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Antagonistic Action of Cholesterol on Mycobacillin

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It was reported earlier that sterols can antagonize the antifungal action of polyene-type antibiotics, e.g., nystatin, filipin, etc. (D. Gottlieb et al., Science **128**:361, 1958). That this antagonizing action is due to the formation of a complex between the sterol and the antibiotic has also been demonstrated by spectrophotometric studies (J. O. Lampen et al., J. Bacteriol. **84**:1152, 1962). There is, however, no report of the antagonistic action of sterols or any other compounds against polypeptide antibiotics, whether antibacterial or antifungal. Mycobacillin (S. K. Mazumder and S. K. Bose, Arch. Biochem. Biophys. **90**:154, 1960; S. K. Mazumder and S. K. Bose, Biochem. J. **74**:596, 1960), an antifungal antibiotic, is a cyclic polypeptide. It acts variously on susceptible microorganisms. This communication describes the antagonistic effect of cholesterol on mycobacillin susceptibility.

TABLE 1. *Effect of cholesterol on the growth-inhibiting property of mycobacillin in solid culture (synthetic medium)*[a]

Reaction system	Zone of inhibition
	mm
Synthetic medium + 0.25 ml of acetone	30
Synthetic medium + no acetone	29
Synthetic medium + 1 mg of cholesterol in 0.25 ml of acetone	22
Synthetic medium + 1.5 mg of cholesterol in 0.25 ml of acetone	18
Synthetic medium + 2 mg of cholesterol in 0.25 ml of acetone	No inhibition

[a] To 15 ml of synthetic agar medium, as described previously (Banerjee and Bose, J. Bacteriol **86**:387, 1963), was added either 0.25 ml of acetone alone or 0.25 ml of acetone containing varying amounts of cholesterol. The amount of inoculum added to each plate was 2×10^4 cells. Mycobacillin (150 μg/ml) was added in 0.1 M $NaHCO_3$ solution.

The paper disc method was used to study the action of cholesterol on the growth-inhibiting property of mycobacillin against a strain of *Candida albicans* (obtained from the School of Tropical Medicine, Calcutta, India). Rate of growth was measured turbidimetrically in a Klett-Summerson colorimeter equipped with a red filter. Agglutination was observed macroscopically (N. Banerjee and S. K. Bose, J. Bacteriol. **86**:387, 1963). Release of ultraviolet-absorbing materials was measured spectrophotometrically by use of a Beckmann DU spectrophotometer.

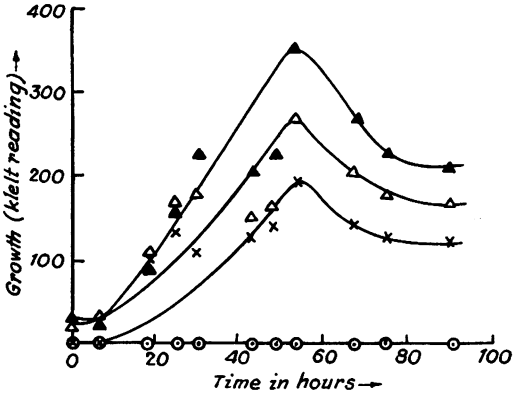

FIG. 1. *Antimycobacillin effect of cholesterol on the growth pattern of Candida albicans. Symbols: ×, $NaHCO_3$ solution + acetone; ⊙, mycobacillin solution + acetone; ▲, mycobacillin solution + cholesterol solution; △, cholesterol solution + $NaHCO_3$ solution. Mycobacillin (150 μg/ml) and cholesterol (200 μg/ml) solutions were made in 0.1 M $NaHCO_3$ solution and acetone, respectively. A 0.1-ml amount of inoculum (24-hr broth culture in synthetic medium) was added to each of the flasks containing 9.5 ml of synthetic medium, 0.5 ml of $NaHCO_3$ solution, or 0.5 ml of mycobacillin solution and 0.3 ml of acetone, or 0.3 ml of cholesterol in acetone. The reaction system was incubated in a shaker at room temperature (31 C).*

The growth-inhibiting action of mycobacillin, as measured by the paper disc method in terms of zone inhibition (Table 1), was completely antagonized by cholesterol. It was also observed that the pattern of growth, as altered by mycobacillin, returned almost to normal in the presence of

TABLE 2. *Effect of cholesterol on the ability of mycobacillin to agglutinate cells*

