(2) J. T. Doluisio, N. F. Billups, L. W. Dittert, E. T. Sugita, and J. F. Swintosky, *J. Pharm. Sci.*, **58**, 1196 (1969).
(3) J. Y. Park, N. F. H. Ho, and W. Morozowich, *J. Pharm. Sci.*, **73**, 1588.
(4) N. F. H. Ho, J. Park, W. Morozowich, and W. I. Higuchi, *J. Theor. Biol.*, **61**, 185 (1976).
(5) M. Hamberg and B. Samuelsson, *J. Biol. Chem.*, **241**, 257 (1966).
(6) K. Desai, Ph.D. thesis, University of Michigan, Ann Arbor, Mich., 1976.

ACKNOWLEDGMENTS

The authors thank Dr. R. S. Hsi, The Upjohn Co., for the generous supply of [^{3}H]dinoprostone.

Influence of Mode of Intravenous Administration and Blood Sample Collection on Rat Pharmacokinetic Data

FRANCIS L. S. TSE ˣ, TSAILING CHANG, BARBARA FINKELSTEIN, FRANCES BALLARD, and JAMES M. JAFFE

Received June 13, 1983, from the *Drug Metabolism Section, Sandoz, Inc., East Hanover, NJ 07936.* Accepted for publication February 16, 1984.

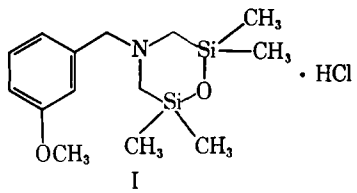
Abstract □ The influence of the mode of intravenous dosing and blood sample collection on the pharmacokinetics of 4-[(3-methoxyphenyl)-methyl]-2,2,6,6-tetramethyl-1-oxa-4-aza-2,6-disilacyclohexane hydrochloride (I) was studied in the rat. Blood samples obtained from the tail and by exsanguination following injection of the ^{14}C-labeled drug into the caudal vein, the jugular vein, and the heart were analyzed for total radioactivity, and the concentration profiles from the different treatments were compared. Dosing and sampling from the tail vein resulted in significantly different blood levels (and related pharmacokinetic parameters) when compared to other methods, probably attributable to a local depot effect. Intracardiac administration tended to cause higher drug levels in the heart than intravenous doses, although no significant differences were found between the respective blood concentrations. The results showed that caudal vein injection is a simple and adequate method of intravenous administration in rats designated for exsanguinated blood and tissue collection. For serial blood sampling in individual animals, the dose may be given *via* the jugular vein and the blood collected from the cut tail. These methods require little or no surgical preparations and are particularly suitable for prolonged sampling in studies where a relatively large number of animals are involved.

Keyphrases □ Pharmacokinetics—influence of mode of intravenous administration and blood sample collection, rat □ Dosing—pharmacokinetics, mode of intravenous administration, blood sample collection

During the developmental process of a potential therapeutic agent, pharmacokinetic studies are often conducted at the same time as toxicity trials. While the oral route of administration is invariably used in these studies in order to be consistent with the toxicity tests, additional experiments employing intravenous doses are usually performed so that important pharmacokinetic parameters such as the volume of distribution and absolute oral bioavailability or absorption can be determined. In the rat, the caudal vein is often used as the site of injection. In contrast, injection *via* the jugular or femoral vein requires surgical exposure of the vein (1) and can be cumbersome in studies involving a large number of animals. Drugs are also administered by simple cardiac puncture, but the results may not be truly representative of an intravenous dose. Potential complications such as embolization and excessive local drug toxicity are further limitations of the intracardiac route. Intraperitoneal injection, commonly used for the administration of compounds to small animals, is different from intravenous dosing in that drug absorption from the peritoneal cavity is slower (2) and entails passage into the portal circulation, resulting in incomplete systemic bioavailability (3).

