

Ascitic Fluid Cephalosporin Concentrations: Influence of Protein Binding and Serum Pharmacokinetics

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Mongrel dogs with ascites created by inferior vena cava ligation were given cephalothin, cephaloridine, cefazolin, and cefamandole to evaluate the effect of protein binding and serum pharmacokinetics on the distribution of cephalosporins into ascitic fluid. Antibiotics were given intramuscularly (15 mg/kg) every 4 h for a total of eight doses. Antibiotic binding to dog serum and ascitic fluid was measured by ultracentrifugation. Binding of the cephalosporins to dog serum ranged from 31% for cephaloridine to 46% for cephalothin, considerably lower than human serum binding for cefazolin, cephalothin, and cefamandole. Antibiotic binding to ascitic fluid was only slightly lower than that to serum. Ascitic fluid antibiotic concentrations, which approached equilibrium at 16 to 28 h, were significantly higher for cefazolin and cephaloridine than for cephalothin and cefamandole. However, serum concentrations were also higher for cefazolin and cephaloridine, and percent penetration (ratio of serum peak to ascites peak \times 100) was not statistically different among the four drugs. Binding of these cephalosporins to extravascular fluid protein was an important factor that determined the total ascitic fluid antibiotic level achieved. A formula utilizing the log mean serum level and binding to serum and extravascular fluid protein was used to accurately predict ascitic fluid drug levels at equilibrium.

We have previously measured ascitic fluid concentrations of three cephalosporins in a dog model after a single parenteral dose (6). In this paper we extend these observations to include cefamandole in addition to cefazolin, cephaloridine, and cephalothin in a multiple-dose study of serum and ascitic fluid pharmacokinetics and protein binding of cephalosporins in dogs. The importance of both the log mean serum concentration and protein binding (to ascites and serum) as determinants of the ascitic fluid antibiotic concentration at equilibrium is emphasized. (This work was presented in part at the 16th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., 27-29 October 1976.)

MATERIALS AND METHODS

Animal model. Ascites was produced in mongrel dogs (initial weight, 14 to 18 kg) by partial ligation of the inferior vena cava as previously described (6). Dietary sodium chloride supplementation (15 g/day) was required in one dog to maintain ascites. A total of five dogs were used in this study. Ascitic fluid volume was measured each time antibiotics were given, using ^{125}I -labeled human serum albumin (6, 10). Ascites volumes in a single dog showed variation of from 1.2 to 3.7 liters during the course of the study. Total protein,

albumin, creatinine, blood urea nitrogen, bilirubin, serum glutamic oxalacetic transaminase, and alkaline phosphatase were determined using the AutoAnalyzer (Technicon, Terrytown, N.Y.). Cell counts were done on a Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.), and differential cell counts were manually performed.

Antibiotic administration. All cephalosporins were administered at a dose of 15 mg/kg (dry weight prior to ascites) by deep intramuscular injection in the gluteus or quadriceps muscles. Ascitic fluid specimens were obtained by midline abdominal paracentesis, and jugular venipuncture was used to obtain blood. Cefamandole was studied in three dogs after single-dose administration, and cefamandole, cefazolin, cephalothin, and cephaloridine were studied in three dogs after multiple doses. In the single-dose study of cefamandole, serum and ascites specimens were obtained at 0, 0.25, 0.5, 1 through 6, and 24 h after administration. In multiple-dose studies, serum and ascites were obtained 20 min and 4 h after each dose. Each dog was given eight injections on a 4-h schedule for a total study time of 32 h. The entire multidose study was completed during a 3-week period, rotating animals so that a minimum of 7 days and a maximum of 15 days elapsed between antibiotic administration to a given dog. Dogs were numbered 1 through 5 and assigned to antibiotics so that each received either two or three different drugs (Table 3). Dry commercial dog food and ad libitum water were allowed during experiments.

Antibiotic assays. All assays were performed in triplicate, using a microbiological disk-plate method with 24-h incubation at 37°C (9, 16). Cefazolin and cefamandole were assayed on antibiotic medium no. 1 (Difco Laboratories, Detroit, Mich.), using *Staphylococcus aureus* ATCC 6538P. *Sarcina lutea* ATCC 9341 was used for the assay of cephaloridine on antibiotic medium no. 1 (Difco). *Bacillus subtilis* ATCC 6633 was the indicator strain for cephalothin, which was assayed on 75% synthetic agar and 25% biochemical medium no. 6 (similar to Difco antibiotic medium no. 5, but with 6 g of yeast extract and 17.5 g of agar per liter). Standard curves in pooled dog ascitic fluid and serum were superimposable and were used interchangeably in the assays. Cefamandole was supplied through the courtesy of Eli Lilly and Co., Indianapolis, Ind.

