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Penetration of Cefazolin into Normal and Osteomyelitic Canine Cortical Bone

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The ability of cefazolin to cross the capillary membranes and its concentrations in the interstitial fluid spaces were studied in normal and osteomyelitic canine bone. The maximum extraction after a single capillary passage and the net extraction after 3 min, determined with triple-tracer indicator-dilution techniques, demonstrated that cefazolin readily traversed the capillaries of normal and osteomyelitic bone. These studies suggest that the altered pathophysiology of osteomyelitic tissue and the complex diffusional characteristics of cefazolin enhanced the ability of this agent to cross the endothelial cells lining the capillaries of osteomyelitic bone. Volume of distribution studies demonstrated that cefazolin was distributed in the plasma and interstitial fluid spaces of normal cortical bone. Although these spaces were increased 330 and 941% in osteomyelitic tissue, the distribution of cefazolin increased proportionally. There was a direct correlation between the calculated concentrations of cefazolin in the interstitial fluid spaces of normal and osteomyelitic cortical bone and the simultaneous serum levels in animals in which a steady-state equilibrium had been achieved. These studies suggest that a physiological barrier or concentration gradient for cefazolin does not exist in normal or osteomyelitic bone. Cefazolin can cross the capillary membranes of bone and achieve bactericidal concentrations in the interstitial fluid space of normal and osteomyelitic tissue.

Cefazolin, a cephalosporin with favorable pharmacokinetic characteristics, is widely utilized as a prophylactic and therapeutic agent in patients having musculoskeletal surgery. The combination of high serum levels and a reduced rate of excretion provides a prolonged half-life with sustained serum levels, which are thought to be responsible for superior tissue penetration (4, 5, 16, 24). Although the ability of cefazolin to enter osseous tissue has been evaluated with bioassay techniques (6, 11, 23, 31, 32, 35), the information obtained fails to fully define the osseous pharmacokinetics. Additionally, the osseous levels determined by bioassay have an erratic relationship to simultaneously determined serum levels (6, 23, 26, 29). Furthermore, the interstitial concentration cannot be surmised from the bioassay of bone.

Since effective antimicrobial prophylaxis and therapy necessitate bactericidal concentrations at the primary sites of drug-microorganism interaction, the interstitial fluid concentration is an important parameter (37). This study was designed to define the osseous capillary permeability, the distribution within the various fluid space compartments, and the interstitial fluid space concentration of cefazolin in both normal and osteomyelitic osseous tissue.

MATERIALS AND METHODS

Osteomyelitic model. A cortical window was created by previously described techniques (R. H. Fitzgerald, Jr., P. J. Kelly, and J. A. Washington II, Abstr. Trans. 25th Annu. Meet. Orthop. Res. Soc. 1979, 4:11) in both upper tibiae of 10 dogs, and the underlying medullary contents were removed. One milliliter of a suspension containing 10^5 colony-forming units of *Staphylococcus aureus* (ATCC 25923) per milliliter was placed in each tibial defect. A cottonoid was added as a foreign body stimulus of infection. The diagnosis of infection was based on clinical, radiological, and bacteriological findings. The mean weight of all 17 dogs utilized for pharmacokinetic studies—10 with infection and 7 normal—was 17.8 kg (range, 11.5 to 25.0 kg).

Capillary permeability. Previously described indicator-dilution techniques (3, 15) were utilized to define the ability of [^{14}C]cefazolin to cross the capillary membrane of the tibiae of five normal dogs and five dogs with osteomyelitis of the proximal tibial metaphysis and the tibial diaphysis. This technique involves the simultaneous injection of [^{14}C]cefazolin, [^{99m}Tc]albumin, and ^{85}Sr into the nutrient artery of the tibia. It permits characterization of the maximum and net

extraction (E_{max} and E_{net}) of cefazolin after a single passage through the osseous capillary network (2). Since ^{85}Sr was injected simultaneously, the maximum extractions could be compared (7).

Once the maximum extraction was determined, it was possible to calculate the permeability-surface area product (PS) as follows:

$$PS = -F_s \log_e (1 - E_{max}) \quad (1)$$

where P is the capillary permeability (centimeters per minute), S is the capillary surface area per unit mass of tissue (square centimeters per 100 g of tissue), and F_s is the flow of the injectate into the nutrient artery (milliliters per minute per 100 g of tissue).

