© Copyright by Arzu Selen 1984

All Rights Reserved

AWPP
SE4d
1984.

DOSE DEPENDENT PHARMACOKINETICS OF PROBENECID FOLLOWING SINGLE AND REPEATED DOSES

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

Arzu Selen

Degree to be awarded: December 19____ May 19____ August 19 84

Approved by Thesis Reading Committee:

Peter G. Welling
Major Professor

June 10, 1984
Date of Examination

Leonard A. Cosmi

Joseph R. Robinson

Dean, Graduate School

DOSE DEPENDENT PHARMACOKINETICS OF PROBENECID FOLLOWING SINGLE AND REPEATED DOSES

by

ARZU SELEN

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
(Pharmacy)

at the

UNIVERSITY OF WISCONSIN-MADISON

1984

Pharmacy
AW
SE4

DOSE DEPENDENT PHARMACOKINETICS OF PROBENECID FOLLOWING SINGLE AND REPEATED DOSES

Arzu Selen

(Under the Supervision of Professor Peter G. Welling)

Probenecid is used for the treatment of hyperuricemia and as adjuvant therapy to decrease the elimination rate of some compounds particularly β-lactam antibiotics (1).

Dose-dependendent elimination half-life of probenecid has been suggested (2, 3). Others have rationalized prolonged clearance of probenecid at high doses in terms of precipitation of the drug in the gastrointestinal tract and delayed absorption governed by the dissolution rate (4). However dose-dependent elimination of probenecid with increasing dose was recently demonstrated (5).

In this thesis possible dose-dependency of probenecid pharmacokinetics were examined in healthy male volunteers. In Study I, single suspension 0.5 and 2.0 gm doses of probenecid were given to avoid a formulation effect interfering with apparent elimination kinetics. Nonlinear kinetics of probenecid were further examined in Study II by giving five 0.5 or 1.0 gm tablet multiple doses every 6 hours. In Study III, nine 1.0 gm tablet multiple doses were administered. Additional to plasma samples, urines were collected in Study I. Probenecid plasma and urine concentrations were determined

by solvent extraction and high pressure liquid chromatography, with detection at 254 nm.

Individual plasma probenecid levels were analyzed initially according to a one-compartment model with first-order absorption and elimination. Dose dependency of dose-normalized area under curve and elimination half-lives prompted further analysis of the data using first-order absorption models with saturable elimination or parallel first-order and saturable elimination.

Plasma data were analyzed by nonlinear regression and numerical integration. Urinary data from Study I were consistent with plasma data suggesting saturation of conjugation and of active secretion of probenecid at the 2.0 gm dose. Studies II and III have provided further evidence for dose dependency of probenecid elimination with accumulation significantly higher than that predicted by first-order elimination model. These studies also demonstrated that probenecid may have autoinhibitory effect on its own absorption as well as elimination following repeated doses in the therapeutic range. Probenecid kinetics although complex are nonlinear and can be adequately described by a one-compartment kinetic model with saturable elimination.

APPROVED: Peter G. Welling, Professor

DATE:

To my parents

Makbule ve Atilla Selen

and

To Professor Peter G. Welling

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Professor Peter G. Welling for his dedicated guidance, trust and enthusiasm throughout my graduate career. He has set a challenging example to follow.

Appreciation is extended to Professors Kenneth A. Connors and Joseph R. Robinson for reading this thesis, and also to the other members of my oral examination committee Professors William A. Craig and William R. Porter.

In addition, I would like to thank all my colleagues and Ms. Barbara A. Johnson for their friendship, Mr. Kenneth W. Berg and Mr. Robert J. Aylesworth for their assistance in many matters and Ms. Ann E. Rohloff and Ms. Wylma C. Robinson for the artwork and typing of this thesis.

Finally, I wish to express my deep gratitude to my parents for their love and encouragement, they made this thesis possible.

TABLE OF CONTENTS

page

Abstract..... ii
Dedication..... iv
Acknowledgements..... v
Table of Contents..... vi

I. Introduction..... 1
A. Background..... 1
Physical Properties..... 1
Clinical Uses of Probenecid..... 2
Interaction of Probenecid with Other Compounds..... 3
Pharmacokinetics of Probenecid..... 7
Absorption..... 7
Distribution..... 8
Metabolism and Elimination..... 10
Dose Dependency of Probenecid Elimination..... 17
B. Study Objectives..... 28

II. Experimental..... 30
A. Materials..... 30
Reagents..... 31
Apparatus..... 31
Preparation of Reagents and Stock Solutions..... 32
B. Methods..... 36
Preparation of Samples..... 36
Plasma..... 36
Urine..... 36
Preparation of Urine Samples for Analysis of Probenecid Following Hydrolyisis of Probenecid-acyl of glucuronide..... 37
Assay..... 37
Extraction of probenecid from plasma samples; the suspension study..... 38
Extraction of probenecid from plasma samples; multiple dose studies..... 39
Extraction of probenecid from urine samples.... 49
Extraction of probenecid from urine samples following hydrolysis of probenecid-acyl glucuronide..... 52
Extraction of probenecid residue remaining in the breakers used for dosing probenecid suspensions..... 54

TABLE OF CONTENTS (Continued)

page

Protocol..... 54
Study I - Probenecid suspension study..... 56
Study II - Probenecid 0.5 and 1.0 gm tablet, multiple dose study..... 57
Study III - Probenecid 1.0 gm tablet, multiple dose study..... 59
Pharmacokinetic and Statistical Analysis..... 64
Study I - Probenecid suspension study..... 64
Study II - Probenecid 0.5 and 1.0 gm tablet, multiple dose study..... 66
Study III - Probenecid 1.0 gm tablet, multiple dose study..... 66

III. Results..... 67
A. Study I - Probenecid Suspension Study..... 67
Plasma..... 67
Statistical Analysis..... 96
Urine..... 113
Statistical Analysis..... 135
B. Study II - Probenecid 0.5 and 1.0 gm Tablet, Multiple Dose Study..... 139
Single Dose..... 139
Statistical Analysis..... 153
Multiple Doses..... 165
Statistical Analysis..... 172
C. Study III - Probenecid 1.0 gm Tablet, Multiple Dose Study..... 191

IV. Discussion..... 232
A. Study I - Probenecid Suspension Study..... 232
Plasma..... 232
Urine..... 235
B. Studies II and III - Multiple Dose Studies..... 237

V. Conclusions..... 241

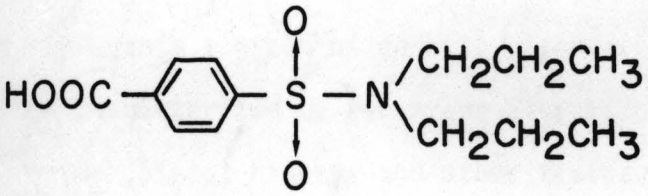
VI. References..... 243

VII. Appendices
I. Glossary..... 250
II. Protocol and Consent Form for Clinical Study I.... 253
III. Protocol and Consent Form for Clinical Study II... 269
IV. Protocol and Consent Form for Clinical Study III.. 285
V. NREG Subroutine for Equation 1..... 301
VI. NREG and DGEAR Subroutines for Equation 2..... 317
VII. NREG and DGEAR subroutines for Equation 3..... 331
VIII. SPSS subroutine..... 345
IX. DGEAR subroutine..... 374

INTRODUCTION

A. Background

Probenecid [p-(di-n-propylsulfamoyl)-benzoic acid] [I] was synthesized by Miller, Ziegler and Sprague as an organic acid to inhibit the renal tubular secretion of penicillin (6), and was patented by Sharp and Dohme in 1952.



Physical Properties

Probenecid is a white, crystalline, odorless powder, practically insoluble in water and dilute acid but soluble in 25 parts alcohol (95%), and 12 parts acetone. Probenecid is also soluble in chloroform and dilute solutions of alkali hydroxides and of sodium hydrogen carbonate (7).

Probenecid is a weak organic acid, and has a pK_a of 3.4 in water (8) and 5.8 in aqueous methanol (9).

Probenecid is highly lipid soluble, with a partition coefficient (K_p) (chloroform: 0.15 M HCl)>2000. At alkaline pH, probenecid is more water soluble, K_p = 0.13(peanut oil: pH 7.4 Sorenson Buffer (10).

Melting point of probenecid is 198-200°C.

Clinical Uses of Probenecid

Although introduced as adjuvant therapy to prolong the therapeutic activity of penicillin (6), uricosuric properties of probenecid were quickly recognized. Probenecid was the first drug commonly accepted as a uricosuric agent for the treatment of gout (11) and is now used primarily for treatment of hyperuricemia associated with gout and gouty arthritis.

Gout represents a group of genetic disorders of purine metabolism or excretion characterized by recurrent attacks of arthritis, urate deposits in the joints, kidneys and other tissues, and elevated blood and urine uric acid levels.

Uric acid is formed by the breakdown of purines and by direct synthesis from 5-phosphoribosyl pyrophosphate and glutamine. The normal blood uric acid level in man is circa 4 mg/dl. Uric acid is filtered, reabsorbed and secreted in the kidney. Elimination of uric acid from the body is by filtration (20%) and tubular secretion (80%) Normally, 98% of the filtered uric acid is reabsorbed (12).

Probenecid is a competitive inhibitor of the active urate reabsorption at the proximal renal tubule. It thereby increases the urinary excretion of uric acid, and lowers serum urate levels below the saturation level (approximately 8 mg/dl), prevents formation of sodium urate crystals and promotes their dissolution.

Competitive inhibition of uric acid secretion by probenecid has been demonstrated in species in which tubular reabsorption of urate is

defective or poorly developed, for example, in humans with Fanconi syndrome or Wilson's disease, or in animals such as the Dalmatian coach hound, rabbit (13), and chicken (14).

The therapeutic plasma probenecid concentration range for uricosuric effect is 100-200 mcg/ml. Probenecid therapy is generally initiated at a dose level of 0.25 gm twice daily, gradually increasing over a 2-week period to a maintenance dose of 0.5 gm two or three times daily. A patient with reduced renal function may require 2.5 to 3.0 gm daily in order to reduce serum urate to satisfactory levels (15).

The inhibitory effect by probenecid on renal tubular secretion is utilized therapeutically by coadministering probenecid with some β-lactam antibiotics to achieve high and prolonged plasma and tissue antibiotic levels. Inhibition of penicillin excretion is achieved with probenecid plasma concentrations of 40-60 mcg/ml.

Probenecid is generally given as 500 mg tablets four times a day to prolong antibiotic activity. For the treatment of gonorrhea, 1 gm probenecid is administered concurrently with 4.8 million units of intramuscular penicillin G procaine, or simultaneously with 3.5 gm oral ampicillin (16).

Interaction of Probenecid with Other Compounds

Probenecid inhibits tubular secretion of other weak acids including p-amino hippurate, phenolsulphaphthalein, salicylate, pantothenic acid (6, 17).

The tubular secretion of organic bases appears not to be affected by probenecid (18).

Probenecid has been claimed to be effective in preventing active removal of antibiotics from the CSF (19) although some investigators have reported equivocal results (20, 21).

Probenecid has been used also to evaluate the rate of dopamine and serotonin metabolism in the central nervous system. Homovanillic acid and 5-hydroxy indoleacetic acid are the main metabolic products of these amines and are removed from the cerebrospinal fluid (CSF) and brain by an active transport mechanism which is shown to be inhibited by probenecid (22). The rate and extent of accumulation of these metabolites in the presence of probenecid reflects the rate of monoamine turnover (23) and has led to the introduction of a "probenecid test," to estimate the relative activity of central monoamine-containing neural systems particularly in depressed patients (22, 24, 25).

Effect of probenecid on cyclic adenosine 3',5'-monophosphate (cAMP) transport has also been examined (26). Cyclic AMP is formed from adenosine triphosphate (ATP) by the action of the enzyme adenylate cyclase, and by the action of the enzyme phosphodiesterase is converted to physiologically inactive 5'-Adenosine monophosphate (5'-AMP). Catecholamines, many hormones, and various other ligands act via receptors on the external surface of the membrane of their target cells to increase intracellular cyclic AMP which mediates their physiologic effects. Particular cyclic AMP-dependent protein

kinases in the cell are activated by cyclic AMP and these then catalyse the phosphorylation of enzymes or other proteins. In the case of enzymes, phosphorylation increases or decreases their activity and thus produces changes in metabolism.

The intracellular concentration of active cyclic AMP depends on the equilibrium between the rate of its production by adenylate cyclase, the rate of its degradation by phosphodiesterase, and the binding to the regulatory units of protein kinases.

Cyclic AMP can be regarded as a "messenger" as it provides the intracellular link between the extracellular chemical messengers and the physiologic response. Intracellularly, the specifity of the response to cyclic AMP is attributed to the nature and amounts of protein kinases and phosphorylatable proteins in the particular cell (27).

Following stimulation of pigeon erythrocytes with epinephrine efflux of cyclic AMP was demonstrated to occur against an apparent concentration gradient, and this efflux which could be inhibited by probenecid, was thus concluded to be an active process.

A probenecid-sensitive, efflux has been demonstrated in rat glial cells (28), human fibroblasts and turkey erythrocytes (29).

Probenecid has been shown also to inhibit extracellular cyclic AMP phosphodiesterase activity in a similar fashion to theophylline and papaverine (30).

Probenecid has been reported to inhibit glycine conjugation of p-aminobenzoic acid and p-aminosalcylate (31), sulfanilamide and choline acetylation (32), and digitoxin hydroxylation (33). Probenecid has been shown to influence the protein binding of digitoxin (34) and also to inhibit accumulation of ouabain in the liver (35).

In the mouse and rat, glucose and digitoxin intestinal resorption were reported to be depressed (36, 37). Inhibition of glucose resorption and accumulation of tetraethylammonium bromide occurs at a probenecid concentration range of $1.75 - 7.0 \times 10^{-4}$ M which is close to the range of inhibition of organic acid transport. Probenecid was shown also to inhibit accumulation of glycine, L-phenylalanine, L-valine, L-arginine, L-lysine, L-pyroline, in rat and mouse kidney cortex slices. Amino acid accumulation in rat and mouse kidney cortex slices, determined by both membrane transport and protein incorporation, appeared to be inhibited by probenecid primarily due to inhibition of protein synthesis rather than transport of the amino acids (38).

Probenecid prolongs and elevates circulating levels of indomethacin (39), naproxen (40), ketoprofen (41), carprofen (42), and clofibrate (43). In each of these interactions, inhibition of acyl glucuronidation of probenecid has been implicated as a causative factor. Inhibition may be due to competition by probenecid for uridinediphosphoglucuronic acid or for glucurosyl transferase enzyme.

Pharmacokinetics of Probenecid

Absorption

Although therapeutic application of probenecid has been investigated in various, diverse areas, its own pharmacokinetics has been examined only recently.

In an early study, efficient oral absorption of probenecid was reported in two dogs following 60 mg/kg oral (p.o.) and intraveneous (i.v.) doses, as similar areas under probenecid plasma curves were obtained (44).

Subsequently, in a study in two subjects following single 2.0 gm p.o. and i.v. doses, similar plasma levels were observed during the post absorptive phase (2).

Another study reported recovery of approximately 80% of the administered radioactivity following a 2.0 gm oral dose of probenecid containing 10μCi of ring ^{14}C, in the 48 hour urine of two subjects (45).

In a previous, five subject study, in which 0.5, 1.0 and 2.0 gm oral doses of probenecid were given in tablet form, maximum drug concentrations, C_{max}, were dose proportional (5). The time at which maximum drug concentrations occurred in plasma, T_{max}, for the three doses showed no significant difference at the 95% confidence level. Similarly, following analysis of probenecid plasma levels obtained from the three different doses, according to a first-order absorption and elimination model, no significant differences ($p<0.05$)

could be detected in the absorption rate constants or the absorption lag times. Although 2.0 gm data were analyzed also by a second model which involved first-order absorption and saturable elimination of probenecid from the body, the absorption parameters were similar to those obtained from the first model. In that study, although no dose-dependency was apparent in parameters indicating rate of absorption, the dose-normalized bioavailability parameter F/V from the 2.0 gm dose was only ca 80% of the value obtained from the lower doses. Since F/V is a composite term, consisting of the fraction of dose absorbed, F, and the apparent distribution volume, V, the decrease in this value following the 2.0 gm dose could be due to a change in one or both of these parameters, and the actual factor causing this could not be determined (5).

Distribution

Probenecid binds extensively to human plasma proteins, 83-95% binding to albumin at drug concentrations of 20 - 170 mcg/ml is reported (10, 2). The binding to human globulins is 28 -33% (2). In the dog probenecid is 72 - 91% bound to plasma proteins at drug concentrations of 28 - 530 mcg/ml (45). In the monkey (Macaca mulatta) probenecid is 78 - 90% bound at drug concentrations of 40 - 800 mcg/ml (46). Thus binding in both species appears to be similar to that in man.

A probenecid-plasma protein binding study in the monkey predicted 6.4 identical binding sites with a binding constant of 2.935×10^3 l/mol, within a probenecid concentration range of 40 - 800 mcg/ml (46).

The major metabolites of probenecid are reported to bind less tightly to human plasma proteins than the parent drug. Binding of the hydroxylated-, carboxylated-, and N-depropyl metabolites has been reported to be 65%, 58% and 81% respectively (10).

Probenecid diffuses freely across the blood-brain barrier, but is actively transported back from the cerebrospinal fluid (CSF) into plasma. The CSF plasma free drug concentration ratio is reported to be 0.2 - 0.6 in man (25, 47-49), 0.3 - 0.4 in rabbits and 0.4 - 1.2 in dogs (50).

During 8 hours following administration of a 0.5 gm i.v. dose of probenecid to three subjects drug distribution volume and total clearance were reported to be 0.14 l/kg and 28.2 ml/min, respectively. Following a 2.0 gm i.v. probenecid dose, the mean distribution volume was 0.16 l/kg while plasma clearance had decreased to 18.3 ml/min (45). However, insufficient plasma samples were taken for accurate description of pharmacokinetic parameters.

In man, renal cortical slices have been shown to concentrate probenecid and this could possibly be related to accumulation of probenecid in renal tubules (51, 52).

Metabolism and Elimination

Although probenecid is actively secreted by the renal tubules, urinary excretion of unchanged probenecid accounts for less than 5% of the administered dose, because of extensive reabsorption of drug into the peritubular capillaries (53).

Probenecid is extensively metabolized, and its metabolism in man involves both side chain oxidation and conjugation of the carboxyl group with glucuronic acid. The major metabolites of probenecid are shown in Figure 1 and some physical properties of probenecid and its metabolites are given in Table I.

Approximately half of the dose of probenecid given to human subjects is claimed to be recovered in the urine in the form of acylglucuronide (17 - 35.6%) and unchanged drug (5.2 - 11%) (54). Monohydroxylated derivatives, formed by the side chain oxidation at the secondary (A) and primary (B) positions, and the carboxy (C) and N-depropyl (D) derivatives, are excreted mostly in the free form. Small amounts of glucuronide conjugates of metabolites A, D are recovered in the urine (46, 54).

Although urinary recovery of unchanged probenecid is reported to be less than 5% a recent study has suggested that even this small urinary recovery may be artifactual, due to rapid hydrolysis of conjugated metabolite at physiological or room temperature, or even during storage. Actual recovery of unchanged probenecid may be negligible (55). However, another study has shown that although

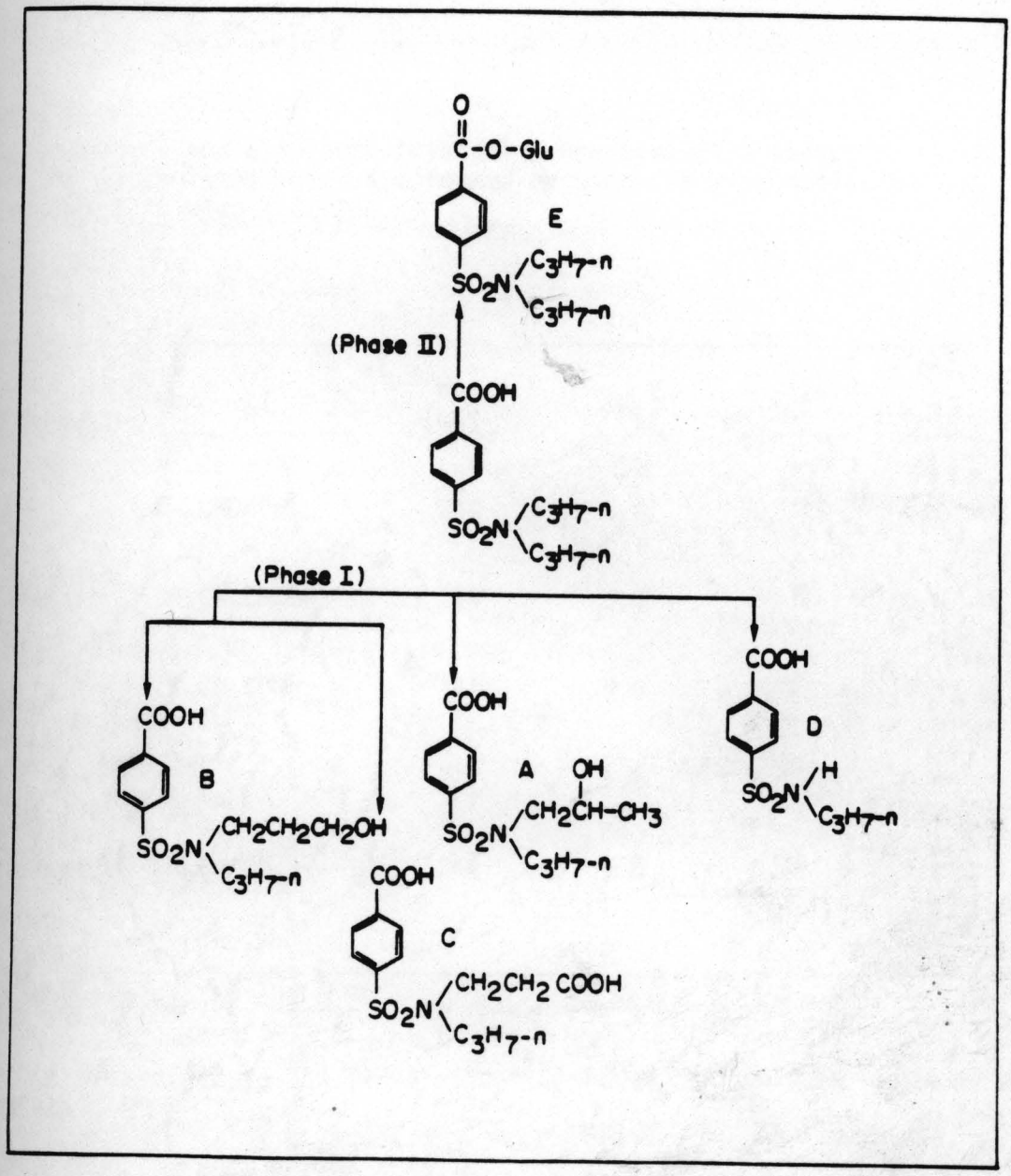


Figure 1: Metabolism of probenecid in man.

Metabolites

A: p-(N-propyl,N-2-hydroxypropyl sulfamoy)benzoic acid
B: p-(N-propyl,N-3-hydroxypropyl sulfamoy)benzoic acid
C: p-(N-propyl,N-2-carboxyethyl sulfamoyl)benzoic acid
D: p-(propyl sulfamoy)benzoic acid
E: Probenecid acyl mono-glucuronide

Table I
The structure and also some physical properties of probenecid and its metabolites which are formed by side-chain oxidation of probenecid *in vivo* (20, 35).

Compound	R[1]	λ_{max} (mμ)	pK_a[2]	K_p[3]	K_p'[3]
A	$CH_2CHOHCH_3$	244	5.8	<0.01	0.3
B	$CH_2CH_2CH_2OH$	244	5.8	<0.01	0.3
C	CH_2CH_2COOH	244	5.9	<0.01	4.5
D	H	227	5.8	<0.01	0.4
Probenecid	$CH_2CH_2CH_3$	244	5.8	0.03	<0.002

[1] $HOOCC_6H_4SO_2N(R)CH_2CH_2CH_3$.

[2] pK_a's determined in 75% (v/v) ethanol, the pK_a of probenecid in water is 3.4.

[3] Kp = Sorensen buffer (pH = 7.4) and peanut oil; (2 ml. of buffer containing 100 mcg./ml. were added to 20 ml. of oil and shaken). Kp' = chloroform-0.15 N HCl; (30 ml. of an HCl solution containing 15 mcg./ml. was shaken with 3 ml. of washed chloroform for 1 h).

probenecid gluruconide is unstable at room temperature, negligible amounts of probenecid gluronide are hydrolyzed to probenecid upon storage at -15°C (46).

No phenolic metabolites of probenecid have been recovered, probably because of deactivation of the phenyl ring by electron withdrawing carboxyl and sulfamoyl constituents (54).

The metabolism of probenecid was studied also in dogs, rats and monkeys. Despite some marked species differences, the pattern of probenecid metabolism in experimental animals appears to be qualitatively similar to that in man. In the rat and monkey, phase I, oxidation reactions predominate, whereas in the dog, phase II, conjugation reactions are more important. A summary of the current information on urinary recovery of probenecid and its metabolites is given in Table II (53, 4, 56).

The rat excretes a smaller percentage of administered compound in urine compared to other species, the balance being voided in bile. Biliary excretion has been shown to be major pathway for excretion of probenecid and metabolites in rats (57). In that study, 64% of a 40 mg/kg i.v. dose of probenecid was recovered in rat bile. The rat and monkey (Cebus apella) with respective doses of 100 mg/kg and 10 mg/kg, excreted only a small proportion of drug as the acylgluruconide (44). This is in contrast to the dog, man, and a different monkey species (Macaca mulatta) in which a large proportion of drug was excreted as the acylglucuronide and a

Table II
Percentage urinary recovery of probenecid and its metabolites.

Urinary Recovery %	Species						
	Man[1]	Man[2]	Monkey[3]	Monkey[4a]	Monkey[4b]	Dog[5]	Rat[6]
Probenecid[7]	3.5- 4.0	5.4-10.3	0.8- 2.9	5.4	6.0- 8.1	2.0	0.4- 1.2
A	7.2-12.5	14.2-18.7	42.0-51.0	A+F: 10.9	A+F: 6.2- 7.2	↑	↑
B	1.6- 3.7	*[8]	14.0-16.0	*		7.0	18.0-34.0
C	6.3- 9.2	14.0-17.1	9.8-18.0	*	4.0- 6.0	↓	↓
D	4.6- 8.0	15.3-15.8	17.0-20.0	D+G: 21.4	7.2- 8.4	3.0	5.0- 7.0
E	42.0-41.0	51.1-38.6	3.5- 5.4	49.1	35.7-33.8	72.8	3.0- 4.0
F	↑ 4.1- 3.4	*	*	*	3.4- 1.5	*	*
G	↓	*	*	*	15.1-15.0	*	*

Table II
(continued)

Urinary Recovery %	Species						
	Man[1]	Man[2]	Monkey[3]	Monkey[4a]	Monkey[4b]	Dog[5]	Rat[6]
H	19.7-33.5	5.4-10.3	82.8-105.0	-[9]	A+D: 13.4-15.6	10.0	23.0-41.0
I	46.1-44.4	51.1-38.6	3.5- 5.4	-	54.2-50.3	72.8	3.0- 4.0
E/H	2.13-1.22	1.04-0.62	0.04-0.05	-	2.17-2.66	7.28	0.13-0.097

A - Secondary hydroxy metabolite
B - Primary hydroxy metabolite
C - carboxy metabolite
D - N-depropyl metabolite
E - probenecid acylmonoglucuronide
F - glucuronide conjugate of A
G - glucuronide conjugate of D
H - Sum of A, B, C, D
I - Sum of glucuronide conjugates

Table II
(continued)

1. Percentage of 2.0 gm, p.o. single dose of ^{14}C-probenecid (given presumably as powder) (54); in 2 subjects in (0-48 h).

2. Percentage of total urinary recovery (85%) from a 2.0 gm solution oral dose of probenecid (4); in 2 subjects.

3. Percentage of total 6 h urinary recovery (59-63%) from a 10 mg/kg dose of ^{14}C-probenecid in monkey (Cebus apella). The dosage route although not indicated was probably intravenous (45).

4. Percentage of total dose administered in one monkey (Macaca mulatta) on two different occasions as i.v. bolus injection, dose administered a: 10 mg/kg; b: 40 mg/kg (46).

5. Percentage of total 5 h urinary recovery (93%) from a 1.5 gm i.v. dose of ^{14}C-probenecid in Mongrel Dog (45).

6. Percentage of total 3.3 day urinary recovery (28-34%) from a 30-40 mg/kg intraperitoneal (i.p.) dose of ^{3}H-probenecid (45).

7. These values may be artifacts due to metabolite hydrolysis during storage (56).

8. * not described.

9. can not be calculated.

smaller proportion as side-chain oxidation metabolites following 40 mg/kg i.v. doses (44, 46).

Biliary recovery of probenecid appears not to have been examined in other species.

Dose Dependency of Probenecid Elimination

Studies in man and dogs have indicated that the metabolism and pharmacokinetics of probenecid may be dose-dependent (2, 3, 4, 5, 58).

In an early study in three healthy male subjects, plasma probenecid levels following a 2.0 gm i.v. dose declined at a slower rate compared to those following a 0.5 gm i.v. dose (2). Unfortunately, plasma sampling following the 2.0 gm dose was not continued for a sufficient period for plasma levels to be measured in the same concentration range from both the high and low doses. Subsequently, further evidence of dose-dependent probenecid elimination was provided by Tjandramaga et al. (3). The elimination half-life ($t_{\frac{1}{2}}$) of probenecid in the same individual was reported to be 9 and 6 hours following 2.0 and 1.0 gm oral doses, respectively.

The apparent plasma $t_{\frac{1}{2}}$ of probenecid increased from 6 to 11 hours in one dog when the dose was increased from 40 mg/kg to 200 mg/kg. A similar increase in $t_{\frac{1}{2}}$ from 4.3 to 8.5 hours was observed when the dose of probenecid was increased from 40 mg/kg to 120 mg/kg in another dog (58).

This somewhat limited and fragmentary evidence of dose-dependent probenecid elimination has been disputed on the basis that the pattern of metabolite formation is dose-independent (4). A summary of the data used by the authors to support this argument is in Table III. The authors claim that the relative quantities of individual metabolites excreted in the urine are essentially constant from 0.5, 1.0 and 2.0 gm doses. They suggest that if there were saturation of any metabolic pathway at the higher doses, this would have caused a change in the pattern of urinary metabolites. They propose that slow dissolution of drug in the gastro-intestinal tract due to its low aqueous solubility gives rise to prolonged absorption at high doses, so that prolonged plasma probenecid levels from high doses is more likely to be an absorption effect rather than saturable elimination.

Considering the difficulty of interpreting such limited data, this argument may be tenuous. In fact, the report of Melethil and Conway (4) can be interpreted as supporting previous claims of dose-dependent probenecid elimination rather than contradicting them. For example, if the major metabolic pathway, glucuronidation, were saturated, then the ratio of side chain oxidation metabolites (A, C and D) (metabolite B was not reported) to the acylglucuronide (E) would increase with increasing dose.

From these data, if the ratio of the sum of the side chain oxidation metabolites (A, C and D) to probenecid acylglucuronide (E)

Table III
Percentage urinary recovery of probenecid and its metabolites in man (4).

Subject	Dose (mg.)	Total excreted % of dose	% of Total Urinary Excretion						
			P	A	C	D	E	F	F/E
1	500	80.7	16.5	16.3	15.0	10.5	41.8	41.8	1
1	1000	76.7	10.7	15.9	15.4	11.0	47.2	42.3	0.9
1	2000	87.8	10.3	18.7	17.1	15.8	38.6	51.6	1.34
2	500	86.4	4.8	15.8	11.0	13.8	55.6	40.6	0.73
2	500	87.3	8.9	15.1	11.6	10.1	54.3	36.8	0.68
2	1000	87.2	4.5	17.0	13.2	14.4	50.8	44.6	0.88
2	2000	84.2	5.4	14.2	14.0	15.3	51.1	43.5	0.85

P: Probenecid
A: Secondary hydroxy metabolite
C: Carboxy metabolite
D: N-depropyl metabolite
E: Probenecid acyl monoglucuronide
F: Sum of A, C, and D

versus dose is plotted for the two subjects, linear regression coefficients of 0.851 for subject 1, 0.637 for subject 2 and 0.977 for the combined values are obtained (Figure 2, Table IV). These results appear to support rather than contradict dose-dependent saturable elimination of probenecid from plasma in man.

Although available evidence thus suggests that probenecid elimination is saturable in man and the dog, the uncertainty in the nature, and possible dose-dependency, of probenecid pharmacokinetics prompted us to examine these questions more closely. In the first study, dose dependency of probenecid pharmacokinetics was examined in five healthy male volunteers following oral 0.5, 1.0 and 2.0 gm single doses to determine if probenecid elimination from plasma is saturable at therapeutic concentrations. Plasma levels of probenecid obtained from the three doses are shown in Figure 3 Results of analysis of individual plasma probenecid profiles according to one compartment model kinetics with first-order absorption and elimination, and also model independent pharmacokinetic paramters C_{max}, T_{max} and dose normalized $AUC^{0\rightarrow 48h}$, $AUC^{0\rightarrow 48h}/D$, are given in Table V. Predicted mean elimination half-lives of probenecid from 0.5 and 1.0 gm doses were 4.2 and 4.9 h respectively. The elimination $t_{\frac{1}{2}}$ for the 2.0 gm dose was 8.5 h which is significantly higher ($p<0.05$) than the values obtained from lower doses. Similarly, dose normalized area under curve values increased with increasing dose. Due to dose-dependency of

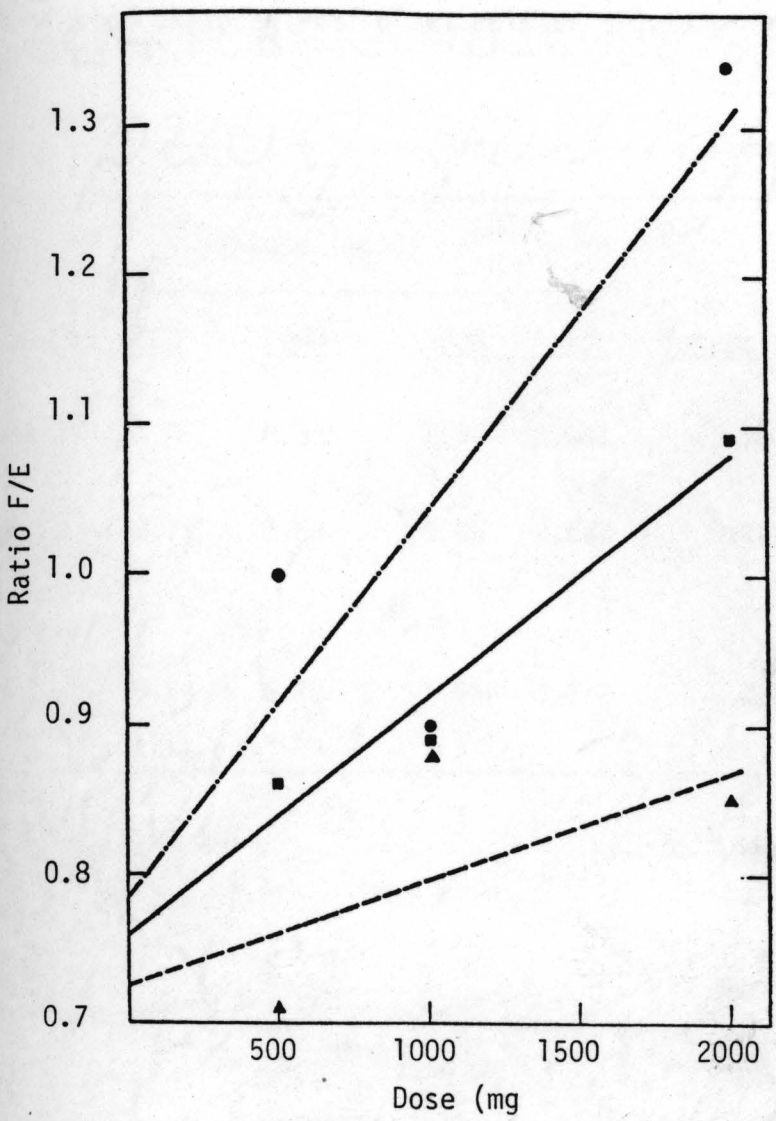


Figure 2: Plot of the ratios of side chain metabolites (F) to acyl glucuronide (E). ●, ▲ denote subject 1 and subject 2, respectively, ■ denotes mean value of subjects 1 and 2.

Table IV
Ratio of side chain oxidation metabolites (F) to probenecid acyl glucuronide (4).[1]

	Dose (mg.)					
	500	1000	2000	r^2	Intercept	Slope
Subject 1	1.0	0.9	1.34	0.851	0.78	2.6×10^{-4}
Subject 2	0.71	0.88	0.85	0.637	0.725	7×10^{-5}
Mean of Subjects 1 and 2	0.86	0.89	1.095	0.977	0.758	1.6×10^{-4}

[1]Data from Table III.

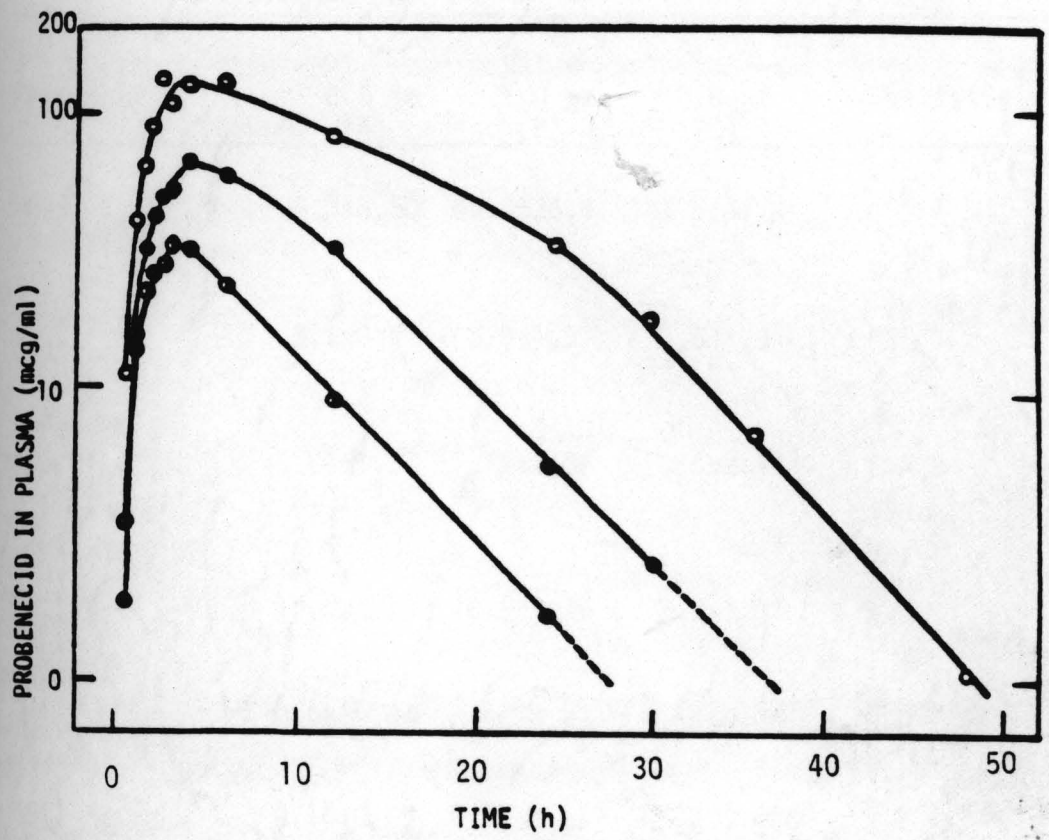


Figure 3: Mean probenecid plasma levels, ◐, ●, o, represent 0.5 gm, 1.0 gm and 2.0 gm tablet doses, respectively (5).

Table V
Pharmacokinetic parameter values (± 1 SD.) for 0.5, 1.0 and 2.0 gm single probenecid tablet doses (5).

Parameter	Value 0.5 gm (A)	1.0 gm (B)	2.0 gm (C)	Statistic[1]
C_{max} (mcg/ml)	35.3±8.50	69.6±26.9	148.6±47.4	C>B>A
T_{max} (h)	3.1±0.9	3.9±1.3	3.5±1.5	NSD[2]
$AUC_{0\rightarrow 48h}/D(10^{-4})$ (h/ml)	5.84±1.31	7.72±2.37	10.5±2.14	C>A,B
$F/V(10^{-5})$ (ml^{-1})	10.0±2.10	10.9±2.20	8.0±1.0	B>C
k_a (h^{-1})	0.98±0.49	0.56±0.41	0.66±0.29	NSD
k_{el} (h^{-1})	0.18±0.05	0.15±0.03	0.08±0.004	A,B>C
$t_{\frac{1}{2}el}$ (h)	4.20±1.10	4.90±0.80	8.5±0.4	C>A,B
t_{lag} (h)	0.80±0.71	1.10±0.60	0.56±0.32	NSD
r^{2}[3]	0.98±0.02	0.98±0.02	0.95±0.07	NSD

1. Differences between doses are significant if $p<0.05$.
2. No significant differences.
3. Coefficient of determination, $r^2=(\Sigma obs^2-\Sigma dev^2)/\Sigma obs^2$

elimination half-lives, and significant increases in dose normalized area under curve values with increasing dose, elimination of probenecid from the body was inferred to follow saturation kinetics. Accordingly, 2.0 gm data were analyzed using a kinetic model with first-order absorption and Michaelis Menten-type elimination. This model successfully described the data obtained from the three doses, thus indicating that the elimination of probenecid from plasma could be saturable at therapeutic doses. Results of the analysis of individual 2.0 gm data according to both models are given in Table VI(5).

Another recent study reported dose-dependent probenecid elimination in monkeys. Following intravenous bolus probenecid doses ranging from 8.2 to 1.64 mg/kg in three monkeys, a decrease in plasma clearances of both total and unbound probenecid was observed with increasing dose. The distribution volume and fraction of metabolites excreted in the urine remained constant with increasing dose. The investigators measured probenecid and its secondary hydroxy (A) and N-depropyl (D) metabolites and their glucuronide conjugates. Carboxy (C) and primary hydroxy (B) metabolites of probenecid are claimed to account collectively for only 4 - 6% of the excreted dose, and the contribution of these metabolites and their conjugates to the dose-dependent elimination was considered insignificant. Thus, further quantitative analysis of the compounds (C, B) was omitted. The urinary excretion rates of probenecid and its metabolites A and

Table VI
Pharmacokinetic parameter values (± 1 S.D.) obtained by analyzing plasma probenecid levels from the 2.0 gm tablet dose according to first-order or saturable kinetic models (5).

Parameter	Value according to Equations 1	2
FD/V (mcg/ml	176 ± 3	160 ± 19
k_a (h^{-1})	0.66 ± 0.29	0.63 ± 0.26
k_{el} (h^{-1})	0.082 ± 0.504	-
K_M (mcg/ml)	-	46.6 ± 26.5
V_M (mcg / ml.h)	-	8.60 ± 2.10
V_M/K_M (h^{-1})	-	0.20 ± 0.06
t_{lag} (h)	0.56 ± 0.32	0.40 ± 0.52
r^2	0.95 ± 0.07	0.96 ± 0.04

D are reported to be urine-flow dependent. The investigators suggest that the dose-dependency of probenecid elimination in plasma is probably not the sole consequence of enzyme saturation of either the conjugation or side chain oxidation pathways, or of decreased uptake of drug at the metabolic site in hepatic cells. The investigators speculate that the glucuronide conjugates of probenecid, of metabolites B and C, and to a lesser extent metabolites B and C, and to a lesser extent metabolite D may be excreted into the bile and stored in the gallbladder. Subsequent emptying of the gallbladder is suggested to maintain high levels of probenecid in the plasma due to intestinal reabsorption of probenecid and hydrolyzed probenecid glucuronide (46).

Although the authors could not explain the observed dose-dependency of probenecid elimination from their rather limited data and study design, they nonetheless have provided another example of dose-dependent probenecid pharmacokinetics. It is unfortunate that the authors had no data on biliary excretion of these compounds, and only one set of data to test their kinetic approach which incorporated a two-compartment pharmacokinetic model with saturable elimination.

B. Study Objectives

Dose-dependent probenecid pharmacokinetics were previously observed following single 0.5, 1.0 and 2.0 gm oral tablet doses (5). The data obtained from the analysis of plasma samples could be successfully described by both first-order absorption and elimination and first-order absorption and saturable elimination models. The superiority of either model in describing the data was equivocal.

The dose-dependency of dose-normalized area under plasma curve values and elimination half-lives, when first-order elimination kinetics were assumed, supported the model incorporating saturation kinetics. Since elimination of probenecid from the body is primarily due to metabolism, the limited available evidence of dose-dependent elimination could be interpreted in terms of saturation of the major metabolic pathway acylglucuronidation.

Although the mechanism causing slower elimination of probenecid at higher plasma levels has not been elucidated, the following have been considered as possible explanations: saturation of one or more steps in probenecid elimination, saturation of a transport step governing access of probenecid to site(s) of metabolism, product inhibition by one or more of the metabolite (18, 22, 23, 24), saturation of a common step in the formation of side chain oxidation metabolites, i.e., these metabolites may be formed through a single intermediate, possibly an epoxide (54), and possibly a change in absorption characteristics (4).

The present studies were designed to further characterize the pharmacokinetics associated with probenecid elimination and accumulation, to identify the associated mechanisms, and to establish whether saturable elimination occurs in therapeutic range.

Three major studies were carried out:

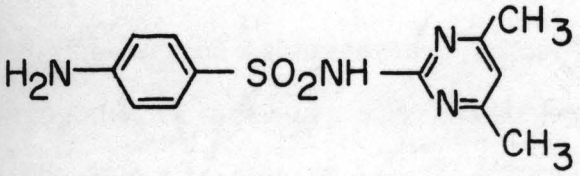
Study I was designed to examine the possible dose-dependency of probenecid elimination following single 0.5 and 2.0 gm probenecid doses administered in suspension form in water. In addition to plasma samples, urine samples were examined for unchanged drug and also probenecid acylglucuronide.

Studies II and III were designed to examine the accumulation characteristics of probenecid following 0.5 and 1.0 gm multiple, oral tablet doses. These studies were based on the fact that, although single dose data can be approximated by a linear kinetic model, multiple dose data should be more sensitive to non-linearity in the system and should thus be a more effective means to determine the "correct" model.

EXPERIMENTAL

A. Materials

Probenecid 500 mg Tablets USP, Lot No. H0953 and J1736 were purchased from Merck Sharp and Dohme, West Point, PA 19486. Lot No. H0953 and J1736 were used for the suspension and multiple dose studies, respectively. Probenecid (Lot L-573,932-00G22) and sulfamethazine (II) (Sigma Lot 78C-0427) of reference standard quality were donated by Merck Sharp and Dohme (West Point, PA 19486) and purchased from Sigma Chemical Co. (St. Louis, MO. 63172), respectively. The purity of probenecid standard had been previously validated by NMR spectroscopy (59).



(II)

Human plasma (for assay verification) was purchased from the American Red Cross, Madison. Non-sterile Vacutainer® 10 ml evacuated blood collection tubes containing 143 USP units of sodium heparin and Vacutainer® 21 gauge 1½" thin wall sample needles, and Luer-Lok BD 10 cc sterile "single use" plastipak syringes were purchased from Becton-Dickinson, Rutherford, NJ 07070.

Intermittent Miniset® vein infusion sets, 21 G x 1.9 cm, were purchased from Travenol Laboratories, Inc., Deerfield, Illinois 60015.

Heparin sodium injection (pig intestine) USP, 5000 USP units/ml, Lot No. 012027, was purchased from Elkins-Sinn Inc., Cherry Hill, NJ 08034. Isotonic sodium chloride injection USP, (8C923H6, 2C815R6) were purchased from Travenol Laboratories, Inc., Deerfield, Illinois 60015.

Reagents

Sodium chloride, citric acid, disodium hydrogen orthophosphate, sodium hydroxide, and potassium dihydrogen orthophosphate were obtained from Mallinckrodt. Anhydrous diethyl ether "Baker Analyzed Reagent" was purchased from J. T. Baker Chemical Co., ultraviolet grade acetonitrile and methanol were purchased from Burdick and Jackson Laboratories, Inc., Muskegon, Michigan 49442.

Hydrochloric acid was purchased from Hi-Pure Chemicals, Inc., P. O. Box 351, Nazareth, Pennsylvania 18064.

All reagents and solvents were used without further purification.

Apparatus

The chromatography system consisted of a solvent pump (Model 110A, Altex Scientific), a fixed volume (20µl), sample injection valve (Model 210A, Altex Scientific) a 10µm particle size reversed-phase octadecyl analytical column (4.6 mm I.D. x 250 mm Ultrasil

ODS, Altex Scientific), and a fixed wavelength (254 nm) UV detector (Model 153, Altex Scientific) with an 8µl analytical cell.

A guard column prepacked with Co-pell ODS (3.2 mm I.D. x 50 mm) was purchased from Alltech Associates. During the analysis of samples, guard column was cleaned and repacked repeatedly with Co-pell ODS. Chromatographs were obtained on a linear strip chart recorder (Model 250, 30 cm) from Linear Instrumental Corp., Irvine, California. Detector output and recorder input were 10 mV and 1 mV, respectively.

Preparation of Reagents and Stock Solutions

Buffer pH 4.0

Disodium hydrogen orthophosphate (35.5 gm) was dissolved in 250 ml of water, and the pH adjusted to 4.0 with 40 gm citric acid, then diluted to 500 ml with water.

Buffer pH 6.0

Potassium dihydrogen orthophosphate (27.2 gm) was dissolved in 500 ml of water and pH adjusted to 6.0 with 1 N sodium hydroxide solution and diluted to 1 l with water.

Mobile Phase

(a) Analysis of plasma samples

Phosphate buffer 50 ml of 0.01 M, pH6 was diluted to 675 ml with water and 325 ml of acetonitrile was added, mixed and degassed for 15 min.

(b) Analysis of urine samples

Phosphate buffer 50 ml of 0.01 M, -H6 was diluted to 690 ml with water and 310 ml of acetonitrile was added, mixed and degassed for 15 min.

Stock Solutions

Probenecid reference standards

Probenecid reference standards were prepared by weighing nine individual probenecid samples which were then dissolved in a known volume of methanol.

Probenecid reference standard, set I (Table VII), was used during the analysis of plasma samples of the suspension study.

Probenecid reference standard, set II (Table VIII), was used during the analysis of plasma samples of multiple dose studies.

Probenecid reference standards for analysis of urine samples

For unchanged probenecid: Reference solution (0.1 ml) (0.962 mg/ml of probenecid in methanol) was used.

For conjugated probenecid: Reference solution (0.1 ml) (1.273 mg/ml of probenecid in methanol) was used.

Internal reference standard

Sulfamethazine (12.4 mg) was dissolved in 250 ml of methanol. This reference standard solution was used in the analysis of probenecid plasma samples obtained from both single and multiple dose studies.

Table VII
Probenecid reference standards, Set I, used during analysis of plasma samples from the suspension study.

Reference Solution	Quantity of probenecid (gm.)	Volume of reference solution (ml)	Concentration of probenecid (mg/ml)
A	0.02035	10	2.035
B	0.01424	10	1.424
C	0.01025	10	1.025
D	0.00765	10	0.765
E	0.00540	10	0.540
F	0.00276	10	0.276
G	0.00149	10	0.149
H	0.00169	25	0.068
I	0.00171	50	0.034

Table VIII
Probenecid reference standards, Set II, used during analysis of plasma samples from multiple dose studies.

Reference Solution	Quantity of probenecid (gm)	Volume of reference solution (ml)	Concentration of probenecid (mg/ml)
A	0.02379	10	2.379
B	0.01558	10	1.558
C	0.01273	10	1.273
D	0.00962	10	0.962
E	0.00455	10	0.455
F	0.00362	10	0.362
G	0.00119	10	0.119
H	0.00093	25	0.037
I	0.00129	50	0.026

No variation was observed in the chromatographic response to this standard solution throughout the entire study.

B. Methods

Preparation of Samples

Plasma:

Probenecid concentrations in plasma were determined by a modification of a previously described high pressure liquid chromatography procedure (60). Plasma (1 ml), pH 4 phosphate buffer (1 ml), sodium chloride (1 gm), internal standard (0.1 - 0.3 ml) (49.6 mcg/ml sulfamethazine in methanol) and diethyl ether (8 ml) were combined in a 15 ml centrifuge tube, vortex mixed for 15 sec and shaken for 30 min on a horizontal shaker. Following centrifugation at 3000 x G for 10 min, 5 ml of the ether layer was transferred to a clean tube and evaporated to dryness in a water bath at 40°C under nitrogen. The residue was reconstituted in 1.0 ml of chromatographic mobile phase by vortex mixing for 30 sec, and then injected on to the column.

Urine:

Probenecid concentrations in urine were determined by a similar high pressure liquid chromatography procedure to that used for plasma. Urine (1 ml), pH 4 phosphate buffer (1 ml) and diethyl ether (8 ml) were combined in a 15 ml centrifuge tube, vortex mixed for 15 sec and shaken for 20 min on a horizontal shaker. Following

centrifugation at 3000 x G for 10 min. 5 ml of ether layer was transferred to a clean tube and evaporated to dryness under nitrogen in a water bath at 40°C. The residue was reconsituted in 1.0 ml of chromatographic mobile phase which consisted of 31% acetonitrile in 0.01 M pH 6 phosphate buffer, by vortex mixing for 30 sec., and then injected on to the column.

Preparation of urine samples for analysis of probenecid following hydrolysis of probenecid-acyl glucuronide:

Urine (1 ml) and 3 N hydrochloric acid (1 ml) were vortex mixed in a 15 ml centrifuge tube for 15 sec. and incubated at 65°C for 28 hrs. At the end of the incubation period, pH 4 phosphate buffer (1 ml) and diethyl ether (8 ml) were added, vortex mixed for 15 sec., and shaken for 20 min on a horizontal shaker. Following centrifugation, samples were handled as described previously and analyzed for probenecid.

The concentration of probenecid-acyl glucuronide was calculated from the difference between concentrations of probenecid before and after hydrolysis.

Assay

The extraction efficiency of probenecid from plasma was 97.4 ± 5.4 (standard deviation) % (n = 20). There is no interference from endogeneous substances or the probenecid metabolites all of which are more polar than the parent compound under the assay conditions (60).

The extraction efficiency of the internal standard sulfamethazine used for the analysis of plasma samples was 80.2 ± 5.8 (standard deviation) % (n = 20). Sulfamethazine could not be used during the analysis of urine samples because of interference by endogeneous compounds. The stability of probenecid that was stored frozen in plasma was examined by analysis of plasma samples containing known quantities of probenecid over a period of three months. No deterioration of samples was observed. Probenecid plasma samples were analyzed within one month of their collection.

The reference standards of sulfamethazine and probenecid in methanol kept under refrigeration showed no deterioration, as their response to the chromatographic conditions remained constant throughout the study.

Extraction of probenecid from plasma samples; the suspension study:

Probenecid reference standards, set I, was prepared to determine probenecid concentrations in the plasma samples from the suspension study. These probenecid reference standards in methanol had a concentration range of 34-2035 mcg/ml. In order to establish the correlation between instrument response and probenecid concentrations, standards were injected on to the column under the chromatographic conditions used for unknown samples. These conditions were: mobile phase: 32.5% acetonitrile in 0.01 M phosphate buffer, pH 6

flow rate: 1 ml/min (1000 psi)

chart speed: 10 cm/hr.

Under these conditions probenecid had a retention time of 7.2 min. The relationship between probenecid peak heights (y) and their corresponding concentrations (x) was given by:

$$y = 0.038 + 0.014\ x - (1.52 \times 10^{-6})\ x^2$$

with coefficient of determination (r^2) and coefficient of correlation (ϱ) values of 0.999.

Chromatographic assay was linearly responsive to probenecid concentrations up to 540 mcg/ml. In this linear range, the relationship between probenecid peak height and concentration was:

$$y = 0.11 + 0.013x$$

$r^2 =$ $= 0.999$

Having thus established the linearity of response to probenecid concentrations below 540 mcg/ml, these reference standards (0.1 ml) and internal standard (0.1) (sulfamethazine 49.6 mcg/ml in methanol) were added to plasma (1 ml) and extracted to construct standard curves.

Three standard curves of probenecid in plasma, in the concentration range 3.4 - 203.5 mcg/ml, were constructed on three separate days. Assay reproducibility was within 10% of the mean within this range (Figure 4, Table IX). These curves were used for the statistical analysis of the linear regression model in terms of pure error and lack of fit variance contributions.

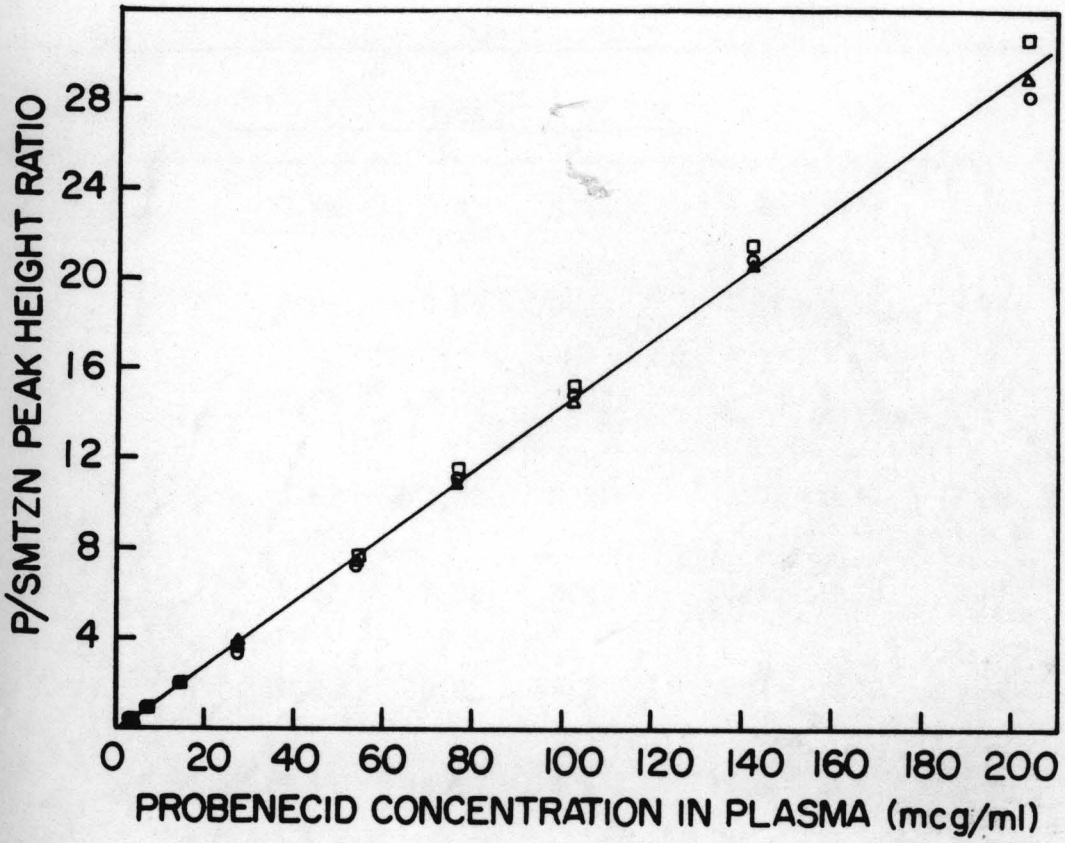


Figure 4: Probenecid standard curve.

Table IX
Probenecid sulfamethazine peak height ratio from Reference Standard, Set I.

Probenecid concentration (mcg/ml)	Runs 1	2	3	$\bar{X}$	SD.	CV%
3.40	0.45	0.43	0.45	0.44	0.01	2.60
6.76	0.89	0.85	0.90	0.88	0.03	3.01
14.9	2.05	2.00	2.07	2.04	0.04	1.77
27.6	3.62	3.90	3.81	3.78	0.14	3.78
54.0	7.27	7.62	7.82	7.57	0.28	3.68
76.5	10.9	10.8	11.5	11.1	0.38	3.42
102.5	15.1	14.5	14.8	14.8	0.30	2.03
142.4	20.8	20.7	21.7	21.1	0.55	2.61
203.5	28.2	29.1	30.9	29.4	1.37	4.68

Pure error and lack of fit are the components of variance that are unexplained by the regression equation. The results obtained by employing linear regression analysis on probenecid concentrations and probenecid, sulfamethazine peak height ratios are shown in Table X. Lack of fit and pure error were calculated accordingly (61). The data showed excellent fit to the regression model and no significant lack of fit was observed.

Probenecid concentrations were determined by the peak height ratio method. The chromatogram in Figure 5 is from a plasma sample obtained 36 hours after administration of a 2.0 gm probenecid suspension dose. Retention times of probenecid and sulfamethazine were 8.4 and 6 min respectively.

Standard curves using pooled predose plasma were run with each batch of postdose samples and probenecid concentrations were determined by using these standards although no day to day or within day variability was detected.

Extraction of probenecid from plasma samples: multiple dose studies:

Probenecid reference standard, set II, was used to determine probenecid concentrations from the multiple dose studies. The concentration range of these standards was 26 - 2397 mcg/ml. In order to examine the linearity of instrument response in this concentration range, standards were injected on to the column under the following chromatographic conditions:

mobile phase: 32.5% acetonitrile in 0.01 M phosphate buffer, pH 6

flow rate: 1 m/min (1000 psi)

Table X
ANOVA for probenecid reference standard, Set I.

Source	D.F.	S.S.	M.S.	F_{ratio}	$F_{(0.95)}$
Total	26	2421.2			
Regression	1	2415.5	2415.4	12077	4.24
Residual	25	5.8	0.2		
Lack of fit	7	0.746	0.107	0.381	2.58
Pure error	18	5.054	0.281		

Coefficient of determination, r^2= 99.8%

Regression equation of the line (value ± SD.):

$$y = - 0.1409 (\pm 0.1369) + 0.1462 (\pm 0.0014)x$$

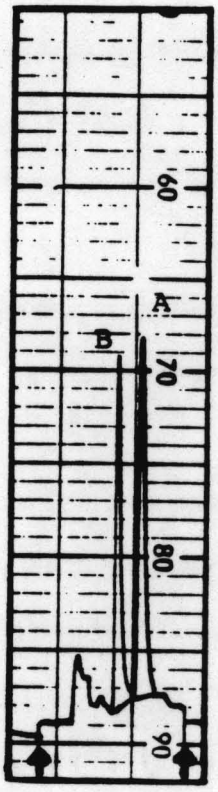


Figure 5: High pressure liquid chromatogram from plasma of subject #4, 36 h after the administration of 2.0 gm probenecid suspension dose. Concentrations of probenecid (A) is 9.52 mcg/ml and sulfamethazine (B) is 4.96 mcg/ml at sensitivity range (r) of 0.02.

chart speed: 20 cm/hr.

The retention time of probenecid under these conditions was 7.2 min.

The relationship between probenecid peak height (y) and concentrations (x) was given by the following equation:

$$y = 0.031 + 0.015\ x - (2.38 \times 10^{-6})\ x^2$$

with coefficient of determination (r^2) and coefficient of correlation (ϱ) values of 0.999.

The assay was linearly responsive to probenecid concentrations <500 mcg/ml, and could be described by the relationship:

$$y = 0.02 + 0.014x$$

Coefficient of determination and coefficient of correlation values were 0.999. These reference standards were used to construct standard curves from plasma in the presence of internal standard, sulfamethazine.

Three standard curves of plasma containing probenecid reference standard (0.1 ml) and sulfamethazine (0.3 ml) were constructed (Figure 6, Table XI) on three separate days. These standard curves were used for statistical analysis of the linear regression model in terms of pure error and lack of fit variance contributions. As previously, data showed good fit to the linear regression model when it was analyzed for pure error and lack of fit (Table XII).

Unknown probenecid concentrations in plasma samples were again determined by peak height ratio method.

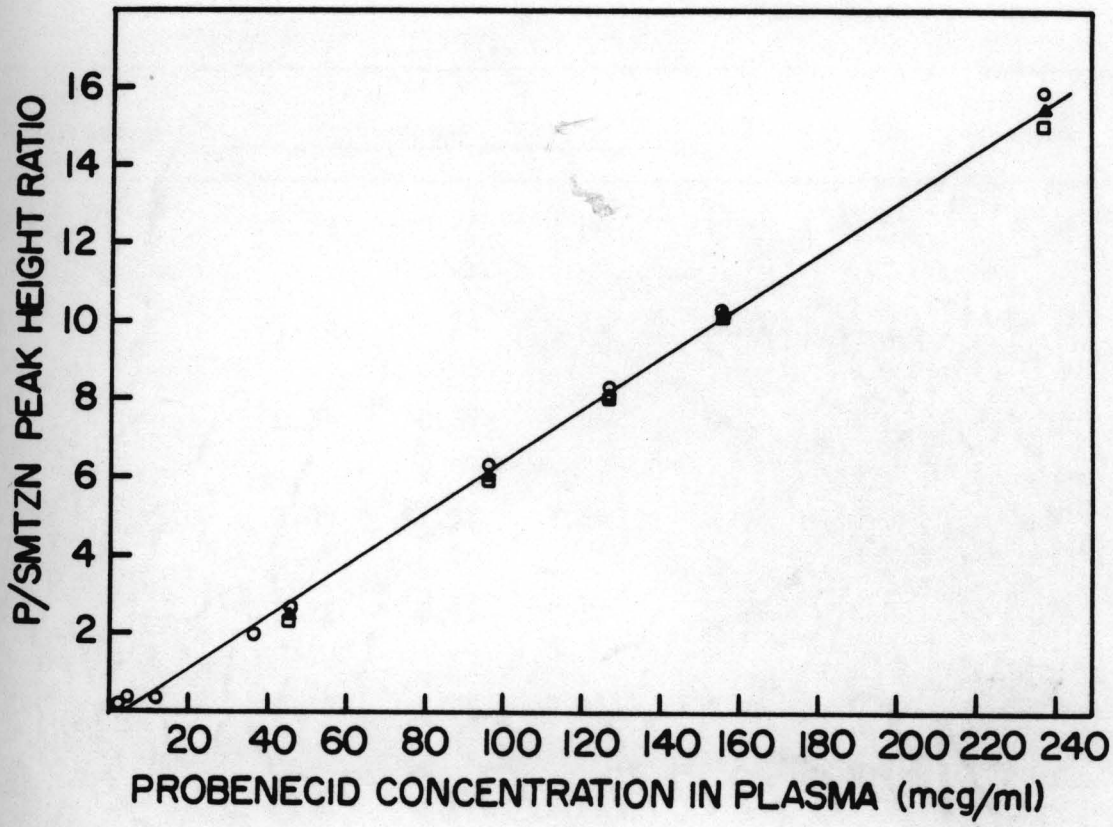


Figure 6: Probenecid standard curve.

Table XI
Probenecid sulfamethazine peak height ratio from Reference Standard, Set II.

Probenecid concentration (mcg/ml)	Runs 1	2	3	$\bar{X}$	SD.	CV%
2.59	0.09	0.10	0.09	0.09	0.01	6.19
3.72	0.13	0.14	0.13	0.13	0.01	4.33
11.9	0.39	0.38	0.35	0.37	0.02	5.57
36.2	2.05	1.98	2.04	2.02	0.04	1.87
45.5	2.72	2.63	2.53	2.63	0.09	3.62
96.2	6.22	6.05	6.02	6.09	0.11	1.77
127.3	8.33	8.11	7.95	8.13	0.19	2.35
155.8	10.3	10.1	10.0	10.1	0.15	1.51
237.9	15.9	15.5	15.0	15.5	0.45	2.92

Table XII
ANOVA for probenecid reference standard, Set II.

Source	D.F.	S.S.	M.S.	F_{ratio}	$F_{(0.95)}$
Total	26	692.25			
Regression	1	691.34	691.34	17283.5	4.24
Residual	25	0.92	0.04		
Lack of fit	7	0.348	0.049	1.56	2.58
Pure error	18	0.572	0.032		

Coefficient of determination, $r^2 = 99.9\%$

Regression equation of the line (value ± SD.):

$$y = -0.2587\ (\pm\ 0.0532) + 0.0661\ (\pm\ 0.005)x$$

The chromatogram in Figure 7 was obtained from analysis of a plasma sample taken 2 hours after administration of the fourth, 1.0 g probenecid tablet dose to subject #6. Retention times of probenecid and sulfamethazine were 7.5 and 6 min. respectively.

Probenecid concentrations were determined according to the standard curve which was constructed with pooled predose plasma during analysis of each batch of postdose plasma samples.

Extraction of probenecid from urine samples:

Sulfamethazine could not be used as internal standard for the analysis of urine samples due to interference from endogeneous substances. In order to improve separation of probenecid the concentration acetonitrile in the mobile phase was reduced by 1.5%.

Probenecid reference standard in urine was prepared by adding a known quantity of probenecid (96.2 mcg) to blank urine. Probenecid peak heights were compared with standard solutions that were injected immediately before and following each set of seven samples according to the method of Harle and Cowen (60).

Unknown probenecid concentrations were determined as follows:

$$\text{probenecid(mcg/ml)} = \frac{\text{sample peak height x standard concentration}}{\text{mean standard peak height}}$$

A typical chromatogram is shown in Figure 8. Chromatographic conditions were:

mobile phase: 31% acetonitrile in 0.01 M phosphate buffer, pH 6

Flow rate: 1 ml/min (1000 psi).

Chart speed: 20 cm/hr.

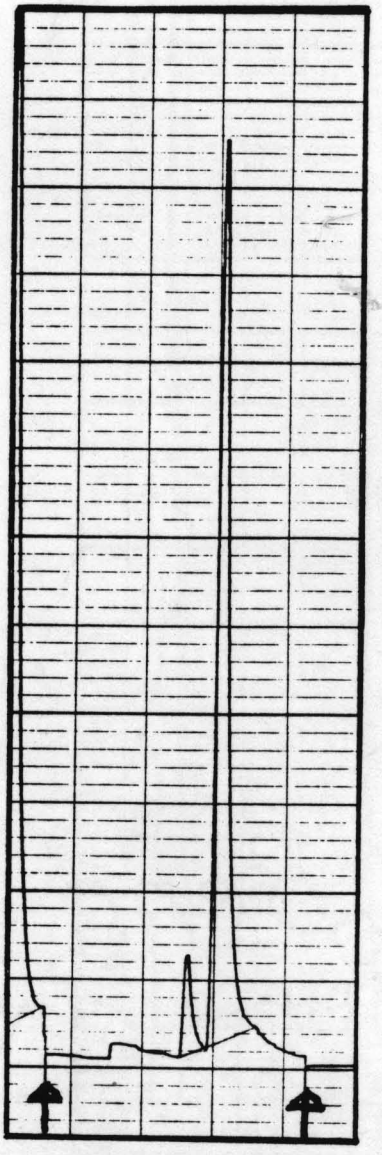


Figure 7: High pressure liquid chromatogram from plasma of subject #6, 2 h after the administration of 4th 1.0 gm probenecid tablet dose. Concentration of probenecid (A) is 175.5 mcg/ml and sulfamethazine (B) is 14.9 mcg/ml at r = 0.16.

