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Penicillin Therapy of Experimental Endocarditis Induced by Tolerant *Streptococcus sanguis* and Nontolerant *Streptococcus mitis*

FRANKLIN D. LOWY,* ELLEN G. NEUHAUS,† DANIEL S. CHANG, AND NEAL H. STEIGBIGEL

Division of Infectious Diseases, Department of Medicine, Montefiore Hospital and Medical Center, and Department of Medicine, The Albert Einstein College of Medicine, Bronx, New York 10467

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The response of tolerant *Streptococcus sanguis* and nontolerant *Streptococcus mitis* infections to penicillin therapy was compared in the rabbit model of endocarditis. The minimal inhibitory and bactericidal concentrations of penicillin were 0.1 and 0.1 μg/ml, respectively, for *S. mitis* and 0.05 and 6.2 μg/ml, respectively, for *S. sanguis*. Time-kill studies done in vitro with penicillin concentrations of 2 and 20 μg/ml demonstrated minimal killing of the tolerant strain, with a 3 log difference in survival between the two strains after 24 and 48 h. Both strains produced endocarditis with comparable bacterial densities on the valvular vegetations. Rabbits were treated with procaine penicillin G in two dosage regimens, 80,000 or 5,000 U/kg given every 8 h. There was no difference between bacterial densities in valvular vegetations removed from rabbits infected with either strain after 2, 4, or 6 days of treatment with the high-dose regimen (serum penicillin concentration at 0.5 h, 9.4 μg/ml), despite the fact that serum bactericidal activity against the tolerant strain at 0.5 h was minimal. With the low-dose penicillin regimen (serum concentration at 0.5 h, 2.5 μg/ml), therapy was significantly less effective in the tolerant group only after 6 days of treatment. Similar results were obtained when penicillin was administered in low and high doses to prevent infection. In this animal model of infection, penicillin tolerance was associated with a diminished response to penicillin therapy only when the dose was severely restricted. In the high-dose regimen, there was no difference in the response to penicillin therapy between animals infected with either strain, despite the presence of only minimal serum bactericidal activity in the rabbits infected with the tolerant strain.

The term penicillin tolerance, as described by Tomasz et al. (26), refers to bacterial strains inhibited by penicillin in vitro but not killed by the usual bactericidal concentrations. This resistance has been related to defects in the bacterial autolytic enzyme system (25, 26).

Several recent clinical reports attributed the failure of penicillin therapy in serious infections to the presence of tolerant bacteria; however, these retrospective analyses were uncontrolled, and the definition of tolerance used varied (17, 18, 22). Therefore, the clinical relevance of this in vitro phenomenon remains uncertain (12).

The purpose of this study was to compare the response to penicillin therapy of endocarditis in rabbits caused by a stable, well-characterized, tolerant bacterium with that caused by a similar, nontolerant organism. The strains used, a tolerant *Streptococcus sanguis* and a nontolerant *Streptococcus mitis*, are both viridans streptococci. The *S. sanguis* species is a common isolate in subacute bacterial endocarditis (14) and by in vitro testing appears to be naturally penicillin tolerant (9–11). Despite this, penicillin treatment of endocarditis caused by this particular type of viridans streptococcus is considered highly effective. The strain (Wicky) chosen for this study has been described in several previously reported in vitro studies on tolerance (9–11). Reports by Horne and Tomasz (11) indicated that of nine *S. sanguis* strains tested, all were tolerant to penicillin. A nontolerant *S. mitis* strain with a minimal inhibitory concentration (MIC) for penicillin similar to that of the Wicky strain of *S. sanguis* was therefore chosen for comparison. *S. mitis* is also often associated with bacterial endocarditis (14).

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† Present address: University of Connecticut Health Center, Farmington, CT 06032.

MATERIALS AND METHODS

Bacterial strains. *S. sanguis* Wicky (kindly provided by A. Tomasz, Rockefeller University, New York, N.Y.) has been well characterized as a tolerant strain in previous in vitro studies (9–11). Because attempts to identify a nontolerant strain of *S. sanguis* were unsuccessful, a closely related *S. mitis* strain was selected. The *S. mitis* strain used was a blood culture isolate obtained from a patient at Montefiore Hospital. Stock cultures of the two strains were prepared by freezing (−70°C) samples consisting of 0.5 ml of overnight growth of the organism in Todd-Hewitt broth (THB) and 0.5 ml of defibrinated sheep blood.

