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Periodicity and Synchronicity of *Plasmodium relictum* in the Pigeon

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Abstract. A strain of *Plasmodium relictum,* isolated from a mourning dove, is being maintained by blood passage through pigeons. Studies concerning the periodicity and synchonicity of this organism indicate the length of the asexual cycle to be 24 hours correlated with a high degree of synchronicity. Segmentation occurs between 5 and 9 PM, with sporulation occurring between 5 AM and 1 PM. The pathogenicity of the strain to pigeons is moderate, with a tendency to kill several days after the crisis of infection has passed. Exoerythryocytic schizonts have been demonstrated in several of the birds that died, with these stages tending to affect the brain more than other organs.

Young chickens, inoculated with infected pigeon blood, remained infected from four to ten days. However, duration of infection decreased with an increase in the age of the bird. Parasites did not develop in either six-week old or twelve-week old chickens.

The taxonomic position of this particular strain of *P. relictum* is discussed.

A number of mourning doves (*Zenaidura macroura carolinensis*) are maintained in pens located at the Green area of the University of Missouri. Routine examination of blood films, made from 33 of these birds, November 22, 1960, revealed one to be infected with a species of *Plasmodium.* Blood from this particular bird was injected into three mourning doves and two pigeons, with all contracting patent infections. Morphological characteristics of gametocytes and asexual stages demonstrable in these five birds closely correspond to the key description of *Plasmodium relictum* presented by Hewitt (1940).

One of the problems associated with investigating natural avian *Plasmodium* infections is in determining the taxonomic status of the isolated organism. A number of strains of *P. relictum* have already been described. These include a matinal strain isolated from a woodthrush by Wolfson (1937) and designated 1T by Huff, Boyd and Manwell (1942), the 1R strain isolated from the American robin by Huff (1937), the Coatney (1938) strain isolated from a pigeon and designated 1P by Huff, Boyd and Manwell (1944), the 1E strain isolated from the pigeon and described by Perez Reyes and Palaez (1953), and the strain isolated by Becker, Hollander and Pattillo (1956) from pigeons designated 1B by Huff, Marchbank and Shiroishi (1959).

The isolation of *P. relictum* leaves the investigator little choice

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but to enter into a comparative study using a number of criteria in an attempt to clarify the position of the particular organism which he has uncovered. These include the morphology of the parasite, host specificity, pathogenicity, invertebrate host preference, and various developmental aspects of the organism.

Accordingly, the following investigation was initiated to determine pathogenicity, host specificity, periodicity, synchronicity, and nature of the developmental aspects of this strain of *P. relictum.*

Materials and Methods

The strain of *P. relictum* used was isolated from one of 33 mourning doves penned in the Green area near Columbia, Missouri. The strain is being maintained in the laboratory by blood passage through pigeons. The pigeons (Modena strain) used during this investigation were obtained from a local breeder, examined carefully for ectoparasites, and kept in a screened animal room. Pre-infection blood films were always obtained from pigeons selected for experiments. To be sure the birds were free of *Plasmodium,* an additional precautionary measure was the use of isodiagnosis as described by Sergent (1920). In no case were parasites found in the pre-infection blood films and no subpatent infections were uncovered by isodiagnosis. Inoculations of all birds were made intravenously, using heparinized blood of previously infected pigeons.

To trace the development of the parasite in the pigeon, blood was taken every four hours, around the clock, by means of toe punctures. Films were then made. After air drying, the slides were stained in Giemsa and later examined microscopically by the use of oil immersion. One-hundred asexual forms were counted at random and grouped according to the following classification:

1. Uninucleate: In which parasite segmentation had not yet begun.
2. Presegmenters: In which nuclear division had occurred, but fully developed merozoites were not yet formed.
3. Mature schizonts: In which parasite segmentation had proceeded to a point where fully developed merozoites were clearly distinguishable.

Macrogametocytes and microgametocytes were enumerated along with the asexual stages and tabulated according to the numbers observed while counting 100 asexual stages. Parasitemia was determined using the method outlined by Gingrich (1932).

