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Relationship of Penicillinase to the Action of Penicillin.

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In a previous report[1] a survey was made of the common pathogenic and non-pathogenic organisms to determine their ability to produce penicillinase, an enzyme which destroys penicillin. Bacteria capable of producing this enzyme were found to be rather common, particularly among the Bacillus, Shigella, and coliform groups of bacteria. Most of the organisms commonly recognized as primary pathogens failed to produce penicillinase, regardless of their sensitivity or insensitivity to penicillin. Furthermore, two penicillinase-positive organisms were encountered that were slightly susceptible to penicillin. Similar observations led Abraham and Chain[2] to conclude that production of penicillinase by bacteria probably was not the sole factor in determining their resistance or sensitivity to penicillin. As a result of these observations further investigation as to the significance of penicillinase as a factor in the mode of action of penicillin appeared warranted.

A number of organisms previously tested for penicillinase production[1] were studied to determine their relative susceptibility to penicillin. To tubes of extract or infusion broth were added dilutions of penicillin to give final concentrations of 5.0, 0.5, and 0.05 units per cc. A set of broth tubes containing these 3 concentrations of penicillin was inoculated with a drop of a 1/100 dilution of a 24-hour broth culture of the organism being studied. The results were read at the end of 24 hours for inhibition of growth.

The relationship between the susceptibility of an organism to penicillin and the ability of the organism to produce penicillinase based on these experiments is shown in Table I. Only a few of the organisms falling into each of 5 distinct categories are listed. Of particular note is the fact that not all penicillinase-negative organisms are susceptible to penicillin. Although organisms such as staphylococci, hemolytic streptococci, and gonococci were susceptible to low concentrations of penicillin, concentrations ten to a hundredfold greater were required to inhibit members of the typhoid-salmonella group. Furthermore, even the highest concentrations used would not inhibit certain other penicillinase-negative organisms such as Pseudomonas and *Brucella melitensis*. It may be concluded, therefore, that the inability of an organism to produce penicillinase is not necessarily a determining factor in its sensitivity to penicillin.

Among the penicillinase-positive organisms, 21 of 23 strains tested were not inhibited by penicillin in a concentration of 5.0 units per cc. The remaining two strains were inhibited by this concentration in spite of their ability to produce penicillinase. No organism was found among the penicillinase-positive cultures that was inhibited by concentrations less than 5.0 units per cc. It appears as though penicillinase production by a bacterium is an important factor in its resistance to penicillin, although not necessarily the only factor.

A number of workers[3,4,5] have succeeded in developing penicillin-resistant strains of bacteria by cultivation in increasing concentrations of penicillin over long periods of time. As yet the nature of this acquired resistance is not known. A possible factor in the development of this resistance could be the acquisition by the organism of the ability to produce

1 Bondi, A., and Dietz, C. C., PROC. SOC. EXP. BIOL. AND MED., 1944, submitted for publication.

2 Abraham, E. P., and Chain, E., *Nature*, Lond., 1940, **146**, 837.

3 McKee, C. M., and Rake, G., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 275.

4 Rammelkamp, C. H., and Maxon, T., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 386.

5 Spink, W. W., Ferris, V., and Vivino, J. J., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 207.

TABLE I.
Relationship of Susceptibility to Penicillin with Destruction of Penicillin by Bacteria.

Organisms*	No. of cultures encountered	Penicillinase	Inhibition of growth Units/ml of penicillin 0.05	0.5	5.0
Staph. aureus Hemo. strept. *N. gonorrheæ*	20	—	+ —	+	+
E. typhosa Salmonella *Prot. vulgaris*	14	—	—	—	+
Pseudomonas *Br. melitensis*	8	—	—	—	—
E. coli *B. cereus* *Shig. dysenteriæ*	21	+	—	—	—
Alk. fecalis *B. anthracis*	2	+	—	—	+

* Organisms listed do not include all the species encountered in each category.

TABLE II.
Failure of Organisms Made Resistant to Penicillin *in Vitro* to Produce Penicillinase.