Antibiotic susceptibility tests. Broth dilution susceptibility testing was performed in duplicate in THB. An average initial bacterial inoculum of 5×10^5 colony-forming units (CFU)/ml, representing a 1:100 dilution of an overnight culture, was used. The MIC was interpreted by visual inspection after 24 h of incubation at 37°C. A 0.1-ml sample from each tube was subcultured onto sheep blood agar plates and incubated for an additional 48 h for measurement of the minimal bactericidal concentration (MBC). The MBC was recorded as that antibiotic concentration which resulted in 99.9% bacterial killing. MIC and MBC determinations were repeated on streptococcal isolates cultured from rabbit valvular vegetations after penicillin therapy for experimental endocarditis. Susceptibility testing was also performed as described above, with pooled, heat-inactivated rabbit serum rather than THB.

Time-kill studies. Time-kill studies were performed at 37°C with either a 1:10 or 1:200 dilution of an overnight growth of the *S. sanguis* or *S. mitis* strain in THB. The bacterial suspension was preincubated for 1.5 h before the penicillin was added (at a concentration of 2 or 20 μg/ml) to assure log-phase growth. Portions were removed for colony counting at 0, 1, 2, 4, 6, 24, and 48 h and treated with *Bacillus cereus* beta-lactamase to minimize carry-over of penicillin activity (21). Serial 10-fold dilutions were plated in duplicate into Todd-Hewitt agar. The plates were incubated for 48 h at 37°C, and then the number of colonies was counted. The results represent the arithmetic mean of two separate studies.

Because of disparities observed between the MIC and MBC results and the time-kill studies, quantitative colony counts were performed on the clear tubes used in the broth dilution susceptibility studies that showed no growth after 24 h. Portions were removed from the clear tubes, serially diluted in saline, and plated in Todd-Hewitt agar containing beta-lactamase. The colonies were counted after 48 h of incubation at 37°C. These determinations were then compared with the data from the time-kill studies.

Rabbit model of endocarditis. Aortic valvular endocarditis was produced in male New Zealand rabbits (Penn Dutch Laboratory Animals, Denver, Pa.) by a modification of the method described by Perlman and Freedman (5, 15). At 48 h after surgery the rabbits were inoculated with appropriate numbers of the test organisms via the marginal ear vein. Therapy was begun 72 h later. In all, 147 rabbits were inoculated in groups of 6 to 10 at each of the 16 sessions. In most sessions, equal numbers of rabbits were infected with the tolerant and the nontolerant strain. Rabbits were assigned randomly to treatment or control groups.

Endocarditis was documented by the presence of positive blood cultures before therapy was instituted and verified by the presence of vegetations at autopsy. Quantitative blood cultures were obtained from groups of infected rabbits on the day after infection and during day 1 or 2 of therapy. Venous blood (1 ml) was collected in heparinized tubes and inoculated into Todd-Hewitt agar. The number of bacterial colonies was counted after 48 h of incubation at 37°C. At the conclusion of therapy, cardiac valvular vegetations were removed from the sacrificed rabbits 12 h after the 6th, 12th, or 18th dose. The vegetations were excised, weighed, homogenized in 1.0 ml of sterile saline, and plated in duplicate into Todd-Hewitt agar plates. Bacterial densities in valvular vegetations that were calculated to be between 0 and $\log_{10}$ 2 CFU/g were reported as $\log_{10}$ 2 CFU/g rather than as 0 because of potential errors associated with the low weight of the valvular tissue (4).

Penicillin serum levels. Serum antibiotic levels were obtained 0.5, 1, 3, 5, and 7 h after intramuscular injection and measured by a microbiological disk assay method with *Sarcina lutea* (21). The highest penicillin serum levels were noted at 0.5 h. Standard solutions of penicillin were prepared in rabbit serum for controls. The results represent the arithmetic mean of at least 10 determinations per group.

