SIDE CHAIN STEREOISOMERISM AND ANTISTAPHYLOCOCCAL POTENCY OF PENICILLINS *

By PAUL D. HOEPRICH

(From the Department of Internal Medicine, University of Utah College of Medicine, and the Salt Lake County General Hospital, Salt Lake City, Utah)

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Penicillins may be regarded as acylation products of 6-aminopenicillanic acid (Figure 1). This bicyclic dipeptide results from cyclization of L-cysteinyl-L-valine (1) and is loosed into the culture medium in good yield when a strain of *Penicillium chrysogenum* (W.51.20) is cultured in the absence of side chain precursors suitable for acylation at the 6-amino grouping (2). With the attainment of commercially feasible fermentive production of 6-aminopenicillanic acid, the organic chemist can now carry out industrially practical terminal synthesis of known or new penicillins.

Alpha-phenoxyethylpenicillin [penicillin B, 6-\((\alpha\text{-phenoxypropioamido})\text{-penicillanate, or phenethicillin—see Figure 1}], the first marketed product of such industrial terminal penicillin synthesis, has not been identified as a naturally occurring penicillin. Special biological properties have been ascribed to this compound which also distinguish it from other penicillins (3–5). These include: 1) resistance to acid (gastric) degradation; 2) excellent absorption from the gastrointestinal tract; 3) greater antibacterial potency. Slow penicillinase inactivation quite reasonably would be reflected in apparent *in vitro* superiority in antibacterial effectiveness against penicillinase-producing bacteria. However, enhanced antibacterial effectiveness against nonpenicillinase-elaborating bacteria would require other explanation.

In part, the augmented antibacterial potency claimed for \(\alpha\)-phenoxyethylpenicillin has been ascribed to the stereoisomerism inherent in the \(\alpha\)-phenoxyethyl side chain characteristic of this penicillin. The L-isomer was reported to be generally more active than the D-isomer, and the racemate\(^1\) more effective than either enantiomorph.

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\(^1\)A product of an organic chemical synthesis, \(\alpha\)-phenoxyethylpenicillin should be 50 per cent levo- and 50 per cent dextro-rotatory, with regard to side chain optical activity. However, federal law requires commercial \(\alpha\)-phenoxyethylpenicillin to have at least 55 per cent L-isomer to a maximum of 75 per cent L-isomer (6). *Racemate, raceme, and racemic*, when applied to \(\alpha\)-phenoxyethylpenicillin in this article, will refer to DL-\(\alpha\)-phenoxyethylpenicillin of legal constitution.
tain to include strains capable of penicillinase elaboration—these are the penicillin-resistant staphylococci of clinical significance (10). Penicillinase production appears to vary quantitatively among staphylococcal strains which are competent in this regard. There are penicillin-resistant staphylococci which have not been shown to elaborate penicillinase and, of course, those staphylococci which are markedly susceptible to penicillins do not make penicillinase. Techniques for detection of penicillinase activity are simple and sensitive; assay for this means of penicillin resistance was carried out with all of the staphylococci under study, using benzylpenicillin, phenoxy methylpenicillin and racemic α-phenoxycetylpenicillin.

MATERIALS AND METHODS

Staphylococci. Human associated staphylococci of community-wide origin (Salt Lake City) were used in testing. The total of 200 tellurite-positive (9) isolates were obtained by culture of: 1) lesions acquired by patients in the course of hospitalization, 50 isolates; 2) anterior nares of normal hospital personnel, 50 isolates; 3) lesions acquired outside the hospital, cultures taken prior to any antibacterial therapy, 50 isolates; 4) anterior nares of normal high school seniors, 50 isolates. Details regarding collection and storage of these isolates are the same as those given in an earlier communication (11).
Penicillins.  d-2 L-2 and racemic 3 α-phenoxyethylpenicillins, as well as phenoxyethylpenicillin, 2 were sterilized by exposure of weighed portions of the respective potassium salts to ethylene oxide gas (12) for 18 hours at room temperature. Potassium benzylpenicillin 2 and di-sodium aminocarboxybutylpenicillin 3 were obtained as preweighed sterile, dry powders in ampules. All of these penicillins were dissolved and diluted for testing in tryptic digest of casein-papaic digest of soybean broth.