Infected birds were kept in a constant-temperature animal room equipped with an automatic light control. This was set

so that the lights in the room came on at 6 AM and remained on until 6 PM.

Tissues for sectioning and for tissue impressions were removed from birds that died or were killed. These included portions of the brain, liver, spleen, kidneys, lungs, heart, and gizzard. Tissue impressions so obtained were stained in the same manner as blood films.

Results and Discussion

Pathogenicity and exoerythrocytic schizogony

Of 38 pigeons inoculated with this strain of *P. relictum*, ten (26.3%) died. As indicated in Table 1, six of these died with lower parasite levels in the blood than they had sustained previously in the infection, while in two (#197 and #497) maximum parasite levels coincided with the death of the birds. Records concerning the other two animals are incomplete. In four, exoerythrocytic forms (phanerozoites) were noted in post mortem tissue impressions of internal organs and the brain.

Table 1. The distribution of exoerythrocytic forms in *Plasmodium relictum* infected pigeons that died.

Bird No.	Parasitemia Pre-Patent	Patent	Maximum	At Death	Affected Tissues Brain	Liver	Lung	Spleen
197	2	20	32.0	32.0	..	..	..	..
452	2	10	20.3	14.2	..	..	..	..
465	2	8	30.5	12.4	..	..	..	..
479	2	8	15.6	8.3	••	••	..	..
497	2	16	29.0	29.0	•	•	..	..
905	2	12	24.7	18.7	..	..	..	..
926	2	6	13.3	11.4	•	..	..	•
3065	2	6	26.6	20.0	•	..	..	•

• EE stages present
•• EE stages present but rare
.. Negative

This strain appears to be moderately pathogenic and more likely to kill some days after the crisis of the infection. Since exoerythrocytic schizonts were observed in four of the ten birds that died, an attempt was made to locate these stages in infected birds killed on the 8th, 11th, 12th, 13th, and 22nd day of infection. Tissues were removed from these birds for tissue impressions and sectioning. Careful examination of stained slides failed to uncover the presence of exorythrocytic schizonts in these particular birds. That these stages do not always develop in infected pigeons is likely. Such a possibility is considered by Manwell (1940) and also by Becker (1961). The latter, investigating the 1B strain, killed four pigeons on the 15th day of infection and eight other pigeons on the 26th day of infection. He examined their tissues for phanerozoites, with negative results.

The morphology of these phanerozoites, including the presence of one or more conspicuous vacuoles, resembles the description of *P. relictum matutinum* phanerozoites by Manwell (1940). This resemblance also extends to these stages of the 1B strain described and illustrated by Becker (1961).

Manwell (1940) refers to the tendency of *P. relictum matutinum* to affect the brain more than other organs. Concerning the 1B strain, Becker (1961) found the brain was not the only favorable site for exoerythrocytic development, since he was able to demonstrate these schizonts as frequently in other tissues as in the brain.

The strain being examined in the present investigation, however, apparently affects the brain more than the other tissues examined.

Host Specificity.

The 1P strain of *P. relictum* isolated from a young mourning dove by Coatney (1937) was found to be highly pathogenic in pigeons. He attempted to transfer this strain to young chickens (Coatney, 1938) with some success.

Since the strain being described here was also isolated from a mourning dove and is moderately pathogenic in pigeons, its adaptability to young chickens was investigated.

Three three-day old chickens, eleven ten-day old chickens, and seven 21-day old chickens were each inoculated with 0.2 ml of infected pigeon blood. The level of parasitemia in the infected blood was 10 parasitized cells in every 100 erythrocytes. Smears were made daily of the infected chicks' blood, and parasitized cells were enumerated while counting 3,000 red blood cells.

In only one (#482) of the three-day and ten-day old birds did parasites fail to develop. The length of patent infections in the other members of these age groups ranged from four to ten days.

These results correspond to those reported by Coatney (1938). In addition, he reported infecting a pigeon using pooled blood from two chicks infected 13 days previously, and in which parasites were demonstrable in blood smears up until the 10th day.

