Reprinted from the Journal of Infectious Diseases
July-August, 1944, Vol. 75, pp. 1-32

THE EFFECT OF IMMUNITY ON THE ASEXUAL REPRODUCTION OF *PLASMODIUM BRASILIANUM*

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This paper presents a detailed study of the modifications observed in the length of the asexual cycle and in the number of merozoites produced by segmenters during infection with *Plasmodium brasilianum*. The work was planned in view of the changes found by Boyd and Allen (1934) and Boyd (1939) in the average number of merozoites per segmenter during the course of infections with *P. cathemerium*. Such changes have to be considered in determining reproductive rates since the basic rate of asexual reproduction of the malarial parasite in the vertebrate host is dependent upon 2 factors—the length of the asexual cycle and the average number of merozoites produced by the segmenters. The present results are of importance in relation to the effects of natural and acquired immunity and the successive types of clinical attacks observed in experimental malaria of man and monkeys.

LITERATURE

As early as 1888, Golgi noted that if all the parasites, which arrived at maturity every 2 days in infections with *P. vivax* and every 3 days in infections with *P. malariae*, should complete their development, the resulting diseases would always progress until pernicious symptoms were evident, but it was not until some 35 years later that the factors which prevent this sequence began to be intensively analyzed.

In 1922, our work on differentiating the parasiticidal and reproduction-inhibiting effects of immunity against trypanosomes necessitated the development of methods of measuring rates of reproduction which were independent of the number of parasites destroyed by parasiticidal factors because net increases in parasites are only valid to express rates of reproduction if all the progeny survive. Applying these ideas to avian malaria, the junior author (1925) pointed out that the periodic synchronous asexual reproduction of plasmodia permits an accurate measure of the rate of reproduction of these parasites. Of the 2 factors necessary to compute the rate, viz., the average number of merozoites per segmenter (as determined by microscopic counts and corrected for that fraction which develop into gametocytes) and the length of the asexual cycle, only the length of the asexual cycle was used because the number of merozoites per segmenter appeared to be constant. This conclusion was based on single determinations of the number of merozoites during the acute and developed periods of the infection. These were obviously insufficient in view of the work of Boyd and Allen. Later, in our study of *P. brasilianum* (1934a) in Central American monkeys, we noted the following facts: the merozoite mean per segmenter varied between 8.5 and 10 and the 3-day periodicity was regular throughout the infection except at the time of the crisis

Received for publication, February 23, 1944.
This work was aided, in part, by a grant from the International Health Division of the Rockefeller Foundation and, in part, by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago. The authors are indebted to Dr. H. C. Clark of the Gorgas Memorial Laboratory for valuable assistance.

or decrease in parasitemia when (1) the asexual cycle sometimes took 4 or 5 days instead of 3 days, (2) the average number of merozoites per segmenter sometimes decreased, and (3) the mean size of the segmenters sometimes decreased (this last finding was undoubtedly correlated with the number of merozoites produced by each parasite). Because we believed that the variations in the merozoite mean per segmenter during the entire infection were minor except at the crisis, we concluded that the basic rate of reproduction in infection with *P. brasilianum* was constant except at the crisis and that the various aspects of the infection were the result of a differential mortality of the parasites during the entire infection on which was superimposed a temporary retardation of reproduction during some crises. The present results furnish more exact data on these points and modify the conclusions to some extent.

In 1934, Boyd and Allen studied in detail the number of merozoites per segmenter and the length of the asexual cycle during the course of infection with *P. cathemerium*. They found that periodicity and synchronism remained constant throughout the infection, but that the merozoite mean per segmenter decreased during the acute rise until the peak in numbers or the beginning of the decrease in numbers was reached and thereafter increased. They also found that the changes in the merozoite mean could be correlated with changes in the size of the adult parasite. These data were consistent with the earlier findings of Hartman (1927) that the size of the adult parasite was inversely proportional to the parasitemia and with the later findings of Wolfson (1937) that the asexual cycle remained constant throughout an infection, including the crisis, of a matinal strain of *P. praecox* (=*relictum*)—see her fig. 1. In 1939, Boyd amplified his and Allen's findings. In general, he found that the values for the merozoite mean were highest at the first or second segmentation in the infection, decreased during one to several subsequent segmentations and increased irregularly thereafter, but only occasionally reached a value as high as obtained at the first or second segmentation. He reasonably took exception to our emphasis of the fact that the rate of reproduction of a given species of plasmodium was essentially constant except at the crisis in some infections of *P. brasilianum* and that the course of the infection could be explained in terms of parasiticidal mechanisms uncomplicated by changes in the rate of reproduction. In 1934, Lourie concluded that the periodicity and the merozoite mean were unchanged in parasites injected into and reproducing in latently infected birds. Later, Boyd and Gilkerson (1942) studied in detail the merozoite mean of parasites after their injection into pairs of birds, one of which was normal and the other of which was latently infected. They found in 18 such pairs that the merozoite mean dropped from 14 in the normal to 10.8 in the latently infected birds during the first reproductive period after injection (no other period could be studied in the latently infected birds because the parasites rapidly disappeared).

The course of *P. brasilianum* in the 6 species of monkeys studied in this paper has already been reported by us (1932a, 1934a, b and c) and by W. H. Taliaferro and Cannon (1936) and W. H. Taliaferro and Klüver (1940). Briefly, *P. brasilianum*, which occurs naturally in cebus, red spider and howler monkeys, varied in these monkeys from low grade, nonfatal long continued infections, in which relapses occurred, to acute, fatal infections. In general, the infections in cebus and red and black

spider monkeys tended more often to be acute and, in howler monkeys, more often to be chronic. In marmosets and night monkeys, the infections tended to be transitory and to decline progressively. The asexual cycle was characterized by a synchronous 72-hour periodicity throughout the entire infection, except that it was less synchronous in marmosets and night monkeys and was sometimes less synchronous and delayed at the crisis or drop in parasitemia in some species and possibly in all species. The delay in segmentation after the peak in numbers was at times slight and caused no change in the over-all periodicity or at times was marked. In the latter event, varying proportions of the population took essentially 4 or 5 days to segment and thereby caused, for example, 1 brood of parasites to split into 2 or more broods of varying proportions. Segmenters contained from 4 to 16 merozoites in the cebus, spider, howler and night monkeys, and from 4 to 14 merozoites in the marmoset. The merozoite mean per segmenter varied slightly between 8.5 and 10 from strain to strain and from day to day in the same infection during either the acute rise or developed infection, but decreased markedly during some intense crises, especially in cebus and spider monkeys. At such times, some parasites, so-called crisis forms, were markedly atypical and degenerate in appearance. These conclusions are amplified in the present paper, especially with regard to the number of merozoites per segmenter.

MATERIALS AND METHODS

The results in the present paper are based on detailed studies of *P. brasilianum* in 4 *Cebus capucinus* monkeys (1 through 4) and on a reexamination of 60 similar infections from the following 6 species: 23 white throated (*C. capucinus*) monkeys, 6 red (*Ateles geoffroyi*) and 9 black (*A. dariensis*) spider monkeys, 10 howler monkeys (*Alouatta palliata*) consisting of the black (*A. p. aequatoralis*) and brown (*A. p. trabeata*) varieties, 6 marmosets (*Leontocebus geoffroyi*), and 6 night monkeys (*Aotus zonalis*). Monkeys 1 through 4 were obtained from a New York dealer. All of the others were furnished by Dr. H. C. Clark of the Gorgas Memorial Laboratory and bear the numbers used by him. The letters W, RS, BS, H, M and NM preceding the numbers represent the above 6 species in order, respectively. The monkeys were kept under ordinary laboratory conditions of light and dark and were fed a varied diet, including cod liver oil and milk.

Monkeys 1 through 4 were infected intravenously from strain 3 of *P. brasilianum* on August 30, 1939, July 14, 1939, April 15, 1941, and May 9, 1941, respectively. The infections in all the other monkeys were either naturally acquired or experimentally induced by the injection of infected blood during 1931, 1932 or 1935 as described previously by us. They represent 14 strains of *P. brasilianum*.

Thin blood films of monkeys 1 through 4 were usually made at 8 and 10 am and at 1 and 5 pm, central standard time. Some were air-dried according to the usual procedure, and others were dipped momentarily in ether, before the blood had dried on them, to facilitate counting the merozoites, as advocated by Boyd (1939). The films were left in ether until about 2 to 5 mm of the outer edge of the blood film appeared yellowish and the rest of the film appeared more or less transparent when removed from ether. Thin air-dried blood films of all the other monkeys were usually made at 4- or 6-hourly intervals from 8 am to 8 pm. All slides were stained with Giemsa.

To reconstruct the various time relationships and the occurrence of major and minor broods in the asexual cycle, we studied samples of 50 or more (usually 100) parasites on the serial blood films and separated the parasite population into the same 5 classes (1, small; 2, intermediate; 3, large uninucleated; 4, 2 to 4-nucleated; and 5, 5 or more nucleated forms and all segmenters) which we used earlier (1934a). To represent the periodicity and synchronism of the asexual cycle as simply and adequately as possible, the per-

centage of class 5 in the parasite populations was plotted in graphs 3 through 4. This class was selected instead of any or all of the others because it reaches a peak just a few hours before the peak in segmentation occurs, and, hence, not only indicates the synchronism of the asexual cycle but the approximate time of segmentation. The resultant curve does not clearly indicate the occurrence of minor broods found by studying the proportion of the above mentioned 5 classes in the population, especially if such broods form only a small proportion of the population. Their occurrence, however, will be pointed out in the text. Parenthetically, it may be mentioned that these procedures, as advocated by us (1934a; cf. also Mulligan, 1935, and Wolfson, 1936a and b), seemed to us better suited to represent the asexual cycle of *P. brasilianum* graphically than other previously devised methods (cf. Stephens and Christophers, 1908, and L. G. Taliaferro, 1925; and Mathis and Leger, 1911, Boyd, 1929a and b and Lourie, 1934), but would have to be modified to study other species. For example, the separation of the parasites into 5 classes and the intervals of 8 and 10 am and 1 and 5 pm, at which blood films were made, are adequate to study a species, such as *P. brasilianum*, which segments into 4 to 16 segmenters every 72 hours except at some crises, but other time intervals and/or other classes would have to be devised to study species with a shorter periodicity, with little synchronism or with fewer or more merozoites per segmenter.

For the daily determinations of the merozoite means per segmenter from monkeys 1 through 4, about half were found on the 8 am blood film and the other half on the 10 am blood film. Samples numbering from 50 to 748, as is noted in the graphs, were studied. The small samples were necessitated by the scarcity of parasites. For such examinations, the film was uniformly spread with oil and the parasites located at a magnification of ×250 and examined at a magnification of ×1250. The large samples were studied for purposes of orientation and various comparisons. Samples of from 200 to 350 were considered adequate. For the daily determinations of the merozoite means per segmenter from the other 60 monkeys, samples of from 25 to 50 were usually made. The standard error was calculated for all merozoite means. The number of merozoites was only recorded from segmenters in which the cytoplasm exhibited compact nuclei and complete cleavage between some of the daughter merozoites. Ether-prepared blood films were used to collect these data (pl. 1) from monkeys 1 through 4 since on such films the component merozoites were more clearly distinguished and more accurately counted. Ordinary thin blood films were used to collect data from the other monkeys.

In using the merozoite means to compute one factor in the rate of asexual reproduction, we did not attempt to correct for that portion of the merozoites which develop into gametocytes. As a rule, such a correction was unnecessary because gametocytes were so scarce.

The important parasitological periods of the initial infection or of any subsequent relapses of *P. brasilianum* in monkeys, as determined by the number of parasites per 10,000 red cells on the stained blood films and as reported by us previously (1932a, 1934a, etc.), are designated in this paper successively as acute rise, crisis or parasite decline, developed infection and latent infection. The step-like increases in number of parasites (acute rise) to a peak in numbers and the subsequent decreases continuing into the developed infection until parasites may not be found by microscopic examination (latency) vary in intensity to such an extent that there is no typical aspect to the number curve (note the difference in the character of the heavy solid line in graphs 1 through 4). The drop in numbers which terminates the acute rises, whether initial or during relapses, assumes singular importance in the present paper. It marks the development of acquired immunity to a degree which arrests the increasing parasitemia. In some acute infections, the resulting drop is sudden and marked and is appropriately termed a crisis. In others, it is gradual. In still others, the immunity of the host keeps the entire patent period low grade with the result that the increase in parasitemia is slight, the point at which the parasitemia begins to decrease is difficult to determine and neither the rate nor amount of decrease is great. In past papers we have used "crisis" for all these decreases because they all seemed to be associated with the same immunological mechanism and we were unwilling to risk the immunological implication of such a term as "lysis" for the more gradual decreases. The use of the term "crisis" in this sense, however, has resulted in some misunderstanding. In the present paper, therefore, we have used the term, crisis, for precipitous decreases in parasitemia, and "parasite decline," for gradual decreases in parasitemia. These shade one into the other. Thus, the crisis or parasite decline is initiated on the third day (occasionally on the day of the peak or on the first or second day) after the highest parasite count of either an initial infection or relapse and lasts 3 to 6 days depending upon the behavior of the parasites with respect to their periodicity, merozoite mean and morphology.

