THE SUSCEPTIBILITY OF PENICILLINASE-PRODUCING BACTERIA TO PENICILLIN

I. Factors Influencing Susceptibility

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Penicillinase, an enzyme that destroys penicillin (Abraham and Chain, 1940), is produced by a wide variety of bacteria (Bondi and Dietz, 1944*a*). Considerable variation is encountered among these organisms as to their susceptibility to penicillin (Bondi and Dietz, 1944*b*). Some strains that produce this enzyme are susceptible to penicillin (Bondi and Dietz, 1946). It appeared worth while to study a wide variety of such organisms to determine further the factors that influence their susceptibility.

EXPERIMENTAL PROCEDURES

The susceptibility of the various organisms studied was determined by a tube dilution technique. Series of tubes of 1.0 per cent tryptone broth carrying two-fold concentrations of penicillin were inoculated with 18- to 24-hour broth cultures of organisms under investigation. Crystalline potassium penicillin G was used throughout the work. Since inoculum size has been shown to influence the susceptibility of these organisms (Kirby, 1945; Luria, 1946), at least three different inocula were tested. The tubes were incubated at 37 C, and growth was read in 24 and 48 hours. All cultures studied were strains that had been maintained in a laboratory stock culture collection for several years on veal infusion agar slants.

EXPERIMENTAL RESULTS

Table 1 shows the results of susceptibility tests on two gram-positive and two gram-negative penicillinase-producing organisms. For comparison, the results of similar tests on one gram-positive and one gram-negative non-penicillinase-producing organisms are shown. A sharp difference in susceptibility is encountered between the two groups of organisms that produce the enzyme. Small inocula of the gram-positive strains are quite susceptible. In fact, individual cells of these organisms have the same order of susceptibility as do those of *Staphylococcus* H, which does not produce penicillinase. Larger inocula are less sensitive because of the more rapid multiplication of the cells resulting in greater production of enzyme and the subsequent rapid destruction of penicillin (Kirby, 1945). Similar results are obtained when these large inocula consist of washed

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TABLE 1

The susceptibility of penicillinase-producing bacteria

ORGANISM	INOCULUM 0.1 ML	INHIBITION OF GROWTH BY PENICILLIN—UNITS/ML										
		None	0.04	0.08	0.16	0.32	0.64	1.25	2.50	5.0	10.0	20	40
Staphylococcus 163	10^{-1}	4*	4	4	4	4	4	4	4	2	—		
	10^{-3}	4	4	4	4	—	—	—	—	—	—		
	10^{-5}	4	4	4	—	—	—	—	—	—	—		
B. anthracis "E"	10^{-1}	4	4	4	4	—	—	—	—	—	—		
	10^{-3}	4	2	—	—	—	—	—	—	—	—		
	10^{-5}	4	—	—	—	—	—	—	—	—	—		
Staphylococcus H†	10^{-1}	4	4	2	—	—	—	—	—	—	—		
	10^{-3}	4	2	—	—	—	—	—	—	—	—		
	10^{-5}	4	2	—	—	—	—	—	—	—	—		
Shigella dysenteriae	10^{-1}	4	4	4	4	4	4	4	4	4	4	2	—
	10^{-3}	4	4	4	4	4	4	4	4	4	4	—	—
	10^{-5}	4	4	4	4	4	4	4	4	4	—	—	—
E. coli	10^{-1}	4	4	4	4	4	4	4	4	4	4	4	2
	10^{-3}	4	4	4	4	4	4	4	4	4	4	4	—
	10^{-5}	4	4	4	4	4	4	4	4	4	4	—	—
E. typhosa†	10^{-1}	4	4	4	4	4	4	4	2	—	—	—	—
	10^{-3}	4	4	4	4	4	4	2	—	—	—	—	—
	10^{-5}	4	4	4	4	4	4	2	—	—	—	—	—

* 4 = no inhibition of growth; 2 = partial inhibition of growth; — = complete inhibition of growth.

† Do not produce penicillinase.

TABLE 2

The susceptibility of penicillinase-producing gram-positive bacteria

ORGANISM	INHIBITING CONCENTRATION OF PENICILLIN UNITS/ML			
	0.1 ml inoculum dilutions			
	Undil.	1×10^{-2}	1×10^{-4}	1×10^{-6}
Staphylococcus 7729	>10.0	5.0	1.28	0.16
Staphylococcus 161	>10.0	5.0	0.40	0.16
B. anthracis E	2.56	0.32	0.02	<0.02
B. anthracis Y	1.28	0.08	0.02	—
B. cereus	>10.0	>10.0	5.0	1.28
Bacillus sp	>10.0	>10.0	2.5	—
Bacillus sp	>10.0	0.64	0.16	—
B. megatherium	1.28	0.02	<0.02	<0.02

cells, indicating, as shown by Luria (1946), that the greater destruction of penicillin is not due to carry-over of penicillinase in the inocula.

The same relationship does not hold true for the gram-negative bacilli that produce penicillinase. Inoculum size does not influence their susceptibility to the extent that it does the gram-positive organisms. Small inocula are not susceptible. Although production of this enzyme probably contributes to the resistance of these organisms, it is not the primary factor. Some other mechanism common to all gram-negative organisms, with the exception of *Neisseria gonorrheae* and *Neisseria intracellularis*, is primarily responsible for their resistance.

