

Effects of Gramicidin and Tyrocidine on Pathogenic Protozoa and a Spirochete.

DAVID WEINMAN.*

From the Department of Comparative Pathology and Tropical Medicine, Schools of Medicine and Public Health, Harvard University, Boston.

Dubos observed that autolyzed cultures of a soil bacillus (*Bacillus brevis*) were highly injurious to other bacteria.¹ From *B. brevis* cultures was first isolated an active crude material, tyrothricin; the latter was then found to be composed of two substances, gramicidin and tyrocidine. While both of these are effective *in vitro* against bacteria in minute amounts, they differ very considerably in specificity. Notably, gramicidin affects almost only gram-positive bacteria, whereas tyrocidine is not so limited and kills bacilli without regard to their gram-staining affinities. Further information is available in the recent summary by Dubos and Hotchkiss.² The effectiveness of these substances on non-bacterial parasites has not been extensively studied.

The present paper reports the action of gramicidin and tyrocidine on the flagellates *Leishmania tropica*, *Trypanosoma cruzi*, and *Trypanosoma lewisi*, on the spirochete *Leptospira icterohemorrhagiae*, and upon *Bartonella bacilliformis*. These organisms were grown in pure culture in semi-solid rabbit serum and hemoglobin agar containing the desired amounts of gramicidin and tyrocidine, incubated at room temperature (ca. 20-21°C), and corked with rubber stoppers. In addition, the effect of tyrothricin upon *Trichomonas vaginalis* both *in vitro* and *in vivo* is reported. This last organism with associated favorable bacteria was cultivated

in Johnson's medium³ containing the test substance in the fluid portion, and incubated at 37°C. Johnson's medium (liver infusion agar slants covered with a mixture of human serum and modified Ringer's fluid) was chosen since in it no bacteria are required for continued growth of the flagellate. Dr. Paul A. Younge selected the cases of "trichomonas vaginitis" for study, directed all therapeutic trials, and from his results kindly furnished the information contained herein.[†]

Results. (I) The effects of gramicidin and tyrocidine upon the organisms grown in pure culture are given in Table I. Gramicidin exerted no apparent harmful effect against any of these in the concentrations employed. Yet, used upon susceptible bacteria, the same concentrations are notably injurious. Bacterial susceptibility, as pointed out, is correlated to a high degree with gram-positive staining. Certain results, however, indicate that other types of gramicidin sensitivity exist. The gram-negative meningococcus and gonococcus are affected by it. Results with mammalian cells have given similar results. Thus bovine spermatozoa are quickly rendered motionless by gramicidin.⁴ Yet when stained, the tails of normal spermatozoa do not retain the violet at all, and while the heads do retain it somewhat, the reaction cannot be compared to the staining of gram-positive bacteria.[‡] However, in so far as the present results are concerned, no atypical susceptibility was found and the general correlation between gram-positiveness and gramicidin sensitivity appears to extend to the protozoa and the leptospira tested.

* Technical assistance rendered by Miss Rebecca Stickney was most helpful.

¹ Dubos, R. J., *J. Exp. Med.*, 1939, **70**, 1.

² Dubos, R. J., and Hotchkiss, R. D., *Trans. and Studies Coll. Phys. Philadelphia*, 1942, **10**, 11.

³ Dubos, R. J., Hotchkiss, R. D., and Coburn, A. F., *J. Biol. Chem.*, 1942, **146**, 421.

⁴ Henle, G., and Zittle, C. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1941, **47**, 193.

⁵ Johnson, G., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **45**, 567.

[†] These studies were carried on at The Free Hospital for Women, Brookline, Mass.

[‡] Personal communication from Dr. Henle.

TABLE I.
Growth in Culture Medium Containing Gramicidin and Tyrocidine.

Organism	Control	Gramicidin (γ per cc of medium)				Tyrocidine (γ per cc of medium)			
		0.005	0.05	0.5	1.0	0.1	0.5	1.0	5.0
<i>Leishmania tropica</i>	++++	++++	++++	++++	++++	++++	++++	++++	0 or +
<i>Trypanosoma cruzi</i>	++++	++++	++++	++++	++++	++++	++++	++++	+
<i>Trypanosoma lewisi</i>	++++	++++	++++	++++	++++	++++	++++	++++	+
<i>Eptospira ictero-hemorrhagiae</i>	++++	++++	++++	++++	++++	++++	++++	++++	++++
<i>Artonella bacilliformis</i>	++++	++++	++++	++++	++++	++++	++++	++++	++++

Tyrocidine, on the contrary, in a concentration of 5 γ per cc, proved to have a markedly adverse effect upon all the flagellates tested. This was evident by the slower rate of development, at times by the complete abolition of growth as in the case of *L. tropica*, as well as by less copious cultures.

Tyrocidine acted by killing the inoculated organisms. The effect was not instantaneous; the flagellates remained active for many hours, but after a lapse of several days had lost their motility. A few usually escaped and thus gave rise to the delayed growth.

