EFFECT OF FOOD ON THE BIOAVAILABILITY AND PHARMACOKINETICS OF ORAL CEPHALOSPORINS IN MAN

A thesis submitted to the Graduate School of the University of Wisconsin-Madison in partial fulfillment of the requirements for the degree of Doctor of Philosophy

by

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Florence W. Kwok

Under the supervision of Professor Peter G. Welling

This study was undertaken to clarify the inconsistencies reported in the literature regarding the effect of food on the bioavailability of oral cephalosporins and to gain insight concerning their absorption kinetics and relevant compartmental models.

The relative bioavailability of cephalexin, cephradine, and cefaclor was examined after single 500 mg oral doses to 8 healthy male volunteers after an overnight fast and immediately following a standard breakfast. Plasma and urinary concentrations were determined by HPLC procedures.

Under fasting conditions, all three cephalosporins were absorbed rapidly, giving rise to mean peak plasma concentrations of 16.8, 19.4, and 10.3 mcg/ml for cephalexin, cephradine, and cefaclor, respectively, at approximately 1 hr. After postprandial doses, C_{max} values were reduced, t_{max} values were increased, and areas under plasma concentration-time curves were unaffected for all three cephalosporins. Under fasting conditions, the total 12 hr urinary recovery of cephalexin, cephradine, and cefaclor was 88.5, 89.1, and 73.7% of the administered dose. Urinary excretion was delayed,

but total urinary recovery was not significantly affected by food. Lower plasma concentrations and reduced urinary recovery of cefaclor are probably due to its chemical instability.

The plasma concentrations of all three cephalosporins were better described by a one-compartment model with zero-order absorption under fasting conditions. However, under nonfasting conditions, plasma concentrations could be adequately described by either the zero-order or first-order absorption model with 'flip-flop' characteristics.

Relative bioavailability was examined by model-independent and model-dependent parameters generated from plasma profiles, cumulative relative fraction absorbed plots, urinary excretion data, the Kwan-Till method, and statistical moment analysis. The results confirm that food causes a delay in absorption of the cephalosporins without significantly affecting their overall absorption efficiency.

The observed changes in plasma cephalosporin concentrations after single doses are likely to occur after repeated doses. The clinical significance of the reduced drug concentration after postprandial doses must be evaluated in light of the microbiological activity of the cephalosporins and the sensitivity of the target organisms.

APPROVED: Peter G. Welling

Peter G. Welling, Professor

DATE: June 27th 1984

To my parents

for

their love

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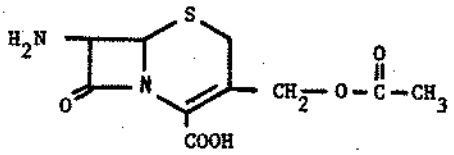
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I. INTRODUCTION

A. Background

The cephalosporins, with the exception of cephamycin derivatives, are a series of antibiotics having 7-aminocephalosporanic acid (I) as the basic nucleus (1).



(I)

Chemical modification of this nucleus has resulted in a relatively large number of semisynthetic cephalosporins that differ in their spectra of activity and pharmacologic properties. Many of these cephalosporins are poorly absorbed from the gastrointestinal tract and as such must be given parenterally. At present, there are four oral cephalosporins which are commercially available and commonly used in the United States. These four oral cephalosporins are cephalexin (Keflex®), cephradine (Velosef®, Anspor®), cefaclor (Ceclor®), and cefadroxil (Duricef®, Ultracef®).

1. Antibacterial Activity and Therapeutic Uses of Oral Cephalosporins

The oral cephalosporins share the mechanism of action of the

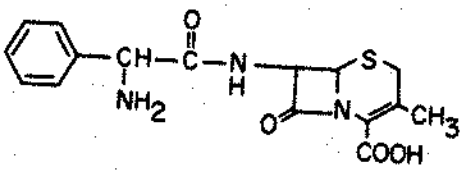
other β-lactam antibiotics. These bactericidal agents interfere with the biosynthesis of the peptidoglycan component of bacterial cell walls by inhibiting the enzymatic activity of transpeptidases and carboxypeptidases (2). The oral cephalosporins are active against both gram-positive and gram-negative bacteria, and are regarded as broad-spectrum antibiotics. The spectrum of antibacterial activity of cephalexin, cephradine, and cefadroxil are similar. They are active against gram-positive pathogens, even penicillinase-producing strains of Staphylococci. Their gram-negative spectrum includes indole-negative Proteus, Haemophilus influenzae, Shigella, Salmonella, many strains of Escherichia coli, Klebsiella and Enterobacter (1). According to several investigations by various authors (3-6), cefaclor is more active than the others against both β-lactamase positive and negative strains of Haemophilus influenzae and certain strains of gram-negative enteric bacilli.

Clinically, these cephalosporins are effective in the treatment of urinary tract infection, upper and lower respiratory tract infections, skin and soft tissue infections. They are almost free of side effects with the most common untoward reactions being gastrointestinal disturbances and hypersensitivity phenomena (1).

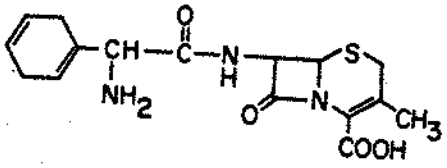
2. Physicochemical Properties of Oral Cephalosporins

The structures of cephalexin, cephradine, cefaclor, and cefadroxil are shown in Figure 1. Consistent with their structural similarities, these oral cephalosporins have almost identical

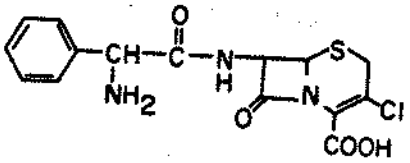
Cephalexin



Cephradine



Cefaclor



Cefadroxil

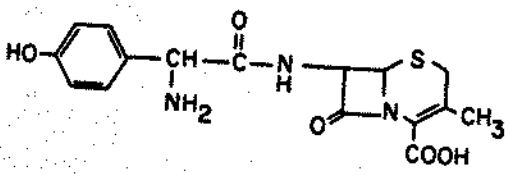


Figure 1. Structures of Oral Cephalosporins.

physicochemical properties.

Cephalexin. Cephalexin is a white crystalline powder with a molecular weight of 365.4 (7). It has an aqueous solubility of 13.5 mg/ml at 37°C (7). At pH 2 and 8, the solubility is increased to approximately 120 and 100 mg/ml, respectively (7). The increase in solubility as a function of pH is due to the zwitterionic nature of the drug (8). The values of pK_1 and pK_2 are 2.7 and 7.0 at 37°C and an ionic strength of 0.5. The drug is most chemically stable at its isoelectric pH of 4.5. Cephalexin retains its activity well in serum and urine as no loss in activity was noted after storage at -20°C for 14 days (7).

Cephradine. Cephradine, a white crystalline powder, has a molecular weight of 349.4 (9). Cephradine, unless specified, is defined as a hydrated form containing 3-6% of water (9). Cephradine is freely soluble in aqueous solvents; 19 mg of drug dissolves in 1 ml of water (10). By titration with sodium hydroxide pK_1 of 2.6 and pK_2 of 7.3 were obtained (9). In the pH range of 3-7, cephradine exists as an internal salt. Cephradine is stable for 24 hr at 25°C within the pH range 2-8 (11). Since it is stable in acidic media there is little loss of activity in the gastric fluid with losses of less than 7% being reported (11).

Cefaclor. Cefaclor is a white crystalline powder with a molecular weight of 385.8 (12). It has an aqueous solubility of 10 mg/ml (12). Similar to the other oral cephalosporins, cefaclor exists as a zwitterion, containing both an acidic carboxyl group

and an alkaline amino group. The pK_1 and pK_2 are 1.5 and 7.2, respectively (12).

Cefaclor is a reasonably stable molecule in the dry state. When cefaclor is present in the monohydrate crystalline form in the dry powder, a two year shelf-life can be easily obtained (12). Nevertheless, cefaclor in solution is prone to degradation. The stability of a cefaclor solution has been shown to be pH dependent with relatively greater stability at pH values lower than 4.5 (13). Cefaclor solutions prepared in pH 2.5 and 4.5 buffers retained at least 90% of their initial activity after 72 hr at 4°C (13). However, in neutral or alkaline solutions, cefaclor underwent a rapid loss of activity. Cefaclor samples prepared in plasma and serum showed 8% loss in activity when incubated for 6 hr at 4°C, 51% loss when incubated for 6 hr at 25°C, and 48% loss when incubated for 2 hr at 37°C (13). When stored in Mueller-Hinton broth at 37°C overnight, 30-60% of the initial activity of the solution was lost (14, 15). On degradation, cefaclor appears to lose HCl quite readily. Further investigation showed that cefaclor can undergo intramolecular nucleophilic attack by the side chain amino group to produce a diketopiperazine derivative (16).

Cefadroxil. Cefadroxil has a molecular weight of 384.4 (17). It is sparingly soluble in aqueous media. The pK_1 and pK_2 for the dissociation of the carboxylic acid and α-amino groups were determined to be 2.6 and 7.3, respectively, at 35°C and an ionic strength of 0.5 (18). The pK_3 for the phenolic group was determined to be

9.7 (18). Cefadroxil is relatively stable in solution at 37°C, in acid or neutral media, with a half-life of more than 24 hr (19).

3. Pharmacokinetics of Oral Cephalosporins

The pharmacokinetic similarities among these oral cephalosporins are more striking than the differences. The pharmacokinetics of cephalexin, cephradine, cefaclor, and cefadroxil will be discussed individually; and then their differences will be compared.

Cephalexin. Cephalexin is reported to be efficiently absorbed after oral doses (20, 21). Peak plasma concentrations generally occur at 1 hr in fasting individuals (20, 22). The time to reach peak concentration may vary among individuals after administration of the drug in capsule form. This finding can be attributed to a lag time in the absorption process due to dosage form effects. Following a 500 mg dose given as a 5% suspension, cephalexin appeared in the serum in 9 min compared with 38 min when given in capsule form (22).

The peak height is dose dependent, typically reaching a level of 18 mcg/ml after a 500 mg dose (22, 23). Single doses of 125, 250, 500, and 1000 mg in 12 fasting subjects produced average peak concentrations of 4.5, 9, 18, and 32 mcg/ml, respectively, at 1 hr (23).

Cephalexin distributes in body tissues to a variable extent at low concentrations (24, 25). A distribution volume of 15 $\pm$ 2.3 ℓ has been reported (26). Plasma protein binding is low (ca 10-15%)

(1, 25).

Cephalexin is not metabolized in the body and is rapidly excreted unchanged, principally by the kidney, although small amounts are also excreted in bile. Studies with radiolabeled ^{14}C-cephalexin in rats and mice showed that the drug is not metabolized and is eliminated unchanged via the kidney (27). Similarly, cephalexin does not undergo enzymatic metabolism in humans (28).

Between 82 and 100% of an orally administered dose appeared in the urine after 250 and 500 mg doses of cephalexin (29, 30). Thornhill et al. (31) found mean urinary concentrations in a 6 hr period of 830 mcg/ml and 1100 mcg/ml after 250 and 500 mg of cephalexin, respectively. Mean urinary concentrations of 2.3 and 5.0 mg/ml were observed in the first 1-2 hr period following administration of 0.5 and 1.0 g of cephalexin, respectively (32).

In subjects with normal renal function, cephalexin had a serum half-life of approximately 0.6-2.1 h after oral administration (20-22, 24-26, 28, 33), with a mean and standard deviation of 1.2 $\pm$ 0.47 hr. The clearance of cephalexin is prolonged in patients with renal dysfunction. Serum half-lives of 7.7, 10.8, and 13.9 hr were observed in subjects with creatinine clearance of 13.5, 9.2, and 4 ml/min, respectively (33).

Owing to its extremely short half-life and relatively long dosing interval of 4-6 hr, little accumulation of cephalexin in serum of patients with normal renal function has been observed (32, 34). Blood level curves and urinary excretion values following

doses of 250 or 500 mg of cephalexin every 6 hr were unchanged after 1, 3, and 5 days (23). Similarly, the blood level curves and mean urinary recovery values in 6 subjects after 1 gm doses every 6 hr were not significantly different from those following a single dose (35). These observations are to be expected from a drug with a short half-life and relatively long dosing interval.

Cephradine. Cephradine is well absorbed after oral administration as evidenced by recoveries of approximately 100% of the administered dose in the urine (36-38).

After oral administration of cephradine, the time of peak serum concentration was 1 hr with peak concentrations ranging from 6 to 7 mcg/ml after a 250 mg capsule (36, 38). After administration of two 250 mg capsules of cephradine, peak serum concentrations were in the range of 11-18 mcg/ml (36, 38, 39). After the administration of 500 mg of cephradine as an oral suspension, a peak serum concentration of 19.5 mcg/ml was obtained at 0.5 h (38). When subjects were given 1 gm of cephradine, the peak concentrations ranged from 15 to 24 mcg/ml (38, 39).

Following oral administration of ^{3}H-cephradine to mice, the drug was distributed throughout body tissues with greater concentrations found in the stomach, small intestine, kidneys, and liver (40). In humans, similar levels to those in serum were found in lung and adipose tissue six hours after oral administration (24). The volume of distribution of cephradine was reported to be 17-22 $\ell/1.73\ m^2$ (24, 41). Cephradine is weakly bound to plasma protein

with studies reported less than 20% binding (10, 11, 24).

A study conducted in mice and dogs using paper chromatography indicated that cephradine was excreted unchanged in the urine (40). A similar study in humans also failed to show any metabolites (36). Cephradine is eliminated from the body by glomerular filtration and tubular secretion (36). The administration of probenecid, a compound which is known to inhibit renal excretion of semisynthetic cephalosporins by inhibiting their tubular secretion, altered the serum levels of cephradine by increasing its peak concentration, delaying the attainment of this peak, decreasing the rate of elimination, and increasing the area under the serum level curve (42).

The recovery of unchanged cephradine in urine ranged from 78 to 96% after oral doses (36-38). The urinary concentration of cephradine in the first 2 hr after oral administration of 500 mg doses was between 1.1 and 3.2 mg/ml (36-38).

The half-lives of elimination of cephradine were 32 min after intravenous administration (24), and approximately 45 min after oral administration (38) in patients with normal renal function. The half-life of elimination is prolonged in patients with renal impairment (24).

Cefaclor. Cefaclor is rapidly and well absorbed when administered orally (6, 43-47). After oral administration to healthy adults in the fasting state, the peak plasma concentration is achieved in approximately one hour and is proportional to the dose. Mean peak plasma concentrations of approximately 6, 13, 25

mcg/ml were obtained one hour after single oral doses of 250, 500, and 1000 mg, respectively (44, 45). The plasma concentration of cefaclor declines rapidly, with no drug detectable in the plasma four to six hours after a single dose (44, 46).

Cefaclor distributes to a variable extent in body tissues. In a study in dogs, mean cefaclor concentrations obtained following 1 gm oral doses in serum, interstitial fluid, renal tissue, hepatic tissue, bile, and urine were 24.6, 4.4, 9.6, 1.1, 66.2, and 402 mcg/ml, respectively (48). Spyker et al. (47) reported a volume of distribution of 26.4 ℓ in humans. The degree of protein binding in man is approximately 25% (49).

The metabolic fate of cefaclor has been studied in rats, mice, and dogs (43). In rats and mice, cefaclor is primarily excreted unchanged in urine. In dogs, however, cefaclor is more susceptible to metabolism and only a portion of the administered antibiotic is eliminated unchanged via the kidney (43).

In man, the major route of cefaclor elimination is renal excretion. Approximately 70% of the active drug is recovered from the urine within 6 hr in patients with normal renal function (46). The mean urinary concentrations of cefaclor following 250 mg oral doses at the 0-2 hr and 4-6 hr urine collection periods were 1017 and 50.3 mcg/ml, respectively (46).

Cefaclor is apparently not metabolized in the liver. However, because of chemical instability, it degrades to inactive products (13, 15, 50). In vitro at 37°C, only 10% of the original

microbiologically active form is found in plasma after 6 hr. The mean plasma half-life in patients with normal renal function is approximately one hour (51, 52). Elimination of the drug is slower in patients with renal impairment. Spyker et al. (47) reported a plasma half-life of approximately 2.3 hr in anephric patients.

Cefadroxil. Cefadroxil is efficiently absorbed after oral administration (53). Following oral doses of 300 mg, 500 mg, and 1000 mg, peak cefadroxil plasma concentrations of 9.8, 16.2, and 33.0 mcg/ml were attained at 1.2, 1.3, and 1.7 hr, respectively (53, 54). The decline in plasma concentration is relatively slow. Plasma concentrations of 33, 5.4, and 2.1 mcg/ml were found 1.7, 6, and 8 hr after oral administration of 1 gm of cefadroxil (54).

Cefadroxil distributes to a variable extent in body tissues (53, 55). Low plasma protein binding of 20% has been reported (53).

Cefadroxil is primarily eliminated unchanged through the kidney. Urinary excretion accounted for 88-92% of the orally administered dose (53, 54, 56). Urine concentration of 1003 mcg/ml was obtained during the 0-4 hr collection period following a single 500 mg oral dose (53).

In subjects with normal renal function, a serum half-life of approximately 1.3 hr has been reported (53, 54). Serum half-life is prolonged in patients with renal dysfunction (55).

Pharmacokinetic Comparisons. The pharmacokinetics of cephalexin, cephradine, cefaclor, and cefadroxil have been compared in healthy, fasted individuals (46, 53, 54, 56-59).

Cephalexin and cephradine, differing in structure by only two hydrogen atoms in a six-membered ring, have almost identical pharmacokinetic properties. Finkelstein et al. (57) studied the pharmacokinetics of cephradine and cephalexin in normal volunteers after the administration of 1 gm doses in a cross-over design. The results of the study show that no significant pharmacokinetic differences exist between these two compounds. The extent of absorption, peak plasma concentrations, times to reach the peak, rates of absorption and elimination, clearances, lack of metabolism, urinary recovery and urinary concentrations are indistinguishable between these two cephalosporins.

Only minor pharmacokinetic differences exist among cefaclor, cephalexin, and cephradine. Cefaclor has a shorter elimination half-life by approximately 15 min as compared with cephalexin and cephradine (46, 47, 58). Dosage adjustments because of this difference are not required. Plasma and urinary concentrations, and cumulative percent of dose excreted in the urine, are lower with cefaclor than with cephalexin and cephradine in healthy volunteers (46, 51, 54, 58).

Welling et al. (58) examined the pharmacokinetics of cefaclor, cephradine, and cephalexin following single 500 mg oral doses to fasted, healthy volunteers. The results of the study showed that absorption of the three compounds was rapid following a brief lag period and peak plasma concentrations were attained in 1-1.5 hr. No difference was observed in the overall bioavailability of the three

compounds. However, peak concentrations and plasma concentrations during the 1-5 hr postdosing period for cefaclor tended to be lower than those of cephradine and cephalexin. The total urinary recovery of cephradine and cephalexin accounted for almost 90% of the dose administered, whereas cefaclor resulted in a urinary recovery of approximately 55%. Similar results have been reported by other investigators (46, 51, 54).

These differences are not due to metabolic loss of the drug in the body; rather, they are caused by an instability which appears to be pH-dependent (13). These differences between cefaclor and cephradine and cephalexin in plasma and urine concentrations are probably of little clinical significance in light of cefaclor's enhanced microbiological activity (3-6). Other pharmacokinetic studies (44, 60), although non-comparative, support these findings. In essence, cefaclor is similar to cephalexin and cephradine with regard to rate and extent of absorption.

Although cefadroxil differs from cephalexin only by the addition of a para-hydroxyl group on the aromatic ring, its rate of elimination is decreased relatively (53, 54, 59). As a result, plasma and urine concentrations of cefadroxil are higher and more prolonged compared with cephalexin and cephradine (53, 54, 56, 59). The total urinary recoveries of cephalexin, cephradine, and cefadroxil are similar (56, 59).

In summary, the oral cephalosporins cephalexin, cephradine, cefaclor, and cefadroxil have similar spectra of antimicrobial

activity. All four compounds are reported to be efficiently absorbed after oral doses, with the majority of administered drug appearing unchanged in urine. While cephalexin and cephradine have similar pharmacokinetic properties, cefaclor gives rise to a relatively lower circulating level of antibiotic activity and a shorter biological half-life. However, these differences are probably of little clinical significance in light of cefaclor's enhanced microbiological activity. Few pharmacokinetic differences exist among cephalexin, cephradine, and cefadroxil. The relatively prolonged plasma half-life and slower urinary excretion rate indicate that cefadroxil can be administered at 12 hr intervals. However, the therapeutic significance of the different pharmacokinetic characteristics of cephalexin, cephradine, and cefadroxil must be evaluated in light of the differences in their pharmacologic activity and the sensitivity of the target organism.

4. Effect of Food on the Bioavailability of Oral Cephalosporins

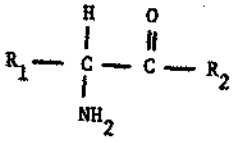
Drug bioavailability studies have routinely been done in fasted individuals. This practice is required by regulatory agencies (61) in order to obtain a controlled study situation. Although this practice is appropriate for the investigation of a new product, or for comparison of different drug products, it bears little relationship to the way in which drugs are prescribed and administered in clinical situations where a patient may frequently ingest drugs and foods in a more random fashion.

There is considerable evidence which indicates that food substances are capable of altering the absorption characteristics, and possibly the therapeutic efficacy, of orally administered compounds (62-65). Depending on the type and degree of interaction, drug absorption may be reduced, delayed, not affected, or increased by concomitant food intake. Suggested mechanisms include delayed gastric emptying, increased intestinal motility, increased splanchnic blood flow, increased gastric secretion, or physicochemical interactions between particular food components and drug molecules (62-65).

Whether the changes in drug absorption due to the presence of food substances are clinically significant depends on the type of drug and on the extent of change. A small change in the absorption characteristics may be critical for a drug with narrow therapeutic index or with a steep dose-response curve. Impaired absorption may be important for antibiotics if plasma concentrations of drug fall below the minimum inhibitory concentration (MIC) for sucseptible organisms.

As discussed previously, a review of the literature indicates that few pharmacokinetic differences exist among the currently available oral cephalosporins. Nevertheless, one of the noted differences is the effect of food on the bioavailability of these agents. Moreover, there is a paucity of data regarding the absorption characteristics of oral cephalosporins, and the existing reports are frequently conflicting.

The physical or chemical characteristics which give rise to efficient absorption of these oral cephalosporins have not been established. However, it is of interest that all these compounds possess the basic structure:



The presence of a free amino group adjacent to a carbonyl group is electronically similar to the free amino group adjacent to a carboxyl group in amino acids. The "amino acid" characteristics of these compounds have been proposed as a possible explanation for their efficient absorption (66). Umeniwa et al. (66) investigated the absorption characteristics of cephalexin, cephradine, and amoxicillin utilizing the recirculating perfusion technique, the loop method, and the everted gut sac technique. Cephradine was found to accumulate in the intestinal tissue and transport against a concentration gradient.

Quay (67) indicated the presence of an active transport mechanism of cephalexin and also demonstrated a competition between cephalexin, glycine, and L-phenylalanylglycine during their absorption from the rat jejunum.

Tsuji et al. (68-72) studied the absorption kinetics of low lipophilic and completely ionized amphoteric β-lactam antibiotics using in situ intestinal loops. The results indicated that the

intestinal absorption of these antibiotics is governed by simple diffusion, following first-order kinetics at a high dose, and is favored by some forms of a carrier-mediated transport process following Michaelis-Menten kinetics at low doses.

Although saturable phenomena and carrier mediated transport systems for oral cephalosporins have been suggested (66-73), the evidence available to prove the existence of these specialized transport mechanisms is insufficient. The absorption characteristics of oral cephalosporins have yet to be elucidated.

It is possible that the absorption characteristics, and hence the therapeutic efficacy, of these oral cephalosporins may be markedly altered by interactions with other substances, including food in the gastrointestinal tract (62-65). While past studies have addressed the problem of factors influencing the absorption of oral cephalosporins, the data are often inconsistent.

Harvengt et al. (37) compared the absorption of cephradine and cephalexin after oral doses to 6 fasted subjects and then to the same subjects 30 min after breakfast. Plasma concentrations of both compounds were depressed at 30 min after the nonfasting dose; but peak plasma concentration, time of peak concentration, and also the total urinary recovery of antibiotic activity were all unaffected.

In another study, Tetzlaff et al. (74) examined the influence of food on the absorption characteristics of cephalexin from capsules and a suspension in children. Plasma antibiotic profiles

indicated very little effect by food on cephalexin absorption from either formulation. The rate of absorption was faster from the suspension than from capsules, and food appeared to delay the absorption from both dosage forms to a small and clinically insignificant extent.

In a subsequent study, Pfeffer et al. (53) compared the effect of food on the bioavailability of cephalexin and cefadroxil following oral doses in 16 volunteers. Plasma concentrations at each sampling time, peak plasma concentration, time of peak concentration, and also the area under the plasma concentration curve were not significantly affected by food for both cephalexin and cefadroxil. Similar results have been obtained in normal volunteers receiving cefadroxil capsules (54), in pediatric patients receiving cefadroxil capsules and suspension (75), in infants and children receiving cefaclor and cefadroxil suspensions (76), and in newborn infants receiving cefaclor suspensions (77).

In contrast to the above findings, various investigators have reported that food substances cause a delay in absorption of cephalexin, cephradine, and cefaclor; and a lowering of plasma antibiotic concentrations (23, 42, 54, 60, 78, 79).

Mischler et al. (42) studied the influence of food on the bioavailability of cephradine capsules in 14 normal subjects. Food altered the cephradine plasma concentration curve by reducing the peak concentration by almost 50 percent and by prolonging the time to peak concentration by approximately 1 hr. Food apparently inter-

fered with the rate of absorption of cephradine but did not affect the total amount absorbed, since the areas under the plasma concentration curves were almost identical for fasting and nonfasting subjects. Complete absorption was further verified by the urinary recovery of 100 percent of the administered dose in both treatment groups.

In another study, Lode et al. (54) reported that administration of cephalexin with simultaneous food intake led to a slower absorption with a delayed and reduced peak plasma concentration. The area under the plasma curve and the urinary recovery were not significantly different between nonfasting and fasting individuals. Almost identical results have been observed for cephalexin following a standard breakfast (23).

Similarly, Glynne et al. (60) showed that food significantly reduced the cefaclor peak plasma concentrations in adult volunteers, but 6 hr urinary excretion was not significantly different in nonfasting and fasting individuals, indicating delayed rather than reduced absorption. In another study in children, food caused a decrease in cefaclor plasma concentration from 10.8 to 6.7 mcg/ℓ at 30 min following a 10 mg/kg dose. However, fasting and nonfasting concentrations were similar at all other sampling times, and areas under the plasma concentration curve were not significantly different between fasting and nonfasting children (78). Shanker et al. (79) also reported similar findings on the effect of food on the bioavailability of cefaclor in children.

In addition to demonstrating delayed drug absorption, several studies have shown that absorption of oral cephalosporins may be significantly reduced with concomitant food intake (21, 76, 78, 81).

Meyer et al. (21) reported that peak plasma concentrations of cephalexin were reduced from 18 mcg/ml to 12 mcg/ml with no prolongation of antibiotic activity in plasma following postprandial doses. The urinary recovery was also reduced from 78-100% to 50-70% of the dose, indicating that the extent of absorption was also reduced with concomitant food intake.

In another study in children, peak concentrations of cephalexin in plasma were diminished by 60% and the area under the plasma concentration curves were reduced by 40% when cephalexin and milk were administered simultaneously (80). Several subsequent investigations further supported these findings (76, 78, 81). McCracken et al. (78) reported that coadministration of cephalexin and milk in children reduced the average area under the plasma concentration-time curve by 43% compared to that of the fasting subjects. Ginsburg et al. (81) also showed that coadministration of cephradine suspension with milk in children resulted in a 50% reduction of peak plasma concentration and a 26% reduction in the area under the plasma concentration-time curve. Ginsburg (76) also compared the effects of milk upon the absorption of cefadroxil, cefaclor, cephalexin, and cephradine in infants and children. The absorption of cefadroxil and cefaclor was not affected. However, both the peak plasma concentrations and areas under the plasma

concentration-time curves for cephalexin and cephradine were significantly reduced after milk ingestion.

In summary, it appears that the absorption of cefadroxil is less affected by the presence of food. However, studies regarding the effect of food on the bioavailability of cephalexin, cephradine, and cefaclor seem to be inconclusive and conflicting. Results have indicated no alteration in absorption, delayed absorption, or decreased absorption of these antibiotics. These numerous inconsistencies between reports show that further study is necessary.

B. Statement of the Problem

While there is a considerable body of knowledge on the pharmacokinetics of oral cephalosporins, there is little comparative information regarding the factors influencing the absorption characteristics of these compounds. The physicochemical characteristics which give rise to the efficient absorption of oral cephalosporins are also not well established. It is probable that the absorption characteristics, and hence the therapeutic efficacy, of these orally administered antibiotics may be markedly altered by interaction with other substances, including food in the gastrointestinal tract (62-65).

The dosage regimens recommended for cephalosporins frequently produce circulating levels of antibiotic that are well above the MIC values for most organisms. However, Wise et al. (82) and others have shown that there is considerable variation in the MIC values of some susceptible strains. As circulating levels of antibiotic in plasma may influence the levels achieved at tissue sites (82), it is important to optimize absorption conditions.

Although past studies have examined the effect of food on the bioavailability of oral cephalosporins, the data are fragmentary and often conflicting. Moreover, the effect of food on the absorption kinetics and compartmental models of oral cephalosporins have never been thoroughly characterized.

Therefore, this study was designed to compare the bioavailability and pharmacokinetics of oral cephalexin, cephradine, and

cefaclor in healthy individuals under fasting and nonfasting conditions. Attention was focused on these three cephalosporins rather than cefadroxil because there are more inconsistencies in data regarding the effect of food on their absorption characteristics. Bioavailability was determined from plasma concentration and urinary excretion data utilizing numerous methods in order to establish a valid conclusion regarding the effect of food on the rate and extent of absorption of oral cephalosporins. Pharmacokinetics were described utilizing both model-independent and model-dependent parameters. Furthermore, plasma concentration data were characterized by two different models in order to gain insight into the effect of food on the absorption kinetics and pharmacokinetic modeling of oral cephalosporins.

This thesis is the result of an in-depth, systematic analysis on the effect of food on the bioavailability and pharmacokinetics of oral cephalexin, cephradine, and cefaclor in man.

It is hoped that the results of this study may contribute to the clarification of the previously reported inconsistencies and to the elucidation of the absorption characteristics of oral cephalosporins; and thereby lead to a rational dosage regimens recommendation to optimize drug bioavailability and therapeutic efficacy.

II. EXPERIMENTAL

A. Materials and Subjects

1. Drugs

A 2 gm sample of cephalexin (potency 940 mcg/mg, lot number 646HD1) and a 25 gm sample of cefaclor (potency 959 mcg/mg, lot number 862AF1) were obtained from Eli Lilly and Company, Indianapolis, IN 46285. A 2 gm sample of cephradine (lot number 31-471-49510-004) was obtained from E. R. Squibb and Sons, Inc., Princeton, NJ 08540. Cephalexin 500 mg capsules (Keflex®, Dista Products Company, lot number 4AX89B), cephradine 500 mg capsules (Velosef®, E. R. Squibb and Sons, Inc., lot number 1B895), and cefaclor 500 mg capsules (Ceclor®, Eli Lilly and Company, lot number 3TS77G) for the oral dose clinical study were obtained commercially.

2. Chemicals

Methanol (distilled in glass) was purchased from Burdick and Jackson Laboratories, Inc., Muskegon, MI 49442. Glacial acetic acid was purchased from Hi-Pure Chemicals, Inc., Nazareth, PA 18064. 8-Hydroxyethyltheophylline was obtained from Sigma Chemical Company, St. Louis, MO 63178. Sodium acetate and potassium phosphate dibasic were purchased from Mallinckrodt Chemical Works,

St. Louis, MO 63160. Potassium phosphate monobasic was obtained from Fisher Scientific Company, Fair Lawn, NJ 07410. All chemicals were either analytical or reagent grade and were used as supplied.

3. Subjects

Eight healthy male subjects participated in the study. All subjects underwent complete physical and laboratory examinations by a qualified physician at the Veterans Administration Hospital, Madison, WI 53705. The laboratory portion included a CBC, urinalysis, and blood chemistry analysis (SMA-12 and SMA-4). Complete drug histories were obtained from all subjects. The following criteria were adopted to exclude subjects from the study:

(i) Any history of drug allergy.

(ii) The taking of any other drugs for two weeks before or during the study.

(iii) Any subject whose vital signs or laboratory values were outside the normal range.

(iv) Any subject with a history of gastrointestinal, hepatic, renal, cardiac, or hematological disease.

(v) Any subject who smoked, or consumed alcohol in excess or had done so in the past.

All eight subjects were in excellent health with biochemistry values within the normal range. Informed consent was obtained from all subjects, and a copy of this form is included for reference in Appendix A. Subject demographic details are given in Table 1.

Table 1

Subject Details

Subject	Age (yr)	Weight (kg)	Height (m)	Body surface area (m^2)
1	39	73.6	1.65	1.81
2	39	77.3	1.80	1.97
3	23	81.8	1.96	2.14
4	32	70.5	1.73	1.84
5	23	70.5	1.78	1.87
6	22	79.5	1.91	2.07
7	22	70.5	1.73	1.84
8	23	70.5	1.80	1.89
Mean	28	74.3	1.80	1.93
SD	7.6	4.6	0.10	0.12
CV%	27.1	6.2	5.6	6.22
Range	22-39	70.5-81.8	1.65-1.96	1.81-2.14

B. Methods

1. Assay

Plasma and urine samples were analyzed for cephalosporins using high pressure liquid chromatographic (HPLC) methods adapted from those described by Carroll et al. (83), Nakagawa et al. (84), and Aziz et al. (85).

a. Sample Preparation

Cephalexin in plasma: One ml of plasma, 0.1 ml of an aqueous 400 mcg/ml solution of internal standard β-hydroxyethyltheophylline, and 1 ml of a 60% methanol/40% 0.2 M sodium acetate solution (pH 5.2) were pipetted into a 15 ml centrifuge tube. The mixture was vortexed for 30 sec and centrifuged at 3000 g for 15 min. The supernatant was filtered through a 0.2 μm, 25 mm Metricel® membrane filter (Gelman Sciences Inc., Ann Arbor, MI 48106), and 20 μl of the filtrate were injected onto the column using a 50 μl Hamilton syringe (Hamilton Company, Reno, NV 89502).

Cephalexin in urine: Urine samples were diluted 10-fold by distilled water. One ml of the diluted urine and 0.1 ml of an aqueous 250 mcg/ml solution of internal standard β-hydroxyethyl-theophylline were pipetted into a test-tube. The mixture was vortexed for 30 sec and filtered through a 0.2 μm, 25 mm Metricel® membrane filter. Twenty μl of the filtrate were injected onto the column using a Hamilton syringe.

Cephradine in plasma: The procedure was identical to the

cephalexin plasma assay, except the internal standard was an aqueous 400 mcg/ml solution of cephalexin.

Cephradine in urine: The procedure was identical to the cephalexin urine assay, except the internal standard was an aqueous 250 mcg/ml solution of cephalexin.

Cefaclor in plasma: The procedure was identical to the cephalexin plasma assay.

Cefaclor in urine: The procedure was identical to the cephalexin urine assay, except all urine samples were diluted 10-fold with 0.1 M pH 4.5 potassium phosphate buffer upon collection. Results obtained from pH studies showed that cefaclor to be markedly more stable under acidic than under alkaline conditions (13). Such a stability pattern indicated that urine samples should be diluted with a pH 4.5 buffer solution.

b. Chromatography

The high pressure liquid chromatographic system consisted of an Altex Model 110A solvent pump (Altex Scientific, Inc., Berkeley, CA 94710), an Altex Model 210 fixed volume (20 µl) injection valve, a Water Associates Model 440 254 nm fixed wavelength absorbance detector (Waters Associates, Inc., Milford, MA 01757), and a Linear Model 255 variable-sensitivity recorder (Linear Instrument, Irvine, CA 92714). The analytical column was a 4.6 x 250 mm, 10 µ particle size, reversed phase, Alltech C-18 µBondapack column (Alltech Associates, Deerfield, IL 60015); which was preceded by a 2.1 x 70 mm,

30-38 μm particle size octadecyl (CO:PELL ODS, Whatman Inc., Clifton, NJ 07014) guard column.

Cephalexin and cefaclor samples were eluted with 15% methanol in 0.01 M sodium acetate buffer (pH 5.2) at a flow rate of 2 ml/min. Detection was at 254 nm and quantification was by the method of peak height ratio. Under these conditions, retention times were 9.5 min for cephalexin and cefaclor, and 12.5 min for the internal standard β-hydroxyethyltheophylline.

Cephradine samples were eluted with 18% methanol in 0.01 M sodium acetate buffer (pH 5.2) at a flow rate of 2 ml/min. Detection was at 254 nm and quantification was by the method of peak height ratio. Under these conditions, cephradine and the internal standard cephalexin had retention times of 7.5 min and 12 min, respectively.

c. Standard Curves

Cephalexin and cephradine stock solutions were prepared in distilled water, were refrigerated, protected from light, and used within one week. Cefaclor stock solution was prepared fresh in a 0.1 M pH 4.5 potassium phosphate buffer daily.

Standard curves of peak height ratio versus concentration were constructed from blank plasma and urine samples containing added known quantities of drug and internal standard. Chromatographic response was linearly related to plasma concentrations of the three cephalosporins of 2.5 to 40 mcg/ml, and to urine concentrations of 25 to 240 mcg/ml. Standard curves were prepared in duplicate along

with each assay batch.

2. Stability Study of Cefaclor

These experiments were carried out to examine the stability characteristics of cefaclor in order to outline procedures for the proper handling, storage, and evaluation of clinical samples of cefaclor.

The stability of cefaclor was examined in 0.1 M pH 4.5 potassium phosphate buffer, pooled human plasma, and pooled human urine from healthy volunteers. Samples were prepared at a concentration of approximately 50 mcg/ml in buffer, plasma, and urine, and stored either at room temperature (ca 25°C), in a refrigerator (ca 4°C), or a freezer (ca -20°C) over maximum periods of 72 hr. Samples were assayed for cefaclor concentration in triplicate at 0, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48, 60, and 72 hr by the HPLC procedure described previously.

3. Clinical Study

Subjects. Eight healthy male volunteers, 22-39 years old (mean 28) and weighing between 70 and 82 kg (mean 74) participated in the study after completing a physical examination. None of the subjects had a history of penicillin or cephalosporin allergy. Informed consent was obtained from each subject. All subjects were ambulatory during the entire clinical study which was conducted in Room 5316 in the School of Pharmacy, University of Wisconsin, Madison, WI 53706.

Protocol. Subjects received single 500 mg oral doses of cephalexin, cephradine, and cefaclor on a fasted stomach or following a standard breakfast which consisted of one glass (4 fl oz) of orange juice, cereal with milk, two poached eggs on two slices of toast and one cup of decaffeinated coffee. The order of dosing was randomized in the form of an eight-subject, six-treatment crossover design as shown in Table 2. The six different treatments were designated as A, B, C, D, E, and F as summarized in Table 3. Treatments were administered at least one week apart in order to ensure complete drug washout and to avoid discomfort due to frequent venipuncture.

Subjects were instructed to take no medication for at least one week before the study, and no medication other than the required cephalosporin doses during the study. Subjects were required not to ingest any caffeine-containing beverages or chocolate for two days before or during the study. Subjects were instructed to eat no food after 8 p.m. and drink no fluid after 10 p.m. on the day preceding a study day. No food, apart from the designated standard breakfast, was permitted on a study day until 4 hr postdosing.

On the morning of a study day, subjects drank 250 ml of water at least 1 hr before dosing. Subjects took the designated medication with 250 ml (fasting treatments) or 25 ml (nonfasting treatments) of water at 8 a.m. Blood samples (ca 10 ml) were taken from a forearm vein into heparinized vacuum tubes (Vacutainers®,

Table 2

Study Design

	Week					
Subject	I	II	III	IV	V	VI
1	A	B	C	D	E	F
2	B	E	F	A	C	D
3	D	A	E	B	F	C
4	C	F	A	E	D	B
5	F	D	B	C	A	E
6	E	C	D	F	B	A
7	A	B	C	D	E	F
8	B	E	F	A	C	D

Table 3

Summary of Treatments

Treatment	Description
A	1 x 500 mg Cephalexin capsule with 250 ml of water after overnight fast
B	1 x 500 mg Cephalexin capsule with 25 ml of water after breakfast
C	1 x 500 mg Cephradine capsule with 250 ml of water after overnight fast
D	1 x 500 mg Cephradine capsule with 25 ml of water after breakfast
E	1 x 500 mg Cefaclor capsule with 250 ml of water after overnight fast
F	1 x 500 mg Cefaclor capsule with 25 ml of water after breakfast

Becton-Dickinson, Rutherford, NJ 07070) immediately before and then at 0.25, 0.5, 0.75, 1 1.5, 2, 2.5, 3, 4, 6, and 8 hr post-dosing. Plasma was separated immediately after venipuncture by centrifugation at 3000 g for 10 min. Approximately 4 ml of plasma was transferred into properly labelled test-tubes and immediately frozen until assayed. Urine was collected immediately before and then quantitatively during 0-2, 2-4, 4-8, and 8-12 hr postdosing. Urine volumes were recorded, pH values were determined by a pH meter (Orion Research, Inc., Cambridge, MA 02139), and 20 ml samples were then frozen until assayed. Cefaclor urine samples were diluted 10-fold with a 0.1 M pH 4.5 phosphate buffer upon collection, and 20 ml samples were frozen immediately until assayed. The time intervals between the collection of biological fluids and quantification of cefaclor were minimized owing to the chemical instability of cefaclor. All cefaclor plasma and urine samples were analyzed within 12 hr and 36 hr, respectively, of being taken.

4. Data Analysis

a. Pharmacokinetic Analysis

Individual plasma drug profiles were examined to obtain maximum drug concentrations, C_{max}, and times of maximum drug concentration, t_{max}. Areas under the plasma drug concentration versus time curves from zero to 8 h, $AUC^{0\rightarrow 8h}$ (which is identical to $AUC^{0\rightarrow\infty}$ in this study), were calculated using the trapezoidal method (86) as shown in Equation 1,

$$AUC_{trap}^{0\to 8h} = \sum_{i=0}^{n-1} \frac{(C_{i+1} + C_i)(t_{i+1} - t_i)}{2} \qquad (1)$$

where C is plasma drug concentration at time t, and n is the number of sample points.

Individual drug plasma concentrations were also plotted versus time on semilogarithmic paper. In all cases, linearity was observed during the elimination phase, which indicated that plasma data could be analyzed utilizing a simple one-compartment model. Therefore, individual plasma drug profiles were analyzed using a one-compartment model with first-order absorption and elimination, and also a one-compartment model with zero-order absorption and first-order elimination in order to define an appropriate apparent absorption model for oral cephalosporins.

In the first-order model, individual data sets were fitted to Equation 2,

$$C = \frac{FD}{V} \left(\frac{k_a}{k_a - k_{el}} \right) \left[e^{-k_{el}(t-t_o)} - e^{-k_a(t-t_o)} \right] \qquad (2)$$

where C is the drug concentration in plasma, F is the fraction of the dose D absorbed, V is the apparent drug distribution volume, k_a is the apparent first-order absorption rate constant, k_{el} is the first-order elimination rate constant, t is the time since dosing, and t_o is the time elapsed between dosing and the time of appearance of measurable drug in plasma.

In the zero-order model, individual data sets were fitted simultaneously to Equations 3 and 4 for times less than and greater than the times of peak plasma concentrations, respectively,

$$C = \frac{Fk_o}{Vk_{el}} [1 - e^{-k_{el}(t-t_o)}] \qquad t \leq t_{max} \qquad (3)$$

$$C = \frac{Fk_o}{Vk_{el}} [1 - e^{-k_{el}(t_{max}-t_o)}]e^{-k_{el}(t-t_{max})} \qquad t > t_{max} \qquad (4)$$

where k_o is the apparent zero-order absorption rate constant, t_{max} is the time of observed peak drug concentration in plasma, and other symbols are as defined for Equation 2.

In the first-order absorption case, initial estimates of k_a, k_{el}, and FD/V were obtained by standard graphical methods. Improved estimates of parameter values were obtained by nonlinear regression analysis using the program NREG (87) on a Univac 1100 digital computer. In some cases, the elimination rate constant k_{el} was fixed during the computer fitting in order to obtain satisfactory convergence.

In the zero-order absorption case, initial estimates of the value Fk_o/V were obtained by dividing computer estimates of FD/V, obtained from the first-order case, by the value $t_{max}-t_o$, which is the apparent drug absorption time. Again, improved estimates of parameter values were obtained using the program NREG on a digital computer.

Urinary excretion data were described in terms of cumulative percent recovery of the unchanged drug in urine.

The relative bioavailability between the fasting and nonfasting treatments for the three cephalosporins were evaluated in terms of maximum drug concentrations in plasma, times at which maximum concentrations occurred, areas under the plasma drug concentrations *versus* time profiles, and cumulative 0-12 h urinary recovery. In addition, the relative absorption efficiency of cephalosporins between fasting and nonfasting treatments was estimated by the four following methods.

Method I assumes that plasma clearance of cephalosporin is constant and is based on Equation 5,

$$\frac{F_{nf}}{F_f} = \frac{D_f (AUC)_{nf}}{D_{nf} (AUC)_f} \qquad (5)$$

where D is the oral dose, AUC is the area under the plasma concentration *versus* time curve, and the subscripts f and nf refer to fasting and nonfasting treatments, respectively.

Method II assumes that non-renal clearance of cephalosporins remains constant, but a correction term is incorporated for changing renal clearance, as in Equation 6,

$$\frac{F_{nf}}{F_f} = \frac{D_f (AUC)_{nf}}{D_{nf} (AUC)_f} - (Cl_{renal\ f} - Cl_{renal\ nf}) \frac{(AUC)_{nf}}{D_{nf}} \qquad (6)$$

where Cl_{renal} is renal clearance of cephalosporin and is calculated

from Equation 7,

$$Cl_{renal} = \frac{A_e}{AUC} \qquad (7)$$

where A_e is the total amount of drug recovered unchanged in 12 h urine.

Method III is based on the dose-corrected amounts of drug excreted in urine as in Equation 8,

$$\frac{F_{nf}}{F_f} = \frac{(A_e)_{nf} D_f}{(A_e)_f D_{nf}} \qquad (8)$$

Method IV estimates the cumulative relative fraction of drug absorbed (CRFA) during 8 hr following each treatment. Cumulative relative fractions of drug absorbed were calculated by means of Equation 9, which was derived from a previously described method for generating percent absorbed *versus* time plots (88),

$$CRFA = \frac{C_t + k_{el_f} (AUC)^{0\to t}}{k_{el_f} (AUC)_f^{0\to\infty}} \qquad (9)$$

where C_t is the concentration of cephalosporin in plasma at time t, $AUC^{0\to t}$ is the area under the plasma concentration *versus* time curve from zero to time t. The values k_{el_f} and $(AUC)_f^{0\to\infty}$ are the first-order elimination rate constant and the area under the plasma concentration *versus* time curve from zero to infinite time, respectively, following the fasting treatment. This method permits

the cumulative absorption of drug from the fasting treatments to be calculated in individual subjects relative to the nonfasting treatments.

In addition to the above methods, plasma data were also analyzed by statistical moments analysis in terms of the mean residence time (MRT) and the variance of the mean residence time (VRT) (89, 90). These values, which represent the first normal and second normal moments of the plasma drug profile, respectively, are defined by Equations 10 and 11.

$$MRT = \frac{\int_0^\infty tCdt}{\int_0^\infty Cdt} \qquad (10)$$

$$VRT = \frac{\int_0^\infty (t - MRT)^2 Cdt}{\int_0^\infty Cdt} \qquad (11)$$

Besides Equation 10, the mean residence time was also calculated by means of Equations 12 and 13,

$$MRT = \frac{1}{k_a} + \frac{1}{k_{el}} \qquad (12)$$

$$MRT = \frac{T}{2} + \frac{1}{k_{el}} \qquad (13)$$

where k_a is the apparent first-order absorption rate constant, k_{el} is the first-order elimination rate constant, and T is the

apparent absorption time.

