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In Vitro Activity of Micoina on *Brucellae*, Compared with that of Terramycin[1]

Milton Thiago de Mello[2]

Section of Bacteriology, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil

Mycoin C, an antibiotic complex that has at least four components C_1, C_2, C_3, and C_4 (*1*), is the basis of the pharmaceutical product *micoina*.[3] The complex was isolated from species of *Penicillium* by Lembke and co-workers (*2, 3*), studied in Germany (*1–5*) and in France (*6–10*), and its *in vitro* and *in vivo* activity against *Brucellae* observed. Preliminary clinical trials with mycoin C, or with some of its fractions, in the treatment of human, bovine, equine, and ovine brucellosis gave promising results (*4, 8–10*) but more work is needed in this field. The fraction C_3 seems to be identical to patulin (*5, 6*).

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[2] Captain, Brazilian Army Veterinary Service.

[3] Supplied as a white powder through courtesy of Cia. Mycoina Panamericana, Montevideo, Uruguay, and Laboratorios Moura Brasil-Orlando Rangel S. A., Rio de Janeiro, Brazil.

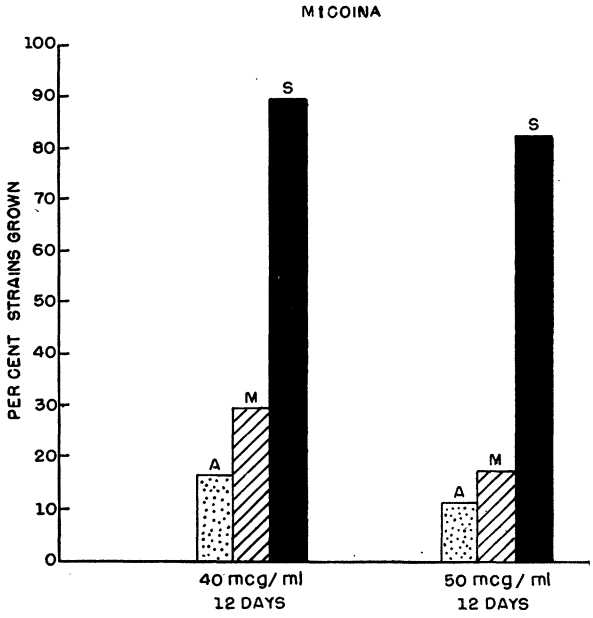

FIG. 1. Per cent of strains of *Brucellae* that survived to a 12 days exposure to 40 and 50 μg of micoina. Dotted, *Br. abortus*; crosshatched, *Br. melitensis*; solid, *Br. suis*. All the other strains died.

We observed striking differences between the action of micoina and Terramycin[4] against the three species of *Brucella*.

Eighty-two strains of *Brucella* from various sources were utilized: they were 36 *Br. abortus* (all aerobic), 29 *Br. suis*, and 17 *Br. melitensis*.

Dextrose veal infusion broth enriched with 0.5% trypticase (pH 6.9) in amounts of 2 ml was inoculated with a loopful of the stock cultures; after 24 or 48 hr of incubation, 0.1 ml of such cultures was transferred

[4] Terramycin hydrochloride for intravenous use, prepared by Chas. Pfizer & Co., Inc., U. S.

TABLE 1

NUMBER OF STRAINS OF *Brucellae* THAT GREW IN THE PRESENCE OF 40 AND 50 μg OF MICOINA*

Concentration of micoina	*Brucella*†	Days of observation			
		1–3	5	6	12
40 μg/ml	*abortus*	0	4(11.1%)	5(13.9%)	6(16.7%)
	melitensis	0	3(17.6%)	4(23.5%)	5(29.4%)‡
	suis	0	22(75.9%)	25(86.2%)	26(89.7%)§
50 μg/ml	*abortus*	0	3 (8.3%)	4(11.1%)	4(11.1%)
	melitensis	0	3(17.6%)	3(17.6%)	3(17.6%)
	suis	0	12(41.4%)	21(72.4%)	24(82.8%)‖

* All the controls grew within 24 hr. The tubes were not observed in the 4th and 11th days. All the strains that did not grow within 12 days, did not develop in subsequent days and were dead when tested after 10 days of further incubation.

† *Br. abortus*, 36 strains; *Br. suis*, 29 strains; *Br. melitensis*, 17 strains.

‡ One strain grew in the 12th day.

§ One strain grew in the 9th day.

‖ Two strains grew in the 7th day and another in the 8th day.

to 2 ml of the same medium and incubated at 37° C during 48 hr to constitute the cultures for the tests.

Stock solutions of the antibiotics were made in distilled water and Seitz filtered; the sterile solutions were mixed with the broth in order to obtain the desired concentrations (2 μg/ml to 50 μg/ml) and distributed in 10 × 120-mm test tubes in amounts of 2 ml; the buffer capacity of the broth maintained its pH (6.9) after the addition of the antibiotic solutions. The final medium was inoculated in the same day.

