EFFECT OF VANCOMYCIN ON THE SYNTHESIS OF THE CELL WALL MUCOPEPTIDE OF STAPHYLOCOCCUS AUREUS

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In a preliminary report Jordan and Inniss (1959) stated that vancomycin caused an inhibition of RNA synthesis in growing cells of S. aureus without a concomitant inhibition of the production of DNA and protein. A continuation of this study has since indicated that although the synthesis of protein as determined by the Folin-Ciocalteu reaction is unaffected by this antibiotic there is nevertheless a blockage in the uptake of C^{14} -amino acids into a "bound" form remaining in the cells after extraction of the intracellular pool constituents and nucleic acids. This suggested an inhibition in the synthesis of a polypeptide devoid of the aromatic amino acids and directed attention to the cell wall mucopeptide.

Methods

The synthetic medium employed consisted of glucose, 5 g; vitamin-free casamino acids, 2 g; DL-tryptophan, 20 mg; thiamine hydrochloride, 2 mg; nicotinamide, 2 mg; K_2HPO_4, 0.5 g; $MgSO_4.7H_2O$, 0.2 g; $(NH_4)_2SO_4$, 0.5 g; and glass-distilled water, 1000 ml. The pH was adjusted to 7.0 with 1.5 N KOH. A 10 ml 18 hr culture of S. aureus 71435 in brain heart infusion (Difco) was added to 90 ml of synthetic medium and incubated for 24 hr at 37°C. The cells were removed by centrifugation,

suspended in 1.8 ml of water and 0.5 ml amounts added to two flasks, each containing 27.5 ml of synthetic medium and 1.0 ml (0.5 μc) glycine -2-C^{14}. After incubation for 10 min 1.0 ml of an aqueous vancomycin solution was added to one flask and an equal volume of water to the other. At various time intervals 5 ml samples were removed and quickly frozen in a mixture of dry ice and alcohol. After rapid thawing the cells were removed by centrifugation, washed, and processed by the method of Park and Hancock (1960). This method, which yields five cellular fractions, was modified to include washing the residue after hot trichloroacetic acid extraction, an extended trypsin-digestion time (4 hr) with more trypsin added after the second hour, and a final washing of the mucopeptide fraction.

For studies on the rate of nucleic acid synthesis the preliminary procedure was the same as that just indicated except that the phosphate level of the synthetic medium was reduced to 0.1 g/liter and inorganic P^{32} (1.38 μc) replaced the C^{14} -glycine. After incubation for 30 min at 37°C vancomycin was added to provide a concentration of 83 μg/mg cell dry weight. At regular intervals 5 ml amounts were removed, frozen, thawed, and the harvested cells fractionated by the method of Hanawalt (1959) modified to include an ethanol-ether (3:1) extraction. Total nucleic acid P^{32} and DNA-P^{32} were measured and the difference in activity was assumed to be that of the RNA-P^{32}.

The radioactivity of all the samples was measured in a flow counter provided with an ultrathin plastic window. The C^{14} was counted at infinite thinness.

Results

Vancomycin had no marked effect on the incorporation of glycine-2-C^{14} into the "ethanol-soluble" protein and lipid

of S. aureus (fraction 2 of Park and Hancock) or on its passage into the intracellular pool (fraction 1). This latter aspect was confirmed by growing cells for various short time intervals in C^{14} -glycine in the presence and absence of vancomycin and determining the C^{14} content of the pool constituents extracted from these cells by the hot water method of Cohen and Rickenberg (1956).

The lack of any effect of vancomycin on the incorporation of glycine-2-C^{14} into trypsin-solubilized protein and its pronounced inhibition of the incorporation into the wall mucopeptide is shown in Fig. 1. Similar results were found when DL-glutamic acid-2-C^{14} and DL-glutamic acid -3, 4-C^{14} replaced the glycine. The inhibition of mucopeptide production occurred

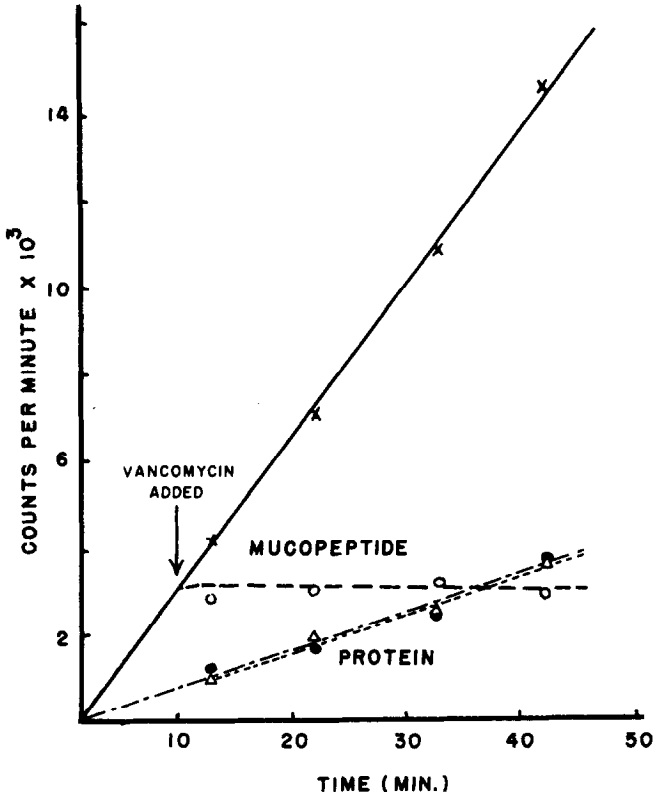

Fig. 1 The effect of vancomycin (83 μg/mg cell dry weight) on the incorporation of glycine-2-C^{14} into the trypsin-solubilized protein and cell-wall mucopeptide of Staphylococcus aureus (x———x, mucopeptide control; o— — —o, mucopeptide plus vancomycin; Δ—·—·—Δ, protein control; ●·····●, protein plus vancomycin.

2 to 5 min after the addition of the vancomycin, while no blockage of RNA synthesis occurred until 20 min later. A slight inhibition of DNA synthesis also was noticed but only after the cells had been in contact with the antibiotic for about 30 min.

These data suggest that the primary effect of vancomycin on S. aureus is the inhibition of the synthesis of cell wall mucopeptide and that the prevention of RNA production as previously reported is a secondary effect, perhaps caused by the initial defect in mucopeptide manufacture.

References

Cohen, G.N. and Rickenberg, H.V. Ann. Inst. Pasteur, 91, 693 (1956).
Hanawalt, P. Science, 130, 386 (1959).
Jordan, D.C. and Inniss, W.E. Nature, 184, 1894 (1959)
Park, J.T. and Hancock, R. J. Gen. Microbiol., 22, 249 (1960)