Serum bactericidal activity. Serum bactericidal levels were measured in the high-dose penicillin treatment groups. Rabbit serum was obtained 0.5 h after the intramuscular injection of penicillin on day 1 or 2 of therapy. The assays were performed as previously described (1). Serial twofold dilutions of 0.5-ml samples of serum were made in THB. The *S. sanguis* strain was used to measure bactericidal activity in serum obtained from rabbits infected with *S. sanguis*, and the *S. mitis* strain was used for the rabbits infected with *S. mitis*. The bactericidal level was determined after 0.1-ml samples from each tube were streaked onto a sheep blood agar plate and incubated at 37°C for 48 h.

Penicillin therapy experiments. Rabbits infected with either the *S. sanguis* or *S. mitis* strain were treated with penicillin for 2, 4, or 6 days. Both groups of infected rabbits were represented in each experiment. The animals were treated with procaine penicillin G (provided by Wyeth Laboratories, Philadelphia, Pa., and Eli Lilly & Co., Indianapolis, Ind.) administered intramuscularly in a dosage of 5,000 or 80,000 U/kg every 8 h. The low-dose regimen was selected to achieve serum penicillin concentrations above the MIC but below the MBC of the tolerant *S. sanguis* strain. The high-dose regimen produced penicillin levels comparable to those commonly achieved in patients.

An additional experiment with a total of 22 rabbits was performed to determine whether a higher proportion of rabbits infected with one of the two bacterial species had positive valve cultures after 6 days of high-dose procaine penicillin G treatment followed by an additional 5 days without therapy. The vegetations were excised and cultured as described above. Any rabbit that died during the 5 days after treatment was stopped was also evaluated for evidence of positive cardiac valve cultures.

Penicillin prophylaxis experiments. Prophylaxis ex-

TABLE 1. Serum and broth dilution susceptibilities of two strains of viridans streptococci to penicillin G

Strain	Medium[a]	MIC (μg/ml)	MBC (μg/ml)	MBC/MIC ratio
S. sanguis	THB	0.05	6.2	128
	Serum	0.1	12.5	128
S. mitis	THB	0.1	0.1	1
	Serum	0.1	0.4	4

[a] Serum, pooled, heat-inactivated rabbit serum.

periments were performed 48 h after surgery for placement of the catheter. Rabbits were given a single intramusclar injection of procaine penicillin G at a dose of either 5,000 or 80,000 U/kg 0.5 h before intravenous injection of either *S. sanguis* or *S. mitis*. Twenty-five rabbits were sacrificed 24 h later, and their cardiac valvular vegetations were excised and processed as described above. Two rabbits were excluded from the evaluation because of improper catheter placement.

Statistical analysis. The Student *t* test was used to determine whether the difference in bacterial densities of valvular vegetations from the two groups were statistically significant. Results were expressed as the mean ± the standard deviation.

RESULTS

Antibiotic susceptibility tests. The MIC and MBC data (Table 1) showed comparable MICs in both broth and serum for the two streptococcal strains but a wide disparity in their respective MBCs. The tolerant *S. sanguis* strain showed a 128-fold difference between the broth MIC and MBC, whereas the comparable value for *S. mitis* was 1.

Time-kill studies. Two initial bacterial inocula were used. The first was the standard concentration of 5×10^5 CFU/ml, and the second was higher, 10^7 CFU/ml, to more closely approximate the bacterial density found in cardiac valvular tissue. At the standard inoculum, there was minimal killing (<1 log) over 24 h of the tolerant *S. sanguis* strain at a penicillin concentration of either 2 or 20 μg/ml (Fig. 1). There was substantial killing of the nontolerant *S. mitis* strain, resulting in a greater than 3 log difference in survival between the two strains at both 24 and 48 h. Results were similar with the higher inoculum.

