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ANTIBIOTICS FROM THE GENUS FUSARIA.

II. The Antituberculous Activity of Enniatin-B

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Abstract

The importance of the genus *Fusarium* Link, as producers of antibictics capable of inhibiting the growth of *Mycobacterium tuberculosis* (Gaumann *et al.*, 1947), the cultural studies and general antimicrobial activity of Enniatin-B (Tirunarayanan and M. Sirsi, 1956), an antibiotic isolated from *Fusarium avanaceum* (CMI 49894) have been described earlier. The *in vitro* antituberculous activity and the effect of this antibiotic on the course of experimental tuberculosis in mice is presented in this communication.

Materials and Methods

'In vitro' *testing*.—These include the effect on pa'hogenic and non-pathogenic mycobacteria. Tne virulent organism was $H_{37}R_v$ strain of *Mycobacterium tuberculosis* maintained by bi-weekly transplanting on modified Proskauer-Beck medium as a oellicle growth. For the '*in vitro*' testing, the surface growth from a 10-day old culture was taken on 3 mm. looo and gently floated on to the fresh sterilized culture tubes, incorporating the required concentration of the drug. The plugs were sealed with paraffin and the tubes inoculated at 37° C. The growth was observed at weekly intervals for a period of three weeks. To establish the bacteriostatic or bactericidal action, the cultures from the drug concentrations showing no growtn or a faint spread, were washed in saline and refloated on fresh media, sealed and incubated as before, and observed for another three weeks.

Mycobacterium lacticola O_{11} and O_{12} strains isolated and identified by Khambata and Bhat 1955[3] and found to be non-pathogenic to laboratory animals (Gangadharam & Sirsi)[1] and grown on nutrient broth were used to assess the effect of Enniatin-B on non-pathogenic Mycobacteria.

'In vivo' *tests.*—A known quantity of the surface culture of $H_{37}R_v$ after ten days' incubation was homogenised in normal saline so as to contain 1 mg./ml. (wet weight) of the bacterial mass. 0·2 ml. of this suspension was injected intravenously into the caudal vein of white mice weighing 18–22 gm. The chemotherapeutic effectiveness was evaluated by noting the weights during the course of infection and by macroscopic and microscopic examination of the lungs at the time of death (Sirsi and De, 1951).[5] The surviving animals were sacrificed at the end of 28 days by which time all the controls had died and the lesions in the organs observed.

Enniatin-B has been found to possess no detectable symptoms of toxicity when administered to mice by intra-peritoneal injection upto dosages of 100 mg./kg. daily for a period of 30 days.

Following infection with 0·2 mg. wet weight of the $H_{37}R_v$ strain of *Mycobacterium tuberculosis*, mice were separated into three groups. One group received no drug and served as the untreated control. The second and the third groups respectively received dihydrostreptomycin and Enniatin-B (in oil) intraperitoneally at dosages of 50 and 100 mg./kg. daily.

OBSERVATIONS

The '*in vitro*' antimicrobial actions on *Mycobacterium tuberculosis* and *Mycobacterium lacticola* are shown in Tables I and II.

TABLE I

Antituberculous activity of Enniatin-B
(*against Mycobacterium tuberculosis var.* hominis *strain* $H_{37}R_v$)

Week	Enniatin-B, 1 part in....parts					
	1,000	10,000	100,000	1,000,000	10,000,000	100,000,000
1st week ..	—	—	—	—	—	++
2nd week ..	—	—	—	—	—	++
3rd week ..	—	—	—	—	—	++

"—" denotes total inhibition; "++" denotes full growth.

Tests relating to the growth-inhibitory effects of Enniatin-B on Mycobacteria have thus shown that this compound possesses exceptionally high activity. Enniatin-B is found to inhibit the growth of the virulent strain of *Mycobacterium tuberculosis* var. *hominis* ($H_{37}R_v$) at dilutions of 1 : 10,000,000 an activity comparable to that of streptomycin. At dilutions of one million and above, the effect was only bacteriostatic, since the inocula when placed once again on fresh Youmans' medium were found to grow, though at a slow rate. At dilutions of less than a million, the effect appeared to be bactericidal.

