A DEMONSTRATION ON THE CULTIVATION OF THE
MALARIAL PARASITES (*PLASMODIUM FALCIPARUM*
AND *PLASMODIUM VIVAX*).

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I have the honour of demonstrating to this Society a series of microscopic slides shewing the development of malarial parasites in culture tubes. The slides illustrating the growth of *plasmodium falciparum* are the most interesting, as it is so seldom we see these undergo segmentation in the peripheral blood. The parasites were incubated at a temperature of about 38° C., and in this particular culture from which the preparations under the microscope were prepared, the growth of the plasmodium was very rapid. Slides are shewn prepared from culture of *P. falciparum* before incubation, after 12 hours', and after 25 hours' incubation. The slide, after 12 hours' incubation, shews the parasites increased in size; the pigment is now evident, being collected into a circular compact mass, and the chromatin is spread out into the protoplasm. At this stage there is a marked clumping of the corpuscles containing the parasites, and I have placed out for your inspection drawings and micro-photographs illustrating the phenomenon. After 25 hours' incubation the parasites had sporulated, and on the slides you now see all stages of segmentation from two spores up to full sporulation, where the number may be as many as 32. I have placed under the microscope a slide which shews the whole field filled with these sporulating forms, and I also place before you micro-photographs and lantern slides taken from these preparations. The large masses of malignant tertian parasites in these preparations seem to be due to a tendency to clumping, and in two recent papers published in 1912 and 1913, in conjunction with Drs. MACLELLAN and D. THOMSON, attention was drawn to this phenomenon, and it was suggested that this might be the possible cause of the pernicious symptoms found in certain cases of malignant tertian malaria. This tendency to clumping also explains why we only see young rings in the peripheral blood of cases of malignant tertian.

While working with my colleagues, Drs. MACLELLAN and D. THOMSON, the malignant tertian parasite has been successfully cultivated from ten cases. The rate of growth varies greatly, and if the temperature is too low, no development takes place. In one case of cultivation full sporulation did not occur until about 52 hours. In this case, however, for the first 12 hours the temperature was about 36° C., and it was only after the incubator had been raised to 40° C. that development occurred.

In this experiment, conducted by Dr. D. THOMSON and myself, we have obtained evidence that it is possible for three generations of parasites to develop in the original tube without any transplantation to a new tube.

The benign tertian parasite was cultivated also at Liverpool by myself and Dr. D. THOMSON, and I have placed some slides under the microscope for your inspection. In this case the evidence is not so convincing owing to the fact that it is possible in cases of benign tertian to find all forms in the peripheral blood from young rings up to the full-grown parasite. In cultivating this parasite, therefore, it is necessary to begin an experiment when only young forms are to be found in the peripheral blood, and careful differential counts must be made. In Liverpool we have successfully cultivated this parasite (1913) on two different occasions, but owing to the fewness of cases, our experiments have been limited.

In conclusion, I shall give you the following summary :—

1. *Plasmodium falciparum* and *plasmodium vivax* are capable of being cultivated up to the stage of sporulation, for at least one generation, and we have obtained evidence that *plasmodium falciparum* may develop for several generations (at least three) in the original culture tube, without transplanting to a new medium.

2. In some cultures the growth was rapid, in others much slower; this is explained by different factors :—(a) The age of the parasite when introduced into the culture tubes. (b) The temperature of the incubator. (c) The amount of glucose used. (d) The influence of previous administration of quinine to the patient.

With respect to (d), we have noted that on several occasions sporulation in the case of malignant tertian fever was not completed when the patient had been treated with quinine, the maximum number

of spores in cultivations prepared from these being about ten. In several cases the parasites refused to develop, and we attributed this to the fact that quinine had been administered before the blood was drawn for cultivation.

3. The cultures of benign tertian differed from those of malignant tertian in that there was no tendency to clumping of parasites in the former, either before or during sporulation.

4. This difference appears to us to explain in a satisfactory manner why only young forms of malignant tertian are found in the peripheral blood, as the clumping tendency of the larger forms causes them to be arrested in the finer capillaries of the internal organs. It also explains the tendency to pernicious symptoms, such as coma, in malignant tertian malaria. All stages of the benign tertian parasites are found in the peripheral blood, and there are seldom pernicious symptoms, because there is no tendency to clumping.

5. The malignant tertian parasite (*P. falciparum*) is capable of producing, in maximum segmentation, 32 spores. On the other hand, benign tertian (*P. vivax*) produces, as a rule, during maximum segmentation, 16 spores; sometimes more may be produced, but the number is never 32.

6. The pigment of *P. falciparum* collects into a definite, circular and very compact mass early in the growth of the parasite. On the other hand, during the growth of *P. vivax*, the pigment remains scattered in definite granules throughout the body of the parasite, till just before segmentation, when it collects into a loose mass of granules in the centre of the full-grown *plasmodium*.

Lastly, I would like to state that in cultures of benign tertian malaria, Dr. D. THOMSON found heavily pigmented parasites, suggesting the formation of gametocytes.

I have to thank Sir EDWIN DURNING-LAWRENCE, whose generosity has made this research possible, and also Sir RONALD ROSS and Dr. FANTHAM. Dr. FANTHAM has drawn the plate illustrating the benign tertian parasite in cultures.

REFERENCES.

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EXPLANATION OF PLATE

(DRS. J. G. THOMSON AND S. W. MACLELLAN).

Micro-photographs shewing development of one generation of *Plasmodium falciparum*.

Figs. 1, 2, 3 and 4 are all of the same magnification. Fig 5 is taken at a higher magnification.

Fig. 1. This photograph shews two parasites before incubation.

Figs. 2 and 3. The parasites after twelve hours' incubation at a temperature of 38° C. These shew increase of size; the pigment is collected together into a circular mass, and the chromatin is spreading out into the protoplasm. The photographs also illustrate the tendency of the parasites to group together.

Fig. 4. Shews schizogony. The chromatin is split up into varying numbers forming merozoites. The pigment is collected into a mass, usually circular. Specimens prepared after twenty-five hours' incubation.

Fig. 5. Schizogony, after twenty-five hours' incubation. The higher magnification shews the daughter nuclei more distinctly. Figs 4 and 5 also shew the tendency of the fully developed schizonts to clump together into masses when a smear is made.