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THE HISTORICAL DEVELOPMENT OF THE CURRENT THEORY ON THE MECHANISM OF INFECTION OF THE MOSQUITO BY THE MALARIA PARASITE

by

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For species of *Plasmodium* which have been studied, the most generally recorded theory of sporogony (e.g., Huff, 1949; Faust & Russell, 1957) is briefly as follows: Following the ingestion of parasitized blood by a mosquito, male and female gametocytes are released from their host erythrocytes, the microgametocyte exflagellates, the macrogametocyte is fertilized by a microgamete, and the resulting sphere is called a zygote. After a variable period of time, the zygote evolves into a migratory elongated organism called an "ookinete" (travelling egg) which moves about in the meal contents. The mature "ookinete" in the shape of a "vermicule" penetrates between the epithelial cells of the mosquito's stomach wall or "rounds up" to form an oocyst. The early oocyst then moves from the internal to the external side of the wall where it grows into the haemocele for a period of 10-12 days. Sporogenesis occurs within the oocyst. Mature sporozoites burst from the oocyst and ultimately pass to the salivary gland.

In order to determine the recorded evidence upon which this theory is based, particularly with reference to the mechanism of infection of the mosquito, the historical development of the current theory has been traced from its beginnings.

Ross's *Memoirs* (1923) present a detailed account of the studies on malaria sporogony up to 1923. From this source, it would appear that Laveran first described the phenomenon of exflagellation in the same year that he discovered the malaria parasite (1880). At that time, the source or significance of exflagellation was not understood. In 1894, Manson suggested that the flagellum might act as a spore to infect the mosquito.

In 1895, Ross worked with Plasmodium falciparum and made the following pertinent observations:

1. When an adult female mosquito was permitted to feed on a human case of malaria whose blood contained many crescents (gametocytes), examination of the stomach blood immediately following engorgement showed roughly the same proportion of parasites as in fresh-drawn finger blood.

2. Within minutes following engorgement, crescents rapidly decreased in number, and in their place spherules appeared.

3. "In pure (fresh) blood, in the marginal clot, the crescents change much more frequently than elsewhere; so I thought that the change in the mosquito might be due to the clotting of blood."

4. "Certainly crescents are very rare in mosquitos' stomachs after half-an-hour."

5. These resulting spherules were "perfectly circular contours" about six microns in diameter.

6. Exflagellation occurred in the mosquito's stomach within a half-hour following engorgement.

From these data, Ross evolved what he called the "crescent-sphere flagellum" metamorphosis of the malaria parasite within the mosquito stomach. At this time, he thought of the flagellum as a spore possibly responsible for infection of the mosquito.

In 1897, Ross discovered the oocysts in Anopheles infected with P. falciparum and also P. vivax. He called these "pigmented cells" and reasoned that they must represent a developmental stage of the malaria parasite because of their pigment, their increase in growth with time, and because they were not present in uninfected control mosquitos.

The earliest oocysts he reported were those of one of the human forms at two days. "It (the mosquito) contained a number of pigmented cells, all the same size and very small, about eight microns; the size of a red corpuscle and of an ordinary crescent-sphere before it emits its "flagella"." He reported the increase in "cell" size as follows: "6μ to 8μ at two days; 12μ-16μ at four days; up to 20μ at five days".

At this point, there were no direct observations on the sequence in the life-cycle between the "flagellum" and the oocysts. It was clear that the midgut wall did become infected, but the mechanism of infection and the parasitic stage at the time of infection were not yet ascertained.

In the same year, MacCallum (1897) published a report from the Johns Hopkins Hospital which seemed to solve the whole matter. He graphically described the process of exflagellation by Haemoproteus as seen in freshly drawn blood from crows, and identified the "flagellum" as the agent which fertilized the spherule. While MacCallum found the significance of exflagellation to be the same in the case of Plasmodium, his additional description of events following the fertilization process in Haemoproteus was generally accepted without experimental confirmation as being equally applicable for Plasmodium. He referred to his organism as the "halteridium of Labbe". The zygote was said to be quiet for 15-20 minutes following fertilization, after which a conical process developed. "This spindle-shaped organism moves forward with a gliding motion, sometimes turning at the same time on its long axis, sometimes going through amoeboid contortions." MacCallum, however, very specifically wrote in his article that he had watched exflagellation in two instances in blood from a woman with "aestive-autumnal fever", "... but although in the two instances in which this process was traced the fertilized form was watched for a long time, no form analogous to the vermiculus was seen".

Nonetheless, this analogy would seem to have appealed to Manson, who published a report on behalf of Ross entitled, "Surgeon-Major Ronald Ross's Recent Investigations on the Mosquito-Malaria Theory" (Manson 1898). He concluded with this statement: "Doubtless, what holds good for halteridium is equally applicable to the closely allied human Plasmodium; indeed though he has not witnessed the transformation of the plasmodial sphere into the travelling vermicule, MacCallum has actually seen and described its impregnation by the flagellum."