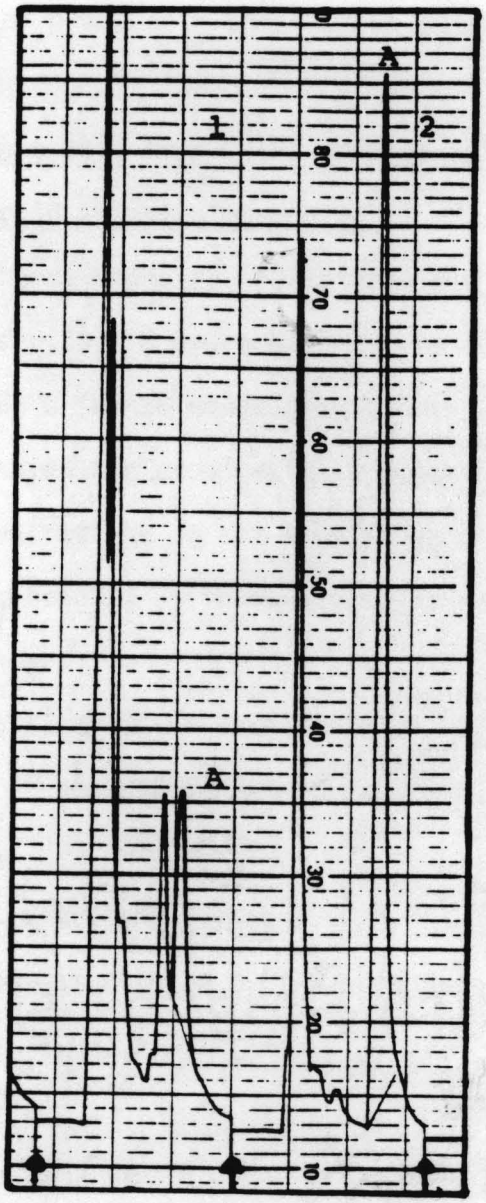


Figure 8: High pressure liquid chromatogram of urine samples before hydrolysis (1) of subject #4 following 2.0 gm suspension dose, during 36-48 h interval, concentrations of probenecid is 210 mcg/ml, (2) probenecid reference standard in urine (96, 2 mcg/ml), both at r = 0.08.

Retention time of probenecid under these conditions was 7.5 min.

Extraction of probenecid from urine samples following hydrolysis of probenecid - acyl glucuronide:

Probenecid reference standard (127.3 mcg) and urine samples (1 ml) were incubated with 3 N hydrochloric acid (1 ml) in 15 ml centrifuge tubes at 65°C for 28 hours. At the end of incubation time samples were assayed for probenecid as described previously.

Probenecid concentrations in the samples were calculated by comparing by the peak heights of unknown samples to the peak height of the reference standard.

Chromatograms obtained after hydrolysis of urine sample in Figure 8(1),and also of probenecid reference standard in urine, are shown in Figure 9.

Chromatographic conditions were:

mobile phase: 31% acetonitrile in 0.01 M phosphate buffer, pH 6.

Flow rate: (1 ml/min) (1000 psi)

Chart speed: 20 cm/hr.

Retention time of probenecid under these conditions was 8.1 min.

Although probenecid samples prepared without incubation have a pH of 6, after hydrolysis, pH of the samples injected on to the column and recovered from the column had a pH of 5.5.

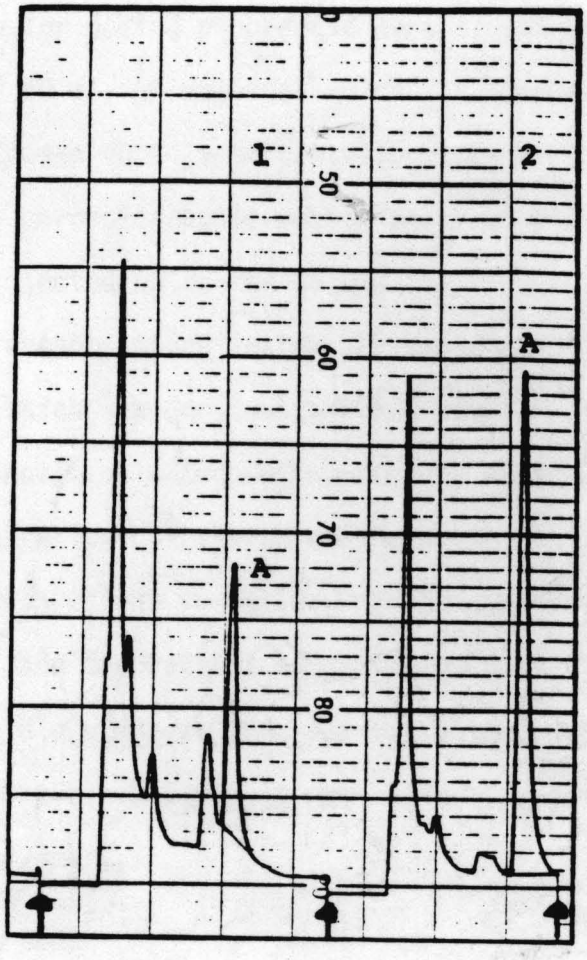


Figure 9: High pressure liquid chromatogram of urine samples after hydrolysis (1) urine sample in Figure 8(1), (2) probenecid reference standard in urine (127.85 mcg/ml), both at r = 0.32.

Extraction of probenecid residue remaining in the beakers used for dosing probenecid suspensions:

Beakers used for dosing probenecid were rinsed repeatedly with a total volume of 40 ml of methanol which was then evaporated to dryness under nitrogen in a 15 ml centrifuge tube. The residue was taken up into chromatographic mobile phase (1 ml), vortex mixed for 30 sec. and injected on to the column.

Probenecid concentrations in the residue were determined from a standard curve which was constructed in a similar fashion by evaporating methanolic solutions of probenecid reference standards and taking up the residue by the chromatographic mobile phase.

The probenecid residue in beakers can be expressed in terms of the percentage of the theoretical administered dose (Table XIII).

The probenecid residue in the beakers following both doses was considered as negligible because it was uniformly less than 0.1% of the theoretical dose.

Protocol

Male volunteers underwent physical examinations and gave informed consent to participate in the study. Physical examinations included blood and urine analyses. The protocol and consent forms are included in Appendix II-IV for the three studies.

Subjects were instructed to take no other medication for one week before or during the study. Subjects were ambulatory during all studies. Blood samples (~8 ml) were drawn from a forearm vein into

Table XIII
Probenecid residue in beakers expressed as a percentage of theoretical administered dose.

Subject	Theoretical Dose	
	0.5 gm	2.0 gm
1	0.083	0.003
2	0.093	0.033
3	0.086	0.028
4	0.098	ND[1]
$\bar{X}$	0.09	0.021
SD.	0.007	0.016
CV%	7.53	75.3

[1]Not determined.

heparinized collection tubes (Vacutainer®) and centrifuged immediately in a table-top centrifuge. Plasma was separated and stored at -20°C until assayed, which was within one month of collection period.

Study I - Probenecid suspension study

The four subjects in this study received single 0.5 and 2.0 gm probenecid suspension doses two weeks apart according to a randomized Latin square design as in the diagram below:

	Study Week	
Subject	1	2
1, 2	0.5 gm	2.0 gm
3, 4	2.0 gm	0.5 gm

All doses were given as finely powdered probenecid tablets in a beaker with 250 ml of water at 8 a.m. following overnight fast. Food was witheld until 4 hours after dosing. Subjects were given 200 ml of water 2.0, 4.0 and 8.0 hr. after dosing.

Although an attempt was made to utilize mini-infusion sets (Butterflies) for blood drawing in all subjects, it was successful in only subjects 3 and 4.

Blood samples were collected immediately before dosing and then at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0, 14.0, 16.0, 20.0, 24.0, 30.0, 36.0 and 48 hours after the dose of probenecid.

In the case of subjects 1 and 2, blood samples were drawn into evacuated blood collection tubes containing sodium heparin. Blood

samples from subjects 3 and 4 were drawn from mini infusion set by a syringe and immediately delivered into a heparinized collection tube for centrifugation and separation of plasma. In order to prevent formation of clots in the mini-infusion set, after each sample collection, tubing was rinsed with 2-3 ml of saline containing 30 USP units of heparin per ml. At each sampling time an initial 4-5 ml of heparin solution with blood was discarded and the blood sample was then drawn with a new disposable syringe.

Subjects were instructed to give a urine sample before receiving probenecid and then to collect urine quantitatively during 0-2, 2-6, 6-12, 12-24, 24-36 and 36-48 hour intervals after receiving probenecid.

Urine volumes and pH were measured and recorded and 20 ml aliquots were immediately frozen. Subject details are given in Table XIV.

Study II - Probenecid 0.5 and 1.0 gm tablet, multiple dose study

Four subjects participated in this study and received 0.5 and 1.0 gm multiple doses ten days apart in a randomized design as follows:

	Study Week	
Subjects	1	2
1, 2	0.5 gm	1.0 gm
3, 4	1.0 gm	0.5 gm

Table XIV
Demographic details of the subjects in the single dose probenecid suspension study.

	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
Age (years)	23	22	25	26	23.8	1.5	6.32
Height (cm.)	173	173	180	160	171.5	8.35	4.87
Weight (kg.)	75.0	68.0	77.0	53.0	68.3	10.9	15.9
Body Surface Area (M^2)	1.88	1.81	1.96	1.53	1.79	0.19	10.4

All doses were given as tablets or multiples of tablets (2 tablets for 1.0 g dose), following overnight fast. Food and water were witheld until 4 hours post dose, when normal eating and drinking were resumed. All 8:00 a.m. doses (3) were given with 250 ml of water after overnight fast, 2:00 p.m. dose (1) was given 2 hours after the midday meal, and the 8:00 p.m. dose (1) was given 2 hours following the evening meal. Blood sampling and dosing times are shown in Table XVI.

In this study, blood samples of all subjects were drawn into heparinized evacuated blood collection tubes (Vacutainer®).

A total of six doses of probenecid were given during 120 hours.

Subject details are given in Table XVI.

Study III - Probenecid 1.0 gm tablet, multiple dose study

Two male subjects received 1.0 gm tablet doses of probenecid, over a period of 96 hours. Subjects were permitted to eat during a 1 hour period commencing 2.5 hours after the administration of each dose.

Subjects received ten doses of probenecid in this study and blood sampling and dosing times are shown in Table 10.

Blood samples were drawn into heparinized evacuated collection tubes, centrifuged to separate the plasma which was then stored at -20°C until assayed.

Subject details are given in Table XVIII.

Table XV
Blood sampling and dosing times of the probenecid 0.5 and 1.0 gm tablet multiple dose study.

DAY	1	2	3	4	5	6
DOSE	8:00 a.m.		8:00 a.m.,2:00 p.m. 8:00 p.m.	2:00 a.m.,8:00 a.m.		
SAMPLING TIMES						
	8:00 a.m.(0.0)	8:00 a.m.(24.0)	8:00 a.m.(48.0)	2:00 a.m.(66.0)	4.00 a.m.(92.0)	8:00 a.m.(120.0)
	8:30 a.m.(0.5)	9:00 p.m.(36.0)	2:00 p.m.(54.0)	8:00 a.m.(72.0)	8:00 a.m.(96.0)	
	9:00 a.m.(1.0)		8:00 p.m.(60.0)	10:00 a.m.(74.0)	8:00 p.m.(108.0)	
	10:00 a.m.(2.0)			12:00 p.m.(76.0)		
	11:00 a.m.(3.0)			4:00 p.m.(80.0)		
	12:00 p.m.(4.0)			8:00 p.m.(84.0)		
	2:00 p.m.(6.0)			12:00 a.m.(88.0)		
	4:00 p.m.(8.0)					
	8:00 p.m.(12.0)					

Table XVI
Demographic details of the subjects in the probenecid multiple dose study.

	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
Age (years)	23	26	25	25	25.8	1.26	5.08
Height (cm.)	173	160	175	180	172.0	8.52	4.96
Weight (kg.)	75.0	53.0	70.5	77.0	68.9	10.9	15.9
Body Surface Area (M^2)	1.9	1.53	1.85	1.96	1.81	0.19	10.6

Table XVII
Blood sampling and dosing times of the probenecid 1.0 gm multiple dose study.

DAY	1	2	3	4	5
DOSE	6:00 a.m.	6:00 a.m.,12:00 p.m. 6:00 p.m.,12:00 a.m.	6:00 a.m.,12:00 p.m. 6:00 p.m.,12:00 a.m.	6:00 a.m.	
SAMPLING TIMES	6:00 a.m.(0.0)	6:00 a.m.(24.0)	6:00 a.m.(48.0)	6:00 a.m.(72.0)	6:00 a.m.(96.0)
	6:30 a.m.(0.5)	8:00 a.m.(26.0)	8:00 a.m.(50.0)	6:30 a.m.(72.5)	
	7:00 a.m.(1.0)	6:00 p.m.(36.0)	6:00 p.m.(60.0)	7:00 a.m.(73.0)	
	8:00 a.m.(2.0)	8:00 p.m.(38.0)	8:00 p.m.(62.0)	8:00 a.m.(74.0)	
	9:00 a.m.(3.0)			9:00 a.m.(75.0)	
	10:00 a.m.(4.0)			10:00 a.m.(76.0)	
	12:00 p.m.(6.0)			12:00 p.m.(78.0)	
	2:00 p.m.(8.0)			1:00 p.m.(79.0)	
	6:00 p.m.(12.0)			2:00 p.m.(80.0)	
				3:00 p.m.(81.0)	
				4:00 p.m.(82.0)	
				6:00 p.m.(84.0)	
				8:00 p.m.(86.0)	
				10:00 p.m.(88.0)	
				12:00 a.m.(90.0)	

Table XVIII
Demographic details of subjects in probenecid multiple dose study.

	Value				
	Subject		$\bar{X}$	SD.	CV%
	5	6			
Age (years)	25	22	23.5	2.12	9.03
Height (cm.)	178	165	171.5	9.19	5.36
Weight (kg.)	68.0	72.0	70.0	2.83	4.04
Body Surface Area (M^2)	1.84	1.79	1.82	0.04	1.95

Pharmacokinetic and Statistical Analysis

In all three probenecid studies, individual plasma probenecid concentration versus time data were first plotted on semi-logarithmic graph paper to obtain graphical estimates of parameters where appropriate. Final parameter estimates were then obtained by non-linear regression analysis of data by using the program NREG (62) on a Univac model 1100 Digital computer. Pharmacokinetic and statistical analysis of data were dictated by the nature of the results.

Study I - Probenecid suspension study

Plasma probenecid concentrations following 0.5 and 2.0 gm suspension doses were analyzed according to three different one compartment kinetic models with first order absorption.

In these three models elimination of drug from plasma was described by:

(i) first-order elimination,

(ii) saturable, Michaelis-Menten type elimination,

(iii) parallel, saturable and first-order elimination model.

Relative changes in plasma profiles with respect to doses, initiated the analysis of data using models (ii) and (iii) in addition to model (i). Observations leading to the use of these models are consistent with results of a previous study (5) where model (ii) successfully described probenecid elimination. Model (iii) was included in an attempt to describe the elimination of probenecid by parallel saturable and first order elimination pathways.

Initial estimates of kinetic parameters for the first model were obtained by usual graphical procedures.

Initial estimates of first order rate constants for appearance of drug, k_a, fraction of the dose absorbed, expressed as concentration in its volume of distribution, FD/V, in models (ii) and (iii) were obtained from the final values of the first model.

In order to obtain estimates for Michaelis-Menten constants V_M and K_M, plasma probenecid concentrations obtained from 4 to 16 hours were used to obtain a second degree polynomial. This equation was used to predict probenecid concentrations at 1 hour intervals. These values and actual concentrations were then used to obtain graphical estimates for parameters V_M and K_M, by the method of Wagner (63).

The computer program for nonlinear regression analysis of data according to models (ii) and (iii) required combination of the program NREG and the numerical integration program DGEAR (64).

Initial estimates of the first order elimination rate constant of model (iii), k', were chosen intuitively relative to the magnitude of the other parameters. For final computer estimation of k', the boundaries were held wide enough so as not to restrict the value of the final estimate.

Subsequently the parameters V_M, K_M and k' obtained from 2.0 gm dose were used to describe 0.5 gm data by models (ii) and (iii).

Statistical analysis of plasma and urine data and parameter estimates were performed by analysis of variance for randomized

complete block design (65). Significant differences were examined by Tukey's Test (66). Significant results were examined further by analysis of variance for factorial experiment (67).

Study II - Probenecid 0.5 and 1.0 gm tablet, multiple dose study

Initial parameter estimates for the analysis of data, according to the three models were obtained as described previously. Statistical analysis of pharmacokinetic parameters was performed by analysis of variance for randomized complete block design. Significant effects were examined by Tukey's Test.

Interesting effects that were observed but could not be detected by two-way analysis of variance were pursued further by multifactorial analysis of the data by the use of program SPSS (68).

Sign tests were performed to differentiate between parameter sets that could best describe the data according to Equation 2.

Study III - Probenecid 1.0 tablet, multiple dose study

Pharmacokinetic analysis of the data was performed as described above.

Since the study consisted of only 2 subjects, further statistical analysis of data beyond the calculation of the mean and standard deviation was not considered.

RESULTS

A. Study I - Suspension single-dose study

Plasma

Plasma levels of probenecid obtained from single oral 0.5 and 2.0 gm suspension doses to four subjects are given in Table XIX and XX. The mean plasma levels and standard deviations from the mean values are presented in Figure 10.

Following the 0.5 gm suspension dose, plasma probenecid levels reached a mean peak value of 41.5 mcg/ml at 2.3 h. Levels then declined in an apparent monoexponential fashion with a mean half-life of 4.7 h. Probenecid could be detected in the plasma of all subjects at 24 h and in three subjects at 30 h after dosing.

Following the 2.0 gm suspension dose, plasma levels of probenecid reached a mean peak value of 219.4 mcg/ml at 2.8 h. After reaching peak values, plasma levels from the 2.0 gm dose declined initially in a slow and erratic manner. The rate of decline was curvilinear and did not become monoexponential until individual levels approached the maximum levels achieved with 0.5 gm suspension dose.

First order elimination kinetics did not appear to occur until 20-24 h after dosing, when probenecid plasma levels had fallen to *circa* 40 mcg/ml. Probenecid could be detected in the plasma of all subjects at 48 h postdosing.

Table XIX
Probenecid plasma levels following single 0.5 gm probenecid suspension dose.

Time[1] (h)	Plasma probenecid (mcg/ml) Subject 1	2	3	4	$\bar{X}$	SD.	CV%
0.0	-	-	-	-[2]	-	-	-
0.5	24.9	18.6	31.3	20.6	23.9	5.62	23.6
1.0	ND[3]	14.5	41.5	45.1	33.7	16.7	49.6
1.5	34.9	22.4	35.5	58.4	37.8	15.0	39.7
2.0	30.3	21.8	33.3	49.2	33.7	11.5	34.0
2.5	31.8	22.9	31.6	42.7	32.3	8.1	25.1
3.0	32.3	23.4	25.7	40.1	30.4	7.50	24.7
4.0	32.3	28.2	23.4	35.7	29.9	5.31	17.8
5.0	25.8	31.2	22.6	30.8	27.6	4.14	15.0
6.0	24.2	23.1	18.9	27.7	23.5	3.63	15.4
8.0	23.4	15.6	13.1	19.9	18.0	4.56	25.4
12.0	11.1	6.04	5.52	10.9	8.39	3.02	36.0
14.0	8.75	4.28	4.72	8.41	6.54	2.37	36.2
16.0	6.60	2.70	3.33	6.31	4.74	2.01	42.4
20.0	4.92	1.55	1.81	4.29	3.14	1.71	54.5
24.0	4.15	1.31	1.48	3.57	2.63	1.44	54.8
30.0	2.28	0.95	-	3.11	1.59	1.38	87.1
36.0	1.83	-	-	2.95	1.19	1.45	121.6
48.0	-	-	-	-	-	-	-

[1]Times indicated are scheduled times. Actual times for individual subjects are shown in Tables XXIX through XXXII.

[2]Below detectable limit (<0.7 mcg/ml).

[3]Sample not taken.

Table XX
Probenecid plasma levels following single 2.0 gm probenecid suspension dose.

Time[1] (h)	Plasma probenecid (mcg/ml) Subject 1	2	3	4	$\bar{X}$	SD.	CV%
0.0	-	-	-	-	-	-	-
0.5	137.1	86.9	142.0	52.9	104.7	42.6	40.7
1.0	220.9	ND	140.3	147.0	169.4	44.7	26.4
1.5	233.9	93.0	143.9	177.7	162.1	59.2	36.5
2.0	214.5	115.3	184.2	222.7	184.2	48.8	26.5
2.5	188.0	105.7	167.4	256.1	179.3	61.9	34.6
3.0	166.2	184.2	177.5	229.7	189.4	27.9	14.7
4.0	188.7	160.3	204.0	175.7	182.2	18.6	10.2
5.0	149.4	151.8	163.5	180.2	161.2	14.1	8.7
6.0	155.1	150.9	152.7	182.6	160.3	14.9	9.3
8.0	153.3	114.2	90.1	148.3	126.5	29.8	23.6
12.0	110.5	92.9	52.1	122.1	94.4	30.7	32.5
14.0	77.9	72.9	55.4	103.4	77.4	19.9	25.7
16.0	65.3	50.9	36.8	78.9	57.9	18.2	31.4
20.0	46.3	38.7	29.2	51.5	41.4	9.69	23.4
24.0	37.6	24.2	18.1	35.4	28.8	9.27	32.2
30.0	28.9	12.5	9.49	21.2	18.0	8.79	48.9
36.0	16.9	4.58	3.79	9.52	8.7	6.04	69.4
48.0	4.73	1.40	1.34	3.01	2.62	1.61	61.3

[1]Times indicated are scheduled times. Actual times for individual subjects are shown in Tables XXXIII through XXXVI.

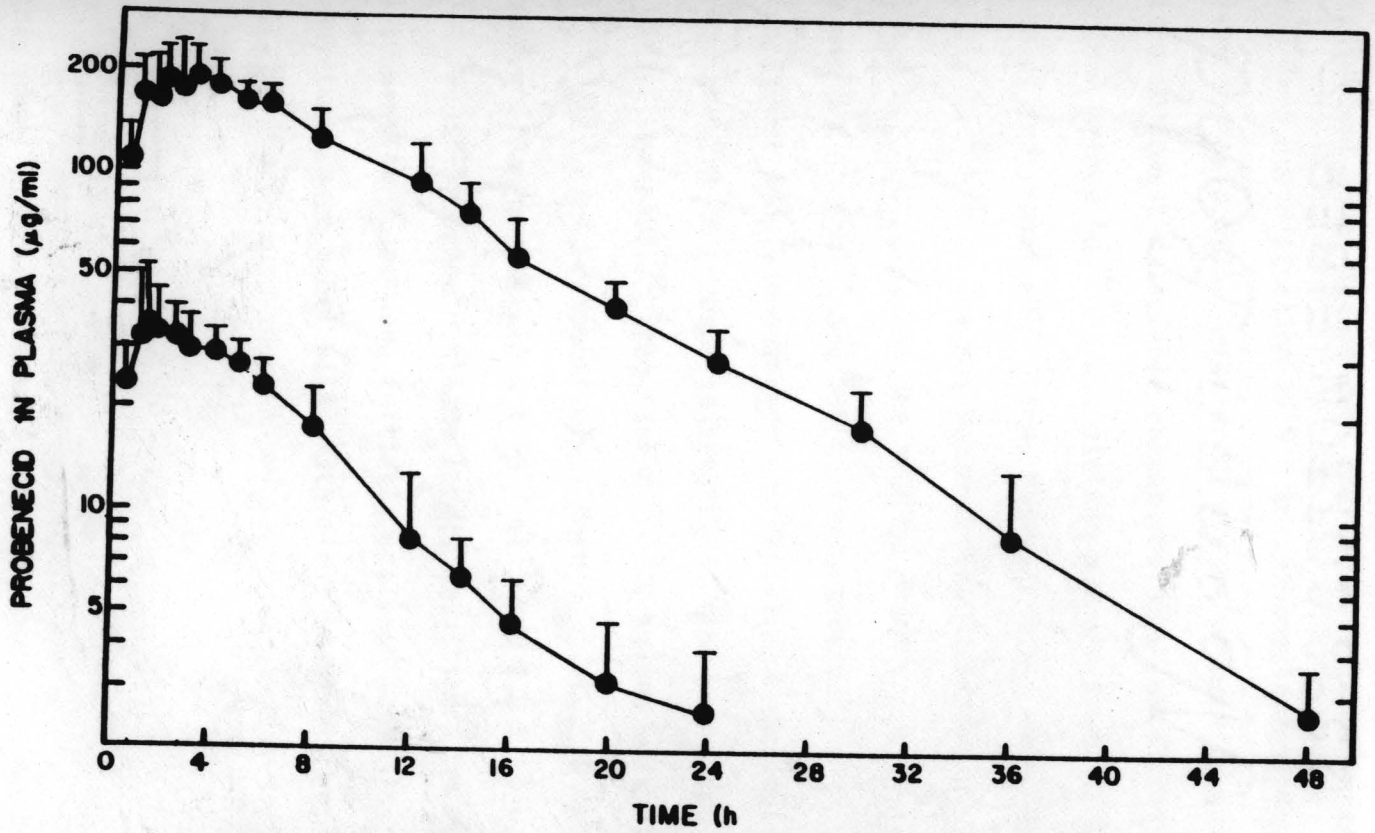


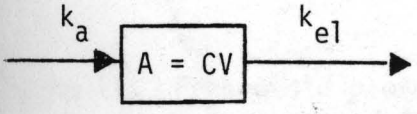
Figure 10: Mean (±SD.) probenecid plasma levels following 0.5 and 2.0 gm probenecid suspension doses.

Individual probenecid plasma concentrations from the 0.5 and 2.0 gm doses are shown in Figures 11-14.

Individual model-independent pharmacokinetic parameter values obtained from administration of 0.5 gm probenecid suspension dose are given in Table XXI. Areas under plasma probenecid curves from time zero to lowest detectable concentrations, calculated by trapezoidal method $AUC^{o\rightarrow t}$ trap, yielded a mean value of 343.8 mcg.h/ml The mean of individual $AUC^{o\rightarrow t}$ trap normalized for dose, $AUC^{o\rightarrow t}/D$, was 6.88×10^{-4} h/ml. The maximum drug concentration, C_{max}, dose normalized C_{max}, C_{max}/D, and the time to reach C_{max}, T_{max} were 40 mcg/ml, 8×10^{-5} ml^{-1} and 2.4 h, respectively.

Individual model-independent pharmacokinetic parameter values following the 2.0 gm probenecid suspension dose are given in Table XXII. Mean $AUC^{o\rightarrow t}$ trap, and $AUC^{o\rightarrow t}/D$ values were 2625 mcg.h/ml and 13.1×10^{-4} h/ml, respectively. Mean C_{max}, C_{max}/D and T_{max} values were 219.4 mcg/ml, 10.9×10^{-5} ml^{-1} and 2.8 h, respectively.

Individual probenecid plasma levels resulting from the 0.5 and 2.0 gm suspension doses were initially analyzed according to a one-compartment open model (III) with first order absorption and elimination.



(III)

With this model plasma probenecid concentrations are described by Equation 1 in which F is the fraction of the oral dose D which is

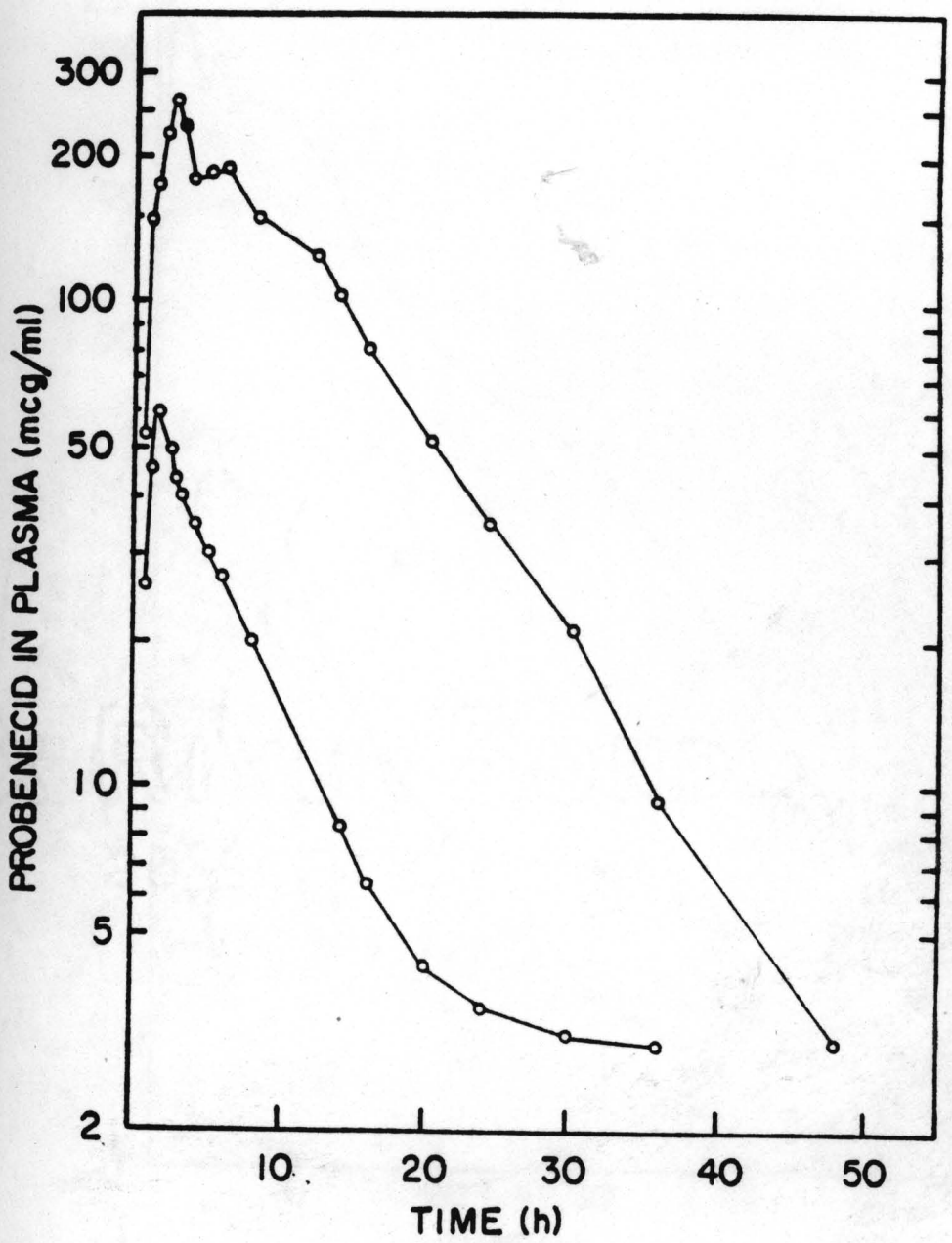


Figure 11: Probenecid plasma levels following single 0.5 and 2.0 gm suspension doses, subject #1.

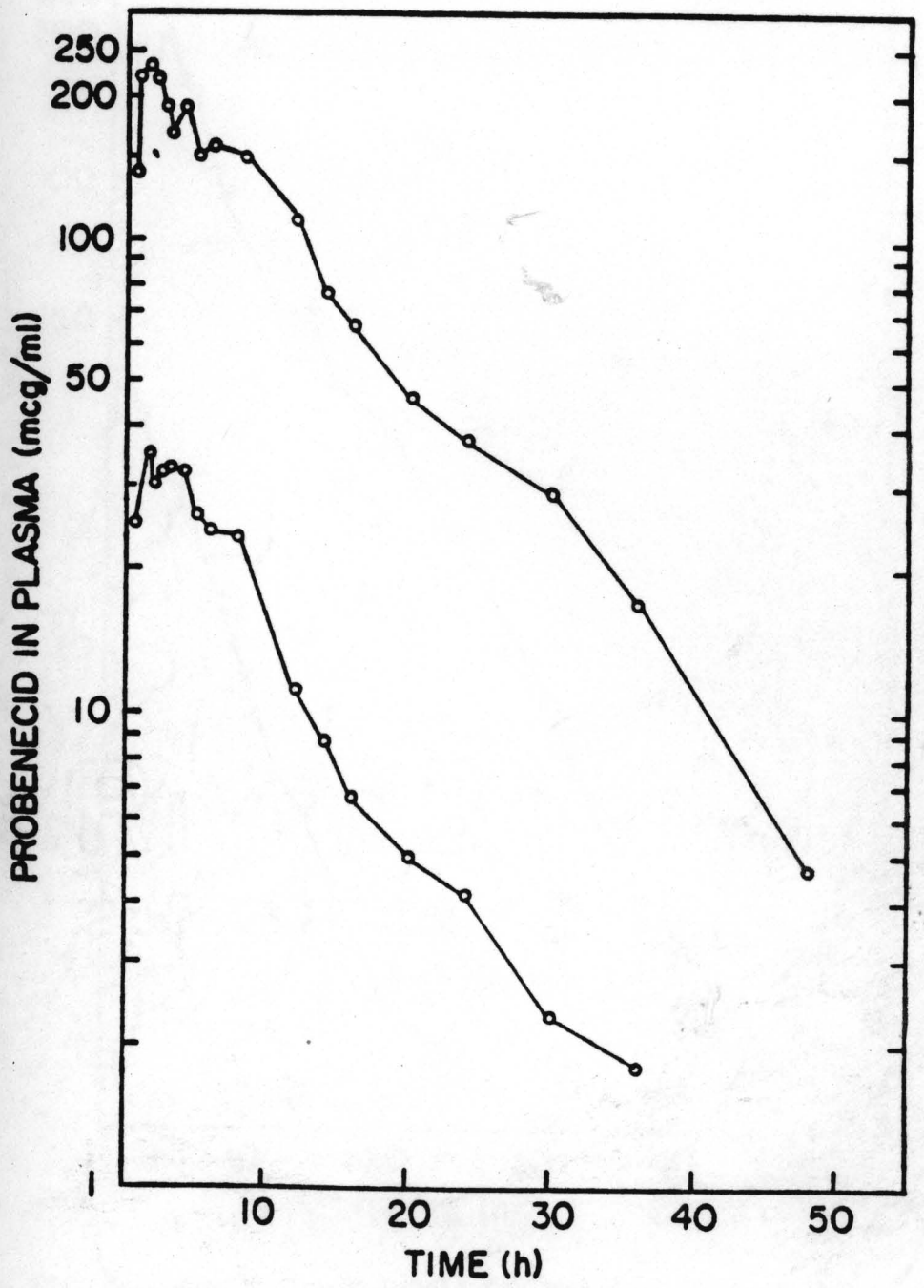


Figure 12: Probenecid plasma levels following single 0.5 and 2.0 gm suspension doses, subject #2.

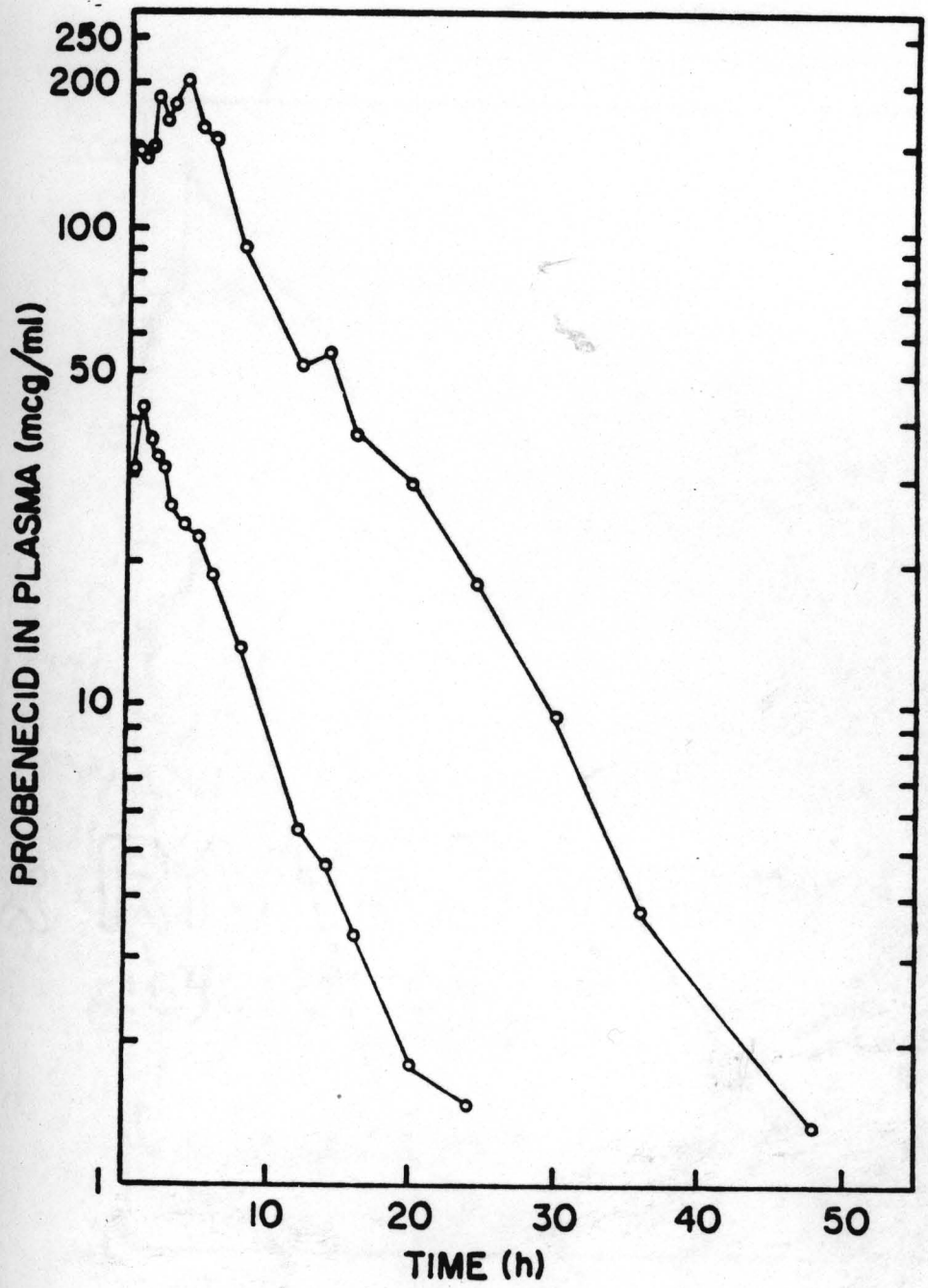


Figure 13: Probenecid plasma levels following single 0.5 and 2.0 gm suspension doses, subject #3.

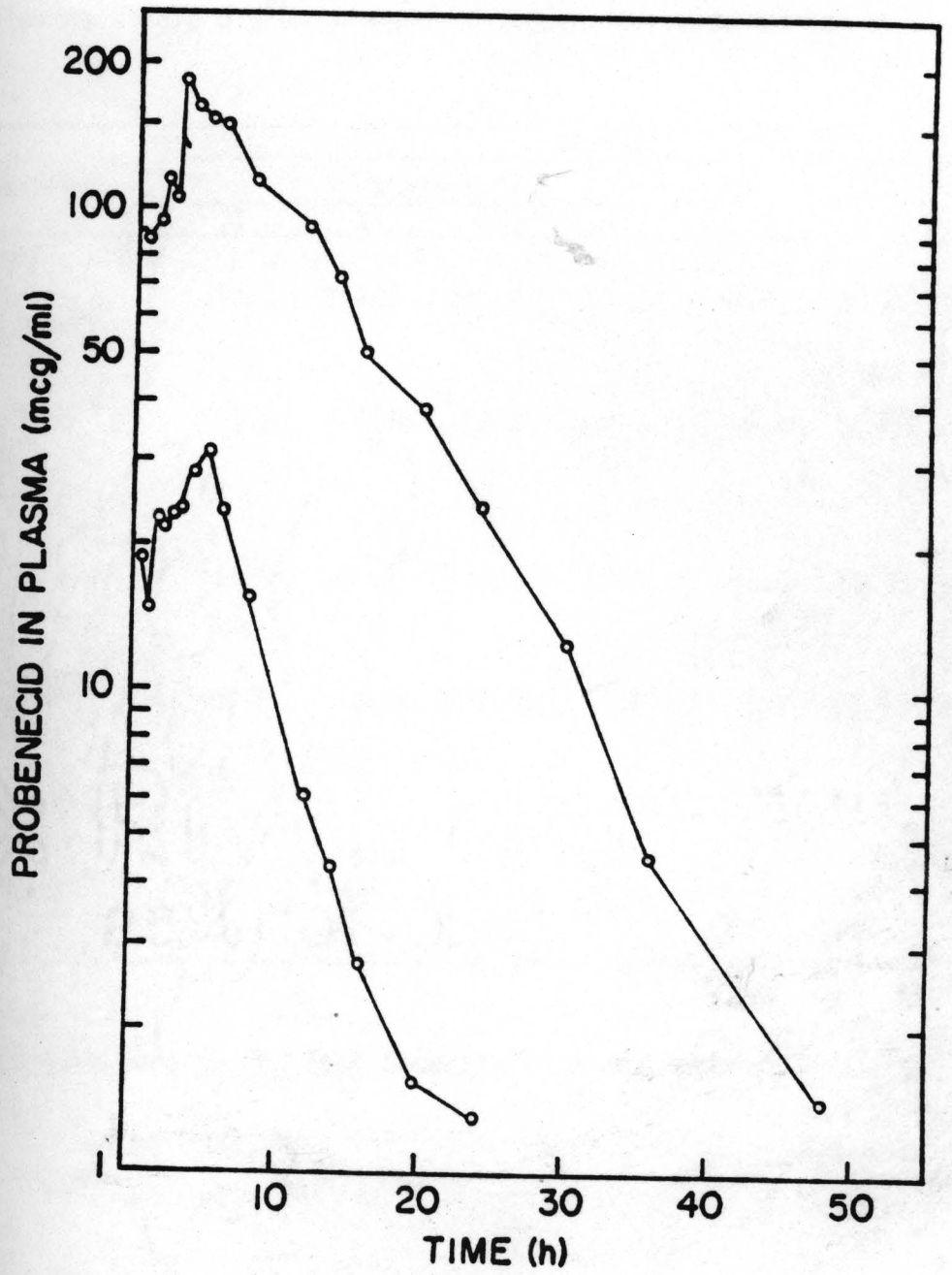


Figure 14: Probenecid plasma levels following single 0.5 and 2.0 gm suspension doses, subject #4.

Table XXI
Individual model-independent pharmacokinetic parameter values following single 0.5 gm probenecid suspension dose.

Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
$AUC_{trap.}^{o \rightarrow t}$[1] (mcg.h/ml)	393.0	259.2	265.8	457.0	343.8	97.4	28.3
$AUC/D(10^{-4})$ (h/ml)	7.86	5.18	5.3	9.1	6.88	1.95	28.3
C_{max} (mcg/ml)	34.9	31.2	41.5	58.4	41.5	12.0	29.0
$C_{max}/D(10^{-5})$ (ml^{-1})	6.98	6.24	7.09	11.7	8.0	2.49	31.2
T_{max} (h)	1.7	5.0	1.0	1.5	2.3	1.8	79.3

[1]From time zero to the last detectable concentration.

Table XXII
Individual model-independent pharmacokinetic parameter values following single 2.0 gm probenecid suspension dose.

Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
$AUC_{trap.}^{o \rightarrow t}$ (mcg.h/ml)	3060	2239	2081	3118	2625	540.7	20.6
$AUC/D(10^{-4})$ (h/ml)	15.3	11.2	10.4	15.6	13.1	2.71	20.6
C_{max} (mcg/ml)	233.4	184.2	204.0	256.1	219.4	31.7	14.5
$C_{max}/D(10^{-5})$ (ml^{-1})	11.7	9.21	10.2	12.8	10.9	1.59	14.5
T_{max} (h)	1.5	3.0	4.0	2.5	2.75	1.04	37.8

absorbed into the systemic circulation, V is the apparent homogeneous distribution volume for probenecid, k_a and k_{el} are first-order rate constants for appearance and loss of drug respectively and t_{lag} is the absorption lag time (69).

$$C = \frac{FD}{V}\left(\frac{k_a}{k_a - k_{el}}\right)\left(e^{-k_{el}(t - t_{lag})} - e^{-k_a(t - t_{lag})}\right) \qquad 1$$

Initial estimates of parameter values were obtained by standard graphical procedures. Final parameter estimates were obtained by nonlinear regression analysis using the program NREG on a digital computer. A summary of these final estimates for 0.5 and 2.0 gm doses are given in Tables XXIII and XXIV respectively.

Analogous to the results of the previous study (5), where $AUC^{o\rightarrow t}/D$ had increased 1.8 fold between 0.5 and 2.0 gm tablet doses, $AUC^{o\rightarrow t}/D$ in this study increased 1.9 fold between the 0.5 and 2.0 gm probenecid suspension doses. In the previous study, the elimination $t_{\frac{1}{2}}$ following 2.0 gm tablet dose was 2.02 - fold greater than the $t_{\frac{1}{2}}$ obtained from the 0.5 gm tablet dose. In this study, Equation 1 predicted an elimination $t_{\frac{1}{2}}$ from the 2.0 gm suspension dose 1.6 - fold greater than the value obtained from 0.5 gm dose. The dose-dependent discrepancies in $AUC^{o\rightarrow t}/D$ and $t_{\frac{1}{2}}$ in both the previous and the present studies, had been recognized as evidence of saturation of elimination (5). The 2.0 gm tablet data were initially analyzed

Table XXIII
Individual pharmacokinetic parameter values following single 0.5 gm probenecid suspension dose according to Equation 1.

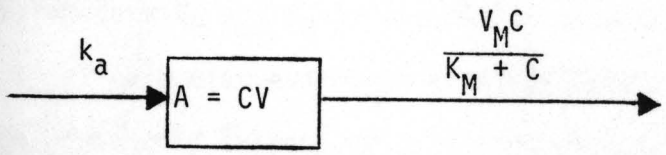
Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
FD/V (mcg/ml)	41.9	50.5	43.9	60.5	49.2	8.38	17.0
$F/V(10^{-5})$ (ml^{-1})	8.38	10.1	8.77	12.1	9.84	1.68	17.1
k_a (h^{-1})	1.48	0.45	4.63	3.02	2.39	1.83	76.3
$t_{1/2\ abs}$ (h)	0.47	1.54	0.15	0.23	0.59	0.64	107.6
k_{el} (h^{-1})	0.11	0.19	0.17	0.15	0.16	0.03	22.0
$t_{1/2\ el}$ (h)	6.48	3.67	4.2	4.53	4.72	1.23	25.9
t_{lag} (h)	0.001	0.001	0.25	0.41	0.17	0.20	121.6
r^2	0.994	0.966	0.997	0.994	0.988	0.015	1.5

[1]Calculated from $t_{1/2\ el} = \ln 2 / k_{el}$

Table XXIV
Individual pharmacokinetic parameter values following single 2.0 gm probenecid suspension dose according to Equation 1.

Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
FD/V (mcg/ml)	231.8	245.9	249.4	269.6	249.2	15.6	6.3
F/V(10^{-5}) (ml)	11.6	12.3	12.5	13.5	12.5	0.78	6.3
k_a (h^{-1})	8.07	0.44	0.99	1.18	2.67	3.61	135.3
$t_{1/2\ abs}$ (h)	0.09	1.58	0.70	0.59	0.74	0.62	83.7
k_{el} (h^{-1})	0.08	0.11	0.12	0.08	0.09	0.02	20.0
$t_{1/2\ el}$ (h)	8.66	6.3	5.78	8.66	7.35	1.53	20.8
t_{lag} (h)	0.46	0.001	0.001	0.28	0.19	0.23	121.5
r^2	0.994	0.975	0.977	0.991	0.984	0.009	0.98

according to a second model incorporating saturable drug elimination from the body. This one-compartment model (IV) was then utilized also to describe the plasma data obtained from 0.5 and 1.0 gm doses. The ability of this model to describe plasma profiles from the three different probenecid doses prompted analysis of the 2.0 gm suspension data according to the same model.



(IV)

This model incorporates first order absorption with an absorption lag time, and saturable elimination described in terms of a Michaelis Menten-type term. The rate equation for time-dependent change in probenecid levels by this model is given by Equation 2.

$$\frac{dc}{dt} = \frac{FD}{V} k_a e^{-k_a(t - t_{lag})} - \frac{V_M C}{K_M + C} \qquad 2$$

The maximum rate of probenecid elimination, V_M, and the drug concentration when the rate of elimination is one-half the maximum value, K_M, are Michaelis Menten-type functions. All other parameters are as defined for Equation 1.

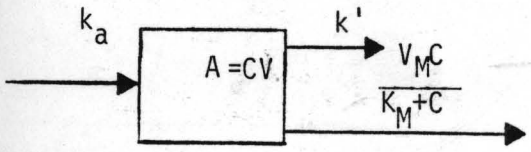
Nonlinear regression analysis of this rate equation was performed by coupling the numerical integration program DGEAR with the program NREG (5). Initial estimates of the value FD/V and k_a were taken from the final parameter estimates obtained from analysis

of the data according to Equation 1. In order to obtain estimates for Michaelis-Menten constants V_M and K_M, plasma probenecid concentrations obtained from 4 to 16 hours were used to obtain a second degree polynominal. This equation was used to predict probenecid concentrations at 1 hour intervals. These values and actual concentrations were then used to obtain graphical estimates for parameters V_M and K_M by the method of Wagner (70).

Final parameter estimates obtained from the analysis of 0.5 and 2.0 gm probenecid suspension data according to equation 2 are given in Tables XXV and XXVI, respectively.

The mean coefficients of determination, r^2, for 0.5 gm probenecid suspension dose according to Equations 1 and 2 were 0.988 and 0.987, respectively. From the analysis of 2.0 gm probenecid suspension data according to Equations 1 and 2, r^2 values of 0.984 and 0.983 were obtained. This indicates that the data from both doses could be adequately described by Equations 1 and 2.

As metabolism of probenecid occurs via two pathways, conjugation and side-chain oxidation, the possibility of describing the data by a third model was examined. This model (V) involved elimination of probenecid from the body by parallel saturable and non-saturable pathways.



(V)

Table XXV
Individual pharmacokinetic parameter values following single 0.5 gm probenecid suspension dose according to Equation 2.

Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
FD/V (mcg/ml)	43.8	42.5	43.8	57.0	46.8	6.84	14.6
$F/V(10^{-5})$ (ml^{-1})	8.76	8.5	8.76	11.4	9.36	1.37	14.6
k_a (h^{-1})	1.29	0.57	4.64	3.5	2.5	1.89	75.8
$t_{1/2\ abs}$ (h)	0.54	1.22	0.15	0.19	0.53	0.49	94.4
K_M (mcg/ml)	99.0	119.5	119.9	119.0	114.4	10.2	8.9
V_M (mcg/ml.h)	15.0	21.0	25.5	21.0	20.6	4.31	20.9
V_M/K_M (h^{-1})	0.15	0.18	0.21	0.18	0.18	0.02	13.6
$t_{1/2\ el}$ (h)	4.62	3.85	3.3	3.85	3.91	0.54	13.9
t_{lag} (h)	0.001	0.001	0.25	0.41	0.17	0.20	121.6
r^2	0.993	0.964	0.996	0.994	0.987	0.015	1.5

[1]Calculated from $t_{1/2\ el} = \ln 2/(V_M/K_M)$.

Table XXVI
Individual pharmacokinetic parameter values following single 2.0 gm probenecid suspension dose according to Equation 2.

Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
FD/V (mcg/ml)	218.5	213.3	224.1	247.1	225.8	14.9	6.6
F/V(10^{-5}) (ml-1)	10.9	11.6	11.2	12.4	11.5	0.65	5.6
k_a (h-1)	9.15	0.56	1.35	1.42	3.12	4.04	129.5
$t_{1/2\ abs}$ (h)	0.08	1.24	0.51	0.49	0.58	0.48	83.2
K_M (mcg/ml)	98.9	120.0	119.9	119.6	114.6	10.5	9.1
V_M (mcg/ml.h)	15.9	20.9	25.7	20.1	20.7	4.02	19.5
V_M/K_M (h-1)	0.16	0.17	0.21	0.17	0.18	0.02	12.5
$t_{1/2\ el}$ (h)	4.33	4.08	3.30	4.08	3.95	0.45	11.3
t_{lag} (h)	0.46	0.001	0.001	0.28	0.19	0.23	121.5
r^2	0.992	0.975	0.977	0.989	0.983	0.009	0.9

The rate equation describing the change in probenecid concentration in plasma with respect to time was developed as follows:

$$\frac{dc}{dt} = \frac{FD}{V} k_a e^{-k_a(t - t_{lag})} - \frac{V_M C}{K_M + C} - k'C \qquad 3$$

The first-order elimination rate constant of the non-saturable pathway is denoted by k' . All other parameters are as defined for Equation 2, and the initial parameter estimates were obtained as described previously. Initial estimates of the first-order elimination rate constant, k', were chosen intuitively, relative to the magnitude of the other parameters. Final parameter estimates were obtained by nonlinear regression analysis of individual data by adaptation of the coupled programs NREG and DGEAR, to accommodate the third model.

The final parameter estimates obtained by analysis of 0.5 and 2.0 gm suspension data according to Equation 3 are given in Tables XXVII and XXVIII, respectively.

Individual plasma probenecid concentrations, and also the concentrations predicted by the analysis of 0.5 gm probenecid suspension data according to Equations 1, 2, and 3, are shown in Tables XXIX-XXXII.

Figures 15-18 depict the predicted concentrations by the three models and the actual data points, following the administration of a single 0.5 gm probenecid suspension dose.

Table XXVII
Individual pharmacokinetic parameter values following single 0.5 gm probenecid suspension dose according to Equation 3.

Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
FD/V (mcg/ml)	48.4	73.6	46.4	60.5	57.2	12.6	21.9
F/V(10^{-5}) (ml-1)	9.68	14.7	9.28	12.1	11.4	2.51	21.9
k_a (h^{-1})	0.97	0.29	4.07	3.1	2.11	1.77	84.1
$t_{1/2\ abs}$ (h)	0.71	2.39	0.17	0.22	0.87	1.04	119.3
K_M (mcg/ml)	120.5	119.0	40.0	107.0	96.6	38.2	39.6
V_M (mcg/ml.h)	20.0	4.99	13.0	2.0	9.99	8.13	81.3
k' (h^{-1})	0.01	0.25	0.01	0.14	0.10	0.12	113.0
V_M/K_M (h^{-1})	0.17	0.04	0.33	0.02	0.14	0.14	102.2
$t_{1/2\ el}$[1] (h)	3.85	2.39	2.04	4.33	3.15	1.11	35.2
t_{lag} (h)	0.001	0.001	0.25	0.41	0.17	0.20	121.6
r^2	0.988	0.968	0.989	0.995	0.985	0.012	1.2

[1]Calculated from $t_{1/2\ el} = \ln 2/(V_M/K_M + k')$.

Table XXVIII
Individual pharmacokinetic parameter values following single 2.0 gm probenecid suspension dose according to Equation 3.

Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
FD/V (mcg/ml)	230.3	237.9	235.2	266.5	242.5	16.3	6.7
$F/V(10^{-5})$ (ml^{-1})	11.5	11.9	11.8	13.3	12.1	0.80	6.6
k_a (h^{-1})	8.34	0.46	1.28	1.19	2.82	3.69	130.9
$t_{1/2\ abs}$ (h)	0.08	1.51	0.54	0.58	0.68	0.59	88.5
K_M (mcg/ml)	119.9	119.9	89.6	106.6	109.0	14.4	13.2
V_M (mcg/ml.h)	20.9	4.38	13.9	2.31	10.4	8.64	83.3
k' (h^{-1})	0.07	0.09	0.05	0.07	0.07	0.02	23.3
V_M/K_M (h^{-1})	0.17	0.04	0.15	0.02	0.10	0.08	79.9
$t_{1/2\ el}$ (h)	2.86	5.66	3.45	7.59	4.89	2.17	44.3
t_{lag} (h)	0.46	0.001	0.001	0.28	0.19	0.23	121.1
r^2	0.994	0.975	0.976	0.991	0.984	0.009	1.00

Table XXIX
Actual and computer predicted probenecid plasma levels following single 0.5 gm probenecid suspension dose according to Equations 1, 2 and 3 for Subject #1.

Time (h)	Plasma probenecid (mcg/ml)			
	Actual	Predicted by equations		
		1	2	3
0.5	24.9	23.8	22.6	20.3
1.7	34.9	34.1	34.1	33.7
2.2	30.3	34.0	34.5	34.7
2.5	31.8	33.4	34.0	34.7
3.0	32.3	32.1	32.9	33.8
4.0	32.3	29.4	30.1	31.0
5.0	25.8	26.5	26.9	27.4
6.0	24.2	23.5	23.6	23.5
8.0	23.4	19.3	18.7	17.8
12.0	11.1	12.4	10.9	9.27
14.0	8.75	10.1	8.34	6.77
16.0	6.60	8.18	6.29	4.86

Table XXX
Actual and computer predicted probenecid plasma levels following single 0.5 gm probenecid suspension dose according to Equations 1, 2 and 3 for Subject #2.

Time (h)	Plasma probenecid (mcg/ml)			
	Actual	Predicted by equations		
		1	2	3
0.5	18.6	9.37	9.81	9.04
1.0	14.5	16.6	17.0	16.2
1.5	22.4	21.6	21.9	21.3
2.0	21.8	24.4	24.4	24.3
2.5	22.9	26.1	25.9	26.2
3.0	23.4	26.8	26.5	27.0
4.0	28.2	26.5	26.0	26.9
5.0	31.2	24.7	24.3	25.2
6.0	23.1	22.1	21.8	22.5
8.0	15.6	16.7	16.9	16.6
12.0	6.04	8.61	9.27	7.76
14.0	4.28	6.07	6.75	5.07
16.0	2.70	4.16	4.79	3.18

Table XXXI
Actual and computer predicted probenecid plasma levels following single 0.5 gm probenecid suspension doese according to Equations 1, 2 and 3 for Subject #3.

Time (h)	Plasma probenecid (mcg/ml)			
		Predicted by equations		
	Actual	1	2	3
0.5	31.3	31.9	31.9	31.5
1.0	41.5	38.7	38.7	40.0
1.5	35.5	36.6	36.6	38.2
2.0	33.3	33.7	33.7	34.9
2.5	31.6	31.2	31.3	32.1
3.0	25.7	28.5	28.5	28.9
4.0	23.4	24.3	24.1	23.7
5.0	22.6	20.7	20.3	19.1
6.0	18.9	17.9	17.2	15.5
8.0	13.1	12.4	11.3	8.69
12.0	5.52	6.41	5.11	2.64
14.0	4.72	4.68	3.48	1.44
16.0	3.33	3.4	2.35	0.77

Table XXXII
Actual and computer predicted probenecid plasma levels following single 0.5 gm probenecid suspension dose according to Equations 1, 2 and 3 for Subject #4.

Time (h)	Plasma probenecid (mcg/ml)			
	Actual	Predicted by equations		
		1	2	3
0.5	20.6	20.2	20.6	19.9
1.0	45.1	48.1	47.6	48.3
1.5	58.4	51.5	50.1	51.6
2.2	49.2	48.5	47.4	48.5
2.5	42.7	45.9	45.3	45.9
3.0	40.1	42.6	42.5	42.6
4.0	35.7	36.6	37.2	36.5
5.0	30.8	31.1	32.2	31.0
6.0	27.7	27.1	28.3	26.8
8.0	19.9	19.5	20.8	19.3
12.0	10.9	10.7	11.2	10.4
14.2	8.41	7.75	7.98	7.49
16.0	6.31	5.81	5.85	5.57

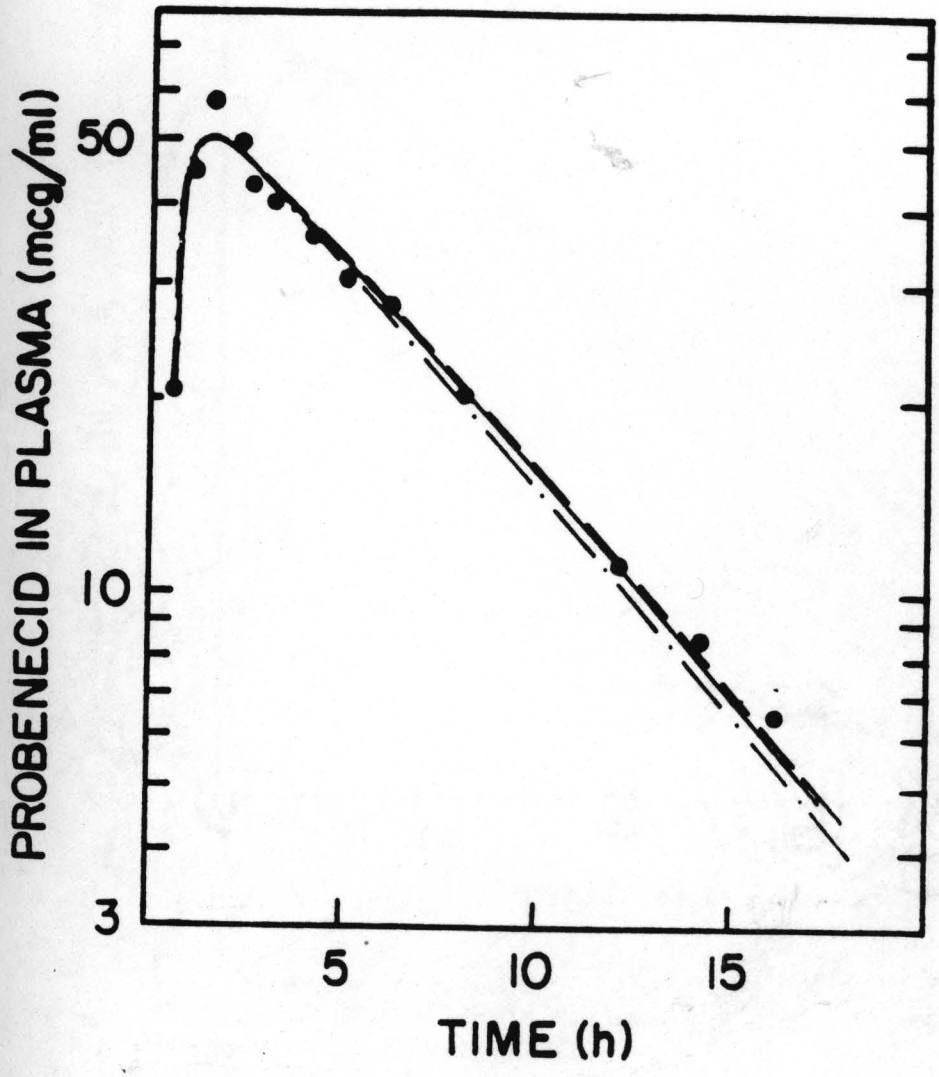


Figure 15: Probenecid plasma level following single 0.5 gm suspension dose, subject #1, and plasma profiles generated by Equation 1 (-), Equation 2 (--), and Equation 3 (-.-).

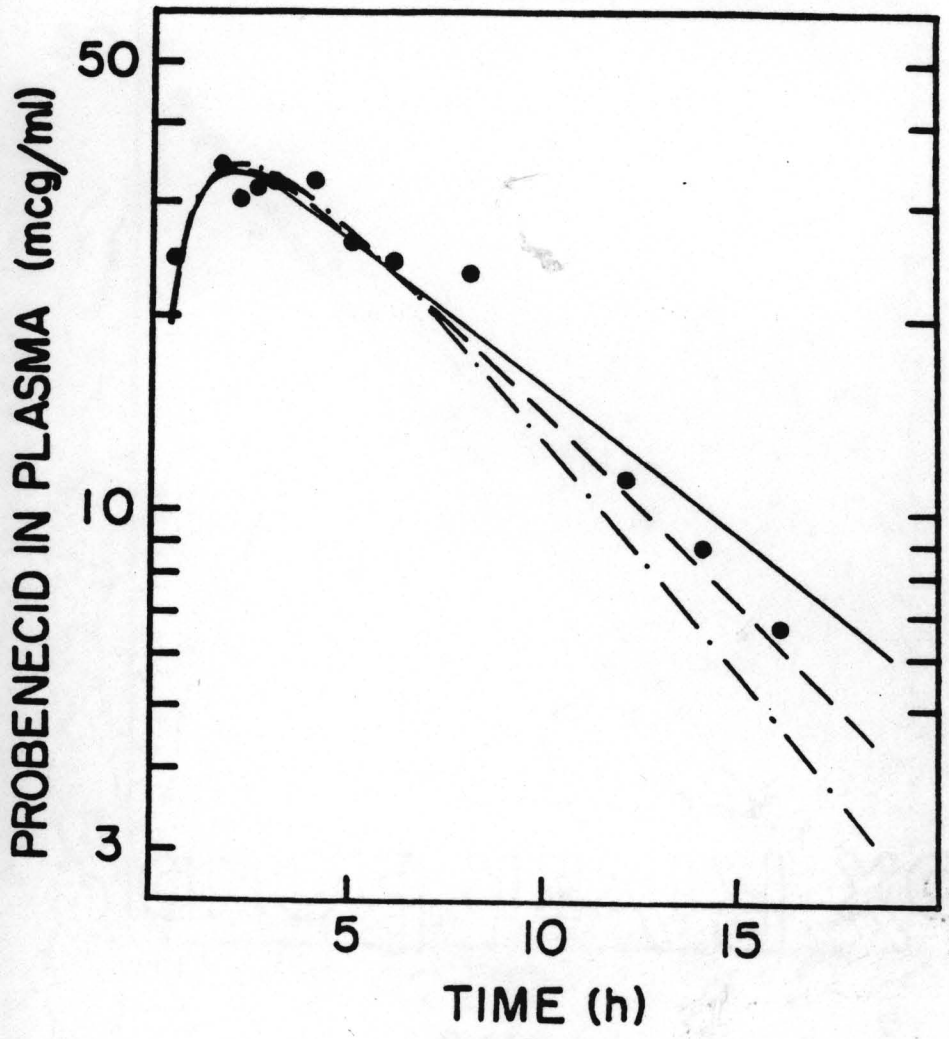


Figure 16: Probenecid plasma levels following single 0.5 gm suspension dose, subject #2.

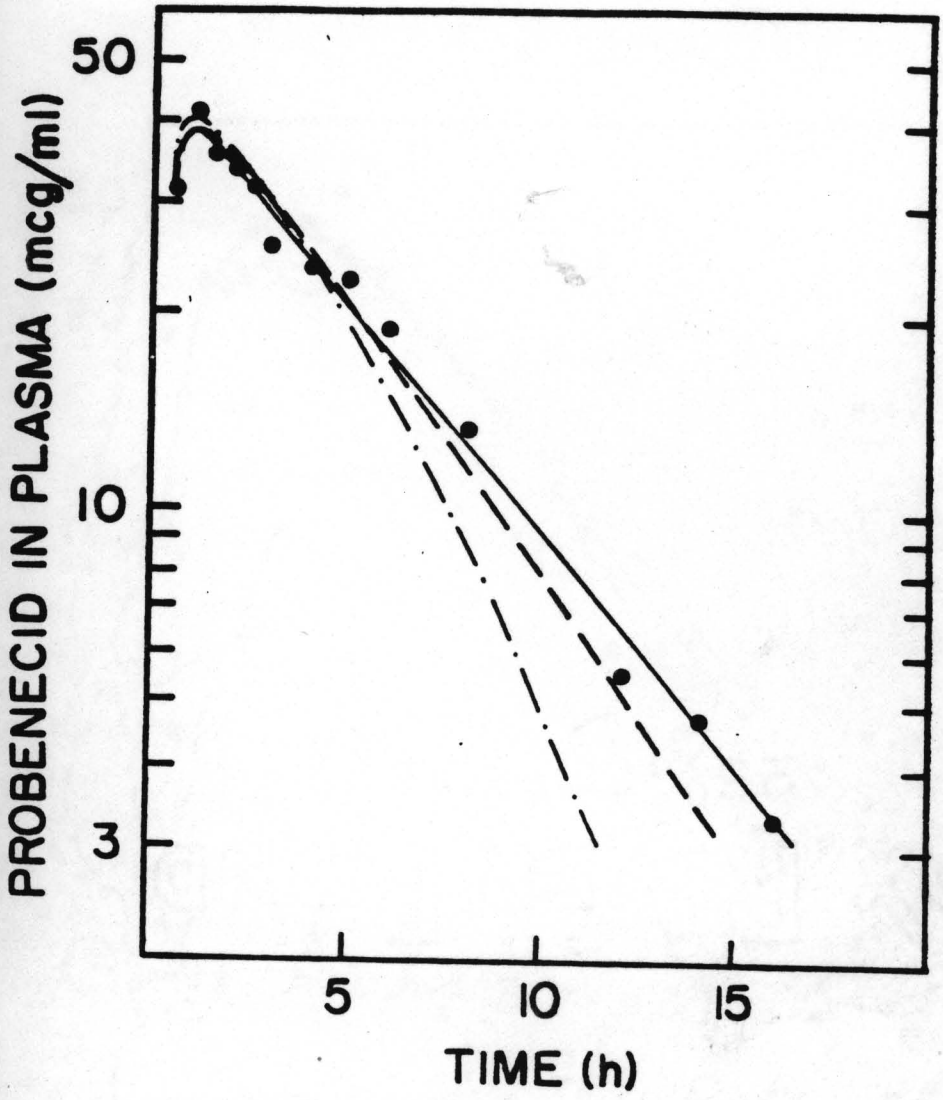


Figure 17: Probenecid plasma levels following single 0.5 gm suspension dose, subject #3.

