The Cultivation of Trypanosoma rhodesiense, *Stephens and Fantham.*

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Employing a strain of *Trypanosoma rhodesiense* kindly supplied to me by Sir Ronald Ross, K.C.B., F.R.S., I have carried out some cultural experiments. The following media were found to be successful. (1) Clegg's *Amœba* agar to which is added twice its amount of rabbits' blood which has been frozen and thawed rapidly so as to cause the hæmoglobin to diffuse into the serum; and (2) the following formula:—agar 15 grm., glucose 10 grm., water 1000 grm., and twice the volume of rabbits' blood added.

The trypanosomes were taken from sub-inoculated rats on the third day of infection. Five drops of blood were placed on the agar, which is a diffluent mass resting at the bottom of the culture tube. These were incubated at 22° to 25° C. The tubes were examined after five days and then every two days, usually, up to the 30th day. It was found that if the culture was successful a multiplication of the trypanosomes occurred, which could be seen actually taking place under the microscope. The trypanosomes were easily recognised as *T. rhodesiense* because of the size and position of the kinetonucleus, the length and breadth of the posterior end, and the position of the trophonucleus. This trypanosome was differentiated from other easily culturable trypanosomes, as for example *T. lewisi*, by inoculation into rats. 0·5 c.c. of the diffluent culture injected intra-peritoneally caused *T. rhodesiense* to appear in the blood 10 days after inoculation and the rat died in a further five days. From these rats the trypanosomes were recovered and again cultivated.

The forms of trypanosomes as commonly seen in these cultures are long and slender with a definite flagellum. They move sinuously, with the flagellar end first. The nucleus is longer and narrower than that seen in the blood, and stains with Giemsa's method a light purple. Volutin-granules are frequently present. The kinetonucleus can be seen separated from the blepharoplast and its usual position is somewhat to the side of the trypanosome. These forms remain actively motile up to the 21st day of cultivation. They are virulent up to the eighth day of culture in the quantity inoculated as mentioned above. In certain cases rosette forms are imitated by the trypanosomes clumping together; however, a little pressure on the cover-glass causes them to separate and to swim away individually. True brood-forms have also been

observed and distinguished from those clumped. In some culture tubes, rounded (involution) forms appear in great numbers on the third day to the fifth day, and the virulence and properties of these forms are now being separately studied.

The percentage of successes varies very much indeed—from 1 tube in 25 to 6 in 6. Numerous other media have been used, giving invariably negative results by the fifth day, including those consisting of frozen and thawed rabbits' blood serum only.

Further Observations on the Recovery of Trypanosoma gambiense *from* Tragelaphus spekei *on the Islands of Lake Victoria Nyanza*

By Dr. H. L. Duke.

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In a paper* dealing with the recovery of a trypanosome from wild antelope on Damba Island, Lake Victoria Nyanza, the diagnosis is discussed at some length with a view to the exclusion of *T. brucei*.

Fifteen subinoculation experiments were considered and the conclusion was arrived at that the available evidence pointed to the organism being *T. gambiense*. After a lapse of some four months this opinion has received considerable support from a more prolonged investigation of the animal reactions of the trypanosome. In the following table many of the original subinoculations are followed to their conclusion on the death of the animal, and several other experiments have been added to the list.

It will be seen that the evidence supplied by the rat experiments is strongly in favour of *T. gambiense*.

In the six experiments conducted with rats the average duration of the disease is 61 days. This constitutes very strong evidence against *T. brucei*. In guinea-pigs (three experiments) the average duration is 67 days: in five completed monkey experiments, 147 days.

* "Antelope and their Relation to Trypanosomiasis," 'Roy. Soc. Proc.,' B, vol. 85, p. 156.