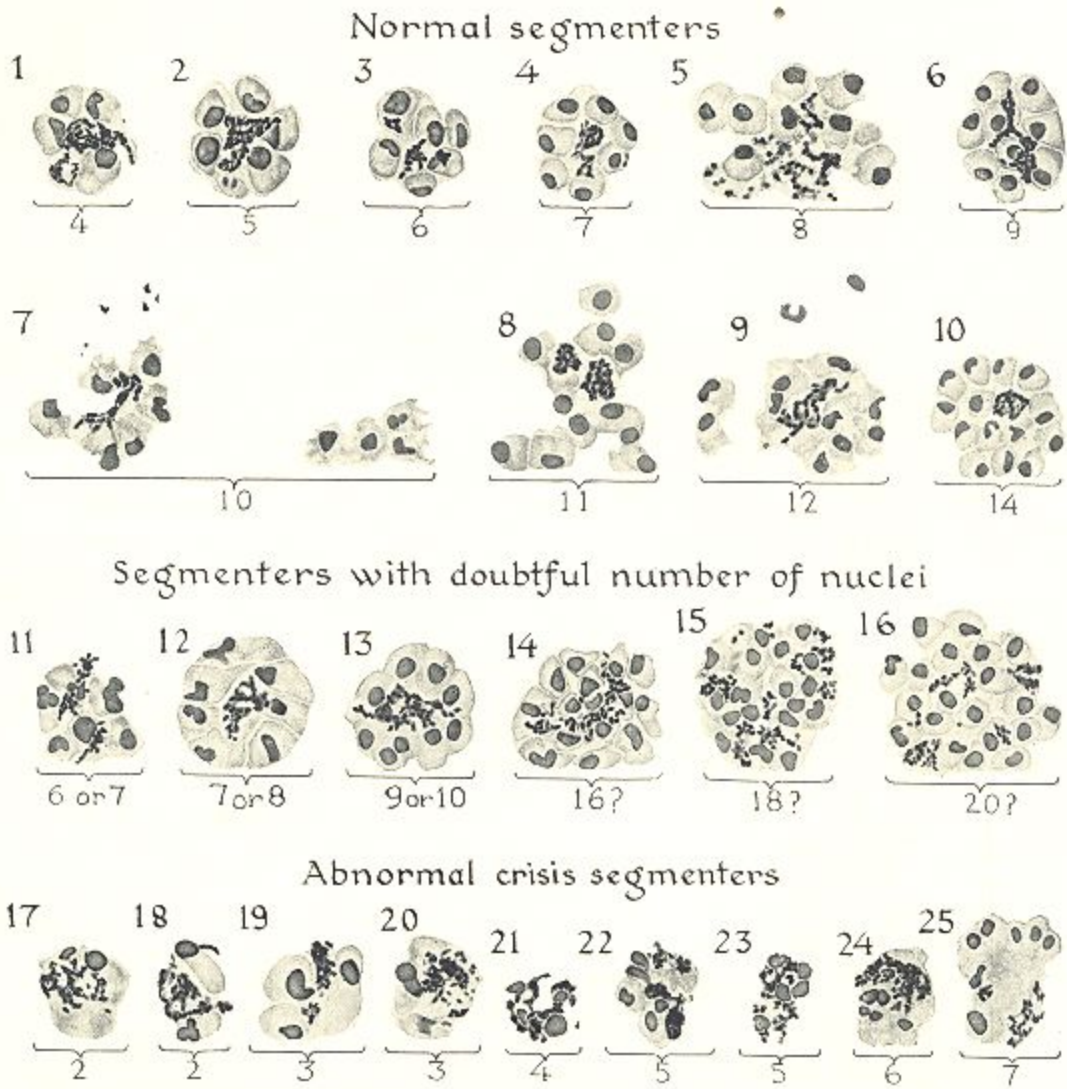


PLATE 1. Segmenters encountered in infections of *P. brasilianum* in cebus monkeys. From blood films dipped in ether and stained with Giemsa. ×2500.

1–10. Segmenters with from 4 to 14 merozoites found during the acute rise or developed infection.

11–13. Segmenters with a doubtful number of merozoites found during the acute rise or developed infection.

14–16. Probably two segmenters in a single red blood cell.

17–25. Crisis segmenters with from 2 to 7 merozoites showing morphological degenerative changes. These were found only around the time of the crisis.

EXPERIMENTAL RESULTS

The number of merozoites in each segmenter was ascertained as accurately as possible, but the following difficulties were encountered and dealt with as follows: (1) The nuclei of some merozoites were often U- or V-shaped or bi- or trilobed (cf. some of the nuclei in pl. 1, fig. 1, 9 and 10). These could usually be satisfactorily analyzed, but occasionally it was impossible to decide whether a particular nuclear arrangement in the segmenter under observation was due to one nucleus or the par-

tial juxtaposition of one nucleus over another (pl. 1, fig. 11). (2) Often, as the cell ruptured in making the smear or in submerging in ether, some of the contained merozoites, were widely scattered. At times, they might not even be seen in the same oil immersion field. Figure 7 in plate 1 was such a segmenter whose 2 components were drawn closer together in the plate to conserve space. This form, when encountered, might be considered at first glance to be 2 segmenters, one containing 6 and the other 4 merozoites. It was recorded as a segmenter with 10 nuclei because in our experience no segmenter is ever entirely devoid of pigment granules as the component containing the 4 merozoites was. (3) As some segmenters were broken and scattered in being spread over the slide, varying numbers up to and including all of the merozoites were often so ruptured that their cytoplasm had completely disappeared and their presence was only indicated by blobs of reddish staining material. Care had to be taken in counting the merozoites of such segmenters since some of the merozoites might be in perfect condition while others might be disintegrated beyond the point of recognition. Occasionally, platelets were closely associated with such distorted material, but were generally easily differentiated. (4) The pigment was often so dense that it could conceivably conceal 1, or more rarely 2 or 3, merozoites (pl. 1, fig. 12). Thus, a segmenter containing 10 merozoites might erroneously be considered to contain 7, 8 or 9 merozoites. At other times the pigment might form a ring, through which a reddish staining nucleus of a merozoite could be faintly seen. The merozoite in the center of figures 6 and 13 in plate 1, for example, might easily be missed in a casual examination. The merozoite number of some forms was not recorded when the number of merozoites was too uncertain. (5) A large, diffuse, lightly staining purplish red blob was a confusing constituent of some segmenters. It was not considered to be one of the merozoites, but its nature was not ascertained. (6) No segmenter was ever found with more than 16 merozoites and undoubted segmenters with 16 merozoites were extremely rare. Doubly infected red cells with two 8-nucleated segmenters, one 8- and one 10-nucleated segmenter, or two 10-nucleated segmenters were, however, occasionally found (pl. 1, fig. 14, 15 and 16), especially when parasites were numerous. (7) Some segmenters which were found around the time of the crisis, so-called crisis forms, were often difficult to classify on account of unequally divided, clumped together or otherwise atypical appearing merozoites (pl. 1, fig. 17–25). In fact, some of these had to be omitted because the nuclear masses were impossible to separate into recognizable nuclei. In many such forms, the cytoplasm did not stain.

The procedure of collecting half the sample of segmenters from the 8 am blood film and the other half from the 10 am blood film to obtain the merozoite means from monkeys 1 through 4 was adopted because a slight decrease of from 0.1 to 0.5 merozoite per segmenter was often found at the 10 am reading. In other words, there was a slight tendency for segmenters with a larger number of merozoites to segment sooner. In this connection, it should be emphasized that the process of segmentation did not take place with mathematical precision. The peak in segmentation might vary by an hour or so, but such irregularities did not affect the over-all occurrence of the process, every third day during the morning hours of the entire infection, except during some crises.

The number of merozoites was re-

corded separately for each segmenter, but these data are presented for conciseness in graphs 1 through 4 as a percentage frequency of the 4 following groups: segmenters (1) with 6 or fewer merozoites, (2) with 7 or 8 merozoites, (3) with 9 or 10 merozoites, and (4) with 11 or more merozoites. During the acute rise and developed infection, segmenters contained from 4 to 14 (rarely 15 or 16) merozoites (pl. 1, fig. 1–10) with the large majority containing 8 or 10 merozoites and about 8% to 15% containing an odd number of merozoites (pl. 1, fig. 2, 4, 6 and 8). During an intense crisis, as found in the cebus and spider monkeys, segmenters contained from 2 to 10 merozoites (pl. 1, fig. 17–25) and occasionally 20% to 35% of them contained odd numbers of merozoites. Often the segmenters with an odd number of merozoites contained 1 merozoite with a noticeably larger nucleus—a fact indicating that the latter may have failed to divide.

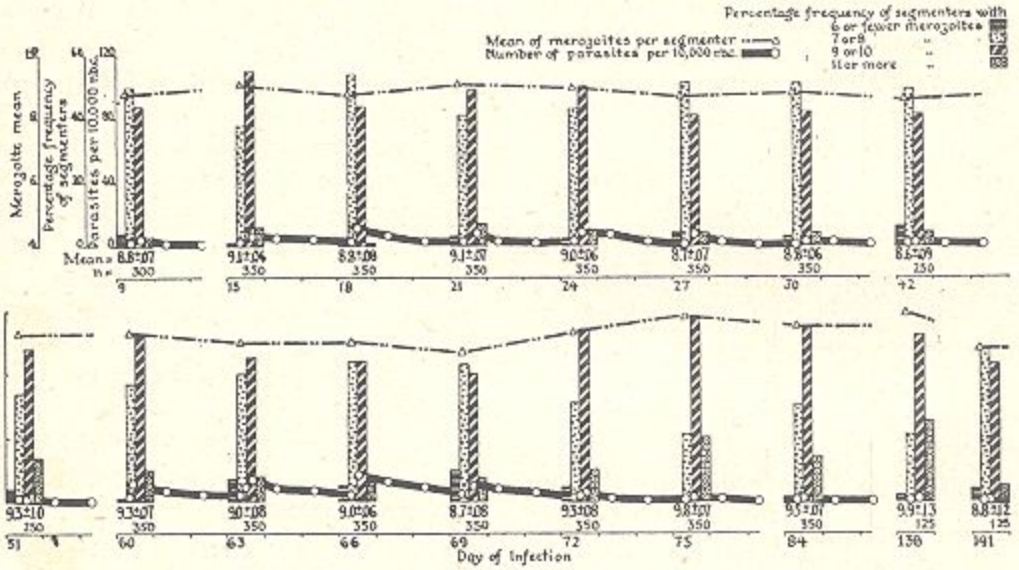
In indicating the significance of differences between individual daily merozoite means or between a daily mean and the mean for a part or all of an infection, we have used the usual formula for the standard error of the difference. In a number of cases, the differences among the daily merozoite means were tested for significance by Fisher's analysis of variance as given by Snedecor (1938). Little additional information was obtained by the latter method because the number of segmenters on which the daily merozoite means were calculated was generally large.

Data from 4 cebus monkeys are given in detail in graphs 1 to 4 because the infections in these animals illustrate the different types of conditions encountered. Thus, the infection in monkey 1 was low grade without a sharp initial acute rise or crisis. There was also a lack of noticeable effects on the periodicity or merozoite mean of the segmenters. The infection in monkey 2 was characterized by a moderate parasitemia, the initial rise of which was terminated by a more or less gradual parasite decline. As in the case of monkey 1, there was no pronounced effect on either the synchronism of reproduction or the number of merozoites per segmenter. The infection in monkey 3 was characterized by an initial rise which reached a high peak for this infection and was terminated by a sharp crisis. In addition, there was a marked effect at the crisis on the rate of reproduction, as evidenced both by a derangement of the asexual cycle and a drop in the average number of merozoites per segmenter. In monkey 4 the initial rise did not reach as high a parasitemia as in monkey 3, but there was a slight derangement of the asexual cycle and a moderate decrease in the number of merozoites per segmenter at the time of the crisis.

Cebus monkey 1 (graph 1).—The infection in monkey 1, as indicated by the number of parasites per 10,000 red cells (heavy solid line in graph 1), consisted of a low grade, fairly constant parasitemia in which there were no marked acute rises or falls. The parasitemia reached a peak of only 11 parasites per 10,000 red cells on the 18th day, dropped to about 3 per 10,000 red cells on the 21st day and continued as a low grade infection until there was a second peak of 17 parasites per 10,000 red cells on the 66th day. The second peak in parasitemia was followed by a decline in numbers on the 69th day. No parasites were found from the 86th to the 137th day, but a low grade relapse occurred on the 138th day. The infection in this monkey illustrates the long-continued low grade infection often encountered in *C. capucinus*, in which the net gains at each segmentation are essentially neutralized by the interseg-

mentation death of parasites and no appreciable peak in numbers is reached. Whether the death of the parasites was the result of a natural immunity in an unimmunized animal or whether in addition there was a superimposed acquired immunity resulting from past infection could not be definitely decided. It was, however, probably the result of natural immunity inasmuch as no parasite was ever found in thick blood films of the monkey prior to its experimental infection. Furthermore, we have observed this type of infection in cebus monkeys either raised in the laboratory (cf. W46 in W. H. and L. G. Taliaferro, 1934c) or after having been found negative for malaria by splenic biopsies (cf. W457 and W470 in table 5 of W. H. Taliaferro and Cannon, 1936).

