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ALTERATION IN THE TIME OF SPORULATION OF *PLASMODIUM BRASILIANUM* IN MONKEYS BY REVERSAL OF LIGHT AND DARK.*

By

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Recent experimental work indicates that although the approximate length of the asexual cycle of the malaria parasites is genetically determined the time of day when sporulation occurs is largely influenced by the habits of the host. The experiments in this paper furnish confirmatory evidence.

In *Plasmodium cathemerium* of the bird, L. G. Taliaferro (1928) showed that the time of sporulation could be proportionately delayed by refrigerating the parasites *in vitro*, but that when such refrigerated parasites were introduced into a normal bird the asexual cycle was speeded up from the ordinary 24-hour cycle to approximately 20–22 hours until the parasites again sporulated at their usual time.

In the next year Boyd (1929) showed that this regulatory process was largely inherent in the habits of the host. By subjecting birds to light at night and darkness during the day, he was able to shift the sporulation time of the plasmodium from 6–8 p.m. to 6–8 a.m. In a second paper (1929b), by making an artificial day of 28 hours, the asexual cycle was lengthened proportionately, but by reducing the periods to 8 hours, its periodicity was deranged. In a subsequent paper (1933) the feeding-time of the bird, though not the only factor involved, appeared to affect the periodicity.

Boughton (1933), working on avian *Isospora*, found that oöcysts which appeared periodically during the afternoon hours in the droppings of sparrows and other birds could be made to appear during the morning hours by alternating the ordinary 12-hour periods of light

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and dark, but appeared normally when the birds were fed exclusively during the morning or afternoon hours or when 6-hour periods of light and dark were alternated. From this, he concluded that oöcyst-production depended upon an antecedent rest period rather than upon nutritional activity.

The work to be reported in this paper forms an integral part of the study of *P. brasilianum* in the monkeys of Panama which has been carried out by the present authors. In a previous paper (1934) the morphology, periodicity and course of infection were described in 7 species of Panamanian monkeys. Suffice it to say here, the parasite is typically quartan in morphology and shows a 3-day periodicity of reproduction in the spiders, howlers and white throated monkeys throughout the entire course of infection when parasites are present in sufficient numbers to study, with sporulation occurring between 8 a.m. and 4 p.m. except that (1) sporulation may be prolonged to 4 days on rare occasions at the first sporulation * due to the manipulations of transfer, or (2) it may more often be temporarily disrupted or prolonged to 4 or 5 days at the time of the crisis (number drop) due to the immune response of the host. Furthermore, it exhibits a course of infection characterized by an acute rise in numbers, a sharp or gradual decrease in numbers (crisis), a low-grade infection, and thereafter, short periods during which no parasites can be found alternate with relapses of varying degrees of intensity. Severe relapses follow in every particular the typical course of an acute initial rise and crisis. Throughout the entire course of infection the segmenters usually form 8 to 12 nuclei (sometimes as few as 4, 6 and 7 or as many as 14 and 16), and the mean-number remains approximately constant (8.5–10) except at the time of the crisis in the spiders (8.3–8.6). Moreover, a tremendous mortality of parasites occurs at sporulation (the numbers generally increase 1 to 3 times instead of 8.5 to 10 times) as well as during intersporulation periods.† In this paper, also, occurs a review of previous literature on *P. brasilianum*, notably by Gonder and von Berenberg-Gossler and Clark, which will not be repeated here.

Materials and methods.

The same plasmodium, *P. brasilianum*, the same technique of inoculating, making smears, staining, the same numbers for the monkeys

* Parasites were often injected intravenously so that they appeared in the blood immediately.

† The reader is referred to the original paper for other conclusions not pertinent to the present experiments.

and initials to identify their species,* etc., were used in this study as were used previously (1934). Number counts per 10,000 red cells were made in accordance with previous practice.

