CLASSICS IN INFECTIOUS DISEASES

A Newly Discovered Parasite in the Blood of Patients Suffering from Malaria. Parasitic Etiology of Attacks of Malaria.

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(1845–1922)

[Editor's note: like many other notables in the history of tropical medicine, Laveran first became acquainted with the problems he was to study throughout his life during his career as a military surgeon. He was born in Paris, the son and the grandson of physicians, and was awarded his degree in medicine by the University of Strasbourg in 1867. After several military assignments in France, he was transferred in 1878 to Algeria, where he became deeply interested in malaria, the cause of which was then being widely discussed. During a series of microscopic blood examinations of malaria patients in Constantine, he discovered spherical pigmented bodies with ameboid movement which he had until then confused with pigmented leukocytes. This was the third time that microorganisms had been found in human blood, the first two being the causative agents of relapsing fever and anthrax. When Laveran's discovery was confirmed, the Academy of Sciences in Paris elected him to honorary membership. In 1904, Laveran retired from military service and joined the Pasteur Institute, where he devoted himself entirely to bacteriologic and parasitologic research. He published more than 600 scientific papers dealing with malaria and many other tropical diseases. In 1907, he received the Nobel Prize as "initiator and pioneer of the pathology of protozoa."

Many others had seen various objects in the peripheral blood of malaria patients—Meckel (1847), Furichs (1858), Planer (1854), Delafield (1872), and Jones (1876)—yet none developed their observations in a systematic manner. Laveran's first communication appeared in the *Bulletin de l'Academie de Médecine*, 19:1235–1236, 1880. The brevity of the original article did not do justice to this monumental discovery. We have reproduced the following article, in full, with its single plate.]

On 20 October of this year, while I was examining microscopically the blood of a patient suffering from malaria, I noticed, among the red corpuscles, elements that seemed to me to be parasites. Since then, I have examined the blood of 44 malaria patients; in 26 cases, these same elements were present. This convinced me of their parasitic nature. These elements were not found in the blood of patients who were not ill with malaria. I will describe these elements as No. 1, No. 2, and No. 3. Eventually, it will become evident that this nomenclature is useful as it makes no assumptions as to the nature of the parasites.

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Description of Parasitic Elements Found in the Blood

No. 1. These are elongated bodies, with ends more or less tapered, often crescent-shaped (see Plate 5, Figs. 3 and 4), but sometimes ovoid (Fig. 5). In length, they measure from 8 to 9 microns and in breadth, they average 3 microns. A very fine line indicates the contour while the body itself is transparent and colorless at the periphery; toward the central part, there is a dark stain due to a series of rounded granulations that are probably pigment granules. Exceptionally, this stain is situated at the periphery. The granulations are often symmetrically disposed, in a crownlike arrangement, similar to the one I shall describe for No. 2. On the concave side of the crescent-shaped bodies, a curved pale line often seems to connect both ends of the crescent. This line is shown on Fig. 4. No. 1 bodies seem motionless; when their outline changes, it does so very slowly.

No. 2. These bodies present different shapes that vary with their being in states of rest or motion. In states of rest, the body is generally round and transparent, finely contoured, measuring 6 microns in diameter. Inside the body (Fig. 6), round pigmented granules of equal size are usually quite regularly arranged in a ring; one might say they look like a necklace of black pearls.

In motion, one sees very transparent filaments that are rapidly moving in all directions. These

movements may be compared to those of nematodes that would have one end attached to the inside of the spherical part. These filaments set the neighboring red blood corpuscles into motion, and this is easily observed. The length of the filaments or mobile appendices is approximately three or four times the diameter of the red corpuscle. I had the impression that three or four of these filaments surround every so-called No. 2 body, but there may be more of them, since only perfectly focused mobile filaments are perceived. These mobile filaments are sometimes regularly spread out on all sides (Fig. 7) or all are sometimes clustered on one side (Fig. 8). The free ends of the moving filaments are swollen, as is indicated on Fig. 7.

While these filaments or motile appendices move around freely, the spherical body on which they seem to be inserted oscillates more or less rapidly and even seems, at times, to move about so that all its parts follow the same direction. The pigmented granules move around freely inside the body and assume various configurations.

No. 2 bodies very often alter their shapes while one observes them. They become longer, flatten out, and become spherical again. In this last instance, the movements recall those of amoebae. Several times, it happened that while I was observing the motion of No. 2 bodies one of the mobile filaments would leave the round body and continue to move around the red corpuscles. Fig. 9 shows one of these filaments that has become free.

In several instances I have also observed in the slides of malaria patients, apart from the elements heretofore described, rounded bodies larger than the No. 2 bodies and usually even larger than leukocytes, which had inside them moving pigmented granulations. These bodies contained no mobile filaments and their motion was Brownian-like (Fig. 10).