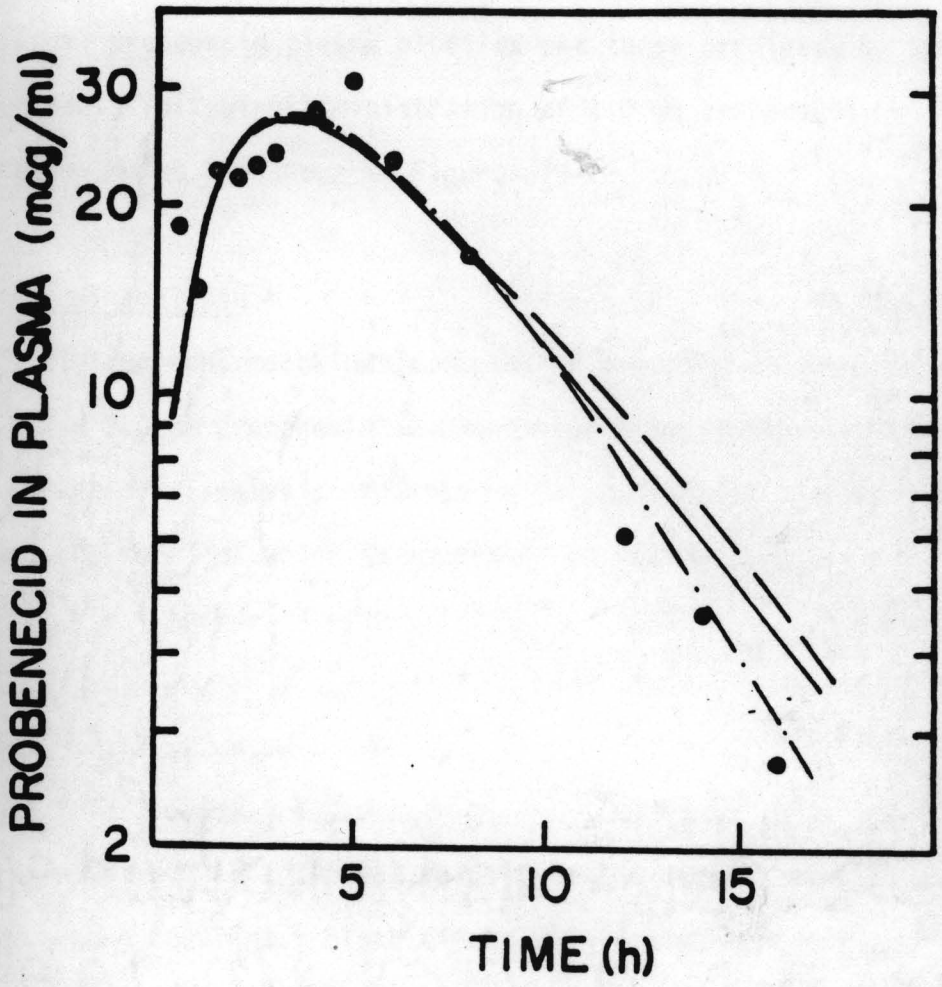


Figure 18: Probenecid plasma levels following single 0.5 gm suspension dose, subject #4.

Similarly, individual actual plasma probenecid concentrations, and those predicted by the three equations following a 2.0 gm probenecid suspension dose are presented in Tables XXXIII - XXXVI. The actual probenecid plasma profiles and those predicted by the three models following administration of 2.0 gm probenecid suspension doses are shown in Figures 19-22.

Statistical analysis

Individual pharmacokinetic parameter values obtained from analysis of 0.5 and 2.0 gm probenecid suspension data according to three models were analyzed by analysis of variance for randomized complete block design, the model of which is developed as follows:

$$X_{ij} = \mu + \beta_i + \tau_j + e_{ij}$$

where $i = 1, 2.....n,$

$j = 1, 2.....k$

In this model x_{ij} is a typical value from the overall population, μ is an unknown constant. Block effect and treatment effect are represented by β_i and τ_j, respectively. Residual component representing all surces of variation other than treatments and blocks is denoted by e_{ij}. The main assumption of this model is that the block and treatment effects are additive. That is, a particular block-treatment combination does not produce an effect that is greater or less than the sum of their individual effects.

Table XXXIII
Actual and computer predicted probenecid plasma levels following single 2.0 gm probenecid suspension dose according to Equations 1, 2 and 3 for Subject #1.

Time (h)	Plasma probenecid (mcg/ml)			
	Actual	Predicted by equations		
		1	2	3
0.5	137.1	137.1	137.9	137.6
1.0	220.9	221.6	211.9	220.9
1.5	233.9	215.8	207.9	215.2
2.0	214.5	207.0	202.1	206.3
2.5	188.0	199.3	196.7	199.3
3.0	166.2	192.5	191.8	192.6
4.0	188.7	178.8	181.6	179.2
5.0	149.4	165.8	171.4	166.4
6.0	155.1	153.7	161.5	154.5
8.0	153.3	132.5	142.8	133.5
12.0	110.5	97.5	107.1	98.3
14.0	77.9	83.7	91.3	84.3
16.0	65.3	71.9	76.7	72.2
20.0	46.3	53.2	52.0	52.9
24.0	37.6	39.3	33.0	38.6
30.0	28.9	24.9	14.9	23.8
36.0	16.9	15.7	6.10	14.5

Table XXXIV
Actual and computer predicted probenecid plasma levels following single 2.0 gm probenecid suspension dose according to Equations 1, 2 and 3 for Subject #2.

Time (h)	Plasma probenecid (mcg/ml)			
	Actual	Predicted by equations		
		1	2	3
0.5	86.9	49.2	52.5	49.8
1.5	93.0	106.9	109.6	107.4
2.0	115.3	126.4	127.7	126.6
2.5	105.7	139.2	139.3	139.2
3.0	184.2	147.9	146.8	147.7
4.0	160.3	154.4	152.0	154.0
5.0	151.8	152.9	150.3	152.4
6.0	150.9	146.2	144.4	145.9
8.0	114.2	126.7	127.4	126.9
12.0	92.9	86.7	90.3	87.4
14.0	72.9	70.2	73.5	70.8
16.0	50.9	56.8	58.9	57.3
20.0	38.7	36.7	35.6	36.5
24.0	24.2	23.7	20.2	23.1
30.0	12.5	12.3	7.96	11.4
36.0	4.58	6.28	2.91	5.41

Table XXXV
Actual and computer predicted probenecid plasma levels following single 2.0 gm probenecid suspension dose according to Equations 1, 2 and 3 for Subject #3.

Time (h)	Plasma probenecid (mcg/ml)			
	Actual	Predicted by equations		
		1	2	3
0.5	142.0	91.9	103.2	104.1
1.0	140.3	146.9	155.3	158.3
1.5	143.9	177.0	178.9	183.7
2.0	184.2	185.1	183.3	188.6
2.5	167.4	186.4	182.5	187.8
3.0	177.5	182.7	178.1	182.9
4.0	204.0	169.5	166.1	169.7
5.0	163.5	153.4	152.6	154.9
6.0	152.7	136.5	138.3	139.4
8.0	90.1	107.5	111.9	111.7
12.0	52.1	66.9	68.7	68.6
14.0	55.4	52.6	51.6	51.9
16.0	36.8	41.2	37.5	38.4
20.0	29.2	25.3	18.3	19.8
24.4	18.1	15.0	7.89	9.07
30.0	9.49	7.59	2.58	3.14
36.0	3.79	3.69	0.85	1.04

Table XXXVI
Actual and computer predicted probenecid plasma levels following single 2.0 gm probenecid suspension dose according to Equations 1, 2 and 3 for Subject #4.

Time (h)	Plasma probenecid (mcg/ml)			
	Actual	Predicted by equations		
		1	2	3
0.5	52.9	47.9	52.6	48.7
1.0	147.0	156.8	160.3	156.9
1.5	177.7	194.6	193.4	194.3
2.0	222.7	213.1	207.9	212.3
2.5	256.1	220.2	212.5	219.4
3.0	229.7	219.7	211.3	218.9
4.0	175.7	209.1	202.2	208.7
5.0	180.2	193.2	189.5	193.4
6.0	182.6	180.3	179.1	180.9
8.2	148.3	150.9	153.8	152.2
12.3	122.1	107.7	111.2	108.9
14.0	103.4	92.7	94.3	93.7
16.0	78.9	78.9	78.1	79.7
20.2	51.5	56.0	49.4	55.9
24.2	35.4	40.2	29.7	39.5
30.0	21.2	24.8	12.9	23.5
36.0	9.52	15.0	5.12	13.5

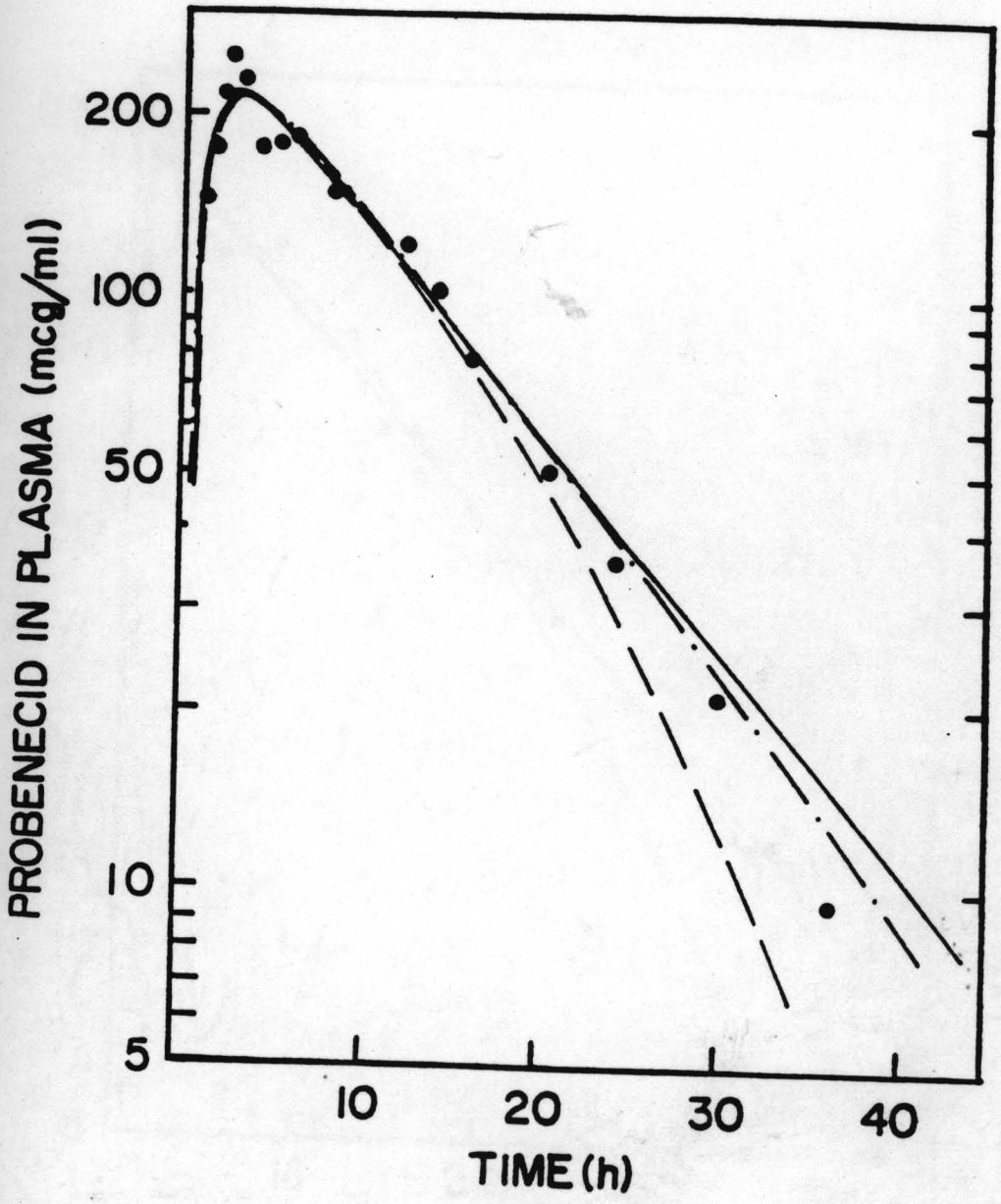


Figure 19: Probenecid plasma levels following single 2.0 gm suspension dose, subject #1.

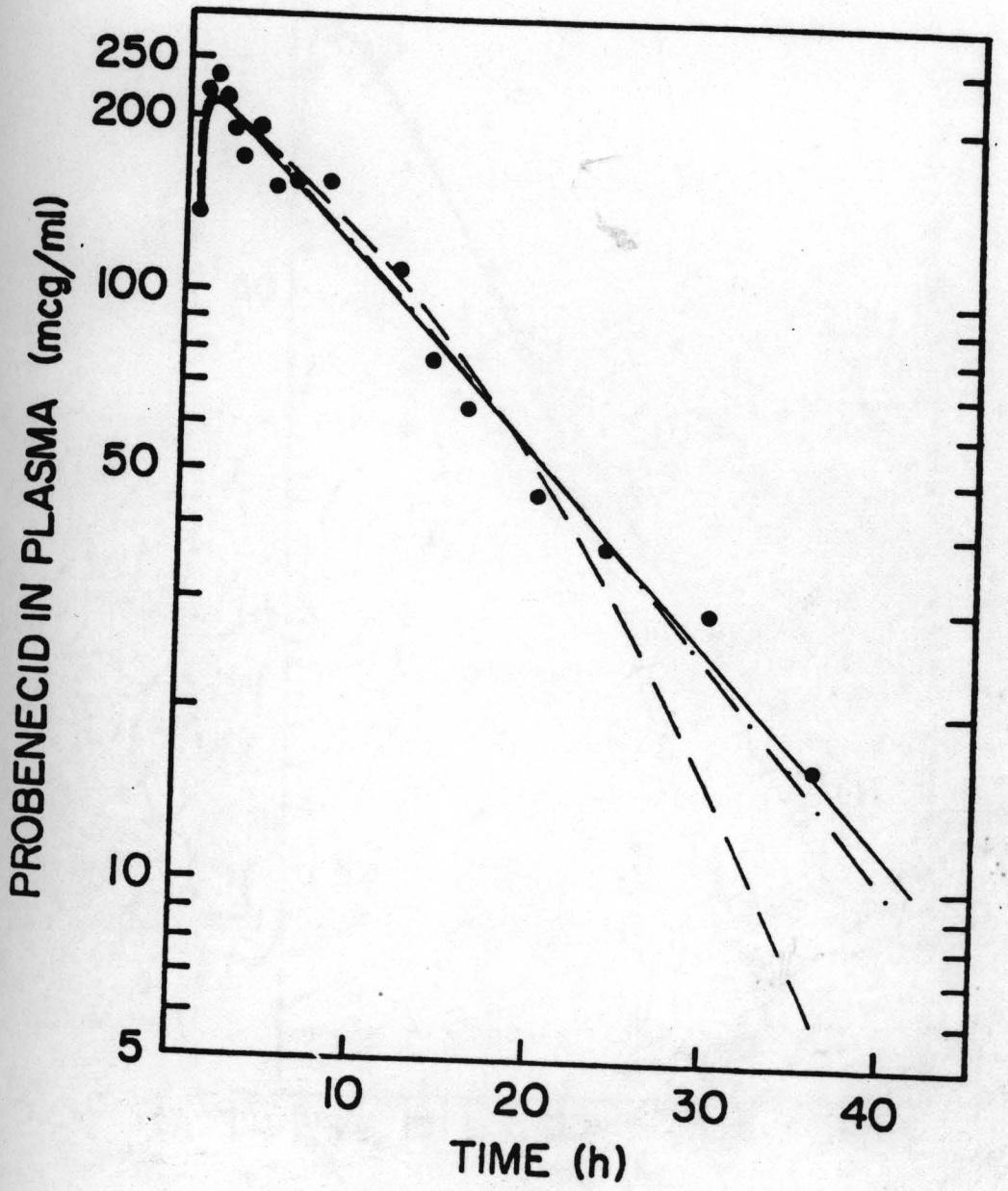


Figure 20: Probenecid plasma levels following single 2.0 gm . suspensiondose, subject #2.

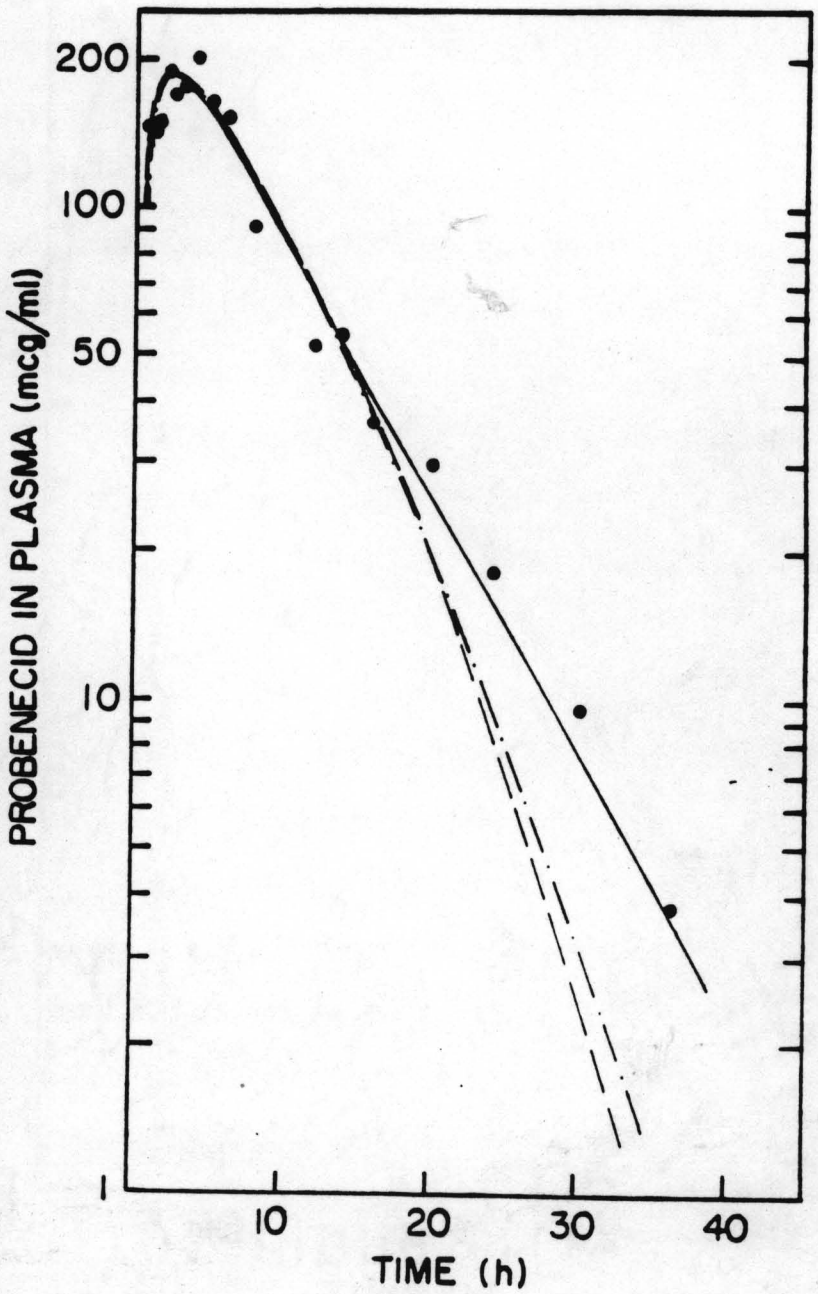


Figure 21: Probenecid plasma levels following single 2.0 gm suspension dose, subject #3.

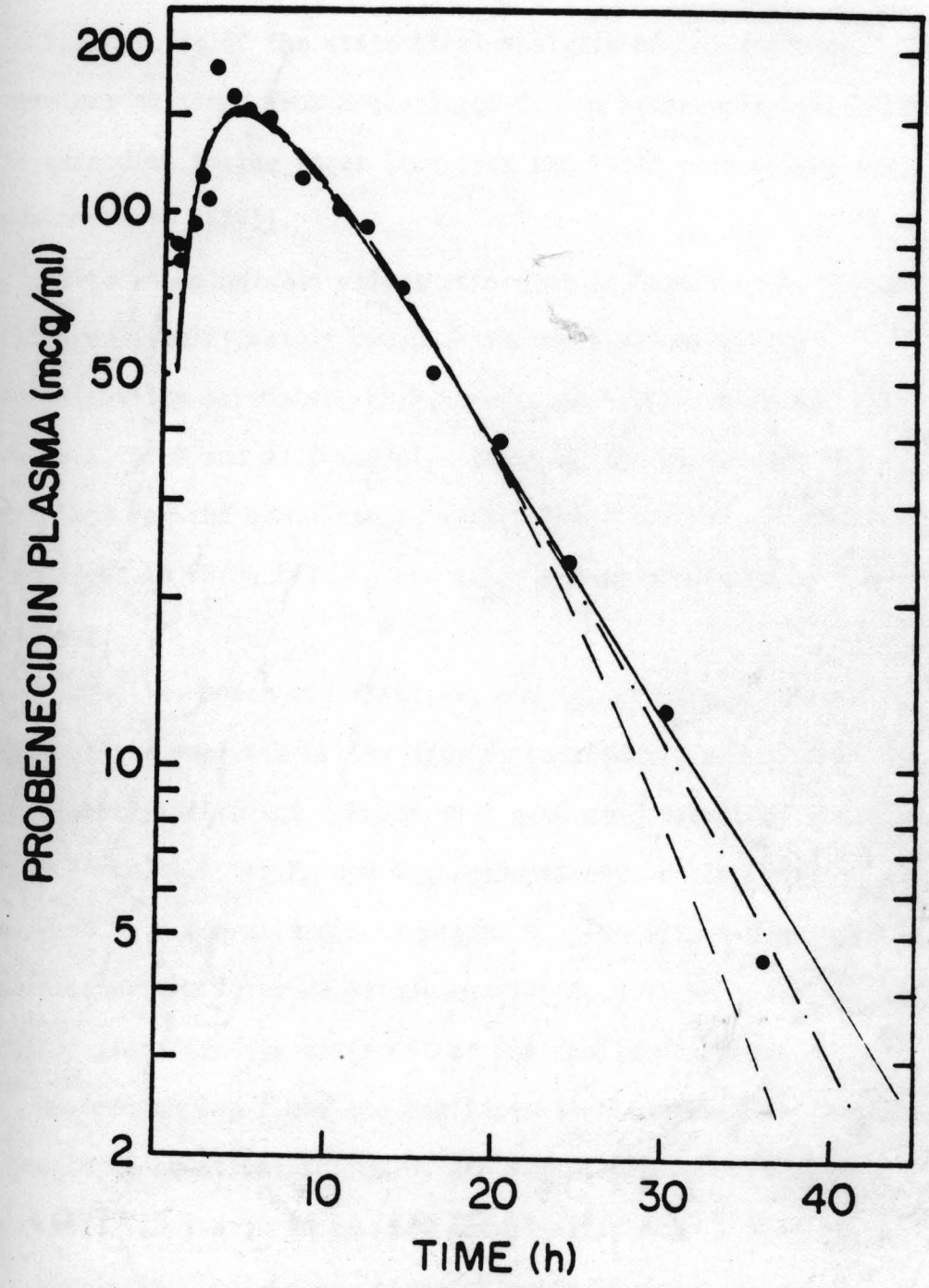


Figure 22: Probenecid plasma levels following single 2.0 gm suspension dose, subject #4.

The results of the statistical analysis of the individual parameters obtained from analysis of 0.5 gm probenecid suspension data according to the three Equations and their mean values are given in Table XXXVII.

Differences between values according to Equations 1, 2 and 3 are considered significant if $p<0.05$. The mean values of the bioavailability parameter, FD/V, predicted by the three equations were 49.2, 46.8 and 57.2 mcg/ml. These values were further normalized for the administered dose. There were no significant differences in FD/V, F/V, k_a and $t_{\frac{1}{2}abs}$ values predicted by the three equations.

Michaelis-Menten constants, V_M and K_M, and their ratios, obtained from analysis of the data by Equations 2 and 3, were not significantly different. Equation 2 predicted values of 114.4 mcg/ml, and 20.6 mcg/ml.h for K_M and V_M, respectively, while Equation 3 predicted 96.6 mcg/ml and 9.99 mcg/ml.h. The elimination half-lives predicted by the three equations were 4.72, 3.91 and 3.15 hours, and were not significantly different at 95% confidence level.

Absorption lag times and coefficients of determination predicted by the three equations following the administration of 0.5 gm probenecid suspension doses were not significantly different.

Similarly, individual pharmacokinetic parameters obtained from the analysis of 2.0 gm probenecid suspension data according to the three equations were examined by analysis of variance for randomized

Table XXXVII
Mean pharmacokinetic parameter values (±1 SD.) obtained by analyzing plasma probenecid levels from the single 0.5 gm probenecid suspension dose according to Equations 1 (A), 2 (B) and 3 (C).

Parameter	Values according to Equations			Statistic
	1 (A)	2 (B)	3 (C)	
FD/V (mcg/ml)	49.2 ± 8.38	46.8 ± 6.84	57.2 ± 12.6	NSD[1]
$F/V(10^{-5})$ (ml^{-1})	9.84 ± 1.68	9.36 ± 1.37	11.4 ± 2.51	"
k_a (h^{-1})	2.39 ± 1.83	2.5 ± 1.89	2.11 ± 1.77	"
$t_{1/2\ abs}$ (h)	0.59 ± 0.64	0.53 ± 0.49	0.87 ± 1.04	"
k_{el} (h^{-1})	0.16 ± 0.03	-[2]	-	-
K_M (mcg/ml)	-	114.4 ± 10.2	96.6 ± 38.2	NSD
V_M (mcg/ml.h)	-	20.6 ± 4.31	9.99 ± 8.13	"
V_M/K_M (h^{-1})	-	0.18 ± 0.02	0.14 ± 0.14	"
k' (h^{-1})	-	-	0.10 ± 0.12	-
$t_{1/2\ el}$ (h)	4.72 ± 1.23	3.91 ± 0.54	3.15 ± 1.11	NSD
t_{lag} (h)	0.17 ± 0.20	0.17 ± 0.20	0.17 ± 0.20	"
r^2	0.988 ± 0.015	0.987 ± 0.015	0.985 ± 0.012	"

[1] No significant differences ($p>0.05$).
[2] Not applicable for this model.

complete block design. The results of the statistical analysis and mean parameter values according to the three equations are shown in Table XXXVIII.

Following administration of the 2.0 gm probenecid suspension dose, FD/V predicted by Equation 2 was significantly smaller than the values predicted by Equations 1 and 3. However, when these values were normalized for the administered dose, no significant difference could be detected in F/V values. The coefficient of variation of F/V values for Equations 1, 2, and 3 were 15, 6, and 22% respectively. The three equations again yielded k_a, $t_{\frac{1}{2}abs}$, t_{lag}, and r^2 values which were not significantly different.

Michaelis-Menten constants V_M and K_M predicted by Equations 2 and 3 did not show a significant difference at 95% confidence level. However, elimination $t_{\frac{1}{2}}$ predicted by Equation 1, 7.35 h, was significantly longer than the value of 3.95 h, predicted by Equation 2. Equation 3, predicted elimination $t_{\frac{1}{2}}$ of 4.89 h which was significantly shorter than the value predicted by Equation 1, at 90% significance level.

Table XXXIX is the comparison of the pharmacokinetic parameters following 0.5 and 2.0 gm probenecid suspension doses using Equations 1, 2, and 3. Dose normalized AUC, AUC/D, values following the administration of 2.0 gm suspension dose was 1.9-fold greater than the value obtained from the 0.5 gm dose. Dose normalized C_{max}, C_{max}/D, from the 2.0 gm dose was 1.4-fold greater than the

Table XXXVIII
Mean pharmacokinetic parameter values (±1 SD.) obtained by analyzing plasma probenecid levels from the single 2.0 gm probenecid suspension dose according to Equations 1 (A), 2 (B), and 3 (C).

Parameter	Values according to Equation			Statistic
	1 (A)	2 (B)	3 (C)	
FD/V (mcg/ml	249.2 ± 15.6	225.8 ± 14.9	242.5 ± 16.3	A, C>B
$F/V(10^{-5})$ (ml^{-1})	12.5 ± 0.78	11.5 ± 0.65	12.1 ± 0.80	NSD
k_a (h^{-1})	2.67 ± 3.61	3.12 ± 4.04	2.82 ± 3.69	"
$t_{1/2\ abs}$ (h)	0.74 ± 0.62	0.58 ± 0.48	0.68 ± 0.59	"
k_{el} (h^{-1})	0.09 ± 0.02	-	-	-
K_M (mcg/ml)	-	114.6 ± 10.5	109.0 ± 14.4	NSD
V_M (mcg/ml.h)	-	20.7 ± 4.02	10.4 ± 8.64	"
V_M/K_M (h^{-1})	-	0.18 ± 0.02	0.10 ± 0.08	"
k' (h^{-1})	-	-	0.07 ± 0.02	-
$t_{1/2\ el}$ (h)	7.35 ± 1.53	3.95 ± 0.45	4.89 ± 2.17	A>B, A>C[1]
t_{lag} (h)	0.19 ± 0.23	0.19 ± 0.23	0.19 ± 0.23	NSD
r^2	0.984 ± 0.009	0.983 ± 0.009	0.984 ± 0.009	"

[1] $0.05<p<0.1$

Table XXXIX
Comparison of pharmacokinetic parameter values (±1 SD.) following single 0.5 and 2.0 gm probenecid suspension doses.

Parameter		Value 0.5 gm (A)	Value 2.0 gm (B)	Statistic
$AUC/D(10^{-4})$ (h/ml)		6.88 ± 1.95	13.1 ± 2.71	B>A
$C_{max}/D(10^{-5})$ (ml^{-1})		8.0 ± 2.49	10.9 ± 1.59	B>A
T_{max} (h)		2.40 ± 1.70	2.75 ± 1.04	NSD
$F/V(10^{-5})$ (ml^{-1})	(i)[1]	9.84 ± 1.68	12.5 ± 0.78	B(i)>A(i),A(ii)
	(ii)[2]	9.36 ± 1.37	11.5 ± 0.65	B(iii)>A(ii)
	(iii)[3]	11.4 ± 2.51	12.1 ± 0.80	
k_a (h^{-1})	(i)	2.39 ± 1.83	2.67 ± 3.61	NSD
	(ii)	2.50 ± 1.89	3.12 ± 4.04	"
	(iii)	2.11 ± 1.77	2.82 ± 3.69	"
$t_{1/2\ abs}$ (h)	(i)	0.59 ± 0.64	0.74 ± 0.62	"
	(ii)	0.53 ± 0.49	0.58 ± 0.48	"
	(iii)	0.87 ± 1.04	0.68 ± 0.59	"
k_{el} (h^{-1})	(i)	0.16 ± 0.03	0.09 ± 0.02	A>B
K_M (mcg/ml)	(ii)	114.4 ± 10.2	114.6 ± 10.5	NSD
	(iii)	96.6 ± 38.2	109.0 ± 14.4	"
V_M (mcg/ml.h)	(ii)	20.6 ± 4.31	20.7 ± 4.02	"
	(iii)	9.99 ± 8.13	10.4 ± 8.64	"
V_M/K_M (h^{-1})	(ii)	0.18 ± 0.02	0.18 ± 0.02	"
	(iii)	0.14 ± 0.14	0.10 ± 0.08	"
k' (h^{-1})	(iii)	0.10 ± 0.12	0.07 ± 0.02	"

Table XXXIX
(continued)

Parameter		Value 0.5 gm (A)	Value 2.0 gm (B)	Statistic
$t_{1/2}$ (h)	(i)	4.72 ± 1.23	7.35 ± 1.53	B(i)>A(i)
	(ii)	3.91 ± 0.54	3.95 ± 0.45	NSD
	(iii)	3.15 ± 1.11	4.89 ± 2.17	"
t_{lag} (h)		0.17 ± 0.20	0.19 ± 0.23	"
r^2	(i)	0.988 ± 0.015	0.984 ± 0.009	"
	(ii)	0.987 ± 0.015	0.983 ± 0.009	"
	(iii)	0.985 ± 0.012	0.984 ± 0.009	"

[1](i) First order absorption and elimination model.

[2](ii) First order absorption and saturable elimination model.

[3](iii) First order absorption and parallel first order and saturable elimination model.

0.5 gm dose. These values were statistically different at 95% significance level. However, observed T_{max} from 0.5 and 2.0 gm doses were 2.4 and 2.8 h, which were not significantly different. The F/V value obtained from 2.0 gm dose according to Equation 1 was 12.5 x 10^{-5} ml^{-1} and this value was statistically greater than 9.84 x 10^{-5} and 9.36 x 10^{-5} ml^{-1} predicted by Equations 1 and 2 from the 0.5 gm suspension dose. Equation 3, from the analysis of 2.0 gm data predicted F/V value of 12.1 x 10^{-5} ml^{-1} which is significantly greater than 9.36 x 10^{-5} ml^{-1} predicted by Equation 2 from 0.5 gm data.

Following administration of 0.5 and 2.0 gm probenecid suspension doses, the mean k_a according to the three equations ranged from 2.11 h^{-1} and 3.12 h^{-1} and no significant difference could be detected in these values. Similarly, no significant difference was observed in $t_{\frac{1}{2}abs}$ and the $t_{\frac{1}{2}abs}$ values ranged from 0.53 to 0.87 h.

First-order elimination rate constants predicted by Equation 1 for 0.5 and 2.0 gm doses were 0.16 and 0.09 h^{-1} and these values were significantly different at 95% confidence level. The Michaelis-Menten constant, K_M, from the 0.5 and 2.0 gm doses was 114.4 and 114.6 mcg/ml using Equation 2 and 96.6 and 109.0 mcg/ml using Equation 3. No significant difference could be detected in these values. Similarly, the V_M from 0.5 and 2.0 gm doses was 20.6 and 20.7 mcg/ml.h using Equation 2 and 9.99 and 10.4 mcg/ml.h using Equation 3. Although the values from Equation 3 appeared to be one

half of those predicted by Equation 2, there was no significant difference in these values due to high individual variation.

There was no significant difference due to dose or models in the ratio V_M/K_M. Equation 2 predicted 0.18 h^{-1} for both the 0.5 and 2.0 gm doses, while Equation 3 predicted slightly smaller but not statistically different values of 0.14 and 0.10 h^{-1}. The first-order elimination rate constants for the 0.5 and 2.0 gm doses yielded by Equation 3 were 0.10 and 0.07 h^{-1}, respectively. The elimination $t_{\frac{1}{2}}$, calculated by Equation 1 for the 2.0 gm dose was 7.35 h, significantly higher than 4.72 h from 0.5 gm dose. Equations 2 and 3 yielded elimination half-lives, ranging from 3.15 to 4.89 h following both 0.5 and 2.0 gm doses and the difference between these values is not statistically significant.

The three equations that were utilized to analyze plasma data involved first-order absorption, and the best description of the data was obtained by including an absorption lag time. The mean lag times following 0.5 and 2.0 gm doses were 0.17 and 0.19 h, respectively. There were no dose or model effect on this value.

Coefficients of determination obtained from nonlinear regression analysis of 0.5 and 2.0 gm probenecid suspension plasma data ranged from 0.983 - 0.988 and there were no significant differences due to dosage or model in this value.

Urine

Volume and pH of urine collected following administration of 0.5 and 2.0 gm single probenecid suspension doses are presented in Tables XL and XLI. The mean (±SD.) total urine volume collected 0-48 h following 0.5 and 2.0 gm doses were 2.6±0.5 and 3.2±0.8 l, respectively.

Urine pH during the 48 hour period had mean values ranging from 5.8 to 6.7 and the coefficient of variation from both doses did not exceed 15.4%.

Amount of unchanged drug (mg) recovered in the urine during collection intervals and the rate of excretion of unchanged drug (mg/h) following the 05. gm probenecid suspension dose are given in Table XLII.

During 0-2 h collection interval, mean amount of unchanged drug in urine was 1.32 mg. This value gradually increased and during the 6-12 h interval, mean amount of unchanged drug was 7.27 mg. The excretion rate of unchanged drug in the 0-2 h interval was 0.66 mg/h, similar to that during 2-6 h, which was 0.85 mg/h. The excretion rate of unchanged probenecid increased 1.4-fold during 6-12 h compared to the 2-6 h collection interval. During 12-24 h, excretion rate of probenecid was significantly reduced to 0.38 mg/h, indicating that most of the drug had been excreted during the first 12h following the dose.

The amount of unchanged probenecid recovered in the urine, and the rate of excretion of unchanged drug, following 2.0 gm probenecid

Table XL
Volume (ml) and pH of urine collected following single 0.5 gm probenecid suspension dose.

Collection interval	Subject 1	2	3	4	$\bar{X}$	SD.	CV%
Control							
pH	5.5	5.2	6.0	6.4	5.8	0.5	9.2
0-2h							
Volume	120.0	85.0	170.0	30.0	101.3	58.9	58.2
pH	5.8	5.2	7.0	6.8	6.2	0.8	13.7
2-6h							
Volume	490.0	150.0	690.0	125.0	363.8	273.9	75.3
pH	6.0	5.0	6.9	6.6	6.1	0.8	13.7
6-12h							
Volume	185.0	340.0	400.0	750.0	418.8	238.7	57.0
pH	5.6	5.2	7.3	6.6	6.2	0.9	15.4
12-24h							
Volume	480.0	800.0	475.0	550.0	576.3	153.0	26.6
pH	6.5	5.6	6.0	6.0	6.0	0.4	6.1
24-36h							
Volume	490.0	450.0	1000.0	845.0	696.3	269.3	38.7
pH	6.8	5.6	6.0	7.0	6.4	0.7	10.4
36-48h							
Volume	350.0	500.0	475.0	375.0	425.0	73.6	17.3
pH	6.2	6.3	5.9	6.2	6.2	0.17	2.8
0-48h (Total)							
Volume	2115.0	2325.0	3210.0	2675.0	2581.3	478.6	18.5

Table XLI
Volume (ml) and pH of urine collected following single 2.0 gm probenecid suspension dose.

Collection interval	Subject 1	2	3	4	$\bar{X}$	SD.	CV%
Control							
pH	6.4	6.2	5.5	5.7	6.0	0.4	7.1
0-2h							
Volume	200.0	190.0	180.0	125.0	173.8	33.5	19.3
pH	7.7	6.2	6.5	6.2	6.7	0.7	10.7
2-6h							
Volume	725.0	445.0	1025.0	675.0	717.5	238.5	33.2
pH	7.6	5.8	7.1	6.0	6.6	0.9	13.1
6-12h							
Volume	325.0	850.0	540.0	195.0	477.5	286.2	59.9
pH	6.1	5.2	6.8	7.5	6.4	1.0	15.4
12-24h							
Volume	1000.0	750.0	790.0	380.0	730.0	257.8	35.3
pH	5.8	5.5	5.6	6.2	5.8	0.3	5.4
24-36h							
Volume	520.0	625.0	700.0	365.0	552.5	145.2	26.3
pH	5.8	5.6	6.7	6.0	6.0	0.5	7.9
36-48h							
Volume	410.0	600.0	720.0	360.0	522.5	167.4	32.0
pH	6.0	6.7	6.7	6.8	6.6	0.4	5.6
0-48h (Total)							
Volume	3180.0	3460.0	3955.0	2100.0	3173.8	784.3	24.7

Table XLII
Quantity of unchanged probenecid (Au) excreted in urine following single 0.5 gm probenecid suspension dose.

Collection interval:	Au (mg)	Au/h (mg/h)
0-2 h		
Subject		
1	-	-
2	1.33	0.66
3	2.86	1.43
4	1.08	0.54
$\bar{X}$	1.32	0.66
SD.	1.18	0.59
CV%	89.5	89.7
2-6 h		
Subject		
1	-	-
2	3.69	0.92
3	7.00	1.75
4	2.88	0.72
$\bar{X}$	3.39	0.85
SD.	2.88	0.72
CV%	84.9	84.9
6-12 h		
Subject		
1	3.77	0.63
2	6.51	1.09
3	14.9	2.49
4	3.9	0.65
$\bar{X}$	7.27	1.22
SD.	5.24	0.88
CV%	72.1	72.1

Table XLII
(continued)

Collection interval:	Au (mg)	Au/h (mg/h)
12-24 h		
Subject		
1	6.99	0.58
2	4.15	0.35
3	4.06	0.34
4	2.71	0.23
$\bar{X}$	4.48	0.38
SD.	1.79	0.15
CV%	40.2	39.2
24-36 h		
Subject		
1	1.82	0.15
2	0.74	0.06
3	0.53	0.04
4	-	-
$\bar{X}$	0.77	0.06
SD.	0.76	0.06
CV%	98.9	101.5
36-48 h		
Subject		
1	0.23	0.02
2	-	-
3	-	-
4	-	-
$\bar{X}$	0.06	0.005
SD.	0.12	0.01
CV%	200.0	200.0

suspension dose are given in Table XLIII.

Following the 2-0 gm dose, during 0-2 h collection interval the mean amount of unchanged probenecid and mean excretion rate was 4.27 mg and 2.13 mg/h, respectively. The excretion rate almost doubled to 4.09 mg/h during the 2-6 h period. The mean excretion rate then gradually decreased to 0.51 mg/h during the 24-48 h collection interval.

The quantity of conjugated probenecid recovered in the urine, and its excretion rate following 0.5 gm probenecid suspension dose are given in Table XLIV. The maximum excretion rate of conjugated probenecid was 14.6 mg/hr during the 2-6 h collection interval. This value decreased to 7.81 mg/h during the 6-12 h collection period.

Table XLV contains the quantity of conjugated probenecid, and its excretion rate, following administration of 2.0 gm suspension dose. The excretion rate of conjugated probenecid was 16.6, 12.5 and 13.9 mg/hr during 0-2, 2-6, and 6-12 h, respectively. Excretion rate of probenecid was fairly constant during 0-36 h with mean values ranging from 12.1 to 16.6 mg/h.

Cumulative percentage recovery of unchanged and conjugated probenecid in urine following the 0.5 gm probenecid suspension dose are given in Table XLVI. The mean cumulative percentage recovery of unchanged and conjugated probenecid (±SD.) from Table XLVI was plotted against the mid-point of collection intervals to obtain the curves in Figure 23.

Table XLIII
Quantity of unchanged probenecid (Au) excreted in urine following single 2.0 gm probenecid suspension dose.

Collection interval:	Au (mg)	Au/h (mg/h)
0-2 h		
Subject		
1	2.96	1.48
2	5.39	2.69
3	3.73	1.86
4	4.98	2.49
$\bar{X}$	4.27	2.13
SD.	1.12	0.56
CV%	26.3	26.3
2-6 h		
Subject		
1	5.76	1.44
2	6.97	1.74
3	40.1	10.0
4	12.7	3.18
$\bar{X}$	16.4	4.09
SD.	16.1	4.01
CV%	98.3	98.1
6-12 h		
Subject		
1	4.54	0.76
2	10.8	1.79
3	40.3	6.71
4	20.4	3.39
$\bar{X}$	19.0	3.16
SD.	15.6	2.60
CV%	82.2	82.2

Table XLIII
(continued)

Collection interval	Au (mg)	Au/h (mg/h)
12-24 h		
Subject		
1	6.44	0.54
2	15.6	1.29
3	17.5	1.45
4	23.5	1.96
$\bar{X}$	15.8	1.31
SD	7.07	0.59
CV%	44.8	44.8
24-36 h		
Subject		
1	7.05	0.59
2	14.8	1.23
3	17.8	1.49
4	13.8	1.15
$\bar{X}$	13.4	1.12
SD.	4.54	0.38
CV%	33.9	33.9
36-48 h		
Subject		
1	4.16	0.35
2	7.78	0.65
3	4.77	0.39
4	7.56	0.63
$\bar{X}$	6.07	0.51
SD.	1.87	0.16
CV%	30.8	31.1

Table XLIV
Quantity of conjugated probenecid (Ac) extreted in urine following single 0.5 gm probenecid suspension dose.

Collection interval:	Ac (mg)	Ac/h (mg/h)
0-2 h		
Subject		
1	13.2	6.62
2	12.3	6.14
3	19.2	9.61
4	16.4	8.19
$\bar{X}$	15.3	7.64
SD.	3.15	1.58
CV%	20.6	20.6
2-6 h		
Subject		
1	43.7	10.9
2	33.3	8.32
3	60.8	15.2
4	95.6	23.9
$\bar{X}$	58.4	14.6
SD.	27.3	6.83
CV%	46.8	46.9
6-12 h		
Subject		
1	30.7	5.12
2	91.9	15.3
3	22.3	3.71
4	42.7	7.11
$\bar{X}$	46.9	7.81
SD.	31.1	5.18
CV%	66.4	66.4

Table XLIV
(continued)

Collection interval:	Ac (mg)	Ac/h (mg/h)
12-24 h		
Subject		
1	36.7	3.06
2	50.5	4.21
3	37.2	3.10
4	38.7	3.23
$\bar{X}$	40.8	3.4
SD.	6.54	0.54
CV%	16.0	16.0
24-36 h		
Subject		
1	4.93	0.41
2	6.95	0.58
3	3.86	0.32
4	4.87	0.41
$\bar{X}$	5.15	0.43
SD.	1.29	0.11
CV%	25.1	25.3
36-48 h		
Subject		
1	1.62	0.13
2	1.58	0.13
3	0.78	0.07
4	0.92	0.08
$\bar{X}$	1.23	0.10
SD.	0.44	0.03
CV%	35.7	31.2

Table XLV
Quantity of conjugated probenecid (Ac) excreted in urine following single 2.0 gm probenecid suspension dose.

Collection interval:	Ac (mg)	Ac/h (mg/h)
0-2 h		
Subject		
1	11.1	5.54
2	54.6	27.3
3	18.0	9.01
4	49.0	24.5
$\bar{X}$	33.2	16.6
SD.	21.8	10.9
CV%	65.7	65.7
2-6 h		
Subject		
1	41.9	10.5
2	100.4	25.1
3	15.2	3.79
4	42.8	10.7
$\bar{X}$	50.1	12.5
SD.	35.9	8.98
CV%	71.7	71.7
6-12 h		
Subject		
1	112.4	18.7
2	139.9	23.3
3	39.3	6.55
4	42.5	7.08
$\bar{X}$	83.5	13.9
SD.	50.5	8.41
CV%	60.5	60.4

Table XLV
(continued)

Collection interval:	Ac (mg)	Ac/h (mg/h)
12-24 h		
Subject		
1	192.8	16.1
2	241.1	20.1
3	154.5	12.9
4	199.8	16.7
$\bar{X}$	197.1	16.5
SD.	35.5	2.95
CV%	18.0	17.9
24-36 h		
Subject		
1	93.8	7.82
2	300.0	25.0
3	32.5	2.71
4	153.9	12.8
$\bar{X}$	145.1	12.1
SD.	114.6	9.55
CV%	78.9	79.0
36-48 h		
Subject		
1	53.1	4.42
2	35.9	2.99
3	21.9	1.83
4	16.5	1.37
$\bar{X}$	31.9	2.65
SD.	16.4	1.36
CV%	51.4	51.3

Table XLVI
Cumulative percentage recovery of unchanged (U) and conjugated (C) probenecid in urine following single 0.5 gm probenecid suspension dose.

Collection interval:	Percentage recovery of probenecid						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
0-2 h							
U	-	0.26	0.58	0.22	0.27	0.24	90.2
C	2.6	2.46	3.84	3.28	3.05	0.64	21.0
2-6 h							
U	-	0.98	1.98	0.78	0.94	0.81	87.2
C	11.32	9.1	16.0	22.4	14.7	5.88	39.9
6-12 h							
U	0.78	2.3	4.92	1.56	2.39	1.79	75.2
C	17.4	27.5	20.4	30.9	24.1	6.23	25.9
12-24 h							
U	2.22	3.14	5.76	2.16	3.32	1.69	50.8
C	24.7	37.6	27.8	38.7	32.2	7.00	21.7
24-36 h							
U	2.58	3.26	5.87	2.16	3.47	1.66	48.0
C	25.7	39.0	28.5	39.7	33.2	7.17	21.6
36-48 h							
U	3.18	3.26	5.87	2.16	3.62	1.58	43.8
C	26.1	39.4	28.6	39.9	33.5	7.18	21.4

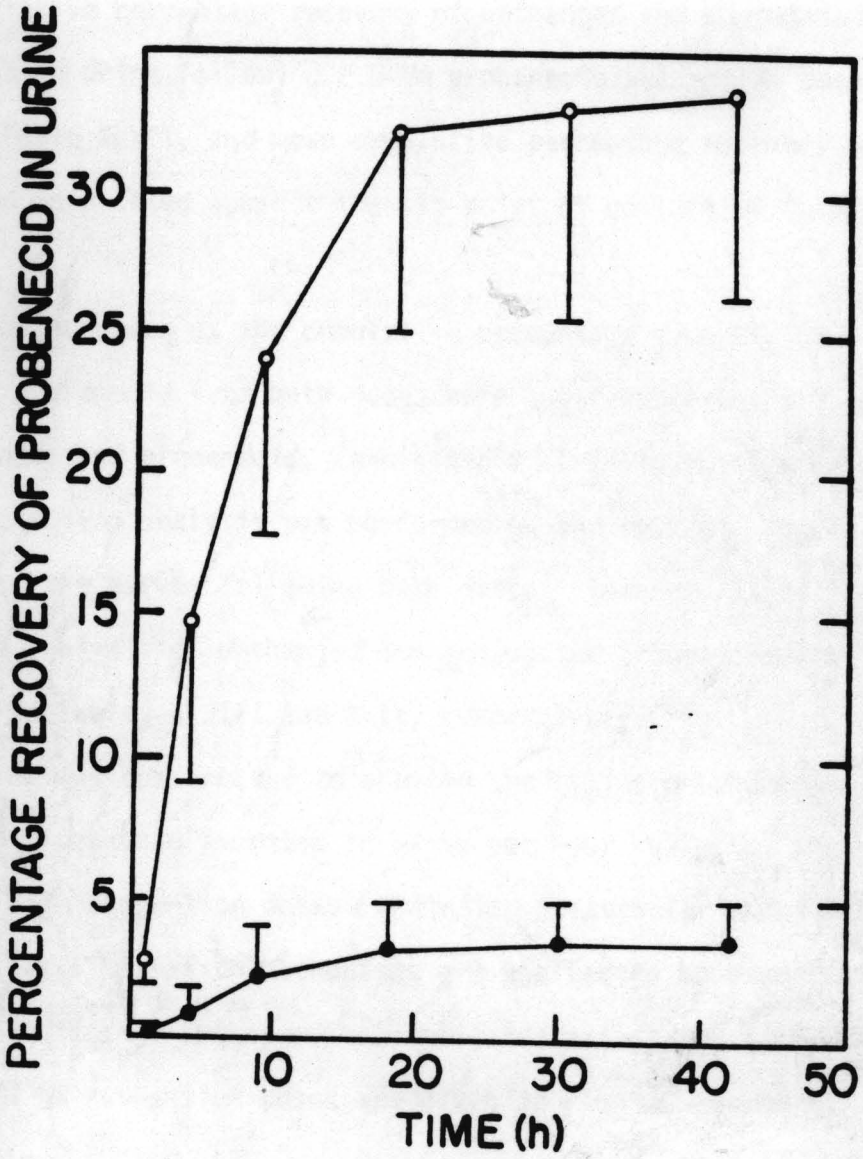


Figure 23: Mean (±SD.) cumulative percentage recovery of probenecid following single 0.5 gm suspension dose, •, o represent conjugated and unchanged probencid, respectively.

Cumulative percentage recovery of unchanged and conjugated probenecid in urine following 2.0 gm probenecid suspension dose are given in Table XLVII, and mean cumulative percentage recovery of probenecid is plotted against the mid-point of collection intervals in Figure 24.

Although curves of the cumulative percentage recovery of unchanged probenecid from both doses were superimposable, the curves of the conjugated probenecid, exhibited a striking difference. Linear regression analysis was performed on the apparent linear segments of the curves following both doses. The results of linear regression analysis of unchanged and conjugated probenecid are presented in Tables XLVIII and XLIX, respectively.

Table L was constructed to examine the ratios of unchanged to conjugated probenecid excreted in urine per hour following the 0.5 and 2.0 gm suspension doses. A similar pattern for both doses would indicate that elimination mechanisms are unaffected by dose size. Individual ratios of unchanged to conjugated probenecid following 0.5 and 2.0 gm suspension doses are given in Figures 25 and 26, respectively.

The maximum mean unchanged to conjugated probenecid ratio following the 0.5 gm suspension dose was 0.24 and it occurred during the 6-12 h interval. Following the 2.0 gm suspension dose, the maximum mean unchanged to conjugated ratio was 0.79 and occurred during the 2-6 h interval.

Table XLVII
Cumulative percentage recovery of unchanged (U) and conjugated (C) probenecid in urine following single 2.0 gm probenecid suspension dose.

Collection interval:	Percentage recovery of probenecid						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
0-2 h							
U	0.14	0.26	0.18	0.24	0.21	0.06	26.9
C	0.56	1.72	0.90	1.46	1.16	0.53	45.4
2-6 h							
U	0.42	0.62	2.18	0.88	1.03	0.79	77.3
C	2.64	6.72	1.66	3.62	3.66	2.19	59.9
6-12 h							
U	0.66	1.16	4.22	1.9	1.99	1.57	79.3
C	8.28	13.74	3.64	5.72	7.85	4.36	55.6
12-24 h							
U	1.02	1.88	5.06	2.98	2.74	1.75	63.8
C	17.9	25.8	11.3	15.7	17.7	6.07	34.3
24-36 h							
U	1.38	2.6	5.9	3.7	3.39	1.92	56.6
C	22.6	40.8	12.9	23.4	24.9	11.6	46.6
36-48 h							
U	1.62	2.96	6.14	4.06	3.69	1.91	51.7
C	25.2	42.6	13.9	24.2	26.5	11.9	44.9

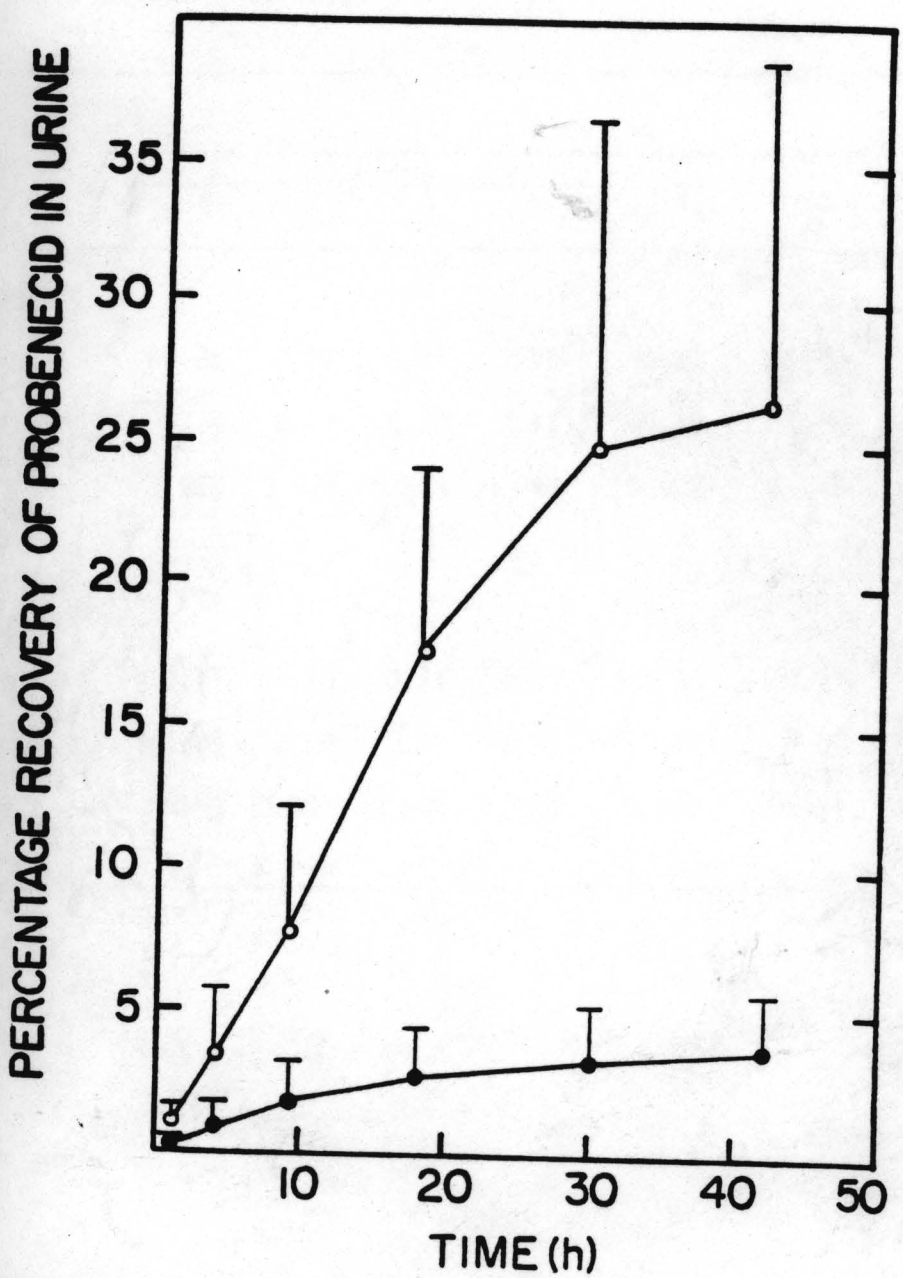


Figure 24: Mean (±SD.) cumulative percentage recovery of probenecid following single 2.0 gm suspension dose, o, o represent conjugated and unchanged probenecid, respectively.

Table XLVIII
Results of linear regression analysis of cumulative percentage recovery of unchanged probenecid in urine, versus mid-point of 0.24 h collection intervals.

Parameter	Value						
	Subject						
	1	2	3	4	$\bar{X}$	SD.	CV%
0.5gm Dose							
intercept	-0.36	0.32	0.87	0.29	0.28	0.50	179.7
slope	0.14	0.17	0.30	0.11	0.18	0.08	46.5
r^2	0.968	0.938	0.864	0.943	0.928	0.045	4.83
2.0gm Dose							
intercept	0.17	0.22	0.75	0.22	0.34	0.27	80.7
slope	0.05	0.09	0.27	0.16	0.14	0.09	67.6
r^2	0.969	0.991	0.846	0.979	0.946	0.067	7.13

Table XLIX
Results of linear regression analysis of cumulative percentage recovery of conjugated probenecid in urine versus mid-point of collection intervals.

Parameter	Value						
	Subject						
	1	2	3	4	$\bar{X}$	SD.	CV%
0.5gm Dose							
intercept	4.31	2.30	7.01	9.10	5.68	2.99	52.6
slope	1.21	2.11	1.25	1.84	1.60	0.44	27.7
r^2	0.924	0.934	0.858	0.808	0.881	0.059	6.72
2.0gm Dose							
intercept	-0.98	0.77	-0.62	-0.03	-0.22	0.76	355.6
slope	1.04	1.40	0.63	0.83	0.98	0.33	33.8
r^2	0.997	0.999	0.953	0.965	0.979	0.023	2.35

Table L
Ratios of unchanged to conjugated probenecid excreted in the urine per hour following single 0.5 gm probenecid suspension dose (A) and 2.0 gm probenecid suspension dose (B).

Collection interval:		Subject 1	2	3	4	$\bar{X}$	SD.	CV%
0-2 h								
	A -	0.0	0.11	0.15	0.07	0.08	0.06	77.5
	B -	0.27	0.09	0.21	0.10	0.17	0.09	52.1
2-6 h								
	A -	0.0	0.11	0.12	0.03	0.07	0.06	91.0
	B -	0.14	0.07	2.66	0.29	0.79	1.25	158.0
6-12 h								
	A -	0.13	0.07	0.67	0.09	0.24	0.29	119.9
	B -	0.04	0.07	1.02	0.48	0.40	0.46	113.8
12-24 h								
	A -	0.19	0.08	0.11	0.07	0.11	0.05	48.3
	B -	0.03	0.07	0.11	0.12	0.08	0.04	49.9
24-36 h								
	A -	0.37	0.10	0.14	0.0	0.15	0.16	102.6
	B -	0.07	0.05	0.54	0.09	0.19	0.24	125.6
36-48 h								
	A -	0.15	0.00	0.0	0.0	0.04	0.08	200.0
	B -	0.08	0.21	0.21	0.28	0.19	0.08	42.8

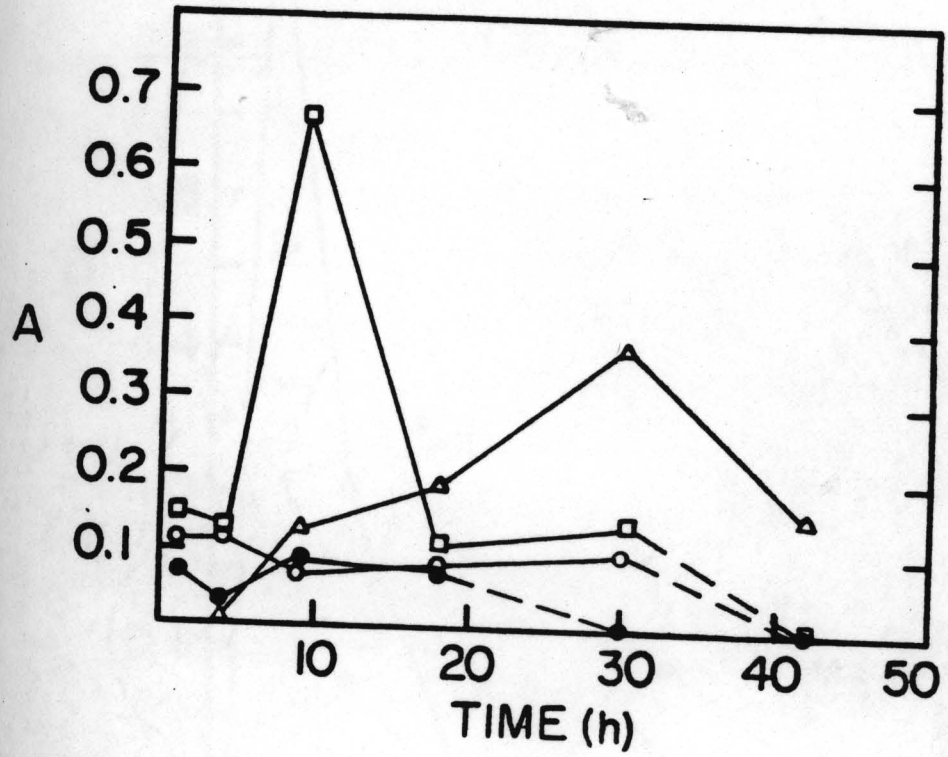


Figure 25: Individual ratios of unchanged to conjugated probenecid (A) excreted in urine per hour following single 0.5 gm suspension dose.

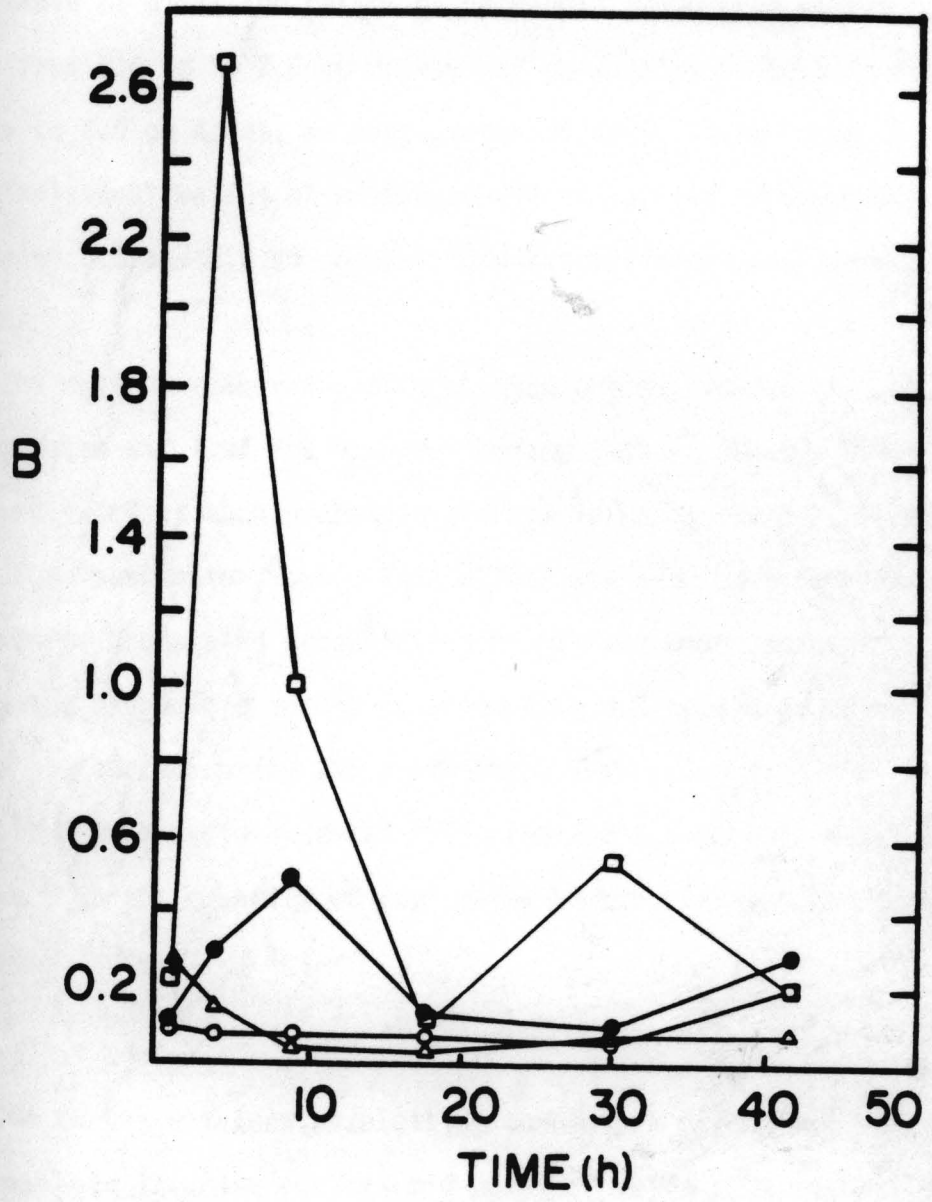


Figure 26: Individual ratios of unchanged to conjugated probenecid (B) excreted in urine per hour following single 2.0 gm suspension dose.

Table LI gives the ratios of unchanged probenecid voided in urine from 0.5 gm to 2.0 gm doses, and conjugated probenecid from 0.5 gm to 2.0 gm doses, as percentages of administered dose.

Individual ratios of unchanged and conjugated probenecid following 0.5 and 2.0 gm suspension doses are shown in Figures 27 and 28.

The maximum mean ratio of unchanged probenecid from 0.5 to 2.0 gm doses was 1.97 and occurred during 6-12 h. Thus, during this interval twice as much unchanged probenecid was excreted following the 0.5 gm suspension dose. This effect was even more magnified in the case of conjugated probenecid. The maximum mean ratio of conjugated probenecid voided in urine from 0.5 to 2.0 gm doses was 7.59 and occurred during 2-6 h interval, indicating that the conjugated probenecid excreted following the 0.5 gm dose was 7.6-fold greater than the quantity of conjugated probenecid excreted from the 2.0 gm dose.

Statistical analysis

The curves obtained by plotting cumulative percentage recovery of probenecid in urine against mid-point of collection intervals are linear during 0-24 h.

The individual slopes of these lines and their coefficients of determination were examined by analysis of variance for randomized complete block design. Slopes obtained from unchanged probenecid following 0.5 and 2.0 gm doses were examined for differences due to

Table LI
Ratios of the percentages of administered probenecid excreted in urine per hour following single 0.5 gm and 2.0 gm probenecid suspension doses.

U - ratio of unchanged probenecid from 0.5 gm to 2.0 gm dose.
C - ratio of conjugated probenecid from 0.5 gm to 2.0 gm dose.

Collection interval:	Subject 1	2	3	4	$\bar{X}$	SD.	CV%
0-2 h							
U	-	1.00	3.22	0.92	1.29	1.37	106.4
C	4.64	0.90	4.27	1.33	2.79	1.94	69.7
2-6 h							
U	-	2.00	0.70	0.88	0.89	0.83	92.6
C	4.19	1.33	16.00	8.85	7.59	6.40	84.4
6-12 h							
U	3.25	2.44	1.44	0.76	1.97	1.09	55.6
C	1.09	2.62	2.24	4.06	2.50	1.22	48.9
12-24 h							
U	4.00	1.17	1.00	0.56	1.68	1.57	93.1
C	0.76	0.84	0.97	0.78	0.84	0.09	11.3
24-36 h							
U	1.00	0.17	0.13	-	0.33	0.46	140.3
C	0.21	0.10	0.43	0.13	0.22	0.15	68.5
36-48 h							
U	2.50	-	-	-	0.63	1.25	200.0
C	0.14	0.20	0.11	0.29	0.19	0.08	42.9

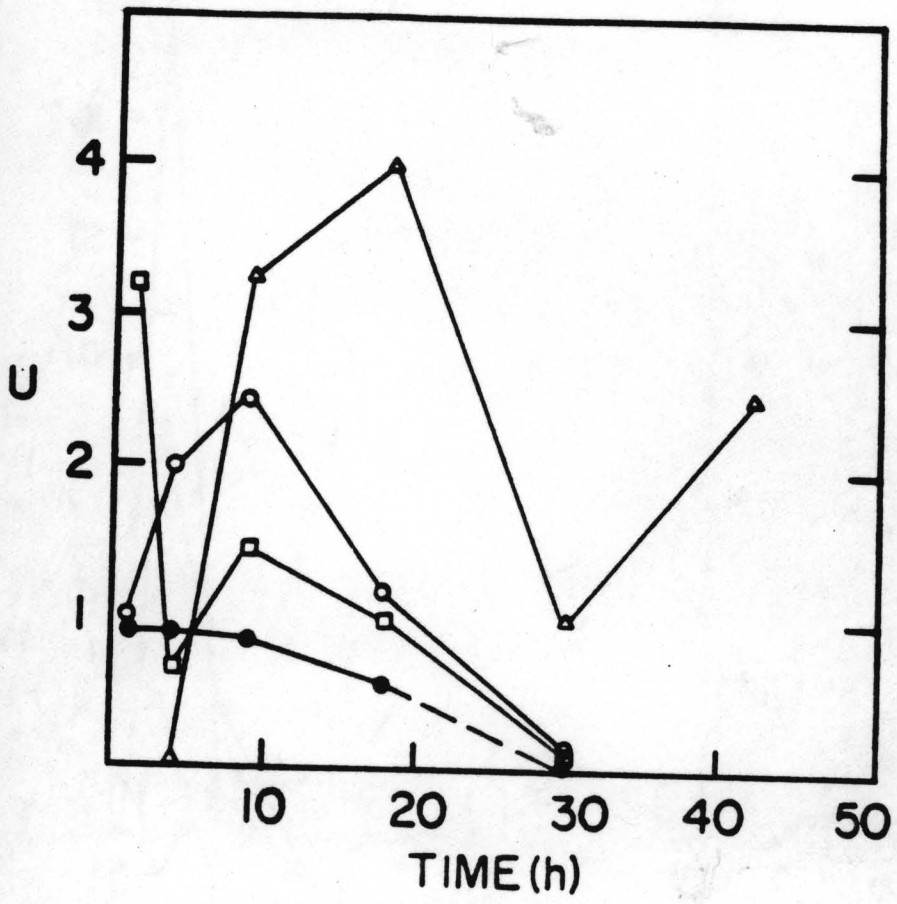


Figure 27: Individual ratios of the percentages of unchanged probenecid (U) from 0.5 to 2.0 gm single suspension doses.