In recent years, numerous sensitive analytical methods that require only microsamples of blood have been developed, and pharmacokinetic data from individual rats or similar small animals can be obtained by serial sample collection. Several methods using a chronic indwelling catheter to facilitate repeated blood collection have been described (4–6), but the animal preparation procedures are elaborate and tedious and are incompatible with prolonged sampling periods in studies involving a large number of animals. Other methods of vascular access and their potential complications have been described in a recent review (7). A simple alternative to cannulation is the nonsurgical method of bleeding the rat from the cut end of its tail. However, due to the relatively low regional blood flow (8, 9), which could result in delayed mixing of the administered drug, the validity of tail concentration data remains unclear.

Using a new skeletal muscle relaxant, 4-[(3-methoxyphenyl)-methyl]-2,2,6,6-tetramethyl-1-oxa-4-aza-2,6-disilacyclohexane hydrochloride[1] (I), as a test compound, the present study was undertaken to examine the effect of the mode of intravenous dosing and blood sample collection on pharmacokinetic data in the rat. Blood was obtained from the tail and by exsanguination following injection of the ^{14}C-labeled drug into the caudal vein, the jugular vein, and the heart. Radioactivity concentration in the heart tissue after intracardiac administration was also measured for comparison with existing intravenous data.



I

EXPERIMENTAL SECTION

Dose Administration and Sample Collection—Male Sprague–Dawley rats, average weight 250 g, were used. They were housed individually in standard cages and had free access to food and water at all times. The rats were divided randomly into three groups of 24 each for drug administration *via* the caudal vein, the jugular vein, and the heart.

Radioactive I was labeled with carbon-14 at the methylene group of the

[1] Compound 58-112 hydrochloride; Sandoz, Inc., East Hanover, N.J.

0022-3549/84/1100-1599$01.00/0

Table I—Summary of Treatments

Treatment	Route of Dosing/Sampling	Dose, mg/kg	No. of Rats
1	Caudal Vein/Tail	10	3
2	Caudal Vein/Vena Cava	10	3/time point
3	Jugular Vein/Tail	10	3
4	Jugular Vein/Vena Cava	10	3/time point
5	Intracardiac/Tail	5	3
6	Intracardiac/Vena Cava	5	3/time point

benzyl moiety. The radiochemical purity of the product was established by the inverse isotope dilution method to be >95%, and the specific activity was 3.25 μCi/mg. The dose was prepared as a 10 mg/mL solution in water. For the intravenous doses (10 mg/kg), each rat received 1 mL of the solution/kg body weight. For injection into the caudal vein, the tail was first immersed for 1–2 min in warm water; for jugular vein dosing, the rats were maintained under light ether anesthesia and the vein was exposed by a small skin incision made in the neck to one side of midline. For intracardiac administration, the dose was reduced from 10 to 5 mg/kg due to frequent deaths at the high dose level. Thus, each rat received 1 mL/kg of the solution (5 mg/mL in normal saline) *via* a single cardiac puncture.

Serial blood samples (250 μL) were collected in heparinized micropipettes[2] from three rats in each dose group *via* the cut tail immediately before and at 5, 15, and 30 min and 1, 3, 5, and 8 h postdose. Of the remaining 21 rats three each were sacrificed at 5, 15, and 30 min and 1, 3, 5, and 8 h postdose. Blood (~5 mL) was collected *via* the vena cava. The heart of each sacrificed rat receiving the intracardiac dose was collected (Table I).

Analysis of Radioactivity—Aliquots of dose preparations, blood, and heart tissue homogenates were air dried and combusted in a sample oxidizer[3] using 9 mL of absorption medium[4] and 12 mL of scintillation cocktail[5]. Radioactivity was measured in a liquid scintillation counter[6]. The quench correction and efficiency of the counter were determined from a standard curve constructed using ^{14}C-labeled hexadecane[7] of known specific activity. The limit of sensitivity of the assay was defined as twice the mean background count. Gross sample counts below this level were considered to be not significantly different from zero. Blood and tissue concentrations of radioactivity were calculated as nanogram equivalents of I per milliliter or gram.

Treatment of Data—The dose-normalized blood levels of radioactivity at each sampling time were examined by analysis of variance. Because of the observed differences in data variance between groups, the statistical tests were performed on log-transformed data (10). If differences ($p < 0.05$) due to treatments were observed, results from individual treatments were compared by the Student's t test.

