ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1991, p. 1089–1092
0066-4804/91/061089-04$02.00/0

Vol. 35, No. 6

Comparative Effect of Protein Binding on the Killing Activities of Teicoplanin and Vancomycin

ELAINE M. BAILEY,[1]* MICHAEL J. RYBAK,[1] AND GLENN W. KAATZ[2]

Antiinfective Research Laboratory, Department of Pharmacy Services, Detroit Receiving Hospital/University Health Center, and College of Pharmacy and Allied Health Professions[1] and Department of Internal Medicine, Division of Infectious Diseases, School of Medicine,[2] Wayne State University, Detroit, Michigan 48201

Received 17 September 1990/Accepted 1 April 1991

The effect of protein binding on the activity of teicoplanin against *Staphylococcus aureus* was evaluated. Bactericidal rates of teicoplanin in cation-supplemented Mueller-Hinton broth (SMHB) and in a 1:1 mixture of pooled human serum and cation-supplemented Mueller-Hinton broth (PHS-SMHB) were compared with those of vancomycin. Eight concentrations of each drug ranging from 15 to 150 μg/ml were studied in two series which correspond to the concentrations in serum achieved with low- (6 mg/kg of body weight once daily) and high-dose (30 mg/kg once daily) teicoplanin. Overall, the bactericidal rate of teicoplanin was lower than that of vancomycin. In the presence of serum, the bactericidal rate of teicoplanin in PHS-SMHB was lower than that in SMHB, often resulting in only one $\log_{10}$ drop in CFU over a 24-h period. There was no statistical difference in the bactericidal rates of high- and low-concentration teicoplanin in either medium. Additionally, concentration-dependent killing in SMHB was not evident with either agent. The bactericidal rates of teicoplanin and vancomycin in a 1:1 mixture of serum ultrafiltrate and SMHB at 60 μg/ml were also studied. It was noted that the bactericidal rate of neither agent was affected by the presence of serum ultrafiltrate. This finding is consistent with teicoplanin's high degree of protein binding (reported to be >90% in undiluted serum) and further substantiates the hypothesis that only the free drug is active against microorganisms. These data support protein binding as being a factor in teicoplanin activity against *S. aureus*.

Teicoplanin is an investigational glycopeptide antibiotic with a spectrum of activity similar to that of vancomycin (1, 10, 25). Its mechanism of action is also similar to that of vancomycin in that it inhibits the polymerization of peptidoglycan by complexing with the terminal D-alanyl-D-alanine precursor (19). Vancomycin also injures protoplasts by altering the permeability of the cytoplasmic membrane and impairing RNA synthesis (8). This additional mechanism of action has not been described with teicoplanin.

Early clinical trials with low-dose teicoplanin (6 mg/kg of body weight) reported high failure rates in the treatment of endocarditis (17). Lagast et al. (15) reported a lower killing rate of *Staphylococcus aureus* by teicoplanin compared with that of vancomycin in an in vitro study which utilized serum samples obtained from volunteers who had received a single dose of either teicoplanin or vancomycin. Chambers and Kennedy (3) also noted inferior results with teicoplanin in the treatment of experimental endocarditis and have suggested that trough concentrations be maintained at 10 times the MIC. There is speculation that besides the inherent differences in the two agents' mechanisms of action, the high degree of serum protein binding may account for some of the lower bactericidal activity of teicoplanin (2).

The effect of protein binding on the bactericidal activity of antimicrobial agents has been a source of controversy since 1947 (5, 6, 16, 18, 22, 24, 26). It is hypothesized that only the free, unbound fraction of the antibiotic is available for interaction with the microorganism. In the treatment of deep-seated infections, protein binding may interfere with the drug's ability to penetrate to the site of the infection (26). Alternatively, protein binding may affect the pharmacokinetics (e.g., increased half-life) of the antibiotic by inhibiting its renal elimination (9).

