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Microbial Resistance to Penicillin as Related to Penicillinase or Penicillin Acylase Activity. (25903)

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Presence of enzyme penicillinase that specifically destroys penicillin activity by degradation to inactive penicilloic acid, offers a ready explanation for resistance of certain microorganisms to this antibiotic. With recent discovery(1) that many microorganisms are able to cleave the acyl group in penicillin G, to yield the relatively inactive moiety 6-aminopenicillanic acid (6-APA), still another mechanism for penicillin resistance became apparent. A number of bacteria are reported here to relate their resistance to penicillin to either penicillinase or penicillin acylase activity.

Results. It is obvious that 6-APA possesses definite antibacterial properties, but to a much less degree than penicillin G (Table I). Comparative values expressed as minimum inhibitory concentration (MIC) in μg/ml in Table I indicate that 6-APA is 4166-fold to 390-fold less active than penicillin G when tested *in vitro* against a series of Gram positive bacterial genera. Although the differences against the Gram negative bacteria are not so striking, 6-APA is 2-fold to 10-fold less active than penicillin G. Differences in activity similar to this were obtained *in vivo*. At concentrations of 200 mg/kg 6-APA failed to protect mice against experimental *Staphylococcus aureus* infections, and levels of 50 mg/kg against *Streptococcus pyogenes* infections offered no protection. In contrast, penicillins G and V were highly effective (Table I).

The relationship of MIC to penicillin, and presence or absence of penicillinase or penicillin acylase activity, for a number of different bacteria are shown in Table II. After an average MIC for penicillin was established, bacterial cells of each culture were examined for presence of penicillin ăcylase activity, *i.e.,* hydrolytic conversion of penicillin G to 6-APA, by the chromatographic procedure described by Batchelor(2). In addition, supernates were examined by several methods for evidence of biological inactivation of penicillin. In one system sterile filtrates of 24 hour cultures were achieved by passage through sin-

TABLE I. Comparison of Activity of 6-Aminopenicillanic Acid and Penicillin G.

I. *In Vitro*	MIC, μg/ml*	
Microorganism	6-Aminopenicillanic acid	Penicillin G
Staphylococcus aureus	125	.03
Streptococcus pyogenes	15.6	.006
" *faecalis*	250	.16
Diplococcus pneumoniae	250	.0078
Erysipelothrix rhusiopathiae	125	.08
Corynebacterium diphtheriae	31.2	.09
Bacillus subtilis	62.5	.02
" *cereus*	125	.04
Aerobacter aerogenes	62.5	25
Escherichia coli	6.25	25
Proteus vulgaris	250	25
Pseudomonas aeruginosa	250	100
Salmonella typhosa	125	12.5
" *pullorum*	62.5	12.5
Klebsiella pneumoniae	62.5	12.5
Neisseria gonorrheae	1.9	.19
Shigella sonnei	125	50
Brucella bronchiseptica	125	100

II. *In Vivo*	PD_{50}, mg/kg			
	6-Aminopenicillanic acid		Penicillin†	
Infection in mice‡	Oral	I.V.	Oral	I.V.
Staphylococcus aureus	>200	>200	1.5	.78
Streptococcus pyogenes	>50	>50	.9	.6

* 2-fold 10 tube serial dilution technic—MIC read after 20 hr incubation at 37°C.

† Penicillin V used orally; penicillin G used intravenously.

‡ Intraper. infection—treatment started ½ hr later—single dose. PD_{50} calculated after 96 hr.

tered glass filters. *Streptococcus pyogenes* ATCC 8668, highly sensitive to penicillin and free from penicillin acylase activity, was suspended in this filtrate. This served as inoculum in a 2-fold serial dilution test with a penicillin concentration of 100 μg/ml in the first tube. If no penicillin-inactivating enzymes were present in the supernatant broth, the resulting MIC of penicillin for this organism was 0.006 μg/ml. If such enzymes were present, the resulting MIC would be considerably higher than 0.006 μg/ml.

A second method made use of a modified Gots technic(3). An inhibitory concentration of penicillin (0.312 μg/ml) was incorporated into brain heart agar plate with *Staph. aureus* (penicillin sensitive). If a sterile paper disc moistened with a broth containing penicillin-inactivating enzymes is placed on the surface of such a plate, enzyme activity diffuses into the medium, penicillin is inactivated, and a halo zone of growth occurs. It is probable that both penicillinase and penicillin acylase activity in a broth would appear as positive by these technics. However, 18-24 hour broths from cultures known to contain penicillin acylase activity as determined by the chromatographic procedure, failed to produce a positive test in either the broth or plates technics. As an explanation, additional studies indicated that under our experimental conditions, penicillin acylase activity is more intracellular than that of penicillinase. Hence, such broth filtrates as above contained little if any, penicillin acylase activity. It is therefore assumed that inactivation of penicillin resulted from the action of penicillinase. In addition, probably the actual quantitative amount of penicillin acylase activity present is important.

