

In Vitro and *In Vivo* Evaluation of Antibiotic Diffusion From Antibiotic-Impregnated Polymethylmethacrylate Beads

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The elution of antibiotics from antibiotic-impregnated polymethylmethacrylate (PMMA) beads was measured in mongrel dogs. The antibiotics, used in mixture with Simplex cement, included cefazolin (Ancef; 4.5 g/40 g cement powder), ciprofloxacin (Cipro; 6 g/40 g powder), clindamycin (Cleocin; 6 g/40 g powder), ticarcillin (Ticar; 12 g/40 g powder), tobramycin (Nebcin; 9.8 g/40 g powder), and vancomycin (Vancocin; 4 g/40 g powder). After a pneumatic drill was used to dredge a trough in the tibia, five beads were implanted. During the next 28 days, seroma samples and serum samples were taken for antibiotic measurements. On Day 28, the dogs were killed, beads removed, and the seroma, serum, bone, and granulation tissue sampled. The results of the study showed that clindamycin, vancomycin, and tobramycin exhibited good elution characteristics and had consistently high levels in bone and granulation tissue.

Local antibiotic delivery systems have improved the management of complex wounds in musculoskeletal surgery. When a trough

debridement is augmented with high-sustained local antibiotic concentration, the development of bone and soft tissue contamination into a regional infection may be prevented.³ At the authors' institution, antibiotic beads are used in the treatment of adult osteomyelitis to sterilize and temporarily maintain dead space after debridement surgery.^{3,5,6} The beads are surgically implanted in the debrided bone and covered with soft tissue. Serum, inflammatory fluid, and antibiotic collects in the space around the beads and is termed seroma. The antibiotics selected for bead delivery must be in powdered form and are selected to correspond to the sensitivities of the wound pathogens.

The diffusion of antibiotics from polymethylmethacrylate (PMMA) beads has been studied *in vitro* in distilled water,^{7,11,23} saline,¹⁶ serum,^{12,27,30} buffered solution,^{1,13,25,29,31} and liquid bacterial cultures.^{10,23,31} *In vivo* studies of various antibiotic-cement combinations have been performed in rabbits,^{4,24,25,32} dogs,^{11,17,28,29} rodents,^{8,21,30} and humans.^{2,8,13,28,29,30} The purpose of this study was to determine diffusion characteristics of the most frequently used antibiotics from PMMA beads, using *in vitro* and *in vivo* studies. Cefazolin (Ancef), ciprofloxacin (Cipro), clindamycin (Cleocin), ticarcillin (Ticar), tobramycin (Nebcin), and vancomycin (Vancocin) were studied.

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MATERIALS AND METHODS

The six antibiotics were each mixed with Simplex cement (Howmedica, Rutherford, New Jersey). The concentrations of antibiotic added to the cement powder were calculated to replicate the bead mixtures used in humans (cefazolin, 4.5 g/40 g powder; ciprofloxacin, 6 g/40 g powder; clindamycin, 6 g/40 g powder; ticarcillin, 12 g/40 g powder; tobramycin, 9.8 g/40 g powder; and vancomycin, 4 g/40 g powder). Methylmethacrylate liquid copolymer was added to the cement-antibiotic mixture and stirred. Half-spherical molds were used to form the cement dough into uniform, 9-mm-diameter beads. For the copolymer to evaporate, the beads were allowed to cure for several hours.

IN VITRO ANALYSIS

In vitro studies were performed in quadruplicate. One bead of each antibiotic cement combination was placed in 2.5 ml of normal dog serum and incubated at 37° for 24 hours. Every 24 hours, the beads were transferred to fresh aliquots of serum and incubated. The serum samples were stored at -70° until microbiologic disk diffusion assays for antibiotic concentration were performed. Aliquots of normal dog serum with beads that did not contain antibiotics served as the controls. All antibiotic concentrations were assayed using agar disk diffusion bioassays.²¹

Normal dog serum was chosen to closely approximate the fluid present in the wound seroma. Aliquots of normal dog serum and control beads in normal dog serum did not inhibit bacterial growth on the microbiologic assay.

