ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1976, p. 307-317

Vol. 10, No. 2
Printed in U.S.A.

# Comparative Pharmacokinetics and Metabolism of Cephapirin in Laboratory Animals and Humans

B. E. CABANA,[1] D. R. VAN HARKEN,* AND G. H. HOTTENDORF
*Research Division, Bristol Laboratories, Syracuse, New York 13201*

Received for publication 17 December 1975

Comparative drug disposition studies in mice, rats, dogs, and humans indicate that cephapirin, a new semisynthetic cephalosporin antibiotic that exhibits broad-spectrum antimicrobial activity, is metabolized to desacetylcephapirin in these species. Pharmacokinetic analyses of the concentrations of cephapirin and desacetylcephapirin in plasma and urine reveal that the rate and extent of deacetylation decreases from rodents to dogs to humans. The kinetic analyses also suggest that the kidney performs a role not only in the excretion but also in the metabolism of cephapirin to desacetylcephapirin.

Cephapirin, sodium 7-(pyrid-4-yl-thioacetamido)cephalosporante (Fig. 1), is a new semisynthetic 7-aminocephalosporanic acid derivative that exhibits a broad spectrum of antimicrobial activity (2, 3, 5) and in humans exhibits a similar pharmacokinetic profile to that of cephalothin (1). These results (1) were based on total bioactivity in serum, as determined by the cup-plate assay (6), and failed to take into account any possible differences in drug metabolism. In mice, rats, dogs, and humans, cephapirin has been shown to be metabolized to a bioactive desacetyl metabolite, and it has been suggested that the kidney performs a role not only in the excretion of the parent compound and desacetyl metabolite but also in the metabolism of cephapirin to desacetylcephapirin (B. E. Cabana and D. R. Van Harken, 5th Int. Congr., Pharmacol., San Francisco, Calif., Abstr. 209, p. 35, 1972; B. E. Cabana, D. R. Van Harken, L. W. Dittert, and J. T. Doluisio, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 12th, Atlantic City, N.J., Abstr. 147, p. 77, 1972). This report consists of a detailed analysis of the comparative metabolic and pharmacokinetic profile of cephapirin in laboratory animals and humans.

## MATERIALS AND METHODS

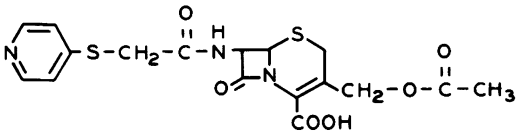
Swiss Webster mice and Miller rats weighing approximately 30 and 200 g, respectively, and four beagle dogs (mean weight, 8.2 kg) were employed in the metabolic studies. Pooled blood samples were taken in fasted mice by exsanguination after decapitation and from the dorsal aorta in fasted rats under ether anesthesia. Urine collection was achieved after water hydration diuresis by use of small rodent metabolism cages. In dogs, blood samples were taken from the saphenous vein by means of an intravenous (i.v.) catheter (Bardic, Intercath). The dogs were fasted overnight and hydrated (30 ml/kg) 15 to 30 min prior to drug administration. Continuous urine collection was achieved by means of Lucite-coated, stainless-steel cannulae surgically implanted within the bladder of the female dogs. The bladder cannulae were permanently implanted in dogs within 1 to 2 months of running the studies. The dogs had normal creatinine clearance (4 ml/min per kg) at the time of the study.

Ten fasted, healthy male volunteers (mean weight, 66 kg) were employed for the human metabolic studies. The drug was administered by the i.v. route into the median cubital vein over a 5-min period. Blood specimens (20 ml) were drawn immediately before and at 5 min, 0.5, 1.0, 1.5, 2, 3, and 4 h after drug administration, and urine was collected at 1, 2, 4, and 6 h after drug administration. A water diuresis was maintained throughout the urine collection period by oral administration of 600 ml of tap water 20 min prior to drug administration and subsequent administration of 300 ml of water at 1 and 3 h after dosing.

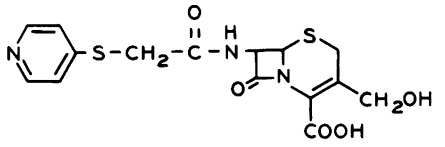
Blood specimens taken from the laboratory animals and men were placed in heparinized tubes (Vacutainers) and kept in an ice bath until centrifuged. Shortly thereafter, the blood specimens were spun down at approximately 1,000 × *g* in a refrigerated centrifuge (5°C), and the plasma samples were quickly frozen in a dry ice-acetone bath and kept frozen until bioassayed. The urine specimens were collected in flasks placed in an ice bath (5°C). All urine specimens were immediately frozen (−20°C) until bioassayed.

Plasma and urine samples were assayed for microbiological activity in terms of the free acid of the parent drug by means of the cup-plate assay (14), employing *Sarcina lutea* as the test organism (6). The identification of the microbiologically active metabolic products of cephapirin present in plasma and urine specimens was obtained by bioautography on *Bacillus subtilis* and *S. lutea* seeded agar plates

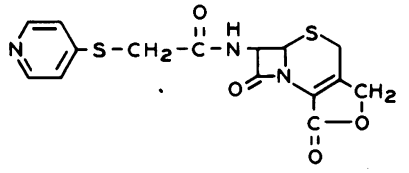
[1] Present address: Division of Biopharmaceutics, Bureau of Drugs, Food and Drug Administration, Rockville, MD 20852.



BL-P1322 (CEPHAPIRIN)



DESACETYL - BL-1322
(DESACETYL-CEPHAPIRIN)



BL-P1322 (LACTONE)

FIG. 1. *Structures of cephapirin and corresponding metabolites isolated from rat urine after parenteral administration of cephapirin.*

at pH 6.0 (Fig. 2) after chromatography on Whatman no. 1 paper strips (100 mm in width) employing *n*-butanol-acetic acid-water (60:15:25) or butanol-ethanol-water (8:2:10, top phase) as the solvent system. The metabolites found in the urine and plasma samples were compared with chemically synthesized products employing the same solvent system. Quantitation of cephapirin and desacetylcephapirin was achieved by differential bioautography as described below.

