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The Course of Columbiform *Plasmodium Relictum* Infections in Canaries[1]

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Abstract. Canaries were inoculated with a strain of *Plasmodium relictum* isolated from a mourning dove and maintained by blood passage through pigeons. Parasitemias reached a peak on the fourth day following inoculation in all but two birds, dropped sharply thereafter and remained at low levels through the tenth day of infection.

An asexual cycle of twenty to twenty-four hours was observed, with matinal and synchronous sporulation. Gametocyte numbers were low throughout the infections and eleven merozoites, on the average, were produced per mature schizont. Signs of pathogenicity were not observed and all infected birds survived the active phase of infection.

The host-specificity of the parasite is discussed.

Introduction

Several years ago Farmer and Moore (1962) isolated a strain of *Plasmodium relictum* from a mourning dove captured in central Missouri. By maintaining the parasite through periodic blood passage in pigeons, they studied several aspects of the strain's behavior. The 24-hour periodicity and fairly strict synchronicity of this strain is similar to the 1T strain of Wolfson (1936, 1937), the 1R strain of Huff (1937) and the 1M strain of Manwell (1940). The exoerythocytic morphology of this strain resembles *P. relictum matutinum* described by Manwell (1940) and the 1B strain of Becker (1956, 1961), but the degree of pathogenicity is similar to the 1P strain of Coatney (1937, 1938).

In many respects, therefore, this organism behaves similarly to previously characterized strains of *P. relictum* isolated from passerine birds. The fact remains, however, that it was isolated from, and is now being maintained in, Columbiform hosts. Of the three best known *P. relictum* strains recorded from pigeons, the strain 1P of Coatney, the strain 1B of Becker, and the 1E strain of Perez Reyes (1953), only the latter demonstrates a strict 24-hour periodicity. However, the strain in question differs from this 1E strain in its time of sporulation. Since the time of sporulation is the only known difference between the two strains, the taxonomic status of Plasmodium isolated by Farmer and Moore needs further clarification. A study of the

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course of infection of this particular parasite in canaries was undertaken because these birds were used as laboratory hosts in much of the initial work on avian malaria, and the behavior of the parasite in the canary is one of the criteria used in characterizing strains (Hewitt, 1940).

Materials and Methods

The canaries used for this investigation were obtained from the American Bird Company, Chicago, Illinois. Each bird was carefully examined for ectoparasites when received and pre-infection blood films were made from those selected for experiments. Sergent's (1920) isodiagnostic technique was carried out to further insure that these birds were free of latent plasmodial infections. The strain of *Plasmodium relictum* used was isolated from a mourning dove that had acquired its infection naturally. This parasite is being maintained in the laboratory by blood passage through pigeons.

Canaries were infected by intravenous inoculation of heparinized infected blood from a donor pigeon. Birds thus inoculated were kept in a constant temperature animal room equipped with automatic light control. The controls were set so that the lights in the room came on at 6:00 AM and remained on until 6:00 PM. Determinations of the course of parasitemia were made by obtaining blood smears from infected birds every 24 hours. These films were methanol-fixed, stained in Giemsa and examined using an oil immersion lens. Parasitemia was calculated according to the procedure of Gingrich (1932). The development and synchonicity of asexual stages was ascertained by taking blood smears from each bird every four hours during a 32-hour period. Since the birds began to show the effect of excessive handling, this procedure was discontinued. These smears were stained and each examined in the following manner. One-hundred asexual stages were enumerated at random, and the number of uninucleate stages, immature schizonts, and mature schizonts recorded. The number of macrogametocytes and microgametocytes observed while counting 100 asexual stages was also recorded. In addition, the numbers of merozoites in each mature schizont seen were tabulated.

Results and Discussion

Each of eight canaries was inoculated with 5 million parasitized erythrocytes of heparinized blood withdrawn from a pigeon infected with *P. relictum*. Six of the birds developed patent infections that remained at low levels until the third day following inoculation. Parasite levels attained their peak on the fourth day following inculation in all birds except X-8 and X-1.

Peak parasite levels in these birds were recorded on the third and fifth days respectively. Parasitemias declined rapidly following these peaks. However, parasite numbers were maintained but at very low levels from the sixth through ninth day following inoculation. The pattern of parasitemia observed in the canaries is illustrated by Fig. 1. It can be seen that bird X-8, unlike the others, has a parasitemia of 4.2% by the second day of infection.

