

use of heavy-metal precipitants,^{16,19,20} high oxygen pressures,²¹ or decreased concentration of nutrients.²²

The preceding observations appear to relegate *Bacterium tularensis* to the poorly defined group of "microaerophiles," as was suggested at one time by Foshay.²³ The species is apparently unusually sensitive to mildly oxidizing intensity in the environment. Our data do not indicate whether this is referable directly to molecular oxygen, or indirectly through the reaction of oxygen with constituents of the medium. As in the case of obligatory anaerobes and of other "micro-

aerophiles," inhibition tends to be abolished by reducing agents, decreased oxygen tension or large inocula. On the other hand, the need for oxygen is indicated by the absence of growth below characteristic levels in agar shake cultures, and stimulation of growth by oxygen is shown by the increased yields obtained in aerated broth cultures.

Summary. 1. *Bacterium tularensis* grew luxuriantly and retained virulence during consecutive transfers in peptone broth and on peptone agar without blood or blood constituents. 2. Large inocula were required with the strains tested. The minimum effective inoculum was decreased by reducing agents or by a lowered oxygen tension over the culture, but growth was found to be dependent upon oxygen and stimulated by increased aeration. 3. *Bacterium tularensis* appears to be an obligatory aerobe sensitive to mildly oxidizing environment.

¹⁹ Burnet, F. M., *J. Path. Bact.*, 1927, **30**, 21.

²⁰ King, J. W., and Rettger, L. F., *J. Bact.*, 1942, **44**, 301.

²¹ Williams, J. W., *Growth*, 1939, **3**, 21.

²² Cahn-Brommer, C. E., *Proc. Soc. Exp. Biol. AND MED.*, 1940, **45**, 454.

²³ Foshay, Lee, *Am. J. Clin. Path.*, 1933, **3**, 379.

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Exudative Trypanosome Pleuritis of Mice Infected Experimentally with *Trypanosoma cruzi*.

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In the course of the routine transfer of a strain of *Trypanosoma cruzi* from mouse to mouse it was found that some of the animals developed a pleuritis which seemed to be of specific origin. Since we were unable to find a description of this pathological condition in the literature, our observations are presented in this note.

The strain of *T. cruzi** was carried in mice since August 1942 and was regularly transferred by subcutaneous, occasionally by intra-abdominal, injection of blood containing the parasites. The virulence of the strain at the time when the occurrence of pleuritis was first noted had been increased by frequent passages through mice of 3 weeks of age

(9-13 g). All the mice injected subcutaneously with 0.05 cc blood containing approximately 50 trypanosomes per microscope field (about 300 $\times$ lin. magnification) died within 10-17 days. In general, trypanosomes appeared in the blood of infected animals 2-8 days after inoculation, according to the number of organisms present in the inoculum.

The first observation was made in a young mouse (No. 234) which was inoculated subcutaneously the 28th of May, 1945 and died the 16th of June. At the autopsy ca. 0.5 cc of a clear pleural exudate was found containing 20-30 trypanosomes per microscope field; 0.1 cc of this exudate was injected intraperitoneally into another mouse which showed prostration 13 days later. Its blood contained at this period a very large number of trypanosomes. The animal was sacrificed and

* Strain of the *T. cruzi* (Chagas 1909) was received from the American Type Culture Collection.

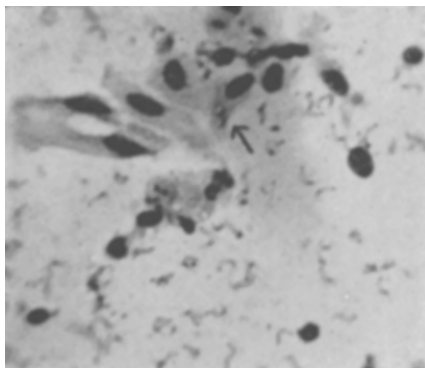


FIG. 1.

Pleural exudate of mouse No. 275, 11 days after subcutaneous infection with *T. cruzi*. Free trypanosome forms. Intracellular leishmania-like bodies in endothelial cells. (←)

the presence of a pleural exudate containing a large number of trypanosomes was noted. No bacteria could be cultured from this exudate on blood agar plates.

A similar observation was made later on in another mouse; No. 247. This animal was infected subcutaneously on the 5th of January, 1946 with 2 drops of blood containing 9 trypanosomes per microscope field. The number of parasites increased progressively in the blood: 6 days after inoculation: 2 trypanosomes per 100 fields; 17 days after inoculation: 4 parasites per one field. The mouse died on the 17th day showing a pleural exudate containing 1-3 trypanosomes per microscope field and a few leishmania-like forms. One drop of the exudate injected subcutaneously to 2 mice lead to a lethal infection. Microscopic sections of the heart and spleen of mouse No. 247 revealed the presence of numerous leishmania-like bodies.

The microphotograph (Fig. 1) of the pleural exudate of a young mouse shows the presence of free trypanosome forms and intracellular leishmania-like bodies. The animal (mouse No. 275; 12 g) died 11 days after subcutaneous infection with an inoculum of 0.05 cc of a suspension containing 50 para-

sites per field. The number of trypanosomes in the peripheral blood increased from about 14/field on the 3rd day to 160/field shortly before the death. Autopsy revealed the presence of 0.1 cc pleural exudate. Smears of the spleen showed numerous leishmania-like forms. Transfers of the exudate on blood agar did not show growth of bacteria.

In order to obtain information on the incidence of pleuritis in mice infected with *T. cruzi*, 26 young and 9 adult mice were infected subcutaneously. They all showed trypanosomes (2-22 per 100 fields) within 3 days, when the first examination was made. From this group of 35 mice, 25 died 9-17 days after infection, in 14 of them pleural exudate containing a large number of trypanosomes was noted. The volume of the exudate varied between 0.1 and 0.5 cc. Eight of the remaining 10 mice were sacrificed 9-11 days after the infection; in 3 of them pleural exudate with numerous trypanosomes was found. Thus, altogether 51.5% of the 33 mice examined presented pleural exudate containing trypanosomes and leishmania-like forms. In the adult group, 3 out of 9 mice developed pleuritis.

A survey recently conducted by G. Soo-Hoo in this laboratory showed that 18 out of 46 young mice (12-14 g) developed pleuritis (39%) after a heavy infection with a suspension containing approximately 50 parasites per microscope field. Out of another group of 56 mice which died after having received a light infection (5-10 parasites per field) only 2 animals (3.6%) showed exudate in the pleural cavity.

Summary. In the course of the infection of young mice with *T. cruzi* the incidence of pleuritis was observed in 3.6 to 50% of the cases depending on the size of the inoculum. The serous exudate which was sterile if examined with bacteriological methods contained numerous trypanosome forms and intracellular leishmania-like bodies.