Since [^{14}C]cefazolin and ^{85}Sr were injected simultaneously, it was possible to compare their permeability-surface area products:

$$\frac{PS_{Cfz}}{PS_{Sr}} = \frac{-F_s \log_e (1 - E_{max\text{-}Cfz})}{-F_s \log_e (1 - E_{max\text{-}Sr})} \quad (2)$$

Furthermore, simultaneous injection would expose both molecules to the same surface area and flow. Thus, the equation becomes:

$$\frac{P_{Cfz}}{P_{Sr}} = \frac{\log_e (1 - E_{max\text{-}Cfz})}{\log_e (1 - E_{max\text{-}Sr})} \quad (3)$$

The permeability ratios were subsequently compared with the ratio of the diffusion coefficients (14, 33) to permit assessment of the mechanism of the capillary passage of cefazolin.

Fluid space distribution. The relative size of the erythrocyte (V_{RBC}), the plasma space (V_P), the vascular space (V_E), and the total exchangeable water space (V_W) were determined in canine cortical bone from the radius (normal bone) and the tibia (osteomyelitic bone) in five volume of distribution experiments with the techniques described by Morris and co-workers (17) and Quinlan and co-workers (W. R. Quinlan, B. B. Hall, R. H. Fitzgerald, Jr., and P. J. Kelly, Fed. Proc. 39:273, 1980). In addition, two dogs without osteomyelitis were investigated by identical techniques to show that the results from normal cortical bone were comparable in the radius and tibia. The vascular space (V_{Bl}), plasma space, and erythrocyte space were determined with ^{99m}Tc-labeled erythrocytes and the hematocrit (Quinlan et al., Fed. Proc. 39:273, 1980). The exchangeable water space was defined by the water of desiccation at 100°C. The extracellular fluid space was determined with the volume of distribution of [^{3}H]sucrose.

The volume of distribution of a substance is the virtual volume in which the virtual concentration is assumed to be the same as that in the reference space but in which the cortical concentration may be more or less. The volume of distribution of a substance was determined by injecting the substance intravenously and allowing it to achieve equilibrium in a steady state. Comparison of the final concentrations of known volumes of bone and serum permitted calculation of the volume of distribution of the substance in milliliters per milliliter of bone. In the same five experiments, the distribution of [^{14}C]cefazolin in the various fluid spaces was determined in normal and osteomyelitic bone.

The technique utilized for the volume of distribution experiments was basically that described by Morris and co-workers (17). The renal pedicles of anesthetized dogs were ligated to expedite the attainment of steady-state conditions. Cannulation of the right carotid artery and left jugular vein created portals for obtaining serum specimens to ensure steady-state conditions and for injecting the specimens to be studied, respectively. The injectate was composed of 90 μCi of [^{14}C]cefazolin (specific activity, 8.77 μCi/mg; Eli Lilly & Co.), 125 μCi of [^{3}H]sucrose (specific activity, 8.50 μCi/mg; New England Nuclear Corp.), and a clinical (unlabeled) dose of cefazolin (15 mg/kg). Before administration of the injectate, a pilot was prepared by adding 0.05 ml of the injectate to 25 ml of triple-distilled water to determine the ratio of labeled to unlabeled cefazolin.

The ^{99m}Tc-labeled erythrocytes were prepared by the techniques previously described by Dewanjee (8) and Albert (1). In previous experiments (15; B. B. Hall, R. H. Fitzgerald, Jr., P. J. Kelly, and J. A. Washington II, Abstr. Trans. 26th Annu. Meet. Orthop. Res. Soc. 1980, 5:32), it was determined that the rapid diffusion of erythrocytes permitted their injection 210 min after the injection of the other isotopes.

Sequential serum samples were collected from the cannula in the carotid artery at 5, 15, 30, 60, 180, 210, 215, 225, and 240 min after the administration of the injectate. Analysis of these samples permits documentation of steady-state conditions of the cefazolin levels.