Ross confirmed MacCallum's observation by recording that he saw six moving vermicules of Halteridium obtained from crows (Ross 1923). But there is no record that Ross saw a moving vermicule in any species of Plasmodium. He wrote to Manson, "You say that if only you could find a vermicule entering a cell ...

there will be no difficulty in tracing the vermicule business by ordinary dissection and washing of the stomach[1]." The related footnote (Memoirs page 281) reads, "Owing to the third interruption, I never had time to do this easy but interesting little piece of work".

Thus, after this detailed historical review of malaria research up to 1923, Ross accepted without direct experimental evidence the theory that the fertilized sphere of human Plasmodium changed into a travelling vermicule which then penetrated the midgut wall. The idea that Ross's theory of penetration was based on reasoned assumptions, rather than on direct evidence, is suggested in the following statement from MacCallum's first edition (1916) Textbook of Pathology: "At this point Ross ... formed the idea that the process of fertilization and formation of a motile zygote described above must occur in the mosquito's stomach, and that the development of cysts in the walls of that organ must be due to the fact that this new active zygote could push its way into that situation and become encapsulated" (MacCallum 1916).

MacCallum's observations were also acknowledged by Ross's principal contemporaries in malaria research, Koch and Grassi. Koch (1899) did not describe the method of his preparations, although in another context he mentions the hanging drop preparation. He described the vermicule of "Halteridium" as having very slow stretching and bending movement with a dragging forward motion ("sehr langsames Strecken und Krummen, sowie ebenso trage Drehbewegungen waren Alles, was sich erkennen liess"). With respect to "Proteosoma" from passerine birds ("Stieglitzen und Sperlingen"), Koch simply stated that the vermicule gradually stretches itself and bends itself ("derselbe verlängert sich immer mehr und krummt sich ..."). Movement in human parasites similar to that of Proteosoma and Halteridium was not described.

In his monograph, "Die Malaria", Grassi (1901) acknowledged the observations of both MacCallum and Koch. This document does not record the method by which vermicules were observed. Grassi's description of P. falciparum vermicules employs two of the same verbs ("krummen, strecken") previously used by Koch when the latter was describing Halteridium and Proteosoma only. (Translation):

"I could observe that the vermicules could make forward motions. The pigment is lacking or spare at the forward end. The vermicule bends itself (krummt sich), stretches itself (streckt sich) and can even ... so far as I could observe ... carry out incomplete forward motions; in any case, it is very difficult to observe the motions in spite of the most careful manipulations ..."

As to the theory of vermicular migration into the stomach wall, Grassi (1901) wrote, (translation): "The kind and manner of penetration is not easy to see, however, in sections it is possible to find vermicules in the cuticular layer (inner brush border) of the epithelium as well as in the epithelium itself or at its base."

"About 40 hours after blood sucking it is already possible to find individual vermicules in this layer which I call the muscle layer (tunica-elastice muscularis). The amphionts (oocysts) lie in this layer which provides an excellent capsule for them."

The illustrations in "Die Malaria" do not picture vermicules within the muscle layer. Fig. 32 of Plate I refers to "amphionts" rather than vermicules. The slender form pictured here does not lie within the muscle layer. It is not clear whether this parasite was intended to illustrate a vermicule within the wall or a flattened variant of two other oval "amphionts" in the same picture.

Grassi's conclusions are pictured in Plate V in the cycle which, aside from minor variations, is still widely accepted. By placing the vermicule sequentially before the oocyst, he followed the lead suggested by Ross (Plate V, Fig. 8, 9).

Three years after the publication of Grassi's monograph, Stephens & Christophers (1904) published "A Practical Study of Malaria". They felt that the use of the term "ookinete" for zygote was justified since the "fertilized egg moves. The vermicular stage can be seen on the slide in the case of Halteridium, but in the case of malaria parasites, only by taking the blood from the stomach of a mosquito after a suitable lapse of time." They implied that the vermicule was motile although they do not record actually having seen it in motion. The concept of motility is repeated in the statement that the "vermicule now finds its way through the epithelium of the stomach".

In his authoritative Protozoology, Wenyon (1926) refers to the "important advance" by MacCallum. His description of human Plasmodium closely followed MacCallum's reference to Halteridium, including the use of similar terms such as "gliding", even though MacCallum's observations were limited to freshly drawn crow blood diluted in saline on a glass slide and did not include in vivo observations on the solid clotted blood meal of a mosquito. Wenyon stated, "the rounded body (recently fertilized zygote) containing two nuclei and the pigment granules, after a period of 10-20 minutes rest, puts out a clear cytoplasmic process or pseudopodium, which increases in size until an elongated vermicule is produced. At the last moment of this process, the vermicule or ookinete glides away ... the ookinete, which measures 18-24 microns in length and 3-5 microns in width, glides about amongst the intestinal contents ... by movements of contraction, bending and gliding, the zygote makes its way through the stomach contents towards the epithelium. Having reached the surface of the intestinal epithelium, it forces its way into a cell ...".

