PERIODICITY OF REPRODUCTION, INFECTION AND RESISTANCE IN BIRD MALARIA

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An analysis of the effects of resistance has been carried out on bird malaria (*Plasmodium praecox* from the English sparrow inoculated into canaries) in a manner similar to the investigations which the present writer carried out in collaboration with W. H. Taliaferro (*Amer. J. Hyg.*, **2**, 1922, 264–319. See also Taliaferro, W. H., *J. Exp. Med.*, **39**, 1924, 171–190 and Coventry, F. A., *Amer. J. Hyg.*, **5**, 1925, 127–144). In this work resistance has been used to denote collectively those factors, either active or passive, which operate adversely against the parasite. Thus, it is easy to see that the number of parasites would increase at a uniform rate if no resistance developed, whereas, if they do not so increase, some type of resistance may be operative. Any effect on the number of parasites, however, may be brought about by one or both of two factors; first, the rate of reproduction of the parasites may be retarded or inhibited, or second, the organisms after they are formed may be destroyed, as the following equation shows:

(1) *Parasites at any time* = (2) *Number produced by reproduction* (1st factor) − (3) *Number destroyed* (2nd factor).

If we can obtain any two of these terms, we can evaluate the third and arrive at a fairly accurate conclusion with regard to which factors in resistance are operative.

In all of this work, the first and second members of the equation have been ascertained and the third evaluated. Thus, the first term was easily obtained by making frequent number counts during the course of an infection. The second member, in the trypanosome work, was obtained by comparing the variability in size of different samples of organisms taken at stated intervals throughout an infection. Such comparisons of variability in size may be used to measure the rate of reproduction of the parasites, because whenever reproduction is going on, there will be a great variability in size, whereas when there is none, the variability will be low. As a measure of this variability, the coefficient of variation for total length was used. The advantage of this measure is that it is independent of the second effect of resistance.

This method of obtaining a measure of the second term of the equation was not available for bird malaria because if reproduction is going on in the

trypanosome infections, all stages of growth and division may be found on a blood smear, whereas in bird malaria, on account of the *periodic cycle* of growth and reproduction, only one particular growth stage may be found at any one time (see fig. 1). Another measure of the rate of reproduction was therefore developed which works out as follows. At the time when large schizonts (asexual stages which later undergo sporulation) are in the blood, the mean size of the parasites is high; as sporulation takes

