8 hours from the time of injection; at the end of this time the response became constant at a level either greater or less than the original. The maximum increase in the respiratory response varied from 30 to 222%. In certain instances the change in respiratory minute volume was also studied at frequent intervals and was found to correlate in a general way with the increased responsiveness of the center to electrical stimulation.

The delay in the onset of action of picrotoxin agrees with the observations of others and may well be due to a delayed passage of this substance into nervous tissue. However, the present prolonged response (average 7.5 hours) is not in accord with the usual beliefs regarding the duration of action of picrotoxin.[3, 4]

Summary. A technique has been described whereby electrical stimulation of the respiratory center can be employed to directly determine the influence on the respiratory center of various drugs affecting respiration. Picrotoxin has been shown to produce a marked and prolonged increase in the sensitivity of the inspiratory center of the cat to direct electrical stimulation. It is believed that the technique which has been described can be used to advantage to contribute additional information to our knowledge of drugs affecting respiration.

[3] Duff, D. M., and Dille, J. M., *J. Pharm. and Exp. Therap.*, 1939, **67**, 353.

[4] Das, S. C., *Quart. J. Exp. Physiol.*, 1939, **29**, 355.

14642

Bacteriostatic Action of Penicillin on Hemolytic Streptococci *in vitro*.*

GLADYS L. HOBBY AND MARTIN H. DAWSON.

From the Departments of Bacteriology and Medicine, College of Physicians and Surgeons, Columbia University, New York, and the Edward Daniels Faulkner Arthritis Clinic, Presbyterial Hospital, New York.

In previous communications[1,2,3] preliminary observations on the effect of penicillin on living cultures of hemolytic streptococci were reported. It was shown that the action of penicillin was either bactericidal or bacteriostatic depending on the experimental conditions. Under the influence of relatively large concentrations of penicillin, organisms when present in small numbers decreased in number at a constant rate until approximately 99% of the organisms originally present were destroyed. Penicillin was not inhibited by para-aminobenzoic acid, blood, serum, or peptone. Additional evidence suggested that penicillin was capable of destroying bacteria only when multiplication took place.

In the present communication evidence will be presented which extends these observations.

Experimental. Hemolytic streptococci (strain C203MV) were used throughout. In the first set of experiments the effect of varying amounts of penicillin was observed. A constant number of organisms was incubated in media containing amounts of penicillin varying from 0.0009 to 9 Oxford units per cc. The number of organisms per cc at intervals was determined by colony counts in pour plates. (Graph I).

With concentrations of 0.9 and 9.0 Oxford units of penicillin per cc the number of viable organisms remained unchanged for the first hour following which there was a steady decrease. With 0.09 units per cc there was an initial lag, followed by a slight increase and then a drop in the number of viable organisms.

* This work was presented in part before the New York and New Jersey Branches of the Society of American Bacteriologists, December, 1942.

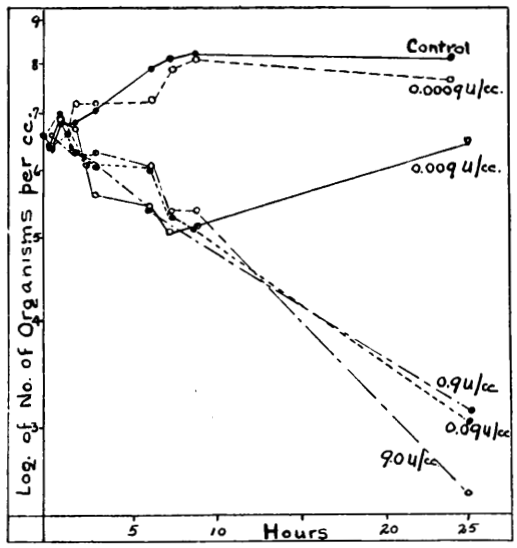
[1] Dawson, M. H., Hobby, G. L., Meyer, K., and Chaffee, E., *J. Clin. Invest.*, 1941, **20**, 434.

[2] Hobby, G. L., Meyer, K., and Chaffee, E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 277.

[3] Dawson, M. H., Hobby, G. L., Meyer, K., and Chaffee, E., *Ann. Int. Med.*, 1944, **19**, 707.

Graph I

Effect of Varying Concentrations of Penicillin on Hemolytic Streptococci in Vitro

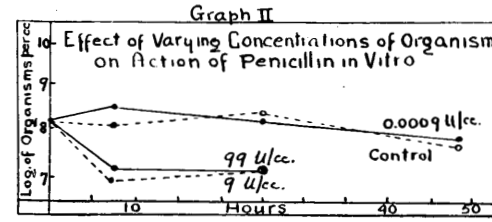


With 0.009 units per cc the results were similar for the first 9 hours of incubation but after 9 hours, multiplication again occurred. Concentrations of penicillin smaller than 0.009 units per cc caused only a slight slowing in the rate of growth.