Segmentation took place regularly during the morning hours of every third day through the entire infection, but the peak of individual segmentations varied from one to several hours. The over-all regularity of the asexual cycle is indicated in graph 1 by the regularity with which the merozoite means per segmenter occurred every 3 days. Even after the long latent period between the 86th and 138th day of the infection, the parasites reappeared and segmented on the day on which it could be calculated they would have segmented provided segmentation had continued regularly.



Graph 1.—Merozoite means per segmenter and the percentage frequency of segmenters containing different numbers of merozoites (both of which indicate the number of progeny formed in the asexual cycle) and the number of parasites in a long continued, low grade infection of *P. brasilianum* in cebus monkey 1. n = the number of segmenters in the sample studied to obtain the corresponding merozoite mean. (The asexual cycle occurred regularly every 3 days.)

Note that asexual reproduction varied less than 15% during the entire infection as is shown by the slight changes in the number of progeny formed and the regularity of the asexual cycle.

In the absence of acute rises and crises, we have compared the variations in the merozoite mean during different parts of the infection with special reference to the periods when the parasitemia decreased. The declines in parasitemia mark the development of immunity analagous to the crisis in the more acute infections. The merozoite

means of the 2 periods of rising parasitemia between the 9th and 18th days and the 60th and 66th days were fairly constant. Thus, the merozoite means were $8.9 \pm .05$ and $9.1 \pm .05$, respectively, for the 2 periods with a greatest variation from the mean in each period of only 2% ($0.2 \pm .07$ merozoites), whereas, the highest and lowest merozoite mean during each of the periods differed by 3% ($0.3 \pm .09$). Similarly, the merozoite means of the 2 segmentations during the decline in parasitemia on the 21st and 69th days did not differ significantly from the rest of the infection. Thus, the composite merozoite mean per segmenter during the entire infection exclusive of the 2 periods of decline was $9.1 \pm .02$ merozoites with a greatest difference from it at the two declines of $0 \pm .07$ and $0.4 \pm .09$ merozoites. In spite of the fact that the larger of these two differences is probably statistically significant, the magnitude expressed as a percentage change in the rate of reproduction is probably of no great biological significance.

Although the changes were small during some sections of the infection and during the parasite declines, there were unquestionably significant increases in the rate during the low grade infection which followed the decline on the 69th day and which probably corresponded to the developed infection of more acute infections. Thus, the merozoite mean of all readings through the 66th day was $8.9 \pm .02$ whereas the composite mean of the 72nd and 75th days was $9.5 \pm .05$ —a difference of $0.6 \pm .06$ or approximately an increase of 6%.

In as much as segmentation was regular throughout this infection, the above percentage differences in the merozoite means represent actual average and maximal differences in the rate of reproduction.

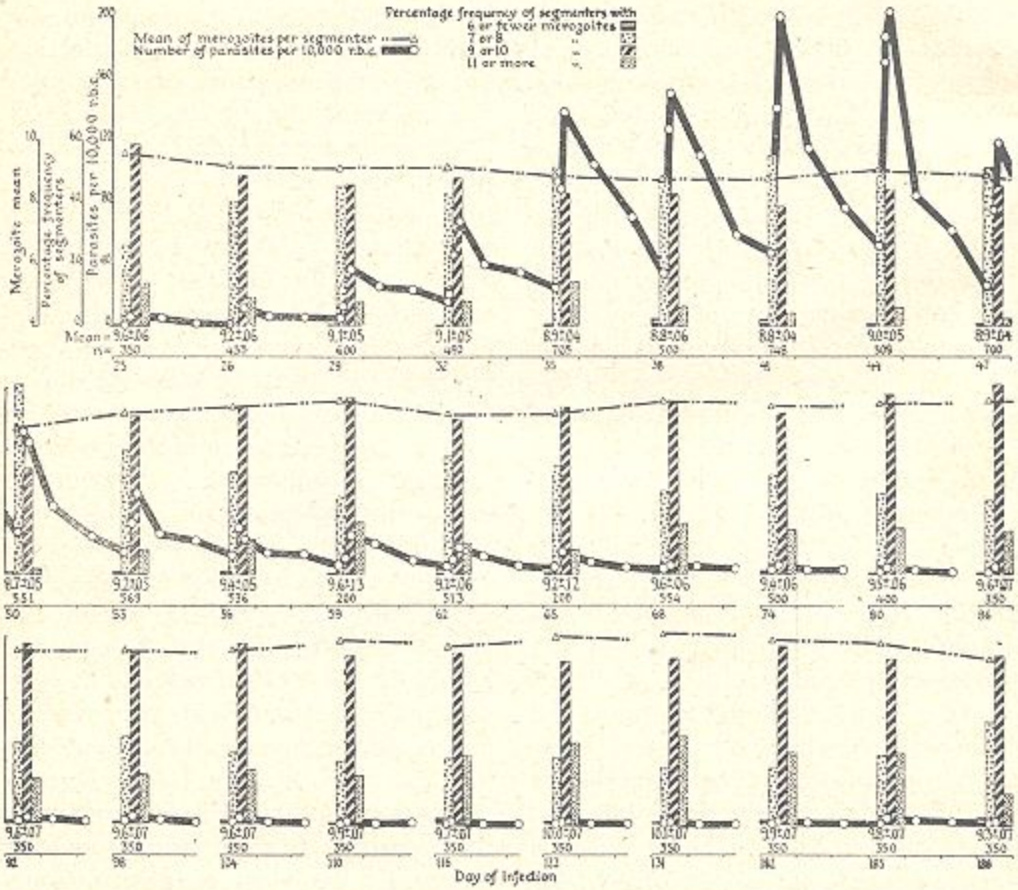
Whenever the merozoite mean was less than 9, segmenters with 7 or 8 merozoites predominated (see the bars in graph 1), but at no time did segmenters with 6 or fewer merozoites predominate. No crisis forms were ever found.

An occasional segmenter, representing a minor brood, was found on the 16th, 19th, etc. days of the infection, but the number of parasites in the brood was too scanty to study statistically.

Cebus monkey 2 (graph 2).—The infection in monkey 2 was also long contined with no sharp crisis, but it differed from that in monkey 1 by reaching a higher level in number of parasites (heavy solid line in graph 2). The steplike number curve, as has been explained in previous papers, was due to a succession of increases associated with periodic segmentation and a more or less constant disappearance of parasites during intersegmentation periods.

The length of the asexual cycle was constant in this infection and the average number of merozoites per segmenter at each segmentation varied essentially within the same range as found in monkey 1. The differences in the merozoite means per segmenter throughout this infection can be seen by noting the changes in the dotted line representing the merozoite mean in graph 2 and the percentage frequency of merozoites per segmenter at each segmentation day. Such differences could be variously considered, but are considered in this and all subsequent infections in terms of maximal and average differences during the various parasitological periods of the infection. Thus, on the one hand, the maximal difference, calculated from the highest and lowest merozoite means, was 8% for the acute rise, was 9% for the acute rise and parasite decline, and was 14% for the whole infection, consisting of acute rise, crisis and developed infection. On the other hand, the aver-

age difference during the acute rise was 6%, as calculated from the greatest variant (9.6±.06) and the composite merozoite mean during the entire acute rise (9.1±.02); the average difference between the parasite decline and acute rise was 3%, as calculated from the composite merozoite mean during the parasite decline (47th and 50th day =8.8±.04) and from the composite merozoite mean during the acute rise (9.1±.02); and the average difference during the developed infection was 5%, as calculated from the greatest variant (10.1±.07) and the composite merozoite mean during the entire developed infection (9.6±.02). During a slight 9-day relapse beginning on the 182nd day of the infection, the merozoite mean decreased 6%, but did not decrease to as low an absolute value as during the parasite decline of the initial infection. Since the asexual cycle was constant, the foregoing percentages represent actual maximal and average differences in the rate of reproduction of the parasite during



Graph 2.—Merozoite means per segmenter and the percentage frequency of segmenters containing different numbers of merozoites (both of which indicate the number of progeny formed in the asexual cycle) and the number of parasites in a long continued infection of *P. brasilianum* in cebus monkey 2. n = number as in graph 1. (The asexual cycle occurred regularly every 3 days.)

Note that asexual reproduction varied less than 15% during the entire infection as is shown by the slight changes in the number of progeny formed and the regularity of the asexual cycle.

the various parasitological stages of this infection. The changes in the merozoite mean were also reflected in the proportion of segmenters with different numbers of merozoites. Thus, segmenters with 7 or 8 merozoites predominated between the 35th through the 50th day of the infection as the infection was approaching and undergoing a decline, whereas segmenters with 9 or 10 merozoites predominated during the rest of the infection (see bars in graph 2). At no time throughout the infection did segmenters with 6 or fewer merozoites form more than 6% of the population and crisis forms were not found.

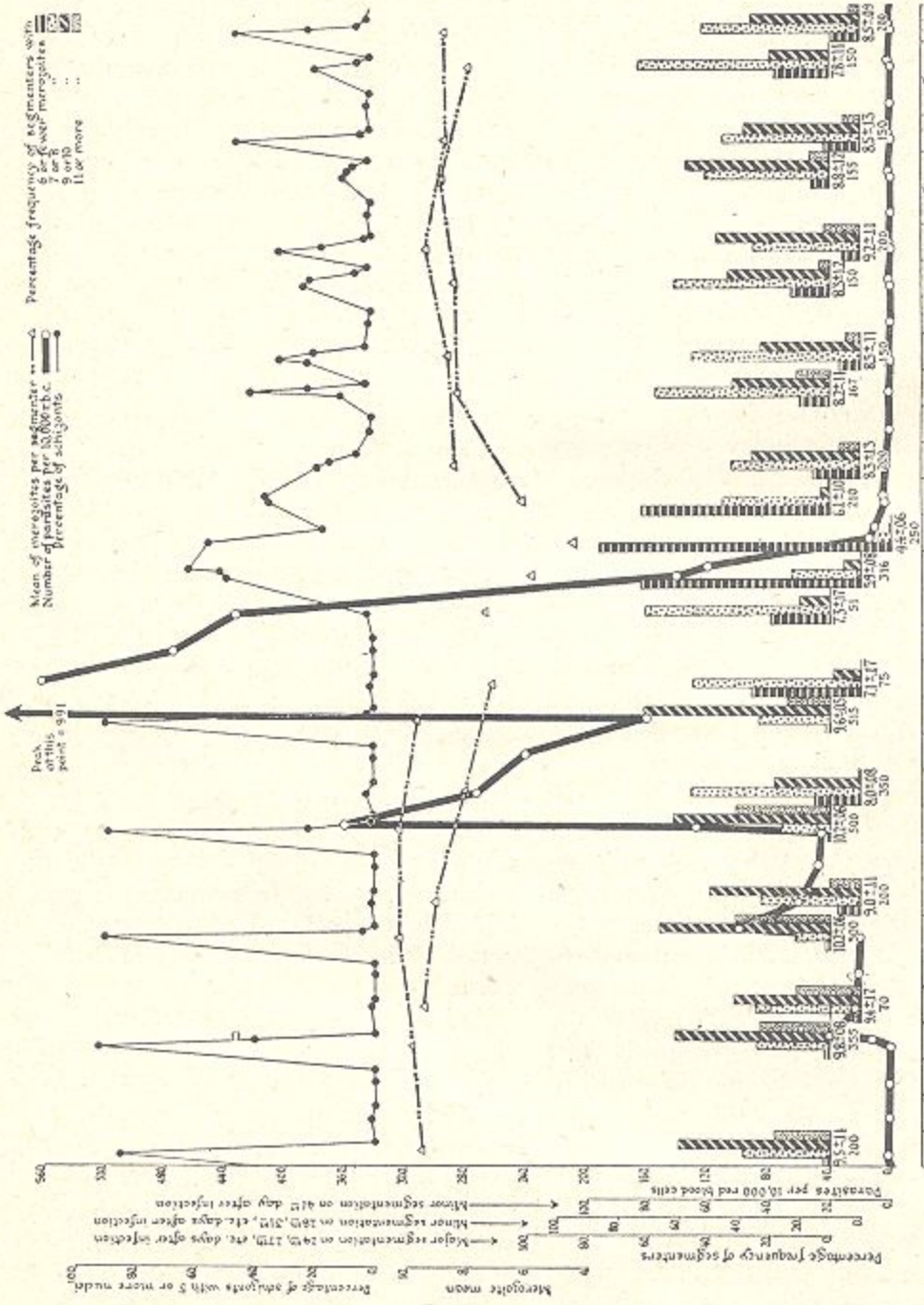
In addition to the brood of parasites which segmented on the 23rd, 26th, etc., days of the infection in this monkey, 2 additional broods of parasites were found, one of which segmented on the 24th, 27th, etc., days of the infection and the other of which segmented on the 25th, 28th, etc., days of the infection. The constituents of these broods were too scarce to be studied statistically.

