18 (64). **"The results of attempts to cultivate trypanosomes from frogs."** A preliminary report: **JOSEPH LEWIS** and **HERBERT U. WILLIAMS.** (Presented by **AUGUSTUS B. WADSWORTH.**)

During the year 1904 an effort was made in the pathological laboratory of the University of Buffalo to make studies on hematozoa in the lower animals. In a considerable number of normal cats, dogs, rabbits and guinea-pigs no hematozoa were found. The results of other examinations were as follows: 51 English sparrows (*Passer domesticus*), half in the winter, half in the spring, all negative; 27 mud-puppies (*Necturus maculatus*) in March, all negative; 40 toads in the summer, all negative. In 140 frogs from the Niagara river there occurred the following infections: 14 with *Trypanosoma*, 5 with *Drepanidium*, 1 with *Filaria*. *Drepanidium* was found both in the summer and fall. The infections with *Trypanosoma* were distributed as follows:

In July, of 15 frogs, 2 showed trypanosomes.

In August, of 26 frogs, 10 showed trypanosomes.

In September, of 14 frogs, 2 showed trypanosomes.

From October to December, of 85 frogs, none showed trypanosomes.

In one case *Trypanosoma* and *Drepanidium* occurred in the same blood. The trypanosomes had the usual characters of *Trypanosoma rotatorium* (*ranarum*). They were in no case numerous; two were rarely seen in one low-power field. The frogs appeared healthy. Eight attempts to inoculate normal frogs by way of the peritoneum with the blood of infected frogs gave negative results.

Attempts at cultivation. — The blood of frogs and toads was taken to make blood-agar (used by Novy and MacNeal for the cultivation of trypanosomes).[1] The blood was first examined carefully to see that it was free from parasites. The animal was etherized and placed in $HgCl_2$ solution 1 to 1,000 for 15 minutes, rinsed with distilled water, opened with all precautions, the blood from the heart taken with a sterile pipette, and mixed rapidly with the water of condensation on slanted agar tubes (made with meat extract and peptone, and slightly alkaline to litmus). Two or three drops of blood were used for each tube. The tubes were sealed with rub-

[1] See page 23 (87).

ber stoppers and allowed to stand five or ten days so that contaminations with bacteria might be detected.

1. The blood of frogs infected with *Trypanosoma rotatorium*, collected in the same manner, was mixed with that in blood-agar tubes prepared and tested as just mentioned. The tubes were kept at the temperature of the room. Cultures made from two infected frogs showed, after two weeks, growths of flagellate protozoa (both on toad's blood-agar and frog's blood-agar). The organisms were of a very long oval form, the bodies of the largest being $2\,\mu \times 18\,\mu$. There was a single flagellum, which was often nearly as long as the body. Only the largest forms showed a trace of an undulating membrane, which never approached the development of this structure in *Trypanosoma rotatorium*, and which did not appear in stained preparations. Motility was not very pronounced. Numerous small forms were seen evidently representing various developmental stages. In preparations stained according to Romanowsky, a blepharoplast (micronucleus, centrosome) was seen at the base of the flagellum and near the *anterior* end. The nucleus appeared to be represented by numerous chromatin granules in the posterior end. It may be noted that Smedly[1] found the centrosome at the anterior end in the cultural forms of the rat trypanosome. Numerous observers have seen typanosomes lacking the undulating membrane under artificial conditions.

The growth in the tubes was never luxuriant. Arrangement in rosettes was not seen. One generation only of subcultures grew. All the cultures soon died. A single attempt to inoculate a normal frog gave a negative result. These experiments were interrupted, as both the authors went out of town.

2. As is mentioned below, the blood of frogs infected with *Drepanidium* was added to blood-agar[2] tubes to see if *Drepanidium* could be made to live or undergo further development. Tubes thus inoculated, showed, in one case, trypanosomes in about ten days. For the moment it appeared as though trypanosomes had developed from *Drepanidium*. Some of the same blood-agar to which no *Drepanidium* blood had been added, was examined again and found to contain the same trypanosomes. They must,

[1] Smedley: *Journal of Hygiene*, January, 1905.