The MBC results suggested greater killing by a given concentration of penicillin (6.2 μg/ml) than did the killing curve results (<1 log killing with 20 μg/ml). This disparity was investigated by performing quantitative colony counts on samples removed from clear tubes after 24 h of incubation during the broth dilution susceptibility studies. Colony counts for *S. sanguis* were 2.9×10^4 CFU/ml in tubes containing 6.25 μg of penicillin per ml when the MBC by the standard tube dilution test method was 6.25 μg/ml. *S. mitis* had counts of 1.7×10^2 CFU/ml in the tube containing 0.05 μg of penicillin per ml when the MBC was 0.05 μg/ml. These studies showed that the two techniques provide different data for

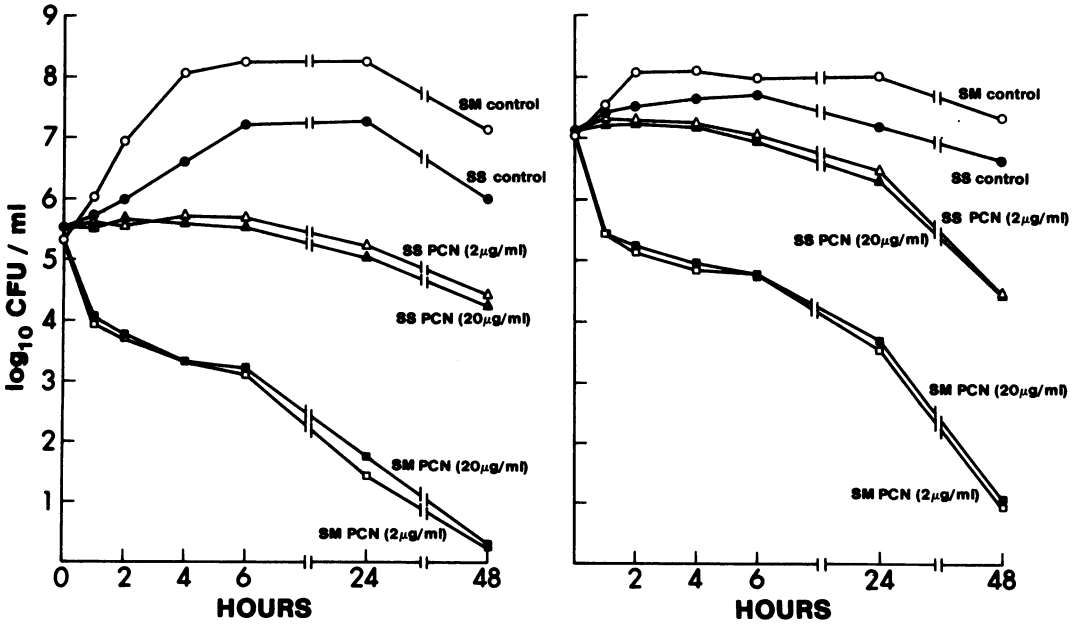

FIG. 1. Time-kill studies comparing the response to penicillin G of the tolerant *S. sanguis* (SS) strain with the nontolerant *S. mitis* (SM) strain, starting with an initial bacterial inocula of 5×10^5 (left) and 10^7 CFU/ml (right). Penicillin (PCN) was used at two concentrations, 2 and 20 μg/ml.

TABLE 2. Bacterial densities in valvular vegetations removed from rabbits treated with procaine penicillin for viridans streptococcal endocarditis

Penicillin regimen[a] (U/kg)	Day of therapy	*S. sanguis* group		*S. mitis* group	
		Log_{10} CFU/g ± SD[b]	No. of sterile vegetations/total	Log_{10} CFU/g ± SD[b]	No. of sterile vegetations/total
5,000	0	9.8 ± 0.5	0/10	9.5 ± 0.4	0/10
	2	8.6 ± 0.8	0/6	7.5 ± 1.1	0/7
	4	7.4 ± 2.4	0/7	6.4 ± 1.7	0/8
	6	8.1 ± 1.4	0/8	4.8 ± 1.2	2/7
80,000	0	9.8 ± 0.5	0/10	9.5 ± 0.4	0/10
	2	5.7 ± 1.0	0/7	6.0 ± 1.6	0/7
	6	3.7 ± 1.0	6/9	3.9 ± 1.0	3/8

[a] Each dose was administered three times daily.
[b] Mean bacterial density of the culture-positive valvular vegetations.

bacterial survival. The differences probably result from continued killing on the agar plates used with the tube dilution studies caused by substantial antibiotic carry-over from the tubes. In contrast, carry-over did not occur with the diluted and beta-lactamase-treated samples used in the time-kill studies.

