Effects of Tyrocidine on *Trypanosoma cruzi* Cultures. (18616)

YOST U. AMREIN. (Introduced by Gordon H. Ball)

From the Department of Zoology, University of California at Los Angeles.

Weinman(1) reported among other findings that cultures of *T. cruzi* when subjected to certain concentrations of tyrocidine exhibited abnormal macroscopic and microscopic cultural characteristics. Instead of the thin whip-like flagellated forms prominent in normal cultures of *T. cruzi,* organisms with enlarged rounded posterior extremities were found in the presence of 5 γ/cc.

It is a question if the changes described above by Weinman were specific for tyrocidine or were the result of other factors, such as the hydrogen ion concentration of the cultures. Furthermore, it was considered important to test the effects of tyrocidine at various concentrations on the growth of the cultures themselves under controlled conditions in order to discover the actual type of effect produced on the trypanosomes.

Materials and methods. The strain of *T. cruzi* employed in these studies was obtained in July, 1947 from the National Institutes of Health, Bethesda, Md. The organisms were grown in the fluid medium with solid base developed by Chang(2,3), cotton stoppered 50 ml Erlenmeyer flasks proving to be the most convenient containers. The usual procedure was to add to 9 ml sterile Seitz-filtered culture fluid 1 ml of the experimental tyrocidine dilutions and 1 ml of trypanosome culture, adjusted with normal saline to give known inoculation densities. Tyrocidine hydrochloride was kindly supplied by Wallerstein Co., N. Y. Counts of the organisms were made with a Spencer bright line haemocytometer with improved Neubauer ruling, using the white blood count technic. Finally, thin films of the parasites were made by the standard thin blood film methods. Giemsa's stain was used after fixation and preliminary staining with Jenner's blood stain.

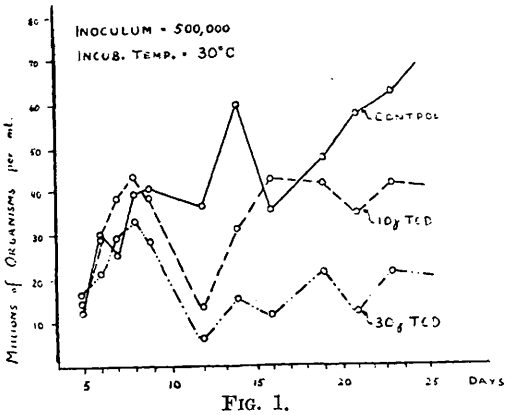

FIG. 1.
Growth curves of *T. cruzi* at 30°C in the presence of 10 γ and 30 γ tyrocidine (TCD) as compared with control cultures.

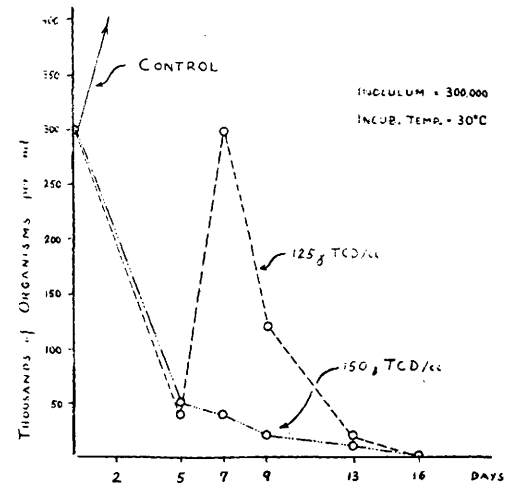

FIG. 2.
Growth curves of *T. cruzi* at 30°C in the presence of 125 γ and 150 γ tyrocidine (TCD).

Experimental results. Fig. 1 represents one of the growth curves for *T. cruzi* cultured at 30°C in the presence of 10 γ/cc and 30 γ/cc of the antibiotic. Initial density was 500000 organisms/cc. It is apparent that there is a clear-cut growth depressing effect of tyrocidine which is roughly proportional to the concentrations used. With initial inoculation densities of 40000 or 60000 organisms per ml the growth never reached sufficient density in the

1. Weinman, D., PROC. SOC. EXP. BIOL. AND MED., 1943, v54, 38.

2. Chang, S. H., *J. Inf. Dis.,* 1947, v80, 164.

3. Chang, S. H. and Negherbon, W. O., *Ibid.,* 1947, v80, 172.

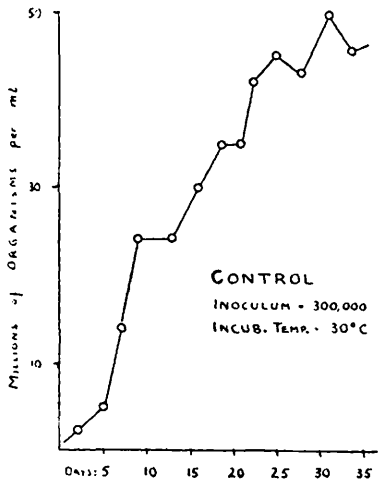

FIG. 3.
Growth curve of control culture of *T. cruzi* at 30°C drawn to different scale than Fig. 2.

control cultures to show any marked differences between these and experimental cultures.

Fig. 2 shows curves of haemoflagellate cultures grown in the presence of 125 γ/cc and 150 γ/cc tyrocidine respectively, initial density being 300000 organisms/cc. The growth depressing effect of tyrocidine is shown clearly with these concentrations of the antibiotic. Fig. 3 represents the control cultures.

