13772

Activity of Penicillin *in vitro*.*

GLADYS L. HOBBY, KARL MEYER AND ELEANOR CHAFFEE.

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

Fleming[1] observed that broth cultures of a certain strain of *Penicillium notatum* contained a substance, penicillin, which inhibited the growth of many Gram positive organisms. Stimulated by the work of Chain, *et al.*,[2] and Florey, *et al.*,[3] who demonstrated the remarkable antibacterial action of impure preparations of penicillin, studies were carried out in this laboratory on its production and extraction and on its chemical and biological properties.[4, 5, 6]

Method. Cultures of Fleming's strain of *P. notatum*† were grown for 3 to 5 days at room temperature on a honey agar medium. Saline suspensions of the spores from the cultures were seeded to 2-liter Erlenmeyer flasks containing 400 cc of a modified Czapek-Dox synthetic medium prepared according to the formula shown in Table I.

Brown sugar was used routinely in a concentration of 2% and

TABLE I.
Modified Czapek-Dox Medium.

	g
Sodium Nitrate ($NaNO_3$)	3.5
Potassium Phosphate (KH_2PO_4)	1.5
Potassium Chloride (KCl)	0.5
Magnesium Sulfate ($MgSO_4 + 7H_2O$)	0.5
Ferrous Sulfate ($FeSO_4 + 7H_2O$)	0.015
Brown Sugar (Jack Frost, dark)	20.0
Distilled H_2O to 1 liter	
Autoclave 15 lbs—15 min.	

* This work has been supported in part by a grant from the John and Mary Markle Foundation.

We are indebted to Dr. Martin H. Dawson for his valuable help and suggestions throughout this work.

1 Fleming, A., *Brit. J. Exp. Path.*, 1929, **10**, 226.

2 Chain, E., *et al.*, *Lancet*, 1940, **2**, 226.

3 Florey, H. W., *et al.*, *Lancet*, 1941, **2**, 177.

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

5 Hobby, G. L., Meyer, K., Dawson, M. H., and Chaffee, E., *J. Bact.*, 1942, **43**, 11.

6 Meyer, K., *et al.*, *Science*, 1942, in press.

† Received through the kindness of Dr. Roger Reid, Johns Hopkins Hospital, Baltimore, Md.

gave a maximum titer of penicillin in 8 days. An equivalent concentration of dextrose required 12 to 14 days.

A temperature of 24°C proved optimal although good yields were obtained at various temperatures between 20° and 26°C.

White mycelial growth with or without early sporulation and accompanied by surface droplets was associated with high yields of penicillin. Excess sporulation was generally accompanied by low yields. There was no parallelism between the amount of yellow pigment produced in the medium and the amount of penicillin.

Titration of Activity. Two methods were used for the titration of the activity of penicillin: (1) the dilution method, and (2) the Oxford plate method.[3]

In the dilution method serial dilutions of penicillin in broth were seeded with a constant amount of a standard strain of hemolytic streptococcus (C203 Mv). For convenience failure to develop turbidity after 24 hours' incubation at 37°C was accepted as evidence of inhibition of growth. Activity was determined by measuring the least amount of penicillin necessary to inhibit growth of 2 to 4 million organisms. This method is rapid and relatively satisfactory if experimental conditions are kept constant.

The Oxford plate method as described by Florey, *et al.*,[3] was employed.‡ The method is based on preliminary dilutions and the error is therefore comparable with that of the dilution method. Certain additional disadvantages are inherent in the plate method. The size of the zone of inhibition is limited by the length of the lag phase of the test organism and by the depth and dryness of the agar. Thus the amount of activity measured is influenced by the extent to which diffusion of the test material has taken place before bacterial multiplication has begun.

Table II shows the results of titration of crude preparations of penicillin by the two methods. Within the limit of error, the two tests give comparable results.

TABLE II.
Titration of Penicillin.

Preparation	Dilution method, γ per cc	Oxford method, units per mg
NH II 2*	.4	42
98 A	.4	45
81 II	.8	25
90 D	.2	106
101 III	.08	240

*Oxford Standard Penicillin.