Equation 12 is derived under the assumption that drug absorption and drug elimination are by first-order processes, while Equation 13 is derived under the assumption that drug absorption is by a zero-order process and drug elimination is by a first-order process.

b. Statistical Analysis

Plasma cephalosporin concentrations at each sampling time, cumulative relative percent absorption, cumulative percent urinary recovery, and all pharmacokinetic parameter values were compared between treatments by analysis of variance for crossover design using the statistical computing system MINITAB (91) on a Univac digital computer. Parameter values generated by computer fitting were similarly compared, when appropriate, between the two absorption models. Values of p less than 0.05 were considered significant. When significant treatment effects were obtained, differences between individual treatments were examined by Tukey's significant difference test (92).

III. RESULTS

A. Assay

Representative chromatograms for the quantification of cephalexin, cephradine, and cefaclor in plasma are shown in Figures 2, 3, and 4, respectively. The chromatographic conditions provided adequate separation of the cephalosporin peaks from ultraviolet absorbing components of the plasma sample. Retention times were 9.5 min for cephalexin and cefaclor, and 12 min for cephradine under the chromatographic conditions specified previously. Standard curves for cephalexin, cephradine, and cefaclor in plasma are given in Figures 5, 6, and 7, respectively. Statistical analysis of the linear regression model was done, and the results of the analysis of variance (ANOVA) of the regression equations for these three curves are given in Tables 4, 5, and 6. In summary, the regression equations of peak height ratio y versus concentration x(mcg/ml) in plasma were:

(a) cephalexin, $y = 0.008+0.027x$, $n=10$, $r^2=0.993$, $p<0.005$,

(b) cephradine, $y = 0.026+0.015x$, $n=10$, $r^2=0.998$, $p<0.005$,

(c) cefaclor, $y = 0.023+0.026x$, $n=10$, $r^2=0.994$, $p<0.005$.

The high coefficients of determination indicated that peak height ratios were linearly proportional to plasma concentrations in the

range of 2.5 to 40 mcg/ml. The day-to-day reproducibility of the plasma assays for the three cephalosporins determined on ten occasions are given in Tables 7, 8, and 9. The coefficients of variation are within 10% in most cases.

Typical chromatograms for the quantification of cephalexin, cephradine, and cefaclor in urine are shown in Figures 8, 9, and 10, respectively. Retention times for cephalexin, cephradine, and cefaclor were identical to those obtained from the plasma assay, and the chromatographic response was linear for all three cephalosporins in the range of 25 to 240 mcg/ml, with coefficients of variation less than 7.5%. Standard curves for cephalexin, cephradine, and cefaclor in urine are shown in Figures 11, 12, and 13, and results of the ANOVA of the regression equations for these three curves are given in Tables 10, 11, and 12. The regression equations of peak height ratio y versus concentration x(mcg/ml) in urine were:

(a) cephalexin, $y=-0.030+0.027x$, $n=5$, $r^2=0.987$, $p<0.005$,

(b) cephradine, $y=0.066+0.013x$, $n=5$, $r^2=0.997$, $p<0.005$,

(c) cefaclor, $y=-0.016+0.031x$, $n=5$, $r^2=0.998$, $p<0.005$.

The day-to-day reproducibility of the urine assays of the three cephalosporins determined on five occasions are given in Tables 13, 14, and 15.

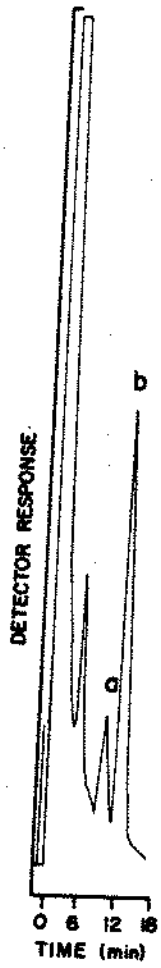


Figure 2. A representative chromatogram of plasma sample containing standard solutions of cephalexin and internal standard β-hydroxyethyltheophylline.
Key: a Cephalexin (10 mcg/ml)
b β-Hydroxyethyltheophylline (40 mcg/ml)

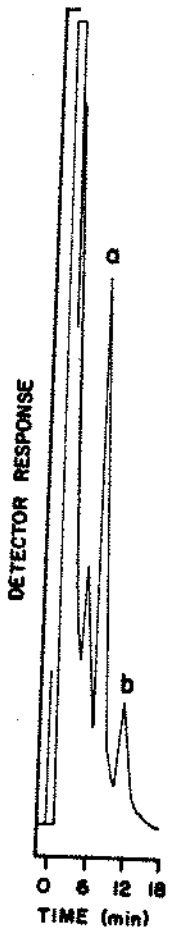


Figure 3. A representative chromatogram of plasma sample containing standard solutions of cephradine and internal standard cephalexin.
Key: a Cephalexin (40 mcg/ml)
b Cephradine (10 mcg/ml)

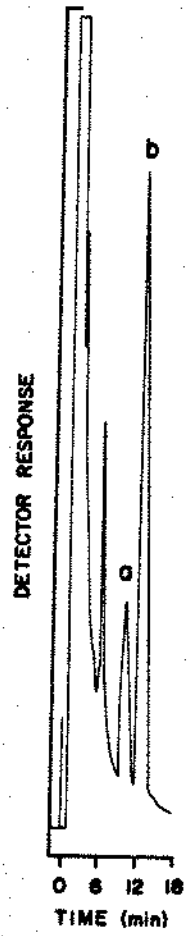


Figure 4. A representative chromatogram of plasma sample containing standard solutions of cefaclor and internal standard β-hydroxyethyltheophylline.
Key: a Cefaclor (10 mcg/ml)
b β-Hydroxyethyltheophylline (40 mcg/ml)

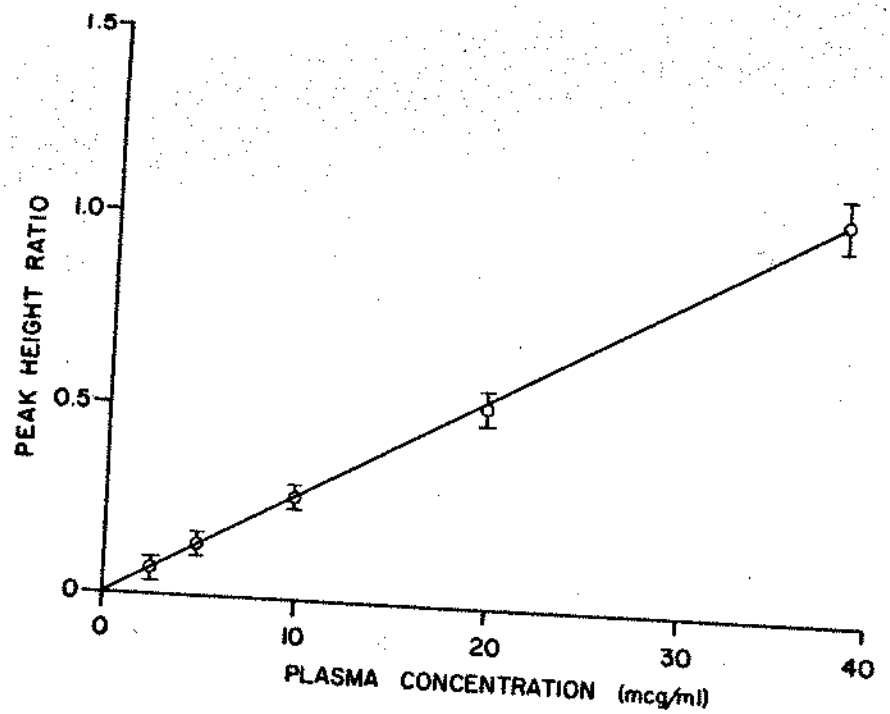


Figure 5. Standard curve relating the mean peak height ratio (±1 SD, n = 10) to the standard concentration of cephalexin in plasma.

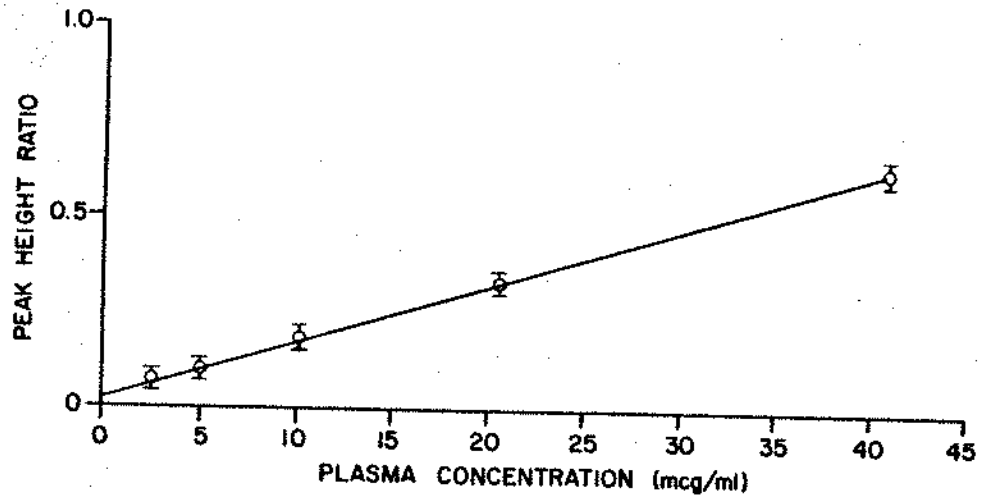


Figure 6. Standard curve relating the mean peak height ratio (±1 SD, n = 10) to the standard concentration of cephradine in plasma.

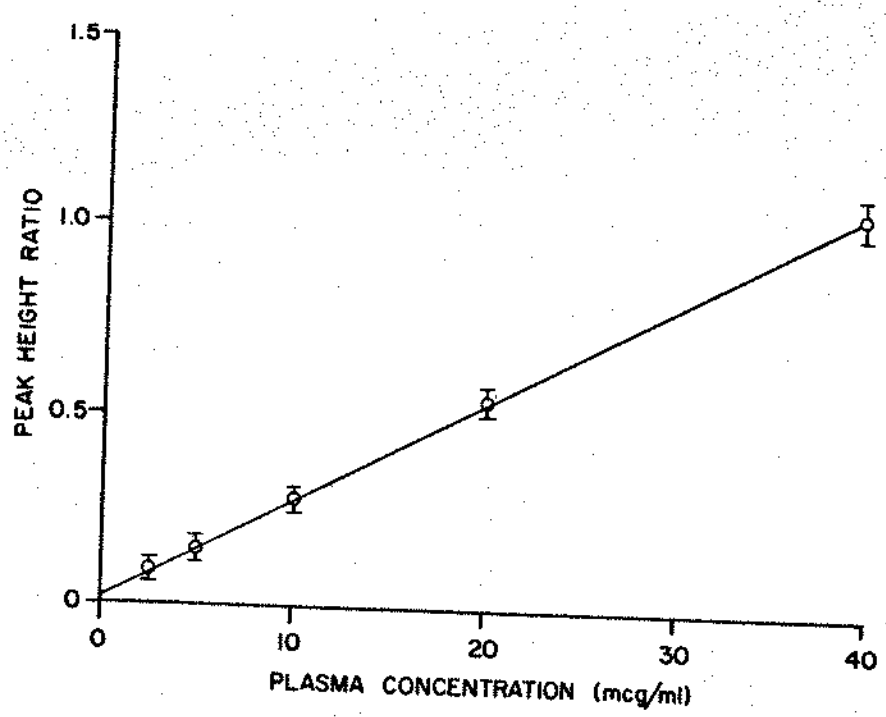


Figure 7. Standard curve relating the mean peak height ratio (±1 SD, n = 10) to the standard concentration of cefaclor in plasma.

Table 4

ANOVA Table for Linear Regression of the Standard Curve of Cephalexin in Plasma

Source of variation	D.F.	S.S.	M.S.	V.R.	$F_{(0.95)}$
Regression	1	6.2815	6.2815	6979.4	4.05
Residual	48	0.0432	0.0009		$p < 0.005$
Total	49	6.3247			

Coefficient of determination, $r^2 = 0.993$.

The regression equation is,
$Y = 0.00849\ (\pm 0.00643) + 0.0266\ (\pm 0.0003)X$ where values in parentheses indicate standard deviation.

Table 5

ANOVA Table for Linear Regression of the Standard Curve of Cephradine in Plasma

Source of variation	D.F.	S.S.	M.S.	V.R.	$F_{(0.95)}$
Regression	1	2.1122	2.1122	21122	4.05
Residual	48	0.0041	0.0001		$p < 0.005$
Total	49	2.1163			

Coefficient of determination, $r^2 = 0.998$.

The regression equation is,
$Y = 0.0262 (\pm 0.0020) + 0.0147 (0.0001)X$ where values in parentheses indicate standard deviation.

Table 6

ANOVA Table for Linear Regression of the Standard Curve of Cefaclor in Plasma

Source of variation	D.F.	S.S.	M.S.	V.R.	$F_{(0.95)}$
Regression	1	6.0451	6.0451	7556.4	4.05
Residual	48	0.0395	0.0008		$p < 0.005$
Total	49	6.0846			

Coefficient of determination, $r^2 = 0.994$.

The regression equation is,
$Y = 0.0232\ (\pm\ 0.00614) + 0.0258\ (\pm\ 0.00030)X$ where values in parentheses indicate standard deviation.

Table 7

Values of the Peak Height Ratios Obtained from Plasma Samples Containing Standard Solutions of Cephalexin and Internal Standard β-hydroxyethyltheophylline

	Peak height ratios				
Set	2.45 (mcg/ml)	4.89 (mcg/ml)	9.78 (mcg/ml)	19.6 (mcg/ml)	39.1 (mcg/ml)
1	0.052	0.12	0.23	0.49	0.97
2	0.053	0.13	0.25	0.49	1.0
3	0.071	0.13	0.25	0.51	1.0
4	0.070	0.13	0.25	0.50	1.0
5	0.070	0.14	0.28	0.55	1.05
6	0.071	0.14	0.28	0.53	1.07
7	0.071	0.15	0.29	0.56	1.05
8	0.085	0.14	0.28	0.56	1.08
9	0.099	0.14	0.29	0.58	1.13
10	0.099	0.15	0.28	0.55	1.12
Mean	0.074	0.14	0.27	0.53	1.05
SD[a]	0.016	0.009	0.021	0.033	0.054
CV%[b]	21.6	6.43	7.78	6.23	5.14

[a]Standard deviation.

[b]Coefficient of variation.

Table 8

Values of the Peak Height Ratios Obtained from Plasma Samples Containing Standard Solutions of Cephradine and Internal Standard Cephalexin

	Peak height ratios				
Set	2.56 (mcg/ml)	5.12 (mcg/ml)	10.2 (mcg/ml)	20.5 (mcg/ml)	41.0 (mcg/ml)
1	0.050	0.10	0.18	0.34	0.64
2	0.056	0.095	0.18	0.34	0.65
3	0.055	0.10	0.18	0.34	0.63
4	0.056	0.098	0.18	0.32	0.62
5	0.057	0.11	0.18	0.33	0.62
6	0.056	0.11	0.17	0.33	0.60
7	0.064	0.11	0.18	0.32	0.63
8	0.065	0.11	0.18	0.32	0.61
9	0.067	0.11	0.17	0.32	0.63
10	0.056	0.10	0.18	0.34	0.64
Mean	0.058	0.10	0.18	0.33	0.63
SD	0.005	0.006	0.004	0.009	0.015
CV%	8.62	6.00	2.22	2.73	2.38

Table 9

Values of the Peak Height Ratios Obtained from Plasma Samples Containing Standard Solutions of Cefaclor and Internal Standard β-hydroxyethyltheophylline

	Peak height ratios				
Set	2.47 (mcg/ml)	4.94 (mcg/ml)	9.87 (mcg/ml)	19.7 (mcg/ml)	39.5 (mcg/ml)
1	0.099	0.17	0.31	0.55	1.07
2	0.074	0.17	0.27	0.54	1.01
3	0.083	0.16	0.27	0.54	0.97
4	0.080	0.15	0.27	0.50	1.04
5	0.068	0.14	0.27	0.53	1.09
6	0.086	0.15	0.29	0.54	1.01
7	0.090	0.14	0.26	0.49	0.97
8	0.066	0.13	0.27	0.56	1.08
9	0.087	0.17	0.33	0.58	1.13
10	0.086	0.15	0.25	0.53	1.04
Mean	0.082	0.15	0.28	0.54	1.04
SD	0.01	0.014	0.024	0.026	0.052
CV%	12.2	9.33	8.57	4.81	5.0

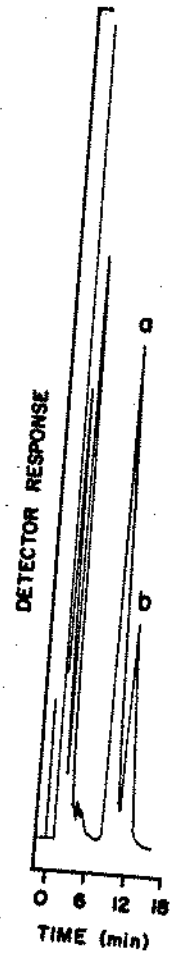


Figure 8. A representative chromatogram of urine sample containing standard solutions of cephalexin and internal standard β-hydroxyethyltheophylline.
Key: a Cephalexin (100 mcg/ml)
b β-Hydroxyethyltheophylline (25 mcg/ml)

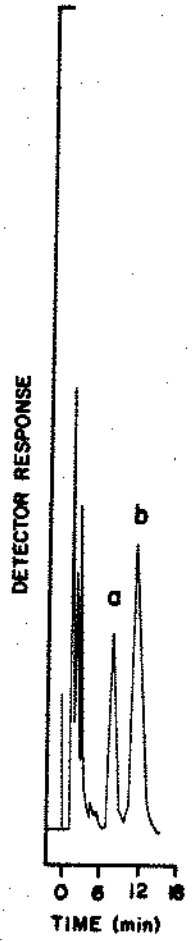


Figure 9. A representative chromatogram of urine sample containing standard solutions of cephradine and internal standard cephalexin.

Key: a Cephalexin (25 mcg/ml)
b Cephradine (100 mcg/ml)

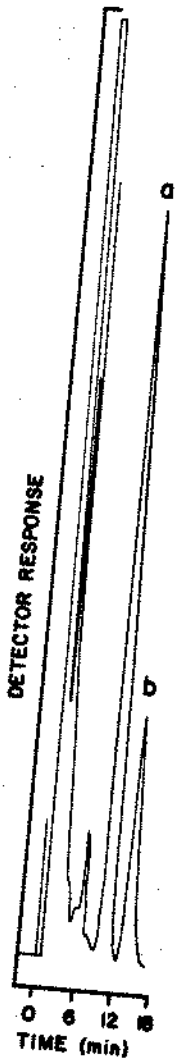


Figure 10. A representative chromatogram of urine sample containing standard solutions of cefaclor and internal standard β-hydroxyethyltheophylline.
Key: a Cefaclor (100 mcg/ml)
b β-Hydroxyethyltheophylline (25 mcg/ml)

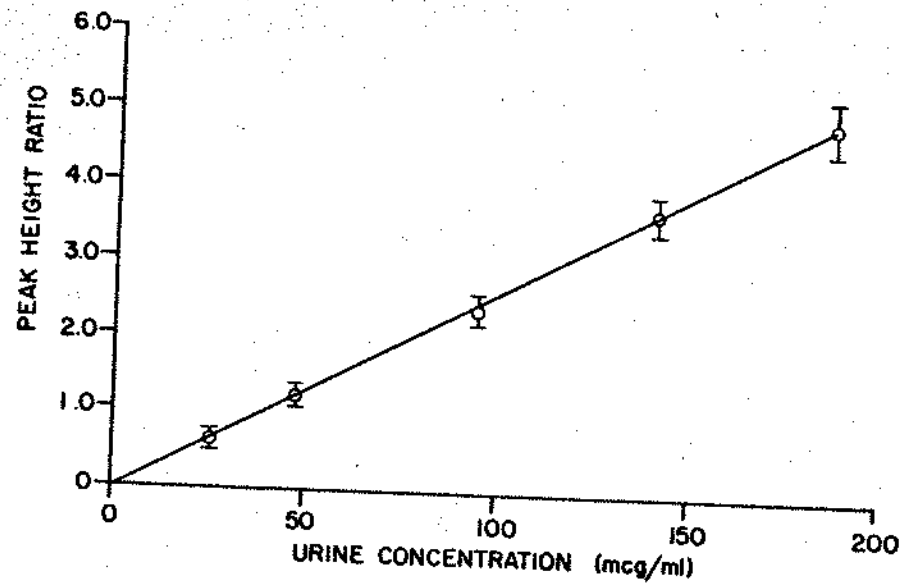


Figure 11. Standard curve relating the mean peak height ratio (±1 SD, n = 5) to the standard concentration of cephalexin in urine.

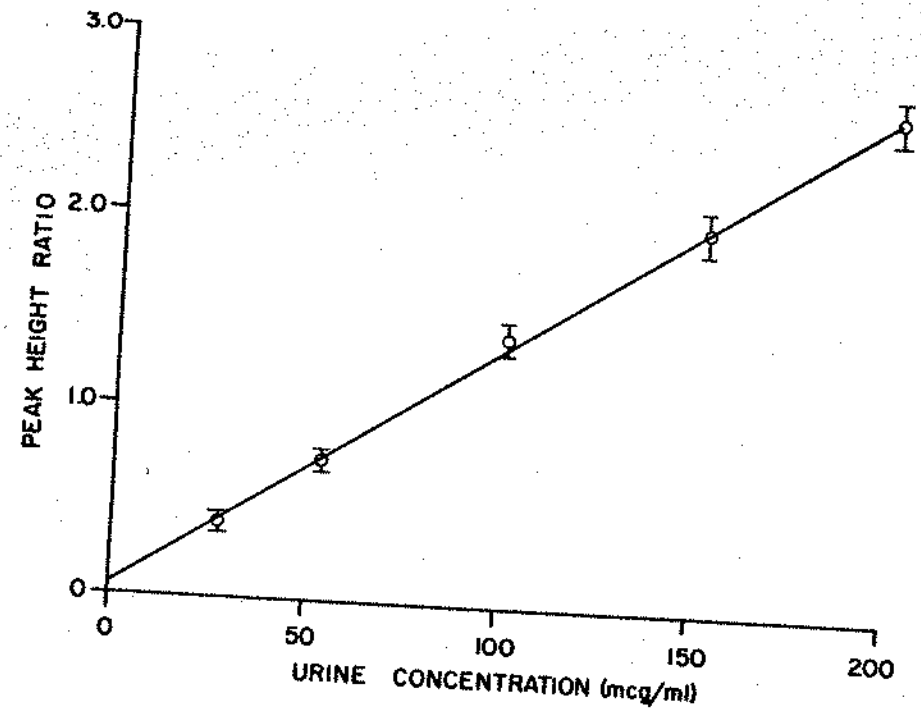


Figure 12. Standard curve relating the mean peak height ratio (±1 SD, n = 5) to the standard concentration of cephradine in urine.

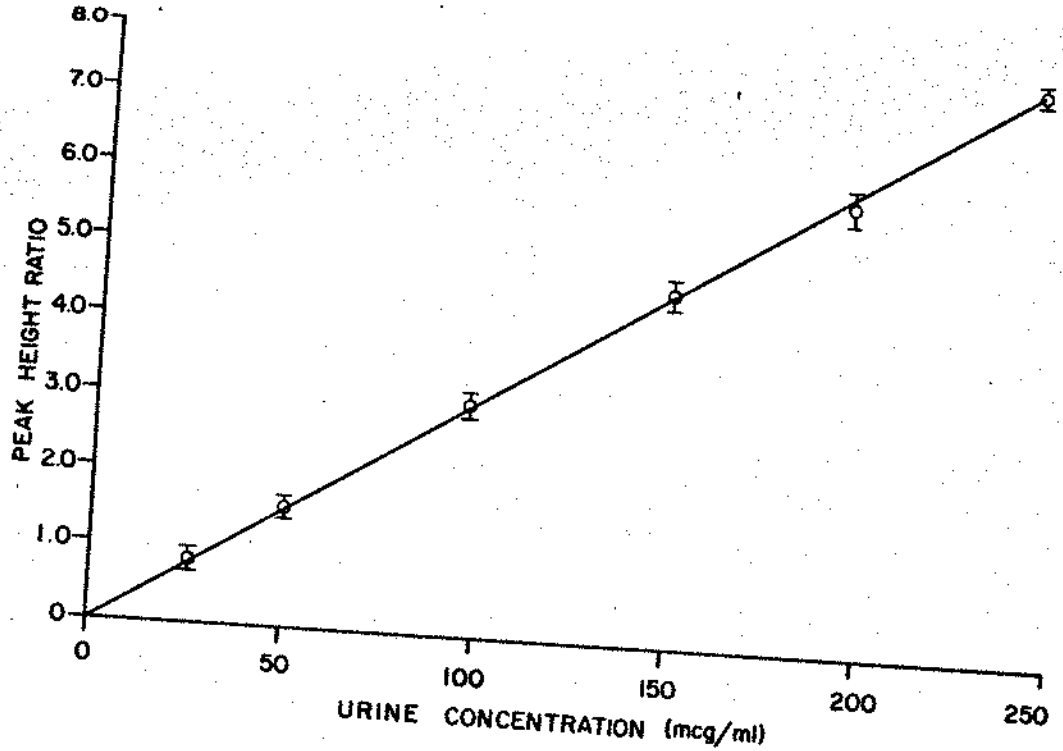


Figure 13. Standard curve relating the mean peak height ratio (±1 SD, n = 5) to the standard concentration of cefaclor in urine.

Table 10

ANOVA Table for Linear Regression of the Standard Curve of Cephalexin in Urine

Source of variation	D.F.	S.S.	M.S.	V.R.	$F_{(0.95)}$
Regression	1	61.775	61.775	1765	4.28
Residual	23	0.815	0.035		$p < 0.005$
Total	24	62.590			

Coefficient of determination, $r^2 = 0.987$.

The regression equation is
$Y = -0.0298 (\pm 0.0732) + 0.0265 (\pm 0.00063)X$ where values in parentheses indicate standard deviation.

Table 11

ANOVA Table for Linear Regression of the Standard Curve of Cephradine in Urine

Source of variation	D.F.	S.S.	M.S.	V.R.	$F_{(0.95)}$
Regression	1	16.176	16.176	8088	4.28
Residual	23	0.044	0.002		$p < 0.005$
Total	24	16.220			

Coefficient of determination, $r^2 = 0.997$.

The regression equation is
$Y = 0.0664 (\pm 0.0171) + 0.0127 (\pm 0.0001)X$ where values in parentheses indicate standard deviation.

Table 12

ANOVA Table for Linear Regression of the Standard Curve of Cefaclor in Urine

Source of variation	D.F.	S.S.	M.S.	V.R.	$F_{(0.95)}$
Regression	1	168.98	168.98	16898	4.20
Residual	28	0.34	0.01		$p < 0.005$
Total	29	169.33			

Coefficient of determination, $r^2 = 0.998$.

The regression equation is
$Y = -0.0158\ (\pm\ 0.0388) + 0.0310\ (0.0003)X$ where values in parentheses indicate standard deviation.

Table 13

Values of the Peak Height Ratios Obtained from Urine Samples Containing Standard Solutions of Cephalexin and Internal Standard β-hydroxyethyltheophylline

	Peak height ratios				
Set	25.3 (mcg/ml)	47.2 (mcg/ml)	94.5 (mcg/ml)	141 (mcg/ml)	187 (mcg/ml)
1	0.65	1.20	2.39	3.65	5.03
2	0.60	1.09	2.22	3.45	4.62
3	0.70	1.21	2.39	3.54	4.54
4	0.67	1.30	2.50	3.94	5.29
5	0.70	1.32	2.58	3.98	5.19
Mean	0.66	1.22	2.42	3.71	4.93
SD	0.042	0.092	0.14	0.24	0.34
CV%	6.36	7.54	5.79	6.47	6.90

Table 14

Values of the Peak Height Ratios Obtained from Urine Samples Containing Standard Solutions of Cephradine and Internal Standard Cephalexin

	Peak height ratios				
Set	27.1 (mcg/ml)	53.6 (mcg/ml)	101 (mcg/ml)	152 (mcg/ml)	201 (mcg/ml)
1	0.41	0.75	1.38	1.97	2.57
2	0.40	0.72	1.39	2.03	2.65
3	0.39	0.73	1.32	1.89	2.51
4	0.42	0.74	1.40	2.05	2.67
5	0.40	0.73	1.39	2.01	2.63
Mean	0.40	0.73	1.38	1.99	2.61
SD	0.011	0.011	0.032	0.063	0.065
CV%	2.75	1.51	2.32	3.17	2.49

Table 15

Values of the Peak Height Ratios Obtained from Urine Samples Containing Standard Solutions of Cefaclor and Internal Standard β-hydroxyethyltheophylline

	Peak height ratios					
Set	25.4 (mcg/ml)	50.3 (mcg/ml)	97.4 (mcg/ml)	149 (mcg/ml)	194 (mcg/ml)	242 (mcg/ml)
1	0.75	1.53	3.01	4.77	5.87	7.54
2	0.82	1.65	3.06	4.75	6.14	7.54
3	0.74	1.44	2.85	4.46	5.65	7.48
4	0.83	1.61	3.03	4.60	5.93	7.55
5	0.79	1.49	3.01	4.42	6.01	7.53
Mean	0.79	1.54	2.99	4.6	5.92	7.53
SD	0.040	0.086	0.082	0.16	0.18	0.028
CV%	5.06	5.58	2.74	3.48	3.04	0.37

B. Stability Study of Cefaclor

The percent residual cefaclor in 0.1 M pH 4.5 potassium monobasic phosphate buffer, plasma, and urine following periods of storage at room temperature, in a refrigerator, and in a freezer are given in Tables 16, 17, 18, and Figures 14, 15, and 16. The points in the figures represent averages of three determinations at each time. The percent degradation in pH 4.5 potassium monobasic phosphate buffer was approximately equal to or less than 10% after storage at room temperature and in the refrigerator for 48 hr. A markedly different stability pattern was observed with cefaclor plasma samples when stored at different temperatures. After storage at room temperature, in the refrigerator, and in the freezer for 24 hr, cefaclor plasma samples showed 90%, 37%, and 11% degradation, respectively. A similar stability pattern was observed with cefaclor urine samples, although cefaclor was more stable in urine than in plasma. After storage at room temperature, in the refrigerator, and in the freezer for 24 hr, cefaclor urine samples showed 38%, 9% and 0% degradation, respectively. The regression equations of ln percent residual cefaclor y(%) versus time x(hr) were:

(a) pH 4.5 buffer at room temperature,

$y = 4.60 - 0.0025x$, $r^2 = 0.934$,

(b) pH 4.5 buffer in the refrigerator,

$y = 4.60 - 0.00085x$, $r^2 = 0.993$,

(c) plasma at room temperature,

$y = 4.61 - 0.093x$, $r^2 = 0.991$,

(d) plasma in the refrigerator,

$y = 4.62 - 0.020x$, $r^2 = 0.996$,

(e) plasma in the freezer,

$y = 4.59 - 0.0031x$, $r^2 = 0.931$,

(f) urine at room temperature,

$y = 4.64 - 0.020x$, $r^2 = 0.996$,

(g) urine in the refrigerator,

$y = 4.60 - 0.0035x$, $r^2 = 0.991$,

(h) urine in the freezer,

$y = 4.61 - 0.0018x$, $r^2 = 0.959$.

The slopes of these regression equations are the negative of the apparent first-order degradation rate constants (hr^{-1}) for cefaclor under the specified storage conditions. Thus, these rate constants indicate that cefaclor is most stable in pH 4.5 buffer and degradation in biological fluids can be minimized by storage in a freezer.

Table 16

Percent Residual Cefaclor in 0.1 M Potassium Monobasic Phosphate (pH 4.5) Stock Solution Following Periods of Storage at Room Temperature and in a Refrigerator

	Percent residual cefaclor in pH 4.5 stock solution													
	0 h	1 h	2 h	3 h	4 h	6 h	8 h	10 h	12 h	24 h	36 h	48 h	60 h	72 h
Room Temperature[a]	100 (0)[b]	100 (2.5)	97.7 (3.0)	97.8 (2.3)	98.0 (1.5)	97.2 (1.0)	98.6 (2.1)	96.8 (0.5)	94.1 (3.2)	93.7 (0.6)	92.8 (1.3)	88.7 (1.7)	83.7 (1.1)	83.7 (2.5)
Refrigerator[a]	100 (0)	103 (5.4)	100 (2.2)	98.6 (2.0)	99.2 (0.8)	101 (3.2)	98.2 (1.5)	98.2 (2.4)	98.7 (2.7)	99.0 (0.4)	-[c]	95.5 (1.2)	-	94.0 (0.5)

[a]Initial concentration of cefaclor in stock solution was 51.3 mcg/ml.

[b]Values in parentheses indicate standard deviation.

[c]Not determined.

Table 17

Percent Residual Cefaclor in Plasma Following Periods of Storage at Room Temperature, in a Refrigerator and in a Freezer

	Percent residual cefaclor in plasma (pH 7.3)													
	0 h	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h	9 h	10 h	24 h	48 h	60 h
Room[a] Temperature	100 (0)[d]	80.8 (9.0)	77.7 (14.1)	76.5 (5.0)	72.0 (10.7)	66.0 (4.3)	58.9 (9.3)	54.0 (1.2)	50.3 (0.4)	44.7 (3.7)	41.1 (2.1)	10.2 (2.8)	-[e]	-
Refrigerator[b]	100 (0)	100 (2.0)	97.0 (5.5)	-	95.2 (1.0)	-	90.0 (4.1)	-	86.1 (2.5)	84.9 (6.4)	82.1 (5.6)	62.7 (0.7)	-	-
Freezer[c]	100 (0)	99.7 (3.4)	97.6 (1.5)	-	100.6 (2.7)	-	94.2 (9.4)	-	100.9 (4.4)	-	96.0 (2.3)	89.1 (4.7)	83.3 (8.5)	83.6 (0.9)

[a] Initial concentration of cefaclor in plasma was 51.2 mcg/ml.

[b] Initial concentration of cefaclor in plasma was 51.8 mcg/ml.

[c] Initial concentration of cefaclor in plasma was 54.0 mcg/ml.

[d] Values in parentheses indicate standard deviation.

[e] Not determined.

Table 18

Percent Residual Cefaclor in Urine Following Periods of Storage at Room Temperature, in a Refrigerator and in a Freezer

	Percent residual cefaclor in urine (pH 6.0)												
	0 h	1 h	2 h	3 h	4 h	8 h	10 h	12 h	24 h	36 h	48 h	60 h	72 h
Room Temperature[a]	100 (0)[c]	97.3 (1.2)	95.2 (1.3)	95.7 (2.0)	95.7 (4.2)	90.3 (0.5)	87.1 (1.4)	84.4 (3.0)	61.8 (5.2)	52.7 (2.8)	39.8 (2.3)	30.7 (0.5)	25.3 (0.9)
Refrigerator[a]	100 (0)	99.7 (2.5)	99.5 (1.5)	99.2 (3.0)	99.3 (2.0)	97.2 (1.3)	-[d]	95.0 (1.5)	91.0 (0.7)	88.0 (2.6)	84.0 (4.7)	79.6 (1.5)	79.0 (0.8)
Freezer[b]	100 (0)	-	100 (2.7)		102 (2.3)	99.8 (5.3)	-	99.7 (3.0)	100 (2.6)	-	92.3 (1.7)	-	-

[a]Initial concentration of cefaclor in urine was 50.4 mcg/ml.

[b]Initial concentration of cefaclor in urine was 50.7 mcg/ml.

[c]Values in parentheses indicate standard deviation.

[d]Not determined.

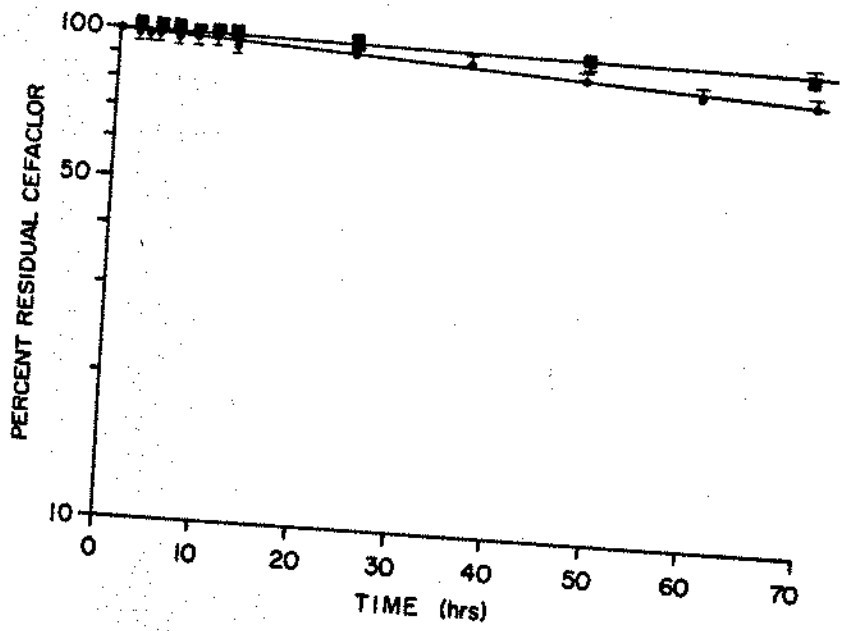


Figure 14. Mean percent residual cefaclor (±1 SE) in 0.1 M potassium monobasic phosphate (pH 4.5) stock solution following periods of storage at room temperature (●) and in a refrigerator (■).

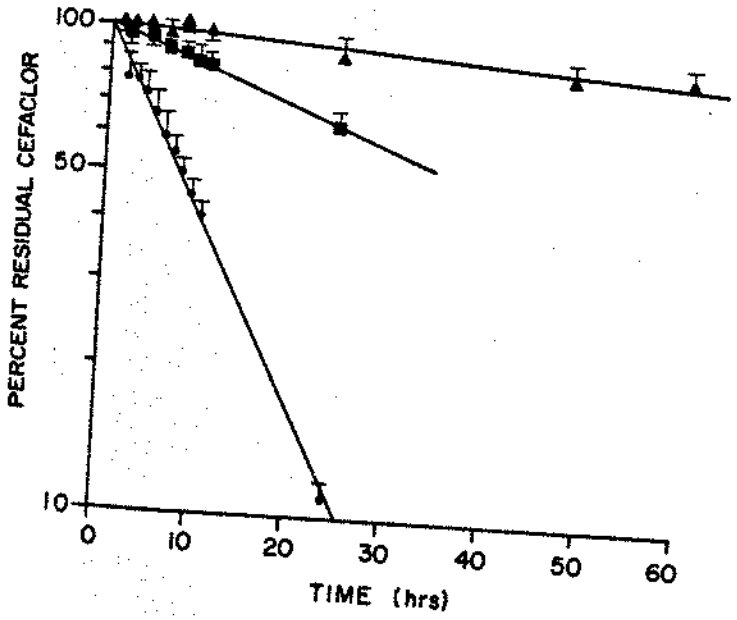


Figure 15. Mean percent residual cefaclor (±1 SE) in plasma following periods of storage at room temperature (●), in a refrigerator (■), and in a freezer (▲).

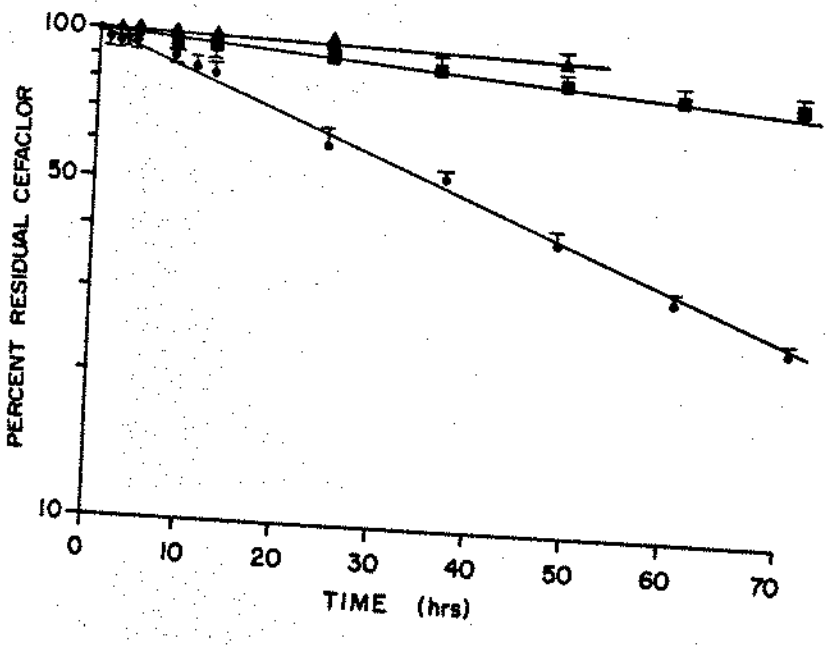


Figure 16. Mean percent residual cefaclor (±1 SE) in urine following periods of storage at room temperature (●), in a refrigerator (■), and in a freezer (▲).

C. Clinical Study and Data Analysis

1. Subjects

All eight subjects completed all phases of the study. There were no reports of side effects or allergic responses to any of the three cephalosporins. There was no evidence of diarrhea nor of any other kind of gastrointestinal disturbance. All subjects conformed exactly to the study protocol.

2. Plasma Concentration Data

Individual plasma cephalosporin concentrations, together with means, standard deviations, and coefficients of variation, are given in Tables 19-24. The mean data are summarized in Figures 17-19, and Table 25. The results of statistical comparisons of cephalosporin concentrations in plasma at each sampling time are also summarized in Table 25. Individual model-independent pharmacokinetic parameters, peak plasma concentrations, times of peak concentrations, and areas under plasma concentration-time curves, are given in Tables 26-28. The mean data associated with each treatment together with statistical comparisons are summarized in Table 29.

All three cephalosporins were absorbed rapidly from the fasting treatments, giving rise to mean peak plasma concentrations of 16.8, 19.4, and 10.3 mcg/ml for cephalexin, cephradine, and cefaclor, respectively, at approximately 1 hr. Plasma concentrations subsequently declined rapidly to approximately 1 mcg/ml, or less, at 4 hr and were undetectable at 6 hr.

Absorption of the three compounds was uniformly delayed after the nonfasting treatments, and mean peak plasma concentrations of 8.7, 11.6, and 5.7 mcg/ml for cephalexin, cephradine, and cefaclor, respectively, were obtained at 1.8 to 2.8 hr. Plasma concentrations were prolonged following postprandial doses. At 4 hr, the mean concentration of cephalexin, cephradine, and cefaclor were 4.6, 3.8, and 1.6 mcg/ml, respectively.

The statistical comparisons in Table 25 show that cephalexin and cephradine concentrations are significantly ($p < 0.05$) higher than those of cefaclor at 1, 1.5, 2, 2.5, 3, and 4 hr. This observation is consistent with previous investigations (46, 54, 58).

The areas under the plasma curves following the fasting treatments were almost identical for cephalexin and cephradine. The value for cefaclor was approximately one-half that of the other two compounds, which is consistent with previous observations (46, 58). When the cephalosporins were taken with food, the areas for all three compounds were almost identical to those obtained following the fasting treatments.

The plasma data thus show that the absorption of cephalexin, cephradine, and cefaclor is delayed by food, but that their overall availability to the systemic circulation is unchanged. The plasma profiles shown in Figures 17-19 indicate that slower absorption of antibiotic from postprandial doses will tend to delay the onset of antibacterial activity, but the activity may be prolonged for a longer period than that achieved when the drugs are taken on an empty stomach.

Table 19

Cephalexin Plasma Concentrations Following Single 500 mg Oral Dose Under Fasting Condition

	Plasma cephalexin (mcg/ml)											
Subject	0.0 h	0.25 h	0.5 h	0.75 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h	4.0 h	6.0 h	8.0 h
1	-[a]	2.8	10.5	ND[b]	17.9	10.0	4.7	4.1	2.9	-	-	-
2	-	-	6.1	16.4	26.1	9.2	6.5	4.8	4.0	2.3	-	-
3	-	-	17.9	ND	11.5	6.9	4.9	3.6	2.8	-	-	-
4	-	-	6.2	18.5	15.4	8.2	4.5	3.0	2.0	-	-	-
5	-	-	-	4.5	7.2	6.3	5.5	6.4	6.3	2.6	-	-
6	-	2.6	13.2	17.1	10.2	8.8	5.6	3.8	3.7	2.4	-	-
7	-	-	13.2	ND	19.9	11.7	5.5	4.3	3.5	1.9	-	-
8	-	-	4.9	9.7	9.7	7.7	5.4	4.4	2.9	-	-	-
Mean	-	0.7	9.0	13.2	14.7	8.6	5.3	4.3	3.5	1.2	-	-
SD[c]	-	1.3	5.7	5.9	6.3	1.7	0.6	1.0	1.3	1.2		
CV%[d]	-	186	63.3	44.7	42.9	19.8	11.3	23.3	37.1	100		

[a]Below limit of detection.

[b]Not determined.

[c]Standard deviation.

[d]Coefficient of variation.

