PROTOZOON-LIKE BODIES IN THE BLOOD AND ORGANS OF YELLOW-FEVER PATIENTS.¹

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(PLATES XXIX.-XXX.)

IN 1909² I published a short account of a supposed parasite which I had found in the blood of yellow-fever patients. During part of the epidemic of the past year I continued my investigations, and I believe it to be time to publish the results I have obtained, although I have not been able to bring forward final and conclusive evidence which would definitely settle the question of the nature of the forms. In fact, I have had scarcely any opportunity of attempting to produce these proofs, that is to say, to infect mosquitoes and follow the supposed evolution of the microbes in their bodies. If attempted, it would be extremely difficult to follow such small corpuscles in the tissues of the mosquito; and it may be impossible, for, as we shall see, there is some reason to believe that the infecting elements are in some stages ultra-microscopic, or nearly so.

I can therefore only amplify my former observations by some new ones, and especially by those made on sections of human organs, and by giving a short description of the formations observed, which can only be appreciated by comparison with the appended coloured figures.

Concerning the technique used, there is little to be said. The blood specimens were mostly fixed in methyl-alcohol, but sometimes in absolute ethyl-alcohol and stained in a strong Giemsa's solution, 15 drops of the original liquid (from Grüber or prepared by ourselves) to 10 c.c. distilled water, for about an hour, or in a weaker solution, 10 drops to 10 c.c. for twenty-four hours; sometimes the Schaudinn modification was used, and in a few cases Ziemann's method. To begin with, some preparations were stained by Wright's method, and later by that of Leishman, which gave better results; they were sometimes more easily obtained entirely without precipitation with Leishman's stain than with the Giemsa preparations. By Manson's method

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² *Berl. klin. Wchnschr.*, 1909, No. 18.

I did not get any good results, and very little by the hæmatein-eosin coloration.

The pieces of tissue were in the first cases fixed in a saturated solution of bichloride of mercury, with the addition of 5 per cent. pure acetic acid, but later I preferred sublimate-alcohol as the fixing fluid. They were embedded and cut in paraffin, and the sections were stained by hæmatoxylin or hæmatein and by Hansen's solution (modified v. Gieson's stain), hæmatein-eosin, Giemsa's, Schaudinn's, Ziemann's, and Leishman's methods; the only satisfactory results were obtained by the last method, although the parasites were also seen in the Giemsa and hæmatein preparations. The Leishman method is somewhat difficult to apply in sections, and requires a strict adherence to the original technique; it was only after a visit to Sir W. B. Leishman that I fully realised its advantages.¹

It has not been possible to demonstrate several phases of evolution as we know them in the malarial parasites and in other protozoa; however, there seem to be differences, not absolutely constant, but apparently nearly so, between the forms we have met with in the various stages of the disease. During the earlier days, in which the initial fever occurs, I have found only extremely small forms with a minute chromatin point and a generally feebly stained blue protoplasm; the chromatin is not very red but rather dark violet. A little later, during the reduction of the temperature, when this occurs on the third or fourth day, or in the period of reactive fever, there are still seen the same small forms; these are usually few in number, but there are in addition others, a little larger, which, whilst they consist chiefly of a rather deeply stained blue protoplasm, contain a still very small, but more purple-stained chromatin point.

Still later, in the last days of the fever, and, as it seems, far into the convalescent stage, we found in the corpuscles chromatin granules, these being well stained single or double, round or pear-shaped, and with a very small border of protoplasm or quite free of protoplasm.

All these forms are intraglobular. Outside the red blood corpuscles very few were observed, of which special mention must be made.