Since, in half of the experiments, blood at each sampling time was collected from different (sacrificed) rats, only mean concentration data were available for pharmacokinetic analysis. For consistency, only mean blood levels from the serially bled animals were used for this purpose. The blood concentration data were fitted to a biexponential function of the form:

$$C = Ae^{-\alpha t} + Be^{-\beta t} \quad (\alpha > \beta) \qquad \text{(Eq. 1)}$$

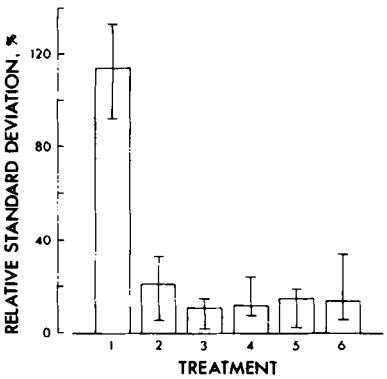


Figure 1—RSD *in blood radioactivity levels following all treatments. Each bar represents the median and range of seven time point observations.*

[2] Microcaps; Drummond Scientific Co., Broomall, Pa.
[3] Model 306; Packard Instrument Co., Downers Grove, Ill.
[4] Carbosorb II; Packard Instrument Co.,
[5] Permafluor V; Packard Instrument Co.
[6] Packard Tri-Carb 460; Packard Instrument Co.
[7] Packard Instrument Co.

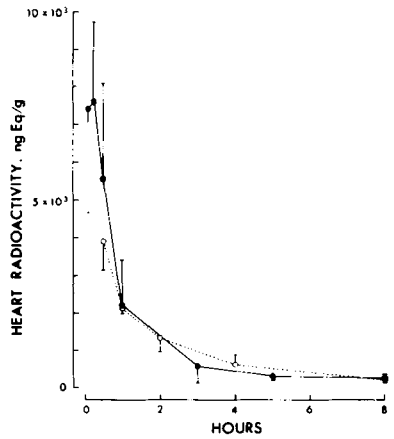


Figure 2—*Mean concentrations of radioactivity in the heart following caudal vein (○) 10 mg/kg, and intracardiac (●), 5 mg/kg injections. Each bar represents one* SD. *The data for caudal vein injection are adapted from Bhuta*[9].

where C is the blood concentration of radioactivity at time, t, A and B are constants, and α and β are composite first-order rate constants. Attempts to fit the data to a triexponential function resulted in greater errors in parameter values with no increase in coefficients of determination. Initial estimates of pharmacokinetic parameters were obtained by standard graphic methods (11) while improved parameter estimates together with statistical analysis were obtained using the iterative nonlinear regression program NONLIN (12) on a computer[8]. The blood level data were weighted by their reciprocals in the regression analysis.

RESULTS

Mean blood levels of radioactivity, together with statistical analyses, are given in Table II. Results of pharmacokinetic analyses are given in Table III. The goodness of fit of the data to Eq. 1 was demonstrated by correlation coefficients of 0.97–1.00.

Comparison of the blood radioactivity levels showed that dosing and sampling, both from the tail vein (treatment 1), resulted in elevated blood level measurements throughout the 8 h sampling period, although the differences were statistically significant only at 0.083, 0.25, and 8 h. Treatment 1 also had a greater interanimal variability in blood levels, as indicated by the large *RSD* values (coefficients of variation) illustrated in Fig. 1. Comparison of the results from treatments 3 *versus* 4 and treatments 5 *versus* 6 also suggested that blood sampling near the site of drug administration yielded higher initial concentrations than at a more distal sampling site, although the differences were not statistically significant.

From Table III, analysis of blood level data obtained from all six treatments yielded similar distribution half-lives of 15–30 min. The elimination half-lives for treatments 2–5 were ~5 h, identical with those observed in previous experiments in which the drug was administered *via* the tail vein and blood was collected from the vena cava for 24 h (~5 half-lives)[9]. Thus, the 8-h sampling period employed in the present study was sufficient to describe the terminal linear phase. For treatments 1 and 6, the half-lives appeared longer. Because of the higher tail blood concentrations obtained following the caudal vein dose (treatment 1), the area under the curve value was almost 10 times greater than those observed for the other treatments. Accordingly, treatment 1 also resulted in markedly reduced plasma clearance and volumes of distribution.