[2] Made from frog blood.

of course, have been derived from the frog from which the blood-agar was made. This frog's blood was examined for parasites before using it to make the medium and just before inoculating it, so that trypanosomes must have been present in numbers too small to show in several large cover-glass preparations, or they existed in some developmental stage not recognized. Novy and MacNeal have also secured cultures of trypanosomes from birds, where none were found by direct examination of the blood with the microscope [page 23 (87)].

In some preparations from the blood-agar tubes as many as four trypanosomes appeared in one field (Zeiss, DD., No. 3 ocular), and there can hardly be any doubt of their having multiplied. The motion of the trypanosomes was active and characteristic. They were much smaller than *Tr. rotatorium*, with rare exceptions, the body being usually about $3\,\mu \times 16\,\mu$. The flagellum was hardly half as long as the body. On the small forms the undulating membrane was not distinct, but the flagellum was plainly marked. Large forms, similar to *Tr. rotatorium*, except that the flagellum was lacking, occurred, but were rare. The nucleus and blepharoplast were placed as in *Tr. rotatorium*, as far as could be determined, but the amount of material was so small that satisfactory, stained preparations could not be secured.

With the trypanosomes there were associated spindle-shaped or crescentic bodies, about $12\,\mu$ in length, looking much like the crescents of æstivoautumnal malaria, except for lack of pigment. These bodies contained several (usually four) shining chromatin granules symmetrically placed in the middle. Motility was doubtful, and in any case slight. Flagella were not seen. The crescentic forms were probably some developmental stage. The crescentic bodies were observed for eight weeks. Motile trypanosomes were observed for five weeks. No growth occurred in subcultures. Two frogs were inoculated by way of the peritoneum from tubes containing the crescentic bodies, with negative results.

3. Attempts to produce development of *Drepanidium* were made from three frogs infected with this parasite, both on frog's and toad's blood-agar. The results were negative, although motile *Drepanidia* were discovered after ten days, and the parasites remained for weeks apparently unaltered within the blood-corpuscles.

Conclusions. — Trypanosomes from the frog may be cultivated on blood-agar, but, in the authors' experience, with considerable difficulty.

From a frog infected with *Tr. rotatorium* a flagellate organism was cultivated, showing important points of difference from *Tr. rotatorium.* It is possible that, owing to the technical difficulties of the experiment, some other organism may have found its way into the tubes. This is improbable.

Undoubted trypanosomes developed in blood-agar prepared from a frog whose blood, during life, showed no trypanosomes. They resembled *Tr. rotatorium,* but were usually much smaller. As this blood-culture medium was inoculated with blood from another source containing *Drepanidium,* it nearly led to the conclusion that *Trypanosoma* might develop from *Drepanidium.* We have here an illustration of the ease with which mistakes may occur in the cultivation of hematozoa which are suspected of passing through cycles. Such a possibility had been pointed out in advance by Novy and MacNeal before this society [page 23 (87)].

There was no evidence from the experiments to show that development of *Drepanidium* can occur on blood-agar.

It is unlikely that material with which further studies may be made can be secured before next summer (1905). As trypanosomes are now exciting so much interest, and are being so widely studied, the authors deemed it best to report their results at this time, although the work is incomplete.

19 (65). **"Experimental measles": LUDVIG HEKTOEN.** (Presented by **EUGENE L. OPIE.**)

The search for the cause of an infectious disease like measles becomes greatly simplified when we learn how to secure the unknown "virus" in relatively pure form unmixed with common microbes. Various methods may now be applied to the investigation of the virus. The transmission of measles from mother to fetus would seem to point to the presence of the cause of the disease in the blood. In the twenty cases of fetal measles collected by Ballantyne, it seemed that the infection of mother and fetus must have been simultaneous, because the eruption in both corresponded in character. In order to learn something further as to the presence