Protein binding. Antibiotic binding to both serum and ascitic fluid was determined by an ultracentrifugation method (15). A minimum of three determinations in ascitic fluid and serum were performed for each antibiotic. Most of the binding determinations were performed in vitro by adding known amounts of drug to pooled ascites or serum; however, in vivo determinations were also performed on specimens obtained from single animals during multidose studies. Both in vivo and in vitro binding was performed for all antibiotics except cephaloridine (serum), with from one to three in vivo determinations in both serum and ascites for each drug.

Prediction of ascitic fluid concentration. Half-life ($t_1/2$) in serum was determined from the slope of the least-squares regression line (7). Log mean serum level (the antibiotic concentration which must be maintained by constant infusion to give the same area under the curve obtained by intermittent administration) was determined by graphically finding the midpoint of a straight line connecting the mean serum peak and trough on semilogarithmic paper. Prediction of antibiotic concentration in ascitic fluid is based on

the assumption that unbound antibiotic diffuses freely between serum and ascites and has the same concentration in both at equilibrium. Total antibiotic concentration (free plus protein bound) in ascitic fluid ($[C_a]$) is determined by the following simple equation: $[C_a] = [C_s] (F_s/F_a)$ (equation 1), where $[C_s]$ = log mean total serum concentration, F_s = free fraction of drug in serum (percent free in serum/100), and F_a = free fraction of drug in ascitic fluid (percent free in ascitic fluid/100). From this formula it can be seen that if a drug is bound equally to serum and ascites, F_s is equal to F_a and the ascitic fluid level is equal to the log mean serum level.

Statistics. Standard error of the mean was determined by the method of Mantel (11). Statistical significance was determined by using the Mann-Whitney U test (1).

RESULTS

Characteristics of ascitic fluid and serum. Ascites and serum cell count, pH, protein, and albumin are shown in Table 1 for four of the five dogs used in this study and did not differ appreciably from values previously reported (6). Blood urea nitrogen, creatinine, bilirubin, serum glutamic oxalacetic transaminase, and alkaline phosphatase were normal in all of the dogs.

Protein binding. Binding of the four cephalosporins to dog serum and ascitic fluid is shown in Table 2. Serum binding was relatively low for all four drugs in dogs. Data for both in vitro and in vivo determinations were pooled (Table 2), since the maximum difference in mean binding by the two methods was 5.8%, well within the accuracy of the bioassay used. Ascitic fluid binding was similar to or slightly lower than serum binding for all of the cephalosporins, as expected

TABLE 1. Characteristics of ascitic fluid and serum from four dogs

Parameter	Leukocytes (per mm ³)	Polymorphonuclears (%)	Total protein (g/dl)	Albumin (g/dl)	pH
Serum					
Mean	14,100	73	4.5	2.2	
Range	9,800-22,000	60-84	3.7-4.8	1.5-2.6	
Ascites					
Mean	1,375	37	3.4	1.7	7.6
Range	900-2,000	16-68	2.8-3.8	1.2-1.9	7.5-7.7

TABLE 2. Serum half-life ($t_{1/2}$) and protein binding to dog serum and ascitic fluid using an ultracentrifugation method

Antibiotic	$t_{1/2}$ (h)	Serum protein binding (%)		Ascites protein binding (%)	
		Mean	Range	Mean	Range
Cefamandole	0.9	41.9	35.5-46.5 (6) ^a	41.4	38.0-46.0 (5)
Cephalothin	0.9	45.7	42.0-50.0 (7)	38.1	33.8-47.0 (6)
Cephaloridine	1.2	31.2	25.0-39.5 (3)	28.4	25.8-31.0 (5)
Cefazolin	1.5	34.2	27.0-43.0 (9)	22.0	13.0-27.0 (5)

^a Number of determinations is given in parentheses.

from the slightly lower ascitic fluid albumin content.