Evidently the observation of such small corpuscles is still more difficult outside than inside the erythrocytes, so that although there have been seen what other observers, to whom I have shown my preparations, and I myself sometimes believed to be, small extraglobular forms, I abstain from reproducing them, because I consider their nature entirely doubtful; I have seen similar formations in many other blood specimens, and am disposed to look upon them as artefacts. I limit myself therefore to the reproductions of the larger extraglobular objects of whose pre-formed and probably protozoic nature there can be little doubt. There may, of course, be some doubt as to their specific nature, as they have as yet been found in very few cases. The two largest forms (Plate XXIX. Figs. 37 and 39), which have

¹ In more recent work I have obtained equally good results with the last technique published by Giemsa (*Deutsche med. Wchnschr.*, 1910).

been found in two different cases, both fatal, and which show a quite characteristic structure and staining, recall somewhat the gametocytes of the malarial parasites, whilst the third shows a slight resemblance to some stages of the evolution of the Leishman - Donovan bodies. Moreover, there have been found in the same cases, and in a few others, similar but less fully developed forms.

The positive findings in the tissues have been obtained principally in the kidneys. They have mostly been localised as a small number of groups in a section, not equally distributed over the whole.

Two or it may be three different kinds of corpuscles have been observed.

Firstly, the most characteristic present themselves as small, oval, faintly, or rather deeply bluish or somewhat reddish stained bodies with sharply defined, very small dark violet spots, generally one, sometimes two or, very seldom, three in each body. They are found in what seem to be but slightly altered or sometimes almost destroyed erythrocytes in capillaries or in renal tubules with degenerated epithelium.

Their dimensions are from one-fifth to one-half of those of a normal red blood corpuscle. These bodies are so sharply limited and on the whole so well defined that I consider there can be little doubt as to their parasitic nature.

Secondly, there were observed also in the renal capillaries very small elements which resemble division forms, being disposed in circles between, but apparently not inside, the blood corpuscles; their number varies from five to nine; they show a uniform violet colour, apparently without chromatin-point, but that may depend upon the technique employed (the sections coming from sublimate with acetic acid and being stained by Giemsa's method, while the other forms described were seen in sublimate-alcohol specimens stained by the Leishman method).

Thirdly, finally, great numbers of a third class of small forms were found within large cells, probably endothelial, in liver and kidney sections; they show a darker spot in their bodies, but do not show such clear-cut outlines or characteristic staining as those of the first class.

This part of the work, the examination of the parasite in the tissues, is rather incomplete. For though I have preserved a great number of pieces of other organs, I have, up to the present, been able to examine only a few. The very irregular, limited distribution in the kidney would suggest that this organ is not the principal site of multiplication of the microbe, and the same seems to be true only in a greater degree in the cases of the liver, pancreas, heart muscle, and spleen, which last organ in yellow fever does not, as a rule, show definite alterations, either macroscopically or microscopically. In freshly made preparations from spleen and bone marrow I have seen

some microbes, and the further study of the latter is evidently suggested; this I have not, so far, attempted.

The principal task would now be to follow out the development in the mosquito; this I have attempted only in a few, very incomplete experiments, and with negative results. However, in such delicate work, this may be a question only of a more elaborate technique than I have been hitherto able to apply in a laboratory overloaded with routine work.

And now a few words about the significance of our observations. The possibility of artefacts I have discussed in my former article, and believe it can be excluded. Neither do I consider that there can be any serious doubt that we have to do with really foreign elements and not with histological constituents, normal or abnormal, of the blood itself. Punctated erythrocytes are quite different from what I have described, and I have never seen any degeneration of these corpuscles really resembling the elements described. Blood platelets, though rather heteromorphous bodies, would seem never to become differentiated to a degree at all approximating the shape of the extraglobular forms I have observed.

And I firmly believe that we can exclude this possibility with still greater confidence when we take into consideration the new facts now recorded regarding the findings in the sections of the organs. No kidney elements that I know of, and certainly no bacteria, would ever present the characteristics described. So I believe that I have demonstrated the presence in the blood and in some organs of micro-organisms which probably have not been seen or so interpreted before.

Regarding the specificity of the supposed microbe, the following data are of interest. In thirty-three cases observed during the last year, after my former article appeared, and which were officially declared to be cases of yellow fever, it was found twenty-five times; but of the negative cases, six occurred during my absence, and the preparations, which I received on my return, did not stain well, so they may well be excluded. We then get twenty-seven cases with twenty-five positive results.

If we include those formerly observed, which formed the basis of the first publication, but which I have not exactly tabulated, we have about eighty cases, in about 90 per cent. of which we have positive findings.