Several reports (3, 11) indicate that the presence of serum may decrease the antibacterial activity of teicoplanin. Results obtained in our laboratory utilizing selected clinical isolates of *S. aureus* have shown that despite the similar MICs of teicoplanin and vancomycin in broth, the serum bactericidal titers against these strains were markedly different with the two drugs at concentrations of ≤30 μg/ml (unpublished data). Since we are now investigating high-dose teicoplanin (i.e., 30 mg/kg once daily) in the treatment of *S. aureus* endocarditis at our institution, one objective of our study was to compare the bactericidal rates (BRs) of teicoplanin in serum at concentrations resulting from this high-dose regimen with those of vancomycin. We also determined the BRs of teicoplanin and vancomycin in the presence and absence of serum to assess the effect of protein binding and to ascertain whether there was a concentration of teicoplanin at which the BR in serum is similar to that seen in broth.

MATERIALS AND METHODS

Drugs. Teicoplanin susceptibility-grade powder (batch 046/3) was supplied by Merrell-Dow Research Institute, Cincinnati, Ohio. Vancomycin susceptibility-grade powder (lot SI-657-8R) was supplied by Eli Lilly & Co., Indianapolis, Ind. Stock solutions were prepared in appropriate amounts of sterile, distilled, and deionized water and then were further diluted in the appropriate medium. We utilized pharmacokinetic data obtained from patients receiving teicoplanin at our institution to simulate achievable drug concentrations in serum which would be seen in patients receiving high- (30 mg/kg once daily) and low-dose (6 mg/kg once daily) teicoplanin (20). Two series of drug concentrations

* Corresponding author.

were studied: 15, 30, 45, and 60 μg/ml (low dose concentration [LDC]) and 75, 85, 100, and 150 μg/ml (high dose concentration [HDC]).

Media. Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) was supplemented with calcium (50 mg/liter) and magnesium (25 mg/liter) (SMHB), and the pH was adjusted to 7.4. Serum samples obtained from 23 healthy volunteers were pooled, divided into 25-ml aliquots, and frozen at −20°C until used. The serum was not heat inactivated. A 1:1 mixture of the pooled human serum (PHS) and SMHB (PHS-SMHB) was prepared, and the pH was adjusted to 7.4.

Bacterial strains. Two clinical isolates of *S. aureus* were obtained from patients being treated for endocarditis at Detroit Receiving Hospital. One strain (MSSA-380) was methicillin sensitive, and one (MRSA-67) was methicillin resistant. MICs and MBCs were determined in SMHB and PHS-SMHB by using a broth microdilution technique (12).

Protein binding. The protein binding of teicoplanin and vancomycin in PHS-SMHB was measured with an Amicon MPS-1 unit with YMT membranes (Amicon Corp., Danvers, Mass.). Teicoplanin and vancomycin were accurately weighed and added to PHS-SMHB. Approximately 1 ml was placed in ultrafiltration units which were then centrifuged for 20 min at 1,000 × *g* (fixed angle) at 37°C, and the ultrafiltrate (UF) was then collected. Previous experimentation in our laboratory has indicated minimal binding of either agent to the membrane (14). The concentration of antibiotic in PHS-SMHB prior to centrifugation (i.e., total) was compared with that in the UF (i.e., free). The free fraction was expressed as the ratio of antibiotic concentration in the UF to that in PHS-SMHB multiplied by 100.

Antibiotic assay. Concentrations of vancomycin in UF were determined by fluorescence polarization immunoassay (21). Antibiotic standards in human serum were obtained from a commercial manufacturer (Abbott Laboratories). We added known quantities of vancomycin to antibiotic-free UF to ascertain that it could be quantified in this medium by fluorescence polarization immunoassay. Multiple replicates indicated greater than 90% recovery rate for all samples. All assays were performed in triplicate. The lower limit of quantitation was 0.6 μg/ml, and the coefficient of variation was less than 5%.