‡ Oxford penicillin NH II 2, having an activity of 42 units per mg, was used as standard. We are indebted to Dr. N. G. Heatley for supplying us with this material.

TABLE III.
Susceptibility of Organisms to Penicillin.

Susceptible strains	Insusceptible strains
Pneumococcus	*H. influenzæ*
Streptococcus hemolyticus	*E. coli*
Staphylococcus	*B. typhosus*
Meningococcus	*B. dysenteriæ*
Gonococcus	*B. proteus*
Streptococcus viridans	*B. paratyphosus* A
B. subtilis	*B. enteritidis*
Cl. welchii	*B. pyocyaneus*
V. septique	*B. fluorescens*
Cl. histolyticus	*B. prodigiosus*
B. sporogenes	Friedländer's bc.
B. oedematiens	*Staphylococcus albus*—1 strain
B. sordelli	*Micrococcus albus*—1 strain
Lactobacillus	*Monilia albicans*
Cryptococcus hominis	*Monilia krusei*
	Monilia candida

Extraction. Penicillin was extracted from the acidified culture fluid with various organic solvents as described by Meyer, *et al.*[6] It was obtained either as the salt or as the free acid.

Preparations of the ammonium salt were obtained routinely with an activity of 0.03-0.1 γ per cc against hemolytic streptococci. Crystalline preparations were obtained which also had an activity of 0.03 γ per cc, equivalent to 240 to 250 Oxford units per mg.

Activity in vitro. Penicillin was tested by the dilution method against a wide variety of organisms. Confirming the work of Florey and his coworkers,[3] the preparations tested were found to exert a remarkable effect *in vitro* on many of the Gram positive organisms, both aerobic and anaerobic, as well as on gonococci and meningococci. The susceptible and insusceptible organisms tested are listed in Table III. Two strains of staphylococci were encountered which proved resistant.

All Gram negative organisms tested were relatively insusceptible to the action of this drug.

Considerable variation was found among different strains of the same organism as well as among the various organisms. The strains of pneumococcus and hemolytic streptococcus tested were 2 to 4 times more sensitive than staphylococcus, and 10 to 20 times more sensitive than some strains of non-hemolytic streptococci. (Table IV.) The effect was either bacteriostatic or bactericidal depending on experimental conditions.

The concentration of penicillin necessary to inhibit growth of or to kill Gram positive bacteria was found to be comparable to that of gramicidin and tyrocidin rather than to that of the sulfonamides.

TABLE IV.
Degree of Sensitivity of Various Organisms.

Organisms	Penicillin preparations Titer in γ per cc				
	NH II 2	101 III	7 II	No. 696	No. 924
Streptococcus hemolyticus (C203Mv)	.46	.08	.25	.12	.6
Staphylococcus albus (Oxford)	.93	.15			
Streptococcus viridans (Dup.)				.25	
Non-hemolytic Streptococcus (Cot)			250		
Lactobacillus (Leale)					2.5

Sterilization of a culture does not always take place with penicillin. Nevertheless there is always a marked decrease in the number of organisms present. Details will be presented in a subsequent paper. This is in sharp contrast to the effect produced by the sulfonamides which act only by decreasing the rate of multiplication.

Inhibition. Titrations of penicillin by the dilution method were carried out in the usual manner, using broth containing para aminobenzoic acid, blood, or serum. As reported by Florey, *et al.*,[3] no inhibition by these substances could be demonstrated. Likewise, the titration of the activity of penicillin in Pappenheimer's synthetic medium[7] containing no peptone indicated that peptone has no inhibiting action.

Confirming the work of Abraham and Chain,[8] it was found that the supernatant broth from cultures *E. coli* does inhibit the activity of penicillin. The nature of this inhibitor will be discussed in another publication.

Summary. The observations of Florey, *et al.*,[3] on the antibacterial activity of penicillin against Gram positive organisms are confirmed and extended. Preparations have been obtained of such activity that 0.03 γ inhibits the growth of 2 to 4 million hemolytic streptococci. This represents an equivalent of 240-250 Oxford units per mg.

[7] Pappenheimer, A. M., Jr., and Hottle, G. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 645.

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