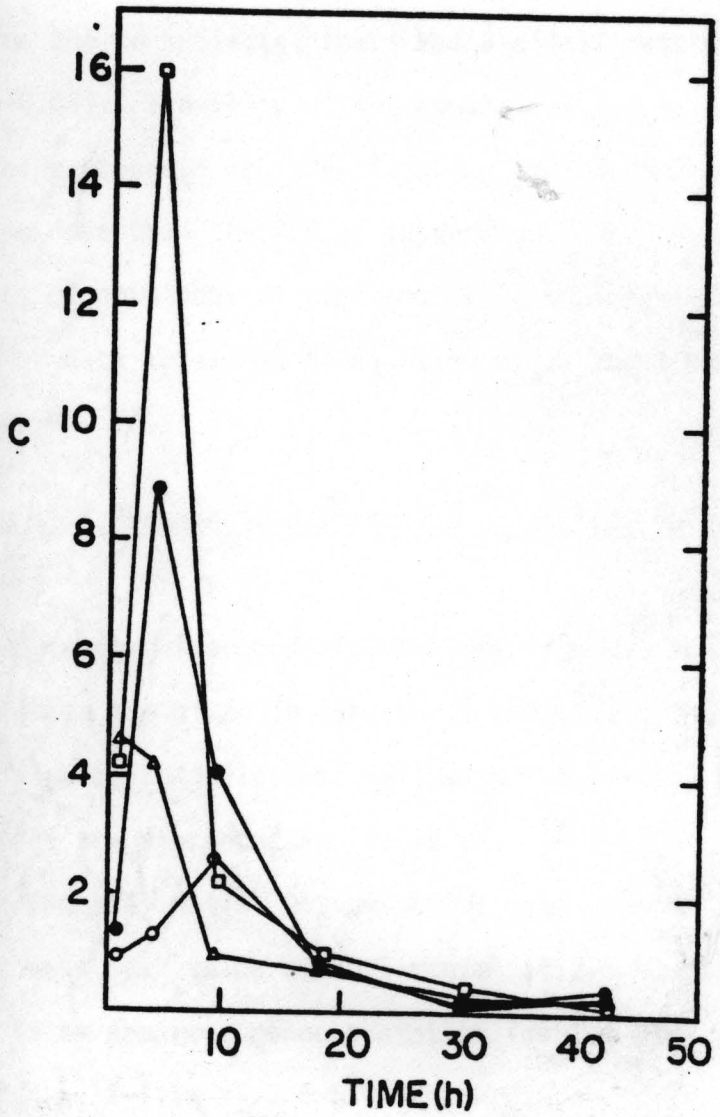


Figure 28: Individual ratios of the percentages of conjugated probenecid (C) from 0.5 to 2.0 gm single suspension doses.

dose and subjects. No significant difference was observed in the slopes for either dose. Similarly, slopes of conjugated probenecid following 0.5 and 2.0 gm suspension doses were examined. Although there was no difference due to subjects, there was a significant difference due to doses ($p<0.05$). The slope of the cumulative percentage recovery of conjugated probenecid was significantly smaller following the 2.0 gm suspension dose than the 0.5 gm suspension dose.

Copies of printouts of programs NREG, NREG coupled DGEAR for the analysis of data according to Equations 1, 2 and 3 are given in the appendices V - VII.

B. Study II - Probenecid 0.5 and 1.0 gm tablet, multiple dose study

Single Dose

Plasma levels of probenecid obtained from 0.5 and 1.0 gm tablet multiple doses are given in Tables LII and LIII. The mean plasma levels and standard deviations following the initial 0.5 and 1.0 gm tablet doses are presented in Figure 29.

Following the initial 0.5 gm tablet dose, plasma probenecid levels reached a mean peak value of 35.5 mcg/ml at 2.5 h. Levels then declined in an apparent monoexponential fashion with a mean elimination half-life of 3.4 h. Probenecid could be detected in the plasma of all subjects at 16.0 h and in one subject at 24.0 h after dosing.

Following the initial 1.0 gm tablet dose, plasma probenecid levels reached a mean peak value of 79.1 mcg/ml at 2.5 h. After

Table LII
Probenecid plasma levels following 0.5 gm tablet multiple doses.

Dose	Time (h)	Plasma probenecid (mcg/ml) Subject 1	2	3	4	$\bar{X}$	SD.	CV%
1	0.0	-[1]	-	-	-	-	-	-
	0.5	2.56	0	4.39	2.68	2.41	1.81	75.2
	1.0	14.4	3.43	11.7	11.4	10.2	4.73	46.2
	2.0	48.7	47.1	20.1	22.0	34.5	15.5	45.1
	3.0	45.9	36.9	16.9	21.0	30.2	13.6	45.0
	4.0	52.6	32.3	16.0	16.6	29.4	17.2	58.6
	6.0	22.4	26.2	11.9	11.9	18.1	7.33	40.5
	8.0	15.9	16.6	9.32	8.17	12.5	4.37	34.9
	12.0	8.45	7.38	4.66	3.27	5.94	2.39	40.2
	16.0	4.52	3.07	2.37	1.99	2.99	1.12	37.5
	24.0	0.76	-	-	-	0.19	0.38	200.0
	36.0	-	-	-	-	-	-	-
2	48.0	-	-	-	-	-	-	-
3	54.0	29.3	19.7	14.2	12.3	18.9	7.63	40.4
4	60.0	56.8	46.9	24.1	18.0	36.5	18.4	50.5
5	66.0	59.4	64.4	26.6	24.4	43.7	21.1	48.4
6	72.0	97.9	90.7	43.3	35.5	66.9	31.9	47.9
	74.0	109.8	95.1	32.2	65.1	75.6	34.4	45.5
	76.0	125.5	117.3	66.5	59.4	92.2	34.0	36.9
	80.0	96.7	93.1	61.9	36.9	72.2	28.2	39.1
	84.0	72.9	64.7	29.5	18.9	46.5	26.3	56.6
	88.0	50.4	40.9	18.5	11.6	30.4	18.3	60.3
	96.0	20.4	20.1	6.0	3.61	12.5	8.97	71.6
	108.0	4.78	4.68	1.92	-	2.85	2.31	81.3
	120.0	-	-	-	-	-	-	-

[1]Below detectable limit.

Table LIII
Probenecid plasma levels following 1.0 gm probenecid tablet multiple doses.

Dose	Time (h)	Plasma probenecid (mcg/ml) Subject 1	2	3	4	$\bar{X}$	SD.	CV%
1	0.0	-	-	-	-			
	0.5	13.7	9.12	-	21.0	10.9	8.79	80.2
	1.0	48.5	31.2	29.4	23.2	33.1	10.8	32.8
	2.0	67.4	87.7	86.2	60.2	75.4	13.7	18.2
	3.0	46.1	75.5	61.4	57.7	60.2	12.1	20.1
	4.0	47.7	65.2	101.1	47.4	65.4	25.2	38.6
	6.0	51.8	53.1	59.4	38.6	50.7	8.74	17.2
	8.0	31.0	51.0	34.7	25.7	35.6	10.9	30.6
	12.0	24.1	28.9	19.5	14.3	21.7	6.25	28.8
	16.0	15.7	20.0	7.22	9.29	13.1	5.87	44.9
	24.0	4.24	9.69	-	4.15	4.52	3.97	87.9
	36.0	-	-	-	-	-	-	-
2	48.0	-	-	-	-	-	-	-
3	54.0	35.4	50.8	58.3	31.8	44.1	12.6	28.5
4	60.0	51.9	91.7	83.5	63.1	72.6	18.3	25.2
5	66.0	98.1	122.4	58.5	79.5	89.6	27.2	30.3
6	72.0	126.8	175.5	172.4	106.0	145.2	34.3	23.6
	74.0	152.7	251.8	167.9	168.1	185.1	45.0	24.3
	76.0	147.7	248.6	237.2	133.2	191.7	59.6	31.1
	80.0	109.8	216.6	157.7	100.5	146.2	53.2	36.4
	84.0	103.1	138.6	138.3	67.3	111.8	34.0	30.4
	88.0	81.3	105.6	75.5	43.8	76.6	25.4	33.2
	96.0	37.4	83.7	38.3	19.2	44.7	27.5	61.5
	108.0	18.7	26.1	14.5	2.96	15.6	9.68	62.2
	120.0	4.62	9.97	-	-	3.65	4.74	129.9

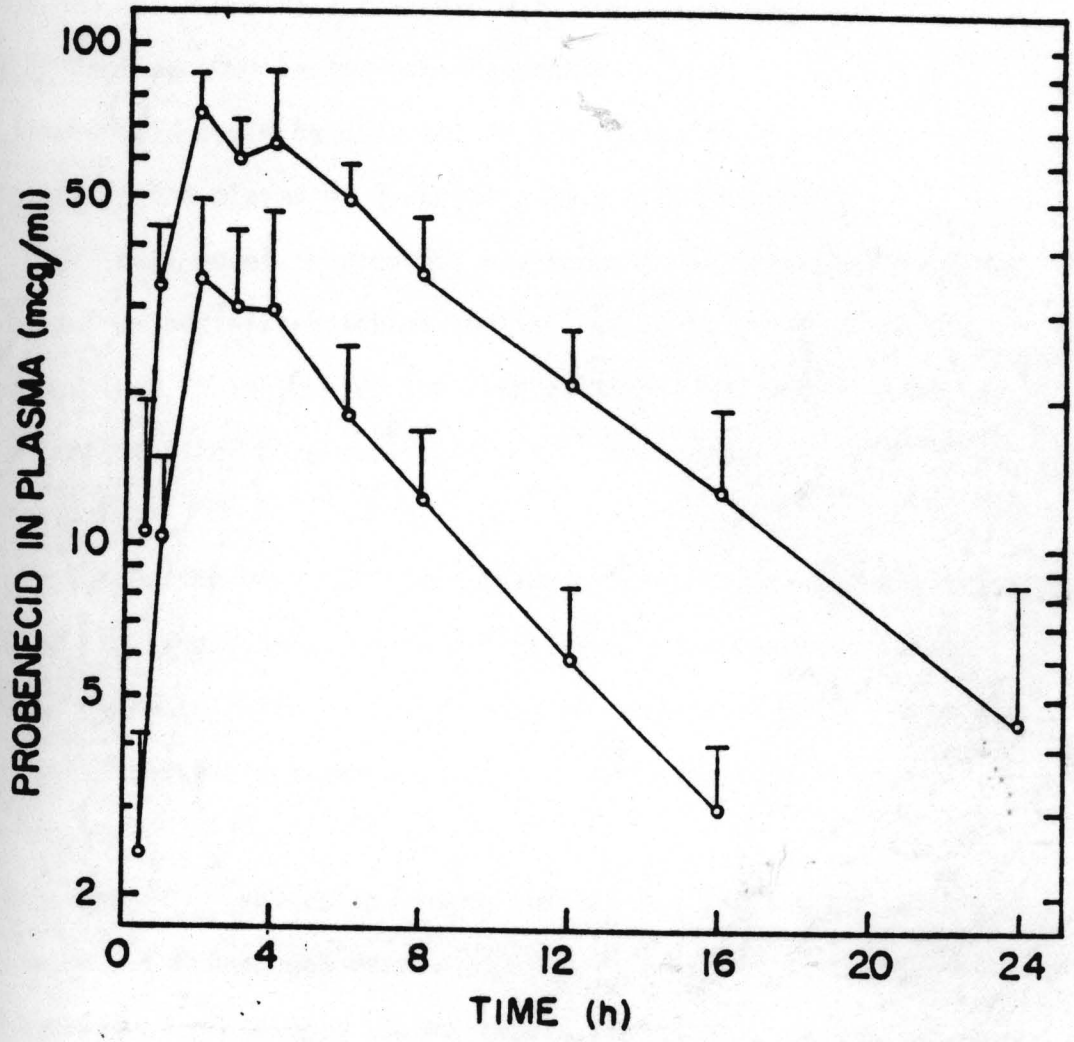


Figure 29: Mean (±SD.) probenecid plasma levels following the initial 0.5 and 1.0 gm tablet doses.

reaching peak values plasma levels declined in a slow and erratic manner. The rate of decline appeared to be curvilinear during 2-8 h postdose, after which time the plasma levels from this dose approached maximum levels achieved with the lower dose, and the rate of decline then became monoexponential.

Probenecid could be detected in the plasma of all subjects at 16 h, and in the plasma of 3 subjects 24.0 h after dosing.

Individual model-independent pharmacokinetic parameter values obtained from administration of initial 0.5 gm probenecid tablet dose are given in Table LIV. The mean of individual $AUC^{o \rightarrow t}$ trap and C_{max} normalized for dose were 4.43×10^{-4} h/ml and $7.08 \times 10^{-5} ml^{-1}$, respectively.

Individual model-independent pharmacokinetic parameter values obtained from administration of initial 1.0 gm probenecid tablet dose are given in Table LV. The mean of individual $AUC^{o \rightarrow t}$ trap and C_{max} normalized for dose were 6.5×10^{-4} h/ml and 7.91×10^{-5} ml^{-1} respectively.

The mean T_{max} values following initial 0.5 and 1.0 gm tablet doses were 2.5 h for both doses.

Individual probenecid plasma levels resulting from the initial 0.5 and 1.0 gm tablet doses were analyzed according to Equation 1 and final parameter estimates were obtained by nonlinear regression analysis of the data by the program NREG.

Table LIV
Model-independent pharmacokinetic parameter values following first 0.5 gm probenecid tablet dose of the multiple dose study.

Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
$AUC^{0\rightarrow t}_{trap.}$ (mcg.h/ml)	320.9	273.8	147.1	143.1	221.2	89.9	40.7
$AUC/D(10^{-4})$ (h/ml)	6.42	5.48	2.94	2.86	4.43	1.8	40.7
C_{max} (mcg/ml)	52.6	47.1	20.1	22.0	35.5	16.8	47.4
$C_{max}/D(10^{-5})$ (ml^{-1})	10.5	9.42	4.0	4.4	7.08	3.36	47.4
T_{max} (h)	4.0	2.0	2.0	2.0	2.5	1.0	40.0

Table LV
Model-independent pharmacokinetic parameter values following first 1.0 gm probenecid tablet dose of the multiple dose study.

Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
$AUC_{trap.}^{o \to t}$ (mcg.h/ml)	632.6	822.4	643.9	500.6	649.9	132.1	20.3
$AUC/D(10^{-4})$ (h/ml)	6.33	8.22	6.44	5.01	6.5	1.32	20.3
C_{max} (mcg/ml)	67.4	87.7	101.1	60.2	79.1	18.7	23.7
$C_{max}/D(10^{-5})$ (ml^{-1})	6.74	8.77	10.1	6.02	7.91	1.87	23.6
T_{max} (h)	2.0	2.0	4.0	2.0	2.5	1.0	40.0

Final estimates for the initial 0.5 and 1.0 gm tablet doses are given in Tables LVI and LVII, respectively.

The $AUC^{0\rightarrow t}/D$ value in this study increased 1.5 fold between 0.5 and 1.0 gm tablet doses. Equation 1 predicted an elimination $t_{\frac{1}{2}}$ from the 1.0 gm tablet dose 1.5-fold greater than the value obtained from 0.5 gm dose. These dose-dependent discrepancies in $AUC^{0\rightarrow t}/D$ and $t_{\frac{1}{2}\ el}$ were again consistent with the results of previous studies, confirming the possibility of saturable elimination. Consequently the plasma data obtained following the initial 0.5 and 1.0 gm tablet doses were then analyzed according to Equations 2 and 3. Initial and final parameter estimates for these models were obtained as described previously.

Final parameter estimates obtained from analysis of first 0.5 and 1.0 gm probenecid tablet doses according to Equation 2 are given in Tables LVIII and LIX, respectively.

From the analysis of plasma data obtained, following the first 0.5 gm probenecid tablet dose, Equations 1 and 2 yielded mean r^2 values of 0.989 and 0.975.

From the analysis of 1.0 gm plasma data by Equations 1 and 2, mean r^2 values of 0.975 and 0.973 were obtained.

Initial 0.5 and 1.0 gm tablet data were analyzed also according to the third model. The final parameter estimates obtained by Equation 3 for 0.5 and 1.0 gm tablet doses are presented in Tables LX and LXI, respectively.

Table LVI
Individual pharmacokinetic parameter values following the first 0.5 gm probenecid tablet dose, according to Equation 1.

Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
FD/V (mcg/ml)	86.0	55.3	25.3	32.9	49.9	27.2	54.6
$F/V(10^{-5})$ (ml^{-1})	17.2	11.1	5.06	6.57	9.98	5.45	54.6
k_a (h^{-1})	0.94	1.89	1.17	0.96	1.24	0.45	35.9
$t_{1/2\ abs}$ (h)	0.74	0.37	0.59	0.72	0.61	0.17	28.1
k_{el} (h^{-1})	0.28	0.19	0.16	0.22	0.21	0.05	24.1
$t_{1/2\ el}$ (h)	2.48	3.65	4.33	3.15	3.4	0.78	22.9
t_{lag} (h)	0.80	0.90	0.35	0.43	0.62	0.27	43.7
r^2	0.982	0.986	0.996	0.995	0.989	0.007	0.7

Table LVII
Individual pharmacokinetic parameter values following the first 1.0 gm probenecid tablet dose, according to Equation 1.

Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
FD/V (mcg/ml)	68.0	99.3	156.6	90.8	103.7	37.7	36.3
$F/V(10^{-5})$ (ml^{-1})	6.8	9.93	15.7	9.08	10.4	3.79	36.5
k_a (h^{-1})	2.44	1.12	0.63	0.68	1.22	0.84	69.3
$t_{1/2\ abs}$ (h)	0.28	0.62	1.10	1.02	0.76	0.38	50.3
k_{el} (h^{-1})	0.09	0.12	0.24	0.19	0.16	0.07	42.4
$t_{1/2\ el}$ (h)	7.70	5.78	2.89	3.65	5.01	2.17	43.4
t_{lag} (h)	0.41	0.46	0.63	0.21	0.43	0.17	40.4
r^2	0.983	0.981	0.958	0.979	0.975	0.012	1.2

Table LVII
Individual pharmacokinetic parameter values following the first 0.5 gm probenecid tablet dose, according to Equation 2.

Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
FD/V (mcg/ml)	50.5	52.1	36.3	27.5	41.6	11.8	28.3
$F/V(10^{-5})$ (ml^{-1})	10.1	10.4	7.26	5.50	8.32	2.35	28.3
k_a (h^{-1})	2.29	1.68	0.63	1.25	1.46	0.70	47.9
$t_{1/2\ abs}$ (h)	0.30	0.41	1.10	0.55	0.59	0.36	60.2
K_M (mcg/ml)	54.0	84.0	68.0	124.0	82.5	30.3	36.7
V_M (mcg/ml.h)	9.0	16.0	23.0	23.3	17.8	6.78	38.0
V_M/K_M (h^{-1})	0.17	0.19	0.34	0.19	0.22	0.08	35.5
$t_{1/2\ el}$ (h)	4.08	3.65	2.04	3.65	3.36	0.89	26.8
t_{lag}[1] (h)	0.80	0.90	0.35	0.43	0.62	0.27	43.7
r^2	0.939	0.988	0.979	0.992	0.975	0.024	2.5

[1]This value fixed during nonlinear regression.

Table LIX
Individual pharmacokinetic parameter values following the first 1.0 gm probenecid tablet dose according to Equation 2.

Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
FD/V (mcg/ml)	65.5	91.9	119.2	75.1	87.9	23.5	26.8
$F/V(10^{-5})$ (ml^{-1})	6.55	9.19	9.19	7.51	8.79	2.34	26.7
k_a (h^{-1})	2.62	1.26	0.97	0.80	1.42	0.83	58.8
$t_{1/2\ abs}$ (h)	0.26	0.55	0.71	0.87	0.59	0.26	43.5
K_M (mcg/ml)	55.2	84.7	67.4	124.4	82.9	30.2	36.4
V_M (mcg/ml.h)	8.66	15.4	23.4	22.9	17.6	6.99	39.7
V_M/K_M (h^{-1})	0.16	0.18	0.35	0.18	0.22	0.09	40.8
$t_{1/2\ el}$ (h)	4.33	3.85	1.98	3.85	3.5	1.04	29.7
t_{lag} (h)	0.41	0.46	0.63	0.21	0.43	0.17	40.4
r^2	0.983	0.977	0.956	0.974	0.973	0.012	1.2

Table LX
Individual pharmacokinetic parameter values following the first 0.5 gm probenecid tablet dose, according to Equation 3.

Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
FD/V (mcg/ml)	71.8	61.0	24.6	33.6	47.8	22.3	46.7
$F/V(10^{-5})$ (ml^{-1})	14.4	12.2	4.92	6.72	9.56	4.47	46.8
k_a (h^{-1})	1.04	1.26	1.23	0.89	1.11	0.17	15.7
$t_{1/2\ abs}$ (h)	0.67	0.55	0.56	0.78	0.64	0.11	16.9
K_M (mcg/ml)	129.9	130.0	85.6	61.6	101.8	33.9	33.4
V_M (mcg/ml.h)	20.4	2.0	2.14	2.0	6.64	9.18	138.3
k' (h^{-1})	0.09	0.17	0.13	0.24	0.16	0.06	40.6
V_M/K_M (h^{-1})	0.16	0.02	0.03	0.03	0.06	0.07	111.4
$t_{1/2\ el}$ (h)	2.77	3.65	4.33	2.57	3.33	0.82	24.5
t_{lag} (h)	0.8	0.9	0.35	0.43	0.62	0.27	43.7
r^2	0.977	0.992	0.996	0.989	0.989	0.008	0.83

Table LXI
Individual pharmacokinetic parameter values following the first 1.0 gm probenecid tablet dose, according to Equation 3.

Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
FD/V (mcg/ml)	66.3	97.3	156.4	79.9	99.9	39.7	39.7
$F/V(10^{-5})$ (ml^{-1})	6.63	9.73	15.6	7.99	9.99	3.95	39.6
k_a (h^{-1})	2.57	1.11	0.63	0.79	1.28	0.89	69.5
$t_{1/2\ abs}$ (h)	0.27	0.62	1.10	0.88	0.72	0.36	49.8
K_M (mcg/ml)	53.2	130.0	80.9	129.6	98.4	37.9	38.6
V_M (mcg/ml.h)	5.18	2.03	25.0	2.00	8.55	11.1	129.4
k' (h^{-1})	0.04	0.10	0.02	0.15	0.08	0.06	76.2
V_M/K_M (h^{-1})	0.09	0.02	0.31	0.02	0.11	0.14	124.9
$t_{1/2\ el}$ (h)	5.33	5.78	2.10	4.08	4.32	1.65	38.1
t_{lag} (h)	0.41	0.46	0.63	0.21	0.43	0.17	40.4
r^2	0.983	0.981	0.925	0.977	0.967	0.028	2.90

The analysis of initial 0.5 and 1.0 gm data by Equation 3 yielded r^2 values of 0.989 and 0.967.

Individual plasma probenecid concentrations, and also the concentrations predicted by the analysis of initial 0.5 gm probenecid tablet data according to Equations 1, 2 and 3 are given in Tables LXII-LXV.

Similarly, individual actual and predicted plasma probenecid concentration predicted by the three equations from the initial 1.0 gm probenecid tablet data are presented in Tables LXVI-LXIX.

Statistical Analysis

Individual pharmacokinetic parameter values obtained from analysis of the first 0.5 gm probenecid tablet data according to the three models were examined by analysis of variance for randomized complete block design. The results of this statistical analysis, and the mean parameter values are given in Table LXX.

There were no significant differences in F/V, k_a, $t_{\frac{1}{2}abs}$, $t_{\frac{1}{2}el}$, t_{lag} and r^2 values predicted by the three equations. The mean k_a values from the initial 0.5 gm dose ranged between 1.11 to 1.46 h^{-1} while the mean $t_{\frac{1}{2}\ el}$ values ranged from 3.33 to 3.4 h.

Michaelis-Menten constants, K_M and V_M obtained from analysis of the 0.5 gm data by Equations 2 and 3, were not significantly different. Equation 2 predicted values of 82.5 mcg/ml and 17.8 mcg/ml.h for K_M and V_M, respectively, while Equations 3 predicted 101.8 mcg/ml and 6.64 mcg/ml.h. However, the ratio V_M/K_M predicted by Equation 2 was

Table LXII
Actual and computer predicted probenecid plasma levels following the first 0.5 gm probenecid table dose of the multiple dose study, according to Equations 1, 2 and 3 for Subject #1.

Time (h)	Plasma probenecid (mcg/ml)			
	Actual	Predicted by equations		
		1	2	3
0.5	2.56	0	0	0
1.0	14.4	14.4	18.3	14.4
2.0	48.7	47.9	43.5	47.9
3.0	45.9	50.9	42.4	50.9
4.0	52.6	44.3	38.8	44.5
6.0	22.4	28.1	31.8	28.2
8.0	15.9	16.6	25.6	16.5
12.0	8.45	5.54	15.7	5.15
16.0	4.52	1.84	9.12	1.55

Table LXIII
Actual and computer predicted probenecid plasma levels following the first 0.5 gm probenecid tablet dose of the multiple dose study, according to Equations 1, 2 and 3 for Subject #2.

Time (h)	Plasma probenecid (mcg/ml)			
	Actual	Predicted by equation		
		1	2	3
0.5	-	0	0	0
1.0	3.43	9.45	7.98	7.18
2.0	47.1	42.4	41.7	43.8
3.0	36.9	40.3	38.3	39.9
4.0	32.3	34.2	33.8	34.1
6.0	26.2	23.7	25.6	23.8
8.0	16.6	16.3	18.9	16.4
12.0	7.38	7.72	9.83	7.72
16.0	3.07	3.66	4.87	3.62

Table LXIV
Actual and computer predicted probenecid plasma levels following the first 0.5 gm probenecid tablet dose of the multiple dose study, according to Equations 1, 2 and 3 for Subject #3.

Time (h)	Plasma probenecid (mcg/ml)			
	Actual	Predicted by equations		
		1	2	3
0.5	4.39	4.04	3.18	3.97
1.0	11.7	12.7	10.9	12.6
2.0	20.1	18.3	18.0	18.2
3.0	16.9	17.9	19.0	17.9
4.0	16.0	16.1	17.4	16.1
6.0	11.9	12.1	11.9	12.1
8.0	9.32	8.86	7.09	8.92
12.0	4.66	4.64	2.11	4.79
16.0	2.37	2.55	0.59	2.57

Table LXV
Actual and computer predicted plasma probenecid levels following the first 0.5 gm probenecid tablet dose of the multiple dose study, according to Equations 1, 2 and 3 for Subject #4.

Time (hr)	Plasma probenecid (mcg/ml)			
	Actual	Predicted by equations		
		1	2	3
0.5	2.68	2.08	2.29	2.10
1.0	11.4	12.9	13.3	12.9
2.0	22.0	20.6	20.0	20.7
3.0	21.0	20.5	19.6	20.6
4.0	16.6	17.9	17.3	18.0
6.0	11.9	12.1	12.6	12.2
8.0	8.17	7.85	8.97	7.82
12.0	3.27	3.22	4.4	3.13
16.0	1.99	1.32	2.12	1.26

Table LXVI
Actual and computer predicted probenecid plasma levels following the first 1.0 gm probenecid tablet dose of the multiple dose study, according to Equations 1, 2 and 3 for Subject #1.

Time (h)	Plasma probenecid (mcg/ml)			
	Actual	Predicted by equations		
		1	2	3
0.5	13.7	13.4	13.7	13.6
1.0	48.5	50.2	49.9	49.9
2.0	67.4	59.5	58.4	58.6
3.0	46.1	55.4	54.9	54.9
4.0	47.7	50.6	50.8	50.4
6.0	51.8	41.9	42.9	42.2
8.0	31.0	34.8	35.7	34.9
12.0	24.1	23.9	23.7	23.4
16.0	15.7	16.5	14.8	15.1

Table LXVII
Actual and computer predicted probenecid plasma levels following the first 1.0 gm probenecid tablet dose of the multiple dose study, according to Equations 1, 2 and 3 for Subject #2.

Time (h)	Plasma probenecid (mcg/ml)			
	Actual	Predicted by equations		
		1	2	3
0.5	9.12	4.61	4.51	4.21
1.0	31.2	43.6	43.6	42.4
2.0	82.7	72.9	70.6	71.6
3.0	75.5	76.1	72.9	75.3
4.0	65.2	71.5	68.6	71.2
6.0	53.1	58.2	56.5	58.7
8.0	51.0	46.3	45.1	47.3
12.0	28.9	29.2	27.1	30.3
16.0	20.0	18.4	15.1	19.4

Table LXVIII
Actual and computer predicted probenecid plasma levels following the first 1.0 gm probenecid tablet dose of the multiple dose study, according to Equations 1, 2 and 3 for Subject #3.

Time (h)	Plasma probenecid (mcg/ml)			
	Actual	Predicted by equations		
		1	2	3
0.5	-	0	0	0
1.0	29.4	31.1	34.2	30.6
2.0	86.2	75.1	75.0	76.9
3.0	61.4	85.9	81.9	93.2
4.0	101.1	81.9	76.8	94.5
6.0	59.4	60.7	57.4	78.9
8.0	34.7	40.3	38.6	57.6
12.0	19.5	16.1	13.9	23.9
16.0	7.22	8.2	4.07	7.88

Table LXIX
Actual and computer predicted probenecid plasma levels following the first 1.0 gm probenecid tablet dose of the multiple dose study, according to Equations 1, 2 and 3 for Subject #4.

Time (h)	Plasma probenecid (mcg/ml)			
	Actual	Predicted by equations		
		1	2	3
0.5	21.0	15.8	15.1	15.9
1.0	23.2	34.7	32.8	34.6
2.0	60.2	52.3	48.9	50.9
3.2	57.7	54.6	51.9	52.7
4.0	47.4	51.6	49.9	49.9
6.0	38.6	39.3	40.6	38.9
8.0	25.7	27.8	30.9	28.9
12.0	14.3	13.2	16.7	15.3
16.0	9.29	6.15	8.56	8.04

Table LXX
Mean pharmacokinetic parameter values (±1 SD.) obtained by analyzing plasma probenecid levels from the first 0.5 gm probenecid tablet dose according to Equations 1 (A), 2 (B) and 3 (C).

Parameter	Value according to Equations			Statistic
	1 (A)	2 (B)	3 (C)	
FD/V (mcg/ml)	49.9 ± 27.2	41.6 ± 11.8	47.8 ± 22.3	NSD
$F/V(10^{-5})$ (ml^{-1})	9.98 ± 5.45	8.32 ± 2.35	9.56 ± 4.47	"
k_{abs} (h^{-1})	1.24 ± 0.45	1.46 ± 0.7	1.11 ± 0.17	"
$t_{1/2\ abs}$ (h)	0.61 ± 0.17	0.59 ± 0.36	0.64 ± 0.11	"
k_{el} (h^{-1})	0.21 ± 0.05	-	-	-
K_M (mcg/ml)	-	82.5 ± 30.3	101.8 ± 33.9	NSD
V_M (mcg/ml.h)	-	17.8 ± 6.78	6.64 ± 9.18	"
V_M/K_M (h^{-1})	-	0.22 ± 0.08	0.06 ± 0.07	B>C
k' (h^{-1})	-	-	0.16 ± 0.06	-
$t_{1/2\ el}$ (h)	3.4 ± 0.78	3.36 ± 0.89	3.33± 0.82	NSD
t_{lag} (h)	0.62 ± 0.27	0.62 ± 0.27	0.62 ± 0.27	"
r^2	0.989 ± 0.007	0.975 ± 0.024	0.989 ± 0.008	"

0.22 h^{-1} which was significantly greater than 0.06 h^{-1} predicted by Equation 3. The ratio V_M/K_M was interestingly close to 0.21 h^{-1}, the value of first-order elimination rate constant predicted by Equation 1.

Individual pharmacokinetic parameter values obtained from analysis of the initial 1.0 gm probenecid tablet data according to the three equations were examined in a similar fashion. The mean parameter values and the results of analysis of variance for randomized complete block design are in Table LXXI.

There were no significant differences in F/V, k_a, $t_{\frac{1}{2}abs}$, $t_{\frac{1}{2}el}$, t_{lag} and r^2 values predicted by the three equations.

The mean k_a values according to the three equations ranged from 1.22 to 1.42 h^{-1} while the mean $t_{\frac{1}{2}\ el}$ values ranged from 3.5 to 5.01 h.

Michaelis-Menten constants K_M and V_M were not significantly different according to Equations 2 and 3. Equations 2 predicted values of 82.9 mcg/ml and 17.6 mcg/ml.h for K_M and V_M, respectively, while Equations 3 predicted 98.4 mcg/ml and 8.55 mcg/ml.h. However, the ratio V_M/K_M predicted by Equation 2, 0.22 h^{-1} was significantly greater than the value 0.11 h^{-1}, predicted by Equation 3.

The mean first-order elimination rate constant from Eqn. 1 was 0.16 h^{-1} yielding a mean $t_{\frac{1}{2}el}$ 1.04-fold greater than the value predicted by Equation 2, however this difference was not significant at 95% confidence level.

Table LXXI
Mean Pharmacokinetic parameter values (±1 SD.) obtained by analyzing plasma probenecid levels from the first 1.0 gm probenecid tablet dose, according to Equations 1 (A), 2 (B) and 3 (C).

Parameter	Value according to Equations			Statistic
	1 (A)	2 (B)	3 (C)	
FD/V (mcg/ml	103.7 ± 37.7	87.9 ± 23.5	99.9 ± 39.7	NSD
$F/V(10^{-5})$ (ml^{-1})	10.4 ± 3.79	8.79 ± 2.34	9.99 ± 3.95	"
k_a (h^{-1})	1.22 ± 0.84	1.42 ± 0.83	1.28 ± 0.89	"
$t_{1/2\ abs}$ (h)	0.76 ± 0.38	0.59 ± 0.26	0.72 ± 0.36	"
k_{el} (h^{-1})	0.16 ± 0.07	-	-	-
K_M (mcg/ml)	-	82.9 ± 30.2	98.4 ± 37.9	NSD
V_M (mcg/ml.h)	-	17.6 ± 6.99	8.55 ± 11.1	"
V_M/K_M (h^{-1})	-	0.22 ± 0.09	0.11 ± 0.14	B>C
k' (h^{-1})	-	-	0.08 ± 0.06	-
$t_{1/2\ el}$ (h)	5.01 ± 2.17	3.5 ± 1.04	4.32 ± 1.65	NSD
t_{lag} (h)	0.43 ± 0.17	0.43 ± 0.17	0.43 ± 0.17	"
r^2	0.975 ± 0.012	0.973 ± 0.012	0.967 ± 0.028	"

Multiple doses

Accumulation factor, R, was calculated for each subject from the results of the first 0.5 and 1.0 gm tablet doses. This value was subsequently used to predict the maximum steady state concentrations of probenecid in Plasma. Tables LXXII and LXXIII give R values, peak concentrations from the first dose, calculated and observed maximum concentrations and their ratios, for 0.5 and 1.0 gm tablet doses. The mean value of observed C_{max} was approximately 2-fold greater than the calculated C_{max} for both doses. This indicates that the extent of accumulation following repeated 0.5 and 1.0 gm tablet doses of probenecid could not be explained by first-order absorption and elimination model. As a consequence, Equation 1 was not used to analyze the plasma data obtained from the final doses of the multiple dose study.

The plasma data obtained following the administration of final 0.5 and 1.0 gm tablet doses were analyzed according to Equations 2 and 3. The pharmacokinetic parameter values obtained from the analysis of plasma data following the final 0.5 and 1.0 gm doses according to Equations 2 are given in Tables LXIV and LXXV.

Individual plasma data following the final 0.5 and 1.0 gm doses were analyzed also according to Equation 3. The pharmacokinetic parameter values predicted by Equation 3 for the final 0.5 and 1.0 tablet doses are in Tables LXXVI and LXXVII.

Individual actual plasma probenecid concentrations and those predicted by Equation 2 and 3, following the final 0.5 gm probenecid

Table LXXII
Actual and predicted maximum probenecid levels, calculated by Equation 1, following 0.5 gm probenecid tablet multiple doses.

	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
R[1]	1.23	1.49	1.65	1.35	1.43	0.18	12.7
$C_{max, first}$ (mcg/ml)	52.6	47.1	20.1	22.0	35.5	16.8	47.4
R.C max, first (mcg/ml)	64.7	70.2	33.1	29.7	49.4	20.9	42.4
$C_{max, final}$ (mcg/ml)	125.5	117.3	66.5	65.1	93.6	32.3	34.5
Ratio[2]	1.94	1.67	2.01	2.19	1.95	0.22	11.1

[1] Calculated by $R = 1/(1 - 2^{-\varepsilon})$ where $\varepsilon = \tau / t_{\frac{1}{2}}$

[2] $(C_{max, final}) / R. C_{max, first}$

Table LXXIII
Actual and predicted maximum probenecid levels, calculated by Equation 1, following 1.0 gm probenecid tablet multiple doses.

	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
R	2.39	2.01	1.31	1.47	1.79	0.49	27.7
$C_{max, first}$ (mcg/ml)	67.4	87.7	101.1	60.2	79.1	18.7	23.7
$R.C._{max, first}$ (mcg/ml)	161.1	176.2	132.5	88.5	139.6	38.6	27.6
$C_{max, final}$ (mcg/ml)	152.7	251.8	237.2	168.1	202.5	49.3	24.4
Ratio	0.95	1.43	1.79	1.89	1.52	0.43	28.1

Table LXXIV
Individual pharmacokinetic parameter values following the final 0.5 gm probenecid tablet dose of the multiple dose study, according to Equation 2.

Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
FD/V (mcg/ml)	74.7	61.9	52.7	55.8	61.3	9.73	15.9
F/V(10^{-5}) (ml-1)	14.9	12.4	10.5	11.2	12.3	1.93	15.8
k_a (h-1)	0.32	0.18	0.54	0.77	0.45	0.26	57.1
$t_{1/2}$ abs (h)	2.17	3.85	1.28	0.90	2.05	1.31	64.0
K_M (mcg/ml)	130.0[1]	130.0[1]	130.0[1]	130.0[1]	130.0	0	0
V_M (mcg/ml.h)	18.3	15.6	19.1	23.4	19.1	3.23	16.9
V_M/K_M (h-1)	0.14	0.12	0.15	0.18	0.15	0.03	16.9
$t_{1/2}$ el (h)	4.95	5.78	4.62	3.85	4.80	0.79	16.7
r^2	0.998	0.981	0.889	0.999	0.967	0.05	5.4

[1]These values were at the upper limit.

Table LXXV
Individual pharmacokinetic parameter values following the final 1.0 gm probenecid tablet dose of the multiple dose study, according to Equation 2.

Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
FD/V (mcg/ml)	43.4	99.3	155.6	85.4	95.9	46.3	48.3
$F/V(10^{-5})$ (ml^{-1})	4.34	9.93	15.6	8.54	9.60	4.65	48.4
k_a (h^{-1})	1.18	1.12	0.27	1.0	0.89	0.42	47.2
$t_{1/2\ abs}$ (h)	0.59	0.62	2.57	0.69	1.12	0.97	86.7
K_M (mcg/ml)	101.8	116.4	109.5	130.0	114.4	11.9	10.5
V_M (mcg/ml.h)	11.0	17.1	25.0	21.8	18.7	6.09	32.5
V_M/K_M (h^{-1})	0.11	0.15	0.23	0.17	0.17	0.05	30.3
$t_{1/2\ el}$ (h)	6.3	4.62	3.01	4.08	4.5	1.37	30.5
r^2	0.997	0.989	0.977	0.996	0.989	0.009	0.93

Table LXXVI
Individual pharmacokinetic parameter values following the final 0.5 gm probenecid tablet dose of the multiple dose study according to Equation 3.

Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
FD/V (mcg/ml)	138.1	79.7	49.6	57.4	81.2	40.0	49.3
$F/V(10^{-5})$ (ml^{-1})	27.6	15.9	9.92	11.5	16.2	7.99	49.2
k_a (h^{-1})	0.19	0.24	0.18	0.76	0.34	0.28	81.6
$t_{1/2\ abs}$ (h)	3.65	2.89	3.85	0.91	2.83	1.34	47.5
K_M (mcg/ml)	130.0	130.0	114.3	130.0	126.1	7.85	6.22
V_M (mcg/ml.h)	4.22	7.1	14.3	23.3	12.2	8.51	69.6
V_M/K_M (h^{-1})	0.03	0.05	0.13	0.18	0.09	0.07	71.7
k' (h^{-1})	0.10	0.07	0.04	0.001	0.05	0.04	80.2
$t_{1/2\ el}$ (h)	5.33	5.78	4.08	3.83	4.76	0.95	19.9
r^2	0.998	0.987	0.871	0.999	0.964	0.062	6.4

Table LXXVII
Individual pharmacokinetic parameter values following the final 1.0 gm probenecid tablet dose of the multiple dose study according to Equation 3.

Parameter	Value						
	Subject				$\bar{X}$	SD.	CV%
	1	2	3	4			
FD/V (mcg/ml)	57.1	148.9	132.4	75.8	103.6	44.0	42.5
$F/V(10^{-5})$ (ml^{-1})	5.71	14.9	13.2	7.58	10.35	4.39	42.4
k_a (h^{-1})	1.81	0.52	0.27	2.8	1.35	1.18	87.3
$t_{1/2\ abs}$ (h)	0.38	1.33	2.57	0.25	1.13	1.07	94.7
K_M (mcg/ml)	63.3	130.0	50.3	130.0	93.4	42.6	45.6
V_M (mcg/ml.h)	9.87	2.0	14.3	12.1	9.57	5.36	56.0
V_M/K_M (h^{-1})	0.16	0.02	0.28	0.09	0.14	0.11	80.6
k' (h^{-1})	0.001	0.06	0.001	0.03	0.02	0.03	122.6
$t_{1/2\ el}$ (h)	4.30	8.66	2.47	5.78	5.30	2.62	49.3
r^2	0.991	0.995	0.954	0.997	0.984	0.020	2.06

tablet dose are presented in Tables LXXVIII - LXXXI.

Similarly, individual actual plasma probenecid concentrations and those predicted by Equations 2 and 3, following the final 1.0 gm tablet dose are given in Tables LXXXII - LXXXV.

Statistical Analysis

Individual pharmacokinetic parameter values obtained from last 0.5 and 1.0 gm probenecid multiple tablet data according to Equations 2 and 3 were examined by analysis of variance for randomized complete block design.

The results of this statistical analysis from the plasma data following the final 0.5 gm dose are given in Table LXXXVI.

There were no significant differences in F/V, k_a, K_M, V_M, V_M/K_M, $t_{\frac{1}{2}el}$ and r^2 values predicted by Equations 2 and 3. The mean k_a was o.45 and 0.34 h^{-1} by Equations 2 and 3. The mean ratio V_M/K_M was 0.15 and 0.09 h^{-1} yielding mean $t_{\frac{1}{2}el}$ values of 4.8 and 4.76 h, respectively.

The mean pharmacokinetic parameter values, and the results of the statistical analysis of plasma data following the final 1.0 gm dose, are presented in Table LXXXVII.

No significant differences were observed in F/V, k_a, K_M, V_M, V_M/K_M, $t_{\frac{1}{2}el}$ and r^2 values predicted by Equations 2 and 3. The mean ratio V_M/K_M was 0.17 and 0.14 h^{-1} yielding mean $t_{\frac{1}{2}el}$ values of 4.5 and 5.3 h by Equations 2 and 3, and these values again were not significantly different.

Table LXXVIII
Actual and computer predicted probenecid plasma levels following final 0.5 gm probenecid tablet dose according to Equations 2 and 3 for Subject #1.

Time (h)	Plasma probenecid (mcg/ml)		
	Actual	Predicted by equations	
		2	3
72.0	97.9	97.9	97.9
74.0	109.8	116.3	116.3
76.0	125.5	117.4	118.1
80.0	96.7	98.9	99.1
84.0	72.9	73.9	72.6
88.0	50.9	51.4	49.4
96.0	20.4	21.2	20.2
108.0	4.78	4.48	4.48

Table LXXIX
Actual and computer predicted probenecid plasma levels following final 0.5 gm probenecid tablet dose according to Equations 2 and 3 for Subject #2.

Time (h)	Plasma probenecid (mcg/ml)		
		Predicted by equations	
	Actual	2	3
72.0	90.7	90.7	90.7
74.0	95.1	96.6	101.9
76.0	117.3	96.4	100.4
80.0	93.1	85.9	81.9
84.0	64.7	69.9	59.6
88.0	40.9	53.4	40.7
96.0	20.1	27.1	17.2
108.0	4.68	7.83	4.19

Table LXXX
Actual and computer predicted probenecid plasma levels following final 0.5 gm probenecid tablet dose according to Equations 2 and 3 for Subject #3.

Time (h)	Plasma probenecid (mcg/ml)		
	Actual	Predicted by equations	
		2	3
72.0	43.3	43.3	43.3
74.0	32.2	66.3	46.5
76.0	66.5	65.1	45.1
80.0	61.9	47.3	35.9
84.0	29.5	30.5	25.2
88.0	18.5	18.6	16.2
96.0	6.0	6.32	5.8
108.0	1.92	1.14	1.04

Table LXXXI
Actual and computer predicted probenecid plasma levels following final 0.5 gm probenecid tablet dose according to Equations 2 and 3 for Subject #4

Time (h)	Plasma probenecid (mcg/ml)		
	Actual	Predicted by equations	
		2	3
72.0	35.5	35.5	35.5
74.0	65.1	65.2	65.9
76.0	59.4	59.2	60.2
80.0	36.9	36.6	37.3
84.0	18.9	20.3	20.7
88.0	11.6	10.7	10.8
96.0	3.61	2.69	2.71

Table LXXXII
Actual and computer predicted probenecid plasma levels following final 1.0 gm probenecid tablet dose according to Equations 2 and 3 for Subject #1.

Time (h)	Plasma probenecid (mcg/ml)		
		Predicted by equations	
	Actual	2	3
72.0	126.8	126.8	126.8
74.0	152.7	152.9	167.6
76.0	147.7	143.5	154.4
80.0	109.8	119.1	126.7
84.0	103.1	96.5	100.9
88.0	81.3	76.4	77.5
96.0	37.4	44.1	39.7
108.0	18.7	15.8	9.41
120.0	4.62	4.81	1.53

Table LXXXIII
Actual and computer predicted probenecid plasma levels following final 1.0 gm probenecid tablet dose according to Equations 2 and 3 for Subject #2.

Time (h)	Plasma probenecid (mcg/ml)		
		Predicted by equations	
	Actual	2	3
72.0	175.5	175.5	175.5
74.0	251.8	241.7	243.1
76.0	248.6	228.1	244.9
80.0	216.6	185.4	202.5
84.0	138.6	145.3	157.3
88.0	105.6	109.6	120.6
96.0	83.7	54.3	69.8
108.0	26.1	13.2	29.9
120.0	9.97	2.55	12.4

Table LXXXIV
Actual and computer predicted probenecid plasma levels following final 1.0 gm probenecid tablet dose according to Equations 2 and 3 for Subject #3.

Time (h)	Plasma probenecid (mcg/ml)		
		Predicted by equations	
	Actual	2	3
72.0	172.4	172.4	172.4
74.0	167.9	204.8	205.0
76.0	237.2	209.6	213.6
80.0	157.7	180.2	196.2
84.0	138.3	133.4	160.8
88.0	75.5	87.5	121.4
96.0	38.3	25.9	51.5
108.0	14.5	2.19	4.09

Table LXXXV
Actual and computer predicted probenecid plasma levels following final 1.0 gm probenecid tablet dose according to Equations 2 and 3 for Subject #4.

Time (h)	Plasma probenecid (mcg/ml)		
		Predicted by equations	
	Actual	2	3
72.0	106.0	106.0	106.0
74.0	168.1	156.9	159.4
76.0	133.2	143.3	138.3
80.0	100.5	102.5	101.7
84.0	67.3	68.3	72.6
88.0	43.8	43.8	50.3
96.0	19.2	19.2	22.2
108.0	2.96	1.99	5.78

Table LXXXVI
Mean pharmacokinetic parameter values (±1 SD.) obtained by analyzing plasma probenecid levels from the final 0.5 gm probenecid tablet dose of the multiple dose study according to Equations 2 (A) and 3 (B).

Parameter	Value according to Equations		Statistic
	2 (A)	3 (B)	
FD/V (mcg/ml)	61.3 ± 9.73	81.2 ± 40.0	NSD
$F/v(10^{-5})$ (ml^{-1})	12.3 ± 1.93	16.2 ± 7.99	"
k_{abs} (h^{-1})	0.45 ± 0.26	0.34 ± 0.28	"
$t_{1/2\ abs}$ (h)	2.05 ± 1.31	2.83 ± 1.34	"
K_M (mcg/ml)	130.1 ± 0.0	126.1 ± 7.85	"
V_M (mcg/ml.h)	19.1 ± 3.23	12.2 ± 8.51	"
V_M/K_M (h^{-1})	0.15 ± 0.03	0.09 ± 0.07	"
k' (h^{-1})	-	0.05 ± 0.04	-
$t_{1/2\ el}$ (h)	4.80 ± 0.79	4.76 ± 0.95	NSD
r^2	0.967 ± 0.05	0.964 ± 0.06	"

Table LXXXVII
Mean Pharmacokinetic parameter values (±1 SD.) obtained by analyzing plasma probenecid levels from the final 1.0 gm probenecid tablet dose of the multiple dose study according to Equations 2 (A) and 3 (B).

Parameter	Value according to Equations		Statistic
	2 (A)	3 (B)	
FD/V (mcg/ml)	95.9 ± 46.3	103.6 ± 44.0	NSD
$F/V(10^{-5})$ (ml^{-1})	9.60 ± 4.65	10.4 ± 4.39	"
k_{abs} (h^{-1})	0.89 ± 0.42	1.35 ± 1.18	"
$t_{1/2\ abs}$ (h)	1.12 ± 0.97	1.13 ± 1.07	"
K_M (mcg/ml)	114.4 ± 11.9	93.4 ± 42.6	"
V_M (mcg/ml.h)	18.7 ± 6.09	9.57 ± 5.36	"
V_M/K_M (h^{-1})	0.17 ± 0.05	0.14 ± 0.11	"
k' (h^{-1})	-	0.02 ± 0.03	-
$t_{1/2\ el}$ (h)	4.50 ± 1.37	5.30 ± 2.62	NSD
r^2	0.989 ± 0.009	0.984 ± 0.02	"

Comparison of pharmacokinetic parameter values following 0.5 and 1.0 gm probenecid multiple doses predicted by Equations 1, 2 and 3 are given in Table LXXXVIII. These parameters were compared for differences due to dose size and dose order (the value predicted from the first dose versus the value predicted from the last dose).

Dose Normalized AUC^{o}_{t} was significantly greater from 1.0 gm dose, than the 0.5 gm dose. Dose normalized C_{max}, T_{max}, F/V, k_a, K_M, V_M, k', $t_{\frac{1}{2}el}$ and r^2 values did not show a significant difference due to dose size or dose order.

However, the absorption $t_{\frac{1}{2}}$ obtained from analysis of final 0.5 gm dose by Equation 3 was significantly greater than the values obtained from the analysis of initial 0.5 and 1.0 gm doses by the three equations.

The ratio V_M/K_M obtained from the analysis of initial 0.5 and 1.0 gm dose was significantly greater than the value predicted by Equation 3 from the initial 0.5 gm dose.

Following the analysis of initial and final dose data according to Equation 2, it became evident that the parameters that gave the best description of the data, particularly k_a and K_M were not constant and appeared to be effected by dose order. Mean parameters obtained from analysis of initial and final, 0.5 and 1.0 gm data according to Equation 2 are given in Table LXXXIX.

The mean k_a value from the initial 0.5 gm dose appeared to be 3.5-fold greater than the mean k_a value from the final 0.5 gm dose.

Table LXXXVIII
Comparison of pharmacokinetic parameter values (±1 SD.) following 0.5 and 1.0 gm probenecid tablet multiple doses.

Parameter		Value 0.5 gm (A)	Value 1.0 gm (B)	Statistics
$AUC/D(10^{-4})$ (h/ml)	(I)[1]	4.43 ± 1.8	6.50 ± 1.32	B>A[2]
$C_{max}/D(10^{-5})$ (ml^{-1})	(I)	7.08 ± 3.36	7.91 ± 1.87	NSD
	(F)[3]	18.7 ± 6.47	20.3 ± 4.93	"
T_{max} (h)	(I)	2.5 ± 1.0	2.5 ± 1.0	"
	(F)	3.5 ± 1.0	2.5 ± 1.0	"
$F/V(10^{-5})$ (ml^{-1})	(I) (i)[4]	9.98 ± 5.45	10.4 ± 3.79	"
	(ii)[5]	8.32 ± 2.35	8.79 ± 2.34	"
	(iii)[6]	9.56 ± 4.47	9.99 ± 3.95	"
	(F)(ii)	12.3 ± 1.93	9.60 ± 4.65	"
	(iii)	16.2 ± 7.99	10.4 ± 4.39	"
k_{abs} (h^{-1})	(I) (i)	1.24 ± 0.45	1.22 ± 0.84	"
	(ii)	1.46 ± 0.7	1.42 ± 0.83	"
	(iii)	1.11 ± 0.17	1.28 ± 0.89	"
	(F)(ii)	0.45 ± 0.26	0.89 ± 0.42	"
	(iii)	0.34 ± 0.28	1.35 ± 1.18	"
$t_{1/2\ abs}$ (h)	(I) (i)	0.61 ± 0.17	0.76 ± 0.38	A(F)(iii)>A(I)(i),
	(ii)	0.59 ± 0.36	0.59 ± 0.26	(ii),(iii);
	(iii)	0.64 ± 0.11	0.72 ± 0.36	A(F)(iii)>B(I)(i),
	(F)(ii)	2.05 ± 1.31	1.12 ± 0.97	(ii),(iii).
	(iii)	2.83 ± 1.34	1.13 ± 1.07	
K_M (mcg/ml)	(I)(ii)	82.5 ± 30.3	82.9 ± 30.2	NSD
	(iii)	101.8 ± 33.9	98.4 ± 37.9	"
	(F)(ii)	130.0 ± 0.0	114.4 ± 11.9	"
	(iii)	126.1 ± 7.85	93.4 ± 42.6	"

Table LXXXVIII
(continued)

Parameter		Value 0.5 gm (A)	Value 1.0 gm (B)	Statistic
V_M (mcg/ml.h)	(I)(ii)	17.8 ± 6.78	17.6 ± 6.99	NSD
	(iii)	6.64 ± 9.18	8.55 ± 11.1	"
	(F)(ii)	19.1 ± 3.23	18.7 ± 6.09	"
	(iii)	12.2 ± 8.51	9.57 ± 5.36	"
V_M/K_M (h-1)	(I)(ii)	0.22 ± 0.08	0.22 ± 0.09	A(I)(ii)>A(I)(iii)
	(iii)	0.06 ± 0.07	0.11 ± 0.14	B(I)(ii)>A(I)(iii)
	(F)(ii)	0.15 ± 0.03	0.17 ± 0.05	A(I)(iii)>A(F)(iii)
	(iii)	0.09 ± 0.07	0.14 ± 0.11	B(F)(iii)
k' (h)	(I)(iii)	0.16 ± 0.06	0.08 ± 0.06	
	(F)(iii)	0.05 ± 0.04	0.02 ± 0.03	
$t_{1/2\ el}$ (h)	(I) (i)	3.4 ± 0.78	5.01 ± 2.17	NSD
	(ii)	3.36 ± 0.89	3.5 ± 1.04	"
	(iii)	3.33 ± 0.82	4.32 ± 1.65	"
	(F)(ii)	4.80 ± 0.79	4.50 ± 1.37	"
	(iii)	4.76 ± 0.95	5.30 ± 2.62	"
r^2	(I) (i)	0.989 ± 0.007	0.975 ± 0.012	"
	(ii)	0.975 ± 0.024	0.973 ± 0.012	"
	(iii)	0.989 ± 0.008	0.967 ± 0.028	"
	(F)(ii)	0.967 ± 0.05	0.989 ± 0.009	"
	(iii)	0.964 ± 0.06	0.984 ± 0.02	"

[1]parameters after the initial dose.

[2]$0.05<p<0.1$

[3]parameters after the final dose.

[4]first-order absorption and elimination model.

[5]first-order absorption and saturable elimination model.

[6]first-order absorption and parallel first-order and saturable elimination model.

Table LXXXVII
Some of the mean (±SD.) pharmacokinetic parameter values obtained from the analysis of initial and final, 0.5 and 1.0 gm probenecid tablet dose data according to Equation 2.

	FD/V	k_a	K_M	V_M	V_M/K_M
0.5 gm Dose					
initial	41.6±11.8	1.46±0.70	82.5±30.3	17.8±30.3	0.22±0.08
final	61.3±9.73	0.45±0.26	130.0±0	19.1±3.23	0.15±0.03
1.0 gm Dose					
initial	87.9±23.5	1.42±0.83	82.9±30.2	17.5±6.99	0.22±0.09
final	95.9±46.3	0.89±0.42	114.4±11.9	18.7±6.09	0.17±0.05

The mean k_a value from the initial 1.0 gm dose appeared to be 1.6-fold greater than the mean k_a value from the final 1.0 gm dose.

However, the mean K_M values obtained from the analysis of the final 0.5 and 1.0 gm doses showed a similar increase of 1.6 and 1.4-fold, respectively. As the V_M values appeared to be relatively constant for both doses, the increases in K_M values were associated with somewhat smaller V_M/K_M values following the lower dose. These apparent differences could not be verified with the statistical model used to analyze the data. Therefore the parameters k_a and V_M/K_M were further examined by means of analysis of variance according to a two-factor completely randomized design.

The model of this analysis design is as follows:

$$X_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

$i = 1, 2, \ldots a$

$j = 1, 2, \ldots b$

$k = 1, 2, \ldots n$

where x_{ijk} represents a typical observation, μ is a constant, and α and β represent effects due to factors A and B, respectively. An effect due to interaction of factors A and B is represented by $(\alpha\beta)$ while e_{ijk} represents the experimental error. Main assumptions involved are that observations in each cell are from a normally distributed population. The populations are all independent and have the same variance (66, 67, 68, 71).

For the analysis of k_a, dose size and dose order were elected to be the two factors, each with a level of two. The results of this analysis are summarixed in Table XC. No significant interaction could be detected between the two factors. There was no significant difference in the values of k_a when grouped by dose. However, significant differences in the k_a values became apparent when these values were grouped by dose order. The k_a values following the last dose were significantly smaller than the first dose.

The ratio V_M/K_M was analyzed in a similar fashion, with dose size and dose order as the two factors. Again, each factor had levels of two. The results of this analysis indicated that there were no significant interactions between dose size and dose order. There were no significant differences in the values of V_M/K_M among doses at 95% confidence level. However differences in V_M/K_M between the first and the last dose were significant at 90% confidence level.

The equivocal results obtained for V_M/K_M prompted further analysis of the parameter V_M/K_M. As the interactions between the main factors were negligible, analysis of this parameter according to a simple model assuming additivity between four factors appeared to be appropriate. The four factors were week of the study, subjects, dose size, and dose order. For this analysis of variance the program SPSS (68) was utilized (Appendix VIII). The results of this statistical analysis are presented in Table XCI. From this table, one can conclude that there is a significant difference in the V_M/K_M

Table XC
ANOVA for k_a following 0.5 and 1.0 gm probenecid tablet doses.

Source	ss	df	MS	F_{ratio}	$R_{(0.95)}$
Dose (A)	0.15	1	0.15	0.42	4.75
Order (B)	2.34	1	2.34	6.5	
Dose-Order(AB)	0.24	1	0.24	0.67	
Treatment	2.73	3			
Residual	4.26	12	0.36		
Total	6.99	15			

Table XCI
ANOVA for V_M/K_M following 0.5 and 1.0 gm probenecid tablet doses.

Source	ss	df	MS	F_{ratio}	$F_{(0.95)}$
Main Effects	0.049	6	0.008	2.257	
Week	0.012	1	0.012	3.224	
Subject	0.008	3	0.003	0.732	3.86
Order	0.028	1	0.028	7.828	5.12
Dose	0.001	1	0.001	0.295	
Explained	0.049	6	0.008	2.257	
Residual	0.032	9	0.004		
Total	0.081	15	0.005		

ratios observed following the initial dose and those obtained after repeated doses.

The parameters obtained from the first and last multiple doses were then used to predict plasma probenecid concentrations starting from the second dose until the last detectable probenecid concentration in plasma. Tables XCII-XCIX indicate actual and predicted probenecid concentrations with parameters FD/V, k_a, K_M and V_M generated from the initial and final doses according to Equation 2, for 0.5 and 1.0 gm probenecid doses. The printout of program DGEAR used to obtained predicted probenecid concentrations in plasma is in Appendix IX.

Figures 30-37 describe actual probenecid data points and the plasma probenecid levels predicted by Equation 2 using parameters from the initial and final doses. Plasma levels of probenecid following the final dose appeared to be described more satisfactorily by multiple dose parameters. This was further examined for significance by sign tests. Plasma probenecid concentrations predicted by multiple dose parameters were significantly closer to the actual values than the values predicted by the single-dose parameters ($p<0.01$) (72).

C. Study III - Probenecid 1.0 gm tablet, multiple dose study

Study III was designed as an extension of Study II. Two subjects received nine - 1.0 gm tablet doses of probenecid every six hours starting 24 h after an initial dose. In study II subjects had

Table XCII
Actual and computer predicted probenecid plasma levels by Equation 2 with parameters from initial dose (I) and from final dose (F) following multiple 0.5 gm probenecid tablet doses to Subject #1.

Dose	Time (h)	Plasma probenecid (mcg/ml) Actual	(I)	(F)
2	48.0	-	-	-
	49.0	*[1]	42.4	19.1
	50.0	*	42.9	31.0
	51.0	*	39.6	37.9
	52.0	*	35.9	41.5
	53.0	*	32.4	42.7
	54.0	29.3	29.2	42.3
3	54.0	29.3[2]	29.3	29.3
	55.0	*	70.1	45.7
	56.0	*	69.5	55.4
	57.0	*	64.9	60.5
	58.0	*	60.2	62.4
	59.0	*	55.6	62.2
	60.0	56.8	51.1	60.4
4	60.0	56.8	56.8	56.8
	61.0	*	96.7	71.3
	62.0	*	95.5	79.4
	63.0	*	90.3	83.1
	64.0	*	84.7	83.8
	65.0	*	79.3	82.3
	66.0	59.4	74.0	79.5
5	66.0	59.4	59.4	59.4
	67.0	*	99.2	73.7
	68.0	*	97.9	81.7
	69.0	*	92.6	85.3
	70.0	*	87.1	85.9
	71.0	*	81.6	84.3
	72.0	97.9	76.3	81.3
6	72.0	97.9	97.9	97.9
	74.0	109.8	135.0	116.6
	76.0	125.5	122.9	117.7
	80.0	96.7	98.7	99.2
	84.0	72.9	76.5	74.1
	88.0	50.9	56.7	51.6
	96.0	20.4	26.2	21.3
	108.0	4.78	5.22	4.51

[1] Plasma samples were not taken at these time points.
[2] Each dosing interval is described with curves starting from the actual probenecid concentrations obtained at the beginning of that interval.

Table XCII
Actual and computer predicted probenecid plasma levels by Equation 2 with parameters from initial dose (I) and from final dose (F) following multiple 0.5 gm probenecid tablet doses to Subject #2.

Dose	Time (h)	Plasma probenecid (mcg/ml) Actual	(I)	(F)
2	48.0	-	-	-
	49.0	*	38.8	9.63
	50.0	*	41.5	16.7
	51.0	*	37.8	21.8
	52.0	*	33.3	25.3
	53.0	*	29.1	27.7
	54.0	19.7	25.2	28.9
3	54.0	19.7	19.7	19.7
	55.0	*	56.7	27.5
	56.0	*	58.0	33.1
	57.0	*	53.1	36.9
	58.0	*	47.4	39.3
	59.0	*	41.9	40.6
	60.0	46.9	36.8	41.0
4	60.0	46.9	46.9	46.9
	61.0	*	82.0	52.8
	62.0	*	81.9	56.7
	63.0	*	75.6	58.9
	64.0	*	68.5	60.0
	65.0	*	61.6	60.0
	66.0	64.4	55.1	59.3
5	66.0	64.4	64.4	64.4
	67.0	*	98.7	69.3
	68.0	*	97.9	72.4
	69.0	*	90.9	73.8
	70.0	*	82.9	74.1
	71.0	*	75.3	73.4
	72.0	90.7	67.9	71.9
6	72.0	90.7	90.7	90.7
	74.0	95.1	122.3	96.4
	76.0	117.3	105.6	96.2
	80.0	93.1	72.9	85.8
	84.0	64.7	46.5	69.9
	88.0	40.9	27.3	53.5
	96.0	20.1	7.52	27.3
	108.0	4.68	0.83	7.86

Table XCIV
Actual and computer predicted probenecid plasma levels by Equation 2 with parameters from initial dose (I) and from final dose (F) following multiple 0.5 gm probenecid tablet doses to Subject #3.

Dose	Time (h)	Plasma probenecid (mcg/ml) Actual	(I)	(F)
2	48.0	-	-	-
	49.0	*	14.5	20.5
	50.0	*	18.9	30.1
	51.0	*	18.7	33.7
	52.0	*	16.5	34.1
	53.0	*	13.7	32.7
	54.0	14.2	10.9	30.5
3	54.0	14.2	14.2	14.2
	55.0	*	25.8	33.1
	56.0	*	28.2	41.6
	57.0	*	26.4	44.3
	58.0	*	22.8	43.8
	59.0	*	18.8	41.6
	60.0	24.0	14.9	38.6
4	60.0	24.0	24.0	24.0
	61.0	*	33.9	42.1
	62.0	*	35.2	49.8
	63.0	*	32.3	51.9
	64.0	*	27.8	50.8
	65.0	*	22.9	48.1
	66.0	26.6	18.3	44.5
5	66.0	26.6	26.6	26.6
	67.0	*	36.2	44.4
	68.0	*	37.1	51.9
	69.0	*	33.9	53.9
	70.0	*	29.2	52.7
	71.0	*	24.1	49.7
	72.0	43.3	19.3	46.1
6	72.0	43.3	43.3	43.3
	74.0	32.2	49.9	66.3
	76.0	66.5	39.1	65.1
	80.0	61.9	16.1	47.3
	84.0	29.5	5.0	30.5
	88.0	18.5	1.37	18.6
	96.0	6.0	0.10	6.32
	108.0	1.92	-	1.14

Table XCV
Actual and computer predicted probenecid plasma levels by Equation 2 with parameters from initial dose (I) and from final dose (F) following multiple 0.5 gm probenecid tablet doses to Subject #4.

Dose	Time (h)	Plasma probenecid (mcg/ml) Actual	(I)	(F)
2	48.0	-	-	-
	49.0	*	17.8	27.5
	50.0	*	20.2	36.6
	51.0	*	18.7	37.8
	52.0	*	16.3	35.6
	53.0	*	13.9	32.1
	54.0	12.3	11.7	28.3
3	54.0	12.3	12.3	12.3
	55.0	*	28.3	38.2
	56.0	*	29.5	46.2
	57.0	*	26.8	46.5
	58.0	*	23.4	43.4
	59.0	*	20.0	39.1
	60.0	18.0	17.1	34.6
4	60.0	18.0	18.0	18.0
	61.0	*	33.4	43.3
	62.0	*	33.9	50.8
	63.0	*	30.8	50.7
	64.0	*	26.9	47.2
	65.0	*	23.1	42.6
	66.0	24.4	19.7	37.7
5	66.0	24.4	24.4	24.4
	67.0	*	39.1	49.0
	68.0	*	39.1	56.1
	69.0	*	35.3	55.4
	70.0	*	30.9	51.6
	71.0	*	26.6	46.5
	72.0	35.5	22.8	41.3
6	72.0	35.5	35.5	35.5
	74.0	65.1	48.1	65.3
	76.0	59.4	38.1	59.3
	80.0	36.9	20.8	36.7
	84.0	18.9	10.7	20.3
	88.0	11.6	5.26	10.7
	96.0	3.61	1.21	2.68

Table XCVI
Actual and computer predicted probenecid plasma levels by Equation 2 with parameters from initial dose (I) and from final dose (F) following multiple 1.0 gm probenecid tablet doses to Subject #1.

Dose	Time (h)	Plasma probenecid (mcg/ml) Actual	(I)	(F)
2	48.0	-	-	-
	49.0	*	57.3	28.5
	50.0	*	57.2	35.1
	51.0	*	53.2	35.1
	52.0	*	49.1	33.2
	53.0	*	45.1	30.8
	54.0	35.4	41.3	28.4
3	54.0	35.4	35.4	35.4
	55.0	*	91.1	61.7
	56.0	*	90.1	66.7
	57.0	*	85.1	65.1
	58.0	*	79.9	61.8
	59.0	*	74.9	57.9
	60.0	51.9	69.9	54.1
4	60.0	51.9	51.9	51.9
	61.0	*	107.3	77.7
	62.0	*	105.9	81.9
	63.0	*	100.6	79.9
	64.0	*	95.1	76.0
	65.0	*	89.7	71.7
	66.0	98.1	84.4	67.3
5	66.0	98.1	98.1	98.1
	67.0	*	152.7	122.4
	68.0	*	150.6	125.5
	69.0	*	144.6	122.3
	70.0	*	138.4	117.2
	71.0	*	132.3	111.7
	72.0	126.8	126.2	106.1
6	72.0	126.8	126.8	126.8
	74.0	152.7	178.8	153.1
	76.0	147.7	165.9	143.7
	80.0	109.8	140.5	119.3
	84.0	103.1	116.3	96.7
	88.0	81.3	93.7	76.5
	96.0	37.4	54.4	44.2
	108.0	18.7	16.4	15.8
	120.0	4.62	3.19	4.84

Table XCVII
Actual and computer predicted probenecid plasma levels by Equation 2 with parameters from initial dose (I) and from final dose (F) following multiple 1.0 gm probenecid tablet doses to Subject #2.

Dose	Time (h)	Plasma probenecid (mcg/ml) Actual	(I)	(F)
2	48.0	-	-	-
	49.0	*	61.3	62.9
	50.0	*	73.1	78.2
	51.0	*	71.3	78.4
	52.0	*	65.9	73.9
	53.0	*	59.8	68.2
	54.0	50.8	53.7	62.3
3	54.0	50.8	50.8	50.8
	55.0	*	108.9	110.4
	56.0	*	118.6	123.6
	57.0	*	114.9	121.9
	58.0	*	107.7	115.6
	59.0	*	99.6	107.9
	60.0	*	91.6	100.1
4	60.0	91.7	91.7	91.7
	61.0	91.7	148.4	149.7
	62.0	*	157.1	161.7
	63.0	*	152.4	158.8
	64.0	*	144.1	151.4
	65.0	*	134.9	142.6
	66.0	122.4	125.7	133.6
5	66.0	122.4	122.4	122.4
	67.0	*	178.2	179.5
	68.0	*	186.3	190.7
	69.0	*	181.1	187.2
	70.0	*	172.2	178.9
	71.0	*	162.4	169.5
	72.0	175.5	152.5	159.7
6	72.0	175.5	175.5	175.5
	74.0	251.8	237.7	241.7
	76.0	248.6	221.9	228.1
	80.0	216.6	179.3	185.4
	84.0	138.6	139.2	145.3
	88.0	105.6	103.1	109.6
	96.0	83.7	46.7	54.3
	108.0	26.1	8.24	13.2
	120.0	9.97	1.07	2.55

Table XCVIII
Actual and computer predicted probenecid plasma levels by Equation 2 with parameters from initial dose (I) and from final dose (F) following multiple 1.0 gm probenecid tablet doses to Subject #3.

