THE CLASSIFICATION AND DIFFERENTIAL DIAGNOSIS OF THE AESTIVO-AUTUMNAL MALARIA PLASMODIA

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The experiences of the various armies with malarial infections during the world war has reawakened interest in the parasites concerned in their etiology, especially with regard to the question of the plurality of species. Several recent observers have recorded what they believed to be transmutations of the species that have been generally accepted by protozoologists and have subscribed to the belief of Laveran, that there is but one species of plasmodium concerned in the etiology of the various clinical types of malaria observed in man.

In this contribution it is not my purpose to discuss the merits of this contention, beyond stating that sufficient evidence has not yet been presented proving that transmutation of species occurs in the malaria plasmodia and none of the evidence so far presented is of such a character as to invalidate, in the least, the facts that prove, in the opinion of most qualified observers, the existence of distinct species of malaria plasmodia. The experimental evidence proving that the direct inoculation, into susceptible individuals, of blood containing any of the well-recognized species of malaria plasmodia is invariably followed by the appearance of the inoculated species in the blood of the inoculated and the occurrence of the characteristic febrile paroxysm of the species inoculated, is amply sufficient to establish the existence of these species, and when there is added to this evidence the results of more than 100 mosquito experiments,

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which are recorded in the literature, in which the species of plasmodia obtained by the mosquito from the infected individual invariably appeared in the blood of individuals bitten by the insects, accompanied by the characteristic clinical symptoms usually produced by that species, one must admit that the proof of the plurality of species is practically incontrovertible.

CLASSIFICATION

At the present time there are three species of malaria plasmodia causing infection in man recognized by the vast majority of authorities, i.e., *Plasmodium malariae* Marchiafava et Celli (1), the cause of quartan malarial fever; *Plasmodium vivax* Grassi et Feletti (2), the cause of tertian malarial fever; and *Plasmodium falciparum* Welch (3), the cause of aestivo-autumnal or subtertian malarial fever.

While practically all authorities accept these three species of malaria plasmodia as distinct, numerous investigators believe that more than one species of plasmodium is concerned in the etiology of the aestivo-autumnal fevers or that sub-species of *Plasmodium falciparum* exist which are morphologically and clinically distinct. Thus Grassi and Feletti (4) recognize two species of aestivo-autumnal or pernicious plasmodia; Mannaberg (5) and Manson (6), three species, a pigmented quotidian, an unpigmented quotidian and the malignant tertian; and Marchiafava and Bignami (7) two species, a quotidian and tertian.

In 1901, as a result of the study of hundreds of malarial infections contracted in Cuba and the Philippine Islands, I accepted Marchiafava and Bignami's classification and described (8) two species of plasmodia associated with the aestivo-autumnal infections that I studied, one sporulating in the infected individual in twenty-four hours and causing a quotidian fever, the other sporulating every forty-eight hours and causing a peculiar type of tertian fever. Further study of these two species convinced me that the evidence was not sufficient to entitle the quotidian plasmodium to *specific* rank, and in 1909 I proposed a classifica-

tion (9) in which the quotidian parasite is considered as a sub-species of *Plasmodium falciparum* and gave to it the name: *Plasmodium falciparum quotidianum*. This classification of the malaria plasmodia is as follows:

Order. *Haemosporidia.*
Genus. *Plasmodium.*
Species. I. *Plasmodium malariae,* Marchiafava et Celli, 1885.
II. *Plasmodium vivax* Grassi et Feletti, 1890.
III. *Plasmodium falciparum* Welch, 1897.
Sub-species. I. *Plasmodium falciparum quotidianum* Craig, 1909.

In descriptions of the aestivo-autumnal plasmodia, published at various intervals (9), (10), (11), (12), I have shown that *Plasmodium falciparum* and *Plasmodium falciparum quotidianum* are distinguishable morphologically, differ in the time consumed in their life-cycle in man, and produce characteristic febrile paroxysms. *Plasmodium falciparum* completes its life-cycle in the blood of man and sporulates every forty to forty-eight hours, the paroxysms of fever occurring every other day, while *Plasmodium falciparum quotidianum* completes its life-cycle in the blood of man in twenty-four hours, producing a paroxysm of fever every day, the temperature curve being a quotidian one. I have not been able to confirm the existence of an unpigmented quotidian plasmodium, as described by Mannaberg and by Manson, although I have examined many thousand blood preparations from aestivo-autumnal infections and have searched carefully for such a parasite.

The majority of textbook writers do not accept more than one variety of aestivo-autumnal plasmodium, although they admit that clinically both the tertian and quotidian types of fever occur. They explain the occurrence of these clinical types by asserting that *Plasmodium falciparum* sometimes sporulates in twenty-four hours and sometimes in forty-eight hours, an assumption that is at variance with the biological laws regarding sporulation as illustrated in the other species of malaria plasmodia. *Plasmodium malariae,* the quartan plasmodium, does

not sporulate sometimes in thirty hours and sometimes in seventy-two hours, but always in seventy-two hours; nor does *Plasmodium vivax*, the tertian plasmodium, sporulate sometimes in twenty-four hours and sometimes in forty-eight hours, but always in approximately forty-eight hours, and to claim that *Plasmodium falciparum* is so marked an exception to the rule as illustrated by sporulation in the other malaria plasmodia is illogical and simply begs the question. Those who believe in only one species of malaria plasmodium use the same argument, claiming that under certain conditions the plasmodium sporulates in twenty-four hours, in forty-eight hours, or in seventy-two hours, but it is almost impossible to believe that such a variation exists in any one species of plasmodium, and, to my mind, the assertion that *Plasmodium falciparum* sporulates sometimes in twenty-four hours and sometimes in forty-eight hours, producing each time a different number of merozoites and a different type of febrile paroxysm, is no more worthy of belief than that there is but one species of plasmodium with three different life-cycles in man, as shown by the variations in the time of sporulation.

Many careful students of the malaria plasmodia believe that at least two varieties or species of *Plasmodium falciparum* exist and the trend of recent scientific opinion is in favor of the recognition of more than one species of this plasmodium. Bass (13), who first cultivated the malaria plasmodia and has had the advantage of studying the morphology and development of the aestivo-autumnal plasmodia in pure cultures, states "that there are at least two and probably more sub-divisions of the aestivo-autumnal parasite" and that a majority of the students of the subject believe that there are at least two different species. Bass accepts the quotidian and tertian species and in the contribution mentioned describes their morphology and development in cultures. In a personal communication he writes that he feels certain that there are two distinct species, differing in their morphology, as observed in blood preparations, and retaining these differences during their development in cultures.

Since the publication of the contributions referred to earlier in this paper I have continued my observations upon the aestivo-autumnal plasmodia, as opportunity offered, and new data regarding the differentiation of *Plasmodium falciparum* and *Plasmodium falciparum quotidianum* have been secured, the results being confirmatory of the existence of the two species mentioned, and it is believed that their publication will be of interest and value to students of malaria. The photomicrographs illustrating this paper are typical pictures of both species as the plasmodia are observed in stained preparations of the blood of infected individuals, and, I think, prove beyond question the difference in the size and other morphological features of the parasites.

DIFFERENTIAL DIAGNOSIS

Morphological

In the differential diagnosis of the aestivo-autumnal plasmodia both morphological and clinical differences are of importance, and while a differential diagnosis of *Plasmodium falciparum* and *Plasmodium falciparum quotidianum* should be based upon a careful study of the morphology and cycle of development of the plasmodia, it is true that in uncomplicated cases a differential diagnosis may be made from a study of the temperature charts of the two infections. For this reason, it is necessary to consider in any adequate presentation of the subject, both the morphology of the plasmodia and some of the clinical phenomena produced by infection with them.

Plasmodium falciparum and *Plasmodium falciparum quotidianum* differ markedly from one another in their morphology, these differences being quite as marked as the morphological differences between *Plasmodium malariae* and *Plasmodium vivax*, and one who is able to differentiate the latter species should have little difficulty in distinguishing the former in suitable preparations. The differentiation into separate species of *Plasmodium malariae* and *Plasmodium vivax* depends chiefly upon differences in the size of the two plasmodia, the number of

merozoites produced at the time of sporulation, and the periodicity of the sporulation, and the same is true of the differentiation of the aestivo-autumnal species, as they differ essentially from one another in all of the characters mentioned.

In differentiating between the aestivo-autumnal plasmodia one should study both living, unstained plasmodia in the blood of infected individuals as well as blood smears stained with some modification of the Romanowsky stain. The numerous excellent modifications of the latter stain have unfortunately resulted in the almost total neglect of the examination of fresh unstained preparations of blood in the study and diagnosis of the malaria plasmodia, but it should not be forgotten that, after a little practice, all species of malaria plasmodia may be as easily recognized in unstained preparations as in stained, and in the study of the morphology and life-cycle of the plasmodia in man the living parasites should always be observed if material is available.

Morphology in unstained preparations

In unstained preparations of blood, the tertian aestivo-autumnal plasmodium, or *Plasmodium falciparum*, in its earliest intracorpuscular stage of development, is noted within or upon the infected erythrocyte as a hyaline "ring" or disc, from 2 to 3 microns in diameter, well defined and sluggishly motile, the periphery of the plasmodium undulating and sending forth minute pseudopodia at irregular intervals. By reason of the amoeboid motion the "ring-forms" frequently become disc-like in shape. The rings are somewhat irregular in shape, one portion of the ring being considerably broader than the remainder, thus giving rise to the so-called "signet-ring" form, an appearance practically never observed in the quotidian aestivo-autumnal "rings." Infection of the erythrocyte with more than one parasite occurs but not as frequently as with the quotidian plasmodium, and it is rather rare to find more than two plasmodia in a corpuscle, while in the quotidian sub-species three, and even four, plasmodia are quite frequently observed within an infected erythrocyte. The ring-forms of this species are

frequently as large as the ring-forms of *Plasmodium vivax* but are distinguished from those of the latter species by the thickening at one portion of the periphery giving rise to the "signet-ring" appearance and by the thicker appearance of the ring-form, the ring-form of the benign tertian plasmodium being very thin and delicate in appearance.