Antibiotic penetrance. Results of single-dose studies of cefazolin, cephaloridine, and cephalothin in this model have been reported previously (6). Serum and ascites levels after a single dose of cefamandole are shown in Fig. 1. Mean peak serum and mean peak ascites levels of cefamandole (16.3 and 1.4 $\mu\text{g/ml}$) were significantly lower ($P < 0.05$) than those of cefazolin (24.0 and 6.2 $\mu\text{g/ml}$) and cephaloridine (27.7 and 5.3 $\mu\text{g/ml}$), but not cephalothin (14.5

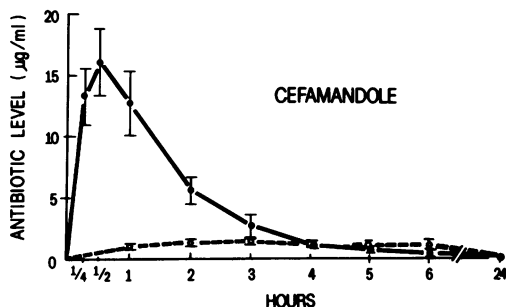


FIG. 1. Cefamandole levels in serum (solid line) and ascitic fluid (broken line) in three dogs. Vertical bars indicate standard error of the mean.

and 3.1 $\mu\text{g/ml}$), in the single-dose study (6). Percent penetration (ratio of ascites peak to serum peak $\times 100$) of cefamandole (9%) was also significantly less than for cefazolin (25.8%) and cephaloridine (19.1%) ($P < 0.05$), but not cephalothin (21.4%). Mean ascites volume (4.0 liters) in the cefamandole studies was lower than in the single-dose studies of the other cephalosporins (5.1 to 6.2 liters) and should not have been responsible for the low ascites levels of cefamandole (6).

Results of multiple-dose studies are shown in Fig. 2. Serum peak concentrations showed some variation, probably as a result of obtaining only a single serum sample 20 min after intramuscular injection. Ascitic fluid levels rose slowly, reaching a plateau at 16 to 28 h in most animals. In dogs with low ascites volumes equilibrium was achieved as early as 8 h. Peak and trough serum concentrations of cefazolin and cephaloridine were higher than for cephalothin and cefamandole and resulted in significantly higher ascites levels at equilibrium ($P < 0.05$).

Mean ascites peak level in the multiple-dose study was higher than in the single-dose study for all four cephalosporins, but this difference was statistically significant only for cefamandole ($P < 0.05$). Similarly, percent penetration (Table