Table 20

Cephalexin Plasma Concentrations Following Single 500 mg Oral Dose Under Nonfasting Condition

	Plasma cephalexin (mcg/ml)											
Subject	0.0 h	0.25 h	0.5 h	0.75 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h	4.0 h	6.0 h	8.0 h
1	-	-	-	ND	6.0	6.2	8.1	7.9	6.4	2.3	-	-
2	-	-	-	ND	-	3.1	4.5	7.2	9.2	7.2	2.1	-
3	-	-	-	-	-	-	5.7	10.8	6.8	4.3	-	-
4	-	-	-	1.6	4.0	6.4	6.8	6.4	6.0	4.2	-	-
5	-	-	-	-	-	1.7	5.1	5.4	6.7	5.9	2.0	-
6	-	-	-	-	2.9	8.4	8.5	7.3	5.9	5.1	-	-
7	-	-	1.6	ND	7.6	11.7	9.7	6.1	5.0	2.6	-	-
8	-	-	-	ND	1.9	5.9	4.7	3.9	7.9	5.2	-	-
Mean	-	-	0.2	0.4	2.8	5.4	6.6	6.9	6.7	4.6	0.5	-
SD			0.6	0.8	2.9	3.8	1.9	2.0	1.3	1.6	0.9	
CV%			300	200	104	70.4	28.8	29.0	19.4	34.8	180	

Table 21

Cephradine Plasma Concentrations Following Single 500 mg Oral Dose Under Fasting Condition

	Plasma cephradine (mcg/ml)											
Subject	0.0 h	0.25 h	0.5 h	0.75 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h	4.0 h	6.0 h	8.0 h
1	-	-	3.6	9.3	14.6	15.4	6.5	3.6	2.6	-	-	-
2	-	-	15.7	27.9	21.0	11.4	7.3	3.5	3.9	2.2	-	-
3	-	-	8.3	11.8	13.6	9.6	3.7	1.1	1.7	1.3	-	-
4	-	-	6.5	ND	25.5	11.8	5.8	1.9	1.0	-	-	-
5	-	-	-	-	4.8	8.4	7.4	9.5	10.6	1.8	0.8	-
6	-	-	3.4	ND	14.7	13.2	5.4	4.0	2.0	1.0	-	-
7	-	-	10.5	22.2	21.9	13.1	7.8	4.2	2.7	-	-	-
8	-	-	2.8	12.9	24.9	8.9	4.5	3.4	1.9	-	-	-
Mean	-	-	6.4	14.0	17.6	11.5	6.1	3.9	3.3	0.8	0.1	-
SD			5.0	9.8	7.0	2.4	1.5	2.5	3.1	0.9	0.3	-
CV%			78.1	70.0	39.8	20.9	24.6	64.1	93.9	113	300	

Table 22

Cephradine Plasma Concentrations Following Single 500 mg Oral Dose Under Nonfasting Condition

	Plasma cephradine (mcg/ml)											
Subject	0.0 h	0.25 h	0.5 h	0.75 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h	4.0 h	6.0 h	8.0 h
1	-	-	-	1.5	2.8	6.1	8.6	16.0	8.8	1.9	-	-
2	-	-	-	-	1.0	4.0	8.6	9.0	15.2	3.7	-	-
3	-	-	-	ND	1.5	1.6	7.1	9.1	11.8	3.6	1.5	-
4	-	-	-	-	5.5	5.1	5.2	6.6	6.4	3.5	-	-
5	-	-	-	ND	0.8	1.9	2.8	2.9	5.2	5.5	1.7	-
6	-	-	1.0	7.0	7.5	11.5	7.9	5.9	2.4	0.4	-	-
7	-	-	-	-	-	4.1	7.3	16.4	9.3	3.7	-	-
8	-	-	-	-	-	2.3	3.6	5.0	10.0	8.0	-	-
Mean	-	-	0.1	1.4	2.4	4.6	6.4	8.9	8.6	3.8	0.4	-
SD			0.4	2.8	2.7	3.2	2.3	5.0	4.0	2.3	0.7	
CV%			400	200	113	69.6	35.9	56.2	46.5	60.5	175	

Table 23

Cefaclor Plasma Concentrations Following Single 500 mg Oral Dose Under Fasting Condition

	Plasma cefaclor (mcg/ml)											
Subject	0.0 h	0.25 h	0.5 h	0.75 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h	4.0 h	6.0 h	8.0 h
1	-	-	10.7	11.5	11.0	6.0	3.8	2.4	1.8	-	-	-
2	-	3.6	9.2	ND	4.0	1.9	1.2	0.9	0.8	-	-	-
3	-	1.1	13.8	14.0	10.5	3.4	2.8	1.2	1.3	0.9	-	-
4	-	-	-	0.6	2.3	6.2	2.7	3.6	1.7	-	-	-
5	-	-	-	1.8	4.8	8.2	8.5	5.2	3.3	-	-	-
6	-	-	11.3	ND	11.0	4.5	2.2	1.1	0.9	0.6	-	-
7	-	-	1.1	8.5	7.8	5.7	2.7	1.9	1.1	-	-	-
8	-	-	2.7	ND	13.3	4.1	1.1	0.5	-	-	-	-
Mean	-	0.6	6.1	7.3	8.1	5.0	3.1	2.1	1.4	0.2	-	-
SD		1.3	5.7	5.9	4.0	1.9	2.3	1.6	1.0	0.4		
CV%		217	93.4	80.8	49.4	38	74.2	76.2	71.4	200		

Table 24

Cefaclor Plasma Concentrations Following Single 500 mg Oral Dose Under Nonfasting Condition

	Plasma cefaclor (mcg/ml)											
Subject	0.0 h	0.25 h	0.5 h	0.75 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h	4.0 h	6.0 h	8.0 h
1	-	-	-	2.8	6.3	4.6	2.3	4.1	2.1	1.4	-	-
2	-	-	1.3	2.5	6.3	6.5	4.3	3.3	2.6	2.0	0.5	-
3	-	-	-	2.7	3.3	4.4	3.0	4.8	3.7	1.9	-	-
4	-	-	-	ND	1.7	7.0	6.9	4.5	4.2	1.3	-	-
5	-	-	1.3	ND	1.6	3.9	3.3	3.3	3.3	2.7	-	-
6	-	-	-	-	1.9	3.8	5.2	3.4	2.1	0.4	-	-
7	-	-	-	-	2.2	7.6	7.7	4.3	2.0	-	-	-
8	-	-	-	2.7	2.7	3.3	4.4	3.1	2.9	2.8	-	-
Mean	-	-	0.3	1.8	3.3	5.1	4.6	3.9	2.9	1.6	0.1	-
SD			0.6	1.4	2.0	1.6	1.9	0.7	0.8	1.0	0.2	
CV%			200	77.8	60.6	31.4	41.3	17.9	27.6	62.5	200	

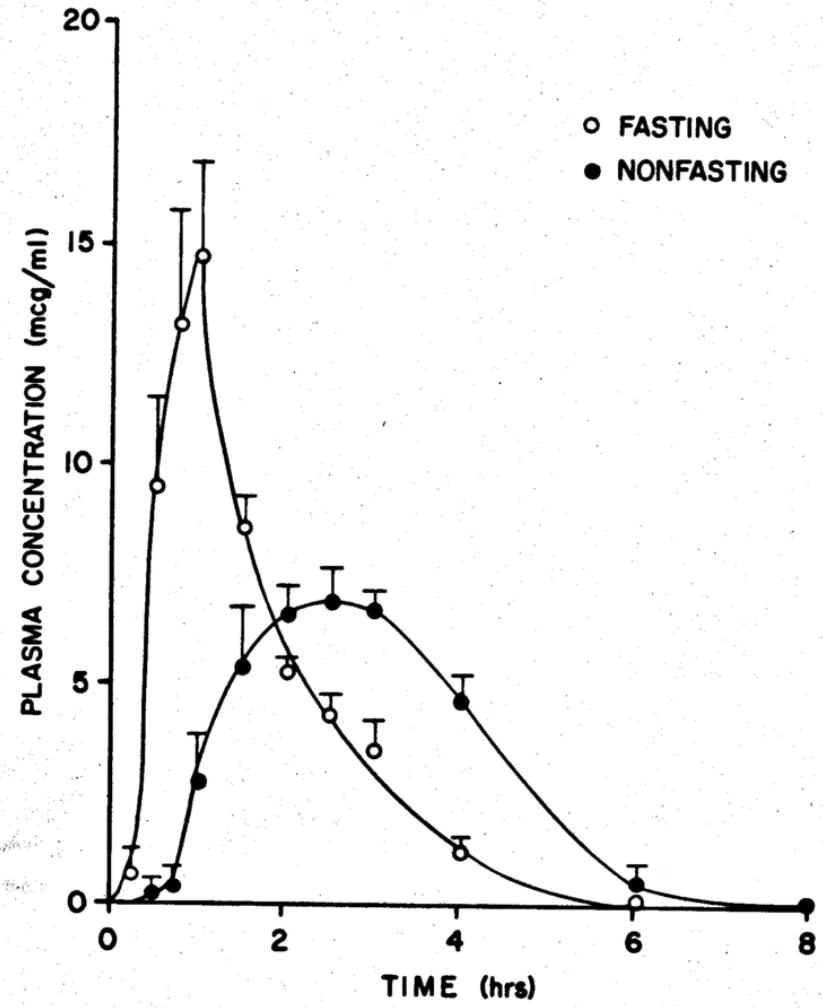


Figure 17. Mean plasma cephalexin concentration (+1 SE) versus time following single 500 mg oral dose under fasting and nonfasting conditions.

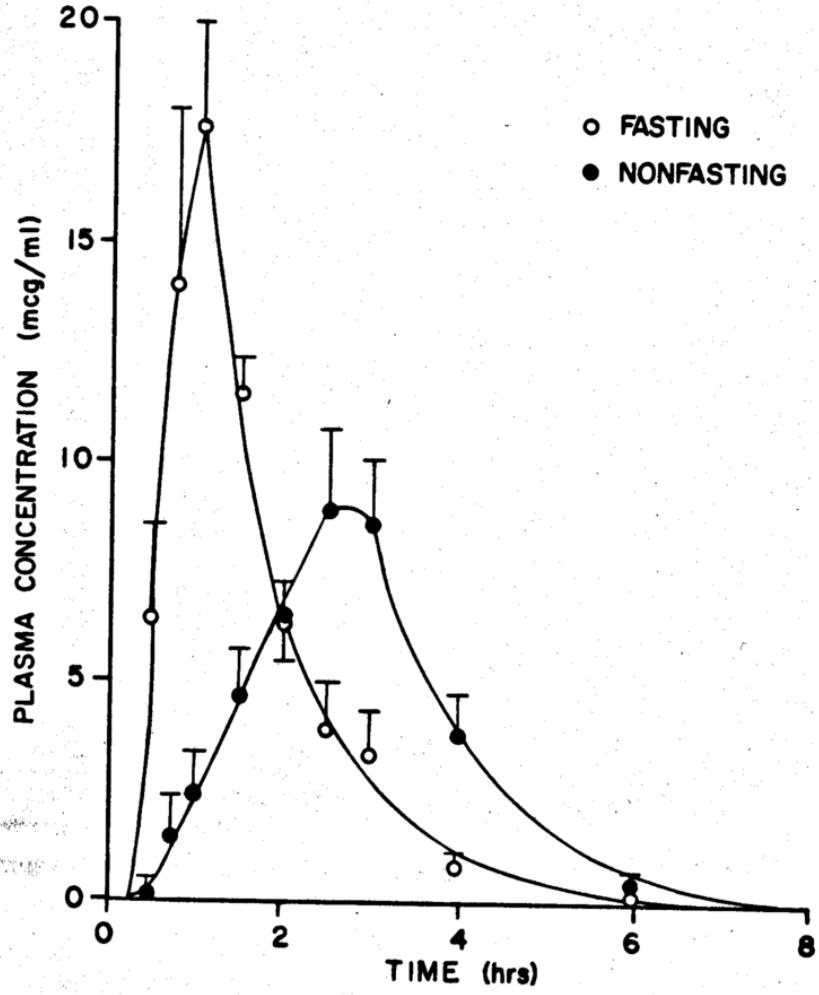


Figure 18. Mean plasma cephradine concentration (±1 SE) versus time following single 500 mg oral dose under fasting and nonfasting conditions.

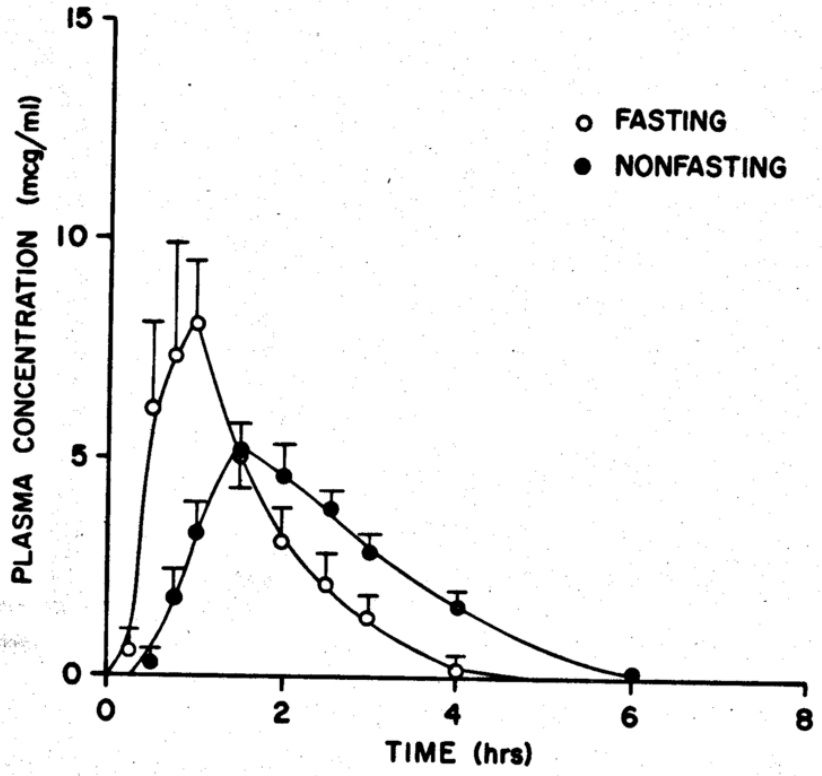


Figure 19. Mean plasma cefaclor concentration (±1 SE) versus time following single 500 mg oral dose under fasting and nonfasting conditions.

2.5 h	4.3 (±1.0)	6.9 (±2.0)	3.9 (±2.5)	8.9 (±5.0)	2.1 (±1.6)	3.9 (±0.7)	D > A,C,E,F B > E
3 h	3.5 (±1.3)	6.7 (±1.3)	3.3 (±3.1)	8.6 (±4.0)	1.4 (±1.0)	2.9 (±0.8)	B,D > A,C,E,F
4 h	1.2 (±1.2)	4.6 (±1.6)	0.8 (±0.9)	3.8 (±2.3)	0.2 (±0.4)	1.6 (±1.0)	B,D > C,E,F B > A
6 h	-	0.5 (±0.9)	0.1 (±0.3)	0.4 (±0.7)	-	0.1 (±0.2)	NSD
8 h	-	-	-	-	-	-	NSD

[a]Data were analyzed by analysis of variance and differences were considered significant when $p < 0.05$. Actual differences between treatments were determined by Tukey's test. The subject factor had a significant effect on the following time periods, 0.75 h, 1.5 h, and 4.0 h.

[b]Below limit of detection.

[c]No significant difference.

Table 26

Values of Model Independent Pharmacokinetic Parameters for Cephalexin Following Single 500 mg Oral Dose Under Fasting and Nonfasting Conditions

	Pharmacokinetic parameters for cephalexin					
	Fasting			Nonfasting		
Subject	C_{max}[a] (mcg/ml)	t_{max} (h)	$AUC^{0\rightarrow 8h}$[c] (mcg h/ml)	C_{max} (mcg/ml)	t_{max} (h)	$AUC^{0\rightarrow 8h}$ (mcg h/ml)
1	17.9	1.0	25.2	8.1	2.0	22.4
2	26.1	1.0	32.2	9.2	3.0	29.2
3	17.9	0.5	22.3	10.8	2.5	19.8
4	18.5	0.75	21.3	6.8	2.0	22.4
5	7.2	1.0	21.6	6.7	3.0	24.0
6	17.1	0.75	27.5	8.5	2.0	25.3
7	19.9	1.0	31.0	11.7	1.5	25.7
8	9.7	1.0	18.2	7.9	3.0	21.9
Mean	16.8	0.88	24.9	8.7	2.4	23.8
SD	5.9	0.19	5.0	1.8	0.58	2.9
CV%	35.1	21.6	20.1	20.7	24.2	12.2

[a]Observed peak plasma concentration.

[b]Observed time to peak plasma concentration.

[c]Area under plasma concentration versus time curve from zero to 8 h as calculated by trapezoidal rule.

Table 27

Values of Model Independent Pharmacokinetic Parameters for Cephradine Following Single 500 mg Oral Dose Under Fasting and Nonfasting Conditions

	Pharmacokinetic parameters for cephradine					
	Fasting			Nonfasting		
Subject	C_{max} (mcg/ml)	t_{max} (h)	$AUC^{0\rightarrow 8h}$ (mcg h/ml)	C_{max} (mcg/ml)	t_{max} (h)	$AUC^{0\rightarrow 8h}$ (mcg h/ml)
1	15.4	1.5	23.4	16.0	2.5	26.3
2	27.9	0.75	36.0	15.2	3.0	28.1
3	13.6	1.0	20.4	11.8	3.0	26.7
4	25.5	1.0	25.7	6.6	2.5	20.5
5	10.6	3.0	26.6	5.5	4.0	19.9
6	14.7	1.0	22.9	11.5	1.5	19.8
7	22.2	0.75	31.0	16.4	2.5	26.4
8	24.9	1.0	23.1	10.0	3.0	24.9
Mean	19.4	1.3	26.1	11.6	2.8	24.1
SD	6.5	0.74	5.1	4.1	0.71	3.4
CV%	33.6	56.9	19.5	35.3	25.4	14.1

Table 28

Values of Model Independent Pharmacokinetic Parameters for Cefaclor Following Single 500 mg Oral Dose Under Fasting and Nonfasting Conditions

	Pharmacokinetic parameters for cefaclor					
	Fasting			Nonfasting		
Subject	C_{max} (mcg/ml)	t_{max} (h)	$AUC^{0\rightarrow 8h}$ (mcg h/ml)	C_{max} (mcg/ml)	t_{max} (h)	$AUC^{0\rightarrow 8h}$ (mcg h/ml)
1	11.5	0.75	17.2	6.3	1.0	12.2
2	9.2	0.5	8.9	6.5	1.5	16.3
3	14.0	0.75	17.1	4.8	2.5	13.6
4	6.2	1.5	8.5	7.0	1.5	15.1
5	8.5	2.0	15.7	3.9	1.5	13.0
6	11.3	0.5	15.1	5.2	2.0	9.1
7	8.5	0.75	11.2	7.7	2.0	12.1
8	13.3	1.0	10.5	4.4	2.0	13.4
Mean	10.3	0.97	13.0	5.7	1.8	13.1
SD	2.7	0.53	3.6	1.3	0.46	2.2
CV%	26.2	54.6	27.7	22.8	25.6	16.8

Table 29

Summary of Mean Values (±1 SD) of Model-Independent Pharmacokinetic Parameters for Cephalexin, Cephradine, and Cefaclor; and Results of Statistical Comparison Between Treatments[a]

Parameter	Cephalexin (fasting) A	Cephalexin (nonfasting) B	Cephradine (fasting) C	Cephradine (nonfasting) D	Cefaclor (fasting) E	Cefaclor (nonfasting) F	Statistical comparison
C_{max} (mcg/ml)	16.8 (±5.9)	8.7 (±1.8)	19.4 (±6.5)	11.6 (±4.1)	10.3 (±2.7)	5.7 (±1.3)	A,C > B,E,F C > D > F
t_{max} (h)	0.88 (±0.19)	2.4 (±0.58)	1.3 (±0.74)	2.8 (±0.71)	0.97 (±0.53)	1.8 (±0.46)	B,D > A,C,E D > F > A,E
$AUC^{0\rightarrow 8h}$ (mcg h/ml)	24.9 (±5.0)	23.8 (±2.9)	26.1 (±5.1)	24.1 (±3.4)	13.0 (±3.6)	13.1 (±2.2)	A,B,C,D > E,F
MRT (h)	1.65 (±0.34)	2.9 (±0.52)	1.64 (±0.44)	2.85 (±0.58)	1.45 (±0.34)	2.38 (±0.24)	B,D > F > A,C,E

[a]Statistical comparisons were carried out as in Table 25. The subject factor had a significant effect on all parameters.

3. Urinary Excretion Data

Concentrations of cephalexin, cephradine, and cefaclor in urine for each collection interval, together with pH values, for all treatments are given in Tables 30-35. Under the fasting treatments, all three cephalosporins yielded high urinary concentrations during the 0-2 hr and 2-4 hr intervals, lower concentrations during the 4-8 hr interval, and zero or low concentrations during the 8-12 hr interval. Under the nonfasting treatments, the highest urinary concentrations for all three cephalosporins occurred during the 2-4 hr interval. Again, zero or low concentrations occurred during the 8-12 hr interval.

Cumulative percent recovery of cephalosporins in urine following the fasting and nonfasting treatments are given in Tables 36-41. The mean data are summarized in Figures 20-22, and the results of statistical comparisons are given in Table 42.

As with the plasma concentrations, urinary excretion of all three compounds was delayed when they were taken after a standard breakfast. This was most evident in the 2 hr urine collection interval. Statistical comparisons showed that cumulative percent urinary recovery of cephalexin and cephradine were significantly greater under the fasting treatment than the nonfasting treatment during the 0-2 hr interval. However, significant difference between fasting and nonfasting treatments was not observed with cefaclor at the same time interval.

The mean total 0-12 hr urinary recovery of cephalexin was

88.5% and 76.7% after fasting and nonfasting treatments, respectively. Recovery of cephradine was also slightly reduced from 89.1% to 86.9% by food, and recovery of cefaclor was reduced from 73.7% to 66%. Statistical comparisons of the cumulative percent urinary recovery at 12 hr showed no significant difference between fasting and nonfasting treatments for all three cephalosporins. Cefaclor yielded a significantly lower total urinary recovery under the nonfasting treatment compared to cephradine and cephalexin.

Table 30

Values of Urine pH and Cephalexin Urine Concentrations Following Single 500 mg Oral Dose Under Fasting Condition

	Urinary data for cephalexin							
	0-2 h		2-4 h		4-8 h		8-12 h	
Subject	pH	Conc. (mcg/ml)	pH	Conc. (mcg/ml)	pH	Conc. (mcg/ml)	pH	Conc. (mcg/ml)
1	6.8	747	6.4	1326	5.4	996	5.2	124
2	5.7	1008	5.7	1942	5.7	226	6.2	32.3
3	6.0	7709	6.0	2854	6.1	328	6.9	21.0
4	6.0	7528	5.8	2192	7.5	187	7.4	63.8
5	5.7	2382	5.3	4308	5.3	594	5.3	113
6	6.5	1810	6.5	1006	6.5	319	5.7	63.1
7	5.3	2938	5.0	1228	5.3	125	5.6	17.8
8	5.7	1397	6.7	815	5.5	97.1	6.0	-[a]
Mean	6.0	3190	5.9	1959	5.9	359	6.0	54.4
SD	0.5	2823	0.6	1167	0.8	301	0.8	45.3
CV%	8.3	88	10	60	14	84	13	83

[a]Below limit of detection.

Table 31

Values of Urine pH and Cephalexin Urine Concentrations Following Single 500 mg Oral Dose Under Nonfasting Condition

	Urinary data for cephalexin							
	0-2 h		2-4 h		4-8 h		8-12 h	
Subject	pH	Conc. (mcg/ml)	pH	Conc. (mcg/ml)	pH	Conc. (mcg/ml)	pH	Conc. (mcg/ml)
1	6.6	545	6.6	2338	5.8	450	5.7	61.7
2	6.4	249	6.1	2228	5.6	682	6.0	99.0
3	6.2	234	6.5	2873	6.2	534	6.5	53.0
4	5.7	757	6.2	1507	6.5	457	7.0	72.2
5	6.1	87.4	6.5	1926	6.2	752	5.6	77.8
6	7.0	923	6.7	851	5.9	546	6.0	87.0
7	5.8	299	6.3	1011	6.2	127	6.8	-
8	7.8	866	7.0	924	6.0	134	6.0	-
Mean	6.5	495	6.5	1707	6.1	460	6.2	56.3
SD	0.7	322	0.3	751	0.3	228	0.5	37.5
CV%	11	65	4.6	44	4.9	50	8.1	67

Table 32

Values of Urine pH and Cephradine Urine Concentrations Following Single 500 mg Oral Dose Under Fasting Condition

	Urinary data for cephradine							
	0-2 h		2-4 h		4-8 h		8-12 h	
Subject	pH	Conc. (mcg/ml)	pH	Conc. (mcg/ml)	pH	Conc. (mcg/ml)	pH	Conc. (mcg/ml)
1	6.7	-	7.2	2488	6.2	818	5.7	38.8
2	6.5	699	6.5	1024	5.4	135	5.6	-
3	6.2	1093	5.6	880	6.5	213	6.0	-
4	5.3	4237	5.1	1265	6.2	181	6.2	23.7
5	5.2	1304	5.2	1221	5.2	427	6.0	7.13
6	6.3	2498	6.1	1168	5.8	360	6.0	17.5
7	5.6	5885	5.5	2667	6.4	255	6.5	-
8	6.0	3250	5.9	890	5.7	121	5.6	-
Mean	6.0	2371	5.9	1450	5.9	314	6.0	10.9
SD	0.6	1993	0.7	712	0.5	230	0.3	14.5
CV%	10	84	12	49	8.5	73	5.0	133

Table 33

Values of Urine pH and Cephradine Urine Concentrations Following Single 500 mg Oral Dose Under Nonfasting Condition

	Urinary data for cephradine							
	0-2 h		2-4 h		4-8 h		8-12 h	
Subject	pH	Conc. (mcg/ml)	pH	Conc. (mcg/ml)	pH	Conc. (mcg/ml)	pH	Conc. (mcg/ml)
1	6.2	-	7.0	824	5.3	831	5.2	58.9
2	6.1	-	6.0	2399	5.6	832	5.7	46.8
3	7.0	392	6.8	2217	5.2	844	6.6	3.27
4	5.4	1142	6.5	2177	6.5	417	5.6	8.28
5	5.5	381	5.9	1515	5.8	1292	5.7	36.7
6	6.6	2744	7.1	1467	5.9	301	5.7	9.81
7	5.4	1531	5.4	3890	7.0	166	5.4	-
8	5.6	247	5.9	2514	5.6	767	5.4	-
Mean	6.0	805	6.3	2125	5.9	681	5.7	20.5
SD	0.6	953	0.6	914	0.6	365	0.4	23.4
CV%	10	118	9.5	43	10	54	7.0	114

Table 34

Values of Urine pH and Cefaclor Urine Concentrations Following Single 500 mg Oral Dose Under Fasting Condition

	Urinary data for cefaclor							
	0-2 h		2-4 h		4-8 h		8-12 h	
Subject	pH	Conc. (mcg/ml)	pH	Conc. (mcg/ml)	pH	Conc. (mcg/ml)	pH	Conc. (mcg/ml)
1	6.5	382	7.0	803	5.6	246	5.3	44.4
2	6.4	2934	6.5	594	6.0	85.5	6.2	-
3	5.5	4617	6.0	699	6.2	42.1	6.1	-
4	5.3	1592	5.3	924	7.0	91.5	7.0	-
5	5.2	1517	5.1	988	5.1	147	6.2	-
6	6.4	2542	6.3	373	6.0	-	6.4	-
7	6.7	1241	7.0	575	6.5	48.7	6.7	-
8	6.5	987	7.1	201	6.7	43.7	6.2	-
Mean	6.1	1977	6.3	645	6.1	88.1	6.3	5.6
SD	0.6	1343	0.8	268	0.6	77.3	0.5	15.7
CV%	9.8	68	13	41.6	9.8	88	7.9	280

Table 35

Values of Urine pH and Cefaclor Urine Concentrations Following Single 500 mg Oral Dose Under Nonfasting Condition

	Urinary data for cefaclor							
	0-2 h		2-4 h		4-8 h		8-12 h	
Subject	pH	Conc. (mcg/ml)	pH	Conc. (mcg/ml)	pH	Conc. (mcg/ml)	pH	Conc. (mcg/ml)
1	6.5	261	5.9	1430	5.2	207	5.2	33.0
2	5.9	1205	6.7	1105	6.2	304	5.7	22.4
3	6.5	653	7.0	1406	7.0	103	7.0	-
4	6.0	1595	6.2	1457	6.8	172	5.6	-
5	5.6	718	6.1	1220	5.6	447	6.3	30
6	7.0	632	6.8	601	6.5	94.6	7.0	-
7	5.8	2430	6.5	1150	6.5	51.1	5.6	-
8	6.3	597	6.8	817	6.5	74.3	5.7	-
Mean	6.2	1011	6.5	1148	6.3	182	6.0	10.7
SD	0.5	705	0.4	306	0.6	135	0.7	15.0
CV%	8.1	70	6.2	27	9.5	74	12	140

Table 36

Cumulative Percent Recovery of Cephalexin in Urine Following Single 500 mg Oral Dose Under Fasting Condition

	Cumulative percent recovery of cephalexin (%)			
Subject	0-2 h	2-4 h	4-8 h	8-12 h
1	30.5	64.9	81.7	96.9
2	52.0	90.5	93.1	94.6
3	64.8	86.4	96.6	98.0
4	76.8	96.5	98.1	99.5
5	37.2	98.3	101	102
6	48.5	65.4	77.2	79.1
7	55.8	70.6	72.8	73.5
8	41.9	60.3	64.7	64.7
Mean	50.9	79.1	85.7	88.5
SD	15.0	15.4	13.5	14.0
CV%	29.5	19.5	15.7	15.8

Table 37

Cumulative Percent Recovery of Cephalexin in Urine Following Single 500 mg Oral Dose Under Nonfasting Condition

	Cumulative percent recovery of cephalexin (%)			
Subject	0-2 h	2-4 h	4-8 h	8-12 h
1	19.6	60.3	81.9	84.4
2	6.0	51.9	64.1	68.9
3	4.9	61.7	79.7	81.6
4	14.5	58.0	82.8	86.4
5	3.5	76.6	91.7	95.0
6	45.0	72.3	95.7	98.2
7	9.3	52.4	57.3	57.3
8	11.4	38.8	42.0	42.0
Mean	14.3	59.0	74.4	76.7
SD	13.5	11.9	18.3	19.3
CV%	94.4	20.2	24.6	25.2

Table 38

Cumulative Percent Recovery of Cephradine in Urine Following Single 500 mg Oral Dose Under Fasting Condition

	Cumulative percent recovery of cephradine (%)			
Subject	0-2 h	2-4 h	4-8 h	8-12 h
1	0	66.2	85.8	87.1
2	71.2	89.9	97.2	97.2
3	28.0	71.4	75.0	75.0
4	74.9	91.2	96.7	97.5
5	29.7	66.4	80.6	80.9
6	59.9	78.4	88.5	88.9
7	53.0	78.0	86.0	86.0
8	81.3	96.7	100	100
Mean	49.8	79.8	88.8	89.1
SD	28.2	11.7	8.8	8.8
CV%	56.6	14.7	9.9	9.9

Table 39

Cumulative Percent Recovery of Cephradine in Urine Following Single 500 mg Oral Dose Under Nonfasting Condition

	Cumulative percent recovery of cephradine (%)			
Subject	0-2 h	2-4 h	4-8 h	8-12 h
1	0	32.6	72.5	74.2
2	0	48.0	77.6	78.3
3	10.8	55.2	71.7	71.9
4	26.0	77.9	99.5	99.8
5	7.9	35.2	75.8	76.4
6	58.1	94.8	102	102.8
7	23.0	88.3	91.1	91.1
8	8.1	73.4	101	101
Mean	16.7	63.2	86.4	86.9
SD	19.2	23.8	13.4	13.1
CV%	115	37.7	15.5	15.1

Table 40

Cumulative Percent Recovery of Cefaclor in Urine Following Single 500 mg Oral Dose Under Fasting Condition

	Cumulative percent recovery of cefaclor (%)			
Subject	0-2 h	2-4 h	4-8 h	8-12 h
1	24.7	61.0	70.4	72.3
2	58.7	67.3	69.3	69.3
3	84.0	93.3	94.5	94.5
4	40.4	82.0	86.9	86.9
5	49.8	79.8	85.4	85.4
6	65.1	73.6	73.6	73.6
7	36.7	48.1	49.4	49.4
8	45.8	56.1	58.3	58.3
Mean	50.7	70.2	73.5	73.7
SD	18.5	14.9	15.1	15.1
CV%	36.5	21.2	20.5	20.5

Table 41

Cumulative Percent Recovery of Cefaclor in Urine Following Single 500 mg Oral Dose Under Nonfasting Condition

	Cumulative percent recovery of cefaclor (%)			
Subject	0–2 h	2–4 h	4–8 h	8–12 h
1	14.4	64.1	76.0	77.3
2	28.4	44.1	55.1	55.7
3	32.7	63.0	69.6	69.6
4	33.8	67.9	74.7	74.7
5	21.1	52.8	64.8	66.1
6	31.7	59.4	64.8	64.8
7	47.6	70.2	70.9	70.9
8	17.4	47.2	49.0	49.0
Mean	28.4	58.6	65.6	66.0
SD	10.7	9.6	9.4	9.5
CV%	37.7	16.4	14.3	14.4

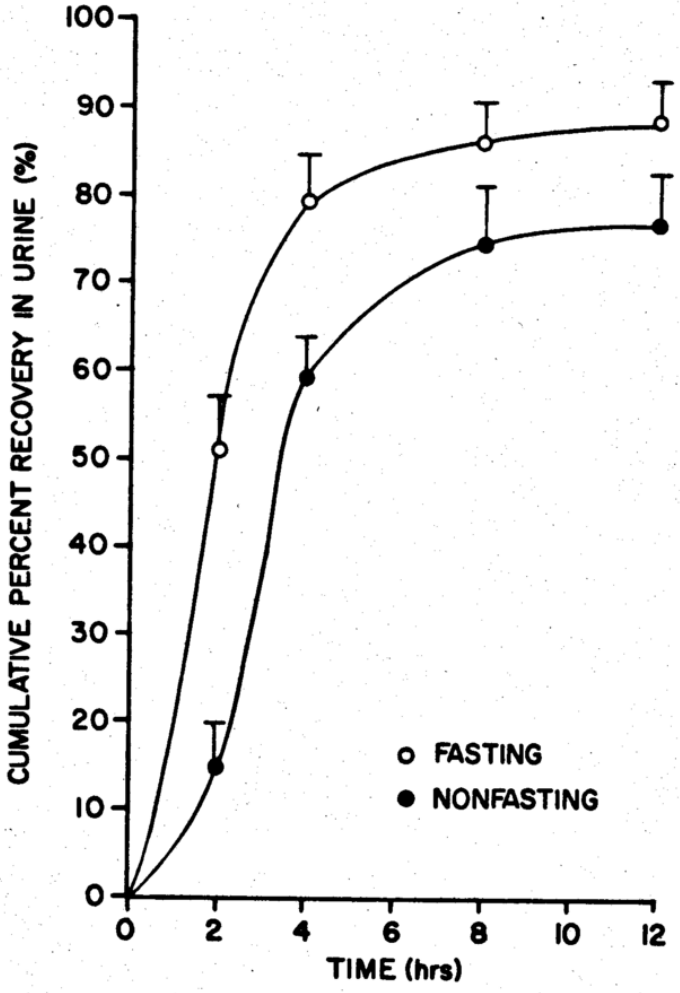


Figure 20. Mean cumulative percent recovery (+1 SE) of cephalexin in urine as a function of time following single 500 mg oral dose under fasting and nonfasting conditions.

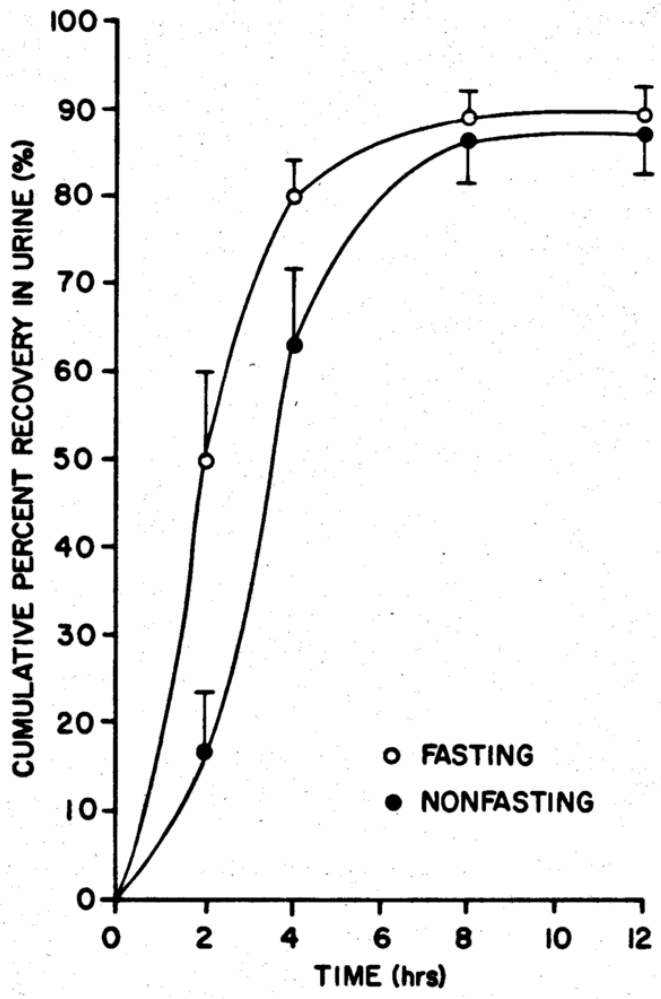


Figure 21. Mean cumulative percent recovery (±1 SE) of cephradine in urine as a function of time following single 500 mg oral dose under fasting and nonfasting conditions.

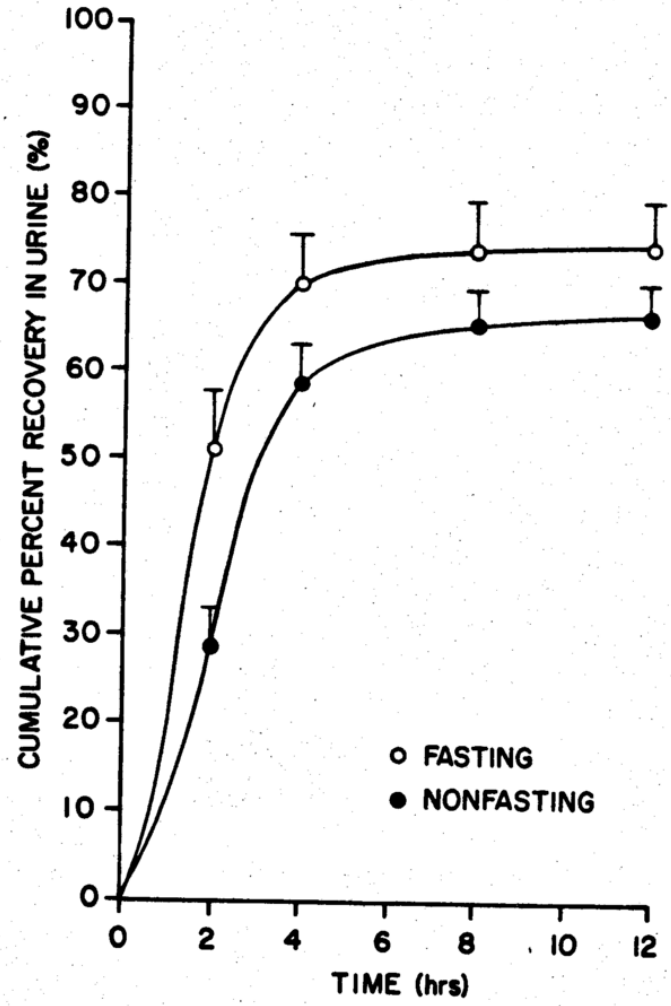


Figure 22. Mean cumulative percent recovery (+1 SE) of cefaclor in urine as a function of time following single 500 mg oral dose under fasting and nonfasting conditions.

Table 42

Summary of Mean Values (±1 SD) of Cumulative Percent Recovery of Cephalexin, Cephradine, and Cefaclor in Urine; and Results of Statistical Comparison Between Treatments[a]

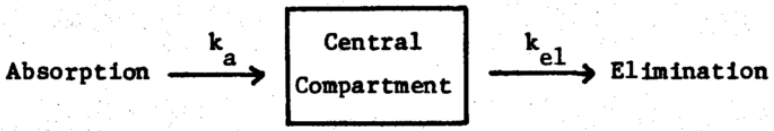
	Mean cumulative percent recovery in urine (%)						
Time Interval	Cephalexin (fasting) A	Cephalexin (nonfasting) B	Cephradine (fasting) C	Cephradine (nonfasting) D	Cefaclor (fasting) E	Cefaclor (nonfasting) F	Statistical Comparison
0-2 h	50.9 (±15.0)	14.3 (±13.5)	49.8 (±28.2)	16.7 (±19.2)	50.7 (±18.5)	28.4 (±10.7)	A,C,E > B,D
2-4 h	79.1 (±15.4)	59.0 (±11.9)	79.8 (±11.7)	63.2 (±23.8)	70.2 (±14.9)	58.6 (± 9.6)	NSD
4-8 h	85.7 (±13.5)	74.4 (±18.3)	88.8 (± 8.8)	86.4 (±13.4)	73.5 (±15.1)	65.6 (± 9.4)	A,C,D > F
8-12 h	88.5 (±14.0)	76.7 (±19.3)	89.1 (± 8.8)	86.9 (±13.1)	73.7 (±15.1)	66.0 (± 9.5)	A,C,D > F

[a]Statistical comparisons were carried out as in Table 25. The subject factor had a significant effect on the following time interval, 0-2 h.

4. Pharmacokinetic Models

a. One-Compartment Model with First-Order Absorption and Elimination

The semilogarithmic plots of cephalosporin plasma concentration versus time for subjects 1 and 6 following 500 mg oral dose under fasting and nonfasting conditions are shown in Figures 23-34. These plots, as well as similar graphs for each of the other subjects, yielded biexponential curves which exhibited linear elimination phases. Therefore, a one-compartment model with first-order absorption and elimination depicted by Scheme I was used to describe individual data sets.



Scheme I. One-compartment model with first-order absorption and elimination

This model can be characterized by Equation 2 as described previously.

$$C = \frac{FD}{V}\left(\frac{k_a}{k_a - k_{el}}\right)\left[e^{-k_{el}(t-t_o)} - e^{-k_a(t-t_o)}\right] \qquad (2)$$

For many drugs administered in their conventional dosage forms, the absorption rate constant is significantly larger than the elimination rate constant. Thus, at some time after administration,

the term $e^{-k_a(t-t_o)}$ approaches zero, whereas the term $e^{-k_{el}(t-t_o)}$ remains finite, and Equation 2 reduces to,

$$C = \frac{FD}{V} \left(\frac{k_a}{k_a - k_{el}} \right) e^{-k_{el}(t-t_o)} \qquad (2a)$$

Equation 2a describes the postabsorptive phase of a plasma concentration versus time curve. Equation 2a written in common logarithms is,

$$\log C = \log \frac{FD\, k_a}{V(k_a - k_{el})} - \frac{k_{el}(t-t_o)}{2.303} \qquad (2b)$$

Therefore an estimate of the elimination rate constant can be obtained from the slope of the linear terminal portion of a semilogarithmic plot of concentration versus time, which is equal to $-k_{el}/2.303$. It follows that the absorption rate constant can be calculated by the method of residuals (93), and the y-intercept is equal to $\log \frac{FD\, k_a}{V(k_a - k_{el})}$. This approach was utilized to generate the initial graphical estimates of k_{el}, k_a, and FD/V for individual subjects for all treatments. Improved estimates including coefficients of determination were obtained using the iterative, nonlinear least squares program, NREG, on a digital computer.

The computer generated curves using the first-order absorption equation for subjects 1 and 6 following all treatments are shown in Figures 23-34. Tables 43-48 give the individual pharmacokinetic

parameters generated by fitting individual data sets to this first-order equation. The high coefficients of determination indicated that plasma profiles can be adequately described by this first-order equation. However, the elimination rate constants and the values FD/V obtained were significantly smaller in the nonfasting treatments than the fasting treatments for all three cephalosporins. These results are deceptive for two reasons. First, the elimination rate constant should remain approximately the same between fasting and nonfasting treatments because the presence of food should exert no influence on the elimination process. Second, it is reasonable to assume that the value FD/V, which is an index of systemic availability should remain the same between fasting and nonfasting treatments since both the plasma and urinary data discussed earlier have indicated that the overall bioavailability is equivalent between the fasting and nonfasting treatments. As a result, individual data sets for the nonfasting treatments were re-analyzed utilizing an alternate approach in which k_a is assumed to be smaller than k_{el} and is commonly known as the 'flip-flop' model.

Such an approach was thought to be appropriate because absorption might be relatively slow in the presence of food compared to the rapid elimination of cephalosporins. Thus, the apparent absorption rate constant might be smaller than the elimination rate constant. In such cases, the time course of drug concentration in plasma can also be described by Equation 2, but the slope of the linear terminal segment of the semilogarithmic plot of concentration

versus time is equal to $-k_a/2.303$ rather than $-k_{el}/2.303$, and the elimination rate constant must be determined by the method of residuals.

Pharmacokinetic parameters for the nonfasting treatment obtained by fitting individual plasma profiles to Equation 2 with 'flip-flop' characteristics are given in Tables 49-51. Statistical comparisons showed that the elimination rate constant, and the values FD/V were not significantly different between the fasting and nonfasting treatments. Therefore, it was concluded that this 'flip-flop' phenomenon existed with the nonfasting treatments.

The mean pharmacokinetic parameters obtained from pharmacokinetic analysis using the first-order absorption model, and the results of statistical comparisons, are summarized in Table 52. The high coefficients of determination indicated that plasma profiles can be adequately described by the first-order absorption equation. The coefficients tend to be lower for the fasting treatments, which may be the result of apparent zero-order absorption characteristics as will be discussed later.

The mean elimination half-lives, which ranged from 0.62-0.97 hr, did not show any significant difference between treatments. Analysis of variance indicated a significant intersubject variability in the elimination half-life.

All three cephalosporins were rapidly absorbed following the fasting treatments with the mean absorption half-lives ranging from 0.33 to 0.38 hr. Absorption for all three compounds was slower

following the nonfasting treatments, and the mean absorption half-lives were 1.28, 1.13, and 0.98 hr for cephalexin, cephradine, and cefaclor, respectively. Analysis of variance showed that the absorption half-lives were significantly longer for the nonfasting treatments than for the fasting treatments.

The absorption lag times were also uniformly prolonged during the nonfasting treatments from 0.19 to 0.86 hr for cephalexin, 0.31 to 1.21 hr for cephradine, and 0.25 to 0.54 hr for cefaclor. Analysis of variance showed a significant difference in the absorption lag time between the fasting and nonfasting treatments for all three cephalosporins.

The quantity FD/V, which is an indicator of systemic availability, was almost identical between the fasting and nonfasting treatments for all cephalosporins. However, the FD/V value for cefaclor was approximately one-half that of cephradine. As the urinary excretion of cefaclor is not significantly reduced compared to the other cephalosporins, the lower FD/V value for cefaclor, at least in part, suggested that cefaclor might have a significantly larger apparent distribution volume in the body than cephradine.

