Effect of Dimetridazole on Growth of *Neurospora sitophila* and *Lactobacillus casei.* (30126)

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Colvin(1) has reported on the toxicity of 1,2-dimethyl-5-nitroimidazole (dimetridazole) in chickens. The drug is recommended by May and Baker, Ltd.(2) for treatment of enterohepatitis in turkeys. Greater than therapeutic doses of the drug were found to have toxic effects on turkeys by Condren (3). Collins *et al*(4) reported on the physiologic side effects of dimetridazole in chicks. The same authors also studied the effects of dimetridazole on the chick embryo. The drug was found to cause a depression of heart rate, blood pressure, respiratory rate, and rectal temperature of chicks and to cause a marked depression of hatchability of fertile eggs. To date, there have been no reports concerning the effects of the drug on bacteria and fungi. The present article reports the effect of dimetridazole on the growth of *Lactobacillus casei* and *Neurospora sitophila.* It also contains preliminary data concerning the detoxication of dimetridazole.

Materials and methods. The two organisms used in these studies were *Neurospora sitophila* 299 and *Lactobacillus casei.* Stock cultures were obtained from the American Type Culture Collection, Washington, D. C. The organisms were maintained on agar slants or stabs as stock cultures. *N. sitophila* was maintained on slants made from Bacto-Neurospora Culture Agar. *L. casei* was maintained as a stab culture on Bacto Micro Assay Culture Agar. Stock cultures were transferred to fresh agar at 2-week intervals and were subcultured 3 times before inoculating into the respective test media.

For the studies involving *N. sitophila,* twenty-five 250 ml Erlenmeyer flasks were divided into 5 groups of 5 flasks each. To each flask was added 5 ml of the media described by Barton-Wright(5) and 5 μg of pyridoxine. *N. sitophila* 299 is a pyridoxine-less mutant. Dimetridazole was added in graded levels from 0.0 to 2560.0 μg per ml. The final volume was 50 ml and the final reaction pH was adjusted to 5.5 with dilute hydrochloric acid where necessary. The flasks were autoclaved for 15 min (15 lb of steam pressure per sq. in., 121°C), cooled, and inoculated with a spore suspension of *N. sitophila* in sterile physiologic saline as described by Barton-Wright(5). After 5 days of incubation, the flasks were autoclaved (5 min, 15 lb of steam pressure per sq. in., 121°C), and the mycelia were harvested, dried, and weighed on a Roller-Smith balance.

For the studies involving *L. casei,* 28 matched micro-assay culture tubes were divided into 7 groups of 4 tubes each. Each tube received 5 ml of Micro Inoculum Broth which is recommended by Difco Laboratories for cultivation of *Lactobacilli*(6). Dimetridazole was added in graded levels from 0.0-3200.0 μg per ml (Table II). The final volume was adjusted to 10 ml either with deionized water or an aqueous solution of dimetridazole. The broth was made double strength to allow for this dilution. The final pH was 7.4. The tubes were capped with metal assay caps, autoclaved according to the procedure outlined for *N. sitophila,* cooled, and inoculated with 2 drops of a suspension of *L. casei* in physiologic saline. After an incubation period of 17 hours (37°C), the growth of the organism was measured by determining the optical density of the solutions.

In the preliminary trials concerning the detoxication of dimetridazole, a series of tubes was made up to contain 0.0, 400.0, 600.0, 800.0, 1200.0, 1600.0, and 1800.0 μg of dimetridazole. In addition the tubes contained 5 ml of the media previously described for cultivation of *L. casei.* Dimetridazole was supplied through the courtesy of Dr. Salsbury's Laboratories, Charles City, Iowa. The final volume was 10 ml. After thorough

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TABLE I. Effect of Dimetridazole on Growth of *N. sitophila* 299.

Treatment	Dimetridazole (μg 1 ml)	Wt*	Inhibition (%)
1 (control)	.0	111.1	.0
2	320.0	107.2	3.6
3	640.0	105.4	5.2
4	1280.0	72.8	34.5
5	2560.0	.0	100.0
		$\pm$ .2	

* Avg of 4 values.

mixing, 0.2 ml of 2 M KCl was added to 1.0 ml of the media containing dimetridazole. The resulting solutions were deoxygenated with nitrogen by conventional techniques and were polarographed from —0.20 to —0.95 volts using a Sargent, Model XV, Polarograph.

To study the time dependence of dimetridazole detoxication, a series of matched microassay culture tubes containing bacterial media and dimetridazole were inoculated from a suspension of *L. casei* as previously described. The amounts of dimetridazole present at start of the experiment and after 24, 48, and 72 hours of bacterial growth were determined by polarographic analysis of the solutions and comparison of the diffusion current with that of known concentrations of the drug.

All experiments reported here were conducted more than once with similar results each time.

Results. The effect of dimetridazole administration on growth of *N. sitophila* is shown in Table I. Levels of less than 640 μg of dimetridazole per ml of media were non-toxic to *N. sitophila*. However, levels of 1280.0 and 2560.0 μg of dimetridazole per ml inhibited the growth of *N. sitophila* to the extent of 34.5 and 100.0%, respectively.