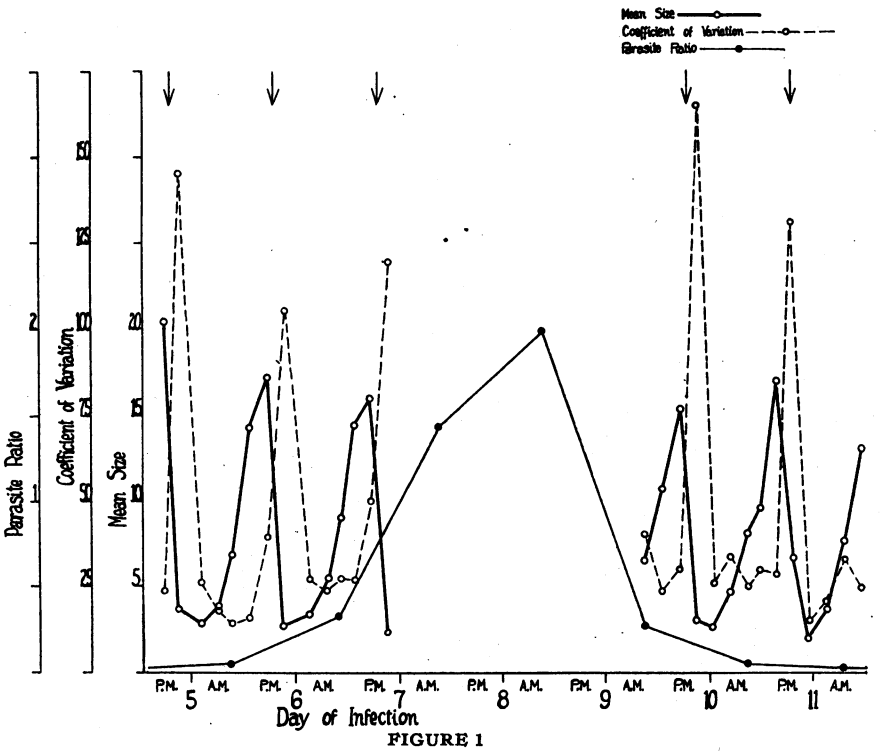

FIGURE 1

Major portion of acute and chronic infection in Bird 61. Number of parasites per 10 red blood cells, and mean size and coefficient of variation for asexual stages during two series of observations. The distance between peaks of the mean is a measure of the rate of reproduction of the parasites which is independent of the number of parasites destroyed.

place, the mean size will immediately drop, after which it will gradually rise as growth ensues until the next period of sporulation. This goes on indefinitely. The length of time between the high values of mean size is actually a measure of the time it takes a merozoite (small form) to become a full-grown schizont (asexual form ready to sporulate), and hence will give us the rate of reproduction of the parasites. Such a measure has the

same advantage that the measure devised for the trypanosome had in that it is independent of any factor which might destroy large numbers of organisms.

The Sargents (*Ann. Inst. Pasteur*, **32**, 1918, 382–388), Ben Harel (*Amer. J. Hyg.*, **3**, 1923, 652–685), and Boyd (MS.) have studied the course of an infection in bird malaria. They found by making daily parasite counts that the typical infection runs somewhat as follows. The parasites, after an incubation period, increase in numbers in the blood and sometimes become very numerous (*acute period*). If the bird does not die, they invariably reach a peak from which they rapidly decrease (*crisis*) and are only found in small numbers thereafter (*chronic period*). Eventually, they disappear (*latent period*) although under unfavorable conditions for the bird they may reappear in small numbers (*relapse*). A number curve for part of the acute period, crisis, and part of the chronic period of an infection is shown in figure 1. The rest of the typical infection (latent and relapse periods), which may cover a year or more, has had to be omitted. Suffice it to say that during these periods the parasites are never very numerous. From this work, it is evident that a sudden decrease of the parasites terminates the acute period of the infection, and as they do not migrate into the tissues, the second factor in resistance (destruction of parasites) must be operative.

The main object of the present work was to ascertain whether or not this destruction was associated with any inhibition of the *rate* of reproduction *per se*. By making several series* of blood smears at 4 hour intervals for a number of birds throughout the infection, and by drawing and measuring 50 parasites on each slide, it was discovered that in a recently isolated strain, a 24 hour cycle of growth and reproduction was taking place *no matter when the observations were made*. The sexual stages which do not reproduce in the bird, but are the infective forms for the mosquito were omitted in these measurements. This cycle is clearly shown in figure 1 (data from Bird 61) which represents graphically (1) the number changes of the parasites during the major part of the acute and chronic periods, (2) two series of data showing the changes in mean size and (3) the coefficient of variation for the same periods. The number curve is typical, as described above. The mean curve, whenever data was drawn up, is high around 5:00 P.M., falls pretty sharply at 9:00 P.M. and gradually rises through the rest of the night and following day. The coefficient of variation for the mean size also shows the cyclic nature of the infections. It is approximately constant except at the time of sporulation. This is due to the fact that growth takes place fairly uniformly—hence, the coefficient of variation, is, generally speaking, constant—but sporulation causes a marked variability in size of the forms encountered (both very large and very small forms occur)—hence, the coefficient of variation will be corre-

spondingly high. The arrows in the figure show the time of sporulation. Detailed studies have been made on 6 birds, and additional observations on 11 others. (In one strain the cycle was of 30 hours duration.) From these data, we may conclude that the reproduction of the parasites progressed at a uniform rate during the acute, crisis, chronic and relapse periods—in fact whenever the parasites were numerous enough to get a statistically valid sample. Hence, in terms of resistance, the host did not develop any resistance directed toward a retardation or inhibition of reproduction.

