THE MODE OF ACTION OF LINCOMYCIN

by

John J. Josten and Paris M. Allen

Research Laboratories, The Upjohn Company, Kalamazoo, Michigan

Received October 31, 1963

The discovery, isolation and antibacterial properties of the antibiotic lincomycin have been reported recently (Mason et al, 1962; Herr et al, 1962; Lewis et al, 1962). This paper is concerned with the mode of action of lincomycin.

METHODS

Staphylococcus aureus strain H was maintained on slants as described by Park (1962). For growth experiments the synthetic medium of Surgalla (1947) was used. Sufficient cells from an overnight culture were inoculated into 500 ml of synthetic medium in a 5-L Fernbach flask to give an initial optical density at 660 mμ of 0.05. The culture was incubated on a rotary shaker at 37°C until the logarithmic growth phase was reached (2-3 hours) At this point the culture was divided into two equal parts and treated as below.

For the incorporation experiments, 5 μc of uniformly labelled C^{14}-L-lysine was added to both parts and lincomycin to one. Incubation was continued for 45 min after which the cells were harvested, washed and fractionated according to Park and Hancock (1960). Radioactivity measurements were made in a Model 314AX Tri-Carb Liquid Scintillation Spectrometer with diotol as the scintillation solvent (Herberg, 1960).

For experiments on nucleic acid synthesis, essentially the same procedure was followed except the culture volume was increased to permit

removal of 200 ml samples at 0, 15, 30, and 60 min after addition of lincomycin. To each sample, 10 ml of 100% (wt/vol) TCA were added and the tubes were allowed to stand at 4°C overnight. The cells were harvested and washed with .05M PO_4 buffer, pH 7.0. The cell pellet was extracted with 75% ethanol for 20 min at room temperature and the residue removed by centrifugation. To this residue, 10 ml of 5% TCA was added followed by hydrolysis for 20 min at 100°C. After cooling, the residue was centrifuged and the supernatant was used for RNA and DNA estimations. Five ml of 1N NaOH was added to the residue and the suspension was incubated at 37° C for 16-20 hr. This fraction was used for protein determinations. The orcinol and diphenylamine reactions were used to estimate RNA and DNA respectively (Schneider, 1957). Protein was determined by the method of Lowry, et al. (1951).

RESULTS

The effect of lincomycin on incorporation of C^{14}-lysine by logarithmic phase cells of S. aureus is shown in Table 1. The total incorporation was inhibited 87% in the presence of lincomycin. However, comparison of the protein and wall ratios shows that incorporation into the protein fraction was inhibited 92% while lysine incorporation into cell wall was apparently stimulated, but probably proceeded at a rate consistent with the enzymic level prior to lincomycin addition. Preliminary experiments, wherein the inhibited culture is allowed to reach the same optical density as the control, have indicated that protein was the only cellular fraction that failed to incorporate radioactivity comparable to the control.

Since protein synthesis was inhibited by lincomycin, it was of interest to determine the effect of this antibiotic on synthesis of RNA and DNA. Figure 1 shows that protein synthesis ceased immediately upon addition of lincomycin while RNA was relatively unaffected. The synthesis of DNA was inhibited, but not until 15 min after addition of

TABLE 1

Effect of Lincomycin on C^{14}-Lysine Incorporation by S. aureus

Addition	O.D.660 mμ Increase	Counts per Minute			Ratios	
		Total*	Protein	Wall	Protein / Total	Wall / Total
None	.310 to .553	40,400	29,000	12,300	.72	.31
Lincomycin 50 μg/ml	.310 to .456	5,150	436	4,190	.08	.80

* Total cpm refers to incorporation exclusive of cold TCA, hot TCA and alcohol soluble material.

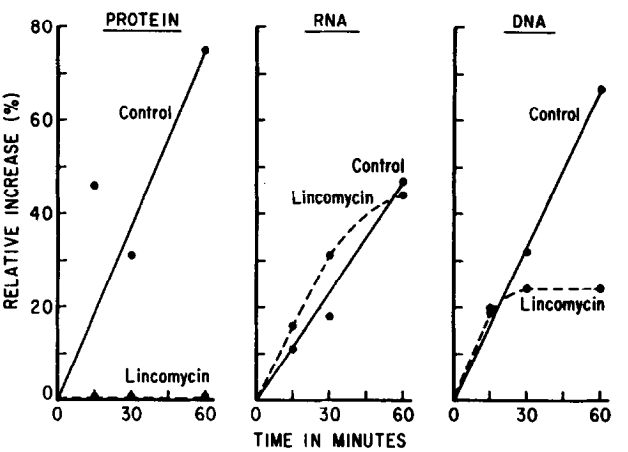

FIG. 1. THE EFFECT OF LINCOMYCIN ON NUCLEIC ACID AND PROTEIN SYNTHESIS IN S. AUREUS.

antibiotic. Similar results have been obtained using radioactive precursors to measure nucleic acid synthesis.

These experiments indicate that the immediate effect of lincomycin on S. aureus is complete inhibition of protein synthesis. Nucleic acid synthesis is not affected for 15 min in the case of DNA and at least

60 min in the case of RNA. These results are comparable to those reported for chloramphenicol (Gale and Folkes, 1953) or puromycin (Yarmolinsky and de la Haba, 1959) and indicate the activation, transfer, or polymerization of amino acids as possible sites of action. Work is in progress on this aspect of lincomycin inhibition.

REFERENCES

Gale, E. F. and Folkes, J. B. Biochem. J., 48, 298 (1953)

Herberg, J. Anal. Chem., 32, 42 (1960)

Herr, R.R. and Bergy, M.E. Antimic. Agents & Chemo., 560 (1962)

Lewis, C., Clapp, H.W., and Grady, J.E. Antimic. Agents & Chemo., 570 (1962)

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. J. Biol. Chem., 193, 265 (1951)

Mason, D.J., Dietz, A., and DeBoer, C. Antimic. Agents & Chemo., 554 (1962)

Park, J.T. J. Biol. Chem., 194, 855 (1952)

Park, J.T. and Hancock, R. J. Gen. Microbiol., 22, 249 (1960)

Schneider, W.C. Methods in Enzymology III, Academic Press, New York, 1957

Surgalla, M. J. Inf. Diseases, 81, 97 (1947)

Yarmolinsky, M.B. and de la Haba, G.L. Proc. Natl. Acad. Sci. U.S., 45, 1721 (1