A NOTE ON THE PATHOGENICITY OF TRYPANOSOMA LEWISI.*

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In the usual scheme of classification of trypanosomes, *Trypanosoma lewisi* occupies the position of the type species of a number of non-pathogenic trypanosomes.[1] While this usage is justifiable in the present state of our knowledge of these organisms, one must not lose sight of the fact that there is abundant evidence to show that *Trypanosoma lewisi* is not strictly non-pathogenic, but occasionally manifests a decided virulence for rats, especially young ones. Apart from such frequent disturbances as fever, anemia, and loss of weight, a considerable mortality may occur among infected rats. Perhaps the best instance that can be cited is that reported by Jürgens[2] who noted a mortality of 29.3 per cent. (16 out of 47) among young rats. Other authors have noted a slight mortality, or no mortality, resulting from infections of *Trypanosoma lewisi.* These differences in pathogenicity indicate that there are strains that differ fundamentally as regards their virulence. Delanoë[3] has added support to this conception of pathogenic and non-pathogenic strains of *Trypanosoma lewisi* by showing that while certain strains, or organisms from certain sources, are incapable of infecting mice, other strains may infect even a considerable percentage (40 per cent.) of the mice inoculated. Further, Roudsky[4] has shown by his "reinforced virus" that the virulence of a given strain is not absolutely fixed, but that it can be markedly increased for both rats and mice. Finally, Wendelstadt and Fellmer[5] have succeeded in

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1 Laveran, C. L. A., and Mesnil, F., Trypanosomes et trypanosomiases, 2d edition, Paris, 1912, 241.

2 Jürgens, *Arch. f. Hyg.*, 1902, xlii, 265.

3 Delanoë, P., *Compt. rend. Soc. de biol.*, 1911, lxx, 649.

4 Roudsky, D., *Compt. rend. Soc. de biol.*, 1910, lxviii, 421, 458; 1911, lxx, 741.

5 Wendelstadt, H., and Fellmer, T., *Ztschr. f. Immunitätsforsch., Orig.*, 1909, iii, 422; 1910, v, 337.

raising the virulence of *Trypanosoma lewisi* by passage through cold-blooded animals.

These facts suffice to show the existence of pathogenic strains of *Trypanosoma lewisi* and indicate that possibly all strains possess potential pathogenic properties. Unfortunately, the known facts regarding its pathogenicity are too meagre to warrant any generalizations. Since the natural host of this organism makes it peculiarly suited to laboratory study, further work upon this subject seems highly desirable, as a clearer comprehension of the conditions governing its pathogenicity would probably aid materially in advancing our knowledge of the more important group of organisms, —the pathogenic trypanosomes.

Recently, we have had under observation a strain of *Trypanosoma lewisi* that, for a short time, showed an unusual increase in its virulence, and an account of the action of this organism is offered as a contribution to the study of its pathogenicity.

A complete genealogy of this strain of *Trypanosoma lewisi* is not available. The organism was isolated from a natural infection in a wild rat and has been carried for several years in white rats with no unusual manifestations of virulence. In October, 1913, for three successive generations, fatal infections resulted from the intraperitoneal injection of one to two drops of tail blood, diluted with one cubic centimeter of a 1 per cent. sodium citrate solution, death taking place eight to eleven days after inoculation. Five rats out of the six inoculated succumbed to this infection. At first it appeared improbable that *Trypanosoma lewisi* was entirely responsible for such an unusually high mortality, but as careful investigation, including cultures from the peritoneal cavity and heart's blood, failed to reveal any other cause, the following test of virulence was applied.

Experiment 1.—Nov. 28, 1913. A large rat infected with strain I of *Trypanosoma lewisi* was bled to death on the twelfth day of infection and the blood defibrinated. At this time there was a heavy blood infection with a few multiplication forms still present. The rat was weak and showed a marked anemia and loss of weight. With aseptic precautions, ten normal rats were injected intraperitoneally with 0.2 c.c. of blood diluted with 0.8 c.c. of an 0.85 per cent. salt solution. Five of the rats weighed between 100 and 170 gm., and the other five between 40 and 70 gm. As a control, four normal rats (two large and two small) of the same lot were kept under observation and two large immune rats were injected intraperitoneally with 0.5 c.c. of blood in 1 c.c. of salt solution.

Nine of the normal rats inoculated showed trypanosomes in their blood within twenty-four hours. The tenth rat, a large one, showed a very few organisms at forty-eight hours, but the infection was transient, disappearing completely within five days without any demonstrable multiplication having taken place; this rat was probably an immune. Another large rat died in forty-eight hours and the autopsy showed a marked bronchiectasis. If we exclude these two rats from the list of normal ones, the eight remaining rats showed a rapid increase in trypanosomes in the blood with definite multiplication on the third day, persisting until the fatal termination of the infection. The rats became torpid and weak, with marked anemia and dyspnea, and slight loss of weight. A few rats showed bloody nasal and lachrymal discharges, and all of them developed a diarrhea with greater or less abdominal distension. Two of the large rats died on the sixth day, and the third died on the seventh day, while all five of the small rats died on the ninth day after inoculation.