During the intracardiac dosing experiments, it was necessary to reduce the dose from 10 to 5 mg/kg because of frequent deaths occurring at the high dose level, apparently due to elevated initial drug concentrations in the heart. This was confirmed by comparing the heart radioactivity concentration obtained following the intracardiac injection to existing data[9] measured after dosing *via* the caudal vein (Fig. 2). Despite a twofold difference in dose level, the 10-mg/kg caudal vein dose and the 5-mg/kg intracardiac dose gave similar radioactivity concentration in the heart at all times.

DISCUSSION

Assuming equal metabolic fate for the drug administered *via* the caudal vein, the jugular vein, and the heart, the blood concentration data of total radioactivity alone would generate sufficient information to answer the

[8] 4341; IBM.
[9] S. I. Bhuta, unpublished results.

Table II—Blood Levels of Radioactivity Obtained from All Treatments, Mean ± *SD*

Treatment	Dose, mg/kg[a]	Blood Concentration of I Equivalents, ng Eq/mL						
		0.083 h	0.25 h	0.5 h	1 h	3 h	5 h	8 h
1	10	23370 ± 21590	23140 ± 29250	12710 ± 16850	5020 ± 5380	4460 ± 5550	3060 ± 3490	2010 ± 2100
2	10	2310 ± 430	1860 ± 380	1160 ± 380	990 ± 210	540 ± 30	440 ± 100	310 ± 90
3	10	1780 ± 340	1420 ± 90	1150 ± 20	980 ± 50	510 ± 70	420 ± 50	280 ± 30
4	10	2260 ± 170	1940 ± 190	1420 ± 140	890 ± 110	500 ± 100	340 ± 80	200 ± 40
5	5	970 ± 160	880 ± 130	665 ± 125	570 ± 65	360 ± 25	285 ± 45	205 ± 5
6	5	1590 ± 95	1785 ± 610	1235 ± 170	625 ± 60	320 ± 85	290 ± 60	175 ± 15
t test among treatments[b]		1 > 2-6	1 > 2-5	NSD[c]	NSD[c]	NSD[c]	NSD[c]	1 > 2-6

[a] For statistical comparisons only, the raw data were dose-normalized and log-transformed. [b] Significant at $p < 0.05$. [c] No significant differences by analysis of variance.

Table III—Pharmacokinetic Parameters Obtained from All Treatments

Parameter[a]	Treatment					
	1	2	3	4	5[b]	6[b]
D, mg/kg	10	10	10	10	5	5
α, h^{-1}	2.4	2.8	1.6	2.0	1.8	1.5
	(−0.81 − 5.6)[c]	(0.11 − 5.5)	(0.012 − 3.2)	(1.2 − 2.8)	(0.29 − 3.3)	(−0.61 − 3.5)
β, h^{-1}	0.086	0.14	0.13	0.17	0.12	0.091
	(−0.32 − 0.50)	(0.22 − 0.27)	(0.011 − 0.25)	(0.093 − 0.25)	(0.042 − 0.19)	(−0.31 − 0.49)
$t_{1/2,\alpha}$, h	0.29	0.25	0.43	0.35	0.38	0.48
$t_{1/2,\beta}$, h	8.1	5.0	5.3	4.1	5.8	7.6
AUC, ng Eq·h/mL	62812	6925	6658	5622	4577	5416
CL_p, L/h/kg	0.16	1.4	1.5	1.8	1.1	0.92
Vd_1, L/kg	0.33	3.7	5.4	3.9	4.7	2.5
Vd_β, L/kg	1.9	10	12	11	9.1	10

[a] D, dose of I; α and β, composite first-order rate constants; $t_{1/2,\alpha}$, half-life of distribution = $0.693/\alpha$; $t_{1/2,\beta}$, half-life of elimination = $0.693/\beta$; AUC, area under concentration *versus* time curve = $A/\alpha + B/\beta$; CL_p, plasma clearance = D/AUC; Vd_1, apparent distribution volume of the central compartment in a two-compartment model = $D/(A + B)$; Vd_β, apparent overall distribution volume = CL_p/β. [b] For comparison between treatments, the data must be dose-normalized by doubling the values of D and AUC for treatments 5 and 6. [c] 95% Confidence interval.