The dogs were sacrificed at 4 h with an overdose of sodium pentothal. Under sterile conditions, deep tissue specimens were obtained and submitted to the microbiology laboratory for aerobic culture on sheep blood agar and in thioglycolate broth. Subsequently, osseous specimens from the radius and tibia were harvested. The cortical bone specimens were stripped of both muscle and periosteum. The metaphyseal-diaphyseal segments were split longitudinally to ensure mechanical removal of all marrow contents. One fragment was used to determine the specific gravity, ρ, of the osteomyelitic tissue (proximal tibial metaphysis) and of normal osseous tissue (radial diaphysis):

$$\rho = \frac{\text{weight in air (g)}}{\text{weight in air (g)} - \text{weight in water (g)}} \quad (4)$$

Approximately 1 g of filed cortical bone was obtained from the other specimen. Quantitation of [^{3}H]sucrose and [^{14}C]cefazolin content in the pilot, osseous, plasma, and erythrocyte samples (all performed in triplicate) was accomplished by tissue oxidation and separation (Packard Tri-Carb oxidizer B-306). The scintillation vials were counted in a Packard Tri-Carb scintillation counter after at least 10 half-lives of ^{99m}Tc (60 h) had passed. The counts were corrected for background and efficiency. Since the oxidizer separates ^{3}H and ^{14}C into individual scintillation vials, correction for spillover was unnecessary.

The quantitation of the ^{99m}Tc content of bone, plasma, and erythrocyte specimens was performed in

a Beckman 310 gamma counter immediately after sacrifice. The counts were corrected for decay and background.

Plasma samples were assayed for cefazolin concentration by the cylinder plate method, using *Sarcina lutea* (ATTC 9341) as the test strain.

Determination of the vascular space of cortical bone required volume correction with the specific gravity, ρ:

$$\frac{\text{cpm of } ^{99m}\text{Tc}}{\text{ml of cortical bone}} = \frac{\text{cpm of } ^{99m}\text{Tc}}{\text{g of cortical bone}} \times \rho\ (\text{g/ml}) \quad (5)$$

$$\frac{\text{cpm of } ^{99m}\text{Tc}}{\text{ml of RBC}} = \frac{\text{cpm of } ^{99m}\text{Tc/ml of whole blood}}{\text{Hct (0.96)}} \quad (6)$$

where Hct is the peripheral hematocrit, 0.96 is the correction factor for trapped plasma, and RBC is erythrocytes (19).

$$V_{RBC} = \frac{\text{cpm of } ^{99m}\text{Tc/ml of cortical bone}}{\text{cpm of } ^{99m}\text{Tc/ml of RBC}} \quad (7)$$

Since V_{RBC} represents a fraction of whole blood, the relative size of the vascular space (V_{Bl}) in cortical bone can be calculated:

$$V_{Bl} = \frac{V_{RBC}}{\text{Hct (0.96)}} \quad (8)$$

The plasma space (V_P) can be derived from these spaces:

$$V_P = V_{Bl} - V_{RBC} \quad (9)$$

The extracellular fluid space (V_E) is equivalent to the volume of distribution of [^{3}H]sucrose:

$$\frac{\text{cpm of [}^3\text{H]sucrose}}{\text{ml of cortical bone}} =$$

$$\frac{\text{cpm of [}^3\text{H]sucrose}}{\text{g of cortical bone}} \times \rho\ (\text{g/ml}) \quad (10)$$

$$V_{D\text{-[}^3\text{H]sucrose}} =$$

$$\frac{\text{cpm of [}^3\text{H]sucrose/ml of cortical bone}}{\text{cpm of [}^3\text{H]sucrose/ml of plasma}} \quad (11)$$

Since the extracellular fluid space of cortical bone is a combination of the plasma and interstitial fluid spaces, the interstitial fluid space (V_{ISF}) can be derived:

$$V_{ISF} = V_E - V_P \quad (12)$$

The fluid space distribution of cefazolin is characterized by its volume of distribution:

$$V_{D\text{-Cfz}} = \frac{\text{(cpm of [}^{14}\text{C]cefazolin/g of cortical bone) (}\rho\text{)}}{\text{cpm of [}^{14}\text{C]cefazolin/ml of plasma}} \quad (13)$$

The volume of distribution of cefazolin in the erythrocyte space ($V_{RBC\text{-Cfz}}$) is similarly calculated:

$$V_{RBC\text{-Cfz}} = \frac{\text{cpm of [}^{14}\text{C]cefazolin/ml of RBC}}{\text{cpm of [}^{14}\text{C]cefazolin/ml of plasma}} \quad (14)$$

Protein binding. The protein binding of cefazolin in serum was determined by the centrifugal ultrafiltration method used by Singhvi et al. (30). Centriflo membrane ultrafilter cones (CF25A [Amicon]) were soaked in distilled water for 1 h to remove the glycerol residue. Subsequently, they were allowed to dry overnight. Serum was prepared by adding [^{14}C]cefazolin and unlabeled cefazolin to dog serum in vitro. Several dilutions were prepared such that the range of concentrations and radioactivity obtained included all those produced by injecting the dogs in volume of distribution experiments. After the addition of 10 ml of serum from each dilution to the cones, they were centrifuged at 900 × *g* for 2 min. The small amount of fluid collected during this time was discarded to rule out the possibility that the pores of the cones were not dry. Then the cones with serum were centrifuged at 900 × *g* for 20 min. Subsequently, the ultrafiltrate was collected.