It is not possible to determine the length of a true prepatent

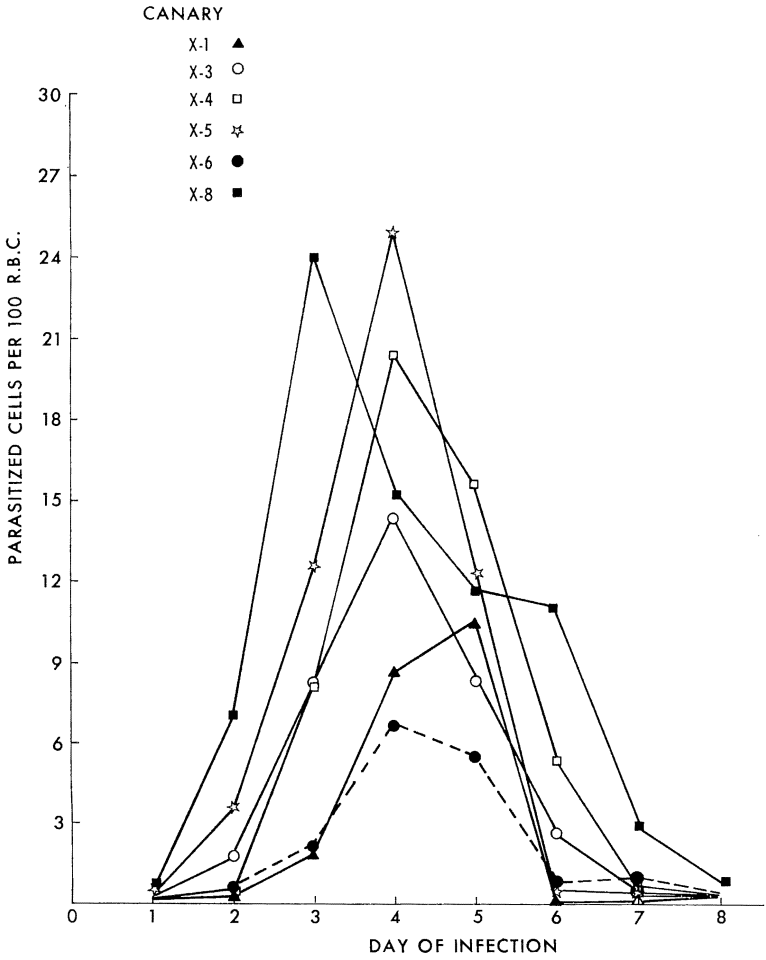

Figure 1. Percent parasitized erythrocytes determined daily from the blood of six canaries infected with *Plasmodium relictum*.

period, since infections were induced by blood inculation. A short developmental period is obvious, however, lasting 48 hours.

The levels of infection and duration of the patent period is evidence that this strain of *Plasodium relictum* can develop in and may be adaptable to passerine birds. Furthermore, it adds weight to the suggestion of Becker (1961), that a *relictum* community exists in nature rather than numerous strains. However, gametocyte counts were consistently low; 9/1000 asexual stages by actual count. It seems unlikely, therefore, that the parasite could survive in nature by mosquito passage between passerine birds if such low gametocyte levels are common. Additional experiments need to be performed to determine the consistency of this lack of gametocytes. Should this appear to be true, serial blood passage of this parasite through canaries might eventually produce a gametocyteless strain that would serve as ideal research tool.

The periodicity and synchronicity of this strain in canaries was established by examining blood smears made from infected birds during a 32-hour period of the fifth and sixth days of the patent periods of infection. The length of the asexual cycle was determined by counting 100 asexual stages, differentiating between their stage of development, and plotting the percent uninucleate forms on the ordinate and hour of the day on the abscissa. Accordingly, the time required to complete an asexual developmental cycle may be estimated by the number of hours between crests or troughs.

Examination of Figs. 2-4 indicates a developmental pattern similar in all birds except X-8. It appears that a twenty to twenty-four hour periodicity is maintained in canaries. The strain behaves similarly in pigeons as reported by Farmer and Moore (1962). It compares with the 1T, 1R strains and *P. relictum matutinum* strain previously described from passerine birds and the 1E strain reported from pigeons. Additional comparison of the development of this strain in pigeons and canaries reveals that in both, sporulation is matinal, and that uninucleate stages reach their highest levels between noon and 4:00 PM. Furthermore, in canaries, a high degree of synchronicity is attained, indicated by the nearly 100 percent uninucleate stages occurring at 4:00 in the afternoon.