questions outlined in the present study. By comparing treatment 1 *versus* 2, treatment 3 *versus* 4, and treatment 5 *versus* 6, the data seem to indicate that blood sampling near the site of drug administration yielded higher initial concentrations than at a more distal sampling site. Nonetheless, tail blood sampling after caudal vein administration (treatment 1) is the only treatment in this study that gave significantly different blood radioactivity levels and related pharmacokinetic parameters. Evidently, dosing *via* the caudal vein results in some residual drug at and/or near the injection site, so that sampling from the same location leads to elevated drug concentrations which are artifacts and which probably do not represent the circulating concentrations in blood. This phenomenon occurred despite the choice of a compound for this study with relatively high aqueous solubility. Furthermore, because it is technically difficult to uniformly dose *via* the tail vein (1), the magnitude of this depot effect can be quite variable, as demonstrated by the large interanimal differences in the blood levels measured for this group (Fig. 1).

However, the artifact which results from high concentrations after caudal vein dosing can be avoided if blood samples are obtained from an appropriate vein such as the vena cava, since the residual drug at the injection site probably represents a minute portion of the dose. Conversely, if the dose is administered *via* a different (*e.g.*, jugular) vein, the tail blood data seem to reflect true kinetics in the rat. These deductions are based on the close similarities in blood levels as well as pharmacokinetic parameters between treatments 2 and 3 and treatment 4. Treatment 4 concerns blood collected from the vena cava following drug administration *via* a major (jugular) vein and, therefore, is believed to provide data which most accurately describe the pharmacokinetics of the drug. From the results of treatments 2 and 4, it can also be deduced that the application of brief ether anesthesia in the jugular vein dosing procedure has no effect on the blood levels and pharmacokinetic parameters of the drug. Previous investigators (13) have reported that ether may interfere with hepatic drug metabolism and should not be used as an anesthetic in kinetic studies of drugs with short elimination half-lives (~15 min), but may be used for drugs with longer half-lives (>1.2 h).

The results of this study also demonstrate the potential differences between intravenous and intracardiac drug administration. After intracardiac dosing, substantial tissue uptake of drug occurred in the heart, resulting in excessive local drug toxicity at the 10-mg/kg dose level. However, the overall amount of drug reaching the systemic circulation appears comparable to that after the intravenous doses, as indicated by the similar blood radioactivity profiles obtained from treatments 2–4, and treatments 5 and 6, after normalizing by the doses.

In summary, dosing and subsequent sampling from the tail vein results in artifactual and variable blood concentration data due probably to a local depot effect. Although intracardiac injection appears to give blood levels similar to those obtained from true intravenous doses, the former tends to cause substantial tissue uptake of drug in the heart and should not be used in tissue distribution studies. For experiments employing a relatively large number of rats designated for exsanguinated blood and tissue collection, caudal vein injection is a convenient and appropriate method of intravenous dosing. On the other hand, if serial blood samples are needed to establish pharmacokinetic profiles in individual animals, they can be obtained simply from the cut tail after administering the dose *via* the jugular vein. Although injection *via* the jugular vein requires surgical preparation of the rat, it appears to be one of the more reliable methods for intravenous drug administration and, unlike most cannulation techiques, imposes no stress which may affect at least some aspects of normal physiology resulting in altered drug kinetics (7, 9, 14, 15).

Although these findings are probably applicable to a variety of drugs, the magnitude of the observed differences could be dependent upon the physicochemical properties of the individual compounds. Thus, further studies using additional compounds with significantly different chemical properties are warranted in order to evaluate the general applicability of these results.

REFERENCES

(1) H. B. Waynforth, "Experimental and Surgical Technique in the Rat," Academic, London, 1980, pp. 39–42.

(2) F. L. S. Tse and P. G. Welling, *J. Parenteral Drug Assoc.*, **34,** 484 (1980).

(3) G. Lukas, S. D. Brindle, and P. Greengard, *J. Pharmacol. Exp. Ther.*, **178,** 562 (1971).