Stock solutions were prepared which were 0.00100 M,

2 d-α-phenoxyethylpenicillin, potassium salt, lot 3168-1184 and L-α-phenoxyethylpenicillin potassium salt, lot 19810, supplied by E. R. Squibb & Sons (Dr. John T. Groel), N. Y.

3 Racemic α-phenoxyethylpenicillin, (batch 02295) with composition given as 35 per cent d-isomer and 65 per cent l-isomer, supplied by Charles Pfizer & Co. (Mr. Andrew J. Schmitz, Jr.), Brooklyn, N. Y. Potassium benzylpenicillin was supplied through the kindness of Dr. Fredrick L. Fink, also of Charles Pfizer & Co.

4 Potassium phenoxyethylpenicillin, lot 75838, supplied by Eli Lilly & Co. (Dr. G. E. Maha), Indianapolis, Ind.

5 Di-sodium aminocarboxybutylpenicillin, supplied as Salmoxin test powder, lot 2085-CP, by Abbott Labs. (Dr. J. T. Sylvester), North Chicago, Ill.

0.0001 M and 0.00001 M for each of the penicillins studied. Preparation for testing involved transfer of 0.1 ml portions of stock solutions to appropriately labeled series of 13 x 100 mm screw-capped culture tubes before storage at −22° C. In use, addition of 0.9 ml tryptic digest of casein-papaic digest of soybean broth, containing the inoculum of test staphylococci, resulted in final test concentrations for each penicillin of 0.001, 0.010, and 0.100 μmole per ml. Some characteristics of the penicillins studied and the relationships of microemles, mass and bioassay units are presented in Table I.

Testing. Test staphyloccoci from frozen storage were grown out overnight in tryptic digest of casein-papaic digest of soybean broth. According to a direct count in the Petroff-Hausser chamber, a dilution yielding 10-000 staphylococci per 0.9 ml was prepared in sufficient quantity (tryptic digest of casein-papaic digest of soybean broth) to permit inoculation of the requisite 19 tubes from a common supply (3 test concentrations per penicillin: 6 penicillins: 1 control tube containing 0.1 ml sterile broth).

After incubation for 24 hours at 37° C the tubes were inspected. Gross turbidity was accepted as evidence of resistance; absence of visible growth was taken to indicate inhibition. All tubes in which there was inhibition of growth were mixed by swirling, before a 3 mm loopful was removed to inoculate an eighth sector of a tryptic

<table>
<thead>
<tr>
<th>Side chain</th>
<th>Optical activity</th>
<th>Salt tested</th>
<th>μmole, μg and bioassay unit equiv. of test concentrations per ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kind</td>
<td></td>
<td>Cation</td>
<td>Mol wt</td>
</tr>
<tr>
<td>Benzyll-</td>
<td>G</td>
<td>K+</td>
<td>372.47</td>
</tr>
<tr>
<td>Phenoxymethyl-</td>
<td>V</td>
<td>K+</td>
<td>388.47</td>
</tr>
<tr>
<td>α-Phenoxyethyl-</td>
<td>B</td>
<td>Levo- or dextro-rotary</td>
<td>402.49</td>
</tr>
<tr>
<td>Aminocarboxybutyl-</td>
<td>N</td>
<td>d-Levorotary</td>
<td>340.37</td>
</tr>
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</table>

**TABLE I**

Some properties of the penicillins studied are listed along with the microgrom and, where appropriate, bioasay unit equivalents of the test concentrations

