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THE TRANSMISSION OF *PLASMODIUM FALCIPARUM* TO THE HOWLER MONKEY, *ALOUATTA SP.* I. GENERAL NATURE OF THE INFECTIONS AND MORPHOLOGY OF THE PARASITES.

THE TRANSMISSION OF *PLASMODIUM FALCIPARUM* TO THE HOWLER MONKEY, *ALOUATTA SP.* I. GENERAL NATURE OF THE INFECTIONS AND MORPHOLOGY OF THE PARASITES.*

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Coincident with a systematic study of the parasites of Panamanian monkeys we became interested in the fate of malarial parasites from man when injected into monkeys. To our surprise we found that temporary, but undoubted, infections with *Plasmodium falciparum,* involving several asexual generations, could be induced in one of the local howler monkeys. In view of the skepticism of protozoölogists as to the possibility of transferring human malaria to the lower monkeys, fully shared by us, we have become convinced of this only after very careful study, not only of the alleged estivo-autumnal infections, but also of the natural parasites of monkeys with which they might be confused. For the same reason we have given our experimental results in detail.

Review of literature.

After the discovery of the human malarial parasites, concerted efforts were made to transmit them to all classes of vertebrates, and in particular to monkeys. The earlier efforts, beginning with that by Laveran in 1883, were consistently negative and for specific references the reader is referred to Laveran (1907). In 1917, Mesnil and Roubaud made a preliminary report of the first successful transmission of *P. vivax* to a chimpanzee. Twelve days after the injection of 8 to 9 cc. of blood infected with all stages of parasites which had not been treated with quinine, young schizonts were found; on the 13th day, gametocytes; on the 14th, young schizonts of the second

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generation; and on the 15th, numerous stages. After the 22nd day, no parasites were found. In 1920, they published further work on this and another chimpanzee. They were unable to reinfect this chimpanzee or to infect the other with *P. falciparum* or *P. vivax* either by inoculation or the bite of a mosquito infected with *P. falciparum*.

Subsequent to this work, the bulk of the findings have been entirely negative or have shown extremely evanescent infections with the exception of the report by Yoshino (1926). This investigator found rings of *P. vivax* in young dogs, rabbits and guinea pigs 2 to 8 days after subcutaneous inoculations. He appended a plate showing the rings, but his descriptions were meager. These successful findings, in conjunction with the stress he laid on transferring infections to young—even newly born—animals, gave impetus to several subsequent but negative investigations.

The negative findings, after 1917, may be briefly listed as follows: Rodhain and Van den Branden (1917) inoculated bats (Pteropidae) with *P. falciparum* or *vivax* and also subjected them to the bites of mosquitoes. Knowles (1919) injected 2 rhesus monkeys with rings and crescents of *P. falciparum* and 2 with crescents alone. Bertarelli (1920) inoculated a *Macacus cynomolgus* twice with *P. vivax* and once with *P. falciparum*. Blacklock and Adler (1924) injected a chimpanzee with *P. falciparum*. Bacchelli (1928) gave 2 monkeys, 2 pigs, 6 guinea pigs, 2 white rats and 2 white mice blood containing *P. vivax* and a similar number of animals blood containing *P. malariae*. Zia and Faust (1928) inoculated *P. vivax* or *P. falciparum* into rabbits, guinea pigs and hamsters about 2 months old some of which had been splenectomized or treated by injections of strychnine. Marginesu (1929) gave *P. vivax* to monkeys and rabbits after splenectomy or "blockade" of the reticulo-endothelial system and later subinoculated their blood into man without producing an infection. Segal (1930) transferred *P. vivax* to new born guinea pigs and 8 to 12 days afterwards transferred blood from the guinea pigs to man without success. Adant (1931) infected 4 monkeys (2 *Cercopithecus sabaeus* and 2 *cynocephalus*), one of which had been splenectomized, with *P. falciparum*. None of these investigators reported the finding of any parasites in the subinoculated animals.

It is of passing interest to note that Knowles (prev. cit.) injected 2 cc. of citrated blood containing crescents of *P. falciparum* into a hununan monkey (*Semnopithecus entellus*) which revealed an intense infection in 23 hours and died in 44 hours. All later authors, how-

ever, are in agreement with Knowles in his conclusion that this could not have been a transfer, but was a relapse of a latent infection in the monkey brought out by the operative shock and injection of foreign blood. Another possibility suggests itself in view of the quickness of the attack and from the fact that we have found it extremely difficult to produce intense relapses in monkeys by the repeated injection of large quantities of human blood. From Knowles' description one wonders if the monkey did not happen to be just at the beginning of a relapse or even have parasites in its blood when the human blood was injected. Knowles simply states that the monkey had been in the laboratory for about a year and was in excellent health, but he does not state how many blood films were examined before the injection.

