MODE OF ACTION OF MYCOBACILLIN, A NEW ANTIFUNGAL ANTIBIOTIC

N. BANERJEE AND S. K. BOSE

Department of Applied Chemistry, Calcutta University, Calcutta, India

Received for publication 12 March 1963

ABSTRACT

BANERJEE, N. (Calcutta University, Calcutta, India) AND S. K. BOSE. Mode of action of mycobacillin, a new antifungal antibiotic. J. Bacteriol. **86**:387–391. 1963.—Mycobacillin agglutinates cells of *Candida albicans*. The agglutination reaction was found to be dependent on temperature, pH level, etc. Experiments have also been planned to assess whether agglutination and antifungal action are causally related. It appears that agglutination may be a secondary late effect of the action of the drug, not causally related to but simply associated with the primary cause responsible for antifungal action.

Mycobacillin (Majumdar and Bose, 1958) is elaborated by a strain of *Bacillus subtilis* and is a cyclic polypeptide composed of 13 residues of 7 different amino acids (Majumdar and Bose, 1960). It is exclusively an antifungal antibiotic. A good number of antifungal antibiotics are known but only a few of them, except those of the polyene type, have been investigated regarding their mode of action. Different polyene antibiotics seem to act by altering cellular permeability, possibly by reacting with the cell membrane of sensitive organisms. Polymyxin, a cyclic polypeptide, is not antifungal but antibacterial. It is supposed to act on the osmotic barrier of sensitive cells (Few and Schulman, 1953). It is of interest to enquire into the mode of antifungal action of mycobacillin. In the present communication, some preliminary experiments have been reported on the mode of action of this antibiotic against a sensitive strain of *Candida albicans*.

MATERIALS AND METHODS

Organisms. The strain of *C. albicans* used in these experiments was obtained through the courtesy of the School of Tropical Medicine, Calcutta.

Media. C. albicans was maintained on glucose-Sabouraud's agar. The synthetic medium used was composed of (per liter): $(NH_4)_2HPO_4$, 2.5 g; KH_2PO_4, 2.0 g; $MgSO_4 \cdot 7H_2O$, 1.0 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; glucose, 3.6 g; pH 6.8.

Mycobacillin solution. Mycobacillin was used in the form of a solution in aqueous $NaHCO_3$ of pH 8.0.

Agglutination reaction. In a typical experiment, cells of the organism were harvested and washed (three times with normal saline) free from the adhering nutrients and resuspended in normal saline. The agglutination reaction was carried out in conical flasks with side tubes. The reaction mixture consisted of 0.8 ml of mycobacillin solution of various concentrations, 3.3 ml of cell suspension of different population densities, and 5.9 ml of synthetic medium. To allow agglutination to occur, all the flasks were shaken for 1 hr at room temperature on a reciprocating shaker and then kept at a given temperature for various lengths of time. Agglutination became macroscopically visible.

RESULTS

Time of agglutination and decrease in viability in a synthetic medium. Action of mycobacillin (Table 1) was not only inhibitory but also fungicidal. This fungicidal action was very rapid during the first few hours, and then slowed down with time. Agglutination, on the other hand, did not occur during first 4 hr of incubation. Thereafter, it appeared slowly, and within 6 to 12 hr it became complete.

Agglutinating power of mycobacillin in different concentrations and viability of agglutinated cells. Minimal concentration of mycobacillin for agglutination (Table 2) was 168 μg/ml, which equaled its inhibitory concentration. The degree of inhibition and also of agglutination increased with an increase in concentration of mycobacillin, within the range studied. It was also observed that agglutinated cells (agglutinating concentration being 168 μg/ml or more) were not all viable, even after thorough dispersion, but the rate of loss of viability was very slow.

TABLE 1. *Effect of mycobacillin on cell suspension of Candida albicans in a synthetic medium**

Time	Agglutination reaction†	Viable count per ml
hr		
0	−	2.5×10^7
0.5	−	2.3×10^7
1	−	2.0×10^7
2	−	1.7×10^7
3	−	1.3×10^7
6	+	1.0×10^7
12	++	6.0×10^6
24	++	2.1×10^5
48	++	4.8×10^4

* Concentration of mycobacillin: 336 μg/ml; temperature: 37 C.