The ring-forms gradually increase in size until, at the end of from sixteen to eighteen hours, they may measure as much as 3.5 microns in diameter, and at this time a few fine grains of reddish-brown or almost black pigment may be observed, generally lying in the enlarged area of the ring-form. The pigment is apparently sluggishly motile, the motility, as in all malaria plasmodia, being due to cytoplasmic currents within the body of the parasite. After the development of pigment the ring-form is soon lost, the parasite increases in size, becomes more clearly defined, the cytoplasm appearing very refractive and slightly granular under very high magnifications. As the parasite develops the pigment tends to collect in a more or less solid mass, at or near the center of the organism. After the development of pigment the amoeboid motility of the parasite is retained for several hours but is sluggish in character and gradually disappears.

In the usual infection only the ring-forms and pigmented ring-forms are observed in the peripheral blood, the larger pigmented forms occurring only in severe infections, unless a very careful and prolonged examination of the blood be made, when, even in average infections, a very few pigmented forms can generally be demonstrated.

When fully developed, just prior to sporulation, the pigmented forms of *Plasmodium falciparum* occupy from two-thirds to three-quarters of the infected erythrocyte and sometimes organisms are noted that practically fill the infected cell. Sporulation occurs every forty-eight hours in uncomplicated infections, but may be delayed for as long as fifty hours or occur as early as forty-four to forty-six hours, but it never occurs in twenty-four hours as claimed by those who believe in only one species of aestivo-autumnal plasmodium. At the time of sporulation the

parasite generally fills from two-thirds to almost the entire erythrocyte, the pigment being collected in a solid, oval or spherical mass at or near the center. The merozoites or spores appear as hyaline, refractive, oval bodies, collected about the mass of pigment in a more or less regular manner. It is difficult to determine their number in the fresh unstained blood preparations but careful examinations made by myself, have shown that they may vary in number from 10 to 30, the average number varying between 18 and 24. Sporulation occurs within the infected erythrocyte but not infrequently the infected cell is so entirely filled with the sporulating organism that very little of it can be observed. The sporulating forms occur very rarely in the peripheral blood, and only in very severe or pernicious infections, but in blood obtained by splenic puncture these forms may be found in large numbers.

The infected erythrocyte, in infections with *Plasmodium falciparum*, is never enlarged, as it invariably is in infections with the benign tertian plasmodium, but is usually slightly smaller than the normal blood corpuscles and of a slightly darker green color. Crenation of the infected cells is frequently observed, especially in those corpuscles containing nearly fully developed or sporulating parasites, but the wrinkled, shrunken appearance so frequently observed in erythrocytes infected with *Plasmodium falciparum quotidianum* is never observed, in my experience.

Bass (14) states that in cultures *Plasmodium falciparum* develops to almost the diameter of the red blood corpuscle and often produces 24 or more merozoites. He also calls attention to the fact that the ring-form is frequently as large as that of the benign tertian plasmodium and may be considerably thicker and heavier in appearance. In fact, most of the ring-forms of this species possess a larger amount of cytoplasm than do those of *Plasmodium vivax*, so that in examining blood containing only these forms, one is very apt to believe them to be tertian plasmodia rather than aestivo-autumnal, owing to the almost universal, although erroneous teaching, that the ring-forms of *Plasmodium vivax* are larger than those of *Plasmodium falciparum*.

Plasmodium falciparum quotidianum, in unstained preparations of blood, examined immediately upon removal of the blood from the infected individual, is first noted in the infected erythrocyte as a very minute hyaline ring, generally a little over 0.5 micron in diameter, though frequently parasites are observed that measure scarcely 0.5 micron in diameter. The very minute size of the ring-forms of this species in their earliest stage of development in man is an important differential point and it is undoubtedly true that the parasite is generally overlooked at this time unless one is an expert in the examination of blood from malarial infections, owing to the minuteness of the rings.

At first the outline of the ring is indistinct but it soon becomes well defined and amoeboid motility develops, the periphery of the ring sending out minute pseudopodia at irregular intervals, the motion being so rapid that only a careful examination will detect it. The typical ring-form is often lost during the periods of amoeboid motility, the parasite appearing triangular or disc-like in shape, but amoeboid activity is not constant and for long periods of time may be absent, the typical ring-form being retained.

In from two to four hours the ring-forms increase in size to about one micron in diameter and become more distinct and refractive. The outline of the ring is more delicate than in the ring-forms of *Plasmodium falciparum*, owing to the smaller amount of cytoplasm, and the "signet-ring" appearance, so common in the ring-forms of the latter species is not observed in the ring-forms of *Plasmodium falciparum quotidianum*, the ring appearing of the same thickness throughout, and consisting of a delicate hyaline ring of cytoplasm enclosing a minute spherical area of the same color as the infected corpuscle. At this time amoeboid activity is not as marked as in the earlier stage of development and is often entirely absent. I have never observed pigment in a ring-form of this species but in infections with *Plasmodium falciparum* the ring-forms are frequently pigmented, as already noted. Double and triple infections of the red blood corpuscle frequently occur and in fatal pernicious cases some of the red blood corpuscles may contain as many as five or six of the ring-forms.

When pigmentation occurs the ring-form is lost, the parasite appearing as a hyaline, oval or spherical disc containing one or two rather coarse granules of dark brown or almost black pigment, situated at the periphery or near the center and perfectly motionless. At this stage of development the organism seldom fills more than one-fifth of the infected erythrocyte, which is shrunken and crenated in appearance and of a dark olive-green color.

After the appearance of pigment the parasite gradually increases in size until at the end of twenty-two to twenty-four hours it fills from one-third to one-half of the infected erythrocyte. The pigment is collected in a very small solid block, spherical or irregular in shape, at or near the center of the organism, which is well defined and refractive. Amoeboid activity has entirely ceased and evidences of sporulation are noted, consisting in delicate striations dividing the parasite into several minute spores or merozoites. Owing to their very minute size, generally less than 0.5 micron in diameter, it is very difficult, and often impossible, to distinguish the number of the merozoites in living, unstained specimens, but in stained preparations they are found to number from 6 to 18, the average being from 12 to 14, as shown by my counts of many hundred sporulating plasmodia of this sub-species.

In *Plasmodium falciparum quotidianum* sporulation occurs at the end of twenty-four hours and I have never observed a case of infection with this plasmodium in which it was delayed for more than an hour or two or occurred earlier than twenty-two hours. In uncomplicated infections sporulation always occurs every twenty-four hours, thus differentiating it from *Plasmodium falciparum*, in which sporulation occurs every forty-eight hours.

At the time of sporulation the plasmodium fills about one-half of the infected erythrocyte, in the vast majority of infections, but sometimes not more than one-third of the infected cell is occupied by the parasite. I have never observed quotidian plasmodia practically filling the erythrocyte as is common in infections with *Plasmodium falciparum*. At this stage the

infected erythrocyte is generally considerably distorted in shape, shrunken in appearance, of a dark olive-green color, and often the hemoglobin appears retracted about the plasmodium.

In infections with the quotidian aestivo-autumnal plasmodium the invaded erythrocyte always presents marked morphological changes due to the growth and development of the organism. These changes are more marked than in infections with *Plasmodium falciparum* and consist in reduction in size, a crenated and shrunken appearance of the cell, and a change in color to a very dark olive-green or "brassy" appearance. The so-called "brassy bodies," i.e., infected erythrocytes presenting a shrunken appearance and of a brass-like color, are more frequently observed in infections with the quotidian aestivo-autumnal plasmodium than with the tertian species, but they are not characteristic of quotidian infections.

Bass (15) states that in cultures the quotidian aestivo-autumnal plasmodium produces about 16 merozoites at the time of sporulation and that the sporulating plasmodia occupy about one-half or slightly more of the infected erythrocyte, while the sporulating forms of the tertian aestivo-autumnal plasmodium fill almost the entire cell. He also calls attention to the very small ring-forms, the ring-forms of the tertian species being much larger.

Stained preparations

The staining reactions of the quotidian and tertian aestivo-autumnal plasmodia are similar, and when Wright's stain or other modification of the Romanowsky stain is employed, the cytoplasm stains a robin's egg blue while the chromatin of the nucleus stains a ruby-red or violet, depending upon the time that the stain is allowed to act.

The young ring-forms of the tertian aestivo-autumnal plasmodium, or *Plasmodium falciparum* present a ring of blue-stained cytoplasm containing one or more dots of chromatin. The comparative thickness of the ring-form of this species is well shown in stained preparations and many of the rings present a marked enlargement, generally situated at a point directly opposite the

dot of chromatin. The chromatin, stained a ruby-red or violet, occurs in the form of one or more spherical dots, situated at the thinnest portion of the ring. While one dot of chromatin is most common, in some infections there occur two dots of this substance, situated close together or at widely separated portions of the periphery of the ring, and rarely more than two dots are observed. Sometimes the two dots of chromatin are in apposition or definitely merged into one another.

In some of the ring-forms of this species pigment may be observed lying in the expanded portion of the ring and appearing as greenish brown, minute granules in stained preparations. Pigmented rings can generally be distinguished in stained preparations if carefully searched for, but such ring-forms are never observed in the quotidian sub-species.

In stained preparations the ring-forms of *Plasmodium falciparium* vary from two to three microns in diameter, but may, in isolated instances, measure as little as one and one-half and as much as three and one-half microns in diameter. They are characterized by their large size, as compared with the quotidian sub-species, the larger amount of cytoplasm, which is expanded at some portion of the periphery of the ring-form, in the vast majority of plasmodia, and by the presence, frequently, of pigment in the expanded portion of the ring.