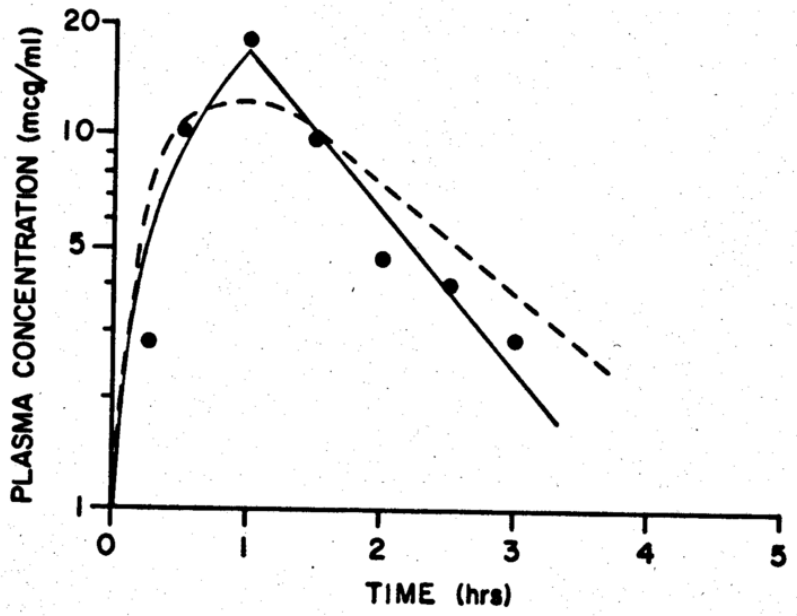


Figure 23. Plasma cephalexin concentration versus time with computer generated curves for subject 1 following single 500 mg oral dose under fasting condition.
Key: • experimentally observed concentration
--- first-order absorption model, $r^2 = 0.889$
___ zero-order absorption model, $r^2 = 0.977$

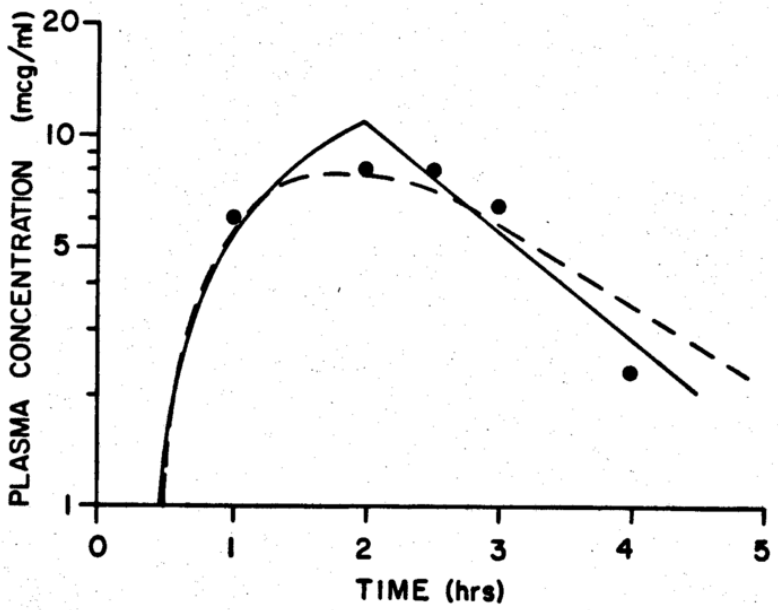


Figure 24. Plasma cephalexin concentration versus time with computer generated curves for subject 1 following single 500 mg oral dose under nonfasting condition.
Key: • experimentally observed concentration
--- first-order absorption model, $r^2 = 0.978$
___ zero-order absorption model, $r^2 = 0.941$

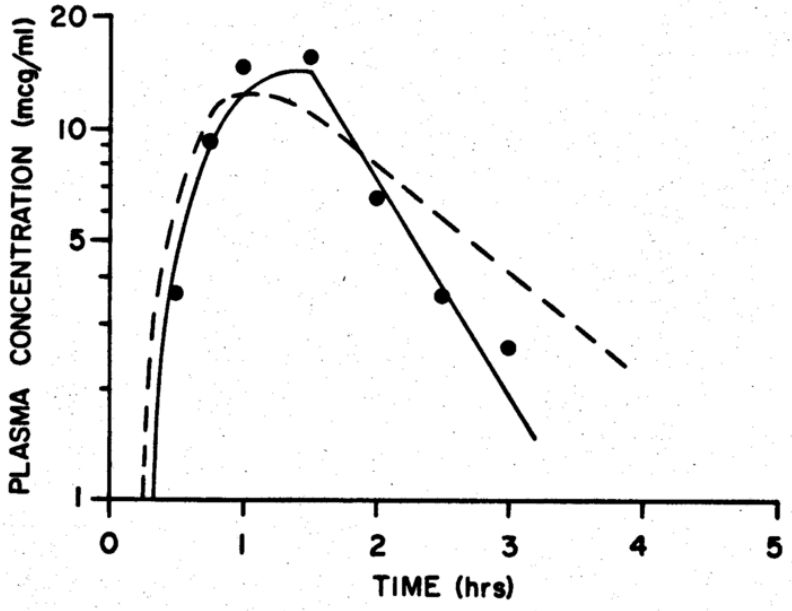


Figure 25. Plasma cephradine concentration versus time with computer generated curves for subject 1 following single 500 mg oral dose under fasting condition.
Key: • experimentally observed concentration
--- first-order absorption model, $r^2 = 0.913$
___ zero-order absorption model, $r^2 = 0.994$

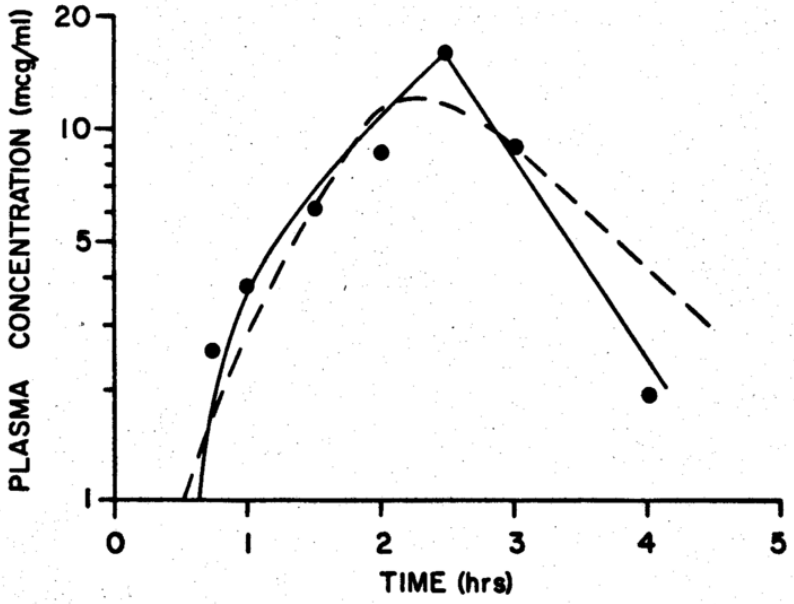


Figure 26. Plasma cephradine concentration versus time with computer generated curves for subject 1 following single 500 mg oral dose under nonfasting condition.
Key: • experimentally observed concentration
--- first-order absorption model, $r^2 = 0.896$
___ zero-order absorption model, $r^2 = 0.997$

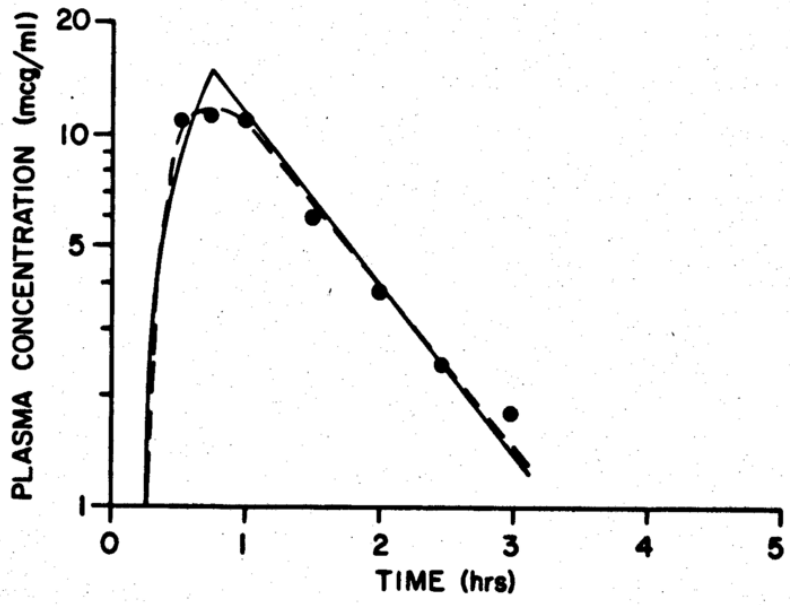


Figure 27. Plasma cefaclor concentration versus time with computer generated curves for subject 1 following single 500 mg oral dose under fasting condition.
Key: • experimentally observed concentration
--- first-order absorption model, $r^2 = 0.998$
___ zero-order absorption model, $r^2 = 0.962$

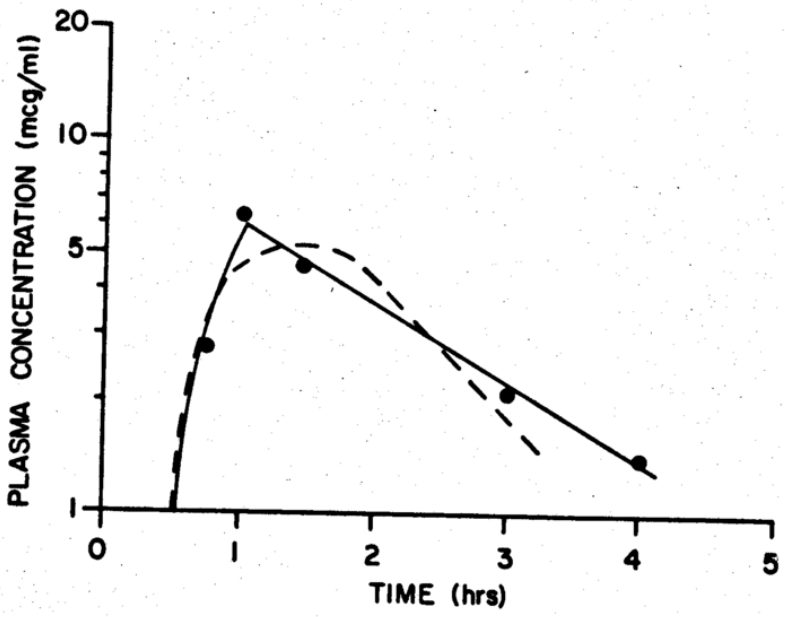


Figure 28. Plasma cefaclor concentration versus time with computer generated curves for subject 1 following single 500 mg oral dose under nonfasting condition.
Key: • experimentally observed concentration
--- first-order absorption model, $r^2 = 0.921$
___ zero-order absorption model, $r^2 = 0.960$

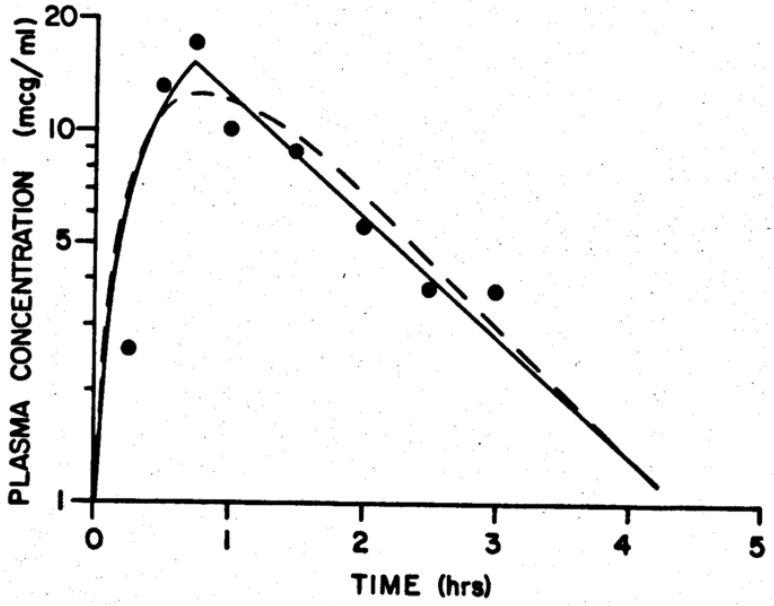


Figure 29. Plasma cephalexin concentration versus time with computer generated curves for subject 6 following single 500 mg oral dose under fasting condition.
Key: • experimentally observed concentration
--- first-order absorption model, $r^2 = 0.914$
___ zero-order absorption model, $r^2 = 0.961$

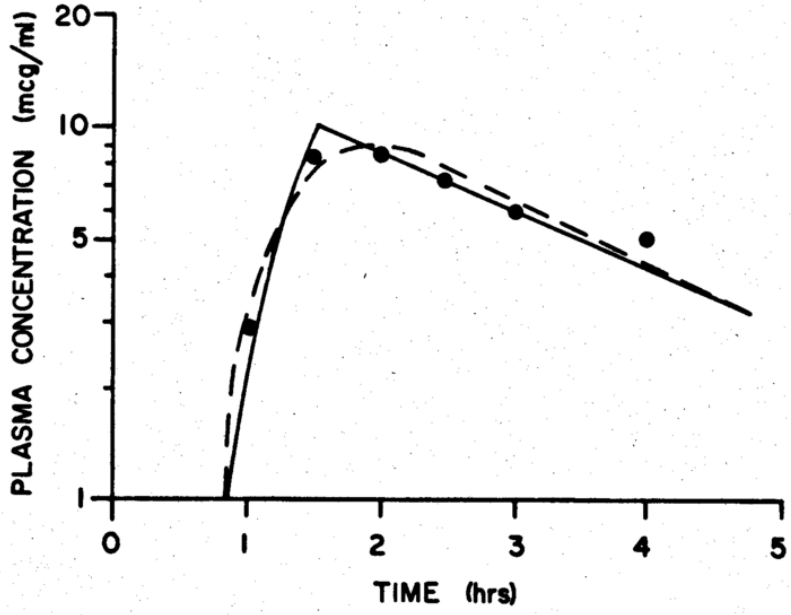


Figure 30. Plasma cephalexin concentration versus time with computer generated curves for subject 6 following single 500 mg oral dose under nonfasting condition.
Key: • experimentally observed concentration
--- first-order absorption model, $r^2 = 0.991$
___ zero-order absorption model, $r^2 = 0.983$

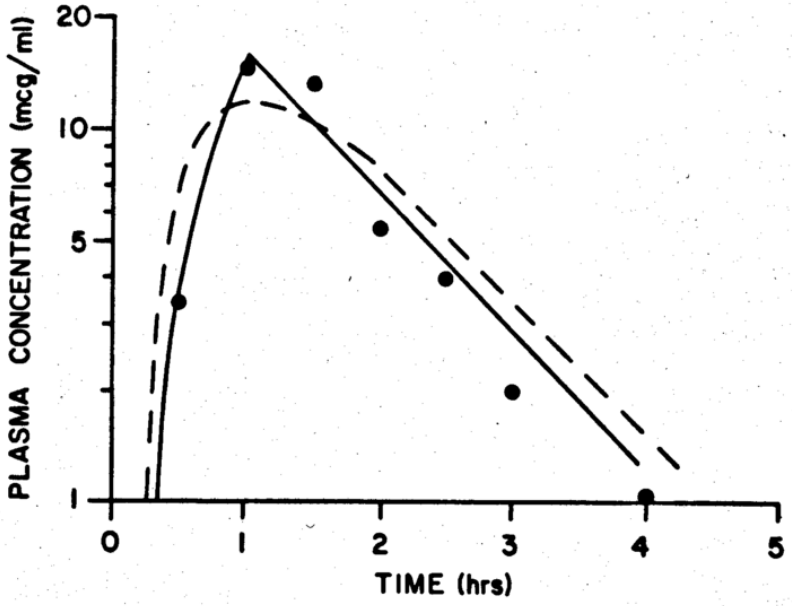


Figure 31. Plasma cephradine concentration versus time with computer generated curves for subject 6 following single 500 mg oral dose under fasting condition.
Key: • experimentally observed concentration
--- first-order absorption model, $r^2 = 0.904$
___ zero-order absorption model, $r^2 = 0.973$

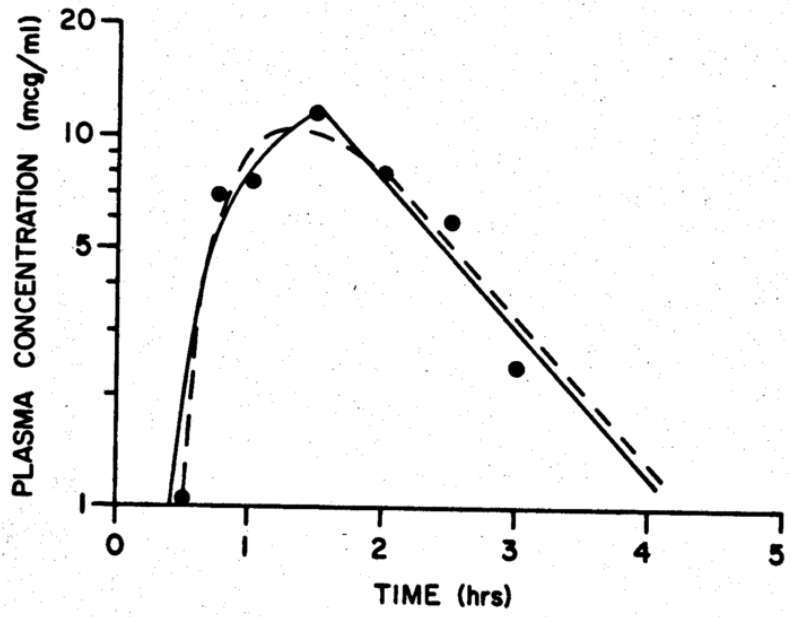


Figure 32. Plasma cephradine concentration versus time with computer generated curves for subject 6 following single 500 mg oral dose under nonfasting condition.
Key: • experimentally observed concentration
--- first-order absorption model, $r^2 = 0.969$
___ zero-order absorption model, $r^2 = 0.981$

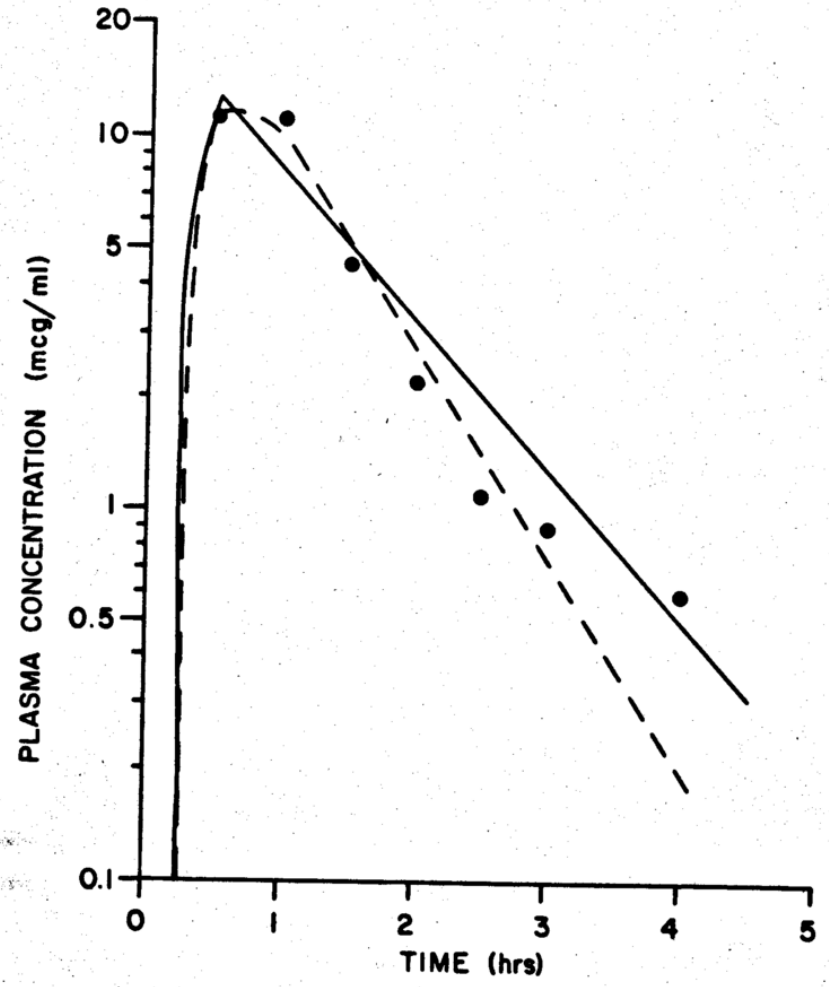


Figure 33. Plasma cefaclor concentration versus time with computer generated curves for subject 6 following single 500 mg oral dose under fasting condition.
Key: • experimentally observed concentration
--- first-order absorption model, $r^2 = 0.990$
___ zero-order absorption model, $r^2 = 0.953$

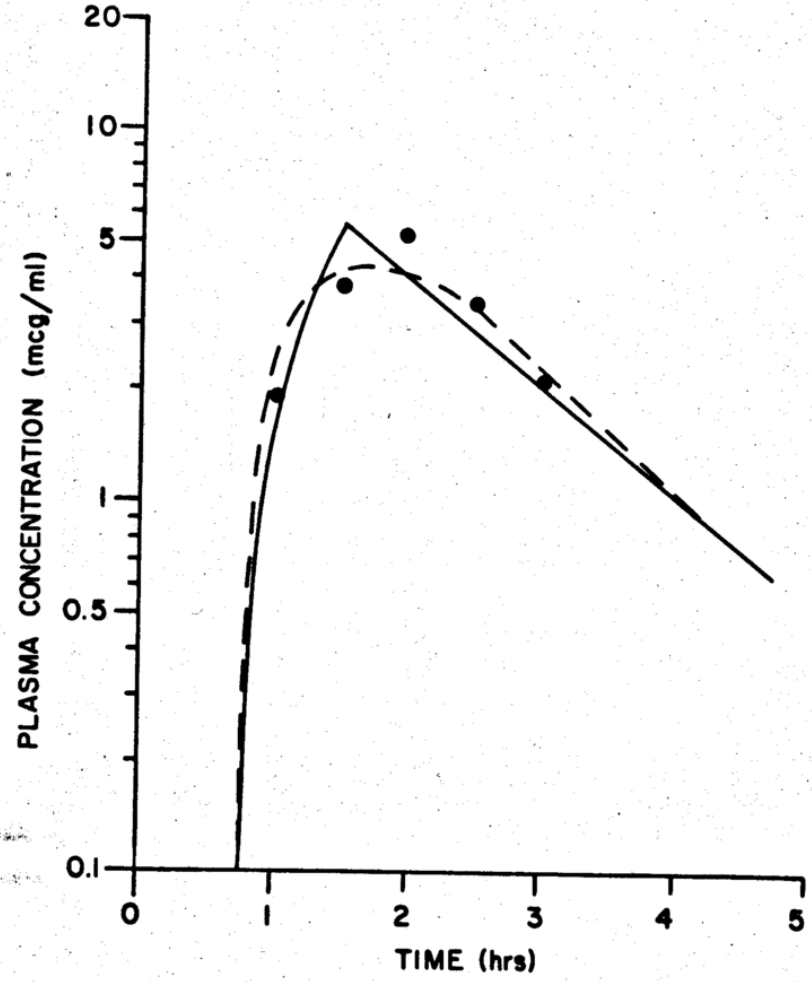


Figure 34. Plasma cefaclor concentration versus time with computer generated curves for subject 6 following single 500 mg oral dose under nonfasting condition.
Key: ● experimentally observed concentration
--- first-order absorption model, $r^2 = 0.960$
___ zero-order absorption model, $r^2 = 0.855$

Table 43

Values of Individual Pharmacokinetic Parameters Obtained From the First-Order Absorption and Elimination Model for Cephalexin Following Single 500 mg Oral Dose Under Fasting Condition

	Pharmacokinetic parameters for cephalexin						
Subject	k_a[a] (h^{-1})	$t_{1/2}(abs)$[b] (h)	k_{el}[c] (h^{-1})	$t_{1/2}(el)$[d] (h)	FD/V[e] (mcg/ml)	t_o[f] (h)	r^2[g]
1	1.50	0.46	0.92	0.75	26.7	0.0	0.889
2	2.61	0.27	0.83	0.83	28.5	0.25	0.866
3	2.14	0.32	0.83	0.83	21.0	0.0	0.791
4	2.81	0.25	1.04	0.67	25.5	0.25	0.926
5	3.43	0.20	0.26	2.68	8.71	0.50	0.976
6	1.83	0.38	0.89	0.78	24.4	0.0	0.914
7	1.98	0.35	1.55	0.45	45.2	0.25	0.983
8	1.80	0.39	0.95	0.73	19.1	0.25	0.991
Mean	2.26	0.33	0.91	0.97	24.9	0.19	0.917
SD	0.64	0.085	0.35	0.70	10.3	0.18	0.068
CV%	28.3	25.8	38.5	72.2	41.4	94.7	7.42

[a] Apparent absorption rate constant.

[b] Absorption half-life.

[c] Elimination rate constant.

[d] Elimination half-life.

[e] Fraction of dose absorbed expressed as a concentration in its apparent distribution volume in the body.

[f] Lag time.

[g] Coefficient of determination.

Table 44

Values of Individual Pharmacokinetic Parameters Obtained From the First-Order Absorption and Elimination Model for Cephalexin Following Single 500 mg Oral Dose Under Nonfasting Condition

	Pharmacokinetic parameters for cephalexin						
Subject	k_a (h^{-1})	$t_{1/2}$(abs) (h)	k_{el} (h^{-1})	$t_{1/2}$(el) (h)	FD/V (mcg/ml)	t_o (h)	r^2
1	0.96	0.72	0.64	1.08	17.5	0.50	0.978
2	0.57	1.22	0.46	1.50	17.6	1.0	0.947
3	1.62	0.43	0.62	1.13	15.3	1.5	0.960
4	1.18	0.59	0.42	1.64	12.2	0.63	0.999
5	0.65	1.07	0.65	1.07	17.4	1.34	0.991
6	1.47	0.47	0.43	1.63	13.6	0.75	0.989
7	1.14	0.61	0.59	1.17	18.5	0.25	0.950
8	0.40	1.73	0.40	1.73	16.2	0.50	0.934
Mean	1.00	0.86	0.53	1.37	16.0	0.81	0.969
SD	0.44	0.45	0.11	0.28	2.20	0.44	0.024
CV%	44.0	52.3	20.8	20.4	13.8	54.3	2.48

Table 45

Values of Individual Pharmacokinetic Parameters Obtained from the First-Order Absorption and Elimination Model for Cephradine Following Single 500 mg Oral Dose Under Fasting Condition

	Pharmacokinetic parameters for cephradine						
Subject	k_a (h^{-1})	$t_{1/2}$(abs) (h)	k_{el} (h^{-1})	$t_{1/2}$(el) (h)	FD/V (mcg/ml)	t_o (h)	r^2
1	1.26	0.55	1.19	0.58	32.1	0.25	0.913
2	4.11	0.17	0.92	0.75	34.8	0.25	0.965
3	2.79	0.25	1.04	0.67	21.8	0.25	0.970
4	1.84	0.38	1.45	0.48	43.5	0.25	0.856
5	1.03	0.68	0.69	1.0	21.3	0.75	0.931
6	1.75	0.40	0.95	0.73	24.6	0.25	0.904
7	2.79	0.25	0.95	0.73	34.0	0.25	0.966
8	1.66	0.42	1.39	0.5	36.5	0.25	0.800
Mean	2.15	0.39	1.07	0.68	31.1	0.31	0.913
SD	1.01	0.17	0.26	0.17	7.84	0.18	0.060
CV%	47.0	43.6	24.3	25	25.2	58.1	6.57

Table 46

Values of Individual Pharmacokinetic Parameters Obtained from the First-Order Absorption and Elimination Model for Cephradine Following Single 500 mg Oral Dose Under Nonfasting Condition

	Pharmacokinetic parameters for cephradine						
Subject	k_a (h^{-1})	$t_{1/2}$(abs) (h)	k_{el} (h^{-1})	$t_{1/2}$(el) (h)	FD/V (mcg/ml)	t_o (h)	r^2
1	1.21	0.57	1.21	0.57	33.6	1.34	0.896
2	0.90	0.77	0.90	0.77	30.1	1.36	0.901
3	1.21	0.57	1.21	0.57	28.9	1.73	0.949
4	1.30	0.53	0.50	1.38	12.1	0.75	0.943
5	0.50	1.39	0.50	1.39	12.4	1.24	0.929
6	1.27	0.55	1.27	0.55	27.5	0.48	0.972
7	1.08	0.64	1.08	0.64	32.5	1.40	0.909
8	0.32	2.19	0.32	2.18	24.0	1.26	0.955
Mean	0.97	0.90	0.87	1.01	25.1	1.20	0.932
SD	0.37	0.59	0.38	0.59	8.48	0.39	0.028
CV%	38.1	65.6	43.7	58.4	33.8	32.5	3.00

Table 47

Values of Individual Pharmacokinetic Parameters Obtained from the First-Order Absorption and Elimination Model for Cefaclor Following Single 500 mg Oral Dose Under Fasting Condition

	Pharmacokinetic parameters for cefaclor						
Subject	k_a (h^{-1})	$t_{1/2}$(abs) (h)	k_{el} (h^{-1})	$t_{1/2}$(el) (h)	FD/V (mcg/ml)	t_o (h)	r^2
1	4.30	0.16	1.06	0.66	18.9	0.25	0.998
2	3.01	0.23	1.39	0.5	12.7	0.0	0.896
3	1.91	0.36	1.32	0.53	24.6	0.0	0.843
4	0.99	0.70	0.83	0.83	9.70	0.5	0.852
5	0.92	0.75	0.88	0.79	18.9	0.5	0.954
6	4.31	0.16	1.39	0.5	21.5	0.25	0.990
7	2.26	0.31	0.88	0.79	11.9	0.25	0.883
8	1.80	0.39	1.80	0.39	23.1	0.25	0.752
Mean	2.44	0.38	1.19	0.62	17.7	0.25	0.896
SD	1.33	0.23	0.34	0.17	5.60	0.19	0.083
CV%	54.5	60.5	28.6	27.4	31.6	76.0	9.26

Table 48

Values of Individual Pharmacokinetic Parameters Obtained from the First-Order Absorption and Elimination Model for Cefaclor Following Single 500 mg Oral Dose Under Nonfasting Condition

	Pharmacokinetic parameters for cefaclor						
Subject	k_a (h^{-1})	$t_{1/2}$(abs) (h)	k_{el} (h^{-1})	$t_{1/2}$(el) (h)	FD/V (mcg/ml)	t_o (h)	r^2
1	3.41	0.20	0.53	1.31	7.11	0.5	0.934
2	1.29	0.54	0.57	1.21	9.83	0.25	0.934
3	0.96	0.72	0.62	1.13	9.84	0.5	0.961
4	1.91	0.36	0.92	0.75	14.2	0.93	0.994
5	1.11	0.63	0.15	4.50	4.59	0.25	0.974
6	1.36	0.51	0.88	0.79	9.52	0.75	0.958
7	1.63	0.43	1.63	0.43	22.2	0.94	0.989
8	3.25	0.21	0.13	5.37	4.14	0.5	0.981
Mean	1.86	0.45	0.68	1.94	10.2	0.58	0.966
SD	0.95	0.19	0.48	1.89	5.84	0.27	0.023
CV%	51.1	42.2	70.6	97.4	57.3	46.6	2.38

Table 49

Values of Individual Pharmacokinetic Parameters Obtained From the First-Order Absorption and Elimination Model with 'Flip-Flop' Characteristic for Cephalexin Following Single 500 mg Oral Dose Under Nonfasting Condition

	Pharmacokinetic parameters for cephalexin						
Subject	k_a (h^{-1})	$t_{1/2}(abs)$ (h)	k_{el} (h^{-1})	$t_{1/2}(el)$ (h)	FD/V (mcg/ml)	t_o (h)	r^2
1	0.66	1.04	1.04	0.67	27.4	0.57	0.978
2	0.43	1.62	0.64	1.08	24.2	1.0	0.946
3	1.20	0.58	0.88	0.79	20.1	1.5	0.963
4	0.42	1.64	1.18	0.59	33.9	0.63	0.999
5	0.42	1.64	0.64	1.08	19.7	1.0	0.966
6	0.67	1.04	1.11	0.63	30.4	0.82	0.991
7	1.96	0.35	0.98	0.71	23.0	0.76	0.990
8	0.30	2.33	0.62	1.13	24.5	0.60	0.934
Mean	0.76	1.28	0.89	0.84	25.4	0.86	0.971
SD	0.56	0.65	0.23	0.22	4.93	0.31	0.023
CV%	73.7	50.8	25.8	26.2	19.4	36.0	2.37

Table 50

Values of Individual Pharmacokinetic Parameters Obtained from the First-Order Absorption and Elimination Model with 'Flip-Flop' Characteristic for Cephradine Following Single 500 mg Oral Dose Under Nonfasting Condition

	Pharmacokinetic parameters for cephradine						
Subject	k_a (h^{-1})	$t_{1/2}$(abs) (h)	k_{el} (h^{-1})	$t_{1/2}$(el) (h)	FD/V (mcg/ml)	t_o (h)	r^2
1	1.12	0.62	1.33	0.52	36.9	1.34	0.896
2	0.90	0.77	0.90	0.77	30.1	1.36	0.901
3	1.23	0.56	1.19	0.58	28.4	1.73	0.949
4	0.57	1.22	0.90	0.77	22.4	0.66	0.950
5	0.40	1.73	0.64	1.08	15.9	1.26	0.927
6	1.04	0.67	1.85	0.38	39.1	0.55	0.969
7	0.53	1.32	1.04	0.67	40.0	1.0	0.853
8	0.32	2.17	0.92	0.75	44.1	1.74	0.944
Mean	0.76	1.13	1.10	0.69	32.1	1.21	0.924
SD	0.35	0.59	0.37	0.21	9.66	0.44	0.038
CV%	46.1	52.2	33.6	30.4	30.1	36.4	4.11

Table 51

Values of Individual Pharmacokinetic Parameters Obtained from the First-Order Absorption and Elimination Model with 'Flip-Flop' Characteristic for Cefaclor Following Single 500 mg Oral Dose Under Nonfasting Condition

	Pharmacokinetic parameters for cefaclor						
Subject	k_a (h^{-1})	$t_{1/2}$(abs) (h)	k_{el} (h^{-1})	$t_{1/2}$(el) (h)	FD/V (mcg/ml)	t_o (h)	r^2
1	1.53	0.45	1.11	0.63	12.4	0.50	0.921
2	0.84	0.83	0.92	0.75	14.9	0.25	0.937
3	0.51	1.36	0.95	0.73	16.7	0.45	0.964
4	1.91	0.36	0.92	0.75	14.2	0.93	0.994
5	0.37	1.87	0.64	1.08	12.5	0.25	0.976
6	1.04	0.66	1.19	0.58	12.6	0.75	0.960
7	0.99	0.70	1.51	0.46	23.3	0.75	0.935
8	0.43	1.60	1.04	0.67	17.3	0.43	0.973
Mean	0.95	0.98	1.04	0.71	15.5	0.54	0.958
SD	0.55	0.56	0.25	0.18	3.68	0.25	0.025
CV%	57.9	57.1	24.0	25.4	23.7	46.3	2.61

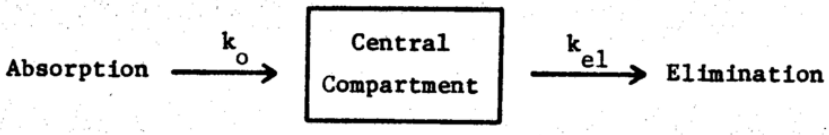
FD/V (mcg/ml)	24.9 (±10.3)	25.4 (±4.93)	31.1 (±7.84)	32.1 (±9.66)	17.7 (±5.6)	15.5 (±3.68)	C,D > E,F
t_o (h)	0.19 (±0.18)	0.86 (±0.31)	0.31 (±0.18)	1.21 (±0.44)	0.25 (±0.19)	0.54 (±0.25)	B,D > A,C,E D > F
r^2[b]	0.917 (±0.068)	0.971 (±0.023)	0.913 (±0.06)	0.924 (±0.038)	0.896 (±0.083)	0.958 (±0.025)	NSD

[a]Statistical comparisons were carried out as in Table 25. The subject factor had a significant effect on the following parameters, k_{el} and $t_{1/2}(el)$.

[b]Coefficient of determination, $r^2 = \frac{(\Sigma\ obs^2 - \Sigma\ dev^2)}{\Sigma\ obs^2}$.

b. One-Compartment Model with Zero-Order Absorption and First-Order Elimination

Individual plasma data sets were also analyzed by means of a one-compartment model with zero-order absorption and first-order elimination as depicted by Scheme II.



Scheme II. One-compartment model with zero-order absorption and first-order elimination

This model can be described by Equations 3 and 4 as discussed earlier.

The computer generated curves using the zero-order absorption equation for subjects 1 and 6 following all treatments are shown in Figures 23-34. Pharmacokinetic parameters obtained by fitting individual plasma profiles to the zero-order absorption equations are given in Tables 53-58. The mean data together with statistical comparisons are summarized in Table 59. The mean elimination rate constants ranged between 0.62-1.2 hr^{-1}, and analysis of variance did not show any significant differences among all treatments.

Table 60 summarizes the mean values and the results of statistical comparison of the elimination rate constants obtained from fitting data to the first-order absorption model and the zero-order absorption model for all three cephalosporins. Under the

fasting treatments, the elimination rate constants for all three compounds obtained utilizing the zero-order absorption model were similar to those obtained utilizing the first-order absorption model. However, under the nonfasting treatments, the elimination rate constants for cephradine and cefaclor obtained utilizing the zero-order absorption model were significantly smaller. As for the nonfasting treatments, the elimination rate constants generated using the first-order absorption model are based on 'flip-flop' characteristics; whereas in the zero-order absorption model, the elimination rate constants are determined from the terminal linear portion of the plasma profiles. Therefore, the model-dependent differences in these values are not unexpected.

Table 59 shows that the apparent zero-order absorption rate was significantly smaller following the nonfasting treatments than the fasting treatments for all three cephalosporins. The times to maximum plasma concentrations were significantly lengthened for cephalexin and cephradine following the nonfasting treatments.

Thus, the model-dependent parameters from both models showed that food delays the rate of drug absorption. However, the overall bioavailability of all three compounds is not significantly affected by food.

In an attempt to differentiate between the two absorption models, the coefficients of determination obtained from fitting plasma data to the first-order absorption equation and the zero-order absorption equation were compared for each treatment. The results

of statistical comparisons of the coefficients of determination are summarized in Table 61. In addition, experimentally observed peak plasma concentrations and peak concentrations predicted by either the first-order absorption model or zero-order absorption model were compared for each treatment. Individual experimentally observed peak concentrations and computer predicted peak concentrations are given in Tables 62-67, and the results of the statistical comparisons of peak concentrations are summarized in Table 68.

Analysis of variance of the coefficients of determination showed that the zero-order absorption equation gave a significantly better fit of the plasma data for all three cephalosporins under the fasting treatments. Furthermore, no significant difference was observed between the experimentally and computer predicted peak concentrations generated by the zero-order equation for all three cephalosporins. Thus, both the coefficients of determination and the computer predicted peak concentrations indicate that the zero-order absorption equation describes the plasma profile significantly better for all three cephalosporins under the fasting treatments.

Under the nonfasting treatments, the coefficients of determination indicated that the first-order model provided a significantly better fit of the plasma data for cephalexin. However, comparison of the coefficients of determination did not show any significant differences between the two models for cephradine and cefaclor. Hence, it appears that the plasma profiles for cephradine and cefaclor can be adequately described by either the zero-order or

first-order absorption equation under the nonfasting treatments.

Table 53

Values of Individual Pharmacokinetic Parameters Obtained from the Zero-Order Absorption and First-Order Elimination Model for Cephalexin Following Single 500 mg Oral Dose Under Fasting Condition

	Pharmacokinetic parameters for cephalexin					
Subject	Fk_o/V (mcg/h ml)	k_{el} (h^{-1})	$t_{1/2}(el)$ (h)	t_{max} (h)	t_o (h)	r^2
1	25.6	0.98	0.71	1.00	0.0	0.977
2	46.9	1.34	0.52	1.08	0.33	0.976
3	78.5	0.85	0.82	0.5	0.25	0.998
4	28.2	1.07	0.65	1.0	0.25	0.998
5	31.5	0.65	1.07	0.71	0.5	0.952
6	27.0	0.74	0.93	0.74	0.0	0.961
7	60.0	1.05	0.66	0.79	0.25	0.995
8	21.0	0.50	1.39	0.80	0.25	0.996
Mean	39.8	0.90	0.84	0.83	0.23	0.982
SD	20.3	0.27	0.28	0.19	0.16	0.018
CV%	51.0	30.0	33.3	22.9	69.6	1.83

Table 54

Values of Individual Pharmacokinetic Parameters Obtained from the Zero-Order Absorption and First-Order Elimination Model for Cephalexin Following Single 500 mg Oral Dose Under Nonfasting Condition

	Pharmacokinetic parameters for cephalexin					
Subject	Fk_o/V (mcg/h ml)	k_{el} (h^{-1})	$t_{1/2}(el)$ (h)	t_{max} (h)	t_o (h)	r^2
1	11.7	0.67	1.03	1.96	0.46	0.941
2	6.37	1.34	0.52	3.04	1.99	0.841
3	4.71	0.62	1.13	3.13	1.5	0.842
4	8.11	0.42	1.64	2.00	0.50	0.970
5	5.03	0.65	1.07	2.95	0.94	0.866
6	10.8	0.36	1.95	1.80	0.80	0.983
7	14.8	0.60	1.16	1.50	0.39	0.997
8	5.03	0.40	1.73	2.47	0.47	0.879
Mean	8.32	0.63	1.28	2.36	0.88	0.915
SD	3.74	0.31	0.46	0.63	0.58	0.065
CV%	45.0	49.2	35.9	26.7	65.9	7.10

Table 55

Values of Individual Pharmacokinetic Parameters Obtained from the Zero-Order Absorption and First-Order Elimination Model for Cephradine Following Single 500 mg Oral Dose Under Fasting Condition

	Pharmacokinetic parameters for cephradine					
Subject	Fk_o/V (mcg/h ml)	k_{el} (h^{-1})	$t_{1/2}(el)$ (h)	t_{max} (h)	t_o (h)	r^2
1	31.4	1.36	0.51	1.37	0.36	0.994
2	71.1	1.08	0.64	0.75	0.25	0.997
3	29.1	1.04	0.67	1.0	0.25	0.972
4	31.6	1.54	0.45	1.27	0.27	0.998
5	9.87	0.74	0.94	2.58	0.58	0.933
6	25.4	0.87	0.80	1.12	0.36	0.973
7	46.8	0.87	0.80	0.86	0.25	0.991
8	43.3	1.68	0.41	1.16	0.41	0.989
Mean	36.1	1.15	0.65	1.26	0.34	0.981
SD	18.1	0.34	0.19	0.57	0.11	0.022
CV%	50.1	29.6	29.2	45.2	32.4	2.24

Table 56

Values of Individual Pharmacokinetic Parameters Obtained from the Zero-Order Absorption and First-Order Elimination Model for Cephradine Following Single 500 mg Oral Dose Under Nonfasting Condition

	Pharmacokinetic parameters for cephradine					
Subject	Fk_o/V (mcg/h ml)	k_{el} (h^{-1})	$t_{1/2}(el)$ (h)	t_{max} (h)	t_o (h)	r^2
1	12.6	1.26	0.55	2.93	0.66	0.997
2	7.44	0.37	1.85	2.81	0.80	0.882
3	9.30	1.21	0.57	3.40	0.89	0.946
4	7.05	0.50	1.38	2.00	0.49	0.896
5	3.36	0.53	1.32	4.08	0.81	0.979
6	18.2	0.91	0.76	1.46	0.41	0.981
7	8.26	0.29	2.42	2.49	0.98	0.847
8	4.16	0.32	2.18	3.86	0.97	0.998
Mean	8.80	0.67	1.38	2.88	0.75	0.941
SD	4.77	0.40	0.72	0.90	0.21	0.058
CV%	54.2	59.7	52.2	31.3	28.0	6.16

Table 57

Values of Individual Pharmacokinetic Parameters Obtained from the Zero-Order Absorption and First-Order Elimination Model for Cefaclor Following Single 500 mg Oral Dose Under Fasting Condition

	Pharmacokinetic parameters for cefaclor					
Subject	Fk_o/V (mcg/h ml)	k_{el} (h^{-1})	$t_{1/2}(el)$ (h)	t_{max} (h)	t_o (h)	r^2
1	37.7	1.06	0.66	0.75	0.25	0.962
2	22.7	1.38	0.50	0.50	0.0	0.975
3	29.5	1.34	0.52	0.75	0.0	0.922
4	7.34	0.80	0.87	1.67	0.67	0.961
5	12.6	0.88	0.79	1.99	0.49	0.959
6	88.2	0.92	0.76	0.42	0.25	0.953
7	11.9	0.83	0.83	1.14	0.40	0.991
8	26.4	2.37	0.29	1.15	0.39	0.999
Mean	29.5	1.20	0.65	1.05	0.31	0.965
SD	25.8	0.52	0.20	0.56	0.23	0.024
CV%	87.5	43.3	30.8	53.3	74.2	2.49

Table 58

Values of Individual Pharmacokinetic Parameters Obtained from the Zero-Order Absorption and First-Order Elimination Model for Cefaclor Following Single 500 mg Oral Dose Under Nonfasting Condition

	Pharmacokinetic parameters for cefaclor					
Subject	Fk_o/V (mcg/h ml)	k_{el} (h^{-1})	$t_{1/2}(el)$ (h)	t_{max} (h)	t_o (h)	r^2
1	8.50	0.49	1.41	1.21	0.46	0.960
2	7.33	0.44	1.56	1.34	0.34	0.987
3	5.33	1.06	0.65	2.61	0.30	0.945
4	7.64	0.49	1.40	1.85	0.77	0.982
5	2.56	0.88	0.79	1.68	0.18	0.990
6	7.59	0.80	0.86	1.78	0.78	0.855
7	9.39	0.68	1.03	1.78	0.75	0.966
8	4.16	0.13	5.37	1.31	0.31	0.964
Mean	6.56	0.62	1.63	1.70	0.49	0.956
SD	2.33	0.29	1.55	0.44	0.24	0.044
CV%	35.5	46.8	95.1	25.9	49.0	4.60

t_o (h)	0.23 (±0.16)	0.88 (±0.58)	0.34 (±0.11)	0.75 (±0.21)	0.31 (±0.23)	0.49 (±0.24)	B > A,C,E D > A
r^2	0.982 (±0.018)	0.915 (±0.065)	0.981 (±0.022)	0.941 (±0.058)	0.965 (±0.024)	0.956 (±0.044)	A > B

[a]Statistical comparisons were carried out as in Table 25. The subject factor had a significant effect on the following parameter, t_{max}.

Table 60

Mean Values (±1 SD) and Results of Statistical Comparison of Elimination Rate Constants Obtained from Fitting Data to the First-Order Absorption Model and the Zero-Order Absorption Model for Cephalexin, Cephradine, and Cefaclor Under Fasting and Nonfasting Conditions[a]

	k_{el} (h^{-1})		
	First-Order Absorption	Zero-Order Absorption	Statistical Comparison
Cephalexin (fasting)	0.91 (±0.35)	0.90 (±0.27)	NSD
Cephalexin (nonfasting)	0.89 (±0.23)	0.63 (±0.31)	NSD
Cephradine (fasting)	1.07 (±0.26)	1.15 (±0.34)	NSD
Cephradine (nonfasting)	1.10 (±0.37)	0.67 (±0.40)	First-order > Zero-order
Cefaclor (fasting)	1.19 (±0.34)	1.20 (±0.52)	NSD
Cefaclor (nonfasting)	1.04 (±0.25)	0.62 (±0.29)	First-order > Zero-order

[a]Data were analyzed by analysis of variance and differences were considered significant when $p < 0.05$. The subject factor had a significant effect on the following treatments, Cephradine (fasting), Cephradine (nonfasting), and Cefaclor (fasting).

Table 61

Mean Values (±1 SD) and Results of Statistical Comparison of Coefficients of Determination Obtained from Fitting Data to the First-Order Absorption Model and the Zero-Order Absorption Model for Cephalexin, Cephradine, and Cefaclor Under Fasting and Nonfasting Conditions[a]

	r^2		
	First-Order Absorption	Zero-Order Absorption	Statistical Comparison
Cephalexin (fasting)	0.917 (±0.068)	0.982 (±0.018)	Zero-Order > First-Order
Cephalexin (nonfasting)	0.971 (±0.023)	0.915 (±0.065)	First-Order > Zero-Order
Cephradine (fasting)	0.913 (±0.06)	0.981 (±0.022)	Zero-Order > First-Order
Cephradine (nonfasting)	0.924 (±0.038)	0.941 (±0.058)	NSD
Cefaclor (fasting)	0.896 (±0.083)	0.965 (±0.024)	Zero-Order > First-Order
Cefaclor (nonfasting)	0.958 (±0.025)	0.956 (±0.044)	NSD

[a]Data were analyzed by analysis of variance and differences were considered significant when $p < 0.05$. The subject factor had no significant effect on all treatments.

Table 62

Experimental Peak Plasma Concentrations Versus Computer Predicted Peak Plasma Concentrations for Cephalexin Following Single 500 mg Oral Dose Under Fasting Condition

	C_{max} (mcg/ml)		
Subject	Experimental[a]	First-Order Absorption[b]	Zero-Order Absorption[c]
1	17.9	12.1	16.3
2	26.1	16.5	24.8
3	17.9	10.9	17.7
4	18.5	14.1	19.0
5	7.2	7.0	6.4
6	17.1	12.3	15.2
7	19.9	17.9	19.7
8	9.7	9.4	10.3
Mean	16.8	12.5	16.2
SD	5.9	3.6	5.7
CV%	35.1	28.8	35.2

[a] Observed peak plasma concentration.