Quantitative differential assays for cephapirin and desacetylcephapirin were achieved by means of descending paper strip chromatography employing butanol-ethanol-water (8:2:10, top phase) as the solvent system, followed by bioautography on *B. subtilis* agar seeded plates at pH 6.0. The size of inoculum and plates employed were similar to the official assay technique (8, 14). The chromatographic system used was Whatman no. 1 paper developed in *n*-butanol-ethanol-water (8:2:10, top phase). When this solvent system was employed, cephapirin and desacetylcephapirin had $R_f$ values of 0.43 and 0.34, respectively (Fig. 2). The plasma samples were diluted with an equal volume of acetone to break protein binding and to reduce plasma esterase activity. The amount spotted on the Whatman paper strips varied from 10 to 100 $\mu$l, depending on the total microbiological activity. The Whatman paper strips were allowed to dry for about 30 to 60 min prior to incubation on the agar plates. Preliminary studies indicated that desacetylcephapirin was very stable, even at room temperature in acetone-precipitated plasma. This was not the case with cephapirin and, therefore, all plasma and urine samples were kept in an ice bath prior to bioassays. The urine samples were diluted with pH 6.0 phosphate buffer (1%) and spotted in 5 to 20-$\mu$l volumes. Quantitation was achieved by plotting the area of zone of inhibition (square millimeters) as a function of log concentration of drug (Fig. 3). The lower limit of detectability by bioautography on *B. subtilis* agar plates was approximately 0.03 $\mu$g for cephapirin and 0.05 $\mu$g for desacetylcephapirin, which generally was spotted in 10-$\mu$l portions. Linear relationships were obtained with both drugs at concentrations of up to 0.1 $\mu$g/$\mu$l (or 1.0 $\mu$g/10 $\mu$l; Fig. 3). In all instances, an attempt was made to dilute the plasma and urine

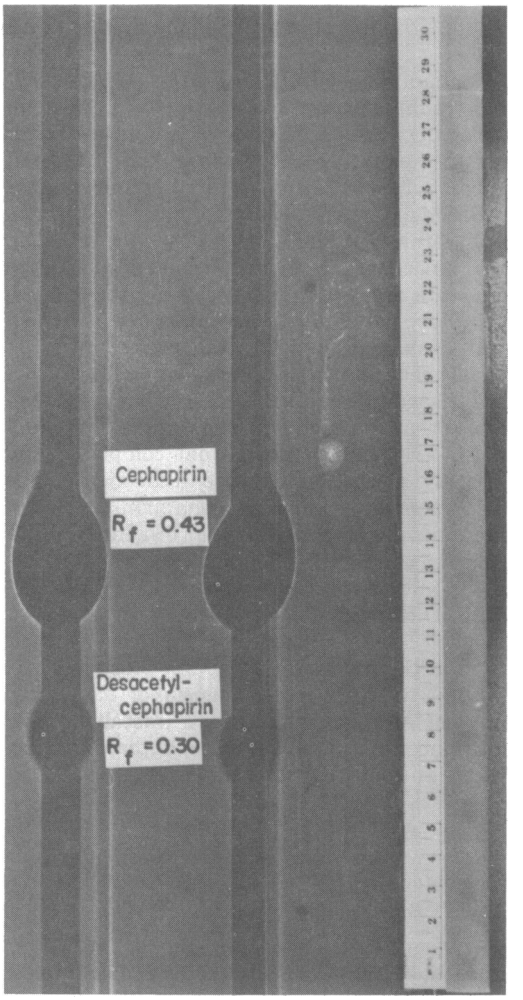



FIG. 2. *Bioautograph of cephapirin and desacetylcephapirin on B. subtilis seeded agar plates employing n-butanol-ethanol-water (8:2:10, top phase) as the chromatographic solvent system.*

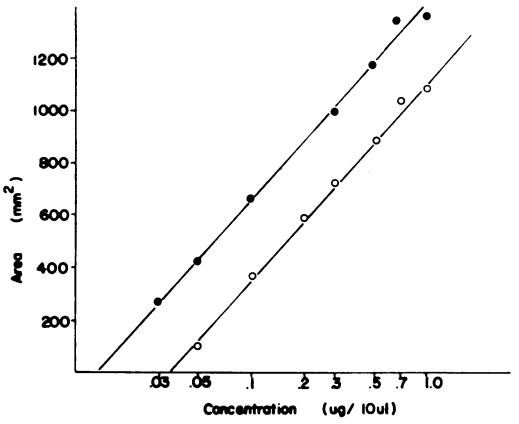



FIG. 3. *Area of zone inhibition on B. subtilis seeded agar plates versus log concentration of cephapirin (●) and desacetylcephapirin (○).*

samples and spot the appropriate portions such that they would approximate a reference standard (0.1 μg/10 μl) for cephapirin and (0.3 μg/10 μl) for desacetylcephapirin. The lower limit of detectability, when expressed in terms of plasma concentrations, was 0.3 μg/ml of plasma for cephapirin and 0.5 μg/ml for desacetylcephapirin. Cephapirin (lot 10611-9-15; bipotency, 942 μg/mg) and desacetylcephapirin (lot 10589-5-II) were employed as standards.

During the course of these studies, it was discovered that the concentration of cephapirin could be determined by the difference between the cup-plate assay (total bioactivity against *S. lutea*) and the desacetylcephapirin (S-335) bioautography assay as illustrated in equation 1. This procedure was facilitated by the fact that desacetylcephapirin had a biopotency of 0.54 ± 0.03 relative to cephapirin when assayed against *S. lutea*.

$$C_{\text{cephapirin}} = \text{cup-plate bioactivity versus } S.\ lutea - 0.54 \times C_{S-335} \quad (1)$$

where $C_{S-335}$ is the plasma concentration of desacetylcephapirin determined from the bioautography assay. The use of equation 1 was shown to give cephapirin plasma concentrations similar to those obtained with direct bioautography. This procedure was used to avoid the necessity of repeating the bioautography assay for higher concentrations of cephapirin and to avoid a saturation in the diffusion process on agar plates with relatively high concentrations of the parent drug. Whenever possible (sufficient sample permitting), the two assay methods were run simultaneously to assure the reliability of the assay procedures.

Kinetic analyses of the plasma concentration and cumulative urinary excretion data were performed with a Fortran 4 computer program (12) run on an IBM 360/30 digital computer and kinetic programs run on a G.E. Mark II Time Sharing computer. Nonlinear regression analyses of the plasma concentration-time curves and the least-squares estimations of the nonlinear parameters were determined by the method of Marquardt (11). The kinetic equations and the model describing the drug concentrations in plasma after i.v. and intramuscular administration were recently published (4, 16, 17, 20). The intrinsic absorption rate was determined by the method of Loo and Riegelman (10).

## RESULTS

**Identification of urinary excretion products.** The pharmacokinetic evaluation of the metabolic characteristics of cephapirin in laboratory animals and humans required identification of the urinary excretion products. Paper strip chromatography followed by bioautography as described above indicated the presence of cephapirin ($R_f$ = 0.43) and desacetylcephapirin ($R_f$ = 0.30) in the urine of mice, dogs, and humans (Fig. 2). The urine of rats contained a trace amount of a third microbiologically active component (cephapirin lactone), having a greater mobility ($R_f$ = 0.65) than cephapirin. The structures of cephapirin, desacetylcephapirin, and cephapirin lactone are presented in Fig. 1.