<table>
<thead>
<tr>
<th>Iso-late no.</th>
<th>μmole ml</th>
<th>Benzyllpenicillin</th>
<th>Phenoxymethylpenicillin</th>
<th>α-Phenoxyethylpenicillin</th>
<th>Aminocarboxybutylpenicillin</th>
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<tr>
<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>163</td>
<td>0.001</td>
<td>R R R R S R C C C C</td>
<td>R R R R R R R R R R R R R R</td>
<td>R R R R R R R R R R R R R R</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.010</td>
<td>C C C C C C C C C C</td>
<td>C C C C C C C C C C C C C C</td>
<td>C C C C C C C C C C C C C C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.100</td>
<td>C C C C C C C C C C</td>
<td>C C C C C C C C C C C C C C</td>
<td>C C C C C C C C C C C C C C</td>
<td></td>
</tr>
<tr>
<td>263</td>
<td>0.001</td>
<td>C C S S S C C C C C C</td>
<td>S S S S S S C C C C C C C C</td>
<td>C C C C C C C C C C C C C C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.010</td>
<td>C C C C C C C C C C</td>
<td>S S S S S S C C C C C C C C</td>
<td>C C C C C C C C C C C C C C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.100</td>
<td>C C C C C C C C C C</td>
<td>S S S S S S C C C C C C C C</td>
<td>C C C C C C C C C C C C C C</td>
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</table>

* There was concordance of susceptibility within replication units 15 times (of 18 trials) with Isolate 163 and 13 times with Isolate 253. By this assessment, a single determination of susceptibility, by the method described, will give the same result as 5 times repeated testing 85% of the time. R: resistant; S: bacteriostatic; C: bactericidal.
digest of casein-papaic digest of soybean agar plate. Following 24 hours at 37° C in a moist air-candle jar, these agar subcultures were examined. Absence of growth was interpreted as indicating a bactericidal effect, growth as evidence of a bacteriostatic effect in the broth test culture from whence the agar subculture was inoculated. Data supporting such loop subculture differentiation of the nature of the observed inhibitory effect have been presented elsewhere (11).

Assessment of the reproducibility of the results of susceptibility testing by the method described was provided by 5 repetitions of testing with 2 staphylococcal isolates. Each test proceeded from an overnight tryptic digest of casein-papaic digest of soybean broth culture inoculated from the frozen storage culture with counting, dilution, inoculation of penicillin tubes, incubation and interpretation as described. Considering 5 tests at a given concentration of a particular penicillin with one strain of staphylococcus to be a unit of testing, there were 18 units per isolate (Table II). There was agreement in 15 units with Isolate 163; 13 units with Isolate 263. Thus, if testing according to the method described be repeated 5 times, there will be accord in results about 88 per cent of the time; i.e., a single test would suffice 88 per cent of the time. The pattern of disagreements was that of stepwise gradation in effectiveness—variation in effect was between resistant and bacteriostatic, or, bacteriostatic and bactericidal; not resistant and bactericidal.

Penicillinase assay. Gots plates (13) were prepared, using a strain of Sarcina lutea* which was inhibited in agar pour plates (Sarcina inoculum, 1:100 final dilution of a 24 hour broth culture) by 5 X 10⁴ μmoles benzylpenicillin per ml. Each staphylococcal isolate was inoculated from an overnight broth culture in the pattern of a small V described on a sixth sector of each of 3 kinds of plates—containing 10⁴ μmoles per ml of either benzyl-, phenoxyethyl- or dl-α-phenoxyethylpenicillin; all plates having been seeded with S. lutea (a 24 hour broth culture in 1:100 final dilution. The plates were incubated at 37° C in moist air-candle jars and inspected at 24 hour intervals for 5 days for appearance of colonies of S. lutea juxtaposed to staphylococcal growth.