Singhvi et al. (31) confirmed the adequacy of the retention of serum proteins by the cone, showing that ultrafiltration removed more than 97% of the serum proteins. Studies have also shown that cefazolin does not bind to the membrane filtration cones (13, 31).

By using the ratio of [^{14}C]cefazolin to the clinical dose (counts per minute/microgram) established with the pilot, oxidation of samples of the ultrafiltrate and unfiltered samples permits calculation of the cefazolin with an isotope assay:

$$\text{Cefazolin}_{\text{ultrafiltrate}}\ (\mu\text{g/ml}) =$$

$$\frac{\text{cpm of [}^{14}\text{C]cefazolin}}{\text{ml of ultrafiltrate}} \times \frac{\text{cpm of [}^{14}\text{C]cefazolin}}{\mu\text{g of cefazolin (pilot)}} \quad (15)$$

Cefazolin concentration in the ultrafiltrate and unfiltered serum was analyzed by isotope and bioassay methods. Standards for the bioassay were prepared with phosphate buffer for the ultrafiltrate samples and with pooled dog serum for the serum samples.

Interstitial fluid concentration. Since the distribution of cefazolin within the interstitial fluid space was determined in the volume of distribution experiments and a ratio of the isotope and clinical dose was established with the pilot, it was possible to determine the cefazolin concentration in the interstitial fluid space of normal and osteomyelitic bone.

The cefazolin concentration in cortical bone was determined by the oxidation of cortical samples:

$$\text{Cefazolin}\left(\frac{\mu\text{g}}{\text{ml of cortical bone}}\right) =$$

$$\left(\frac{\text{cpm of [}^{14}\text{C]cefazolin}}{\text{g of cortical bone}}\right)\left(\rho\ \frac{\text{g}}{\text{ml}}\right)$$

$$\left(\frac{\mu\text{g of cefazolin}}{\text{cpm of [}^{14}\text{C]cefazolin}}\right) \quad (16)$$

If the concentration of cefazolin in the vascular space of cortical bone is subtracted from the overall concentration, only the extravascular concentration remains:

$$\frac{\mu g \text{ of cefazolin}}{\text{ml of extravascular space of cortical bone}} = \left[\frac{\mu g \text{ of cefazolin}}{\text{ml of cortical bone}}\right] - \left[V_P\ (\text{cefazolin}_{\text{serum}}\ [\mu g/ml])\right] \quad (17)$$

Since the size of the interstitial fluid space of normal and osteomyelitic bone was equivalent to the volume of distribution of cefazolin, the interstitial fluid concentration of cefazolin was calculated:

$$ISF_{Cfz}\ (\mu g/ml) = \frac{\text{cefazolin}_{\text{extravascular}}\ (\mu g/ml)}{V_{ISF}\ (ml/ml)} \quad (18)$$

Because this value was determined with an isotopic assay, it was corrected for the protein binding determined above.

RESULTS

Extraction studies. The maximum instantaneous fractional extractions of the two test tracers in normal and osteomyelitic cortical bone after a single transcapillary passage are shown in Table 1. When the extraction of ^{85}Sr, a small ion of known permeability, was compared with the extraction of [^{14}C]cefazolin, using [^{99m}Tc]albumin as a reference tracer, the small bone-seeking ion, ^{85}Sr, was extracted to a greater degree. This was true throughout the experiment, as well as at the maximum extractions. There was no significant difference in the maximum extraction of [^{14}C]cefazolin or ^{85}Sr between normal and osteomyelitic bone (Table 2).

The ratio of the capillary permeability of [^{14}C]cefazolin to the capillary permeability of ^{85}Sr (P_{Cfz}/P_{Sr}) was 0.66 ± 0.042 for normal and 0.77 ± 0.050 for osteomyelitic bone (Table 1). The difference between these figures was statistically significant ($P < 0.001$; Table 2).