The larger, pigmented plasmodia of this species present a blue-stained cytoplasm in which may be observed a small amount of chromatin in the form of fine grains collected within an unstained vesicular area representing the nucleus. The pre-sporulating plasmodia consist of a mass of blue-stained cytoplasm in which lie small, irregular clumps of ruby-red chromatin and a small amount of greenish black pigment collected in an irregular mass near the center of the parasite.

The sporulating forms, in stained preparations, almost fill the infected erythrocyte and measure from 5 to 6 microns in diameter. The spores, or merozoites, are arranged more or less regularly around a mass of greenish pigment, each merozoite consisting of a small blue-stained, oval or spherical mass of cytoplasm containing a spherical ruby-red dot of chromatin. The

merozoites can be easily counted and vary in number from 10 to 30, the average running between 18 and 24.

The infected erythrocyte, in stained preparations, may or may not appear crenated and stains a pink color. A few basophilic granules may be noted frequently, the so-called Maurer's dots. As a rule, the infected erythrocyte is not reduced in size or distorted in shape as observed in most stained preparations.

Plasmodium falciparum quotidianum, in stained preparations, may be readily distinguished from *Plasmodium falciparum*. The ring-forms are characterized by the small amount of cytoplasm and the relatively large amount of chromatin, while the larger forms are distinguished by their small size and the smaller number of merozoites in the sporulating plasmodia.

The smallest intracorpuscular forms of this sub-species are so very minute that they may be overlooked, in stained preparations, even by those who are more or less expert in examining malarial blood. They measure as little as 0.5 micron, or even less, in diameter, the smallest consisting of a minute granular mass of chromatin, stained pink or red in color, enclosed in a very minute amount of cytoplasm, stained blue, but so small in quantity that it requires the most careful examination to detect it. In this stage of development these plasmodia may be mistaken for a very small blood-plate or mere cellular detritus, as the true ring-form is not present, and it is undoubtedly the fact that at this time the plasmodium is very frequently overlooked or mistaken for something else.

At a slightly later stage of development the true ring-form is present but is often so very minute that very careful examination is necessary in order to demonstrate it. In these minute rings the cytoplasm is represented by a delicate blue-stained ring which contains somewhere along its circumference an irregular, semi-lunar mass of red or violet stained chromatin. The amount of chromatin is very large as compared with the amount of cytoplasm and ring-forms are frequently observed in which the chromatin comprises one-half or even more of the ring, and sometimes almost the entire plasmodium appears to be composed of chromatin.

In this sub-species the chromatin is not arranged in the form of a spherical dot or dots at some portion of the circumference of the ring, but usually occurs as a semi-lunar shaped mass forming a portion of the ring, in some instances almost the entire ring being formed of one or more of these semi-lunar masses of chromatin, practically no cytoplasm being visible. The center of the ring, i.e., the portion of cytoplasm enclosed by the ring-form, is absolutely colorless and the plasmodia look as though they were stamped into the substance of the infected erythrocyte with a punch. This appearance is very characteristic of the ring-forms of *Plasmodium falciparum quotidianum* in stained preparations, the ring-forms of *Plasmodium falciparum* presenting an entirely different appearance, the center of the ring staining pink, as does the remainder of the erythrocyte. In pernicious infections with the quotidian aestivo-autumnal plasmodium almost every infected corpuscle will present this colorless spot surrounded by the cytoplasm and chromatin of the parasite and when multiple infection of the erythrocytes occurs, as is frequently observed in such infections, the invaded erythrocytes present a very characteristic appearance, looking as though they were filled with small holes, each surrounded by the cytoplasm and chromatin of the tiny plasmodium. The fully developed ring-forms do not show the unstained central portion mentioned, the enclosed portion of the erythrocyte staining pink. The fully developed ring-forms present a larger amount of cytoplasm forming a portion of the ring but even in these forms the relative amount of chromatin to cytoplasm is greater, and it appears as irregular ragged masses at some portion of the periphery of the ring or as a semilunar shaped mass or masses forming a considerable portion of the ring. As in the smaller ring-forms, some of the fully developed rings appear to be composed almost entirely of chromatin, due to the large size of the semi-lunar mass or the merging of two such masses. Such forms are illustrated in figures 1, 3, 5 and 9.

This peculiar richness of the ring-forms of the quotidian subspecies in chromatin, and its arrangement within the ring, is a most important differential feature, for in the ring-forms of the

tertian species the chromatin is very small in amount in comparison with the amount of cytoplasm and is arranged in the form of one or two perfectly spherical dots at some portion of the periphery of the ring and never in irregular clumps or semilunar masses which comprise a large portion of the ring, as in the quotidian sub-species. The photomicrographs accompanying this contribution well illustrate the differences in the amount and arrangement of the chromatin in the two aestivo-autumnal plasmodia.

The pigmented and pre-sporulating forms of the quotidian plasmodium contain a comparatively small amount of blue-stained cytoplasm and a relatively large amount of chromatin, arranged in the form of irregular granular clumps or threads scattered throughout the cytoplasm. Pigmented ring-forms are never observed, as in the tertian species, the ring-form being lost before pigmentation begins. In the pre-sporulating plasmodia the chromatin is situated irregularly throughout the cytoplasm while the pigment is collected in a small spherical or irregular mass at or near the center of the plasmodium. The pigmented and pre-sporulating forms are small, seldom exceeding three microns in diameter, generally measuring about two microns in diameter and filling less than one-half of the infected erythrocyte.

The sporulating quotidian plasmodia measure from two and a half to three microns in diameter, in the vast majority of instances, but smaller and slightly larger forms may be observed in some infections. They fill about one-half or a little more of the infected erythrocyte and appear to be composed mostly of chromatin owing to the fact that the merozoites are composed very largely of this material. The plasmodia at this stage of development are seen to consist of numerous oval or spherical collections of very finely granular chromatin, stained a pink or reddish-violet, each imbedded in a very minute mass of blue-stained cytoplasm. The pigment appears almost black in stained preparations and is collected in a solid block or irregular clump at or near the center of the sporulating plasmodium.

When sporulation is complete the merozoites, or spores, are visible as distinct oval or round, very minute bodies, stained a dark red or violet and surrounded by a minute amount of blue-stained cytoplasm. They are very small, not measuring more than one-half micron in diameter in stained preparations, and it is often very difficult to determine their exact morphology owing to their minute size. They are characterized by the relatively large amount of chromatin, the merozoites generally appearing to be composed almost entirely of this substance, and the amount of cytoplasm is so small that it is often almost invisible. The fact that the merozoites of *Plasmodium falciparum quotidianum* are so largely composed of chromatin distinguishes them from the merozoites of the tertian aestivo-autumnal plasmodium, in which the chromatin is limited to a small dot situated in a relatively large amount of cytoplasm.

The number of merozoites varies considerably in different plasmodia but in the counts that I have made of many hundred sporulating plasmodia of this sub-species, I have never observed more than eighteen merozoites nor less than 6, the average running between 12 and 14, the latter number being most frequently encountered in individual plasmodia. As already stated the sporulating plasmodia seldom fill more than one-half of the infected erythrocyte while the sporulating forms of *Plasmodium falciparum* fill practically the entire blood corpuscle in the majority of instances and as many as 24 merozoites may be commonly observed.

In stained preparations the erythrocytes infected with the quotidian sub-species are almost always distorted in shape and the cytoplasm stains poorly, basophilic granulation often being present. The characteristic unstained portion of the cell surrounded by the ring-form has already been noted, and is probably due to a vacuole. It constitutes one of the most important differential points in the diagnosis of the quotidian aestivo-autumnal plasmodium at this early stage of development, as it is never observed in the cells infected with the ring-forms of the tertian aestivo-autumnal plasmodium. In the latter the substance of the erythrocyte enclosed by the ring-form may stain

poorly but there is never the absolute lack of color and the "hole-like" appearance that is noted in infections with the quotidian sub-species.

Double, triple, and quadruple infections of the erythrocyte with quotidian ring-forms are observed and double and triple infections are very common, much more so than in infection with the tertian species. Owing to their distorted shape it is often impossible to determine whether the erythrocytes infected with the quotidian species are reduced in size but in cells that have preserved their shape it is noted that there is a considerable reduction in size, as compared with uninfected cells surrounding them.

The *gametes* of *Plasmodium falciparum* and *Plasmodium falciparum quotidianum* are practically indistinguishable except in size. They are crescentic in shape, the so-called "crescents" and in both fresh and stained preparations of blood their morphology and staining reactions are the same. However, the gametes of the quotidian sub-species are smaller than those of the tertian species, by at least one-third, and are more plump, both the microgametocyte and the macrogametocyte being kidney-bean in shape rather than crescentic.

SUMMARY

The differential diagnostic points in the morphology and development of *Plasmodium falciparum* and *Plasmodium falciparum quotidianum*, as observed in the blood of man and mentioned in this contribution are best summarized in tabular form and the following table has been prepared and will be found useful in differentiating the aestivo-autumnal plasmodia. It should be remembered that the differentiation of these plasmodia does not rest upon any single morphological peculiarity but rather upon the summation of all the differential points considered.

