

Stimulation of Sporogenic and Nonsporogenic Bacteria by Traces of Penicillin or Streptomycin

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The remarkable therapeutic efficacy of penicillin and streptomycin is dependent upon their low toxicity to tissues and their unusual capacity to inhibit or kill, *in vivo*, a wide variety of pathogenic organisms.

That the biological activity of these antibacterial agents is not limited to their bactericidal or bacteriostatic properties may be readily demonstrated, although this fact seems to have been overlooked by most microbiologists and clinicians. The bacteria-controlling property of these drugs has for the most part obscured the phenomenon of growth stimulation associated with low concentrations of the drugs. Evidence of a so-called biphasic action of penicillin has been reported by Miller, Green, and Kitchen¹ who noted that certain concentrations of penicillin in broth containing *Staphylococcus aureus* induced upon incubation, increased turbidity over the control containing no penicillin. The effect was irregular at 37°C but quite consistent at 24°C.

It is the purpose of this paper to show that suitably diluted solutions of either penicillin or streptomycin, when incorporated in a nutrient medium, exert a definite stimulating effect upon the growth of many bacterial species. Stimulation of this nature was first observed in a spore-forming species with tomatin as the test substance; our observations were then extended to penicillin and streptomycin and widened to include a rather large group of spore-forming species and 2 nonspore-forming pathogenic species.

When spore-forming species were used, washed spores were seeded uniformly into tubes of sterile (autoclaved) skim milk to yield a final concentration of approximately 50,000 viable spores per milliliter. The in-

oculated samples were heated at 95°C for 15 minutes and cooled, after which distilled water dilutions of the antibiotics*† were thoroughly mixed with the inoculated samples. These samples together with the controls, which received no antibiotics but were manipulated similarly, were then incubated, usually at 30°C and observed at frequent intervals for the first visible indication of growth. In the nonsporing species a small loopful of a 17-hour tomato broth culture served as inoculum for each tube of medium. The requisite dilutions of reagents were made up from the powder just prior to use. Two or more controls were included in each series to take account of chance variations. The data were essentially reproducible although in some samples unexplained discrepancies occurred.

Examination of the data (Table I) shows that both penicillin and streptomycin were for most strains stimulatory at one or more concentration levels. The degree of stimulation varied from a few hours to several days as measured by first visible evidence of growth‡ between the test and control samples. Film preparations made at one or more stages in the development of the cultures, in most instances revealed a positive correlation between visible indication of stimulation and cell numbers. Greatest stimulation was found in *Bacillus megatherium*, while in *Bacillus mycoides* and certain strains of *Bacillus cereus* it was not apparent. In *Clostridium perfringens* (2 cultures) stimulation was slight and frequently irregular. The

* Penicillin sodium obtained from Chas. Pfizer & Co., Brooklyn, N.Y.

† Streptomycin hydrochloride obtained from Merek & Co., Rahway, N.J.

‡ Perceptible thickening, surface ring of peptonized milk or gas bubble.

¹ Miller, W. S., Green, C. A., and Kitchen, H., *Nature*, 1945, **155**, 210.

TABLE I. Effect of Traces of Penicillin or Streptomycin upon the Growth of Bacteria in Milk.

Organism	No.	Incubation temp., °C	Penicillin u/ml				Streptomycin u/ml			
			0.01	0.001	0.0001	0.00001	0.05	0.005	0.0005	
<i>B. cereus</i>	369	30	—	+ 2 hr	+ 2 hr	+	+	+	+	+ 8 hr
"	401	30	0	+ 3 hr	+ 3 hr	+	0	+	0	0
"	232	30	+ sl	+ sl	0	0	0	+	+	+ 2 hr
<i>B. mycoides</i>	6462	30	0	—	—	0	—	0	—	—
<i>B. megatherium</i>	696	30	+	+ 3 days	—	0	+	+	+	+ 5 days
<i>B. subtilis</i>	L.B.	30	+	+ 4 hr	+	0	+	+	+	+ 6 hr
"	6051	30	—	—	0	+	+	+	+	+ 4 hr
"	230	30	—	—	0	+	+	+	+	+
<i>B. stearothermophilus</i>	1518	52	—	—	—	—	+	+	+	+
"	67	52	—	—	—	—	+	+	+	+
"	846	37	—	—	—	—	+	+	+	+
<i>C. perfringens</i>	3626	37	—	—	—	—	+	+	+	+
"	3679*	30	+	+ 12 days	+	+	+	+	+	+
<i>Strep. agalactiae</i>		37	+	+	+	+	+	+	+	+
<i>Staph. aureus</i>		37	—	+	+	0	+	+	+	+

* A proteolytic anaerobe.

0 = first visible evidence of growth approx. equal in time to, — greater than (retardation), and + — less than 2 hours.

For the level of concentration which showed maximum stimulation, the time has been indicated.