**Metabolism and urinary excretion of cephapirin in mice.** Summarized in Table 1 are the mean concentrations of cephapirin, desacetylcephapirin, and cup-plate bioactivity obtained in plasma of 30 mice 30 min after subcutaneous administration of 200-mg/kg doses of cephapirin. Also included in Table 1 are the mean cumulative urinary excretions of the same agents obtained in two groups of 15 mice over a 20-h period after subcutaneous administration of 200 mg of cephapirin per kg. It should be particularly noted that the rate of deacetylation of cephapirin in mice was very rapid, resulting in plasma levels of desacetylcephapirin approximately threefold that of the parent compound within 30 min of drug administration. It should

TABLE 1. *Plasma concentrations and urinary excretion of cephapirin and desacetylcephapirin in mice after subcutaneous administration of a 200-mg/kg dose of cephapirin*

| Determination | Plasma concn[a,b] | Urinary excretion (% of dose)[c] |
|---|---|---|
| Cephapirin | 33.1 ± 1.1 | 20.0 |
| Desacetylcephapirin | 106.7 ± 9.3 | 39.8 |
| Cup-plate bioactivity[d] | 105 | 31.3 |

[a] Time = 30 min.
[b] Micrograms per milliliter ± standard error; $N$ = 30.
[c] Percentage of dose excreted in 24 h.
[d] Bioactivity is expressed in terms of cephapirin.

also be noted that the use of cephapirin as a standard to determine cup-plate bioactivity (6) in plasma and urine specimens underestimated the actual amounts of antibiotic present in plasma and urine specimens. Thus, for instance, the cumulative urinary excretion determined by the cup-plate assay (6) accounted for only 31% of the dose (Table 1), but the differential bioautography assay indicated that about 60% of the dose was actually excreted in the urine as microbiologically active products. Of the total amount of drug excreted in mice, cephapirin and desacetylcephapirin accounted for about 20 and 40% of the dose, respectively.

**Metabolism and urinary excretion of cephapirin in rats.** The intraperitoneal administration of cephapirin to rats at doses of 200 and 1,000 mg/kg resulted in mean plasma concentrations of 58 and 753 μg/ml, respectively, 30 min after dosing, which declined exponentially over a 4- to 6-h period (Table 2). The plasma concentrations of desacetylcephapirin during the same period were approximately 40% those of cephapirin. The plasma half-lives of cephapirin and its desacetyl metabolite in rats were approximately 0.6 to 0.9 h. Significantly higher plasma concentrations of cephapirin were achieved with the 1,000-mg/kg dose than expected from comparison of the peak plasma levels obtained after the 200-mg/kg dose. The higher plasma concentrations observed were not due to a greater bioavailability but rather presumed to be due to a decrease in metabolic clearances. This hypothesis is supported by the fact that the cumulative percent excretion of total drug was the same for both doses (Table 3). Approximately 30 to 34% of the administered dose of cephapirin was excreted in the urine of rats over an 8-h period at both doses. Assuming equal bioavailabilities, the plasma clearances ($Cl_p$) over a 6-h period after intraperitoneal administration of 200- and 1,000-mg/kg doses were 41.5 and 22.3 ml/min per kg, respectively. During that same period, the renal clearances of cephapirin were 2.2 and 1.8 ml/min per kg, respectively. The excretion of desacetylcephapirin accounted for approximately 80% of the total excretion. When expressed in terms of cephapirin employing the cup-plate assay, only approximately 18 to 22% of the administered dose can be accounted for in the urine of rats rather than 30 to 34% actually present. The difference is due to the fact that desacetylcephapirin has about 54% of the bioactivity of cephapirin when assayed against *S. lutea*. Despite the much greater concentrations of cephapirin (twofold relative to desacetylcephapirin) in plasma (Table 2), significantly more desacetylcephapirin was excreted (Table 3) in the rats.

**Pharmacokinetics and metabolism of cephapirin in beagle dogs.** After i.v. administration of cephapirin to beagle dogs at doses of 30 mg/kg, the concentrations of cephapirin in plasma decreased biexponentially very rapidly over the next 2 to 3 h (Table 4 and Fig. 4). Simultane-

TABLE 2. *Plasma concentrations of cephapirin and desacetylcephapirin in rats after intraperitoneal administration of cephapirin at doses of 200 and 1,000 mg/kg*

| Dose (mg/kg) | Time (h) | Plasma concn[a] | | |
|---|---|---|---|---|
| | | Cephapirin | Desacetyl-cephapirin | Cup-plate bioactivity[b] |
| 200 | 0.5 | 58.4 ± 10.8 | 24.6 ± 9.2 | 71.6 ± 12.8 |
| | 1.0 | 30.8 ± 2.3 | 16.8 ± 2.0 | 39.9 ± 2.8 |
| | 2.0 | 13.9 ± 3.6 | 8.9 ± 4.1 | 18.8 ± 5.5 |
| | 4.0 | 3.3 ± 2.3 | 1.9 ± 0.7 | 3.9 ± 1.8 |
| 1,000 | 0.5 | 753.2 ± 29.0 | 353.8 ± 17.5 | 945.0 ± 22.5 |
| | 1.0 | 304.6 ± 45.1 | 167.5 ± 23.2 | 395.0 ± 50.6 |
| | 2.0 | 85.6 ± 33.5 | 44.0 ± 18.7 | 108.8 ± 42.4 |
| | 4.0 | 6.7 ± 2.3 | 5.3 ± 1.0 | 7.8 ± 2.2 |
| | 6.0 | 1.5 ± 0.8 | 0.8 ± 0.3 | 1.8 ± 0.6 |

[a] Micrograms per milliliter ± standard error; $N = 8$.
[b] Bioactivity is expressed in terms of cephapirin.

TABLE 3. *Cumulative urinary excretion of cephapirin and desacetylcephapirin in rats after intraperitoneal administration of cephapirin at doses of 200 and 1,000 mg/kg*

| Dose (mg/kg) | Time (h) | Urinary excretion[a] | | |
|---|---|---|---|---|
| | | Cephapirin | Desacetyl-cephapirin | Cup-plate bioactivity[b] |
| 200 | 24 | 5.2 ± 0.7 | 28.1 ± 4.4 | 17.8 ± 3.1 |
| 1,000 | 24 | 8.1 ± 0.8 | 26.3 ± 4.4 | 22.4 ± 1.8 |

[a] Percentage of dose ± standard error; $N = 16$.
[b] Bioactivity is expressed in terms of cephapirin.

