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Production of Penicillinase by Bacteria.

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Abraham and Chain[1] first reported the production by *Escherichia coli* and other bacteria of an agent which destroys penicillin. Because of its enzymic nature these workers termed the agent, penicillinase. Hobby *et al.*[2] confirmed the production of this agent by *E. coli.* Harper[3] reported the use of a cellular extract of a paracolon bacillus as a constituent of media for cultivation of bacteria in body fluids of patients treated with penicillin. The penicillin was destroyed, allowing growth of organisms which otherwise would have been inhibited. Lawrence[4] recently reported that the action of takadiastase and clarase in destroying penicillin may be attributed entirely to the presence of contaminating bacteria since penicillinase production is a common property among air contaminants. Studies were carried out in this laboratory to determine the nature and significance of this agent produced by bacteria.[5]

In this study the test used for determining the production of penicillinase by bacteria is similar to that used by Abraham and Chain.[1] It entails the addition of penicillin to growing broth cultures of bacteria or their supernates, and the subsequent testing of the mixture for residual penicillin activity by the Oxford Cup method using *Staphylococcus aureus H* as the test organism. Destruction of the penicillin by a culture serves as an index of penicillinase production by that organism.

Broth cultures of the organisms studied are grown for a minimum of 4 days. To 1.4 cc volumes of these broth cultures is added 0.1 cc of a dilution of penicillin. One cup on each of 3 agar plates previously seeded with *Staph. aureus H* is filled with the penicillin-culture mixture without initial incubation. Five or 6 cultures may be tested on a given plate, although each organism was always run in triplicate. Plates are immediately placed in the 37°C incubator and read at the end of 18 to 24 hours for absence of a zone of inhibition of the *Staph. aureus* indicating destruction of penicillin. The same quantity of penicillin added to a tube of sterile broth and likewise tested serves as a control. The concentration of penicillin is not important as long as the control penicillin broth produces a zone of inhibition on the plate of from 20 to 25 mm in diameter. This concentration must be the same for each culture so studied.

The destruction of penicillin is a rapid process; incubation of the mixture for periods of time ranging from one to six hours before the cups are filled does not materially affect the results. Furthermore, additional incubation actually does occur in the cups.

The results of a typical experiment are shown in Table I. Penicillin-culture mixtures of the penicillinase-positive organisms show either no zone of inhibition or a zone of inhibition considerably less than the control indicating destruction of the penicillin. Sim-

TABLE I.
Detection of Bacterial Penicillinase by an Oxford Plate Technic.

Organism + Penicillin	Zone of inhibition* in mm	Penicillinase
Staph. aureus	24.8	—
Esch. coli	0	+
E. typhosa	25.2	—
Sal. enteriditis	24.9	—
Shig. dysenteriæ	0	+
Shig. paradysenteriæ	14.2	+
Broth control	25.1	

* Inhibition of *Staph. aureus*—average of triplicate tests.

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

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

3 Harper, G. J., *Lancet*, 1943, **2**, 569.

4 Lawrence, C. A., *Science*, 1944, **99**, 15.

5 Bondi, A., and Dietz, C. C., *J. Bact.*, 1944, **47**, 20.

ilar mixtures of penicillinase-negative organisms show zones of inhibition comparable to that of the control indicating that penicillin was not destroyed by those organisms.

A list of organisms studied by this technic for evidence of penicillinase production is shown in Table II. Among the gram-positive and gram-negative cocci studied no culture was encountered that produced penicillinase. One might have expected these results inasmuch as most infections produced by these organisms respond effectively to penicillin therapy. No doubt penicillinase-producing strains may be found among the cocci, as Abraham and Chain[1] reported finding a Micrococcus which was capable of destroying penicillin. In the group of gram-positive bacilli studied, many of the aerobic spore-formers were positive. All 6 cultures of Clostridia studied were negative.

In the group of gram-negative bacilli, penicillinase-producing cultures were more frequently encountered. All strains of coliform bacilli tested were positive. With the exception of one strain of *Shigella paradysenteriæ* all other members of the genus *Shigella* tested were positive. In contrast, members of the typhoid-salmonella group were uniformly negative. This sharp difference between the two groups might eventually prove useful as a differential aid. It is interesting to note that *Shigella newcastle* whose place in the genus *Shigella* has been questioned by many workers is penicillinase-positive.

Three strains of *Proteus vulgaris* were uniformly penicillinase-negative. Likewise, all non-intestinal, gram-negative bacilli tested, such as Brucella and Hemophilus were also negative.