Autopsies on these rats showed an acute enteritis, marked splenic enlargement and hyperplasia of the bone marrow, and a few foci of necrosis in the liver. Several rats showed a moderate pulmonary congestion but no bronchiectasis or pneumonia. Cultures made immediately after death from the peritoneal cavity and from the heart's blood of three rats remained sterile. No cultures were taken from the other rats.

The normal and immune controls remained unaffected for one month, when observations were discontinued.

The existence of nasal and lachrymal discharges and diarrhea in these rats would naturally incline one to suspect some condition complicating the trypanosomiasis. In an attempt to clear up the relation of these conditions to the trypanosomal infection, a number of observations have been made on normal and immune rats inoculated with different strains of *Trypanosoma lewisi.* These observations show that the above conditions may recur frequently in a given series of inoculations while they do not appear in control immunes inoculated from the same source, in uninoculated normal rats from the same lot, or in rats inoculated with another strain of *Trypanosoma lewisi.* Likewise, normal and immune rats kept in

contact with infected rats showing these conditions, with a single exception, have not developed these symptoms except as the normal rats became infected with trypanosomes by natural means. The fact that the same symptoms occur in the course of other trypanosomal infections in the rat indicates that they may be a part of the morbid manifestations of trypanosomiasis. Whether these conditions are the result of an uncomplicated trypanosomal infection or the result of a latent infection whose development is favored by the trypanosomiasis is not clear. In either case, however, it is evident that it is the trypanosome that is directly or indirectly responsible for the condition.

The series of fatal infections in the stock transfers of this organism was interrupted by inoculating two young rats weighing seventy grams, with one drop of tail blood, taken on the tenth day of infection, from the rat killed for the above experiment. One of the rats was extremely ill for about twenty days but finally recovered. The infection in the other rat was much less severe. On the next transfer from the first of these rats, one large and one small rat were inoculated and the resulting infections were still further decreased in severity.

The small rat of this series was then sacrificed for a second test of the virulence of this strain of *Trypanosoma lewisi,* in this instance contrasted with another strain, recently isolated from a natural infection in a white rat.

Experiment 2.—Dec. 17, 1913. A young rat infected with strain I of *Trypanosoma lewisi* and another young rat infected with strain V were bled from the heart on the eighth day of infection and the blood was collected and defibrinated under aseptic conditions. The blood of the two rats showed about an equal number of trypanosomes with many multiplication forms. From each of these rats, five rats weighing 80 to 90 gm. were inoculated intraperitoneally with 0.2 c.c. of blood diluted with 0.8 c.c. of salt solution.

The five rats infected with strain I all showed an incubation period of less than twenty-four hours. Three of the five rats developed a severe infection with weakness, a high grade anemia, and loss of weight. The other two showed only a moderate infection. None of the rats died and trypanosomes were present in the blood of four on the thirtieth day, when observations were discontinued. Of the five rats infected with strain V, the incubation period of four was

less than twenty-four hours, of the fifth between thirty-six and forty-eight hours. Only one of these rats showed any appreciable disturbance from the infection. This rat showed an extreme blood infection, with weakness, torpor, anemia, and loss of weight, persisting for about three weeks. Trypanosomes disappeared from the blood of two of these rats within twenty days and from a third by the twenty-sixth day.

Although none of the ten rats died from the infection, the difference in the severity of the infection produced by the two strains of *Trypanosoma lewisi* was sufficiently well marked to show that strain I was decidedly more virulent than strain V.

This series of observations lends support to the idea that there are strains of *Trypanosoma lewisi* that differ fundamentally as to their pathogenicity, but what is of even greater importance is that any particular strain is subject to marked fluctuations of virulence. From these facts it would seem important to determine the conditions that give rise to such alterations of virulence as have been described, the extent to which these variations of virulence more or less permanently modify the characteristics of the strain, and, finally, whether there are differences in morphology corresponding with variations in virulence or with the differences in pathogenicity exhibited by different strains of *Trypanosoma lewisi.* These subjects are now under investigation.

CONCLUSIONS.

1. Some strains of *Trypanosoma lewisi* may, at times, produce rapidly fatal infections in a large percentage of the rats infected.

2. In such strains of *Trypanosoma lewisi,* a sufficient degree of pathogenicity may persist to warrant the designation of these strains as pathogenic.

3. The pathogenicity of a given strain of *Trypanosoma lewisi* is not constant, but is subject to marked and even sudden variations.