Sterile liquefied antibiotic Medium 1 (Difco, Detroit, Michigan) was used for these studies. A 0.1-mm aliquot of *Bacillus subtilis* spore suspension (Difco) was added per 100 ml of the antibiotic Medium 1. Five milliliters of this seeded agar was aseptically pipetted into petri dishes (100 × 15 mm). The dishes were gently swirled to ensure uniform distribution of the agar. Standard twofold serial dilutions were made for all six antibiotics, beginning at 1000 µg/ml. Samples of normal dog serum, which previously tested negative for inhibition of *B. subtilis* and were then frozen, were used as the diluent for the standards. Twenty microliters of each standard was added to each of four sterile disks (6 mm; Difco), and these were plated to each of four seeded plates. Twenty microliters of each *in vitro* sample was added to each of four disks and placed on the seeded plates. The plates were incubated overnight at 37°C. The diameter of the zones of inhibition for each standard was measured and averaged. The means were drawn

onto semilog paper to obtain a standard curve for each antibiotic. The unknown concentration for the *in vitro* samples were determined by comparing their respective zone size means with the standard curve.

The bioassay for clindamycin differed slightly from that used for the other five antibiotics: To each 18 ml of sterile liquefied antibiotic Media 1, 0.4 ml of an overnight culture of *Sarcina lutea* (American Type Culture Collection ATCC No. 9341, Rockville, Maryland), was added. Eighteen milliliters of this seeded agar was aseptically pipetted into 150- × 15-mm plates and gently swirled to insure a uniform distribution. The remainder of the assay was performed as described for the other antibiotics.

IN VIVO ANALYSIS

Control and antibiotic-impregnated beads were implanted into the ventral aspect of the left tibia of large mongrel dogs (more than 18 kg). Three dogs were used for each antibiotic. Prior to use, the animals, which were all in good health, were treated for worms and vaccinated. After they were anesthetized with sodium pentobarbital (25 mg/kg of body weight), a preoperative serum sample was drawn for use as a control. Systemic perioperative antibiotics were not used. The left leg was aseptically cleaned and draped, and a longitudinal trough was fashioned in the proximal tibia, using a Midas Rex pneumatic burr (Midas Rex, Dallas, Texas). Cancellous bone was removed, and five beads were placed into each defect (Fig. 1). Each defect contained beads of only one antibiotic type or contained nonimpregnated cement. The deep periosteum, fascia, and skin were carefully approximated. No drains were placed in the incision. After surgery, serum samples were drawn at three, six, and 12 hours and on Days 1, 3, 9, 14, 21, and 28 for antibiotic determinations. Deep wound seroma aspirates were collected by percutaneous punctures on postoperative Days 1, 3, 9, 14, and 21, to determine bead seroma antibiotic concentrations. On Day 28, the dogs were killed, the seroma was aspirated under direct inspection, and the beads were removed. Samples of the granulation tissue encasing the beads and the adjacent cortical bone were harvested.

Seroma fluid was assayed as described for the *in vitro* studies. The granulation tissue taken from the surgical trough at autopsy was emulsified, suspended in equal parts of distilled water and serum, and stirred for two hours. This suspension was centrifuged and the supernatant was assayed for antibiotic concentrations per gram of tissue. The cortical bone samples were ground to a powder

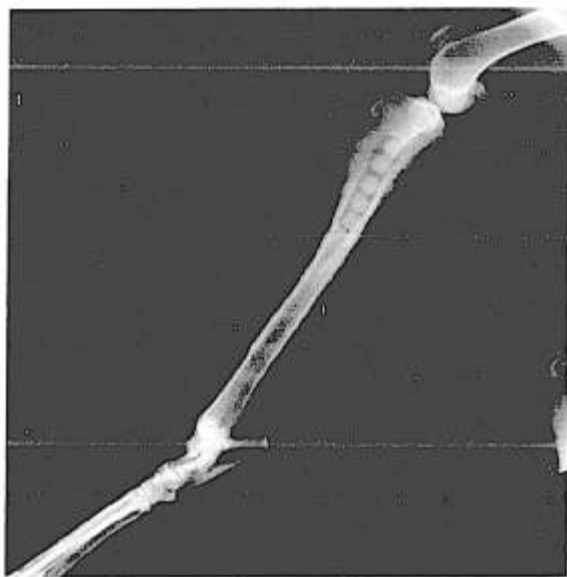


FIG. 1. Roentgenogram of the left tibia showing five antibiotic-impregnated polymethylmethacrylate (PMMA) beads placed in the surgical defect. Fluid accumulates around the implanted PMMA beads in the seroma cavity.

under refrigerated conditions (5°) and similarly processed in an identical manner to granulation tissue for assay. Control tissue and bone samples were collected from the contralateral tibial cortex, periosteum, and aponeurotic tendon.