Cebus monkey 3 (graph 3).—The infection in monkey 3 was acute, reached an unusually high degree of parasitemia for *P. brasilianum* and then suddenly decreased at a sharp crisis. It progressed by typical step-like stages during the acute rise of the infection until approximately 10% of the peripheral red cells were infected on the 36th day of the infection. During the subsequent days of the crisis, the infection decreased in numbers until only 0.04% of the red cells were infected on the 42nd day of the infection, and, thereafter, continued at about the same level (heavy solid line in graph 3).

A casual inspection of the blood films, as indicated by the percentage of schizonts with 5 or more nuclei in the top section of graph 3, during the acute rise of the infection gave the impression that there was only a single brood of parasites with a highly synchronous quartan periodicity that segmented on the 24th, 27th, etc., days after infection. Detailed study of the slides, however, revealed the presence of a second brood which formed less than 1% of the total population and segmented the day after the main brood, i.e., on the 28th, 31st, etc., days after infection. The parasites of both of these broods were studied to determine the number of merozoites per segmenter.

Of the 2 factors involved in asexual reproduction, the asexual cycle of both the major and minor broods of parasites was essentially constant in length during the acute rise of the infection. The most striking finding was that the merozoite means of the minor brood were consistently lower than those of the major brood, although the nearest approach to equality on the 27th (9.8 $\pm$.08) and 28th (9.4$\pm$.17) days of the infection gave a difference of only 0.4 $\pm$.18 merozoites, which was probably not significant. On the other hand, the greatest discrepancy on the 33rd (10.2 $\pm$.06) and 34th (8.0$\pm$.08) days of the infection gave a difference of 22% which probably represented in part an actual difference between the major brood and minor brood and in part a beginning action of the immune phenomena of the crisis.

At the time of the crisis (37th through the 43rd day) there were marked derangements in the length of the asexual cycle, the synchronism of asexual development, the average number of progeny produced by the segmenters and the cytology of the parasites. For example, there were many immature forms at 9 am on the 37th day, and the major brood did not segment as was to be expected during the morning hours of the 39th day. In fact, the population at this time contained such a small proportion of schizonts with 5 or more nu-



Graph 3 (See facing page for legend)

clei that only 51 were found during an exhaustive search of 12 ether-prepared smears. The majority of the parasites were immature 2- or 4-nucleated schizonts. Most of this brood segmented during the 40th day, but a few did not segment until the 41st day of the infection. Segmentation, therefore, instead of occurring during the morning hours of the 39th day, occurred continuously from 8 am on the 39th day through 8 pm on the 41st day of the infection. Segmentation of the minor brood was also delayed and prolonged. These conclusions were reached by studying the various forms present on the slides throughout this period, but only the fact that segmentation was taking place in an irregular way is indicated by the percentage of schizonts with 5 or more nuclei in graph 3.

The average number of merozoites formed by the segmenters were markedly decreased from the 37th day through the 42nd day. In fact, it was only at this period in the infection that segmenters with less than 4 merozoites were ever found (pl. 1, fig. 17–20). On the 40th, 41st and 42nd day, segmenters with 6 or fewer merozoites predominated (see bars in graph 3) for the first time in any infection so far described, and on the 41st day of the infection, the merozoite mean of $4.4 \pm .06$ was the lowest ever found. This merozoite mean represented a difference of $5.8 \pm .07$ merozoites or a 57% decrease from the highest merozoite mean ($10.2 \pm .05$) during the acute rise and the merozoite mean ($6.2 \pm .06$) of all segmenters during the crisis represented a 31% decrease from the composite merozoite mean of the entire acute rise. Because the merozoite means during this period were associated with a disruption of the cycle and were derived from members of both broods, they are not connected with the preceding and following ones in graph 3.

Not only was the merozoite mean per segmenter lower during the crisis than during the acute rise of the infection, but many of the segmenters were crisis forms. The variations from the normal were as follows: Many of the segmenters were small (pl. 1, fig. 17, 21–24) and none contained more than 10 merozoites. Twenty to 35% of the segmenters sometimes had an odd number of merozoites. The nuclei of the individual merozoites of a given segmenter were often small (cf. fig. 21–25 with fig. 1–4 in pl. 1) or unequal in size (pl. 1, fig. 21, 24 and 25). The cytoplasm of the individual merozoites was sometimes lacking or, at least, did not stain (pl. 1, fig. 21 and 23). Frequently it stained irregularly (pl. 1, fig. 22 and 25). These cytological findings indicated that many of the parasites were injured and arrested in their growth and development. In fact, every gradation of injury appeared to be sustained. A few parasites segmented uninterruptedly, the majority segmented from several hours up to 2 days late, whereas still others were ap-

Graph 3.—Percentage of segmenters with 5 or more nuclei (which indicates the reproductive interval in the asexual cycle); the merozoite means per segmenter and the percentage frequency of segmenters containing different numbers of merozoites (both of which indicate the number of progeny formed in the asexual cycle); and the number of parasites in an acute intense infection of *P. brasilianum* in cebus monkey 3.

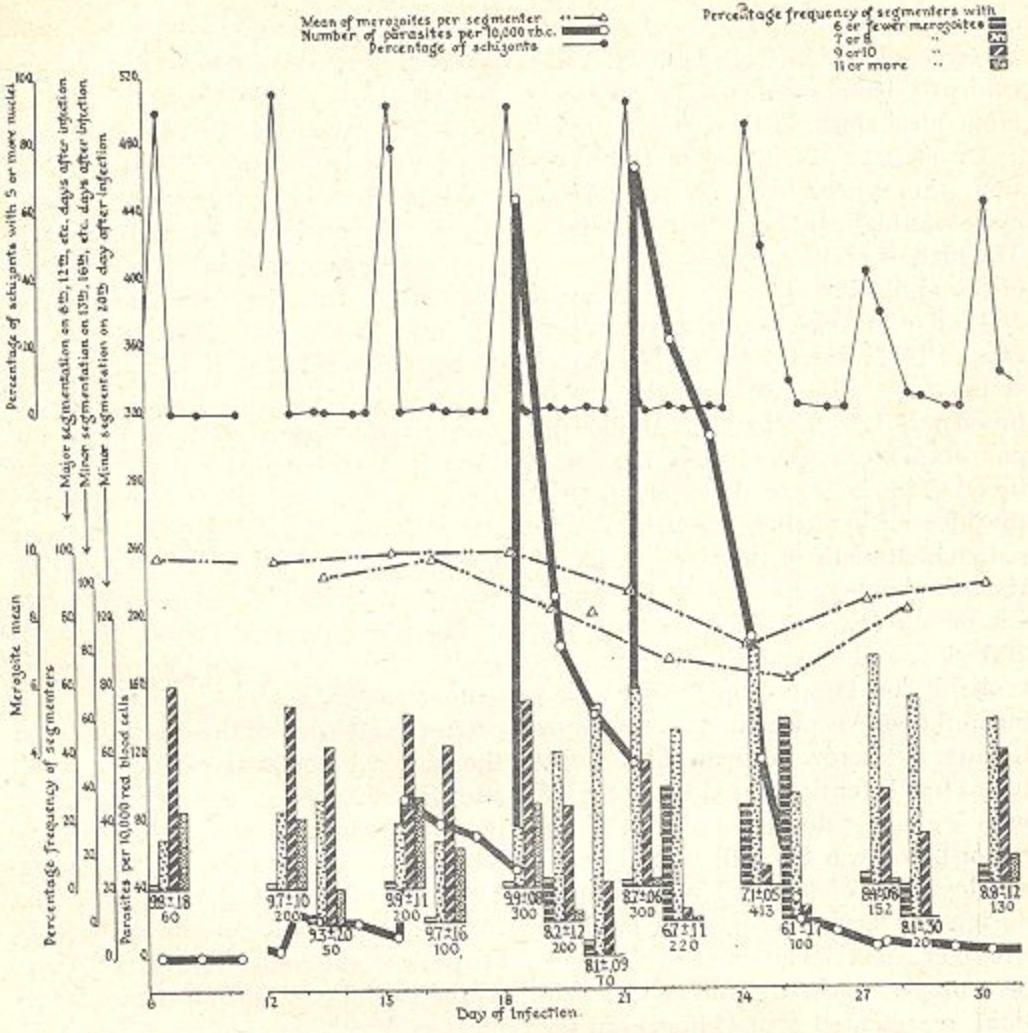
Note that asexual reproduction varied about 15% during the acute rise of the infection, as is shown by the regularity of the asexual cycle and changes in the number of progeny formed, but markedly decreased during the crisis (37th–43rd day) as shown by a lengthening and irregularity in the asexual cycle and marked decreases in the number of progeny formed.

parently unable to complete segmentation and lingered on until they were removed by phagocytosis.

Crisis segmenters were first found on the 36th day of the infection. At that time and through the 39th day of the infection, 1% of the segmenters were crisis forms, but on the 40th and 41st days of the infection, about one-third of the segmenters were markedly abnormal, and thereafter gradually decreased until on the 48th day of the infection, none was found. Segmenters were not the only forms which were abnormal. Essentially similar proportions of all other stages, including gametocytes, were also abnormal throughout the same time intervals. The abnormality, however, was progressively more noticeable as the parasites grew, and since the segmenters seemed unable to rupture the red cell, they lingered on in the blood cells from 24 to 48 hours and became more and more atypical (pl. 1, fig. 21 and 23).

There is no question that the rate of reproduction of some segmenters was markedly inhibited during the crisis, but the degree of this retardation in the population as a whole could not be exactly determined. Certain figures are, however, interesting. (1) For example, the segmenters on the 41st day of the infection had the lowest merozoite mean ($4.4 \pm .06$) encountered. If, as seems probable, these schizonts took 5 days to develop, their rate of reproduction might be expressed as 4.4/5 or an average increase of 0.88 per day. Contrasted with the average increase of $9.5/3 = 3.16$ for the broods segmenting prior to the crisis, the rate of reproduction of some segmenters decreased 72%. (2) Or if we assume that all the segmenters during the crisis had a merozoite mean of 6.5, as indicated by the composite merozoite mean for the entire crisis, and took, on the average, 4 days to develop, their rate of reproduction would give an increase of 1.6 per day as compared with the average increase of 3.16 for all broods prior to the crisis, i.e., a 49% decrease in the daily rate of reproduction during the entire crisis. (3) Or the surviving segmenters segmented within the regular 3-day interval. Under this condition and assuming the merozoite mean of 6.5 to be approximately valid, the rate of reproduction decreased 31%. (4) Or the segmenters which segmented regularly may have been more viable than those that were delayed and the longer the segmenters took to segment, the less vitality—and hence the less chance to survive—they may have had. In other words, almost all of the progeny after the crisis may have come from segmenters which only took 3 days to segment and negligible parts may have come from segmenters taking from 4 through 5 days to segment. Under these conditions it might be more valid to assume that the majority of the segmenters on the 41st day did not survive and, therefore, that the merozoite mean of 4.4 should be omitted in calculating the composite merozoite mean of the crisis. Thus, assuming a regular periodicity and a merozoite mean for viable parasites of 6.94, the rate of reproduction of viable parasites decreased only 27%.

Beginning on the 45th day of the infection, segmentation again occurred regularly during the morning hours and the merozoite means were higher than during the crisis but lower than before the crisis. The remarkable finding was that two major broods (cf. percentages of schizonts with 5 or more nuclei in graph 3) and one minor brood (too scarce to obtain valid data) occurred at this time instead of the one major and two minor broods. This realignment was definitely brought about by the disruption of the asexual cycle at the crisis,



Graph 4.—Percentage of segmenters with 5 or more nuclei (which indicates the reproductive interval in the asexual cycle); the merozoite means per segmenter and the percentage frequency of segmenters containing different numbers of merozoites (both of which indicate the number of progeny formed in the asexual cycle); and the number of parasites in an acute intense infection of *P. brasilianum* in cebus monkey 4.

Note that asexual reproduction varied about 15% during the acute rise of the infection, as is shown by the regularity of the asexual cycle and changes in the number of progeny formed, but markedly decreased during the crisis (22nd, possibly 19th–25th day) as shown by a slight irregularity in the asexual cycle and marked decreases in the number of progeny formed.

and each of the broods after the crisis probably contained varying proportions of all of the broods existing before the crisis.

Cebus monkey 4 (graph 4).—The infection in monkey 4 reached a parasitemia of about half the severity of that in monkey 3 (heavy solid line in graph 4). During the acute rise of the infection, a major and a minor brood of parasites segmented. A third brood was also present, but only occurred in sufficient numbers to be studied on one day, i.e., on the 20th day of the infection. Although parasites were more numerous on the 23rd day than on the 20th day

of the infection, the progeny of the third brood was less numerous because it was evidently being acted upon by the immune mechanism of the crisis.