[b] Computer predicted peak plasma concentrations based on the assumption of a one-compartment model with first-order absorption and elimination.

[c] Computer predicted peak plasma concentrations based on the assumption of a one-compartment model with zero-order absorption and first-order elimination.

Table 63

Experimental Peak Plasma Concentrations Versus Computer Predicted Peak Plasma Concentrations for Cephalexin Following Single 500 mg Oral Dose Under Nonfasting Condition

	C_{max} (mcg/ml)		
Subject	Experimental	First-Order Absorption	Zero-Order Absorption
1	8.1	7.8	10.7
2	9.2	7.2	7.1
3	10.8	8.6	9.7
4	6.8	6.9	9.0
5	6.7	5.8	7.5
6	8.5	8.5	10.1
7	11.7	11.5	12.0
8	7.9	6.0	6.9
Mean	8.7	7.8	9.1
SD	1.8	1.8	1.8
CV%	20.7	23.1	19.8

Table 64

Experimental Peak Plasma Concentrations Versus Computer Predicted Peak Plasma Concentrations for Cephradine Following Single 500 mg Oral Dose Under Fasting Condition

	C_{max} (mcg/ml)		
Subject	Experimental	First-Order Absorption	Zero-Order Absorption
1	15.4	12.1	14.3
2	27.9	22.5	27.6
3	13.6	12.1	15.2
4	25.5	17.6	24.5
5	10.6	9.4	11.0
6	14.7	11.9	15.9
7	22.2	19.3	24.1
8	24.9	14.5	24.2
Mean	19.4	14.9	19.6
SD	6.5	4.5	6.1
CV%	33.6	30.2	31.1

Table 65

Experimental Peak Plasma Concentrations Versus Computer Predicted Peak Plasma Concentrations for Cephradine Following Single 500 mg Oral Dose Under Nonfasting Condition

	C_{max} (mcg/ml)		
Subject	Experimental	First-Order Absorption	Zero-Order Absorption
1	16.0	12.1	16.0
2	15.2	11.1	11.8
3	11.8	10.6	11.8
4	6.6	6.4	7.4
5	5.5	4.5	5.5
6	11.5	10.0	11.9
7	16.4	10.0	11.6
8	10.0	8.4	10.3
Mean	11.6	9.1	10.8
SD	4.1	2.6	3.2
CV%	35.3	28.6	29.6

Table 66

Experimental Peak Plasma Concentrations Versus Computer Predicted Peak Plasma Concentrations for Cefaclor Following Single 500 mg Oral Dose Under Fasting Condition

	C_{max} (mcg/ml)		
Subject	Experimental	First-Order Absorption	Zero-Order Absorption
1	11.5	11.8	14.7
2	9.2	6.5	8.2
3	14.0	10.6	13.9
4	6.2	3.9	5.8
5	8.5	7.1	10.4
6	11.3	11.6	12.6
7	8.5	6.5	9.0
8	13.3	8.1	13.3
Mean	10.3	8.3	11.0
SD	2.7	2.8	3.1
CV%	26.2	33.7	28.2

Table 67

Experimental Peak Plasma Concentrations Versus Computer Predicted Peak Plasma Concentrations for Cefaclor Following Single 500 mg Oral Dose Under Nonfasting Condition

	C_{max} (mcg/ml)		
Subject	Experimental	First-Order Absorption	Zero-Order Absorption
1	6.3	5.1	6.0
2	6.5	5.2	6.9
3	4.8	4.3	5.2
4	7.0	7.0	7.6
5	3.9	3.4	3.6
6	5.2	4.3	6.6
7	7.7	6.8	8.4
8	4.4	3.8	3.8
Mean	5.7	5.0	6.0
SD	1.3	1.3	1.7
CV%	22.8	26.0	28.3

Table 68

Mean Values (±1 SD) and Results of Statistical Comparison of Experimentally Observed Peak Plasma Concentrations and Computer Predicted Peak Plasma Concentrations for Cephalexin, Cephradine, and Cefaclor Under Fasting and Nonfasting Conditions[a]

	C_{max} (mcg/ml)			
	Experimental	First-Order Absorption	Zero-Order Absorption	Statistical Comparison
Cephalexin (fasting)	16.8 (±5.9)	12.5 (±3.6)	16.2 (±5.7)	Exp., Zero-Order > First-Order
Cephalexin (nonfasting)	8.7 (±1.8)	7.8 (±1.8)	9.1 (±1.8)	Zero-Order > First-Order
Cephradine (fasting)	19.4 (±6.5)	14.9 (±4.5)	19.6 (±6.1)	Exp., Zero-Order > First-Order
Cephradine (nonfasting)	11.6 (±4.1)	9.1 (±2.6)	10.8 (±3.2)	Exp., Zero-Order > First-Order
Cefaclor (fasting)	10.3 (±2.7)	8.3 (±2.8)	11.0 (±3.1)	Exp., Zero-Order > First-Order
Cefaclor (nonfasting)	5.7 (±1.3)	5.0 (±1.3)	6.0 (±1.7)	Exp., Zero-Order > First-Order

[a]Data were analyzed by analysis of variance and differences were considered significant when $p < 0.05$. Actual differences between experimentally observed and computer predicted values were determined by Tukey's test. The subject factor had a significant effect on all treatments.

5. Clearance

Cefaclor has been reported to be chemically unstable in plasma (13). This stability pattern was consistent with the results of the in vitro stability study of cefaclor in this present investigation. Therefore, it is reasonable to assume that cefaclor will have a larger elimination rate constant in vivo as compared with the other two chemically stable cephalosporins. However, the clinical study showed that the elimination rate constants of the three cephalosporins were not significantly different from each other. In order to investigate this further, plasma and renal clearances, which are useful parameters for evaluation of an elimination process, were calculated for the three cephalosporins.

Plasma clearance was calculated by means of Equation 14 under the assumption that the fraction of dose absorbed is unity.

$$Cl_{plasma} = \frac{D}{AUC} \qquad (14)$$

Renal clearance was calculated by dividing the total amount of drug excreted unchanged in urine by AUC as in Equation 7.

Individual values of plasma and renal clearances are given in Tables 69-71. The mean values and the results of statistical comparison are summarized in Table 72. Statistical comparison showed that both the plasma and renal clearances were significantly larger for cefaclor than cephalexin and cephradine.

Table 69

Values of Plasma Clearances and Renal Clearances for Cephalexin Following Single 500 mg Oral Dose Under Fasting and Nonfasting Conditions[a]

	Plasma and renal clearances for cephalexin			
	Fasting		Nonfasting	
Subject	Cl_{plasma}[b] (l/h)	Cl_{renal}[c] (l/h)	Cl_{plasma} (l/h)	Cl_{renal} (l/h)
1	19.9	19.3	22.3	18.8
2	15.5	14.7	17.1	11.8
3	22.4	22.0	25.2	20.6
4	23.5	23.4	22.3	19.3
5	23.1	23.5	20.9	19.8
6	18.2	14.4	19.8	19.4
7	16.1	11.8	19.5	11.2
8	27.5	17.8	22.8	9.59
Mean	20.8	18.4	21.2	16.3
SD	4.10	4.45	2.47	4.58
CV%	19.7	24.2	11.7	28.1

[a] Data from Ref. 1.

[b] Calculated from the equation, $Cl_{plasma} = D/AUC$, based on the assumption that the fraction of dose absorbed is unity.

[c] Calculated from the equation, $CL_{renal} = A_e/AUC$, where A_e is the amount of drug excreted unchanged in urine.

Table 70

Values of Plasma Clearances and Renal Clearances for Cephradine Following Single 500 mg Oral Dose Under Fasting and Nonfasting Conditions

	Plasma and renal clearances for cephradine			
	Fasting		Nonfasting	
Subject	Cl_{plasma} (l/h)	Cl_{renal} (l/h)	Cl_{plasma} (l/h)	Cl_{renal} (l/h)
1	21.4	18.6	19.0	14.1
2	13.9	13.5	17.8	13.9
3	24.5	18.4	18.7	13.4
4	19.5	19.0	24.4	24.4
5	18.8	15.2	25.2	19.2
6	21.8	19.4	25.3	26.0
7	16.1	13.9	18.9	17.2
8	21.6	21.7	20.1	20.3
Mean	19.7	17.5	21.2	18.6
SD	3.41	2.92	3.21	4.83
CV%	17.3	16.7	15.1	26.0

Table 71

Values of Plasma Clearances and Renal Clearances for Cefaclor Following Single 500 mg Oral Dose Under Fasting and Nonfasting Conditions[a]

	Plasma and renal clearances for cefaclor			
	Fasting		Nonfasting	
Subject	Cl_{plasma} (l/h)	Cl_{renal} (l/h)	Cl_{plasma} (l/h)	Cl_{renal} (l/h)
1	29.2	21.1	41.0	31.7
2	56.4	39.1	30.7	17.1
3	29.2	27.6	36.8	25.6
4	58.9	51.2	33.1	24.8
5	31.9	27.2	38.4	25.4
6	33.0	24.3	54.9	35.6
7	44.6	22.0	41.4	29.3
8	47.6	27.8	37.3	18.3
Mean	41.4	30.0	39.2	26.0
SD	12.2	10.2	7.31	6.28
CV%	29.5	34.0	18.6	24.2

Table 72

Summary of Mean Values (±1 SD) of Plasma Clearance and Renal Clearance for Cephalexin, Cephradine, and Cefaclor; and Results of Statistical Comparison Between Treatments[a]

	Cephalexin (fasting) A	Cephalexin (nonfasting) B	Cephradine (fasting) C	Cephradine (nonfasting) D	Cefaclor (fasting) E	Cefaclor (nonfasting) F	Statistical Comparison
Plasma Clearance (l/h)	20.8 (±4.10)	21.2 (±2.47)	19.7 (±3.41)	21.2 (±3.21)	41.4 (±12.2)	39.2 (±7.31)	E,F > A,B,C,D
Renal Clearance (l/h)	18.4 (±4.45)	16.3 (±4.58)	17.5 (±2.92)	18.6 (±4.83)	30.0 (±10.2)	26.0 (±6.28)	E > A,B,C,D F > B,C

[a]Statistical comparisons were carried out as in Table 25. The subject factor had no significant effect on any of the parameters.

6. Apparent Volume of Distribution

The apparent volume of distribution is a parameter that relates the plasma concentration of a drug to the total amount of drug in the body.

The quantity FD/V, generated by computer fitting earlier, suggested that cefaclor might have a larger apparent volume of distribution. In order to explore this further, apparent volumes of distribution were calculated by the following two equations,

$$V = \frac{FD}{k_{el}AUC} \tag{15}$$

and

$$V_{dss} = \frac{FD}{AUC} \left(\frac{AUMC}{AUC} - \frac{1}{k_a} \right) \tag{16}$$

where V is the apparent volume of distribution, F is the fraction of dose absorbed, k_{el} is the first-order elimination rate constant, AUC is the area under the plasma concentration-time curve, V_{dss} is the apparent volume of distribution at steady state, AUMC is the area under the first moment versus time curve and k_a is the apparent first-order absorption rate constant.

Equation 15 can be applied to plasma data obtained from a drug that confers upon the body the characteristics of a one-compartment model with first-order elimination. Equation 16 was first described by Benet et al. (94) utilizing a model-independent approach for an instantaneous input process, and later derived by Perrier et al.

(95) for a first-order input process.

Individual values of V and V_{dss} are shown in Tables 73-75. The mean data and the results of statistical comparisons are summarized in Table 76. Statistical comparisons showed that the apparent volume of distribution for cefaclor was significantly larger than cephradine. The values of apparent volumes of distribution obtained were similar irrespective to the methods of calculation.

Table 73

Values of Apparent Volumes of Distribution for Cephalexin Following Single 500 mg Oral Dose Under Fasting and Nonfasting Conditions

	Apparent volumes of distribution for cephalexin			
	Fasting		Nonfasting	
Subject	V^a (l)	V^b_{dss} (l)	V (l)	V_{dss} (l)
1	20.8	13.9	18.1	17.1
2	17.7	20.0	18.4	14.6
3	26.4	19.4	23.5	45.5
4	22.5	23.4	16.4	5.79[c]
5	36.3	49.5	31.0	25.6
6	16.2	17.1	17.5	23.9
7	7.65	13.5	11.4	18.9
8	18.70	18.7	15.6	-4.62[c]
Mean	20.8	21.9	19.0	24.3
SD	8.30	11.6	5.90	11.2
CV%	40.0	53.0	31.1	46.1

[a] Calculated from the equation, $V = \frac{FD}{AUC\ k_{el}}$.

[b] Calculated from the equation, $V_{dss} = \frac{FD}{AUC}\left(\frac{AUMC}{AUC} - \frac{1}{k_a}\right)$.

[c] Values not included in the calculation of the mean.

Table 74

Values of Apparent Volumes of Distribution for Cephradine Following Single 500 mg Oral Dose Under Fasting and Nonfasting Conditions

	Apparent volumes of distribution for cephradine			
	Fasting		Nonfasting	
Subject	V (l)	V_{dss} (l)	V (l)	V_{dss} (l)
1	15.7	13.9	10.6	22.8
2	14.6	18.1	15.5	23.9
3	17.7	22.1	11.3	32.2
4	13.1	14.3	27.1	21.8
5	22.0	25.8	30.0	23.7
6	20.4	21.6	14.1	22.0
7	14.6	14.1	16.6	15.5
8	15.6	16.8	22.0	3.33[c]
Mean	16.7	18.3	18.4	23.1
SD	3.09	4.42	7.22	4.91
CV%	18.5	24.1	39.2	21.3

Table 75

Values of Apparent Volumes of Distribution for Cefaclor Following Single 500 mg Oral Dose Under Fasting and Nonfasting Conditions

	Apparent volumes of distribution for cefaclor			
	Fasting		Nonfasting	
Subject	V (l)	V_{dss} (l)	V (l)	V_{dss} (l)
1	20.0	23.4	28.6	48.9
2	28.2	28.5	18.5	21.5
3	20.9	23.1	27.0	13.3
4	61.5	48.0	26.8	45.5
5	31.1	23.9	39.6	-0.39[c]
6	17.5	26.3	29.9	44.6
7	25.2	22.3	19.4	28.8
8	15.5	16.1	17.6	5.94[c]
Mean	27.5	26.5	25.9	33.8
SD	14.7	9.41	7.36	14.7
CV%	53.5	35.5	28.4	43.5

Table 76

Summary of Mean Values (±1 SD) of Apparent Volumes of Distribution for Cephalexin, Cephradine, and Cefaclor; and Results of Statistical Comparison Between Treatments[a]

	Cephalexin (fasting) A	Cephalexin (nonfasting) B	Cephradine (fasting) C	Cephradine (nonfasting) D	Cefaclor (fasting) E	Cefaclor (nonfasting) F	Statistical Comparison
V(1)	20.8 (±8.30)	19.0 (±5.90)	16.7 (±3.09)	18.4 (±7.22)	27.5 (±14.7)	25.9 (±7.36)	E > C
V_{dss}(1)	21.9 (±11.6)	24.3 (±11.2)	18.3 (±4.42)	23.1 (±4.91)	26.5 (±9.41)	33.8 (±14.7)	NSD

[a]Statistical comparisons were carried out as in Table 25. The subject factor had a significant effect on the following parameter, V.

7. Relative Bioavailability

The relative bioavailability between fasting and nonfasting treatments for all three cephalosporins estimated by comparison of the total area under the plasma concentration-time curve (Equation 5), comparison of the total area under the plasma concentration-time curve with correction for changing renal clearance (Equation 6), comparison of the total amount of drug excreted in urine (Equation 8) and comparison of the cumulative relative fraction absorbed at 8 hr are given in Tables 77-80.

Individual estimates of cumulative relative fraction absorbed for each sampling time to 6 hr for all treatments are shown in Tables 81-86. The mean estimates of cumulative relative fraction absorbed between the fasting and nonfasting treatments are summarized in Figures 35-37 and Table 87. Under the fasting treatment, the cumulative percents absorbed at 1 hr were 88%, 85%, and 83% for cephalexin, cephradine, and cefaclor, respectively. Under the nonfasting treatment, the cumulative relative percents absorbed at 1 hr were 13%, 12%, and 29% for cephalexin, cephradine, and cefaclor, respectively. These values indicate that all three cephalosporins are more rapidly absorbed under the fasting than nonfasting treatments. However, the cumulative relative percent absorbed between the fasting and nonfasting treatments were not significantly different after 3 hr, which confirmed that the overall systemic availability for the three cephalosporins was not significantly affected by food.

The mean relative bioavailability between the fasting and nonfasting treatments for the three cephalosporins, as calculated by means of four methods, and the results of statistical comparison are summarized in Table 88. The relative bioavailability is not significantly different from each other, or from unity when calculated by the four methods, which further substantiates the fact that the extent of absorption of the three cephalosporins is similar between the fasting and nonfasting treatments. As the ratio obtained from method I and the cumulative relative fraction absorbed method are based on plasma concentration data, while method III is based on urinary excretion data, it can be concluded that for the three cephalosporins used in this study, urine and plasma data gave equivalent estimates of relative bioavailability. Method II, based on both plasma and urinary excretion data, tends to reduce the variability of the data. It appears that method II is a good method for estimating the relative bioavailability of cephalosporins between the fasting and nonfasting treatments.

Table 77

Relative Bioavailability Between Fasting and Nonfasting Conditions for Cephalexin, Cephradine, and Cefaclor Estimated by Comparison of the Total Area Under the Plasma Concentration versus Time Curve

Subject	F_{nf}/F_f[a]		
	Cephalexin	Cephradine	Cefaclor
1	0.89	1.12	0.71
2	0.91	0.78	1.84
3	0.89	1.31	0.79
4	1.05	0.80	1.78
5	1.11	0.75	0.83
6	0.92	0.86	0.60
7	0.83	0.85	1.08
8	1.20	1.08	1.28
Mean	0.98	0.94	1.11
SD	0.13	0.20	0.48
CV%	13.3	21.3	43.2

[a]Calculated from the equation, $\frac{F_{nf}}{F_f} = \frac{D_f\ (AUC)_{nf}}{D_{nf}\ (AUC)_f}$.

Table 78

Relative Bioavailability Between Fasting and Nonfasting Conditions for Cephalexin, Cephradine, and Cefaclor Estimated by Comparison of the Total Area Under the Plasma Concentration *versus* Time Curve with Correction for Changing Renal Clearance

Subject	F_{nf}/F_f[b] Cephalexin	Cephradine	Cefaclor
1	0.87	0.89	0.97
2	0.74	0.81	1.12
3	0.83	1.05	0.74
4	0.87	1.02	0.98
5	0.93	0.91	0.78
6	1.17	1.12	0.81
7	0.79	1.03	1.25
8	0.85	1.01	1.02
Mean	0.88	0.98	0.96
SD	0.13	0.10	0.18
CV%	14.8	10.2	18.8

[b]Calculated from the equation,

$$\frac{F_{nf}}{F_f} = \frac{D_f(AUC)_{nf}}{D_{nf}(AUC)_f} - (Cl_{renal\ f} - Cl_{renal\ nf})\frac{AUC_{nf}}{D_{nf}}.$$

Table 79

Relative Bioavailability Between Fasting and Nonfasting Conditions for Cephalexin, Cephradine, and Cefaclor Estimated by Comparison of the Total Amount of Drug Excreted in Urine

Subject	F_{nf}/F_f[c] Cephalexin	Cephradine	Cefaclor
1	0.87	0.85	1.07
2	0.73	0.81	0.80
3	0.83	0.96	0.74
4	0.87	1.02	0.86
5	0.93	0.94	0.77
6	1.24	1.16	0.88
7	0.78	1.06	1.44
8	0.65	1.01	0.84
Mean	0.86	0.98	0.93
SD	0.18	0.11	0.23
CV%	20.9	11.2	24.7

[c]Calculated from the equation, $\frac{F_{nf}}{F_f} = \frac{D_f(A_e)_{nf}}{D_{nf}(A_e)_f}$.

Table 80

Relative Bioavailability Between Fasting and Nonfasting Conditions for Cephalexin, Cephradine, and Cefaclor Estimated by Comparison of the Cumulative Relative Fraction Absorbed

Subject	F_{nf}/F_f[d]		
	Cephalexin	Cephradine	Cefaclor
1	0.85	1.07	0.70
2	0.91	0.81	1.82
3	0.85	1.31	0.79
4	1.01	0.74	1.60
5	0.98	0.73	0.75
6	0.96	0.85	0.61
7	0.82	0.81	1.09
8	1.13	0.98	1.11
Mean	0.94	0.91	1.06
SD	0.10	0.20	0.44
CV%	10.6	22.0	41.5

[d]Calculated from the equation, $\frac{F_{nf}}{F_f} = \frac{C_{nf}^{8h} + k_{el}(AUC)_{nf}^{8h}}{k_{el}(AUC)_f^{8h}}$.

Table 81

Cumulative Percent of Cephalexin Absorbed at Various Times Following Single 500 mg Oral Dose Under Fasting Condition

Subject	Cumulative percent of cephalexin absorbed (%)										
	0.0 h	0.25 h	0.5 h	0.75 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h	4.0 h	6.0 h
1	0.0	12.8	50.1	ND[a]	100	100	100	100	100	100	100
2	0.0	0.0	24.8	71.4	123	88.3	90.5	92.7	96.5	100	100
3	0.0	0.0	97.9	ND	96.5	92.7	95.0	97.0	100	100	100
4	0.0	0.0	30.5	98.1	100	100	100	100	100	100	100
5	0.0	0.0	0.0	31.9	55.5	64.2	71.3	89.5	100	100	100
6	0.0	12.2	63.8	94.2	77.9	89.5	89.6	90.5	100	100	100
7	0.0	0.0	33.5	ND	75.0	83.5	84.6	90.0	94.6	100	100
8	0.0	0.0	29.5	64.1	76.6	87.7	92.0	100	100	100	100
Mean	0.0	3.1	41.3	71.9	88.1	88.2	90.4	95.0	98.9	100	100
SD	0.0	5.8	29.5	26.7	20.8	11.3	9.3	4.8	2.1	0.0	0.0
CV%	0.0	187	71.4	37.1	23.6	12.8	10.3	5.1	2.1	0.0	0.0

[a]Not determined.

Table 82

Cumulative Relative Percent of Cephalexin Absorbed at Various Times Following Single 500 mg Oral Dose Under Nonfasting Condition

	Cumulative relative percent of cephalexin absorbed (%)										
Subject	0.0 h	0.25 h	0.5 h	0.75 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h	4.0 h	6.0 h
1	0.0	0.0	0.0	ND	29.8	42.3	63.3	77.5	84.6	84.6	84.6
2	0.0	0.0	0.0	ND	0.0	14.0	24.9	43.9	63.5	81.2	90.8
3	0.0	0.0	0.0	0.0	0.0	0.0	34.3	76.4	74.5	85.4	85.4
4	0.0	0.0	0.0	7.9	21.3	43.6	60.5	73.5	85.6	101	101
5	0.0	0.0	0.0	0.0	0.0	12.7	42.1	55.1	76.2	97.6	97.6
6	0.0	0.0	0.0	0.0	13.3	47.0	62.8	72.6	79.1	96.3	96.3
7	0.0	0.0	4.0	ND	24.3	48.9	62.2	67.6	74.4	81.6	81.6
8	0.0	0.0	0.0	ND	12.7	43.9	51.2	57.7	94.1	113	113
Mean	0.0	0.0	0.5	2.0	12.7	31.6	50.2	65.5	79.0	92.6	93.8
SD	0.0	0.0	1.4	4.0	11.9	19.3	14.8	12.1	9.2	11.3	10.4
CV%	0.0	0.0	280	200	93.7	61.1	29.5	18.5	11.6	12.2	11.1

Table 83

Cumulative Percent of Cephradine Absorbed at Various Times Following Single 500 mg Oral Dose Under Fasting Condition

	Cumulative percent of cephradine absorbed (%)										
Subject	0.0 h	0.25 h	0.5 h	0.75 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h	4.0 h	6.0 h
1	0.0	0.0	14.3	40.9	71.6	100	100	100	100	100	100
2	0.0	0.0	53.9	100	100	100	100	100	100	100	100
3	0.0	0.0	44.1	73.1	97.2	107	95.1	88.4	94.8	100	100
4	0.0	0.0	20.2	ND	100	100	100	100	100	100	100
5	0.0	0.0	0.0	0.0	26.6	56.7	65.2	90.0	100	100	100
6	0.0	0.0	17.1	ND	87.3	100	100	100	100	100	100
7	0.0	0.0	37.4	87.0	100	100	100	100	100	100	100
8	0.0	0.0	10.3	50.1	100	100	100	100	100	100	100
Mean	0.0	0.0	24.7	58.5	85.3	95.5	95.0	97.4	99.4	100	100
SD	0.0	0.0	18.5	36.2	25.8	15.9	12.2	4.8	1.8	0.0	0.0
CV%	0.0	0.0	74.9	61.9	30.2	16.6	12.8	4.9	1.8	0.0	0.0

Table 84

Cumulative Relative Percent of Cephradine Absorbed at Various Times Following Single 500 mg Oral Dose under Nonfasting Condition

	Cumulative relative percent of cephradine absorbed (%)										
Subject	0.0 h	0.25 h	0.5 h	0.75 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h	4.0 h	6.0 h
1	0.0	0.0	0.0	6.0	12.7	33.4	57.4	108	109	107	107
2	0.0	0.0	0.0	0.0	3.4	16.2	39.4	53.2	89.5	80.7	80.7
3	0.0	0.0	0.0	ND	8.8	12.9	49.8	78.8	117	116	131
4	0.0	0.0	0.0	0.0	17.3	26.1	36.3	51.1	62.9	74.1	74.1
5	0.0	0.0	0.0	ND	5.0	12.7	21.4	26.8	46.0	66.5	72.7
6	0.0	0.0	4.8	36.2	46.2	84.6	89.1	94.7	87.6	84.7	84.7
7	0.0	0.0	0.0	0.0	0.0	16.3	35.0	81.8	78.6	80.6	80.6
8	0.0	0.0	0.0	0.0	0.0	9.6	20.0	33.6	65.4	97.8	97.8
Mean	0.0	0.0	0.6	7.0	11.7	26.5	43.6	66.0	82.0	88.4	91.1
SD	0.0	0.0	1.7	14.5	15.2	24.8	22.3	29.2	23.9	17.0	19.9
CV%	0.0	0.0	283	207	130	93.6	51.1	44.2	29.1	19.2	21.8

Table 85

Cumulative Percent of Cefaclor Absorbed at Various Times Following Single 500 mg Oral Dose Under Fasting Condition

	Cumulative percent of cefaclor absorbed (%)										
Subject	0.0 h	0.25 h	0.5 h	0.75 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h	4.0 h	6.0 h
1	0.0	0.0	66.0	86.3	100	100	100	100	100	100	100
2	0.0	33.9	97.3	ND	91.9	91.7	94.2	100	100	100	100
3	0.0	5.7	75.3	97.3	99.8	88.2	94.6	93.2	100	100	100
4	0.0	0.0	0.0	7.6	32.6	100	100	100	100	100	100
5	0.0	0.0	0.0	12.9	36.6	76.2	100	100	100	100	100
6	0.0	0.0	64.8	ND	101	95.4	95.7	96.0	100	100	100
7	0.0	0.0	11.5	89.7	100	100	100	100	100	100	100
8	0.0	0.0	16.5	ND	100	100	100	100	100	100	100
Mean	0.0	4.9	41.4	58.8	82.7	93.9	98.1	98.7	100	100	100
SD	0.0	11.9	38.5	44.5	29.9	8.5	2.7	2.6	0.0	0.0	0.0
CV%	0.0	243	93.0	75.7	36.2	9.1	2.8	2.6	0.0	0.0	0.0

Table 86

Cumulative Relative Percent of Cefaclor Absorbed at Various Times Following Single 500 mg Oral Dose Under Nonfasting Condition

	Cumulative relative percent of cefaclor absorbed (%)										
Subject	0.0 h	0.25 h	0.5 h	0.75 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h	4.0 h	6.0 h
1	0.0	0.0	0.0	17.2	42.6	49.2	46.5	65.5	63.7	69.8	69.8
2	0.0	0.0	12.7	27.9	70.6	109	121	135	145	167	182
3	0.0	0.0	0.0	14.2	21.6	38.4	43.1	63.1	70.8	79.4	79.4
4	0.0	0.0	0.0	ND	25.6	115	149	149	168	160	160
5	0.0	0.0	9.1	ND	15.0	37.5	43.5	52.6	61.6	74.9	74.9
6	0.0	0.0	0.0	0.0	11.1	30.0	52.2	57.9	60.9	61.0	61.0
7	0.0	0.0	0.0	0.0	22.3	93.1	126	118	109	109	109
8	0.0	0.0	0.0	16.9	23.1	39.5	62.4	73.2	85.9	111	111
Mean	0.0	0.0	2.7	12.7	29.0	64.0	80.5	89.3	95.6	104	106
SD	0.0	0.0	5.1	10.9	19.2	35.5	43.8	38.4	41.3	40.8	44.3
CV%	0.0	0.0	189	85.8	66.2	55.5	54.4	43.0	43.2	39.2	41.8

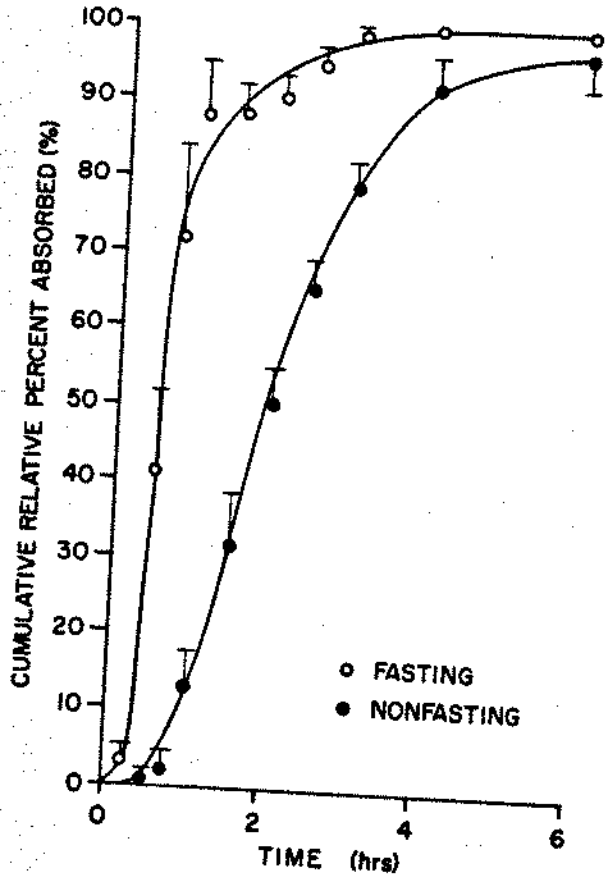


Figure 35. Mean cumulative relative percent of cephalexin absorbed (±1 SE) as a function of time following single 500 mg oral dose under fasting and nonfasting conditions.

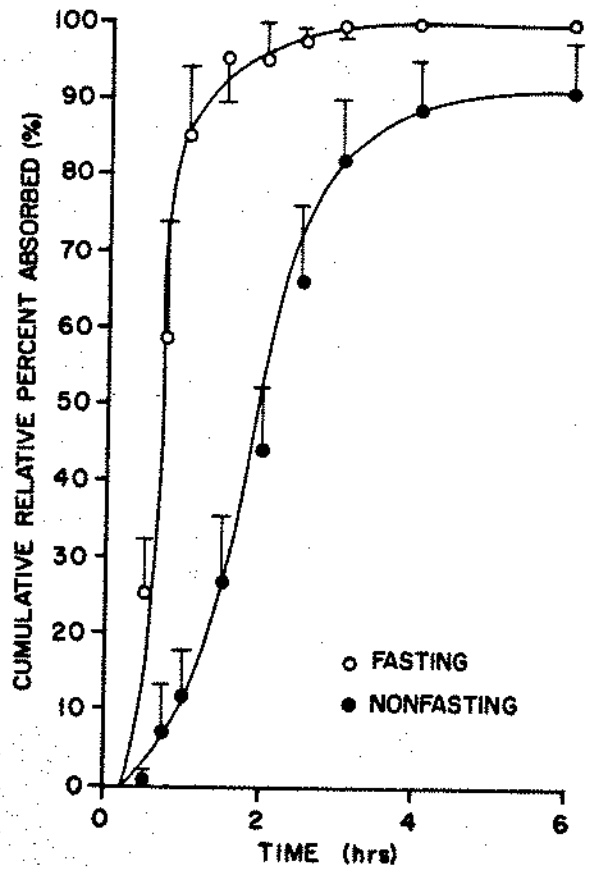


Figure 36. Mean cumulative relative percent of cephradine absorbed (±1 SE) as a function of time following single 500 mg oral dose under fasting and nonfasting conditions.

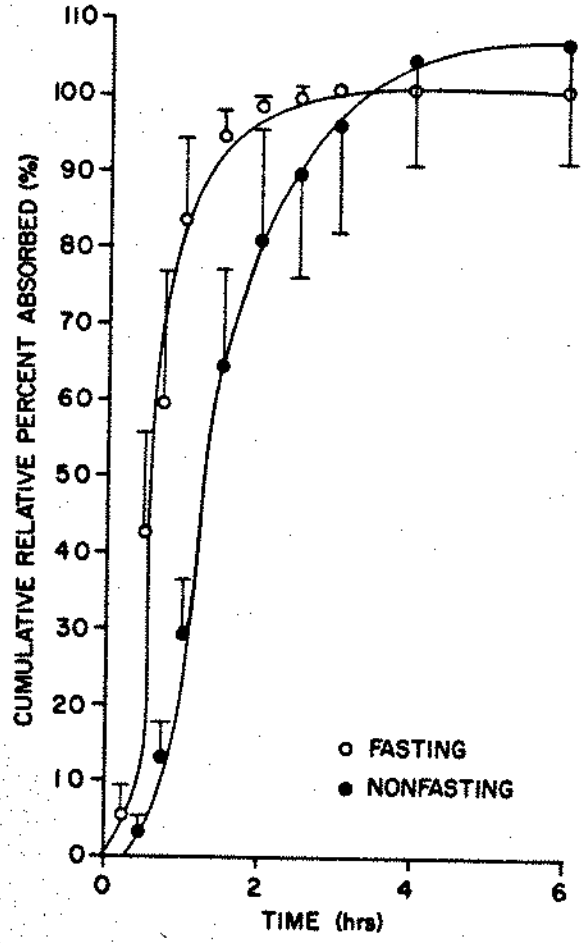


Figure 37. Mean cumulative relative percent of cefaclor absorbed (±1 SE) as a function of time following single 500 mg oral dose under fasting and nonfasting conditions.

Table 87

Summary of Mean Values (±1 SD) of Cumulative Relative Percent of Cephalexin, Cephradine, and Cefaclor Absorbed at Various Times Following Single 500 mg Oral Dose; and Results of Statistical Comparison Between Treatments[a]

	Cumulative relative percent absorbed (%)						
Time	Cephalexin (fasting) A	Cephalexin (nonfasting) B	Cephradine (fasting) C	Cephradine (nonfasting) D	Cefaclor (fasting) E	Cefaclor (nonfasting) F	Statistical comparison
0.0 h	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)	NSD
0.25 h	3.1 (±5.8)	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)	4.9 (±11.9)	0.0 (±0.0)	NSD
0.5 h	41.3 (±29.5)	0.5 (±1.4)	24.7 (±18.5)	0.6 (±1.7)	41.4 (±38.5)	2.7 (±5.1)	A,E > B,D,F
0.75 h	71.9 (±26.7)	2.0 (±4.0)	58.5 (±36.2)	7.0 (±14.5)	58.8 (±44.5)	12.7 (±10.9)	A,C,E > B,D,F
1.0 h	88.1 (±20.8)	12.7 (±11.9)	85.3 (±25.8)	11.7 (±15.2)	82.7 (±29.9)	29.0 (±19.2)	A,C,E > B,D,F
1.5 h	88.2 (±11.3)	31.6 (±19.3)	95.5 (±15.9)	26.5 (±24.8)	93.9 (±8.5)	64.0 (±35.5)	A,C,E,F > B,D C,E > F

2.0 h	90.4 (±9.3)	50.2 (±14.8)	95.0 (±12.2)	43.6 (±22.3)	98.1 (±2.7)	80.5 (±43.8)	A,F,E > B,D F > D
2.5 h	95.0 (±4.8)	65.5 (±12.1)	97.4 (±4.8)	66.0 (±29.2)	98.7 (±2.6)	89.3 (±38.4)	C,E > B,D
3.0 h	98.9 (±2.1)	79.0 (±9.2)	99.4 (±1.8)	82.0 (±23.9)	100 (±0.0)	95.6 (±41.3)	NSD
4.0 h	100 (±0.0)	92.6 (±11.3)	100 (±0.0)	88.4 (±17.0)	100 (±0.0)	104 (±40.8)	NSD
6.0 h	100 (±0.0)	93.8 (±10.4)	100 (±0.0)	91.1 (±19.9)	100 (±0.0)	106 (±44.3)	NSD

[a]Statistical comparisons were carried out as in Table 25. The subject factor had a significant effect on the following time periods, 0.5 h, 1.0 h, and 1.5 h.

Table 88

Summary of Relative Bioavailability (±1 SD) between Fasting and Nonfasting Conditions for Cephalexin, Cephradine, and Cefaclor Estimated from Four Different Methods; and Results of Statistical Comparison

	F_{nf}/F_f			
	Cephalexin	Cephradine	Cefaclor	Statistical comparison
Method I[a]	0.98 (±0.13)	0.94 (±0.20)	1.11 (±0.48)	NSD[e]
Method II[b]	0.88 (±0.13)	0.98 (±0.10)	0.96 (±0.18)	NSD
Method III[c]	0.86 (±0.18)	0.98 (±0.11)	0.93 (±0.23)	NSD
Method IV[d]	0.94 (±0.10)	0.91 (±0.20)	1.06 (±0.44)	NSD

[a]Estimated by comparison of the total area under the plasma concentration versus time curve.

[b]Estimated by comparison of the total area under the plasma concentration versus time curve with correction for changing renal clearance.

[c]Estimated by comparison of the total amounts of drug excreted in urine.

[d]Estimated by comparison of the cumulative relative fraction absorbed.

[e]No significant differences due to subjects, methods, and drugs by analysis of variance.

8. Statistical Moments

Individual values of the mean residence times calculated by the three methods (Equations 10, 12, 13) for cephalexin, cephradine, and cefaclor under fasting and nonfasting treatments are tabulated in Tables 89-91. The mean values and the results of statistical comparisons are summarized in Table 92.

Under the fasting treatments, the mean residence times calculated by method I were 1.65, 1.64, and 1.45 hr for cephalexin, cephradine, and cefaclor, respectively. Under the nonfasting treatments, the mean residence times were uniformly prolonged to 2.9, 2.85, and 2.38 hr for cephalexin, cephradine, and cefaclor, respectively. Statistical comparisons showed that the mean residence times were significantly lengthened following the nonfasting treatments for all three cephalosporins. The MRT values of cephalexin and cephradine were significantly larger than cefaclor following the nonfasting treatment. The values of the mean residence time calculated by three different methods were not significantly different from each other within each treatment. However, methods II and III tend to give more variability in the data.

Individual values of the variances of the mean residence time are given in Table 93. Statistical comparisons did not show any significant difference among all treatments. It has been pointed out by Gibaldi et al. (96) and Riegelman et al. (90) that numerical estimation of the VRT values might lead to an unacceptable level of computational error. Therefore, VRT values will not be used for data interpretation in the present study.

Table 89

Values of Mean Residence Times Calculated from Three Different Methods for Cephalexin Following Single 500 mg Oral Dose Under Fasting and Nonfasting Conditions

	Mean residence times for cephalexin (h)					
	Fasting			Nonfasting		
Subject	Method[a] I	Method[b] II	Method[c] III	Method I	Method II	Method III
1	1.39	1.75	1.53	2.41	2.47	2.47
2	1.74	1.59	1.29	3.58	3.90	2.26
3	1.35	1.67	1.43	3.05	1.98	3.19
4	1.36	1.32	1.43	2.67	3.22	3.37
5	2.39	4.15	1.90	3.66	3.93	3.01
6	1.74	1.68	1.71	2.73	2.40	3.71
7	1.65	1.15	1.35	2.21	1.53	2.42
8	1.61	1.61	2.41	2.89	4.99	3.73
Mean	1.65	1.87	1.63	2.90	3.05	3.02
SD	0.34	0.95	0.37	0.52	1.17	0.58
CV%	20.6	50.8	22.7	17.9	38.4	19.2

[a]Calculated from the equation, $MRT = \frac{\int_0^\infty t\,Cdt}{\int_0^\infty Cdt}$, based on an non-compartmental approach.

[b]Calculated from the equation, $MRT = 1/k_a + 1/k_{el}$, based on the assumption that the drug follows one-compartment model with first-order absorption and elimination.

[c]Calculated from the equation, $MRT = T/2 + 1/k_{el}$, based on the assumption that the drug follows one compartment model with zero-order absorption and first-order elimination.

Table 90

Values of Mean Residence Times Calculated from Three Different Methods for Cephradine Following Single 500 mg Oral Dose Under Fasting and Nonfasting Conditions

	Mean residence times for cephradine (h)					
	Fasting			Nonfasting		
Subject	Method I	Method II	Method III	Method I	Method II	Method III
1	1.54	1.64	1.42	2.51	1.64	2.26
2	1.58	1.33	1.30	2.83	2.22	4.08
3	1.56	1.32	1.46	3.21	1.65	2.52
4	1.29	1.23	1.29	2.66	2.88	2.98
5	2.67	2.42	2.65	3.72	4.05	3.94
6	1.69	1.63	1.71	1.81	1.50	1.83
7	1.38	1.41	1.57	2.80	2.86	4.73
8	1.38	1.33	1.18	3.29	4.21	5.08[d]
Mean	1.64	1.54	1.57	2.85	2.63	3.19
SD	0.44	0.39	0.47	0.58	1.07	1.07
CV%	26.8	25.3	29.9	20.4	40.7	33.5

[d]Value not included in the calculation of the mean.

Table 91

Values of Mean Residence Times Calculated from Three Different Methods for Cefaclor Following Single 500 mg Oral Dose Under Fasting and Nonfasting Conditions

	Mean residence times for cefaclor (h)					
	Fasting			Nonfasting		
Subject	Method I	Method II	Method III	Method I	Method II	Method III
1	1.34	1.18	1.32	2.20	1.56	2.64
2	1.06	1.05	0.98	2.45	2.27	2.93
3	1.36	1.28	1.12	2.48	3.01	2.25
4	1.95	2.22	2.09	2.36	1.61	2.95
5	1.96	2.22	2.13	2.69	4.26	1.98
6	1.32	0.95	1.30	2.21	1.80	2.14
7	1.45	1.59	1.77	1.99	1.67	2.37
8	1.14	1.12	1.00	2.64	3.28	8.41[d]
Mean	1.45	1.45	1.46	2.38	2.43	2.47
SD	0.34	0.51	0.47	0.24	0.99	0.38
CV%	23.4	35.2	32.2	10.1	40.7	15.4

Table 92

Summary of Mean Values (± 1 SD) of Mean Residence Times Calculated from Three Different Methods for Cephalexin, Cephradine, and Cefaclor Under Fasting and Nonfasting Conditions; and Results of Statistical Comparison Between Different Methods of Calculating Mean Residence Time[a]

	MRT (h)			
	Method I	Method II	Method III	Statistical comparison
Cephalexin (fasting)	1.65 (±0.34)	1.87 (±0.95)	1.63 (±0.37)	NSD
Cephalexin (nonfasting)	2.90 (±0.52)	3.05 (±1.17)	3.02 (±0.58)	NSD
Cephradine (fasting)	1.64 (±0.44)	1.54 (±0.39)	1.57 (±0.47)	NSD
Cephradine (nonfasting)	2.85 (±0.58)	2.63 (±1.07)	3.19 (±1.07)	NSD
Cefaclor (fasting)	1.45 (±0.34)	1.45 (±0.51)	1.46 (±0.47)	NSD
Cefaclor (nonfasting)	2.38 (±0.24)	2.43 (±0.99)	2.47 (±0.38)	NSD

[a]Data were analyzed by analysis of variance and differences were considered significant when $p < 0.05$. The subject factor had a significant effect on the following treatments, Cephalexin (fasting), Cephradine (fasting), Cephradine (nonfasting), and Cefaclor (fasting).

Table 93

Values of Variances of the Mean Residence Time for Cephalexin, Cephradine, and Cefaclor Following Single 500 mg Oral Dose Under Fasting and Nonfasting Conditions

	Variances of the mean residence time[a]					
	Fasting			Nonfasting		
Subject	Cephalexin	Cephradine	Cefaclor	Cephalexin	Cephradine	Cefaclor
1	0.55	0.45	0.56	0.82	0.60	1.15
2	1.12	1.09	0.63	1.55	0.57	1.91
3	0.66	1.01	1.04	0.54	1.50	1.06
4	0.50	0.31	0.42	1.03	0.99	0.73
5	1.07	1.32	0.42	1.66	1.75	1.18
6	1.36	0.76	0.83	0.98	0.59	0.57
7	1.06	0.48	0.47	0.99	0.56	0.31
8	0.61	0.44	0.16	0.95	0.60	1.21
Mean	0.87	0.73	0.57	1.07	0.90	1.02
SD	0.32	0.37	0.27	0.37	0.48	0.49
CV%	36.8	50.7	47.4	34.6	53.3	48.0

[a]Calculated from the equation, $VRT = \frac{\int_0^\infty (t - MRT)^2 Cdt}{\int_0^\infty Cdt}$.

IV. DISCUSSION

An attempt will be made in this discussion to integrate all of the information obtained in the above studies and to present a summary on the effect of food on the bioavailability and pharmacokinetics of oral cephalosporins. The philosophical approach of this discussion is not merely to enumerate the pharmacokinetic parameter values associated with cephalosporins, but also to provide a systematic approach for the evaluation of bioavailability data. The methods developed here may thus be applicable in studies concerning other drugs with pharmacokinetic characteristics similar to those of the cephalosporins.

A. Assay

Cephalexin, cephradine, and cefaclor can be quantified utilizing microbiological, iodometric, spectrophotometric, colorimetric, or high pressure liquid chromatographic (HPLC) procedures (7, 9, 12). The HPLC technique is a more rapid and accurate method of analysis compared to the other methods, and thus was used in the present study. The assay in this study proved to be rapid, sensitive, and specific. The procedure involved a single-step protein precipitation or simple dilution for sample preparation. The sample volume

was only 20 µl. The total analysis time after sample preparation was approximately 15 min per sample. The sensitivity was adequate, and the reproducibility was excellent with coefficients of variation less than 10%. It can be concluded that the HPLC methods described in this study are good for routine therapeutic drug monitoring or pharmacokinetic studies of cephalosporins in terms of reliability, sensitivity, specificity, and simplicity.

B. Stability Study of Cefaclor

The results of the stability study are in general agreement with the findings reported by Foglesong et al. (13). The stability of cefaclor is pH and temperature dependent. Cefaclor is markedly more stable under acidic conditions than at neutral pH. It is relatively unstable in human plasma and urine in vitro. Temperature of course influenced the stability of cefaclor, and greater stability can be achieved at lower temperatures.

Figure 15 shows that approximately 50% degradation occurred when plasma samples were stored at room temperature for 7.5 hr. However, only 2.3% of degradation occurred when the same samples were stored in a freezer. Although urine samples are more stable than plasma samples, a similar stability pattern is observed. Cefaclor urine samples are more stable at lower temperatures than at room temperature (Figure 16). These results thus show that plasma and urine samples of cefaclor should be stored immediately in a refrigerator or freezer upon collection in order to minimize degradation.