Teicoplanin in UF was quantified by microbiological assay using *Bacillus subtilis* ATCC 6633 as the indicator organism (7). Antibiotic standards were prepared in human serum. The lower limit of detectability was 0.19 μg/ml, and the coefficient of variation was less than 10%.

Rate of killing. The BRs of teicoplanin and vancomycin at eight concentrations were studied in both media against MRSA-67 and MSSA-380. The test strains were grown overnight at 37°C in SMHB and serially diluted to yield an initial inoculum (mean ± standard deviation) of $\log_{10}$ 6.30 ± 0.31 CFU/ml. Sufficient stock antibiotic solution was added to achieve the desired concentration in a final volume of 10 ml. Controls were prepared in a similar fashion with substitution of the appropriate medium in place of the stock antibiotic solution. All tubes were incubated at 37°C with constant rotation for a 24-h period. At 0, 2, 4, 6, 8, 12, and 24 h, duplicate samples (0.1 ml each) were removed, diluted at least 250-fold in 0.15 M NaCl to reduce antibiotic carryover, and then plated on Trypticase soy agar (Difco). Colony counts were determined after incubation for 18 to 24 h. The lowest reliably detectable viable cell count was 30 CFU/ml by this method. All time-kill curves were performed in duplicate. A similar methodology was followed for the determination of killing rate in an UF of the pooled serum. The killing activities of vancomycin and teicoplanin, each at a concentration of 60 μg/ml, in SMHB and UF-SMHB, were compared.

Statistics. For each sample tested, the $\log_{10}$ CFU of viable bacteria per milliliter was plotted versus time of exposure to drug. Multiple linear regression analysis was used to determine the line of best fit between 2 and 12 h. The BRs ($-\log_{10}$ CFU per milliliter per hour) of teicoplanin and vancomycin were defined as the slope of the regression line. The mean BRs for LDC and HDC were calculated. The effects of drug, concentration, and medium on BR were compared by three-way analysis of variance with Tukey's test for multiple comparisons. The relationship of concentration of the respective antibiotic to BR in SMHB was evaluated by multiple linear regression. The effect of UF-SMHB versus SMHB on the BR was tested by a Student's *t* test. A *P* value of <0.05 was considered significant in all instances.

RESULTS

The MIC of vancomycin in SMHB was 0.4 μg/ml for both strains, and the MBC was 0.5 to 0.6 μg/ml. In the presence of 50% serum, the MIC of vancomycin was 0.8 μg/ml and the MBC was 1.6 μg/ml for both strains. For teicoplanin, the MIC and MBC in SMHB were 0.4 and 0.6 μg/ml, respectively, for both strains. In the presence of 50% serum, the MIC rose 2- to 4-fold to 0.8 μg/ml and the MBC rose 5- to 10-fold to 3 to 4 μg/ml.

Protein binding of teicoplanin at 60 and 150 μg/ml in the PHS-SMHB mixture was 83.5 and 80%, respectively. Protein binding of vancomycin at 60 μg/ml in this medium was 45.3%.

Representative time-kill curves with teicoplanin and vancomycin at 45 μg/ml in the presence and absence of serum are shown in Fig. 1. The mean coefficient of determination of the calculated slopes of the BR for all time-kill curves was 0.92 ± 0.13. Since there were no apparent differences noted in the terminal CFU titers between the two strains for the respective drugs or a statistical difference in the BRs, the data collected by evaluation of time-kill curves for both MRSA-67 and MSSA-380 were combined for purposes of statistical evaluation (Table 1). Growth of control organisms (unexposed to antibiotic) was not affected by the presence of serum. The killing activity of vancomycin was superior to that of teicoplanin at all concentrations, regardless of the medium used. The BR of teicoplanin in PHS-SMHB was lower than the corresponding BR in SMHB for both LDC and HDC. There was no statistical difference between the BRs of HDC and LDC teicoplanin in either medium. At 24 h, there was no visible growth on any plates in the vancomycin group regardless of the medium used, whereas for teicoplanin the final values ($\log_{10}$ CFU per milliliter [mean ± standard deviation]) were 3.26 ± 2.69 in SMHB and 3.92 ± 1.15 in PHS-SMHB. Regrowth was not seen at any time during the entire experiment for either agent. The BR of HDC vancomycin in PHS-SMHB was significantly higher than that of LDC vancomycin in SMHB (-0.4052 h^{-1} in PHS-SMHB versus -0.3247 h^{-1} in SMHB). The BR of teicoplanin at 60 μg/ml in SMHB was not different from that in UF-SMHB (-0.233 versus -0.213 h^{-1}). Vancomycin activity was also not affected by the presence of UF (-0.313 versus -0.289 h^{-1}). We were unable to simulate the killing activity of teicoplanin at 15 μg/ml in SMHB at any concentration in PHS-SMHB. A linear plot of BR in SMHB versus