Through the 20th day of the infection, the synchronism of the asexual cycle was high and periodicity constant. The highest and lowest merozoite mean of the major brood gave a difference of 2% and of the minor brood gave a difference of 16%, whereas the highest merozoite mean (9.9±.08) of the major brood was 18% higher than the lowest merozoite mean (8.1±.09) of the minor brood. The last two differences, as in the infection in monkey 4, were probably not characteristic of the acute period of the infection because they were dependent on the low merozoite mean of the 20th day, and the merozoite mean was probably low because the crisis was being initiated. Average differences were as follows: the merozoite mean of the major brood for the entire period was 9.8±.05 with a greatest deviation of 1%, of the minor brood was 8.8±.08 with a greatest deviation of 9% and of all broods during the acute period was 9.3±.04 with a greatest deviation of 13%. These percentages, as in the foregoing infections, represented actual changes in the rate of reproduction because the 3-day asexual cycle was regular.

The crisis in this infection (21st through 25th day) was not as intense as in monkey 3. It may have been initiated on the 19th day of the infection, as indicated by the marked decrease in the merozoite mean, but in any case was operative on the day of the peak in numbers. Thus, on the 21st day, the peak was not much higher than the number count 3 days previously, an appreciable delay in segmentation was noted in some parasites, the merozoite mean was decidedly lower than previously and 15% of the segmenters were crisis forms. On the 22nd and 24th day, parasites were again delayed in segmenting, the merozoite mean markedly decreased and segmenters with 6 or fewer merozoites markedly increased. On the 25th day, the merozoite mean reached the low value of 6.1±.17 and the majority of the segmenters had 6 or fewer merozoites. Knowing that segmentation took 3 days during the acute rise and approximately 3½ days during the crisis, the lowest merozoite mean of any day during the crisis (6.1/3.5) represented a possible maximal decrease of 47% in the rate of reproduction from the highest merozoite mean during the acute rise (9.9/3), and the composite merozoite mean of the entire crisis (7.3/3.5) represented an average decrease in the rate of reproduction of 32% from the composite merozoite mean of the entire acute rise (9.3/3).

On the 21st day of the infection when the major brood was segmenting, all segmenters appeared normal during the morning hours, but on the following day 8% of the segmenters were crisis forms. These were forms which had lingered on from the previous day and were not segmenters of the minor brood. Similarly, on the 24th day of the infection, all segmenters were normal at first, but <1% of crisis forms appeared at 1:30 pm, proportionately increased the rest of the day (since the normal segmenters were completing their development and breaking up) and lingered on through the following day. Such forms had disappeared by the 26th day of the infection. During the following two segmentations on the 27th and 28th days of the infection, an occasional crisis form was found, but none was found on the 30th day of the infection. Some crisis segmenters appeared to be incapable of breaking the red cell and, within the old cell, were incapable of continuing their development and, hence, degenerated.

During the developed infection, as represented by the period from the 27th day through the 30th day of the infection, a delay of several hours in the segmentation of some of the forms was still evident, but the merozoite means of both broods rose and segmenters with 6 or fewer merozoites markedly decreased.

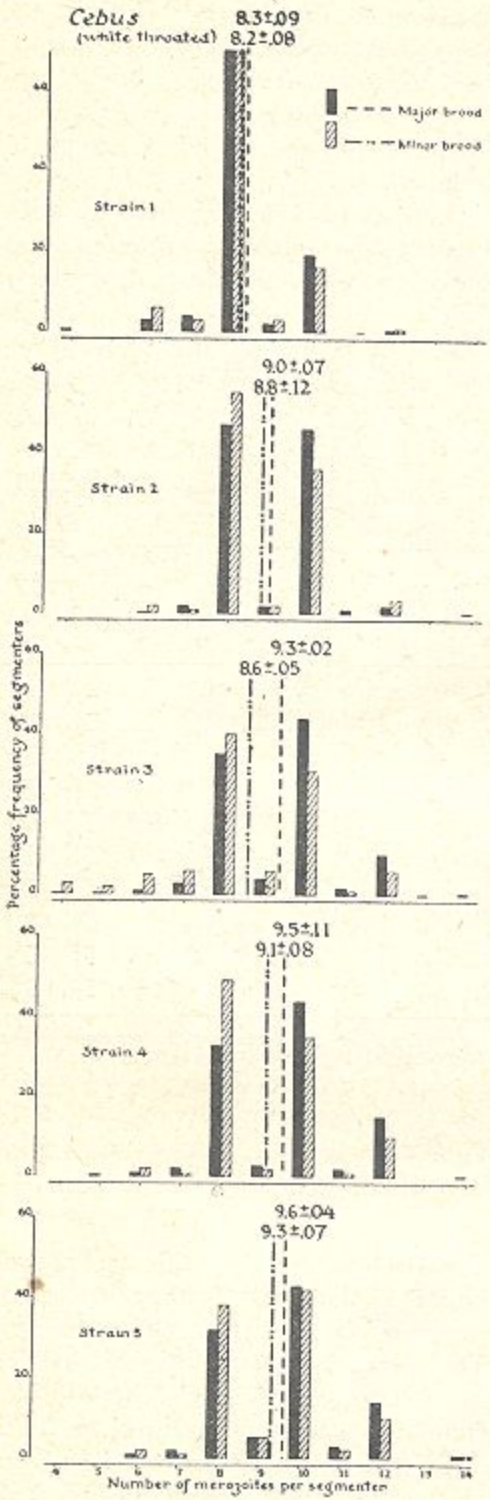
Other cebus monkeys.—Infections of *P. brasilianum* in 23 additional cebus monkeys were studied with respect to parasite count, periodicity and synchronism of the asexual cycle, number of merozoites formed and occurrence of crisis forms, and data from 16 of them are given in table 1 for 17 days of the initial infection. Data from monkeys 1 through 4 were included in this table to show how the data were recorded. Relapses were also studied, if they occurred, but such data were omitted from the table to conserve space. They, however, were similar to those collected and tabulated for the spider monkeys in table 3.

The data in table 1 are oriented with respect to the highest parasite count and the immediately ensuing crisis or parasite decline because the most pronounced changes, if they occurred, occurred just after the peak in numbers. The merozoite means are recorded for the 3 days of the crisis or parasite decline (if segmenters occurred on all 3 days) and for the major brood and one of the two minor broods (if any were found) for three 3-day intervals immediately before and for two 3-day inter-

TABLE 1.—*Merozoite means per segmenter during the major part of infection with P. brasilianum in 20 cebus monkeys (cf. the tabulation of data from monkeys 1 through 4 here and in graphs 1 through 4) and the percent change in the merozoite mean at the crisis or parasite decline as compared to the acute rise of the infection*

Monkey number	Strain of parasite	Acute rise						Crisis or parasite decline			Developed infection				% change in merozoite mean at crisis	
		3-day period of segmentation														
		1st		2nd		3rd		4th			5th		6th			
		Segmentation broods														
		Mj	Mn	Mj	Mn	Mj Peak	Mn	Mj[1]	Mn	Mn	Mj	Mn	Mj	Mn	Max	Av
1	3			9.1		8.8		9.1			9.0		8.7		3	+ 2
2	3	8.8		8.8		9.0		8.9[4]			9.2		9.4		− 9	− 3
3	3	10.2	9.0	10.2	8.0	9.6	7.1[3]	7.3[3]	5.9	4.4[4]	8.2	8.5	8.3	9.2	−57	−31
4	3	9.9	9.7	9.9	8.2	8.7[2]	6.7	7.1[3]	6.1		8.4	8.1	8.8	8.4	−38	−28
21	7	8.0	9.3	9.0	8.6	9.3	7.9[2]	8.2[3]	8.5	7.5					−30	−13
101	1	8.4	8.7	8.3	8.2	8.5	8.5	8.0	8.2	7.9					− 9	− 4
119	9			8.9	8.1	8.7	7.2[2]	7.9[3]	7.7						−19	−11
128	13	8.6	9.0	8.8	8.2	8.7	8.2	7.0[3]	7.4						−22	−16
396	6			9.1		8.3	8.3	9.0	8.1	9.3[4]	9.8	10.4	9.1	10.6	−11	+ 2
421	6	9.3	9.1	9.0	9.3	9.2	9.6	8.7	8.6	9.2	9.4	9.4	9.4	10.2	− 7	− 3
346A	5	9.0	8.4	8.7	9.6	9.5	9.1	9.0			9.1		9.1	9.3	−10	− 2
349A	5	8.9		9.1	9.1	9.5	10.1	6.8[3]	7.2	8.4					−32	−19
354A	5	10.5	10.1	9.8	8.6	9.9	9.0	8.6	8.5	9.6	10.7	10.0			−20	− 8
360A	5			9.6	8.3	9.1	9.2	9.1[3]	8.9	9.4	9.1	8.6	9.8		− 7	+ 1
363A	5	9.9	10.3	10.7	9.0	9.2	8.0[2]	5.7[3]	8.2						−47	−20
365A	5			9.5	9.9	9.1	9.3	8.8	8.6	10.0	9.5	8.8	8.3		−10	− 3
378A	5	9.4	9.0	8.7	8.8	8.8		8.2	8.5	8.5[4]	9.0	8.1	9.0		−15	− 7
112B	4	9.4	8.9	9.6	8.9	9.2	8.3	9.8[3]	8.6						−15	− 2
113B	4	9.4	9.7	10.3	9.7	8.8[2]	8.4	7.5							−27	−20
120B	4	9.7	9.3	9.9	9.1	9.5	8.0	6.0[3]	6.6[4]						−39	−23

[1] This brood is the same as the one designated major during the acute rise of the infection, but may become numerically a minor brood during the derangement of the parasite decline and the realignment during the developed infection.
[2] The crisis or parasite decline began on this day.
[3] This asexual cycle was delayed during this segmentation.
[4] The crisis or parasite decline continued for 3 more days.
Mj and Mn = major and minor segmentation broods, respectively.
Peak = the day of peak in number of parasites.
Max = the % decrease in the lowest merozoite mean during the crisis or parasite decline as compared to the highest merozoite mean during the acute rise.
Av = % change in the composite merozoite mean during the crisis or parasite decline as compared to the composite merozoite mean during the acute rise.



Graph 5.—Frequency distribution of merozoites per segmenter and merozoite means in major and minor broods during the acute rise in the infection of 5 strains of *P. brasilianum* in the cebus monkey. The constants for the 5 strains were calculated from counts from 1, 1, 4, 3 and 11 monkeys, respectively.

Note that the merozoite mean varied from 8.2 to 9.6 when the 5 strains were grown in the same species of monkey.

vals after the crisis or parasite decline. Footnotes indicate irregularities in the crisis or parasite decline, such as (1) its initiation on the day of or the day after the peak in the number count rather than on the third day after the peak, (2) an appreciable delay in the 3-day cycle at the crisis, or (3) an extension of the crisis or parasite decline over 3 additional days (cf. the way in which the data in graphs 1 through 4 are recorded in table 1). In the 2 last columns are tabulated the maximal and average per cent differences in the merozoite mean during the crisis or parasite decline as compared to the acute rise. The maximal per cent difference was obtained by using the lowest merozoite mean during the crisis or parasite decline and the highest merozoite mean during the acute rise, and the average per cent difference was obtained by using the composite merozoite mean during the crisis or parasite decline and the composite merozoite mean during the acute rise. The same values for initial infections, as well as for relapses, of *P. brasilianum* are given in table 3 for spider monkeys and in table 4 for howler, marmoset and night monkeys.

Of the 23 additional cebus monkeys examined, 2 could be studied during the acute rise, 10 through the acute rise and decrease in parasitemia, 11 through the acute rise, decrease in parasitemia and developed stages of the initial infection, and two during the various stages of the initial infection and relapse. The data from these infections corroborated and amplified the conclusions drawn from

the data from monkeys 1 through 4 in the following respects.