Positive cultures of *Leishmania tropica* appeared normal in every respect, *Trypanosoma lewisi* colonies were somewhat more diffuse and at a deeper level, but were otherwise similar to the controls, whereas *Trypanosoma cruzi* cultures differed in both macroscopic and microscopic appearance.

Normally, in semi-solid medium, *T. cruzi* produces a thick, white, cloudy growth in the topmost centimeter of the medium, very scanty growth elsewhere. In the 5 γ tcd. (= 5 γ tyrocidine per cc) tubes, after an initial delay, growth appeared throughout the whole depth of the tubes as scattered white colonies reaching a relatively large size (0.2 to 0.3 mm). Those colonies which developed at a level lower than in the control tubes supported a certain degree of anaerobiosis, since in this medium methylene blue is decolorized to within about 1 cm of the surface.

This abnormal macroscopic growth corresponded to morphological peculiarities. In a normal culture of *T. cruzi*, long, thin, whip-like crithidial forms are numerous and prominent. They were extremely rare in the 5 γ

tcd. cultures and instead one usually encountered crithidia with enlarged rounded posterior extremities. These stubby crithidia seemed healthy since they were actively motile, multiplied, and had a longevity fully equal to the normal elongate forms.

The second peculiarity consisted in the immaturity of the trypanosomes. Although in 40- to 60-day cultures the trypanosomes were not uncommon, in almost every instance they contained a rod-like relatively small kinetoplast, situated near the nucleus. In contrast, in normal cultures of equal age, trypanosomes resembling those found in the blood, that is to say, with a large round kinetoplast occurring near the posterior end, were far more frequent.

This variant growth which continued as long as the organism was maintained in the tyrocidine medium, was not a permanent modification, for upon successive transfers in normal, semi-solid medium, the cultures gradually reverted to a more typical appearance.

It seemed of interest to determine whether the dissemination of the colonies throughout the medium was correlated with a change in the metabolic requirements of *T. cruzi*, particularly in regard to growth under anaerobic conditions. To test this, organisms obtained from the bottom of 5 γ tcd. tubes were inoculated into semi-solid medium containing 0.1% of sodium thioglycollate. The organisms survived the oxygen deprivation, but colonies were scanty and small so that the result indicated survival rather than growth. Control anaerobic culture with organisms never treated with tyrocidine developed similarly. Therefore, there seemed to be no increased tolerance to reduced oxygen.

(II) *Trichomonas vaginalis* in culture was treated with varying concentrations of tyrothricin, which contains 10 to 20% gramicidin and 40 to 60% tyrocidine.³ Concentrations of 50 γ per cc of culture fluid were invariably effective in killing the flagellates. It is probable that the effect was direct, rather than by the intermediary of the associated bacteria, since the medium employed supports growth of *T. vaginalis* in pure culture. It is also probable that the effective substance in the tyrothricin was the tyrocidine; gramicidin not being known to affect flagellates adversely.

Since these substances have been effectively employed in treating surface infections, it was thought that "trichomonas vaginitis" might be amenable to tyrothricin treatment. Accordingly, Dr. Paul Younge organized a small scale clinical trial. To avoid conjugal reinfection patients were requested to refrain from intercourse during treatment and all stated that they did so. Tyrothricin was used in a concentration of $\frac{1}{2}$ mg (500 γ) per cc. The vagina was thoroughly scrubbed with this solution, and subsequently a vaginal tampon wet with the same solution was put in place and left for 24 hours.

The immediate effect was a dramatic disappearance of the trichomonads. After an interval of a few days, however, the organisms reappeared and the relapse forms were apparently uninfluenced by the same tyro-

thricin treatment which had previously been so effective. Several possibilities may explain this result: first, an acquired resistance to tyrothricin, and second, multiplication of those few trichomonads which may have been tyrothricin-resistant from the outset with destruction of the non-resistant forms. Also both of these possibilities apply to the associated vaginal bacteria which favored the trichomonads.

Summary. 1. The effects of substances derived from *Bacillus brevis* were tested on protozoa, a leptospira, and on *Bartonella bacilliformis*. 2. Tyrocidine in a concentration of 5.0 γ per cc markedly inhibited the growth of *Leishmania tropica*, *Trypanosoma cruzi*, and *Trypanosoma lewisi* but not that of either *Leptospira icterohemorrhagiae* or *Bartonella bacilliformis*. 3. Tyrothricin killed *Trichomonas vaginalis* in culture at a concentration of 50 γ per cc of fluid medium. In patients with "trichomonas vaginitis" tyrothricin caused an initial dramatic disappearance of the trichomonads; relapse forms were uninfluenced by further applications. 4. Gramicidin in concentrations of 1.0 γ per cc of culture medium, or less, exerted no apparent adverse effect on the hemoflagellates nor upon *Leptospira icterohemorrhagiae* or *Bartonella bacilliformis*. These organisms are all gram-negative. The results obtained extend the correlation between gram-positive staining and gramicidin sensitivity.