This study did not examine the stability pattern of cefaclor in plasma at 37°C because it is thought that chemical degradation in vivo at 37°C is insignificant. A review of the literature has shown that the major site for elimination of cefaclor is the kidney, which is well perfused with blood, and therefore rapidly accessible to drug in the systemic circulation for elimination. It has also been established that oral cephalosporins, including

cefaclor, have short elimination half-lives. Since renal clearance occurs at a much faster rate than chemical degradation, it is reasonable to assume that most of the drug in plasma will be eliminated by the kidney before it can undergo chemical degradation *in vivo*. In light of the rapid clearance of cefaclor by the kidney, chemical degradation *in vivo* at 37°C will be unimportant, and therefore chemical degradation was not examined at 37°C *in vitro*.

The intent of the stability study was to examine the stability characteristics of cefaclor in order to outline procedures for the proper handling and storage of clinical samples of cefaclor. In summary, cefaclor is most stable when prepared in a pH 4.5 buffer solution and stored in a refrigerator. Therefore, for analytical work, cefaclor stock solution should be prepared fresh daily in a pH 4.5 buffer. When dealing with plasma samples, which do not possess the desired acidic pH, the optimal stability of samples can be obtained by keeping samples in a refrigerator and assaying the samples on the same day they are collected. Samples that are to be assayed at a later time should be deep-frozen. Urine samples should be diluted with a pH 4.5 buffer solution upon collection. Samples should be kept in a refrigerator and assayed on the same day they are collected. Samples that are to be assayed at a later time should be frozen. These procedures will enhance the *in vitro* stability of cefaclor in biological fluids, and hence permit proper evaluation of clinical samples.

C. Plasma Concentration and Urinary Excretion Data

The plasma profiles and urinary excretion of cephalexin, cephradine, and cefaclor observed in this study are consistent with results obtained previously when these compounds were studied separately, or in pairs (37, 46, 53, 58). Although the three compounds are rapidly and efficiently absorbed following oral doses, plasma concentrations and urinary excretion of cefaclor tend to be lower than those of cephradine and cephalexin. The peak concentrations, times to peak concentrations, areas under the plasma concentration-time curves, and cumulative percent urinary recovery for all three cephalosporins following oral doses under fasting conditions from various studies are compared with values obtained in this present investigation in Table 94. The values obtained following the nonfasting treatments are not used for comparison because of inconsistencies among various studies. Although Table 94 shows a scattering in the data and differences among laboratories, the values obtained in this present investigation appear to be reasonably consistent with others.

This study shows that similar plasma drug profiles were obtained following cephalexin and cephradine doses to fasted subjects, with mean C_{max} values of 16.8-19.3 mcg/ml occurring at approximately 1 hr. Under the same conditions, cefaclor yielded a mean C_{max} of 10.3 mcg/ml, occurring also at 1 hr. After postprandial doses, C_{max} values of all three cephalosporins were reduced and t_{max} values were increased. However, statistical

comparison cannot discern any significant difference in the C_{max} and t_{max} values for cefaclor between the fasting and nonfasting conditions. Areas under the plasma concentration-time curves were almost identical in fasting and nonfasting treatments for all three compounds, with the AUC values of cefaclor being approximately one-half those of the other two compounds.

As with the plasma concentrations, urinary excretion of all three compounds was delayed when they were taken after a standard breakfast. This was most evident in the 2 hr urine collection interval. Interestingly, significant difference between the fasting and nonfasting treatments was not observed with cefaclor at the same time interval. The total 12 hr urinary recovery of cephalexin, cephradine, and cefaclor following fasting treatments was 88.5%, 89.1%, and 73.7%, respectively, of the administered dose. The total 12 hr urinary recovery was only slightly, not significantly, reduced by food for all three compounds. However, cefaclor yielded a significantly lower total urinary recovery under the nonfasting treatment compared to cephradine and cephalexin.

Bioavailability studies usually examine parameters such as peak concentrations, times to peak concentrations, areas under the plasma concentration-time curves, and total urinary recovery for significant differences in the rate and extent of drug absorption. With these parameters as criteria, this study shows that administration of food immediately before oral cephalexin, cephradine, and

cefaclor definitely altered the plasma concentration profiles by lowering the peak concentrations and delaying the times to peak concentration. Food apparently interfered with the rate of absorption of cephalexin, cephradine, and cefaclor, but did not affect the total amount absorbed, since the area under the plasma concentration-time curves were almost identical following fasting and nonfasting treatments. Similar urinary recovery between fasting and nonfasting treatments further verified that the extent of absorption is not affected by food. The effect of food on the circulating antibiotic concentrations was remarkably consistent for cephalexin and cephradine. However, the presence of food tends to affect the rate of absorption for cefaclor to a lesser extent. Although food delays the absorption of cefaclor and lowers its peak concentration, the differences between the fasting and nonfasting treatments are not statistically significant. A review of the literature shows that there is no other comparative study regarding the effect of food on the absorption of these three cephalosporins. However, our finding of delayed absorption confirms the observation of several other investigations when these compounds were studied separately (42, 54, 60).

The observed delay in cephalosporin absorption is probably due to a decreased stomach emptying rate and/or a delayed dissolution process brought about by the ingestion of food. The three compounds examined are stable in solution at acidic pH, and no degradation of drug occurred as a result of prolonged residence in the stomach.

Hence, the extent of absorption is not affected for the three compounds.

The observed changes in plasma and urinary profiles after single doses are likely to occur also after multiple doses as very little drug accumulation would occur with a 6 hr dosage interval, whether the drugs are administered on an empty stomach or postprandially. The clinical significance of the observed changes in plasma profiles is uncertain. Following the single doses used in this study, food lowered C_{max} values to an extent that might be important for less sensitive organisms. Comparative clinical studies are necessary for a conclusive assessment.

Table 94

Maximum Plasma Concentrations, Times to Maximum Plasma Concentrations, Areas Under Plasma Concentration-Time Curves, and Cumulative Percent Urinary Recovery Following 500 mg Oral Doses of Cephalexin, Cephradine, and Cefaclor Under Fasting Conditions

Drug	Dose	C_{max} (mcg/ml)	t_{max} (h)	AUC (mcg h/ml)	Cumulative percent urinary recovery (%)	Reference
Cephalexin	500	16.8 (±5.9)	0.88 (±0.19)	24.9 (±5.0)	88.5 (±14.0) in 12h	Present Study
	500	14.2 (±3.2)	1.3 (±0.6)	32.0 (±5.8)	85.2 (±9.3) in 6 h	58
	500	20.7 (±1.5)	0.71 (±0.06)	29.0 (±2.5)	-[b]	53
	500	17.9[c]	0.43	33.4	-	35
	500	17.3	1.4	31.8	-	22
	500	18.7 (±1.0)	1.0	-	88.8 in 24 h	37
	2x250	18.9 (±4.7)	0.75-1.0	-	96.3 in 6 h	46
	1/2x1000[a]	15.3 (±1.8)	1.0 (±0.2)	26.4 (±4.7)	90.6 (±8.7) in 6 h	57
	1/2x1000[a]	19.4 (±4.1)	0.93 (±0.36)	-	91.2 (±18.5) in 24 h	54
Cephradine	500	19.4 (±6.5)	1.3 (±0.74)	26.1 (±5.1)	89.1 (±8.8) in 12 h	Present Study
	500	13.2 (±1.4)	1.3 (±0.6)	27.4 (±3.5)	86.3 (±14.9) in 6 h	58
	500	17.7 (±1.2)	0.80 (±0.08)	27.5 (±2.2)	-	53

	500	11.0 (±1.25)	1.0	-	92.1 in 24 h	38
	5[illegible]	18.3 (±2.0)	1.0	-	88.6 in 24 h	37
	500	19.8	0.8	28	100 in 24 h	42
	1/2x1000[a]	16.4 (±4.0)	1.2 (±0.37)	30.9 (±5.6)	85.5 (±9.5) in 6 h	57
Cefaclor	500	10.3 (±2.7)	0.97 (±0.53)	13.0 (±3.6)	73.7 (±15.1) in 12 h	Present Study
	500	9.9 (±1.4)	1.0 (±0.3)	16.1 (±2.5)	53.8 (±14.8) in 6 h	58
	500	10.2 (±4.8)	1.0	-	72 in 6 h	60
	500	12.5	1.0	-	42 (±27) in 8 h	44
	2x250[a]	12.0 (±1.1)	1.0	-	70.1 in 6 h	46
	1/2x1000[a]	17.3 (±3.9)	1.0	-	52.7 (±6.9) in 24 h	54

[a] C_{max} and AUC values adjusted to a dose of 500 mg.

[b] Value not reported in the study.

[c] Standard deviation not reported in the study.

D. Pharmacokinetic Models

Previous reports in the literature concerning the pharmacokinetics of cephalosporins have used both the one-compartment model (20-22, 26, 28, 35, 57-59) and two-compartment model (24, 97, 98) to describe the time course of drug in the body. The absorption of oral cephalosporins has frequently been described by first-order kinetics. However, a recent report on the pharmacokinetics of orally administered cephradine in man suggests that absorption is best described by a zero-order process (98). Owing to the diverse reports with regard to the pharmacokinetic characterization of oral cephalosporins, individual plasma profiles of all subjects were examined in order to determine the most appropriate method of data analysis.

1. One-Compartment Model with First-Order Absorption and Elimination

Individual plasma drug concentrations were plotted versus time on semilogarithmic paper. In most cases, linearity was observed during the elimination phase. In some cases, the later sampling points were disregarded in the construction of the plot due to the questionable accuracy of assay at such low concentrations. When this was taken into consideration, monoexponential elimination phases were obtained in all subjects, indicating that the simplest model that adequately describes the plasma concentration profile is possibly a one-compartment model with first-order absorption and

elimination as characterized by Equation 2. As a result, individual plasma data sets were fitted to Equation 2. The high coefficients of determination obtained by fitting plasma data to this equation indicate that plasma profiles can be adequately described by this model. The elimination rate constants (k_{el}) obtained were 0.91 ($\pm$0.35), 0.53 ($\pm$0.11), 1.07 ($\pm$0.26), 0.87 ($\pm$0.38), 1.19 ($\pm$0.34), and 0.68 ($\pm$0.48) h^{-1} for cephalexin (fasting), cephalexin (nonfasting), cephradine (fasting), cephradine (nonfasting), cefaclor (fasting), and cefaclor (nonfasting), respectively. These elimination rate constants are consistent with values reported in the literature utilizing either the one-compartment or two-compartment model. However, it is of interest to note that the elimination rate constants are uniformly smaller in the nonfasting than the fasting treatments for all three compounds. Similarly, the quantity FD/V is smaller in the nonfasting than the fasting treatments for all three cephalosporins. These observations lead to further inquiry into the validity of using Equation 2 to analyze the plasma concentration data in the nonfasting treatments.

In a one-compartment model with first-order absorption and elimination, the determination of the absorption and elimination rate constants using only plasma concentration data depends on the magnitude of the difference between k_a and k_{el}. When $k_a >> k_{el}$, k_{el} can be readily determined from the slope of the terminal phase of the log C versus time plot as in Equation 2b. This approach allows estimation of k_{el} for all three cephalosporins following the

fasting treatments.

However, following the nonfasting treatments, absorption may be relatively slow compared to elimination, and the absorption rate constant may be either identical to or smaller than the elimination rate constant. Thus, the plasma data for the nonfasting treatments were re-examined both under the assumption that $k_a = k_{el} = K$, and also that $k_{el} >> k_a$.

In the first case, k_a is assumed to be equal or close to k_{el}, thus absorption continues throughout the elimination phase. This would cause the slope of the terminal portion of the plasma concentration-time curve to represent a combination of the absorption and elimination of the drug from the body. In this situation, Equation 2, which is used to characterize a one-compartment model with first-order absorption and elimination, is indeterminable mathematically and therefore, cannot be used to determine the relevant pharmacokinetic parameters.

When $k_a = k_{el} = K$, the drug concentration in the body can be characterized by Equation 17 or 18 (99),

$$C = \frac{FD}{V} K t e^{-Kt} \tag{17}$$

$$\log C = \log \frac{FD}{V} Kt - \frac{Kt}{2.303} \tag{18}$$

Equation 18 indicates that when $k_a = k_{el}$, a semilogarithmic plot of C versus time will contain no linear segments. Since the

semilogarithmic plots of plasma concentration versus time contain linear segments during the elimination phase, this observation indicates that Equation 18 may not be the appropriate equation to analyze the plasma concentration data initially. In order to ascertain this, two alternative approaches were taken. By differentiating Equation 17 with respect to time and setting $dC/dt = 0$ at t_{max}, simple relationships can be derived for the parameters t_{max} and C_{max} as in Equation 19 and 20, respectively.

$$t_{max} = \frac{1}{K} \tag{19}$$

$$C_{max} = \frac{FD}{V} e^{-1} \tag{20}$$

The product of $t_{max} \cdot C_{max}$ is equal to $AUC \cdot e^{-1}$ as shown in Equation 21.

$$t_{max} \cdot C_{max} = \frac{FD}{VK} e^{-1} = AUC \cdot e^{-1} \tag{21}$$

Thus, whenever $t_{max} \cdot C_{max}$ can be shown to be equal to $AUC \cdot e^{-1}$, the rate constants must be equal and Equation 17 should be used to describe the plasma data.

Both Equations 19 and 21 were used to re-analyze the plasma data following the nonfasting treatments. The elimination rate constants calculated using Equation 19 for cephalexin, cephradine, and cefaclor following the nonfasting treatments are shown in

Table 95. The elimination rate constants were 0.45 (±0.12), 0.39 (±0.12), and 0.61 (±0.19) h^{-1} for cephalexin, cephradine, and cefaclor, respectively. Again, these values are smaller than those obtained using Equation 2 for the three cephalosporins under the fasting treatments, which show that Equation 19 does not give an accurate estimate of k_{el}.

The quantities $C_{max} \cdot t_{max}$ and $AUC \cdot e^{-1}$ for all three cephalosporins following the nonfasting treatments are tabulated in Table 96. Table 96 shows that $C_{max} \cdot t_{max}$ is not equal or close to $AUC \cdot e^{-1}$ for all three cephalosporins. This further substantiates that k_a is not equal or close to k_{el} for the nonfasting treatments.

In the second case, k_{el} is assumed to be greater than k_a. Under this situation, the time course of drug concentration in plasma can be described by Equation 2, but the slope of the linear segment of the semilogarithmic plot of concentration versus time is equal to $-k_a/2.303$ rather than $-k_{el}/2.303$. This 'flip-flop' phenomenon has been described previously in Section III of this thesis. This approach was thought to be appropriate because absorption might be slow in the presence of food compared to the rapid elimination of cephalosporins. The results of the analysis confirm the existence of this 'flip-flop' phenomenon following the nonfasting treatments. The elimination rate constants obtained, 0.89 h^{-1} for cephalexin, 1.10 h^{-1} for cephradine, and 1.04 h^{-1} for cefaclor, were not significantly different from those obtained following the fasting treatments for all three cephalosporins. Similarly, the

Table 95

Values of Elimination Rate Constants Calculated Based on the Assumption that $k_a = k_{el}$ for Cephalexin, Cephradine, and Cefaclor Following Single 500 mg Oral Dose Under Nonfasting Condition

Subject	Elimination rate constant[a] (h^{-1})		
	Cephalexin	Cephradine	Cefaclor
1	0.5	0.4	1.0
2	0.33	0.33	0.67
3	0.4	0.33	0.4
4	0.5	0.4	0.67
5	0.33	0.25	0.67
6	0.5	0.67	0.5
7	0.67	0.4	0.5
8	0.33	0.33	0.5
Mean	0.45	0.39	0.61
SD	0.12	0.12	0.19

[a]Calculated from the equation, $K = 1/t_{max}$.

Table 96

Values of $t_{max} \cdot C_{max}$ and $AUC \cdot e^{-1}$ Calculated for Cephalexin, Cephradine, and Cefaclor Following Single 500 mg Oral Dose Under Nonfasting Condition

	Cephalexin		Cephradine		Cefaclor	
Subject	$t_{max} \cdot C_{max}$ (mcg h/ml)	AUC/e (mcg h/ml)	$t_{max} \cdot C_{max}$ (mcg h/ml)	AUC/e (mcg h/ml)	$t_{max} \cdot C_{max}$ (mcg h/ml)	AUC/e (mcg h/ml)
1	16.2	8.2	40.0	9.7	6.3	4.5
2	27.6	10.7	45.6	10.3	9.8	6.0
3	27.0	7.3	35.4	9.8	12.0	5.0
4	13.6	8.2	16.5	7.5	10.5	5.6
5	20.1	8.8	22.0	7.3	5.9	4.8
6	17.0	9.3	17.3	7.3	10.4	3.3
7	17.6	9.5	41.0	9.7	15.4	4.5
8	23.7	8.1	30.0	9.2	8.8	4.9
Mean	20.4	8.8	31.0	8.9	9.9	4.8
SD	5.2	1.1	11.3	1.3	3.1	0.8

quantities FD/V obtained for the nonfasting treatments using this approach were almost identical to those obtained following the fasting treatments. Although this 'flip-flop' phenomenon exists in a majority of the subjects under the nonfasting treatments, there are individual exceptions. Since the existence of this 'flip-flop' model depends on the extent to which the rate of drug absorption is reduced, intersubject variation is not unexpected.

In summary, the high coefficients of determination indicate that plasma profiles can be adequately described by a one-compartment model with first-order absorption and elimination. The absorption and elimination rate constants obtained, which are in general agreement with values reported in the literature, further substantiate the validity and practicality of utilizing a one-compartment model to describe the plasma concentration data for oral cephalosporins.

Although the pharmacokinetics of cephalexin and cephradine have been described by a two-compartment model following intravenous administration (24), this study shows that a one-compartment model should describe the plasma data adequately following oral administration as the rapid α-distribution phase probably would be obscured by the absorption phase. Greene et al. (97) have extensively studied the pharmacokinetics of cephalexin by one- and two-compartment model analysis and concluded that, for practical purposes, the pharmacokinetics could be described adequately by a one-compartment model. Plasma concentration profiles do not exhibit

two-compartment characteristics in this study. Furthermore, pharmacokinetic parameters obtained in this study are not appreciably different from those reported by Greene et al. (97) after extensive two-compartment analysis. Thus, it is concluded that plasma concentration data of oral cephalosporins are best described by a simple one-compartment model.

2. One-Compartment Model with Zero-Order Absorption and First-Order Elimination

The rate of appearance of drug in the systemic circulation after oral administration is frequently described by assuming simple first-order kinetics. Although the assumption of first-order absorption in pharmacokinetics is almost axiomatic, several exceptions have been reported in the literature. The absorption of ethanol (100), sulfisoxazole (101), hydroflumethiazide (102, 103), griseofulvin (104), and erythromycin (105) has been found to be best described by assuming zero-order rather than first-order kinetics. In these cases, the use of a concentration-independent zero-order absorption process was explained in terms of a rate-limiting dissolution process, low aqueous solubility of the drug, or membrane permeability-limited absorption.

Previous studies have reported analysis of cephalosporin plasma concentration curves following oral dosing with first-order absorption. However, a recent report on the pharmacokinetics of cephradine following oral doses showed that the concentration of

cephradine in plasma could be adequately described by a zero-order absorption process (98). Specifically, drug concentrations in plasma after oral administration rose to a sharp peak and quickly declined with no plateau region. Similar plasma concentration-time profiles were observed for the three cephalosporins, when they were administered after an overnight fast (Figures 17-19). As a result, individual plasma profiles were fitted to a one-compartment model with zero-order absorption and first-order elimination. This model, which can be characterized by Equations 3 and 4, has been described previously. The high coefficients of determination obtained by fitting data to the zero-order absorption model showed that absorption of oral cephalosporins can be adequately described by zero-order kinetics.

Interestingly, a comparison of the coefficients of determination shows that the zero-order absorption equation gives a significantly better fit of the plasma data for all three cephalosporins under the fasting treatments. Furthermore, there is no significant difference between the experimental and predicted C_{max} using the zero-order absorption model, which describes the peak concentrations adequately and provides excellent agreement with observed values. The sharpness with which the blood concentrations rise to a peak and immediately decline without a plateau region further substantiates zero-order absorption characteristics under the fasting treatments. Therefore, it is concluded that the zero-order absorption model is the appropriate model to describe the

absorption kinetics of oral cephalosporins following the fasting treatments in this study.

However, this study has not determined the most appropriate model to describe the circulating antibiotic levels of all three cephalosporins under the nonfasting treatments. The first-order model appears to be more appropriate for cephalexin utilizing the coefficients of determination as a criterion. However, the plasma profile for cephradine and cefaclor can be adequately described by either the zero-order or first-order absorption equation following the nonfasting treatments.

The gastrointestinal absorption of drugs is complex and involves several processes, including dissolution, absorption from different sites, and gastric emptying, that occur both simultaneously and sequentially. The apparent zero-order process could be due to a saturable and/or a carrier-mediated transport process. The possibility of saturable kinetics governing the intestinal absorption of cephalosporins in rats and the presence of a carrier-mediated mechanism have been postulated (66-73). Whether or not such mechanisms are present in man cannot be readily determined from the present data.

Although, in the present study, the oral absorption process is best described by a zero-order function under the fasting treatment for all three cephalosporins, this may not necessarily be the case. A first-order process could be present but indistinct under the fasting treatment. Owing to the complexity of the

absorption process, it is possible that absorption of oral cephalosporins can be governed by both first-order and zero-order processes. Following the fasting treatments, the rate-limiting step of absorption of oral cephalosporins is best described by zero-order kinetics. It is probable that the rate-limiting step of absorption is altered by the presence of food. As a result, plasma data can be adequately described by both first-order and zero-order models. Mechanistic characterization of the observed changes is necessary to better understand the absorption kinetics.

E. Clearance

The total clearance of drug from the body almost always involves more than one organ. By definition, systemic clearance is the sum of all individual organ clearances that contribute to the overall elimination of a drug and is equal to the ratio of overall elimination rate, dx/dt, to drug concentration, C, in plasma as in Equation 22.

$$Cl_{plasma} = \frac{\frac{dx}{dt}}{C} \qquad (22)$$

Therefore, clearance is a parameter which relates the concentration to the rate of drug elimination.

By integrating the right-hand side of Equation 22 with respect to time from t = 0 to t = ∞, Equation 23 is obtained.

$$Cl_{plasma} = \frac{\int_0^{\infty} \left(\frac{dx}{dt}\right) dt}{\int_0^{\infty} C dt} \qquad (23)$$

The term $\int_0^{\infty} (dx/dt)dt$ is equal to the total amount of drug ultimately eliminated, while the term $\int_0^{\infty} Cdt$ is equivalent to the total area under the drug plasma concentration-time curve AUC.

Therefore, clearance can be estimated in the form of Equation 14 described previously. Equation 14 is based on the assumption that the fraction of the administered dose absorbed is unity after

oral doses. This is thought to be appropriate as these oral cephalosporins are reported to be efficiently absorbed. The plasma clearances calculated using Equation 14 are consistent with values obtained in the literature (24, 47, 55, 57).

This study shows that cefaclor has a significantly larger plasma clearance than the other two cephalosporins, which indicates that the volume of plasma that is cleared of cefaclor per unit time is apparently larger than for the other two compounds. In other words, cefaclor is eliminated from the body more efficiently than are cephalexin and cephradine. This observation is not unexpected as cefaclor is relatively unstable in plasma, and can undergo chemical degradation. Thus, cefaclor is cleared by both renal and nonrenal routes simultaneously, whereas cephalexin and cephradine are cleared solely by the renal route. Therefore, it is not unexpected that cefaclor should have a larger clearance value compared to cephalexin and cephradine.

Since no metabolites have been identified in humans for these cephalosporins, renal and plasma clearance could be expected to be identical, with the exception of cefaclor. This expectation was found to be true for cephalexin and cephradine. The slightly larger plasma clearance value is probably due to an overestimation of this parameter by assuming 100% absorption of the antibiotics.

As for cefaclor, plasma clearance is significantly larger than renal clearance, which is consistent with the fact that cefaclor is

eliminated by both renal and nonrenal routes as discussed earlier.

Interestingly, renal clearance of cefaclor is also larger than cephalexin and cephradine. Since these antibiotics are cleared by both glomerular filtration and active tubular secretion in the kidney, it is possible that cefaclor may have a greater affinity for the tubular secretion mechanism, and is therefore more readily eliminated in the urine than are cephalexin and cephradine.

Although clearance of cefaclor is significantly larger than that of cephalexin and cephradine, the elimination rate constants obtained from curve fitting are not significantly different among the three cephalosporins. The value of the rate constant depends upon the values of both clearance and volume of distribution as in Equation 24.

$$k_{el} = \frac{\text{Clearance}}{V} \tag{24}$$

Even though cefaclor has a larger clearance, it appears also to have a larger volume of distribution as compared to the other two cephalosporins as discussed in the next section. The net result is that the elimination rate constant of cefaclor is not significantly different from those of cephalexin and cephradine.

F. Apparent Volume of Distribution

The apparent volume of distribution is a mathematical parameter which estimates the extent to which the body is exposed to the drug; it is a proportionality constant relating drug concentration in plasma to the amount of drug in the body.

The quantity, FD/V, generated by computer fitting earlier, suggested that cefaclor might have a larger apparent volume of distribution. The fact that cefaclor has an elimination rate constant similar to cephalexin and cephradine despite its relatively larger clearance values also suggested that cefaclor might have a larger apparent volume of distribution. Therefore, the apparent volumes of distribution were calculated by two different approaches.

A more general approach for estimating V is to use the well-developed relationship between the total area under the drug concentration versus time curve and the dose administered, as in Equation 15, and discussed earlier. This volume term is sometimes called V_{area}. It can be applied to plasma data obtained from a drug that confers upon the body the characteristics of a one-compartment model with first-order elimination. Since these oral cephalosporins may be considered to follow a simple one-compartment model with first-order elimination, this approach is thought to be appropriate.

The volume of distribution was calculated also utilizing a model-independent approach which assumes that all dispositional processes are linear, with elimination occurring from the rapidly equilibrating compartment (i.e., the plasma in this study). This

approach may also be termed an area analysis since it is based on the determination of the area under the plasma concentration-time curve and the area under the first moment of the plasma concentration-time curve as in Equation 16. This approach is thought to be advantageous because there is no need to ascribe the data to a specific compartmental model, and no sophisticated computer and nonlinear regression curve fitting procedures are required. The parameter V_{dss} obtained by this approach is also termed the apparent volume of distribution at steady state. As its name implies, V_{dss} relates the amount of drug in the body to the drug concentration in the plasma at steady state, which is independent of drug elimination.

As the body may be viewed as a single compartment with respect to the distribution and elimination kinetics of these oral cephalosporins, these two volume terms, V and V_{dss}, are expected to be equivalent.

The values of apparent volume of distribution shown in Table 76 are consistent with this expectation. The values of apparent volumes of distribution obtained were similar irrespective of the method of calculation. The values for cephalexin and cephradine are in general agreement with values reported by other investigators (24, 55, 57).

The apparent volume of distribution of cefaclor is larger than those of cephalexin and cephradine, as suspected. This observation has never been reported in the literature, although it may have

been present. In fact, the volume of distribution of cefaclor is probably being underestimated in this study because the fraction of dose (F) absorbed is calculated utilizing urinary excretion data on the assumption that the dose absorbed is excreted completely unchanged in the urine. Although this is a valid assumption for cephalexin and cephradine, this is unlikely for cefaclor. In light of the chemical degradation that occurs in plasma, the percent of intact cefaclor recovered in the urine is probably smaller than the actual amount absorbed. As a result, total urinary recovery will not give an accurate estimate of the fraction of cefaclor absorbed. Thus, it is concluded that the volume of distribution of cefaclor is underestimated in this study. A review of the literature shows that cefaclor is efficiently absorbed (47, 55, 106), and 90% absorption can be assumed. An average of 70% absorption was assumed utilizing urinary recovery data in the calculation of the volume of distribution in the present study. As a result, the volume of distribution of cefaclor is probably being underestimated by an average of approximately 28% in this study. Likewise, the low values of distribution volume of cefaclor reported in the literature may be due to similar computational errors.

It is of interest to note that when using the model-independent approach to calculate V_{dss}, unrealistic volumes of distribution were obtained in a few subjects. In light of the other values obtained in this study and those commonly reported in the literature for these oral cephalosporins, these unrealistic values of volumes

of distribution were not included in the calculation of the mean. The small values obtained may be due to inherent errors in the method used to estimate the areas, and/or due to an inaccurate estimate of the k_a values in these few subjects.

Since Equations 15 and 16 give similar estimates of the volume of distribution for cephalexin, cephradine, and cefaclor, it is concluded that for drugs that conform to a one-compartment model with first-order elimination, Equation 15 provides a simple and direct approach for estimating the apparent volume of distribution, and therefore is preferred.

G. Relative Bioavailability

Bioavailability refers to the rate and extent of drug absorption. The rate at which a drug reaches the systemic circulation is an important consideration for drugs used to treat acute conditions. On the other hand, the extent of absorption is usually the more important factor for drugs that are administered repetitively for the treatment of subchronic or chronic conditions.

Relative bioavailability refers to the comparative bioavailability of a drug from different formulations, or the same drug from different routes of administration. Bioavailability studies are often carried out in place of clinical effect studies to determine therapeutic equivalence.

In the present study, the relative bioavailability was examined for three different drugs as well as for the same drug administered under different conditions (i.e., fasting vs. nonfasting conditions).

The rate and extent of drug absorption are usually determined by C_{max}, t_{max}, AUC, and urinary excretion data in routine bioavailability studies.

The concept of utilizing AUC as the major criterion in assessing the extent, or relative extent, of drug absorption is generally well accepted. It is also well established that the total amount of drug eventually eliminated in the urine can be correlated with the amount absorbed or the extent of absorption provided a constant fraction of the available dose is eliminated

primarily unchanged in the urine, and the study is conducted long enough to allow for complete elimination (generally, 5 to 7 half-lives). Both the AUC and urinary excretion data were utilized to examine the relative extent of drug absorption in this study.

Although pharmacokinetic theory is well developed for the determination of the extent of drug absorption, the approaches for the evaluation of the rate of drug absorption still deserve further examination. It is recognized that C_{max} and t_{max} are useful descriptive parameters for evaluating the rate of drug absorption. It is thought that the characterization of absorption kinetics based on pharmacokinetic models can also provide useful insight regarding the relative differences in absorption rates. A common method of evaluating absorption kinetics is to assume that the drug concentration-time profile can be described by a pharmacokinetic compartment model and to fit the data to an equation consistent with the assumed model by means of the method of residuals and a nonlinear least-squares regression program. This model-dependent approach, together with the model-independent parameters t_{max} and C_{max}, were utilized to characterize the absorption kinetics of oral cephalosporins in the present study.

The model-independent parameters show that food causes a delay in absorption by prolonging the times to maximum concentrations (t_{max}) and a lowering of the peak plasma concentration (C_{max}) for all three cephalosporins. However, the overall bioavailability of all three compounds is not significantly affected by food as

indicated by the area under the plasma concentration versus time curves (AUC) and similar urinary recovery for all three cephalosporins. These model-independent parameters further indicate that cephalexin and cephradine have similar plasma profiles; however, cefaclor gives rise to a lower plasma concentration. The model-dependent parameters (k_a in the first-order absorption model and k_o in the zero-order absorption model) also indicate that food reduces the rate of drug absorption. However, the extent of drug absorption, as indicated by the availability index FD/V, is not significantly different between the fasting and nonfasting treatments for all three cephalosporins. The availability index FD/V is equal to AUC$\cdot k_{el}$; it is therefore inherently corrected for the intrasubject variation in the elimination rate constant and is a good parameter for the evaluation of systemic availability.

In order to explore further the various approaches to evaluate bioavailability data, the relative bioavailability between the fasting and nonfasting treatments was estimated by four different methods (two of which are routinely used in bioavailability studies) and also the method of statistical moments (to be discussed in the next section).

Method I (Equation 5) is the most commonly used method for estimating availability. It is based on the ratio of the area under the plasma concentration-time curve corrected for the dose administered. This method depends on the assumption that average drug clearance is the same in each of the comparative treatments.

This is unlikely to be the case if different subjects are used for each treatment as intersubject variability in drug clearance can be pronounced. This variability can be reduced by using the same subjects and by alternating the order of drug administration in a crossover design as in the present study. Hence, the average values determined in this study should give a good estimate of the true value. However, intrasubject variability in clearance may still exist between treatments.

Therefore, various approaches are devised to reduce the intrasubject variability in bioavailability studies. This would be accomplished if the clearance in the same subject from one treatment to another can be accounted for. If one can assume that the volume of distribution in a given subject is invariant from treatment to treatment, and then correct for differences in the elimination rate constant as in the quantity FD/V, which is equal to $AUC \cdot k_{el}$, intrasubject variability can be reduced. This correction method assumes that a change in the elimination rate constant from one treatment to the next in the same subject reflects solely a change in clearance and is not mediated by a change in apparent volume of distribution.

Method II (Equation 6) is an alternative approach to correct for intrasubject variability and was first suggested by Kwan et al. (107). It requires both plasma concentration and urinary excretion data. This method assumes that changes in total clearance are solely the result of changes in renal clearance and that nonrenal

clearance remains constant from treatment to treatment. It appears to be most useful for but not limited to drugs that are substantially excreted unchanged in the urine. Even if the assumption regarding the constancy of nonrenal clearance from one treatment to the next was incorrect, it would be of little consequence if the drug was substantially excreted unchanged, since nonrenal clearance would represent a small fraction of systemic clearance. As these oral cephalosporins are mainly excreted unchanged in the urine, this method is thought to be an appropriate method to correct for intrasubject variability in the present study.

Method III (Equation 8) is based on dose corrected urinary excretion data. The basis for this determination is that the ratio of the total amount of unchanged drug excreted in the urine after oral administration is a measure of the absorption of the drug. Method III is valid for all linear models and is a commonly used method in bioavailability studies. The principal drawback in using urinary excretion data for estimating availability is the need for collecting urine until virtually all of the drug has been excreted. This does not present a problem for drugs with short elimination half-lives such as the oral cephalosporins.

Method IV (Equation 9) is based on the construction and evaluation of percent absorbed-time plots (88), which does not require the assumption of zero- or first-order absorption. Method IV estimates the cumulative relative fraction of drug absorbed (CRFA) at various times following drug administration. This method relates

the cumulative amount of drug absorbed at various times to the amount of drug ultimately absorbed, rather than to the dose administered. An important characteristic of this method for the evaluation of absorption data is that no model is assumed for the absorption process. One limitation of this method is that it applies rigorously only to drugs with one-compartment characteristics, and is only an approximation in all other cases. In this study, this method is applicable because these oral cephalosporins are found to obey simple one-compartment model kinetics. Therefore, this method was utilized to compare the cumulative percent absorption of drug from the nonfasting treatments relative to the fasting treatments.

The cumulative relative percent absorbed plots (Figures 35-37) indicate that all three cephalosporins are more rapidly absorbed under the fasting than nonfasting treatments. However the cumulative relative percent absorbed between the fasting and nonfasting treatments is not significantly different after 3 hr, which confirms the initial conclusion that the overall systemic availability for the three cephalosporins is not significantly affected by food.

The mean relative bioavailability values between the fasting and nonfasting treatments for the three cephalosporins calculated by the four methods are not significantly different from each other, or from unity (Table 88). It is therefore concluded that plasma and urine data yield equivalent estimates of relative bioavailability, and the extents of absorption are similar between the

fasting and nonfasting treatments for the three cephalosporins. By utilizing both plasma and urine data and applying a correction factor for changing renal clearance as discussed previously, Method II tends to reduce the apparent variability of the estimate. It appears that when large intrasubject variability exists in bioavailability studies, Method II may present a reasonable approach to reduce the variability.

Owing to the inconsistencies reported in the literature regarding the effect of food on the bioavailability of oral cephalosporins, this study has utilized a systematic and more stringent approach to evaluate the bioavailability data. All these methods have confirmed that food delays but does not affect the extent of absorption of cephalexin, cephradine, and cefaclor.

H. Statistical Moments

Statistical moments arise naturally as a means of describing the features of an input function, or the resulting responses, when it is recognized that these processes are essentially stochastic in nature. Moment analysis has been used extensively in chemical engineering. The application of statistical moments to pharmacokinetics was first reported in 1979 by Yamaoka et al. (89) and Culter (108). In 1980, Riegelman and Collier (90) applied statistical moment theory to the evaluation of drug absorption.

Since the movement of individual drug molecules through a body compartment is governed by probability, as they will not all be metabolized or excreted at the same time, the time course of drug concentration in plasma can be regarded as a frequency distribution curve. Analysis of this distribution function can be made by the method of moments. The zero, first, and second moments are termed the area under the concentration-time curve (AUC), the mean residence time (MRT), and the variance of the mean residence time (VRT), respectively. MRT and VRT have been defined previously as in Equations 10 and 11, and can be calculated by numerical integration using the trapezoidal rule from the plasma concentration data.

The concept of utilizing the zero moment as the measurement of the area under the plasma concentration-time curve from zero to infinity to estimate the extent of drug absorption is well recognized and has been widely used in bioavailability studies.

However, the first and second moments have not been commonly used in pharmacokinetic analysis. The application of AUC to bioavailability studies has been discussed. The utility of MRT and VRT in bioavailability studies is discussed in this section.

When an amount of drug is administered, some of the drug molecules will be excreted, while some molecules will remain in the body. As a result, the behavior of an individual drug molecule cannot be predicted. However, the overall collective behavior of this population of drug molecules administered can be observed and described. Therefore, the residence time of the drug in the body can be regarded as a statistically random variable, with the mean and variance reflecting the overall behavior of the population of drug molecules.

The mean residence time can therefore be defined as the mean time for the intact drug molecule to pass through the body; it involves a composite of kinetic processes, including in vivo release from the dosage form, absorption into the body, and all disposition processes. By assuming that the disposition function of the drug follows linear kinetics or exponential decline, a log-normal distribution is appropriate to describe the frequency function. In theory, the mean occurs at 63.2% for the log-normally distributed cumulative curve. Therefore, the MRT represents the time for 63.2% of the administered dose to be eliminated by all processes.

The second moment of the concentration-time curve (VRT), as its name implies, is used to define the variance of the mean resi-

dence time. As with general moment analysis, higher moments can be applied theoretically to define the skewness and kurtosis of the distribution. However, studies by numerical estimation of the VRT values indicate that this higher moment leads to serious, unacceptable computational errors. Therefore, VRT values are not used for data interpretation in this study.

While MRT can be calculated theoretically from any concentration-time curve using the trapezoidal rule, mathematical relationships have also been developed that connect model-dependent pharmacokinetic parameters with statistical moments. Compartment models in pharmacokinetics can be expressed by simultaneous ordinary differential equations which can be solved by Laplace transforms (109). A convenient relationship between the statistical moments and the Laplace-transformed equation has been developed (110). According to Yamaoka et al. (89), the theorems associated with the use of Laplace transforms (111) and MRT can be related as in Equation 25,

$$MRT = \frac{\lim_{s \to 0} \frac{d\bar{C}}{ds}}{\lim_{s \to 0} \bar{C}} \qquad (25)$$

where $\bar{C}$ is the Laplace transform of the equation, equal to $\int_0^\infty e^{-st} C(t)\,dt$, and s is the Laplace variable with respective to time t. Equation 25 allows the MRT to be calculated in terms of pharmacokinetic parameters. Therefore, for each representative

pharmacokinetic model, the Laplace-transformed equation for the model can be obtained, and the MRT of the plasma concentration-time curve can be calculated in terms of the model parameters according to Equation 25.

Utilizing this approach, all MRT values were calculated by Equations 12 and 13 based on the assumption of a one-compartment model with first-order and zero-order input, respectively.

The values of mean residence times (Table 92) calculated by Equation 10 show that under the fasting treatments, the mean residence times were 1.65, 1.64, and 1.45 hr for cephalexin, cephradine, and cefaclor, respectively. The mean residence time for cefaclor was approximately 12 min shorter than the other two cephalosporins, which is probably due to the relatively faster clearance of cefaclor in the body. These values of the mean residence time indicate that approximately 63% of the three cephalosporins were eliminated from the body at approximately 1.5 hr. Under the nonfasting treatments, the mean residence times were uniformly prolonged to 2.9, 2.85, and 2.38 hr for cephalexin, cephradine, and cefaclor, respectively. Using the simple relationship, $\Delta MRT = MRT_{nf} - MRT_{f}$, where the subscripts nf and f denote nonfasting and fasting treatments, the ΔMRT values were 1.25, 1.21, and 0.93 hr for cephalexin, cephradine, and cefaclor, respectively. Thus, it can be concluded that food lengthens the mean residence time of the three cephalosporins in the body by ca one hour.

Interestingly, the MRT values calculated by the three equations

(Equations 10, 12, and 13) are similar. As it is likely that neither pure zero-order or first-order absorption actually occurs in the body fluids, both Equation 12 (first-order absorption) and Equation 13 (zero-order absorption) yield similar values as those obtained by Equation 10 (theoretical definition of MRT).

In summary, this study has demonstrated the utility of the moment analysis method in examining bioavailability data. It should be emphasized that, because statistical moments are additive for linear systems and can be obtained without the use of a pharmacokinetic model, data evaluation can be performed by simple arithmetic operations.

In addition to describing the overall characteristics of the drug plasma concentration-time profile (AUC), the first moment (MRT) provides information as to whether a drug passes through the body quickly or over a long period of time. Therefore, it is evident that statistical moments (AUC and MRT) are excellent parameters to characterize the pharmacokinetics of a drug. However, the use of the second moment (VRT) in pharmacokinetics may need future examination as higher moments are prone to an unacceptable level of computational error. Applying the methods of statistical moments analysis, this study shows that oral administration of cephalexin, cephradine, and cefaclor following food ingestion prolongs the mean residence times of the three cephalosporins in the body without reducing their extents of systemic availability. These results are consistent with those obtained by the various approaches previously

discussed in this study. In light of the practicability and applicability of this noncompartmental approach based on statistical moment theory, it is recommended that MRT should be used as adjunct to the conventional parameters, AUC, C_{max}, and t_{max}, for the routine evaluation of bioavailability data.

I. Clinical Implications

This study has shown that food causes a delay in the absorption of oral cephalexin, cephradine, and cefaclor, but does not reduce their overall availability to the systemic circulation.

The clinical significance of the above interactions is difficult to assess. Studies comparing clinical efficacy under fasting and nonfasting conditions have not been carried out. The clinical effect of delayed absorption depends on the microbiological activity of the cephalosporins, the sensitivity of the target organisms, and the extent to which plasma concentrations are reduced. Reduced plasma concentrations are likely to be clinically significant in situations where circulating drug concentrations are close to the minimum inhibitory concentrations.

For antibiotic therapy, the peak plasma concentration may have a significant influence on the interstitial concentrations achieved at the infected area (112, 113). On the other hand, Bergan (114) has called attention to the fact that both high peak concentrations and a large area under the plasma concentration-time curve are necessary for effective antibiotic therapy. At present, this question has not yet been definitively clarified.

The dosage regimens recommended for cephalosporins frequently produce circulating concentrations of antibiotic activity that are well above the MIC values for most organisms. However, there is considerable variation in the MIC values of some susceptible strains as shown in Table 97. As circulating levels of antibiotic in plasma

Table 97

Susceptibility of Microorganisms to Cephalexin, Cephradine, and Cefaclor

Micro-organism	Inoculum Size (10^2cfu/µl)	Strains tested	Geometric mean MIC (mcg/ml)		
			Cephalexin	Cephradine	Cefaclor
E. coli β-[a]	22	6	8.5	2.4	
E. coli β+[b]	28	6.7	8	2.8	
Klebsiella spp	50	4.6	8	1.9	
P. mirabilis β-	37	10	14	1.8	
P. mirabilis β+	15	32	48	14	
Indole positive Proteus	20	84	51	28	
B. fragilis	10	28	21	128	
N. gonorrhoeae β-	31	0.26	0.29	0.035	
Grp. D. Streptococci	15	95	52	128	
Grp. A. Streptococci	19	0.41	0.3	0.39	
S. aureus β-	23	1.7	1.6	1.8	
S. aureus β+	25	1.8	1.5	1.6	
Enterobacter spp	12	24	24	13	
H. influenzae β-	44	8	13.9	3.0	
H. influenzae β+	11	8.5	11.3	2.4	
Strept. pneumoniae	30	0.9	0.46	0.4	

[a] Non-β-lactamase producer

[b] β-Lactamase producer

(Adapted from reference 82)

may influence the levels achieved at tissue sites, it is important to optimize absorption conditions.

It is the author's view that the therapeutic significance of the effect of food on the pharmacokinetic characteristics of these oral cephalosporins must be evaluated in light of their relative antibacterial activity and the sensitivity of the target organism. In order to obtain maximum circulating drug concentrations for a given dose, and to maintain reproducible drug concentrations between doses, oral cephalexin, cephradine, and cefaclor should be taken on an empty stomach, or at least one hour before meals whenever possible. Alternatively, if a prolonged plasma concentration is desired in the eradication of susceptible organisms with low MIC, it might be more advantageous to administer these cephalosporins with food. Comparative clinical studies are necessary to make a conclusive assessment.

V. SUMMARY OF CONCLUSIONS

A summary of the conclusions of this thesis are enumerated as follows:

1. HPLC procedures have been developed for the quantification of cephalexin, cephradine, and cefaclor in human plasma and urine. These procedures are suitable for routine drug monitoring and pharmacokinetic studies in terms of specificity, reliability, sensitivity, and simplicity.
2. The stability of cefaclor is pH and temperature dependent. Cefaclor is most stable when prepared in a pH 4.5 buffer solution and stored in a refrigerator. Therefore, for analytical work, cefaclor stock solution should be prepared fresh daily in a pH 4.5 buffer. Plasma samples should be kept in a refrigerator and assayed on the same day they are collected. Samples that are to be assayed at a later time should be frozen. Urine samples should be diluted with a pH 4.5 buffer solution upon collection, kept in a refrigerator, and assayed on the same day they are collected. Samples that are to be assayed later should be frozen. These procedures will enhance the *in vitro* stability of cefaclor in biological fluids.
3. After single 500 mg oral doses to man, similar plasma drug profiles were obtained following cephalexin and cephradine

doses to fasted subjects, with mean C_{max} values of 16.8–19.3 mcg/ml occurring at approximately 1 hr. Under the same conditions, cefaclor yielded a mean C_{max} of 10.3 mcg/ml, occurring also at 1 hr. After postprandial doses, C_{max} values of all three cephalosporins were reduced, and t_{max} values were increased. Areas under the plasma concentration-time profiles were unaffected by food for all three compounds, with the AUC values of cefaclor being approximately one-half those of the other two compounds. The total 12 hr urinary recovery of cephalexin, cephradine, and cefaclor following fasting treatment was 88.5%, 89.1%, and 73.7%, respectively, of the administered dose. The total 12 hr urinary excretion of unchanged cephalosporin was delayed, but total 12 hr urinary recovery was only slightly, not significantly, reduced by food for all three compounds. Thus, the plasma concentration and urinary excretion data show that ingestion of food causes a delay in absorption of oral cephalexin, cephradine, and cefaclor. The overall absorption efficiency of all three compounds is not significantly affected.

4. In order to define an appropriate pharmacokinetic model for oral cephalosporins, plasma profiles were analyzed utilizing a one-compartment model with first-order absorption and elimination, and a one-compartment model with zero-order absorption and first-order elimination. Following the nonfasting treatments, 'flip-flop' characteristics exist in the first-order

absorption model as a result of the relatively slow absorption compared to the rapid elimination of cephalosporins. Comparison of the coefficients of determination shows that the zero-order absorption equation gives a significantly better fit of the plasma data for all three cephalosporins under the fasting treatments. The sharpness with which the blood concentrations rise to a peak and immediately decline without a plateau region further suggests zero-order absorption characteristics under the fasting treatments. However, with the nonfasting treatments, plasma concentration profiles can be adequately described by either the zero-order or first-order absorption equation. Mechanistic characterization of the observed changes would be necessary to better understand the absorption kinetics. The pharmacokinetic parameters generated from these models are consistent with the plasma concentration and urinary excretion data in reflecting delayed absorption of the three cephalosporins when taken on a nonfasted stomach, with no significant changes in the overall availability of drug to the systemic circulation.