TABLE II

Activity of Enniatin-B against Mycobacterium lacticola in vitro

Test organisms	Enniatin-B, 1 part in....parts				
	1,000	10,000	100,000	1,000,000	10,000,000
Mycobacterium lacticola 0–11	—	—	—	++	++
0–12	—	—	+	++	++

"—" denotes inhibition; "++" denotes full growth; "+" denotes slight growth.

The inhibitory effect on the growth of these non-pathogenic Mycobacteria was markedly less than those on the virulent tubercle bacilli.

The effect of Enniatin-B on experimental tuberculosis in mice.—The growth response curves of the untreated, Enniatin and streptomycin treated mice infected with *Mycobacterium tuberculosis* are shown in Fig. 1.

While a continuous loss in weight was noticed with the untreated group, the other two groups showed an initial increase in weight during the 1st week and the weight loss during the next two weeks was slight and more gradual indicating that the progress of the disease which is usually responsible for the rapid loss in weight during the 2nd week was checked to a certain extent.

The survival periods and the gross amount of lesions in the lungs of the three groups are seen in Table III and Fig. 2 (Plate VIII).

The mean loss or gain in weight, the numbers surviving, the degree of gross lesions and the type of lesions as observed by histopathological studies are presented in Table IV.

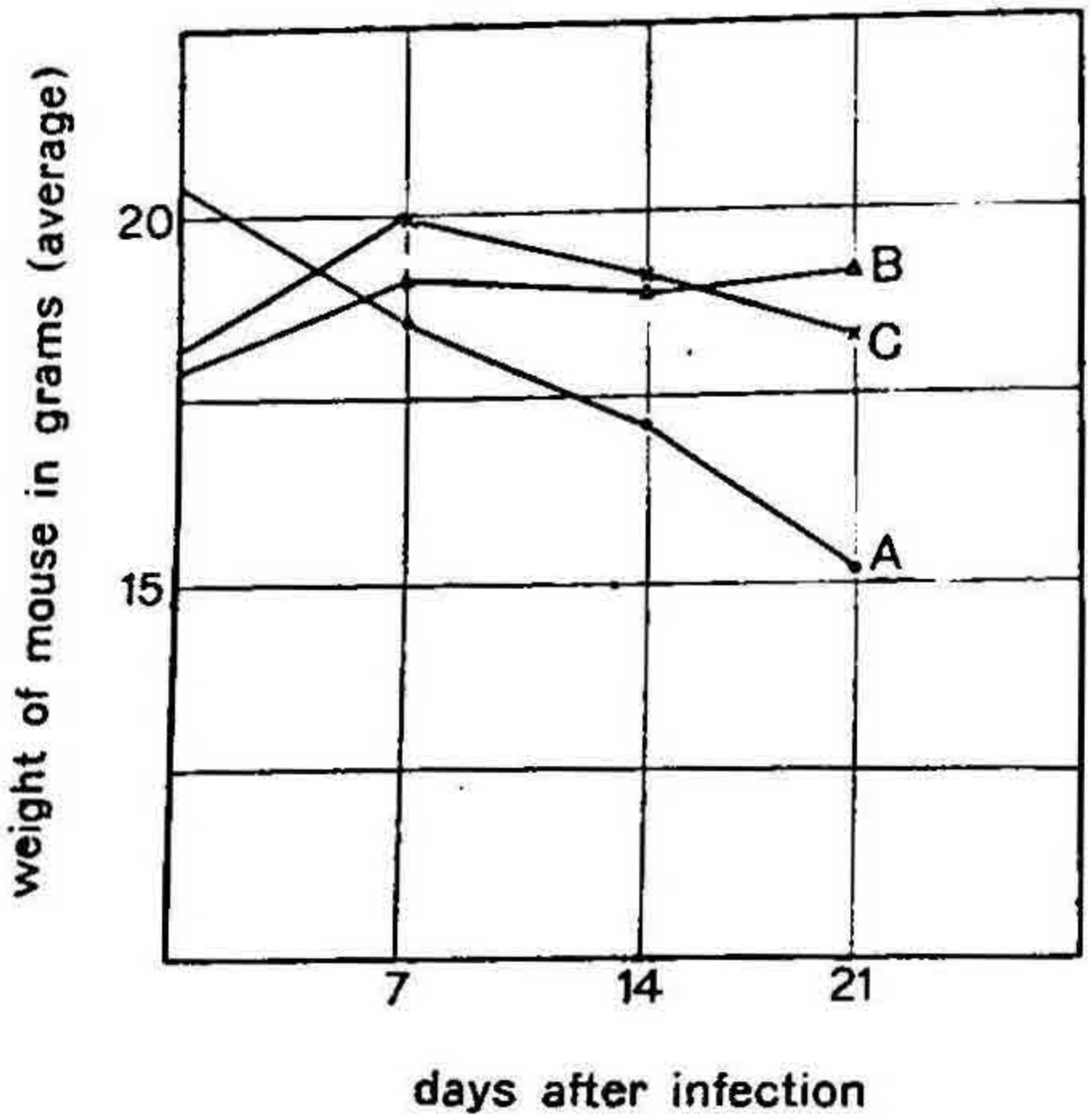

FIG. 1. Effect of Enniatin-B on murine tuberculosis. Macroscopic appearance of the lungs of mice from A — Untreated
B — Dihydro Streptomycin treated, and,
C — Enniatin treated groups.

TABLE III

Activity of enniatin-B in tuberculosis of mice

Animal No.	Group	Survival time in days	Gross amount of lesions in lungs
1	A. Untreated (control) ..	19	3·6+
2	,, ..	21	3·5+