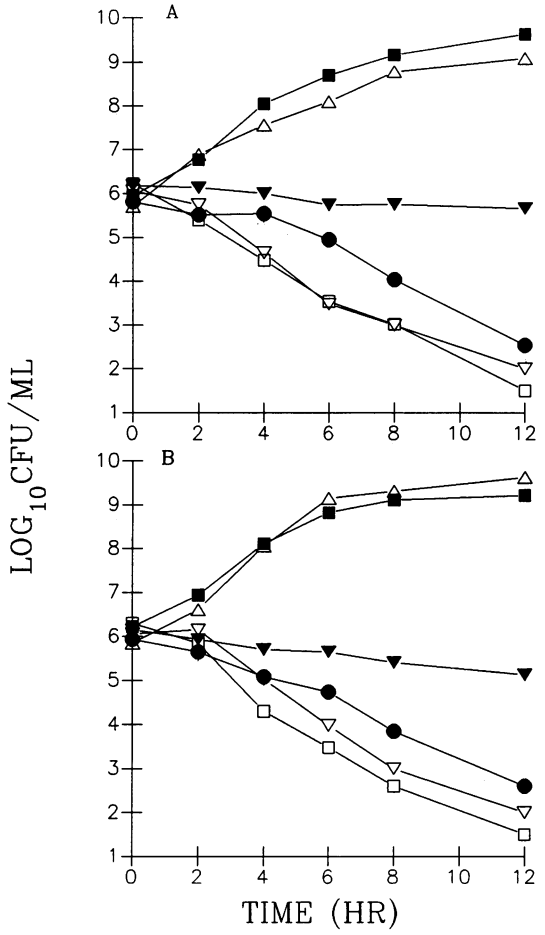

FIG. 1. Representative time-kill curves determined for SMHB and PHS-SMHB in the presence of teicoplanin and vancomycin at 45 μg/ml against MRSA-67 (A) and MSSA-380 (B). Symbols: ●, teicoplanin and SMHB; ▽, vancomycin and SMHB; ▼, teicoplanin and PHS-SMHB; □, vancomycin and PHS-SMHB; ■, control and SMHB; △, control and PHS-SMHB.

concentration revealed coefficients of determination of 0.02 for teicoplanin and 0.05 for vancomycin (Fig. 2).

DISCUSSION

There was a significant difference in the bactericidal activity of teicoplanin versus that of vancomycin in the different media we tested. The inherent difference in the bactericidal activity between teicoplanin and vancomycin may be due in part to selected mechanisms by which vancomycin inhibits and kills bacteria (8). This difference in bactericidal activity may not result in a clinically significant change in the response of patients with gram-positive infections (i.e., change in duration of fever, chills, or bacteremia) with the possible exception of deep-seated infections such as endocarditis. There has been one report of resistance to teicoplanin developing during therapy of *S. aureus* endocarditis. The possible role of low teicoplanin concentrations at the site of infection has been suggested with recommendations made to maintain higher concentrations in serum in serious systemic *S. aureus* infections (13).