RESULTS

Since antistaphylococcal effectiveness was observed at three concentrations for each of the six


![Graph](image-url)

**FIG. 2.** THE TOTAL HEIGHT OF THE COLUMNS INDICATES THE EXTENT OF INHIBITION OF GROWTH (BACTERIOSTATIC PLUS BACTERICIDAL EFFECT) OF 200 CLINICAL ISOLATES OF TELLURITE-POSITIVE STAPHYLOCOCCI BY 6 PENICILLINS APPLIED AT 3 TEST CONCENTRATIONS. Aminocarboxybutyrylpenicillin was least potent. Benzylo-

penicillin and d-α-phenoxyethylpenicillin were about equally effective; both were superior to aminocarboxybutyrylpenicillin but were less potent than phenoxyethylpenicillin, l-α-phenoxyethylpenicillin or dl-α-phenoxyethylpenicillin; the latter 3 penicillins were nearly identical in potency. Bactericidal potency, the stippled area of each column, varied in the same manner as did inhibition of growth. All of the penicillins tested were more effective when present in high concentrations.
penicillins studied, the results of susceptibility
testing are presented (Figure 2) as three groups of
data corresponding to the three concentrations
employed. It is apparent that with the decimally-
stepped increments in penicillin concentration, dif-
ferences in effectiveness tended to decrease, if
not disappear. Thus, aminocarboxybutylenpicillin
was virtually impotent at 0.001 \( \mu \)mole per ml, ef-
fecting just bacteriostasis with only 2 of 200 iso-
lates. But this same penicillin, when present in a
concentration of 0.100 \( \mu \)mole per ml, inhibited the
growth of 177 of these same 200 staphylococcal
isolates. The other penicillins studied were much
more effective than aminocarboxybutylenpicillin,
even at 0.001 \( \mu \)mole per ml, but all displayed in-
creased effectiveness at greater concentrations.

Since all six penicillins were effective against
most of the test staphylococci at some concentra-
tion, differences in effectiveness were quantita-
tive; i.e., potency varied. In order to determine
whether or not potency could be related to side
chain stereoisomerism, chi square comparison of
inhibitory effectiveness (bacteriostatic plus bac-
tericidal effect) and of bactericidal effectiveness
was carried out. These results (Figure 3) are
best considered one test concentration group at a
time.

0.001 \( \mu \)mole per ml. Using phenoxymethylpeni-
cillin, a clinically and pharmacologically well
known penicillin as basis for comparison, benzyl-
and \( \alpha \)-phenoxycetyl- and aminocarboxybutylenpicil-
lin were all significantly (\( p \leq 0.050 \)) less
effective inhibitors for 200 tellurite-positive staphy-
lococci of clinical origin. Racemic and \( \alpha \)-phenox-
yethylenicillins were also significantly more active
than the \( \alpha \)-isomer of penicillin B.

No difference in over-all inhibitory potency
distinguished phenoxymethylpenicillin, \( \alpha \)-phe-
noxylethylpenicillin and \( \alpha \)-phenoxymethylpenicil-
lin. However, detailed comparison revealed that,
while 51 of the 200 isolates were resistant, 117
were susceptible to these three penicillins. The
remaining 32 isolates were resistant to one or two
of the three penicillins, with 4 resistant to phe-
oxymethylpenicillin alone, 5 resistant only to
\( \alpha \)-phenoxymethylpenicillin and 6 resistant just to
\( \alpha \)-phenoxymethylpenicillin (Figure 4).

In terms of bactericidal action, the same po-
tency relationships prevailed as were described for
inhibition of growth.

0.010 \( \mu \)mole per ml. Both aminocarboxybutylen-
cillin and \( \alpha \)-phenoxymethylpenicillin were
again significantly less effective inhibitors of
staphylococci than was phenoxymethylpenicillin.
At this test concentration, however, benzylpeni-
cillin was not markedly inferior to phenoxymethyl-
penicillin. Again, \( \alpha \)- and \( \alpha \)-phenoxymethylpenicil-
lin were significantly more effective than the \( \alpha \)-isomer of this penicillin.