5. Plasma clearance of cefaclor is significantly larger than those of the other two compounds, which can be explained in view of its stability characteristics. There is no significant difference in clearances between the fasting and nonfasting treatments for all three cephalosporins.

6. The apparent volume of distribution is similar for cephalexin

and cephradine. However, cefaclor has a relatively larger apparent volume of distribution than the other two cephalosporins. The two volume terms, V and V_{dss}, are not significantly different from each other. It is concluded that for drugs that conform to a one-compartment model with first-order elimination, the equation $V = FD/AUC \cdot k_{el}$ provides a simple and direct approach for estimating the apparent volume of distribution.

7. The relative bioavailability between the fasting and nonfasting treatments for cephalexin, cephradine and cefaclor is evaluated in light of the model-independent parameters (AUC, C_{max}, t_{max} and t_o), cumulative urinary excretion data, and model-dependent parameters (k_a and FD/V). All these parameters indicate that food delays drug absorption, but does not reduce the extent of absorption. The relative bioavailability between the fasting and nonfasting treatments for the three cephalosporins is evaluated also by four different methods which yield equivalent estimates indicating that the extent of absorption is similar. By utilizing both plasma and urinary data, and applying a correction factor for changing renal clearance, the method devised by Kwan et al. (107) tends to reduce the apparent variability of the estimate. It is also recognized that the cumulative relative percent absorbed plot, which relates the relative amount of drug absorption at various times to the amount ultimately absorbed, is a useful means to

characterize the relative rates of drug absorption in bioavailability studies.

8. The utility of the first moment, the mean residence time, in evaluating bioavailability data is demonstrated in this study. Food uniformly prolongs the mean residence time of cephalexin, cephradine, and cefaclor in the body without reducing the extent of systemic availability. It is recommended that mean residence time should be used as an adjunct parameter to evaluate bioavailability data.

9. The observed changes in plasma cephalosporin concentrations after single doses are likely to occur also after repeated doses. The clinical significance of the reduced drug concentrations after postprandial dose is difficult to assess. The clinical effect of delayed absorption must be evaluated in light of the microbiological activity of cephalosporin, the sensitivity of the target organisms, and the extent to which plasma concentration is reduced. In order to obtain maximum circulating drug concentration for a given dose and to maintain reproducible drug concentration between doses, oral cephalexin, cephradine, and cefaclor should be taken on an empty stomach whenever possible. Alternatively, if a prolonged plasma concentration is desired in the eradication of susceptible organisms with low MIC, it might be more advantageous to administer these cephalosporins with food. Comparative clinical studies are necessary to make a conclusive assessment.

10. This study has confirmed that food delays but does not affect the extent of absorption of oral cephalexin, cephradine, and cefaclor in man. More information is needed regarding mechanistic characterization of the observed changes and their clinical significance. Only when sufficient data are accumulated can patterns and mechanisms of physicochemical or physiological interactions be established that will enable dosage regimens to be optimized for maximum therapeutic benefits.

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APPENDIX A

Glossary of Terms

A_e	Total amount of drug recovered unchanged in 12 hr urine.
AUC	Area under the plasma drug concentration *versus* time curve.
$AUC^{0\rightarrow t}$	Area under the plasma drug concentration *versus* time curve from time zero to time t.
$AUC^{0\rightarrow\infty}$	Area under the plasma drug concentration *versus* time curve from time zero to time infinity.
AUMC	Area under the (first) moment *versus* time curve.
C	Drug concentration in plasma.
C_{max}	Maximum drug concentration in plasma.
C_t	Drug concentration in plasma at time t.
Cl_{plasma}	Plasma clearance.
Cl_{renal}	Renal clearance.
CRFA	Cumulative relative fraction of dose absorbed.
CV	Coefficient of variation.
D	Dose of cephalosporin administered.
F	Fraction of the administered dose absorbed.
FD/V	Fraction of the administered dose absorbed expressed as a concentration term in its distribution volume in the body.
F_{nf}/F_f	Fraction of the administered dose absorbed following the nonfasting treatments relative to the fasting treatments.
k_a	Apparent first-order absorption rate constant.
k_{el}	First-order elimination rate constant.
k_o	Apparent zero-order absorption rate constant.

MIC	Minimum inhibitory concentration.
MRT	Mean residence time.
r^2	Coefficient of determination.
SD	Standard deviation.
t	Time since dosing.
$t_{1/2}(abs)$	Apparent absorption half-life.
$t_{1/2}(el)$	Elimination half-life.
t_{max}	Time of maximum drug concentration in plasma.
t_o	Time period (lag time) between dosing and appearance of measurable drug concentration in plasma.
T	Apparent absorption time.
V	Apparent volume of distribution.
V_{dss}	Apparent volume of distribution at steady state.
VRT	Variance of the mean residence time.

APPENDIX B

Consent Form for the Study of Effect of Food on the Bioavailability and Pharmacokinetics of Oral Cephalosporins in Man

CONSENT FORM

for the study of

EFFECT OF FOOD ON THE BIOAVAILABILITY AND

PHARMACOKINETICS OF ORAL CEPHALOSPORINS IN MAN

A study is being conducted by the School of Pharmacy, University of Wisconsin-Madison, on the possible effects of food on the absorption of three orally dosed cephalosporin antibiotics from the gastrointestinal tract.

Several reports in the medical and scientific literature have shown considerable differences in the absorption of these drugs when taken under different conditions.

As the therapeutic effect of antibiotics is often related to circulating levels of drug in the body, which are in turn related to how well the drug is absorbed, their clinical usefulness may also be affected by changes in absorption efficiency.

These studies are designed to obtain as much information as possible about the above factors, and it will be obtained using the encapsulated form of the cephalosporins. The information obtained will enable the physician to prescribe, and the patient to take, these compounds under the best possible conditions for efficiency of absorption and consistency of therapeutic effect.

A complete protocol for the study will be given to you to read

(and to keep if you wish) but the study details are also briefly described here.

For your information the uses and side effects of these cephalosporin antibiotics are listed here. Many of the reported side effects have occurred in very few cases, and they are not expected to occur with the doses used in this study. However, having this information will help you recognize the side effects if they should occur.

It is very important that during your medical examination, which is carried out to ensure that you are fit, you give the physician complete information regarding any drug reactions you may have had in the past. It is extremely important also to report any allergic reaction immediately after the first symptom, regardless of how mild it is.

Uses of Cephalexin, Cephradine, and Cefaclor

The cephalosporin antibiotics, of which the above three compounds are members, are closely related to the penicillins, and are used for a variety of infections. They are used commonly to treat infections of the respiratory tract and also the urinary tract.

Side Effects

There is considerable evidence that people who are sensitive to penicillins will also be sensitive to cephalosporins. If you have any history of allergy to penicillins or to other cephalosporins, you will not be permitted to take part in this study.

Apart from the important possibility of a reaction, other adverse effects most frequently reported following oral administration of cephalosporins include nausea, vomiting, and diarrhea.

STUDY PROGRAMS AND DETAILS

Before being permitted to participate in any of the studies, you will be required to undergo a full physical examination at the University Hospitals. These will be carried out by Dr. William A. Craig, or another suitably qualified physician, and will be at no cost to you.

Provided you pass this examination you may participate in the study.

In the study, you will take a single 500 mg oral dose of capsules of cephalexin, cephradine, and cefaclor. Each medication will be taken:

A. On an empty stomach, with 250 ml water.

B. Immediately following a standard breakfast with 25 ml water.

Thus you will receive each drug twice in each phase of this study, i.e., three different cephalosporins and two different dosing conditions.

The general procedures of the study will be as follows:

The fasting period for all parts will begin at 8 p.m. the night before dosing. Fluid intake must stop at 10 p.m.

You will drink 200 ml of water on arising, at least one hour before dosing, on the day of a study.

Drugs will be administered, according to the appropriate controlled treatment, at 8 a.m.

You will not be permitted to eat any additional food, or drink any additional fluid until 4 hours after taking the drug.

You will give 12 blood samples (8-10 ml each time) from a forearm vein before and during the eight hours after taking each dose. Your urine will be collected, quantitatively, before and during 0-2, 2-4, 4-8, and 8-12 hours after dosing.

The blood and urine samples you provide will be analyzed in our laboratories in order for us to determine how much drug is absorbed, and how fast it is absorbed, from each dose. In this way we can determine the effect of food on the absorption of the oral cephalosporins, and what is the best way they should be dosed in order to get maximum absorption.

The following points are most important:

1. You must have taken no drugs for seven days before each study and you must take no drugs other than the one administered to you during the study. In addition, you will not be permitted to ingest any caffeine-containing beverages and chocolate for two days before and during the entire blood sampling period following each dose.
2. You must follow closely the instructions regarding the dosing and sampling procedures.
3. There will be no immediate benefits to you by taking

part in this study. However, depending on the outcome of the results, there may be long term benefits to you, and to others, if we can establish the best conditions for absorption of these antibiotics.

4. Apart from the drug-related risks already described, the only other major risk to you will be that of infection due to repeated blood sampling. This risk is very small as all blood samples will be drawn under aseptic conditions by fully qualified personnel.

5. You will receive a copy of this consent form, which you may retain.

6. You are free to withdraw your consent and to discontinue your participation in the study at any time.

7. If there is anything you do not understand about this study, please ask Dr. Welling or Dr. Craig to explain it to you.

8. You will receive $4 for each blood sample and $3 for each complete urine collection pertaining to the drug studies.

9. There is a small risk that you may experience a toxic side-effect to cephalosporin. If you experience <u>any adverse reaction</u> or feel unwell after you have received a dose of cephalosporin, report it to the investigators immediately. You will then receive appropriate advice, and you may be required to withdraw from the study.

10. You will be given a card containing the names and both the

office and private telephone numbers of the investigators. You are free to call these at any time.

11. The policy of the University of Wisconsin is that, in the event of physical injury as a result of research carried out on humans, the University does not automatically provide compensation. In the case of this study the University does not take responsibility for untoward side effects.

Peter G. Welling, D. Sc. 608-263-3986

William A. Craig, M.D. 608-256-1901

Florence W. Kwok, M.Sc. 608-262-5819

CONSENT

I have read the information contained in this consent form.

I hereby give my consent for Dr. William A. Craig, or a qualified assistant, to administer the drugs indicated in this study to me at such times as are described in the study protocol. I consent also to Dr. Craig, or a qualified assistant, to obtain venous blood samples from me, and also to provide urine samples at the times and using the procedures described to me.

I understand that participation in these studies will be of no direct benefit to me. I understand further that I am free to withdraw from participation in the study at any time.

_____ _____

Subject Date

_____ _____

Witness Date

APPENDIX C

Typical Computer Output Using the Program NREG

One-Compartment Model with First-Order Absorption and Elimination

```
 * * * * * *  SPERRY 1100 TIME/SHARING EXEC === MULTI-PROCESSOR SYSTEM === VER,  MACC 36.41  SITE *  MACC      * * * * * *
 RUN:# 405668          PROJECT# 0100                USER# 9000100793          CREATED ON MAY 23, 1980 AT 21120115    INPUT DEVICE# MACR3
                  FILE NAME# PR#D00405668            PART NUMBER# 00          PRINTED ON MAY 23, 1980 AT 21121123    OUTPUT DEVICE# MAPR3


@RUN KNOX,81000,9000100793,5M,500
@FCP,SI      ,*MAIN
FORTRAN-MACC 1.175-05/23/80-21120115
00101     1.            PARAMETER                  MAIN
00101     2.           1NEXPO = 90,
00101     3.           2NVAR = 1,
00101     4.           3NPO = 7,
00101     5.           4NROBS = NEXPO,
00101     6.           5NPRO = NEXPO,
00102     7.           6NS=(17+NEXPO*NVAR)*NPO+2*NPRO*NVAR+2*(NPO+1)*(NPO+2)+1
00103     8.            REAL THINT(NPO),THFIN(NPO),YOBS(NROBS,NVAR),YPRO(NROBS,NVAR),S(NS)
00103     9.           1,TOL(4),T(NEXPO),BNDLO(NPO),BNDHI(NPO),NAMES(NPO),DIFF(NPO),FIX(NP
00104    10.           2O)
00134    11.            INTEGER ITEMS(12),INFO(7),IDENT(13),NEXP,NP
00135    12.            COMMON T
00106    13.            COMMON/LIM/NEXP1,NEXP2,K
00110    14.            DATA FIX/7*1,0/
00112    15.            DATA DIFF/7*0.005/
00114    16.            DATA BNDLO/7*1.0E-4/
00116    17.            DATA BNDHI/7*1.0E4/
00120    18.            DATA NAMES/6HASS/1 ,6HM+E/1 ,6HFOV/1 ,6HLAG/1 ,3*6H      /
00122    19.            DATA TOL/1.5E-6,1.0E-9,0.0,1.0E-16/
00126    20.            DATA INFO/1,10,5*1/
00126    21.            DATA ITEMS/
00126    22.           1,2*VAR=1,*ROWS,*RPRO,3,*ARO*,*NONE*,*NREG*,*CENT*,*REL*,50/
00134    23.            READ(5,110) (IDENT(I), I=1,13)
00135    24.      110   FORMAT(13A6)
00142    25.            READ (5,120) NEXP1,NEXP2,NP
00143    26.      120   FORMAT(2X,I3,2X,I3,3X,I2)
00140    27.            NEXP = NEXP1 + NEXP2
00145    28.            ITEMS(1) = NEXP
00146    29.            ITEMS(3)=NP
00150    30.            READ(5,100)( THINT(I), I=1,NP )
00152    31.            READ (5,100) (YOBS(I,1),I=1,NEXP1)
00170    32.            READ (5,100) (T(I), I = 1,NEXP1)
00171    33.            K = NEXP1+ 1
00172    34.            IF (K.GE.NEXP) GO TO 66
00201    35.            READ (5,100) (YOBS(I,1),I=K,NEXP)
00207    36.            READ (5,100) (T(I),I=K,NEXP)
00210    37.       66   CONTINUE
00211    38.      100   FORMAT(8F10.5)
00212    39.            EXTERNAL F,DEFIV
00213    40.            CALL SPECIFY(INT,NAMES,BNDLO,BNDHI,FIX,DIFF,THFIN,YOBS,YPRO,
00213    41.           1'NONE',F,'NONE',ITEMS,TOL,INFO,IDENT,S)
00214    42.            CALL OUTPUT(NEXP, NEXPO,THFIN,NVAR,NP,NRPRO,YPRO,YOBS)
00214    43.            END
      END OF COMPILATION:  NO  DIAGNOSTICS.
@ADD,SI    , SUBF
FORTRAN-MACC 1.175-05/23/80-21120117
00101     1.            SUBROUTINE F(T,Y,NEXP,NVAR,NP,NRPRO)
00103     2.            DIMENSION T(1),Y(NNP+0,1),F(1)
00103     3.            COMMON T
```

```
00104      4.          COMMON/LIM/NEXP1,NEXP2,K
00105      5.          TH1 = TH(1)
00106      6.          TH2 = TH(2)
00107      7.          TH3 = TH(3)
00110      8.          TH4 = TH(4)
00111      9.          DO 10 I = 1,NEXP1
00112     10.          IF ((T(I)-TH4).LT.0) GO TO 11
00113     11.          Y(I,1) = (TH3*TH1/(TH1-TH2))*(EXP(-TH2*(T(I)-TH4))-EXP(-TH1*(T(I)-
                      1TH4)))
00117     13.          GO TO 10
00120     14.       11 Y(I,1) = 0.0
00121     15.       10 CONTINUE
00123     16.          IF( K.EQ.NEXP ) GO TO 30
00125     17.          DO 20 I = K,NEXP
00130     18.       20 Y(I,1) = TH4+(1-1/(TH1-TH2)*(TH1*EXP(-TH2*T(I))-TH2*EXP(-TH1*T(I))
                      1)
00132     20.       30 CONTINUE
00133     21.          RETURN
00134     22.          END

        END OF COMPILATION:        NO  DIAGNOSTICS.
#FOR,SI      ,OTP
FORTRAN-VACC 1.175-05/23/74-21920218          OTP
00101      1.          SUBROUTINE OUTPUT(NEXP,NEXPO,THFIN,NVAR,KP,NRPRO,YPRO,YOBS)
00102      2.          DIMENSION T(1),YOBS(1),YPRO(1)
00103      3.          COMMON T
00104      4.          COMMON/LIM/NEXP1,NEXP2,K
00105      5.          K = 0
00106      6.       200 CONTINUE
00107      7.          SD2 = 0.0
00110      8.          SPP = 0.0
00111      9.          SC = 0.0
00112     10.          SYOBS = 0.0
00113     11.          SDEV = 0.0
00114     12.          COR = 0.0
00115     13.          RS = 0.0
00116     14.          YOM = 1.0
00117     15.          YPM = 0.0
00120     16.          K = K + 1
00121     17.          IF (K.EQ.1) GO TO 210
00123     18.          LL = NEXP1 + 1
00124     19.          LU =  EXP
00125     20.          GO TO 250
00126     21.       210 LL = 1
00127     22.          LU = NEXP1
00130     23.       250 DO 10 I = LL,LU
00133     24.          SYOBS = SYOBS + YOBS(I)**2
00134     25.          SDEV = SDEV + (YOBS(I)-YPRO(I))**2
00135     26.          YOM=YOM+YOBS(I)
00136     27.          YPM=YPM+YPRO(I)
00137     28.       10 CONTINUE
00141     29.          RS = (SYOBS-SDEV)/SYOBS
00142     30.          YOM=YOM/NEXP
00143     31.          YPM=YPM/NEXP
00144     32.          DO 20 I=1,NEXP
00147     33.          SC = SC+(YOBS(I)-YOM)*(YPRO(I)-YPM)
00150     34.          SD2 = SD2 + (YOBS(I)-YOM)**2
```

```
00151     35.           SP2 = SP2 + (YPRD(I)-YP)**2
00152     36.        20 CONTINUE
00154     37.           COR = SC/(SQRT(SP2)*SQRT(SP21))
00155     38.           WRITE(6,100) A,B,C,COR
00165     39.       100 FORMAT(1H0,'R-SQUARED FOR FUNCTION=',I1,' A=',F6.4/1X,'COR FOR FUNCT
00165     40.          1ION=',I1,' A=',F6.4)
00166     41.           IF (NEXP2.EQ.0) GO TO 66
00166     42.           IF(R.GT.1) GO TO 300
00170     43.           SYDBS = SYUSS
00171     44.           SDEU = SDEJ
00172     45.           GO TO 220
00173     46.       300 SYDBS = SYDBS + SYDSQ
00173     47.           SDEU = SDEU + SDEJ
00175     48.           RS = (SYDSS-SDEU)/SYDSS
00176     49.           WRITE(6,111) RS
00201     50.       111 FORMAT(1X,'GIVEN ALL R-SQUARED=',F6.4)
00202     51.        66 CONTINUE
00213     52.           CALL PRPL (NEXP,NEXP1,THFIX,NVAR,NP,NPRD,YPRD,YUBS)
00214     53.           RETURN
00214     54.           END

      END OF COMPILATION:        NO  DIAGNOSTICS.
$ENTRY,SI      ,PRPL
$FORTRAN-H-ACC 1.175-MVS/23/AM=2112)(=            PRPL
00101     1.            SUBROUTINE PRPL (NEXP,NEXP1,THFIX,NVAR,NP,NPRD,YPRD,YUBS)
00102     2.            DIMENSION T(1),YUBS(NPRD,1),YPRD(NPRD,1)
00103     3.            COMMON /
00104     4.            COMMON/ALL/M,NEXP1,NEXP2,K
00105     5.            M = 1
00106     6.            CALL DEVSET(5,PRINTER)
00107     7.        220 CONTINUE
00110     8.            M = M + 1
00111     9.            IF(M.EQ.13) GO TO 50
00113     10.           DO 140 I = 1,NEXP2
00116     11.           MM = NEXP1 + I
00117     12.           YOBS(I,1) = YUBS(M,1)
00124     13.       140 T(I) = T(MM)
00122     14.           LL = NEXP2
00123     15.           GO TO 60
00124     16.        50 CONTINUE
00125     17.           LL = NEXP1
00126     18.        60 CONTINUE
00127     19.           YMAX = YOBS(1,1)
00130     20.           DO 10 I = 2,LL
00133     21.        10 YMAX = AMAX1(YMAX,YOBS(I,1))
00135     22.           IF( M.EQ.2 ) GO TO 170
00137     23.           YMAX = T(1)
00140     24.           DO 15 I = 2,NEXP
00143     25.        15 YMAX = AMAX1(T(LK),T(I))
00145     26.           YMAX = YMAX/(NEXP1+NEXP1+NEXP2)
00146     27.           MM = NEXP1 + NEXP2 + 1
00147     28.           LL = NEXP1 + NEXP2 + 2
00150     29.           T(LL) = 0.0
00151     30.           DO 30 I = MM2,NEXPO
00154     31.           J = I - L
00155     32.        30 T(I) = T(J) + YMAX
00157     33.           IF ( M.EQ.1 ) GO TO 180
```

```
00161    54.          GO TO 170
00162    55.      160 NEXI = NEXP1
00163    56.          NEXP1 = NEXPO
00164    57.          K = NEXPO
00165    58.          GO TO 180
00166    59.      170 CONTINUE
00167    60.          NEXP1 = 1
00170    61.          K = 1
00171    62.          NEXP = NEXPO
00172    63.      180 CONTINUE
00173    64.          CALL F(TMFI,YPRO,NEXPO,NYAR,NP,NRPRO)
00174    65.          NEXP1 = NEXI
00175    66.          DO 20 I = 1,NEXPO
00200    67.       20 YMAX = AMAX1(YMAX,YPRO(I,1))
00202    68.          YSCLE = YMAX/8.8
00203    69.          TSCLE = TMAX/8.8
00204    70.          CALL GRAPH(T,SMLINEAR,YPRO,SMLINEAR,SNEXPO,15,SMBLANK,'TIMESS',1FU
00204    71.         1NCTION VALUESSS',SMAXES,'PLOT OF OBSERVED,O''S AND PREDICTED,P''S
00205    72.         2VALUESSS',12,0,12,0)
00206    73.          CALL GRAPHE('SCALI',YOBS,'SCALI',LU,18,SMBLANK)
00210    74.          IF (NEXPZ.EQ.1) GO TO 66
00212    75.          IF(M.EQ.1) GO TO 200
00213    76.       66 CONTINUE
00214    77.          RETURN
                     END

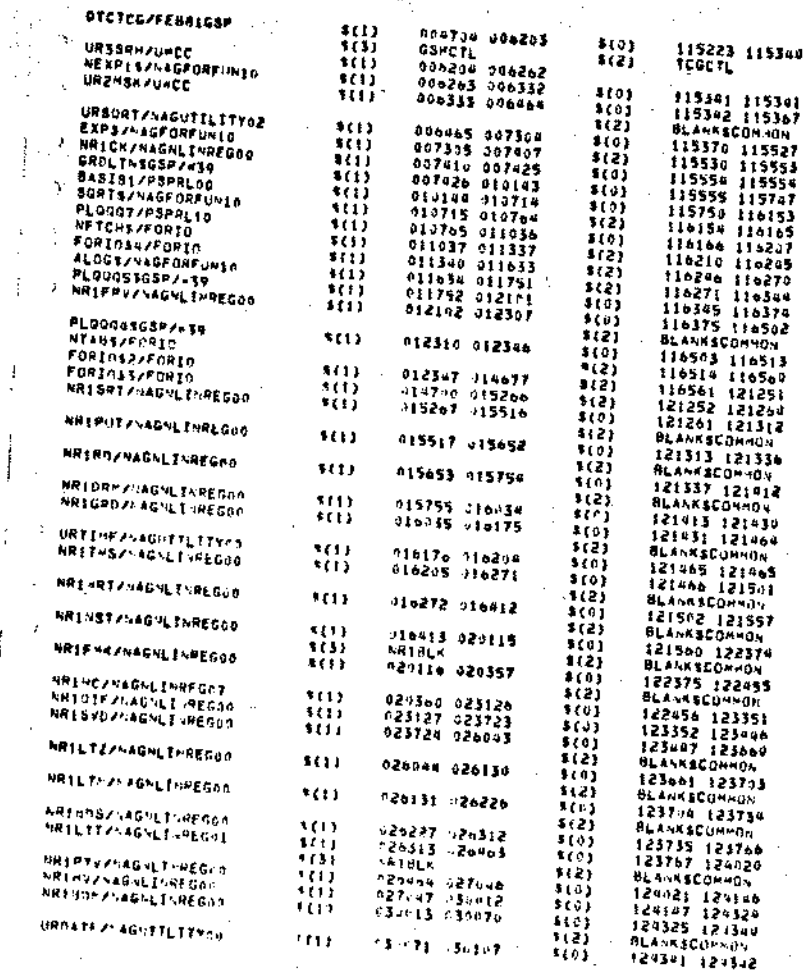
        END OF COMPILATION:          NO  DIAGNOSTICS.
$MAP
MAP 10R21108 SLIB 2 05/23/84 21:20:21
AFCW STATUS OF OUTPUT FILE IS UNKNOWN
QUARTER/THIRD WORD INSENSITIVE


$BANK NAME(R21)
$IBANK(4)              ADDRESS LIMITS
$DBANK(5)              001261 113537      35240 IBANK WORDS DECIMAL
STARTING ADDRESS  113342  114000 145501   13920 DBANK WORDS DECIMAL


          SEGMENT STATUS          001000 113537     114000 145501

UR1TR[/UNCC01
DSHCLR/GSPREFPSP          $(1)   001000 001105
                          $(1)   001106 001225
$LOAD/GSPV3A
NEFIT=/FURTS              $(1)   001226 001523
SMIMIZ/MAGNETAREGOO       $(1)   001524 002567
NR1=RL/=AGNETAREGOO       $(1)   002570 003333
                          $(1)   003334 003622
NR1S=R/=AGNETIAREGOO      $(1)   003623 004240
NEXPAX/=AGFSRF=412        $(1)   004241 004476
SYMBOL/=AYAIGSP           $(1)   004477 004660
MCOFF/GSPV03              $(1)   004661 004743

$(0)   114000 114006
$(2)   000000
$(0)   114007 114042
$(2)   114043 114260
$(0)   114261 115033
$(0)   115034 115054
$(2)   BLANKCOMMON
$(0)   115055 115122
$(2)   BLANKCOMMON,
$(2)   115123 115202
$(0)   115203 115222
$(2)   GSPCTL
```



```
DTCTCG/FE$81GSP

URSSRH/UNCC
NEXP1$/NAGFORFUN10
URZMSK/UNCC

URSORT/NAGUTILITY02
EXP3/NAGFORFUN10
NR1CK/NAGNLINREG00
GRDLTNSGSP/#39
BASIS1/PSPRL00
SQRT$/NAGFORFUN10
PL0007/PSPRL10
NFTCH$/FORIO
FORIO34/FORIO
ALOG1/NAGFORFUN10
PL0005$GSP/-39
NR1FRV/NAGNLINREG00

PL0004$GSP/#39
NTA$$/FORIO
FORIO32/FORIO
FORIO33/FORIO
NR1SRT/NAGNLINREG00

NR1PUT/NAGNLINREG00

NR1RD/NAGNLINREG00

NR1DRM/NAGNLINREG00
NR1GRD/NAGNLINREG00

URTIMF/NAGUTILITY03
NR1TM$/NAGNLINREG00

NR1ART/NAGNLINREG00

NR1NST/NAGNLINREG00

NR1FM4/NAGNLINREG00

NR1MC/NAGNLINREG07
NR1O1F/NAGNLINREG00
NR1SYD/NAGNLINREG00

NR1LTZ/NAGNLINREG00

NR1LT$/NAGNLINREG00

NR1RS$/NAGNLINREG00
NR1LTT/NAGNLINREG01

NR1PTV/NAGNLINREG00
NR1HV/NAGNLINREG00
NR1SP$/NAGNLINREG00

URDAT$/NAGUTILITY00

$(1)    006730 006203
$(3)    GSHCTL
$(1)    006204 006262
$(1)    006263 006332
$(1)    006333 006464

$(1)    006465 007308
$(1)    007309 007407
$(1)    007410 007425
$(1)    007026 010143
$(1)    010144 010714
$(1)    010715 010784
$(1)    010785 011036
$(1)    011037 011337
$(1)    011340 011633
$(1)    011634 011751
$(1)    011752 012101
$(1)    012102 012307

$(1)    012310 012346

$(1)    012347 014677
$(1)    014700 015266
$(1)    015267 015516

$(1)    015517 015652

$(1)    015653 015756

$(1)    015755 016034
$(1)    016035 016175

$(1)    016176 016204
$(1)    016205 016271

$(1)    016272 016412

$(1)    016413 020115
$(3)    NR1BLK
$(1)    020116 020357

$(1)    020360 023126
$(1)    023127 023723
$(1)    023724 026043

$(1)    026044 026130

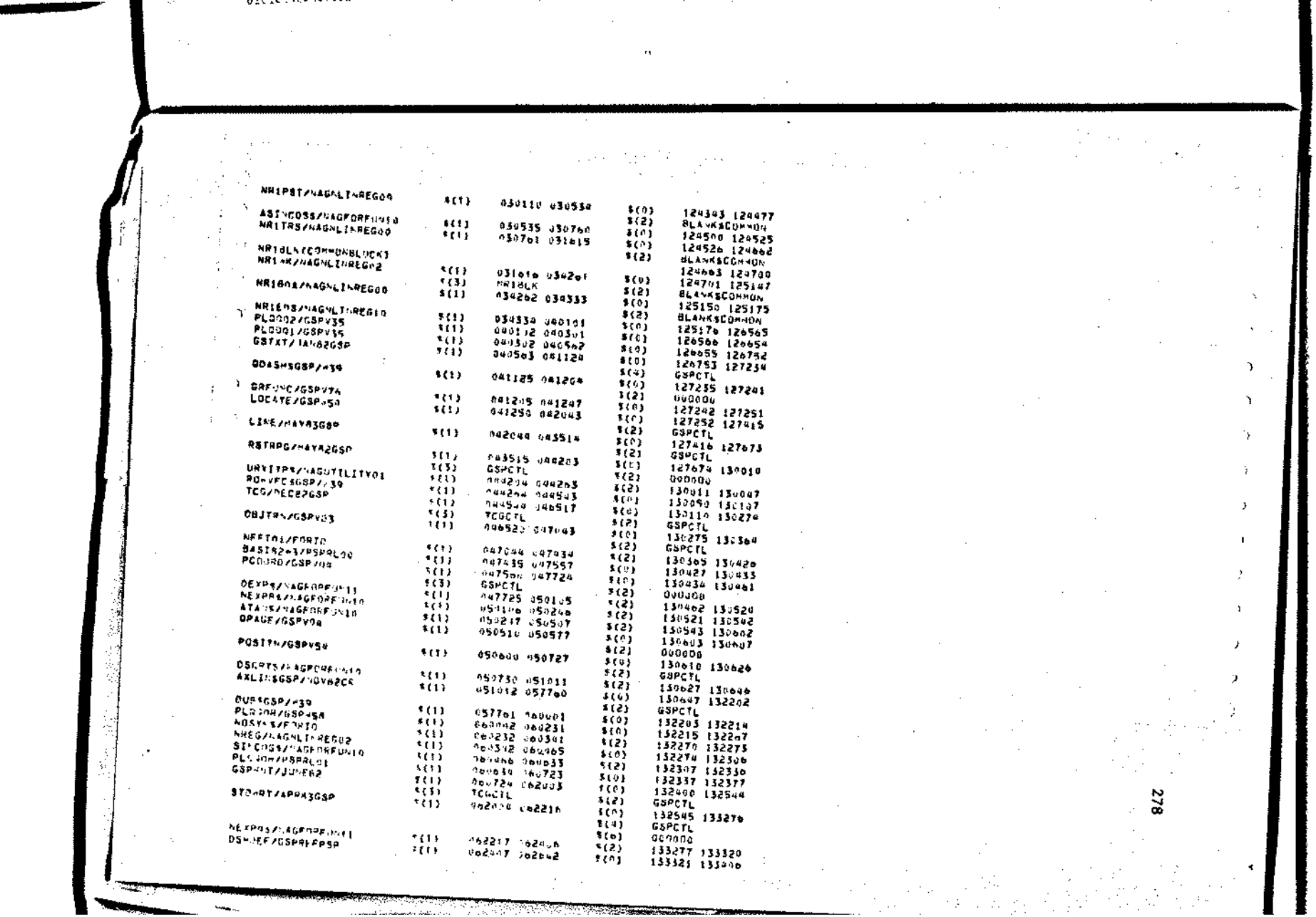
$(1)    026131 026226

$(1)    026227 026312
$(1)    026313 026405
$(3)    NR1BLK
$(1)    026406 027046
$(1)    027047 030012
$(1)    030013 030070

$(1)    030071 030107

$(0)    115223 115340
$(2)    TCGCTL

$(0)    115341 115341
$(0)    115342 115367
$(2)    BLANK$COMMON
$(0)    115370 115527
$(2)    115530 115553
$(0)    115554 115554
$(0)    115555 115747
$(0)    115750 116153
$(2)    116154 116165
$(0)    116166 116207
$(2)    116210 116245
$(2)    116246 116270
$(2)    116271 116344
$(0)    116345 116374
$(0)    116375 116502
$(2)    BLANK$COMMON
$(0)    116503 116513
$(2)    116514 116560
$(2)    116561 121251
$(2)    121252 121260
$(0)    121261 121312
$(2)    BLANK$COMMON
$(0)    121313 121336
$(2)    BLANK$COMMON
$(0)    121337 121412
$(2)    BLANK$COMMON
$(0)    121413 121430
$(0)    121431 121464
$(2)    BLANK$COMMON
$(0)    121465 121465
$(0)    121466 121501
$(2)    BLANK$COMMON
$(0)    121502 121557
$(2)    BLANK$COMMON
$(0)    121560 122374
$(2)    BLANK$COMMON
$(0)    122375 122455
$(2)    BLANK$COMMON
$(0)    122456 123351
$(0)    123352 123466
$(0)    123467 123660
$(2)    BLANK$COMMON
$(0)    123661 123733
$(2)    BLANK$COMMON
$(0)    123734 123736
$(2)    BLANK$COMMON
$(0)    123735 123766
$(0)    123767 124020
$(2)    BLANK$COMMON
$(0)    124021 124146
$(0)    124147 124320
$(0)    124325 124340
$(2)    BLANK$COMMON
$(0)    124341 124342
```



NR1PST/NAGNLI%REG04 $(1) 030110 030534
ASI%COSS/%AGFORFUN10 $(0) 124343 124477
NR1TRS/%AGNLI%REG00 $(1) 030535 030760 $(2) BLANK$COMMON
$(1) 030761 031615 $(0) 124500 124525
NR1BLK[COMMON$BLOCK] $(0) 124526 124662
NR1**/%AGNLI%REG02 $(2) BLANK$COMMON
$(1) 031616 034267 124663 124700
NR1BOX/%AGNLI%REG00 $(3) NR1BLK $(0) 124701 125147
$(1) 034262 034333 $(2) BLANK$COMMON
NR1ERS/%AGNLI%REG10 $(0) 125150 125175
PL0002/GSPV35 $(1) 034334 040101 $(2) BLANK$COMMON
PL0001/GSPV35 $(1) 040102 040302 $(0) 125176 126565
GSTXT/'IA%02GSP $(1) 040302 040602 $(0) 126566 126654
$(1) 040603 041120 $(0) 126655 126752
$(0) 126753 127234
$(4) GSPCTL
$(0) 127235 127241
$(2) 000000
$(0) 127242 127251
$(0) 127252 127415
$(2) GSPCTL
$(0) 127416 127673
$(2) GSPCTL
$(1) 127674 130010
$(2) 000000
$(2) 130011 130087
$(0) 130090 130107
$(0) 130110 130274
$(2) GSPCTL
$(0) 130275 130364
$(2) GSPCTL
$(2) 130365 130426
$(0) 130427 130433
$(0) 130434 130461
$(2) 000000
$(2) 130462 130520
$(2) 130521 130542
$(2) 130543 130602
$(0) 130603 130607
$(2) 000000
$(0) 130610 130626
$(2) GSPCTL
$(2) 130627 130646
$(0) 130647 132202
$(2) GSPCTL
$(0) 132203 132214
$(0) 132215 132267
$(2) 132270 132275
$(0) 132276 132326
$(2) 132327 132336
$(0) 132337 132377
$(0) 132400 132544
$(2) GSPCTL
$(0) 132545 133276
$(4) GSPCTL
$(6) 000000
$(2) 133277 133320
$(0) 133321 133406

GD&SMSGSP/*39
GRF%%C/GSPV7A
LOC4TE/GSP%50
LINE/MAYA3G80
R8TRPG/%AYA2GSP
URYITP%/%ASUTILITY01
RO%VFC$GSP//39
TCG/%EC82GSP
OBJT%%/GSPV33
NFFT01/FORTD
BASIS2%3/PSPRL00
PC0%RD/GSP/08
OEXP%/%AGFORF%%11
NEXPR%/%AGFORF%%10
ATA%%/%AGFORF%%10
OPAGE/GSPV08
POSITN/GSPV50
DSCRTS/%AGFORF%%10
AXLI%$GSP/%0V82C5
OUR$GSP/*39
PLR%OR/GSP*58
NOSY%$/FORTD
NREG/%AGNLI%REG02
SI%COS%/%AGFORFUN10
PL%00%/PSPRL01
GSP%UT/JUMP62
STO%RT/APP%3GSP
NEXPR%/%AGFORF%%11
DSMJEF/GSPRIFPSP

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GSPSET/JANB4GSP
                    $(1)    062643 063351
                    $(3)    TCGCTL
TRHRIT/NOVR2GSP
                    $(1)    063352 063554
PAGDEF/MAY82GSP
                    $(1)    063555 063766
                    $(3)    GSPCTL
PAGE/MAY82GSP
                    $(1)    063767 064412
                    $(3)    GSPCTL
ARGSET/GSPV34
                    $(3)    064413 064531
WRTEXT/APR83GSP
                    $(1)    064532 065324
URRNTD/UNCC
ERUS
                    $(1)    065325 065333
PLOUGE/JAN82GSP
                    $(1)    065334 065710
STDUGE4/DEC83GSP
                    $(1)    065711 067512
                    $(3)    000000
TCGCTL(COMMONBLOCK)
SHOIMG/JAN84GSPCR
                    $(1)    067513 077360
                    $(3)    TCGCTL
VECTOR/GSP84A
                    $(1)    077361 100110
UNERRS/MAGPOPEINA2
DLOGS/MAGFUNFUN10
SCALE/83SP84A
                    $(1)    100111 100721
                    $(1)    100722 101054
                    $(1)    101055 103320
DORUDC(COMMONBLOCK)
GSPCTL(COMMONBLOCK)
GRAPH1/NOV82GSP
                    $(1)    103327 107122
FORT%1/F(R)0
                    $(3)    000036
LIM(COMMONBLOCK)
                    $(1)    107123 112314
BLANK$COMMON(COMMONBLOCK)
PRPL
                    $(1)    112315 112600
OTP
                    $(3)    LIM
                    $(1)    112601 113158
SURF
                    $(3)    LIM
                    $(1)    113157 113341
MAIN
                    $(3)    LIM
                    $(1)    113342 113537
                    $(3)    LIM