A few investigators noted parasites during the course of the first few hours after the transfusion of plasmodia-containing blood into the various animals. Thus, Bass (1922) found several rings in one guinea pig inoculated intravenously with *P. falciparum*, although several other guinea pigs, one *M. rhesus* and 4 rabbits never showed parasites. An interesting feature of his work is that in cultures, the *P. falciparum* grew when overlaid with donor's serum, but failed to grow when overlaid with serum from the test animals. Franchini (1927) reported the finding of 2 gametocytes in a thick blood film from one of 9 pigs 19 days after infection with *P. vivax*. Four other pigs were inoculated intramuscularly 6 times with *P. falciparum*, but exhibited no parasites in their blood at any time. Massa (1929) recovered infections of *P. vivax* in man after leaving them in two rabbits for 4 hours and 3 days, respectively, according to Marginesu. Steinfeld (1929) found a pigmented leucocyte in a splenectomized Java monkey infected with *P. malariae* and upon autopsy 12 days after infection found pigment in its kidney and liver, but no parasites. Another "blockaded" monkey, infected with *P. falciparum*, failed to show any parasites. Stradomsky, Petrowsky, Popow and Rudnew (1930) found an occasional parasite in only a small number of the dogs and guinea pigs inoculated with *P. vivax* and *P. falciparum* and then only in the first few hours after subcutaneous or intravenous injections. Bodechtel (1930), in experiments similar to those of Massa, recovered *P. vivax* in 7 out of 8 humans after leaving it in "blockaded"' mice 24 to 48 hours. The blood from 2 or 3 mice was pooled for the inoculation into each human.

Materials and methods.

This study involved inoculations of *P. falciparum* into 10 monkeys, 9 from human beings and 1 from an infected monkey. Subsequently one of these was infected with *P. brasilianum*, the quartan species found naturally in monkeys. Work on monkey 68A was done in the spring of 1932; work on the remainder in the spring of 1931.

All of the monkeys were infant brown howlers weighing 440 to 645 grams and captured from Herrera, Panama. Although they have been tentatively identified as *Alouatta palliata palliata* by Clark and Dunn (1933), Prof. Thomas Barbour of Harvard University (personal communication) believes that they represent an undescribed species. The use of these monkeys obviated the possibility of confusing the experimentally administered infection with a natural infection since only three of several hundreds of them brought into the Gorgas Memorial Laboratory from this locality have been found infected with malaria (*P. brasilianum*) as ascertained by daily examinations for a week and once a week thereafter until they died. In fact, the great disadvantage of using these animals was that they did not stand captivity well. Therefore, the death of 6 of our monkeys during early stages of the malarial infection need not be attributed to the experimental procedure *per se*, since normal monkeys were dying equally rapidly.

The procedure was simple. Fifteen to 20 cc. of blood were taken from the femoral vein of a patient showing several to many rings per microscopic field of *P. falciparum* in a thick film. The blood was poured into four times its volume of 0.4 per cent citrate and centrifuged. After decanting the supernatant fluid, the blood cells were mixed with about an equal quantity of saline and were injected as soon as possible into the tail vein of a monkey in whose blood parasites had never been found. The transfer generally took about an hour—never more than two hours. Thick and thin blood smears were made immediately and then at about 12-hour intervals until the monkey was killed or died. Since the monkeys were so small, the infecting dose of 7 to 10 cc. of washed, packed red blood cells was an enormous addition of blood into the blood stream. In fact, in one case (no. 206), enough parasites were introduced so that immediately after inoculation a number count showed that there were 380 rings per 10,000 red blood cells or approximately 1 infected cell in every 27 cells.

Number counts were made frequently from the thin films. These consisted simply in ascertaining the ratio between the parasites and

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Page 321, 2nd line of 3rd paragraph:
for "the femoral vein," *read* "a cubital vein."

red cells in a number of microscopic fields on a stained film and calculating the proportion of parasites per 10,000 red cells. In the majority of cases the size of the sample used in determining this ratio was such that the probable error did not exceed 15 per cent of the total observed value (Hartman, 1927). Various red cell counts per cmm. of blood showed a variation from three and a half to six millon per cmm. Such a difference might appear to indicate that there would be fairly large discrepancies when the parasite number counts were referred to an absolute number of red cells, but in several monkeys where frequent red cell counts per cmm. were made and the parasite counts plotted per 10,000 red cells and per cmm. of blood, the trends in the number counts were only inappreciably changed. In view, therefore, of the large number of parasite counts necesssary in this and other work carried on simultaneously, the number of parasites per 10,000 red cells was considered adequate and sufficiently accurate. Examinations of thick films were resorted to when the parasites dwindled in number to such an extent that thin films were practically useless in disclosing them. From parallel number counts on thick and thin films a given number in thick films is equivalent to a tenth of that number per 10,000 red cells in thin films.