RESULTS

IN VITRO MODEL

The *in vitro* results for each antibiotic are depicted in Figures 2 and 3. Cefazolin serum concentrations diffused from PMMA measured $250 \mu\text{g/ml}$ at 24 hours, $10 \mu\text{g/ml}$ on Day 7, and slowly decreased to $3 \mu\text{g/ml}$ on Day 28. Ciprofloxacin serum concentrations from PMMA measured $74.5 \mu\text{g/ml}$ at 24 hours, $11.5 \mu\text{g/ml}$ on Day 7, and slowly decreased to $5.2 \mu\text{g/ml}$ on Day 28. Serum concentrations from the clindamycin-impregnated PMMA beads measured $407 \mu\text{g/ml}$ at 24 hours, $115 \mu\text{g/ml}$ on Day 7, and

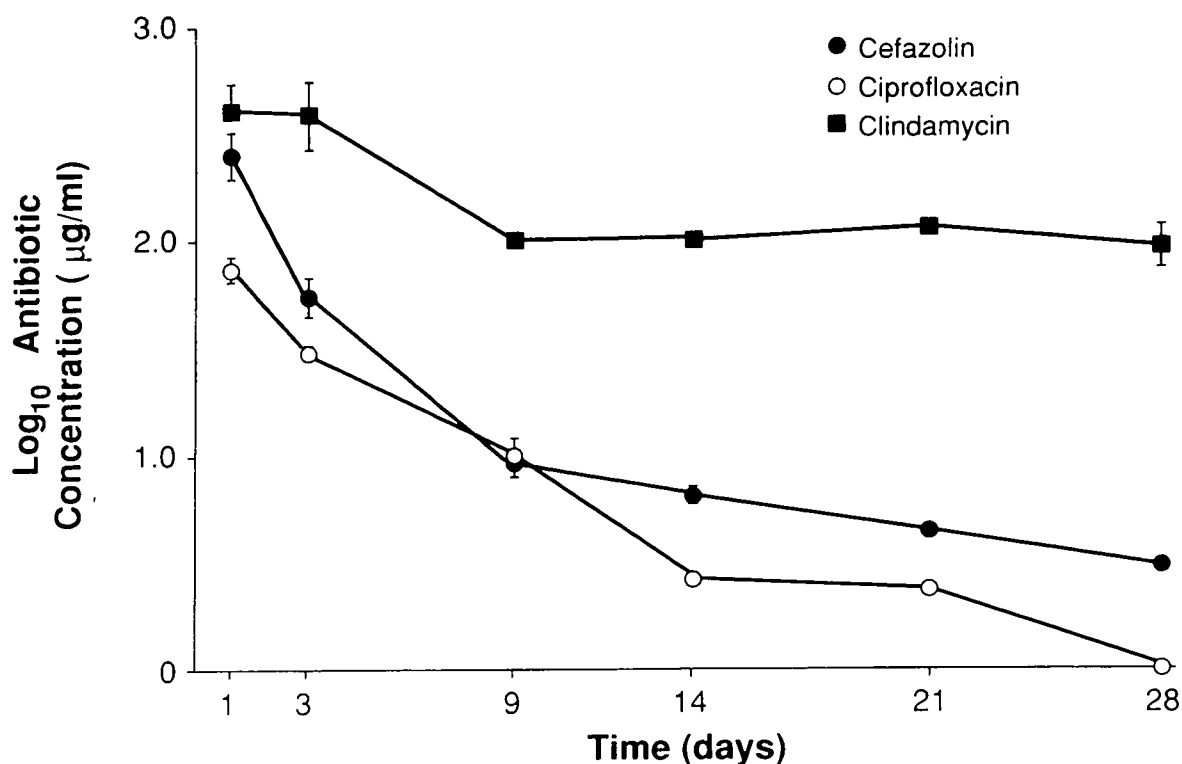


FIG. 2. *In vitro* cefazolin, ciprofloxacin, and clindamycin concentrations, each eluted from one PMMA bead. Each antibiotic study was performed in quadruplicate. Each point represents the mean; the brackets, \pm SEM.