† Agglutination was observed macroscopically. The viability was determined simultaneously by the plate count method, samples from experimental flasks being collected after thorough dispersion which was ensured by optical density measurement. In practice, the optical density after thorough dispersion agreed very nearly to that at the zero hour.

TABLE 2. *Agglutinating power of mycobacillin for Candida albicans in different concentrations and viability of cells**

Concn of mycobacillin	Agglutination reaction	Viable cells† collected at intervals of	
		24 hr	48 hr
μg/ml			
0.0	−	9.0×10^9	8.6×10^9
42.0	−	7.0×10^9	7.2×10^9
84.0	−	4.0×10^9	4.5×10^9
168.0	+	8.0×10^6	4.2×10^6
252.0	++	3.5×10^6	6.0×10^6
336.0	++	2.5×10^5	5.0×10^4

* Temperature, 37 C; number of viable cells, 2.8×10^7/ml.

† Viability was determined as usual by the plate count method at intervals of 24 and 48 hr.

Effect of temperature on agglutination. Agglutinating ability was studied at two temperatures, viz., 4 and 37 C. The conditions for agglutination were kept identical, including the initial preincubation shaking at room temperature for 1 hr. Macroscopically, agglutination did not occur with cold incubation at 4 C.

Viability of nonagglutinated cells in the presence of mycobacillin. Since mycobacillin did not agglutinate cells in the cold at 4 C, viability of these nonagglutinated cells represents an interesting point of investigation. The results (Table 3) indicate that nonagglutinated cells, on exposure to 37 C, agglutinate and lose their viability quickly, whereas those kept continuously in the cold neither were agglutinable nor was their decrease in viable count at all comparable with that of cells agglutinated by exposure to 37 C.

Effect of pH on agglutination. The various concentrations of mycobacillin required to agglutinate cells at different pH values were determined in the usual synthetic medium. The results (Table 4) indicate that the amount of mycobacillin required for agglutination was minimal at pH 7.0 to 7.5, which shows that agglutination is relatively insensitive to pH, with a slight optimum appearing at pH 7.0 to 7.5.

Effect of mycobacillin on agglutination of killed cells. Saline suspensions of *C. albicans* in Pyrex test tubes (6 × ¾ in.) were exposed to boiling-water temperature for 10, 20, 30, and 60 min.

TABLE 3. *Viability of nonagglutinated cells of Candida albicans in the presence of mycobacillin**

Time	Viable count per ml	
	A†	B‡
hr		
0	2.6×10^7	2.7×10^7
1	2.1×10^7	2.4×10^7
24	1.8×10^7	2.0×10^7
30	1.8×10^7	1.1×10^7
36	1.7×10^7	6.2×10^6
48	1.4×10^7	2.0×10^5

* Concentration of mycobacillin: 336 μg/ml.

† Data indicate viable count when the tubes for agglutination were kept, after initial shaking for 1 hr at room temperature, continuously in the cold at 4 C.

‡ Data indicate viable count when the tubes, after pretreated as above, were kept in the cold at 4 C for 24 hr, then exposed to 37 C for 24 hr.

TABLE 4. *Effect of pH on agglutination of Candida albicans*

pH values	Minimal concn of mycobacillin yielding visible agglutination
	μg/ml
6.0	200
6.5	200
7.0	150
7.5	150
8.0	175

Agglutination tests were carried out as usual in synthetic medium, the concentration of mycobacillin being 336 μg/ml. No agglutination occurred with killed cells.

Morphological changes. A cell suspension treated in the usual way for agglutination was continuously observed under a microscope. During the first 4 hr, no visible change occurred. Thereafter, the cells were found to form clumps, and the size of clumps increased with time, but no change in individual morphology was noted (Fig. 1, 2, and 3) throughout the period.

Discussion

The present work involves the mode of action of mycobacillin on a sensitive strain of *C. albicans*. In the course of the investigation, it was observed that mycobacillin brought about agglutination of sensitive cells. This agglutination, however, did not take place immediately after the addition of mycobacillin, but required a preagglutination period of 5 to 6 hr, during which time a decrease in the viable count occurred very quickly (to the extent of 60%). After agglutination occurred, the viable count declined.