The ratio of the diffusion coefficients of [^{14}C]cefazolin and ^{85}Sr (D_{Cfz}/D_{Sr}) was 0.36 (14, 34). This figure is significantly different from the permeability ratios for both normal and osteomyelitic bone ($P < 0.001$; Table 2), indicating that cefazolin crosses the capillary membrane without difficulty. Since strontium is known to cross the osseous capillary membrane passively (i.e., passing through the intercellular pores of the endothelial cells lining the membrane), these data indicate that cefazolin passes through these pores, and quite possibly, its lipophilic characteristics at physiological pH permit passage through the intracytoplasmic vesicular system.

Volume of distribution studies. The relative sizes of the various fluid spaces—exchangeable water, vascular and its components, extracellular, and interstitial fluid spaces—in the radius were quite similar to those reported in the tibia by Morris and co-workers (17) and in the radius by Quinlan and co-workers (Fed. Proc. 39:273, 1980) (Table 3). Comparison of the results of the tibia and radius in the normal dogs (A-388 and Z-559) in this study demonstrates that the two sites were similar (Table 4). It is reasonable to assume, therefore, that the cortical tissue from these two sites is comparable. In the statistical analysis, the results from the radius and tibia of the normal dogs were averaged and considered as one, so as not to overweight these experiments.

The values of each of the fluid spaces in osteomyelitic tissue were statistically increased ($P < 0.005$) compared with their counterparts in normal bone (Tables 3–5).

The volume of distribution of cefazolin (V_D) in normal cortical bone was 0.0662 ± 0.0168 ml/ml

TABLE 1. Comparison of maximum extractions (E_{max}) of ^{85}Sr and [^{14}C]cefazolin in normal and osteomyelitic dog tibiae

Statistical datum	E_{max} (normal)		E_{max} (osteomyelitic)		P_{Cfz}/P_{Sr}[a]
	Sr	[^{14}C]cefazolin	Sr	[^{14}C]cefazolin	
Mean	0.51	0.38			0.66
SD	0.036	0.031			0.042
SEM	0.016	0.014			0.019
No.	5	5			5
Mean			0.49	0.40	0.77
SD			0.104	0.082	0.050
SEM			0.047	0.037	0.022
No.			5	5	5

[a] P, Permeability.

TABLE 2. Results of statistical analysis (Student's t test) of extraction data

Difference between values of:	t value	P
E_{max-Sr}: normal and osteomyelitic bone	0.595	NS[a]
$E_{max-Cfz}$: normal and osteomyelitic bone	0.600	NS[a]
Normal bone: P_{Cfz}/P_{Sr} and D_{Cfz}/D_{Sr}[b]	11.51	<0.001
Osteomyelitic bone: P_{Cfz}/P_{Sr} and D_{Cfz}/D_{Sr}	15.57	<0.001
P_{Cfz}/P_{Sr}: normal and osteomyelitic bone	3.772	<0.01

[a] NS, No significant difference.
[b] P, Permeability; D, diffusion coefficient.

of bone and in infected cortical bone, 0.572 ± 0.0756 ml/ml of bone (Table 6). The difference between these values is statistically significant ($P < 0.001$; Table 6). The volume of distribution of cefazolin in the erythrocyte space ($V_{RBC-Cfz}$) was 0.00930 ± 0.00335 ml/ml of erythrocytes (Table 6); the result of $V_{RBC-Cfz}$ from dog X-510 was not reliable due to technical problems encountered during the preparation of the ^{99m}Tc-labeled erythrocytes.

Protein binding. Simultaneous microbiological and isotopic assays were carried out on dog serum and serum ultrafiltrate in an attempt to quantitate the in vivo protein binding of cefazolin and to correlate our results with clinical bioassay determinations of serum antibiotic concentration. Several dilutions of cefazolin were prepared in serum to include the range of concentrations and radioactivity observed in the volume of distribution experiments. The protein binding can be expected to be similar in these dilutions as long as the protein is not saturated (25); therefore, they are comparable.

The serum protein binding of cefazolin (Table 7) determined by isotopic methods was 35.8 ± 2.64%, and that determined by bioassay was 38.8 ± 2.51%. These figures are not significantly different. The close correlation between isotopic and microbiological assays is evident in Table 7.