Since the cycle of growth and division is of prime importance in this study where it occurs every 48 hours instead of every 72 hours as in our strain of malaria of monkeys, the two measures we have used to express it are worthy of individual consideration.

In the first place, the changes in mean size give an accurate measure of the cycle, provided the samples are taken at least at 12-hour intervals. The mean size of various samples of parasites was obtained by drawing the outlines of 50 or 100 parasites with a camera lucida, measuring and calculating the mean ($\Sigma x/n$). The drawings were $\times 3000$. They were measured as follows: A series of circles 3, 6, 9 up to 27 mm. in diameter were heavily outlined in India ink and fixed over a museum jar in which was put an electric light. The diameters of these circles, therefore, represented 1, 2, 3 up to 9 μ. The drawing of each malaria parasite was then superimposed over various of these circles until it was found to fit between two circles. Its size was then considered the smaller of the circles. For example, a parasite intermediate in size between circles 5 and 6 was considered equal in size to circle 5 whose diameter was equivalent to 5 μ. This method was adapted from one used by one of us (1925) in work on avian malaria and though accurate is extremely time-consuming. It was used to study the infection in monkey 171.

In the second place, the changes in the percentage of forms showing division of the nucleus will also give an accurate measure of the cycle provided also the samples are taken at least at 12-hour intervals. In our work 50 or 100 parasites were inspected, were divided into several classes and where division occurred the number of nuclei was counted as accurately as possible. In tabulating the data, two classes were recognized, one consisting of all the growth stages and those with 2, 3 or 4 nuclei and the second consisting of those with 5 or more nuclei. This criterion was selected because it seemed an easy point of demarcation in the whole process of division and included a wide enough range of division forms so that examination of 50 or 100 forms was sufficient to obtain an adequate sample. Thus, it was sometimes difficult to be sure whether a form was uninucleated or binucleated since an apparently uninucleated form might be due to the juxtaposition of 2 nuclei or to the partial superimposition of one over the other. By the time the third division of the nucleus was inaugurated, however, there was always a complete separation of some of the parts from which the juxtaposition or superimposition of others could be inferred. Thereafter, the increasing number of nuclei sometimes made exact, accurate counts impossible. Such a procedure is simpler, less time consuming and just as accurate as drawing and measuring samples of parasites. It is similar to the one developed by Boyd (1929) except that as he was studying very heavy infections, he could look at much larger samples in a reasonable length of time, and hence used, as his criterion, the percentage of forms in 500 or even 1000 forms showing actually mature segmenters, i.e., segmenters with fully formed merozoites just ready to break out of the cell. This method was used in studying all of the infections reported in this paper. When parasites became too scarce to find on thin films, they were studied in thick films.

Both of these measures are somewhat inadequate due to localization of the larger schizonts and segmenters in the capillaries. In the monkeys, however, they can be relied upon to give an indication of the occurrence of sporulation since a larger proportion of growth stages (necessary for the first method) and of segmenters (necessary for the second method) occur in the peripheral blood of the monkey than in the routine infections of this species encountered in man.

In 5 monkeys (nos. 156, 198, 199, 204 and 206) a small piece of spleen weighing from 0.043 to 0.25 grams was removed prior to infection in order to obtain a picture of that organ before and during or after infection. To prevent excessive bleeding of the spleen after the

excision of the piece, the cut surface was cauterized by rolling a hot glass rod over it.

Thin blood films were stained with Giemsa's or Wright's stain or with tetrachrome by the method of Gingrich (1932). Thick blood films were made and stained by the method of Barber and Komp (1924).

Experimental results.

Morphology.