Table 2 shows the susceptibility of a number of additional gram-positive bacteria. The effect of inoculum is evident. A wide variation in susceptibility is apparent. Some cultures, such as *Bacillus megatherium* and *Bacillus anthracis*, are highly susceptible when smaller inocula are tested. Others, such as *Bacillus cereus*, are resistant.

TABLE 3

The relationship of susceptibility to quantitative penicillinase production

ORGANISM	INOCULUM 0.1 ML	PENICILLIN—UNITS/ML		
		Inhibiting concentration	Destruction/ml*	
			24-hr culture	96-hr culture
Staphylococcus 161	10^{-2}	5.0	15.36	22.0
	10^{-4}	0.40		
	10^{-6}	0.16		
B. megatherium	10^{-2}	0.02	7.67	76.8
	10^{-4}	<0.02		
	10^{-6}	<0.02		
B. cereus	10^{-2}	>10.0	7.68	29.4
	10^{-4}	5.0		
	10^{-6}	1.28		

* Incubation for 1 hour at 37 C.

It appeared likely that susceptibility was related directly to the ability of an organism to produce penicillinase quantitatively. To obtain evidence of this, 24- and 96-hour broth cultures of three organisms with varying degrees of susceptibility were assayed for penicillinase by a method previously described (Bondi and Dietz, 1944*a*). These results are shown in table 3. Enzyme production is expressed in terms of the amount of penicillin destroyed by 1.0 ml of the culture. The susceptibilities of these cultures are likewise shown for comparison. It is apparent that there is not a direct relation between the amount of penicillinase produced by an organism and its susceptibility to penicillin. *Bacillus megatherium*, which is the best producer of the enzyme, is the most susceptible of the three cultures tested. The resistance of these organisms does not appear to be directly related to their ability potentially to produce large amounts of the enzyme.

Organisms vary considerably in the speed with which they produce penicillinase. As shown in table 3, the yield of penicillinase from a culture of *B. megatherium* was increased tenfold by incubation for 4 days. It seemed likely that the speed with which an organism produced this enzyme and the subsequent rapid destruction of penicillin had a direct bearing on its susceptibility to penicillin. Evidence for this is shown in table 4. At intervals shortly after a series of tubes containing twofold concentrations of penicillin had been inoculated with varying inocula of these organisms for susceptibility testing, assays were made from certain of the tubes for residual penicillin. The results of the assays from the tubes containing 5.0 units per ml 1, 2, and 4 hours after inoculation are shown. It is evident that there is a correlation between resistance and the rapidity of penicillin destruction. *B. megatherium*, the most susceptible of the organisms

TABLE 4

The relationship of susceptibility to speed of production of penicillinase

ORGANISM	INOCULUM 0.1 ML	INHIBITING CONCENTRATION, UNITS/ML	PENICILLIN—5.0 UNITS/ML BROTH		
			Units/ml left		
			1 hr	2 hr	4 hr
Staphylococcus 161	Undil.	>10.0	2.9	None	None
	10^{-2}	5.0	4.9	4.6	4.15
	10^{-4}	.40	5.2	5.0	5.10
B. megatherium	Undil.	1.28	4.7	4.2	3.0
	10^{-2}	.02	5.0	4.9	4.8
	10^{-4}	<.02	5.3	5.1	4.9
B. cereus	Undil.	>10.0	1.75	None	None
	10^{-2}	>10.0	4.80	4.9	None
	10^{-4}	5.0	5.30	5.2	4.7

studied in this fashion, destroyed relatively small amounts of penicillin during the short intervals tested. *B. cereus*, however, which is resistant, rapidly destroyed the 5.0 units present.

DISCUSSION

Undoubtedly, penicillinase production contributes to the resistance of a gram-positive organism to penicillin. The degree of its resistance depends upon the ability of the organism to produce the enzyme rapidly rather than upon its ability potentially to produce large amounts of it. If a sufficient amount is produced by an organism to destroy the antibiotic present soon after contact, growth rather than inhibition of the organism takes place.

Individual cells of many gram-positive penicillinase-producing organisms are as susceptible to penicillin as are those of organisms that do not produce the enzyme. The therapeutic effectiveness of penicillin in the treatment of an infection due to such an organism, apart from dosage, will depend not only upon the

number of organisms present but upon the speed with which they produce the enzyme. Anthrax, caused by a gram-positive penicillinase-producing organism, has responded well to penicillin therapy (Murphy, LaBoccetta, and Lockwood, 1944). *In vitro* tests have shown that *B. anthracis* is a very slow producer of penicillinase and consequently is susceptible, particularly when small inocula are tested.

Penicillinase production likewise contributes to the resistance of the gram-negative penicillinase-producing organisms. However, some other basic factor common to most gram-negative organisms is primarily responsible for their resistance. The nature of this resistance is unknown.

SUMMARY

The susceptibility to penicillin of a wide variety of penicillinase-producing organisms is reported.

Considerable variation in the susceptibility of the gram-positive organisms is observed; individual cells of some of these cultures are highly sensitive. The degree of resistance of these organisms is related to the speed with which they produce the enzyme rather than to the amount that they produce.

Although production of this enzyme may increase the resistance of gram-negative organisms, it is not the primary factor in their resistance to penicillin.

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