MOSQUITO TRYPANOSOMES.

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In a previous paper on Bird Trypanosomes it was pointed out that these organisms grew readily in the test-tube on blood agar and that the resulting forms resembled the flagellates which Schaudinn found in the gut of mosquitoes which had fed on owls infected with *Halteridium* and with *H. Ziemanni*. In other words, the position taken was that the flagellates observed in the mosquitoes did not represent stages in the life-history of intracellular parasites but were actually cultures *in vivo* of trypanosomes present in the blood of the birds used. In confirmation of this position it was desirable to show that trypanosomes could actually grow and multiply in the gut of mosquitoes and that such forms actually did correspond to those which would be obtained *in vitro*.

Accordingly, large numbers of mosquitoes were captured along the river-bank and allowed to feed on perfectly clean animals, such as rats, guinea-pigs and pigeons. At varying intervals, 36 to 72 hours after feeding, the contents of the stomachs of the mosquitoes were examined in living and in stained preparations and cultures on blood agar were made at the same time. Of more than 800 mosquitoes which were examined in this way about 120 or 15 per cent. were found to have a flagellate infection of the intestinal tract. In some, this was very marked; large masses of rosettes, flagella inside, completely filling the lumen of the tube.

Several distinct forms of the trypanosomes were met with, the most common of these was a *Herpetomonas* (probably *Herpetomonas subulata*) and *Chrithidia fasciculata*.

Owing to the large numbers of bacteria usually present much difficulty was experienced in obtaining cultures of these flagellates. Eventually, however, the *Herpetomonas* was isolated in mixed culture associated with a minute coccus, while the *Chrithidia* was obtained in association with a yeast. These mixed cultures have now been grown in the laboratory for some six months. Several other cultures were obtained but these were soon outgrown by the accompanying bacteria.

The cultural form of these two organisms was exactly the same as that seen in the gut of the mosquito, thus confirming the view expressed that the flagellates found growing in the intestinal tube of insects represent cultural forms *in vivo*, and, as such, correspond to those *in vitro*. In both conditions not only was the form and size the same but the blepharoplast was anterior to the nucleus. The *Herpetomonas* was characterized by the presence of two diplosomes in the posterior part of the cell. These bodies were found in the parasites within the mosquitoes as well as those grown in culture. Animals inoculated with the cultures failed to show an infection.

When mosquitoes are allowed to feed on *Tr. Brucei* or *Tr. Lewisi* these parasites may be detected in the blood in the intestine of the mosquito 24 hours after feeding, and even later, and rats inoculated with such stomach contents develop typical infection.

The trypanosomes which have been met with by various investigators in the stomachs of Tsetse-flies, lice, leeches, etc. are distinctly "cultural forms" since they show the blepharoplast in a position anterior to the nucleus. This fact indicates that all such forms can be cultivated in the test-tube. The *Herpetomonas* forms found in flies and mosquitoes are true cultural trypanosomes, and, without doubt, future studies will reveal the blood parasite from which they are derived. The *Chrithidia* show no undulating membrane, in the ordinary truncated form, and on account of their peculiarity, for the present at least, are to be considered as representing a distinct genus.

ISOLATION OF TRYPANOSOMES FROM ACCOMPANYING BACTERIA.

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In general, it may be said that bacteria once introduced into a culture of trypanosomes tend to outgrow and check the development of the flagellates. In exceptional instances, however, the bacteria thus introduced exert little or no interference and may be even apparently beneficial. While in the former case the trypanosomes soon die out, in the latter instance the mixed cultures may be kept for six months or longer.

The isolation of the trypanosomes in pure form from such mixed cultures is a matter of some importance, especially when it is desired to study the pathogenic action of the flagellates. The need for some method of separation was particularly felt in connection with the study of the mosquito trypanosomes which, since they are present in the intestinal canal, are always accompanied by various bacteria and yeasts. After many ineffectual attempts the following method was successfully employed for the isolation of pure cultures of *Herpetomonas* and *Chrithidia*.

By means of a small glass spatula, made by drawing out the end of a glass rod, a little of the mixed culture was spread in a series of streaks over six Petri dishes containing solidified blood agar. The Petri dish known as the "Kriegsministeriums-Modell," made by Greiner and Friedrichs, is particularly adapted for this purpose inasmuch as it can be sealed effectually by means of a wide rubber band. The sealed dishes are then set aside at room temperature for 10 to 12 days. The last plate or two of the series will be found to show isolated colonies of trypanosomes which can then be transplanted in the usual way to the test-tube. This method will undoubtedly be found useful in future studies of the flagellates found in the intestinal canal of insects and other sanguivora. The intestinal contents can be spread directly over the plates in the manner indicated.