Therefore, two pigeons were intravenously inoculated with blood from chick #480, 15 days after it had been initially infected with *P. relictum*. The pigeons remained under observation for 21 days during which time blood smears from these birds remained negative.

Failure of parasites to develop beyond the 5th day of in-

Table 2. Number of parasitized cells per 3,000 red blood cells in chickens injected with *P. relictum*.

		DAY OF INFECTION									
Age	No.	1	2	3	4	5	6	7	8	9	10
3 days	480	6	30	57	126	168	31	1	0	1	1
3 days	481	1	2	1	0	0	0	1	3	3	0
3 days	482	0	0	0	0	0	0	0	0	0	0
10 days	292	6	12	10	1	3	3	0	0	0	0
10 days	293	9	20	18	8	5	3	0	0	0	0
10 days	294	9	18	4	1	0	0	0	0	0	0
10 days	836	..	23	9	1	0	0	0	0	0	..
10 days	837	..	2	6	3	0	0	0	0	0	..
10 days	838	..	5	3	2	1	1	0	0	0	..
10 days	839	..	4	2	0	0	0	0	0	0	..
10 days	840	..	6	9	3	1	0	0	0	0	..
10 days	841	..	2	2	0	0	0	0	0	0	..
10 days	842	..	3	8	2	1	2	1	0	0	..
10 days	843	..	5	11	2	0	0	0	0	0	..
21 days	181	..	1	1	1	0	0	0	0	0	0
21 days	182	..	3	9	6	1	0	0	0	0	0
21 days	396	..	6	11	6	1	0	0	0	0	0
21 days	397	..	9	13	8	2	0	0	0	0	0
21 days	398	..	7	7	6	1	0	0	0	0	0
21 days	399	..	16	8	3	0	0	0	0	0	0
21 days	400	..	2	3	0	0	0	0	0	0	0

fection was observed in all seven of the 21-day old chicks. In addition, two six-week old chickens and two 12-week old chickens were injected with parasitized pigeon blood. Blood smears from birds remained parasite-free for 11 days following intravenous inoculation, at which time daily blood films were no longer taken. Blood films obtained on the 14th, 17th, and 21st days after exposure remained negative.

Periodicity and Synchronicity

To investigate the periodicity of this strain of *P. relictum,* blood films were made from infected pigeons every four hours around the clock. To determine the length of the asexual cycle, counts were made of the number of segmenters in 100 asexual forms. Graphs were made with the percents of segmenters on the ordinates and hours of the day on the abscissas. The length of a complete asexual cycle may be estimated by the number of hours from trough to trough or crest to crest as indicated by the graphs. The length of the asexual cycle of this strain, determined from counts made from 15 infected pigeons, is nearly constant at 24 hours.

A typical example of the periodicity of this strain, as illustrated by counts made from pigeons #465 and #919, are presented in Figs. 1 and 2. This compares with the periodicity of the 1T strain reported by Wolfson (1936, 1937), with that of the 1R strain of Huff (1937), with that of *P. relictum matutinum* of Manwell (1940), and with that of the 1E strain described by Perez Reyes and Palaez (1953). The only pigeon strain to maintain a 24-hour cycle is the 1E strain described by these Mexican workers. Other pigeon and dove strains of *P. relictum* include the 1P strain of Coatney (1938) and the 1B strain of Becker (1956). The former exhibited an asexual cycle of 27 hours dura-

tion, the latter an average periodicity of 25.5 hours (Becker, 1961).

The cyclic behavior of this particular strain follows an almost identical pattern in each of the 15 pigeons. The onset of segmentation apparently occurs between 5 PM and 9 PM. Since the lights of the animal room were turned off at 6 PM, a correlation between onset of darkness and segmentation was suspected. Sporulation is matinal, occurring between 5 AM and 1 PM. Maximum levels of uninucleate stages may be observed between 1 and 5 PM. Since, at any one time, the majority of parasites are in the same stage of development, this strain possesses a high degree of synchonicity. This is indicated in Figs. 1 and 2. The crests occur near 5 AM each day, with the numbers of segmenters ranging between 88 and 100 percent at this particular time.