TABLE 4. *Mean concentration of cephapirin and desacetylcephapirin in plasma after i.v. administration of cephapirin to beagle dogs at a dose of 30 mg/kg*

| Time | Concn[a] | | |
|---|---|---|---|
| | Cephapirin | Desacetyl-cephapirin | Cup-plate bioactivity[b] |
| 5 min | 92.3 ± 14.6 | 9.9 ± 4.6 | 104.0 ± 11.4 |
| 15 min | 52.0 ± 6.8 | 14.5 ± 3.6 | 56.8 ± 5.8 |
| 30 min | 26.9 ± 2.9 | 13.9 ± 2.5 | 33.5 ± 3.7 |
| 45 min | 15.1 ± 0.8 | 14.2 ± 1.8 | 20.6 ± 2.3 |
| 1.0 h | 10.5 ± 2.2 | 11.9 ± 2.3 | 12.6 ± 2.6 |
| 1.5 h | 3.8 ± 0.8 | 7.5 ± 0.9 | 5.6 ± 0.6 |
| 2.0 h | 2.1 ± 0.5 | 4.0 ± 1.2 | 3.2 ± 0.5 |
| 3.0 h | 0.6 ± 0.2 | <1.0 | 0.6 ± 0.1 |
| 4.0 h | 0.2 ± 0.1 | <1.0 | 0.2 ± 0.1 |

[a] Micrograms per milliliter ± standard error.
[b] Bioactivity is expressed in terms of cephapirin.

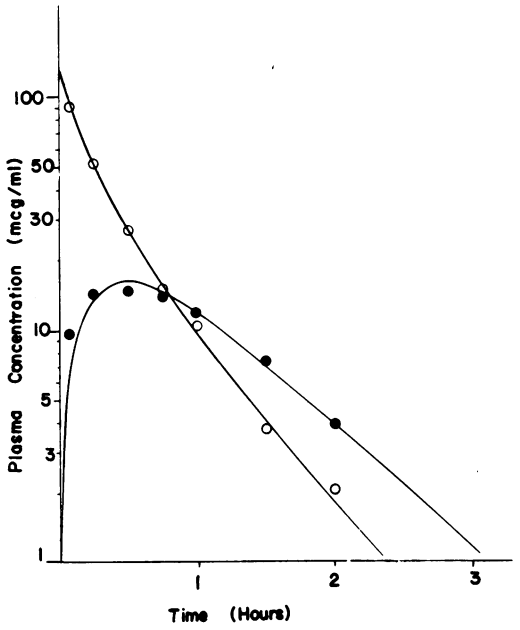



FIG. 4. *Mean concentrations of cephapirin* (○) *and desacetylcephapirin* (●) *in plasma of beagle dogs after i.v. administration of cephapirin at doses of 30 mg/kg.*

ously, the concentrations of desacetylcephapirin in plasma rapidly increased, achieving peak levels of 14 to 15 μg/ml within 30 min of dosing. Beyond 1 h, the concentrations of desacetylcephapirin in plasma were about twice those of cephapirin. Despite the lower plasma levels of desacetylcephapirin during h 1, the urinary excretion rate of the desacetyl metabolite significantly exceeded that of cephapirin (Table 5). Approximately 32 and 66% of the administered dose was excreted as cephapirin and desacetylcephapirin, respectively, in 8 h after i.v. administration of cephapirin, thus accounting for about 98% of the dose. It should be noted that the total microbiological activity grossly underestimated the actual amount of antibiotics excreted (Table 5).

Kinetic analyses of the plasma concentration data revealed that the mean plasma concentrations of cephapirin were best described by the following biexponential equation:

$$C_{\text{cephapirin}} = A_1 e^{-\alpha t} + B_1 e^{-\beta t} \qquad (2)$$

where $A_1$ = 76.69 μg/ml, $\alpha$ = 5.77 $h^{-1}$, $B_1$ = 51.55 μg/ml, and $\beta$ = 1.67 $h^{-1}$.

The biexponential equation 2 can best be interpreted in terms of the two-compartmental, open-system model (Fig. 5). The model consists of a central plasma compartment ($V_p$) and a peripheral tissue compartment ($V_T$) between which cephapirin is in a state of complete equilibrium only under steady-state conditions (18).

The kinetic model invokes not only the drug distribution profile of cephapirin but also its metabolism. Summarized in Table 6 are the rate constants governing distribution ($K_1$ and $K_{-1}$) to and from tissue fluids, the rate constant governing drug elimination ($K_2$), and the respective volumes of drug distribution, $V_p$ and $V_t$ (illustrated). Also included in Table 6 are the total area under the plasma concentration-time curve, the plasma half-life of cephapirin, and the apparent volume of drug distribution ($V_D$). Thus, after i.v. administration to beagle dogs, cephapirin appears to be rapidly distributed (half-life for drug distribution = 0.12 h) within the extracellular fluids ($V_p$ = 23% of body volume) and to some extent within the peripheral tissue compartment ($V_T$ = 9% of body volume). The apparent volume of drug distribution was approximately 32% of the body volume and the plasma half-life in the dogs was approximately 0.4 h. The plasma half-life of desacetylcephapirin during the β-elimination phase was about 0.6 h, and the area under the plasma concentration-time curve was about 25 μg·h per ml or about 60% that of cephapirin (Table 6).

Given the following kinetic model (Fig. 5), the rate constants governing the urinary excretion (*ke*) and metabolism (*km*) of cephapirin (Table 6) are given by the following equations:

$$ke = fe \cdot K_2 = \frac{Xe}{\text{dose}} \cdot K_2 \qquad (3)$$

and

$$km = fm \cdot K_2 = \frac{Xme}{\text{dose}} \cdot K_2 \qquad (4)$$

where *Xe* and *fe* are the total amounts and

TABLE 5. *Cumulative urinary excretion of cephapirin and desacetylcephapirin after i.v. administration of cephapirin to beagle dogs at a dose of 30 mg/kg*

| Time (h) | %[a] of dose excreted ± SE | | |
|---|---|---|---|
| | Cephapirin | Desacetyl-cephapirin | Cup-plate bioactivity[b] |
| 0.5 | 20.9 ± 3.1 | 27.9 ± 4.6 | 35.2 ± 4.0 |
| 1.0 | 27.0 ± 4.1 | 44.3 ± 4.3 | 48.4 ± 5.5 |
| 1.5 | 28.8 ± 3.9 | 53.5 ± 5.8 | 54.6 ± 6.5 |
| 2.0 | 30.3 ± 3.9 | 60.4 ± 4.7 | 58.8 ± 6.2 |
| 4.0 | 31.0 ± 3.9 | 64.8 ± 4.8 | 61.5 ± 6.5 |
| 6.0 | 31.1 ± 3.9 | 65.8 ± 4.8 | 61.9 ± 6.5 |
| 8.0 | 32.0 ± 4.0 | 65.9 ± 4.9 | 62.0 ± 6.5 |

[a] Percent molar excretion is expressed in terms of the administered dose. SE, Standard error.