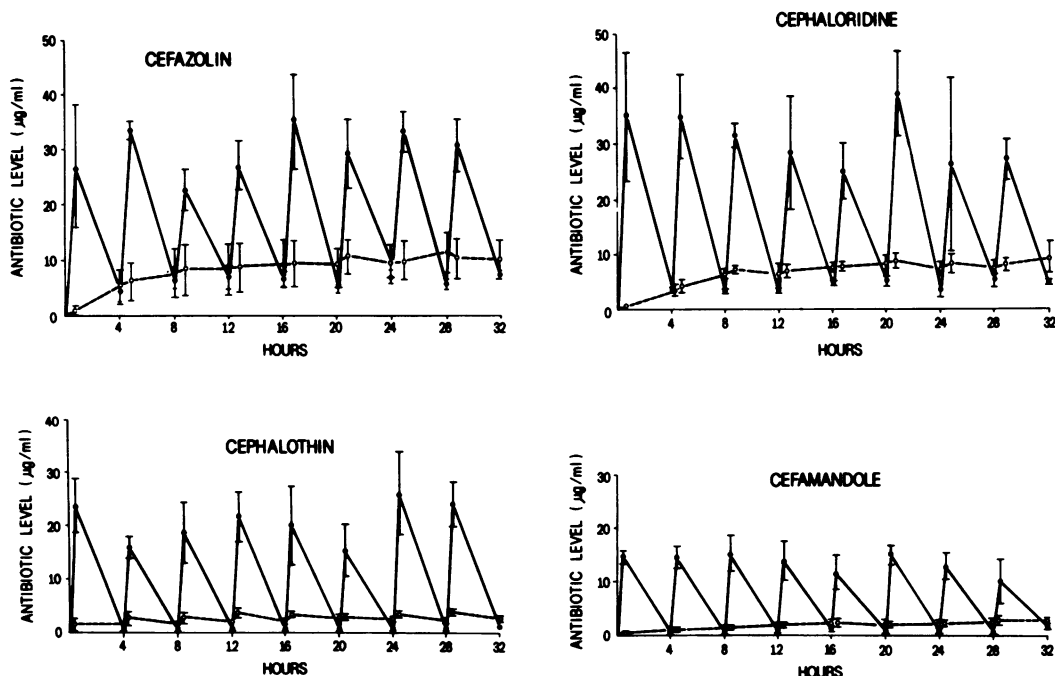


FIG. 2. Mean serum (solid line) and ascitic fluid (broken line) levels of cefazolin, cephaloridine, cephalothin, and cefamandole after intramuscular administration every 4 h in three dogs each. Vertical bars indicate standard error of the mean.

3) was statistically greater in the multiple-dose study than in the single-dose study only for cefamandole ($P < 0.05$). Although percent penetration among the cephalosporins varied from 16% for cephalothin to 37% for cefazolin (Table 3), these differences were not statistically significant.

Calculation of predicted ascites antibiotic concentration. Serum t_1 (Table 2) was shorter for cefamandole and cephalothin than for cephaloridine and cefazolin. Mean peak and trough serum levels from the multidose study are shown in Table 4 together with the log mean serum levels. Equation 1 was used to calculate a predicted ascitic fluid level for each drug based on protein binding to serum and ascites (Table 2) and the log mean serum level. This predicted ascites level is in good agreement with the measured ascites level at equilibrium (Table 4).

DISCUSSION

Serum protein binding of cephalosporins, especially cefazolin, and its effect on extravascular fluid antibiotic concentration have been studied in several models (2, 3, 5, 6, 14, 17, 19). In general, despite high binding to human serum, cefazolin concentrations in extravascular fluids have nevertheless been high, with the exception of observations made using the skin-window technique (17). These high extravascular cefazolin concentrations fail to support the contention that high serum binding inhibits the distribution of drug into tissue fluids. Failure to take into account two important variables, species differences in serum protein binding and binding to extravascular fluid protein in addition to serum, can account for these apparently discrepant results.

The first of these variables, species differences in serum protein binding, is a major factor influencing cephalosporin kinetics, particularly in canine experiments. Whereas cefazolin is 86% bound to human serum (8), binding to dog serum was only 34% by ultracentrifugation in this study compared to 88% in human serum by the same method (15). Previous studies are not in agreement on the binding of cefazolin to canine serum. Naber and Madsen and Nishida et al. found cefazolin binding to serum by slow dialysis and ultrafiltration to be only 24 and 20% (12, 13), whereas Watermann et al., using ultrafiltration, found cefazolin was 80% serum bound in dogs (20). Similarly, cefamandole binding to human serum (80%) is approximately twice as high as in dogs (15, 18). Canine serum binding of cephaloridine (31%) in this study was higher than previously reported in dogs (10%), but cephalothin binding (46%) agreed with previous reports (13, 20), although it was considerably lower than the 79% reported in humans (13, 15). Thus, for three of the four cephalosporins studied in these canine experiments, serum protein binding was markedly lower than in humans, and therefore an inhibitory effect of high serum protein binding on extravascular distribution should not be expected in dogs.

The second variable that serves to explain high extravascular levels of highly bound antibiotics is binding to extravascular fluid protein. Our measurements in dogs (Table 2) show that cephalosporins are bound only slightly less to ascitic fluid than to serum proteins. This appreciable binding of cephalosporins to ascitic fluid serves to explain why total concentrations of these drugs in ascitic fluid may be quite high.

TABLE 3. Mean and range of ascites volume and percent penetration (ratio of ascites peak to serum peak \times 100) for four cephalosporins after intramuscular administration every 4 h for eight doses

Antibiotic	Dogs studied ^a	Ascites volume (liters)		Penetration (%)	
		Mean	Range	Mean	Range
Cefamandole	1, 2, 3	8.7	5.6-12.4	24	19-28
Cephalothin	2, 4, 5	6.2	1.1-9.2	16	10-30
Cephaloridine	3, 4, 5	3.8	1.0-6.3	25	22-33
Cefazolin	1, 2, 4	8.7	3.6-11.7	37	21-56

^a Dogs were numbered 1 through 5.