By use of the same test one may estimate quantitatively the amount of penicillinase produced by different bacteria. The technic is the same except that serial two-fold dilutions of the culture supernates are tested for their ability to destroy penicillin. Because bacteria remaining in the filtrates may continue to produce penicillinase, cultures to be assayed

TABLE II.
Production of Penicillinase by Bacteria.

Organism	Penicillinase
Staph. aureus (5*)	—
Hemo. streptococci (3) (Group A)	—
Viridans streptococci (2)	—
Enterococci (2)	—
Pneumococci (2)	—
N. catarrhalis (2)	—
N. intracellularis	—
N. gonorrheæ	—
B. cereus	+
B. subtilis	—
B. megatherium	+
B. anthracis (2)	+
Clostridia (6)	—
C. diphtheriæ	—
Diphtheroid	—
List. monocytogenes (2)	—
Esch. coli (2)	+
Aerob. aerogenes	+
Paracolon (2)	+
E. typhosa (3)	—
Salmonella (6)	—
Shig. dysenteriæ	+
" *paradysenteriæ* (3)	+
" *paradysenteriæ*	—
" *sonnei* (2)	+
" *newcastle*	+
Proteus vulgaris (3)	—
Alk. fecalis	+
Pseudomonas (3)	—
" (3)	+
Br. bronchiseptica	—
" *melitensis*	—
Hemo. influenzæ (2)	—
" *parapertussis*	—

* Number in parenthesis indicates number of strains tested, otherwise only the single strain was tested.

TABLE III.
Quantitative Estimation of Penicillinase Produced by Different Bacteria.

Organism*	Dilution†	Zone of inhibition in mm
Penicillin control		20.0
Alk. fecalis	1/2	—
	1/4	—
	1/8	13.0
Esch. coli	1/8	—
	1/16	—
	1/32	15.5
B. cereus	1/16	—
	1/32	—
	1/64	15.8
Paracolon	1/32	—
	1/64	—
	1/128	16.2
B. megatherium	1/64	—
	1/128	—
	1/256	14.0

* Ninety-six-hour cultures.
† Two-fold dilutions in 1.5 ml volumes.

must be filtered through bacterial filters or a growth-inhibiting agent must be added. In the experiment shown in Table III, 0.25% phenolized saline was used as the diluting fluid. Phenol in such a concentration did not affect the activity either of penicillin or of penicillinase. These results show that bacteria vary considerably as to the amount of penicillinase they produce.

Discussion. Bacteria capable of producing penicillinase, an enzyme destroying penicillin, are widely distributed in nature. They are common among the gram-negative bacilli normally found in the intestinal tract. With the exception of the Shigella group of organisms most penicillinase-positive organisms are not primary pathogens, a fact of considerable importance.

The relationship of penicillinase production by a bacterium to its resistance to penicillin is a subject of another report.[6] Although there is some correlation between the two phenomena, it is worthy of note at this time that *B. anthracis* which has been reported as susceptible to penicillin is a penicillinase producer.

Otherwise, the significance of penicillinase production by bacteria as it relates to penicillin therapy is open to conjecture. It is conceivable that infected foci secondarily invaded with penicillinase-producing bacteria, such as the coliform bacilli and Pseudomonas, might not respond favorably to penicillin therapy due to destruction of penicillin *in situ*. Further clinical evidence is necessary, however, in order to determine the importance of penicillinase in relation to penicillin therapy. In the meanwhile, it is highly recommended that mixed infections treated with penicillin be tested for the presence of penicillinase-producing bacteria.

As suggested by Rammelkamp and Keefer,[7] penicillinase production in the intestine probably is of considerable importance. It is doubtful whether penicillin could ever be used for treatment of intestinal infections for this reason. Furthermore, it is conceivable that the rapid loss of penicillin following its introduction into patients might be accounted for in part by carriage by the blood stream to the intestinal tract where it is destroyed by bacterial penicillinase.

To date there is no readily available substance that can be used for experimental purposes to destroy penicillin or to inhibit its action. Penicillinase eventually may prove to be this badly needed agent. From a practical point of view a preparation of penicillinase should be as valuable for culturing body fluids containing penicillin as is para-aminobenzoic acid for culturing specimens containing sulfonamides. In this laboratory, culture filtrates containing penicillinase have been used for this purpose with moderate success.

Conclusions. A simple test for the qualitative and quantitative determination of penicillinase produced by bacteria is described. Bacteria capable of producing penicillinase are widely distributed in nature; such organisms vary considerably as to the amount of penicillinase they produce. The possible significance of penicillinase and penicillinase-producing bacteria in relation to penicillin therapy is discussed.

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

[7] Rammelkamp, C. H., and Keefer, C. S., *J. Clin. Invest.*, 1943, **22**, 425.