2 nonspore-formers,[§] *Streptococcus agalactiae* and *Staphylococcus aureus*, were chosen because of their causal relationship to bovine mastitis. The latter evidenced rather slight and at times irregular stimulation at the 0.001 and 0.0001 levels (u/ml) of penicillin and in most of the streptomycin concentrations. *Strep. agalactiae* was appreciably stimulated through a wider range of penicillin and streptomycin. In general, streptomycin was more frequently stimulatory than was penicillin. It is perhaps significant that the stimulatory effects of penicillin and streptomycin were generally more apparent in the cultures incubated at 30°C than at higher temperatures, which accords with the previously mentioned observation of Miller *et al.*¹ upon the staphylococcus.

In view of the limited number of drug levels used, it should not be supposed that the most favorable concentrations were attained in these experiments or that cultures negative in reaction would not respond at other drug levels. The recorded data were obtained in a skim milk medium; when this medium was enriched by the addition of glucose and yeast extract the stimulating properties of the antibiotics were usually greatly reduced or entirely eliminated.

Welch, Price, and Randall² recently reported that streptomycin at certain concentration levels when injected intraperitoneally in mice infected with *Eberthella typhosa* increased rather than decreased the fatality rate. This suggested to the authors the possibility of stimulation of the infective agent by streptomycin; further strengthening this view was their observation that certain levels of streptomycin in broth, though inhibiting any visible evidence of growth, induced, nevertheless, a greater multiplication of cells than did samples containing lower concentrations of the drug. This report, together with that of Miller *et al.*,¹ came to our attention after most of our observations were completed. The former observations together

§ Recently isolated from acute bovine mastitis by L. A. Burkey, this Bureau.

² Welch, H., Price, C. W., and Randall, W. A., *J. Am. Pharm. Assn.*, 1946, **35**, 155.

with those here reported indicate that the stimulating activity of penicillin and streptomycin evident *in vitro* is probably operative *in vivo*.

Discussion. By evincing, in suitable concentrations, bacteriostatic or bactericidal activity and at certain much lower concentration levels a stimulatory effect, penicillin and streptomycin conform to a familiar behavior pattern of drugs and chemical reagents. The biphasic action of sulphonamides has been reported.¹ Recognition of the potential stimulatory activity of these drugs further increases the problems of initial dosage and maintenance in the body fluids of proper drug levels. The importance of achieving complete destruction of the infective agent by the initial dosages receives added emphasis. It is apparent also that concentrations of these drugs efficacious as bactericidal or bacteriostatic agents for one species of micro-organism, for another, perhaps coexistent infective agent, may be stimulatory. Thus, Eagle *et al.*³ found that 0.01-0.02 u/ml of penicillin was actively spirochaeticidal, a zone not infrequently stimulating to the spore-forming species employed in our study.

³ Eagle, H., Magnuson, H. J., and Fleischman, R., *Johns Hop. Hosp. Bull.*, 1946, **79**, 168.

Rammelkamp and Keefer⁴ reported maximum antibacterial action for *Streptococcus haemolyticus* when the concentration of penicillin in blood and serum was 0.019 u/ml. Evidence^{5,6} seems to indicate that part of the therapeutic activity of penicillin is associated with accompanying impurities, a fact which has necessitated in recent years the administration of larger doses to offset the greater refinement of the product; conversely it is not unlikely that the stimulatory concentration level of penicillin and streptomycin would be lowered by increased purification.

Summary. Suitable, low concentrations of penicillin or streptomycin in sterile milk (autoclaved) stimulated the growth of many spore-formers and *Staphylococcus aureus* and *Streptococcus agalactiae*. The degree of stimulation differed with the organism and with some cultures was not apparent in the range of concentrations used; some of the practical implications of growth stimulation by penicillin and streptomycin are briefly discussed.

⁴ Rammelkamp, C. H., and Keefer, C. S., *J. Clin. Invest.*, 1943, **22**, 649.

⁵ Smith, W. J., *Science*, 1946, **104**, 411.

⁶ Comm. Med. Res., U. S. P. H. and F. D. A., *J. Med. Assn.*, 1946, **131**, 271.

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Studies on Auricular Tachycardia Caused by Aconitine Administration.

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Aconitine in minute amounts provokes abnormal stimulus formation in the heart. It has been demonstrated that with particular precautions a long-lasting, regular, bigeminal rhythm can be obtained after the intravenous administration of aconitine in the experimental animal.¹ Studies on the effect of

topical application of aconitine on the dog's heart are reported in this paper.

Method. The experiments were performed on 23 dogs. Morphine-nembutal anesthesia was used. The heart was exposed in the usual way. The vagi were severed in the neck. If necessary, the peripheral end was stimulated with the aid of a Cambridge inductorium.

The electrocardiograms were registered in lead II.

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¹ Scherf, D., *Z. f. d. ges. exp. Med.*, 1929, **65**, 198, 222.