Since the cycle and its periodicity is so dependent upon methods of measuring, however, the method used to study the infections reported in this paper will be cursorily described. This method † consists in classifying the growth stages in a random sample of 50 parasites (or more) from thin or thick films at 12 or 24-hourly intervals into rings, young schizonts less than half the size of the blood cell, large schizonts half or more than half the size of the blood cell, and multinucleated stages according to their number of nuclei. From such data the cycle and its synchronicity can be readily ascertained. In presenting these data in the accompanying graphs, they are summarily shown as the percentage of parasites containing 5 or more nuclei because the latter occur roughly for 12 hours during the 72-hour cycle. By this procedure the actual peak in sporulation (concomitant occurrence of sporulators and rings) follows by about four hours, according to previous work, the peak in forms with 5 or more nuclei. This grouping does not show the presence of several broods when only one daily blood film is made as clearly as do the more detailed data, but it lends itself to graphic treatment. Furthermore, slides were generally made at intervals of 12 hours or more frequently. To facilitate phraseology, the term segmenter will be used to denote all parasites with 5 or more nuclei.

To reverse light and dark, the monkeys were placed in a wire cage of about a cubic yard capacity. This cage was covered with several layers of heavy denim cloth and heavy paper. A small electric fan was used to force air through a two inch pipe at the top with an outlet at the bottom. The covering was completely light-tight since several bends were made in both the air intake and outlet. During the night the coverings at the sides and door were removed, and two 100 watt gas-filled Mazda electric lights illuminated the cage. The lights were filtered through a "Dalite" filter. Observations of the monkeys during the day and night clearly indicated that after the first few days they slept during the day and were more or less active at night.

* W = white throated monkey (*Cebus capucinus*); RS = red spider (*Ateles geoffroyi*); BS = black spider (*Ateles dariensis*).

† Determinations by another method (see paper on *P. falciparum* in monkeys by W. H. and L. G. Taliaferro, 1934b) depending on measurements of the diameter of parasites, which furnished corroboratory evidence will be omitted from this paper since they were made during only a part of the infection in W10.

Experimental work.

The data presented in this paper involve infections in 3 white throated monkeys belonging to the species *Cebus capucinus*, Linn. 1758. After a few normal sporulations they were put in cages, previously