Only in one case was the blood given me before I saw the patient; in this I found the forms seen in Plate XXIX., Figs. 20, 21, and 25, and made the diagnosis of yellow fever, which was at first doubted by the physicians in charge but afterward fully agreed to. The patient entered the hospital on the third day of his disease, and the preparations were taken the same day.

In another case, which was diagnosed as typhoid fever, the same corpuscles were observed; but I think that I am able to uphold the clinical diagnosis of yellow fever and will give, with that purpose, a short résumé of the clinical history, for which I am indebted to Dr. Vargas, director of the lazaret, and to Dr. Vega, to whose service in the hospital the patient was afterwards transferred.

CASE. — M. B., æt. 25, went into the lazaret 12th December 1909. His disease began the same morning with headache and pains in the back; temperature (axillary) in the afternoon, after admission, $39^{\circ}4$ C. (103° F.); the symptoms increased and the temperature went up the third day to $39^{\circ}7$, its highest point, from which it descended to $37^{\circ}2$ on the sixth day, rising in the seventh again to $38^{\circ}9$; from the eighth day it was about or below 37° . The pulse, which was during the first days about 100 per minute, went down afterwards, as low as 40 on the eighth and 38 on the twelfth day. There was abdominal pain, first rather diffuse, but soon localised to the epigastrium, especially on pressure. From the third day there was jaundice, first conjunctival, afterward diffuse. The stools were mostly pale, but during several days with melæna. The urine contained albumin, casts, bile pigments, and indican; diazo reaction negative.

The Widal reaction (with formalised cultures after Dreyer's method) showed agglutination only in a dilution of 1 to 20; 1 to 50 and those higher were negative, so it cannot be considered as conclusively demonstrative of typhoid fever. In the service of the hospital to which the patient was transferred a diagnosis of bilious catarrh was made, but by this time all the febrile symptoms had subsided.

This case, therefore, if it be not considered wholly conclusive, can be taken in no wise as demonstrative against the specificity of the microbe; from my point of view, and I followed the clinical course of the malady, it may be looked upon as showing the usefulness of the microscopical examination in difficult cases.

Another case was observed where influenza was diagnosed, principally because the patient, a negro from Jamaica, was considered immune to yellow fever. Here the corpuscles were also found.

Serious doubts arose when I found them also in two individuals who certainly did not suffer from yellow fever—in one a young man with a chronic (parenchymatous) nephritis, and in the other a girl with chronic digestive, somewhat sprue-like, troubles. Both were natives of Yucatán. In the first case there were small forms, in the second only a single free form was found almost identical with that seen in Plate XXIX. Fig. 39.

If we consider that the immunity of natives against yellow fever is due, on the consensus of medical opinion, to one or more previous attacks of the disease, atypical and not recognised as such; and moreover, that the malarial parasites and some piroplasmas, in cattle for instance, are known to persist for a considerable time without producing any symptoms, then the findings referred to do not seem very much out of line.

In the case of nephritis, more especially, it would perhaps not be too remote to admit the possibility of yellow fever as an etiological

factor, as there was no history of any of the ordinary conditions likely to give rise to nephritis. When I first saw the patient the case looked rather serious, but afterwards it took a surprisingly benign course.

It would evidently be premature to pretend to determine the precise zoological place of the microbe. There can be little doubt as to its protozoic nature, considering its shape and staining reactions, but the forms observed do not suffice to enable us to make an exact diagnosis. The want of pigment and some of the morphological details, such as the double forms and the chromatin bodies almost without protoplasm, would suggest an affinity to the genus *Babesia* (*Piroplasma*),¹ whilst the aspect of the parasites in the kidney, and it may be the one apparently flagellated form observed, would seem to indicate a relation to the Leishman-Donovan bodies (*Herpetomonas*); in other details there is some resemblance to the malarial parasites.