No. 3. These bodies are round, larger in diameter (8 to 10 microns and sometimes greater), than No. 2 bodies, slightly granular, motionless, without any apparent peripheral filaments. Inside the bodies one sees pigmented granules sometimes arranged like those in No. 2 (Fig. 11) but more often disposed haphazaradly and in variable numbers. These bodies alter in shape increasingly and so tend to become very different from the type I just described (Fig. 12 and Fig. 13).

In addition to No. 1, 2, and 3 bodies, one nearly always finds small, rounded, mobile, and bright bodies and crimson red or light blue pigment granules. These pigmented granules are free or are included in No. 3 bodies or in leukocytes. The crimson red pigment seems to undergo transformation, and the result would seem to be the blue pigment.

Refutation of Some Objections. Methods of Blood Examination

Before I delve into the nature of the parasitic elements and their pathologic role, I wish to answer two objections made to me several times which probably will be presented to me again:

(*1*) Were the above-described parasitic elements really found in the blood of the patients? Were they not accidentally introduced into the slides?

(*2*) Were altered blood elements mistaken for parasitic elements?

The technique I used in all my preparations should shield me from the first of these objections. The glass slides were carefully washed in alcohol; the patient's finger was swabbed with alcohol; pure blood was examined without the addition of another fluid; and finally the slides were sealed with paraffin. It is true that all these precautions would not prevent floating air particles from entering the preparation, but how could one possibly maintain that the complicated parasites I have described were floating in the air only while I was examining the blood of malaria patients? How could one correlate the relationship I have many times ascertained between the abundance or rarity of parasites and the degree of sickness or apparent recovery of malaria patients if the inclusion of the parasites was due only to chance? Moreover, I was always careful to prepare the slides in different locations in the wards or in my laboratory, and the results were identical. I beg to dispose of this objection and to maintain that the parasites were truly in the blood of patients.

The second point seems even easier to deal with than the first. It is impossible to confuse No. 2 bodies equipped with mobile filaments with any possible normal or pathologic element of the blood. The swift movements of the filaments have nothing in common with the slow motions of leukocytes, and the pigmented granules, mobile or

arranged in a ring, should not be confused with the granulation of leukocytes. All my colleagues to whom I was able to show these No. 2 bodies in motion did not hesitate for a single second in recognizing that what was moving was indeed a parasite. One would only need a glance at Figs. 7 and 8 to become convinced that such bodies cannot be confused with leukocytes, however altered these may be. Even when they are motionless, No. 2 bodies resemble leukocytes only vaguely. They are usually smaller, the granulations are dark or crimson red and arranged in a ring, and they have no inner nuclei.

It would be just as impossible to confuse No. 1 bodies with blood elements. Erythrocytes, sometimes similar in shape to the bodies, never have any internal pigmented granules. It is true that No. 3 bodies look very much like pigmented leukocytes. They are alike in shape and measurements. Yet, the differences are great. Pigmented granules have a regular arrangement in No. 3 bodies (Fig. 11); there are no internal nuclei, and these elements do not take carmine dye as do leukocytes. Moreover, it is easy to ascertain that No. 3 bodies are the result of transformation of No. 2 bodies, the living nature of which is incontestable.

Nature and Pathologic Role Played By Parasitic Elements Found in the Blood

Bodies Nos. 1, 2, and 3 seem to represent different aspects or different phases of the evolution of the same parasite. It is evident that No. 3 bodies are the result of a transformation of No. 2 bodies after their death. It is easy to convince oneself in the following fashion. One looks for a No. 2 body with mobile filaments and after having found one that is characteristic and very lively, the slide is placed on the microscope and is observed from time to time. After a variable period (from some minutes to several hours), the movements cease and the filaments become invisible. The body changes from its Fig. 7 or 8 aspect to the one shown in Fig. 6. After a while, No. 2 body enlarges as if flattening out while the pigmented granules disassociate, forming a widening circle (Fig. 11). Finally, the parasitic organism alters its shape to the point where it is no longer recognizable. The pigmented granules become arranged irregularly. They accumulate at one point or disappear altogether with the exception of one or two. The same procedure, when applied to No. 1 bodies, give similar results. The bodies become shapeless after a while, although not so rapidly as No. 2 bodies. No. 1 bodies become first ovoid and then spherical and irregular.

Often, one finds in the slides ovoid bodies that appear to be intermediate between No. 1 and No. 2 bodies. Nevertheless, I have never witnessed a No. 1 body transforming itself into a No. 2 body even after a 36- or 48-hour period of observation.