(1) During the acute rise of the initial infection: (1a) The periodicity of the asexual cycle was regular and took essentially 3 days. (1b) The asexual cycle was synchronous. Thus, in general, within a given brood, 95 to 100% of the forms had 5 or more nuclei at 8 am, the peak in segmentation occurred from 10 am to noon and 95 to 100% were rings at 2 pm every third day. An occasional segmentation, however, took from one to several hours more or less than 3 days, but such an irregularity never effectively changed the periodicity because the next segmentation usually occurred at the regular time. (1c) Minor broods were common. For example, 8 monkeys had 2 minor broods, 10 monkeys had one minor brood of parasites and 5 had only 1 major brood. The minor broods usually formed 1% or less of the population, but sometimes formed larger parts of the population. In strain 1, there were 3 broods of equal size. (1d) The daily merozoite mean of the major broods during the acute rise varied in a given monkey. Thus, in the major brood, the highest and lowest merozoite means in a given monkey might differ by as much as 15%. In the majority of the monkeys, this difference was approximately 8%. In general, the same values obtained for the minor broods. (1e) In all broods considered together in the 23 monkeys, the highest merozoite mean per segmenter differed from 3 to 22% (average = 12%) from the lowest merozoite mean, whereas the most variable merozoite mean of all broods differed from 1 to 13% (average = 6%) from the composite merozoite mean of the acute rise. As previously described in monkeys 3 and 4, however, the variation from 16 to 22% was found to be due to 1 or 2 low merozoite means at the end of the acute rise and, hence, was not strictly within the range of the acute rise but rather within the range of the crisis. (1f) The foregoing range in percentage differences in the merozoite mean represented data from 14 strains of *P. brasilianum*. A similar range occurred in monkeys infected with the same strain of parasite. In fact, the variations seemed to be more directly associated with the monkey than with the strain of parasite. (1g) The absolute value of the composite merozoite mean was, on the whole, slightly different from strain to strain. Thus, the composite merozoite means of major and minor broods during the acute rise for all the cebus monkeys examined in 6 strains are shown in graph 5 and in one section of graph 6 and were, in general, small in 1 strain, were intermediate in 3 strains and large in 2 strains. The merozoite means of the two broods closely approximated each other, but that of the major brood was usually 0.1 to 0.7 merozoite larger than the minor brood (graph 5). In graph 5, the differences between the merozoite mean of the major and minor brood of strain 1 and 2 cannot be considered statistically valid, whereas those of strains 3, 4 and 5 are probably highly valid. The data from all but 3 of 21 of these cebus monkeys during the acute period of the infection showed that the major brood had a higher merozoite mean than the minor brood. The mean differences between the 21 broods was 0.348 merozoites in favor of the major brood. Furthermore, there was a high statistical significance when the broods were analyzed by Student's method of paired comparisons. Thus, t was found to be 3.9 and $P = .0008$. Moreover, the major and minor broods were analyzed in the acute rise of the infection in a total of 38 cebus, spider and howler monkeys. The analysis was limited to these species because of the atypical in-

fection encountered in the night monkey and marmoset. Among these animals, the data from only 5 showed a minor brood with a higher mean that the major brood. The mean difference in favor of the major brood for all infections was 0.474 merozoites. When analyzed by Student's method of paired comparisons, t equaled 5.4, and $P<.0002$, i.e., the difference between the 2 broods was statistically highly significant. (1h) Simwere common and segmenters with 10 merozoites often predominated. Segmenters with 4 to 16 merozoites occurred, but those containing an odd number of nuclei were comparatively infrequent and those containing 4 or 5 and especially 15 or 16 were rare (graph 5).

From the foregoing values, it may be seen that the merozoite means in various segmentations in the acute infec-

TABLE 2.—*Percent change in the rate of reproduction during the initial acute rise (italics) and during the crisis or parasite decline as compared to the acute rise (boldface) of P. brasilianum in Central American monkeys. The rate of reproduction is based on both the number of merozoites formed per segmenter*[1] *and the periodicity of the asexual cycle*

Species of monkey	Percent change						Number of monkeys studied
	+ − 4–0–5	− 6–15	− 16–25	− 26–35	− 36–45	− 46–55	
Cebus	*14* **8**	*13* **6**	**5**	**2**	**2**	**2**	27 25
Ateles	*2*	*12* **1**	**3**	**4**	**3**	**1**	14 12
Alouatta	*2*	*6*	**1**	**1**			8 2
Leontocebus	*2*	*3* **1**	**3**				5 4
Aotus	**1**	*3*	**1**	**1**			3 3

[1] This value for the acute rise was the percent difference between the most variable merozoite mean during the acute rise and the composite merozoite mean during the acute rise and for the parasite decline was the percent difference between the composite merozoite mean during the crisis and a similar one during the acute rise.

ilar differences occurred in one strain from monkey to monkey. Thus, the merozoite distribution in segmenters of strain 5 in graph 5 represents readings from 11 cebus monkeys which were as low as 8.4 and 8.1 in one monkey and as high as 10.3 and 9.6 in another monkey for the major and minor broods, respectively (cf. the merozoite means from the several cebus monkeys injected with strains 3, 4 and 5 in table 1). (1i) Daily merozoite means of the major broods in cebus monkeys, comprising all 14 strains, varied from 8.0 to 10.7 with an ordinary range from 8.5 to 9.5 and of the minor broods varied from 8.0 to 10.3 with an ordinary range from 8.4 to 9.3. (1j) Segmenters with 8 merozoites tion differed from each other within a range of 3 to 22% in the 27 cebus monkeys examined, but that the upper limits of the variation from 16 to 22% obtained in some of the monkeys were due to low merozoite means when the crisis or parasite decline was probably being initiated (cf. monkeys 3 and 4). From these facts, in conjunction with the constancy of the asexual cycle, the rate of reproduction seemed to vary within a range of 3 to 15% in the normal unimmunized animal (cf. values in italics in table 2).

(2) During the crisis or parasite decline following the initial acute rise of the infection, the periodicity and synchronism of the asexual cycle, the num-

ber of merozoites per segmenter and the occurrence of crisis forms varied within the limits defined by monkeys 1 through 4. Thus, (2a) the periodicity of the asexual cycle in about half of the infections was as regular as during the acute rise or was sometimes less synchronous temporarily without any after-effects, but was differentially disrupted in the other half of the infections during 1 or 2 asexual cycles to such a marked extent that the whole or part of a brood was delayed from about one half to 2 days (footnote 3 in table 1). (2b) The lowest daily merozoite mean per segmenter during the crisis or parasite decline in about half of the infections did not vary more than 15% from the highest daily merozoite mean during the acute rise: it decreased in the other half from 16 to 57% (next to last column in table 1). (2c) The merozoite mean per segmenter during the crisis or parasite decline ranged from 4.4 to 10.0 (table 1) with segmenters forming from 2 to 14 (sometimes only 2 to 10) merozoites. (2d) A rare crisis segmenter was found during a segmentation of the acute rise in an occasional infection, but, in general, crisis segmenters occurred only during the parasite decline in a few infections, particularly when there was a sharp crisis. The maximal percentage of crisis segmenters varied from <1 to 33% of the total population or from <1 to 80% of segmenters alone. The higher percentages were due to the fact that crisis segmenters lingered on in the blood, whereas the rest of the population was rapidly decreasing and the normal segmenters were disappearing because they ruptured the red cells and continued their development as merozoites. Only from <1 to 10% (occasionally up to 30%) of the total population of any particular asexual cycle, therefore, ever degenerated into crisis segmenters. The crisis segmenters originated from morphologically degenerated schizonts. In fact, all stages of the parasite, including gametocytes, could be found which looked as if they were degenerating as determined by vacuolation, abnormally stunted size, irregular contours, darkly staining pigment, which sometimes clumped prematurely, poor staining of the cytoplasm and irregular divisions of the nuclei of the schizonts. The bizarre and abnormal appearance of the crisis segmenters has already been described (pl. 1, fig. 17–25).

These data appear to be correlated with each other, with the decrease in parasite number and with the rate of reproduction roughly as follows: (A) The infections with marked decreases in the merozoite mean did not always exhibit a marked disruption in the asexual cycle and vice versa (in table 1, cf. infections with and without footnote 3 and with more than and less than a 15% maximal change in the merozoite mean in next to last column). Specifically, of the 21 cebus monkeys with infections including the crisis or parasite decline together with monkeys 1 through 4, 8 varied within the limits found during the acute rise, 2 had a disruption of the cycle, 6 had a decrease of more than 15% in the lowest merozoite mean at the parasite decline as compared to the highest merozoite mean during the acute rise, and 9 had both a disruption of the cycle and a maximal decrease of more than 15% in the merozoite mean. Of the 15 last mentioned infections with maximal decreases of more than 15% in the merozoite mean, only 7 had average decreases of more than 15% in the merozoite mean, i.e., the per cent difference between the composite merozoite mean during the crisis or parasite decline and the composite merozoite mean during the acute rise (cf. the 2 last columns in table 1). (B) Infections with a marked

disruption in the asexual cycle and/or a marked decrease in the merozoite mean per segmenter occurred more often in infections with an intense rise in numbers to a high peak and a sharp number crisis (cf. monkeys 3 and 4) than in infections with intermediate or low peaks in numbers which receded gradually (cf. monkeys 1 and 2). (C) Infections with a marked disruption in the asexual cycle and/or a marked decrease in the merozoite mean per segmenter at the crisis more often were characterized by the occurrence of crisis forms than were infections whose asexual cycle proceeded uninterruptedly during the parasite decline with no pronounced decrease in merozoite mean per segmenter. (D) Infections with a marked disruption of the cycle and/or a marked decrease in the merozoite mean per segmenter generally had especially marked decreases in the rate of reproduction at the crisis. Although various calculations could be used to arrive at this conclusion (cf. monkey 3, on page 14), it was decided that a rough approximation could be gotten by obtaining the per cent difference between (1) the composite merozoite mean for the entire crisis or parasite decline divided by an approximation of the length of the asexual cycle (estimated at 3, 3½ or 4 days) and (2) the composite merozoite mean for the entire acute rise divided by the regular 3-day asexual cycle (cf. the second calculation for monkeys 3 and 4 on pages 14 and 16). No other approximation seemed to be more adequate although the error in this approximation probably increased as the percentage difference increased because segmenters may be proportionately less viable as their segmenting time is increased and because segmentation is not delayed in any population for a definite period of time. By this procedure, the rate of reproduction in 14 of the infections did not vary more than 15% (boldface numerals in table 2). This difference, as already noted, represented the base line of variation encountered in the unimmunized monkey. The rate of reproduction in the other 11 infections decreased from 16 to 49% (boldface numerals in table 2).

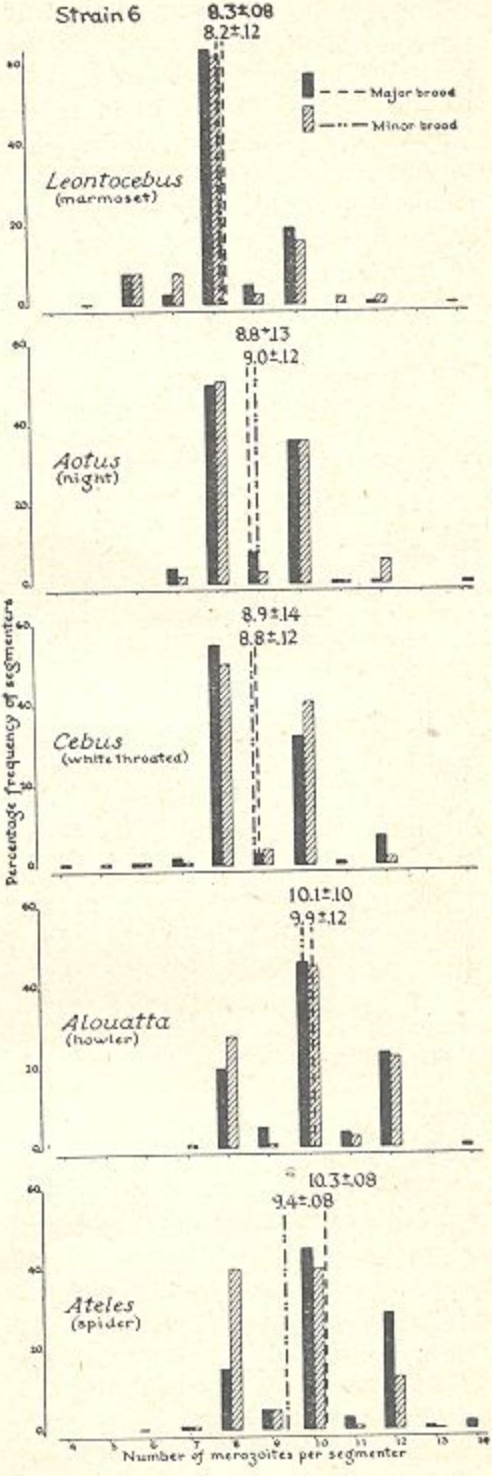
(3) During the developed period of the initial infection, the derangements undergone by the parasites during the time of the crisis or parasite decline straightened out after varying lengths of time until eventually (generally from one to several segmentations) the regularity of the 3-day asexual cycle and the range in values for the merozoite mean observed during the acute rise were reestablished except that the merozoite mean per segmenter sometimes rose slightly higher than during the acute rise. Therefore, the maximal and average differences were somewhat larger during the developed infection than during the acute rise in long continued infections (see graphs 1 and 2). If the delay in segmentation at the time of the crisis or parasite decline was markedly irregular, the percentage of the parasite population and the merozoite means in the major and minor broods in the developed infection were often different from those in the acute period. In other words, a major brood of 99% of the population and a minor brood of 1% of the population with merozoite means of 10 and 9, respectively, before the crisis or parasite decline might become realigned thereafter into 2 major broods of 49% and one minor brood of 2% with merozoite means of 9.5, 9 and 8.5, respectively. (Cf. the somewhat similar percentages of schizonts with 5 or more nuclei and merozoite means in graph 3 before and after the crisis).