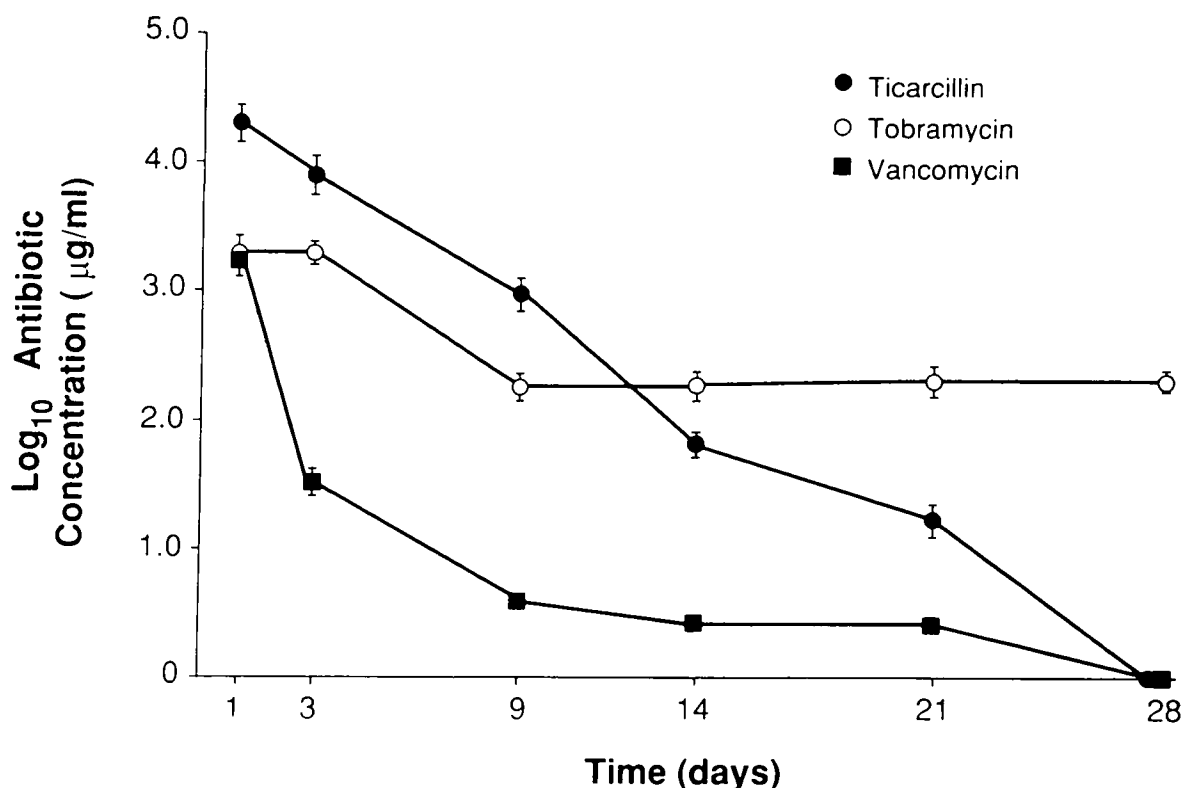


FIG. 3. *In vitro* ticarcillin, tobramycin, and vancomycin concentrations, each eluted from one PMMA bead. Each antibiotic study was performed in quadruplicate. Each point represents the mean, the brackets, \pm SEM.

decreased to 93 $\mu\text{g/ml}$ on Day 28. The serum concentrations measured from the ticarcillin PMMA beads measured 20,000 $\mu\text{g/ml}$ at 24 hours and decreased rapidly by Day 7 to 1000 $\mu\text{g/ml}$. By Day 28, there were no detectable ticarcillin concentrations. Tobramycin serum concentrations from PMMA were 2000 $\mu\text{g/ml}$ at 24 hours, 200 $\mu\text{g/ml}$ at Day 7, and stayed at 200 $\mu\text{g/ml}$ through Day 28. Vancomycin serum concentrations from PMMA were 1700 $\mu\text{g/ml}$ at 24 hours, 4 $\mu\text{g/ml}$ on Day seven, and 0 $\mu\text{g/ml}$ on Day 28.

IN VIVO MODEL

Results for each antibiotic, converted to \log^{10} , can be found in Figures 4–9. Seroma concentrations of cefazolin (Fig. 4) measured 153.3 $\mu\text{g/ml}$ at Day 1, 78.7 $\mu\text{g/ml}$ at Day 3, 53 $\mu\text{g/ml}$ at Day 9, 10.8 $\mu\text{g/ml}$ at Day 14, 0

$\mu\text{g/ml}$ at Day 21, and 0.7 $\mu\text{g/ml}$ at Day 28. Tissue and bone concentrations at autopsy measured 87.7 $\mu\text{g/ml}$ and 2.7 $\mu\text{g/ml}$, respectively.