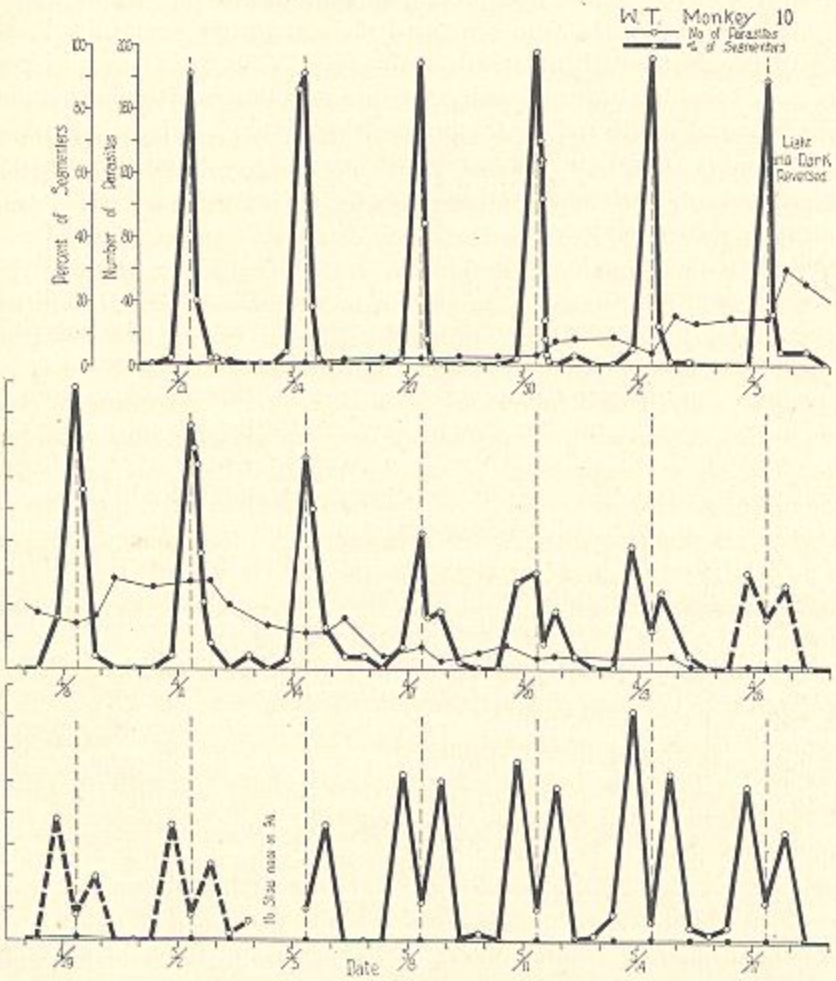

FIG. 1. The number of parasites per 10,000 red cells and percentage of segmenters in the acute rise, crisis and developed infection during a relapse of *P. brasilianum* following splenectomy in experimentally infected white throated monkey 10. The narrow, vertical dotted lines occur at 8 a.m. throughout. Note, as shown by the percentage of segmenters, that before the reversal of light and dark on 4/5 one brood of parasites sporulates during the morning, whereas after the reversal 2 broods eventually are formed, one of which sporulates 12 hours before and the other 12 hours after the original brood had sporulated.

described, so that they could be kept in complete (but ventilated) darkness from 8 a.m. to 8 p.m., and in electric light from 8 p.m. to 8 a.m. They were fed at 8 p.m. and food was available throughout the balance of the light period. Data on the reversal of light and dark on W10 were presented in a preliminary manner by W. H. Taliaferro (1932) and now with the other two will be reported more fully.

W10 was infected intraperitoneally on 4/21/30 with 1 cc. of blood from BS11 which in turn had received its infection in a similar manner from naturally infected RS4. Sixteen days later, a typical acute rise (presumably initial) in the number of parasites was followed in turn by a crisis, and thereafter a low-grade infection was intermittently interspersed by relapses of varying severity. On 3/19/31, when there were very few parasites in thick film, this animal was splenectomized. The ensuing relapse from 3/20 through 5/18 may be seen in figure 1. On 4/5, this monkey was placed on the reversed light and dark schedule and maintained on it until its death (50 days). The number of parasites increased in step-like progression from 3/19 for 17 days, due to the increases at sporulation every 3 days, until they reached a peak of 60 per 10,000 red cells. Thereafter, a gradual crisis took place as evidenced by the greater decreases during sporulation and intersporulation so that eventually a low grade infection was present on 4/25 and was maintained throughout the balance of the infection during which the parasites numbered less than 1 per 10,000 red cells.

Prior to the reversal of light and dark six regular sporulations occurred during which from 95 to 100 per cent of the forms sporulated every 3 days from 8 a.m. to 4 p.m. with only an extremely occasional form sporulating on the alternate 2 days. Directly after the reversal of light and dark 1 sporulation was fairly typical in all respects, but beginning with the sporulation period of April 11, sporulating forms were more numerous during the whole day. On 4/17, 4/20 and 4/23 they were continuously present in appreciable numbers for more than 36 hours, and never exceeded 40 per cent. Here appeared for the first time two slight peaks of sporulating forms before and after 8 a.m. instead of one peak at 8 a.m. These peaks * gradually became accen-

* The percentage of segmenters does not adequately show the reproductive cycle from 4/25 through 5/3 since only one slide was made daily at 8 a.m. but detailed study of the various growth and division stages clearly revealed that the disorganized cycle was gradually developing into 2 broods of parasites. To show this in the graph, the percentage of forms with 2 to 4 nuclei on the morning slides (which according to our previous work take 12 hours to develop into segmenters) have been considered as the percentage of segmenters which would have been found on the evening slides. (No slides were made on 5/4.)

tuated until from 5/5 through 5/18 (thereafter parasites were too scarce to find 50) two pronounced peaks in segmenters occurred regularly, one of which preceded by 12 hours and the other of which followed by 12 hours the ordinary time at which the preponderance of segmenters had occurred before the reversal in light and dark. In other words, by shifting day and night periods, one synchronous brood of parasites became irregular and then reassembled into two broods, one sporulating 12 hours previous to, and the other 12 hours following the usual time of maximum sporulation. The objection might be raised that the changed cycle was observed during a low grade infection where the synchronicity may ordinarily alter. Work on low grade normal infections, however, demonstrates this not to be the case.