The forms found in the blood of these monkeys during the 48-hour cycle are shown by camera lucida drawings in plate 1 and by photographs in plate 2.* As an examination of these plates shows, they are typical subtertian forms. Small typical rings occurred immediately after segmentation (pl. 1; 1–2 and pl. 2; 1–2). Some possessed slight pseudopods (pl. 1; 3); some showed two chromatin dots (pl. 1; 3–4; pl. 2; 1); some were situated on the border of the cell (pl. 1; 2, and pl. 2; 1–2) and often doubly or triply infected a cell (pl. 1; 4 and pl. 2; 1–2). After several sporulations in the monkey, the rings were approximately typical, but frequently showed 2 or 3 chromatin dots (pl. 1; 3 and 5), more often possessed pseudopods and seemed to stain more delicately. A gradual growth in the ring forms took place. This was represented, during the first 8 to 30 hours, not so much by an increase in circumference as by a denser amount of cytoplasm (pl. 1; 4–8). Occasionally, in very deeply stained cells containing half-grown schizonts, Maurer's dots were seen (pl. 2; 3–4). These were typical—irregular in shape, brick red in color and larger in size than Schüffner's dots. Within 30 to 40 hours, an actual increase in size took place together with an increase in the nuclear material (pl. 1; 9–11) and the accumulation of pigment granules (pl. 1; 10–12). The pigment granules were quite large (pl. 1; 12–13), and early in the process of division began to clump so that at the two-nucleated stage they might be clumped (pl. 1; 14), and thereafter were almost invariably clumped. Division was initiated when the parasite itself did not nearly half fill the cell (pl. 1; 13 and pl. 2; 5) and even at the four-nucleated stage, growth was not pronounced (pl. 1; 16 and pl. 2; 6). Concomitantly with division, growth proceeded, until, when division was

* In plate 1, figs. 1, 8–12, 14, 16–23 are from monkey 171; figs. 13–15 are from monkey 167; and figs. 2–7, 24 and 25 are from monkey 204. In plate 2, figs. 1, 2, 6–8 are from monkey 171, figs. 3, 4 and 9 from monkey 198, and figs. 5 and 10 from monkey 204.

completed, the parasite sometimes filled or almost filled the cell (pl. 1; 17–25 and pl. 2; 8–10).

For comparative purposes plate 1 contains five typical forms of *P. falciparum* from a human placenta * and 5 typical forms from the peripheral blood of a monkey infected with *P. brasilianum*. The ring stages of *falciparum* (pl. 1; 1–2) show considerably less cytoplasm than *brasilianum* (pl. 1; 31). The uninucleated form of *P. falciparum* (pl. 1; 11, 12 and 26) does not grow as large before division is initiated as does *P. brasilianum* (pl. 1; 32). The two-nucleated human form is much smaller and already shows the pigment clumped (pl. 1; cf. 13 and 27 with 33). The 8-nucleated form of *P. brasilianum* frequently does not show the pigment clumped (pl. 1; 34). Finally, the human form in both the monkey and in the human placenta forms from 16 to 24 or more nuclei (pl. 1; 19–25, 29–30), whereas *P. brasilianum* forms ordinarily from 8 to 12, rarely up to 16 nuclei (pl. 1; 35).

The similarity between *P. falciparum* in the monkey and in man is brought out in another way by the camera lucida drawings. In plate 1, figs. 4, 9, 10, 13, 15, 16, 22, 24 and 25 and in plate 2, figs. 3, 4, 5, 9 and 10 are in human blood cells, at least they have been in the monkey less than 48 hours, whereas figs. 1, 2 and 3 have undergone one sporulation in the monkey and the balance in plate 1, figs. 5–8, 11, 12, 14, 17–21, 23, and plate 2, figs. 1, 2, 6, 7 and 8, have undergone two sporulations in the monkey.

The actual sizes of the parasites at sporulation and at 12-hourly intervals have been obtained from drawings and measurements of parasites in monkey 171 and may be found in table 1. (The data represent random samples meeting the special requirements of each column.) The rings just after sporulation show a mean of 2.8 μ. Growth is very gradual and slow for 12, 24 and 36 hours as represented by the measurements 3.0, 3.6 and 3.9 μ respectively, but for the following 12 hours, when division is occurring, is fairly rapid until at sporulation multinucleated forms show a mean of 6.3 μ.

The peculiar presence of segmenters led to an intensive study of them. One hundred segmenters with 2 or more nuclei were drawn and the nuclei counted on June 8 at 8 a.m. from monkey 171. Their mean diameter was 6.3 μ. Of these 53 had 12 or more nuclei, and it is interesting to note that their mean diameter was 6.9 μ while the size of the blood cells in which they were found was 7.28 $\pm$ .06μ.

* This placenta was obtained in Puerto Rico in 1928 and besides the heavy concentration of asexual forms, a few crescents were found.

Moreover, the mean diameter of 200 uninfected red cells from this monkey was $7.73 \pm .02\ \mu$. These data which may be found in table 1 indicate that the mature segmenter almost filled the red cell, but did not enlarge it. In fact, during the final stages just before division, the diameter of the red cell as compared with the normal appears to contract slightly since the difference is $0.45 \pm .06\ \mu$. These conclusions are also shown in plate 1. Figures 1 to 3 and 5 to 8, showing red cells containing rings and therefore presumably normal in size, may be compared with figs. 11, 12, 14, 17–21 and 23, showing red cells containing segmenting forms. All of these probably represent red cells from monkeys since they occurred during the second or third sporulation in the monkeys. One in each of these groups (7 and 19) measures $7\ \mu$ and all of the remainder measure $8\ \mu$.