(4) During the acute rise, parasite decline and developed infection of relapses: conditions were in general similar to conditions found during similar peri-

ods of the initial infection except that the parasite decline of the relapse was liable to be less intense than that of the initial infection and that of each succeeding relapse was liable to be progressively more gradual. For example, W350A had an initial infection followed by 2 relapses, all of which reached peaks of approximately 150 parasites per 10,000 red cells on the 13th, 25th and 40th days of its infection, respectively. At the parasite decline, the asexual cycle took 4 days during the initial infection, but was only partially delayed to the extent that minor broods developed during the 2 relapses. In addition, the lowest merozoite means during the 3 parasite declines were 24, 22 and 12%, respectively, lower than the highest merozoite means of the immediately preceding acute periods.

Ateles (spider) monkeys.—Six red and 9 black spider monkeys were studied and representative data from them may be found in table 3. These data corroborated the conclusions drawn from the cebus monkeys except that periodicity was more often disrupted at the crisis, and the merozoite means were slightly higher and more variable during the various stages of the infection. These differences modified to some extent conclusions 1d, e and i and 2a, b and c, as described for the cebus monkeys.

Specific differences for the spider monkeys were as follows: During the acute and developed infection, the merozoite mean per segmenter on different segmentation days ranged from 8.2 to 11.8 for



Graph 6.—Frequency distribution of merozoites per segmenter and merozoite means in major and minor broods during the acute rise of the infection of strain 6 of *P. brasilianum* in cebus, spider, howler, marmoset and night monkeys. The constants were calculated from counts from 2 or 3 members of each species.

Note that the merozoite means of this strain were smallest when grown in the marmoset, intermediate in the night and cebus monkeys and largest in the howler and spider monkeys.

the major brood and from 8.0 to 11.6 for the minor broods with a usual range from 9.4 to 10.8 in both broods (table 3). Segmenters formed from 4 to 16 segmenters as in the cebus monkeys. Hence, the higher merozoite means in the spider monkeys were accounted for by the fact that more segmenters formed from 10 to 16 merozoites and fewer formed from 4 to 8 merozoites (cf. strain 6 when

ing the developed infection although they were sometimes higher in long continued infections. The basic rate of reproduction during the acute rise and developed infection, therefore, may have varied slightly more than 15%, i.e., through 16 or 17%. The findings during the crisis in the spider monkeys may be briefly summarized as follows: The merozoite mean ranged from 4.7 to

TABLE 3.—*Merozoite means per segmenter during the major part of infection with P. brasilianum in 5 red spiders and 5 black spiders and the percent change in the merozoite mean at the crisis or parasite decline as compared to the acute rise of the infection*

Monkey number	Strain of parasite	Acute rise						Crisis or parasite decline			Developed infection				% change in merozoite mean at crisis	
		3-day period of segmentation														
		1st		2nd		3rd		4th			5th		6th			
		Segmentation broods														
		Mj	Mn	Mj	Mn	Mj Peak	Mn	Mj[1]	Mn	Mn	Mj	Mn	Mj	Mn	Max	Av
Red spiders																
16	7			10.3	10.0	9.2	9.6	8.2[2]			9.9		10.7		−20	−16
95	13	9.2	9.8	8.3	9.2	9.1	9.3	10.1[2]	9.9		9.3				− 5	+ 4
297A	5					9.1	9.3	7.9[2]	8.2	7.3	10.1	9.5	10.1	8.4	−22	−15
356A	14	9.5	9.8	10.6	10.3	10.2	10.3	9.9[2]	8.9	9.8	9.5		9.8		−16	− 5
104B	4			11.1	9.3	10.6	9.1	8.5[2]	6.7	4.7	8.6		10.5		−57	−29
		9.5	8.9	8.9	9.7	10.4	9.2	9.6[2]	8.2	9.0	11.3	9.0			−36	−15
		9.7	8.6	10.0	8.4	9.9	8.8	9.1	8.4	9.4	9.6	8.6	10.1	8.2	−16	− 3
Black spiders																
91	7	11.3		10.3		9.4		9.0			9.4		10.1		−20	− 1
395	6	10.5		10.6	9.1	9.4	8.5	8.0[2]	7.2	7.53	8.4	10.0			−32	−21
397	6	10.4	10.1	10.4	9.6	10.4	8.8	8.0[2]	8.1[2]		11.8	9.4	10.8	9.5	−24	−19
471	6	9.8	10.8	10.8	8.0	11.2	8.7	8.4[2]	8.3[3]						−19	−14
351A	5	11.0		11.0		10.7	10.1	9.3[2]	7.2	6.3[2]	10.0	9.6	10.7	10.5	−43	−27
				11.4	11.6	10.9	10.7	8.4[2]	8.4						−23	−22
				10.8	9.1	9.5	8.3	8.3	9.1	9.0	10.8	9.1	9.3		−23	− 8

[1,2] and [3] correspond to [1,3] and [4] in Table 1.

grown in cebus and in spider monkeys in graph 6). The maximal difference between the highest and lowest merozoite means of a given infection of all broods separately ranged from 1 to 20% or together ranged from 3 to 23% (average =14%) during the acute infection. The average difference between the most variable merozoite mean during the acute rise and the composite merozoite mean of the acute period ranged from 1 to 14% (average = 8%) (values in italics in table 2). Essentially similar maximal and average differences were found dur-

10.1. Of the 12 infections which underwent declines in parasitemia, 3 had a disruption of the cycle, 1 had more than a 15% decrease in the lowest merozoite mean at the crisis as compared to the highest merozoite mean during the acute rise, and 8 had both a disruption of the cycle and a similar decrease in the merozoite mean. Of the 9 last mentioned infections with maximal decreases of more than 15% in the merozoite mean, only 5 had decreases of more than 15% by comparing the composite merozoite mean during the parasite decline with

the composite merozoite mean during the acute rise (cf. footnote 3 and the last 2 columns in table 3). Calculations from these data, by the method used for the cebus monkeys, indicated that the rate of reproduction was within the range of the acute rise in only one animal and varied from 19 to 46% in the other 11 monkeys (boldface numerals in table 2). per segmenter and the retardation of reproduction were successively less pronounced with each succeeding parasite decline.

*Howler, marmoset and night monkeys.** —The infection of *P. brasilianum* in 10 howlers, 6 marmosets and 6 night monkeys was often so transient that the data are not as conclusive as those from

TABLE 4.—*Merozoite means per segmenter during the major part of infection with P. brasilianum in 5 howlers, 4 marmosets and 3 night monkeys and the percent change in the merozoite mean during the parasite decline as compared to the acute rise of the infection*

Monkey number	Strain of parasite	Acute rise						Parasite decline			Developed infection				% change in merozoite mean at crisis	
		3-day period of segmentation														
		1st		2nd		3rd		4th			5th		6th			
		Segmentation broods													Max	Av
		Mj	Mn	Mj	Mn	Mj	Mn	Mj[1]	Mn	Mn	Mj	Mn	Mj	Mn		
Howlers																
H 40	10	8.0	8.2	9.2	8.0	8.3	7.8									
H149	11	8.8	8.9	9.8	8.5	9.0	8.6									
H152	14	9.2	10.1	11.0	10.2	10.0	9.4									
H432	6	9.7	9.1	9.7	9.0	10.5	9.3	9.9[3]	8.5	8.1[4]	8.2	9.1			−23	− 8
H433	6	11.1	11.0	9.9	9.1[2]	8.9	8.6	9.5[3]	8.6	8.7	9.9	9.6	10.0	10.4	−22	−12
Marmosets																
M473	6	8.0	8.1	7.8	7.6	7.8	7.2	7.2[3]	6.3	8.2	10.4	9.4	8.2	7.7	−19	− 4
M480	6			8.8	8.6	8.6	7.0[2]	7.5[3]	8.8	8.8[4]	8.6	9.2	8.7	9.0	−20	− 8
M372A	12	10.0	9.0	10.0	9.8	9.3	8.0[2]	9.4	7.4	8.2					−28	−14
						8.8	9.5	8.6	7.8	8.2	9.2	9.3	8.5	9.0	−18	−11
M373A	12	8.7		9.7	7.9	8.9	8.2	7.7	7.9	8.3	8.4	8.3	8.3	8.7	−21	− 8
Night monkeys																
NM378	6			9.3	8.4	8.4	8.7	8.3	8.8						−10	− 2
					9.5	10.3	8.7[2]	9.1[3]	9.6	9.3	11.3	10.6			−16	+ 4
NM370A	12			10.3		9.6[2-4]		8.0	7.8	6.5					−37	−19
NM377A	5	11.2		9.6	9.2	11.2	9.0	8.2[3]	8.3	9.0					−27	−15

[1,2,3] and [4] same as in table 1.

The 2 infections in red spider 104B and in black spider 351A were particularly interesting because they were long continued and underwent several declines in parasitemia (table 3). They were both characterized by an initial intense acute rise and by two relapses, data for the major part of each of which are tabulated in table 3 on separate lines. In both infections, the merozoite mean decreased less at each successive parasite decline as compared to the acute rise and the delay in segmentation was progressively less. In other words, the decrease in number of merozoites the cebus and spider monkeys. As far as they go, however, they (tables 2 and 4) corroborate the conclusions reached formerly for the cebus monkeys with the following exceptions affecting conclusions 1b, i and j and 2b, c and d.

Specific differences were as follows: The asexual cycle was characteristically less synchronous during the entire infec-

* In 1934, we (1934a) noted that *P. brasilianum* was often fatal to night monkeys and marmosets. Since that time, more data indicate that these monkeys died because they were not acclimatized to laboratory conditions rather than because the infection was fatal.

tion in the marmoset and night monkeys. Although the peak in segmentation occurred as usual in the morning, 2 to 4% of segmenters were frequently found through the late afternoon in the marmosets and through the afternoon and evening in the night monkeys. During the acute and developed infection, the range in the merozoite mean per segmenter (table 4 and graph 6) in the howlers extended over the range found in both the cebus and spider monkeys, in the night monkeys resembled that of the cebus monkeys, and in the marmosets was lower than that of the cebus monkeys. In the marmosets, 4-nucleated segmenters were very commonly found and 15- and 16-nucleated ones were never found. The initial rise of the infection was never as acute. Also the parasite decline was never as sharp or as sudden in these monkeys as in some cebus and spider monkeys. Correlated with this finding, crisis forms were scarce, the lowest merozoite mean encountered was 6.3 and the asexual cycle was never as completely delayed, as in some cebus and spider monkeys. As a consequence, 35% was the maximal decrease found in the rate of reproduction at the crisis (table 2).

DISCUSSION

The overall changes in the synchronism and length of the asexual cycle, the number of merozoites per segmenter and the morphology of the parasites, all of which are associated with the reproduction of *P. brasilianum*, will now be considered in relation to the responses of the host. The antiparasitic factors during the acute rise of the infection represent, for the most part, nonspecific factors of natural immunity and host differences, whereas those operative at the crisis or parasite decline represent the first important action of acquired immunity which is superimposed on the nonspecific factors of natural immunity. These two factors continue to act in varying degrees during the developed infection and latency in such a way that a balance is more or less established between the antiparasitic effects of immunity and the reproductive activity of the parasite. The changes in the rate of reproduction during the acute rise are chiefly of interest because they form a base line upon which the effects of acquired immunity during the crisis and subsequent stages of the infection can be evaluated.

Synchronism, in all strains of *P. brasilianum* studied, is much more exact in the cebus, spider and howler monkeys than in the marmoset and night monkey. In the cebus, spider and howler monkeys, however, an occasional entire segmentation during the acute rise or developed infection may take a few hours more or less than 72 hours. These irregularities have not been definitely associated with any factors connected with the host, but seem most likely to be associated with activity and body temperature. For example, under laboratory conditions, the cebus, spider and howler monkeys are largely active periodically during the day, the marmoset is less active and the night monkey is sluggish.

The merozoite mean per segmenter of the minor broods in all species of monkeys is usually lower than the major brood during the acute rise and developed infection. This fact coupled with the fact that the minor brood generally segments on the day after the major brood segments might lead one to suppose that the minor brood is composed of degenerating forms unable to segment within the regular length of time, but the actual occurrence of appropriate stages on the blood films points incontrovertably to the independent development of minor broods. These same facts

might lead one to invoke the idea of atrepsy except for the fact that the merozoite mean of the minor brood is lower than that of the major brood even when the parasite count is at an extremely low level. Furthermore, the alternate occurrence of major and minor broods disposes of the view that the smaller merozoite mean of the minor brood may be due to the gradual onset of the crisis. At present, therefore, we are unable to account for fewer merozoites, in general, forming in the segmenters of the minor brood than in those of the major brood.

The merozoite mean of the major brood at the first segmentation of the acute rise may be lower than at subsequent segmentations (graphs 1 and 3). Such differences are in the order of 3%. Furthermore, one merozoite mean on one segmentation day may differ by 15% from another on another segmentation day during the acute rise in a given monkey of any species or may differ up to 17% in the red and black spider monkeys. In general, smaller variations (up to 8%) are more common in all species of monkeys. These values represent the variability of asexual reproduction (since segmentation is regular) in the unimmunized monkey and are associated with the nonspecific factors of natural immunity. In addition to these differences in individual monkeys of the same species, the composite merozoite mean of all broods of a given strain of *P. brasilianum* is smaller by about 0.5 merozoite when grown in the marmoset and is larger by more than 1.0 merozoite when grown in the howler and spider monkeys than the merozoite mean of 8.8 to 9.0 when grown in the night monkey and cebus monkey. Since these differences are statistically valid for the acute rise of the infection, they indicate possibly that the marmoset, on the one hand, is not as satisfactory as the howler or spider monkey, on the other hand, as a culture medium for the parasite.

