

Synergistic Action of Nicotinamide Upon Penicillin.

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In the course of some studies dealing with the mechanism of penicillin action, it was observed that relatively high concentrations of nicotinamide appeared to increase measurably the effectiveness of penicillin against the Oxford strain of *Staphylococcus aureus*. Since, of the B vitamins, only para-aminobenzoic acid has been shown to affect penicillin activity,^{1,2} this synergistic action has been investigated in more detail.

Experimental Methods. In testing the effectiveness of compounds under investigation for their synergistic action, it was found most convenient to compare the concentrations of penicillin necessary to produce half-maximum inhibition of the test organisms in the presence and absence of the agent under study. The organisms being investigated were grown in a modified proteose-peptone medium, with added glucose and yeast extract. Short (5-7 hour) growth periods were used. Growth levels were estimated turbidimetrically, using a Klett-Summerson photoelectric colorimeter.

In most of the experiments, Lederle penicillin (potency 400 Oxford units per mg) was used. Crystalline sodium penicillin G was obtained through the courtesy of Merck and Co. Eastman Kodak Company nicotinamide and nicotinic acid were used without purification, except as noted below.

Results. The addition of nicotinamide to the culture medium in concentrations less than about 250 γ per ml has no apparent effect upon the inhibition of *Staphylococcus aureus* by penicillin. Beyond this point, however, significant effects are observed. Representative data are shown in Fig. 1. It is evident that the concentration of penicillin necessary for half-maximum inhibition decreases with increasing concentration of the amide. Con-

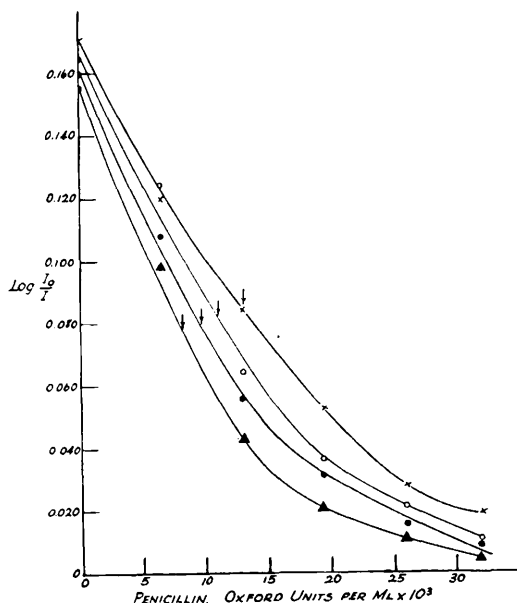


FIG. 1.

Effect of increasing concentrations of nicotinamide upon penicillin inhibition of *Staphylococcus aureus* (Oxford). The concentrations of nicotinamide, in mg/ml, are as follows: $\times$ —0.00; $\circ$ —0.40; $\bullet$ —0.80; $\blacktriangle$ —1.60. The vertical arrows indicate half-maximum inhibition points.

centrations of nicotinamide greater than 1.7 mg/ml were not studied.

Despite the fact that nicotinic acid is frequently interchangeable with nicotinamide in bacterial nutrition,³ it was found that sodium nicotinate was unable to duplicate the action of nicotinamide when used in comparable concentrations.

The relatively high nicotinamide levels necessary to produce observable synergism suggested the possibility that the effects noted were in reality due to an impurity in the preparation used, although two different lots of nicotinamide, obtained from the same manufacturer at different times, gave identical

¹ Ungar, J., *Nature*, 1943, **152**, 245.

² Hobby, G. L., and Dawson, M. H., *Proc. Soc. Exp. Biol. and Med.*, 1944, **56**, 184.

³ Peterson, W. H., and Peterson, M. S., *Bact. Rev.*, 1945, **9**, 49.

results. That this was not the case was shown by the fact that purified nicotinamide, recrystallized from either dioxane or alcoholic benzene, gave results which were indistinguishable from those obtained with the cruder preparation. Likewise, identical results were had when the impure penicillin preparations were replaced by crystalline penicillin at comparable concentrations.

The possibility that the effects observed were due to a slow reaction of penicillin with nicotinamide to form a product superior in antibacterial activity to penicillin itself was next investigated. On the basis of this hypothesis, the high nicotinamide concentrations required are necessary to force the reaction measurably toward completion in the relatively short periods involved. Accordingly, mixtures of nicotinamide and penicillin (including mole ratios from 4:1 to 4,000:1) were incubated at 37°. After 18 hours, the solutions were diluted so that the residual nicotinamide concentrations were less than 20 γ per ml, and inhibition curves determined. These were compared with curves for a solution of penicillin alone which had been treated similarly. No enhancement of penicillin action could be observed under these conditions.

In an effort to determine the effect of nicotinamide upon penicillin inhibition of other organisms, experiments similar to those described above were performed using *Streptococcus pyogenes* C-203, *E. coli*, and two other strains of *Staphylococcus aureus*. Penicillin inhibition of *S. pyogenes* and *E. coli* was unaffected by the presence of even large amounts of nicotinamide. However, the other strains of *Staphylococcus aureus* studied responded to combinations of penicillin and nicotinamide

in a manner qualitatively and quantitatively analogous to the response of the Oxford staphylococcus. In the presence of 1.5 mg of nicotinamide per ml, the concentration of penicillin required for half-maximum inhibition of the latter organism is approximately 0.7 that required when no nicotinamide is present. Ratios for the other strains studied were 0.64 and 0.67 respectively. It should be noted that these 3 strains had significantly different penicillin sensitivities.

It is well known that penicillin is most effective under conditions favoring rapid growth of the organism, and the theory that penicillin acts primarily upon actively multiplying cells is supported by a good deal of evidence.⁴ It seems unlikely, however, that the phenomenon under discussion can be explained by an increased rate of cellular multiplication. Although detailed studies of the effect of nicotinamide upon the rate of growth of the staphylococcus have not been made, a single experiment indicated that a slight decrease in rate occurred upon using concentrations of nicotinamide comparable to those indicated above.

Summary. High concentrations of nicotinamide exert a synergistic effect upon penicillin inhibition of *Staphylococcus aureus*. Nicotinic acid was inactive in this respect. No evidence for a reaction between nicotinamide and penicillin could be found. Although the effect of nicotinamide appeared to be general for the 3 strains of staphylococcus tested, no effect could be demonstrated upon either *Streptococcus pyogenes* or *E. coli*.

⁴ Chain, E., and Duthie, E. S., *Lancet*, 1945, 1, 652.