It may be mentioned in this connection that datemycin (Nakajima, 1959), an antibiotic specific against *C. albicans*, was reported to exert its antifungal action by way of agglutination. This antibiotic also induces mycelial formation. In the case of mycobacillin, however, no change in individual morphology, except for the formation of clumps, has been observed. The agglutinating property of streptomycin against gram-positive and gram-negative bacteria has been reported, although the role of agglutination in its antibiotic action has not been assessed. Polymyxin, a cyclic polypeptide with a side chain unlike mycobacillin, brings about agglutination of *Staphylococcus albus* (*S. epidermidis*) and *Escherichia coli*. It is difficult to assess from these reports the role of agglutination in the antifungal action exerted by mycobacillin.

Experiments relating the various concentrations of the drug to its agglutinating power do not throw any more light on the role of agglutination in antifungal action than the mere confirmation of the already observed fact that the agglutinated cells remain nonviable even after thorough dispersion.

Some other characteristics of the agglutination reaction, viz., effect of pH, temperature, etc., have also been studied. The effect of temperature on agglutination is worth mentioning. Agglutination does not occur in the presence of mycobacillin upon cold incubation at 4 C, although it does occur in parallel experiments where preagglutination incubation is carried out at 37 C. This might indicate the possibility of isolating the agglutinating effect of the drug from that of its effect on viability. It was observed, on the contrary, that a further decrease in the viable count did not occur in tubes where agglutination was absent owing to cold incubation. On exposure to 37 C, the nonagglutinated cells agglutinated and, simultaneously, the viable count dropped quickly. This seems to indicate a direct correlation between agglutination and antifungal action. This correlation, however, does not hold for the preagglutination period, when major fungicidal effect to the extent of 60% is exerted in the absence of observable microscopic or macroscopic agglutination. These views, apparently contradictory, may be reconciled on the assumption that agglutination may be a secondary, late effect of the drug not causally related to, but simply associated with, its antifungal activity whose primary mechanism of action may be a process that is thermosensitive.

It may be concluded that mycobacillin is fungicidal, and that it agglutinates cells of a sensi-

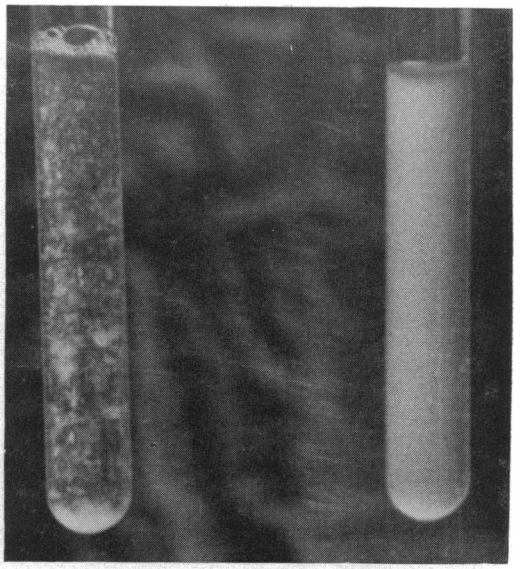

FIG. 1. *Cell suspension of Candida albicans with (left) and without (right) mycobacillin.*