Rabbit model of endocarditis. Ninety-five percent of the animals inoculated with either strain developed endocarditis. The average intravenous bacterial inoculum (± standard deviation) used to produce endocarditis with the *S. sanguis* strain was 1.7 (± 1.3) × 10^8 CFU/ml, compared with 1.3 (± 1.0) × 10^8 CFU/ml for the *S. mitis* strain ($P > 0.30$). The level of bacteremia for the two strains 1 day after infection was 66.8 ± 204 CFU/ml for *S. sanguis* and 176.6 ± 292 CFU/ml for *S. mitis* ($P < 0.05$). There were no differences between the two groups in the frequency of positive blood cultures among those taken 1 day after institution of therapy. Bacterial densities in valvular vegetations removed from both control groups of untreated rabbits 72 h after infection were comparable (Table 2). Infected rabbits left untreated died within 3 weeks (mean, 15.2 versus 8.7 days for the *S. sanguis* and *S. mitis* groups, respectively). There was a higher mortality in the nontolerant *S. mitis* group (19 of 82, versus 5 of 65 rabbits for the *S. sanguis* group) in the 72 h before the institution of therapy; however, bacterial densities in vegetations removed from these groups were comparable to the controls sacrificed 72 h after infection.

Penicillin serum levels. Serum penicillin concentrations for the high-dose regimen (80,000 U/kg) were 9.4 ± 6.3 and 2.5 ± 1.5 μg/ml at 0.5 and 7 h, respectively, after intramuscular injection. The 0.5- and 7-h levels for the low-dose regimen (5,000 U/kg) were 1.3 ± 0.4 and <0.1 μg/ml, respectively. There were no differences in penicillin levels between the groups of rabbits infected with each strain.

Serum bactericidal activity. Serum bacteriostatic and bactericidal levels obtained with the high-dose regimen 0.5 h after intramuscular penicillin injection showed comparable bacteriostatic levels for both strains but a marked difference in bactericidal activity (Table 3). Only minimal serum bactericidal activity was demonstrated against the tolerant strain. The ratio of serum bactericidal to bacteriostatic levels was comparable to the MBC/MIC ratios for the two strains.

Penicillin therapy experiments. In the low-dose treatment group there was no difference in results for the tolerant and nontolerant strains after 2 or 4 days of penicillin therapy; however, there was a difference after 6 days (Table 2). At that time, the bacterial densities in vegetations obtained from rabbits infected with the tolerant *S. sanguis* strain were significantly greater than those obtained from the nontolerant *S. mitis* strain ($P < 0.001$). There were no differences

TABLE 3. Serum bacteriostatic and bactericidal levels in rabbits treated for viridans streptococcal endocarditis with the high-dose penicillin regimen[a]

Strain	Median bacteriostatic levels (range)[b]	Median bactericidal levels (range)[b]	Median ratio (bactericidal/bacteriostatic)
S. sanguis	1/128 (1/8–1/256)	<1/2 (<1/2–1/16)	≥128
S. mitis	1/64 (1/64–1/128)	1/64 (<1/2–1/128)	1

[a] The high-dose regimen was 80,000 U/kg administered intramuscularly three times daily.
[b] The results represent eight determinations per group. The levels were obtained 0.5 h after the penicillin injection.

TABLE 4. Bacterial densities in valvular vegetations removed from rabbits given an intramuscular injection of procaine penicillin 0.5 h before the intravenous injection of either the *S. sanguis* or the *S. mitis* strain

Penicillin regimen (U/kg)	Strain inoculated	Average inoculum (log_{10} CFU/ml ± SD)	Bacterial density (log_{10} CFU/g ± SD)[a]	No. of sterile vegetations/total
5,000	*S. sanguis*	8.1 ± 0.3	7.9 ± 0.9	0/6
	S. mitis	7.9 ± 0.2	5.9 ± 1.0	1/6
80,000	*S. sanguis*	7.6 ± 1.2	3.5 ± .1	4/6
	S. mitis	7.8 ± 0.3	2.6 ± 0	6/7

[a] Mean bacterial density of the culture-positive valvular vegetations.

between the two groups when the high-dose penicillin regimen was used.