In another series of experiments the effect of penicillin on varying concentrations of hemolytic streptococci was determined. Varying dilutions of an 18-hour culture were employed. The undiluted culture contained 200,000,000 organisms per cc, and the dilutions 2,000,000 and 40 organisms respectively. Penicillin was added to each dilution in amounts varying from 0.0009 to 0.9 Oxford units per cc and the cultures were incubated at 37°C. Controls without penicillin were incubated simultaneously. The number of organisms per cc at various intervals was determined as before by colony counts.

In plain broth with an initial concentration of 200,000,000 organisms per cc, there was no significant change in the number of viable organisms during the first 24 hours. After 48 hours the number of organisms diminished significantly. With 0.0009 to 0.09 Oxford units per cc similar results were obtained. In the presence of 0.9 to 99 units per cc the number of organisms decreased during the

Graph II



first 7 hours, then remained stationary. (Graph II.)

When the initial concentration of organisms per cc was lower—concentrations which permitted rapid multiplication in plain broth—no effect was observed with 0.0009 Oxford units per cc. With 0.009 units per cc a slowing of the rate of growth occurred and in the presence of larger amounts of penicillin direct killing took place. (Graphs III, IV.)

From the graphs it is apparent that the dilutions containing the greatest number of organisms showed killing as well as those containing fewer organisms—provided a sufficient amount of penicillin was used.

It is also apparent that the retardation of growth with small amounts of penicillin resembled that produced by sulfonamides in much greater concentrations. Penicillin in a concentration of 0.0009 Oxford units per cc produced an effect comparable with that of 100 micrograms of sulfadiazine per cc. In terms of pure penicillin[4,5] this represented an amount of 0.0005 μg of penicillin.

This bacteriostatic or bactericidal effect was apparent only with organisms sensitive to penicillin. A number of resistant strains of staphylococcus, pneumococcus, and *E. coli* were isolated. Resistant strains of staphylococci and *E. coli* were grown in broth for 18 hours at 37°C. The organisms were centrifuged, frozen, dried by the lyophile process, and ground with steel balls at a temperature of approximately –70°C. The resulting extracts were suspended in saline, again frozen and dried, and stored in dry ice. Solutions of these extracts were sterilized by Seitz filtration and tested for their effect upon penicillin. It was found that one microgram of freshly

[4] Coghill, Robt., *Chem. and Eng. News*, 1944, **22**, 588.

[5] Hunter, A. C., *J. Bact.*, 1944, **47**, 24.