Additions to reaction system[a]	Agglutination after 18 hr		
	At 37 C	At 4 C	Flasks at 4 C removed to 37 C[b]
1. $NaHCO_3$, 0.5 ml.....	−[c]	−	−
2. $NaHCO_3$, 0.5 ml, + 0.3 ml of acetone...	−	−	+
3. Mycobacillin in $NaHCO_3$, 0.5 ml, + 0.3 ml of acetone...	+++	−	+++
4. Mycobacillin in $NaHCO_3$, 0.5 ml, + 0.3 ml of a solution of cholesterol in acetone.....	−	−	+
5. $NaHCO_3$, 0.5ml, + 0.3 ml of a solution of cholesterol in acetone.....	−	−	+

[a] Each set of additions was made to 5.6 ml of synthetic medium (system 1, 5.9 ml) plus 3.6 ml of cell suspension. Mycobacillin (150 μg/ml) was added in solution with 0.1 M $NaHCO_3$, and cholesterol (200 μg/ml), in acetone solution. Cells freed from medium by centrifugation and washing were suspended in 0.85% saline. After the additions, flasks were shaken for 1 hr at 37 C and then incubated for 18 hr at 37 or 4 C.

[b] Flasks preincubated at 4 C were removed to 37 C, and, after 18 hr, the observation was made.

[c] Symbols: +, agglutination; −, no agglutination.

cholesterol (Fig. 1), in the sense that the lag induced by mycobacillin (N. Banerjee and S. K. Bose, Indian J. Med. Res. **52**:1062, 1964) was eliminated and growth was enhanced. Mycobacillin agglutinates susceptible cells of *C. albicans* at 37 C but not at 4 C (N. Banerjee and S. K. Bose, J. Bacteriol. **86**:387, 1963). The presence of cholesterol, however, prevented this agglutinating action at 37 C (Table 2). Mycobacillin causes release of ultraviolet-absorbing materials from *C. albicans* (N. Banerjee and S. K. Bose, Intern.

TABLE 4. *Antimycobacillin effect of cholesterol on the Gram reaction of Candida albicans*

Additions to reaction system[a]	Per cent of gram-negative organisms
1. $NaHCO_3$, 0.5 ml.....	2.5
2. Mycobacillin in $NaHCO_3$, 0.5 ml, + 0.3 ml of acetone.....	20.0
3. Mycobacillin in $NaHCO_3$, 0.5 ml, + 0.3 ml of a solution of cholesterol in acetone.....	2.1
4. $NaHCO_3$, 0.5 ml, + 0.3 ml of a solution of cholesterol in acetone.....	2.9

[a] Each set of additions was made to 5.6 ml of synthetic medium (system 1, 5.9 ml) plus 3.6 ml of 24-hr-old washed cell suspension. Mycobacillin and cholesterol were added in concentrations of 250 and 300 μg/ml, respectively. The flasks were incubated on a shaker for 20 hr, and the cells were centrifuged and stained as usual. The count was made directly from microscopic observations. The percentage calculation was based on the average of five different counts.

TABLE 3. *Effect of cholesterol on the ability of mycobacillin to release ultraviolet-absorbing materials from Candida albicans*[a]

Flask no.	Reagents added (ml) to 9.2 ml of cell suspension of *C. albicans*		Absorbancy at	
	Zero-time	After 20 hr	260 mμ	280 mμ
1	$NaHCO_3$, 0.5	Acetone, 0.3 Mycobacillin, 0.5 Cholesterol, 0.3	0.080	0.074
2	$NaHCO_3$, 0.5 Acetone, 0.3	Mycobacillin, 0.5 Cholesterol, 0.3	0.105	0.091
3	Acetone, 0.3 Mycobacillin, 0.5	$NaHCO_3$, 0.5 Cholesterol, 0.3	0.176	0.173
4	Mycobacillin, 0.5 Cholesterol, 0.3	$NaHCO_3$, 0.5 Acetone, 0.3	0.051	0.070
5	Cholesterol, 0.3 $NaHCO_3$, 0.5	Mycobacillin, 0.5 Acetone, 0.3	0.052	0.049

[a] Flasks containing the ingredients listed in the zero-time column were incubated on a shaker for 20 hr at 31 C. Substances listed under "After 20 hr" were then added, and the contents of each flask was centrifuged immediately. Beckman readings were made of the supernatant fluids (10× dilution). For a base reading, all constituents were added simultaneously and centrifuged, and the supernatant fluid (10× diluted) was used. The concentrations of mycobacillin and cholesterol were 150 and 200 μg/ml, respectively.

Symp. Mechanism Antibiot. Fungicides, Reinhardsbrunn, Germany, 1966, *in press*). This releasing action was prevented by cholesterol (Table 3). Mycobacillin also causes the Gram-staining character of *C. albicans* to change from positive to negative, the maximal change being 20%. Cholesterol prevented this change (Table 4). It was thus observed that all the reactions of cells susceptible to mycobacillin were inhibited by cholesterol.