[b] Bioactivity is expressed in terms of cephapirin.

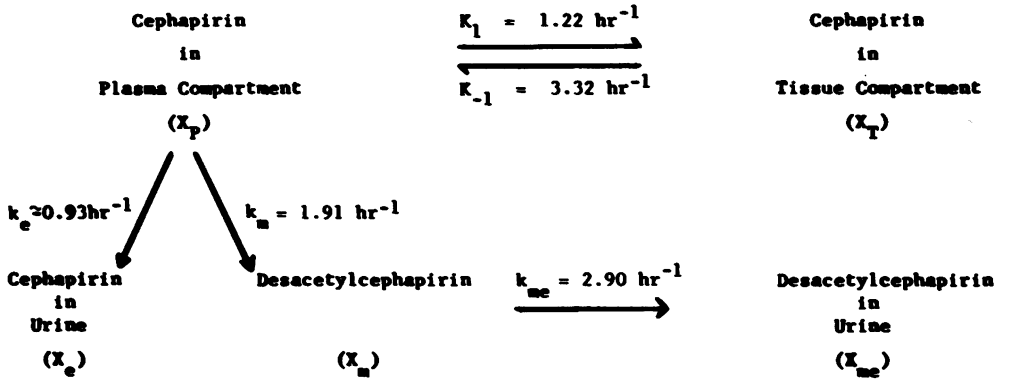



FIG. 5. *Two-compartment, open-system model describing the distribution and elimination of cephapirin in beagle dogs.*

TABLE 6. *Parameters for the two-compartment, open-system model estimated from concentration of cephapirin in plasma after i.v. administration of 30 mg/kg to dogs*[a]

| Parameter | Value |
|---|---|
| $K_1$ ($h^{-1}$) | 1.22 |
| $K_{-1}$ ($h^{-1}$) | 3.32 |
| $K_2$ ($h^{-1}$) | 2.90 |
| $Km$ ($h^{-1}$) | 0.91 |
| $Ke$ ($h^{-1}$) | 0.93 |
| $Kme$ ($h^{-1}$) | 2.90 |
| $V_P$ (liters/kg) | 0.23 |
| $V_T$ (liters/kg) | 0.09 |
| $V_D$ (liters/kg) | 0.32 |
| Half-life (h) | 0.41 |
| Area ($\mu$g·h per ml) | 44.16 |

[a] Equation: $C_{\text{cephapirin}} = 76.69e^{-5.77t} + 51.55e^{-1.67t}$

molar fraction of cephapirin excreted at time infinity, respectively, and $Xme$ and $fm$ are the total amounts and molar fraction of cephapirin metabolized to descetylcephapirin. The use of equation 4 assumes that the desacetylcephapirin formed in the dogs is totally recovered in the urine. This assumption was considered valid since about 98% of the dose was recovered in the urine (Table 5). Therefore, in the beagle dogs, the rate constants governing excretion ($ke$) and metabolism ($km$) of cephapirin were 0.93 $h^{-1}$ and 1.91 $h^{-1}$, respectively. It would appear that the deacetylation of cephapirin to desacetylcephapirin plays a major role in the elimination of cephapirin in the dogs.

The urinary excretion rate of cephapirin in the dogs is given by:

$$\frac{dXe}{dt} = ke \cdot Xp \qquad (5)$$

where $Xp$ is the total amount of cephapirin in the central plasma compartment at any given time $t$.

Since $Xp$ at any time $t$ is given by:

$$Xp = \text{dose}\,(C_1 e^{-\alpha t} + C_2 e^{-\beta t}) \qquad (6)$$

where $C_1 = A_1/Cp^0$ and $C_2 = B_1/Cp^0$ (from equation 2) and $Cp^0$ (zero time plasma concentration $= A_1 + B_1$) = 128.24 $\mu$g/ml, equation 4 may be written as:

$$\frac{dXe}{dt} = ke\ \text{dose}\,(C_1 e^{-\alpha t} + C_2 e^{-\beta t}) \qquad (7)$$

Integration of equation 7, solving for the constant of integration and rearranging, yields:

$$Xe = ke\ \text{dose}\,\frac{C_1}{\alpha}(1 - e^{-\alpha t}) + \frac{C_2}{\beta}(1 - e^{-\beta t}) \qquad (8)$$

where $Xe$ is the cumulative amount of cephapirin excreted at any time $t$. Although the plasma half-life of desacetylcephapirin was similar to that previously reported for cephapirin ($t_{1/2}$ = 0.5 h), an attempt was made to compute the excretion rate ($kme$) of desacetylcephapirin in dogs by plotting the rate of excretion ($dXme/dt$) in milligrams per hour against the mean interpolated amount of metabolite ($Xme$) present in the plasma during the excretion period (Fig. 6) as described by Martin (13). With the exception of the value obtained during the initial 30 min, the excretion rate of desacetylcephapirin was directly proportional to the plasma concentration. The regression coefficient describes the renal clearance of desacetylcephapirin ($Cl_R$) in the dogs, which was approximately 6 liters/h of 100 ml/min. The renal clearance of desacetylcephapirin ($Cl_R$) in the dogs was also computed from its area under the plasma concentration-time curve (area = 25.1 $\mu$g·h per ml) employing the following equation:

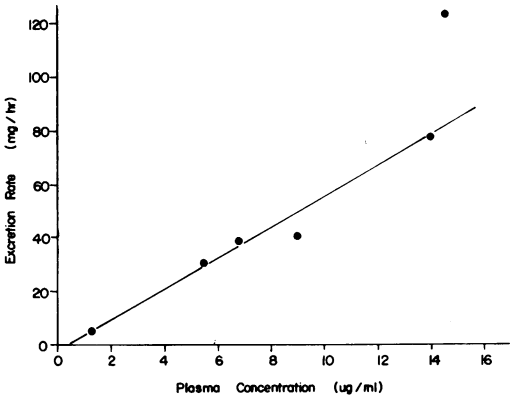



FIG. 6. *Plot of the urinary excretion rate of desacetylcephapirin in beagle dogs as a function of the corresponding plasma concentration. The regression line is equal to the renal clearance value in liters per hour.*

$$Cl_R = \frac{Xme}{\text{area}} \quad (9)$$

A value of 5.8 liters/h or 96.7 ml/min was obtained by this method.