As indicated for bacteria presented in section I of Table II, penicillinase activity was not detected. However, all contained penicillin acylase activity. Therefore, resistance to penicillin in these cultures can be explained by conversion of the highly-active penicillin to the considerably less active 6APA. As shown for these bacteria in section II of Table II, penicillin acylase activity was not detected. However, in each instance penicillinase activity was present. Penicillinase production by Gram negative bacteria including the representative genera in Table II has been reported by a number of authors(4,5).

TABLE II. Penicillinase and Penicillin Acylase Profiles of Microorganisms Resistant to Penicillin.

Microorganism	No. of strains	Avg MIC, μg/ml. penicillin	Presence of: Penicillinase activity	Presence of: Penicillin acylase activity
Section I				
Aerobacter aerogenes	3	25	Neg	Pos
Escherichia coli	5	25-50	"	"
Proteus vulgaris	4	25-50	"	"
Pseudomonas aeruginosa	2	25-50	"	"
Pseudomonas phaseolicola	1	100	"	"
Section II				
Escherichia coli	5	25-50	Pos	Neg
Pseudomonas aeruginosa	2	25-50	"	"
Aerobacter aerogenes	1	25	"	"
Section III				
Proteus vulgaris	3	25	Neg	"
Escherichia coli	2	25-50	"	"
Pseudomonas aeruginosa	3	25-50	"	"
Section IV				
Staphylococcus aureus, penicillin resistant	1	>100	"	"
Staphylococcus aureus, clinical isolates, penicillin resistant	10	>100	Pos	"
Streptococcus pyogenes	2	.006	Neg	"
Staphylococcus aureus	3	.0156	"	"
Diplococcus pneumoniae	3	.0078	"	"

Although one might suspect the presence of both penicillinase and penicillin acylase activities in the same microorganism, such was not detected in any of those listed. Since there seems to be no logical explanation that would negate such a possibility, our studies are continuing.

The fact that still another metabolic mechanism or mechanisms in addition to that of penicillinase and penicillin acylase activity, confers resistance to penicillin is shown by the bacteria in section III of Table II. Although

resistant to large concentrations of penicillin, neither penicillinase nor penicillin acylase activity was detected in these microorganisms. In contrast, the usual Gram positive bacteria have a similar profile, neither penicillinase nor penicillin acylase activity. However, these microorganisms are sensitive to low concentrations of penicillin (section IV, Table II).

It is apparent that presence or absence of penicillinase and penicillin acylase activity is not strictly related to MIC values for penicillin. These values remain constant for different genera listed in categories I, II, and III, and appear independent of presence or absence of penicillinase or penicillin acylase activity. Probably the final effect of penicillin (as indicated by the MIC value), penicillinase and penicillin acylase actions depends upon the killing rate of penicillin and enzyme production rate. The latter must not be rapid enough and is probably not produced in sufficient amount to interfere drastically with penicillin action.

Penicillin resistant *Staph. aureus,* IV of Table II, is resistant to penicillin and was obtained by selection in the laboratory technic of serial transfer in presence of gradually-increasing concentrations of penicillin. Such strains usually differ from penicillin-resistant clinical isolates because they fail to show the presence of penicillinase. Penicillin-resistant strains obtained from patients do contain penicillinase(6). This is shown in IV, Table II. The presence of penicillin acylase activity was anticipated in such laboratory-developed penicillin-resistant strains. Such activity has not been detected. Some mechanism other than penicillinase or penicillin acylase activity is indicated.

It is of interest to note the strain specificity relevant to the source of penicillinase or penicillin acylase activity. Various strains of *Escherichia coli* and *Pseudomonas* appear in 3 of the classifications in Table II; *Aerobacter aerogenes* appears in 2, etc.

Summary. 1. The penicillin moiety, 6-aminopenicillanic acid, has antibacterial activity but is considerably less active than penicillin. 2. Resistance to penicillin in one group of bacteria could be explained logically by presence of penicillinase activity, *i.e.,* conversion of penicillin to the inactive penicilloic acid. 3. Resistance to penicillin in a second group of bacteria could be explained logically by presence of penicillin acylase activity, *i.e.,* conversion of penicillin to the relatively inactive 6-aminopenicillanic acid. 4. In a third group of bacteria resistant to penicillin, neither penicillinase nor penicillin acylase activity could be detected. Still another metabolic mechanism other than penicillinase or penicillin acylase activity must be present in selected bacteria.

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