Seroma concentrations of ciprofloxacin (Fig. 5) measured 7.5 $\mu\text{g/ml}$ at Day 1, 3.6 $\mu\text{g/ml}$ at Day 3, 0.9 $\mu\text{g/ml}$ at Day 9, 0.4 $\mu\text{g/ml}$ at Day 14, 0.2 $\mu\text{g/ml}$ at Day 21, and 0.1 $\mu\text{g/ml}$ at Day 28. Tissue and bone concentrations at autopsy measured 37.7 $\mu\text{g/ml}$ and 1.8 $\mu\text{g/ml}$, respectively.

Clindamycin seroma concentrations (Fig. 6) measured 1516.7 $\mu\text{g/ml}$ at Day 1, 1013.3 $\mu\text{g/ml}$ at Day 3, 102.2 $\mu\text{g/ml}$ at Day 9, 22.4 $\mu\text{g/ml}$ at Day 14, 0.3 $\mu\text{g/ml}$ at Day 21, and 36 $\mu\text{g/ml}$ at Day 28. The concentrations measured in tissue and bone were 33.7 $\mu\text{g/ml}$ and 29.6 $\mu\text{g/ml}$, respectively.

Ticarcillin seroma concentrations (Fig. 7) measured 6100 $\mu\text{g/ml}$ at Day 1, 5250 $\mu\text{g/ml}$

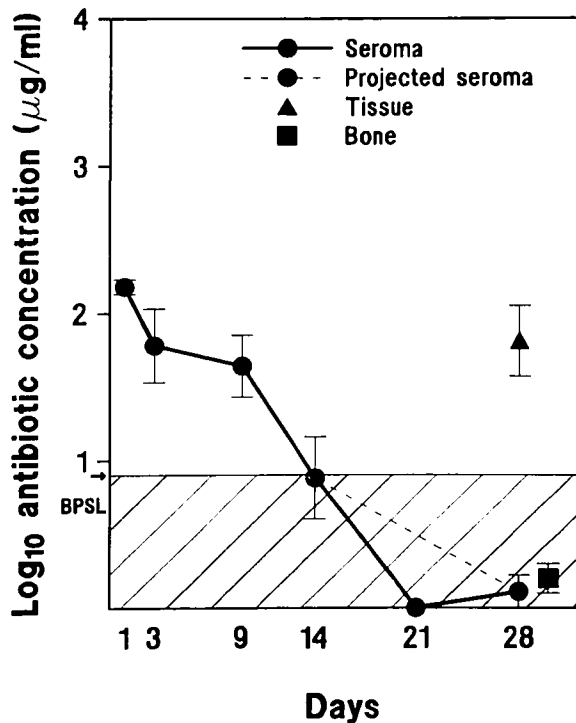


FIG. 4. Cefazolin concentrations found in the seroma fluid, granulation tissue, and bone. Each point represents the mean for three animals. The brackets indicate \pm SEM. The projected seroma concentrations between Day 14 and 28 are shown by the dashed line. The shaded area represents the break point sensitivity (BPSL) for cefazolin.

at Day 3, 460 $\mu\text{g/ml}$ at Day 9, 6.7 $\mu\text{g/ml}$ at Day 14, and dropped to 0 $\mu\text{g/ml}$ through Day 28. Tissue concentrations at autopsy measured 5.8 $\mu\text{g/ml}$, with no detectable concentrations found in bone.

Tobramycin seroma concentrations (Fig. 8) measured 93 $\mu\text{g/ml}$ at Day 1, 154.7 $\mu\text{g/ml}$ at Day 3, 107.7 $\mu\text{g/ml}$ at Day 9, 30.1 $\mu\text{g/ml}$ at Day 14, 4.3 $\mu\text{g/ml}$ at Day 21, and 3.9 $\mu\text{g/ml}$ at Day 28. At autopsy, the tissue concentrations measured 64.2 $\mu\text{g/ml}$, but no detectable concentrations were found in the bone.

Vancomycin seroma concentrations (Fig. 9) measured 10.2 $\mu\text{g/ml}$ at Day 1, 9.3 $\mu\text{g/ml}$ at Day 3, 3 $\mu\text{g/ml}$ at Day 9, 0.5 $\mu\text{g/ml}$ at Day 14, 1.6 $\mu\text{g/ml}$ at Day 21, and increased to 17.9 $\mu\text{g/ml}$ at Day 28. Tissue and bone concentrations measured 48.1 $\mu\text{g/ml}$ and 15.4 $\mu\text{g/ml}$, respectively.

There were no detectable serum concentrations at any time point for cefazolin, ciprofloxacin, clindamycin, or vancomycin. At three and six hours, tobramycin and ticarcillin were detected, but all measurements were well below breakpoint sensitivity and toxicity limits.