Assuming that the plasma compartment volume for desacetylcephapirin is about the same as that previously determined for cephapirin ($V_p$ = 23% of body volume in the dogs), an estimate of the rate constant (*kme*) for excretion of desacetylcephapirin is given by:

$$kme = \frac{Cl_R}{V_p} \quad (10)$$

where $Cl_R$ is the renal clearance of desacetylcephapirin in milliliters per hour. Under the assumption, the rate constant *kme* would be 2.90 $h^{-1}$. Thus, it would appear that desacetylcephapirin is cleared by the kidneys at approximately the same rate as cephapirin and, therefore, would not be a rate-limiting step in the kinetic model.

Having thus defined the rate constants that govern the distribution of cephapirin to and from tissue fluids ($K_1$, $K_{-1}$) and its excretion (*ke*) as well as the rate constants that govern the rate of formation (*km*) and excretion (*kme*) of desacetylcephapirin in dogs, it is possible to test the appropriateness of the kinetic model (Fig. 5) by the combined use of digital and analogue computers employing previously described equations. Figure 7 illustrates the use of the kinetic model in describing the pharmacokinetic profile of cephapirin in dogs. It should be particularly noted that the observed plasma levels of cephapirin and the urinary excretion of cephapirin and desacetylcephapirin were in excellent agreement with the predicted computer plots, indicating that the kinetic model adequately described the kinetic profile of cephapirin in dogs. However, it should also be noted that there is a significant disagreement between the observed plasma levels of desacetylcephapirin and the computer-predicted plots. The latter findings suggest that the present kinetic model (Fig. 5) is inadequate in describing the kinetic profile of desacetylcephapirin. The present kinetic model predicted significantly higher plasma levels of desacetylcephapirin than were observed during the initial h 1. The tendency would be to assume that the observed discrepancy is due to faulty computation of the rate constants that govern the rate of formation (*km*) and elimination (*kme*) of desacetylcephapirin. It should be noted, however, that the magnitude of *km* is governed strictly by the molar fraction (*fm*) of desacetylcephapirin recovered in the urine (equation 3), and the rate constant (*kme*) was computed from renal clearance values for desacetylcephapirin determined by two separate methods. An alternate explanation might be a source of desace-

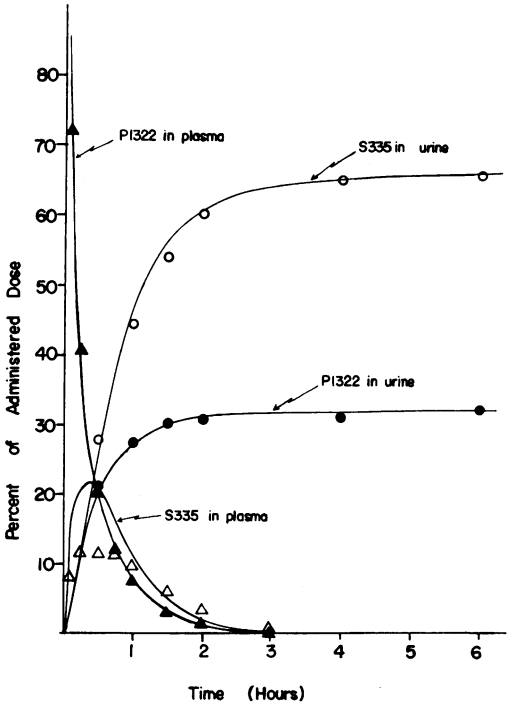



FIG. 7. *Observed and computer-predicted plots of the concentrations of cephapirin (P1322) (▲) and desacetylcephapirin (S335) (△) in plasma and cumulative urinary excretion of cephapirin (●) and desacetylcephapirin (○) in beagle dogs after rapid i.v. bolus.*

tylcephapirin that does not originate within the plasma compartment ($V_p$). The lack of proportionality between the excretion rate and the plasma concentrations during the initial 30 min suggests the possibility of a first pass effect occurring within the kidney, which results in the metabolism of cephapirin to desacetylcephapirin within the canine kidney. This hypothesis is further supported by the fact that, despite the lower plasma levels of metabolite during h 1, significantly more metabolite than parent drug was excreted during this initial period. This hypothesis will be further supported and discussed at length in dealing with human excretion data.

**Pharmacokinetics and metabolism of cephapirin in humans.** Summarized in Table 7 and illustrated in Fig. 8 are the mean concentrations of cephapirin and desacetylcephapirin in plasma of 10 human volunteers administered a 1.0-g i.v. bolus of cephapirin. It can be readily seen that the levels of desacetylcephapirin in plasma were extremely low and in some instances undetectable and that the concentrations of cephapirin in the plasma closely approximated the cup-plate activity (Table 7). Despite the extremely low levels of desacetylcephapirin in plasma, approximately 45.3% of the dose was excreted as the desacetyl metabolite (Table 8). The urinary excretion of cephapirin accounted for about 48.5% of the dose. The cup-plate assay (expressed in terms of parent drug) grossly underestimated the total amounts of antibiotics excreted, accounting for only 69.2% of the dose, whereas bioautographic assays, in fact, accounted for 93.8% of the dose. The large discrepancy was due to the presence of desacetylcephapirin in the urine, which has about 54% of the bioactivity of cephapirin, employing *S. lutea* as the test organism.

Kinetic analyses of the plasma concentrations of cephapirin beyond the early drug distribution phase revealed that the plasma half-life of cephapirin in humans was about 0.5 h. Similarly, the half-life of desacetylcephapirin was about 0.43 h, and the "apparent" volume of distribution ($V_D$) of cephapirin in humans was about 0.23 liter/kg. The renal clearance ($Cl_R$) of cephapirin (computed from an equation analogous to equation 9) was about 342 ml/min, indicating that cephapirin was secreted by the renal tubules. A similar attempt with the desacetylcephapirin excretion data revealed a total lack of proportionality in the excretion rate and observed plasma concentrations of desacetylcephapirin. Kinetic analysis of the cumulative excretion of desacetylcephapirin during h 1 after drug administration indicated that the effective renal clearance value for that period was about 2,700 ml/min. The mean renal clearance over the 6-h period was about 1,800 ml/min. This renal clearance

TABLE 7. *Mean concentration of cephapirin and desacetylcephapirin in plasma after i.v. administration of a 1.0-g dose of cephapirin to humans*

| Time | Concn[a] | | |
|---|---|---|---|
| | Cephapirin | Desacetyl-cephapirin[b] | Cup-plate bioactivity[c] |
| 5 min | 63.6 ± 5.2 | 2.5 | 65.0 ± 5.3 |
| 0.5 h | 10.2 ± 0.8 | 1.9 | 11.1 ± 0.8 |
| 1.0 h | 3.0 ± 0.3 | 1.0 | 3.5 ± 0.4 |
| 1.5 h | 1.7 ± 0.2 | 0.3 | 1.9 ± 0.2 |
| 2.0 h | 0.9 ± 0.1 | ND[d] | 0.9 ± 0.1 |
| 3.0 h | 0.4 ± 0.05 | | 0.4 ± 0.05 |
| 4.0 h | 0.2 ± 0.02 | | 0.2 ± 0.02 |

[a] Micrograms per milliliter ± standard error; $N$ = 8.