Over-all, \( \alpha \)-phenoxymethylpenicillin, \( \alpha \)-phe-
noxylethylpenicillin and benzylpenicillin were not
significantly different from phenoxymethylpenicil-
lin in effectiveness as inhibitors of staphylococci.
From isolate-by-isolate comparison, there were
182 of the 200 isolates tested which were suscep-
tible, and 1 which was resistant, to all of these
four penicillins. Seventeen were resistant to one
or more, but not to all four penicillins. One iso-
late was resistant to phenoxymethylpenicillin alone,
1 was resistant only to \( \alpha \)-phenoxymethylpenicillin
and 10 were resistant just to benzylpenicillin (Fig-
ure 4).

In terms of bactericidal action, phenoxymethylpeni-
cillin was significantly superior to aminocar-
boxybutylenpicillin, but was not more active than
the other penicillins tested. The pure diastereo-
isothers and the DL-mixture of \( \alpha \)-phenoxymethyl-
penicillin were not significantly variable in poten-
tial for lethal effect.

0.100 \( \mu \)mole per ml. Aminocarboxybutylenpicil-
lin was significantly less effective in securing
either inhibition or death of staphylococci than was
any of the five other penicillins—all of which were
equally effective. Only one isolate was resistant
to all six penicillins; another isolate, resistant to
benzylpenicillin as well as to aminocarboxybuty-
penicillin, was susceptible to the remaining four
penicillins tested (Figure 4).

These data suggest the following sequence of
antistaphylococcal potency for the penicillins
tested:

\[
\text{phenoxymethylpenicillin} > \text{L-\( \alpha \)-phenoxymethylpenicillin} \geq \text{benzyl} \geq \text{D-\( \alpha \)-phenoxymethyl} > \text{aminocarboxybutylenpenicillin}
\]
FIG. 3. THE ANTISTAPHYLOCCAL POTENCY OF AMINOCARBOXYBUTYL PENICILLIN (N), BENZYL-PENICILLIN (G), D-α-PHENOXYETHYL-PENICILLIN (d-B), DL-α-PHENOXYETHYL-PENICILLIN (dL-B) AND L-α-PHENOXYETHYL-PENICILLIN (L-B) WAS EVALUATED BY COMPARISON WITH PHENOXY-METHYL-PENICILLIN (V). Two hundred recent clinical isolates of tellurite-positive staphylococci were exposed to all 6 penicillins at the same time, under identical test conditions.

**TOP.** (0.001 μmole penicillin per ml) These were the relationships whether over-all inhibition of growth or bactericidal effect was considered: penicillin V was significantly more effective than penicillins N, G and d-B; penicillins L-B and dL-B were more effective than d-B; penicillins V, L-B and dL-B were not significantly different.

**MIDDLE.** (0.010 μmole penicillin per ml) Inhibitory effect: penicillin V was significantly more effective than penicillins N and d-B; penicillins L-B and dL-B were significantly more effective than d-B; penicillins V, L-B and dL-B were not significantly different. Bactericidal effect: penicillin V was significantly more potent than was penicillin N, but was not significantly more often associated with lethal antistaphylococcal effect than were penicillins G, d-B, dL-B or d-B.
STEREOISOMERISM AND POTENCY OF PENICILLINS

G

0.70>P>0.50

p=0.000

p=1

p=1

0.70>P>0.50

0.70>P>0.50

0.70>P>0.50

0.70>P>0.50

0.50>P>0.30

0.50>P>0.30

DL-B

BACTERICIDAL

INHIBITORY

0.100 micromol per ml.

Fig. 3—Continued

BOTTOM. (0.100 μmole penicillin per ml). In terms of both inhibitory and bactericidal effect, penicillin N was significantly less effective than penicillins V, G, d-B, dl-B and L-B.