W396 and W421 were both infected from naturally infected RS1 through RS110 and BS395. On 3/26/32 at noon, W396 was injected intravenously with 5 cc. of infected blood from BS395 so that immediately afterward it showed 53 parasites per 10,000 red cells in its blood. A typical increase in parasites to 75 and then to 134 occurred at the two following sporulations, but thereafter the parasites tended to decrease slowly although each sporulation was marked by a slight gain in numbers. On 3/16/32, W421 was injected intravenously with a smaller dose of parasites from BS395 so that immediately afterward it showed less than 1 parasite per 10,000 red cells in its blood. For several sporulations parasites were few, but by 4/2 at 8 p.m. they had increased to 76 per 10,000 red cells and thereafter slowly decreased. On 4/5, during the crisis, both of these animals were subjected to a reversal of light and dark which was maintained for 47 days in the case of W396 and for 36 days in the case of W421. The latter, however, showed so few parasites in its blood after 4/26 that observations had to be discontinued.

The number counts and percentages of segmenters for these monkeys will be found in figures 2 and 3. Prior to 4/1 only daily thin films were made at 8 a.m., but thereafter thick and thin films were made daily at 8 a.m., 2 and 8 p.m. Both of these infections before the experiment started showed a main brood of parasites sporulating at 8 a.m. on 3/30 and 4/2, etc., and a subsidiary brood sporulating at 8 a.m. the day before on 3/29 and 4/1, etc. Following the reversal of light and dark on 4/5, one sporulation period and the accompanying minor one were similar to previous ones, then followed several sporulations during which segmenters were present continuously in larger numbers for longer periods, until gradually recombinations in segmenters showed peaks at 8 p.m. instead of 8 a.m.

In W421 (fig. 2) the percentage of segmenters between 4/14 and 4/26 showed clearly one major and two unequal minor broods each sporulating after 8 p.m. except on 4/23 at 8 p.m. when the parasites were so scanty as to make it probable that segmenters were not encountered in the observed sample.

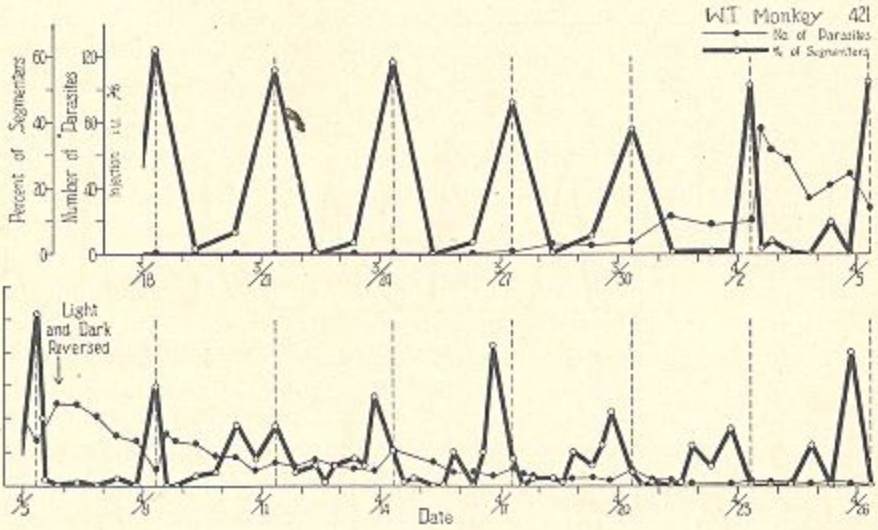

FIG. 2. The number of parasites per 10,000 red cells and the percentage of segmenters in the acute rise, crisis and developed infection during an initial experimental infection of *P. brasilianum* in white throated monkey 421. The narrow, vertical dotted lines occur at 8 a.m. throughout. Note, as shown by the percentage of segmenters, that before the reversal of light and dark on 4/5, one major and one minor brood of parasites sporulate during the morning, whereas after the reversal, 3 broods of unequal proportions are eventually formed, all sporulating during the evening, i.e., each brood had split into 2 broods sporulating 12 hours before and after the original brood.