(4) R. S. Pope, *J. Appl. Physiol.*, **24,** 276 (1968).

(5) B. Scharschmidt and P. D. Berk, *Proc. Soc. Exp. Biol. Med.*, **143,** 364 (1973).

(6) R. A. Upton, *J. Pharm. Sci.*, **64,** 112 (1975).

(7) D. M. Cocchetto and T. D. Bjornsson, *J. Pharm. Sci.*, **72,** 465 (1983).

(8) R. P. Rand, A. C. Burton, and T. Ing, *Can. J. Physiol. Pharmacol.*, **43,** 257 (1965).

(9) W. M. Johannessen, I. M. Tyssebotn, and J. Aarbakke, *J. Pharm. Sci.*, **71,** 1352 (1982).

(10) B. J. Winer, "Statistical Principles in Experimental Design," McGraw-Hill, New York, N.Y., 1971, p. 397.

(11) J. G. Wagner, "Fundamentals of Clinical Pharmacokinetics," 1st ed., Drug Intelligence, Hamilton, Ill., 1975, pp. 59–63.

(12) C. M. Metzler, G. L. Elfring, and A. J. McEwen, *Biometrics*, **30,** 562 (1974).

(13) W. Johannessen, G. Gadeholt, and J. Aarbakke, *J. Pharm. Pharmacol.*, **33,** 365 (1981).

(14) W. F. Bousquet, B. D. Rupe, and T. S. Miya, *J. Pharmacol. Exp. Ther.*, **147**, 376 (1965).
(15) R. E. Stitzel and R. L. Furner, *Biochem. Pharmacol.*, **16**, 1489 (1967).

ACKNOWLEDGMENTS

The authors thank Mr. Kenrick Talbot for preparing the radioactive drug, Ms. Constance Farley for technical assistance, and Mr. Edward Barry for helpful discussions.

Nonaqueous Cephalosporin Suspension for Parenteral Administration: Cefazolin Sodium

KENNETH S. E. SU ˣ, JOHN F. QUAY, KRISTINA M. CAMPANALE, and JOHN F. STUCKY

Received December 16, 1983, from *Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.* Accepted for publication February 24, 1984.

Abstract □ The flocculation-deflocculation behavior of cefazolin sodium (I) in nonaqueous media and the effect of surfactants as measured by zeta potential, sedimentation, and porosity were studied. A significant difference in zeta potential was observed when the particles were suspended in different nonaqueous media. The addition of surfactant produced a deflocculated state. The surfactant deflocculated the particles by a process of supersaturation and crystallization involving a surfactant-cefazolin complex. The shielding effect of the surfactant on the surface of the particles also apparently affected their electrophoretic properties. Kinetic studies on the stability of the drug as a function of temperature were conducted; it appears that the chemical stability in ethyl oleate at room temperature is adequate for a reasonable shelf life. The efficiency of absorption of the drug from the ethyl oleate suspension was evaluated after intramuscular administration in dogs. The area under the plasma concentration *versus* time curve and urinary recovery indicated that cefazolin was 100% bioavailable from this nonaqueous preparation.

Keyphrases □ Cefazolin sodium—surfactants, stability, bioavailability □ Cephalosporin—nonaqueous suspension, stability, bioavailability

Parenteral pharmaceutical suspensions have been used for a long time (1–4). In general, this dosage form is comprised of a physiologically active agent and a vehicle. The vehicle is typically comprised of a suspending liquid, an emulsifying agent, a surfactant, density- and viscosity-adjusting substances, and preservatives. While water is generally the preferred suspending liquid, some physiologically active agents such as the cephalosporin antibiotics are not chemically stable in water-based parenteral pharmaceutical suspensions. Therefore, to achieve a ready-to-use cephalosporin preparation which can be stored at room temperature, it is desirable to develop a satisfactory suspension utilizing a nonaqueous liquid as the suspending medium. This paper describes the preparation of a unique, simple flocculated ready-to-use cefazolin sodium suspension which is dispersed in a nonaqueous medium. The efficiency of absorption of cefazolin from the nonaqueous system administered intramuscularly in dogs is also reported.