3	,, ..	25	3·6+
4	,, ..	22	3·0+
5	,, ..	23	3·2+
6	B. Streptomycin treated ..	—	1·0+
7	,,	—	2·0+
8	,, ..	—	1·5+
9	,, ..	—	3·0+
10	,, ..	—	2·0+
11	C. Enniatin-B treated ..	—	2·8+
12	,, ..	27	4·0+
13	,, ..	—	3·0+
14	,, ..	—	3·0+
15	,, ..	—	3·0+

"—" denotes alive at the end of 28 days

TABLE IV

Evaluation of Enniatin-B activity in experimental tuberculosis of mice

Group	No. of mice	Mean loss or gain in weight gm.	No. surviving at the end of 28 days	Mean gross lesions	Nature of lesions
Untreated ..	5	−5·2	0	3·4	EN +
Streptomycin treated ..	5	+1·4	5	2·0	P+
Enniatin-B treated	5	+1·8	4	3·16	P+EN few

EN = Exudative necrotic lesion; P = proliferative lesion.

These results indicate that Enniatin-B in the dose administered prolongs the survival period, diminishes the mortality and arrests the progress of the disease but is inferior to streptomycin in its efficacy as could be gauged by the amount of gross lesion and the presence of exudative necrotic lesions.

SUMMARY

Enniatin-B, an antibiotic isolated from *Fusarium avenaceum* (CMI 49894) inhibits '*in vitro*' the growth of *Mycobacterium tuberculosis* $H_{37}R_r$, in 1 in 10 million dilution, and proves bactericidal in concentrations upto 1/100,000. The bacteriostatic effect of the antibiotic on non-pathogenic *Mycobacterium lacticola* is considerably less than on the virulent strain. In a dosage of 100 mg./kg. daily, given intraperitoneally, Enniatin-B arrests the progress of the experimental tuberculosis in mice but not to the same extent as dihydrostreptomycin.

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EXPLANATION OF PLATE VIII

Infection: *Mycobacterium tuberculosis* $H_{37}R_r$ (i.v.)
Dihydrostreptomycin: 50 mg./kg. daily I.P.
Enniatin-B: 100 mg./kg. daily I.P.