Penicillinase activity was first perceptible after 48 hours' incubation of the Gots plates. The Sarcina colonies were tiny at this time and were little pigmented. While the total number of positive reactions was greater at 72 than at 48 hours' incubation, there was little increase thereafter. However, observation at 120 hours was considered most reliable, since the deep golden color of the Sarcina colonies was then fully developed. Under the test conditions described, penicillinase activity was evident with: 122 isolates against benzylpenicillin; 131 isolates against phenoxy-methylpenicillin; 134 isolates against dl-α-phenoxymethylpenicillin. The differences between these three values are not significant (0.50 > p > 0.30).

DISCUSSION

Molar description of penicillin concentrations used for susceptibility testing may seem an undue refinement; yet, penicillins are compounds of number of staphylococcal isolates inhibited by all penicillins of a group increased also. Although aminocarboxybutylpenicillin never achieved statistical parity in effectiveness with the other penicillins tested, the major difference between these penicillins in antistaphylococcal effectiveness appears to be quantitative.
known chemical constitution which become irreversibly fixed to bacteria to an extent directly proportional to susceptibility to lethal injury (14). Bound penicillins appear to work their effects by bringing about serious enzymatic dislocations which are only partially understood. Penicillins are antimetabolites and as such are effective as whole molecules. It follows that rational assessment of potency must proceed from description of concentration in molar terms.

In 1957 Sheehan and Henery-Logan (15) reported the laboratory synthesis of phenoxyethylpenicillin. Of little significance in terms of commercial manufacture of penicillins, this chemical milestone was a feat of great significance in permitting demonstration of the essential nature of certain structural features of the bicyclic ring nucleus common to all penicillins. It was known that lysis of the C$_5$—C$_6$ bond of the β-lactam ring (see Figure 1) to produce a penicillic acid, as accomplished by bacterial penicillinases (16), resulted in inactivation of penicillins. From comparison of synthetic with natural penicillins, it became clear that an intact 6-aminopenicillanic acid nucleus was also required in terms of the 5-membered ring and its substituents. More pertinent to the present study was the finding that, for antibacterial activity, all of the optically active centers of 6-aminopenicillanic acid must have the configuration uniformly present in natural penicillins. These observations with 6-aminopenicillanic acid, together with the demonstrated essentiality of particular optical configuration with chloramphenicol (17) and cycloserine (18), are precedent for the affirmation of significant variation in antistaphylococcal effectiveness of penicillins, differing only in optical configuration in the side chain.

Widely publicized in advertisements, the report of Gourevitch, Hunt and Lein (3) indicated that the L-isomer of α-phenoxyethylpenicillin displayed in vitro greater antibacterial activity than did the D-isomer against three of five strains of Staphylococcus aureus designated: 52-34, 52-75, WR 188, BRL J, BRL O. The superiority of L over D-α-phenoxyethylpenicillin was attested by respective minimal inhibitory concentrations in micrograms per milliliter: 1.6 vs 3.1, 3.1 vs 6.2, 3.1 vs 3.1, 0.8 vs 0.8, 0.8 vs 1.6. In the same report, a DL- mixture of α-phenoxyethylpenicillin was: 1) also more active than D-α-phenoxyethylpenicillin with four of the five test strains of staphylococcus—respective minimal inhibitory concentrations in micrograms per milliliter: 0.8 vs 3.1, 3.1 vs 6.2, 1.6 vs 3.1, 0.8 vs 0.8, 0.8 vs 1.6; 2) more active than L-α-phenoxyethylpenicillin with strains 52-34 and WR 188—respective minimal inhibitory concentrations in micrograms per milliliter: 0.8 vs 1.6, 1.6 vs 3.1; 3) equally as active as L-α-phenoxyethylpenicillin with strains 52-75, BRL J and BRL O (3.1, 0.8 and 0.8 μg per ml, respectively).