There was a significant difference in the bactericidal activity of teicoplanin at both the LDC and HDC in PHS-SMHB when compared with activity in SMHB. Additionally, the BR of teicoplanin in UF was similar to that in SMHB. Together, these findings support our hypothesis that the high degree of protein binding may be a factor affecting the killing activity of teicoplanin, at least against *S. aureus*.

It was interesting to note that the BR for HDC vancomycin in PHS-SMHB was significantly better than that of LDC vancomycin in SMHB. The reason for this finding is not understood. Preliminary data from our laboratory offer evidence that pH influences the antibacterial activity of teicoplanin (unpublished data). Factors which may cause alterations of antibiotic activity in serum other than proteins and pH include osmolality and salt concentration, phosphates, and divalent cations (23). Further investigations to deter-

TABLE 1. BRs of teicoplanin and vancomycin in different media[a]

Medium and drug	Mean BR (SD)
SMHB	
Teicoplanin	
LDC	0.2061 (0.086)
HDC	0.2255 (0.094)
Vancomycin	
LDC	0.3247 (0.060)
HDC	0.3430 (0.070)
PHS-SMHB	
Teicoplanin	
LDC	0.0874 (0.047)[b]
HDC	0.0869 (0.037)[b]
Vancomycin	
LDC	0.3914 (0.041)
HDC	0.4052 (0.055)[c]

[a] BR = $-\log_{10}$ CFU per milliliter per hour. The data for MSSA-380 and MRSA-67 were combined. The BR of teicoplanin was lower than that of vancomycin at all concentrations in both media ($P < 0.05$).

[b] Significantly different from both concentrations of teicoplanin in SMHB ($P < 0.05$).

[c] Significantly different from LDC vancomycin in SMHB ($P < 0.05$).

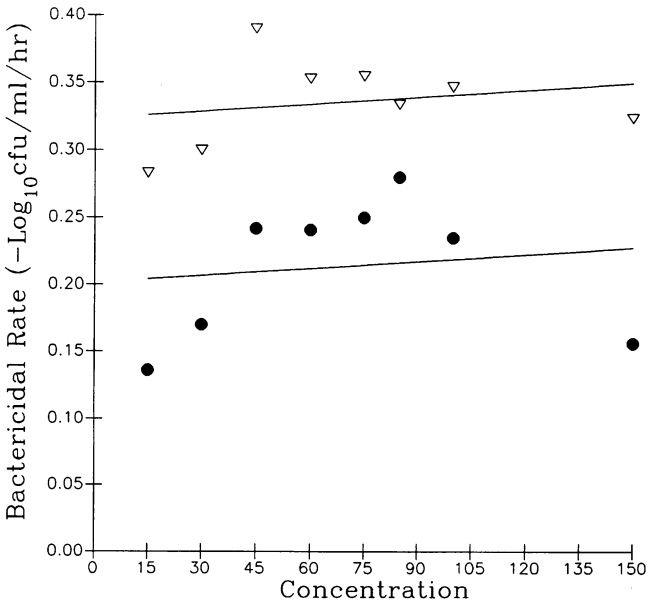

FIG. 2. Correlation of antibiotic concentration with BR. The line of best fit is indicated for each antimicrobial agent. Symbols: ●, teicoplanin ($r^2 = 0.02$); ▽, vancomycin ($r^2 = 0.05$).

mine the effect of these additional factors on teicoplanin bactericidal activity are necessary.

Although the activity of teicoplanin in UF appears similar to that in broth, we were unable to compensate for protein binding in serum by increasing teicoplanin concentrations. This would suggest that the impact of the presence of protein is more complex than the simple association between concentration of free drug and killing activity. In terms of in vivo activity, protein binding may affect distribution of teicoplanin. Poor penetration of [^{14}C]teicoplanin beyond the periphery of cardiac vegetation in rabbits has been reported (4). This factor, along with its slow accumulation and reduced activity in the presence of serum, supports current clinical studies which utilize higher doses in the treatment of *S. aureus* endocarditis.