[b] Desacetylcephapirin was detectable in three patients at 5 min, five patients at 0.5 h, and two patients at 1.5 h.

[c] Bioactivity is expressed in terms of cephapirin.

[d] ND, Not determined.

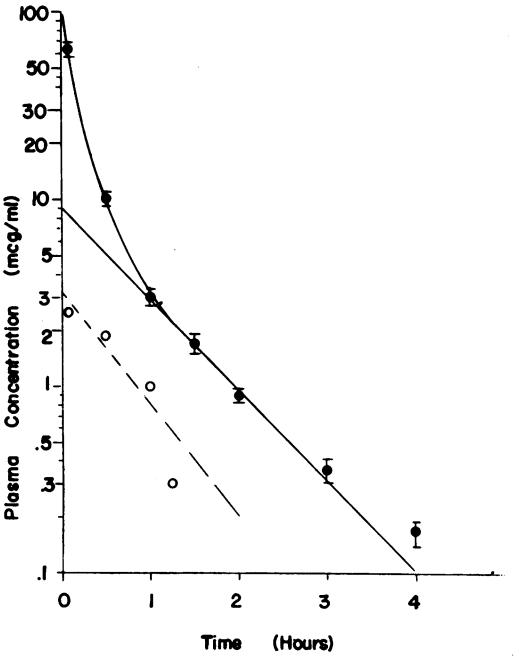



FIG. 8. *Semilogarithmic plots of the concentration of cephapirin (●) and desacetylcephapirin (○) in plasma of human volunteers after a rapid i.v. bolus of a 1.0-g dose of cephapirin.*

TABLE 8. *Cumulative urinary excretion of cephapirin and desacetylcephapirin after i.v. administration of cephapirin to humans*

| Time (h) | Excretion (%)[a] ± SE | | |
|---|---|---|---|
| | Cephapirin | Desacetyl-cephapirin | Cup-plate bioactivity[b] |
| 1.0 | 40.5 | 32.4 | 55.8 |
| 2.0 | 46.4 | 40.7 | 64.9 |
| 4.0 | 48.3 | 44.6 | 68.6 |
| 6.0 | 48.5 | 45.3 | 69.2 |

[a] Percent excretion is expressed in terms of administered dose (1 g). SE, Standard error. $N = 8$.

[b] Bioactivity is based on a cephapirin reference standard.

value is totally unphysiological, even assuming renal tubular secretion, since renal plasma flow in humans is about 600 to 800 ml/min. In view of the total lack of proportionality between urinary excretion of desacetylcephapirin and the corresponding plasma concentration, no attempt was made to fit the data to the previously described model (Fig. 5).

## DISCUSSION

Comparative metabolic studies performed in mice, rats, dogs, and humans suggest that the biotransformation of cephapirin was qualitatively similar in these species. The fate of cephapirin was similar to that reported for cephalothin (17, 18) and involved metabolism to its desacetyl metabolite and renal excretion of the parent drug and metabolite. Circulating desacetylcephapirin was found in plasma of all species in varying amounts after parenteral administration of cephapirin.

A method was devised, employing paper chromatography followed by bioautography on *B. subtilis* seeded agar plates, that permitted quantitation of both cephapirin and desacetylcephapirin in plasma and urine. The pharmacokinetic data presented herein conclusively showed that, with the exception of human plasma which contained very little desacetyl metabolite, the use of the cup-plate assay on *S. lutea* seeded agar plates grossly underestimated the actual amounts of antibiotics present in the plasma and urine specimens. This was particularly evident in dealing with urinary recovery of antibiotics when in some instances the cup-plate assay accounted for only about 50 to 60% of the actual amount of drug present, as determined by the bioautography assay (Tables 1 and 5). The large discrepancy observed was due to the fact that the desacetylcephapirin present in the urine specimens had a 54% activity relative to cephapirin when assayed on *S. lutea* plates. The use of the bioautography assay permitted the pharmacokinetic characterization of cephapirin in the laboratory animals and humans.

Although no qualitative differences in the biotransformation of cephapirin were noted among species, pharmacokinetic analyses of the plasma concentration and urinary excretion data revealed that the rate and extent of metabolism of cephapirin to its desacetyl metabolite decreased from rodents to dogs to humans. The kinetic analyses also suggested that, in rats, dogs, and humans, the kidney performed a role not only in excretion but also in the metabolism of cephapirin to desacetylcephapirin. These conclusions concerning rats and dogs are largely based on the disproportionate amount of desacetylcephapirin excretion in these species relative to plasma concentrations (see Tables 2 to 5). Although the circulating desacetylcephapirin was cleared at approximately the same rate as cephapirin ($t_{1/2} = 0.5$ h) in dogs, significantly more desacetyl metabolite was excreted (Table 5) in the urine. The renal metabolism hypothesis is further supported by recent published data obtained in rats by Shimizu (19) on cephapirin and cephalothin. Significantly higher concentrations (threefold) of desacetyl metabolites of these two cephalosporin antibiotics were found in the kidney when compared with the serum levels (19).

A kinetic model was used to describe the distribution and elimination of cephapirin in beagle dogs (Fig. 6). Although the model was adequate in describing the concentrations of cephapirin in plasma and the urinary excretion of cephapirin and desacetylcephapirin in the dogs (Fig. 7), it failed to adequately predict the concentrations of desacetylcephapirin in plasma, which were disproportionately low relative to its corresponding urinary excretion. After an i.v. bolus of cephapirin to beagle dogs at a dose of 30 mg/kg, cephapirin was rapidly distributed to the central plasma compartment and tissue fluids, achieving an "apparent" volume of distribution ($V_d$) of approximately 320 ml/kg or 32% of the body volume. Cephapirin is thereafter eliminated by excretion and metabolism (the latter occurring in the plasma compartment and presumably in the kidneys) at a rapid rate (half-life = 0.44 h). The renal clearances ($Cl_r$ = 100 ml/min) of cephapirin and desacetylcephapirin were similar in dogs and exceeded the glomerular filtration rate (4 ml/min per kg), indicating ac-

tive renal tubular secretion.

The kinetic data obtained in human volunteers suggest that cephapirin is handled in a similar manner as in dogs. As in the beagle dogs, the plasma half-life of cephapirin was about 0.5 h and the renal clearance of cephapirin ($Cl_r$) = 342 ml/min) exceeded glomerular filtration, indicating that active tubular secretion was occurring. The "apparent" volume of drug distribution of cephapirin ($V_d$ = 230 ml/kg) comprised about 23% of the body volume, suggesting that cephapirin is distributed primarily in the extracellular fluids and to some small extent within tissue fluids.