DISCUSSION

At the authors' institution, antibiotic-impregnated PMMA beads are used to sterilize and maintain dead space.^{3,5,6} Antibiotic-impregnated beads are usually placed at the first debridement surgery. Three to four weeks later, the beads are removed and the space is filled with cancellous bone graft.^{3,5,6} The antibiotic used in the PMMA beads should provide seroma concentrations above breakpoint sensitivities for three to four weeks and should achieve adequate granulation tissue

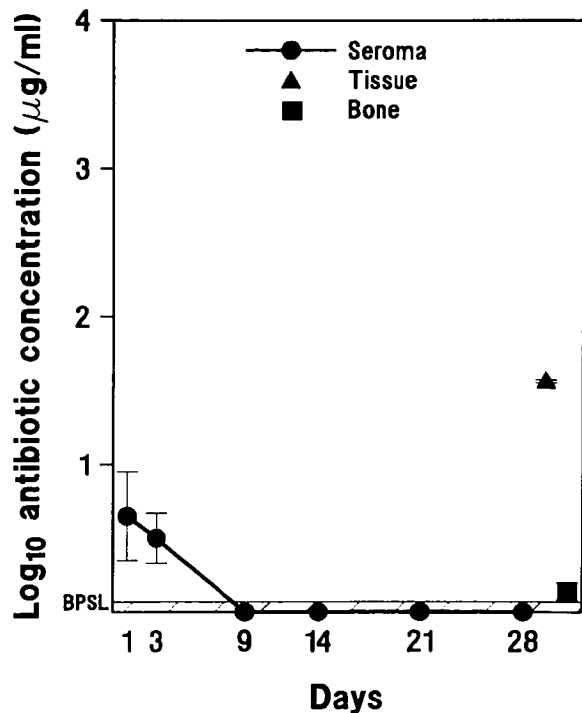


FIG. 5. Ciprofloxacin concentrations found in the seroma fluid, granulation tissue, and bone. Each point represents the mean for three animals. The brackets indicate \pm SEM. The shaded area represents the BPSL for ciprofloxacin.

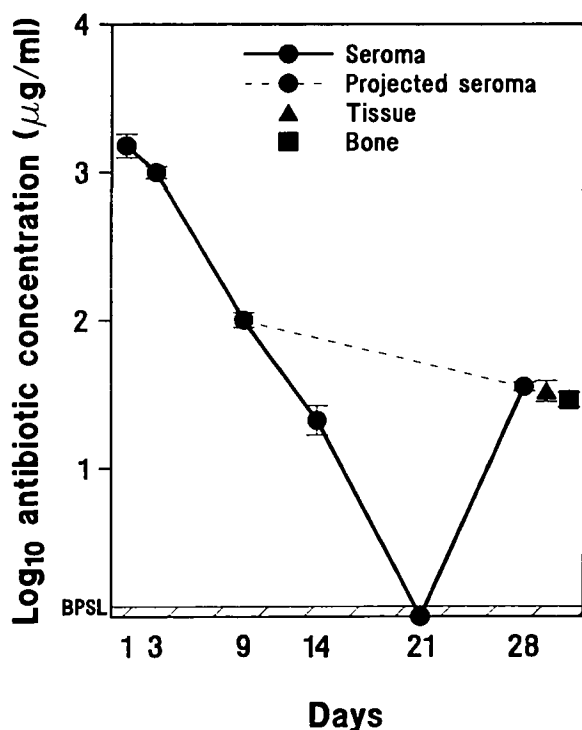


FIG. 6. Clindamycin concentrations found in the seroma fluid, granulation tissue, and bone. Each point represents the mean for three animals. The brackets indicate \pm SEM. The projected seroma concentrations between Day 9 and 28 are shown by the dashed line. The shaded area represents the BPSL for clindamycin.

and bone concentrations. The antibiotic should not produce toxic serum concentrations.

The results of this study show that high concentrations of antibiotics are eluted from antibiotic-impregnated acrylic beads. When the beads are formed in the dough phase, their surface-to-mass ratio favors low central core temperatures during polymerization. Essentially all the antibiotic mixed and transported in the acrylic remains biologically active.³³ The breakpoint sensitivity limits for the antibiotics used are as follows: cefazolin, 8 $\mu\text{g/ml}$; ciprofloxacin, 1 $\mu\text{g/ml}$; clindamycin, 1 $\mu\text{g/ml}$; ticarcillin, 64 $\mu\text{g/ml}$; tobramycin, 4 $\mu\text{g/ml}$; and vancomycin, 5 $\mu\text{g/ml}$.²¹ A breakpoint sensitivity limit designates the transition concentration between bacterial

killing and resistance to an antibiotic. In the *in vitro* studies, the only antibiotics that did not maintain concentrations above the breakpoint sensitivity concentrations through Day 28 were ticarcillin and vancomycin. Tobramycin beads sustained the highest *in vitro* concentrations throughout the 28-day study.