TABLE 4. Comparison of the fluid space volumes, volume of distribution of cefazolin, concentration of cefazolin, and interstitial fluid concentrations of cefazolin in the normal radii and tibiae of two dogs

Parameter[a]	Dog A-388		Dog Z-559	
	Radius	Tibia	Radius	Tibia
V_{RBC}	0.00546	0.00816	0.00612	0.00697
V_P	0.00707	0.0106	0.0109	0.0125
V_{BI}	0.0125	0.0187	0.0170	0.0194
V_E	0.0841	0.0869	0.0601	0.0746
V_{ISF}	0.0770	0.0764	0.0492	0.0622
V_D	0.0924	0.0774	0.0485	0.0589
Cefazolin (μg/g)	2.31	1.96	1.84	1.56
ISF_{Cfz} (μg/ml)	53.44	45.34	46.12	45.54

[a] For definitions of abbreviations, see Tables 2 and 3.

Interstitial fluid concentrations. The concentration of cefazolin in cortical bone (Table 8) was 1.88 ± 0.57 μg/g in normal bone and 17.61 ± 2.97 μg/g in infected bone. These values were significantly different ($P < 0.001$). The serum concentration of cefazolin averaged 48.87 μg/ml in the seven dogs.

The calculated interstitial fluid concentrations of cefazolin were 55.56 ± 19.01 and 50.09 ± 6.91 μg/ml in normal and infected cortical bone, respectively (Table 9). The difference between these figures was not statistically significant. There was a close correlation between these values and the total serum level of cefazolin (48.87 ± 6.44 μg/ml; Table 9, Fig. 1).

DISCUSSION

We have previously demonstrated that the techniques applied in this study will help to delineate a number of important parameters of

TABLE 3. Erythrocyte space (V_{RBC}), plasma space (V_P), vascular space (V_{BI}), volume of distribution of sucrose (V_E), and interstitial fluid space (V_{ISF}) in normal (radius) cortical bone[a]

Statistical datum	V_{RBC}	V_P	V_{BI}	V_E	V_{ISF}
Mean	0.00530	0.00824	0.0135	0.0625	0.0542
SD	0.00234	0.00292	0.0051	0.0181	0.0159
SEM	0.00088	0.00110	0.0019	0.0068	0.0060
No.	7	7	7	7	7

[a] Values represent the mean of the right and left sides of each dog and are given in milliliters per milliliter of bone.

TABLE 5. Erythrocyte space (V_{RBC}), plasma space (V_P), vascular space (V_{BI}), volume of distribution of sucrose (V_E), and interstitial fluid space (V_{ISF}) in infected (tibia) cortical bone[a]

Statistical datum	V_{RBC}	V_P	V_{BI}	V_E	V_{ISF}
Mean	0.0160	0.0271	0.0431	0.537	0.510
SD	0.00566	0.0107	0.0153	0.0911	0.109
SEM	0.00253	0.0048	0.0068	0.0407	0.049
No.	5	5	5	5	5

[a] Values represent the mean of the right and left sides of each dog and are given in milliliters per milliliter of bone.

TABLE 6. Volume of distribution of [^{14}C]cefazolin in infected (tibia)[a] and some normal (radius) cortical bone (V_D) and in the erythrocyte space ($V_{RBC-Cfz}$)

Statistical datum	V_D[b] (infected)	V_D[b] (normal)	$V_{RBC-Cfz}$[c]
Mean	0.572	0.0662	0.00930
SD	0.0756	0.0168	0.00335
SEM	0.0338	0.0063	0.00137
No.	5	7	6

[a] Values represent the mean of the right and left sides of each dog.
[b] Milliliters per milliliter of bone.
[c] Milliliters per milliliter of erythrocytes.

the osseous pharmacokinetics in normal cortical bone. This study extends the application of these techniques to the study of cefazolin in osteomyelitic bone.

The extraction studies indicate that cefazolin readily traverses the osseous capillary membrane of normal and osteomyelitic bone. Since the permeability ratio of cefazolin to strontium (P_{Cfz}/P_{Sr} = 0.66 ± 0.042) was greater than the ratio of their diffusion coefficients (D_{Cfz}/D_{Sr} = 0.36), one can surmise that the weak lipophilic properties of cefazolin may allow it to pass through the intracellular vesicular system, in addition to the intracellular pores or clefts; this may differ from the passive diffusion of the hydrophilic molecule strontium. These findings indicate that, in bone, the capillary membrane does not present a significant barrier to the passage of cefazolin from the vascular space to the extravascular space.