Inasmuch as the number of merozites developing in a mature schizont is a characteristic used for strain differentiation, a tabulation of merozoites per mature segmenter was made. The average numbers of merozoites per mature schizont at 10:30 AM on the 4th, 5th, 6th, and 7th days of infection respectively

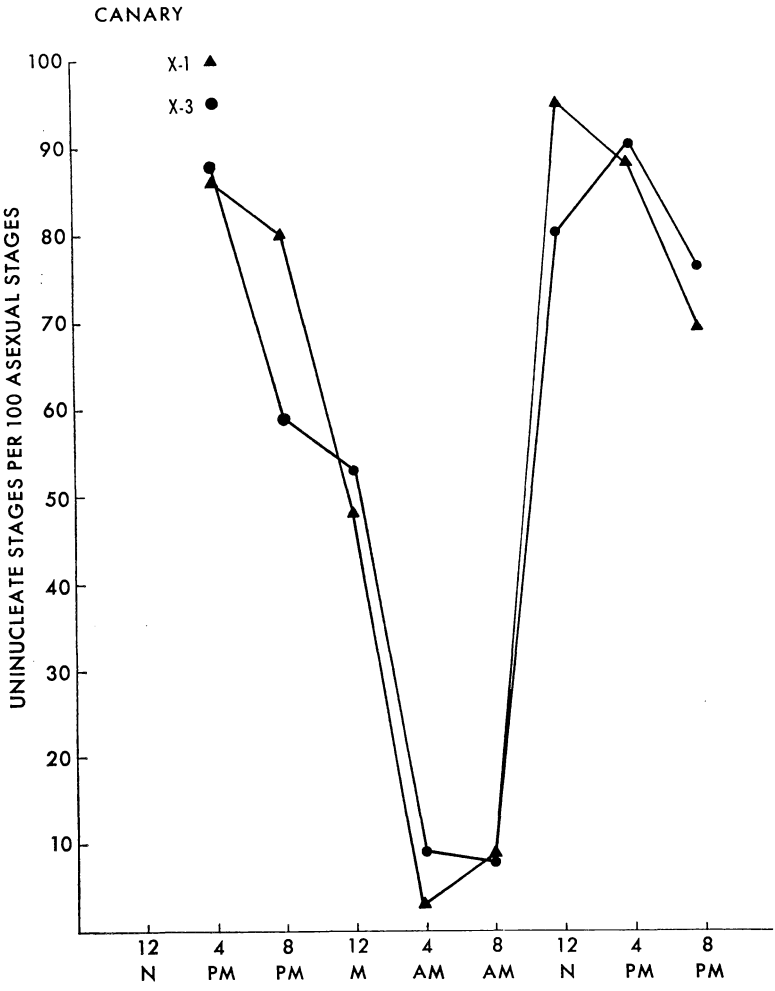

Figure 2. Uninucleate stages of *Plasmodium relictum* per 100 asexual stages observed in the infected blood of canaries X1 and X3.

were 8.0, 11.0, 13.9, and 12.0 with a range of 8, 10-12, 9-20 and 10-14.

Conclusions

The ease with which this columbiform strain of *P. relictum* developed in passerine birds lends support to the postulate of Becker that a *relictum* community exists in nature rather than numerous strains. Apparent strain differences may only reflect a physiological adjustment of the parasite to a particular host