The crisis or parasite decline following the acute rise of infections of *P. brasilianum* is not stereotyped. It may begin at any parasite level or on any of the three days of a sexual cycle. In other words, it may begin when parasites have reached a peak of only 11 per 10,000 red cells (18th day in graph 1) or not until they have reached a peak of 991 per 10,000 red cells (36th day in graph 3); it may begin on the day of the peak in numbers (21st day in graph 4), or possibly even before (graph 2), or on any of the several succeeding days after the peak in numbers (37th day in graph 3). It may proceed precipitously or slowly. Furthermore, during all types of parasite decline, the merozoite mean per segmenter does not vary more than 15% in about one half of the monkeys. In the other half of the monkeys, especially those with sharp crises, it decreases roughly from 16 to 35%, rarely up to 50%, i.e., from 1 to 20% or rarely to 35% beyond the 15% normal range. The exact extent, however, is difficult to evaluate. In the first place, there are always some parasites, even in the most severe crises, which apparently develop normally. Their occurrence always raises the question of the relative importance of normal segmenters versus retarded segmenters in carrying on the infection. In the second place, some parasites are so degenerate in appearance that they are obviously dying. To the extent that they do not resume reproduction, they simply represent the persistence of dying organisms and do not actually affect the basic rate of reproduction. Although the exact extent of the decrease in the rate of reproduction could not be ascertained, the data are consistent in indicating that appreciable decreases take place in some immune animals at the crisis.

There are two ways in which antibodies might inhibit the reproduction of the parasites at the time of the crisis: (1) by the action of a specific reproduction-inhibiting antibody or ablastin, such as the senior author (1924 and 1932b) has described in infections with *Trypanosoma lewisi;* and (2) by some nonlethal effect of opsonins, agglutinins and precipitins—the first of which is certainly and all of which are probably fundamentally parasiticidal in action.

Of these possibilities, there is little evidence that a specific ablastin is present. The anti*lewisi* ablastic antibody is so specific in its action that it inhibits growth and reproduction without killing the parasites or producing noticeable effects on their vitality, motility or infectivity. In the present infection, however, there are obvious deleterious effects on the parasite.

The parasiticidal factors may act on the rate of reproduction in several ways. As noted in the presentation of the experimental data, the rate of reproduction is lowered by (1) a decrease in merozoites per segmenter and (2) a retardation in the growth of individual segmenters to varying degrees which deranges the periodicity of the asexual cycle.

The lowering of the number of merozoites per segmenter and the delay in development may reasonably be due to an antiparasitic action of some serological factor. Thus, at the crisis, in addition to increasing phagocytosis, the opsonin or some other antibody may depress the general metabolic functions of developing parasites, including those upon which division, growth and other factors of reproduction depend. The occurrence of crisis forms suggests some such harmful type of generalized serological effect on the parasite. In addition to the direct antibody effects, the lengthening of the asexual cycle may be due to indirect effects. Thus, animals with an intense crisis are frequently ill and may die as a result of what we believe to be an intense antigen-antibody reaction. Some of the changes in the asexual cycle of the parasites at the time of sharp, sudden crises are somewhat similar to the changes in the asexual cycle during the transitional period of alternating light and darkness in infections of *P. brasilianum*, as reported by W. H. and L. G. Taliaferro (1934b). Furthermore, Stauber (1939) has furnished evidence that the periodicity of malaria is dependent upon the activity and body temperature of the host. It seems possible, therefore, that the obvious illness of the animal which is accompanied by low activity and low body temperature during the crisis of the infection is a primary factor involved in the delay in segmentation and the lack of synchronism. The illness of the host may also account, in part, for the lowering of the merozoite mean per segmenter since we know that other nonspecific effects cause the merozoite mean per segmenter to vary during the acute rise. The fact that the inhibition of reproduction is to a large extent temporary seems to indicate that the nonspecific factors are the most important, but it is entirely possible that a higher antibody concentration occurs at the crisis than during the developed infection. Upon this last point, we have no experimental data, but those parts of the derangement of the cycle which are toxic manifestations of the antigen-antibody reaction would obviously be greatest during the crisis when a large amount of antigen is present.

The marked inhibition of reproduction which occurs at the crisis does not continue through the developed infection although we know that acquired immunity to superinfection does persist. A certain residual effect, however,

does persist in some animals. Thus, in monkeys 3 and 4, the merozoite mean for several segmentations during the developed infection was on the whole lower than that of the acute rise of the infection. Significantly, this lowering was largely the result of the occurrence of many more segmenters with 6 or fewer merozoites (cf. also the experiments of Boyd and Gilkersen, 1942). In addition, a slight derangement in the synchronism of the asexual cycle probably persisted in agreement with the findings of *P. relictum* by L. G. Taliaferro (1925). Later in the developed infection, the merozoite mean per segmenter reaches essentially the same values as during the acute rise in those infections in which there is little derangement at the parasite decline (graphs 1 and 2) as well as in those infections in which the derangement is marked (RS104B in table 3). In long infections the merozoite mean per segmenter may become slightly higher than during the acute rise of the infection.

The question arises as to why some animals undergo rapidly developing acute heavy parasitemias with sharp crises during which there are pronounced antiparasitic effects, whereas others develop slowly progressing low grade parasitemias with gradual parasite declines during which the changes are no more marked than they are during the remainder of the infection. The extreme variants in these respects were found in monkeys 1 through 4. These monkeys were all young, were all kept under the same laboratory conditions and were infected in pairs from the same monkey 2 years apart. Yet the 2 pairs differed from each other to the extent that 1 and 2 varied as little as any of the other cebus monkeys studied, whereas monkeys 3 and 4 varied as greatly as any studied. In other words, the conditions were as radically different in these 4 monkeys as any encountered during three 3-month periods in the space of 5 years, i.e., the time during which data from all the other monkeys were collected. The differences as far as they were analyzed were as follows: The first pair of monkeys had been in the laboratory a year and were fully acclimatized before they were infected: the second pair had only been in the laboratory a few weeks before they were infected. Moreover, no parasites or other infectious agents were found in the first pair: in the second pair a rare trypanosome of the *T. lewisi* type was found and a nonhemolytic staphylococcus produced a septicemia which reached a climax on the 63rd day of the malarial infection in monkey 3 and on the 31st day of the malarial infection in monkey 4. It would seem that the sharpness of the crisis and the extent of the effects at the crisis are determined to a large extent by the degree of the initial acute parasitemia with resulting antigen absorption. The histories of monkeys 1 through 4 also suggest that this degree of parasitemia may be related to intercurrent infection and other factors which affect natural immunity.

Irrespective of the mechanism controlling the changes, as described, the data may explain why the fever curve, which depends on the asexual cycle, may change during infection. Thus, in graph 3, a predominating major brood and scarcely perceptible minor brood before the crisis became reorganized after the crisis into two broods of nearly equal size and a barely perceptible third brood.

The findings in *P. brasilianum* were different from those in *P. cathemerium*, as found by Boyd and his coworkers. In the latter infection, the periodicity of the asexual cycle continued uninterruptedly throughout the infection including the period of the crisis, according to

Boyd and Allen (1934) and Boyd (1939) whereas the merozoite mean per segmenter decreased more or less regularly through the acute rise, did not markedly decrease at the crisis or parasite decline, and increased during the developed infection nearly up to or occasionally up to the point attained on the first day of the acute rise. The factors responsible for this difference are not known.

SUMMARY

A study of asexual reproduction in major and minor broods during initial infections and relapses of 14 strains of the quartan parasite, *P. brasilianum*, in 64 monkeys belonging to 6 species of Central American monkeys indicated the following with respect to the length and synchronism of the asexual cycle and the number of merozoites formed by the segmenters:

Significant changes occurred (1) in the synchronism of *P. brasilianum* in all marmosets and night monkeys during the entire infection and in some cebus, spider and howler monkeys during the parasite decline; (2) in the average number of merozoites per segmenter in major and minor broods during the entire malarial infection in the majority of the monkeys of all species (they were even more marked during the crises in some monkeys) and (3) in the periodicity and morphology of the parasites at the time of the crisis or parasite decline in some monkeys of all species.

The above changes may be related specifically to the host, to the parasite, to the parasitological stages of the malarial infection, and to immunity and nonspecific factors as follows:

The merozoite mean per segmenter varied within a given parasitological stage of the infection when the same strain or different strains of the parasite were grown in different individuals of the same species of monkey (graph 5), but was smallest within a given strain when grown in marmosets, intermediate when grown in cebus and night monkeys and largest when grown in howler and spider monkeys (graph 6).

During the *initial acute rise* of *P. brasilianum*, the 3-day periodicity of the asexual cycle remained constant; the number of merozoites formed per segmenter varied within or between major and minor broods and within the same (graphs 1 through 4) or different strains of the parasite (graph 5) from 3 through 15% ordinarily or up to 17% in the spiders. Similar results were obtained in most *developed infections* and during *some crises* or *parasite declines*. During the *parasite declines* in practically all spiders and in about half of the other monkeys, however, the periodicity of the asexual cycle was temporarily delayed and/or the number of merozoites per segmenter markedly decreased. The extent of these derangements varied markedly from monkey to monkey. Thus, varying proportions of the population might take approximately from 3½ to 4 days (rarely 5 days) to segment and the merozoite mean per segmenter might decrease from 16 to 35% (occasionally to 50% in the spider and cebus monkeys). These two effects did not always occur concomitantly, but one or both tended to occur more generally in infections with an intense rise in numbers to a high peak and a sharp number crisis, as in graphs 3 and 4, than in infections with intermediate or low peak in numbers which receded gradually, as in graphs 1 and 2. The marked decrease in the merozoite mean per segmenter at the crisis was frequently accompanied by a morphological degeneration of the segmenters (crisis forms) and was brought about not only by a shift in the mean but by a shift in the range of merozoites per seg-

menter from 4 to 16 to lower values of 2 to 10. The lengthening of the asexual cycle, if marked, sometimes caused the percentage population of the brood or broods to be different after the crisis than before the crisis. For example, one brood before the crisis might realign after the crisis into two broods—a finding which may explain the change in the type of fever curve often observed in malaria of man. In some infections, after a marked derangement at the crisis, a residual lowering of the merozoite mean per segmenter and lack of synchronism persisted for varying periods of time during the developed infection.

Similar findings were obtained during the *acute rise*, *parasite decline* and *developed infection* of *relapses* except that the derangements at each successive parasite decline in a given infection were generally progressively less marked.

From these data, it was calculated that the rate of reproduction of *P. brasilianum* varied from 3 to 15% in cebus, howler, marmoset and night monkeys and up to 17% in the spider monkeys during the acute and developed stages of the initial infection and relapses and in some monkeys in each species except the spiders during the parasite decline. In the spider monkeys and in about half of the monkeys in the other 4 species studied, it may have decreased to varying degrees up to 50% (usually up to 35%). In addition to these changes in the rate of reproduction, large numbers of parasites disappeared during the acute rise of the infection and larger numbers disappeared thereafter, especially at the crisis.

The foregoing data, in conjunction with previous data obtained by us on parasiticidal effects, may be interpreted in terms of immunity as follows: (1) *Natural immunity* is associated with a marked death of the parasites and may be associated with a sporadic variation in asexual reproduction up to about 15% (usually less than 8%) as indicated by the regularity of the asexual cycle and the sporadic variations in the merozoite mean of the major and minor broods during the entire acute rise of the infection. (2) *Acquired immunity*, in conjunction with natural immunity, results in a heightened death of the parasites and, especially during sharp crises, with a marked lowering of the rate of asexual reproduction.

The marked lowering of the asexual reproduction in a few animals during sharp crises is due to (1) a derangement of the asexual cycle involving a lack of synchronism and a lengthening of the cycle in some parasites and (2) a decrease in the number of merozoites in some segmenters as well as the appearance of obviously degenerate "crisis" forms. It is suggested that these changes may be, in part, the generalized deleterious effects of fundamentally parasiticidal antibodies. In other words, many metabolic functions, including those on which growth and reproduction are dependent, may be depressed and cause the death of some parasites, as indicated by crisis forms, the variable retardation of the growth of others, which brings about a delay in the average length of the asexual cycle and in the loss of synchronism, and a lowering of the mean number of merozoites per segmenter. It is further suggested that the changes, especially the loss of synchronism, may in part be due to such nonspecific factors as the loss of activity and the lowered body temperature of the host, which are a manifestation of the general toxicity of the intense antigen-antibody reaction during the crisis. There is no evidence that the temporary delay in reproduction at the crisis is associated with an ablastin of the *T. lewisi* type.

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