TABLE 4. Predicted cephalosporin ascitic fluid concentration at equilibrium determined from the log mean serum level, protein binding to ascites and serum (Table 2), and equation 1

Antibiotic	Mean serum peak ($\mu\text{g/ml}$)	Mean serum trough ($\mu\text{g/ml}$)	Log mean serum level ($\mu\text{g/ml}$)	Predicted ascites level at equilibrium ($\mu\text{g/ml}$)	Measured ascites level at equilibrium ($\mu\text{g/ml}$)
Cefamandole	13.5	1.1	3.8	3.8	3.3
Cephalothin	20.8	0.8	4.0	3.7	3.4
Cephaloridine	32.7	4.3	11.8	11.3	8.2
Cefazolin	29.8	6.3	13.6	11.4	10.3

Although free (unbound) drug levels may be relatively low in ascitic fluid, the total drug concentration (bound and unbound) may be very high if large amounts are bound to the protein in the extravascular fluid.

Unfortunately, both the factor of lowered serum protein binding and the factor of substantial extravascular binding are operative in the dog model, making it impossible to discern the relative importance of each. However, in humans, where cefazolin serum binding is high, high binding to extravascular fluid protein is very likely the explanation for reported high cefazolin concentrations in ascitic fluid and pleural fluid (3, 4). In contrast, saline-filled skin windows which contain low amounts of protein also have very low total concentrations of cefazolin, nearly all of which is probably free drug (17).

It is also clear from our data that cephalosporin serum kinetics (peak and trough levels, t_4) in dogs are the major determinant of the equilibrium level achieved in ascitic fluid. The low serum peak levels and short t_4 of cefamandole and cephalothin, compared to cephaloridine and cefazolin, resulted in markedly lower log mean serum levels of the former drugs when given every 4 h (Table 4).

Equation 1 was developed to incorporate the effects of both serum kinetics and protein binding on extravascular penetrance of antibiotics. It is based on the assumption that unbound antibiotic diffuses freely into extravascular body fluids but is bound to these extravascular fluids in proportion to the amount of protein (albumin) present in the fluid. The equilibrium level of drug achieved in ascitic fluid is then determined by only two factors, the drug level maintained in the serum (log mean serum level) and the relative binding of the drug to serum and ascitic fluid (ratio of free drug fraction in serum to free drug fraction in ascites). We emphasize that the equation applies only to steady-state or equilibrium conditions. This is particularly important when working with a large extravascular volume such as ascites, in which equilibrium may not occur until multiple doses of drug have been given (Fig. 2).

Although equation 1 gave a reasonably close prediction of ascites levels, it was about 10% high for cefamandole, cephalothin, and cefazolin and 25% high for cephaloridine (Table 4). These small errors may be due to the inherent inaccuracies of the bioassay technique used in determining antibiotic levels and protein binding, or to failure to obtain peak levels in ascitic fluid which may occur at some time between the 20-min and 4-h sampling times used in this study (6).

In addition to ascites, we expect equation 1 to be applicable to any extravascular fluid into which antibiotic can diffuse freely, such as pleural effusions, joint effusions, and serous wound exudates. A previous study of penicillins in dog ascites has shown good predictability of equation 1 for that class of antibiotics, several of which are highly protein bound (D. N. Gerding, L. R. Peterson, J. K. Salomonson, W. H. Hall, and E. A. Schierl, *J. Infect. Dis.*, in press). It may seem paradoxical that very highly protein-bound drugs actually have the potential to achieve the highest extravascular fluid concentrations due to binding to protein in the extravascular fluid. Of course the free drug concentration of these highly bound drugs will still be low, but as long as it exceeds the minimum inhibitory concentration of susceptible organisms, the drug should be effective. There may even be some advantage in having large amounts of locally bound antibiotic in dynamic equilibrium with free drug at the site of infection, for the protein-bound drug may act as a reservoir to maintain adequate local free drug levels during periods when serum free drug levels are lower than extravascular fluid free drug concentrations.

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Evelyn C. Glatt prepared the manuscript.