Finally, our finding that there was no correlation with concentration and killing activity of either antibiotic in SMHB suggests that these drugs do not exhibit concentration-dependent killing. This would indicate that there is a maximum effective concentration for both glycopeptides and that higher dosages may improve efficacy primarily by ensuring adequate concentration at the site of infection or reducing the likelihood of the emergence of glycopeptide-resistant mutants. For teicoplanin, the higher dosages would also offset the degree of protein binding by increasing the concentrations of free unbound drug. Further investigations are warranted to determine the effect of protein binding on efficacy as it relates to tissue penetration.

ACKNOWLEDGMENTS

This work was funded in part by American College of Clinical Pharmacy—Miles Pharmaceuticals Infectious Diseases Research Fellowship.

We acknowledge Abbott Diagnostics for the use of the TDX analyzer for the assay of vancomycin. We also thank Merrell Dow Research Institute and Eli Lilly & Co. for supplying teicoplanin and vancomycin, respectively.

REFERENCES

1. **Bibler, M. R., P. T. Frame, D. N. Hagler, R. B. Bode, J. L. Staneck, V. Thamlikitkul, J. E. Harris, A. Haregewoin, and W. E. Bullock.** 1987. Clinical evaluation of efficacy, pharmacokinetics, and safety of teicoplanin for serious gram-positive infections. Antimicrob. Agents Chemother. **31**:207–212.
2. **Calain, P., K. H. Krause, P. Vaudaux, R. Auckenthaler, D. Lew, F. Waldvogel, and B. Hirschel.** 1987. Early termination of a prospective, randomized trial comparing teicoplanin and flucloxacillin for treating severe staphylococcal infections. J. Infect. Dis. **155**:187–191.
3. **Chambers, H. F., and S. Kennedy.** 1990. Effects of dosage, peak and trough concentrations in serum, protein binding, and bactericidal rate on efficacy of teicoplanin in a rabbit model of endocarditis. Antimicrob. Agents Chemother. **34**:510–514.
4. **Cremieux, A. C., B. Maziere, J. M. Vallois, M. Ottaviani, A. Azancot, H. Raffoul, A. Bouvet, J. J. Pocidalo, and C. Carbon.** 1989. Evaluation of antibiotic diffusion into cardiac vegetations by quantitative autoradiography. J. Infect. Dis. **159**:938–944.
5. **Drusano, G. L.** 1988. Role of pharmacokinetics in the outcome of infections. Antimicrob. Agents Chemother. **32**:289–297.
6. **Dudley, M. N., J. Blaser, D. Gilbert, and S. H. Zinner.** 1990. Significance of "extravascular" protein binding for antimicrobial pharmacodynamics in an in vitro capillary model of infection. Antimicrob. Agents Chemother. **34**:98–101.
7. **Erickson, R. C., A. R. Hildebrand, P. F. Hoffman, and C. B. Gibson.** 1989. A sensitive bioassay for teicoplanin in serum in the presence or absence of other antibiotics. Diagn. Microbiol. Infect. Dis. **12**:235–241.
8. **Fekety, R.** 1990. Vancomycin and teicoplanin, p. 317–323. *In* G. L. Mandell, R. G. Douglas, and J. E. Bennett (ed.), Principles and practice of infectious diseases. Churchill Livingstone, New York.
9. **Gibaldi, M. (ed.).** 1984. Drug disposition—elimination, p. 181–205. *In* Biopharmaceutics and clinical pharmacokinetics. Lea & Febiger, Philadelphia.