The results of broth dilution susceptibility studies performed on *S. sanguis* and *S. mitis* isolates (seven determinations per strain) obtained from valvular vegetations after penicillin therapy were unchanged.

An additional group of animals was treated with the high-dose regimen for 6 days and then observed for an additional 5 days to determine whether there was any difference in the proportion of positive valve cultures between rabbits infected with either strain. There were two of seven rabbits with positive cultures in the *S. sanguis* group (mean for the positive valve cultures, log_{10} 6.4 CFU/g), and one of eight was positive in the *S. mitis* group (log_{10} 2.6 CFU/g).

Penicillin prophylaxis experiments. A significant difference ($P < 0.05$) in valvular bacterial densities between the groups infected by the two strains was demonstrable only with the low-dose regimen (Table 4). There were no significant differences among groups which received the high-dose penicillin prophylaxis. The latter regimen was more effective in protecting the valves from seeding by either strain. There were no differences between the two groups in the number of sterile vegetations.

DISCUSSION

These results showed that penicillin therapy was less effective against the tolerant than the nontolerant streptococcus in the rabbit model of endocarditis only when the dose was severely limited. At the higher dose of penicillin, no differences were demonstrable in the prophylaxis or therapy studies despite the poor bactericidal activity obtained both in vivo and in vitro against the *S. sanguis* strain.

Ideally, two *S. sanguis* strains would have been compared, a tolerant versus a nontolerant strain. Attempts in our laboratory to identify a nontolerant *S. sanguis* strain were unsuccessful. Reports by Horne and Tomasz (11) indicated that of nine strains tested, all were tolerant to penicillin. The *S. mitis* strain chosen was similar to the *S. sanguis* strain not only in the degree of susceptibility to growth inhibition by penicillin but also in biochemical characteristics (differing in fermentation of raffinose and arginine) (8). In fact, it has been suggested, on the basis of these similarities and on DNA base sequence studies, that the *S. mitis* species be included in the type B *S. sanguis* biotype (8). Clinically, both strains produce comparable forms of endocarditis, with a similar incidence of complications and response to antibiotic therapy (14).

In the present study both organisms consistently produced ultimately fatal endocarditis with similar initial cardiac valvular bacterial densities in rabbits. However, *S. mitis* produced a higher initial mortality and a higher level of bacteremia. Despite this suggestion of greater virulence for the *S. mitis* strain, it was more effectively treated by the low dose of penicillin.

The response to penicillin for both strains was consistent in the in vitro time-kill studies and in the serum bactericidal studies performed on samples taken from rabbits being treated for endocarditis at a time when the serum penicillin levels averaged 9.4 μg/ml. The nontolerant *S. mitis* strain was readily killed by penicillin concentrations of 2 μg/ml in vitro, and median serum bactericidal levels against it were 1/64. In contrast, the tolerant *S. sanguis* strain showed little killing by penicillin concentrations of 20 μg/ml in vitro, and minimal serum bactericidal activity (<1/2) was developed against it. In addition, there was no change in MIC and MBC results in bacterial isolates obtained from vegetations of penicillin-treated animals.

S. sanguis appears to be a naturally occurring tolerant species (9–11). Horne and Tomasz (11) studied nine strains of *S. sanguis*, including five obtained from patients with endocarditis, and found that all were penicillin tolerant in vitro. These strains were resistant to the bactericidal activity of penicillin unless they were first exposed to exogenous autolysin (11). It is of interest that *S. sanguis* is among the most common isolates from patients with viridans streptococcal endocarditis, an infection in which the use of bactericidal antibiotics such as penicillin in therapy generally achieves cure (14, 24).