Discussion. When *T. cruzi* is grown in Chang's serum-containing medium with a tyrocidine concentration above 100 γ/cc, very marked growth depression is evidenced. 200 γ/cc kills the organisms within a few hours. With 175 γ/cc living flagellates can be detected for but a day or two, while a concentration of 150 γ/cc reduces the culture to zero over a period of two weeks. In 125 γ/cc *T. cruzi* appears to show an abortive recovery peak after the initial shock of exposure to tyrocidine, yet these cultures too die out in two and a half weeks. Hotchkiss (4) has suggested that tyrocidine acts like a cationic detergent in altering the cellular membrane of bacteria; the same mechanism may be operating on the protozoan cell. Even though noticeable interference with normal growth of *T. cruzi* cultures in the presence of tyrocidine in concentrations below 100 γ/cc does occur, such inhibition can only be demonstrated clearly when relatively high initial densities of *T. cruzi* are used. If the inocula consist of less than 100000 organisms/cc, the cultures never really get a good start. Apparently the shock of subculturing to a new medium affects adversely a certain percentage of the flagellate population. Therefore, the higher the initial density, the higher the number of surviving and actively multiplying organisms.

Vacuolated and rounded leptomonad stages could first be detected on slides made after the 10th day. Usually the cultures with antibiotic substance showed this change in morphology a few days earlier than the controls. Such abnormal forms appeared when the hydrogen-ion concentration of the culture media dropped to 6 or below. This apparent close correlation between pH and morphological changes was observed both in the antibiotic-containing cultures and in the controls. These findings raise the question if the hydrogen-ion concentration *per se* is the sole causative agent for such drastic morphological changes or if these alterations are due in part to the actions of metabolites of an ageing culture.

No significant differences in growth characteristics could be observed in these experiments when inactivated instead of normal serum was used. The growth curves show that the effects of tyrocidine are only slightly influenced by temperature between 20°C and 30°C. Finally, by the time the cultures were three weeks old, no further conclusive effect of tyrocidine could be demonstrated; apparently by that time the activity of the antibiotic had practically completely vanished.

Summary. Effects of various concentrations of tyrocidine, an antibiotic substance produced by *Bacillus brevis*, have been tested on *in vitro* cultures of *Trypanosoma cruzi*. Concentrations of 100 γ tyrocidine/cc and over show a growth-depressing effect on *T. cruzi* cultures even in the presence of serum, such an effect being increased proportionately with antibiotic concentration, until with 200 γ/cc and a few hours' exposure, the lethal dosage is reached. The effects of tyrocidine *in vitro* on *T. cruzi* are only slightly influenced

4. Hotchkiss, R. D., *Adv. Enzymology*, 1944, v4, 153.

by temperature over a range of 20°C to 30°C. When the pH of the culture medium reaches 6.0 or lower, morphological changes in the flagellates are produced. Vacuolation and rounding up occur in both control and antibiotic-containing cultures and do not seem to be due to the influence of tyrocidine, but rather to a change in the hydrogen-ion concentration of the culture medium, with possibly the actions of metabolites in an ageing culture.

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Water, Sodium and Potassium Content of Normal and Encephalomyelitic Mouse Brain.* (18617)

FRANK GOLLAN† AND MAURICE B. VISSCHER.
From the Department of Physiology, University of Minnesota.

Changes in the water and electrolyte content of brain tissue have been described in a variety of conditions. By using the method of extracellular electrolyte depletion described by Yannet and Darrow(1), Swinyard, Toman and Goodman(2) were able to show that a marked reduction occurred in electroshock and chemoshock threshold in the first 4 hours and that the threshold returned to normal in 24 hours. In a later study from the same laboratory(3) this phenomenon was further investigated and it could be demonstrated that a small but not significant increase in total brain water occurred concomitantly with a decrease in electroshock seizure threshold and that an increase in extracellular sodium and chloride concentration of cerebral cortical tissue was more closely correlated with the electroshock seizure threshold than with other factors examined. In micro-incinerated preparations and by employing a microcrystallographic method(4), changes of local distribution of potassium and sodium in the cerebral cortex in relation to experimental convulsions could be detected. It was further shown that these neuron changes involved a loss of potassium and a gain in sodium. These changes were found to be consistent in animals given metrazol or electroshock convulsions, but were absent in asphyxia, exercise, ether anesthesia and did not depend on cortical topography. In experimental cerebral concussion in the cat an increase in the volume of the brain up to 5.5% could be found(5). In dogs, however, there was no indication of swelling and there was no shifting of water from the extracellular to the intracellular phase or vice versa as determined by the water, chloride, sodium, potassium, calcium, magnesium and total nitrogen content of cerebral tissue(6). Furthermore, the ionic environment has a pronounced influence on brain tissue respiration and it could be shown(7) that potassium caused an increase in aerobic glycolysis and inhibited anaerobic glycolysis, whereas sodium lacked these effects(8).

In view of clinical data suggesting that salt depletion and hemoconcentration may be predisposing factors to the invasion of the poliomyelitis virus(9) and the low serum potassium levels found in patients with severe infantile paralysis(10), studies on the water and electrolyte content of brain tissue of mice infected by the intraperitoneal route with a

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† Present address, Samuel S. Fels Research Institute, Yellow Springs, O.

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3. Swinyard, E. A., *Am. J. Physiol.*, 1949, v156, 163.

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7. Ashford, C. A. and Dixon, K. C., *Biochem. J.*, 1935, v29, 157.

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