In vivo, high concentrations of antibiotics are generated in the seroma fluid, granulation tissue, and bone surrounding antibiotic-impregnated acrylic beads. Each *in vivo* antibiotic diffusion profile was similar to its respective *in vitro* profile. The antibiotic concentrations are not comparable, however, because the *in vitro* results represent one bead in 1 ml serum, and the *in vivo* results represent five beads in an unknown amount of seroma fluid, which probably varied over time. Because granulation tissue filled the seroma cavity after Day 9, seroma fluid became

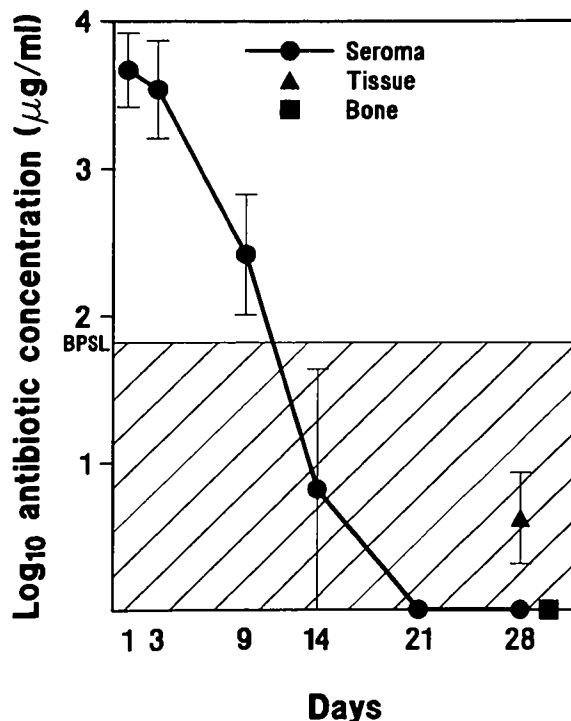


FIG. 7. Ticarcillin concentrations found in the seroma fluid, granulation tissue, and bone. Each point represents the mean for three animals. The brackets indicate \pm SEM. The shaded area represents the BPSL for ticarcillin.

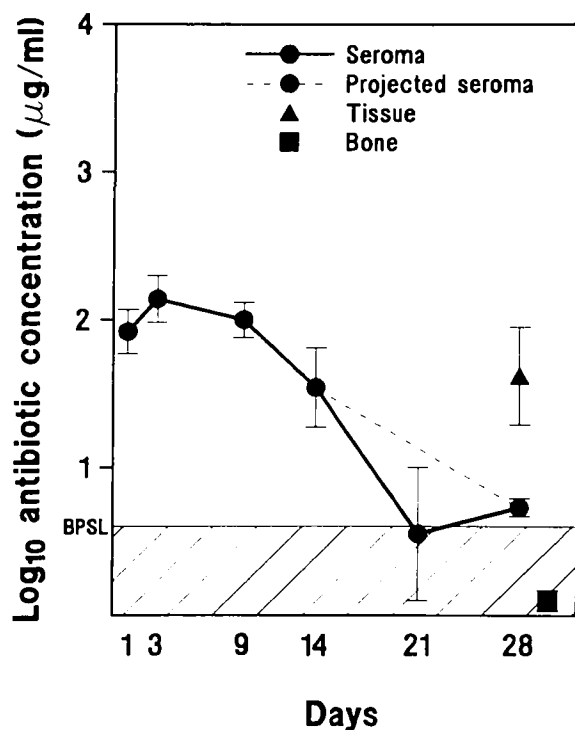


FIG. 8. Tobramycin concentrations found in the seroma fluid, granulation tissue, and bone. Each point represents the mean for three animals. The brackets indicate \pm SEM. The projected seroma concentrations between Day 14 and 28 are shown by the dashed line. The shaded area represents the BPSL for tobramycin.

scarce, making seroma fluid sampling difficult and antibiotic determinations unreliable. At death, however, the seroma cavity was visualized through an incision in the skin without disturbing the underlying subcutaneous tissue. This enabled the seroma fluid to be completely removed and assayed. Even though there was no significant rise in the concentrations of cefazolin, ciprofloxacin, or ticarcillin at Day 28, the increased concentrations of clindamycin, tobramycin, and vancomycin in the seroma fluid at Day 28 could relate also to a disturbance of granulation tissue at the time of death, leading to a release of drug into the seroma fluid.