Dose	Time (h)	Plasma probenecid (mcg/ml) Actual	(I)	(F)
2	48.0	-	-	-
	49.0	*	65.8	33.6
	50.0	*	81.5	54.7
	51.0	*	79.3	67.3
	52.0	*	71.0	73.9
	53.0	*	61.0	76.3
	54.0	58.3	50.9	75.7
3	54.0	58.3	58.3	58.3
	55.0	*	118.7	85.4
	56.0	*	131.5	102.2
	57.0	*	126.7	111.4
	58.0	*	115.7	115.1
	59.0	*	102.8	114.9
	60.0	83.5	89.6	111.8
4	60.0	83.5	83.5	83.5
	61.0	*	142.6	108.9
	62.0	*	154.5	124.3
	63.0	*	148.9	132.4
	64.0	*	137.0	135.1
	65.0	*	123.2	133.9
	66.0	58.5	108.9	129.8
5	66.0	58.5	58.5	58.5
	67.0	*	118.9	85.6
	68.0	*	131.7	102.3
	69.0	*	126.9	111.6
	70.0	*	115.9	115.3
	71.0	*	102.9	115.1
	72.0	172.4	89.8	111.9
6	72.0	172.4	172.4	172.4
	74.0	167.9	238.7	206.1
	76.0	237.2	217.1	211.2
	80.0	157.7	151.1	181.8
	84.0	138.3	91.5	134.7
	88.0	75.5	45.3	88.6
	96.0	38.3	5.12	26.4
	100.0	14.5	0.1	2.19

Table XCIX
Actual and computer predicted probenecid plasma levels by Equation 2 with parameters from initial dose (I) and from final dose (F) following multiple 1.0 gm probenecid tablet doses to Subject #4.

Dose	Time (h)	Plasma probenecid (mcg/ml) Actual	(I)	(F)
2	48.0	-	-	-
	49.0	*	37.7	50.1
	50.0	*	50.2	63.2
	51.0	*	51.9	63.3
	52.0	*	49.1	59.0
	53.0	*	44.5	53.4
	54.0	31.8	39.5	47.7
3	54.0	31.8	31.8	31.8
	55.0	*	66.1	78.9
	56.0	*	76.2	90.1
	57.0	*	75.9	88.5
	58.0	*	71.2	82.5
	59.0	*	64.8	75.3
	60.0	63.1	58.0	67.9
4	60.0	63.1	63.1	63.1
	61.0	*	95.0	108.2
	62.0	*	103.3	117.8
	63.0	*	101.4	114.8
	64.0	*	95.1	107.4
	65.0	*	87.1	98.8
	66.0	79.5	78.7	89.9
5	66.0	79.5	79.5	79.5
	67.0	*	110.5	123.8
	68.0	*	117.9	132.7
	69.0	*	115.3	128.9
	70.0	*	108.3	120.9
	71.0	*	99.6	111.7
	72.0	106.0	90.4	102.2
6	72.0	106.0	106.0	106.0
	74.0	168.1	142.1	157.1
	76.0	133.2	130.2	143.5
	80.0	100.5	90.2	102.7
	84.0	67.3	56.6	63.4
	88.0	43.8	32.8	42.6
	96.0	19.2	9.06	13.9
	108.0	2.96	1.04	2.06

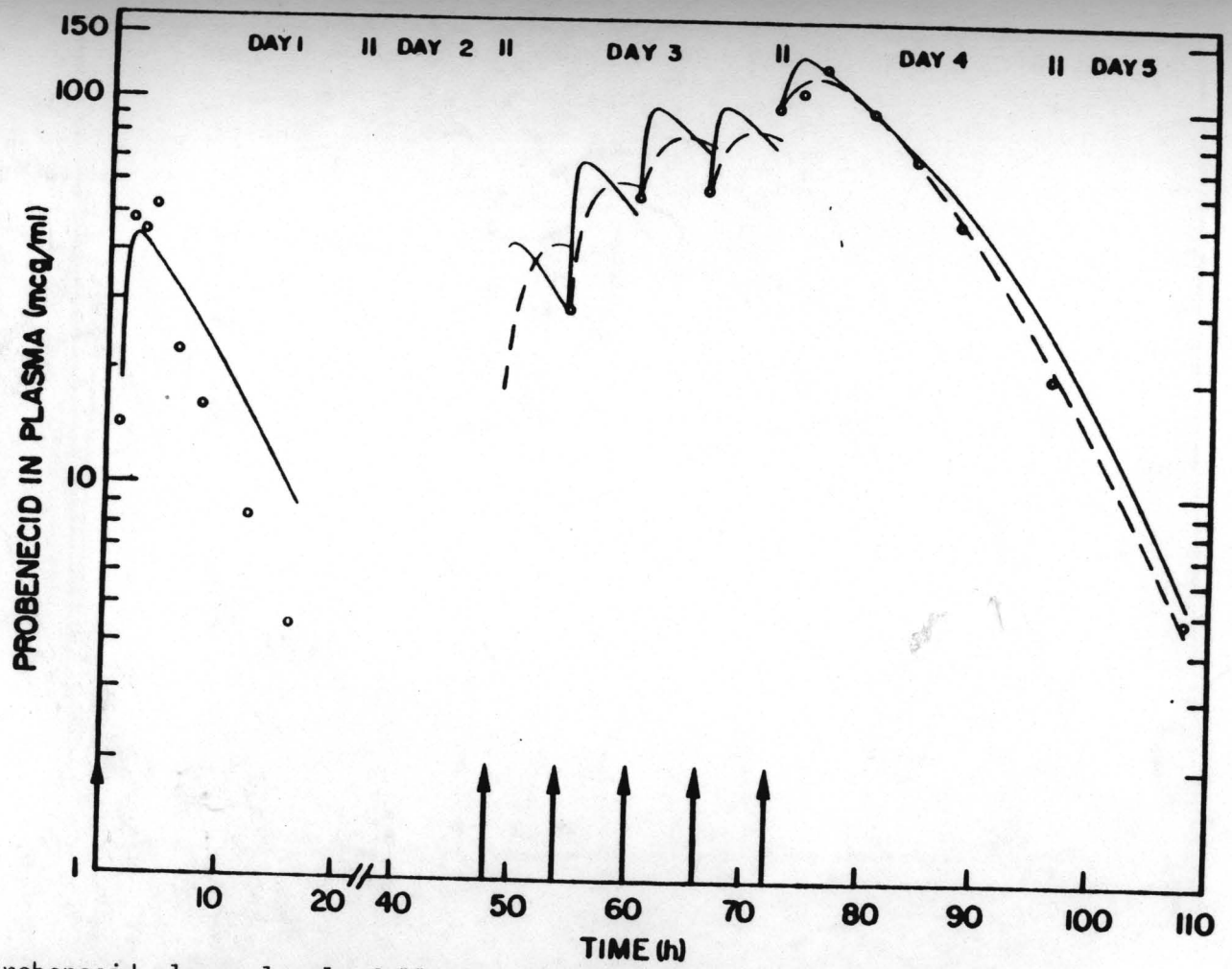


Figure 30: Probenecid plasma levels following 0.5 gm probenecid tablet multiple doses, subject #1, and plasma profiles generated by Equation 2 with parameters from the initial dose (-), and from the last dose (--).

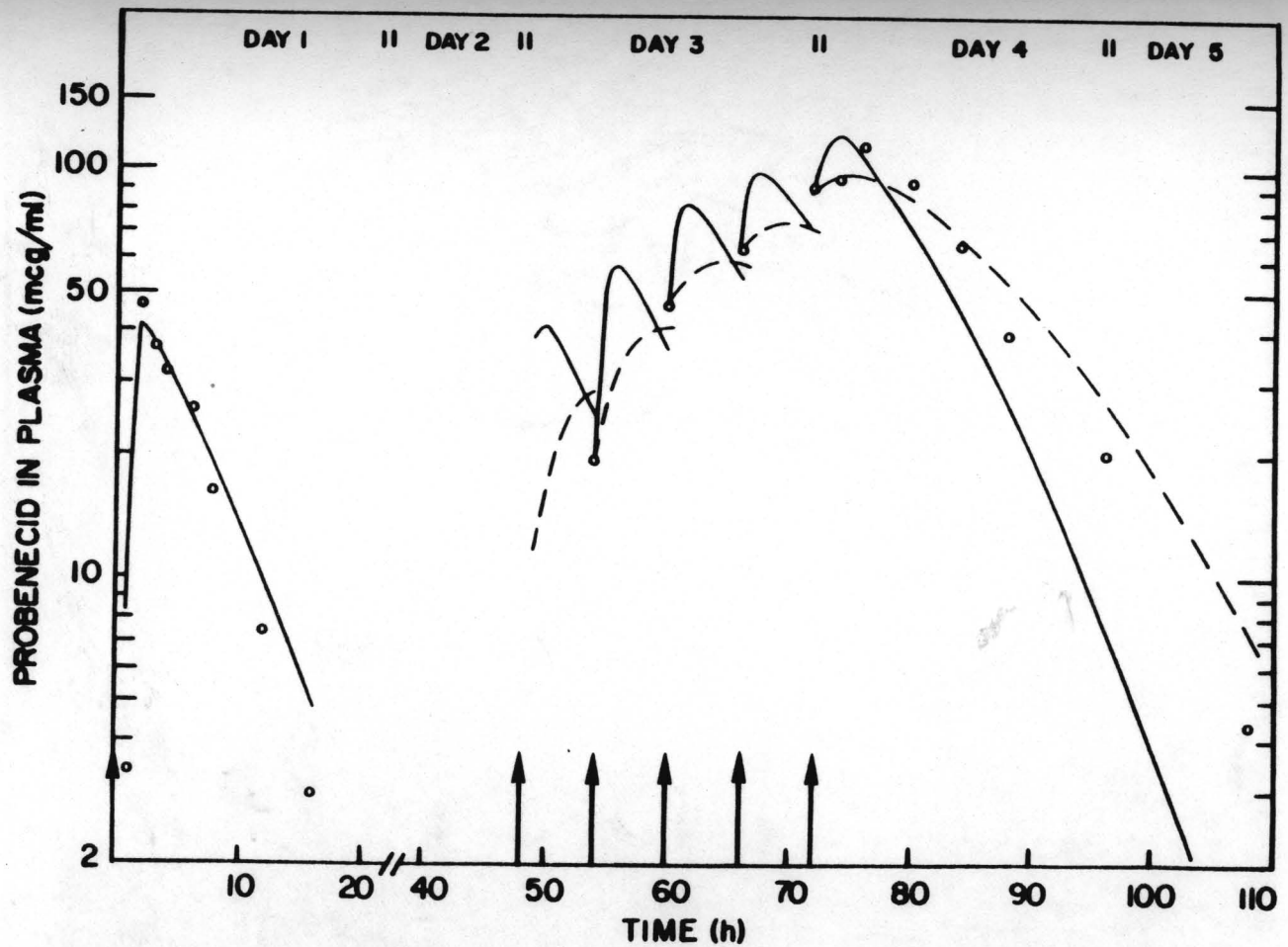


Figure 31: Probenecid plasma levels following 0.5 gm probenecid tablet multiple doses, subject #2.

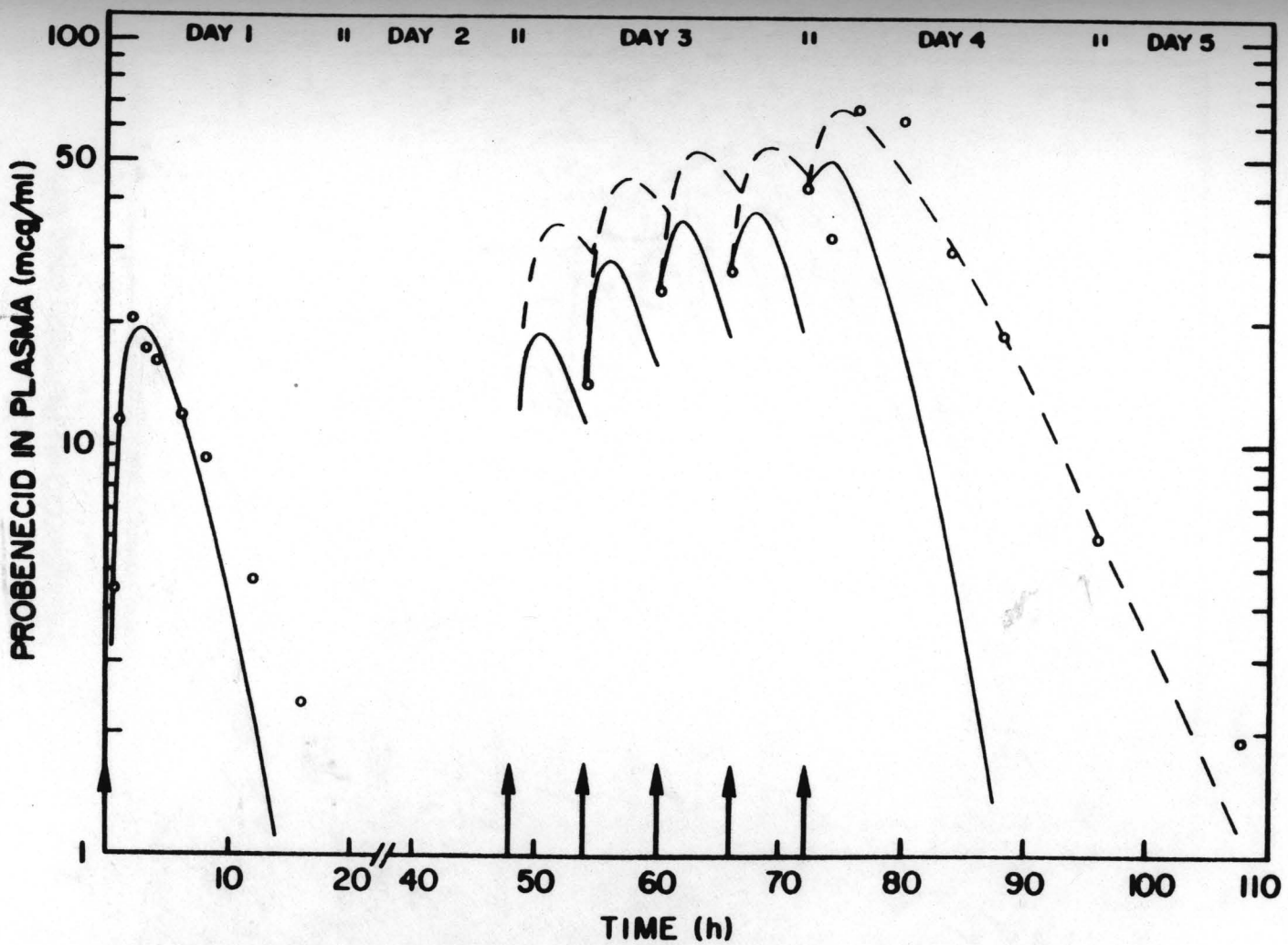


Figure 32: Probenecid plasma levels following 0.5 gm probenecid tablet multiple doses, subject #3.

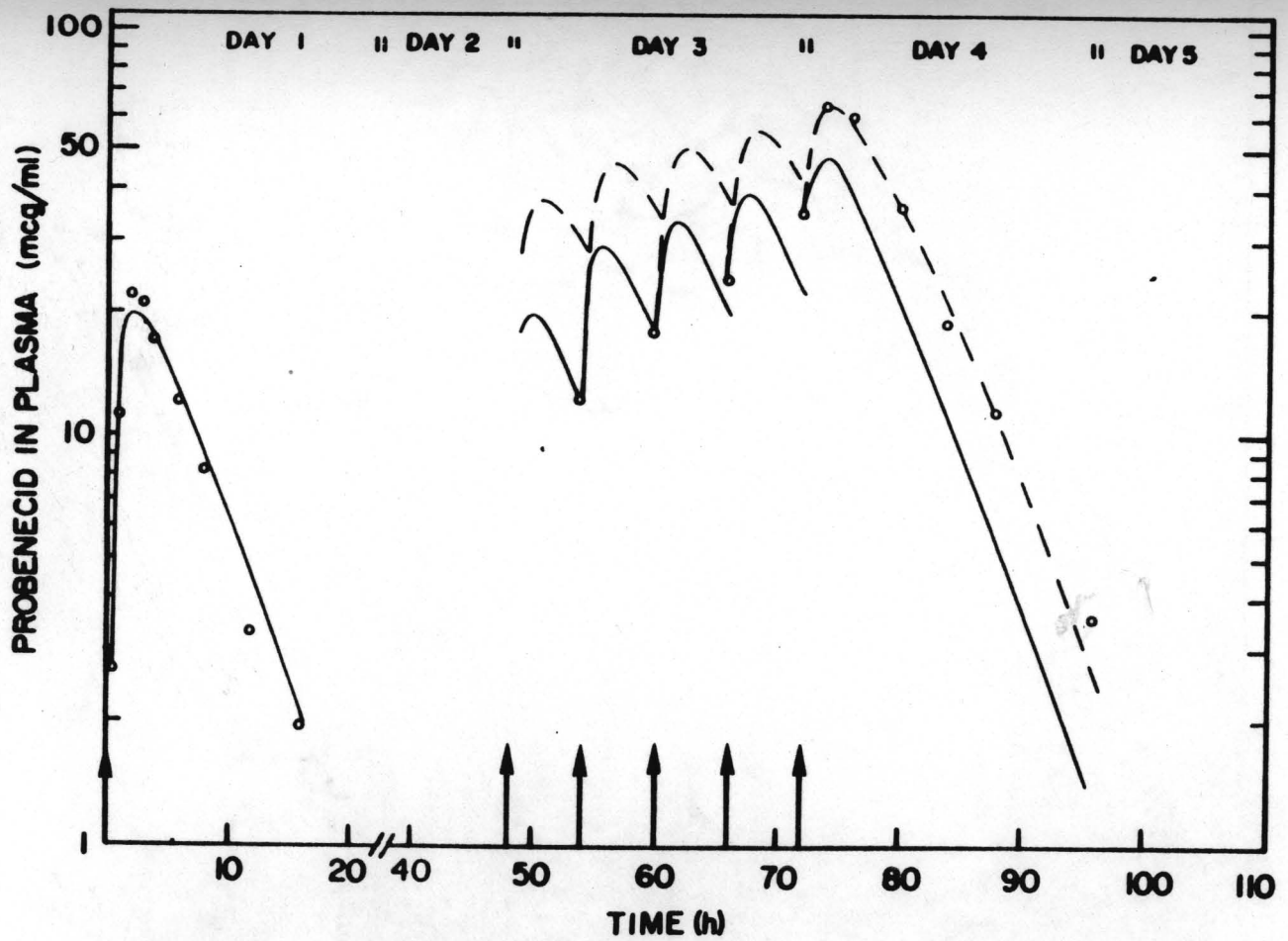


Figure 33: Probenecid plasma levels following 0.5 gm probenecid tablet multiple doses, subject #4.

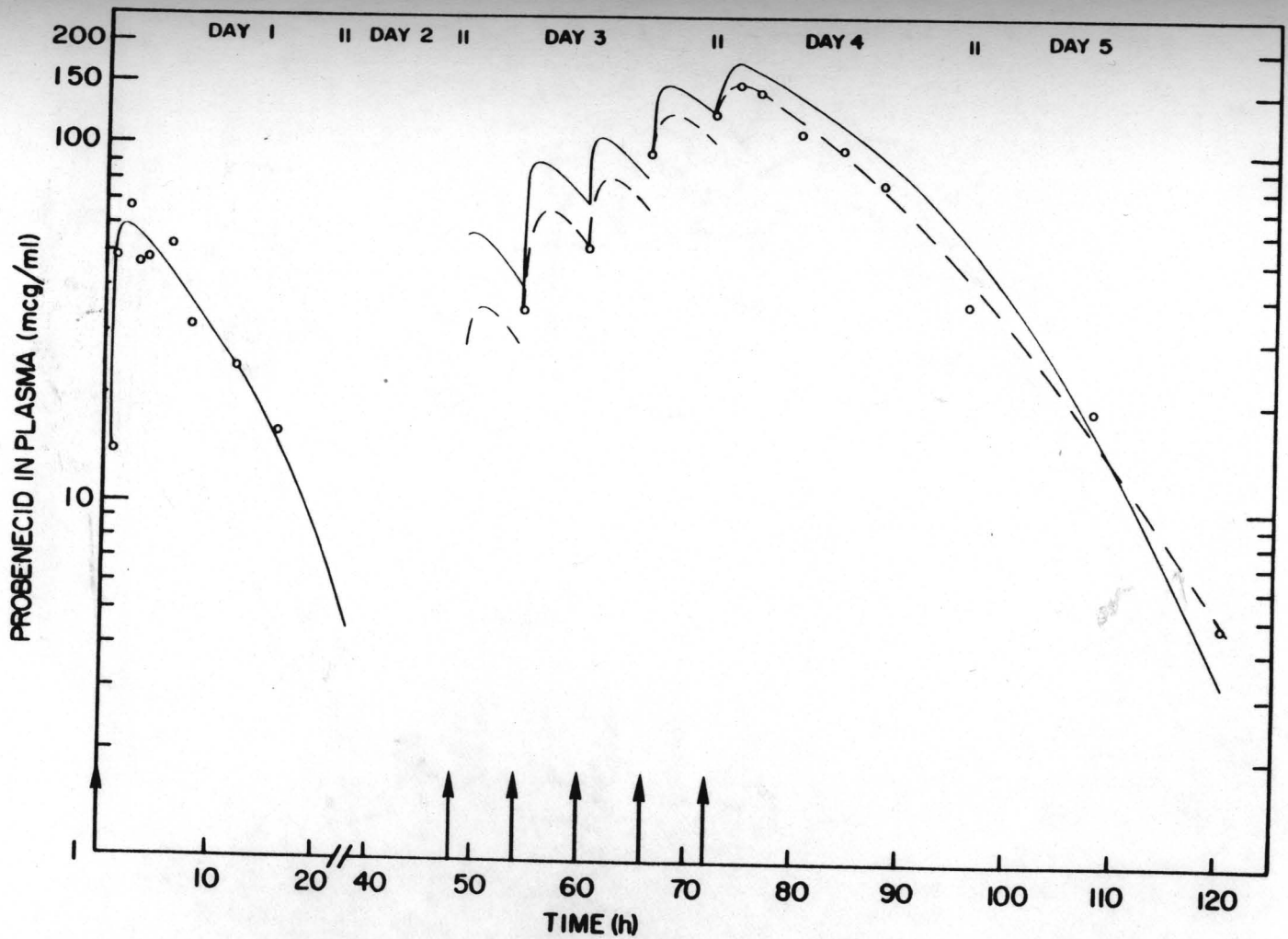


Figure 34: Probenecid plasma levels following 1.0 gm probenecid tablet multiple doses, subject #1.

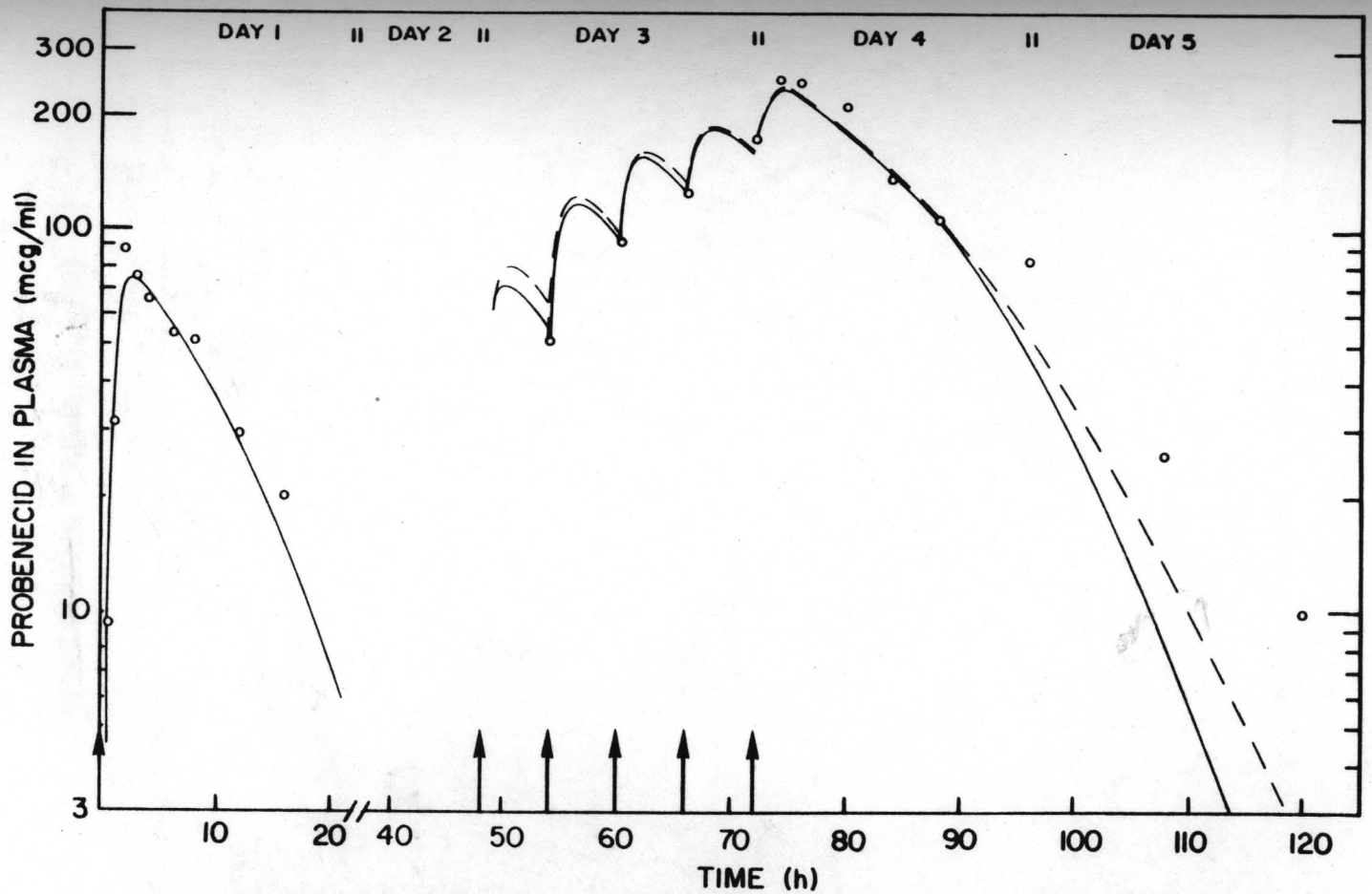


Figure 35: Probenecid plasma levels following 1.0 gm, probenecid tablet multiple doses, subject #2.

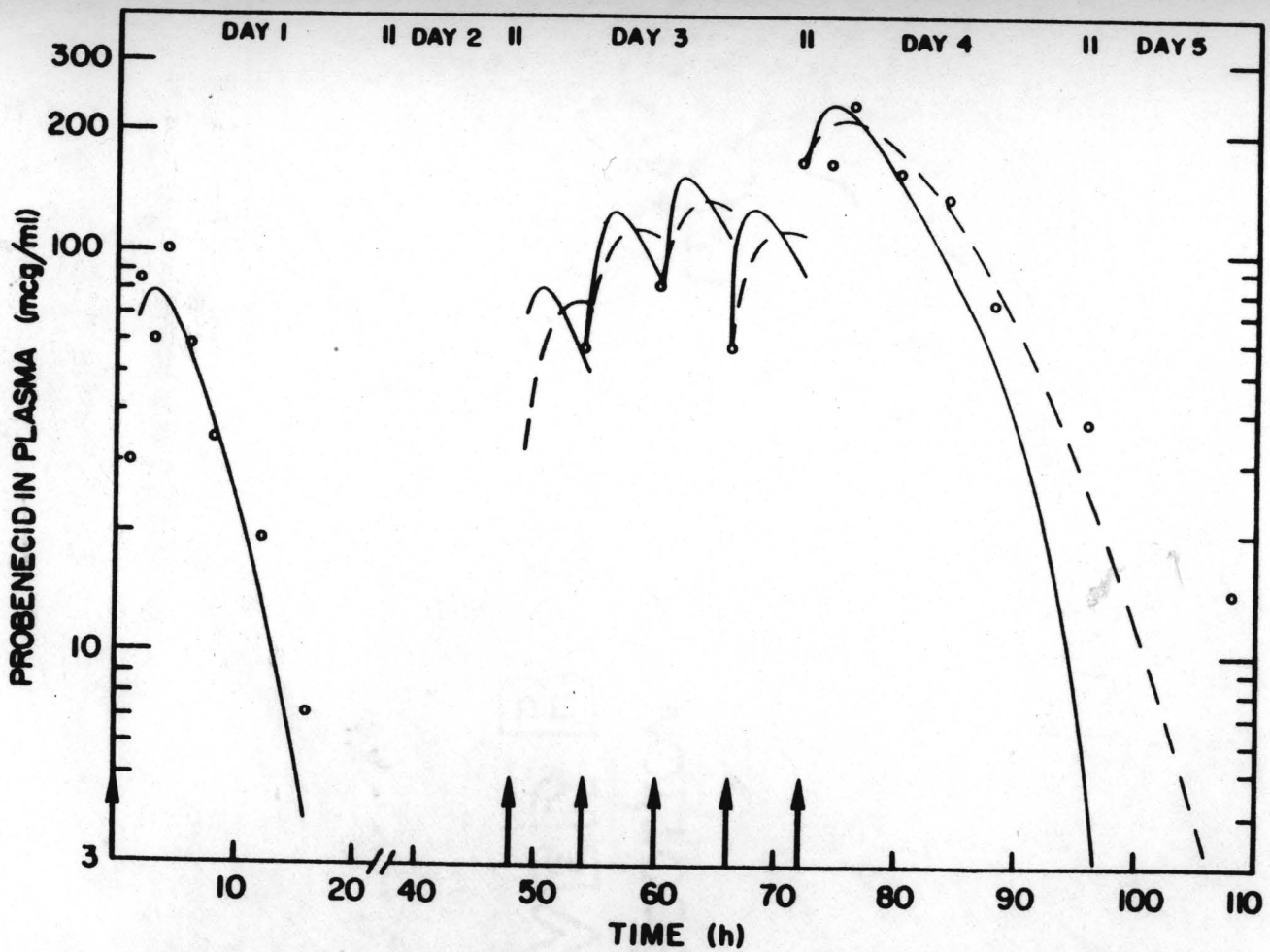


Figure 36: Probenecid plasma levels following 1.0 gm, probenecid tablet multiple doses, subject #3.

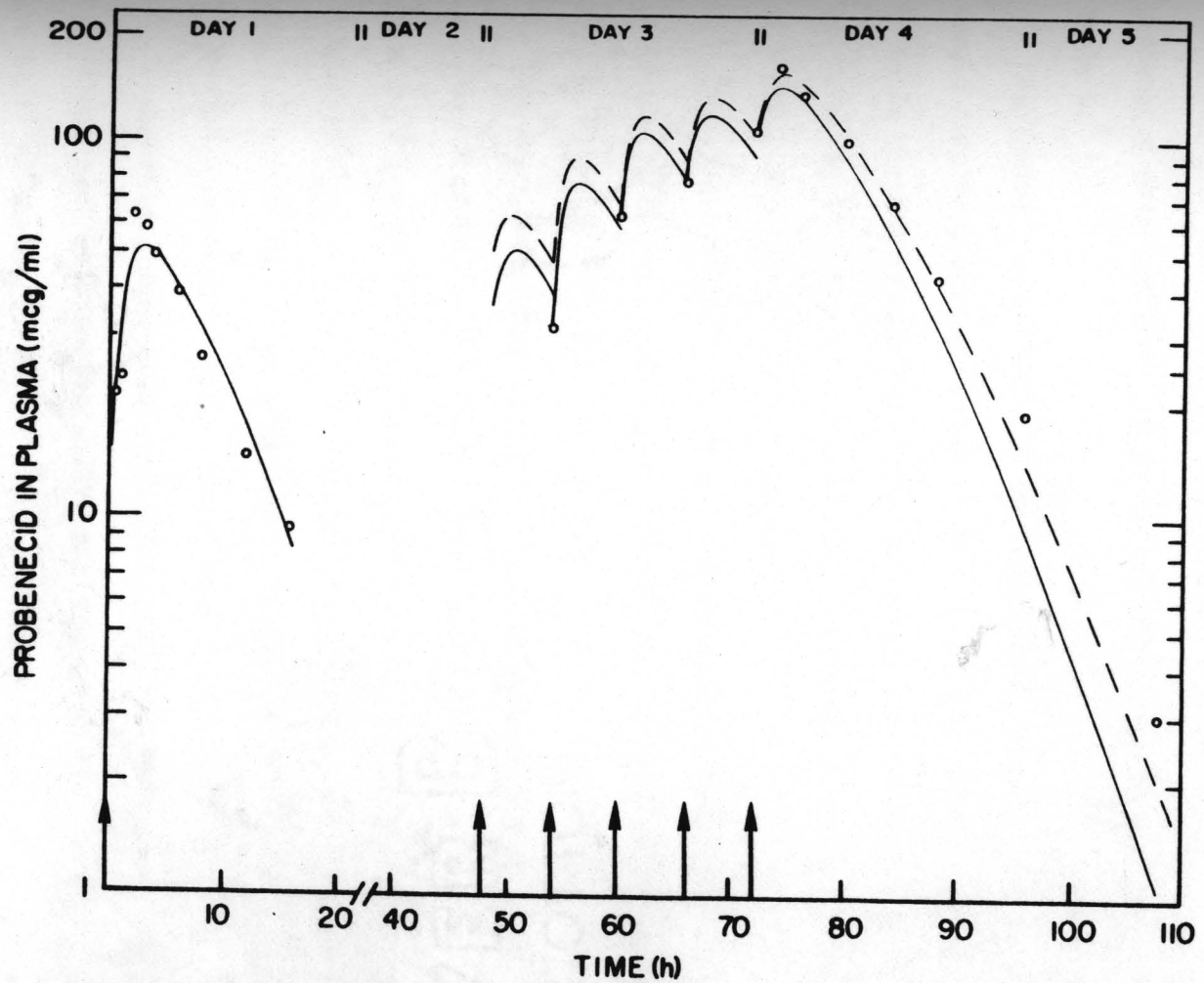


Figure 37: Probenecid plasma levels following 1.0 gm, probenecid tablet multiple doses, subject #4.

received five 0.5 or 1.0 gm tablet doses of probenecid every six hours starting 48 h after an initial dose. Plasma levels of probenecid obtained from 1.0 gm tablet multiple doses are given in Table C.

Following the initial 1.0 gm tablet dose, plasma probenecid levels reached a maximum value of 76.4 mcg/ml in one subject at 4h while maximum concentration in the other subject was 78.6 mcg/ml and occurred at 2 h postdose. The mean probenecid concentration 24 h after the first dose was 5.41 mcg/ml.

Individual model-independent pharmacokinetic parameter values obtained following the administration of initial 1.0 gm tablet dose are in Table CI.

The mean dose normalized $AUC^{0\rightarrow t}$ value was calculated by trapezoidal method as 6.91×10^{-4} h/ml and this is similar to the value of 6.5×10^{-4} h/ml obtained in Study II. The mean dose normalized C_{max} values in studies II and III were 7.91×10^{-5} ml^{-1} and 7.75×10^{-5} ml^{-1}. In addition to the values of these parameters, T_{max} values obtained in both studies appeared to be similar.

Probenecid plasma levels resulting from the initial 1.0 gm tablet doses of both subjects were analyzed according to Equation 1 and final parameter estimates were obtained by nonlinear regression analysis of data by the program NREG.

A summary of these final estimates following the initial 1.0 gm dose according to Equation 1 is given in Table CII.

Table C
Probenecid plasma levels following 1.0 gm probenecid tablet multiple doses.

Dose	Time (h)	Plasma probenecid (mcg/ml) Subject 5	Subject 6	$\bar{X}$	SD.	CV%
1	0.0	-	-	-	-	-
	0.5	-	63.5	31.8	44.9	141.2
	1.0	-	SL[1]	-	-	-
	2.0	22.3	78.6	50.5	39.8	78.9
	3.0	41.2	61.8	51.5	14.6	28.3
	4.0	76.4	57.5	66.9	13.4	19.9
	6.0	52.7	50.5	51.6	1.56	3.0
	8.0	43.7	28.5	36.1	10.7	29.8
	12.0	24.9	23.9	24.4	0.71	2.9
2	24.0	6.99	3.82	5.41	2.24	41.5
	26.0	73.4	54.9	64.2	13.1	20.4
3	30.0	*	*	*	*	*
4	36.0	97.8	79.9	88.9	12.7	14.2
	38.0	160.4	175.5	167.9	10.7	6.4
5	42.0	*	*	*	*	*
6	48.0	201.2	192.6	196.9	6.08	3.1
	50.0	334.5	239.9	287.2	66.9	23.3
7	54.0	*	*	*	*	*
8	60.0	270.9	151.7	211.3	84.3	39.9
	62.0	428.6	263.9	346.3	116.5	33.6
9	66.0	*	*	*	*	*
10	72.0	242.2	194.8	218.5	33.5	15.3
	72.5	285.3	272.1	278.7	9.33	3.35
	73.0	364.2	257.4	310.8	75.5	24.3
	74.0	227.3	252.9	240.1	18.1	7.53
	75.0	280.6	301.5	291.1	14.8	5.1
	76.0	306.8	271.2	289.0	25.2	8.71
	78.0	216.7	271.7	244.2	38.9	15.9
	79.0	270.5	205.8	238.2	45.7	19.2
	80.0	265.8	188.7	227.3	54.5	23.9
	81.0	201.9	193.4	197.7	6.01	3.04
	82.0	233.1	174.0	203.6	41.8	20.5
	84.0	143.6	163.7	153.7	14.2	9.3
	86.0	98.4	126.5	112.5	19.9	17.7
	88.0	80.5	117.2	98.9	25.9	26.3
	90.0	78.6	94.3	86.5	11.1	12.8
	96.0	46.4	59.3	52.9	9.12	17.3

[1]Sample broken in the centrifuge.

Table CI
Model-independent pharmacokinetic parameter values following first 1.0 gm probenecid tablet dose of the multiple dose study.

Parameter	Value				
	Subject		$\bar{X}$	SD.	CV%
	5	6			
$AUC^{0\rightarrow t}_{trap}$ (mcg.h/ml)	667.6	713.4	690.5	32.4	4.69
AUC/D(10^{-4}) (h/ml)	6.68	7.13	6.91	3.18	4.61
C_{max} (mcg/ml)	76.4	78.6	77.5	1.56	2.01
$C_{max}/D \cdot 10^{-5}$) (ml^{-1})	7.64	7.86	7.75	0.16	2.01
T_{max} (h)	4.0	2.0	3.0	1.41	47.1

Table CII
Individual pharmacokinetic parameter values following the first 1.0 gm probenecid tablet dose according to Equation 1.

Parameter	Value				
	Subject		$\bar{X}$	SD.	CV%
	5	6			
FD/V (mcg/ml)	86.9	90.6	88.8	2.62	2.94
k_a (h^{-1})	0.55	2.45	1.50	1.34	89.6
$t_{1/2}$ (h)	1.26	0.28	0.77	0.69	89.9
k_{el} (h^{-1})	0.12	0.13	0.13	0.007	5.7
$t_{1/2\ el}$ (h)	5.78	5.33	5.56	0.32	5.7
t_{lag} (h)	1.1	0.001	0.55	0.78	141.2
r^2	0.954	0.995	0.975	0.029	2.98

First-order absorption rate constant for one of the subjects was 4.5-fold greater than the value for the other subject. However, first-order elimination rate constants did not show this variability and were 0.12 and 0.13 h^{-1}, yielding elimination half-lives of 5.78 and 5.33 h. In Study II, following the initial 1.0 gm dose mean elimination $t_{\frac{1}{2}}$ by Equation 1 was predicted as 5.01 h.

Similar pharmacokinetic parameters were obtained from Study III and Study II by means of Equation 1. The plasma data from Study III were further analyzed according to Equations 2 and 3.

The final parameter estimates obtained following analysis of initial 1.0 gm plasma data by Equations 2 and 3 are presented in Tables CIII and CIV.

Individual actual and predicted plasma probenecid concentrations of the three equations are given in Tables CV and CVI.

From the results of the initial 1.0 gm dose, an accumulation factor according to first-order absorption and elimination model was calculated, and used to predict maximum steady state probenecid concentrations.

The observed and calculated probenecid C_{max} values after repeated dosing are given in Table CVII. The mean ratio of observed and predicted maximum probenecid concentrations in plasma by Equation 1 was 2.21. Again, observed maximum probenecid concentration in plasma could not be explained by the first-order absorption and elimination model. Subsequently, the plasma data

Table CIII
Individual pharmacokinetic parameter values following the first 1.0 gm probenecid tablet dose according to Equation 2.

Parameter	Value				
	Subject		$\bar{X}$	SD.	CV%
	5	6			
FD/V (mcg/ml)	104.9	85.9	95.4	13.4	14.1
k_a (h^{-1})	0.44	3.41	1.93	2.1	109.1
$t_{1/2\ abs}$ (h)	1.58	0.20	0.89	0.98	109.6
K_M (mcg/ml)	103.6	129.9	116.8	18.6	15.9
V_M (mcg/ml.h)	25.0	21.5	23.3	2.47	10.6
V_M/K_M (h^{-1})	0.24	0.17	0.21	0.05	24.1
$t_{1/2\ el}$ (h)	2.89	4.08	3.49	0.84	24.1
t_{lag} (h)	1.1	0.1	0.60	0.71	117.9
r^2	0.957	0.994	0.976	0.026	2.68

Table CIV
Individual pharmacokinetic parameter values following the first 1.0 gm probenecid tablet dose according to Equation 3.

Parameter	Value				
	Subject		$\bar{X}$	SD.	CV%
	5	6			
FD/V (mcg/ml)	87.5	88.9	88.2	0.7	0.79
k_a (h^{-1})	0.54	3.13	1.84	1.83	99.8
$t_{1/2\ abs}$ (h)	1.28	0.22	0.75	0.75	99.9
K_M (mcg/ml)	69.7	126.6	98.2	40.2	40.9
V_M (mcg/ml.h)	10.0	3.57	6.79	4.55	67.0
V_M/K_M (h^{-1})	0.14	0.03	0.09	0.08	91.5
k' (h^{-1})	0.05	0.10	0.08	0.04	47.1
$t_{12/\ el}$ (h)	3.65	5.33	4.49	1.19	26.5
t_{lag} (h)	1.1	0.10	0.60	0.71	117.9
r^2	0.953	0.994	0.974	0.029	2.98

Table CV
Actual and computer predicted probenecid plasma levels following the first 1.0 gm probenecid tablet dose of the multiple dose study according to Equations 1, 2 and 3 for Subject #5.

Time (h)	Plasma probenecid (mcg/ml)			
	Actual	Predicted by equations		
		1	2	3
0.0	-	0	0	0
0.5	-	0	0	0
1.0	-	0	0	0
2.0	22.3	31.9	31.3	31.2
3.0	41.2	49.4	49.4	47.9
4.0	76.4	56.1	56.9	54.3
6.0	52.7	54.7	56.2	52.5
8.0	43.7	46.8	46.7	43.8
12.0	24.9	30.6	25.4	25.9
24.0	6.99	7.59	1.91	3.35

Table CVI
Actual and computer predicted probenecid plasma levels following the first 1.0 gm probenecid tablet dose of the multiple dose study, according to Equations 1, 2 and 3 for Subject #6.

Time (h)	Plasma probenecid (mcg/ml)			
	Actual	Predicted by equations		
		1	2	3
0.0	-	0	0	0
0.5	63.5	61.2	62.0	61.6
1.0	ND	NP[1]	NP	NP
2.0	78.6	73.1	72.3	73.4
3.0	61.8	64.9	64.9	65.3
4.0	57.5	57.4	58.1	57.8
6.0	50.5	44.9	45.8	45.3
8.0	28.5	35.1	35.6	35.3
12.0	23.9	21.5	20.6	21.4
24.0	3.82	4.93	3.25	4.53

[1]Not predicted.

Table CVII
Actual and predicted maximum probenecid levels by Equation 1 following 1.0 gm probenecid tablet multiple doses.

	Value				
	Subject		$\bar{X}$	SD.	CV%
	5	6			
R	1.99	1.89	1.94	0.07	3.6
$C_{max, first}$ (mcg/ml)	76.4	78.6	77.5	1.56	2.0
$R.C_{max, first}$ (mcg/ml)	152.0	148.5	150.3	2.47	1.65
$C_{max, final}$ (mcg/ml)	364.2	301.5	332.9	44.3	13.3
Ratio	2.39	2.03	2.21	0.25	11.5

following the final probenecid dose were analyzed according to Equations 2 and 3.

The final parameter estimates predicted by these Equations following the final 1.0 gm dose are given in Tables CVIII and CIX. Individual actual and predicted plasma probenecid concentrations are given in Tables CX and CXI.

The mean parameter values predicted by Equation 2 following the initial and final 1.0 gm doses are summarized in Table CXII.

The previous trend of decreasing values of k_a with repeated dosing was again apparent. Probenecid concentrations in plasma using values of FD/V, k_a, K_M and V_M calculated from the initial and final 1.0 gm doses were predicted using the program DGEAR. The actual concentrations and those predicted by the two sets of parameters are given in Tables CXII and CXIV. The program DGEAR has also provided probenecid plasma levels during intermediate dosage intervals. Figures 38 and 39 depict the actual probenecid concentrations and those probenecid plasma levels predicted by Equation 2 with parameters from the initial and final 1.0 gm tablet doses.

Due to the small sample size, parameters predicted by the three equations were not statistically examined. However, a summary of the mean pharmacokinetic parameter values (±1SD.) are given in Table CXV.

Following the initial dose, parameters FD/V, k_a, $t_{\frac{1}{2}abs}$ appear to be similar by the three equations. The k_a value according to

Table CVIII
Individual pharmacokinetic parameter values following the final 1.0 gm probenecid tablet dose according to Equation 2.

Parameter	Value				
	Subject		$\bar{X}$	SD.	CV%
	5	6			
FD/V (mcg/ml)	120.5	134.6	127.6	9.97	7.82
k_a (h^{-1})	0.42	0.88	0.65	0.33	50.0
$t_{1/2\ abs}$ (h)	1.65	0.79	1.22	0.61	49.8
K_M (mcg/ml)	110.8	130.0	120.4	13.6	11.3
V_M (mcg/ml.h)	21.5	23.5	22.5	1.41	6.28
V_M/K_M (h^{-1})	0.19	0.18	0.19	0.01	3.82
$t_{1/2\ el}$ (h)	3.65	3.85	3.75	0.14	3.77
r^2	0.965	0.992	0.979	0.019	1.95

Table CIX
Individual pharmacokinetic parameter values following the final 1.0 gm probenecid tablet dose according to Equation 3.

Parameter	Value				
	Subject		$\bar{X}$	SD.	CV%
	5	6			
FD/V (mcg/ml)	166.8	89.3	128.1	54.8	42.8
k_a (h^{-1})	0.44	3.12	1.78	1.89	106.5
$t_{1/2\ abs}$ (h)	1.58	0.22	0.90	0.96	106.9
K_M (mcg/ml)	130.0	60.6	95.3	49.1	51.5
V_M (mcg/ml.h)	25.0	13.3	19.2	8.27	43.2
V_M/K_M (h^{-1})	0.19	0.22	0.21	0.02	10.3
k' (h^{-1})	0.02	0.001	0.01	0.01	127.9
$t_{1/2\ el}$ (h)	3.30	3.14	3.22	0.11	3.5
r^2	0.974	0.989	0.982	0.011	1.08

Table CX
Actual and computer predicted probenecid plasma levels following final 1.0 gm probenecid tablet dose according to Equations 2 and 3 for Subject #5.

Time (h)	Plasma probenecid (mcg/ml)		
	Actual	Predicted by equations	
		2	3
72.0	242.2	242.2	242.2
72.5	285.3	257.4	264.7
73.0	364.2	268.2	280.2
74.0	227.3	279.7	295.7
75.0	280.6	281.9	297.3
76.0	306.8	278.2	290.4
78.0	216.7	260.4	262.9
79.0	270.5	248.7	246.1
80.0	265.8	236.2	228.4
81.0	201.9	223.1	210.5
82.0	233.1	209.8	192.8
84.0	143.6	183.3	158.9
86.0	98.4	157.7	128.0
88.0	80.5	133.5	100.8
90.0	78.6	110.9	77.4
96.0	46.4	56.5	30.1

Table CXI
Actual and computer predicted probenecid plasma levels following final 1.0 gm probenecid tablet dose according to Equations 2 and 3 for Subject #6.

Time (h)	Plasma probenecid (mcg/ml)		
	Actual	Predicted by equations	
		2	3
72.0	194.8	194.8	194.8
72.5	272.1	235.6	260.3
73.0	257.4	258.6	269.4
74.0	252.9	257.1	261.9
75.0	301.5	272.5	250.9
76.0	271.2	262.1	240.1
78.0	271.7	234.4	218.7
79.0	205.8	219.8	208.4
80.0	188.7	205.3	197.9
81.0	193.4	191.1	187.6
82.0	174.0	177.3	177.4
84.0	163.7	151.1	157.5
86.0	126.5	126.9	138.4
88.0	117.2	104.8	120.0
90.0	94.3	84.9	102.6
96.0	59.3	40.2	53.4

Table CXII
Some of the mean (±SD.) pharmacokinetic parameter values predicted by Equation 2 following the initial and final 1.0 gm probenecid tablet multiple doses.

	FD/V	k_a	K_M	V_M	V_M/K_M
Initial					
1.0 gm dose	95.4±13.4	1.93±2.1	116.8±18.6	23.3±2.47	0.21±0.05
Final					
1.0gm dose	127.6±9.97	0.65±0.33	120.4±12.6	22.5±1.41	0.19±0.01

Table CXIII
Actual and computer predicted probenecid plasma levels by Equation 2 with parameters from initial dose (I) and from final dose (F) following multiple 1.0 gm probenecid tablet doses to Subject #5.

Dose	Time (h)	Plasma probenecid (mcg/ml)		
		Actual	(I)	(F)
2	24.0	6.99	6.99	6.99
	25.0	*	39.6	44.1
	26.0	*	55.6	64.2
	27.0	*	62.0	73.7
	28.0	*	62.6	76.7
	29.0	*	59.7	75.6
3	30.0	*	54.9	72.0
	31.0	*	82.3	103.8
	32.0	*	94.7	120.1
	33.0	*	98.1	126.6
	34.0	*	95.9	126.8
	35.0	*	90.5	123.1
	36.0	97.8	83.3	116.9
4	36.0	97.8	97.8	97.8
	37.0	*	122.3	128.3
	38.0	*	132.5	143.5
	39.0	*	133.9	149.1
	40.0	*	129.8	144.4
	41.0	*	122.5	143.9
5	42.0	*	113.3	136.9
	43.0	*	137.0	165.9
	44.0	*	146.6	179.9
	45.0	*	147.4	184.3
	46.0	*	142.8	182.6
	47.0	*	134.9	177.0
	48.0	201.2	125.1	168.9
6	48.0	201.2	201.2	201.2
	49.0	*	221.9	228.4
	50.0	*	228.9	241.0
	51.0	*	227.1	244.1
	52.0	*	220.0	241.0
	53.0	*	209.6	234.1

Table CXIII
(continued)

Dose	Time (h)	Plasma probenecid (mcg/ml) Actual	(I)	(F)
7	54.0	*	197.2	224.6
	55.0	*	217.9	251.3
	56.0	*	224.9	263.5
	57.0	*	223.4	266.2
	58.0	*	216.3	262.7
	59.0	*	205.9	255.4
	60.0	270.9	193.6	245.5
8	60.0	270.9	270.9	270.9
	61.0	*	290.2	296.9
	62.0	*	295.8	308.4
	63.0	*	292.9	310.5
	64.0	*	284.4	306.3
	65.0	*	272.6	298.3
9	66.0	*	258.8	287.7
	67.0	*	278.2	313.5
	68.0	*	284.1	324.7
	69.0	*	281.3	326.5
	70.0	*	273.1	322.2
	71.0	*	261.5	313.9
	72.0	242.2	247.8	303.2
10	72.0	242.2	242.2	242.2
	72.5	285.3	254.1	257.6
	73.0	364.2	261.9	268.6
	74.0	227.3	267.9	280.4
	75.0	280.6	265.4	282.8
	76.0	306.8	257.5	279.1
	78.0	216.7	232.9	261.3
	79.0	270.5	218.4	249.6
	80.0	265.8	203.4	236.9
	81.0	201.9	188.1	223.9
	82.0	233.1	172.9	210.6
	84.0	143.6	143.5	184.1
	86.0	98.4	116.1	158.4
	88.0	80.5	91.2	134.1
	90.0	78.6	69.5	111.6
	96.0	46.4	27.8	57.0

Table CXIV
Actual and computer predicted probenecid plasma levels by Equation 2 with parameters from initial dose (I) and from final dose (F) following multiple 1.0 gm probenecid tablet doses to Subject #6.

Dose	Time (h)	Plasma probenecid (mcg/ml) Actual	(I)	(F)
2	24.0	3.82	3.82	3.82
	25.0	*	80.1	76.6
	26.0	*	74.8	99.7
	27.0	*	67.3	102.8
	28.0	*	60.2	98.2
	29.0	*	53.7	90.6
3	30.0	*	47.6	82.2
	31.0	*	121.2	149.6
	32.0	*	113.7	169.2
	33.0	*	104.0	169.4
	34.0	*	94.7	161.8
	35.0	*	85.9	151.3
	36.0	79.9	77.6	139.8
4	36.0	79.9	79.9	79.9
	37.0	*	151.8	147.5
	38.0	*	143.1	167.1
	39.0	*	132.1	167.4
	40.0	*	121.5	159.9
	41.0	*	111.3	149.4
5	42.0	*	101.6	138.0
	43.0	*	172.7	203.3
	44.0	*	163.2	221.3
	45.0	*	151.5	219.9
	46.0	*	140.2	210.8
	47.0	*	129.2	198.8
	48.0	192.6	118.7	185.7
6	48.0	192.6	192.6	192.6
	49.0	*	261.4	256.5
	50.0	*	249.8	273.2
	51.0	*	235.8	270.7
	52.0	*	222.1	260.5
	53.0	*	208.7	247.3

Table CXIV
(continued)

Dose	Time (h)	Plasma probenecid (mcg/ml) Actual	(I)	(F)
7	54.0	*	195.6	232.9
	55.0	*	264.4	296.1
	56.0	*	252.7	312.0
	57.0	*	238.7	308.9
	58.0	*	224.9	298.1
	59.0	*	211.4	284.1
	60.0	151.7	198.2	269.1
8	60.0	151.7	151.7	151.7
	61.0	*	221.4	216.6
	62.0	*	210.6	234.2
	63.0	*	197.6	232.5
	64.0	*	184.8	223.2
	65.0	*	172.3	210.8
9	66.0	*	160.2	197.4
	67.0	*	229.7	261.1
	68.0	*	218.8	277.7
	69.0	*	205.5	275.2
	70.0	*	192.5	264.9
	71.0	*	179.8	251.6
	72.0	194.8	167.5	237.2
10	72.0	194.8	194.8	194.8
	72.5	272.1	258.1	235.4
	73.0	257.4	263.6	258.6
	74.0	252.9	251.9	275.4
	75.0	301.5	237.9	272.9
	76.0	271.2	224.2	262.8
	78.0	271.7	197.6	235.1
	79.0	205.8	184.7	220.5
	80.0	188.7	172.3	206.1
	81.0	193.4	160.2	191.9
	82.0	174.0	148.5	178.1
	84.0	163.7	126.4	151.8
	86.0	126.5	106.1	127.5
	88.0	117.2	87.7	105.3
	90.0	94.3	71.4	85.4
	96.0	59.3	34.9	40.6

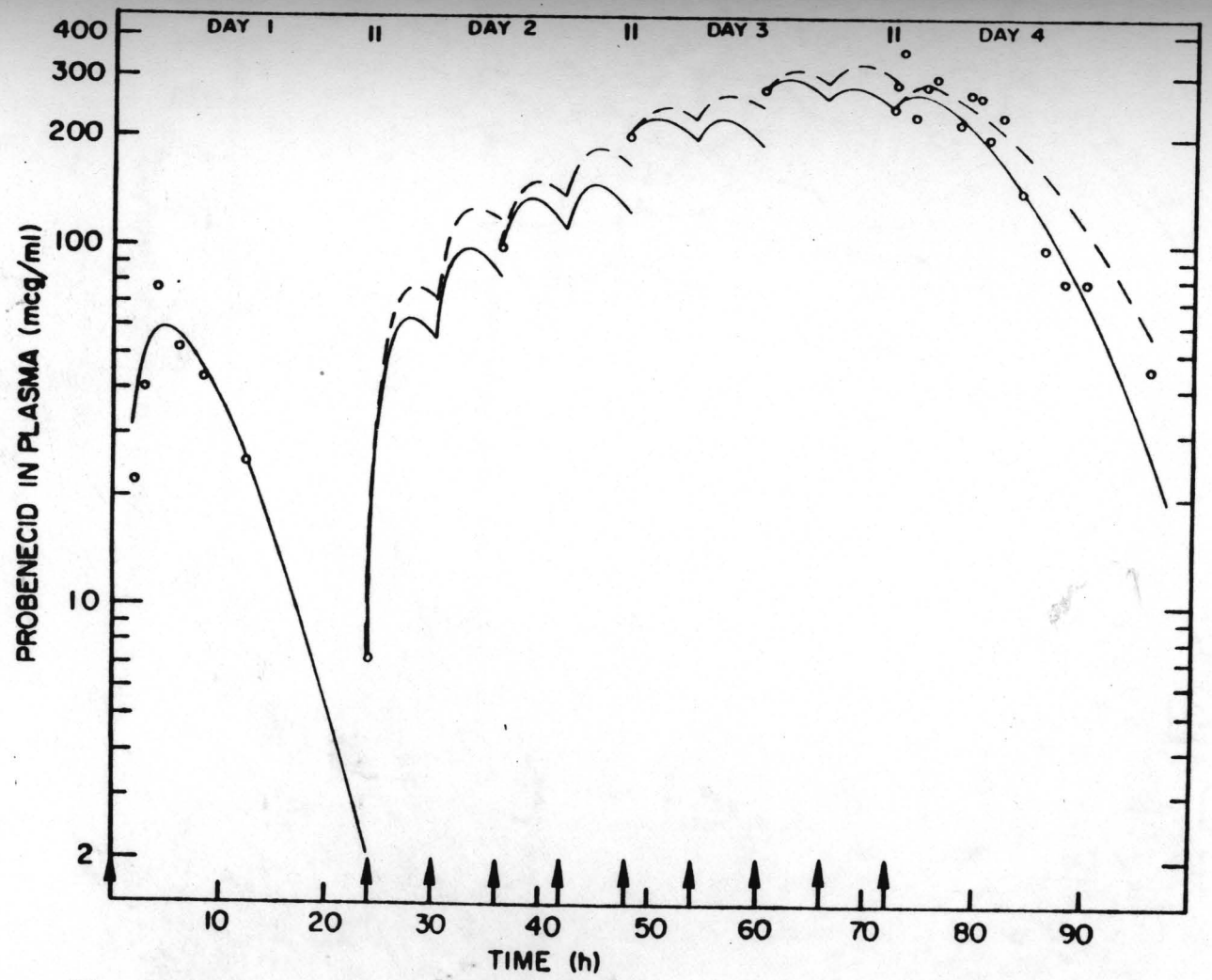


Figure 38: Probenecid plasma levels following 1.0 gm, probenecid tablet multiple doses, subject #5.

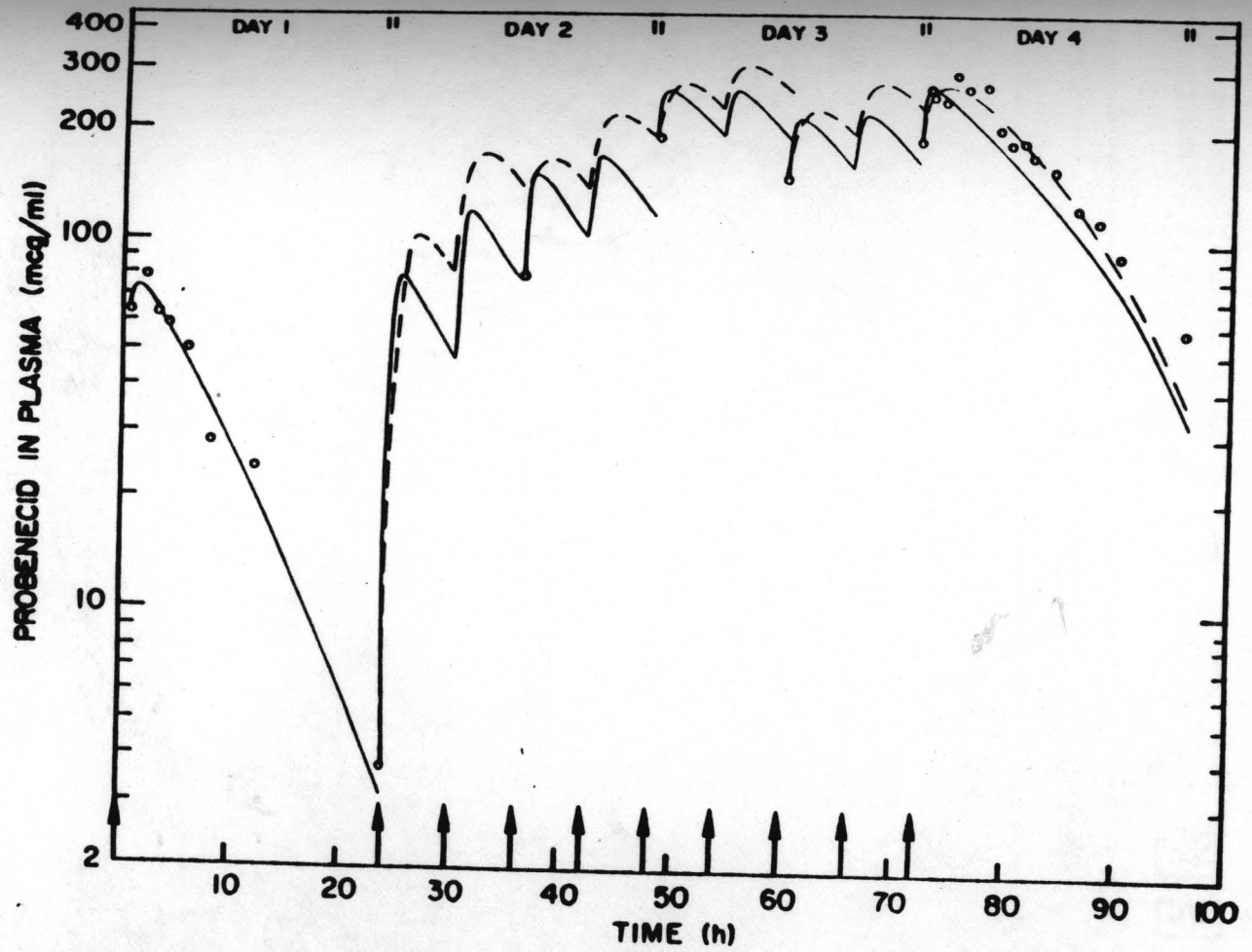


Figure 39: Probenecid plasma levels following 1.0 gm probenecid tablet multiple doses, subject #6.

Table CXV
Summary of the pharmacokinetic parameter values (± 1 SD.) following 1.0 gm probenecid tablet multiple doses to subjects 5 and 6 according to Equations 1, 2, and 3.

Parameter	Values according to Equations		
	1	2	3
FD/V (mcg/ml)	(I) 88.8 ± 2.62	(I) 95.4 ± 13.4 (F) 127.6 ± 9.97	(I) 88.2 ± 0.7 (F) 128.1 ± 54.8
k_a (h^{-1})	(I) 1.50 ± 1.34	(I) 1.93 ± 2.1 (F) 0.65 ± 0.33	(I) 1.84 ± 1.83 (F) 1.78 ± 1.89
$t_{1/2\ abs}$ (h)	(I) 0.77 ± 0.69	(I) 0.89 ± 0.98 (F) 1.22 ± 0.61	(I) 0.75 ± 0.75 (F) 0.90 ± 0.96
k_{el} (h^{-1})	(I) 0.13 ± 0.007	-	-
K_M (mcg/ml)	-	(I) 116.8 ± 18.6 (F) 120.4 ± 13.6	(I) 98.2 ± 40.2 (F) 95.3 ± 49.1
V_M mg/ml.h)	-	(I) 23.3 ± 2.47 (F) 22.5 ± 1.41	(I) 6.79 ± 4.55 (F) 19.2 ± 8.27
V_M/K_M (h^{-1})		(I) 0.21 ± 0.05 (F) 0.19 ± 0.01	(I) 0.09 ± 0.08 (F) 0.21 ± 0.02
k' (h^{-1})	-	-	(I) 0.08 ± 0.04 (F) 0.01 ± 0.01
$t_{1/2\ el}$ (h)	(I) 5.56 ± 0.32	(I) 3.49 ± 0.84 (F) 3.75 ± 0.14	(I) 4.49 ± 1.19 (F) 3.22 ± 0.11
t_{lag}	(I) 0.55 ± 0.78	(I) 0.60 ± 0.71	(I) 0.60 ± 0.71
r^2	(I) 0.975 ± 0.029	(I) 0.976 ± 0.026 (F) 0.979 ± 0.019	(I) 0.974 ± 0.029 (F) 0.982 ± 0.011

Equation 3 is relatively constant, between the initial and the final doses. However, according to Equation 2, k_a value is greatly influenced by the order of the dose and this value shows a 3-fold decrease following the final dose. The values K_M, V_M, and the ratio V_M/K_M appear not to be effected by the order of the dose according to Equation 2. The K_M values are slightly lower in Equation 3 following both the initial and final doses, than the values predicted by Equation 2. The value of V_M according to Equation 3 increased approximately 3-fold after repeated dosing, yielding higher V_M/K_M ratios following the final dose.

DISCUSSION

A. Study I - Suspension single dose study plasma

Dose dependency of probenecid pharmacokinetic following 0.5 and 2.0 gm suspension doses were again evident. Dose normalized areas under curve and elimination half-life values following 2.0 gm suspension dose were significantly higher than the values obtained from the 0.5 gm suspension dose. Similarly dose normalized C_{max} value following the 2.0 gm suspension dose was significantly higher when compared to the 0.5 gm suspension dose. These results indicate saturation of metabolizing enzymes and/or transport of probenecid to the site(s) of metabolism.

Close agreement of these results with previous studies that suggested dose-dependent elimination of probenecid (2, 3, 5) prompted analysis of plasma data according to a one compartment kinetic model with first-order absorption and saturable elimination as well as first-order absorption and elimination model. In addition to these two models, plasma data were analyzed also using a third model involving parallel first-order and saturable elimination of probenecid from the body.

Analysis of plasma data by three models showed significant differences only in the parameters F/V, k_{el} and $t_{\frac{1}{2}}$. Under the assumption of saturable elimination F/V was not influenced by dose

size. In a previous study, similar F/V values were predicted by Equation 1, from 0.5 and 2.0 gm tablet doses (5). In the present study giving the probenecid dose in suspension form caused an increase in F as might be expected but the increase in the dose normalized area under curve value was still higher than predicted by the increased value of F/V.

Equations 2 and 3 predict elimination half-lives that are not significantly different following 0.5 and 2.0 gm suspension doses below levels of saturation. Although these models did not provide a superior fit of the data they are conceptually attractive as they can accommodate the increase in dose normalized area under curve values and prolonged elimination half-lives following the higher doses.

Although theoretically promising Equation 3 did not provide a superior fit to the data and all three equations were equally successful in describing the data.

Applicability of the information criterion proposed by Akaike (AIC) to numerically express the amount of information and to statistically determine the number of parameters in an equation that represents experimental data was examined. Although Akaike had proposed this method for analysis of linear pharmacokinetic models that can be expressed as a sum of exponential functions, an attempt to apply this method to Equation 2 and 3, as well as Equation 1 was made. AIC is calculated as follows:

$$AIC = N \ln R_e + 2p$$

where N is the number of experimental data points, R_e is the residual sum of squares and p is the number of parameters (73). This equation or Akaike's assumption that random errors obey Gaussian distribution appear to be acceptable propositions for Equations 2 and 3. AIC values were calculated from the analysis of 0.5 and 2.0 gm suspension plasma data according to the three models. The three equations gave similar AIC numbers, again indicating that none of the models provided a superior fit of the data. Due to "principle of parsimony" Equations 1 and 2 may be considered more successful than Equation 3 as they could describe the data similarly with 4 parameters instead of 5. Equation 3 may be regarded as a theoretical exercise and the results of the analysis of data according to Equation 3 are included as an extension of the work but will not be considered as the major part of the discussion.

In the present study the analysis of data again suggests dose dependent pharmacokinetics as demonstrated by prolonged apparent elimination half-lives following the high dose and disproportionate increase in dose normalized area under curve values. Although the data could be adequately described by both first-order absorption and elimination and first-order absorption and saturable elimination models, saturable elimination model is favored as it can provide an explanation for the dose dependent changes in the pharmacokinetic parameters.

The data here provide further evidence that probenecid pharmacokinetics, probenecid disposition does not obey simple first-order

kinetics. As in the previous study saturable elimination kinetics requiring the use of V_M, K_M were observed following 2.0 gm doses. Although a possible contribution due to prolonged absorption may have influenced the previous data and considered a contributing factor in the previous study, this is unlikely in the present study as suspension doses are used.

Urine

Mean cumulative percentage recovery of unchanged and conjugated probenecid following both suspension doses is in excellent agreement with the values reported by other researchers (4, 53, 54). Similar percentages of total unchanged probenecid were recovered from both the 0.5 and 2.0 gm doses, and the excretion pattern appeared not to be affected by dose size. However, the percentage of total conjugated probenecid and also its excretion rate appeared to be dose-dependent. Excretion of 95% of conjugated probenecid was complete at the end of 24 h and 36 h following the administration of 0.5 and 2.0 gm suspension doses, respectively (Tables XLVI and XLVII). This steady and more prolonged excretion of probenecid acylglucuronide following the 2.0 gm dose is apparent in Figure 24 and Table XLVII. When cumulative percentage recovery of conjugated probenecid was plotted against mid-point of collection intervals following the 2.0 gm dose a slope of approximately 1 was obtained, whereas the slope from the 0.5 gm dose was significantly greater (Table XLIX).

The steady and prolonged excretion of probenecid acyl-glucuronide may possibly be due to saturation of glucuronidation, transport to the site (s) of metabolism, saturation of active secretion, or decreased uptake of the drug at the metabolic site with increased dose as proposed by several researchers (18, 22, 23, 24, 54).

Other indications of dose dependent changes in elimination of probenecid associated with probenecid acylglucuronide are:

(1) Ratio of unchanged to conjugated probenecid excreted in urine increased 4-fold with a 4-fold increase in dose size (Table L). Despite the increase in unchanged probenecid excreted in the urine, the quantity of conjugated probenecid from both doses was constant.