Differential diagnosis of the aestivo-autumnal malarial plasmodia (stained preparations)

PERIOD OF DEVELOPMENT	PLASMODIUM FALCIPARUM	PLASMODIUM FALCIPARUM QUOTIDIANUM
1. Length of cycle in man	Forty-eight hours	Twenty-four hours
2. Earliest intracorpuscular stage	Ring-form. Average diameter, 1.5 microns	Minute oval body. Average diameter 0.5 micron
3. Morphology of ring-form		
a. Size	1.5 to 3.5 microns in diameter	0.5 to 1 micron in diameter
b. Cytoplasm	Well defined. Relatively large in amount	Poorly defined. Relatively very small in amount
c. Chromatin	Relatively small in amount, composed of one or two spherical dots	Relatively large in amount, composed of irregular or semi-lunar masses, forming a large part of the ring-form
d. Pigment	Present. Fine grains in expanded portion of ring	Never present in the ring-form
e. Effect on erythrocyte	Slightly reduced in size	Reduced in size. Distorted in shape frequently. Peculiar hole-like appearance in portion enclosed by ring-form
4. Morphology of pigmented and presporulating forms		
a. Size	1.5 to 6 microns in diameter	1 to 3 microns in diameter
b. Cytoplasm	Large in amount. Well defined	Small in amount and poorly defined
c. Chromatin	Relatively small in amount	Relatively large in amount
d. Pigment	Small granules and irregular clumps	Smaller in amount. One or two solid blocks
e. Effect on erythrocyte	Reduced in size	Reduced in size and distorted in shape

PERIOD OF DEVELOPMENT	PLASMODIUM FALCIPARUM	PLASMODIUM FALCIPARUM QUOTIDIANUM
5. Morphology of sporulating forms		
a. Size	5 to 6.5 microns in diameter	3 to 3.5 microns in diameeter
b. Cytoplasm	Well defined. Large in amount	Poorly defined owing to small amount
c. Chromatin	Each merozoite has a small spherical dot	Each merozoite is composed almost entirely of chromatin
d. Pigment	Irregular mass	Solid minute block
e. Effect on erythrocyte	Reduced in size. Sporulating plasmodium almost fills erythrocyte	Distorted in shape. Slightly reduced in size. Sporulating plasmodium fills only about one-half or slightly more of the erythrocyte
f. Number of merozoites	10 to 30	6 to 18

DIFFERENTIAL DIAGNOSIS

Clinical

As I have stated, not only do *Plasmodium falciparum* and *Plasmodium falciparum quotidianum* differ markedly morphologically but they also differ in the clinical symptoms produced in the human host by their presence. In fact, there is just as much difference in the clinical type of infection produced by these two parasites as there is in the clinical infections due to *Plasmodium vivax*, the benign tertian plasmodium, and *Plasmodium malariae*, the quartan plasmodium. The clinical symptoms produced by the latter plasmodia do not differ essentially except in the time at which the febrile paroxysms occur and the character of the temperature curve, and the same statement is true of infections with the two aestivo-autumnal plasmodia, except that the character of the temperature curve differs even more strikingly in infections with these plasmodia than in those with the benign tertian and quartan plasmodia.

Relative frequency of occurrence

Just as infections with the quartan malaria plasmodium are rare as compared with infections with the benign tertian parasite, so are infections with the quotidian aestivo-autumnal plasmodium as compared with infections with the tertian species. In 1662 aestivo-autumnal infections that I personally studied, 1473 were caused by *Plasmodium falciparum* and only 189 by *Plasmodium falciparum quotidianum.* These infections were all observed in patients coming from localities where both species were present but there are many localities where the tertian aestivo-autumnal plasmodium occurs and where the quotidian sub-species has not been observed. Thus, at Camp Stotsenburg, Philippine Islands, both aestivo-autumnal plasmodia were present, although the quotidian sub-species was comparatively rare, but at Camp McKinley, some sixty miles from Stotsenburg, the quotidian sub-species was never found although the tertian aestivo-autumnal species caused a considerable proportion of the malarial infections at that post.

The comparative rarity of the quotidian sub-species is one of the chief reasons for its not having been generally recognized as a distinct plasmodium, just as the rarity of the quartan plasmodium caused it to be overlooked for long periods of time in various localities. For instance, during the early years of work upon the Panama Canal it was officially reported repeatedly that the quartan plasmodium did not occur in the Canal Zone but later it was found that, while rare, as it is in almost every locality, it was present but had remained unrecognized. The same condition prevails today in many localities as regards the recognition of the quotidian aestivo-autumnal plasmodium and if one remembers the exceedingly minute size of the "ring-forms" of this sub-species, which are the forms usually noted in the peripheral blood, and the infrequency of infections with this parasite, I think that it is evident why so many observers in malarial regions have failed to note its presence or have not recognized it, when present.

Time of febrile paroxysm

In uncomplicated acute infections with *Plasmodium falciparum* the plasmodia sporulate every forty-eight hours and thus cause a febrile paroxysm every other day, while in uncomplicated acute infections with *Plasmodium falciparum quotidianum* sporulation occurs at the end of every twenty-four hours and the febrile paroxysm is quotidian in character. Unfortunately, it is rather exceptional to observe absolutely regular paroxysms in aestivo-autumnal infections, as previous treatment with quinine or the natural tendency of these infections to irregularity, due to anticipation or retardation of sporulation, often obscures the real periodicity of the paroxysms. However, in typical uncomplicated cases, *Plasmodium falciparum* causes a febrile paroxysm every forty-eight hours and *Plasmodium falciparum quotidianum* one every twenty-four hours.

In infections with the tertian aestivo-autumnal plasmodium sporulation may be observed as early as forty-two or forty-six hours and it may be retarded as long as fifty hours but I have never observed a case of infection with this species in which sporulation occurred in twenty-four hours, as it does in the quotidian sub-species. In infections with the latter plasmodium sporulation sometimes occurs as early as twenty-two hours or as late as twenty-six hours but I have never observed an infection with this plasmodium in which sporulation occurred later than twenty-six hours. In cultures Chamberland (16) states that *Plasmodium falciparum* sporulates at forty to forty-eight hours and Bass states that sporulation occurs at the end of forty-eight hours.

Character of temperature curve

Aside from the marked difference in the time of occurrence of the febrile paroxysm, the most typical clinical difference between acute infections with the two aestivo-autumnal plasmodia consists in the character of the temperature curve produced by infection with them. In uncomplicated cases the type of temperature curve produced by *Plasmodium falciparum* is absolutely

characteristic and unlike that met with in any other febrile condition, while that produced by the quotidian sub-species is a typical quotidian curve which differs in no particular from quotidian curves observed in many infections.

The peculiar and characteristic temperature curve noted in acute uncomplicated infections with *Plasmodium falciparum* was first carefully described by Marchiafava and Bignami and is well illustrated in the temperature charts that accompany this contribution, which were obtained from soldiers suffering from tertian aestivo-autumnal fever and who volunteered to go without quinine treatment in order that typical temperature charts might be secured.

Upon reference to these charts it will be noted that, at the onset of the fever, corresponding to the sporulation of the plasmodia, the temperature rises rapidly to 102°, 103° F. or higher. Following this rapid rise there occur slight oscillations in the temperature covering several hours, during which time it may fall from 0.5° to 1°. This period of oscillation is followed by a distinct fall, or pseudo-crisis, the temperature generally dropping from 1.5° to 2°, or even 3°, and this fall in the temperature is often mistaken for the true crisis. However, the temperature again rapidly rises, often to a height greater than that attained during the initial rise and then falls rapidly to normal or even below normal. When the pseudo-crisis is very marked the temperature may fall to almost normal and in these cases the temperature curve may resemble that due to the quotidian sub-species, there being apparently a quotidian rise in temperature.

From this brief description of the temperature curve in typical tertian aestivo-autumnal infections, it may be noted that it can be divided into five quite distinct stages, as follows: 1, the initial rise; 2, the period of oscillation; 3, the pseudo-crisis; 4, the pre-critical rise, and 5, the true crisis. It will also be observed, from a study of the temperature charts, that the actual febrile period in this type of infection is much longer than it is in infections with any other species of malaria plasmodium, covering practically the greater portion of forty-eight hours. In most acute

uncomplicated infections with this plasmodium the temperature actually remains elevated for from thirty-eight to forty hours, and sometimes even longer, whereas in other malarial infections the temperature is seldom above normal for as long as twenty-four hours unless there be double or triple infections. This point is of great value in differentiating infection with *Plasmodium falciparum* from infections with other malaria plasmodia.

The temperature curve in acute uncomplicated infections with *Plasmodium falciparum quotidianum* is of an entirely different character, consisting of a daily abrupt rise in temperature to 103° F. or more, succeeded by as abrupt a fall to normal, or below, the entire febrile paroxysm only lasting from eight to ten hours, in the vast majority of infections. However, the temperature curve in this type of infection often becomes irregular, the paroxysms tending to run into one another, thus giving rise to an irregular or almost continuous fever. The typical quotidian curve produced by this plasmodium is similar to the curves observed in a double infection with the benign tertian plasmodium and only a microscopical examination of the blood will show its true etiological nature. Thus, while it would be possible to diagnose a typical infection with *Plasmodium falciparum* by the character of the temperature curve alone, it would be impossible to do so in infection with the quotidian sub-species, as exactly similar curves may occur in multiple infection with either the benign tertian or the quartan plasmodium.

It should be remembered that while the temperature curves here described as characteristic of the two aestivo-autumnal plasmodia are those typical of each infection, marked variations in the curves are commonly observed which destroy their value for differential purposes. These deviations from the typical are due to many factors, the most important of which are insufficient medication with quinine; mixed infections with both aestivo-autumnal plasmodia or with the benign tertian or quartan plasmodium; double infections; anticipation or retardation of the paroxysms; slight elevations of temperature between the paroxysms, or complication with other acute or chronic infections.