As might be expected, the permeability of the osseous capillary membrane to cefazolin, relative to strontium, was increased in the presence of acute osteomyelitis. The more complex diffusional characteristics of cefazolin, as well as its larger size, probably contribute to a greater change in its permeability during inflammation compared with that of strontium.

The increased capillary permeability to cefazolin and the increased maximum extraction of cefazolin in infected bone may explain a previous report of increased uptake of cefazolin and other antibiotics in inflamed bone (31). It may be postulated that the increased extraction results from the increased permeability, and both may be due to the neovascularization associated with inflammation.

Once an antimicrobial agent leaves the vascular space, it is distributed in some or all of the various fluid spaces of bone, depending on the characteristics of the agent and the availability of the space. These spaces include the interstitial fluid space, the cellular space, and the water of the hydration shell of the crystalline structures of cortical bone. A thorough description of osseous fluid spaces and their measurement has been reported (P. J. Kelly, *In* F. M. Abboud and J. T. Shepherd, ed., *Handbook of Physiology: Peripheral Circulation and Organ Blood Flow*, in press).

The relative sizes of the fluid spaces in acute osteomyelitic bone measured in this study correlate closely with those of other studies in our laboratory (Quinlan et al., Fed. Proc. **39**:273, 1980). The erythrocyte space, V_{RBC}, increased from 0.0053 ml/ml of bone in normal cortical bone to 0.0160 ml/ml of bone in osteomyelitic cortical bone, an increase of 302%. The plasma

TABLE 7. Serum concentration of cefazolin by isotopic (S^I) and bioassay (S^B) methods and serum protein binding of cefazolin using ultrafiltration with isotopic (PB^I) and bioassay (PB^B) methods

Trial	S^I (μg/ml)	S^B (μg/ml)	PB^I (%)	PB^B (%)
1	32.8	28.7	38.4	49.5
2	41.9	31.6	31.8	33.5
3	49.0	40.0	36.7	34.5
4	58.3	44.0	37.5	38.9
5	65.8	54.0	34.5	37.4
Mean			35.8	38.8
SD			2.64	2.51
SEM			1.18	1.12

TABLE 8. Concentration of [^{14}C]cefazolin in infected (tibia)[a] and normal (radius) cortical bone

Concn of cefazolin in:	Mean	SD	SEM	No.
Infected bone	17.61	2.97	1.33	5
Normal bone	1.88	0.57	0.22	7

[a] Values represent the mean of the right and left sides of each dog and are given in micrograms of cefazolin per gram of bone.

TABLE 9. Concentration of [^{14}C]cefazolin (ISF_{Cfz}) in infected (tibia)[a] and normal (radius) bone and in serum

Concn of cefazolin in:	Mean	SD	SEM	No.
ISF_{Cfz} (infected bone)	50.09	6.91	3.09	5
ISF_{Cfz} (normal bone)	55.56	19.01	7.19	7
Serum	48.87	6.44	2.43	7

[a] Values represent the mean of the right and left sides of each dog and are given in micrograms per milliliter.

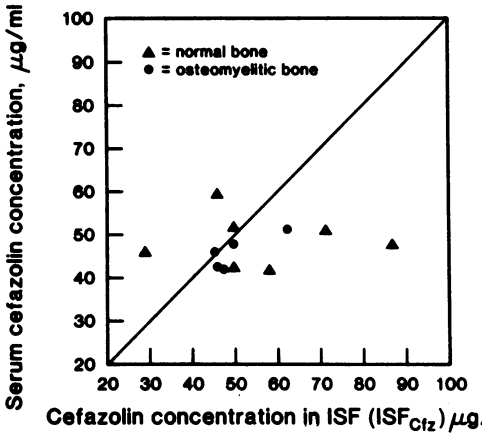

FIG. 1. Relationship of the serum concentration of cefazolin to the calculated interstitial fluid concentration of cefazolin in normal and osteomyelitic bone. There was a close correlation between the two concentrations except for two normal dogs, in which there was an apparent concentration of cefazolin in the interstitial fluid space.

space, V_P, increased from 0.0082 to 0.0271 ml/ml of bone, an increase of 330%. The vascular space, V_{BI}, increased from 0.0135 to 0.0431 ml/ml of bone, an increase of 319%; the extracellular space, V_E, increased from 0.0625 to 0.537 ml/ml of bone, an increase of 859%; and the interstitial fluid space, V_{ISF}, increased 941%, from 0.0542 to 0.510 ml/ml of bone.