In addition to indicating that L- and DL-α-phenoxyethylpenicillin were superior in antistaphylococcal effectiveness to D-α-phenoxyethylpenicillin, these meager data were represented as indicating that D- and L-stereoisomers worked in complementary fashion with each other so that a DL-mixture was superior to either single enantiomorph in antistaphylococcal effect. While mouse protection, following on Smith strain staphylococcus infection, was reported to be more successful with

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*Both L- and DL- mixtures were more often solely effective than was the D-isomer. However, since the DL-mixture was not significantly more often alone effective than was the L-form, there is no indication from these data that mixing stereoisomers augments antistaphylococcal potency.*
rarefied than with either pure diastereoisomer of α-phenoxyethylpenicillin, the authors noted that the differences were not statistically significant.

Determination of the susceptibility of a significant number of clinical isolates of tellurite-positive staphylococci, as described in the present study, indicates that both L- and DL-α-phenoxyethylpenicillins are superior to D-α-phenoxyethylpenicillin.

There was no support from these data for the claim of augmented antistaphylococcal potency on mixing stereoisomers. Over-all, DL-α-phenoxyethylpenicillin was not significantly different in potency from L-α-phenoxyethylpenicillin at any of the concentrations tested (Figure 2). In addition, when isolate-by-isolate comparison of the effectiveness of these three α-phenoxyethylpenicillins was carried out to determine just how often each was the sole inhibitor of a staphylococcal isolate (Table III), no differences were found that were not apparent from the over-all susceptibility data (Figure 2). If complementary effectiveness were gained from mixing D- and L-isomers, the DL-mixture should have been most often the sole effective form of α-phenoxyethylpenicillin. This was not so—these data do not support the notion of augmented potency ascribed to DL-mixture of α-phenoxyethylpenicillin.

Yet, in affirming that the L-form was more potent than the D-form, the critical importance of optical configuration is asserted even in the side chain of penicillin. This importance is likely not critical in terms of clinical usage of α-phenoxyethylpenicillin since, generally, overtreatment is clinical practice. However, because chemical variation in acyl radical appears to be the only feasible approach to synthesis of new penicillins, side chain stereoisomerism should be considered in designing new penicillins for synthesis.

Garrod (19) compared in vitro the antistaphylococcal activity of benzylpenicillin, phenoxymethylpenicillin and α-phenoxyethylpenicillin (since isomeric designation was not made, it is assumed that a DL-mixture of α-phenoxyethylpenicillin was tested). With 36 "penicillin-sensitive" strains, there were no significant differences in effectiveness of the three penicillins by either plate or tube-dilution testing.

Thirty-eight penicillin-resistant (penicillinase-forming) strains were also tested as part of the same study. When a small inoculum (an 0.02 ml drop of a 1:500 dilution in saline of a broth culture) was applied to an agar culture medium containing penicillin in plate-dilution testing, the three kinds of penicillin were again effective to the same extent. However, by tube-dilution tests in which a large inoculum (an 0.02 ml drop of an undiluted broth culture) was used, both phenoxymethylpenicillin and α-phenoxyethylpenicillin were superior to benzylpenicillin. Moreover, α-phenoxyethylpenicillin was generally more effective than was phenoxymethylpenicillin.

Preparation of inocula in the present study proceeded from broth cultures which by direct count usually had around 10⁶ staphylococci per ml; accordingly, dilution to achieve the inoculum, 10,000 staphylococci per 0.9 ml, was at least 100,000-fold. If Garrod's broth cultures for inoculation had attained bacterial populations of density similar to ours—say 5 × 10⁸ per ml—his small inoculum would have delivered about 200,000 and his large inoculum about 250 million staphylococci (as added to 2.5 ml broth, there would have been about 10 million staphylococci per ml). From these considerations, our test situation was more nearly comparable (staphylococci versus penicillins and penicillins versus preformed penicillinase) to Garrod's small inoculum plate-dilution trials than to his large inoculum tube-dilution system. Even so, there remain differences—inoculum size, relative accessibility of penicillins to staphylococci, agar versus broth culture—of such significance that disagreement in results, if not inevitable, is at least not surprising.

