14500 P

Action of Penicillin and Other Antibiotics on *Treponema Pallidum.*

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The demonstration that penicillin is effective in the treatment of syphilis[1] emphasizes the need for an *in vitro* test for the action of penicillin* and other antibiotic agents on the *Treponema pallidum*. Preliminary tests employing penicillin *in vitro*, with 240 Oxford units per 0.8 ml, failed to reveal any action on the spirochetes. This was in spite of the fact that the concentration employed, which was equivalent to 300,000 O.U. per kg, was considerably greater than appears to be required to protect rabbits from infection. This may be due to the fact that the test period is necessarily brief, as the spirochetes become immotile within a few hours outside the body under optimal conditions, and to the fact that the only criterion for the action of a drug in such a test is the rendering of the spirochetes immotile. Tests were, therefore, performed in which the drug was used in still higher concentrations, up to 3200 O.U. per 0.8 ml. These gave satisfactory results.

Employing the technic of Eagle,[2] the testes of rabbits in the stage of acute orchitis following inoculation with the Nichols strain were ground and suspended in a mixture of equal parts of normal rabbit serum and physiological saline solution. This was centrifuged at 1500 r.p.m. for 10 minutes and the supernate withdrawn for use in the tests. Serial dilutions of the antibiotic agent in the solvent required for the preparation of the original solution were mixed with equal volumes of the suspension and placed in an atmosphere of hydrogen at approximately 22°C for 2 hours. Dark field examinations were then made and the percentage of immotile spirochetes determined. Control tubes containing only suspension and the solvent employed for the antibiotic agent were also included in the tests. The percentage of spirochetes immobilized by the antibiotic agent was calculated by means of the following formula:

$$\%\ \text{Immobilized by Drug} = \frac{\text{Drug \% Immotile} - \text{Control \% Immotile}}{100 - \text{Control \% Immotile}} \times 100$$

Besides the method of computing the results, the only other modification of Eagle's technic consisted of putting mineral oil around the edges of the coverslip, which prevented the formation of currents and so aided in determining true motility of the organisms when this was slight.

The mean of the results of the tests with penicillin dissolved in physiological saline solution is shown by the solid line in Fig. 1. This indicates that under the conditions of the test there is a relatively sharp increase in the action on the spirochetes between a concentration of 800 and 1600 O.U. per 0.8 ml.

The results obtained with certain other antibiotic agents are given in Table I. Gliotoxin† had a marked spirocheticidal action, while aspergillic acid[3] and bromo-aspergillic acid[4] were less effective. Fumigacin[5] showed the least activity.

During certain studies with penicillin on the prophylaxis of syphilitic skin infections in rabbits, it was noted that some chancres

1 Mahoney, J. F., Arnold, R. C., and Harris, A., *Am. J. Pub. Health*, 1943, **33**, 1387.

* Part of the penicillin used in this study was allocated by the War Production Board for company research.

2 Eagle, Harry, *J. Pharm. and Exp. Therap.*, 1940, **69**, 342.

† Obtained through the courtesy of Dr. J. D. Dutcher, Division of Organic Chemistry, The Squibb Institute for Medical Research.