Organism	No. of transplants in penicillin	Units/ml of penicillin inhibiting growth	Penicillinase production
Staph. aureus (X-3)	0	0.045	—
	6	0.09	—
	10	0.19	—
	17	0.75	—
	22	3.0	—
	27	12.0	—
E. typhosa (Panama)	0	1.5	—
	6	6.0	—
	12	24.0	—
	23	48.0	—
P. vulgaris (T 3028)	0	3.0	—
	4	6.0	—
	10	12.0	—
	18	48.0	—

penicillinase, although evidence to the contrary has been reported.[4]

In order to confirm the latter report, strains of *Staph. aureus, Eberthella typhosa*, and *Proteus vulgaris* were rendered resistant *in vitro* by cultivation in increasing concentrations of penicillin. Transplants were made at 2- to 3-day intervals in tubes of broth containing increasing amounts of penicillin over a period of 6 to 8 weeks.

Isolations made at different stages of the development of resistance were stored under mineral oil and tested at the same time for susceptibility to penicillin. All such isolations were tested for their ability to produce penicillinase as shown in Table II. In spite of the acquisition of resistance, none of the cultures produced penicillinase.

Discussion. The development of sulfonamide-fast bacteria has been reported by many workers.[6,7,8,9] In the case of *Staph. aureus* this resistance has been associated with an

[6] MacLeod, C. M., and Doddi, G., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 69.

[7] Westphal, L., Charles, R. L., and Carpenter, C. M., *Ven. Dis. Inform.*, 1940, **21**, 183.

[8] Strauss, E., Dingle, J. H., and Finland, M., *J. Immunol.*, 1941, **42**, 331.

[9] Vivino, J. J., and Spink, W. W., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 336.

increased synthesis of para-aminobenzoic acid.[10] That the same mechanism is not involved in the development of penicillin-fastness is evidenced by susceptibility of sulfonamide-fast strains of staphylococci and pneumococci[5,11] to penicillin. Furthermore, it is evident that penicillinase does not hold analogous relationship to penicillin-fast staphylococci as para-aminobenzoic acid does to sulfonamide-fast staphylococci. Other factors as yet unknown must be concerned in the development of resistance by bacteria to penicillin.

That development of resistance to penicillin is not dependent on penicillinase is not surprising inasmuch as inability to produce penicillinase does not appear to be the determining factor in the sensitivity of an organism to penicillin. The factors concerned in the development of resistance to penicillin by bacteria no doubt are related to the factors which determine their susceptibility to penicillin.

It is to be expected that organisms producing penicillinase would not be highly susceptible to penicillin. The susceptibility of the two penicillinase-positive organisms to higher concentrations of this antibiotic may be attributed to production of penicillinase in smaller amounts. Furthermore, 24-hour cultures were used in the susceptibility tests whereas cultures 4 days or older contain much greater amounts of penicillinase.

Conclusions. Inability to produce penicillinase is not a determining factor in the sensitivity of an organism to penicillin. However, penicillinase-producing bacteria are not likely to be highly susceptible to penicillin. Development of resistance to penicillin by a bacterium is not associated with acquisition by the bacterium of the ability to produce penicillinase.

10 Landy, M., Larkum, N. W., Oswald, E., and Streightoff, F., *Science*, 1943, **97**, 265.

11 Powell, H. M., and Jamieson, W. A., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 387.

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Production of Labyrinthine Paralysis by Application of Local Anesthetics to External and Middle Ear.*

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In the course of experiments dealing with other problems of abnormal labyrinthine excitability, methods of eliminating the impulses from the labyrinth were developed that may be able to make more radical measures (Dandy,[1] Putnam,[2] Mollison[3]), at least in some instances of Ménière syndrome, unnecessary. While Brown-Séquard's[4] procedure of producing symptoms of labyrinthine paralysis by instillation of chloroform into the external auditory canal in guinea pigs is not applicable to cats or dogs, paralysis of the labyrinth was obtained, in cats, by electrosmosis of solutions of local anesthetics introduced into the external auditory meatus. Two per cent solutions of cocain, pontocain, or metycain in 80% alcohol were instilled into the external meatus, and the meatus was plugged by an electrode that was wrapped by cotton soaked with the

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1 Dandy, W. E., *Acta Otolaryngol.*, 1934, **20**, 1.

2 Putnam, T. J., *Arch. Otolaryngol.*, 1938, **27**, 161.

3 Mollison, W. W., *J. Laryng. and Otol.*, 1936, **51**, 38.

4 Brown-Séquard, C. E., *C. R. Soc. de biol.*, Ser. 7, 1880, **2**, 383.