10. **Glupczynski, Y., H. Lagast, P. Van Der Auwera, J. P. Thys, F. Crokaert, E. Yourassowsky, F. Meunier-Carpentier, J. Klastersky, J. P. Kains, E. Serruys-Schoutens, and J. C. Legrand.** 1986. Clinical evaluation of teicoplanin for therapy of severe infections caused by gram-positive bacteria. Antimicrob. Agents Chemother. **29**:52–57.
11. **Greenberg, R. N., and C. A. Benes.** 1990. Time-kill studies with oxacillin, vancomycin, and teicoplanin versus *Staphylococcus aureus*. J. Infect. Dis. **161**:1036–1037.
12. **Jones, R. B., A. L. Barry, T. L. Gavan, and J. A. Washington II.** 1985. Susceptibility tests: microdilution and macrodilution broth procedures, p. 972–977. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
13. **Kaatz, G. W., S. M. Seo, N. J. Dorman, and S. A. Lerner.** 1990. Emergence of teicoplanin resistance during therapy of *Staphylococcus aureus* endocarditis. J. Infect. Dis. **162**:103–108.
14. **Kaatz, G. W., S. M. Seo, V. N. Reddy, E. M. Bailey, and M. J. Rybak.** 1990. Daptomycin compared with teicoplanin and vancomycin for therapy of experimental *Staphylococcus aureus* endocarditis. Antimicrob. Agents Chemother. **34**:2081–2085.
15. **Lagast, H., P. Dodion, and J. Klastersky.** 1986. Comparison of pharmacokinetics and bactericidal activity of teicoplanin and vancomycin. J. Antimicrob. Chemother. **18**:513–520.
16. **Lam, Y. W. F., M. H. Duroux, J. G. Gambertoglio, S. L. Barriere, and B. J. Guglielmo.** 1988. Effect of protein binding on serum bactericidal activities of ceftazidime and cefoperazone in healthy volunteers. Antimicrob. Agents Chemother. **32**:298–302.
17. **Leport, C., C. Perronne, P. Massip, P. Canton, P. Leclercq, E. Bernard, P. Lutun, J. J. Garaud, and J. L. Vilde.** 1989. Evaluation of teicoplanin for treatment of endocarditis caused by gram-positive cocci in 20 patients. Antimicrob. Agents Chemother. **33**:871–876.
18. **Merrikin, D. J., J. Briant, and G. N. Rolinson.** 1983. Effect of protein binding on antibiotic activity in vivo. J. Antimicrob. Chemother. **11**:233–238.
19. **Parenti, F.** 1988. Glycopeptide antibiotics. J. Clin. Pharmacol. **28**:136–140.
20. **Rybak, M. J., S. A. Lerner, D. P. Levine, L. M. Albrecht, P. L. McNeil, G. A. Thompson, M. T. Kenny, and L. Yuh.** 1991. Teicoplanin pharmacokinetics in intravenous drug abusers being treated for bacterial endocarditis. Antimicrob. Agents Chemother. **35**:696–700.
21. **Schwenzer, K. S., C.-H. J. Wang, and J. P. Anhalt.** 1983. Automated florescence polarization immunoassay for monitoring vancomycin. Ther. Drug Monit. **5**:341–345.
22. **Shyu, W. C., R. Quintiliani, C. H. Nightingale, and M. N. Dudley.** 1988. Effect of protein binding on drug penetration into blister fluid. Antimicrob. Agents Chemother. **32**:128–130.
23. **Stratton, C. W., and L. B. Reller.** 1977. Serum dilution test for bactericidal activity. I. Selection of a physiologic diluent. J. Infect. Dis. **136**:187–195.
24. **Tompsett, R., S. Shultz, and W. McDermott.** 1947. The relation of protein binding to the pharmacology and antibacterial activity of penicillins X, G, Dihydro F, and K. J. Bacteriol. **53**:581–595.
25. **Williams, A. H., R. N. Gruneberg, A. Webster, and G. L. Ridgway.** 1986. Teicoplanin in the treatment of infection caused by gram-positive organisms. J. Hosp. Infect. **7**(Suppl. A):101–103.
26. **Wise, R.** 1986. The clinical relevance of protein binding and tissue concentrations in antimicrobial therapy. Clin. Pharmacokinet. **11**:470–482.