Most clinical reports of infections that are

poorly responsive to penicillin therapy and caused by tolerant bacteria have been those due to staphylococci, although group C and nonenterococcal group D streptococci have also been cited (17, 18, 22, 23). Such studies are difficult to interpret because they involve small numbers of retrospectively analyzed clinical cases in which therapy as well as definitions of tolerance have varied. In only one study was tolerance correlated with a decrease in autolytic activity in the isolates (22). In addition, penicillin tolerance in staphylococci is manifested differently than it is in *S. sanguis*. Results for staphylococci are not reliably reproducible in vitro, and the tolerance mechanism is less clearly understood (2, 3, 13, 16, 19). These problems have all contributed to the confusion about the clinical importance of tolerance (12, 20).

Goldman and Petersdorf (6) compared the response to methicillin therapy of single strains of tolerant and nontolerant *Staphylococcus aureus* in the rabbit endocarditis model. No differences between the two strains were found in this model in either response to treatment with or prevention of an experimental infection by pharmacological doses of methicillin. Similar results were reported by Guze et al. (7), who compared the response of tolerant and nontolerant staphylococci to methicillin therapy in a rat model of hematogenous pyelonephritis. In neither study was tolerance documented with serum bactericidal titers. These studies are difficult to interpret because of the inconsistency of tolerance often shown by staphylococci in vitro (2, 13, 16).

The present study investigated tolerance by using a well-characterized, stable, tolerant viridans streptococcus and compared the results with those for a similar nontolerant organism. Our data indicated that there were no differences in the response to penicillin therapy for experimental endocarditis between the two groups with the high-dose penicillin regimen, despite the differences in serum bactericidal activity. In the studies investigating the frequency of bacteriological relapse, it was of interest that the bacterial densities in the *S. sanguis* group were higher in the few rabbits with positive cultures. However, the overall results of our studies suggest the possibility that factors other than bactericidal antibiotic activity may have an important role in eradicating infection in this animal model. The nonlethal activity of penicillin may have an important effect on bacteria-host tissue interactions (11). In addition, response to therapy may occur as a result of the slow, gradual bacterial killing by penicillin per se over a prolonged time period that may occur even with tolerant bacteria.

Although it is unlikely that a clinical situation would arise in which penicillin would now be used in a dosage comparable to that used for the low-dose group in the present treatment study, the low-dose situation might arise during antibiotic prophylaxis for endocarditis before dental procedures are performed. In this setting a tolerant *S. sanguis* strain might be more capable of colonizing a valvular vegetation and producing endocarditis than a comparable nontolerant strain. This possibility is suggested by the study on prophylaxis with the low dose of penicillin reported here.

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LITERATURE CITED

1. **Barry, A. L., and L. D. Sabath.** 1979. Special tests: bactericidal activity and activity of antimicrobics in combination, p. 431–435. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
2. **Bradley, H. E., J. G. Wetmur, and D. S. Hodes.** 1980. Tolerance in *Staphylococcus aureus*: evidence for bacteriophage role. J. Infect. Dis. **141**:233–237.
3. **Bradley, J. J., C. G. Mayhall, and H. P. Dalton.** 1978. Incidence and characteristics of antibiotic-tolerant strains of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **13**:1052–1057.
4. **Carrizosa, J., W. D. Kobasa, and D. Kaye.** 1979. Effectiveness of nafcillin, methicillin, and cephalothin in experimental *Staphylococcus aureus* endocarditis. Antimicrob. Agents Chemother. **15**:735–737.
5. **Durack, D. T., and P. B. Beeson.** 1972. Experimental bacterial endocarditis. I. Colonization of a sterile vegetation. Br. J. Exp. Pathol. **53**:44–49.
6. **Goldman, P. L., and R. G. Petersdorf.** 1979. Significance of methicillin tolerance in experimental staphylococcal endocarditis. Antimicrob. Agents Chemother. **15**:802–806.
7. **Guze, P. A., G. M. Kalmanson, and L. G. Guze.** 1982. The role of antibiotic tolerance in the response to treatment of pyelonephritis due to *Staphylococcus aureus* in rats. J. Infect. Dis. **145**:169–173.
8. **Hamada, S., and H. D. Slade.** 1980. Biology, immunology, and cariogenicity of *Streptococcus mutans*. Microbiol. Rev. **44**:331–384.
9. **Horne, D., and A. Tomasz.** 1977. Tolerant response of *Streptococcus sanguis* to beta-lactams and other cell wall inhibitors. Antimicrob. Agents Chemother. **11**:888–896.
10. **Horne, D., and A. Tomasz.** 1979. Release of lipoteichoic acid from *Streptococcus sanguis*: stimulation of release during penicillin treatment. J. Bacteriol. **137**:1180–1184.
11. **Horne, D., and A. Tomasz.** 1980. Lethal effect of a heterologous murein hydrolase on penicillin-treated *Streptococcus sanguis*. Antimicrob. Agents Chemother. **17**:235–246.
12. **Kaye, D.** 1980. The clinical significance of tolerance in *Staphylococcus aureus*. Ann. Intern. Med. **93**:924–926.
13. **Mayhall, C. G., and E. Apollo.** 1980. Effect of storage and changes in bacterial growth phase antibiotic concentration on antimicrobial tolerance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **18**:784–788.
14. **Murray, H. W., K. C. Gross, H. Masur, and R. B. Roberts.** 1978. Serious infections caused by *Streptococcus*