TABLE 1.

The mean diameter in microns: 1, of parasites during the 48-hour cycle of sporulation; 2, of infected; and 3, of uninfected red blood cells.

Diameter in microns	Parasites:						Red blood cells:	
	After sporulation				At sporulation		Containing parasites with 12 or more nuclei	Uninfected
	Ring-forms	12 hr.	24 hr.	36 hr.	2 or more nuclei	12 or more nuclei		
2	52	6	1					
3	134	39	79	24				
4	7	5	118	69				
5			2	4	26	1		
6				3	47	13	6	4
7					37	29	27	61
8					14	10	19	120
9							1	15
Total	193	50	200	100	124	53	53	200
Mean	2.77	2.98	3.61	3.86	6.31	6.90	7.28	7.73

One fully formed segmenter showing complete division of the cytoplasm contained 18 merozoites. Further work on the number of merozoites formed was undertaken as follows: The nuclei in 75 parasites from each of 3 monkeys (171, 198, and 204) in which there were 8 or more nuclei were counted and tabulated in table 2. For purposes of comparison, not only has a similar sample been tabulated from *P. falciparum* in a human placenta (mentioned previously),

TABLE 2.

Frequency distribution of the number of nuclei in 75 segmenting parasites of P. falciparum *containing 8 or more nuclei: 1–3, in 3 monkeys: 4, in one human placenta. Also, the frequency distribution of the number of nuclei: 5, in 50 parasites of* P. falciparum *where division is complete and fully formed merozoites occur.*

	Number of nuclei in dividing parasites																						Mean of numbers of nuclei
	8	9	10	11	12	13	14	15	16	17	18	20	22	23	24	25	26	27	28	32	34	36	40	
1. Monkey 171	8		3	3	12	4	5	6	19	1	4	6	1		2					1				14.6
2. Monkey 198	11	2	14	4	12	5	6	7	10	1	1		1					1						12.4
3. Monkey 204	7	2	6	3	4	13	12	2	15						2	1			1	2	1	1	3	15.6
4. Human placenta	7	1	15	4	14	2	4	3	3		2	5	6		8	1								14.4
5. Human placenta											1	11	10	3	23	1	1							22.6

but also a sample of 50 segmenters from the same placenta with fully formed merozoites just ready to rupture the blood cell. In the monkeys and in the human placenta, parasites having from 8 to 14 nuclei did not look like completely formed segmenters as their nuclei were large and often irregularly shaped, whereas parasites with 16 to 24 nuclei appeared quite regularly, and in monkey 204 an unusual predominance of parasites with 28 to 40 nuclei appeared (pl. 1; 24–25 and pl. 2; 10). The question arises as to whether or not these were due to doubly or even triply infected cells. In this connection, of 500 infected cells found on June 10 at 4 p.m. in this monkey (i.e., the young rings which later formed the segmenters under consideration), 14 were doubly and 1 triply infected. In other words, 3 per cent of the infected cells were doubly (rarely triply) infected, whereas, 6 per cent of the segmenters contained 40 nuclei and 14 per cent contained 32 or more. These data seem to indicate that the number of nuclei formed in a segmenter in this infection may be as high as 40, but additional work would be necessary to make this conclusion more than tentative. In the human placenta among the mature segmenters 20 to 24 merozoites occurred generally, although there was one each containing 18, 25 and 26 merozoites, respectively (table 2). From these data, it appears that the segmenters form approximately the same number of nuclei in the monkeys as in the human placenta with the exception of monkey 204 (table 2).

General course of infections and asexual periodicity.

The most intense infection was encountered in monkey 171, the graph of which may be found in fig. 1. This infection persisted for 5⅔ days, included 3 sporulation periods and was characterized by several decreases and enormous increases in numbers of parasites which in turn were probably terminated by a crisis. In fact, at the peak of the infection, (3⅔ days) there were 915 parasites per 10,000 red cells, i.e., every eleventh cell was parasitized. This transcends the number found in any other *P. falciparum* or *P. brasilianum* infection which we have encountered. When the monkey was killed, a few parasites were still present.

The percentage of segmenters with 5 or more nuclei shows plainly that sporulation occurred approximately every second day. Thus, on June 4 at 8 a.m. (no other slide was made on this day) 8 per cent occur; on June 6 at 9 a.m. and 2 p.m., 6 and 1 per cent occur; and on June 8 at 8 a.m., noon and 4 p.m., 21, 1 and 4 per cent occur, respectively.