SYS$*RLIB$, LEVEL 34
END MAP,  ERRORS: 0  TI-E: 24.%09  STORAGE: 20085/7/040777/0100777
*XQT
                    $(2)    000000
                    $(0)    133407 134237
                    $(2)    GSPCTL
                    $(4)    000000
                    $(0)    134240 134332
                    $(4)    TCGCTL
                    $(6)    GSPCTL
                    $(0)    134333 134414
                    $(2)    000000
                    $(0)    134415 134541
                    $(2)    000000
                    $(0)    134542 134567
                    $(0)    134570 134675
                    $(2)    GSPCTL
                    $(0)    134676 134676
                    $(0)    134677 135001
                    $(2)    GSPCTL
                    $(0)    135002 135175
                    $(2)    GSPCTL
                            135176 135203
                    $(0)    135204 136461
                    $(2)    GSPCTL
                    $(0)    136462 136520
                    $(2)    GSPCTL
                    $(2)    136521 137160
                    $(2)    137161 137266
                    $(0)    137267 140043
                            140044 140125
                            140126 140572
                    $(0)    140573 142077
                    $(2)    GSPCTL
                    $(2)    142100 143174
                            143175 143177
                            143200 143261
                    $(0)    143202 143365
                    $(2)    BLANK$COMMON
                    $(0)    143366 143455
                    $(2)    BLANK$COMMON
                    $(0)    143456 143511
                    $(2)    BLANK$COMMON
                    $(0)    143512 145341
                    $(2)    BLANK$COMMON
```

MADISON ACADEMIC COMPUTING CENTER - SUBROUTINE NREG
CEPHALEXIN NONFASTING MEAN

05/23/84 TRANSMITTAL NO. AU5066 CALL 1 PAGE 1

0----F----0----T----0----(13A6)(2X,I3,2X,I3,3X,I2

S U M M A R Y O F T H E D A T A

ITEMS

ITEMS(1): NEXP = 11 NUMBER OF EXPERIMENTS
ITEMS(2): NVAR = 1 NUMBER OF VARIABLES
ITEMS(3): NP = 4 NUMBER OF PARAMETERS

ITEMS(4): NROBS = 11 ROW DIMENSION OF ARRAY YOBS
ITEMS(5): NRPRD = 11 ROW DIMENSION OF ARRAY YPRD
ITEMS(6): NRWTS = 11 ROW DIMENSION OF ARRAY WTS

ITEMS(7): METHOD = 'MARQ' MARQUARDT'S METHOD
ITEMS(8): KWTS = 'NONE' NO WEIGHTING
ITEMS(9): KDERIV = 'NREG' NREG CALCULATED DERIVATIVES
ITEMS(10): KSYM = 'CENT' CENTRAL DIFFERENCES
ITEMS(11): KDIFF = 'REL' RELATIVE DIFFERENCES

ITEMS(12): MAXIT = 50 LIMIT ON NUMBER OF ITERATIONS

TOLERANCES

TOL(1) = 1.5000000-06 REL. CHANGE IN A PARAMETER
TOL(2) = 1.0000000-09 REL. CHANGE IN SUM OF SQUARES
TOL(3) = 0.0000000 RATIO TO INITIAL SUM OF SQUARES
TOL(4) = 1.0000000-16 PIVOT TOLERANCE

SUNDRIES

NUMBER OF ACTIVE PARAMETERS = 4
NUMBER OF CELLS OF SCRATCH REQUIRED = 195

PARAMETERS

NO.	NAME	INITIAL VALUE	PROPORTION FOR DERIV. ESTIMATE	LOWER BOUND	UPPER BOUND
1	ABS/1	7.0000000-01	5.0000000-03	1.0000000-04	1.0000000+04
2	M+E/1	8.0000000-01	5.0000000-03	1.0000000-04	1.0000000+04
3	FDV/1	2.5000000+01	5.0000000-03	1.0000000-04	1.0000000+04
4	LAG/1	8.0000000-01	5.0000000-03	1.0000000-04	1.0000000+04

S I N G U L A R V A L U E S A N D V E C T O R S A T I N I T I A L P O I N T

STG. VALUES		1.7669810+01	1.0789050+01	7.0477818+00	1.0222198-02
PAR.	1 ABS/1	-.3967880	.4474988	-.7999597	.0278822
	2 M+E/1	-.9204786	-.8759040	-.4808530	-.0538351
	3 FDV/1	-.0142898	.1842879	-.0058044	-.9990383
	4 LAG/1	.9167454	.1754057	-.3589017	-.0004907

SETUP TIME = .050 SECONDS

(13A6)(2X,I3,2A,I3,3X,I2

THE ITERATIONS

ITERATION NO. 1

	BASE POINT	TEST POINT	TEST POINT	TEST POINT	TEST POINT	TEST POINT
SUM OF SQUARES	1.967214E+01	1.984649E+02	1.986501E+02	1.986522E+02	1.986915E+01	2.007940E+00
L	0	0	1	2	3	4
LAMBDA	0.0000000	0.0000000	1.0449429-04	3.1348557-04	7.3148965-04	1.5676700-03
GAMMA	1.000000E+00	1.9667573-01	3.8932634-01	7.7895504-01	1.0000000+00	1.0000000+00
ANGLE IN DEGREES	.0000	89.8884	89.7965	89.8121	89.2437	88.5072
MAX. PIVOT REDUCTION	1.000000E+00	1.3722471-03	2.1403884-03	4.2644638-03	8.4645390-03	1.6678228-02
PAR. 1 ABS/I	7.0000000-01	1.4231513+00	1.3774223+00	1.2859819+00	9.6043856-01	7.5285288-01
2 THETA/I	8.1999999-01	4.2124115-02	5.4482751-02	7.9195544-02	4.0090469-01	8.7708501-01
3 FOV/I	2.5400000+01	1.0000000-04	1.0000000-04	1.0000000-04	9.0839438+00	1.7238001+01
4 LAG/I	4.0000000-01	8.3588674-01	8.2025540-01	8.0049321-01	7.9222104-01	7.9622769-01

CUMULATIVE NO. OF FUNCTION CALLS = 6

ITERATION TIME = .038 SECONDS

CUMULATIVE TIME = .084 SECONDS

ITERATION NO. 10

	BASE POINT	TEST POINT	TEST POINT
SUM OF SQUARES	2.317217E+00	2.3174268+00	2.3171718+00
L	0	5	6
LAMBDA	0.1911287-03	3.1951475-03	6.1965906-03
GAMMA	1.000000E+00	1.0000000+00	1.0000000+00
ANGLE IN DEGREES	89.593	87.9090	87.8563
MAX. PIVOT REDUCTION	2.4778397-02	1.2503873-02	2.4156818-02
PAR. 1 ABS/I	7.0763775-01	7.1796166-01	7.2298973-01
2 THETA/I	7.2140577-01	7.3006530-01	7.2597235-01
3 FOV/I	1.0427343+01	1.0270887+01	1.0949085+01
4 LAG/I	7.8377116-01	7.8349078-01	7.8350030-01

CUMULATIVE NO. OF FUNCTION CALLS = 26

ITERATION TIME = .000 SECONDS

CUMULATIVE TIME = .220 SECONDS

THE LAST ITERATION

		BASE POINT		TEST POINT
ITERATION NO. 15				
SUM OF SQUARES		2.3170514+00		2.3172378+00
L		13		14
LAMBDA		3.3831525+01		1.1850266+02
GAMMA		1.2500000-01		6.2500000-02
ANGLE IN DEGREES		26.6987		16.3497
MAX. PIVOT REDUCTION		9.9437214-01		1.7003120+00
PAR. 1	ABS/1	7.2397270+01		7.2397231+01
2	=+F/1	7.2505640-01	8	7.2565640-01
3	#OV/1	1.8923966+01		1.8923966+01
4	LAG/1	7.8367040-01		7.8366967-01

CUMULATIVE NO. OF FUNCTION CALLS = 48 ITERATION TIME = .016 SECONDS CUMULATIVE TIME = .358 SECONDS

ITERATION TERMINATES:
MAX. RELATIVE CHANGE IN A PARAMETER .LT. TOL(1) = 1.5000030-06

FORMAT(1H0,15A6/(2X,13,2X,13,3X,E2

FINAL PARAMETER VALUES

			FINAL VALUE
AR,	1	ABS/1	7.239727D+01
	2	M+E/1	7.25[illegible]D+01
	3	FOV/1	1.892396D+01
	4	LAG/1	7.63[illegible]D+01

SUM OF SQUARES = 2.317051D+00

ITERATION SUMMARY

ITER NO.	SUM OF SQUARES	L	LAMBDA	GAMMA	ANGLE	NO. PIVOT FAILURES	NO. BOUNDS FAILURES	MAX. PIVOT REDUCTION	TIME IN SECONDS
0	1.047213D+01	3	0.0000D+00						
1	2.[illegible]D+00	4	1.5[illegible]D-03	1.000000D+00	.0000	0	0	1.0000000D+00	.000
2	2.467[illegible]D+00	5	3.[illegible]D-03	1.[illegible]D+00	85.5072	0	0	1.6078228D-02	.034
3	2.35[illegible]D+00	6	6.5272D-03	1.0303000D+00	89.1786	0	0	1.1513751D-02	.039
4	2.33[illegible]D+00	5	3.[illegible]D-03	1.0303000D+00	89.2506	0	0	1.5806925D-02	.019
5	2.32[illegible]D+00	6	6.24[illegible]D-03	1.0000000D+00	89.4365	0	0	1.2116487D-02	.014
6	2.31[illegible]D+00	7	1.2[illegible]D-02	1.0000000D+00	89.3188	0	0	1.3297121D-02	.015
7	2.317[illegible]D+00	8	2.[illegible]D-02	1.0000000D+00	89.2986	0	0	4.0191780D-02	.012
8	2.317[illegible]D+00	9	4.[illegible]D-02	1.0000000D+00	89.2930	0	0	1.1910018D-02	.010
9	2.317[illegible]D+00	8	1.9722691D-02	1.0000000D+00	89.2930	0	0	2.3881119D-02	.000
10	2.317[illegible]D+00	9	3.[illegible]D-02	1.0000000D+00	85.7239	0	0	2.4738397D-02	.028
11	2.317[illegible]D+00	10	6.[illegible]D-02	1.0000000D+00	89.0693	0	0	2.4136814D-02	.000
12	2.317[illegible]D+00	11	1.[illegible]D-01	1.0000000D+00	87.8505	0	0	8.6024829D-02	.032
13	2.317[illegible]D+00	12	2.[illegible]D-01	1.0000000D+00	87.6088	0	0	1.3931088D-01	.029
14	2.317[illegible]D+00	13	3.[illegible]D-01	1.4000000D+00	89.5705	0	0	2.5218744D-01	.015
15	2.317[illegible]D+00	12	1.[illegible]D-02	1.2500000D+01	81.7208	0	0	9.9437214D-01	.021
				6.2500000D+02	28.6987	0	0	1.7003126D+00	.016
					10.3497	0	0		

SINGULAR VALUES AND VECTORS AT TERMINAL POINT

SING. VALUES			1.403267D+01	1.252131D+01	5.436669D+00	1.9755768D-08
PAR,	1	ABS/1	-.5295883	.3806156	-.7571075	-.0381593
	2	M+E/1	-.4254524	.4293511	-.3639186	.0382529
	3	FOV/1	-.5187677	-.0018863	-.0149890	.9985392
	4	LAG/1	.1805819	-.8191428	-.5023345	.0000050

MADISON ACADEMIC COMPUTING CENTER - SUBROUTINE NREG
CEPHALEXIN NONFASTING MEAN

05/23/84 TRANSMITTAL NO. A05668 CALL 1 PAGE 5

NORMALIZING ELEMENTS AND CORRELATION MATRIX

PARAMETER NO.		1	2	3	4
NORM. ELTS.		1.9515539+02	1.9362886+02	5.0588186+03	1.2204585+01
PAR. 1	ABS/1	1.0000000			
2	M+E/1	+.9999994	1.0000000		
3	FDV/1	+.9999997	+.9999999	1.0000000	
4	LAG/1	+.2048189	+.2055199	+.2053006	1.0000000

CONFIDENCE LIMITS ON LINEAR HYPOTHESIS

		LOWER LIMIT	FINAL VALUE	UPPER LIMIT
PAR. 1	ABS/1	-2.2153312+02	7.2397270-01	2.2298106+02
2	M+E/1	-2.2207685+02	7.2505644-01	2.2352696+02
3	FDV/1	-5.7971173+03	1.8923964+01	5.8348652+03
4	LAG/1	6.4254621-01	7.8567046-01	9.2479472-01

EXPLORATION

			LOWER TEST	SUM OF SQUARES	LINEAR EST. OF SUM OF SQUARES	UPPER TEST	SUM OF SQUARES	LINEAR EST. OF SUM OF SQUARES
PAR. 1	ABS/1	B	1.0000000-04	1.9455347+02	5.2035143+01	5.0288245+01	2.4022722+02	1.2243442+05
2	M+E/1	B	1.0000000-04	8.1832098+02	1.9581407+01	5.0425533+01	1.8878085+02	1.0106266+05
3	FDV/1	B	1.0000000-04	1.9666797+02	8.8849438+00	1.4729093+03	1.1355503+06	1.9220581+08
4	LAG/1		7.4838968-01	2.4422816+00	2.4588340+00	8.1895152-01	2.4764225+00	2.4588340+00

(13A6)(2X,I3,2A,13,5X,12

F I N A L F U N C T I O N V A L U E S A N D R E S I D U A L S

ROOT MEAN SQUARE RESIDUAL = 5.7533237-01
THIS IS THE SCALE UNIT IN THE GRAPH OF THE RESIDUALS.

NO. DEGREES OF FREEDOM = 7

EXPT. NO.	VAR. NO.	PREDICTION	OBSERVATION	RESIDUAL	GRAPH OF RESIDUAL
1	1	0.0000000	0.0000000	0.0000000	
2	1	0.0000000	0.0000000	0.0000000	*
3	1	0.0000000	2.0000000-01	2.0000000-01	*
4	1	0.0000000	4.0000000-01	4.0000000-01	****
5	1	2.5336697+00	2.8000000+00	2.6613030-01	********
6	1	5.[illegible]	2.8000000+00	-4.4086509-01	******
7	1	6.0334339+00	5.4000000+00	-3.3345390-01	*********
8	1	6.7800941+00	6.0000000+00	1.1910588-01	******
9	1	6.[illegible]	6.9000000+00	6.0466334-01	***
10	1	6.2361834+00	6.7300000+00	3.1391655-01	**********
11	1	1.0521817+00	5.0000000-01	-1.1321817+00	******

-2 -1 0 +1 +2

TIME SINCE END OF THE LAST ITERATION = .141 SECONDS TOTAL TIME = .477 SECONDS

R-SQUARED FOR FUNCTION = .9881
COR FOR FUNCTION = .9864

TIME

One-Compartment Model with Zero-Order Absorption and First-Order Elimination

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12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890**SEQ NO, 01
12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890**SEQ NO, 01
12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890**SEQ NO, 01
12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890**SEQ NO, 01
*  *  *  *  *  *  SPERRY 1180 TIME/SHARING EXEC --- MULTI-PROCESSOR SYSTEM --- VER.  MACC 38.21  SITE *  MACC     *  *  *  *  *  *
RUNID: 816134          PROJECT: 41000       USER: 9000100793          CREATED ON MAY 23, 1984 AT 21:44:26    INPUT DEVICE: CR
            FILE NAME: PR000816134            PART NUMBER: 00          PRINTED ON MAY 23, 1984 AT 21:45:36   OUTPUT DEVICE: PR2

@RUN KWOK,41000,9000100793,$M,500
@FOR,SI      ,MAIN
FORTRAN-MACC 1.175-05/23/84-21:44:27            MAIN
00101     1.          PARAMETER
00101     2.         1NEXPD = 50,
00101     3.         2NVAR = 1,
00101     4.         3NPD = 7,
00101     5.         4NROBS = NEXPD,
00101     6.         5NRPRD = NEXPD,
00101     7.         6NS=(17+NEXPD+NVAR)+NPD+2+NRPRD+NVAR+2*(NPD-1)*(NPD+2)+1
00102     8.          REAL THINT(NPD),THFIN(NPD),YOBS(NROBS,NVAR),YPRD(NROBS,NVAR),S(NS)
00102     9.         1,TOL(4),T(NEXPD),BNDLO(NPD),BNDHI(NPD),NAMES(NPD),DIFF(NPD),FIX(NP
00102    10.         2D)
00103    11.          INTEGER ITEMS(12),INFO(7),IDENT(13),NEXP,NP
00104    12.          COMMON T
00105    13.          COMMON/LIN/NEXP1,NEXP2,K
00106    14.          COMMON/TAA/TAA
00107    15.          DATA FIX/7*1.0/
00111    16.          DATA DIFF/7*0.005/
00113    17.          DATA BNDLO/7*1.0E-4/
00115    18.          DATA BNDHI/2*1.0E4,1.0E6,4*1.0E4/
00117    19.          DATA NAMES/6HKO    ,6HK1    ,6HV     ,6HFFO/1 ,3*6H      /
00121    20.          DATA TOL/1.5E-6,1.0E-9,0.0,1.0E-16/
00123    21.          DATA INFO/1,10,5*1/
00125    22.          DATA ITEMS/
00125    23.         1 1,NVAR,1,NROBS,NRPRD,0,'NARG','NONE','NREG','CENT','REL',50/
00127    24.          READ(5,110) (IDENT(I), I=1,13)
00135    25.      110  FORMAT(13A6)
00136    26.          READ,NEXP1,NEXP2,NP
00143    27.      120 FORMAT(2X,I3,2X,I3,3X,I2)
00144    28.          NEXP = NEXP1 + NEXP2
00145    29.          ITEMS(1) = NEXP
00146    30.          ITEMS(3)=NP
00146    31.      C   TAA IS THE CRITICAL TIME UNCORRECTED FOR LAG
00147    32.      121 FORMAT(F10.5)
00150    33.          READ(5,100) (THINT(I), I=1,NP )
00156    34.          READ 100,(YOBS(I,1),I=1,NEXP1)
00164    35.          READ (5,100) (T(I), I = 1,NEXP1)
00172    36.          K = NEXP1+ 1
00173    37.          IF (K.GE.NEXP) GO TO 44
00175    38.          READ (5,100) (YOBS(I,1),I=K,NEXP)
00203    39.          READ (5,100) (T(I),I=K,NEXP)
00211    40.       44 CONTINUE
00212    41.      100  FORMAT(8F10.5)
00213    42.          EXTERNAL F,DERIV
00214    43.          CALL NREG(THINT,NAMES,BNDLO,BNDHI,FIX,DIFF,THFIN,YOBS,YPRD,
00214    44.         1'NONE',F,'NONE',ITEMS,TOL,INFO,IDENT,S)
00215    45.          CALL OUTPUT(NEXP,NEXPD,THFIN,NVAR,NP,NRPRD,YPRD,YOBS)
00216    46.          END

      END OF COMPILATION:        NO  DIAGNOSTICS.
@FOR,SI    , SUBF
FORTRAN-MACC 1.175-05/23/84-21:44:28            SUBF
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```
00101      1.          SUBROUTINE F(TH,Y,NEXP,NVAR,NP,NRPRD)
00102      2.          REAL KO,KI
00103      3.          DIMENSION TH(1),Y(NRPRD,1),T(1)
00104      4.          COMMON T
00105      5.          COMMON/LIN/NEXP1,NEXP2,K
00106      6.          COMMON/YAA/TAA
00107      7.          TH1 = TH(1)
00110      8.          TH2 = TH(2)
00111      9.          TH3 = TH(3)
00112     10.          TAA=TH3
00113     11.          TH4 = TH(4)
00114     12.          KO=TH1
00115     13.          KI=TH2
00116     14.          TLAG=TH4
00117     15.          TAU=TAA-TLAG
00117     16.     C    TAU IS THE CRITICAL TIME CORRECTED FOR LAG
00120     17.          V=1.0
00121     18.          DO 10 I = 1,NEXP1
00124     19.          IF(T(I).GT.TLAG) GO TO 3
00126     20.          Y(I,1)=0.0
00127     21.          GO TO 10
00130     22.        3 Q=T(I)-TLAG
00131     23.          IF(T(I).GT.TAU) GO TO 5
00133     24.          Y(I,1)=(KO/(KI*V))*(1.0-EXP(-KI*Q   ))
00134     25.          GO TO 10
00135     26.        5 Y(I,1)=(KO/(KI*V))*(1.0-EXP(-KI*TAU))*EXP(-KI*(Q   -TAU))
00136     27.       10 CONTINUE
00140     28.          RETURN
00141     29.          END

      END OF COMPILATION:        NO  DIAGNOSTICS.
@FOR,SI     ,OTP
FORTRAN=HACC 1.175-05/23/84-21:44:30                OTP
00101      1.          SUBROUTINE OUTPUT(NEXP,NEXPD,TNFIN,NVAR,NP,NRPRD,YPRD,YOBS)
00102      2.          DIMENSION T(1),YOBS(1),YPRD(1)
00103      3.          COMMON T
00104      4.          COMMON/LIN/NEXP1,NEXP2,K
00105      5.          K = 0
00106      6.      200 CONTINUE
00107      7.          SO2 = 0.0
00110      8.          SP2 = 0.0
00111      9.          SC = 0.0
00112     10.          SYOBS = 0.0
00113     11.          SDEV = 0.0
00114     12.          COR = 0.0
00115     13.          RS = 0.0
00116     14.          YON = 0.0
00117     15.          YPN = 0.0
00120     16.          K = K + 1
00121     17.          IF (K.EQ.1) GO TO 210
00123     18.          LL = NEXP1 + 1
00124     19.          LU = NEXP
00125     20.          GO TO 250
00126     21.      210 LL = 1
00127     22.          LU = NEXP1
00130     23.      250 DO 10 I = LL,LU
00133     24.          SYOBS = SYOBS + YOBS(I)**2
```

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00134     25.          SDEV = SDEV + (YOBS(I)-YPRD(I))**2
00135     26.          YOM=YOM+YOBS(I)
00136     27.          YPM=YPM+YPRD(I)
00137     28.       10 CONTINUE
00140     29.          RS = (SYOBS-SDEV)/SYOBS
00142     30.          YOM=YOM/NEXP
00143     31.          YPM=YPM/NEXP
00144     32.          DO 20 I=1,NEXP
00147     33.          SC = SC+(YOBS(I)-YOM)*(YPRD(I)-YPM)
00150     34.          SO2 = SO2 + (YOBS(I)-YOM)**2
00151     35.          SP2 = SP2 + (YPRD(I)-YPM)**2
00152     36.       20 CONTINUE
00154     37.          COR = SC/(SQRT(SO2)*SQRT(SP2))
00155     38.          WRITE(4,100) K,RS,K,COR
00163     39.      100 FORMAT(1HO,'R-SQUARED FOR FUNCTION',I1,' =',F6.4/1X,'COR FOR FUNCT
00163     40.         1ION',I1,' =',F6.4)
00164     41.          IF (NEXP2.EQ.0) GO TO 66
00166     42.          IF(K.GT.1) GO TO 300
00170     43.          SYOBS1 = SYOBS
00171     44.          SDEV1 = SDEV
00172     45.          GO TO 200
00173     46.      300 SYOBS = SYOBS + SYOBS1
00174     47.          SDEV = SDEV + SDEV1
00175     48.          RS = (SYOBS-SDEV)/SYOBS
00176     49.          WRITE(4,111) RS
00201     50.      111 FORMAT(1X,'OVER ALL R-SQUARED=',F6.4)
00202     51.       66 CONTINUE
00203     52.          CALL PRPL (NEXP,NEXPO,THFIN,NVAR,NP,NRPRO,YPRD,YOBS)
00204     53.          RETURN
00205     54.          END

      END OF COMPILATION:      NO  DIAGNOSTICS.
@FOR,S1      ,PRPL
FORTRAN-HACC 1.175-05/23/84-21:44:13                    PRPL
00101     1.          SUBROUTINE PRPL (NEXP,NEXPO,THFIN,NVAR,NP,NRPRO,YPRD,YOBS)
00102     2.          DIMENSION T(1),YOBS(NRPRO,1),YPRD(NRPRO,1)
00103     3.          COMMON T
00104     4.          COMMON/LIM/NEXP1,NEXP2,K
00105     5.          NN = 0
00106     6.          CALL DEVSET(4HPRNTER)
00107     7.       200 CONTINUE
00110     8.          NN = NN + 1
00111     9.          IF(NN.EQ.1) GO TO 50
00113     10.         DO 140 I = 1,NEXP2
00114     11.         NN = NEXP1 + 1
00117     12.         YOBS(I,1) = YOBS(NN,1)
00120     13.      140 T(I) = T(NN)
00122     14.         LU = NEXP2
00123     15.         GO TO 60
00124     16.      50 CONTINUE
00125     17.         LU = NEXP1
00126     18.      60 CONTINUE
00127     19.         YMAX = YOBS(1,1)
00130     20.         DO 10 I = 2,LU
00133     21.      10 YMAX = AMAX1(YMAX,YOBS(I,1))
00135     22.         IF( NN.EQ.2 ) GO TO 170
00137     23.         YMAX = T(1)
```

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00190     24.          DO 15 I = 2,NEXP
00193     25.       15 THAX = AMAX1(THAX,T(I))
00195     26.          BMAX = THAX/(NEXPO-NEXP1-NEXP2)
00196     27.          NM1 = NEXP1 + NEXP2 + 1
00197     28.          NM2 = NEXP1 + NEXP2 + 2
00150     29.          T(NM1) = 0.0
00151     30.          DO 30 I = NM2,NEXPO
00154     31.          J = I - 1
00155     32.       30 T(I) = T(J) + BMAX
00157     33.          IF ( KK.EQ.1 ) GO TO 160
00161     34.          GO TO 170
00162     35.      160 NEXI = NEXP1
00163     36.          NEXP1 = NEXPO
00164     37.          K = NEXPO
00165     38.          GO TO 180
00166     39.      170 CONTINUE
00167     40.          NEXP1 = 1
00170     41.          K = 1
00171     42.          NEXP = NEXPO
00172     43.      180 CONTINUE
00173     44.          CALL F(THFIN,YPRO,NEXPO,NVAR,NP,NMPRO)
00174     45.          NEXP1 = NEXI
00175     46.          DO 20 I = 1,NEXPO
00200     47.       20 THAX = AMAX1(THAX,YPRO(I,1))
00202     48.          YSCLE = YMAX/8.8
00203     49.          TSCLE = THAX/8.8
00204     50.          CALL GRAPHIT,6HLINEAR,YPRO,6HLINEAR,*NEXPO,1S,5HBLANK,*TIMES$*,*FU
00204     51.         1NCTION VALUES$$*,4HAXES,*PLOT OF OBSERVED,O**S AND PREDICTED,P**S
00204     52.         2VALUES$$*,12.0,12.0)
00205     53.          CALL GRAPHMIT,*SCALI*,YOBS,*SCALI*,LW,I4,5HBLANK)
00204     54.          IF (NEXP2.EQ.0) GO TO 66
00210     55.          IF(NM.EQ.1) GO TO 200
00212     56.       66 CONTINUE
00213     57.          RETURN
00214     58.          END

      END OF COMPILATION:        NO  DIAGNOSTICS.
BMAP
MAP 30R1U08 5L18 2 05/23/84 21:44:33
AFCM STATUS OF OUTPUT ELEMENT=UNKNOWN
QUARTER/THIRD WORD INSENSITIVE


BANKNAME(BDI)        ADDRESS LIMITS
$(BANKIN)            001000 113607     38280 (BANK WORDS DECIMAL
$DBANK(S)            114000 145354     13037 DBANK WORDS DECIMAL
STARTING ADDRESS  113415


          SEGMENT $MAIN$          001000 113607     114000 145354

UR1TR1/UNCC01              $(1)    001000 001165
NR1PTV/NAGNLINREGOO        $(1)    001164 001550     $(0)    114000 114125
NR1TR5/NAGNLINREGOO        $(1)    001551 002405     $(1)    114126 114262
                                                     $(2)    BLANKSCOMMON
```

```
NR1SWP/NAGNLINREGOO      $(1)    002404 003031
                                 003032 003110
UR35RH/UNCC              $(1)    003111 003160
NEXP1$/NAGFORFUN10       $(1)    003161 003312
UR2MSK/UNCC              $(1)

URSORT/NAGUTILITY02      $(1)    003313 004132
NR1CK/NAGNLINREGOO       $(1)    004133 004150
NR1SRT/NAGNLINREGOO      $(1)    004151 004400

NR1DIF/NAGNLINREGOO      $(1)    004401 005176
NR1SVD/NAGNLINREGOO      $(1)    005174 007315

DUB#GSP/M39              $(1)    007316 007336
NEXP9$/NAGFORFUN11       $(1)    007337 007526
DEXP$/NAGFORFUN11        $(1)    007527 007707
NEXP8$/NAGFORFUN10       $(1)    007710 010050
ATAN$/NAGFORFUN10        $(1)    010051 010311
NR1BOS/NAGNLINREGOO      $(1)    010312 010375
NR1LIT/NAGNLINREGOI      $(1)    010376 010546

ARGSET/GSPV38            $(3)    NR1BLK
NR1FNK/NAGNLINREGOO      $(1)    010547 010645
                         $(1)    010644 011127

NR1HV/NAGNLINREGOO       $(1)    011130 012073
NR1BOM/NAGNLINREGOO      $(1)    012074 012151

URDATE/NAGUTILITY00      $(1)    012152 012170
QPAGE/GSPV04             $(1)    012171 012260

ASINCOS$/NAGFORFUN10     $(1)    012261 012504
LOCATE/GSP#50            $(1)    012505 013300

NR1NK/NAGNLINREG02       $(1)    013301 015744
DSMCLR/GSPREFPSP         $(3)    NR1BLK
                         $(1)    015745 016004

NR1BDA/NAGNLINREGOO      $(1)    016005 016056

DLOG$/NAGFORFUN10        $(1)    016057 016211
DSQRT$/NAGFORFUN10       $(1)    016212 016273
SCALE/GSPV38             $(1)    016274 020545
PLOGAG/GSPV38            $(1)    020546 021043
NR1HO/NAGNLINREGOO       $(1)    021044 021607
NR1RD/NAGNLINREGOO       $(1)    021610 021711

NR1ORM/NAGNLINREGOO      $(1)    021712 021771
NR1MRT/NAGNLINREGOO      $(1)    021772 022112

NR1BLK[COMMONBLOCK]
NR1NST/NAGNLINREGOO      $(1)    022113 023615

NR1MSG/NAGNLINREGOO      $(3)    NR1BLK
                         $(1)    023616 024104

NFFT1$/FOR10             $(1)    024105 025150
NEXP6$/NAGFORFUN12       $(1)    025151 025400
SYMBOL/NAT81GSP          $(1)    025401 025542
                         $(0)    114243 114330
                         $(2)    BLANK$COMMON

                         $(0)    114331 114331
                         $(0)    114332 114357
                         $(2)    BLANK$COMMON
                         $(0)    114360 114517
                         $(0)    114520 114520
                         $(0)    114521 114562
                         $(2)    BLANK$COMMON
                         $(0)    114553 114647
                         $(0)    114650 115041
                         $(2)    BLANK$COMMON
                         $(0)    115042 115073
                         $(2)    115074 115115
                         $(2)    115116 115154
                         $(2)    115155 115174
                         $(2)    115177 115234
                         $(0)    115237 115270
                         $(0)    115271 115322
                         $(2)    BLANK$COMMON
                         $(0)    115323 115350
                         $(0)    115351 115431
                         $(2)    BLANK$COMMON
                         $(0)    115432 115607
                         $(0)    115610 115623
                         $(2)    BLANK$COMMON
                         $(0)    115624 115625
                         $(0)    115626 115632
                         $(2)    000000
                         $(0)    115633 115660
                         $(0)    115661 116025
                         $(2)    GSPCTL
                         $(0)    116025 116273
                         $(2)    BLANK$COMMON
                         $(0)    116274 116302
                         $(2)    000000
                         $(0)    116303 116330
                         $(2)    BLANK$COMMON
                         $(2)    116331 116436
                         $(2)    116437 116456
                         $(0)    116457 117233
                         $(0)    117234 117267
                         $(0)    117270 120012
                         $(0)    120013 120064
                         $(2)    BLANK$COMMON
                         $(0)    120067 120104
                         $(0)    120105 120162
                         $(2)    BLANK$COMMON
                                 120163 120200
                         $(0)    120201 121015
                         $(2)    BLANK$COMMON
                         $(0)    121016 121066
                         $(2)    BLANK$COMMON
                         $(2)    121067 121304
                         $(2)    121305 121344
                         $(0)    121345 121404
```

```
MCORE/GSPV94          $(1)    025563 025605
DTCTCG/FEB81GSP       $(1)    025606 027105
                      $(3)    GSPCTL
URTINF/NAGUTILITY00   $(1)    027106 027114
NRITNS/NAGNLINREG00   $(1)    027115 027201

EXPS/NAGFORFUN10      $(1)    027202 027304
PLQQQ7/PSPRL10        $(1)    027305 027354
PLQQQ5$GSP/W39        $(1)    027357 027506
PLQQQ2/GSPV35         $(1)    027507 027706
PLQQQ1/GSPV35         $(1)    027707 030167
POSITN/GSPV59         $(1)    030170 030317

GADLIN$GSP/W39        $(1)    030320 031035
BASI51/PSPRL00        $(1)    031036 031604
SQRTS/NAGFORFUN10     $(1)    031607 031656
NFTCN$/FORIO          $(1)    031657 032157
FORIO$4/FORIO         $(1)    032160 032453
ALOG$/NAGFORFUN10     $(1)    032454 032571
NRIFPV/NAGNLINREG00   $(1)    032572 032777

PLQQQ4$GSP/W39        $(1)    033000 033036
FORIO$3/FORIO         $(1)    033037 033425
NRIPUT/NAGNLINREG00   $(1)    033426 033561

NRIGRD/NAGNLINREG00   $(1)    033562 033722

NRILTN/NAGNLINREG00   $(1)    033723 034020

NRIMC/NAGNLINREG07    $(1)    034021 036567
NRIPST/NAGNLINREG00   $(1)    036570 037214

NRILTZ/NAGNLINREG00   $(1)    037215 037301

NRIEOS/NAGNLINREG10   $(1)    037302 043047
GSTXT/JAN82GSP        $(1)    043050 043411

GD4SH$GSP/W39         $(1)    043412 043471

GRFUNC/GSPV76         $(1)    043472 043534
LINE/MAY83GSP         $(1)    043535 045205

RSTRP$/MAY82GSP       $(1)    045206 045674
                      $(3)    GSPCTL
URXITP$/NAGUTILITY01  $(1)    045675 045754
ROWVEC$GSP/W39        $(1)    045755 046234
TCG/DEC82GSP          $(1)    046235 050210
                      $(3)    TCGCTL
OBJTRN/GSPV03         $(1)    050211 050534

NFFTO$/FOH10          $(1)    050535 051125
BASIS2=3/PSPRL00      $(1)    051124 051250
PCOORD/GSPV04         $(1)    051251 051415
                      $(3)    GSPCTL
AXLINS$GSP/NOV82CR    $(1)    051416 060344

                      $(2)    GSPCTL
                      $(0)    121405 121522
                      $(2)    TCGCTL
                      $(0)    121523 121523
                      $(0)    121524 121537
                      $(2)    BLANK$COMMON
                      $(2)    121540 121563
                      $(0)    121564 121605
                      $(0)    121606 121635
                      $(0)    121636 121724
                      $(0)    121725 122022
                      $(0)    122023 122041
                      $(2)    GSPCTL
                      $(0)    122042 122234
                      $(0)    122235 122440
                      $(2)    122441 122452
                      $(2)    122453 122510
                      $(2)    122511 122533
                      $(2)    122534 122607
                      $(0)    122610 122715
                      $(2)    BLANK$COMMON
                      $(0)    122716 122726
                      $(2)    122727 122735
                      $(0)    122736 122761
                      $(2)    BLANK$COMMON
                      $(0)    122762 123015
                      $(2)    BLANK$COMMON
                      $(0)    123016 123046
                      $(2)    BLANK$COMMON
                      $(0)    123047 123742
                      $(0)    123743 124077
                      $(2)    BLANK$COMMON
                      $(0)    124100 124122
                      $(2)    BLANK$COMMON
                      $(0)    124123 125512
                      $(0)    125513 125774
                      $(4)    GSPCTL
                      $(0)    125775 126001
                      $(2)    000000
                      $(0)    126002 126011
                      $(0)    126012 126267
                      $(2)    GSPCTL
                      $(0)    126270 126404
                      $(2)    000000
                      $(2)    126405 126443
                      $(0)    126444 126503
                      $(0)    126504 126670
                      $(2)    GSPCTL
                      $(0)    126671 126740
                      $(2)    GSPCTL
                      $(2)    126741 127022
                      $(0)    127023 127027
                      $(0)    127030 127065
                      $(2)    000000
                      $(0)    127056 130411
                      $(2)    GSPCTL
```

```
PLOGGR/GSPWS8
MOSYMS/FORIO
NREG/NAGNL[NREGO2
S[NCOSS/NAGFORFUN10
PLOGGH/PSPALOI
GSPRNT/JUNE82

STOWRT/APR83GSP


OSHDEF/GSPNEFPSP

GSPSET/JAN84GSP


TAWRIT/NOV82GSP


PAGDEF/MAY82GSP

PAGE/MAY82GSP

URRNID/UWCC
UWERRS/NAGFORFUNO2
PLOGGE/JAN82GSP

WRTEXT/APR83GSP

STOOGEN/DEC83GSP

TCGCTL[COMMONBLOCK]
SHOIMG/JAN84GSPCR

VECTOR/GSPWN4

OOOOOO[COMMONBLOCK]
GSPCTL[COMMONBLOCK]
GRAPH/NOV82GSP

NTABS/FORIO
FORIOS2/FORIO
ERUS
NISYMS/FORIO
FORIOSI/FORIO
TAA[COMMONBLOCK]
LIM[COMMONBLOCK]
BLANK$COMMON[COMMONBLOCK]
PRPL

OTP

SUBF


MAIN
```

```
$(1)    040365 040614
$(3)    040615 040724
$(1)    040725 041050
$(3)    041061 041214
$(1)    041217 041306
$(1)    041307 042346
$(3)    TCGCTL
$(1)    042347 042401

$(3)    042402 043035

$(1)    043036 043544
$(3)    TCGCTL

$(1)    043545 043747

$(1)    043750 044161
$(3)    GSPCTL
$(1)    044162 044405
$(3)    GSPCTL
$(1)    044406 044614
$(3)    044615 045925
$(1)    045926 046002

$(1)    046003 046575

$(1)    046576 070377
$(3)    000000

$(1)    070400 100246
$(3)    TCGCTL
$(1)    100246 100776

$(1)    100776 104571
$(3)    000000

$(1)    104672 107122

$(1)    107123 107220
$(1)    107221 112412

$(1)    112413 112756
$(3)    LIM
$(1)    112757 113254
$(3)    LIM
$(1)    113255 113414
$(3)    LIM

$(1)    113415 113607
$(3)    LIM
```

```
$(0)    130412 130464
$(2)    130465 130470
$(0)    130471 130503
$(2)    130504 130533
$(0)    130534 130574
$(0)    130575 130741
$(2)    GSPCTL
$(0)    130742 131473
$(4)    GSPCTL
$(6)    000000
$(0)    131474 131661
$(2)    000000
$(0)    131662 132412
$(2)    GSPCTL
$(4)    000000
$(0)    132413 132505
$(4)    TCGCTL
$(6)    GSPCTL
$(0)    132506 132567
$(2)    000000
$(0)    132570 132714
$(2)    000000
$(0)    132715 132715
$(2)    132716 133355
$(0)    133356 133460
$(2)    GSPCTL
$(0)    133461 133566
$(2)    GSPCTL
$(0)    133567 133762
$(2)    GSPCTL
        133763 133770
$(0)    133771 135246
$(2)    GSPCTL
$(0)    135247 135305
$(2)    GSPCTL
        135306 135367
        135370 136034
$(0)    136035 137341
$(2)    GSPCTL
$(2)    137342 137406
$(2)    137407 142077

$(2)    142100 142104
$(2)    142106 143201
        143202 143202
        143203 143205
        143206 143267
$(0)    143270 143373
$(2)    BLANK$COMMON
$(0)    143374 143463
$(2)    BLANK$COMMON
$(0)    143464 143522
$(2)    BLANK$COMMON
$(4)    TAA
$(0)    143523 145364
$(2)    BLANK$COMMON
$(4)    TAA
```

```
SYS$*ALIB$.LEVEL 19
END MAP. ERRORS: 0  TIME: 26.069  STORAGE: 20485/6/0040777/0100777
@XQT
```

S U M M A R Y O F T H E D A T A

ITEMS

ITEMS(1): NEXP	= 10	NUMBER OF EXPERIMENTS	
ITEMS(2): NVAR	= 1	NUMBER OF VARIABLES	
ITEMS(3): NP	= 4	NUMBER OF PARAMETERS	
ITEMS(4): NROBS	= 10	ROW DIMENSION OF ARRAY YOBS	
ITEMS(5): NRPRD	= 10	ROW DIMENSION OF ARRAY YPRD	
ITEMS(6): NRWTS	= 10	ROW DIMENSION OF ARRAY WTS	
ITEMS(7): METHOD	= 'MARQ'	MARQUARDT'S METHOD	
ITEMS(8): KWTS	= 'NONE'	NO WEIGHTING	
ITEMS(9): KDERIV	= 'NREG'	NREG CALCULATED DERIVATIVES	
ITEMS(10): KSYM	= 'CENT'	CENTRAL DIFFERENCES	
ITEMS(11): KDIFF	= 'REL'	RELATIVE DIFFERENCES	
ITEMS(12): MAXIT	= 60	LIMIT ON NUMBER OF ITERATIONS	

TOLERANCES

TOL(1) =	1.5000000-06	REL. CHANGE IN A PARAMETER
TOL(2) =	1.0000000-09	REL. CHANGE IN SUM OF SQUARES
TOL(3) =	0.0000000	RATIO TO INITIAL SUM OF SQUARES
TOL(4) =	1.0000000-16	PIVOT TOLERANCE

SUNDRIES

NUMBER OF ACTIVE PARAMETERS = 4
NUMBER OF CELLS OF SCRATCH REQUIRED = 189

PARAMETERS

NO.	NAME	INITIAL VALUE	PROPORTION FOR DERIV. ESTIMATE	LOWER BOUND	UPPER BOUND
1	KO	3.9800000+01	5.0000000-03	1.0000000+04	1.0000000+04
2	K1	9.0000000-01	5.0000000-03	1.0000000+04	1.0000000+04
3	V	8.3000000-01	5.0000000-03	1.0000000+04	1.0000000+04
4	FFD/1	2.3000000-01	5.0000000-03	1.0000000+04	1.0000000+04

S I N G U L A R V A L U E S A N D V E C T O R S A T I N I T I A L P O I N T

SING. VALUES		7.8434050+01	4.0181825+01	1.2582298+01	1.7444519-01
PAR.	1 KO	-.0091178	-.0091986	-.0011493	-.9999989
	2 K1	.1678413	.1509624	.9941861	-.0010105
	3 V	-.7023372	-.6750791	-.2264259	.0095058
	4 FFD/1	.6917142	-.7221272	.0072754	-.0032837

SETUP TIME = .041 SECONDS

(6G6G6G6G6G6G-6G6G6G6(1)A4(2X,13,2X,13,3X,I2

THE ITERATIONS

ITERATION NO. 1	BASE POINT	TEST POINT	TEST POINT	TEST POINT	TEST POINT	TEST POINT
SUM OF SQUARES	9.9902029+01	9.0511574+01	8.9429889+01	8.8483377+01	8.7933680+01	8.7891390+01
L	0	0	1	2	3	4
LAMBDA	0.0000000	0.0000000	3.0595158-02	9.2993331-02	2.2722817-01	5.6900138-01
GAMMA	1.0000000+00	1.0000000+00	1.0000000+00	1.0000000+00	1.0000000+00	1.0000000+00
ANGLE IN DEGREES	.0000	88.2710	86.5717	83.2868	77.5166	70.1396
MAX. PIVOT REDUCTION	1.0000000+00	1.0044904-01	1.5410044-01	2.1426267-01	4.5165002-01	9.6487177-01
PAR. 1 KO	3.9800000+01	3.6648311+01	3.8227836+01	3.9021781+01	3.9424791+01	3.9428564+01
2 K1	9.0000000-01	7.0760799-01	7.0924936-01	7.1012843-01	7.1071354-01	7.1139758-01
3 V	8.3000000-01	7.2735821-01	7.1235302-01	7.0982642-01	7.0102124-01	6.9908640-01
4 FFD/1	2.3000000-01	2.3071444-01	2.3590140-01	2.3850921-01	2.3984003-01	2.4055174-01

ITERATION NO. 1	TEST POINT	TEST POINT	TEST POINT	TEST POINT
SUM OF SQUARES	8.8013158+01	1.0156570+02	1.2864002+02	1.7010478+01
L	5	4	7	8
LAMBDA	2.5257082+00	6.6972199+01	3.5758258+02	1.4045882+03
GAMMA	1.0000000+00	1.0000000+00	1.0000000+00	1.0000000+00
ANGLE IN DEGREES	65.9254	59.2326	40.7999	20.8952
MAX. PIVOT REDUCTION	4.7053569-01	6.5837374-01	7.8152804-01	1.1239456+00
PAR. 1 KO	3.9761391+01	3.9797554+01	3.9798877+01	3.9799274+01
2 K1	7.1401544-01	7.7241562-01	8.5342366-01	8.9255084-01
3 V	6.9859944-01	7.1374281-01	7.4007373-01	7.6651219-01
4 FFD/1	2.4095307-01	2.4160142-01	2.4429078-01	2.4857695-01

CUMULATIVE NO. OF FUNCTION CALLS = 10 ITERATION TIME = .001 SECONDS CUMULATIVE TIME = .122 SECONDS

(6666666666"666666(13A6)(2X,I3,2X,I3,3X,I2

THE LAST ITERATION

ITERATION NO. 9		BASE POINT		TEST POINT
SUM OF SQUARES		1.4377882+01		1.4377882+01
L		21		21
LAMBDA		1.3986293+07		1.3986293+07
GAMMA		1.2500000-01		1.2500000-01
ANGLE IN DEGREES		22.9806		22.9806
MAX. PIVOT REDUCTION		1.5862458+00		1.5862458+00
PAR. 1	KO	3.9798877+01		3.9798877+01
2	KI	8.7739425-01		8.7739425-01
3	V	7.4858858-01		7.4858858-01
4	FFD(1	2.4858817-01	5	2.4858817-01

CUMULATIVE NO. OF FUNCTION CALLS = 92 ITERATION TIME = .016 SECONDS CUMULATIVE TIME = .284 SECONDS

ITERATION TERMINATES:
MAX. RELATIVE CHANGE IN A PARAMETER .LT. TOL(1) = 1.5000000+04

(6666E6666E-666666(13A4(2X,I3,2X,I3,3X,I2

FINAL PARAMETER VALUES

			FINAL VALUE
AR.	1	KD	3.9798877+01
	2	KI	8.7739425-01
	3	V	7.4858858-01
	4	FFD/I	2.4858617-01

SUM OF SQUARES = 1.4377882+01

ITERATION SUMMARY

ITER NO.	SUM OF SQUARES	L	LAMBDA	GAMMA	ANGLE	NO. PIVOT FAILURES	NO. BOUNDS FAILURES	MAX. PIVOT REDUCTION	TIME IN SECONDS
0	9.9902029+01	0	0.0000000						
1	1.7010478+01	8	1.9045882+03	1.0000000+00	.0000	0	0	1.0000000+00	.000
2	1.4717262+01	9	2.3192239+03	1.0000000+00	20.4952	0	0	1.1239456+00	.081
3	1.4483559+01	9	2.0120539+03	1.0000000+00	27.1260	0	0	1.3820689+00	.028
4	1.4407710+01	13	9.4076587+04	1.0000000+00	74.8725	0	0	1.0007859+00	.017
5	1.4389369+01	15	1.8022238+05	1.0000000+00	75.5333	0	0	2.3554351-02	.000
6	1.4380310+01	16	3.6186878+05	1.0000000+00	70.2672	0	0	8.8644722-02	.041
7	1.4377993+01	18	1.9584048+06	1.0000000+00	47.7552	0	0	1.6581778-01	.024
8	1.4377918+01	21	1.3986243+07	2.5000000-01	40.0192	0	0	9.8383425-01	.016
9	1.4377882+01	21	1.3986243+07	1.2500000-01	22.9880	0	0	1.5862365+00	.018
					22.9806	0	0	1.5862458+00	.018

SINGULAR VALUES AND VECTORS AT TERMINAL POINT

			SING. VALUES	9.6484704+03	9.6490786+01	1.0889344+01	1.6786588-01
PAR.	1	KD		-.0000471	.0121681	-.0012318	-.9999252
	2	KI		.0002030	-.2799369	.9615949	-.0045235
	3	V		.3124983	.9133908	.2606694	.0107795
	4	FFD/I		-.9499183	.3004224	.0859574	.0035948

NORMALIZING ELEMENTS AND CORRELATION MATRIX

PARAMETER NO.		1	2	3	4
NORM. ELTS.		6.3390175+00	9.3026078-02	7.4976616-02	2.4952477-02
PAR. 1	KO	1.0000000			
2	K1	.3080022	1.0000000		
3	V	-.9187110	.0059195	1.0000000	
4	FFD/I	-.9125479	.0027730	.9999980	1.0000000

CONFIDENCE LIMITS ON LINEAR HYPOTHESIS

PAR.		LOWER LIMIT	FINAL VALUE	UPPER LIMIT
1	KO	2.0188719+01	3.9798877+01	5.9409034+01
2	K1	5.8938494-01	8.7739425-01	1.1654036+00
3	V	5.1646051-01	7.4858858-01	9.8071665-01
4	FFD/I	1.7133515-01	2.4858817-01	3.2584120-01

EXPLORATION

PAR.		LOWER TEST	SUM OF SQUARES	LINEAR EST. OF SUM OF SQUARES	UPPER TEST	SUM OF SQUARES	LINEAR EST. OF SUM OF SQUARES
1	KO	3.4896338+01	2.0021896+01	2.3829266+01	4.4701416+01	2.7626737+01	2.3829266+01
2	K1	8.0539192-01	1.3519441+01	1.5794876+01	9.4939658-01	1.7723316+01	1.5794876+01
3	V	6.9055456-01	8.8387782+01	7.1248958+03	8.0662059-01	3.0708565+01	7.1248958+03
4	FFD/I	2.2927492-01	1.5785329+01	7.2872844+03	2.6790143-01	1.2293029+02	7.2872830+03

NOTICE

THE SUM OF SQUARES AT THE LOWER TEST POINT 2 IS SMALLER THAN THE FINAL SUM OF SQUARES.

F I N A L F U N C T I O N V A L U E S A N D R E S I D U A L S

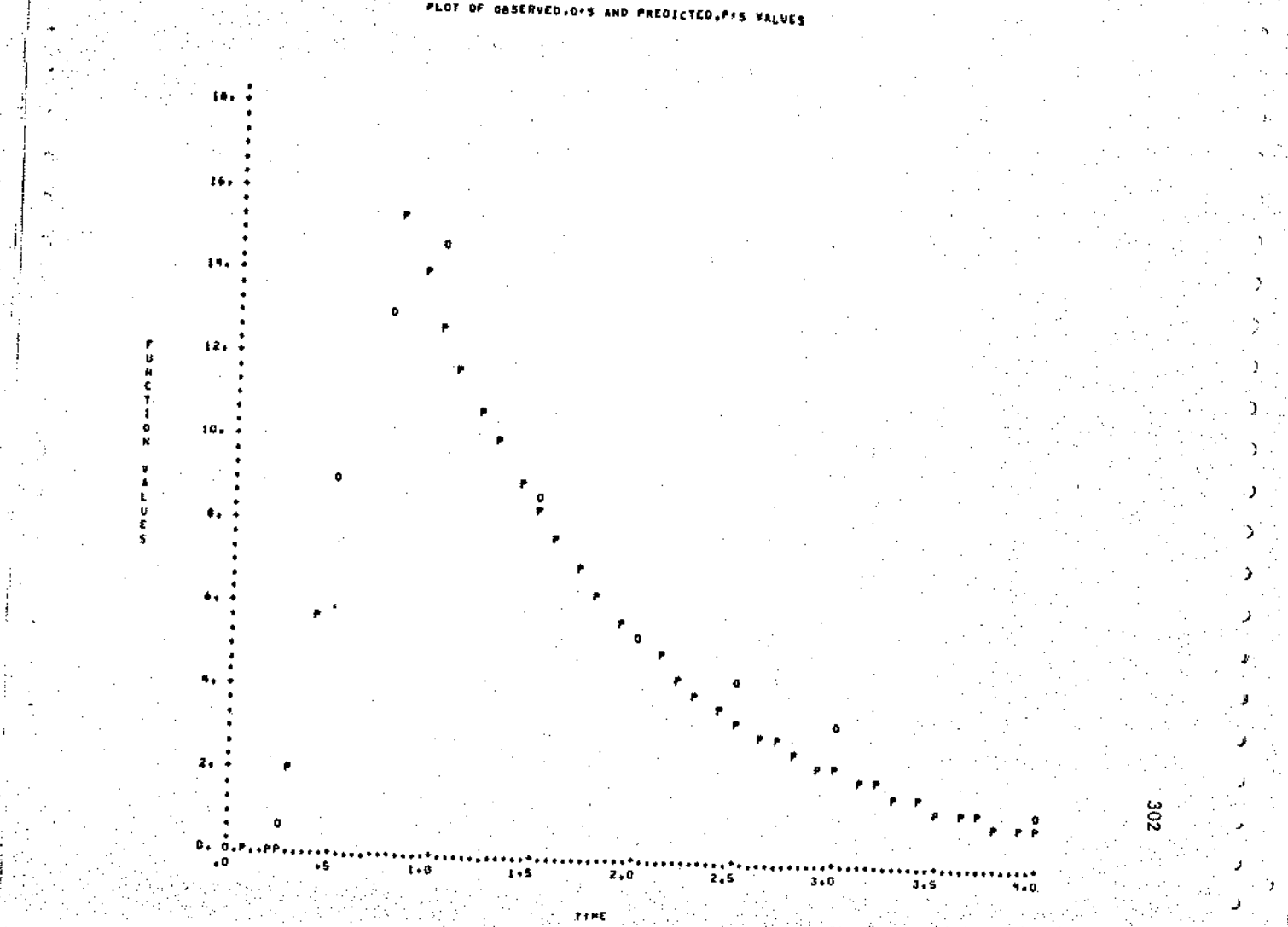
ROOT MEAN SQUARE RESIDUAL = 1.5980031+00
THIS IS THE SCALE UNIT IN THE GRAPH OF THE RESIDUALS.

NO. DEGREES OF FREEDOM = 4

EXPT. NO.	VAR. NO.	PREDICTION	OBSERVATION	RESIDUAL	GRAPH OF RESIDUAL (-2 -1 0 +1 +2)
1	1	0.0000000	0.0000000	0.0000000	
2	1	5.4159191+02	7.0000000+01	6.4384581-01	*
3	1	8.9791813+00	9.0000000+00	2.0818710-02	****
4	1	1.6088606+01	1.3200000+01	-2.8884065+00	*
5	1	1.2719823+01	1.4700000+01	1.7801772+00	**********
6	1	8.3316845+00	8.5999999+00	2.6831543-01	**********
7	1	5.3729039+00	5.3000000+00	-7.2903931-02	***
8	1	3.4648571+00	4.3000000+00	8.3514285-01	*
9	1	2.2349034+00	3.5000000+00	1.2655966+00	******
10	1	9.2921048-01	1.2000000+00	2.7078950-01	*********
					***

TIME SINCE END OF THE LAST ITERATION = .133 SECONDS TOTAL TIME = .919 SECONDS

R-SQUARED FOR FUNCTION: = .9763
COR FOR FUNCTION: = .9764


PLOT OF OBSERVED,O'S AND PREDICTED,P'S VALUES
FUNCTION VALUES
TIME
18.
16.
14.
12.
10.
8.
6.
4.
2.
0.
.0
.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0