Table I—Flocculation-Deflocculation Behavior of Cefazolin Sodium in Peanut Oil [a]

Conc. of Polysorbate 80, %	Sedimentation Volume, H_u/H_o %[b]	Number of Turns to Redisperse
0	48	268
0.17	87	>3500
0.50	82	∞
3.30	63	∞

[a] In the presence of polysorbate 80. [b] Ultimate settled height (H_u) is based on the sedimentation height on day 33.

EXPERIMENTAL SECTION

The following chemicals were used: sterile micronized cefazolin sodium[1]; polysorbate 80, commercial grade[2]; lecithin[3]; ethyl oleate[4]; peanut oil[5]; cefazolin sodium[6]. For *in vivo* studies, sterile micronized cefazolin sodium and ethyl oleate were prepared under sterile conditions. The sterile cefazolin sodium was aseptically micronized[7] and ethyl oleate was sterilized in a preheated oven at 155°C for 5 h. For *in vitro* studies, the materials were used without further sterilization.

Particle Size Distribution—The determination of particle size distribution was conducted by the sedimentation method using a micromerograph[8]. The mean particle size diameter of the micronized material was 7 μm.

Preparation of Suspensions—For *in vitro* studies, suspensions were prepared using an electric mixer[9] to disperse 12.5 g of micronized cefazolin sodium in 50 mL of peanut oil in the presence of various amounts of polysorbate 80 [*i.e.*, 0, 0.17, 0.50, and 3.3% (w/v), respectively]. Suspensions containing lecithin in ethyl oleate were prepared in the same manner. However, the concentration of lecithin added was 0, 0.2, 0.4, 0.6, 0.8, and 1.0% (w/v). For *in vivo* studies, the suspension was aseptically prepared by dispersing 1 g-equivalent activity of sterile micronized cefazolin sodium in 3.0 mL of sterile ethyl oleate with an electric mixer. The suspension was then passed through a 150-mesh sterile stainless steel screen and mixed until uniform. For *in vivo* control studies, aqueous solutions of cefazolin sodium were prepared for intravenous and intramuscular administration by dissolving the sodium salt[6] in isotonic saline to a final concentration of 100 mg/mL as cefazolin.

Sedimentation Volume—The sedimentation volumes were recorded in terms of the ultimate settled height, H_u, to the original height, H_o, as described in the literature (5, 6):

$$\text{Sedimentation Volume} = H_u/H_o \qquad \text{(Eq. 1)}$$

Redispersibility—The number of revolutions necessary to restore the suspension to homogeneity was recorded by means of a multi-purpose rotator[10] with the modification described by Matthews and Rhodes (7).

Turbidity—The turbidity of the supernatant of the suspensions was measured by an analytical nephelometer[11] after cefazolin sodium suspensions had been kept at 25°C for 1 month. The procedure was carried out as described by the manufacturer (8).

Crystal Growth—Crystal size changes with time were microscopically examined. Cefazolin sodium (I) in the dry state was compared with I in a suspension at initial time, and in a suspension stored for 3 months at 25°C.

Viscosity—The viscosities of ethyl oleate and peanut oil were measured with a viscometer[12].

Porosity—The porosity of a sedimentation bed was determined by the method of Kaneniwa and Takamura (9).

[1] Lilly Research Laboratories, Indianapolis, Ind.
[2] Tween 80; ICI America Inc., Wilmington, Del.
[3] American Lecithin Company, Atlanta, Ga.
[4] Pfaltz and Bauer, Inc., Stamford, Conn.
[5] Sessions Oil Mills, Interprise, Ala.
[6] KEFZOL; Eli Lilly and Company, Indianapolis, Ind.
[7] Sturtvant Mill; Sturtvant Mill Company, Boston, Mass.
[8] Sharples, Pennwalt Corp., Warminster, Pa.
[9] Lightnin' Mixing Equipment Co., Inc., Rochester, N.Y.
[10] Scientific Industries, Inc., Bohemia, N.Y.
[11] Hach Chemical Company, Ames, Iowa.
[12] Brookfield Engineering Laboratories, Inc., Stoughton, Mass.

0022-3549/84/1100-1602$01.00/0