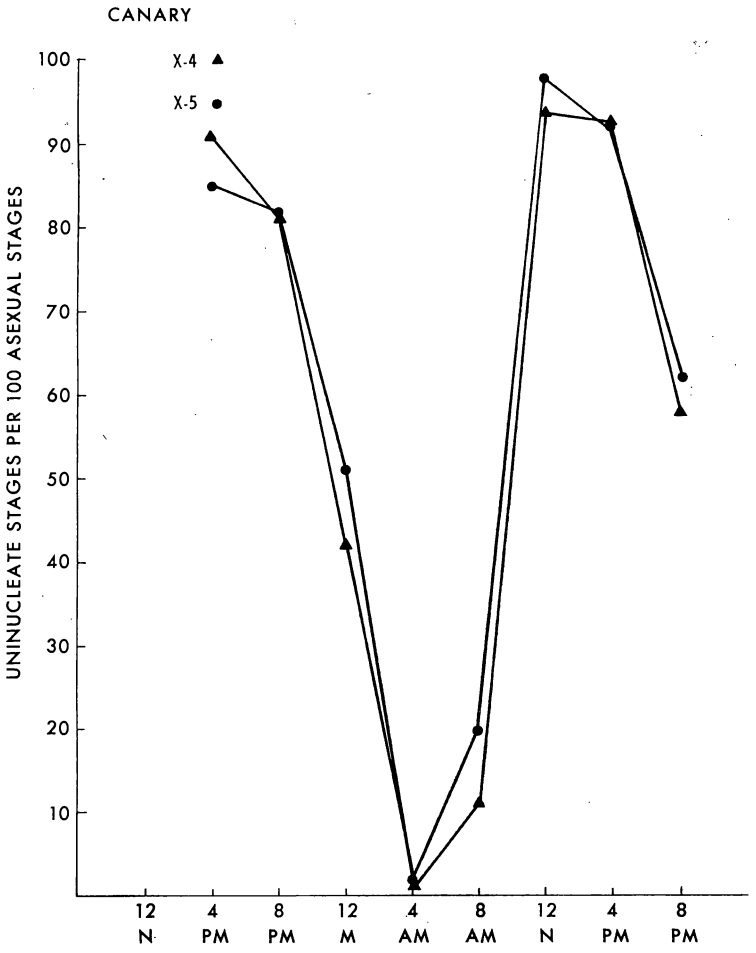

Figure 3. Uninucleate stages of *Plasmodium relictum* per 100 asexual stages observed in the infected blood of canaries X4 and X5.

species. In this particular case, such adjustment may have affected only the production of gametocytes. Perhaps, as Rollo (1964) has suggested, gametocytes are differentiated in response to enforced changes in metabolic patterns. However, considering the limited success of Moore (1963) in adapting strain to sparrows, another explanation for low gametocyte levels may be that columbiform and not passerine birds represent the preferred natural hosts.

Demonstration of the existence of a latent phase of this strain

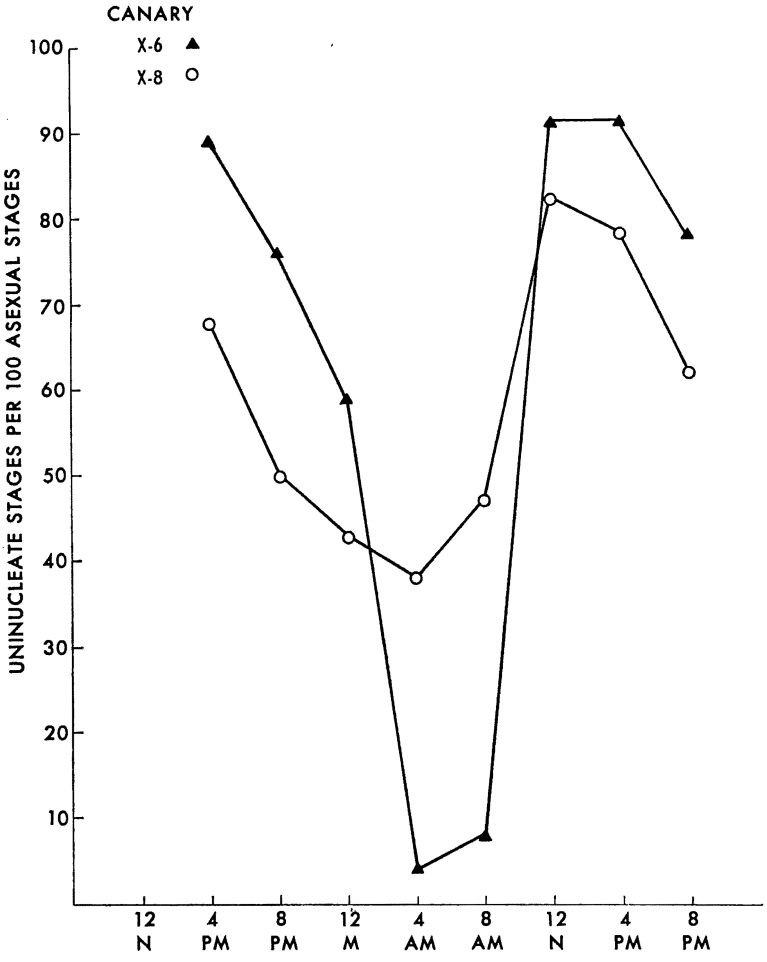

Figure 4. Uninucleate stages of *Plasmodium relictum* per 100 asexual stages observed in the infected blood of canaries X6 and X8.

of *P. relictum* in canaries would be additional evidence for the lack of host specificity exhibited by this strain. Work is in progress in an attempt to elucidate this phase of the problem.

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