The concentrations of cefazolin in normal bone determined by the bioassay or isotope assay outlined in this study are similar to those reported in other investigations (6, 28). However, these values bear little relationship to simultaneous serum concentrations (6, 27–29). This variance has supported the thesis that osseous tissue somehow inhibits the penetration of antibiotics. Since the bioassay of bone frequently requires crushing or grinding of osseous specimens before elution in a buffer solution or distilled water, the mechanical alteration of the chemical structure may account for the ability of this technique to recover from 38 to 84% of agents from standards (6, 23, 26, 27, 29). Although there are no known physiological phenomena which would inhibit the penetration of osseous tissue by antimicrobial agents, the variance of the different bioassay techniques forms the basis to suggest the superiority of one agent over another in the treatment or prevention of osseous infections (27–29).

Three observations from this study suggest that cefazolin is distributed within the interstitial fluid spaces of normal and osteomyelitic cortical bone in a concentration which is equivalent to the plasma concentration. First, the indicator-dilution studies indicate that cefazolin traversed the capillary membranes of bone. Second, the volume of distribution of cefazolin was equivalent to that of sucrose, which occupies the plasma and interstitial fluid spaces of normal and osteomyelitic bone, even though they were increased 330 and 941%, respectively, in osteomyelitic tissue. Third, the calculated concentrations of cefazolin in the interstitial fluid spaces of normal and osteomyelitic bone were equivalent even though the size of the two spaces varied by 941%.

The failure of bioassay techniques to relate the measured concentrations to the interstitial fluid space, which occupies a relatively small proportion of the volume of osseous tissue, accounts for the variance of osseous and serum concentrations. Once the actual size of the plasma and interstitial fluid spaces was determined, it was possible to convert the cefazolin concentration from micrograms per gram of bone to micrograms per milliliter of interstitial fluid space. Thus, we were able to define a close relationship between the serum and interstitial fluid space concentrations in all but two normal osseous specimens (Fig. 1). The reasons for the elevated levels of cefazolin in the interstitial fluid space of these two dogs are unknown. Since the extracellular fluid volumes and concentrations of cefazolin in the cortical bone were comparable to those of the other dogs, unidentified technical factors may have contributed to the elevated concentrations.

Since the portion of antibiotic bound to proteins is unavailable for antibacterial activity, it is important to consider protein binding when determining the concentration of an antibiotic attained in infected tissue (12, 21, 25, 31). Cefazolin binds to serum proteins to a different extent in dogs and humans. In humans, cefazolin is highly protein bound, with reports ranging from 73 to 92% (9, 10, 12, 13, 22, 24, 30, 31), whereas in dogs reports have varied from 32.5% (20) to 47% (13) to 80% (37). The results of this study indicate that serum protein binding of cefazolin in dogs is relatively low, 35.8% by isotope assay and 38.8% by bioassay.

The rate of diffusion of a drug out of the vascular space increases with the free serum concentration of the drug, whereas the opportunity for an equilibrium to be reached between serum and interstitial fluid increases with the serum half-life of the drug (25). This can be illustrated with cefazolin. Protein binding will tend to increase the serum half-life of cefazolin, which is eliminated by glomerular filtration and tubular secretion. Cefazolin has the highest sustained serum levels, the highest protein binding, and the greatest tissue penetration of the cepha-

losporins (10, 24). Although some physicians have considered cefazolin to be the drug of choice for prophylactic use in patients undergoing total hip arthroplasty, others question its prophylactic value since its rate of diffusion into the interstitial fluid space is relatively slow (5, 23, 35).

Since the osseous interstitial fluid space contains significant amounts of protein (18), the issue of concern to the clinician desiring adequate serum levels for prophylaxis or treatment of osteomyelitis is how the free antibiotic concentration in the interstitial fluid compares with that of the serum, assuming an adequate time and dosage schedule for equilibrium. Although previous investigators have demonstrated differences in the protein binding of antibiotics in interstitial fluid versus serum (12, 36–38), Rolinson (25) showed that the unbound serum concentration of a drug will equal the unbound concentration of the drug in interstitial fluid regardless of the relative protein binding. Since cefazolin appears to assume equal total concentrations in osseous interstitial fluid and serum, the percentage of protein-bound drug in these two fluid spaces is probably equal. On the other hand, if we assume that protein binding in the interstitial fluid is equal to that in the serum, the fact that the total concentration of cefazolin is the same in both indicates that the free concentration of cefazolin in interstitial fluid will equal that of serum.

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