The infection in W396 (fig. 3) which could be observed for a longer period than W421 since parasites continued in larger numbers for a longer time showed that the one major and one minor brood sporulating after 8 a.m. split up into one major and 2 minor broods sporulating after 8 p.m. This is brought out in table 1, where the percentage of segmenters at 8 a.m., 2 p.m. and 8 p.m. for one sporulation period before (3/30, 8 p.m.–4/2, 2 p.m.) and 4 sporulation periods thereafter (4/8, 8 p.m.–4/11, 2 p.m.; 4/17, 8 p.m.–4/20, 2 p.m.; 5/2, 8 p.m.–5/5, 2 p.m.; 5/14, 8 p.m.–5/17, 2 p.m.) are shown. A comparison between the first line of data and the 4 succeeding lines shows the

gradual shifts and recombinations of the segmenters from morning to the preceding or following evening. For example, normally, at 8 a.m. (first line, next to last column) 42 per cent of segmenters occur, but after the reversal of dark and light this gradually diminishes (succeeding lines, same column). In the preceding column (8 p.m.), the reciprocal change from few to many takes place.

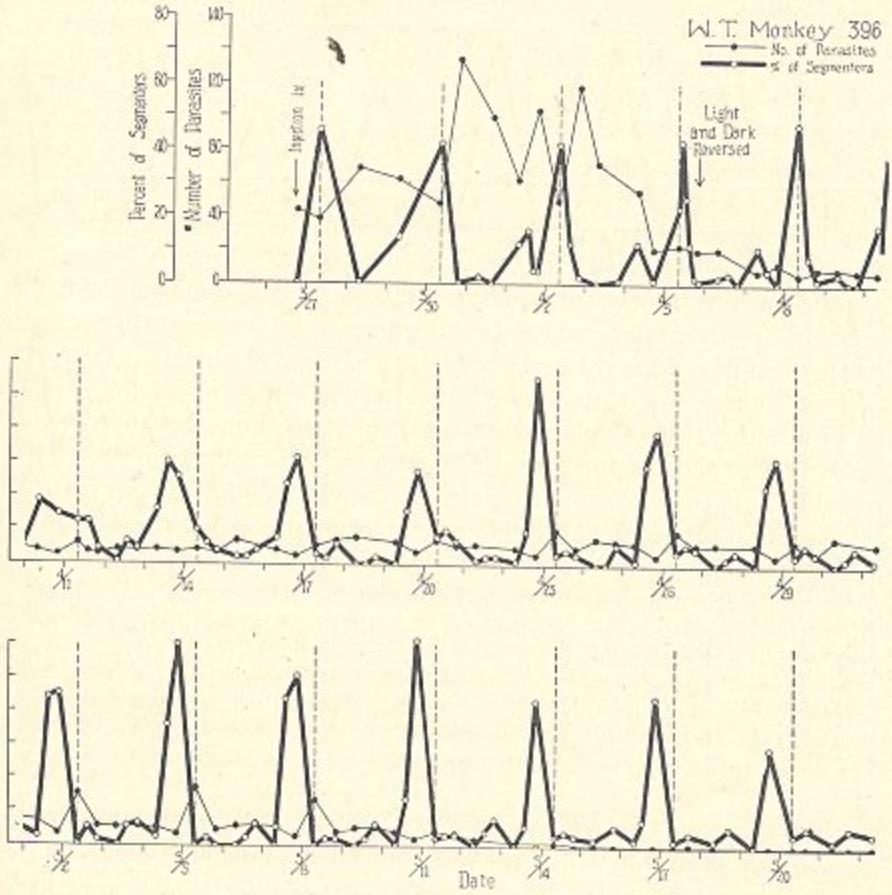

FIG. 3. The number of parasites per 10,000 red cells and the percentage of segmenters in the acute rise, crisis and developed infection during an initial experimental infection of *P. brasilianum* in white throated monkey 396. The narrow, vertical dotted lines occur at 8 a.m. throughout. Note, as shown by the percentage of segmenters, that before the reversal of light and dark on 4/5, one major and one minor brood of parasites sporulate during the morning, whereas after the reversal one major and 2 minor broods are eventually formed, all sporulating during the evening, i.e., each brood has split into 2 broods, sporulating 12 hours before and after the original brood.

TABLE 1.

Percentage of segmenters during 5 three-day intervals from the infection of P. brasilianum *in W396 showing the shift in the occurrence of segmenters from morning to evening as the result of the reversal of light and dark which was begun on April 5.*

3-day intervals beginning:	Percentage of segmenters								
	8 p.m.	8 a.m.	2 p.m.	8 p.m.	8 a.m.	2 p.m.	8 p.m.	8 a.m.	2 p.m.
3/30 8 p.m.	0	2		0	12	16	4	42	12
4/8 8 p.m.	2	3	0	0	18		12	12	
4/17 8 p.m.	6	0	0	2	0	16	24	8	10
5/2 8 p.m.	1	0	5	6	2	36	60	0	2
5/14 8 p.m.	3	2		6	2	8	45	2	4