(2) The ratio of urinary excretion of conjugated probenecid from the 0.5 gm dose compared to the 2.0 gm dose was higher during 2-6 h urine collection interval than 0-2 h interval. The ratio declined in subsequent urine collection intervals.

These data suggest that one or more steps associated with urinary excretion of conjugated probenecid is saturated at high circulating drug levels.

From Table LI the ratio of percent unchanged probenecid excreted in urine following 0.5 and 2.0 gm doses during 0-6 h approximated unity. However, during 6-12, 12-24 h intervals, unchanged probenecid excreted from the 0.5 gm dose was higher than that from 2.0 gm dose.

This could be due to saturation of active secretion of probenecid from the kidney tubules at high concentrations.

These urinary data are in excellent agreement with plasma data, and are also consistent with previous reports that have explained dose-dependent pharmacokinetics of probenecid in man and experimental animals in terms of saturable elimination. As the present results were obtained following suspension doses, and the dose-dependent effects observed persisted for at least 12 h postdose, the contribution of absorption factors appears not to be important.

B. Studies II and III - Multiple dose studies

More rigorous examination of nonlinear pharmacokinetics of probenecid was performed by giving multiple 0.5 and 1.0 gm tablet doses. Similar dose-dependent changes in dose-normalized areas under curves and half-lives following initial 0.5 and 1.0 gm doses were again observed, consistent with previous work (2, 3, 5, 46).

When accumulation of probenecid following multiple doses was examined, the first-order elimination model predicted plasma levels that were only approximately one-half of the observed values from both doses. However, steady-state values, although reached from 0.5 gm dose in 30 h, were not achieved in this time period with 5 1.0 gm doses of probenecid. Study II was therefore extended to Study III in which 9 consecutive 1.0 gm doses of probenecid were administered every six hours. The results of Study III showed that

accumulation of probenecid was much higher than predicted by Equation 1, and the time to reach steady-state following the 1.0 gm dose is long compared to the 0.5 gm dose.

The saturable elimination model indicated the complex nature of probenecid pharmacokinetics by predicting two different sets of parameter values that could describe the data following either the initial or the last dose. Examples of these are in Figures 30-39. The striking dose-order dependence of parameters k_a and V_M/K_M is apparent. With repeated dosing, values of k_a decreased when compared to the value from the initial dose. This may possibly be due to "autoinhibition" of probenecid absorption at high doses, as probenecid is known to interfere with active absorption of other compounds such as cardiac glycosides and glucose (34, 36, 37). Although no absorption effect was observed from single dose data, absorption of probenecid was influenced with repeated dosing. In similar fashion the decrease in V_M/K_M observed after repeated dosing is possibly due to autoinhibition in the metabolism rate of probenecid after certain dry levels are achieved. Saturation could be at one or more steps. From previous urinary excretion data the conjugated form of probenecid appears to exhibit saturable elimination. Since the other metabolites of probenecid were not examined the possibility of saturation of other routes with similar V_M and K_M values cannot be excluded.

In order to elucidate the inference of changing parameter values on multiple dose kinetics the accuracy of a model-independent

steady-state prediction method of accelerated convergence was examined (74). Although this is a robust method, it could not be used successfully to predict steady-state probenecid levels after multiple probenecid doses, providing additional, albeit indirect, evidence of changing kinetic parameter values with repeated doses.

The effect of protein binding on dose-dependent changes in probenecid pharmacokinetics was reported to be negligible as similar dose-dependent changes in dose-normalized area under curve values were observed from both free and total drug in plasma (46).

Biliary recycling of probenecid occurs in experimental animals. It has been suggested that probenecid glucuronide conjugate may be stored in the gall bladder and hydrolyzed to the parent drug after it is released into the intestine. Reabsorption of the parent drug back into the systemic circulation would thus give rise to prolonged plasma levels (46).

This hypothesis may possibly represent one of the many factors that appear to influence probenecid pharmacokinetics. More detailed and controlled studies concerning probenecid GI absorption, membrane transport, metabolism and elimination are required to explain and characterise the time-dependent and concentration-dependent changes in probenecid disposition in the body, and also to establish the most appropriate kinetic model. In view of the complex interplay between the various factors that influence probenecid absorption, distribution, metabolism and excretion, it is unlikely that complete identification

of probenecid characteristics can be achieved in an *in vivo* system without surgical intervention.

The saturable elimination model used for the analysis of plasma data in this study has been successful in demonstrating the diverse nature of probenecid pharmacokinetics, and establishing that probenecid elimination kinetics are nonlinear and saturable following repeated doses in the therapeutic range.

CONCLUSIONS

(1) Previous studies in this laboratory and elsewhere (2, 3, 5, 46, 58) suggested that probenecid may undergo saturable elimination. In the studies described in this thesis, further evidence of dose-dependent pharmacokinetics of probenecid was presented following single 0.5 and 2.0 gm probenecid suspension doses. The plasma data from both doses could be successfully described by a saturable elimination model. The validity of the parameters V_M and K_M obtained from the 2.0 gm dose was substantiated in their ability to describe also the 0.5 gm dose data.

(2) Total percentage recovery of unchanged probenecid in urine was similar following 0.5 and 2.0 gm suspension doses. Although following the 2.0 gm dose saturation of active secretion of unchanged probenecid was observed during the initial 12 hours of urine collection, similar percentages of unchanged drug was recovered in the urine from both the 0.5 and 2.0 gm doses during the entire 48 h collection period.

Conjugated probenecid excreted in urine is influenced by dose-size as more conjugated probenecid was excreted from 0.5 gm dose than the 2.0 gm dose during 12 hours postdose. However, total percentage recovery of conjugated probenecid is again similar from both doses.

(3) Repeated dose plasma data confirmed dose-dependent pharmacokinetics of probenecid within the therapeutic range. Application of a saturable elimination model to describe plasma data has facilitated recognition of the complex nature of probenecid kinetics. The probenecid absorption rate constant, and also V_M/K_M, were influenced by dose-order. Although the most accurate model to describe probenecid kinetics requires more qualitative and quantitative information regarding its absorption, distribution, and elimination, this information is likely to be difficult to obtain. The present saturable elimination model represents a simple alternative that can provide information on probenecid kinetics following single and repeated doses within the therapeutic range.

REFERENCES

1. W. P. Boger, W. P. Forrest, and M. E. Gallagher: The Influence of a New Benzoic Acid Derivative on the Metabolism of Para-Aminosalicyclic Acid (PAS) and Penicillin. Ann. Int. Med. 33: 18-31, 1950.

2. P. G. Dayton, T. F. Yu, W. Chen, L. Berger, L. A. West, and A. B. Gutman: The Physiological Disposition of Probenecid, Including Renal Clearance, in Man, Studied by an Improved Method for its Estimation in Biological Materic. J. Pharmacol. Exptl. Therap., 140: 278-286, 1963.

3. T. B. Tjandramaga, S. A. Cucinell, Z. H. Israili, J. M. Perel, P. G. Dayton, T. F. Yu, and A. B. Gutman: Observations on the Disposition of Probenecid in Patients receiving Allopurinol. Pharmacology, 8: 259-272, 1972.

4. S. Melethil, and W. D. Conway: Urinary Excretion of Probenecid and its Metabolites in Humans as a Function of Dose. J. Pharm. Sci., 65: 861-865, 1976.

5. A. Selen, G. L. Amidon, and P. G. Welling: Pharmacokinetics of Probenecid following Oral Doses to Human Volunteers. J. Pharm. Sci., 71: 1238-1242, 1982.

6. W. P. Boger, W. P. Forrest, and M. E. Gallagher: Benemid and Carinamide: Comparison of Effect on Para-Aminosalicylic acid (PAS) Plasma Concentrations. J. Lab. Clin. Med., 36: 276-282, 1950.

7. A. A. Al-Badr, and H. A. El-Obeid: in Analytical Profiles of Drug Substances, 10, Academic Press Inc., pp. 639-663, 1981.

8. P. A. Shore, B. B. Brodie, and C. A. M. Hogben: The Gastric Secretion of Drugs: A pH Partition Hypothesis. J. Pharmacol. Exptl. Therap., 119: 361-369, 1957.

9. P. G. Dayton, M. M. Weiss, and J. M. Perel: The Effect of Probenecid, Phenylbutazone, and their Analogs on the Excretion of L-Ascorbic Acid in Rats. J. Med. Chem., 9 941-966, 1966.

10. Z. H. Israili, J. M. Perel, R. F. Cunningham, P. G. Dayton, T. F. Yu, A. B. Gutman, K. R. Long, R. C. Long, Jr., and J. H. Goldstein: Metabolites of Probenecid, Chemical, Physical, and Pharmacological Studies. J. Med. Chem., 15: 709-713, 1972.

11. V. J. Zarro: Clinical Therapeutics, Lowenthal and Mayor (Eds.), Grune and Stratton, New York, pp. 343-346, 1978.

12. W. F. Ganong: Review of Medical Physiology, 10th Edition, Lange Medical Publications, Los Altos, California, pp. 235, 1981.

13. H. Poulsen: Inhibition of Uric Acid Excretion in Rabbits Given Probenecid or Salicylic Acid. Acta Pharmacol. Toxicol., 11: 272-286, 1955.

14. L. Berger, T. F. Yu, and A. B. Gutman: Effect of Drugs that Alter Uric Acid Excretion in Man on Uric Acid Clearance in the Chicken. Am. J. Physiol., 198: 575-580, 1960.

15. L. H. Smith, Jr., Cecil Text Book of Medicine, P. L. Beeson, W. McDermott and W. B. Wyngaarden (Eds.), Saunders Company, Philadelphia, Pennsylvania, 1982.

16. 1983 USP DI, Vol. I, Drug Information for the Health Care Provider: United States Pharmacopeial Convention, Inc. Rockville, Maryland, pp. 716, 1982.

17. A. B. Gutman, T. F. Yu, and J. H. Sirota: A Study, by Simultaneous Clearance Techniques, of Salicylate Excretion in Man. Effect of Alkalinization of the Urine by Bicarbonate Administration; Effect of Probenecid. J. Clin. Invest., 34: 711-721, 1955.

18. I. M. Weiner, J. A. Washington II, and G. H. Mudge: On the Mechanism of Action of Probenecid on Renal Tubular Secretion. Bull. John Hopkins Hospital, 106: 333-346, 1960.

19. J. C. Craft, W. E. Feldman, and J. D. Nelson: Clinicopharmacological Evaluation of Amoxicillin and Probenecid Against Bacterial Meningitis. Antimicrob. Ag. Chemother., 16: 346-352, 1979.

20. C. Liu, D. R. Hinthorn, G. R. Hodges, J. L. Harms, G. Couchonnal, and D. L. Dworzack: Penetration of Cefoxitin into Human Cerebrospinal Fluid: Comparison with Cefamandole, Ampicillin and Penicillin. Rev. Inf. Dis., 1: 127-131, 1979.

21. S. R. Nair, C. E. Cherubin, and M. Weinstein: Penetration of Cefoxitin into Cerebrospinal Fluid and Treatment of Meningitis Caused by Gram-Negative Bacteria. Rev. Inf. Dis., 1: 134-141, 1979.

22. N. R. Tamarkin, F. K. Goodwin, and J. Axelrod: Rapid Elevation of Biogenic Amine Metabolites in Human CSF Following Probenecid. Life Sci., 9: 1397-1408, 1970.

23. B. E. Roos, N. E. Anden, and B. Werdinus: Effect of Drugs on the Levels of Indole and Phenolic Acids in the Central Nervous System. Int. J. Neuro-Pharmacol., 3: 117-122, 1964.

24. H. M. Van Praag, J. Korf, L. C. W. Dols, and T. Schut: A Pilot Study of the Predictive Value of the Probenecid Test in Application of S-Hydroxytryptophan as Antidepressant. Psychopharmacologia, 25: 14-21, 1972.

25. J. M. Perel, J. Levitt, and D. L. Dunner: Plasma and Cerebral Spinal Probenecid Concentrations as Related to Accumulation of Acidic Biogenic Amine Metabolites in Man. Psychopharmacologia, 35: 83-90, 1974.

26. P. R. Davoren, and E. W. Sutherland: The Effect of L-Epinephrine and Other Agents on the Synthesis and Release of Adenosine 3',5'-Phosphate by Whole Pigeon Erythrocytes. J. Biol. Chem., 238: 3009-3015, 1963.

27. W. F. Ganong: Review of Medical Physiology, 10th Edition, Lange Medical Publications, Los Altos, California, pp. 26, 187, 277, 1981.

28. J. Penit, S. Jard, and P. Benda: Probenecid Sensitive 3'-5'-Cyclic AMP Secretion by Isoproterenol Stimulated Glial Cells in Culture. FEBS Lett., 41: 156-160, 1974.

29. M. J. Rindler, M. M. Bashor, N. Spitzer and M. H. Saier: Regulation of Adenosine 3'-5'-Monophosphate Efflux from Animal Cells. J. Biol. Chem., 253: 5431-5436, 1978.

30. R. A. Podevin, E. F. Boumendil-Podevin, J. Bujoli-Roche, and C. Priol: Effects of Probenecid on Transport and Metabolism of Cyclic AMP by Isolated Rabbit Renal Tubules. Biochimica et Biophysica Acta, 629: 135-142, 1980.

31. K. H. Beyer, V. D. Wiebelhaus, E. K. Tillson, H. F. Russo, and K. M. Wilhoyte: "Benemid," p-(di-n-propylsulfamyl)-Benzoic Acid: Inhibition of Glycine Conjugative Reactions. Proc. Soc. Exp. Biol. Med., 74: 772-775 (1950).

32. K. Kuriaki, and H. Marumo: On the Effects of SKF 525-A, Carinamide, Probenecid, Salicylate and Aspirin on the Bioacetylation of Sulfanilamide and Choline. Jap. J. Pharmacol., 8: 96-104, 1959.

33. K. H. Damm, and R. Erttmann: Interaction of Probenecid with Digitoxin Metabolism in Rats. Toxicol. appl. Pharmacol., 33: 246-257, 1975.

34. K. H. Damm, W. Braun, and H. Heckert: Die wirkung von Probenecid auf die verteilung von Digitoxin, Digoxin und Ouabain an der Maus. Naunyn-Schmiedeberg's Arch. Pharmacol., 277: 267-279, 1973.

35. R. Erttmann, and K. H. Damm: Probenecid and Hepatic Transport of ^{3}H-Ouabain in Rats. Arch. Int. Pharmacodyn., 211: 197-210, 1974.

36. K. H. Damm, and V. Woermann: The Effect of Probenecid on the in vitro Absorption of Cardiac Glycosides. Europ. J. Pharmacol., 28: 157-163, 1974.

37. H. J. Binder, L. A. Katz, R. P. Spencer and H. M. Spiro: The Effect of Inhibitors of Renal Transport on the Small Intestine. J. Clin. Invest., 45: 1854-1858, 1966.

38. A. Wack, C. Woermann, and W. Braun: A Study of the Probenecid Effect on Amino Acid Accumulation in Kidney Cortex Slices. Arch. int. Pharmacodyn, 256: 292-300, 1982.

39. N. Baber, L. Halliday, R. Sibieon, T. Littler, and M. L. E. Orme: The Interaction Between Indomethacin and Probenecid, A Clinical and Pharmacokinetic Study. Clin. Pharmacol. Ther., 24: 298-307, 1978.

40. R. Runkel, E. Mroszczak, M. Chaplin, H. Svelius, and E. Segre: Naproxen-Probenecid Interaction. Clin. Pharmacol. Ther., 24: 706-713, 1978.

41. R. A. Upton, R. L. Williams, J. B. Buskin, and S. Riegelman. World Conf. on Clinical Pharmacology and Therapeutics, London, 1980 (abstrs.).

42. T. F. Yu, and J. Perel: Pharmacokinetic and Clinical Studies of Carprofen in Gout. J. Clin. Pharmacol., 20: 347-351, 1980.

43. J. R. Veendal, P. M. Brooks, and P. Meffin: Probenecid-Clofibrate Interaction. Clin. Pharmacol. Ther., 29: 351-358, 1981.

44. E. K. Tillson, G. S. Schuchardt, J. K. Fishman, and K. H. Beyer: The Determination of Probenecid (Benemid) in Body Fluids. J. Pharmacol. Exptl. Therap., 111: 385-394, 1954.

45. J. M. Perel, P. G. Dayton, T. F. Yu, and A. B. Gutman: Studies of the Renal Excretion of Probenecid Acyl Glucuronide in Man. Europ. J. Clin. Pharmacol., 3: 106-112, 1971.

46. C. N. Chiang, and L. Z. Benet: Dose-Dependent Kinetics of Probenecid in Rhesus Monkeys--Intravenous Bolus Studies. Pharmacology, 23: 326-336, 1981.

47. N. H. Neff, T. N. Tozer, and B. B. Brodie: Application of Steady-State Kinetics to Studies of the Transfer of S-Hydroxyindoleacetic Acid from Brain to Plasma. J. Pharmacol. Exptl. Therap., 158: 214-218, 1967.

48. B. Werdinius: Effect of Probenecid on the Levels of Monoamine Metabolites in the Rat Brain. Acta Pharmacol. Toxicol., 25: 18-23, 1967.

49. R. Sjöström: Steady-State Levels of Probenecid and Their Relation to Acid Monoamine Metabolites in Human Cerebrospinal Fluid. Psychopharmacologia, 25: 96-100, 1972.

50. W. L. Shei, J. Y. Mu, R. F. Cunningham, Z. H. Israili, and P. G. Dayton: Probenecid in CSF and Plasma of Rabbits and Dogs Measured by Radioimmunoassay. Psychopharmacologia, 53: 315-318, 1977.

51. W. O. Berndt: Probenecid Binding by Renal Cortical Slices and Homogenates. Proc. Soc. Exptl. Biol. Med., 126: 123-126, 1967.

52. W. Braun, and H. Schniewind: Die Anreicherung von Probenecid (^{35}S) in Nierenschnitten des Meerschweinchens. Naunyn-Schmiedeberg's Archiv fur Experimentelle Pathologie und Pharmakologie, 243: 429-438, 1962.

65. J. Neter, and W. Wasserman. Applied Linear Statistical Models, Richard D. Irwin, Inc., Homewood, Illinois, pp. 474-477, 1974.

66. Wayne W. Daniel. Biostatistics: A Foundation for Analysis in the Health Sciences. Second Edition. John Wiley & Sons, Inc., New York, pp. 232-241, 1970.

67. B. J. Winer. Statistical Principles in Experimental Design. Second Edition, McGraw-Hill, Inc., New York, pp. 431-487, 1962.

68. Statistical Package for the Social Sciences, Madison Academic Computer Center, University of Wisconsin.

69. J. G. Wagner: Fundamentals of Clinical Pharmacokinetics, Drug Intelligence Publications, Hamilton, Illinois, pp. 74, 1979.

70. J. G. Wagner: Pharmacokinetic Notes, J. M. Richards Laboratory, Grosse Pointe Park, Michigan, 1969.

71. N. Krishnan Namboodiri, Lewis F. Carter, and Hubert M. Blalock, Jr. Applied Multivariate Analysis and Experimental Designs. Mcgraw-Hill, Inc., New York, pp. 356-367, 1975.

72. "Scientific Tables" Documenta, 6th Edition, Konrad Diem (Ed.), Geigg Pharmaceuticals, Ardsley, New York, pp. 105, 1962.

73. K. Yamaoka, T. Nakagawa, and T. Uno. Application of Akaike's Information Criterion (AIC) in the Evaluation of Linear Pharmacokinetic Equations. J. Pharm. Biopharm., 6: 165-175, 1978.

74. G. L. Amidon, M. J. Paul, and P. G. Welling: Model-Independent Prediction Methods in Pharmacokinetics: Theoretical Considerations. Math. Biosc., 25: 259-272, 1975.

APPENDIX I

Glossary

A	Amount of probenecid in the body.
A_c	Amount of conjugated probenecid excreted in urine.
A_u	Amount of unchanged probenecid in the urine.
$AUC^{0\rightarrow t}$	Area under plasma probenecid curve from time zero to time t.
$AUC^{0\rightarrow\infty}$	Area under plasma probenecid curve from time zero to infinite time.
$AUC^{0\rightarrow t}/D$	Dose normalized ara under plasma probenecid curve from time zero to time t.
C	Concentration of probenecid in plasma.
C_{max}	Maximum probenecid concentration.
D	Dose of probenecid administered
F	Fraction of administered dose of probenecid that is available to the systemic circulation.
K_M	Michaelis constant
k_a	First-order rate constant for appearance of probenecid into the systemic circulation.
k_{el}	First-order for elimination of probenecid by all routes.
k'	First-order rate constant for elimination of probenecid from the nonsaturable pathway in Equation 3.
R	Accumulation factor during repeated probenecid dose.
t	Time elapsed since a single probenecid dose.
$t_{\frac{1}{2}abs}$	Absorption half-life of probenecid.
$t_{\frac{1}{2}}$	Elimination half-life of probenecid.
t_{lag}	Time period between the administration of dose and appearance of probenecid in plasma.
T_{max}	Time after dosing when C_{max} occurs.
τ	Dose interval

V	Volume
V_M	Maximum rate of elimination of probenecid in a saturable system.

APPENDIX II

Protocol and Consent Form for Clinical Study I

PROTOCOL FOR THE STUDY OF PROBENECID PHARMACOKINETICS IN MAN

Arzu Selen[1], M.S.; Peter G. Welling[1], Ph.D.; and
William A. Craig[2], M.D.

School of Pharmacy[1], Veterans Administration Hospital[2] and
School of Medicine[2], University of Wisconsin, Madison, Wisconsin

Drug 256

Probenecid 500 mg tablets (Benemid®) obtained commercially.

Subjects

Subjects will be healthy male volunteers aged between 20 and 40 years and with body weight:height relationships within ± 10% of normal. Subjects will be students (not those of the investigators) and technical and administrative staff at the University of Wisconsin.

Physical Examination

Before being admitted to the study, subjects will undergo a complete physical examination, including gross examination, blood and urine analysis, and drug history. Participation is dependent upon all values being within normal limits. The physical examination will be at no cost to the subjects.

Consent

Having successfully completed the physical examination, subjects will be asked to read and, if agreeable, to sign the consent form. The consent form will have been previously approved by the University of Wisconsin Committee for Protection of Human Subjects (CPHS). Anything in the consent form that is not fully understood by the subject, including all risks and benefits, is explained to him. The subject is given a copy of the consent form to retain.

Confidentiality of Records

Confidentiality of records will be maintained in accordance with standard procedures at the University of Wisconsin. Medical records of all subjects are kept in a locked cabinet in Dr. Craig's office in the Veterans Administration Hospital. For the study, subjects are assigned a code number known only to the investigators.

Risks, Benefits and Withdrawal 257

Potential risks to subjects consist of side effects from probenecid and from multiple blood sampling procedures. These risks are minimized by administering therapeutic doses of probenecid under medical supervision and by using experienced personnel and standard aseptic procedures for blood sampling.

Subjects are advised that they will derive no direct benefits from the study. However the results obtained may result in indirect benefits to them and to the public at large in characterizing the pharmacokinetics of probenecid and its known metabolites, thereby providing a scientific basis for the method of administration of this medicinal agent to patients.

Subjects are advised that they are free to withdraw from the study at any time.

Side Effects of Probenecid

Several side effects have been reported for probenecid. They include stomachache, vomiting, flushing, dizziness, skin rashes, dermatitis, fever, and sore gums. Subjects will be advised of these side effects so that they will recognize them if they occur.

Restrictions

Participating subjects must take no other medication for one week, and no enzyme stimulating agents such as phenobarbital for one month, before and during a study. Coffee, tea, and other caffeine or xanthine-containing beverages or foods will not be permitted for two days before or during a study.

Treatment of Adverse Effects

If a side effect is experienced as a result of the study, the condition will be treated by Dr. Craig or a medically qualified colleague at no cost to the subject. The subjets are advised that the policy of the University of

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

Subjects may be withdrawn from the study by Dr. Craig if he considers it appropriate. Subjects are provided with names and telephone numbers of all investigators.

Protocol

There will be two separate studies. Subjects will be invited to participate in both, but this is not necessary. Having completed the first study, subjects will be invited to continue with the second, but the decision to continue or withdraw is that of the subject. The two studies will be three months apart.

Single Dose-study

Four male volunteers will each receive single oral doses of 0.5 and 2.0 g probenecid, as 500 mg Benemid tablets, according to a randomized design. The doses will be administered two weeks apart. Subjects will report to Room 5316 at the School of Pharmacy at 7:40 a.m. on the designated days. Appropriate probenecid doses will be taken as powdered tablets in 250 ml water at 8:00 a.m. following overnight (10:00 p.m.) fast. Food will be further withheld until 12 noon on the day of medication, after which time normal diets are resumed. Subjects will be given 200 ml of water 2.0, 4.0, and 8.0 hr after dosing.

Blood samples (~10 ml) will be taken by heparinized Vacutainer from a forearm vein immediately before (2) and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0, 14.0, 16.0, 20.0, 24.0, 30.0, 36.0, and 48.0 hours after

dosing. Plasma will be separated and stored at -20°C for subsequent assay. Subjects will then give 190 ml of blood during a 48-hour period.

Urine will be collected in provided containers immediately before and then quantitatively during 0-2, 2-6, 6-12, 12-24, 24-36, and 36-48 hours after dosing. Urine volumes and pH values will be recorded, divided into two 20 ml aliquots and stored at -20°C until assayed. Subjects will be confined to the study site during the first 12 hr after each probenecid dosage but will be permitted to leave the site between plasma sampling times subsequently.

Analytical

Plasma and urine samples will be assayed for probenecid and metabolites by a high pressure liquid chromatography procedure developed in the School of Pharmacy, University of Wisconsin.

Data Analysis

Plasma levels and urinary excretion data will be analyzed from pharmacokinetic and statistical viewpoints to establish basic kinetic parameters and also the nature and mechanism of probenecid elimination in healthy individuals.

Multiple-Dose Study

The objective of this study is to examine the accumulation characteristics of probenecid and its metabolites following repeated doses and also to determine the ability of kinetic models that were developed from single doses to predict circulating drug and metabolite levels and excretion patterns following repeated doses of probenecid.

Subjects will receive 1.0 g (2 x 500 mg tablets) oral doses of probenecid at 8:00 a.m. and additional 2 x 500 mg doses of probenecid at 2:00 p.m. and 8:00 p.m. on Day 1. On Days 2-4, subjects will receive 2 x 500 mg doses of

probenecid at 8:00 a.m., 2:00 p.m. and 8:00 p.m. All 8:00 a.m. doses will be given with 250 ml of water after overnight fast. All 2:00 p.m. doses will be given 2 hours after the midday meal. All 8:00 p.m. doses will be given 2 hr following the evening meal. A light snack will be permited 2 hr after the 8:00 p.m. dose.

Plasma and urine will be collected during 48 hr following the 8:00 a.m. probenecid dose on Days 1 and 10 at the same times, in identical fashion, and under the same conditions to that in the single-dose study. The volume and pH of each urine collection will be determined. All plasma samples and urine aliquots will be stored at -20°C until assayed.

Data Analysis

Plasma level and urinary excretion data will be examined from pharmacokinetic and statistical viewpoints to determine the degree of drug and metabolite accumulation and also urinary metabolite excretion patterns with repeated doses. This study will determine also the ability of a kinetic model incorporating saturable or parallel saturable and nonsaturable elimination developed from single-dose data to predict drug accumulation and elimination patterns following repeated doses.

Arzu Selen

Peter G. Welling

William A. Craig

CONSENT FORM FOR THE STUDY OF PROBENECID PHARMACOKINETICS IN MAN

Arzu Selen[1], M.S.; Peter G. Welling[1], Ph.D.; and A. Craig[2], M.D.

School of Pharmacy[1], Veterans Administration Hospital[2], and School of Medicine[2], Univerisity of Wisconsin, Madison, Wisconsin

CONSENT FORM FOR THE STUDY OF PROBENECID PHARMACOKINETICS IN MAN

A study is being conducted by the School of Pharmacy, University of Wisconsin, on probenecid. We wish to study the pharmacokinetics of probenecid after single doses and to examine the elimination characteristics of probenecid and its major metabolite at two different doses.

This consent form concerns the single dose study which will then be followed by a multiple dose study. The first study involves you taking two separate single doses of probenecid and giving blood samples and urine collections after each dose. The second study involves you taking repeated doses of probenecid and giving blood samples and urine collections after the first and last dose and some additional blood samples following intermediate doses. You may volunteer to participate in the single-dose study or the repeated-dose study or both. If you volunteer for one study, you do not have to volunteer for the other.

Principal Investigators

The principal investigators carrying out the study are: Arzu Selen, School of Pharmacy, 608-263-4159; Dr. Peter G. Welling, School of Pharmacy, 608-263-3986; and Dr. William A. Craig, School of Medicine, 608-256-1901 (572).

Where the Study Will be Conducted

The study will be conducted in Room 5316, School of Pharmacy, Madison, Wisconsin 53703.

Therapeutic Uses of Probenecid

Probenecid is used for gout and gouty arthritis. It is also in combination with some other antibacterial drugs like penicillins, to prolong their action by slowing down their rate of excretion.

Side Effects of Probenecid

Several side effects have been reported for probenecid; they include stomachache, vomiting, flushing, dizziness, skin rashes, dermatitis, fever and sore gums. Probenecid can also react with other drugs which a patient may be taking to cause an adverse side effect.

Reported side effects of probenecid invariably occur after repeated doses. They are, therefore, more likely to occur during the repeated-dose study than during the single-dose study. However reactions of the above types could occur in either study so it is important that you be aware of this possibility. If you do experience such a reaction, you must report it to Dr. Craig or a medically qualified colleague. If the reaction is considered by the physician to be dose-related, he/she will administer the appropriate treatment.

Dosage

In the single-dose study, you will receive single oral doses of 0.5 and 2.0 g probenecid (as 500 mg Benemid® tablets) on two separate occasions two weeks apart.

In the repeated-dose study, you will receive oral doses of 1.0 g probenecid (as 2 x 500 mg Benemid® tablets) three times each day during a 7-day period.

Study Program and Details

If after reading and fully understanding this consent form you wish to take part in the study, you will receive a complete physical examination which will be conducted by Dr. Craig or a fully qualified clinical associate at the Clinical Science Center. During this examination, you will be required to give a blood sample (10 ml) and a urine sample.

You will also be asked whether you are receiving any other medications, whether you have any allergic reactions to drugs and also your smoking and drinking habits.

Having successfully completed the physical examination, you will be invited to sign this consent form and thereby become a participant in one or both of the studies.

If you participate in a study, you will be permitted to take no other medication for one week and no enzyme stimulating agents such as phenobarbital for one month before or during a study. Coffee, tea, and other caffeine or xanthine-containing beverages or foods will not be permitted for two days before and during a study.

Single-Dose Study

You will receive the two different dosages of probenecid one week apart according to a predetermined schedule.

The day before medication, you will eat no food and drink no liquid after 10:00 p.m. On a study day, you wil report to Room 5316 at 7:40 a.m. You will receive the appropriate probenecid dose at 8:00 a.m. Probenecid doses will be taken as powdered tablets in 250 ml of water. You will not be permitted to eat until 12 noon when you will resume your normal diet. You will drink 200 ml of water 2.0, 4.0, and 8.0 hr after dosing.

Blood samples (about 10 ml) will be taken by means of a Vacutainer from your forearm vein immediately before (2 x 10 ml) and then at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0, 14.0, 16.0, 20.0, 24.0, 30.0, 36.0, and 48.0 hr after you receive the probenecid. Plasma will be separated from your blood, divided into two aliquots and the plasma will be deep frozen until analyzed for probenecid and its metabolites.

You will be required to void a urine sample immediately before you receive probenecid. You will then collect your urine quantitatively (all of it!) during 0-2, 2-6, 6-12, 12-24, 24-36, and 36-48 hr after receiving probenecid. The volume and the acidity of your urine will be measured and two 20 ml aliquots of each collection will be retained for future analysis.

Each time you receive probenecid, you will give 19 x 10 ml blood samples = 190 ml during a 48-hr period. As you will receive two doses of probenecid, you will give a total of 380 ml or less than a pint during a four-week period.

Analysis

Analysis of your plasma and urine samples for concentrations of probenecid and its metabolites will help us to determine how probenecid is handled in the body, how it is metabolized, and how rapidly it is excreted from the two different doses.

Repeated-Dose Study

The design of the repeated-dose study will be governed by the results of this and other previous studies. Details of the repeated-dose study will be described to you in the future if you would like to take part in that too.

PLEASE NOTE THE FOLLOWING IMPORTANT POINTS 266

1. Participating in this study will be of no benefit to you. Overall benefits may be obtained by the public at large and persons likely to receive probenecid therapy, in that the study will determine the basic pharmacokinetics of probenecid in the body.
2. You are free to withdraw from the study at any time.
3. If there is anything you don't understand about the study, ask one of the investigators to explain it to you before you volunteer.
4. There is a risk that you might experience an adverse reaction to probenecid. If you experience an adverse reaction or feel unwell after you have received a single dose or during repeated doses of probenecid, report it to our investigator immediately and you may withdraw from the study. There is also a small risk to you of infection due to vein puncture. This risk is minimized by fully qualified personnel using strict aseptic procedures.
5. You will receive a copy of this consent form which you may keep.
6. In order to ensure confidentiality, your name will not appear on any documents.
7. You will be paid $5.00 for each blood sample and $3.00 for each complete urine collection.
8. You will be given a card containing the names of both office and private telephone numbers of the investigators. You are free to call these at any time.
9. The policy of the University of Wisconsin is that in the event that physical injury occurs 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.

CONSENT

(Single-Dose Probenecid Study)

I have read and fully understand the details in this consent form. Anything that I did not understand has been explained to me. I agree to adhere to the protocol of the single-dose probenecid study as outlined above, to undergo a physical examination, and to give a blood sample and urine sample at that examination. I consent to receive the doses of probenecid and to give blood samples and to collect urine as indicated in the protocol. I consent also to adhere to all diet, fluid intake, and other medication restrictions in the protocol.

I understand that participation in this study will result in no direct benefit to me and that I am free to cease participation in, and to withdraw from, the study at any time.

_____ _____

Signature of Subject Date

_____

Print Name

_____ _____

Signature of Witness Date

_____

Print Name

APPENDIX III

Protocol and Consent Form
for Clinical Study II

PROTOCOL FOR THE STUDY OF PROBENECID PHARMACOKINETICS IN MAN

Arzu Selen[1], M. S.; Peter G. Welling[1], D.Sc.; and William A. Craig[2], M.D.

School of Pharmacy[1], Veterans Administration Hospital[2] and School of Medicine[2], University of Wisconsin, Madison, Wisconsin.

PROTOCOL FOR THE STUDY OF PROBENECID PHARMACOKINETICS IN MAN

Objectives

To examine the pharmacokinetics and accumulation characteristics of probenecid following single and repeated 0.5 and 1.0 g oral doses to healthy male volunteers.

Background

Conflicting reports have appeared in the pharmacokinetics of probenecid. In particular, while some reports have demonstrated that probenecid elimination from the body is saturable at therapeutic dose levels, another report has disputed this on the basis of unchanged metabolite patterns at different dose levels.

Preliminary studies in this laboratory have confirmed that probenecid elimination is dose-dependent and that the decline in probenecid plasma levels from high therapeutic doses can be accurately described by a kinetic model incorporating Michaelis Menten-type elimination.

The studies described in this protocol are designed to further characterize the pharmacokinetics of probenecid following repeated doses and

thus examine the extent of accummulation at therapeutic doses.

Investigators

Arzu Selen, M.S., School of Pharmacy, 608-263-4159.

Dr. Peter G. Welling, Ph.D., School of Pharmacy, 608-263-3986.

Dr. William A. Craig, M.D., School of Medicine, 608-256-1901 (572).

Site of Study

The clinical study will be conducted in Room 5316, School of Pharmacy, Madison. All analytical work will also be done in the School of Pharmacy.

Drug

Probenecid 500 mg tablets (Benemid®) obtained commercially.

Subjects

Subjects will be healthy male volunteers aged between 20 and 40 years and with body weight:height relationships within ± 10% of normal. Subjects will be students (not those of the investigators) and technical and administrative staff at the University of Wisconsin.

Physical Examination 273

Before being admitted to the study, subjects will undergo a complete physical examination, including gross examination, blood and urine analysis, and drug history. Participation is dependent upon all values being within normal limits. The physical examination will be at no cost to the subjects.

Consent

Having successfully completed the physical examination, subjects will be asked to read and, if agreeable, to sign the consent form. The consent form will have been previously approved by the University of Wisconsin Committee for Protection of Human Subjects (CPHS). Anything in the consent form that is not fully understood by the subject, including all risks and benefits, is explained to him. The subject is given a copy of the consent form to retain.

Confidentiality of Records

Confidentiality of records will be maintained in accordance with standard procedures at the University of Wisconsin. Medical records of all subjects are kept in a locked cabinet in Dr. Craig's office in the Veterans Administration Hospital. For the study, subjects are assigned a code number known only to the investigators.

Risks, Benefits and Withdrawl

Potential risks to subjects consist of side effects from probenecid and from multiple blood sampling procedures. These risks are minimized by administering therapeutic doses of probenecid under medical supervision and by using experienced personnel and standard aseptic procedures for blood sampling.

Subjects are advised that they will derive no direct benefits from the study. However the results obtained may result in indirect benefits to them and to the public at large in characterizing the pharmacokinetics of probenecid, thereby providing a scientific basis for the method of administration of this medicinal agent to patients.

Subjects are advised that they are free to withdraw from the study at any time.

Side Effects of Probenecid

Several side effects have been reported for probenecid. They include stomachache, vomiting, flushing, dizziness, skin rashes, dermatitis, fever, and sore gums. Subjects will be advised of these side effects so that they will recognize them if they occur.

Restrictions

Participating subjects must take no other medication for one week, and no enzyme stimulating agents such as phenobarbital for one month, before and during a study. Coffee, tea, and other caffeine or xanthine-containing

beverages or foods will not be permitted for two days before or during study.

Treatment of Adverse Effects

If a side effect is experienced as a result of the study, the condition will be treated by Dr. Craig or a medically qualified colleague at no cost to the subject. The subjects are advised that the policy of the University of Wisconsin is that, in the event that physical injury ocurs as a result of research carried out on humans, the University does not automatically provide compensation. In the case of the present study, the University does not take responsibility for untoward side effects.

Subjects may be withdrawn from the study by Dr. Craig if he considers it appropriate. Subjects are provided with names and telephone numbers of all investigators.

Protocol

This study consists of two phases which will be conducted ten days apart. Each phase will last for five days, during which blood samples will be collected according to a predetermined schedule. Six doses of probenecid will be given in each phase.

Multiple-Dose Study

Four male subjects will each receive 0.5 and 1.0 g oral doses of probenecid according to a randomized design. Subjects will report to Room

5316 at the School of Pharmacy at 7:40 a.m. on Day 1. Probenecid doses will be given as tablets with 250 ml of water at 8:00 a.m. following an overnight (10:00 p.m.) fast. Food will be further withheld until 12 noon on the day of medication, after which time normal diets are resumed. All 8:00 a.m. doses will be given with 250 ml of water after overnight fast. All 2:00 p.m. doses will be given 2 hours after the midday meal. All 8:00 p.m. doses will be given 2 hr following the evening meal. A light snack will be permitted 2 hr after the 8:00 p.m. dose.

Blood samples (10 ml) will be taken by heparinized vacutainer from a forearm vein immediately before dosing on Day 1. The blood sampling and dosing times of probenecid will be according to the following schedule:

Blood sampling and dosing times starting from time zero

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
8:00AM (0.0)*	8:00AM (24.0)	8:00AM (48.0)*	2:00AM (66.0)*	4:00AM (92.0)	8:00AM (120.0)
8:30AM (0.5)	8:00PM (36.0)	2:00PM (54.0)*	8:00AM (72.0)*	8:00AM (96.0)	
9:00AM (1.0)		8:00PM (60.0)*	10:00AM (74.0)	8:00PM (108.0)	
10:00AM (2.0)			12:00AM (76.0)		
11:00AM (3.0)			4:00PM (80.0)		
12:00AM (4.0)			8:00PM (84.0)		
2:00PM (6.0)			12:00PM (88.0)		
4:00PM (8.0)					
8:00PM (12.0)					

*Dosing of probenecid

Plasma will be separated and stored at -20°C for subsequent assay. Subjects will then give a total of 250 ml of blood during a 120-hour period.

Subjects will be confined to the study site during the first 12 hr on Day 1 and will be permitted to leave the site between plasma sampling times subsequently.

Analytical

Plasma samples will be assayed for probenecid by a high pressure liquid chromatography procedure developed in the School of Pharmacy, University of Wisconsin.

Data Analysis

Plasma levels of probenecid will be examined from pharmacokinetic and statistical viewpoints to determine the degree of drug accumulation with repeated doses. This study will determine also the ability of a kinetic model incorporating saturable or parallel saturable and nonsaturable elimination developed from single-dose data to predict drug accumulation and elimination patterns following repeated doses.

Arzu Selen

Peter G. Welling

William A. Craig

CONSENT FORM FOR THE STUDY OF PROBENECID PHARMACOKINETICS IN MAN

Arzu Selen,[1] M.S.; Peter G. Welling,[1] D.Sc.;
and William A. Craig,[2] M.D.

School of Pharmacy,[1] Veterans Administration Hospital,[2] and School of Medicine[2], University of Wisconsin, Madison, Wisconsin.

CONSENT FORM FOR THE STUDY OF PROBENECID PHARMACOKINETICS IN MAN

A study is being conducted by the School of Pharmacy, University of Wisconsin, on Probenecid Pharmacokinetics. We wish to examine the accumulation characteristics of probenecid at two different therapeutic doses.

There are two phases of this repeated dose study. Each phase will last five days, and the two phases are ten days apart. You will be given a single dose of probenecid on Day 1 and repeated doses of the drug on Day 3. You will give a total of 25 blood samples according to a predetermined schedule, during each phase.

Principal Investigators

The principal investigators carrying out the study are: Arzu Selen, School of Pharmacy, 608-263-4159; Dr. Peter G. Welling, School of Pharmacy, 608-263-3986; and Dr. William A. Craig, School of Medicine, 608-256-1901 (ext. 572).

Where the Study Will be Conducted

The study will be conducted in Room 5316, School of Pharmacy, Madison, Wisconsin 53703.

Therapeutic Uses of Probenecid

Probenecid is used for gout and gouty arthritis. It is also administered in combination with some other antibacterial drugs like penicillins, to prolong their action by slowing down their rate of excretion.

Side Effects of Probenecid

Several side effects have been reported for probenecid; they include stomachache, vomiting, flushing, dizziness, skin rashes, dermatitis, fever and sore gums. Probenecid can also react with other drugs which a patient may be taking to cause an adverse side effect.

If you do experience such a reaction, you must report it to Dr. Craig or a medically qualified colleague. If the reaction is considered by the physician to be dose-related, he/she will administer the appropriate treatment.

Dosage

You will receive 0.5 g and 1.0 g oral doses of probenecid (as 500 mg Benemid® tablets) on two separate occasions, ten days apart. The sequence of dosing and blood sampling are given in "Study Program and Details."

Study Program and Details

If after reading and fully understanding this consent form you wish to take part in the study, you will receive a complete physical examination which will be conducted by Dr. Craig or a fully qualified clinical associate at the Clinical Science Center. During this examination, you will be required to give a blood sample (10 ml) and a urine sample.

You will also be asked whether you are receiving any other medications, whether you have any allergic reactions to drugs and also your smoking and drinking habits. And we will ask you to complete a subject information sheet.

Having successfully completed the physical examination, you will be invited to sign this consent form and thereby become a participant in the study.

If you participate in a study, you will be permitted to take no other medication for one week and no enzyme stimulating agents such as phenobarbital for one month before or during a study. Coffee, tea, and other caffeine or xanthine-containing beverages or foods will not be permitted for two days before and during a study.

You will receive the two different dosages of probenecid ten days apart according to the following diagram.

The day before medication, you will eat no food and drink no liquid after 10:00 p.m. On Day 1 you will report to Room 5316 at 7:40 a.m. You will receive the appropriate probenecid dose at 8:00 a.m. Probenecid doses will be given as tablets with 250 ml of water. You will not be permitted to eat until 12 noon, when you will resume your normal diet. All 8:00 a.m. doses will be given with 250 ml of water after overnight fast. All 2:00 p.m. doses will be given 2 hours after the mid-day meal. All 8:00 p.m. doses will be given 2 hours following the evening meal. A light snack will be permitted 2 hours after the 8:00 p.m. dose.

Blood samples (about 10 ml) will be taken by means of a vacutainer from your forearm vein immediately before dosing and as scheduled during the study. Plasma will be separated from the blood and deep frozen until the analysis for probenecid.

Analysis

Analysis of your plasma samples for concentrations of probenecid will help us to determine its elimination and accumulation characteristics following single and repeated oral doses at two different dosages.

Blood sampling and dosing times starting from time zero

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
8:00 a.m. (0.0)*	8:00 a.m. (24.0)	8:00 a.m. (48.0)*	2:00 a.m. (66.0)*	4:00 a.m. (92.0)	8:00 a.m. (120.0)
8:30 a.m. (0.5)	8:00 p.m. (36.0	2:00 p.m. (54.0)*	8:00 a.m. (72.0)*	8:00 a.m. (96.0)	
9:00 a.m. (1.0)		8:00 p.m. (60.0)*	10:00 a.m. (74.0)	8:00 p.m. (108.0)	
10:00 a.m. (2.0)			12:00 p.m. (76.0)		
11:00 a.m. (3.0)			4:00 p.m. (80.0)		
12:00 p.m. (4.0)			8:00 p.m. (84.0)		
2:00 p.m. (6.0)			12:00 a.m. (88.0)		
4:00 p.m. (8.0)					
8:00 p.m. (12.0)					

* Dosing of Probenecid

PLEASE NOTE THE FOLLOWING IMPORTANT POINTS

1. Participating in this study will be of no benefit to you. Overall benefits may be obtained by the public at large and persons likely to receive probenecid therapy, in that the study will determine the basic pharmacokinetics of probenecid in the body.
2. You are free to withdraw from the study at any time.
3. If there is anything you don't understand about the study, ask one of the investigators to explain it to you before you volunteer.
4. There is a risk that you might experience an adverse reaction to probenecid. If you experience an adverse reaction or feel unwell after you have received a single dose or during repeated doses of probenecid, report it to our investigator immediately and you may withdraw from the study. There is also a small risk to you of infection due to vein puncture. This risk is minimized by fully qualified personnel using strict aseptic procedures.
5. You will receive a copy of this consent form which you may keep.
6. In order to ensure confidentiality, your name will not appear on any documents.
7. You will be paid $200 for participating in this study.
8. You will be given a card containing the names of both office and private telephone numbers of the investigators. You are free to call these at any time.
9. The policy of the University of Wisconsin is that in the event that physical injury occurs 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.

CONSENT

(Multiple-Dose Probenecid Study)

I have read and fully understand the details in this consent form. Anything that I did not understand has been explained to me. I agree to adhere to the protocol of the multiple-dose probenecid study as outlined above, to undergo a physical examination, and to give a blood sample and urine sample at that examination. I consent to receive the doses of probenecid and to give blood samples as indicated in the protocol. I consent also to adhere to all diet, fluid intake, and other medication restrictions in the protocol.

I understand that participation in this study will result in no direct benefit to me and that I am free to cease participation in, and to withdraw from, the study at any time.

_____ _____

Signature of Subject Date

_____

Print Name

_____ _____

Signature of Witness Date

_____

Print Name

APPENDIX IV

Protocol and Consent Form for Clinical Study III

PROTOCOL FOR THE STUDY OF PROBENECID PHARMACOKINETICS IN MAN AFTER REPEATED DOSING.

Arzu Selen[1], M. S.; Peter G. Welling[1], D.Sc.;
and William A. Craig[2], M.D.

School of Pharmacy[1], Veterans Administration Hospital[2] and School of Medicine[2], University of Wisconsin, Madison, Wisconsin.

PROTOCOL FOR THE STUDY OF PROBENECID PHARMACOKINETICS IN MAN FOLLOWING REPEATED DOSING.

Objectives

To examine the pharmacokinetics and accumulation characteristics of probenecid following single and repeated 1.0 g oral doses to healthy male volunteers.

Background

Conflicting reports have appeared in the pharmacokinetics of probenecid. In particular, while some reports have demonstrated that probenecid elimination from the body is saturable at therapeutic dose levels, another report has disputed this on the basis of unchanged metabolite patterns at different dose levels.

Preliminary studies in this laboratory have confirmed that probenecid elimination is dose-dependent and that the decline in probenecid plasma levels from high therapeutic doses can be accurately described by a kinetic model incorporating Michaelis Menten-type elimination.

The studies described in this protocol are designed to further characterize the pharmacokinetics of probenecid following repeated doses and thus examine the extent of accummulation at therapeutic doses.

Investigators

Arzu Selen, M.S., School of Pharmacy, 608-263-4159.

Dr. Peter G. Welling, D.Sc., School of Pharmacy, 608-263-3986.

Dr. William A. Craig, M.D., School of Medicine, 608-256-1901 (572).

Site of Study

The clinical study will be conducted in Room 5316, School of Pharmacy, Madison. All analytical work will also be done in the School of Pharmacy.

Drug

Probenecid 500 mg tablets (Benemid®) obtained commercially.

Subjects

Subjects will be healthy male volunteers aged between 20 and 40 years and with body weight:height relationships within ± 10% of normal. Subjects will be students (not those of the investigators) and technical and administrative staff at the University of Wisconsin.

Physical Examination

Before being admitted to the study, subjects will undergo a complete physical examination, including gross examination, blood and urine analysis, and drug history. Participation is dependent upon all values being within normal limits. The physical examination will be at no cost to the subjects.

Consent

Having successfully completed the physical examination, subjects will be asked to read and, if agreeable, to sign the consent form. The consent form will have been previously approved by the University of Wisconsin Committee for Protection of Human Subjects (CPHS). Anything in the consent form that is not fully understood by the subject, including all risks and benefits, is explained to him. The subject is given a copy of the consent form to retain.

Confidentiality of Records

Confidentiality of records will be maintained in accordance with standard procedures at the University of Wisconsin. Medical records of all subjects are kept in a locked cabinet in Dr. Craig's office in the Veterans Administration Hospital. For the study, subjects are assigned a code number known only to the investigators.

Risks, Benefits and Withdrawal

Potential risks to subjects consist of side effects from probenecid and from multiple blood sampling procedures. These risks are minimized by administering therapeutic doses of probenecid under medical supervision and by using experienced personnel and standard aseptic procedures for blood sampling.

Subjects are advised that they will derive no direct benefits from the study. However the results obtained may result in indirect benefits to them and to the public at large in characterizing the pharmacokinetics of probenecid, thereby providing a scientific basis for the method of administration of this medicinal agent to patients.

Subjects are advised that they are free to withdraw from the study at any time.

Side Effects of Probenecid

Several side effects have been reported for probenecid. They include stomachache, vomiting, flushing, dizziness, skin rashes, dermatitis, fever, and sore gums. Subjects will be advised of these side effects so that they will recognize them if they occur.

Restrictions

Participating subjects must take no other medication for one week, and no enzyme stimulating agents such as phenobarbital for one month, before and during a study. Coffee, tea, and other caffeine or xanthine-containing beverages or foods will not be permitted for two days before or during study.

Treatment of Adverse Effects

If a side effect is experienced as a result of the study, the condition will be treated by Dr. Craig or a medically qualified colleague at no cost to the subject. The subjects are advised that the policy of the University of Wisconsin is that, in the event that physical injury ocurs as a result of research carried out on humans, the University does not automatically provide compensation. In the case of the present study, the University does not take responsibility for untoward side effects.

Subjects may be withdrawn from the study by Dr. Craig if he considers it appropriate. Subjects are provided with names and telephone numbers of all investigators.

Protocol

This study will last for five days, during which blood samples will be collected according to a predetermined schedule. Ten doses of probenecid will be given during the study.

Multiple-Dose Study

Two male subjects will each receive 1.0 g oral doses of probenecid. Subjects will report to room 5316 at the School of Pharmacy at 5:45 a.m. on

Day 1. Probenecid doses will be given as tablets with 250 ml of water at 6:00 a.m. following overnight fast. Food will be further withheld until 8:30 a.m. Food can be consumed during a 1-hour period, 2.5 hrs after administration of each dose, until the administration of last dose on Day 4. Normal diets will be resumed 2.5 hrs after the last dose of probenecid on Day 4.

Blood samples (~ 6 ml) will be taken by heparinized vacutainer from a forearm vein immediately before dosing on day 1 and as scheduled during the study.

The blood sampling and dosing times of Probenecid will be according to the following schedule:

Blood sampling and dosing times starting from time zero

DAY	1	2	3	4	5
DOSE	6:00 a.m.	6:00 a.m., 12:00 p.m. 6:00 p.m., 12:00 a.m.	6:00 a.m., 12:00 p.m. 6:00 p.m., 12:00 a.m.	6:00 a.m.	
SAMPLING TIMES	6:00 a.m. (0.0) 6:30 a.m. (0.5) 7:00 a.m. (1.0) 8:00 a.m. (2.0) 9:00 a.m. (3.0) 10:00 a.m. (4.0) 12:00 p.m. (6.0) 2:00 p.m. (8.0) 6:00 p.m. (12.0)	6:00 a.m. (24.0) 8:00 a.m. (26.0) 6:00 p.m. (36.0) 8:00 p.m. (38.0)	6:00 a.m. (48.0) 8:00 a.m. (50.0) 6:00 p.m. (60.0) 8:00 p.m. (62.0)	6:00 a.m. (72.0) 6:30 a.m. (72.5) 7:00 a.m. (73.0) 8:00 a.m. (74.0) 9:00 a.m. (75.0) 10:00 a.m. (76.0) 12:00 p.m. (78.0) 1:00 p.m. (79.0) 2:00 p.m. (80.0) 3:00 p.m. (81.0) 4:00 p.m. (82.0) 6:00 p.m. (84.0) 8:00 p.m. (86.0) 10:00 p.m. (88.0) 12:00 a.m. (90.0)	6:00 a.m. (96.0)

Plasma will be separated and stored at -20°C for subsequent assay. Subjects will then give a total of 198 ml of blood during a 96-hour period.

Subjects will be confined to the study site during the first 6 hr on day 1 and will be permitted to leave the site between plasma sampling times subsequently.

Analytical

Plasma samples will be assayed for probenecid by a high-pressure liquid chromatography procedure developed in the School of Pharmacy, University of Wisconsin.

Data Analysis

Plasma levels of probenecid will be examined from pharmacokinetic and statistical viewpoints to determine the degree of drug accumulation with repeated doses. This study will determine also the ability of a kinetic model incorporating saturable or parallel saturable and nonsaturable elimination developed from single-dose data to predict drug accumulation and elimination patterns following repeated doses.

Arzu Selen

Peter G. Welling

William A. Craig

CONSENT FORM FOR THE STUDY OF PROBENECID PHARMACOKINETICS IN MAN AFTER REPEATED DOSING

Arzu Selen,[1] M.S.; Peter G. Welling,[1] D.Sc.;
and William A. Craig,[2] M.D.

School of Pharmacy,[1] Veterans Administration Hospital,[2] and School of Medicine[2], University of Wisconsin, Madison, Wisconsin.

CONSENT FORM FOR THE STUDY OF PROBENECID PHARMACOKINETICS IN MAN AFTER REPEATED DOSING

A study is being conducted by the School of Pharmacy, University of Wisconsin, on Probenecid Pharmacokinetics. We wish to examine the accumulation characteristics of probenecid at a therapeutic dose.

You will be given 1.0 g doses of probenecid for 4 days and the study will last for five days. You will give a total of 33 blood samples according to a predetermined schedule.

Principal Investigators

The principal investigators carrying out the study are: Arzu Selen, School of Pharmacy, 608-263-4159; Dr. Peter G. Welling, School of Pharmacy, 608-263-3986; and Dr. William A. Craig, School of Medicine, 608-256-1901 (ext. 572).

Where the Study Will be Conducted

The study will be conducted in Room 5316, School of Pharmacy, Madison, Wisconsin 53703.

Therapeutic Uses of Probenecid

Probenecid is used for gout and gouty arthritis. It is also administered in combination with some other antibacterial drugs like penicillins, to prolong their action by slowing down their rate of excretion.

Side Effects of Probenecid

Several side effects have been reported for probenecid; they include stomachache, vomiting, flushing, dizziness, skin rashes, dermatitis, fever and sore gums. Probenecid can also react with other drugs which a patient may be taking to cause an adverse side effect.

If you do experience such a reaction, you must report it to Dr. Craig or a medically qualified colleague. If the reaction is considered by the physician to be dose-related, he/she will administer the appropriate treatment.

Dosage

You will receive 1.0 g oral doses of probenecid (as 500 mg Benemid® tablets). The sequences of dosing and blood sampling are given in "Study Program and Details."

Study Program and Details

If after reading and fully understanding this consent form you wish to take part in the study, you will receive a complete physical examination which will be conducted by Dr. Craig or a fully qualified clinical associate at the Clinical Science Center. During this examination, you will be required to give a blood sample (10 ml) and a urine sample.

You will also be asked whether you are receiving any other medications, whether you have any allergic reactions to drugs and also your smoking and drinking habits. And we will ask you to complete a subject information sheet.

Having successfully completed the physical examination, you will be invited to sign this consent form and thereby become a participant in the study.

If you participate in a study, you will be permitted to take no other medication for one week and no enzyme stimulating agents such as phenobarbital for one month before or during the study. Coffee, tea, and other caffeine or xanthine-containing beverages or foods will not be permitted for 2 days before and during the study.

You will receive 1.0 g doses of probenecid according to the following diagram.

You will eat no food and drink no liquid after 10:00 p.m. on the day before the study. On Day 1 you will report to Room 5316 at 5:45 a.m. You will receive two probenecid tablets with 250 mls of water, at 6:00 a.m. Food will be withheld until 8:30 a.m. Until the administration of last dose on Day 4, food can be consumed during a 1-hour period, 2.5 hours after the administration of each dose.

Normal diets will be resumed after 8:30 a.m. on Day 4.

Blood samples (about 6 ml) will be taken by means of a vacutainer from your forearm vein immediately before dosing and as scheduled during the study. Plasma will be separated from the blood and deep frozen until the analysis for probenecid.

Analysis

Analysis of your plasma samples for concentrations of probenecid will help us to determine its elimination and accumulation characteristics following single and repeated oral doses.

Blood sampling and dosing times starting from time zero

DAY	1	2	3	4	5
DOSE	6:00 a.m.	6:00 a.m., 12:00 p.m. 6:00 p.m., 12:00 a.m.	6:00 a.m., 12:00 p.m. 6:00 p.m., 12:00 a.m.	6:00 a.m.	
SAMPLING TIMES	6:00 a.m. (0.0) 6:30 a.m. (0.5) 7:00 a.m. (1.0) 8:00 a.m. (2.0) 9:00 a.m. (3.0) 10:00 a.m. (4.0) 12:00 p.m. (6.0) 2:00 p.m. (8.0) 6:00 p.m. (12.0)	6:00 a.m. (24.0) 8:00 a.m. (26.0) 6:00 p.m. (36.0) 8:00 p.m. (38.0)	6:00 a.m. (48.0) 8:00 a.m. (50.0) 6:00 p.m. (60.0) 8:00 p.m. (62.0)	6:00 a.m. (72.0) 6:30 a.m. (72.5) 7:00 a.m. (73.0) 8:00 a.m. (74.0) 9:00 a.m. (75.0) 10:00 a.m. (76.0) 12:00 p.m. (78.0) 1:00 p.m. (79.0) 2:00 p.m. (80.0) 3:00 p.m. (81.0) 4:00 p.m. (82.0) 6:00 p.m. (84.0) 8:00 p.m. (86.0) 10:00 p.m. (88.0) 12:00 a.m. (90.0)	6:00 a.m. (96.0)

PLEASE NOTE THE FOLLOWING IMPORTANT POINTS

1. Participating in this study will be of no benefit to you. Overall benefits may be obtained by the public at large and persons likely to receive probenecid therapy, in that the study will determine the basic pharmacokinetics of probenecid in the body.
2. You are free to withdraw from the study at any time.
3. If there is anything you don't understand about the study, ask one of the investigators to explain it to you before you volunteer.
4. There is a risk that you might experience an adverse reaction to probenecid. If you experience an adverse reaction or feel unwell after you have received a single dose or during repeated doses of probenecid, report it to our investigator immediately and you may withdraw from the study. There is also a small risk to you of infection due to vein puncture. This risk is minimized by fully qualified personnel using strict aseptic procedures.
5. You will receive a copy of this consent form which you may keep.
6. In order to ensure confidentiality, your name will not appear on any documents.
7. You will be paid $165 for the study.
8. You will be given a card containing the names of both office and private telephone numbers of the investigators. You are free to call these at any time.
9. The policy of the University of Wisconsin is that in the event that physical injury occurs 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.

CONSENT

(Multiple-Dose Probenecid Study)

I have read and fully understand the details in this consent form. Anything that I did not understand has been explained to me. I agree to adhere to the protocol of the multiple-dose probenecid study as outlined above, to undergo a physical examination, and to give a blood sample and urine sample at that examination. I consent to receive the doses of probenecid and to give blood samples as indicated in the protocol. I consent also to adhere to all diet, fluid intake, and other medication restrictions in the protocol.

I understand that participation in this study will result in no direct benefit to me and that I am free to cease participation in, and to withdraw from, the study at any time.