In the study of malarial infections due to the aestivo-autumnal plasmodia, especially those due to *Plasmodium falciparum*, no dependence, so far as differential diagnosis is concerned, can be placed upon a temperature chart that records only the morning and evening temperature, for such charts are worse than useless because they are always misleading. In the study of these fevers the temperature should be taken and recorded every three or four hours and only by so doing can one secure a picture of the actual temperature curve in these cases. It is also necessary to omit the use of quinine if it is desired to obtain a typical chart and all of the temperature curves reproduced in this contribution were obtained from volunteers who received no quinine until the exact nature of the temperature was ascertained. As this procedure cannot be followed in actual practice, and as quinine, if given improperly, as it so often is, will render the most typical malarial temperature atypical, it is easily understood why so few really typical temperature curves of the two types of aestivo-autumnal infection are observed in general practice.

CONCLUSION

The evidence that the aestivo-autumnal malaria plasmodia comprise at least two distinct forms rests upon differences in morphology, in the length of the life-cycle, and in the clinical picture of the infections which are produced by them. The morphological differences between *Plasmodium falciparum* and *Plasmodium falciparum quotidianum* are as constant and distinctive as those between *Plasmodium vivax* and *Plasmodium malariae*, while the striking difference in the temperature curve caused by these two plasmodia still further serves to differentiate them. The quotidian plasmodium causes a simple intermittent quotidian curve, indistinguishable from that observed in a double infection with the benign tertian plasmodium, while the tertian aestivo-autumnal plasmodium causes a temperature curve which is most typical and which differs markedly from that observed in any other type of malarial infection and which is diagnostic in itself. When, in addition, we find always asso-

ciated with infections characterized by these varying temperature curves, distinctive plasmodia that differ morphologically and in their time of sporulation, these differences being constant and easily recognized by the trained observer, it must be admitted that the evidence is most convincing as to their specific character.

Unfortunately, there is no direct experimental evidence that these two plasmodia are distinct species, as we have no records of the experimental production of quotidian aestivo-autumnal malaria by direct inoculation of blood containing *Plasmodium falciparum quotidianum* into susceptible individuals or through the bites of experimentally infected mosquitoes, for while there are numerous successful experiments recorded of the production of aestivo-autumnal malaria both by direct inoculation of infected blood and by the bites of infected mosquitoes, no effort was apparently made to ascertain the exact species of aestivo-autumnal plasmodium used in the experiments, it apparently being taken for granted that there is but one species of this plasmodium.

In a recent publication Darling (17) describes two cases of experimental infection with the tertian aestivo-autumnal plasmodium in which the initial paroxysms were tertian or subtertian in character while subsequent ones became quotidian and uses these cases as an argument in support of the nonexistence of the quotidian sub-species. An inspection of the charts published in his paper shows, that beyond some irregularity, as would be expected in experimental infections, the temperature curves are essentially those of tertian aestivo-autumnal malaria, and that the administration of quinine in all probability produced the atypical features of the temperature noted after its administration. There is absolutely nothing in the charts of these experimental cases which would negative the existence of the tertian and quotidian aestivo-autumnal plasmodia, as he has evidently mistaken the typical temperature curve of tertian aestivo-autumnal infection, which practically covers forty-eight hours, with, in these cases, a very marked remission at the end of twenty-four hours (probably due to quinine) for quotidian periodicity. His statement that the

diagnosis of *Plasmodium falciparum quotidianum* was chiefly made before the Romanowsky stains were in general use is also hardly correct, as Marchiafava and Bignami used these stains in differentiating the two species and I have never employed any other stain than some modification of the Romanowsky, chiefly Wright's stain, in studying the malaria plasmodia.

I believe that the evidence summarized in this contribution is almost sufficient to entitle *Plasmodium falciparum quotidianum* to *full* specific rank, in which case, of course, the name would have to be changed, but until this can be absolutely proven by rigid experimental evidence I believe that this plasmodium should be regarded as a sub-species of *Plasmodium falciparum.*

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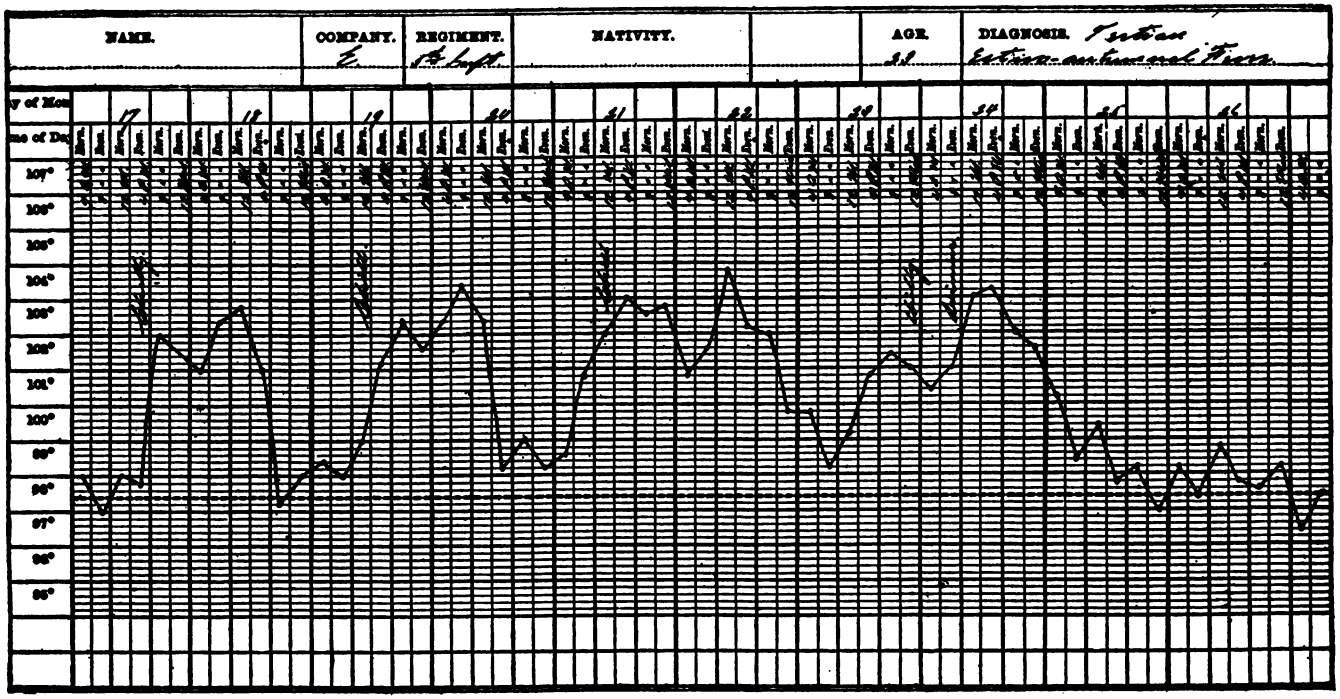