Similarly, the data obtained by treatment of *L. casei* with dimetridazole are shown in Table II. As in *N. sitophila*, dimetridazole was toxic to the organism at most levels used. The levels of 100 and 200 μg of dimetridazole per ml of media were only slightly toxic to the organism. A level of 400 μg of dimetridazole per ml of media gave a growth inhibition of 23.9%. Levels of 800, 1600, and 3200 μg of dimetridazole per ml of media inhibited the growth of *L. casei* to the extent of 35.7, 54.4, and 69.5%, respectively.

Values for the diffusion current obtained by polarographic analysis of bacterial culture media containing various concentrations of dimetridazole are shown in Table III. There

TABLE II. Effect of Dimetridazole on Growth of *L. casei*.

Treatment	Dimetridazole (μg 1 ml)	O.D.* (17 hrs)	Inhibition (%)
1	.0	.272	.0
2	100.0	.263	3.3
3	200.0	.259	4.8
4	400.0	.207	23.9
5	800.0	.175	35.7
6	1600.0	.124	54.4
7	3200.0	.083	69.5

* Avg of 4 values.

TABLE III. Diffusion Currents for Various Concentrations of Dimetridazole in Bacterial Culture Media.

Dimetridazole	Diffusion current*
.0	.0
400.0	34.8
600.0	49.5
800.0	61.5
1200.0	92.8
1600.0	122.5
1800.0	140.5 $\pm$ .6

* Sensitivity = .600 4 amps/mm.

was a rectilinear relationship between diffusion current and dimetridazole concentration. Recovery was better at higher than at lower concentrations. Table IV shows that as the length of the incubation period increased, the height of the polarographic wave also increased. The half wave potential for dimetridazole in bacterial culture media was found to be —0.71 volt. This increased to —0.59 volt after 72 hours of incubation in the presence of *L. casei*. The height of the polarographic wave was unaffected by incubation in the absence of *L. casei*. Growth of the organism (optical density) also increased as length of incubation period was increased.

Discussion. These results illustrate that dimetridazole is toxic to at least 2 types of micro-organisms. In *N. sitophila* the drug is completely toxic at a level of 2560 μg per ml of media. By interpolation, a comparable

TABLE IV. Optical Density and Polarographic Wave Height After Various Lengths of Incubation of *L. casei* in the Presence of Dimetridazole.

Lot No.	Incubation time (hr)	Wave height (mm)	E½ (volts)	O.D.*
1	0†	.0	.00	.000
2	24‡	.0	.00	.444
3	48‡	.0	.00	.432
4	72‡	.0	.00	.456
5	0§	136.0	−.71	.000
6	24‖	135.5	−.71	.000
7	48‖	136.9	−.71	.000
8	72‖	135.5	−.71	.000
9	24¶	155.3	−.65	.229
10	48¶	165.6	−.62	.272
11	72¶	177.0	−.59	.292

* All values represent average of 4 determinations.
† 0.0 mg dimetridazole/10 ml.
‡ *Idem*
§ 16.0 mg dimetridazole/10 ml.
‖ Uninoculated, 16 mg dimetridazole/10 ml.
¶ Inoculated, 16 mg dimetridazole/10 ml.

level administered to *L. casei* would be less than 70% inhibitory. Hence, the 2 microorganisms are not affected to the same extent by the drug. Half maximal inhibition for *L. casei* was found at a concentration of 1600 μg of dimetridazole per ml of media. This is more than 5 times the value for half maximal inhibition of *E. coli* as reported by Woolley(7). The point of half maximal inhibition of *N. sitophila* was at a level of 1280 μg of dimetridazole per ml of media. The mode of action of the drug in these and other organisms is not presently known. Kane(8) reported that the height of the polarographic wave of dimetridazole was unaffected by pH changes from 1.0 to 10.0. Since this would cover the pH range in media inoculated with *L. casei*, the increase in the polarographic wave height must be due to some alteration of the dimetridazole molecule. It would seem that detoxication of dimetridazole would lead to a diminution of the wave height. However, the tracings obtained after incubation with *L. casei* were not typical dimetridazole polarograms. This suggests the production of some metabolite of the drug which had a reinforcing effect on the diffusion current of dimetridazole. Kane(8) also alluded to such difficulties in determining dimetridazole in various biological materials. Kane reported that the half-wave potential for dimetridazole varied with pH. Perhaps the increases in half-wave potential found with changes in incubation time were at least partially due to changes in the pH of the media. It should also be noted that there were no biological compounds in the culture media which gave polarographic waves at the half-wave potential of dimetridazole nor were any produced during the incubation period either in presence or absence of *L. casei*. This further supports the supposition that *L. casei* can alter the dimetridazole molecule to some extent.

Summary. Dimetridazole has been shown to be toxic to turkeys(3), chickens(1), and chick embryos(4). It has also been shown to decrease certain physiologic values in chicks(4). The data presented here indicate that the drug inhibits growth of *N. sitophila* and *L. casei* and that it can be, to some extent, metabolized by *L. casei*.

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