Mobile No. 2 bodies are mostly found in the

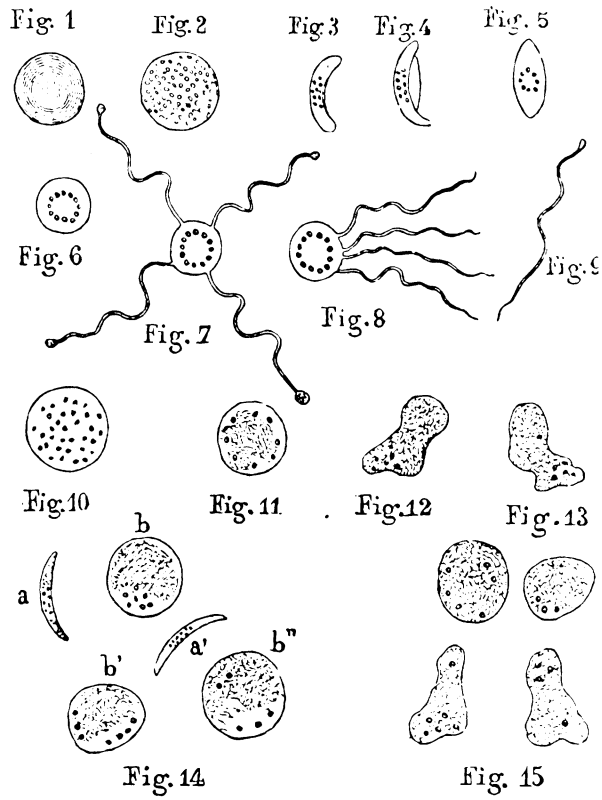

Plate 5. Key to drawings. *Fig. 1.* Blood red corpuscle. *Fig. 2.* Polymorphonuclear leukocyte. (These elements serve as points of comparison to give an idea of the measurements of the other elements; all diameters magnified [greatly].) *Figs. 3* and *4.* No. 1 bodies. *Fig. 5.* Ovoid intermediate body between No. 1 and No. 2 bodies. *Fig. 6.* No. 2 body, motionless. *Fig. 7.* No. 2 body with mobile peripheral filaments bulging at the free end. *Fig. 8.* No. 2 body with mobile filaments grouped laterally. *Fig. 9.* Mobile filament freed from the body. *Fig. 10.* Spherical body filled with mobile pigmented granules. *Fig. 11.* No. 3 body. *Figs. 12* and *13.* Distorted No. 3 body. *Fig. 14.* Pigmented elements from the blood of a man who died of pernicious fever: (*a*, *a'*) elements similar to No. 1 body; (*b*, *b'*, *b''*) elements similar to No. 3 bodies. *Fig. 15.* Elements from the spleen of a man who died of pernicious fever; elements similar to No. 3 bodies.

blood of patients who have suffered a recurrence of fever and who do not take quinine sulfate regularly.

The very fact that the parasitic organisms above described are found in an alkaline medium such as blood leads one to think that the parasites are of animal and not vegetable origin. The rapid and very varied movements of the filaments of No. 2 bodies, as well as the modifications of form they go through, lead the researcher to think of an organism like an infusoria. Is it as I first thought, an amoeba, or could bodies No. 1 and No. 2 be the result of an agglutination of cystlike parasites formed by normal elements in the blood? Could these parasites, fully developed, be the mobile filaments of No. 2 bodies, that sometimes leave the bodies to lead independent lives? This last hypothesis seems to me the most probable one. Once free, the mobile filaments are very much like filariae; and several researchers, Hallier among them, think filariae play an important part in the pathology of swamp fevers. The small, mobile, bright bodies, almost always present in the preparations, may be the first phase of an evolution of an organism. Quite often, one of these little bodies attaches itself to a red corpuscle and makes the effort, if I may say so, to penetrate into the interior.

The important role played by the parasites above described in the pathogenesis of swamp fevers may be evaluated as follows:

(*1*) These parasites are found only in the blood of patients suffering from malaria. It is fair to add that they are not always found there but, since only one or two drops of blood are examined, it is obvious that when the parasites are very scarce, their presence is difficult to establish.

(*2*) These parasites, while abundant in the blood of patients who have suffered from the fever for some time and who received no regular treatment, vanish from the blood of those treated for a long time with quinine sulfate, and who may be considered cured. Many of the patients I examined had received quinine sulfate for several days, and that could explain the high percentage of negative results I obtained.

(*3*) In the blood of patients who have died of pernicious fever, one finds a great number of pigmented elements that look very much like No. 3 bodies or, in rarer instances, No. 1 bodies. The presence of these elements in capillaries of all tissues and of all organs, particularly of the spleen and liver, is characteristic of acute malarial infection. Fig. 14 shows pigmented bodies found in the blood of a man who died of pernicious fever, and Fig. 15, similar bodies found in spleen tissue in another case of pernicious fever. The resemblance of these bodies to those I described as No. 1 bodies and No. 3 bodies, and whose parasitic nature I believe to have established, is striking.

From where come these parasitic elements found in the blood of malaria patients? How do they get into the human system? How do they cause intermittent fever and other signs of malaria? Only now is one able to pose these important questions.

Conclusion

Parasitic elements are found in the blood of patients who are ill with malaria. Up to now, these elements were thought incorrectly to be pigmented leukocytes. The presence of these parasites in the blood probably is the principal cause of malaria.