To test the hypothesis that onset of darkness initiates segmentation, four pigeons were parasitized and their blood taken every four hours, around the clock. The photoperiod for these

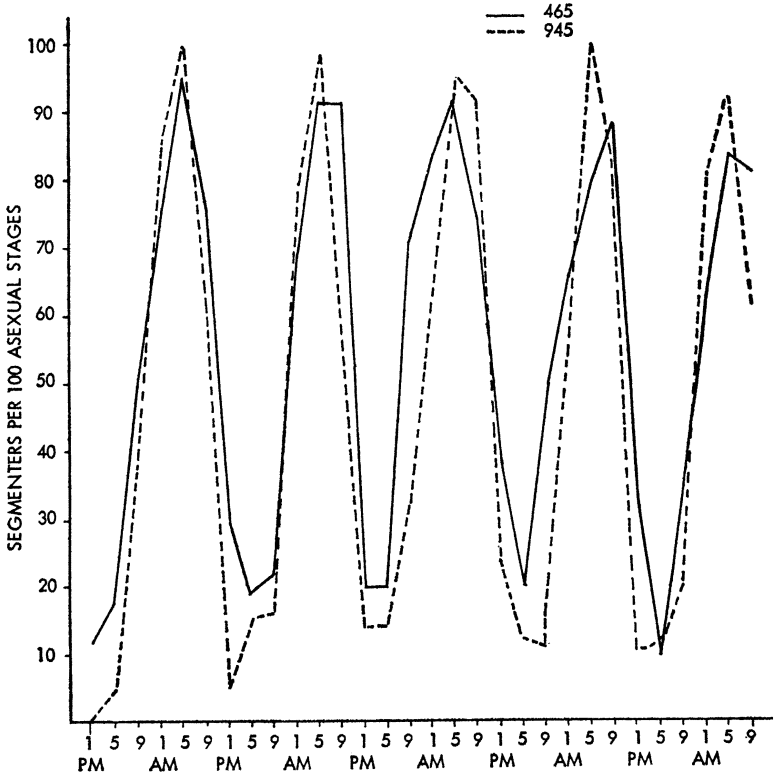

Figure 1. Percent segmenters observed in infected pigeons, #465 and#945, subjected to varied photoperiods.

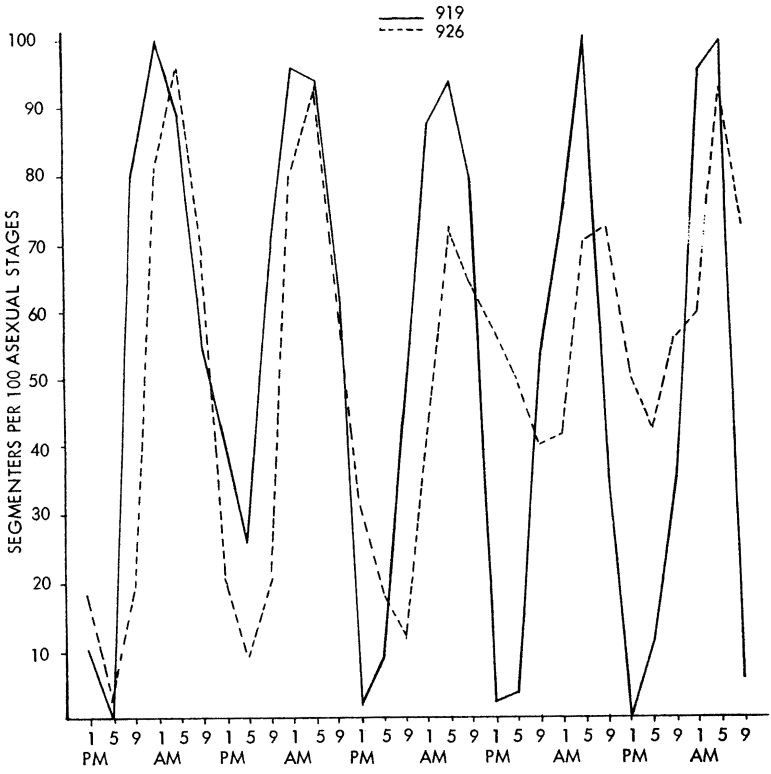

Figure 2. Percent segmenters observed in infected pigeons, #919 and #926, subjected to varied photoperiods.