We must now consider the question, how do our observations correspond with the already established facts regarding the epidemiology of yellow fever? In the chief points they evidently agree perfectly well: the parasite is found in the blood, and the disease is transmitted by a blood-sucking insect. But there are two circumstances that do not seem to conform with the accepted theory. According to the observations of the American Yellow Fever Commission and others, the microbe ought to be ultra-microscopical, or nearly so, because of its ability to pass through Berkefeld filters; this may be quite true in the sense that there may be an ultra-microscopical stage in its development, as such is suggested in connection with other diseases, e.g. *kála-ázar*, instanced by Manson, and this solution would accord with the fact that the microbes are found generally only in small numbers in the blood; it might be that the visible bodies are present in a small minority in comparison with the invisible forms.

The other circumstance to which I formerly alluded is, that the germ can only be transmitted during the first three or four days of the disease, whilst the microbes are found for a much longer period. There may be two explanations of this, the first of which is essentially the same as that which has now been suggested,—namely, that the ultra-microscopic stage, on which the transmission depends, exists, or is present in the blood, only during the first few days, although the larger forms may remain a much longer period. It ought to be remembered, however, that the observations on which the axiom mentioned has been founded are not very numerous, so that it can as yet scarcely be admitted as an established fact.

In my earlier papers I mentioned previous work on the same question; I was not cognisant of any observations which have given the same results, except possibly those of Thayer in a single case at post-mortem examination. Neither since my previous report have I

¹ In cases of a disease in imported cattle, very much like a piroplasmosis, I found very numerous naked chromatin bodies.

become acquainted with other works on the same subject, although at least two observers have told me personally that they had already seen some of the same forms which I have described.

I would not omit to refer to a paper in which some bodies are described,¹ which show a great similarity to those I have described. These have been seen in quite another disease, namely, *typhus exanthematicus*, and the authors do not discuss the nature of their microbe. If both are confirmed there would be a still further increase in the already numerous protozoal diseases, which in recent years, and more especially in tropical countries, are rapidly acquiring an importance almost as great as that of the bacterial infections.

It is known that *Babesia* have been described in human diseases (Wilson and Chowning, Andersen, Gotschlich, Darling), but these observations have not as yet been confirmed.

DESCRIPTION OF PLATES.

PLATE XXIX.

Figs. 12, 15, 16, 20, 21, and 25 are from cases the third day of the disease.

Figs. 7, 18, 19, 31, 37, and 40 from the fourth day.

FIG. 14 from the fifth day.

Figs. 29, 34, and 36 from the sixth day.

Figs. 17, 23, 28, 30, and 32 from the seventh day.

Figs. 8, 9, 10, and 11 from the eighth day.

FIG. 27 from the fourteenth day, in a somewhat protracted case with fever continuing till the eighth day, and a new, sudden elevation of temperature on the eleventh, going down again on the twelfth (relapse).

Figs. 1, 2, 3, 4, 5, 6, 13, 22, 24, 26, 33, 35, 38, and 39 cannot be exactly classified.

Figs. 28, 30, and 32 are from preparations stained after Schaudin's method; Figs. 34 and 36 by Leishman's method, and all others from Giemsa preparations.

PLATE XXX.

Figs. 41, 42, and 44.—Corpuscles in kidney (convoluted tubules). Leishman's stain.

FIG. 45.—The same (glomerulus cell). Giemsa's stain.

FIG. 43.—"Division forms" in kidney capillary. Giemsa.

FIG. 46.—Corpuscles in spleen. Hæmatein-Hansen solution.

FIG. 47.—The same. Hæmatoxylin-Hansen solution.

Figs. 48 and 49.—The same. Leishman.

FIG. 50.—Corpuscles in liver, in erythrocyte. Leishman.

(All figures $\times 1400$.)

All figures in the two plates have been prepared by Mrs. Margrethe Seidelin with Zeiss's apochr. immers. 3 mm. num. ap. 1.40 and comp. oc. 12 by the aid of the Abbé apparatus; exception made only for Nos. 13 and 14, in which cases Zeiss's achr. immers. $\frac{1}{4}$ and comp. oc. 8 have been used.

¹ Krompecher, Goldzieher u. Augyán, *Centralbl. f. Bakteriöl. u. Parasitenk.*, Jena, 1909, Abth. 1, Orig. Bd. I. S. 612.

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