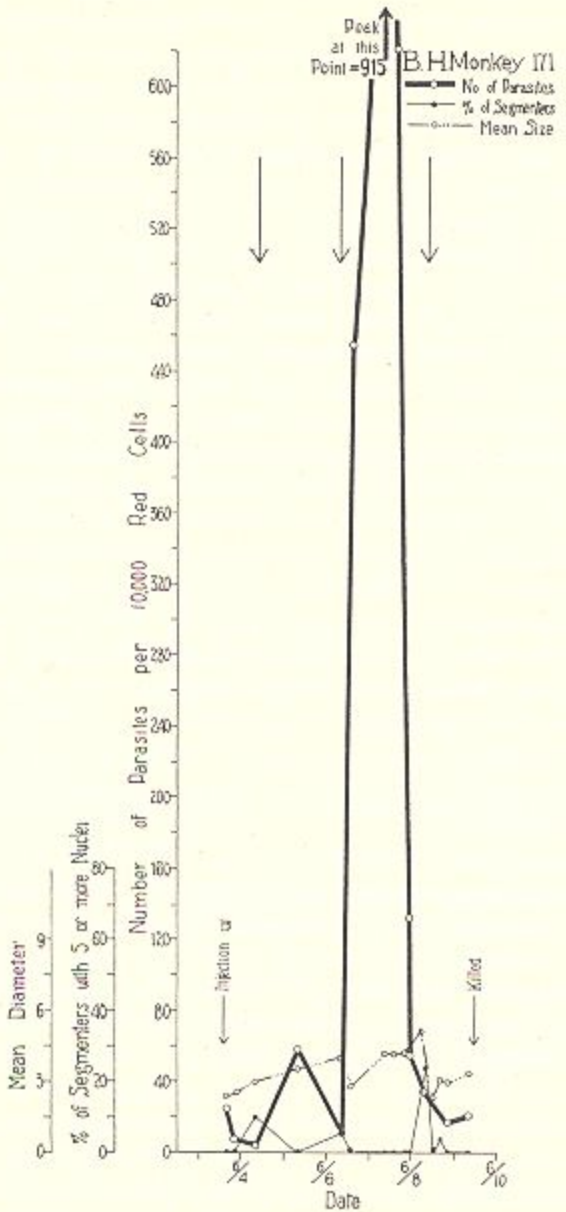

FIG. 1. The infection of *P. falciparum* in monkey 171 showing: 1, the number of parasites; 2, the percentage of segmenters (as indicative of sporulation: see arrows); and 3, the mean diameter of parasites in microns.

Throughout the balance of the infection, whenever slides were made, no segmenters occurred (fig. 1). It is interesting to note that these percentages, which are not very high, are, nevertheless, rarely found in man. Their occurrence may be correlated with the abnormality of the host or the susceptibility of such normal untreated infants. The mean size of the parasites does not indicate one of the sporulation periods due to the fact that on June 4 no blood film was made at 8 p.m. (fig. 1).

The scarcity of segmenters in the blood at any time, coupled with the enormous increases in numbers of parasites even after segmenters had disappeared from the peripheral blood, indicate that a differential percentage of segmenters must exist in the peripheral blood and in the capillaries of the deeper organs. Thus, on June 6 at 9 a.m., there were only 11 parasites per 10,000 red cells and of these only 6 per cent were schizonts with 5 or more nuclei. Nevertheless, in 24 hours the parasites had increased to 915 per 10,000 red cells, although if all the schizonts had sporulated and produced 20 progeny, there could not have been more than 13.2 parasites per 10,000 red cells. Such localizations made it impossible to calculate the proportion of progeny surviving at each sporulation, as has previously been done for *P. brasilianum* (Taliaferro, 1932) and *P. cathemerium*, the avian parasite (Taliaferro, 1929). Moreover, the fact that the largest percentage of segmenters occurred during the crisis (June 8) seems to indicate that adverse conditions elsewhere tend to drive them into the blood.

For comparative purposes, the graph of an infection of *P. brasilianum* the simian form, is given in fig. 2. This is also characterized by a series of decreases and increases in numbers of parasites, but sporulation occurs every *third* day instead of every *second*, and is so synchronous that at the peaks of sporulation 60–70 per cent of all the parasites in the blood have five or more nuclei.