_____ _____

Signature of Subject Date

_____

Print Name

_____ _____

Signature of Witness Date

_____

Print Name

APPENDIX V

Example of Computer Output of First-Order Absorption and Elimination Model, Using the Program, NREG.

```
@RUN SELEN,20000,3908464260,5M,500
@SYM PRINT$,,XEROX1
@FOR,SI       ,MAIN
FORTRAN-MACC 1.17S-06/13/84-18:11:51                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/LIM/NEXP1,NEXP2,K
00106      14.            DATA FIX/7*1.0/
00110      15.            DATA DIFF/7*0.005/
00112      16.            DATA BNDLO/7*1.0E-4/
00114      17.            DATA BNDHI/7*1.0E4/
00116      18.            DATA NAMES/6HABS/1  ,6HM+E/1  ,6HFDV/1  ,6HLAG/1  ,3*6H       /
00120      19.            DATA TOL/1.5E-6,1.0E-9,0.0,1.0E-16/
00122      20.            DATA INFO/1,10,5*1/
00124      21.            DATA ITEMS/
00124      22.           11,NVAR,1,NROBS,NRPRD,0,'MARQ','NONE','NREG','CENT','REL',50/
00126      23.            READ(5,110) (IDENT(I), I=1,13)
00134      24.       110  FORMAT(13A6)
00135      25.            READ (5,120) NEXP1,NEXP2,NP
00142      26.        120 FORMAT(2X,I3,2X,I3,3X,I2)
00143      27.            NEXP = NEXP1 + NEXP2
00144      28.            ITEMS(1) = NEXP
00145      29.            ITEMS(3)=NP
00146      30.            READ(5,100)( THINT(I), I=1,NP )
00154      31.            READ (5,100) (YOBS(I,1),I=1,NEXP1)
00162      32.            READ (5,100) (T(I), I = 1,NEXP1)
00170      33.            K = NEXP1+ 1
00171      34.            IF (K.GE.NEXP) GO TO 66
00173      35.            READ (5,100) (YOBS(I,1),I=K,NEXP)
00201      36.            READ (5,100) (T(I),I=K,NEXP)
00207      37.         66 CONTINUE
00210      38.       100  FORMAT(8F10.5)
00211      39.            EXTERNAL F,DERIV
00212      40.            CALL NREG(THINT,NAMES,BNDLO,BNDHI,FIX,DIFF,THFIN,YOBS,YPRD,
00212      41.           1'NONE',F,'NONE',ITEMS,TOL,INFO,IDENT,S)
00213      42.            CALL OUTPUT(NEXP,NEXPD,THFIN,NVAR,NP,NRPRD,YPRD,YOBS)
00214      43.            END

      END OF COMPILATION:        NO  DIAGNOSTICS.
@FOR,SI    , SUBF
FORTRAN-MACC 1.17S-06/13/84-18:11:52                SUBF
00101       1.            SUBROUTINE F(TH,Y,NEXP,NVAR,NP,NRPRD)
00102       2.            DIMENSION TH(1),Y(NRPRD,1),T(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
00114      10.                IF ((T(I)-TH4).LT.0) GO TO 11
00116      11.                Y(I,1)= (TH3*TH1/(TH1-TH2))*(EXP(-TH2*(T(I)-TH4))-EXP(-TH1*(T(I)
00116      12.               1-TH4)))
00117      13.                GO TO 10
00120      14.             11 Y(I,1)= 0.0
00121      15.             10 CONTINUE
00123      16.                RETURN
00124      17.                END

       END OF COMPILATION:          NO  DIAGNOSTICS.
@FOR,SI       ,OTP
FORTRAN-MACC 1.17S-06/13/84-18:11:54                OTP
00101       1.                SUBROUTINE OUTPUT(NEXP,NEXPD,THFIN,NVAR,NP,NRPRD,YPRD,YOBS)
00102       2.                DIMENSION T(1),YOBS(1),YPRD(1)
00103       3.                COMMON T
00104       4.                COMMON/LIM/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.                YOM = 0.0
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 = 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
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
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)*(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(6,100) 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.            SDEU1 = SDEU
00172     45.            GO TO 200
00173     46.        300 SYOBS = SYOBS + SYOBS1
00174     47.            SDEU = SDEU + SDEU1
00175     48.            RS = (SYOBS-SDEU)/SYOBS
00176     49.            WRITE(6,111) RS
00201     50.        111 FORMAT(1X,'OVER ALL R-SQUARED=',F6.4)
00202     51.         66 CONTINUE
00203     52.            CALL PRPL (NEXP,NEXPD,THFIN,NVAR,NP,NRPRD,YPRD,YOBS)
00204     53.            RETURN
00205     54.            END

      END OF COMPILATION:        NO  DIAGNOSTICS.
@FOR,SI      ,PRPL
FORTRAN-MACC 1.17S-06/13/84-18:11:55            PRPL
00101      1.            SUBROUTINE PRPL (NEXP,NEXPD,THFIN,NVAR,NP,NRPRD,YPRD,YOBS)
00102      2.            DIMENSION T(1),YOBS(NRPRD,1),YPRD(NRPRD,1)
00103      3.            COMMON T
00104      4.            COMMON/LIM/NEXP1,NEXP2,K
00105      5.            MM = 0
00106      6.            CALL DEVSET(6HPRNTER)
00107      7.        200 CONTINUE
00110      8.            MM = MM + 1
00111      9.            IF(MM.EQ.1) GO TO 50
00113     10.            DO 140 I = 1,NEXP2
00116     11.            MN = NEXP1 + I
00117     12.            YOBS(I,1) = YOBS(MN,1)
00120     13.        140 T(I) = T(MN)
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( MM.EQ.2 ) GO TO 170
00137     23.            TMAX = T(1)
00140     24.            DO 15 I = 2,NEXP
00143     25.         15 TMAX = AMAX1(TMAX,T(I))
00145     26.            BMAX = TMAX/(NEXPD-NEXP1-NEXP2)
00146     27.            NM1 = NEXP1 + NEXP2 + 1
00147     28.            NM2 = NEXP1 + NEXP2 + 2
00150     29.            T(NM1) = 0.0
00151     30.            DO 30 I = NM2,NEXPD
00154     31.            J = I - 1
00155     32.         30 T(I) = T(J) + BMAX
00157     33.            IF ( MM.EQ.1 ) GO TO 160
00161     34.            GO TO 170
00162     35.        160 NEX1 = NEXP1
00163     36.            NEXP1 = NEXPD
00164     37.            K = NEXPD
```

```
00165     38.            GO TO 180
00166     39.         170 CONTINUE
00167     40.            NEXP1 = 1
00170     41.            K = 1
00171     42.            NEXP = NEXPD
00172     43.         180 CONTINUE
00173     44.            CALL F(THFIN,YPRD,NEXPD,NVAR,NP,NRPRD)
00174     45.            NEXP1 = NEX1
00175     46.            DO 20 I = 1,NEXPD
00200     47.          20 YMAX = AMAX1(YMAX,YPRD(I,1))
00202     48.            YSCLE = YMAX/8.8
00203     49.            TSCLE = TMAX/8.8
00204     50.            CALL GRAPH(T,6HLINEAR,YPRD,6HLINEAR,+NEXPD,15,5HBLANK,'TIME$$','FU
00204     51.           1NCTION VALUES$$',4HAXES,'PLOT OF OBSERVED,O''S AND PREDICTED,P''S
00204     52.           2VALUES$$',12.0,12.0)
00205     53.            CALL GRAPHM(T,'SCAL1',YOBS,'SCAL1',LU,14,5HBLANK)
00206     54.            IF (NEXP2.EQ.0) GO TO 66
00210     55.            IF(MM.EQ.1) GO TO 200
00212     56.         66 CONTINUE
00213     57.            RETURN
00214     58.            END

      END OF COMPILATION:        NO  DIAGNOSTICS.
@MAP
Collector 31R1U04 1984 Jun 13 Wed 1811:58
AFCM status of output element is UNKNOWN
Quarter/Third-word insensitive


BANKNAME(BDI)          ADDRESS LIMITS
$IBANK(4)              001000 113462      38195 IBANK WORDS DECIMAL
$DBANK(5)              114000 145333      13020 DBANK WORDS DECIMAL
STARTING ADDRESS  113265



          SEGMENT $MAIN$          001000 113462      114000 145333

UR3SRH/UWCC              $(1)     001000 001056
NEXP1$/NAGFORFUN10       $(1)     001057 001126     $(0)    114000 114000
RSTRPG/MAY82GSP          $(1)     001127 001615     $(0)    114001 114115
                         $(3)     GSPCTL            $(2)    000000
UR2MSK/UWCC              $(1)     001616 001747     $(0)    114116 114143
                                                    $(2)    BLANK$COMMON
UR1TRI/UWCC01            $(1)     001750 002135
NR1FMK/NAGNLINREG00      $(1)     002136 002377     $(0)    114144 114224
                                                    $(2)    BLANK$COMMON
NR1PTV/NAGNLINREG00      $(1)     002400 002762     $(0)    114225 114352
PAGDEF/MAY82GSP          $(1)     002763 003174     $(0)    114353 114434
                         $(3)     GSPCTL            $(2)    000000
NR1TRS/NAGNLINREG00      $(1)     003175 004031     $(0)    114435 114571
                                                    $(2)    BLANK$COMMON
NR1WSQ/NAGNLINREG00      $(1)     004032 004320     $(0)    114572 114642
                                                    $(2)    BLANK$COMMON
NR1SWP/NAGNLINREG00      $(1)     004321 004744     $(0)    114643 114710
                                                    $(2)    BLANK$COMMON
```

```
NEXP6$/NAGFORFUN12      $(1)    004745 005174
DEXP$/NAGFORFUN11       $(1)    005175 005355
DTCTCG/FEB81GSP         $(1)    005356 006655
                        $(3)    GSPCTL
URSORT/NAGUTILITY02     $(1)    006656 007475
NR1CK/NAGNLINREG00      $(1)    007476 007513
PLQQQ7/PSPRL10          $(1)    007514 007565
PLQQQ5$GSP/W39          $(1)    007566 007715
NR1SRT/NAGNLINREG00     $(1)    007716 010145

PLQQQ2/GSPV35           $(1)    010146 010345
PLQQQ1/GSPV35           $(1)    010346 010626
NR1PUT/NAGNLINREG00     $(1)    010627 010762

NR1RD/NAGNLINREG00      $(1)    010763 011064

NR1DRM/NAGNLINREG00     $(1)    011065 011144
NR1GRD/NAGNLINREG00     $(1)    011145 011305

URTIMF/NAGUTILITY00     $(1)    011306 011314
OBJTRN/GSPVD3           $(1)    011315 011640

PCOORD/GSPV04           $(1)    011641 012005
                        $(3)    GSPCTL
NR1HC/NAGNLINREG07      $(1)    012006 014554
QDASH$GSP/W39           $(1)    014555 014634

NR1WRT/NAGNLINREG00     $(1)    014635 014755

NR1DIF/NAGNLINREG00     $(1)    014756 015552
GRFUNC/GSPV76           $(1)    015553 015615
NR1SVD/NAGNLINREG00     $(1)    015616 017735

NR1LTZ/NAGNLINREG00     $(1)    017736 020022

DUB$GSP/W39             $(1)    020023 020043
NEXP9$/NAGFORFUN11      $(1)    020044 020233
DSHDEF/GSPREFPSP        $(1)    020234 020467

NEXPB$/NAGFORFUN10      $(1)    020470 020630
ATAN$/NAGFORFUN10       $(1)    020631 021071
TRWRIT/NOV82GSP         $(1)    021072 021274


NR1LTN/NAGNLINREG00     $(1)    021275 021372

PAGE/MAY82GSP           $(1)    021373 022016
                        $(3)    GSPCTL
NR1BDS/NAGNLINREG00     $(1)    022017 022102
NR1LIT/NAGNLINREG01     $(1)    022103 022253
                        $(3)    NR1BLK
ARGSET/GSPV38           $(1)    022254 022372
NR1HV/NAGNLINREG00      $(1)    022373 023336
NR1BDM/NAGNLINREG00     $(1)    023337 023414

URDATE/NAGUTILITY00     $(1)    023415 023433
URRNID/UWCC             $(1)    023434 023442

                        $(2)    114711 114770
                        $(2)    114771 115027
                        $(0)    115030 115145
                        $(2)    TCGCTL
                        $(0)    115146 115305
                        $(0)    115306 115306
                        $(0)    115307 115330
                        $(0)    115331 115360
                        $(0)    115361 115412
                        $(2)    BLANK$COMMON
                        $(0)    115413 115501
                        $(0)    115502 115577
                        $(0)    115600 115623
                        $(2)    BLANK$COMMON
                        $(0)    115624 115677
                        $(2)    BLANK$COMMON
                        $(0)    115700 115715
                        $(0)    115716 115751
                        $(2)    BLANK$COMMON
                        $(0)    115752 115752
                        $(0)    115753 116042
                        $(2)    GSPCTL
                        $(0)    116043 116070
                        $(2)    000000
                        $(0)    116071 116764
                        $(0)    116765 116771
                        $(2)    000000
                        $(0)    116772 117047
                        $(2)    BLANK$COMMON
                        $(0)    117050 117144
                        $(0)    117145 117154
                        $(0)    117155 117366
                        $(2)    BLANK$COMMON
                        $(0)    117367 117411
                        $(2)    BLANK$COMMON
                        $(0)    117412 117423
                        $(2)    117424 117445
                        $(0)    117446 117533
                        $(2)    000000
                        $(2)    117534 117555
                        $(2)    117556 117615
                        $(0)    117616 117710
                        $(4)    TCGCTL
                        $(6)    GSPCTL
                        $(0)    117711 117741
                        $(2)    BLANK$COMMON
                        $(0)    117742 120066
                        $(2)    000000
                        $(0)    120067 120120
                        $(0)    120121 120152
                        $(2)    BLANK$COMMON
                        $(0)    120153 120200
                        $(0)    120201 120356
                        $(0)    120357 120372
                        $(2)    BLANK$COMMON
                        $(0)    120373 120374
                        $(0)    120375 120375
```

```
QPAGE/GSPVD4,                $(1)    023443 023532
                                                          $(0)    120376 120402
                                                          $(2)    000000
ASINCOS$/NAGFORFUN10         $(1)    023533 023756        $(0)    120403 120430
LOCATE/GSPW50                $(1)    023757 024552        $(0)    120431 120574
                                                          $(2)    GSPCTL
NR1WK/NAGNLINREG02           $(1)    024553 027216        $(0)    120575 121043
                             $(3)    NR1BLK               $(2)    BLANK$COMMON
DSHCLR/GSPREFPSP             $(1)    027217 027256        $(0)    121044 121052
                                                          $(2)    000000
NR1BDA/NAGNLINREG00          $(1)    027257 027330        $(0)    121053 121100
                                                          $(2)    BLANK$COMMON
POSITN/GSPV54                $(1)    027331 027460        $(0)    121101 121117
                                                          $(2)    GSPCTL
DLOG$/NAGFORFUN10            $(1)    027461 027613        $(2)    121120 121225
UWERR$/NAGFORFUN02           $(1)    027614 030424        $(2)    121226 121665
DSQRT$/NAGFORFUN10           $(1)    030425 030506        $(2)    121666 121705
STDOGEN/DEC83GSP             $(1)    030507 032310        $(0)    121706 122101
                             $(3)    000000               $(2)    GSPCTL
SCALE/GSPV38                 $(1)    032311 034562        $(0)    122102 122656
TCG/DEC82GSP                 $(1)    034563 036536        $(0)    122657 123043
                             $(3)    TCGCTL               $(2)    GSPCTL
PLQQAG/GSPV38                $(1)    036537 037034        $(0)    123044 123077
NFFTI$/FORIO                 $(1)    037035 040100        $(2)    123100 123315
NR1HO/NAGNLINREG00           $(1)    040101 040644        $(0)    123316 124040
NR1BLK(COMMONBLOCK)                                               124041 124056
NR1NST/NAGNLINREG00          $(1)    040645 042347        $(0)    124057 124673
                             $(3)    NR1BLK               $(2)    BLANK$COMMON
SHOIMG/JAN84GSPCR            $(1)    042350 052215        $(0)    124674 126151
                             $(3)    TCGCTL               $(2)    GSPCTL
NFTCH$/FORIO                 $(1)    052216 052516        $(2)    126152 126207
MCORE/GSPV94                 $(1)    052517 052541
SYMBOL/MAY81GSP              $(1)    052542 052723        $(0)    126210 126227
                                                          $(2)    GSPCTL
NR1TMS/NAGNLINREG00          $(1)    052724 053010        $(0)    126230 126243
                                                          $(2)    BLANK$COMMON
NTAB$/FORIO                                               $(2)    126244 126310
EXP$/NAGFORFUN10             $(1)    053011 053113        $(2)    126311 126334
FORIO$2/FORIO                $(1)    053114 055444        $(2)    126335 131025
GRDLIN$GSP/W39               $(1)    055445 056162        $(0)    131026 131220
BASIS1/PSPRL00               $(1)    056163 056733        $(0)    131221 131424
SQRT$/NAGFORFUN10            $(1)    056734 057003        $(2)    131425 131436
GSPSET/JAN84GSP              $(1)    057004 057512        $(0)    131437 132267
                             $(3)    TCGCTL               $(2)    GSPCTL
                                                          $(4)    000000
NR1PST/NAGNLINREG00          $(1)    057513 060137        $(0)    132270 132424
                                                          $(2)    BLANK$COMMON
FORIO$4/FORIO                $(1)    060140 060433        $(2)    132425 132447
ALOG$/NAGFORFUN10            $(1)    060434 060551        $(2)    132450 132523
STDWRT/APR83GSP              $(1)    060552 060764        $(0)    132524 133255
                                                          $(4)    GSPCTL
                                                          $(6)    000000
NR1FPV/NAGNLINREG00          $(1)    060765 061172        $(0)    133256 133363
                                                          $(2)    BLANK$COMMON
TCGCTL(COMMONBLOCK)                                               133364 133371
GSPRNT/JUNE82                $(1)    061173 062252        $(0)    133372 133536
                             $(3)    TCGCTL               $(2)    GSPCTL
PLQQQ4$GSP/W39               $(1)    062253 062311        $(0)    133537 133547
```

```
FORIO$3/FORI0                $(1)    062312 062700    $(2)    133550 133556
NR1EDS/NAGNLINREG10          $(1)    062701 066446    $(0)    133557 135146
GSTXT/JAN82GSP               $(1)    066447 067010    $(0)    135147 135430
                                                      $(4)    GSPCTL
LINE/MAY83GSP                $(1)    067011 070461    $(0)    135431 135706
                                                      $(2)    GSPCTL
URXITP$/NAGUTILITY01         $(1)    070462 070541    $(2)    135707 135745
ROWVEC$GSP/W39               $(1)    070542 071021    $(0)    135746 136005
SINCOS$/NAGFORFUN10          $(1)    071022 071167    $(2)    136006 136035
VECTOR/GSPWN6                $(1)    071170 071717    $(0)    136036 136074
                                                      $(2)    GSPCTL
ERU$/SYS75R1UW1
NFFTO$/FORIO                 $(1)    071720 072310    $(2)    136075 136136
BASIS2-3/PSPRL00             $(1)    072311 072433    $(0)    136137 136143
WRTEXT/APR83GSP              $(1)    072434 073226    $(0)    136144 136251
                                                      $(2)    GSPCTL
AXLIN$GSP/NOV82CR            $(1)    073227 102175    $(0)    136252 137605
                                                      $(2)    GSPCTL
PLQQQR/GSPW58                $(1)    102176 102425    $(0)    137606 137660
NOSYM$/FORIO                 $(1)    102426 102535    $(2)    137661 137664
NREG/NAGNLINREG02            $(1)    102536 102661    $(0)    137665 137677
PLQQQH/PSPRL01               $(1)    102662 102751    $(0)    137700 137740
PLQQQE/JAN82GSP              $(1)    102752 103326    $(0)    137741 140043
                                                      $(2)    GSPCTL
000000(COMMONBLOCK)                                           140044 140125
GSPCTL(COMMONBLOCK)                                           140126 140572
GRAPH/NOV82GSP               $(1)    103327 107122    $(0)    140573 142077
                             $(3)    000000           $(2)    GSPCTL
FORIO$1/FORIO                $(1)    107123 112314    $(2)    142100 143174
LIM(COMMONBLOCK)                                              143175 143177
BLANK$COMMON(COMMONBLOCK)                                     143200 143261
PRPL                         $(1)    112315 112660    $(0)    143262 143365
                             $(3)    LIM              $(2)    BLANK$COMMON
OTP                          $(1)    112661 113156    $(0)    143366 143455
                             $(3)    LIM              $(2)    BLANK$COMMON
SUBF                         $(1)    113157 113264    $(0)    143456 143503
                             $(3)    LIM              $(2)    BLANK$COMMON
MAIN                         $(1)    113265 113462    $(0)    143504 145333
                             $(3)    LIM              $(2)    BLANK$COMMON

END MAP.  ERRORS: 0  TIME: 24.006  STORAGE: 22211/12/042577/0107077
@XQT
```

SUMMARY OF THE DATA

ITEMS

ITEMS(1): NEXP = 15 NUMBER OF EXPERIMENTS
ITEMS(2): NVAR = 1 NUMBER OF VARIABLES
ITEMS(3): NP = 4 NUMBER OF PARAMETERS

ITEMS(4): NROBS = 15 ROW DIMENSION OF ARRAY YOBS
ITEMS(5): NRPRD = 15 ROW DIMENSION OF ARRAY YPRD
ITEMS(6): NRWTS = 15 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 = 219

PARAMETERS

NO.	NAME	INITIAL VALUE	PROPORTION FOR DERIV. ESTIMATE	LOWER BOUND	UPPER BOUND
1	ABS/1	1.7800000+00	5.0000000-03	1.0000000-04	1.0000000+04
2	M+E/1	1.4000000-01	5.0000000-03	1.0000000-04	1.0000000+04
3	FDV/1	5.8620000+01	5.0000000-03	1.0000000-04	1.0000000+04
4	LAG/1	2.5000000-01	5.0000000-03	1.0000000-04	1.0000000+04

SINGULAR VALUES AND VECTORS AT INITIAL POINT

SING. VALUES		3.8597618+02	6.3432537+01	8.6288011+00	8.9926292-01
PAR.	1 ABS/1	-.0025735	.2148638	-.9703844	.1103681
	2 M+E/1	-.9997671	-.0211090	-.0024497	-.0037557
	3 FDV/1	.0038157	.0089798	-.1110493	-.9937670
	4 LAG/1	.0210835	-.9763745	-.2145139	.0152293

SETUP TIME = .035 SECONDS

THE ITERATIONS

ITERATION NO. 1		BASE POINT		TEST POINT		TEST POINT		TEST POINT		TEST POINT
SUM OF SQUARES		1.9069411+02		5.3095730+02		4.5119124+02		3.0263364+02		1.1780500+02
L		0		0		1		2		3
LAMBDA		0.0000000		0.0000000		1.0643851+00		5.8713429+00		5.7475320+01
GAMMA		1.0000000+00		1.0000000+00		1.0000000+00		1.0000000+00		1.0000000+00
ANGLE IN DEGREES		.0000		86.0573		83.7746		82.3875		82.0706
MAX. PIVOT REDUCTION		1.0000000+00		2.7586349-01		3.0530372-01		3.1924941-01		4.4446477-01
PAR. 1	ABS/1	1.7800000+00		3.2851246+00		3.1243699+00		2.9721675+00		2.4905939+00
2	M+E/1	1.4000000-01	S	1.3952472-01		1.4434906-01		1.4682407-01		1.4659246-01
3	FDV/1	5.8620000+01		5.6496291+01		5.7782604+01		5.8478502+01		5.8669482+01
4	LAG/1	2.5000000-01		5.6430078-01	S	5.4065005-01	S	5.1349961-01	S	4.0925180-01

CUMULATIVE NO. OF FUNCTION CALLS = 5 ITERATION TIME = .044 SECONDS CUMULATIVE TIME = .079 SECONDS

ITERATION NO. 10		BASE POINT		TEST POINT
SUM OF SQUARES		7.8015876+01		7.8015855+01
L		0		0
LAMBDA		0.0000000		0.0000000
GAMMA		1.0000000+00		1.0000000+00
ANGLE IN DEGREES		83.0660		83.1820
MAX. PIVOT REDUCTION		3.3803096-01		3.3815692-01
PAR. 1	ABS/1	3.0204893+00		3.0191432+00
2	M+E/1	1.5334921-01	S	1.5335977-01
3	FDV/1	6.0468792+01		6.0471290+01
4	LAG/1	4.1344995-01		4.1338697-01

CUMULATIVE NO. OF FUNCTION CALLS = 14 ITERATION TIME = .000 SECONDS CUMULATIVE TIME = .221 SECONDS

THE LAST ITERATION

ITERATION NO. 14		BASE POINT		TEST POINT
SUM OF SQUARES		7.8015847+01		7.8015850+01
L		2		3
LAMBDA		3.5384394+00		8.3050507+00
GAMMA		1.0000000+00		1.0000000+00
ANGLE IN DEGREES		80.2208		78.6217
MAX. PIVOT REDUCTION		3.6679685-01		4.5402055-01
PAR. 1	ABS/1	3.0195515+00	S	3.0195515+00
2	M+E/1	1.5335730-01		1.5335718-01
3	FDV/1	6.0470698+01		6.0470670+01
4	LAG/1 S	4.1340647-01		4.1340645-01

CUMULATIVE NO. OF FUNCTION CALLS = 24 ITERATION TIME = .013 SECONDS CUMULATIVE TIME = .316 SECONDS

ITERATION TERMINATES:
MAX. RELATIVE CHANGE IN A PARAMETER .LT. TOL(1) = 1.5000000-06

FINAL PARAMETER VALUES

SUM OF SQUARES = 7.8015848+01

			FINAL VALUE
AR.	1	ABS/1	3.0195515+00
	2	M+E/1	1.5335730-01
	3	FDV/1	6.0470698+01
	4	LAG/1	4.1340647-01

ITERATION SUMMARY

ITER NO.	SUM OF SQUARES	L	LAMBDA	GAMMA	ANGLE	NO. PIVOT FAILURES	NO. BOUNDS FAILURES	MAX. PIVOT REDUCTION	TIME IN SECONDS
0	1.9069411+02	0	0.0000000	1.0000000+00	.0000	0	0	1.0000000+00	.000
1	1.1780500+02	3	5.7475320+01	1.0000000+00	82.0706	0	0	4.4446477-01	.044
2	8.1790005+01	2	5.8713429+00	1.0000000+00	82.6750	0	0	4.5349982-01	.036
3	7.8661952+01	1	1.0643851+00	1.0000000+00	87.4563	0	0	2.8007397-01	.017
4	7.8047891+01	0	0.0000000	1.0000000+00	82.4305	0	0	2.8672995-01	.013
5	7.8021959+01	0	0.0000000	1.0000000+00	87.3749	0	0	3.3670465-01	.019
6	7.8017261+01	0	0.0000000	1.0000000+00	81.4272	0	0	3.3892511-01	.012
7	7.8016171+01	0	0.0000000	1.0000000+00	83.8433	0	0	3.3774808-01	.000
8	7.8015920+01	0	0.0000000	1.0000000+00	82.6874	0	0	3.3829552-01	.032
9	7.8015876+01	0	0.0000000	1.0000000+00	83.0660	0	0	3.3803096-01	.012
10	7.8015855+01	0	0.0000000	1.0000000+00	83.1820	0	0	3.3815692-01	.000
11	7.8015855+01	2	4.5767764+00	1.0000000+00	76.3405	0	0	3.8662998-01	.039
12	7.8015852+01	1	1.2875632+00	1.0000000+00	79.4846	0	0	3.3221741-01	.025
13	7.8015847+01	2	3.5384394+00	1.0000000+00	80.2208	0	0	3.6679685-01	.019
14	7.8015850+01	3	8.3050507+00	1.0000000+00	78.6217	0	0	4.5402055-01	.013

SINGULAR VALUES AND VECTORS AT TERMINAL POINT

SING. VALUES			3.6444465+02	1.2018584+02	4.6154328+00	1.0847225+00
PAR.	1	ABS/1	-.0023207	.0523603	-.9809399	.1871095
	2	M+E/1	-.9994493	-.0329185	-.0001857	-.0041577
	3	FDV/1	.0040116	.0033278	-.1872022	-.9823076
	4	LAG/1	.0328553	-.9980800	-.0520791	.0066779

NORMALIZING ELEMENTS AND CORRELATION MATRIX

PARAMETER NO.			1	2	3	4
NORM. ELTS.			2.7372582-01	4.7211508-03	9.0649206-01	1.5303410-02
PAR.	1	ABS/1	1.0000000			
	2	M+E/1	-.5050874	1.0000000		
	3	FDV/1	-.5948026	.8114387	1.0000000	
	4	LAG/1	.8251478	-.2922611	-.3689059	1.0000000

CONFIDENCE LIMITS ON LINEAR HYPOTHESIS

			LOWER LIMIT	FINAL VALUE	UPPER LIMIT
PAR.	1	ABS/1	1.5616075+00	3.0195515+00	4.4774954+00
	2	M+E/1	1.2821107-01	1.5335730-01	1.7850353-01
	3	FDV/1	5.5642456+01	6.0470698+01	6.5298941+01
	4	LAG/1	3.3189602-01	4.1340647-01	4.9491692-01

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	2.6550655+00	8.8615296+01	8.6100544+01	3.3840375+00	8.5706209+01	8.6100544+01
	2	M+E/1	1.4707074-01	8.3375304+01	8.3259835+01	1.5964386-01	8.2924319+01	8.3259835+01
	3	FDV/1	5.9263638+01	8.4104457+01	8.4105124+01	6.1677759+01	8.4105816+01	8.4105124+01
	4	LAG/1	3.9302886-01	8.3667624+01	8.4050487+01	4.3378408-01	8.4462969+01	8.4050487+01

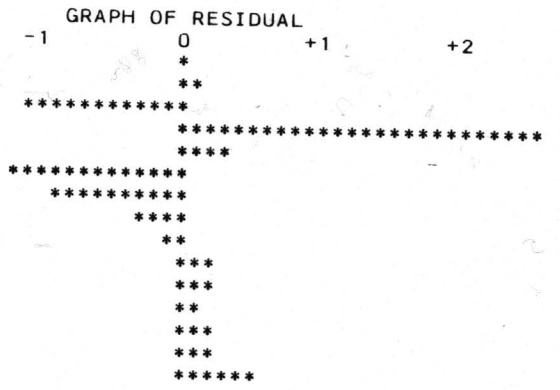
F I N A L F U N C T I O N V A L U E S A N D R E S I D U A L S

ROOT MEAN SQUARE RESIDUAL = 2.6631466+00
THIS IS THE SCALE UNIT IN THE GRAPH OF THE RESIDUALS.

NO. DEGREES OF FREEDOM = 11

EXPT. NO.	VAR. NO.	PREDICTION	OBSERVATION	RESIDUAL
1	1	0.0000000	0.0000000	0.0000000
2	1	2.0210463+01	2.0560000+01	3.4953713-01
3	1	4.8059132+01	4.5140000+01	-2.9191322+00
4	1	5.1456695+01	5.8410000+01	6.9533052+00
5	1	4.8474948+01	4.9190000+01	7.1505117-01
6	1	4.5941336+01	4.2720000+01	-3.2213359+00
7	1	4.2625600+01	4.0100000+01	-2.5256000+00
8	1	3.6584317+01	3.5660000+01	-9.2431688-01
9	1	3.1144088+01	3.0820000+01	-3.2408762-01
10	1	2.7045946+01	2.7690000+01	6.4405346-01
11	1	1.9539143+01	1.9970000+01	4.3085670-01
12	1	1.0694457+01	1.0950000+01	2.5554276-01
13	1	7.7498638+00	8.4100000+00	6.6013622-01
14	1	5.8087392+00	6.3099999+00	5.0126070-01
15	1	3.0880243+00	4.2900000+00	1.2019757+00

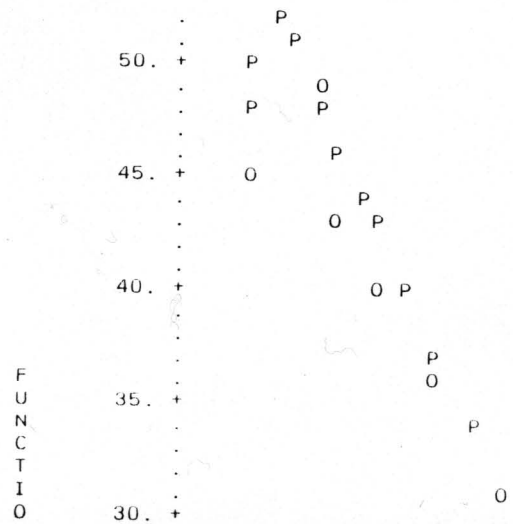
GRAPH OF RESIDUAL

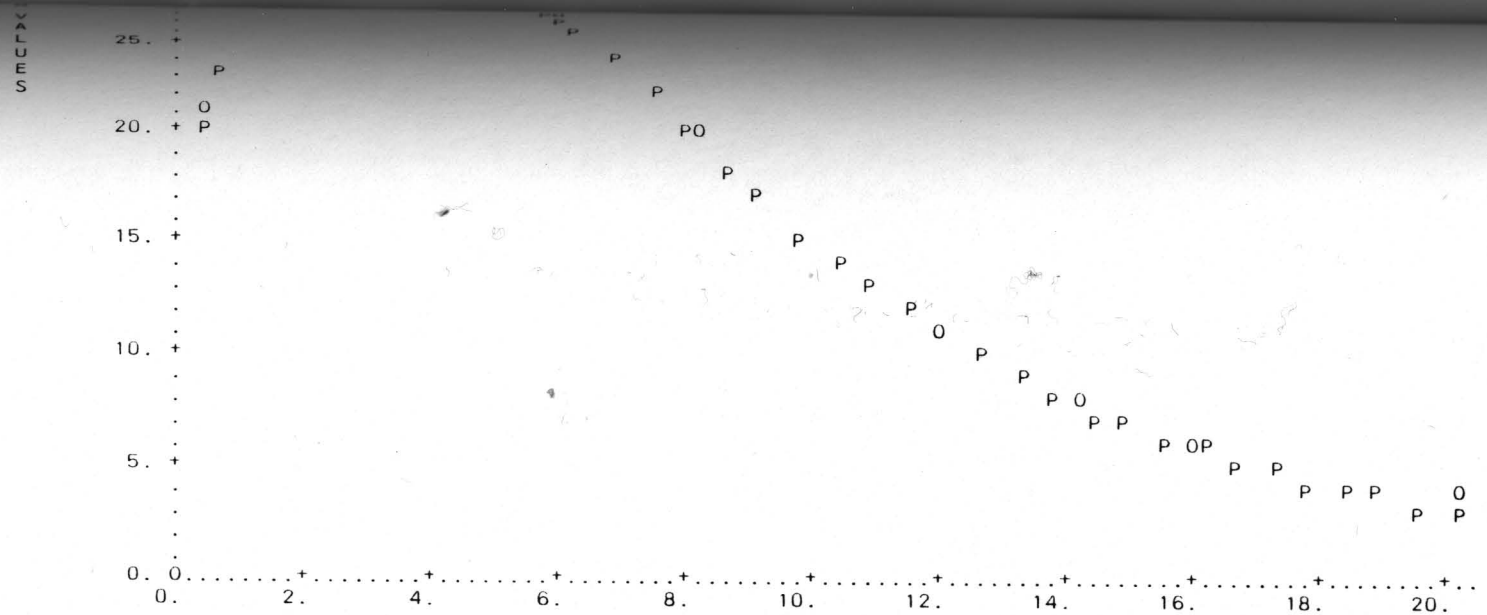


TIME SINCE END OF THE LAST ITERATION = .166 SECONDS TOTAL TIME = .482 SECONDS

R-SQUARED FOR FUNCTION1 = .9949
COR FOR FUNCTION1 = .9917

PLOT OF OBSERVED,O'S AND PREDICTED,P'S VALUES






```
@FIN

    RUNID:  AD2502   PROJECT: 20000            USER: 3908464260
18:12:22    AD2502 FIN

  ITEM                                AMOUNT       COST

CPU TIME                        00:00:02.795        $0.08
EXEC CHARGES                              10        $0.11
SOFTWARE SUPPORT                          22        $0.25
FILE I/O REQUESTS                        891        $0.15
FILE I/O WORDS                       1138685        $0.08
MEMORY USAGE                           0.285        $0.12
CARDS IN                                 188        $0.03
PAGES PRINTED                             14        $0.25
SHARED FILES                               1        $0.07
JOB CHARGE                                 1        $0.04

TOTAL COST                                          $1.18

USER BALANCE       $5.47

RUN TIME: 18:11:50 --> 18:12:22 JUN 13,84.  PREV: 10:39:02 JUN 13,84
THE $ AMOUNTS ARE APPROXIMATE
RATE MULTIPLIERS    0.50 OVERNIGHT
                    1.00 UW MADISON
```

APPENDIX VI

Example of Computer Output of First-Order Absorption and Saturable Elimination Model, Using the Coupled Programs NREG and DGEAR.

```
* * * * * * SPERRY 1180 TIME/SHARING EXEC --- MULTI-PROCESSOR SYSTEM --- VER.  MACC 38.22  SITE *  MACC      * * * * * *
RUNID: AD2489        PROJECT: 05994        USER: 3908464260          CREATED ON JUN 12, 1984 AT 21:51:30    INPUT DEVICE: NACR3
          FILE NAME: PR@000AD2489          PART NUMBER: 00          PRINTED ON JUN 12, 1984 AT 21:51:57   OUTPUT DEVICE: NAPR2


@RUN SELEN,5994,3908464260,5M,500
@SYM PRINT$,,XEROX1
@ASG,AX NREG.
@FOR,SI MAIN
FORTRAN-MACC 1.17S-06/12/84-21:51:31 (,0)       MAIN
00101      1.            PARAMETER NEXP=7,NVAR=1,NP=4,NROBS=NEXP,NRPRD=NEXP,
00101      2.           *NS=(17+NEXP*NVAR)*NP+2*NEXP*NVAR+2*(NP+1)*(NP+2)+1
00102      3.            REAL THINT(NP),THFIN(NP),YOBS(NROBS),YPRD(NRPRD),S(NS),TOL(4)
00103      4.            INTEGER ITEMS(12),INFO(7),ITITLE(1)
00104      5.            REAL DIFF(NP)
00105      6.            DIMENSION X(7),FIX(4),BNDLO(4),BNDHI(4)
00106      7.            COMMON /XX/X,ITEST
00107      8.            COMMON/LIM/NEXP1,NEXP2,K
00110      9.            DATA FIX/4*1.0/
00112     10.            DATA TOL/1.0E-5,0.0,0.0,1.0E-5/
00114     11.            DATA DIFF/4*0.005/
00116     12.            DATA BNDLO/70.0,0.8,50.0,2.0/
00120     13.            DATA BNDHI/100.0,1.0,130.0,30.0/
00122     14.            DATA ITEMS/NEXP,NVAR,NP,NROBS,NRPRD,0,'GAUSS','NONE',
00122     15.           *'NREG','SIDE','REL',50/
00124     16.            DATA INFO/1,1,5*1/
00126     17.            DATA ITITLE/'TEST$$'/
00130     18.            DATA (X(I),I=1,7)/2.0,4.0,8.0,12.0,16.0,24.0,36.0/
00132     19.            DATA (YOBS(I),I=1,7)/168.09,133.18,100.52,67.33,43.82,19.23,2.96/
00134     20.            NEXP1=NEXP
00135     21.            NEXP2=0
00136     22.            K=1
00137     23.            EXTERNAL F
00140     24.            READ(-,-)THINT,ITEST
00147     25.            CALL NREG(THINT,'OMIT',BNDLO,BNDHI,FIX,DIFF,THFIN,YOBS,YPRD,
00147     26.           *WTS,F,DERIV,ITEMS,TOL,INFO,ITITLE,S)
00150     27.            CALL OUTPUT(NEXP,NEXP,THFIN,NVAR,NP,NRPRD,YPRD,YOBS)
00151     28.            STOP
00152     29.            END

      END OF COMPILATION:        NO  DIAGNOSTICS.
@FOR,SI F
FORTRAN-MACC 1.17S-06/12/84-21:51:33 (,0)       F
00101      1.            SUBROUTINE F(TH,Y,NEXP,NVAR,NP,NRPRD)
00102      2.            REAL TH(4),Y(1)
00103      3.            REAL THO(4)
00104      4.            REAL X(7)
00105      5.            REAL WK(12)
00106      6.            COMMON/T/THO
00107      7.            COMMON /XX/X,ITEST
00110      8.            EXTERNAL FCN,FCNJ
00111      9.            DO 9 J=1,4
00114     10.          9  THO(J)=TH(J)
00116     11.            XX=0.0
00117     12.            YY=106.03
00120     13.            H=1.0E-5
00121     14.            INDEX=1
00122     15.            TOLC=0.0008
```

```
00123      16.            METH=2
00124      17.            MITER=1
00125      18.            DO 10 I=1,NEXP
00130      19.            XFIN=X(I)
00131      20.            CALL DGEAR(1,FCN,FCNJ,XX,H,YY,XFIN,TOLC,METH,MITER,INDEX,
00131      21.           *IWK,WK,IER)
00132      22.            IF(IER .GT.128)GOTO 100
00134      23.            Y(I)=YY
00135      24.        10  CONTINUE
00137      25.            IF(ITEST .NE. 0)GO TO 20
00141      26.            WRITE(-,-)(Y(I),I=1,7)
00147      27.            STOP
00150      28.        20  CONTINUE
00151      29.            RETURN
00152      30.        100 WRITE(6,110)IER
00155      31.        110 FORMAT(' ....ERROR',I5)
00156      32.            RETURN
00157      33.            END

      END OF COMPILATION:         NO  DIAGNOSTICS.
@FOR,SI FCN
FORTRAN-MACC 1.17S-06/12/84-21:51:34 (,0)        FCN
00101       1.            SUBROUTINE FCN(N,X,Y,DY)
00102       2.            DIMENSION DY(N),Y(N)
00103       3.            REAL TH(4)
00104       4.            COMMON/T/TH
00105       5.            TH1=TH(1)
00106       6.            TH2=TH(2)
00107       7.            TH3=TH(3)
00110       8.            TH4=TH(4)
00111       9.            DY(1)=TH1*TH2*EXP(-TH2*X)-TH4*Y(1)/(TH3+Y(1))
00112      10.            RETURN
00113      11.            END

      END OF COMPILATION:         NO  DIAGNOSTICS.
@FOR,SI FCNJ
FORTRAN-MACC 1.17S-06/12/84-21:51:35 (,0)        FCNJ
00101       1.            SUBROUTINE FCNJ(N,X,Y,PD)
00102       2.            REAL Y(N),PD(N,N)
00103       3.            REAL TH(4)
00104       4.            COMMON/T/TH
00105       5.            TH3=TH(3)
00106       6.            TH4=TH(4)
00107       7.            PD(1,1)=-TH3*TH4/((TH3+Y(1))**2)
00110       8.            RETURN
00111       9.            END

      END OF COMPILATION:         NO  DIAGNOSTICS.
@FOR,SI      ,OTP
FORTRAN-MACC 1.17S-06/12/84-21:51:36             OTP
00101       1.            SUBROUTINE OUTPUT(NEXP,NEXPD,THFIN,NVAR,NP,NRPRD,YPRD,YOBS)
00102       2.            DIMENSION T(1),YOBS(1),YPRD(1)
00103       3.            COMMON /XX/T,ITEST
00104       4.            COMMON/LIM/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.            YOM = 0.0
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 = 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
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
00141     29.            RS = (SYOBS-SDEV)/SYOBS
00142     30.            YOM=YOM/NEXP
00143     31.            DO 20 I=1,NEXP
00146     32.            SC = SC+(YOBS(I)-YOM)*(YPRD(I)-YPM)
00147     33.            SO2 = SO2 + (YOBS(I)-YOM)**2
00150     34.            SP2 = SP2 + (YPRD(I)-YPM)**2
00151     35.         20 CONTINUE
00153     36.            COR = SC/(SQRT(SO2)*SQRT(SP2))
00154     37.            WRITE(6,100) K,RS,K,COR
00162     38.        100 FORMAT(1HO,'R-SQUARED FOR FUNCTION',I1,' =',F6.4/1X,'COR FOR FUNCT
00162     39.           1ION',I1,' =',F6.4)
00163     40.            IF (NEXP2.EQ.0) GO TO 66
00165     41.            IF(K.GT.1) GO TO 300
00167     42.            SYOBS1 = SYOBS
00170     43.            SDEU1 = SDEU
00171     44.            GO TO 200
00172     45.        300 SYOBS = SYOBS + SYOBS1
00173     46.            SDEU = SDEU + SDEU1
00174     47.            RS = (SYOBS-SDEU)/SYOBS
00175     48.            WRITE(6,111) RS
00200     49.        111 FORMAT(1X,'OVER ALL R-SQUARED=',F6.4)
00201     50.         66 CONTINUE
00202     51.            RETURN
00203     52.            END

      END OF COMPILATION:        NO  DIAGNOSTICS.
@PACK NREG.
FURPUR 28R3A U02 SLIB34 06/12/84 21:51:38
END PACK. TEXT=15,TOC=1,ABS=1
@MAP ,NREG.ABS
Collector 31R1U04 1984 Jun 12 Tue 2151:38
      1.                  LIB MACC*IMSL.

AFCM status of output element is UNKNOWN
Quarter/Third-word insensitive
```

```
BANKNAME(BDI)          ADDRESS LIMITS
$IBANK(4)              001000 047663      19892 IBANK WORDS DECIMAL
$DBANK(5)              050000 065414       6925 DBANK WORDS DECIMAL
STARTING ADDRESS  047600


          SEGMENT $MAIN$          001000 047663      050000 065414

UR3SRH/UWCC              $(1)    001000 001056
NEXP1$/NAGFORFUN10       $(1)    001057 001126    $(0)    050000 050000
UR2MSK/UWCC              $(1)    001127 001260    $(0)    050001 050026
                                                  $(2)    BLANK$COMMON
UR1TRI/UWCC01            $(1)    001261 001446
NR1FMK/NAGNLINREG00      $(1)    001447 001710    $(0)    050027 050107
                                                  $(2)    BLANK$COMMON
NR1PTV/NAGNLINREG00      $(1)    001711 002273    $(0)    050110 050235
NR1TRS/NAGNLINREG00      $(1)    002274 003130    $(0)    050236 050372
                                                  $(2)    BLANK$COMMON
NR1WSQ/NAGNLINREG00      $(1)    003131 003417    $(0)    050373 050443
                                                  $(2)    BLANK$COMMON
NR1SWP/NAGNLINREG00      $(1)    003420 004043    $(0)    050444 050511
                                                  $(2)    BLANK$COMMON
URSORT/NAGUTILITY02      $(1)    004044 004663    $(0)    050512 050651
NR1CK/NAGNLINREG00       $(1)    004664 004701    $(0)    050652 050652
NR1SRT/NAGNLINREG00      $(1)    004702 005131    $(0)    050653 050704
                                                  $(2)    BLANK$COMMON
NR1PUT/NAGNLINREG00      $(1)    005132 005265    $(0)    050705 050730
                                                  $(2)    BLANK$COMMON
NR1RD/NAGNLINREG00       $(1)    005266 005367    $(0)    050731 051004
                                                  $(2)    BLANK$COMMON
NR1DRM/NAGNLINREG00      $(1)    005370 005447    $(0)    051005 051022
NR1GRD/NAGNLINREG00      $(1)    005450 005610    $(0)    051023 051056
                                                  $(2)    BLANK$COMMON
URTIMF/NAGUTILITY00      $(1)    005611 005617    $(0)    051057 051057
NR1HC/NAGNLINREG07       $(1)    005620 010366    $(0)    051060 051753
NOSYM$/FORIO             $(1)    010367 010476    $(2)    051754 051757
NR1WRT/NAGNLINREG00      $(1)    010477 010617    $(0)    051760 052035
                                                  $(2)    BLANK$COMMON
NR1DIF/NAGNLINREG00      $(1)    010620 011414    $(0)    052036 052132
NR1SVD/NAGNLINREG00      $(1)    011415 013534    $(0)    052133 052344
                                                  $(2)    BLANK$COMMON
NR1LTZ/NAGNLINREG00      $(1)    013535 013621    $(0)    052345 052367
                                                  $(2)    BLANK$COMMON
NR1LTN/NAGNLINREG00      $(1)    013622 013717    $(0)    052370 052420
                                                  $(2)    BLANK$COMMON
NR1BDS/NAGNLINREG00      $(1)    013720 014003    $(0)    052421 052452
NR1LIT/NAGNLINREG01      $(1)    014004 014154    $(0)    052453 052504
                         $(3)    NR1BLK           $(2)    BLANK$COMMON
NR1HV/NAGNLINREG00       $(1)    014155 015120    $(0)    052505 052662
NR1BDM/NAGNLINREG00      $(1)    015121 015176    $(0)    052663 052676
                                                  $(2)    BLANK$COMMON
URRNID/UWCC              $(1)    015177 015205    $(0)    052677 052677
URDATE/NAGUTILITY00      $(1)    015206 015224    $(0)    052700 052701
ASINCOS$/NAGFORFUN10     $(1)    015225 015450    $(0)    052702 052727
```

```
NR1WK/NAGNLINREG02          $(1)    015451 020114    $(0)    052730 053176
                            $(3)    NR1BLK           $(2)    BLANK$COMMON
NR1BDA/NAGNLINREG00         $(1)    020115 020166    $(0)    053177 053224
                                                     $(2)    BLANK$COMMON
UWERR$/NAGFORFUN02          $(1)    020167 020777    $(2)    053225 053664
NR1HO/NAGNLINREG00          $(1)    021000 021543    $(0)    053665 054407
NR1BLK(COMMONBLOCK)                                          054410 054425
NR1NST/NAGNLINREG00         $(1)    021544 023246    $(0)    054426 055242
                            $(3)    NR1BLK           $(2)    BLANK$COMMON
NFFTI$/FORIO                $(1)    023247 024312    $(2)    055243 055460
NEXP6$/NAGFORFUN12          $(1)    024313 024542    $(2)    055461 055540
NTAB$/FORIO                                          $(2)    055541 055605
NR1TMS/NAGNLINREG00         $(1)    024543 024627    $(0)    055606 055621
                                                     $(2)    BLANK$COMMON
EXP$/NAGFORFUN10            $(1)    024630 024732    $(2)    055622 055645
FORIO$2/FORIO               $(1)    024733 027263    $(2)    055646 060336
SQRT$/NAGFORFUN10           $(1)    027264 027333    $(2)    060337 060350
NR1PST/NAGNLINREG00         $(1)    027334 027760    $(0)    060351 060505
                                                     $(2)    BLANK$COMMON
BLANK$COMMON(COMMONBLOCK)
NR1FPV/NAGNLINREG00         $(1)    027761 030166    $(0)    060506 060613
                                                     $(2)    BLANK$COMMON
FORIO$3/FORIO               $(1)    030167 030555    $(2)    060614 060622
NR1EDS/NAGNLINREG10         $(1)    030556 034323    $(0)    060623 062212
URXITP$/NAGUTILITY01        $(1)    034324 034403    $(2)    062213 062251
ERU$/SYS75R1UW1
NFFTO$/FORIO                $(1)    034404 034774    $(2)    062252 062313
NEXP5$/NAGFORFUN11          $(1)    034775 035104    $(2)    062314 062323
NREG/NAGNLINREG02           $(1)    035105 035230    $(0)    062324 062336
FORIO$1/FORIO               $(1)    035231 040422    $(2)    062337 063433
LUDATF/IMSL07               $(1)    040423 041451    $(0)    063434 063540
LUELMF/IMSL07               $(1)    041452 041712    $(0)    063541 063604
DGRIN/IMSL07                $(1)    041713 042025    $(0)    063605 063634
                                                     $(2)    GEAR
DGRPS/IMSL07                $(1)    042026 042312    $(0)    063635 063677
                                                     $(2)    GEAR
DGRCS/IMSL07                $(1)    042313 042772    $(0)    063700 064175
DGRST/IMSL07                $(1)    042773 045317    $(0)    064176 064266
                                                     $(2)    GEAR
UGETIO/IMSL07               $(1)    045320 045370    $(0)    064267 064275
UERTST/IMSL07               $(1)    045371 045727    $(0)    064276 064377
GEAR(COMMONBLOCK)                                            064400 064531
DGEAR/IMSL0701              $(1)    045730 047061    $(0)    064532 064600
                                                     $(2)    GEAR
T(COMMONBLOCK)                                               064601 064604
LIM(COMMONBLOCK)                                             064605 064607
XX(COMMONBLOCK)                                              064610 064617
OTP                         $(1)    047062 047323    $(0)    064620 064710
                            $(3)    LIM              $(2)    XX
FCNJ                        $(1)    047324 047356    $(0)    064711 064723
                                                     $(2)    T
FCN                         $(1)    047357 047431    $(0)    064724 064743
                                                     $(2)    T
F                           $(1)    047432 047577    $(0)    064744 065030
                            $(3)    XX               $(2)    T
MAIN                        $(1)    047600 047663    $(0)    065031 065414
                            $(3)    LIM              $(2)    XX
```

```
END MAP.  ERRORS: 0  TIME: 15.641  STORAGE: 20611/9/042677/0103677
@XQT NREG.ABS
```

SUMMARY OF THE DATA

ITEMS

```
ITEMS(1): NEXP   =     7   NUMBER OF EXPERIMENTS
ITEMS(2): NVAR   =     1   NUMBER OF VARIABLES
ITEMS(3): NP     =     4   NUMBER OF PARAMETERS

ITEMS(4): NROBS  =     7   ROW DIMENSION OF ARRAY YOBS
ITEMS(5): NRPRD  =     7   ROW DIMENSION OF ARRAY YPRD
ITEMS(6): NRWTS  =     7   ROW DIMENSION OF ARRAY WTS

ITEMS(7): METHOD = 'GAUSS'   GAUSS'S METHOD
ITEMS(8): KWTS   = 'NONE'    NO WEIGHTING
ITEMS(9): KDERIV = 'NREG'    NREG CALCULATED DERIVATIVES
ITEMS(10): KSYM  = 'SIDE'    ONE SIDED DIFFERENCES
ITEMS(11): KDIFF = 'REL'     RELATIVE DIFFERENCES

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

TOLERANCES

```
TOL(1) =  1.0000000-05  REL. CHANGE IN A PARAMETER
TOL(2) =  0.0000000     REL. CHANGE IN SUM OF SQUARES
TOL(3) =  0.0000000     RATIO TO INITIAL SUM OF SQUARES
TOL(4) =  1.0000000-05  PIVOT TOLERANCE
```

SUNDRIES

```
NUMBER OF ACTIVE PARAMETERS =   4
NUMBER OF CELLS OF SCRATCH REQUIRED =   171
```

PARAMETERS

NO.	INITIAL VALUE	PROPORTION FOR DERIV. ESTIMATE	LOWER BOUND	UPPER BOUND
1	7.5400000+01	5.0000000-03	7.0000000+01	1.0000000+02
2	8.1999999-01	5.0000000-03	8.0000000-01	1.0000000+00
3	1.3000000+02	5.0000000-03	5.0000000+01	1.3000000+02
4	2.0000000+01	5.0000000-03	2.0000000+00	3.0000000+01

SINGULAR VALUES AND VECTORS AT INITIAL POINT

SING. VALUES		4.1124445+01	7.2249629+00	4.9066493-01	4.8345479-02
PAR.	1	-.0280335	.1375427	-.9846792	.1034552
	2	-.9977494	-.0641917	.0190916	-.0033078
	3	-.0069144	.0912664	.1168463	.9889234
	4	.0605171	-.9841910	-.1280207	.1063790

SETUP TIME = .029 SECONDS

THE ITERATIONS

ITERATION NO. 1	BASE POINT		TEST POINT
SUM OF SQUARES	5.0827386+02		4.3804062+02
GAMMA	1.0000000+00		1.5106161-01
ANGLE IN DEGREES	.0000		89.7813
MAX. PIVOT REDUCTION	1.0000000+00		4.5845442-03
PAR. 1	7.5400000+01		7.1277552+01
2	8.1999999-01		1.0000000+00
3	1.3000000+02	S	1.0730405+02
4	2.0000000+01		1.7433109+01

CUMULATIVE NO. OF FUNCTION CALLS = 2 ITERATION TIME = .161 SECONDS CUMULATIVE TIME = .189 SECONDS

ITERATION NO. 2	BASE POINT		TEST POINT
SUM OF SQUARES	4.3804062+02		3.7581532+02
GAMMA	1.0000000+00		1.3630521-01
ANGLE IN DEGREES	89.7813		82.7047
MAX. PIVOT REDUCTION	4.5845442-03		1.2755963-02
PAR. 1	7.1277552+01		7.4171602+01
2	1.0000000+00	B	1.0000000+00
3	S 1.0730405+02	S	1.3000000+02
4	1.7433109+01		1.9772416+01

CUMULATIVE NO. OF FUNCTION CALLS = 3 ITERATION TIME = .131 SECONDS CUMULATIVE TIME = .321 SECONDS

ITERATION NO. 3	BASE POINT		TEST POINT
SUM OF SQUARES	3.7581532+02		2.6714646+02
GAMMA	1.0000000+00		1.0000000+00
ANGLE IN DEGREES	82.7047		2.4874
MAX. PIVOT REDUCTION	1.2755963-02		4.7746508-03
PAR. 1	7.4171602+01		8.5947979+01
2	B 1.0000000+00	B	1.0000000+00
3	S 1.3000000+02	B	1.3000000+02
4	1.9772416+01	S	2.1913694+01

CUMULATIVE NO. OF FUNCTION CALLS = 4 ITERATION TIME = .133 SECONDS CUMULATIVE TIME = .453 SECONDS

THE ITERATIONS

ITERATION NO. 4	BASE POINT	TEST POINT
SUM OF SQUARES	2.6714646+02	2.6688514+02
GAMMA	1.0000000+00	1.0000000+00
ANGLE IN DEGREES	2.4874	62.3769
MAX. PIVOT REDUCTION	4.7746508-03	2.6961226-02
PAR. 1	8.5947979+01	8.5460772+01
2	B 1.0000000+00	B 1.0000000+00
3	B 1.3000000+02	SB 1.3000000+02
4	S 2.1913694+01	2.1809531+01

CUMULATIVE NO. OF FUNCTION CALLS = 5 ITERATION TIME = .150 SECONDS CUMULATIVE TIME = .603 SECONDS

ITERATION NO. 5	BASE POINT	TEST POINT	TEST POINT	TEST POINT	TEST POINT
SUM OF SQUARES	2.6688514+02	2.6689787+02	2.6688973+02	2.6688691+02	2.6678589+02
GAMMA	1.0000000+00	1.0000000+00	5.0000000-01	2.5000000-01	1.2500000-01
ANGLE IN DEGREES	62.3769	54.1509	54.1509	54.1509	54.1509
MAX. PIVOT REDUCTION	2.6961226-02	2.9402721-02	2.940272f-02	2.9402721-02	2.9402721-02
PAR. 1	8.5460772+01	8.5542625+01	8.5501698+01	8.5481235+01	8.5471003+01
2	B 1.0000000+00	B 1.0000000+00	B 1.0000000+00	B 1.0000000+00	B 1.0000000+00
3	SB 1.3000000+02	B 1.3000000+02	B 1.3000000+02	B 1.3000000+02	B 1.3000000+02
4	2.1809531+01	S 2.1824891+01	S 2.1817211+01	S 2.1813371+01	S 2.1811451+01

CUMULATIVE NO. OF FUNCTION CALLS = 9 ITERATION TIME = .215 SECONDS CUMULATIVE TIME = .818 SECONDS

ITERATION NO. 6	BASE POINT	TEST POINT	TEST POINT	TEST POINT	TEST POINT
SUM OF SQUARES	2.6678589+02	2.6688693+02	2.6688564+02	2.6688518+02	2.6678391+02
GAMMA	1.2500000-01	2.5000000-01	1.2500000-01	6.2500000-02	3.1250000-02
ANGLE IN DEGREES	54.1509	75.7430	75.7430	75.7430	75.7430
MAX. PIVOT REDUCTION	2.9402721-02	4.6057184-02	4.6057184-02	4.6057184-02	4.6057184-02
PAR. 1	8.5471003+01	S 8.5429196+01	S 8.5450100+01	S 8.5460551+01	S 8.5465777+01
2	B 1.0000000+00	B 1.0000000+00	B 1.0000000+00	B 1.0000000+00	B 1.0000000+00
3	B 1.3000000+02	B 1.3000000+02	B 1.3000000+02	B 1.3000000+02	B 1.3000000+02
4	S 2.1811451+01	2.1808532+01	2.1809991+01	2.1810721+01	2.1811086+01

CUMULATIVE NO. OF FUNCTION CALLS = 13 ITERATION TIME = .227 SECONDS CUMULATIVE TIME = 1.045 SECONDS

THE ITERATIONS

ITERATION NO. 7	BASE POINT	TEST POINT	TEST POINT
SUM OF SQUARES	2.6678391+02	2.6688593+02	2.6677679+02
GAMMA	3.1250000-02	6.2500000-02	3.1250000-02
ANGLE IN DEGREES	75.7430	64.6134	64.6134
MAX. PIVOT REDUCTION	4.6057184-02	1.1387231-01	1.1387231-01
PAR. 1	S 8.5465777+01	S 8.5417952+01	S 8.5441865+01
2	B 1.0000000+00	B 1.0000000+00	B 1.0000000+00
3	B 1.3000000+02	B 1.3000000+02	B 1.3000000+02
4	2.1811086+01	2.1806003+01	2.1808544+01

CUMULATIVE NO. OF FUNCTION CALLS = 15 ITERATION TIME = .149 SECONDS CUMULATIVE TIME = 1.194 SECONDS

ITERATION NO. 8	BASE POINT	TEST POINT	TEST POINT	TEST POINT	TEST POINT	TEST POINT
SUM OF SQUARES	2.6677679+02	2.6688781+02	2.6688608+02	2.6688519+02	2.6688514+02	2.6688486+02
GAMMA	3.1250000-02	6.2500000-02	3.1250000-02	1.5625000-02	7.8125000-03	3.9062500-03
ANGLE IN DEGREES	64.6134	60.9574	60.9574	60.9574	60.9574	60.9574
MAX. PIVOT REDUCTION	1.1387231-01	1.1363727-01	1.1363727-01	1.1363727-01	1.1363727-01	1.1363727-01
PAR. 1	S 8.5441865+01	S 8.5396307+01	S 8.5419086+01	S 8.5430476+01	S 8.5436171+01	S 8.5439018+01
2	B 1.0000000+00	B 1.0000000+00	B 1.0000000+00	B 1.0000000+00	B 1.0000000+00	B 1.0000000+00
3	B 1.3000000+02	B 1.3000000+02	B 1.3000000+02	B 1.3000000+02	B 1.3000000+02	B 1.3000000+02
4	2.1808544+01	2.1803632+01	2.1806088+01	2.1807317+01	2.1807930+01	2.1808238+01

ITERATION NO. 8	TEST POINT	TEST POINT
SUM OF SQUARES	2.6688511+02	2.6688509+02
GAMMA	1.9531250-03	9.7656250-04
ANGLE IN DEGREES	60.9574	60.9574
MAX. PIVOT REDUCTION	1.1363727-01	1.1363727-01
PAR. 1	S 8.5440442+01	S 8.5441154+01
2	B 1.0000000+00	B 1.0000000+00
3	B 1.3000000+02	B 1.3000000+02
4	2.1808391+01	2.1808468+01

CUMULATIVE NO. OF FUNCTION CALLS = 22 ITERATION TIME = .285 SECONDS CUMULATIVE TIME = 1.479 SECONDS

ITERATION TERMINATES:
MAX. RELATIVE CHANGE IN A PARAMETER .LT. TOL(1) = 1.0000000-05

FINAL PARAMETER VALUES

FINAL VALUE SUM OF SQUARES = 2.6677679+02

PAR.	1	8.5441865+01
	2	1.0000000+00
	3	1.3000000+02
	4	2.1808544+01

ITERATION SUMMARY

ITER NO.	SUM OF SQUARES	GAMMA	ANGLE	NO. PIVOT FAILURES	NO. BOUNDS FAILURES	MAX. PIVOT REDUCTION	TIME IN SECONDS
0	5.0827386+02	1.0000000+00	.0000	0	0	1.0000000+00	.000
1	4.3804062+02	1.0000000+00	89.7813	0	0	4.5845442-03	.161
2	3.7581532+02	1.0000000+00	82.7047	0	1	1.2755963-02	.131
3	2.6714646+02	1.0000000+00	2.4874	0	2	4.7746508-03	.133
4	2.6688514+02	1.0000000+00	62.3769	0	2	2.6961226-02	.150
5	2.6678589+02	1.2500000-01	54.1509	0	2	2.9402721-02	.215
6	2.6678391+02	3.1250000-02	75.7430	0	2	4.6057184-02	.227
7	2.6677679+02	3.1250000-02	64.6134	0	2	1.1387231-01	.149
8	2.6688509+02	9.7656250-04	60.9574	0	2	1.1363727-01	.285

SINGULAR VALUES AND VECTORS AT TERMINAL POINT

SING. VALUES		3.8322920+01	5.1374125+00	6.1894527-01	1.7625988-01
PAR.	1	-.0190305	.1427132	-.9237097	-.3550086
	2	.9822010	.1873497	.0037383	.0129362
	3	-.0048400	.0819535	.3686290	-.9259443
	4	.1868038	-.9684093	-.1042067	-.1281743

NORMALIZING ELEMENTS AND CORRELATION MATRIX

PARAMETER NO.		1	2	3	4
NORM. ELTS.		2.5069284+00	8.6079755-02	5.2869670+00	7.6987422-01
PAR.	1	1.0000000			
	2	-.7221405	1.0000000		
	3	.7312760	-.8380056	1.0000000	
	4	.8863483	-.9225278	.9131652	1.0000000

CONFIDENCE LIMITS ON LINEAR HYPOTHESIS

		LOWER LIMIT	FINAL VALUE	UPPER LIMIT
PAR.	1	3.8161012+01	8.5441865+01	1.3272272+02
	2	-6.2347052-01	1.0000000+00	2.6234705+00
	3	3.0287413+01	1.3000000+02	2.2971259+02
	4	7.2886598+00	2.1808544+01	3.6328429+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		7.3621652+01	6.1736274+02	4.6241148+02		9.7262078+01	6.3571369+02	4.6241148+02
	2	B	8.0000000-01	3.8931264+02	3.2348706+02	B	1.0000000+00	2.6677679+02	2.6677679+02
	3		1.0507185+02	5.9267449+02	4.4721173+02	B	1.3000000+02	2.6677679+02	2.6677679+02
	4		1.8178573+01	1.1020034+03	1.2682821+03		2.5438515+01	8.2953298+02	1.2682821+03

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 = 9.4300369+00 NO. DEGREES OF FREEDOM = 3
THIS IS THE SCALE UNIT IN THE GRAPH OF THE RESIDUALS.

EXPT. NO.	VAR. NO.	PREDICTION	OBSERVATION	RESIDUAL	GRAPH OF RESIDUAL (-2 -1 0 +1 +2)
1	1	1.5693401+02	1.6809000+02	1.1155989+01	**********
2	1	1.4332911+02	1.3318000+02	-1.0149115+01	**********
3	1	1.0251081+02	1.0052000+02	-1.9908066+00	***
4	1	6.8255339+01	6.7330000+01	-9.2533875-01	**
5	1	4.2463935+01	4.3820000+01	1.3560648+00	**
6	1	1.3598977+01	1.9230000+01	5.6310232+00	*******
7	1	1.9856865+00	2.9600000+00	9.7431356-01	**

TIME SINCE END OF THE LAST ITERATION = .438 SECONDS TOTAL TIME = 1.917 SECONDS

R-SQUARED FOR FUNCTION1 = .9958
COR FOR FUNCTION1 = .1229
@FIN

RUNID: AD2489 PROJECT: 05994 USER: 3908464260
21:51:56 AD2489 FIN

ITEM	AMOUNT	COST
CPU TIME	00:00:03.152	$0.09
EXEC CHARGES	13	$0.14
SOFTWARE SUPPORT	24	$0.27
FILE I/O REQUESTS	677	$0.11
FILE I/O WORDS	793376	$0.05
MEMORY USAGE	0.209	$0.08
CARDS IN	148	$0.02
PAGES PRINTED	12	$0.21
SHARED FILES	2	$0.22
JOB CHARGE	1	$0.04
TOTAL COST		$1.23

RUN TIME: 21:51:30 --> 21:51:56 JUN 12,84. PREV: 21:50:25 JUN 12,84
THE $ AMOUNTS ARE APPROXIMATE
RATE MULTIPLIERS 0.50 OVERNIGHT
1.00 UW MADISON

EOF 13 PAGES COST $0.65 AD2489 56789012345678901234567890123456789O

APPENDIX VII

Example of Computer Output of First-Order Absorption and Parallel First-Order and Saturable Elimination Model, Using the Coupled Programs NREG and DGEAR.

```
@RUN SELEN,5994,3908464260,5M,500
@SYM PRINT$,,XEROX1
@ASG,AX NREG.
@FOR,SI MAIN
FORTRAN-MACC 1.17S-06/12/84-21:57:22 (,0)        MAIN
00101      1.            PARAMETER NEXP=7,NVAR=1,NP=5,NROBS=NEXP,NRPRD=NEXP,
00101      2.           *NS=(17+NEXP*NVAR)*NP+2*NEXP*NVAR+2*(NP+1)*(NP+2)+1
00102      3.            REAL THINT(NP),THFIN(NP),YOBS(NROBS),YPRD(NRPRD),S(NS),TOL(4)
00103      4.            INTEGER ITEMS(12),INFO(7),ITITLE(1)
00104      5.            REAL DIFF(NP)
00105      6.            DIMENSION X(7),FIX(5),BNDLO(5),BNDHI(5)
00106      7.            COMMON /XX/X,ITEST
00107      8.            COMMON/LIM/NEXP1,NEXP2,K
00110      9.            DATA FIX/5*1.0/
00112     10.            DATA TOL/1.0E-5,0.0,0.0,1.0E-5/
00114     11.            DATA DIFF/5*0.005/
00116     12.            DATA BNDLO/20.0,0.1,20.0,2.0,0.001/
00120     13.            DATA BNDHI/180.0,5.0,130.0,25.0,1.0/
00122     14.            DATA ITEMS/NEXP,NVAR,NP,NROBS,NRPRD,0,'GAUSS','NONE',
00122     15.           *'NREG','SIDE','REL',50/
00124     16.            DATA INFO/1,1,5*1/
00126     17.            DATA ITITLE/'TEST$$'/
00130     18.            DATA (X(I),I=1,7)/2.0,4.0,8.0,12.0,16.0,24.0,36.0/
00132     19.            DATA (YOBS(I),I=1,7)/168.09,133.18,100.52,67.33,43.82,19.23,2.96/
00134     20.            NEXP1=NEXP
00135     21.            NEXP2=0
00136     22.            K=1
00137     23.            EXTERNAL F
00140     24.            READ(-,-)THINT,ITEST
00147     25.            CALL NREG(THINT,'OMIT',BNDLO,BNDHI,FIX,DIFF,THFIN,YOBS,YPRD,
00147     26.           *WTS,F,DERIV,ITEMS,TOL,INFO,ITITLE,S)
00150     27.            CALL OUTPUT(NEXP,NEXP,THFIN,NVAR,NP,NRPRD,YPRD,YOBS)
00151     28.            STOP
00152     29.            END

      END OF COMPILATION:        NO  DIAGNOSTICS.
@FOR,SI F
FORTRAN-MACC 1.17S-06/12/84-21:57:24 (,0)        F
00101      1.            SUBROUTINE F(TH,Y,NEXP,NVAR,NP,NRPRD)
00102      2.            REAL TH(5),Y(1)
00103      3.            REAL THO(5)
00104      4.            REAL X(7)
00105      5.            REAL WK(12)
00106      6.            COMMON/T/THO
00107      7.            COMMON /XX/X,ITEST
00110      8.            EXTERNAL FCN,FCNJ
00111      9.            DO 9 J=1,5
00114     10.          9  THO(J)=TH(J)
00116     11.            XX=0.0
00117     12.            YY=106.03
00120     13.            H=1.0E-5
00121     14.            INDEX=1
00122     15.            TOLC=0.0008
```

```
00123      16.            METH=2
00124      17.            MITER=1
00125      18.            DO 10 I=1,NEXP
00130      19.            XFIN=X(I)
00131      20.            CALL DGEAR(1,FCN,FCNJ,XX,H,YY,XFIN,TOLC,METH,MITER,INDEX,
00131      21.           *IWK,WK,IER)
00132      22.            IF(IER .GT.128)GOTO 100
00134      23.            Y(I)=YY
00135      24.        10  CONTINUE
00137      25.            IF(ITEST .NE. 0)GO TO 20
00141      26.            WRITE(-,-)(Y(I),I=1,7)
00147      27.            STOP
00150      28.        20  CONTINUE
00151      29.            RETURN
00152      30.        100 WRITE(6,110)IER
00155      31.        110 FORMAT(' ....ERROR',I5)
00156      32.            RETURN
00157      33.            END

       END OF COMPILATION:         NO  DIAGNOSTICS.
@FOR,SI FCN
FORTRAN-MACC 1.17S-06/12/84-21:57:26 (,0)        FCN
00101       1.            SUBROUTINE FCN(N,X,Y,DY)
00102       2.            DIMENSION DY(N),Y(N)
00103       3.            REAL TH(5)
00104       4.            COMMON/T/TH
00105       5.            TH1=TH(1)
00106       6.            TH2=TH(2)
00107       7.            TH3=TH(3)
00110       8.            TH4=TH(4)
00111       9.            TH5=TH(5)
00112      10.            DY(1)=TH1*TH2*EXP(-TH2*X)-(TH4*Y(1)/(TH3+Y(1)))-TH5*Y(1)
00113      11.            RETURN
00114      12.            END

       END OF COMPILATION:         NO  DIAGNOSTICS.
@FOR,SI FCNJ
FORTRAN-MACC 1.17S-06/12/84-21:57:27 (,0)        FCNJ
00101       1.            SUBROUTINE FCNJ(N,X,Y,PD)
00102       2.            REAL Y(N),PD(N,N)
00103       3.            REAL TH(5)
00104       4.            COMMON/T/TH
00105       5.            TH3=TH(3)
00106       6.            TH4=TH(4)
00107       7.            TH5=TH(5)
00110       8.            PD(1,1)=-TH5-TH3*TH4/((TH3+Y(1))**2)
00111       9.            RETURN
00112      10.            END

       END OF COMPILATION:         NO  DIAGNOSTICS.
@FOR,SI       ,OTP
FORTRAN-MACC 1.17S-06/12/84-21:57:28             OTP
00101       1.            SUBROUTINE OUTPUT(NEXP,NEXPD,THFIN,NVAR,NP,NRPRD,YPRD,YOBS)
00102       2.            DIMENSION T(1),YOBS(1),YPRD(1)
00103       3.            COMMON /XX/T,ITEST
00104       4.            COMMON/LIM/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.            YOM = 0.0
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 = 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
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
00141     29.            RS = (SYOBS-SDEV)/SYOBS
00142     30.            YOM=YOM/NEXP
00143     31.            DO 20 I=1,NEXP
00146     32.            SC = SC+(YOBS(I)-YOM)*(YPRD(I)-YPM)
00147     33.            SO2 = SO2 + (YOBS(I)-YOM)**2
00150     34.            SP2 = SP2 + (YPRD(I)-YPM)**2
00151     35.         20 CONTINUE
00153     36.            COR = SC/(SQRT(SO2)*SQRT(SP2))
00154     37.            WRITE(6,100) K,RS,K,COR
00162     38.        100 FORMAT(1H0,'R-SQUARED FOR FUNCTION',I1,' =',F6.4/1X,'COR FOR FUNCT
00162     39.           1ION',I1,' =',F6.4)
00163     40.            IF (NEXP2.EQ.0) GO TO 66
00165     41.            IF(K.GT.1) GO TO 300
00167     42.            SYOBS1 = SYOBS
00170     43.            SDEU1 = SDEU
00171     44.            GO TO 200
00172     45.        300 SYOBS = SYOBS + SYOBS1
00173     46.            SDEU = SDEU + SDEU1
00174     47.            RS = (SYOBS-SDEU)/SYOBS
00175     48.            WRITE(6,111) RS
00200     49.        111 FORMAT(1X,'OVER ALL R-SQUARED=',F6.4)
00201     50.         66 CONTINUE
00202     51.            RETURN
00203     52.            END

      END OF COMPILATION:        NO  DIAGNOSTICS.
@PACK NREG.
FURPUR 28R3A U02 SLIB34 06/12/84 21:57:30
END PACK. TEXT=15,TOC=1,ABS=1
@MAP ,NREG.ABS
Collector 31R1U04 1984 Jun 12 Tue 2157:32
        1.                  LIB MACC*IMSL.