CHART I. TEMPERATURE CURVE OF TERTIAN AESTIVO-AUTUMNAL MALARIAL FEVER

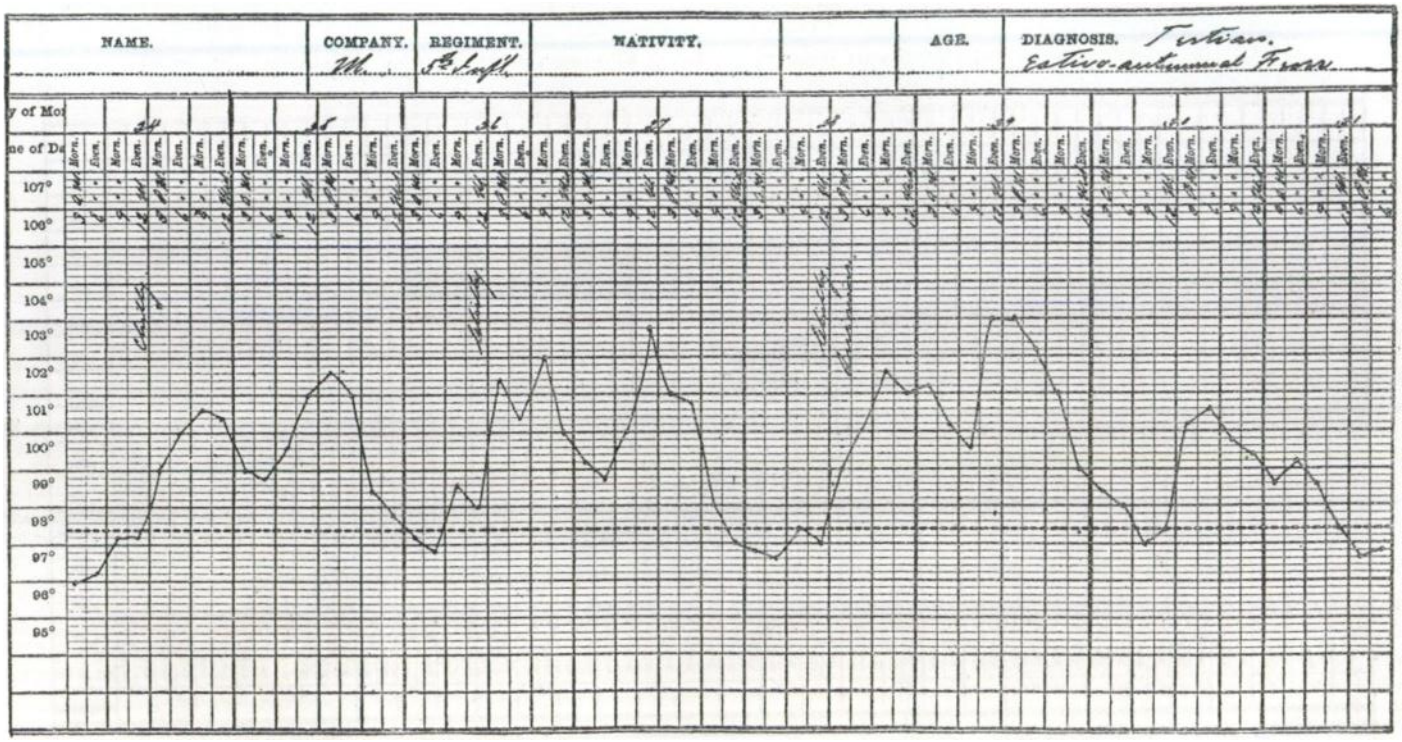


CHART II. TEMPERATURE CURVE OF TERTIAN AESTIVO-AUTUMNAL MALARIAL FEVER

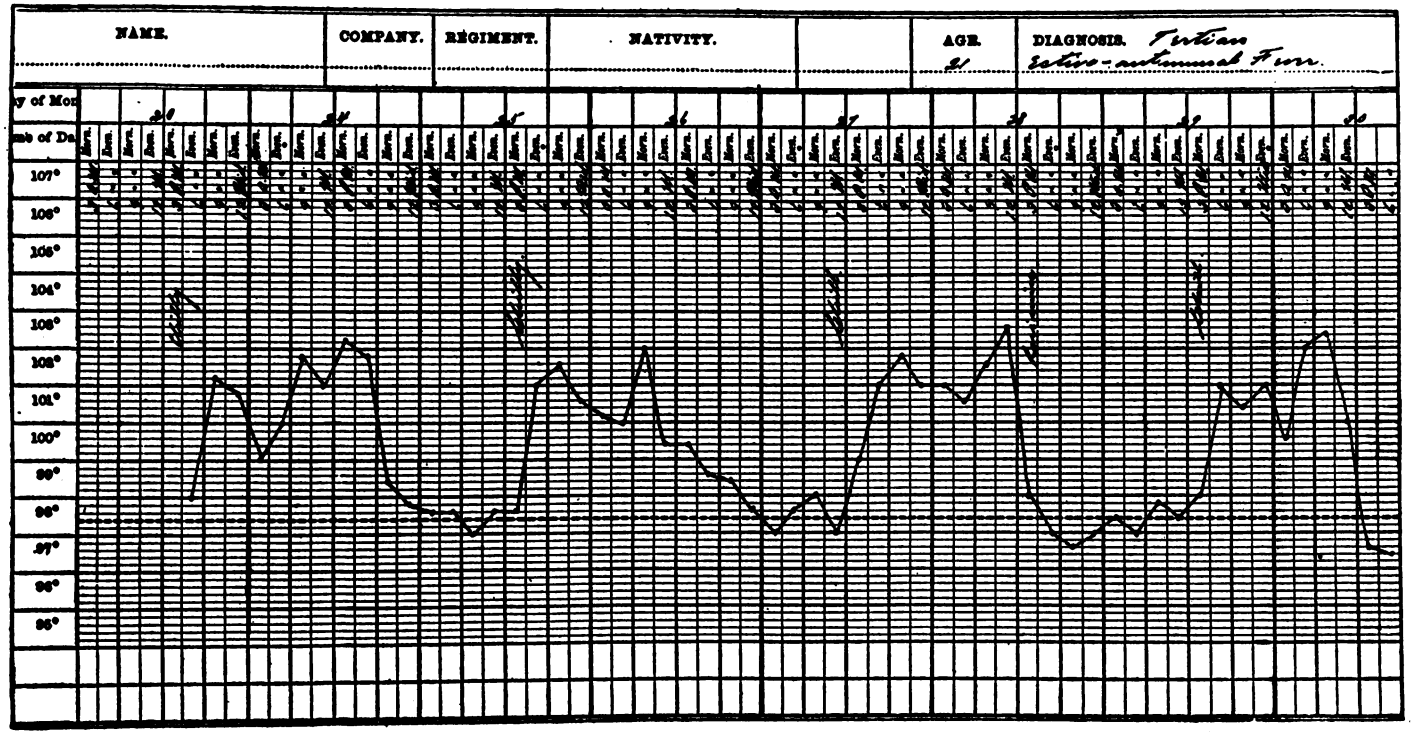


CHART III. TEMPERATURE CURVE OF TERTIAN AESTIVO-AUTUMNAL MALARIAL FEVER

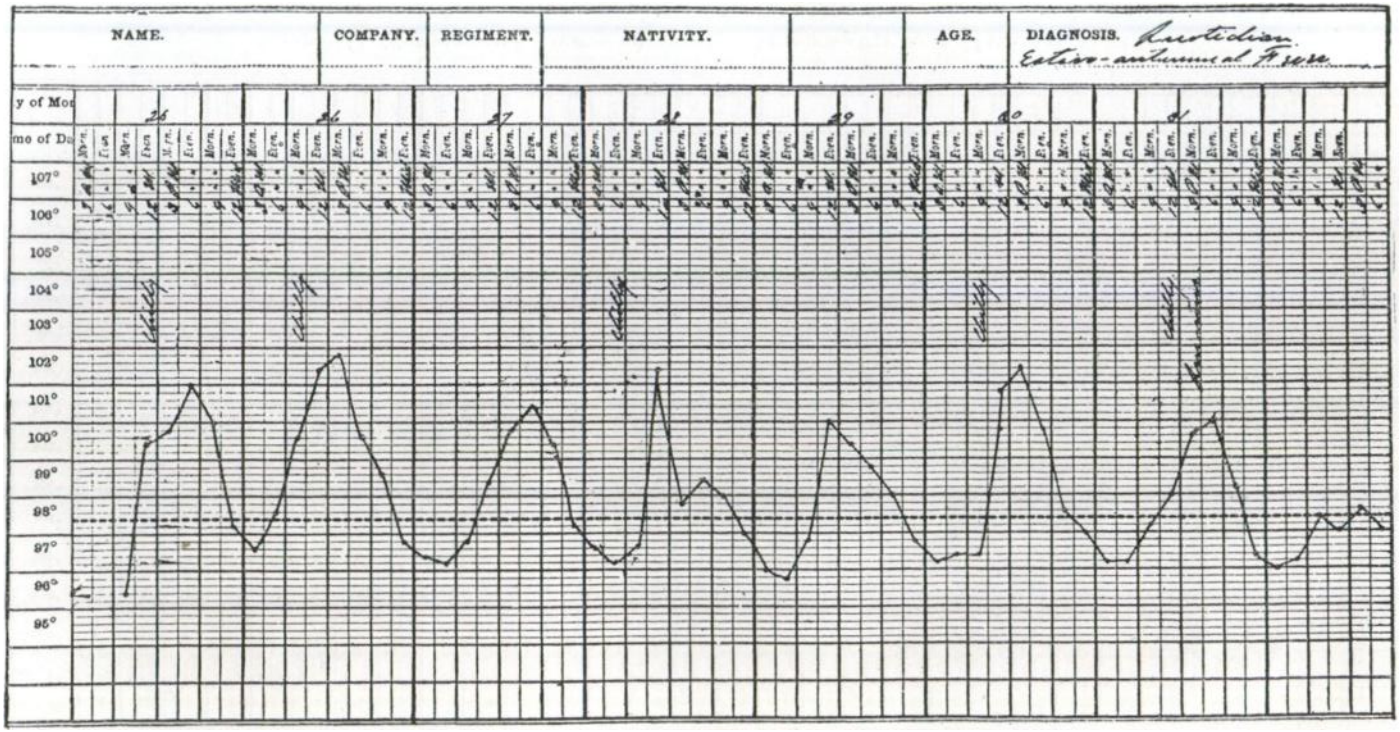


CHART IV. TEMPERATURE CURVE OF QUOTIDIAN AESTIVO-AUTUMNAL MALARIAL FEVER

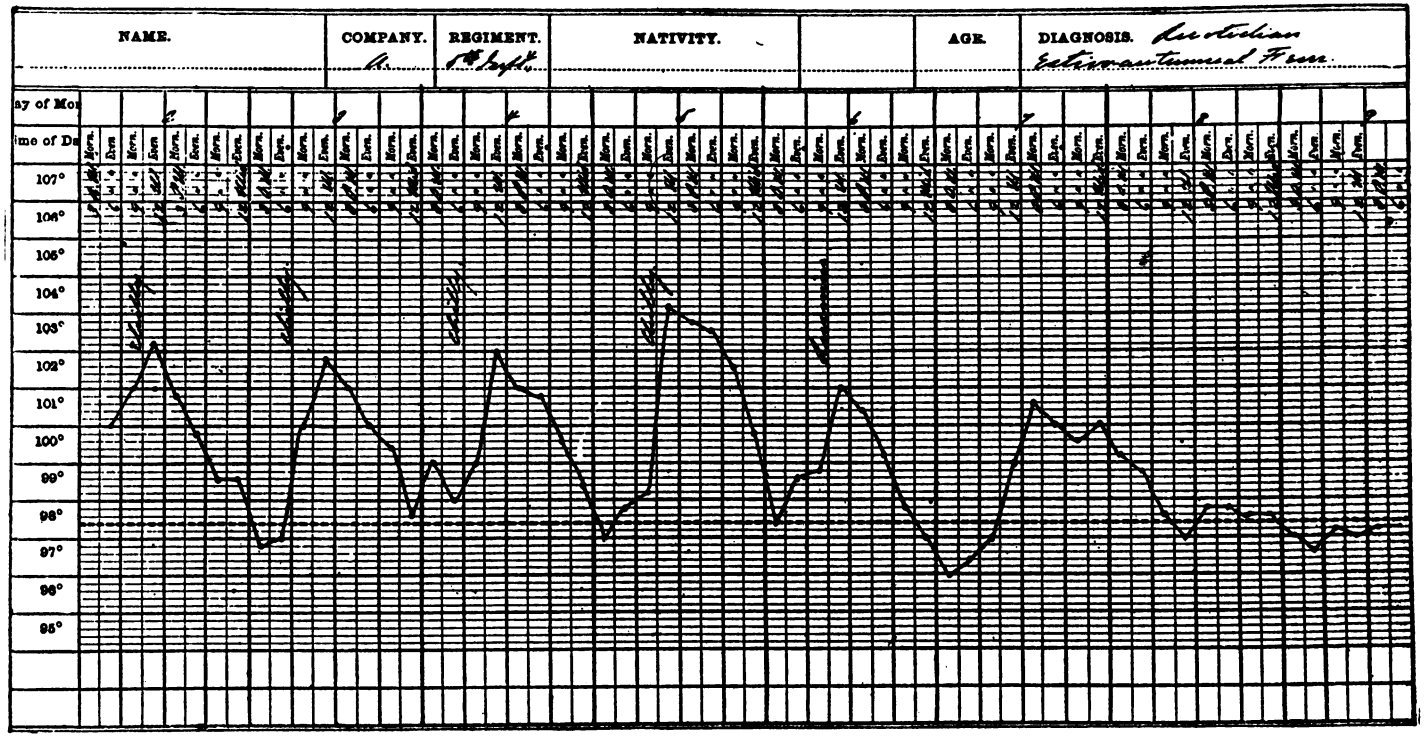


CHART V. TEMPERATURE CURVE OF QUOTIDIAN AESTIVO-AUTUMNAL MALARIAL FEVER

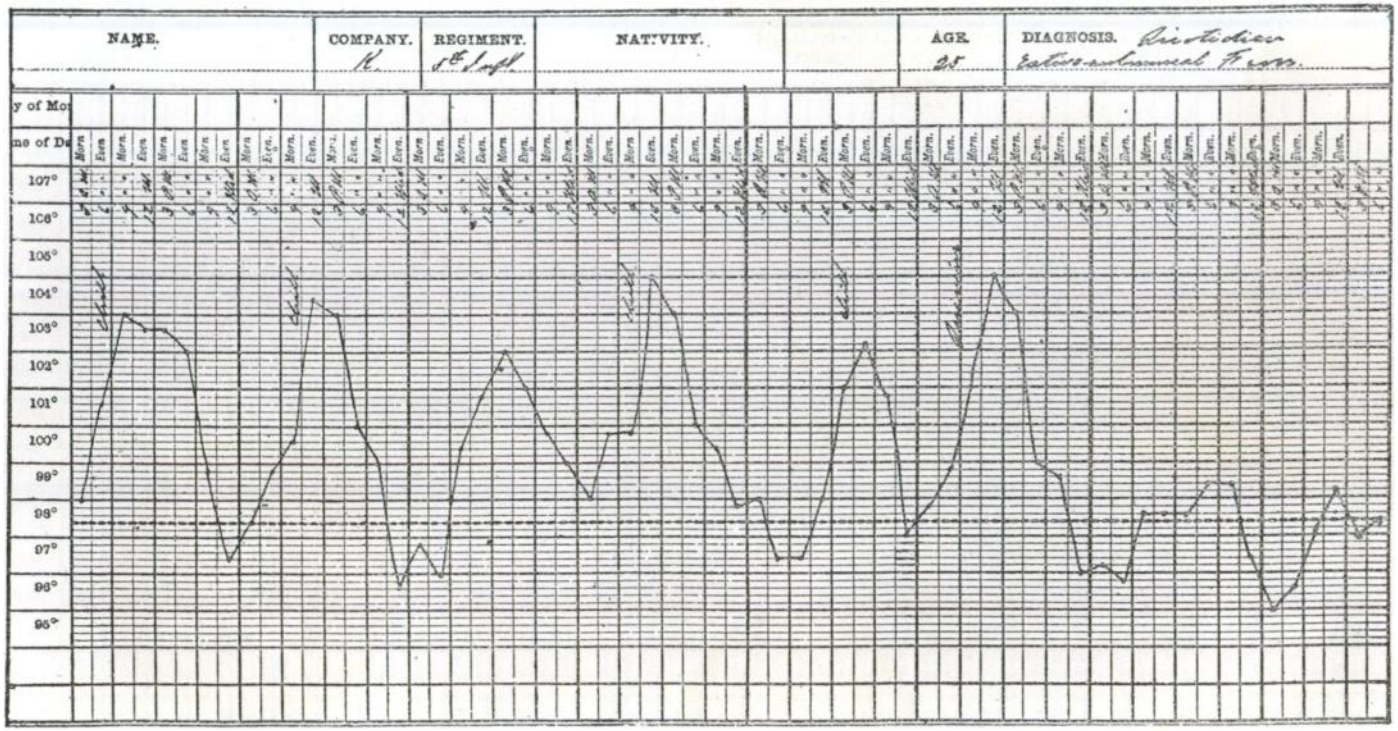


CHART VI. TEMPERATURE CURVE OF QUOTIDIAN AESTIVO-AUTUMNAL MALARIAL FEVER

EXPLANATION OF PLATES

The photomicrographs are of plasmodia observed in the peripheral blood of infections with the tertian and quotidian aestivo-autumnal parasites and are chosen to illustrate the chief morphological differences between them.

FIG. 1. *Plasmodium falciparum quotidianum.* Ring-forms. Note relatively large amount of chromatin, one of the "rings" appearing to be composed almost entirely of this material. (In all the photomicrographs the very dark staining portion of the plasmodia represents the chromatin.) Note minute size of parasite. × 1600.

FIG. 2. *Plasmodium falciparum.* Ring-form. Note dot of chromatin, and expanded portion of the "ring." Note large size of the ring compared with the ring-forms in figure 1. × 1600.

FIG. 3. *Plasmodium falciparum quotidianum.* Ring-forms. Note minute size and large amount of chromatin, comprising a large portion of the ring. Also "hole-like" appearance of portion of erythrocyte enclosed by the ring-forms. Triple infection of one erythrocyte. × 1600.

