PRELIMINARY NOTE ON THE CULTIVATION OF THE
LEISHMAN BODY.

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With a view to confirming Rogers' experiment of June last, when he stated that he had grown flagellated organisms from Leishman bodies, punctures were made, forty minutes after death, into the spleen and liver in a case of Dum-Dum fever; the blood and tissues so derived were placed, under aseptic precautions, in tubes containing a small quantity of 4 per cent. solution of sodium citrate.

Private R., the case from which this material was derived, had long been considered one of Dum-Dum fever. From a year after he went to India (1898) up till 1901, when in Allahabad and Calcutta, he suffered from attacks of what was described as "ague." It was in Bombay, however, in 1902, that he had the most fever. The first attack of definite remittent fever appears to have taken place in Bombay in 1902, the fever continuing till he was invalided home in February. From his admission to Netley in March, till the time of his death from pneumonia, on December 4th, 1904, he had fever continuously, with the exception of several apyretic periods, the longest a month. The fever was chiefly of an intermittent type, the temperature seldom rising above 102° F. The other symptoms have been emaciation, anaemia, great enlargement of the spleen and enlargement of the liver; the spleen, during nearly the whole of his stay at Netley, extended down into the pelvis and to the right of the umbilicus. No other of the known symptoms of Dum-Dum fever were present.

Repeated examinations of the blood made during life, show the red cells to have been reduced; they varied from two to two and a half millions per cubic millimetre; the white cells were greatly reduced, varying from 1,500 to 2,500 per cubic millimetre, thus showing an absolute as well as a relative leukopenia. The large mononuclears varied from 19 per cent. to 25 per cent. of the total number; this increase was at the expense of the polymononuclears. Splenic puncture was not obtainable during life.

The patient contracted pneumonia towards the end of November, and died at 11.30 p.m. on the 4th of this month (December). Forty minutes later the punctures and inoculations above referred to were
made, and the citrated splenic and hepatic blood incubated at 20°C. Smears of the blood obtained from these punctures and stained by Leishman's method, showed the presence of numerous Leishman bodies, chiefly intracellular.

The post-mortem examination, besides indicating that the patient had died from a double pneumonia, showed the great size of the liver (106 ozs.) and the spleen (87 ozs.).

No macroscopic changes were found in any part of the gastrointestinal tract, and there was no enlargement of the mesenteric glands. In smears taken at the time and in sections cut afterwards, the presence of the Leishman body has been demonstrated in the spleen, liver, lungs and, in small numbers, in the suprarenal bodies; this part of the examination is, however, not yet completed.

On the morning of December 6th, after thirty-six hours' incubation, three blood smears were taken from the cultures and stained by Leishman's method. In the slides so prepared a few cells containing the bodies were noticed, as well as some free forms. Many of the free Leishman bodies had increased considerably in size, their protoplasm stained a blue colour and was much vacuolated, while the much enlarged macronucleus stained faintly and had a less definite outline; the micronucleus, however, persisted more or less unchanged.

A second examination, made on December 7th, showed that the bodies had further increased in size, 5-7 μ, some were somewhat oval in shape; there was, however, so much vacuolation and the macronucleus stained so faintly, that I had begun to think that these were degenerating forms.

On December 8th definite flagellated bodies, 10-20 μ in length, were seen, and on further examination parasites of many shapes and sizes, varying from the original Leishman body to a long trypanosoma-like form, were found. Many of these forms showed longitudinal fission, while several instances of parasites developing in the splenic cells were noticed. Hanging drop preparations of the cultures have shown the parasite to be motile.

From December 8th till the 14th repeated examinations have served to show that growth was proceeding in most of the cultures, the parasites seen being much the same as those found on the 8th, with the exception that some extremely small and slender forms approximating somewhat to spirillae were noticed.

Subcultures have been made into fresh citrated blood, and in these, as well as in similar cultures made by Major Leishman, very definite growth has been obtained. The organism appears to grow
in greyish-white granular masses on the white corpuscular layer of the citrated blood. Examination of these growths has shown the presence of parasites similar to those found in the original tubes.

Pond-water and tap-water inoculated with the cultures have been incubated at 20° C. and repeatedly examined, but although a few parasites have been found within the first few days, there are no evidences of growth in this medium and no flagellated forms have been seen.

Further work on this subject, in conjunction with Major Leishman, is proceeding, and it is hoped that it may be possible to make a fuller communication at a later date.

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