On June 7 at 4 p.m. at the peak of the infection in monkey 171, 5 cc. of cardiac blood were taken from this monkey and injected intravenously into monkey 200 so that immediately afterwards there were 95 parasites per 10,000 red cells. At 8 p.m. only 2 parasites and at 11 p.m. only one parasite was found in 5,500 red cells. Thick films made subsequently were negative for five days, at which time the monkey was killed for examination of its tissues. This infection was exceedingly transient. In view of the fact that it was subinoculated from monkey 171 during the crisis (in 24 hours the parasites in 171 had dropped from 915 to 33 per 10,000 red cells),

the question arises: Were the parasites already opsonized and hence more easily phagocytosed? Further work on this problem is in progress in *P. brasilianum*.

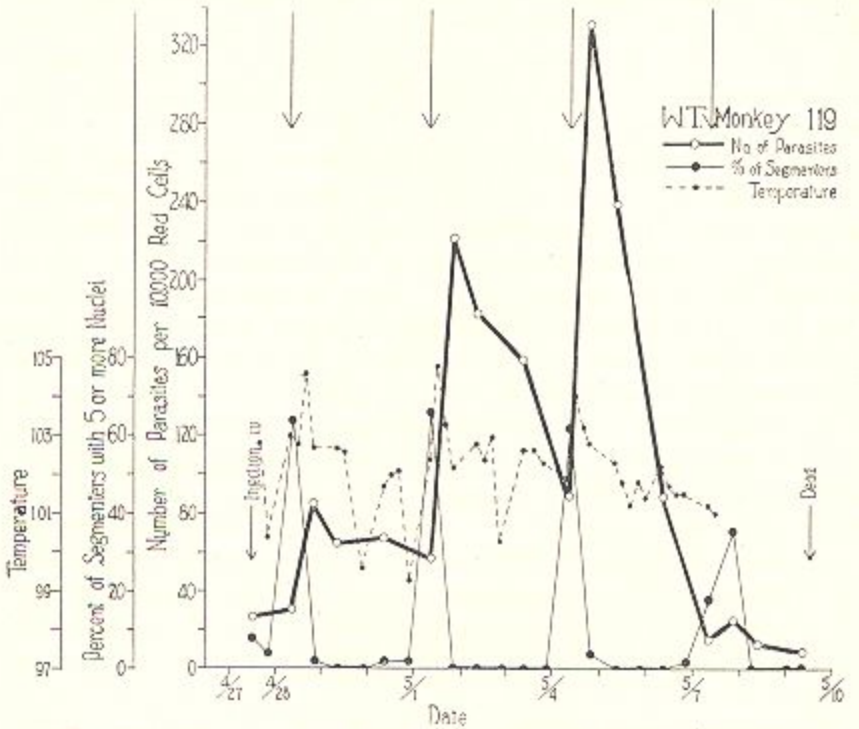

FIG. 2. The infection of *P. brasilianum* in white throated monkey 119 for comparison with figs. 1 and 3. Note quartan periodicity and pronounced synchronicity as compared to *P. falciparum*.

Monkey 204 elicited a lighter infection than monkey 171 in spite of the fact that immediately after infection there were twice as many parasites in its blood (52 as compared to 25 parasites per 10,000 red cells). It persisted for 8 days at which time the animal died (fig. 3). From June 10 through June 15 parasites were fairly numerous and showed two sporulation periods, the first on June 12 around 8 a.m. and the second on June 14 around 8 a.m. Sporulation occurred, however, over a considerable period. Thus, at the first sporulation, 2 per cent of parasites with 5 or more nuclei occurred at 8 p.m. on June 11, 13 per cent at 8 a.m. on June 12, and 1 per cent at noon on the same day, and at the second sporulation, 2 per cent at 10 p.m. on June 13 and 4 per cent at 8 a.m. on June 14. As noted

previously, a comparatively large number of parasites were found with from 32 to 40 nuclei at the sporulation period occurring on June 12. Comparisons between fig. 3 and fig. 2 again illustrate the differences encountered between the two species of malaria in the monkey.

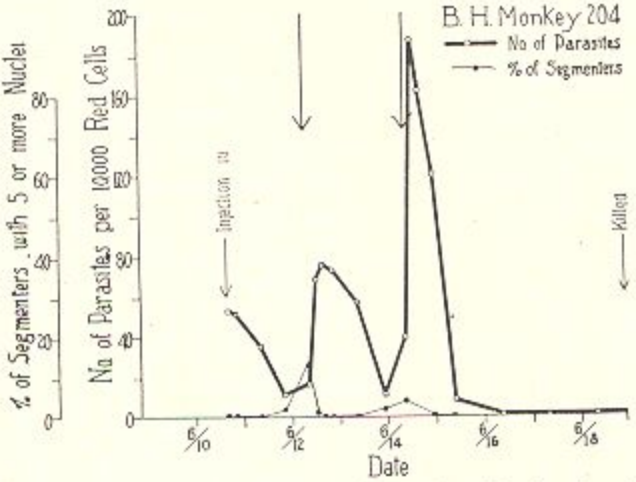

FIG. 3. The infection of *P. falciparum* in monkey 204 showing: 1, the number of parasites; and 2, the percentage of segmenters (as indicative of sporulation: see arrows). (In this monkey the parasites were too scarce to ascertain the percentage of segmenters after June 15, 9 a.m.)