The tubes were inoculated with 0.1 ml of the 48-hr-broth cultures and incubated at 37° C. Daily observations of them were made with indirect illumination against a dark background. Turbidity of the medium was a signal of growth and recorded as positive. Sometimes a discrete turbidity was observed, mainly in the first 24 hr of incubation, but since it could not be ascertained as growth, the result was considered doubtful. Total absence of growth was considered negative. The results were recorded daily and tabulated in percentage of strains grown (cumulative numbers). All the controls, without antibiotics, yielded vigorous growth in 24 hr.

The positive tubes were discarded and the remaining ones were observed until growth appeared. After 2 or 3 wk the contents of the tubes in which any growth could be detected were inoculated in trypticase agar and observed for one week more.

The results obtained in several series of tests showed that micoina had more effective activity against *Br. abortus* than against *Br. suis*; just the contrary was observed with Terramycin which was more potent against *Br. suis* than against *Br. abortus* (Fig. 1). The behavior of the strains of *Br. melitensis* was intermediate for both antibiotics.

With 5 μg of micoina/ml of medium, 72% of the strains of *Br. abortus* were inhibited in 24 hr whereas only 27% of the strains of *Br. suis* were inhibited. With 10 μg/ml all the strains tested were inhibited during 24 hr, in general.

More striking results were obtained with larger doses of antibiotics. The strains of *Br. suis* were the first to initiate growth in presence of micoina, and developed in the presence of higher concentrations of the drug. With Terramycin, *Br. abortus* was first to initiate growth and supported larger doses of the antibiotic.

The presence of normal horse serum apparently did not enhance or diminish the activity of micoina. Preincubation of the medium with the antibiotic for more than 8 days inactivated the drug even in the higher concentrations, and the microorganisms then inoculated in that medium could develop in the same manner as in the controls without antibiotics.

As a bacteriostatic agent, Terramycin was more potent than micoina in delaying growth, but when the incubation was prolonged, all the strains tested grew in even the highest concentration of antibiotic used (20 μg of Terramycin/ml of medium); *Br. suis* being the species most retarded. In high concentrations, micoina was markedly bactericidal, at least against *Br. abortus*. In concentrations of 40 and 50 μg/ml, almost all the strains of *B. abortus* were killed (respectively 30 and 32 out of 36 strains), whereas only a few of *Br. suis* failed to survive (respectively 3 and 5 out of 29 strains) (Table 1 and Fig. 1). According to Criscuolo and co-workers (*11*), *Br. abortus* grows even in the presence of 50 μg of Terramycin/ml of medium, after 8 days of incubation.

Figure 2 is representative of tests with both antibiotics. It can be seen that, in a general manner, the bacteriostatic activity of micoina per weight was one-fourth of that of Terramycin, when all other conditions of the tests were maintained. It can be observed also that micoina is more inhibitory to *Br. abortus*, and Terramycin more inhibitory to *Br. suis*.

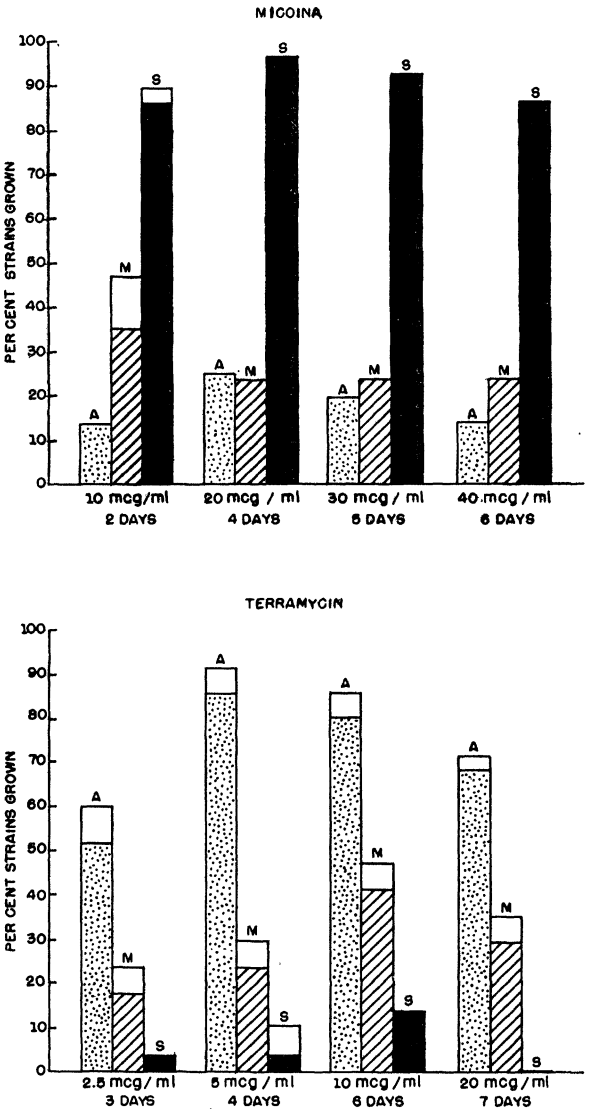

FIG. 2. Per cent of strains of *Brucellae* grown in presence of micoina and Terramycin. Dotted, *Br. abortus*; cross-hatched, *Br. melitensis*; solid, *Br. suis*; open, doubtful growth.

