

Infectivity of *Trypanosoma cruzi* after Cultivation for Thirteen Years *in vitro* Without Animal Passage.

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It is an established fact that when *Trypanosoma lewisi* is cultured on N.N. (Novy and MacNeal) medium¹ and subcultured at 3- or 4-week intervals the strain remains infective to rats during the first year of cultivation. However, at the end of 2 years' *in vitro* cultivation the strain loses its ability to infect rats, even if a massive inoculum prepared from several cultures is injected into young or splenectomized rats.²⁻⁴

The question whether or not *Trypanosoma cruzi* also becomes attenuated after a similar period of *in vitro* cultivation had not been investigated previous to this study. Since we have in our possession a strain of *Tr. cruzi* which has been kept *in vitro* for nearly 13 years without animal passage, this problem was studied.

Methods and Materials. A naturally infected *Triatoma geniculatus* received from Panama was crushed and the intestinal contents of the insect were inoculated into a guinea pig. On June 20, 1932, a strain of *Tr. cruzi* was cultured from the guinea pig's blood on N.N. medium.⁴ This culture has been maintained on N.N. medium at room temperature or at 25°C. Subcultures were made usually every 4 to 8 weeks; occasionally a longer interval was allowed to elapse before transfers were made.⁵ During this 13-year period, the strain was subcultured 81 times without any animal passage.

The 81st subculture of this strain of *Tr. cruzi* was inoculated to 8 mice (*Mus musculus*). The blood of these animals was examined microscopically at various intervals following inoculation, devoting about 5 minutes to each preparation. Xenodiagnosis (which consists of feeding normal *Triatoma* on the animals and subsequently examining the insect for infection with flagellates), was used on 2 mice. At varying times after inoculation, the mice were sacrificed and the blood was inoculated to N.N. medium. The tubes were covered with rubber caps, incubated at 25°C and examined after about 3 weeks for motile flagellates.

Results. The trypanosomes were demonstrated by direct microscopic examination in 4 out of 8 mice from 27 to 48 days after inoculation.

One of the xenodiagnostic tests was positive 50 days after injection, while the other was found negative 46 days following inoculation.

The mice were killed from 34 to 53 days after inoculation, and the blood of all 8 animals gave positive cultures for flagellates.

Summary. A strain of *Trypanosoma cruzi* cultured from experimentally-infected guinea pig on N.N. medium was subcultured at various intervals on N.N. medium for 13 years without animal passage. At the end of this period the strain was found to be still capable of producing infection in experimental animals.

The presence of *Tr. cruzi* was microscopically demonstrated in the circulating blood of 50% of the inoculated animals. However, cultures made from the heart blood of all the animals on N.N. medium were positive for *Tr. cruzi*.

¹ Novy, F. G., and MacNeal, W. J., *J. Infect. Dis.*, 1904, **1**, 1.

² Novy, F. G., Perkins, W. A., and Chambers, R., *J. Infect. Dis.*, 1912, **11**, 411.

³ Behrens, C. A., *J. Infect. Dis.*, 1914, **15**, 24.

⁴ Packchanian, A., *Science*, 1934, **80**, 407.

⁵ Packchanian, A., *J. Parasitol.*, 1943, **29**, 275.