Number counts of all the infections are summarized in table 3. Some, which were made at extremely frequent intervals, have been omitted for the sake of economy of space. Those not hitherto described will now be taken up.

The infection in monkey 167 was light, never exceeded 20 parasites per 10,000 red cells, and disappeared, as far as thick and thin blood films could show, in 5 days (table 3). Nevertheless, on the morning of June 3 and June 5, 6 and 2 per cent of sporulating forms were found, respectively, and corroborated the conclusion reached from the study of the other infections, i.e., that the reproductive cycle is 48 hours. Seven days after infection with *P. falciparum* and 2 days after the infection had disappeard, this monkey was injected (June 9) with the simian strain, *P. brasilianum*. The infection seemed to progress in a normal manner, but unfortunately the monkey died before some of the questions regarding sporulation time, cross-infection, etc., could be studied.

The infection in monkey 206 was remarkable in that the number

TABLE 3.

Results of the intravenous injection of large numbers of P. falciparum *into howler monkeys.*

Monkey no.	Date of: Partial splenectomy	Date of: Infection	Number of parasites per 10,000 red cells: Hours after infection 1/12	4	18	Days after infection 1	1¼	1⅔	2¼	2⅔	3⅔	4	5	5⅔	7	8
156	5–27	6–15 5P	③	0	D											
68A		5–27 N	③		①	—		—	—		—	—	—	—	—	—
199	6–8	6–10 4P	140	115	101	63K										
198	6–8	6–8 4P	157	138	104	50	9	12	D							
206	6–6	6–8 4P	380	206	41	51	101	142		D						
146		6–6 11A	47	28		①		①	—	−D						
200*		6–7 4P	95	⑧	—			—		—		—	K			
171		6–3 4P	25	8	4			58		11	915	540	17	21 K		
204	6–8	6–10 4P	53	52	35	17	11	17	74	57	39	162	8	⑧	①	① D
167†		6–2 5P	16	11	6			20	9	3		2	—	—	—	
167†		6–9 N	9	4	4			6		4 D						

0 = none seen in thin film; — = none seen in thick film; numbers encircled = number seen in thick film; D = died; K = killed.

* = This monkey received infected blood from monkey 171.

† = *P. falciparum* disappeared in 72 hours: on 6–9 infected with *P. brasilianum*.

count immediately after the intravenous injection of 8 cc. of infected blood cells (at 4 p.m. on June 8) was 380 per 10,000 red cells. It was equally remarkable for the rate at which the parasites disappeared. Thus, at 8 p.m. the same day (4 hours after infection) there were 206 parasites per 10,000 red cells, at 8 a.m. the following morning (16 hours after infection) there were 41, and at noon (20 hours after infection), when sporulation was beginning to take place, there were 24. As a result of sporulation, the parasites increased to 142 per 10,000 red cells, but further study was prevented by the death of the animal. On June 9 when sporulation occurred, there were no sporulating forms at 8 a.m., 6 per cent at noon, 5 per cent at 4 p.m. and none at 9 p.m.

The infection encountered in monkey 198 was quite similar to that just described except that fewer parasites were injected initially. It also showed one sporulation period. In fact, at 8 a.m. on June 10, 24 per cent of segmenters were found.

Monkey 199 was killed after it had been infected 24 hours, and hence, no sporulation period was encountered. One hundred and forty parasites per 10,000 red cells were found immediately after inoculation. These rapidly decreased until when it was sacrificed there were only 63 per 10,000 red cells.

So few parasites were injected into monkey 68A that immediately after infection there were only 0.3 parasite per 10,000 red cells and in 24 hours none could be found. Nor could any be found in thick film for 25 days thereafter, at which time the animal died.

A similarly small infective dose was given monkey 156, and when it died in twelve hours no parasites could be found in thin films.

The infection in monkey 146 was of such a low grade that no data could be accumulated on its sporulation time. Parasites were present in very small numbers for 2 days, but subsequently, could not be found in thick film for 2 days at which time the monkey died.

Discussion, summary and bibliography.

The discussion, summary and bibliography of this section are given at the end of section II.