Only a year later, Knowles & Senior-White (1927) repeated Wenyon's exact phrasing without recording new evidence. "By movements of contraction, bending and gliding with a worm-like slow movement, the zygote ... makes its way through the contents of the mosquito's stomach to the gut epithelium." They also added that the zygote "bores its way into the epithelial cell".

Without describing additional evidence, the following reviews and texts refer to the active movement and migratory nature of the ookinete: Manwell (1941), Huff (1949), Brumpt (1949), Russell (1952), Garnham (1952), Belding (1952), Faust & Russell (1957). In referring to Plasmodium vivax, Brumpt (1949) attributed a "gregarinoid movement" to the ookinete. Shortt (1951) affirmed Wenyon's early concept that the ookinete "glides" among the stomach contents.

While affirming zygote motility, Garnham (1952) at least posed the question: "What is the nature of this motility? We don't know ... How the microgametes, the ookinetes, and the sporozoites move is a mystery; there are no muscle fibres, organs of locomotion or amoeboid movement."

DISCUSSION

From the viewpoint of the transmission of malaria, it was of the greatest significance that Ross had been able to trace the cycle through the mosquito. At the time, it undoubtedly seemed less important to determine the precise mechanism by which the "spherule" passed into the midgut wall. When his contemporary, MacCallum, reported the apparently motile zygote of Haemoproteus, Ross proposed the theory of vermicular penetration in the case of Plasmodium, although by his own admission, neither penetration or vermicular motility had been observed.

Among the authors in this review, Grassi alone has recorded that he himself saw a motile vermiculus of Plasmodium. However, it is noteworthy that even he made specific reference to the great difficulty in observing motility "in spite of the most careful manipulation".

Huff (1934) and Mackerras et al. (1948) recorded that they were unable to find vermicules penetrating the midgut wall in avian or human Plasmodium. Mackerras loc. cit. stated, "we have not detected vermicules in or between cubical cells in sections, but minute cysts already completely external to the epithelial and muscle layers were already found quite regularly at 48 hours, and their measurements did not indicate that much, if any, growth had occurred since the zygote state". Although in paraffin sections, a few vermicules were found to be pointing at right angles from the meal into the brush border, Mackerras et al. note that they could not provide direct evidence that this represented an act of penetration.

Other interpretations of movement have been derived largely from the study of fixed material, as for example, Reichenow's view (1932) that vermicules appear just internal to the peritrophic membrane because they are unable to penetrate through it, and Stohler's thesis (1957) that vermicules cannot penetrate through the peritrophic membrane after a certain stage of membrane development.

Ball & Chao (1960) have reported the in vitro development of all mosquito stages of P. relictum. Since this cycle was demonstrated in separate successive stages, it was not possible to directly observe the progression of morphological change with the certainty that a particular organism actually progressed through the zygote-vermicule-oocyst sequence.

On the basis of this review, it would appear that four basic points regarding the sequence and mechanism of infection still need to be experimentally demonstrated for species of Plasmodium.

1. Can it be shown by continuous in vivo or in vitro observation that a particular individual spherical zygote evolves into the elongated organism referred to as the vermicule?

2. Ruling out the influence of passive forces, can it be demonstrated in vivo or in vitro that vermicules are innately motile?

3. Can it be shown by continuous observation in vivo or in vitro that a particular individual vermicule evolves into a spherical oocyst?

4. Can it be shown by in vivo or in vitro methods that vermicules penetrate the midgut wall? If vermicular motility cannot be demonstrated, or if motility represents only a very low order of movement, by what process does infection of the wall actually take place?

SUMMARY

The principal literature on the mechanism and sequence of infection of the mosquito by Plasmodium has been reviewed with the purpose of following the historical development of evidence upon which the current theory is based.

Ross originated the theory that the mosquito becomes infected by means of a migrating vermicule which evolves from the initially fertilized spherical zygote and penetrates the midgut wall to become the early oocyst. Ross credited the role of the Plasmodium vermicule to MacCallum who, however, recorded vermicular formation and motility only in the case of Haemoproteus. Ross recorded that he himself did not observe Plasmodium vermicules in motion or in the process of penetration.

Except for the record by Grassi, the 28 authors in this review have not recorded that they themselves observed innate motility or the process of penetration for vermicules of Plasmodium.

Considering the limited published evidence upon which the current theory of infection is based, this survey suggests that experimental confirmation may still be necessary before the present theory can be considered proven.

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