Granulation tissue antibiotic concentrations were high for all the antibiotics evaluated, except ticarcillin. Vancomycin and

clindamycin were the only two antibiotics to achieve high concentrations in cortical bone. With the exception of tobramycin and ticarcillin at three and six hours after bead placement, serum antibiotic concentrations were undetectable. However, these tobramycin and ticarcillin serum concentrations were below the breakpoint sensitivity limits. The serum concentration of tobramycin was also lower than the toxic trough concentration of $2 \mu\text{g/ml}$. It has been the authors' clinical experience that the implantation of eight or more beads results in detectable, but low, serum antibiotic concentrations with vancomycin and tobramycin.^{3,5}

Pharmacologically, clindamycin was the best antibiotic used in these PMMA bead studies. Clindamycin demonstrated high ser-

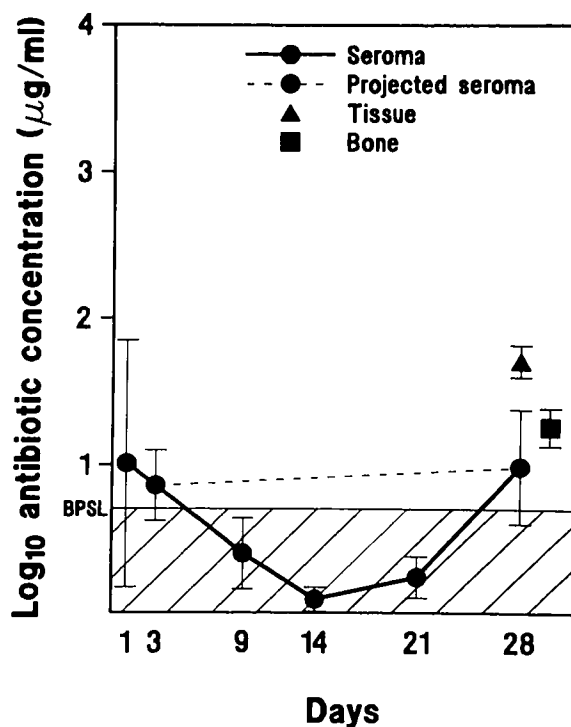


FIG. 9. Vancomycin concentrations found in the seroma fluid, granulation tissue, and bone. Each point represents the mean for three animals. The brackets indicate \pm SEM. The projected seroma concentrations between Day 3 and 28 are shown by the dashed line. The shaded area represents the BPSL for cefazolin.

oma, granulation tissue, and bone concentrations. The use of clindamycin in beads is attractive because of its action on *S. aureus*, *S. epidermidis*, nonenterococcal streptococcus species, and anaerobes.^{14,15} In addition, clindamycin may reduce glycocalyx formation.^{18,19} Glycocalyx reduction facilitates antibiotic and phagocytic cell access to bacterial organisms that produce glycocalyx, thus suppressing infection by these organisms.^{9,20,22,26}

Although tobramycin and vancomycin produced good results, each had a deficiency. Tobramycin produced excellent seroma and granulation tissue concentrations but exhibited low bone concentrations; vancomycin provided poor seroma concentrations but produced excellent granulation tissue and bone concentrations. Sampling difficulties could have led to lower vancomycin seroma concentrations Days 9–21.

In these studies, beads impregnated with cefazolin, ciprofloxacin, and ticarcillin showed unfavorable characteristics. All three antibiotics failed to maintain seroma concentrations above breakpoint sensitivities for more than two weeks, and all had low bone concentrations. Cefazolin and ciprofloxacin, however, did produce good granulation tissue concentrations.

Although antibiotic-impregnated beads have not proved superior to other methods used to manage dead space, including local and free muscle flaps, cancellous bone grafting, and primary closure,^{3,5,6} antibiotic beads allow the surgeon to maintain dead space until definite dead space management can be achieved.³ This form of dead space management may allow the dead space to mature, and may convert a long, complicated surgical procedure into two shorter and technically easier procedures.⁵ Along with good debridement surgery, beads can be used to maintain dead space temporarily, until a definitive surgical procedure is performed. Antibiotic-impregnated beads appear to be a safe method of obtaining higher-than-normal concentrations of antibiotics in closed seromas.

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