birds was changed so that they were subjected to 16 hours of light and eight hours of darkness. On the third day, the light period was extended to 21 hours with only three hours of darkness. This continued for three more days. The results from three of the four days are similar to those indicated for pigeon #945 in Fig. 1. During the first two days the onset of segmentation was not delayed. Extension of the light period to 21 hours appears to lengthen the period during which uninucleate stages are most numerous, while segmentation is slightly delayed. That interference with the photoperiod does interfere with the developmental pattern of the parasite is illustrated in Fig. 2. In pigeon #926 segmentation was definitely delayed. In addition, during the extended light period, the parasite remained in a state of segmentation for over 48 hours with the number of segmenters failing to drop below 40% at any one time.

Summary and Conclusions

In comparing periodicity of the various strains of *P. relictum*, the organism being investigated resembles the 1T strain isolated

from the woodthrush by Wolfson (1937), the 1R strain of Huff (1938) uncovered in the American robin, *P. relictum matutinum* of Manwell (1940) isolated from an English sparrow and the 1E strain of Perez Reyes and Pelaez (1953) isolated from the pigeon. Furthermore, both Wolfson (1937) and Huff (1938) emphasize the matinal nature of their respective strains, a characteristic also exhibited by this present strain.

On the basis of exoerythrocytic morphology and development, this strain resembles *P. relictum matutinum* of Manwell (1940) and the 1B strain described by Becker (1961).

Finally, on the basis of pathogenicity and adaptability to young chickens, the strain compares favorably with the 1P strain of Coatney (1938) isolated from a young mourning dove.

Consideration of exoerythryocytic morphology, the circumstances associated with the death of infected pigeons (i.e., some birds would die post-crisis) a 24-hour asexual cycle and the matinal nature of sporulation would justify naming this strain *P. relictum matutinum.* However, such a conclusion leaves one in a peculiar position of apparently isolating from a columbiform host, a strain of *P. relictum* previously described only from passerine hosts. Is this *P. relictum matutinum* restricted only to columbiform hosts? Or, is it so well adapted that it may develop in passerine and, possibly gallinaceous hosts as well?

The possibility that a reservoir of *P. relictum* exists in nature is not overlooked. The introduction of this organism into a variety of hosts may well result in a variety of strikingly similar strains that differ only slightly in their characteristics. However, before attempting to pin down this so-called reservoir, one may well wonder whether a typical representative of *P. relictum matutinum* exists. As Becker has suggested, "The broader and more fundamental problem would seem to be the *relictum*-community."

Accordingly, studies are soon to be initiated to determine the adaptability of this strain in passerine hosts.

In summary:

1. A strain of *P. relictum,* isolated from one of 33 mourning doves, is being maintained by blood passage through pigeons.
2. A quotidian asexual exactly 24 hours in length was exhibited by infections in 15 pigeons. A short pre-patent period of two days was indicated.
3. Since the onset of segmentation occurred between 5 and 9 PM and sporulation occurred between 5 AM and 1 PM, this strain was considered to be highly synchronous.
4. Pathogenicity of this strain was moderate, with a tendency

to kill several days after the crisis of the infection has passed.

5. Exoerythrocytic schizonts have been demonstrated in four of ten birds that died, with these stages tending to affect the brain more than other organs.
6. Twenty-one chickens ranging in age from three to 21 days, were inoculated with infected pigeon blood; twenty of these developed patent infections of four to ten days duration. However, parasites failed to develop into chickens six and twelve weeks of age.
7. Although similar in many respects to *P. relictum matutinum* of passerine birds, the fact remains that this organism was isolated and reared in columbiform hosts. Thus, the taxonomic position of this strain remains unclear.

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