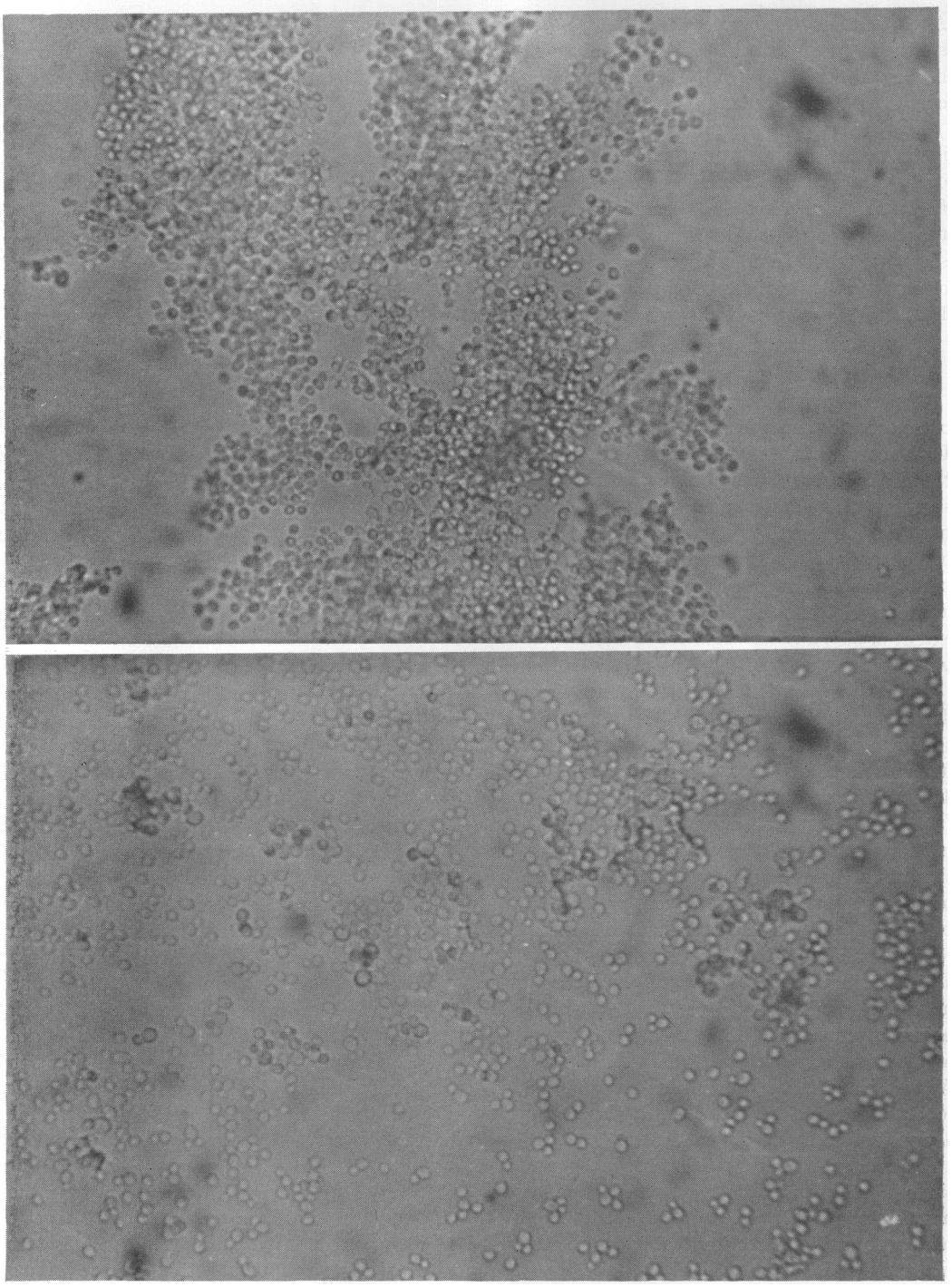

FIG. 2. (top) *Photomicrograph of cells of Candida albicans after exposure to mycobacillin.*
FIG. 3. (bottom) *Photomicrograph of cells of Candida albicans not exposed to mycobacillin.*

tive organism, but it is too early to speculate on how these two actions are related.

ACKNOWLEDGMENT

We gratefully acknowledge the financial support given to one of us (N. Banerjee) by the Council of Scientific and Industrial Research, Government of India, New Delhi.

LITERATURE CITED

FEW, A. V., AND J. H. SCHULMAN. 1953. The absorption of polymyxin E by bacteria and bacterial cell walls and its bactericidal action. J. Gen. Microbiol. **9**:454–466.

MAJUMDAR, S. K., AND S. K. BOSE. 1958. Mycobacillin, a new antifungal antibiotic produced by *B. subtilis*. Nature **181**:134–135.

MAJUMDAR, S. K., AND S. K. BOSE. 1960. Amino acid sequence in mycobacillin. Biochem. J. **74**:596–599.

NAKAJIMA, S. 1959. Mode of action of datemycin on the growth of *Candida albicans*. Tohoku J. Exptl. Med. **70**:103–113.