FIG. 4. *Plasmodium falciparum.* Ring-form. Note two small dots of chromatin, expanded portion of cytoplasm of the "ring," and the large size as compared with the ring-forms of the quotidian sub-species. The ring-form in this species fills almost as much of the erythrocyte as the three ring-forms of the quotidian parasite shown in figure 3. × 1600.

FIG. 5. *Plasmodium falciparum quotidianum.* Ring-forms. Note minute size, and large amount of chromatin. × 1600.

FIG. 6. *Plasmodium falciparum.* Typical ring-forms. Compare with ring-forms of quotidian plasmodium. × 1600.

FIG. 8. *Plasmodium falciparum.* Double infection of erythrocyte with ring-forms. Compare with Figure 7, noting relative size of plasmodia and morphology of the "ring." × 1600.

FIG. 9. *Plasmodium falciparum quotidianum.* Typical ring-forms. Note triple infection of erythrocyte and large amount of chromatin, arranged as a semi-lunar mass forming a considerable portion of the "ring." × 1800. Although magnified 200 diameters more than the ring-forms of *Plasmodium falciparum,* shown in Figure 8, the "rings" are still much smaller than those of the latter species and entirely different in morphology.

FIG. 10. *Plasmodium falciparum quotidianum.* Sporulating form and ring-forms. Note that sporulating form fills only about half of the erythrocyte. × 1600.

FIG. 11. *Plasmodium falciparum.* Half-grown pigmented form. Note that though only half grown it fills as much of the erythrocyte as the sporulating form of Plasmodium falciparum quotidianum. × 1600.

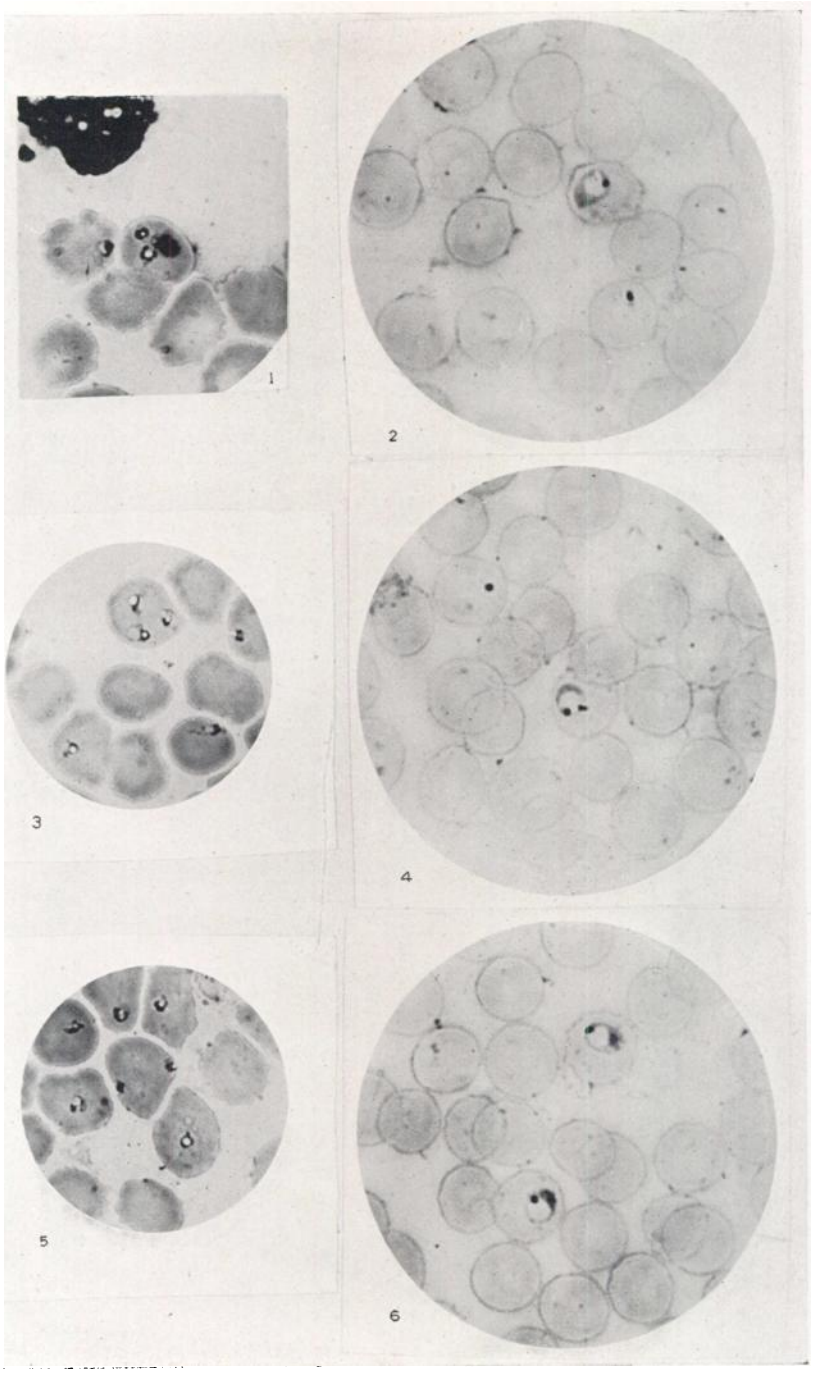
FIG. 12. *Plasmodium falciparum quotidianum.* Sporulating and ring-forms. Note that only half of the erythrocyte is filled by the sporulating form and the extremely minute size of the ring-form in the erythrocyte below and to the left of the sporulating form. × 1500.