These data are strikingly different from all the uninfluenced infections in this species reported previously (1934). Normally, sporulation occurred regularly every 3 days between 8 a.m. and 4 p.m. It is true that an unusual number of segmenters quite frequently persisted in the blood for from 12 to 48 hours at the time of the crisis instead of for 8 hours so that all or only a part of the parasites might take 4 or even 5 days to sporulate, but in all such infections, sporulation if delayed was delayed for 24 or 48 hours and not for 12 hours so that eventually it took place within the habitual time-limits. Nevertheless, the variations in the derangement of the cycle at the crisis in different monkeys, in conjunction with the fact that the reversal of light and dark covered the complete period of the crisis in W10 and the latter part of the crisis in W396 and 421, may account for the fact that one brood broke up into 2 more or less equal broods in W10, whereas one major and one minor brood broke up into one predominant brood and 2 minor broods of unequal proportions in W421 and broke up into one major and 2 more or less equal minor broods in W396.

The fact that after the reversal of light and dark, the one brood of parasites in W10 split into 2 broods and the 2 broods in W396 and 421 split into 3 broods leads to the interesting theoretical conclusion that if an infected monkey with a single quartan brood were subjected to a reversal of light and dark and then to a resumption of the usual night and day periods, the single brood showing sporulation every third day would eventually become rearranged into three broods showing sporulation every day. The procedure would be as follows: The original brood would sporulate at 8 a.m. on the following days: 1, 4, 7, etc. After alternating the normal day and night periods, the single brood

would split up into two broods with sporulation at 8 p.m. on the following days: 1, 3, 4, 6, 7, etc. With resumption of the normal day and night these two broods would split up into 3 broods sporulating every day at 8 a.m.

Conclusions.

By subjecting 3 monkeys (*Cebus capucinus*) infected with *P. brasilianum* to a reversal of the normal 12-hour periods of light and dark for from 21 to 43 days, the customary periodicity of the reproductive cycle was altered so that within 2 to 3 weeks sporulation occurred at a maximum rate just after 8 p.m. instead of just after 8 a.m. In one of these monkeys (W10) the one brood of parasites present split into 2 broods of approximately equal numbers, one of which sporulated 12 hours before, and the other 12 hours after the original brood had sporulated (fig. 1). In the other two monkeys (W396 and 421) one major and one minor brood of parasites split into one major and two minor broods of parasites all sporulating after 8 p.m. instead of after 8 a.m. (figs. 2 and 3 and table 1).

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