG. 2.—Graph illustrating the type of number curve usually encountered in the course of an infection of bird malaria in the canary.

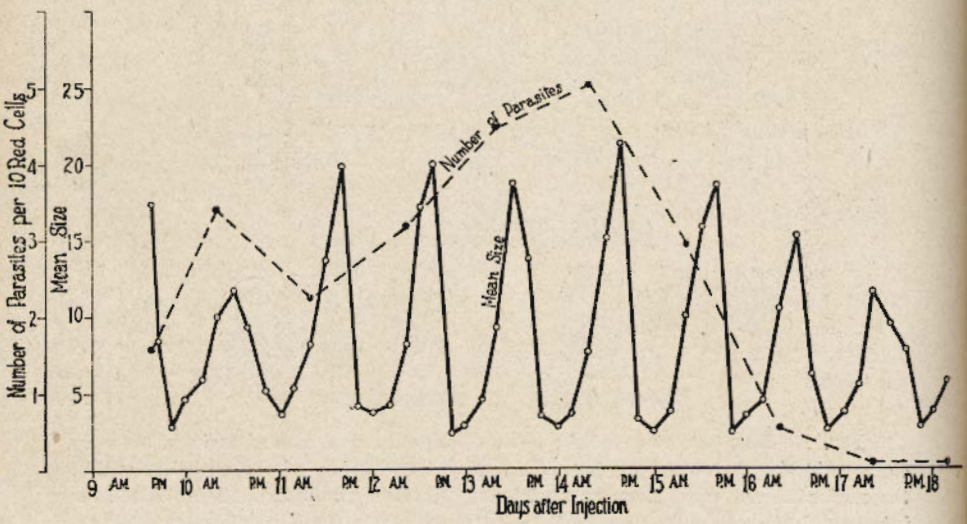

FIG. 3.—Graph showing the changes in mean size of the asexual forms and the number curve through the acute period and crisis in an infection with *P. praecox* in a canary. The uniform rate of reproduction (as shown by the regularity in the mean-size curve) and the type of number curve (as seen in the preceding figure) indicate that there is a parasiticidal but no reproduction-inhibiting resistance developed. (From data by L. G. Taliaferro.)

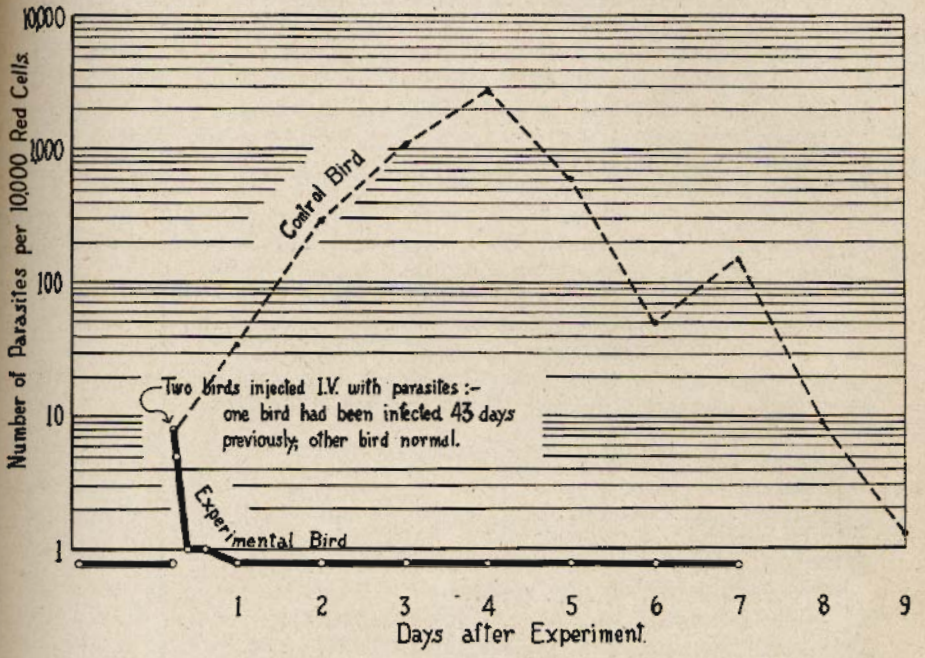

FIG. 4.—Experiment showing the disappearance of washed parasitized cells from the blood of a bird in the latent infection and the survival of the same type of cells in a control uninfected bird. Both birds (one with latent infection and the other uninfected) were injected intravenously with enough washed infected cells so that a few minutes later both showed an infection of about eight parasites per ten thousand red cells. In the bird with the latent infection these were removed from the circulation within twelve hours, but in the uninfected bird they survived and progressively increased in numbers.

[1] The subject matter of the present paper is essentially that of a lecture given at the School of Tropical Medicine, February 13, 1928.

[2] Most of our work at the University of Chicago has been done with a strain of bird malaria which Dr. E. Hartman isolated from an English sparrow in Baltimore. In all of our previous papers this has been termed *Plasmodium praecox*, but Hartman (1927, Arch. Protistenk. 60: 1) has adduced evidence that this name should be restricted to the type of sparrow parasite isolated and used by Whitmore. Furthermore, Hartman has proposed the name *P. cathemerium* for his strain.

[3] There has been considerable discussion in the past as to whether malarial parasites were intra- or extra-cellular. The recent work of Ratcliffe (1927, Amer. Jour. Hyg. 7: 383) leaves little doubt that the parasite of bird malaria and *P. vivax* of man are actually within the cell.

[4] Ann. Inst. Pasteur 32: 382.

[5] Amer. Jour. Hyg. 3: 652.

[6] Amer. Jour. Hyg. 5: 742.

[7] Amer. Jour. Hyg. 5: 818.

[8] Amer. Jour. Hyg. 7: 407.

[9] Mrs. Taliaferro thought it was a little longer *i. e.*, 24 and 1 minute, but Hartman (1927) has shown that it is exactly 24 hours.

[10] This demonstrates that the parasites which die in each asexual cycle do not perish as merozoites, but throughout the various stages of growth.

[11] Jour. Parasit. 13: 217.

[12] The same type of disappearance has already been noted for those parasites which die in the acute period as shown by the work of Hartman.

[13] Jour. Exp. Med. 16: 567.

[14] Amer. Jour. Hyg. 6: 602.

[15] Amer. Jour. Hyg. 7: 635.

[16] Amer. Jour. Trop. Dis. 1: 240.

[17] Centrals. Bakter. Orig. 66: 105.

[18] Jour. Prev. Med. 1: 343. Also see Ibid. 2: 147.

[19] Jour. Trop. Med. & Hyg. 30: 57.

[20] Centralb. Bakter. Orig. 96: 356, 360, 362, 440, 444 and 451.

[21] Bull. Acad. Roy. Méd. Belgique (Feb. 26, 1927) : 142.

[22] Jour. Roy. Army Med. Corps. 29: 379.

[23] A Manual of the Parasitic Protozoa of Man. Philadelphia p. 569.

[24] Jour. Exp. Med. 5: 429.

[25] Byam and Archibald: The Practice of Medicine in the Tropics. 2: 1516.

[26] The Prevention of Malaria, New York, 1910. pp. 669.

[27] Ann. Trop. Med. & Parasit. 4: 267–306.

[28] Bull. Johns Hopkins Hosp. 29: 62.

[29] Jour. Trop. Med. & Hyg.