LITERATURE CITED

1. Auble, D. 1953. Extended tables for the Mann-Whitney statistic. Institute of Educational Research, School of Education, Indiana University, Bloomington.
2. Carbon, C., A. Contrepoint, N. Brion, and S. Lamotte-Barillon. 1977. Penetration of cefazolin, cephaloridine, and cefamandole into interstitial fluid in rabbits. *Antimicrob. Agents Chemother.* 11:594-598.
3. Cole, D. R., and J. Pung. 1977. Penetration of cefazolin into pleural fluid. *Antimicrob. Agents Chemother.* 11:1033-1035.
4. Gerding, D. N., W. H. Hall, and E. A. Schierl. 1977. Antibiotic concentrations in ascitic fluid of patients with ascites and bacterial peritonitis. *Ann. Intern. Med.* 86:708-713.
5. Gerding, D. N., W. H. Hall, E. A. Schierl, and R. E. Manion. 1976. Cephalosporin and aminoglycoside concentration in peritoneal capsular fluid in rabbits. *Antimicrob. Agents Chemother.* 10:902-911.
6. Gerding, D. N., J. P. Kromhout, J. J. Sullivan, and W. H. Hall. 1976. Antibiotic penetrance of ascitic fluid in dogs. *Antimicrob. Agents Chemother.* 10:850-855.
7. Goldstein, A., L. Aronow, and S. Kolman. 1974. The time course of drug action, p. 301-355. *In Principles of drug action: the basis of pharmacology*, 2nd ed. John Wiley & Sons, Inc., New York.
8. Kirby, W. M. M., and C. Regamey. 1973. Pharmacokinetics of cefazolin compared with four other cephalosporins. *J. Infect. Dis.* 128:S341-S346.
9. Lilly Research Laboratories. 1970. Paper disk or cup

- plate antibiotic assay procedures. Eli Lilly and Co., Indianapolis.
10. **Luttwak, E. M.** 1973. Quantitative studies of ascitic fluid. *Surg. Gynecol. Obstet.* **136**:269-272.
 11. **Mantel, N.** 1951. Rapid estimation of standard errors of means for small samples. *Am. Stat.* **5**:26-27.
 12. **Naber, K. G., and D. O. Madsen.** 1976. Renal lymph concentrations of cephalosporins: the importance of protein binding and renal excretion. *Infection* **4**(Suppl. 2):S131-S136.
 13. **Nishida, M., T. Matsubara, T. Murakawa, Y. Mine, Y. Yokota, S. Kuwahara, and S. Goto.** 1970. In vitro and in vivo evaluation of cefazolin, a new cephalosporin C derivative, p. 236-243. *Antimicrob. Agents Chemother.* 1969.
 14. **Nishida, M., and T. Murakawa.** 1977. Exudate levels and bactericidal activity of cefazolin in a new local infection system using rat granuloma pouches. *Antimicrob. Agents Chemother.* **11**:1042-1048.
 15. **Peterson, L. R., W. H. Hall, H. H. Zinneman, and D. N. Gerding.** 1977. Standardization of a preparative ultracentrifuge method for quantitative determination of protein binding of seven antibiotics. *J. Infect. Dis.* **136**:778-783.
 16. **Sabath, L. D., J. I. Casey, P. A. Ruch, L. L. Stumpf, and M. Finland.** 1971. Rapid microassay for circulating nephrotoxic antibiotics, p. 83-89. *Antimicrob. Agents Chemother.* 1970.
 17. **Tan, J. S., and S. J. Salstrom.** 1977. Levels of carbenicillin, ticarcillin, cephalothin, cefazolin, cefamandole, gentamicin, tobramycin, and amikacin in human serum and interstitial fluid. *Antimicrob. Agents Chemother.* **11**:698-702.
 18. **Watermann, N. G., H. U. Eickenberg, and L. Scharfenberger.** 1976. Concentration of cefamandole in serum interstitial fluid, bile, and urine. *Antimicrob. Agents Chemother.* **10**:733-735.
 19. **Watermann, N. G., M. J. Raff, L. Scharfenberger, and P. A. Barnwell.** 1976. Protein binding and concentrations of cephaloridine and cefazolin in serum and interstitial fluid in dogs. *J. Infect. Dis.* **133**:642-647.
 20. **Watermann, N., L. Scharfenberger, and M. J. Raff.** 1974. Rate of binding of antibiotics to canine serum protein. *Antimicrob. Agents Chemother.* **5**:294-295.