Finally, this study seems to have a direct bearing on the problem of relapse. Among the many hypotheses advanced (see Hegner and Taliaferro, W. H., *Human Protozoölogy*, 1924, pp. 349–351), Bignami, Ross, Whitmore and others believe that the asexual cycle continues throughout the latent period although the parasites are too few to find until some condition adverse to the host causes a relapse. This view is borne out by the present study. I found that not only were the parasites reproducing at the same rate whenever found, but various parts of the cycle occurred at exactly the same time after as before the latent period. It seems safe to suppose then that even when the parasites are too few to find, they are continuing their cycle of growth and sporulation uninterruptedly.

In the various periods of the infection outlined previously, the acute part of the infection undoubtedly presents the optimum conditions for the life of the parasite. Consequently, it is interesting to ascertain just what proportion of the parasites survive during this period. Since the reproduction rate is constant for a given strain, and since microscopic examination indicates that, on the average, each schizont produces 15.5 merozoites, we can compare the number of merozoites actually produced with the number which must survive to give us the observed increases of the parasites in the blood. The results indicate that the majority of merozoites produced at each sporulation period perish, and conversely, that only a small but fairly constant proportion survive and complete their development.

Our tentative conclusion in regard to an infection with bird malaria may be briefly summarized as follows: The asexual stages of the parasite are found in the peripheral blood of an infected bird in varying numbers and undergo their cycle of development and reproduction at the same rate throughout the entire course of the infection. From the beginning the bird seems to possess a natural resistance because only a part of the merozoites which are produced have been found to survive. At or near the crisis, the bird develops a resistance directed toward a destruction of the parasites which becomes more potent as time goes on although it may be temporarily minimized. As a consequence, at the crisis, a large number of parasites are destroyed; during the chronic period, the number destroyed equals or slightly exceeds the number produced by reproduction; during

the latent period, the destruction of the parasites keeps the number in the blood below the number at which they may be found by microscopical examination (they are still present in small numbers as shown by Whitmore, *Johns Hopkins Hosp. Bull.*, **29**, 1918, 62–67); and during the relapse, the destruction is temporarily lessened so they accumulate in the blood. At no time is there evidence of any retardation of the rate of reproduction as found by Taliaferro, W. H. (1924, *loc. cit.*) in *Trypanosoma lewisi*.

* A bird has such a relatively small amount of blood that it is not possible to make blood smears at four hour intervals throughout a single infection. Therefore, several series of slides were made from each bird allowing several days for recuperation. From the birds as a whole, however, data was obtained covering every period of the infection except the latent period, of course, when no parasites can be found.

THE CHROMOSOMES OF ZEA MAYS

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Zea Mays L. offers an interesting field for the study of chromosome behavior, particularly in view of the importance the plant has assumed in problems of genetics.

Determinations of the chromosome number in several varieties of maize have been made by Kuwada. In 1911, he[1] reported that the haploid number varies from 9 to 12 in different races, at the same time expressing his view that 12 is to be considered as the original number. In 1919, Kuwada[2] concluded that, with the exception of the "sugar corns," the chromosome numbers are 10 (haploid) and 20 (diploid) and that the plants most closely related to Zea as possible ancestral species have a somatic number of 20. The sugar corns, according to Kuwada, show considerable variation, from 20 to 25 somatic chromosomes being present. In the majority of plants of the sugar-corn type, the haploid number was found to be 12.

Longley,[3] in a study of maize and maize relatives in 1924, reported that, in the four varieties of corn studied, his investigations failed to show any deviation from 10 as the haploid chromosome number. Hybrids between *Zea Mays* and *Euchlaena mexicana* show 10 bivalents in the heterotypic division. The number of chromosome elements in a cross between *Zea Mays* and *Euchlaena perennis* is 30, and their distribution at meiosis is very irregular.

Since the writing of this paper, an article has appeared by Kiesselbach and Petersen[4] giving the results of chromosome counts, by the aceto-carmine method, of a number of commercial and inbred strains of dent maize,