Comparison of aminocarboxybutylpenicillin with benzylpenicillin by in vitro assay of antistaphylococcal effectiveness, using 30 isolates (20) and 7 isolates (21), indicated that penicillin N was markedly less inhibitory for staphylococci than was penicillin G. The data of the present study, relating to 200 staphylococcal isolates of recent, community-representative origin, support these findings—benzylpenicillin had greater antistaphylococcal potency than had aminocarboxybutylpenicillin. In addition, data were presented which assert that phenoxymethyl- and the
α-phenoxyethylpenicillins are also, individually, more potent antistaphylococcal penicillins than is amincarboxybutylpenicillin. However, penicillin N is remarkably more active than penicillin G against many species of gram-negative bacilli. Moreover, in addition to being an unusual penicillin in origin from Cephalosporium species—a non-Penicillium genus of the class Fungi imperfecti—the side chain of penicillin N is a hydrophilic, aminoacyl chain which is optically active (8). Unfortunately, we can only speculate on what would be the antistaphylococcal activity of penicillins enantiomorph and racemic to the tested d-aminocarboxybutylpenicillin. Application of dense inocula of staphylococci to agar media containing low concentrations of penicillins (about five times the minimal concentration inhibitory for the indicator strain of S. lutea which was heavily seeded in the agar media) provided settings designed for demonstration of even meager penicillinase production. On the other hand, the broth susceptibility testing method used exposed small inocula of staphylococci, virtually without preformed penicillinase, to 10-, 100- and 1,000-fold greater concentrations of penicillins than were present in the tests for penicillinase activity. Thus, susceptibility testing and penicillinase assay conditions were sufficiently disparate in design (as in aim) to make reasonable the finding that fewer of the 200 staphylococcal isolates tested were inhibited by even 0.001 μmole of the penicillins per ml than were found to be capable of inactivating these same penicillins. However, assay for penicillinase activity against benzyl-, phenoxymethyl- and dL-α-phenoxyethylpenicillin was carried out to determine whether or not there were differences among these penicillins in terms of susceptibility to staphylococcal penicillinase. Under the conditions of testing employed, there were no significant qualitative differences—results which are in agreement with those reported by McCarthy, Hirsch and Finland (5).

SUMMARY

On the basis of broth tube-dilution susceptibility testing with 200 recent clinical isolates of teallurite-positive staphylococci, the potency of d-, l- and dL-α-phenoxyethylpenicillins was compared with simultaneously and identically tested benzyl-, phenoxymethyl- and amincarboxybutylpenicillins.

Differences in antistaphylococcal potency were noted which were quantitative: all of the penicillins were more effective when applied in higher concentrations. A potency sequence of the penicillins tested was apparent:

\[
\text{phenoxymethyl-} \quad \text{L-α-phenoxyethyl-} \quad \text{DL-α-phenoxyethyl-} \quad > \text{benzyl-} > \text{D-α-phenoxyethyl-} > \text{aminocarboxybutylpenicillin}
\]

While both L- and DL-α-phenoxyethylpenicillin were superior to D-α-phenoxyethylpenicillin, there was no indication of augmented potency on the part of the DL-mixture in comparison with pure L-α-phenoxyethylpenicillin.

Although side chain optical configuration of α-phenoxyethylpenicillin had significant reflection in antistaphylococcal potency in vitro, the major therapeutic implication of this observation lies in the designing of new penicillins for terminal synthesis.

Alpha-phenoxyethylpenicillin, in any of its optical forms, was not a more potent inhibitor of staphylococci than was phenoxymethylpenicillin. Benzylpenicillin, phenoxymethylpenicillin and DL-α-phenoxyethylpenicillin did not differ qualitatively in susceptibility to inactivation by staphylococcal penicillinase.

Despite the presence of an asymmetric carbon atom in the aminoacyl side chain of amincarboxybutylpenicillin, the single enantiomorph studied was least effective of the penicillins tested as an inhibitor of staphylococci.

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REFERENCES


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