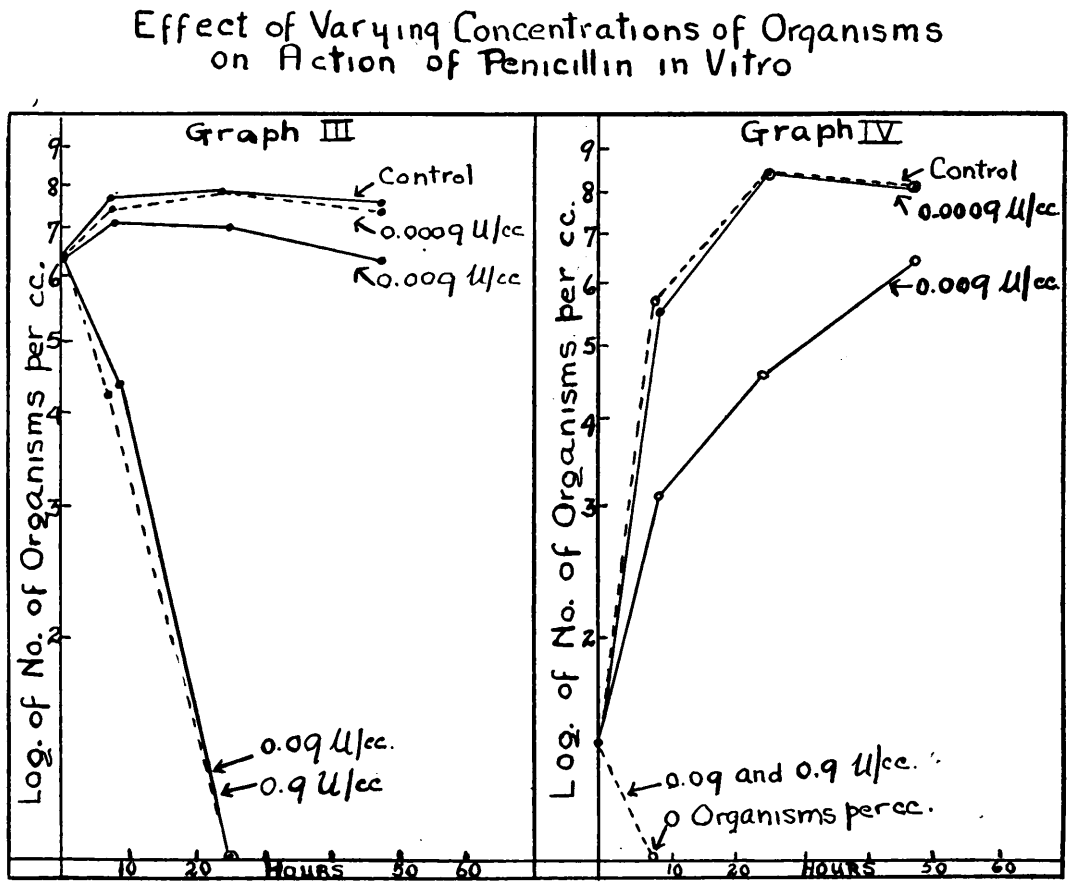


TABLE I.
Inhibition of Activity of Penicillin by Extracts of Bacteria.

Inhibitor			No. of organisms (× 1000) per cc			
Source	Amt (mg per cc)	Amt of penicillin (units per cc)	0 hr	2 hr	4½ hr	29 hr
—	—	—	2242	31000	31000	139000
—	—	2	2242	106	21	0
Pneumococcus	3.7	—	2242	19900	118500	221000
”	3.7	2	2242	2550	46	0
E. coli	3.7	—	2242	13700	130000	297000
” ”	3.7	2	2242	15600	38000	130000

prepared crude extract from *E. coli* inhibited 0.01-0.1 unit of penicillin. The amount of inhibiting substance necessary to inactivate each unit of penicillin is therefore extremely small. (Table I.) Preliminary experiments showed that the inhibiting substance was partially or completely inactivated at 80°C for one hour, and that it was destroyed by M/200 sodium fluoride. A similar substance was isolated in 1941 by Abraham and Chain[6] who suggested that it was probably an enzyme. Although the number of strains tested was small, the inhibiting substance was found in

[6] Abraham, E. P., and Chain, E., *Nature*, 1940, **146**, 837.

all resistant strains, both Gram-positive and Gram-negative, and not in sensitive ones.

Summary. The effect of penicillin *in vitro* varies with the concentration of penicillin, the number of organisms present, and the rate of growth of the organisms. It is most effective when rapid multiplication takes place.

Penicillin is inhibited by a substance found in resistant strains of bacteria and not in the sensitive strains tested. The presence of this inhibiting substance in resistant strains and its absence in sensitive strains suggests that resistance to penicillin may depend upon the capacity of an organism to elaborate this substance. The degree of sensitivity of any strain not producing the inhibiting substance appears to be correlated with the rate of growth exhibited by that strain.

14643

Effect of Rate of Growth of Bacteria on Action of Penicillin.*

GLADYS L. HOBBY AND MARTIN H. DAWSON.

From the Departments of Bacteriology and Medicine, College of Physicians and Surgeons, Columbia University, New York, and the Edward Daniels Faulkner Arthritis Clinic, Presbyterian Hospital, New York.

In previous communications[1, 2] the effect of growth conditions of hemolytic streptococci on the action of penicillin were described. In the present report further observations of this nature will be reported.

Experimental. Hemolytic streptococcus (strain C203MV) was used throughout. Unless otherwise specified organisms were grown in a beef infusion phosphate buffered medium.

1. Conditions which enhance growth: Hemolytic streptococci were incubated in plain broth, in broth containing varying concentrations of defibrinated rabbits' blood, and in whole defibrinated rabbits' blood. A constant amount of penicillin was added to each of these. The number of organisms per cc was determined at various intervals.

In the presence of defibrinated rabbits' blood there was an increase in the rate of growth of hemolytic streptococci and a corresponding increase in the rate at which the organisms were destroyed by penicillin. The effect of 100% defibrinated rabbits' blood in contrast to plain broth is shown in Table I.

In a further series of experiments, the effect of serum, para-aminobenzoic acid, and dextrose was observed. Each of these substances also increased the rate of growth of hemolytic streptococci and caused a corresponding increase in the rate at which the organisms were destroyed by penicillin.

2. Conditions which inhibit growth: Hemolytic streptococci were incubated in media containing a constant amount of penicillin and varying concentrations of physiological saline. One volume of nutrient broth was diluted with one and with two volumes of physiological saline. A constant amount of penicillin and a constant number of organisms per cc were added. The number of organisms per cc was again determined at various intervals. Under these conditions there was a decrease in the rate of growth of hemolytic streptococci. There was likewise a decrease in the rate at which the organisms were destroyed by the penicillin. (Table II.)

Similar experiments were carried out in broth containing both penicillin and sulfadiazine. Sufadiazine was added to nutrient broth in amounts sufficient to give a concentration of 100 μg per cc. Penicillin was then added giving a concentration of 6.4 Oxford units per cc. Controls were run with nutrient

* This work was presented in part before the New York and New Jersey Branches of the Society of American Bacteriologists, December, 1942.

1 Hobby, G. L., and Dawson, M. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 178.

2 Hobby, G. L., Meyer, K., and Chaffee, E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 281.