FIG. 13. *Plasmodium falciparum.* Sporulating form. Note that the infected erythrocyte is almost entirely filled by the plasmodium. × 1500.

FIG. 14. *Plasmodium falciparum quotidianum.* An unusually large sporulating plasmodium filling slightly more than one-half of the erythrocyte. × 1800.

FIG. 15. *Plasmodium falciparum.* This plasmodium is near the pre-sporulating stage but almost fills the infected erythrocyte. It is shown for comparison with the quotidian plasmodium in figure 14 to illustrate the difference in the size and the amount of erythrocyte occupied by the parasites. Although the tertian parasite is only about two-thirds grown it occupies more of the infected erythrocyte than the sporulating quotidian parasite and is about the same size. × 1800.

NOTE: In the photomicrographs of the ring-forms of *Plasmodium falciparum* the deep staining of the expanded portion of the "ring" is not due to the presence of chromatin but to the comparatively large amount of cytoplasm which takes a deep blue stain. The chromatin in these ring-forms is shown as a spherical dot or dots generally opposite the expanded portion of the "ring."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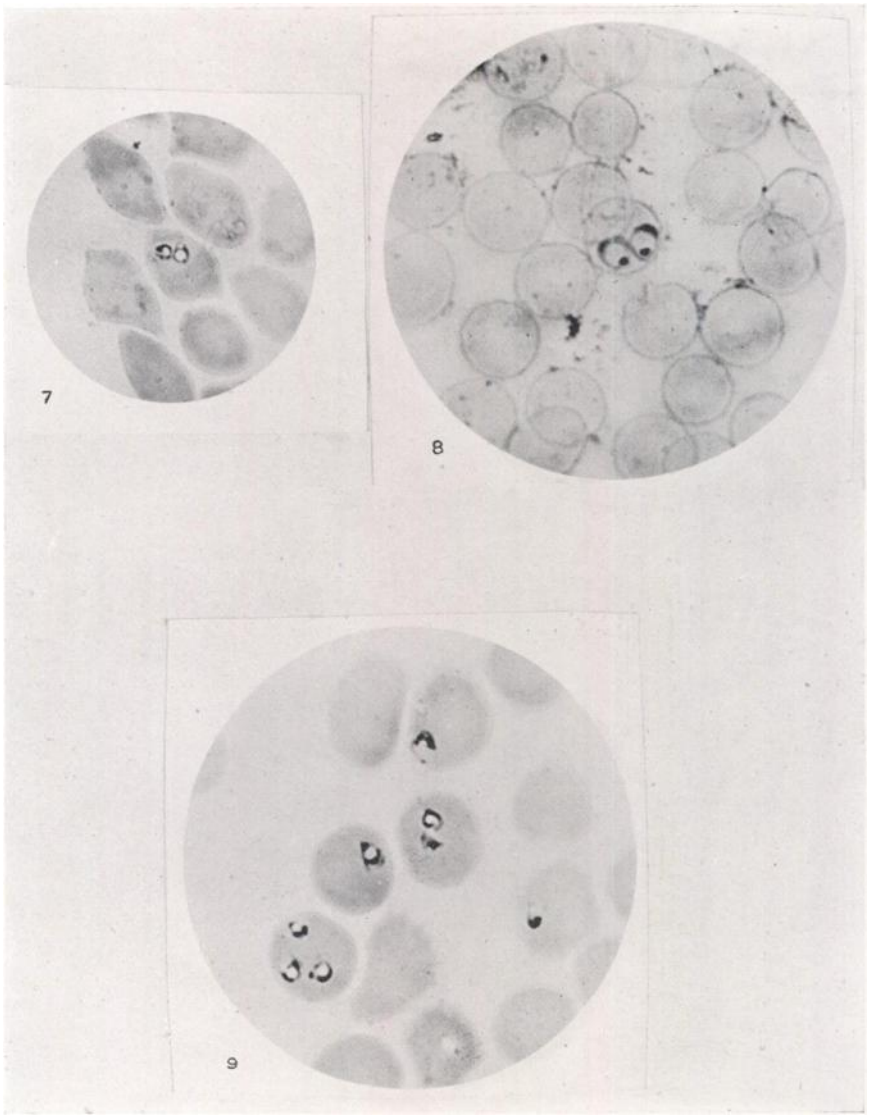
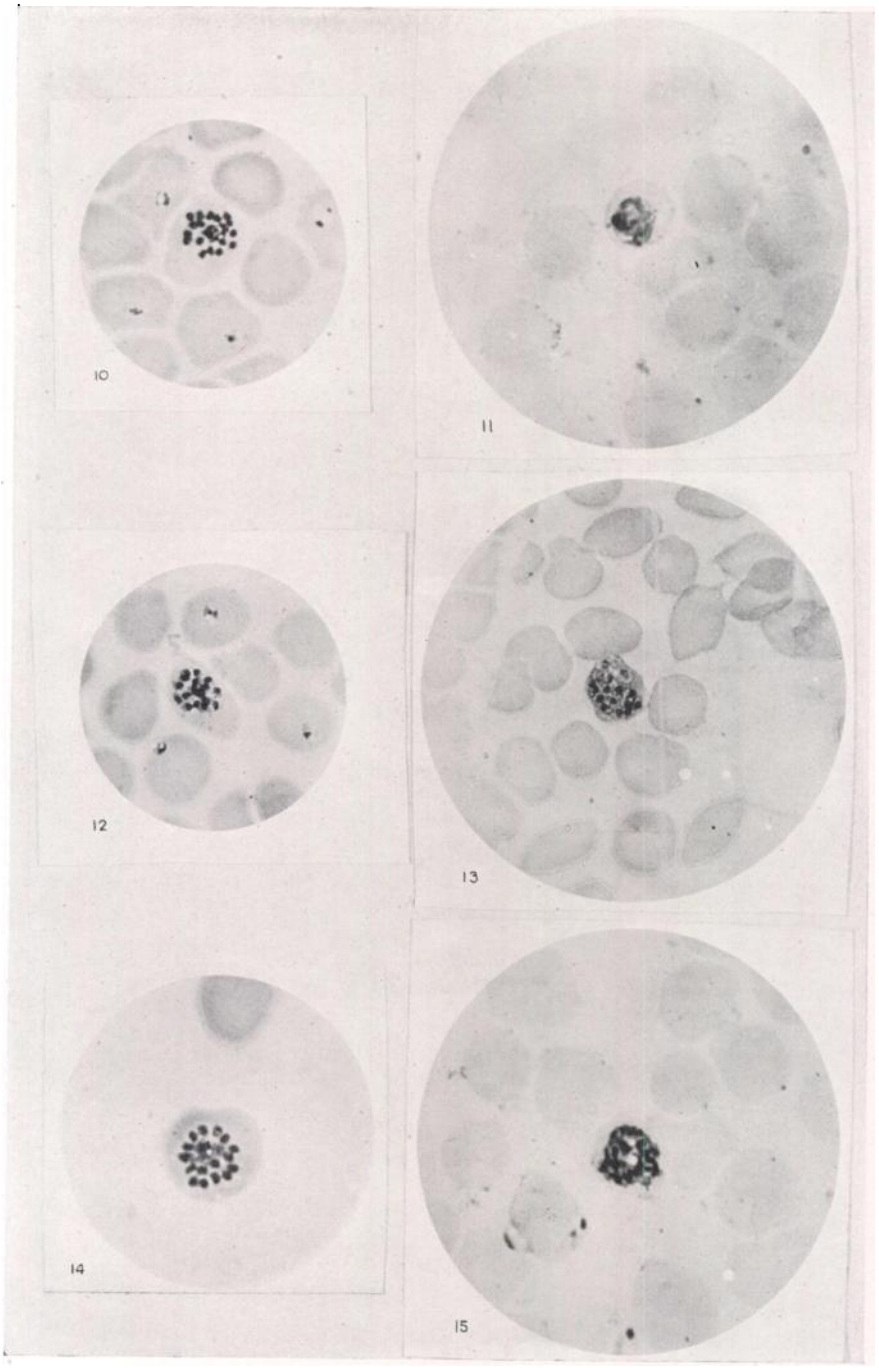
AESTIVO-AUTUMNAL MALARIA PLASMODIA

CHARLES F. CRAIG

PLATE II

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