Although qualitative similarity exists in the kinetic profile of cephapirin in humans and in common laboratory animals, there are subtle unique differences in humans. A major difference is the rate of deacetylation of cephapirin within the central plasma compartment. Extremely low levels (<2 μg/ml) of desacetylcephapirin were obtained in plasma after i.v. administration of a 1.0-g bolus. The cephapirin plasma concentrations approximate the cup-plate bioactivity only in humans (Table 7) and not in dogs and rodents. Despite the low levels of circulating desacetylcephapirin, approximately 45% of the dose was excreted as the desacetyl metabolite in the urine. In contrast to cephapirin, which was cleared at a rate of 342 ml/min, the renal clearance of desacetylcephapirin ($Cl_r \simeq$ 1,800 ml/min) significantly exceeded renal plasma flow (600 to 800 ml/min). Kinetic analyses of the human data showed that the previously described kinetic model (Fig. 6) was totally inadequate in describing the metabolic profile of cephapirin in humans. A suitable kinetic model (6) was devised that takes into account not only tissue distribution and urinary excretion of cephapirin but also its deacetylation within the kidneys.

Kidney metabolism of drugs has recently been shown by Wan and Riegelman (21, 22) and Wan et al. (23) for a number of drugs, and Rennick (15) has recently postulated that renal metabolism may be an integral part of the renal tubular transport system for these drugs. The results reported herein indicate that renal metabolism of cephapirin does occur in humans, and comparative metabolic studies in rats and dogs suggest that a similar drug elimination mechanism may occur in these species. Kirby et al. (7) have recently reported that, in contrast to normal volunteers, high levels of circulating desacetylcephalothin were obtained in uremic patients. Those findings suggest that deacetylation of cephalothin must occur to a great extent within the plasma compartment (i.e., via plasma or liver esterases) and manifests itself particularly in uremic patients. The present findings suggest that renal metabolism of cephapirin must play a major role in the elimination of this drug in normal subjects. The effects of uremia on cephapirin elimination remain as yet undefined.

## LITERATURE CITED


1. **Axelrod, J., R. R. Meyers, and S. Z. Hirschman.** 1972. Cephapirin: pharmacology in normal human volunteers. J. Clin. Pharmacol. 12:84–88.
2. **Bodner, S. J., and M. G. Koenig.** 1972. Clinical and *in vitro* evaluation of cephapirin: a new parenteral cephalosporin. Am. J. Med. Sci. 263:43–51.
3. **Bran, J. L., M. E. Levison, and D. Kaye.** 1972. Clinical and in vitro evaluation of cephapirin, a new cephalosporin antibiotic. Antimicrob. Agents Chemother. 1:35–40.
4. **Cabana, B. E., D. R. Van Harken, G. H. Hottendorf, J. T. Doluisio, W. O. Griffen, Jr., D. W. A. Bourne, and L. W. Dittert.** 1975. The role of the kidney in the elimination of cephapirin in man. J. Pharmacokinet. Biopharm. 3:419–438.
5. **Chisholm, D. R., F. Leitner, M. Misiek, G. E. Wright, and K. E. Price.** 1970. Laboratory studies with a new cephalosporanic acid derivative, p. 244–246. Antimicrob. Agents Chemother. 1969.
6. **Grove, D. C., and W. A. Randall.** 1955. Assay methods of antibiotics—a laboratory manual. Medical Encyclopedia, Inc., New York.
7. **Kirby, W. M. M., J. D. DeMaine, and W. S. Serill.** 1971. Pharmacokinetics of the cephalosporins in healthy volunteers and uremic patients. Postgrad. Med. J. 47 (Suppl):41–46.
8. **Kirshbaum, A., and B. Arret.** 1967. Outline of details for official microbiological assays of antibiotics. J. Pharm. Sci. 56:511–515.
9. **Lee, C. C., E. B. Herr, Jr., and R. C. Anderson.** 1963. Pharmacological and toxicological studies on cephalothin. Clin. Med. 70:1123–1138.
10. **Loo, J. C. K., and S. Riegelman.** 1968. New method for calculating the intrinsic absorption rate of drugs. J. Pharm. Sci. 57:918–927.
11. **Marquardt, D. W.** 1963. An algorithm for least squares estimation of non-linear parameters. J. Soc. Ind. Appl. Math. 11:431.
12. **Marquardt, D. W.** 1966. Least squares estimation of non-linear-parameters. IBM Share Program Library, no. 3094.
13. **Martin, B. K.** 1967. Treatment of data from urinary excretion. Nature (London) 214:247–249.
14. **Office of Federal Register.** 1972. Code of federal regulations, Title 21, p. 234–240. U.S. Government Printing Office, Washington, D.C.
15. **Rennick, B. R.** 1972. Renal excretion of drugs: tubular transport and metabolism. Annu. Rev. Pharmacol. 12:141–156.
16. **Riegelman, S., J. Loo, and M. Rowland.** 1968. Shortcomings in pharmacokinetic analysis by conceiving the body to exhibit properties of a single compartment. J. Pharm. Sci. 57:117–127.
17. **Riegelman, S., J. Loo, and M. Rowland.** 1968. Concept of a volume of distribution and possible errors in evaluation of the parameter. J. Pharm. Sci. 57:128–133.
18. **Riggs, D. S.** 1963. Mathematical approach to physiolog-

ical problems, p. 203-209. The Williams & Wilkins Co., Baltimore.
19. **Shimizu, T.** 1975. Serum level and urinary excretion of parent antibiotics and desacetyl forms after parenteral administration of cephalothin and cephapirin. Jpn. J. Antibiot. 28:628-633.
20. **Wagner, J. G., and J. I. Northam.** 1967. Estimation of volume of distribution and half-life of a compound after rapid intravenous injection. J. Pharm. Sci. 56:529-531.
21. **Wan, S. H., and S. Riegelman.** 1972. Renal contribution to overall metabolism of drugs. I. Conversion of benzoic acid to hippuric acid. J. Pharm. Sci. 61:1278-1284.
22. **Wan, S. H., and S. Riegelman.** 1972. Renal contribution to overall metabolism of drugs. II. Biotransformation of salicylic acid to salicyluric acid. J. Pharm. Sci. 61:1284-1287.
23. **Wan, S. H., B. Von Lehmann, and S. Riegelman.** 1972. Renal contribution to overall metabolism of drugs. III. Metabolism of p-aminobenzoic acid. J. Pharm. Sci. 61:1288-1292.