milleri. Am. J. Med. 64:759–764.
15. **Perlman, B. B., and L. R. Freedman.** 1971. Experimental endocarditis. II. Staphylococcal infection of the aortic valve following placement of a polyethylene catheter in the left side of the heart. Yale J. Biol. Med. 44:206–213.
16. **Peterson, L. R., D. N. Gerding, W. H. Hall, and E. A. Schierl.** 1978. Medium-dependent variation in bactericidal activity of antibiotics against susceptible *Staphylococcus aureus*. Antimicrob. Agents Chemother. 13:665–668.
17. **Portnoy, D., I. Wink, G. K. Richard, and M. Z. Blanc.** 1980. Bacterial endocarditis due to a penicillin-tolerant group C streptococcus. Can. Med. Assoc. J. 122:69–75.
18. **Rajasheshekaraiah, K. R., T. Rice, V. S. Rao, D. Marsh, B. Ramakrishna, and C. A. Kallnick.** 1980. Clinical significance of tolerant strains of *Staphylococcus aureus* in patients with endocarditis. Ann. Intern. Med. 93:796–801.
19. **Raynor, R. H., D. F. Scott, and G. K. Best.** 1979. Oxacillin-induced lysis of *Staphylococcus aureus*. Antimicrob. Agents Chemother. 16:134–140.
20. **Reyman, M. T., H. P. Holley, Jr., and C. G. Cobbs.** 1978. Persistent bacteremia in staphylococcal endocarditis. Am. J. Med. 65:729–737.
21. **Sabath, L. D., J. I. Casey, P. A. Ruch, L. L. Stumpf, and M. Finland.** 1971. Rapid microassay of gentamicin, kanamycin, neomycin, streptomycin and vancomycin in serum or plasma. J. Lab. Clin. Med. 78:457–463.
22. **Sabath, L. D., N. Wheeler, M. Laverdiere, D. Blazevic, and B. J. Wilkinson.** 1977. A new type of penicillin resistance of *Staphylococcus aureus*. Lancet i:443–447.
23. **Savitch, C. D., A. L. Barry, and P. D. Hoeprich.** 1978. Infective endocarditis caused by *Streptococcus bovis* resistant to the lethal effect of penicillin. Arch. Intern. Med. 138:931–934.
24. **Scheld, W. M., and M. A. Sande.** 1979. Endocarditis and intravascular infections, p. 653–690. *In* G. L. Mandell, R. G. Douglas, Jr., and J. E. Bennett (ed.), Principle and practice of infectious diseases. John Wiley & Sons, New York.
25. **Tomasz, A.** 1979. From penicillin-binding proteins to the lysis and death of bacteria: a 1979 view. Rev. Infect. Dis. 1:434–467.
26. **Tomasz, A., A. Albino, and E. Zanati.** 1970. Multiple antibiotic resistance in a bacterium with suppressed autolytic system. Nature (London) 227:138–140.