3 White, E. C., *Science*, 1940, **92**, 127.

4 Dutcher, J. D., Rake, G., Wintersteiner, O. P., and Jones, H. P., to be published.

5 Menzel, A. E. O., Wintersteiner, O. P., and Hoogerheide, J. C., *J. Biol. Chem.*, 1944, **152**, 419.

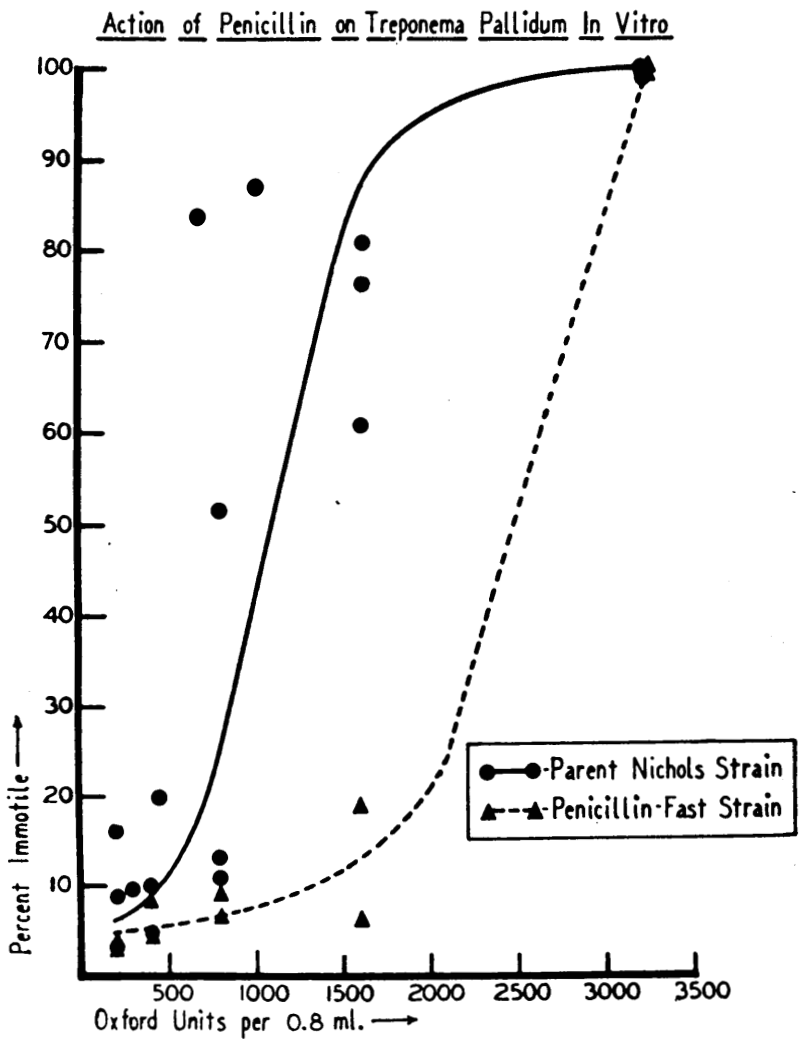

FIG. 1.

TABLE I.
Action of Aspergillic Acid, Bromo-Aspergillic Acid, Fumigacin, and Gliotoxin on *T. pallidum in vitro.*

Agent	mg/0.8 ml	Solvent	% immobilized by agent
Aspergillic Acid	.5	1% $NaHCO_3$	100
	.1		10
	.05		13
	.01		6
Bromo-Aspergillic Acid	.5	1% $NaHCO_3$	26
	.1		21
	.05		15
	.01		2
Fumigacin	2.0	0.3% $NaHCO_3$ and 0.15% Na_2CO_3	34
	1.0		6
	0.4		0
Gliotoxin	.04	2% Alcohol	93

developed late. These lesions were smaller than is usual. A suspension of the popliteal lymph nodes of one of these rabbits was used to inoculate the testes of two other rabbits, one of which later developed an orchitis less severe than that ordinarily observed with the Nichols strain. The testes were ground and an *in vitro* test was performed to determine whether any alteration had occurred in the resistance of the spirochetes to penicillin. Using this suspension another intratesticular passage was made in rabbits and the action of penicillin on the spirochetes again determined. The mean of the results of both tests is shown by the broken line in Fig. 1. These clearly show that the strain obtained from the insufficiently treated rabbit was more resistant to the action of penicillin than was the original Nichols strain. The tests in which 1600 O.U. were present in 0.8 ml of final mixture showed that 62 to 81% of the spirochetes of the unaltered Nichols strain were immobilized, while only 6 to 18% of the penicillin-fast spirochetes were rendered immotile. This finding indicates that in the treatment of human syphilis, care must be exercised to administer a sufficient quantity of penicillin to cure the patient rapidly. Otherwise, there will be the possibility of producing a penicillin resistant strain.

Summary. The test for spirocheticidal action of drugs *in vitro* (Eagle[2]) has been used with certain antibiotic substances. Penicillin, originally presumed to be inactive *in vitro*, has proved to be active under these conditions in the relatively high concentration of 800 to 1600 Oxford units per 0.8 ml. Inadequate therapy of syphilis in a rabbit resulted in the production of a more resistant strain of *Treponema pallidum* and emphasizes the importance of adequate initial therapy.

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Influence of Vitamin K Preparations on Blood Pressure in Hypertensive Rats.*

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While we have been occupied with the preparation of kidney extracts capable of lowering the blood pressure, it has become increasingly clear that the hypertension resulting from inadequate blood supply to the kidney might be due to faulty deamination of certain amino acids. Holtz *et al.*[1] found that in anaerobic conditions, such as may exist in renal ischemia, decarboxylation without subsequent deamination can occur, thus leading to the accumulation of pressure amines, and Bing and co-workers[2,3] demonstrated that experimental hypertension can be produced by injection of amino acids into the ischemic kidney of cats. Under normal aerobic conditions, on the other hand, such amines do not accumulate since they are readily destroyed by the catalytic action of amino oxidase. Friedman, Soloway, Marrus and Oppenheimer[4] demonstrated that certain quinones, which may inactivate pressure amines, may have well-defined blood pressure reducing qualities in hypertensive rats. Grollman and Harrison,[5] found some blood pressure lowering substance in body and liver oils of fish and assumed that this substance might also be a quinone. It was, therefore, suggestive to investigate the

* The authors wish to express their gratitude to the American Philosophical Society for financial support from the Daland Fund.

1 Holtz, P., Heise, R., and Luedtke, K., *Arch. Exp. Path. u. Phar.*, 1938, **191**, 87.

2 Bing, R. J., *Am. J. Phys.*, 1941, **132**, 497.

3 Bing, R. J., and Zucker, M. B., *J. Exp. Med.*, 1941, **74**, 235.

4 Friedman, B., Soloway, S., Marrus, J., and Oppenheimer, B. S., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 195.

5 Grollman, A., and Harrison, T. R., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 162.