This work suggests that micoina and Terramycin interfere with distinct metabolic mechanisms of *Br. abortus* and *Br. suis*.

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Observations on the Chemical Constitution of Inflammatory Exudate in Normal and Hypophysectomized Rats[1]

V. W. Adamkiewicz, A. Horava, and E. Salgado

Institut de Médecine et de Chirurgie expérimentales
Université de Montréal, Montreal, Canada

Chemical data on the inflammatory exudates, though numerous, are conflicting because it is difficult to induce exudate formation in animals, in an exactly reproducible manner, and avoid general systemic stress. Usually inflammatory exudate is obtained after inflammation has been caused by the injection of irritants into large body cavities. Animals that survive such treatment are ill and stressed. This technique can be applied only with difficulty to hypophysectomized animals whose resistance to stress is particularly low. The extent to which stress influences, directly or through humoral substances, the course of inflammation has been clearly demonstrated in numerous publications (*1*). It is evident therefore that we should attempt to learn more about the fundamental development of inflammation when it is free from interfering factors.

A method was recently devised (*1–3*) that overcomes most of the difficulties. This method, the "granuloma pouch" technique is applicable to small laboratory animals. Inflammation can be initiated in a reproducible and quantitative manner and is well tolerated even by hypophysectomized rats; therefore, it is relatively stress free, yet it is responsive to humoral influences (*3*). The exudate is abundant, homogeneous, and well delimited by the inflammatory granulomatous tissue.

Two groups of male Sprague-Dawley rats were used. Group I consisted of 6 normal rats and group II of 5 rats that had been hypophysectomized 120 days prior to the initiation of inflammation. Inflammation was obtained in both groups by injecting 25 ml of air under the dorsal skin, followed by 1 ml of a 1% solution of croton oil (irritant) in Mazola oil (vehicle). Thus an *in vivo* ampulla was formed. Fourteen days later, it had a granulomatous wall (Fig. 1) and was filled with a hemorrhagic exudate. At this stage, the rats were killed, the exudate was collected and analyzed. The completeness of hypophysectomy was carefully verified.

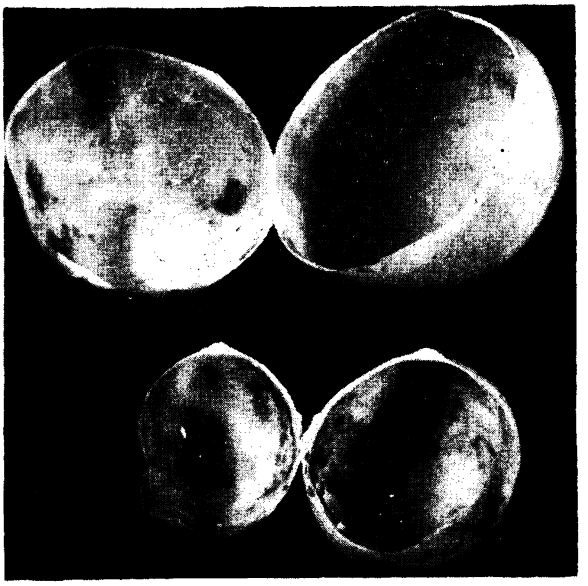

FIG. 1. (Upper) Granuloma pouch in a control rat. (Lower) Granuloma pouch in a hypophysectomized rat.

The pH of the exudate was measured on a Beckman pH meter, model G, and the sodium and potassium on the Beckman flame spectrophotometer, model DU. The sugars were determined by the Folin and Malmros micromethod (*4*), the NPN (nonprotein nitrogen) by Folin and Wu (*5*), the inorganic phosphates by Fiske and SubbaRow (*6*), chlorides by Van Slyke (*7*), and iron by Kennedy's method (*8*). The total proteins were determined by an adaptation of the micro-Kjeldahl procedure (*9*). The total fat was estimated by the following gravimetric method. Five milliliters of the exudate were adsorbed on Whatman filter paper No. 40 and dried in an oven at 75° C for 48 hr. The filter papers were then folded, weighed to constant weight, and extracted in a Soxhlet apparatus under reflux with 3:1, ethanol: ethyl ether mixture for 8 hr. The filter papers were then removed from the Soxhlet, again dried in an oven at 75° C for 48 hr, and reweighed to constant weight. The loss of weight of the filter papers during extraction was equal to the total ether-soluble fraction (fatty substances) of the exudate. Table 1 summarizes the results obtained.

The rats of the control group gained an overall 81 g in body weight during the 14 days the experiment lasted. The gain was due to normal somatic growth and to the weight of the granuloma pouch.

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