AFCM status of output element is UNKNOWN
```

```
BANKNAME(BDI)          ADDRESS LIMITS
$IBANK(4)              001000 047673      19900 IBANK WORDS DECIMAL
$DBANK(5)              050000 065507       6984 DBANK WORDS DECIMAL
STARTING ADDRESS  047610


          SEGMENT $MAIN$          001000 047673      050000 065507

UR3SRH/UWCC                $(1)   001000 001056
NEXP1$/NAGFORFUN10         $(1)   001057 001126   $(0)   050000 050000
UR2MSK/UWCC                $(1)   001127 001260   $(0)   050001 050026
                                                  $(2)   BLANK$COMMON
UR1TRI/UWCC01              $(1)   001261 001446
NR1FMK/NAGNLINREG00        $(1)   001447 001710   $(0)   050027 050107
                                                  $(2)   BLANK$COMMON
NR1PTV/NAGNLINREG00        $(1)   001711 002273   $(0)   050110 050235
NR1TRS/NAGNLINREG00        $(1)   002274 003130   $(0)   050236 050372
                                                  $(2)   BLANK$COMMON
NR1WSQ/NAGNLINREG00        $(1)   003131 003417   $(0)   050373 050443
                                                  $(2)   BLANK$COMMON
NR1SWP/NAGNLINREG00        $(1)   003420 004043   $(0)   050444 050511
                                                  $(2)   BLANK$COMMON
URSORT/NAGUTILITY02        $(1)   004044 004663   $(0)   050512 050651
NR1CK/NAGNLINREG00         $(1)   004664 004701   $(0)   050652 050652
NR1SRT/NAGNLINREG00        $(1)   004702 005131   $(0)   050653 050704
                                                  $(2)   BLANK$COMMON
NR1PUT/NAGNLINREG00        $(1)   005132 005265   $(0)   050705 050730
                                                  $(2)   BLANK$COMMON
NR1RD/NAGNLINREG00         $(1)   005266 005367   $(0)   050731 051004
                                                  $(2)   BLANK$COMMON
NR1DRM/NAGNLINREG00        $(1)   005370 005447   $(0)   051005 051022
NR1GRD/NAGNLINREG00        $(1)   005450 005610   $(0)   051023 051056
                                                  $(2)   BLANK$COMMON
URTIMF/NAGUTILITY00        $(1)   005611 005617   $(0)   051057 051057
NR1HC/NAGNLINREG07         $(1)   005620 010366   $(0)   051060 051753
NOSYM$/FORIO               $(1)   010367 010476   $(2)   051754 051757
NR1WRT/NAGNLINREG00        $(1)   010477 010617   $(0)   051760 052035
                                                  $(2)   BLANK$COMMON
NR1DIF/NAGNLINREG00        $(1)   010620 011414   $(0)   052036 052132
NR1SVD/NAGNLINREG00        $(1)   011415 013534   $(0)   052133 052344
                                                  $(2)   BLANK$COMMON
NR1LTZ/NAGNLINREG00        $(1)   013535 013621   $(0)   052345 052367
                                                  $(2)   BLANK$COMMON
NR1LTN/NAGNLINREG00        $(1)   013622 013717   $(0)   052370 052420
                                                  $(2)   BLANK$COMMON
NR1BDS/NAGNLINREG00        $(1)   013720 014003   $(0)   052421 052452
NR1LIT/NAGNLINREG01        $(1)   014004 014154   $(0)   052453 052504
                           $(3)   NR1BLK          $(2)   BLANK$COMMON
NR1HV/NAGNLINREG00         $(1)   014155 015120   $(0)   052505 052662
NR1BDM/NAGNLINREG00        $(1)   015121 015176   $(0)   052663 052676
                                                  $(2)   BLANK$COMMON
URRNID/UWCC                $(1)   015177 015205   $(0)   052677 052677
```

```
URDATE/NAGUTILITY00     $(1)    015206 015224    $(0)    052700 052701
ASINCOS$/NAGFORFUN10    $(1)    015225 015450    $(0)    052702 052727
NR1WK/NAGNLINREG02      $(1)    015451 020114    $(0)    052730 053176
                        $(3)    NR1BLK           $(2)    BLANK$COMMON
NR1BDA/NAGNLINREG00     $(1)    020115 020166    $(0)    053177 053224
                                                 $(2)    BLANK$COMMON
UWERR$/NAGFORFUN02      $(1)    020167 020777    $(2)    053225 053664
NR1HO/NAGNLINREG00      $(1)    021000 021543    $(0)    053665 054407
NR1BLK(COMMONBLOCK)                                      054410 054425
NR1NST/NAGNLINREG00     $(1)    021544 023246    $(0)    054426 055242
                        $(3)    NR1BLK           $(2)    BLANK$COMMON
NFFTI$/FORIO            $(1)    023247 024312    $(2)    055243 055460
NEXP6$/NAGFORFUN12      $(1)    024313 024542    $(2)    055461 055540
NTAB$/FORIO                                      $(2)    055541 055605
NR1TMS/NAGNLINREG00     $(1)    024543 024627    $(0)    055606 055621
                                                 $(2)    BLANK$COMMON
EXP$/NAGFORFUN10        $(1)    024630 024732    $(2)    055622 055645
FORIO$2/FORIO           $(1)    024733 027263    $(2)    055646 060336
SQRT$/NAGFORFUN10       $(1)    027264 027333    $(2)    060337 060350
NR1PST/NAGNLINREG00     $(1)    027334 027760    $(0)    060351 060505
                                                 $(2)    BLANK$COMMON
BLANK$COMMON(COMMONBLOCK)
NR1FPV/NAGNLINREG00     $(1)    027761 030166    $(0)    060506 060613
                                                 $(2)    BLANK$COMMON
FORIO$3/FORIO           $(1)    030167 030555    $(2)    060614 060622
NR1EDS/NAGNLINREG10     $(1)    030556 034323    $(0)    060623 062212
URXITP$/NAGUTILITY01    $(1)    034324 034403    $(2)    062213 062251
ERU$/SYS75R1UW1
NFFTO$/FORIO            $(1)    034404 034774    $(2)    062252 062313
NEXP5$/NAGFORFUN11      $(1)    034775 035104    $(2)    062314 062323
NREG/NAGNLINREG02       $(1)    035105 035230    $(0)    062324 062336
FORIO$1/FORIO           $(1)    035231 040422    $(2)    062337 063433
LUDATF/IMSL07           $(1)    040423 041451    $(0)    063434 063540
LUELMF/IMSL07           $(1)    041452 041712    $(0)    063541 063604
DGRIN/IMSL07            $(1)    041713 042025    $(0)    063605 063634
                                                 $(2)    GEAR
DGRPS/IMSL07            $(1)    042026 042312    $(0)    063635 063677
                                                 $(2)    GEAR
DGRCS/IMSL07            $(1)    042313 042772    $(0)    063700 064175
DGRST/IMSL07            $(1)    042773 045317    $(0)    064176 064266
                                                 $(2)    GEAR
UGETIO/IMSL07           $(1)    045320 045370    $(0)    064267 064275
UERTST/IMSL07           $(1)    045371 045727    $(0)    064276 064377
GEAR(COMMONBLOCK)                                        064400 064531
DGEAR/IMSL0701          $(1)    045730 047061    $(0)    064532 064600
                                                 $(2)    GEAR
T(COMMONBLOCK)                                           064601 064605
LIM(COMMONBLOCK)                                         064606 064610
XX(COMMONBLOCK)                                          064611 064620
OTP                     $(1)    047062 047323    $(0)    064621 064711
                        $(3)    LIM              $(2)    XX
FCNJ                    $(1)    047324 047361    $(0)    064712 064725
                                                 $(2)    T
FCN                     $(1)    047362 047441    $(0)    064726 064750
                                                 $(2)    T
F                       $(1)    047442 047607    $(0)    064751 065035
                        $(3)    XX               $(2)    T
```

```
MAIN                    $(1)    047610 047673      $(0)    065036 065507
                        $(3)    LIM                $(2)    XX
END MAP.  ERRORS: 0  TIME: 15.645  STORAGE: 20611/9/042677/0103677
@XQT NREG.ABS
```

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

ITEMS

```
ITEMS(1): NEXP   =     7  NUMBER OF EXPERIMENTS
ITEMS(2): NVAR   =     1  NUMBER OF VARIABLES
ITEMS(3): NP     =     5  NUMBER OF PARAMETERS

ITEMS(4): NROBS  =     7  ROW DIMENSION OF ARRAY YOBS
ITEMS(5): NRPRD  =     7  ROW DIMENSION OF ARRAY YPRD
ITEMS(6): NRWTS  =     7  ROW DIMENSION OF ARRAY WTS

ITEMS(7): METHOD = 'GAUSS'  GAUSS'S METHOD
ITEMS(8): KWTS   = 'NONE'   NO WEIGHTING
ITEMS(9): KDERIV = 'NREG'   NREG CALCULATED DERIVATIVES
ITEMS(10): KSYM  = 'SIDE'   ONE SIDED DIFFERENCES
ITEMS(11): KDIFF = 'REL'    RELATIVE DIFFERENCES

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

TOLERANCES

```
TOL(1) =  1.0000000-05  REL. CHANGE IN A PARAMETER
TOL(2) =  0.0000000     REL. CHANGE IN SUM OF SQUARES
TOL(3) =  0.0000000     RATIO TO INITIAL SUM OF SQUARES
TOL(4) =  1.0000000-05  PIVOT TOLERANCE
```

SUNDRIES

```
NUMBER OF ACTIVE PARAMETERS =   5
NUMBER OF CELLS OF SCRATCH REQUIRED =   219
```

PARAMETERS

NO.	INITIAL VALUE	PROPORTION FOR DERIV. ESTIMATE	LOWER BOUND	UPPER BOUND
1	9.0839999+01	5.0000000-03	2.0000000+01	1.8000000+02
2	6.8000000-01	5.0000000-03	1.0000000-01	5.0000000+00
3	4.0650000+01	5.0000000-03	2.0000000+01	1.3000000+02
4	1.0500000+01	5.0000000-03	2.0000000+00	2.5000000+01
5	9.9999999-03	5.0000000-03	1.0000000-03	1.0000000+00

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		2.3587121+03	3.9395394+01	2.6129180+00	5.3015345-01	7.5598835-02
PAR.	1	-.0006543	.0165956	-.0473030	.9695376	.2397568
	2	.0050835	.9997447	-.0133623	-.0174485	-.0012641
	3	-.0004370	-.0042735	-.0936128	-.2432354	.9654298
	4	.0068058	.0137865	.9943714	.0229915	.1022758
	5	.9999636	-.0051673	-.0067717	.0004603	-.0001109

SETUP TIME = .035 SECONDS

THE ITERATIONS

ITERATION NO. 1		BASE POINT		TEST POINT
SUM OF SQUARES		1.4081758+03		3.3198186+02
GAMMA		1.0000000+00		1.0000000+00
ANGLE IN DEGREES		.0000		89.9768
MAX. PIVOT REDUCTION		1.0000000+00		1.9106014-03
PAR.	1	9.0839999+01		7.2429123+01
	2	6.8000000-01		1.3954383+00
	3	4.0650000+01		8.8022294+01
	4	1.0500000+01	S	7.5657009+00
	5	9.9999999-03		4.6582187-02

CUMULATIVE NO. OF FUNCTION CALLS = 2 ITERATION TIME = .194 SECONDS CUMULATIVE TIME = .229 SECONDS

ITERATION NO. 2		BASE POINT		TEST POINT
SUM OF SQUARES		3.3198186+02		2.6622606+02
GAMMA		1.0000000+00		4.1806927-01
ANGLE IN DEGREES		89.9768		89.9827
MAX. PIVOT REDUCTION		1.9106014-03		2.0549398-04
PAR.	1	7.2429123+01		7.5695669+01
	2	1.3954383+00		2.4200826+00
	3	8.8022294+01	S	1.3000000+02
	4	7.5657009+00		1.3835788+01
	5	4.6582187-02		1.9328437-02

CUMULATIVE NO. OF FUNCTION CALLS = 3 ITERATION TIME = .147 SECONDS CUMULATIVE TIME = .376 SECONDS

ITERATION NO. 3		BASE POINT		TEST POINT		TEST POINT		TEST POINT
SUM OF SQUARES		2.6622606+02		1.0233375+03		3.4691809+02		2.1652809+02
GAMMA		1.0000000+00		1.0000000+00		5.0000000-01		2.5000000-01
ANGLE IN DEGREES		89.9827		89.9772		89.9772		89.9772
MAX. PIVOT REDUCTION		2.0549398-04		2.6804688-03		2.6804688-03		2.6804688-03
PAR.	1	7.5695669+01		5.7738116+01		6.6716893+01		7.1206282+01
	2	2.4200826+00		3.9420375+00		3.1810600+00		2.8005713+00
	3	1.3000000+02	B	1.3000000+02	B	1.3000000+02	B	1.3000000+02
	4	1.3835788+01	S	8.9548811+00	S	1.1395335+01	S	1.2615561+01
	5	1.9328437-02		4.2893544-02		3.1110991-02		2.5219714-02

CUMULATIVE NO. OF FUNCTION CALLS = 6 ITERATION TIME = .158 SECONDS CUMULATIVE TIME = .534 SECONDS

THE ITERATIONS

ITERATION NO. 4	BASE POINT	TEST POINT	TEST POINT	TEST POINT	TEST POINT
SUM OF SQUARES	2.1652809+02	1.5355218+03	4.6977211+02	2.1942754+02	1.9158232+02
GAMMA	2.5000000-01	5.0000000-01	2.5000000-01	1.2500000-01	6.2500000-02
ANGLE IN DEGREES	89.9772	89.9781	89.9781	89.9781	89.9781
MAX. PIVOT REDUCTION	2.6804688-03	1.4525084-05	1.4525084-05	1.4525084-05	1.4525084-05
PAR. 1	7.1206282+01	1.0640571+02	8.8805997+01	8.0006139+01	7.5606210+01
2	2.8005713+00	2.8516228+00	2.8260970+00	2.8133342+00	2.8069527+00
3	B 1.3000000+02	1.2942738+02	1.2971369+02	1.2985685+02	1.2992842+02
4	S 1.2615561+01	S 8.8528374+00	S 1.0734200+01	S 1.1674881+01	S 1.2145221+01
5	2.5219714-02	4.7608544-02	3.6414129-02	3.0816921-02	2.8018318-02

CUMULATIVE NO. OF FUNCTION CALLS = 10 ITERATION TIME = .265 SECONDS CUMULATIVE TIME = .798 SECONDS

ITERATION NO. 5	BASE POINT	TEST POINT
SUM OF SQUARES	1.9158232+02	1.8893179+02
GAMMA	6.2500000-02	4.4086675-03
ANGLE IN DEGREES	89.9781	89.9869
MAX. PIVOT REDUCTION	1.4525084-05	4.5979041-04
PAR. 1	7.5606210+01	7.5797577+01
2	2.8069527+00	2.8040951+00
3	1.2992842+02	1.3000000+02
4	S 1.2145221+01	S 1.2142785+01
5	2.8018318-02	2.8081718-02

CUMULATIVE NO. OF FUNCTION CALLS = 11 ITERATION TIME = .154 SECONDS CUMULATIVE TIME = .952 SECONDS

ITERATION NO. 6	BASE POINT	TEST POINT	TEST POINT	TEST POINT	TEST POINT	TEST POINT
SUM OF SQUARES	1.8893179+02	3.9878182+02	2.3909117+02	2.1080553+02	2.0466101+02	1.9205783+02
GAMMA	1.2500000-01	2.5000000-01	1.2500000-01	6.2500000-02	3.1250000-02	1.5625000-02
ANGLE IN DEGREES	89.9869	89.9494	89.9494	89.9494	89.9494	89.9494
MAX. PIVOT REDUCTION	4.5979041-04	4.1902110-04	4.1902110-04	4.1902110-04	4.1902110-04	4.1902110-04
PAR. 1	7.5797577+01	8.4885065+01	8.0341321+01	7.8069448+01	7.6933513+01	7.6365544+01
2	2.8040951+00	2.1497768+00	2.4769360+00	2.6405156+00	2.7223054+00	2.7632003+00
3	1.3000000+02	SB 1.3000000+02	SB 1.3000000+02	SB 1.3000000+02	SB 1.3000000+02	SB 1.3000000+02
4	S 1.2142785+01	1.2897107+01	1.2519946+01	1.2331365+01	1.2237075+01	1.2189930+01
5	2.8081718-02	2.5770095-02	2.6925907-02	2.7503812-02	2.7792765-02	2.7937242-02

THE ITERATIONS

ITERATION NO. 6	TEST POINT	TEST POINT	TEST POINT	TEST POINT	TEST POINT	TEST POINT
SUM OF SQUARES	1.9425596+02	1.9673876+02	1.9282790+02	1.8908151+02	1.8900591+02	1.9247131+02
GAMMA	7.8125000-03	3.9062500-03	1.9531250-03	9.7656250-04	4.8828125-04	2.4414063-04
ANGLE IN DEGREES	89.9494	89.9494	89.9494	89.9494	89.9494	89.9494
MAX. PIVOT REDUCTION	4.1902110-04	4.1902110-04	4.1902110-04	4.1902110-04	4.1902110-04	4.1902110-04
PAR. 1	7.6081560+01	7.5939569+01	7.5868572+01	7.5833075+01	7.5815326+01	7.5806451+01
2	2.7836477+00	2.7938714+00	2.7989833+00	2.8015392+00	2.8028172+00	2.8034562+00
3	SB 1.3000000+02	SB 1.3000000+02	SB 1.3000000+02	SB 1.3000000+02	SB 1.3000000+02	SB 1.3000000+02
4	1.2166357+01	1.2154571+01	1.2148678+01	1.2145731+01	1.2144258+01	1.2143521+01
5	2.8009480-02	2.8045599-02	2.8063658-02	2.8072688-02	2.8077203-02	2.8079461-02

ITERATION NO. 6	TEST POINT	TEST POINT	TEST POINT	TEST POINT	TEST POINT
SUM OF SQUARES	1.8895019+02	1.9168951+02	1.9180329+02	1.8893420+02	1.8893275+02
GAMMA	1.2207031-04	6.1035156-05	3.0517578-05	1.5258789-05	7.6293945-06
ANGLE IN DEGREES	89.9494	89.9494	89.9494	89.9494	89.9494
MAX. PIVOT REDUCTION	4.1902110-04	4.1902110-04	4.1902110-04	4.1902110-04	4.1902110-04
PAR. 1	7.5802013+01	7.5799795+01	7.5798686+01	7.5798131+01	7.5797853+01
2	2.8037756+00	2.8039354+00	2.8040152+00	2.8040552+00	2.8040752+00
3	SB 1.3000000+02	SB 1.3000000+02	SB 1.3000000+02	SB 1.3000000+02	SB 1.3000000+02
4	1.2143153+01	1.2142969+01	1.2142877+01	1.2142831+01	1.2142808+01
5	2.8080589-02	2.8081154-02	2.8081436-02	2.8081577-02	2.8081648-02

CUMULATIVE NO. OF FUNCTION CALLS = 27 ITERATION TIME = .613 SECONDS CUMULATIVE TIME = 1.565 SECONDS

ITERATION TERMINATES:
MAX. RELATIVE CHANGE IN A PARAMETER .LT. TOL(1) = 1.0000000-05

FINAL PARAMETER VALUES

FINAL VALUE SUM OF SQUARES = 1.8893179+02

		FINAL VALUE
PAR.	1	7.5797577+01
	2	2.8040951+00
	3	1.3000000+02
	4	1.2142785+01
	5	2.8081718-02

ITERATION SUMMARY

ITER NO.	SUM OF SQUARES	GAMMA	ANGLE	NO. PIVOT FAILURES	NO. BOUNDS FAILURES	MAX. PIVOT REDUCTION	TIME IN SECONDS
0	1.4081758+03	1.0000000+00	.0000	0	0	1.0000000+00	.000
1	3.3198186+02	1.0000000+00	89.9768	0	0	1.9106014-03	.194
2	2.6622606+02	1.0000000+00	89.9827	0	0	2.0549398-04	.147
3	2.1652809+02	2.5000000-01	89.9772	0	1	2.6804688-03	.158
4	1.9158232+02	6.2500000-02	89.9781	0	0	1.4525084-05	.265
5	1.8893179+02	1.2500000-01	89.9869	0	0	4.5979041-04	.154
6	1.8893275+02	7.6293945-06	89.9494	0	1	4.1902110-04	.613

SINGULAR VALUES AND VECTORS AT TERMINAL POINT

SING. VALUES		2.5223722+03	1.2204633+01	4.3280371+00	2.4982066-01	2.7559545-02
PAR.	1	-.0010448	.0753648	-.0139127	.9922365	-.0979399
	2	-.0126007	.9838978	.1606569	-.0745205	-.0205499
	3	-.0004657	.0438303	-.0987262	.0929847	.9897907
	4	-.0011185	-.1555384	.9819569	.0355987	.1014876
	5	-.9999193	-.0123240	-.0030625	-.0001808	-.0002132

NORMALIZING ELEMENTS AND CORRELATION MATRIX

PARAMETER NO.		1	2	3	4	5
NORM. ELTS.		5.3295777+00	8.0799535-01	3.5916560+01	3.6922412+00	7.8780111-03
PAR.	1	1.0000000				
	2	.3403127	1.0000000			
	3	-.6590398	-.9266390	1.0000000		
	4	-.6363171	-.9321748	.9976647	1.0000000	
	5	.5862920	.9233374	-.9829587	-.9881319	1.0000000

CONFIDENCE LIMITS ON LINEAR HYPOTHESIS

		LOWER LIMIT	FINAL VALUE	UPPER LIMIT
PAR.	1	-2.7802557+01	7.5797577+01	1.7939771+02
	2	-1.2902295+01	2.8040951+00	1.8510485+01
	3	-5.6817172+02	1.3000000+02	8.2817172+02
	4	-5.9629634+01	1.2142785+01	8.3915202+01
	5	-1.2505668-01	2.8081718-02	1.8122012-01

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		4.9897544+01	1.7148078+03	5.4593270+03		1.0169761+02	2.1264463+03	5.4593270+03
	2	B	1.0000000-01	5.9876372+03	8.6335453+03	B	5.0000000+00	1.7996740+02	5.7577482+03
	3	B	2.0000000+01	4.1302739+03	2.2573986+04	B	1.3000000+02	1.8893179+02	1.8893179+02
	4	B	2.0000000+00	1.3729672+04	3.2366866+03	B	2.5000000+01	4.4814860+03	5.0862627+03
	5	B	1.0000000-03	5.6135478+03	4.8544581+03		6.6366318-02	2.4147884+03	9.5128073+03

N O T I C E

THE SUM OF SQUARES AT THE UPPER 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 = 9.7193568+00 NO. DEGREES OF FREEDOM = 2
THIS IS THE SCALE UNIT IN THE GRAPH OF THE RESIDUALS.

EXPT. NO.	VAR. NO.	PREDICTION	OBSERVATION	RESIDUAL	GRAPH OF RESIDUAL -2 -1 0 +1 +2
1	1	1.5940796+02	1.6809000+02	8.6820393+00	**********
2	1	1.3831733+02	1.3318000+02	-5.1373272+00	******
3	1	1.0169438+02	1.0052000+02	-1.1743784+00	**
4	1	7.2585937+01	6.7330000+01	-5.2559366+00	******
5	1	5.0261606+01	4.3820000+01	-6.4416060+00	********
6	1	2.2178112+01	1.9230000+01	-2.9481118+00	****
7	1	5.7835034+00	2.9600000+00	-2.8235034+00	****

TIME SINCE END OF THE LAST ITERATION = .495 SECONDS TOTAL TIME = 2.060 SECONDS

R-SQUARED FOR FUNCTION1 = .9970
COR FOR FUNCTION1 = .1122
@FIN

RUNID: AD2490 PROJECT: 05994 USER: 3908464260
21:57:53 AD2490 FIN

ITEM	AMOUNT	COST
CPU TIME	00:00:03.304	$0.09
EXEC CHARGES	13	$0.14
SOFTWARE SUPPORT	25	$0.27
FILE I/O REQUESTS	690	$0.11
FILE I/O WORDS	844183	$0.06
MEMORY USAGE	0.214	$0.08
CARDS IN	150	$0.02
PAGES PRINTED	12	$0.21
SHARED FILES	2	$0.22
JOB CHARGE	1	$0.04
TOTAL COST		$1.24

RUN TIME: 21:57:21 --> 21:57:53 JUN 12,84. PREV: 21:51:57 JUN 12,84
THE $ AMOUNTS ARE APPROXIMATE
RATE MULTIPLIERS 0.50 OVERNIGHT
1.00 UW MADISON

EOF 13 PAGES COST $0.65 AD2490 56789012345678901234567890123456789O

APPENDIX VIII

Example of Computer Output of the Program, SPSS.

```
@RUN SELEN,5994,3908464260,5M,500
@SYM PRINT$,,XEROX1
@SPSS
```

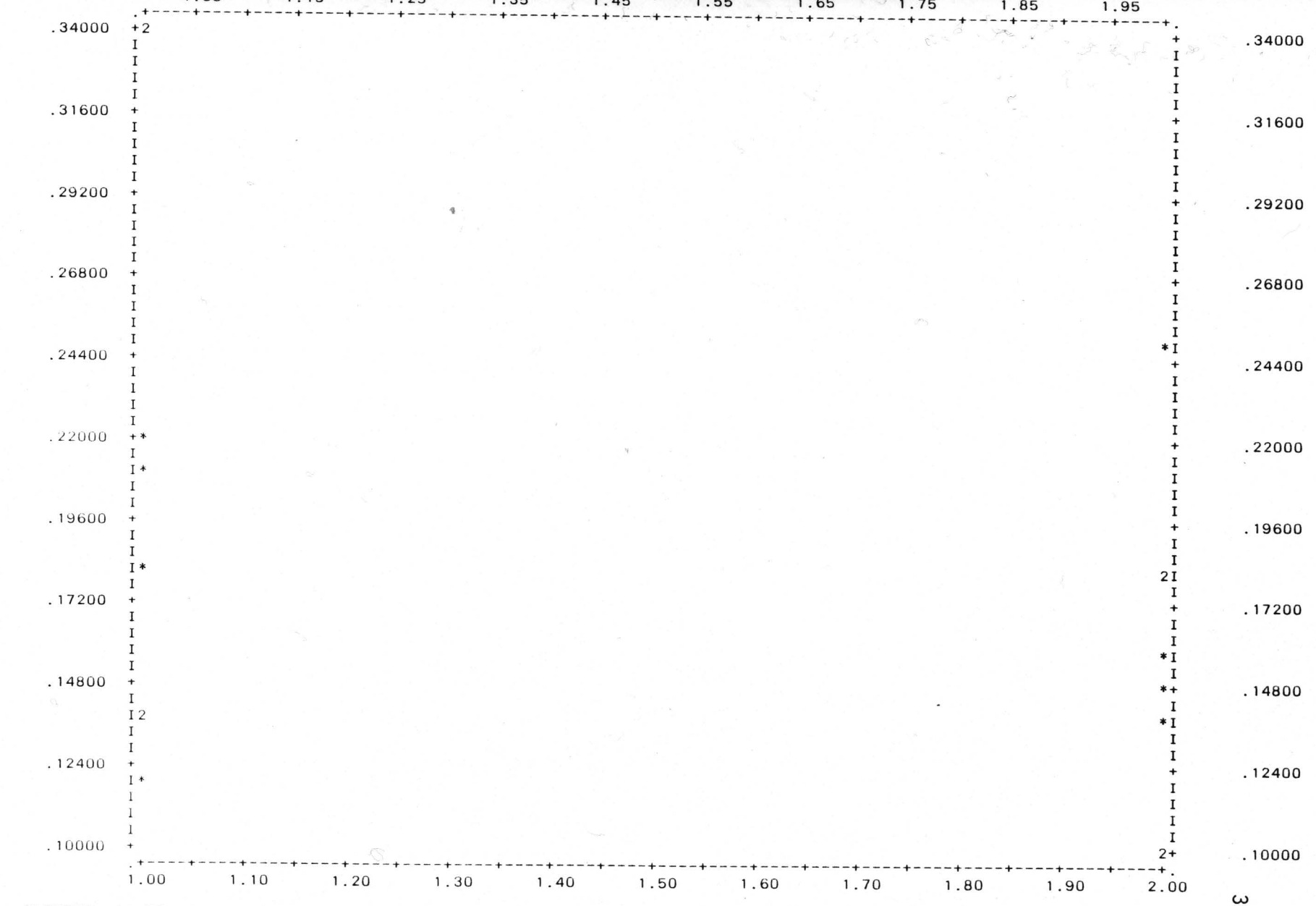
SPSS FOR SPERRY UNIVAC 1100 EXEC 8, VERSION H, RELEASE 9.0-UW2.0, FEBRUARY 1983

```
SPACE ALLOCATION..          ALLOWS FOR..    37 TRANSFORMATIONS
WORKSPACE      7875 WORDS                   150 RECODE VALUES + LAG VARIABLES
TRANSPACE      1125 WORDS                   300 IF/COMPUTE OPERATIONS

  1.  RUN NAME        COMPARISON OF V MAX TO KM RATIO FOLLOWING 0.5 AND 1.0 G
  2.                  TABLET DOSES AFTER THE FIRST AND LAST DOSE.
  3.  VARIABLE LIST   WEEK,SUBJ,ORDR,DOSE,VAL
  4.  INPUT FORMAT    FIXED (4F1.0,T6,F4.3)


     ACCORDING TO YOUR INPUT FORMAT, VARIABLES ARE TO BE READ AS FOLLOWS

     VARIABLE  FORMAT  RECORD    COLUMNS

     WEEK      F 1. 0      1      1-    1
     SUBJ      F 1. 0      1      2-    2
     ORDR      F 1. 0      1      3-    3
     DOSE      F 1. 0      1      4-    4
     VAL       F 4. 3      1      6-    9

THE INPUT FORMAT PROVIDES FOR    5 VARIABLES.    5 WILL BE READ
IT PROVIDES FOR    1 RECORDS ('CARDS') PER CASE.  A MAXIMUM OF     9 'COLUMNS' ARE USED ON A RECORD.

  5.  INPUT MEDIUM    CARD
  6.  N OF CASES      16
  7.  VAR LABELS      WEEK WEEK OF STUDY/
  8.                  SUBJ SUBJECT NUMBER/
  9.                  ORDR FIRST OR LAST DOSE/
 10.                  DOSE 0.5 OR 1.0 G TABLET DOSE/
 11.                  VAL V MAX TO KM RATIO/
 12.  PRINT FORMATS   VAL(3)/
 13.  TASK NAME       LIST OF RAW DATA
 14.  LIST CASES      CASES=16/VARIABLES=ALL
 15.  READ INPUT DATA
```

CASE-NO	SEQNUM	SUBFILE	CASWGT	WEEK	SUBJ	ORDR	DOSE	VAL
1	1.	NONA	1.0000	1.	1.	1.	1.	.340
2	2.	NONA	1.0000	1.	1.	2.	1.	.140
3	3.	NONA	1.0000	1.	2.	1.	1.	.210
4	4.	NONA	1.0000	1.	2.	2.	1.	.120
5	5.	NONA	1.0000	1.	3.	1.	2.	.340
6	6.	NONA	1.0000	1.	3.	2.	2.	.220
7	7.	NONA	1.0000	1.	4.	1.	2.	.180
8	8.	NONA	1.0000	1.	4.	2.	2.	.140
9	9.	NONA	1.0000	2.	1.	1.	2.	.150
10	10.	NONA	1.0000	2.	1.	2.	2.	.100
11	11.	NONA	1.0000	2.	2.	1.	2.	.180
12	12.	NONA	1.0000	2.	2.	2.	2.	.100
13	13.	NONA	1.0000	2.	3.	1.	1.	.160
14	14.	NONA	1.0000	2.	3.	2.	1.	.140
15	15.	NONA	1.0000	2.	4.	1.	1.	.250
16	16.	NONA	1.0000	2.	4.	2.	1.	.180

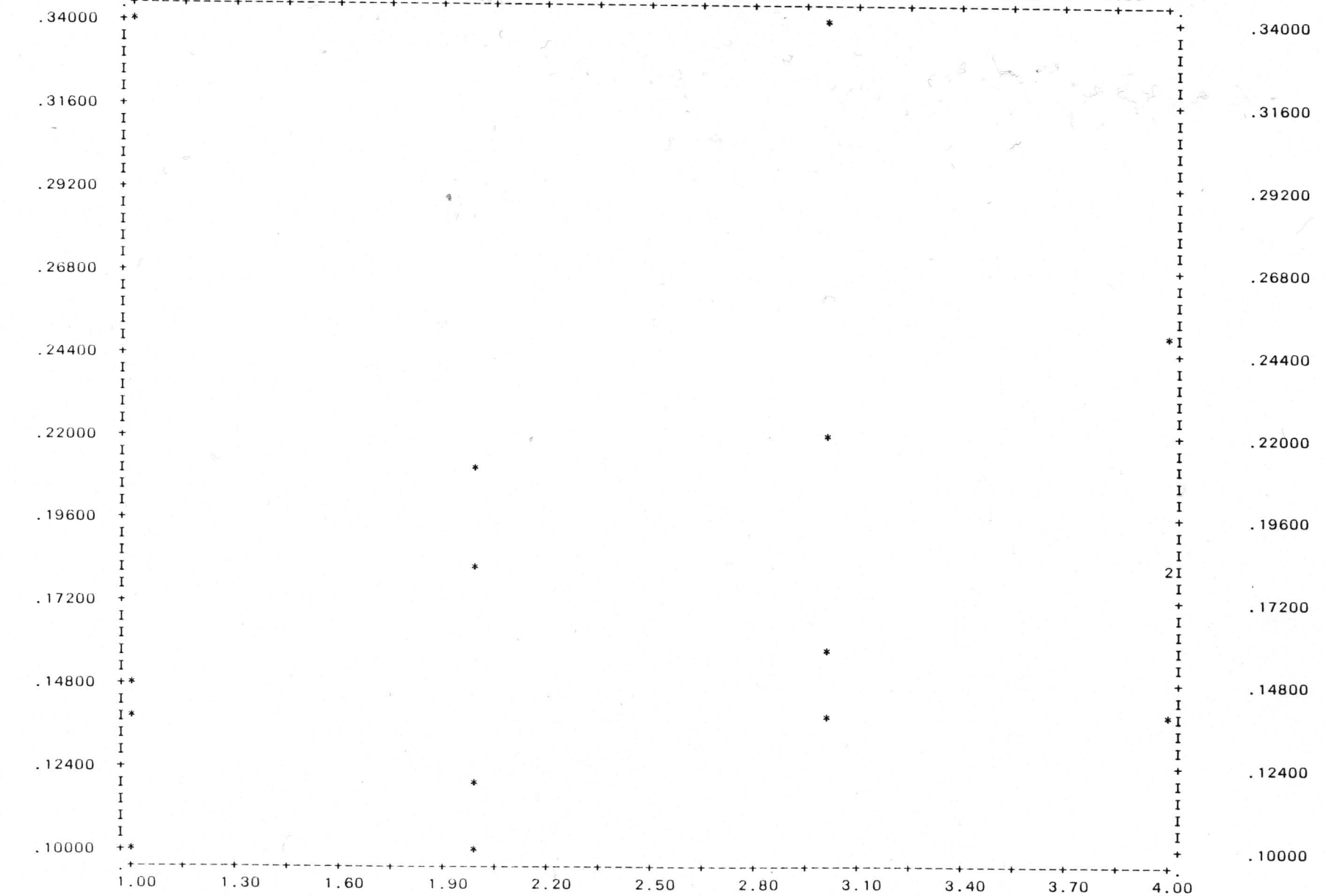
```
16.  END INPUT DATA
17.  TASK NAME        PLOT OF DATA AGAINST THE CLASSIFICATION VARIABLES
18.  SCATTERGRAM      VAL WITH WEEK TO DOSE/
19.  OPTIONS          4

***** GIVEN WORKSPACE ALLOWS FOR   1310 CASES FOR SCATTERGRAM PROBLEM *****
```

PLOT OF DATA AGAINST THE CLASSIFICATION VARIABLES
FILE NONAME (CREATION DATE = 06/12/84)
SCATTERGRAM OF (DOWN) VAL V MAX TO KM RATIO (ACROSS) WEEK WEEK OF STUDY



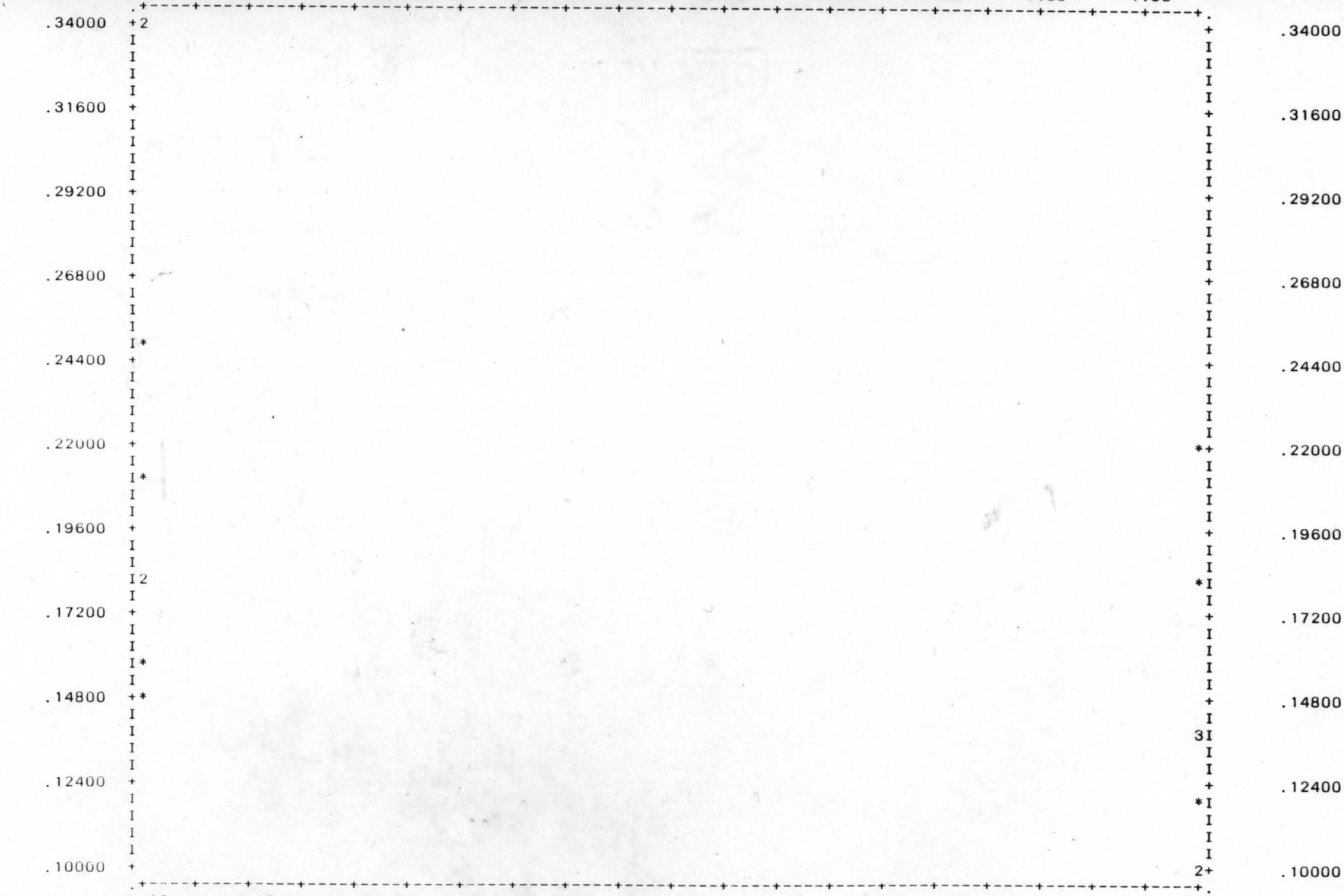
PLOTTED VALUES - 16 EXCLUDED VALUES- 0 MISSING VALUES - 0

SCATTERGRAM OF (DOWN) VAL V MAX TO KM RATIO (ACROSS) SUBJ SUBJECT NUMBER



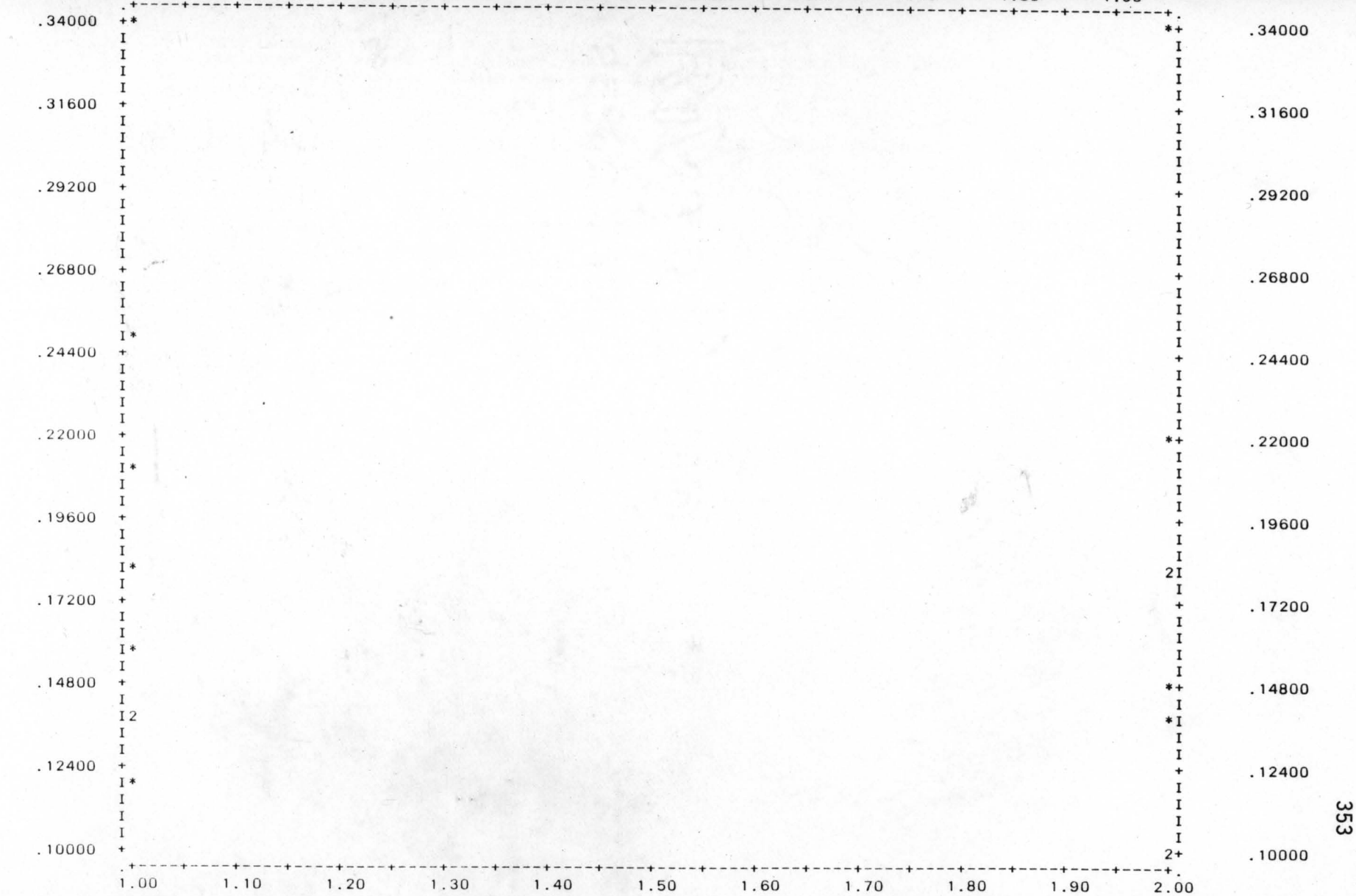
PLOTTED VALUES - 16 EXCLUDED VALUES- 0 MISSING VALUES - 0

PLOT OF DATA AGAINST THE CLASSIFICATION VARIABLES
FILE NONAME (CREATION DATE = 06/12/84)
SCATTERGRAM OF (DOWN) VAL V MAX TO KM RATIO (ACROSS) ORDR FIRST OR LAST DOSE



PLOTTED VALUES - 16 EXCLUDED VALUES- 0 MISSING VALUES - 0

PLOT OF DATA AGAINST THE CLASSIFICATION VARIABLES
FILE NONAME (CREATION DATE = 06/12/84)
SCATTERGRAM OF (DOWN) VAL V MAX TO KM RATIO (ACROSS) DOSE 0.5 OR 1.0 G TABLET DOSE



PLOTTED VALUES - 16 EXCLUDED VALUES- 0 MISSING VALUES - 0

CPU TIME REQUIRED.. .66 SECONDS

```
20.  TASK NAME      MEANS FOR ALL CLASSIFICATION VARIABLES
21.  BREAKDOWN      TABLES=VAL BY WEEK /VAL BY SUBJ/ VAL BY ORDR/ VAL BY DOSE/
22.                 VAL BY ORDR BY WEEK/ VAL BY ORDR BY DOSE/
23.  STATISTICS     1
```

***** GIVEN WORKSPACE ALLOWS FOR 874 CELLS AND 2 DIMENSIONS FOR SUBPROGRAM BREAKDOWN *****

DESCRIPTION OF SUBPOPULATIONS

CRITERION VARIABLE VAL V MAX TO KM RATIO
BROKEN DOWN BY WEEK WEEK OF STUDY

VARIABLE	CODE	VALUE LABEL	SUM	MEAN	STD DEV	VARIANCE	N
FOR ENTIRE POPULATION			2.9500	.1844	.0734	.0054	(16)
WEEK	1.		1.6900	.2112	.0868	.0075	(8)
WEEK	2.		1.2600	.1575	.0486	.0024	(8)

TOTAL CASES = 16

ANALYSIS OF VARIANCE

VARIABLE	CODE	VALUE LABEL	SUM	MEAN	STD DEV	SUM OF SQ	N
WEEK	1.		1.6900	.2112	.0868	.0527	(8)
WEEK	2.		1.2600	.1575	.0486	.0166	(8)
	WITHIN GROUPS TOTAL		2.9500	.1844	.0703	.0692	(16)

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	SIG.
BETWEEN GROUPS	.012	1	.012	2.337	.1486
WITHIN GROUPS	.069	14	.005		

ETA = .3782 ETA SQUARED = .1430

MEANS FOR ALL CLASSIFICATION VARIABLES
FILE NONAME (CREATION DATE = 06/12/84)

DESCRIPTION OF SUBPOPULATIONS

CRITERION VARIABLE VAL V MAX TO KM RATIO
BROKEN DOWN BY SUBJ SUBJECT NUMBER

VARIABLE	CODE	VALUE LABEL	SUM	MEAN	STD DEV	VARIANCE	N
FOR ENTIRE POPULATION			2.9500	.1844	.0734	.0054	(16)
SUBJ	1.		.7300	.1825	.1072	.0115	(4)
SUBJ	2.		.6100	.1525	.0512	.0026	(4)
SUBJ	3.		.8600	.2150	.0900	.0081	(4)
SUBJ	4.		.7500	.1875	.0457	.0021	(4)

TOTAL CASES = 16

- A N A L Y S I S O F V A R I A N C E -

VARIABLE	CODE	VALUE LABEL	SUM	MEAN	STD DEV	SUM OF SQ		N
SUBJ	1.		.7300	.1825	.1072	.0345	(	4)
SUBJ	2.		.6100	.1525	.0512	.0079	(	4)
SUBJ	3.		.8600	.2150	.0900	.0243	(	4)
SUBJ	4.		.7500	.1875	.0457	.0063	(	4)
	WITHIN GROUPS TOTAL		2.9500	.1844	.0780	.0729	(	16)

A N A L Y S I S O F V A R I A N C E

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	SIG.
BETWEEN GROUPS	.008	3	.003	.432	.7342
WITHIN GROUPS	.073	12	.006		

ETA = .3121 ETA SQUARED = .0974

DESCRIPTION OF SUBPOPULATIONS

CRITERION VARIABLE VAL V MAX TO KM RATIO
BROKEN DOWN BY ORDR FIRST OR LAST DOSE

VARIABLE	CODE	VALUE LABEL	SUM	MEAN	STD DEV	VARIANCE	N
FOR ENTIRE POPULATION			2.9500	.1844	.0734	.0054	(16)
ORDR	1.		1.8100	.2262	.0767	.0059	(8)
ORDR	2.		1.1400	.1425	.0406	.0016	(8)

TOTAL CASES = 16

- A N A L Y S I S O F V A R I A N C E -

VARIABLE	CODE	VALUE LABEL	SUM	MEAN	STD DEV	SUM OF SQ		N
ORDR	1.		1.8100	.2262	.0767	.0412	(	8)
ORDR	2.		1.1400	.1425	.0406	.0115	(	8)
	WITHIN GROUPS TOTAL		2.9500	.1844	.0614	.0527	(	16)

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	SIG.
BETWEEN GROUPS	.028	1	.028	7.448	.0163
WITHIN GROUPS	.053	14	.004		

ETA = .5893 ETA SQUARED = .3473

MEANS FOR ALL CLASSIFICATION VARIABLES
FILE NONAME (CREATION DATE = 06/12/84)

DESCRIPTION OF SUBPOPULATIONS

CRITERION VARIABLE VAL V MAX TO KM RATIO
BROKEN DOWN BY DOSE 0.5 OR 1.0 G TABLET DOSE

VARIABLE	CODE	VALUE LABEL	SUM	MEAN	STD DEV	VARIANCE	N
FOR ENTIRE POPULATION			2.9500	.1844	.0734	.0054	(16)
DOSE	1.		1.5400	.1925	.0730	.0053	(8)
DOSE	2.		1.4100	.1762	.0778	.0061	(8)

TOTAL CASES = 16

- A N A L Y S I S O F V A R I A N C E -

VARIABLE	CODE	VALUE LABEL	SUM	MEAN	STD DEV	SUM OF SQ		N
DOSE	1.		1.5400	.1925	.0730	.0374	(	8)
DOSE	2.		1.4100	.1762	.0778	.0424	(	8)
	WITHIN GROUPS TOTAL		2.9500	.1844	.0755	.0797	(	16)

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	SIG.
BETWEEN GROUPS	.001	1	.001	.185	.6733
WITHIN GROUPS	.080	14	.006		

ETA = .1143 ETA SQUARED = .0131

DESCRIPTION OF SUBPOPULATIONS

CRITERION VARIABLE VAL V MAX TO KM RATIO
BROKEN DOWN BY ORDR FIRST OR LAST DOSE
BY WEEK WEEK OF STUDY

VARIABLE	CODE	VALUE LABEL	SUM	MEAN	STD DEV	VARIANCE	N
FOR ENTIRE POPULATION			2.9500	.1844	.0734	.0054	(16)
ORDR	1.		1.8100	.2262	.0767	.0059	(8)
WEEK	1.		1.0700	.2675	.0846	.0072	(4)
WEEK	2.		.7400	.1850	.0451	.0020	(4)
ORDR	2.		1.1400	.1425	.0406	.0016	(8)
WEEK	1.		.6200	.1550	.0443	.0020	(4)
WEEK	2.		.5200	.1300	.0383	.0015	(4)

TOTAL CASES = 16

- A N A L Y S I S O F V A R I A N C E -

VARIABLE	CODE	VALUE LABEL	SUM	MEAN	STD DEV	SUM OF SQ		N
ORDR	1.		1.8100	.2262	.0767	.0412	(	8)
ORDR	2.		1.1400	.1425	.0406	.0115	(	8)
	WITHIN GROUPS TOTAL		2.9500	.1844	.0614	.0527	(	16)

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	SIG.
BETWEEN GROUPS	.028	1	.028	7.448	.0163
WITHIN GROUPS	.053	14	.004		

ETA = .5893 ETA SQUARED = .3473

DESCRIPTION OF SUBPOPULATIONS

CRITERION VARIABLE VAL — V MAX TO KM RATIO
BROKEN DOWN BY ORDR — FIRST OR LAST DOSE
BY DOSE — 0.5 OR 1.0 G TABLET DOSE

VARIABLE	CODE	VALUE LABEL	SUM	MEAN	STD DEV	VARIANCE	N
FOR ENTIRE POPULATION			2.9500	.1844	.0734	.0054	(16)
ORDR	1.		1.8100	.2262	.0767	.0059	(8)
DOSE	1.		.9600	.2400	.0762	.0058	(4)
DOSE	2.		.8500	.2125	.0862	.0074	(4)
ORDR	2.		1.1400	.1425	.0406	.0016	(8)
DOSE	1.		.5800	.1450	.0252	.0006	(4)
DOSE	2.		.5600	.1400	.0566	.0032	(4)

TOTAL CASES = 16

ANALYSIS OF VARIANCE

VARIABLE	CODE	VALUE LABEL	SUM	MEAN	STD DEV	SUM OF SQ	N
ORDR	1.		1.8100	.2262	.0767	.0412	(8)
ORDR	2.		1.1400	.1425	.0406	.0115	(8)
	WITHIN GROUPS TOTAL		2.9500	.1844	.0614	.0527	(16)

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	SIG.
BETWEEN GROUPS	.028	1	.028	7.448	.0163
WITHIN GROUPS	.053	14	.004		

ETA = .5893 ETA SQUARED = .3473

MEANS FOR ALL CLASSIFICATION VARIABLES

CPU TIME REQUIRED.. .32 SECONDS

```
24.  TASK NAME     ANALYSIS OF LATIN SQUARE DESIGN
25.  ANOVA         VAL BY WEEK(1,2),SUBJ(1,4)ORDR(1,2),DOSE(1,2)/
26.  OPTIONS       4
27.  STATISTICS    1,3
```

'ANOVA' PROBLEM REQUIRES 811 WORDS OF SPACE.

ANALYSIS OF LATIN SQUARE DESIGN
FILE NONAME (CREATION DATE = 06/12/84)

	ORDR 1	ORDR 2
WEEK		
1	.27	.15
	(4)	(4)
2	.18	.13
	(4)	(4)

	DOSE 1	DOSE 2
WEEK		
1	.20	.22
	(4)	(4)
2	.18	.13
	(4)	(4)

	ORDR 1	ORDR 2
SUBJ		
1	.25	.12
	(2)	(2)
2	.19	.11
	(2)	(2)
3	.25	.18
	(2)	(2)
4	.21	.16
	(2)	(2)

	DOSE 1	DOSE 2
SUBJ		
1	.24	.13
	(2)	(2)
2	.16	.14
	(2)	(2)
3	.15	.28
	(2)	(2)

4	.21	.16
	(2)	(2)

	DOSE	
	1	2
ORDR		
1	.24	.21
	(4)	(4)
2	.14	.14
	(4)	(4)

ANALYSIS OF LATIN SQUARE DESIGN
FILE NONAME (CREATION DATE = 06/12/84)

* * * * * * * * * * A N A L Y S I S O F V A R I A N C E * * * * * * * * * *

VAL V MAX TO KM RATIO
BY WEEK WEEK OF STUDY
SUBJ SUBJECT NUMBER
ORDR FIRST OR LAST DOSE
DOSE 0.5 OR 1.0 G TABLET DOSE

* *

SOURCE OF VARIATION	SUM OF SQUARES	DF	MEAN SQUARE	F	SIGNIF OF F
MAIN EFFECTS	.049	6	.008	2.257	.131
WEEK	.012	1	.012	3.224	.106
SUBJ	.008	3	.003	.732	.559
ORDR	.028	1	.028	7.828	.021
DOSE	.001	1	.001	.295	.600
EXPLAINED	.049	6	.008	2.257	.131
RESIDUAL	.032	9	.004		
TOTAL	.081	15	.005		

16 CASES WERE PROCESSED.
0 CASES (.0 PCT) WERE MISSING.
DUE TO EMPTY CELLS OR A SINGULAR MATRIX,
HIGHER ORDER INTERACTIONS HAVE BEEN SUPPRESSED.

* * * M U L T I P L E C L A S S I F I C A T I O N A N A L Y S I S * * *

VAL V MAX TO KM RATIO
BY WEEK WEEK OF STUDY
SUBJ SUBJECT NUMBER
ORDR FIRST OR LAST DOSE
DOSE 0.5 OR 1.0 G TABLET DOSE

* *

GRAND MEAN = .18

VARIABLE + CATEGORY	N	UNADJUSTED DEV'N	UNADJUSTED ETA	ADJUSTED FOR INDEPENDENTS DEV'N	ADJUSTED FOR INDEPENDENTS BETA	ADJUSTED FOR INDEPENDENTS + COVARIATES DEV'N	ADJUSTED FOR INDEPENDENTS + COVARIATES BETA
WEEK							
1	8	.03		.03			
2	8	-.03		-.03			
			.38		.38		
SUBJ							
1	4	.00		.00			
2	4	-.03		-.03			
3	4	.03		.03			
4	4	.00		.00			
			.31		.31		
ORDR							
1	8	.04		.04			
2	8	-.04		-.04			
			.59		.59		
DOSE							
1	8	.01		.01			
2	8	-.01		-.01			
			.11		.11		
MULTIPLE R SQUARED					.601		
MULTIPLE R					.775		

ANALYSIS OF LATIN SQUARE DESIGN

CPU TIME REQUIRED.. .20 SECONDS

28. FINISH

NORMAL END OF JOB.
28 CONTROL CARDS WERE PROCESSED.
0 ERRORS WERE DETECTED.

APPENDIX IX

Example of Computer Output For Prediction of Plasma Levels According to Equation 2, Using the Program DGEAR.

```
*  *  *  *  *  *  SPERRY 1180 TIME/SHARING EXEC --- MULTI-PROCESSOR SYSTEM --- VER.  MACC 38.22  SITE *  MACC     *  *  *  *  *  *
RUNID: AD2493         PROJECT: 05994       USER: 3908464260          CREATED ON JUN 12, 1984 AT 22:08:08    INPUT DEVICE: NACR3
            FILE NAME: PR@000AD2493          PART NUMBER: 00        PRINTED ON JUN 12, 1984 AT 22:08:21   OUTPUT DEVICE: NAPR2


@RUN SELEN,5994,3908464260,5M,500
@SYM PRINT$,,XEROX1
@ASG,U PROBENECID.
@ERS PROBENECID.
FURPUR 28R3A U02 SLIB34 06/12/84 22:08:09
END ERS.
@FOR,IS ,PROBENECID.DIFEQ
FORTRAN-MACC 1.17S-06/12/84-22:08:10                DIFEQ
00101       1.              INTEGER N,METH,MITER,INDEX,IWK(1),IER,K
00102       2.              REAL Y(6),WK(19),X,TOL,XEND,H,YY(1)
00103       3.              DIMENSION X(7)
00104       4.              DATA X/0.0,1.0,2.0,3.0,4.0,5.0,6.0/
00106       5.              EXTERNAL FCN,FCNJ
00107       6.              N=1
00110       7.              TOL=0.001
00111       8.              H=0.01
00112       9.              METH=1
00113      10.              MITER=1
00114      11.              YY(1)=0.0
00115      12.              INDEX=1
00116      13.              DO 10 K=1,6
00121      14.              XX=X(K)
00122      15.              XEND=X(K+1)
00123      16.              CALL DGEAR (N,FCN,FCNJ,X,H,YY,XEND,TOL,METH,MITER,INDEX,IWK,WK,
00123      17.             *IER)
00124      18.              INDEX=2
00125      19.              WRITE(-,-)K,XEND,YY(1)
00132      20.              Y(K)=YY(1)
00133      21.          10  CONTINUE
00135      22.              STOP
00136      23.              END

       END OF COMPILATION:         NO  DIAGNOSTICS.
@FOR,IS ,PROBENECID.SUBFCN
FORTRAN-MACC 1.17S-06/12/84-22:08:11                SUBFCN
00101       1.              SUBROUTINE FCN(N,X,YY,DY)
00102       2.              DIMENSION DY(1),YY(1)
00103       3.              TH1=85.44
00104       4.              TH2=1.0
00105       5.              TH3=130.0
00106       6.              TH4=21.81
00107       7.              DY(1)=TH1*TH2*EXP(-TH2*X)-(TH4*YY(1))/(TH3+YY(1))
00110       8.              RETURN
00111       9.              END

       END OF COMPILATION:         NO  DIAGNOSTICS.
@FOR,IS ,PROBENECID.SUBFCNJ
FORTRAN-MACC 1.17S-06/12/84-22:08:12                SUBFCNJ
00101       1.              SUBROUTINE FCNJ(N,X,YY,PD)
00102       2.              REAL YY(N),PD(N,N)
00103       3.              TH3=130.0
00104       4.              TH4=21.81
00105       5.              PD(1,1)=(-TH4*TH3)/((TH3+YY(1))**2)
```

```
00106       6.            RETURN
00107       7.            END

      END OF COMPILATION:         NO  DIAGNOSTICS.
@MAP,IN ,PROBENECID.PROGRAM
Collector 31R1U04 1984 Jun 12 Tue 2208:13


END MAP.  ERRORS: 0  TIME: 7.527  STORAGE: 19459/7/042677/0101477
@P
 Ready
@XQT PROBENECID.PROGRAM
 *** WARNING WITH FIX ERROR  (IER =  66) FROM IMSL ROUTINE DGEAR
               1     1.0000         50.0776
               2     2.0000         63.2122
               3     3.0000         63.3380
               4     4.0000         59.0363
               5     5.0000         53.4375
               6     6.0000         47.6962
@FIN

    RUNID:  AD2493   PROJECT: 05994              USER: 3908464260
22:08:20    AD2493 FIN

  ITEM                                  AMOUNT       COST

CPU TIME                        00:00:00.509         $0.01
EXEC CHARGES                               9         $0.10
SOFTWARE SUPPORT                          15         $0.16
FILE I/O REQUESTS                        299         $0.05
FILE I/O WORDS                        328288         $0.02
MEMORY USAGE                           0.081         $0.03
CARDS IN                                  52         $0.01
PAGES PRINTED                              1         $0.02
SHARED FILES                               2         $0.22
JOB CHARGE                                 1         $0.04

TOTAL COST                                           $0.66

RUN TIME: 22:08:08 --> 22:08:20 JUN 12,84.  PREV: 21:59:25 JUN 12,84
THE $ AMOUNTS ARE APPROXIMATE
RATE MULTIPLIERS    0.50 OVERNIGHT
                    1.00 UW MADISON
```

```
*  *  *  *  *  *  SPERRY 1180 TIME/SHARING EXEC --- MULTI-PROCESSOR SYSTEM --- VER.  MACC 38.22  SITE *  MACC     *  *  *  *  *  *
RUNID: AD2495        PROJECT: 05994       USER: 3908464260         CREATED ON JUN 12, 1984 AT 22:09:55    INPUT DEVICE: NACR3
          FILE NAME: PR@000AD2495         PART NUMBER: 00       PRINTED ON JUN 12, 1984 AT 22:10:06   OUTPUT DEVICE: NAPR2


@RUN SELEN,5994,3908464260,5M,500
@SYM PRINT$,,XEROX1
@ASG,AX PROBENECID.
@ERS PROBENECID.
FURPUR 28R3A U02 SLIB34 06/12/84 22:09:56
END ERS.
@FOR,IS ,PROBENECID.DIFEQ
FORTRAN-MACC 1.17S-06/12/84-22:09:56              DIFEQ
00101       1.            INTEGER N,METH,MITER,INDEX,IWK(1),IER,K
00102       2.            REAL Y(6),WK(19),X,TOL,XEND,H,YY(1)
00103       3.            DIMENSION X(7)
00104       4.            DATA X/0.0,1.0,2.0,3.0,4.0,5.0,6.0/
00106       5.            EXTERNAL FCN,FCNJ
00107       6.            N=1
00110       7.            TOL=0.001
00111       8.            H=0.01
00112       9.            METH=1
00113      10.            MITER=1
00114      11.            YY(1)=31.77
00115      12.            INDEX=1
00116      13.            DO 10 K=1,6
00121      14.            XX=X(K)
00122      15.            XEND=X(K+1)
00123      16.            CALL DGEAR (N,FCN,FCNJ,X,H,YY,XEND,TOL,METH,MITER,INDEX,IWK,WK,
00123      17.           *IER)
00124      18.            INDEX=2
00125      19.            WRITE(-,-)K,XEND,YY(1)
00132      20.            Y(K)=YY(1)
00133      21.        10  CONTINUE
00135      22.            STOP
00136      23.            END

      END OF COMPILATION:         NO  DIAGNOSTICS.
@FOR,IS ,PROBENECID.SUBFCN
FORTRAN-MACC 1.17S-06/12/84-22:09:57              SUBFCN
00101       1.            SUBROUTINE FCN(N,X,YY,DY)
00102       2.            DIMENSION DY(1),YY(1)
00103       3.            TH1=85.44
00104       4.            TH2=1.0
00105       5.            TH3=130.0
00106       6.            TH4=21.81
00107       7.            DY(1)=TH1*TH2*EXP(-TH2*X)-(TH4*YY(1))/(TH3+YY(1))
00110       8.            RETURN
00111       9.            END

      END OF COMPILATION:         NO  DIAGNOSTICS.
@FOR,IS ,PROBENECID.SUBFCNJ
FORTRAN-MACC 1.17S-06/12/84-22:09:58              SUBFCNJ
00101       1.            SUBROUTINE FCNJ(N,X,YY,PD)
00102       2.            REAL YY(N),PD(N,N)
00103       3.            TH3=130.0
00104       4.            TH4=21.81
00105       5.            PD(1,1)=(-TH4*TH3)/((TH3+YY(1))**2)
```

```
00106       6.              RETURN
00107       7.              END

       END OF COMPILATION:          NO  DIAGNOSTICS.
@MAP,IN ,PROBENECID.PROGRAM
Collector 31R1U04 1984 Jun 12 Tue 2209:59


END MAP.  ERRORS: 0  TIME: 7.530  STORAGE: 19459/7/042677/0101477
@P
 Ready
@XQT PROBENECID.PROGRAM
                1      1.0000          78.9446
                2      2.0000          90.1084
                3      3.0000          88.5027
                4      4.0000          82.5274
                5      5.0000          75.2777
                6      6.0000          67.8990
@FIN

    RUNID:  AD2495   PROJECT: 05994                  USER: 3908464260
22:10:05    AD2495 FIN

  ITEM                                    AMOUNT       COST

CPU TIME                          00:00:00.508         $0.01
EXEC CHARGES                                 9         $0.10
SOFTWARE SUPPORT                            15         $0.16
FILE I/O REQUESTS                          299         $0.05
FILE I/O WORDS                          328289         $0.02
MEMORY USAGE                             0.081         $0.03
CARDS IN                                    52         $0.01
PAGES PRINTED                                1         $0.02
SHARED FILES                                 2         $0.22
JOB CHARGE                                   1         $0.04

TOTAL COST                                             $0.66

RUN TIME: 22:09:55 --> 22:10:05 JUN 12,84.  PREV: 22:08:21 JUN 12,84
THE $ AMOUNTS ARE APPROXIMATE
RATE MULTIPLIERS    0.50 OVERNIGHT
                    1.00 UW MADISON
```

```
@RUN SELEN,5994,3908464260,5M,500
@SYM PRINT$,,XEROX1
@ASG,AX PROBENECID.
@ERS PROBENECID.
FURPUR 28R3A U02 SLIB34 06/12/84 22:10:40
END ERS.
@FOR,IS ,PROBENECID.DIFEQ
FORTRAN-MACC 1.17S-06/12/84-22:10:40                DIFEQ
00101       1.              INTEGER N,METH,MITER,INDEX,IWK(1),IER,K
00102       2.              REAL Y(6),WK(19),X,TOL,XEND,H,YY(1)
00103       3.              DIMENSION X(7)
00104       4.              DATA X/0.0,1.0,2.0,3.0,4.0,5.0,6.0/
00106       5.              EXTERNAL FCN,FCNJ
00107       6.              N=1
00110       7.              TOL=0.001
00111       8.              H=0.01
00112       9.              METH=1
00113      10.              MITER=1
00114      11.              YY(1)=63.06
00115      12.              INDEX=1
00116      13.              DO 10 K=1,6
00121      14.              XX=X(K)
00122      15.              XEND=X(K+1)
00123      16.              CALL DGEAR (N,FCN,FCNJ,X,H,YY,XEND,TOL,METH,MITER,INDEX,IWK,WK,
00123      17.             *IER)
00124      18.              INDEX=2
00125      19.              WRITE(-,-)K,XEND,YY(1)
00132      20.              Y(K)=YY(1)
00133      21.          10  CONTINUE
00135      22.              STOP
00136      23.              END

       END OF COMPILATION:         NO  DIAGNOSTICS.
@FOR,IS ,PROBENECID.SUBFCN
FORTRAN-MACC 1.17S-06/12/84-22:10:41                SUBFCN
00101       1.              SUBROUTINE FCN(N,X,YY,DY)
00102       2.              DIMENSION DY(1),YY(1)
00103       3.              TH1=85.44
00104       4.              TH2=1.0
00105       5.              TH3=130.0
00106       6.              TH4=21.81
00107       7.              DY(1)=TH1*TH2*EXP(-TH2*X)-(TH4*YY(1))/(TH3+YY(1))
00110       8.              RETURN
00111       9.              END

       END OF COMPILATION:         NO  DIAGNOSTICS.
@FOR,IS ,PROBENECID.SUBFCNJ
FORTRAN-MACC 1.17S-06/12/84-22:10:42                SUBFCNJ
00101       1.              SUBROUTINE FCNJ(N,X,YY,PD)
00102       2.              REAL YY(N),PD(N,N)
00103       3.              TH3=130.0
00104       4.              TH4=21.81
00105       5.              PD(1,1)=(-TH4*TH3)/((TH3+YY(1))**2)
```

```
00106      6.            RETURN
00107      7.            END

       END OF COMPILATION:        NO  DIAGNOSTICS.
@MAP,IN ,PROBENECID.PROGRAM
Collector 31R1U04 1984 Jun 12 Tue 2210:43


END MAP.  ERRORS: 0  TIME: 7.528  STORAGE: 19459/7/042677/0101477
@P
 Ready
@XQT PROBENECID.PROGRAM
                1      1.0000        108.1784
                2      2.0000        117.8013
                3      3.0000        114.7823
                4      4.0000        107.4108
                5      5.0000         98.7531
                6      6.0000         89.9466
@FIN

    RUNID:  AD2496   PROJECT: 05994              USER: 3908464260
22:10:50    AD2496 FIN

  ITEM                                  AMOUNT       COST

CPU TIME                        00:00:00.506        $0.01
EXEC CHARGES                               9        $0.10
SOFTWARE SUPPORT                          15        $0.16
FILE I/O REQUESTS                        299        $0.05
FILE I/O WORDS                        328289        $0.02
MEMORY USAGE                           0.081        $0.03
CARDS IN                                  52        $0.01
PAGES PRINTED                              1        $0.02
SHARED FILES                               2        $0.22
JOB CHARGE                                 1        $0.04

TOTAL COST                                          $0.66

RUN TIME: 22:10:38 --> 22:10:50 JUN 12,84.  PREV: 22:10:07 JUN 12,84
THE $ AMOUNTS ARE APPROXIMATE
RATE MULTIPLIERS     0.50 OVERNIGHT
                     1.00 UW MADISON
```

```
*  *  *  *  *  *  SPERRY 1180 TIME/SHARING EXEC --- MULTI-PROCESSOR SYSTEM --- VER.  MACC 38.22  SITE *  MACC     *  *  *  *  *  *
RUNID: AD2497        PROJECT: 05994       USER: 3908464260          CREATED ON JUN 12, 1984 AT 22:11:24    INPUT DEVICE: NACR3
          FILE NAME: PR@000AD2497          PART NUMBER: 00          PRINTED ON JUN 12, 1984 AT 22:11:36   OUTPUT DEVICE: NAPR2


@RUN SELEN,5994,3908464260,5M,500
@SYM PRINT$,,XEROX1
@ASG,AX PROBENECID.
@ERS PROBENECID.
FURPUR 28R3A U02 SLIB34 06/12/84 22:11:25
END ERS.
@FOR,IS ,PROBENECID.DIFEQ
FORTRAN-MACC 1.17S-06/12/84-22:11:25              DIFEQ
00101       1.            INTEGER N,METH,MITER,INDEX,IWK(1),IER,K
00102       2.            REAL Y(6),WK(19),X,TOL,XEND,H,YY(1)
00103       3.            DIMENSION X(7)
00104       4.            DATA X/0.0,1.0,2.0,3.0,4.0,5.0,6.0/
00106       5.            EXTERNAL FCN,FCNJ
00107       6.            N=1
00110       7.            TOL=0.001
00111       8.            H=0.01
00112       9.            METH=1
00113      10.            MITER=1
00114      11.            YY(1)=79.5
00115      12.            INDEX=1
00116      13.            DO 10 K=1,6
00121      14.            XX=X(K)
00122      15.            XEND=X(K+1)
00123      16.            CALL DGEAR (N,FCN,FCNJ,X,H,YY,XEND,TOL,METH,MITER,INDEX,IWK,WK,
00123      17.           *IER)
00124      18.            INDEX=2
00125      19.            WRITE(-,-)K,XEND,YY(1)
00132      20.            Y(K)=YY(1)
00133      21.         10 CONTINUE
00135      22.            STOP
00136      23.            END

      END OF COMPILATION:         NO  DIAGNOSTICS.
@FOR,IS ,PROBENECID.SUBFCN
FORTRAN-MACC 1.17S-06/12/84-22:11:27              SUBFCN
00101       1.            SUBROUTINE FCN(N,X,YY,DY)
00102       2.            DIMENSION DY(1),YY(1)
00103       3.            TH1=85.44
00104       4.            TH2=1.0
00105       5.            TH3=130.0
00106       6.            TH4=21.81
00107       7.            DY(1)=TH1*TH2*EXP(-TH2*X)-(TH4*YY(1))/(TH3+YY(1))
00110       8.            RETURN
00111       9.            END

      END OF COMPILATION:         NO  DIAGNOSTICS.
@FOR,IS ,PROBENECID.SUBFCNJ
FORTRAN-MACC 1.17S-06/12/84-22:11:28              SUBFCNJ
00101       1.            SUBROUTINE FCNJ(N,X,YY,PD)
00102       2.            REAL YY(N),PD(N,N)
00103       3.            TH3=130.0
00104       4.            TH4=21.81
00105       5.            PD(1,1)=(-TH4*TH3)/((TH3+YY(1))**2)
```

```
00106       6.              RETURN
00107       7.              END

      END OF COMPILATION:         NO  DIAGNOSTICS.
@MAP,IN ,PROBENECID.PROGRAM
Collector 31R1U04 1984 Jun 12 Tue 2211:29


END MAP.  ERRORS: 0  TIME: 7.530  STORAGE: 19459/7/042677/0101477
@P
 Ready
@XQT PROBENECID.PROGRAM
                1       1.0000          123.7476
                2       2.0000          132.6851
                3       3.0000          128.9983
                4       4.0000          120.9873
                5       5.0000          111.6740
                6       6.0000          102.1961
@FIN

    RUNID:  AD2497   PROJECT: 05994              USER: 3908464260
22:11:35    AD2497 FIN

  ITEM                                  AMOUNT       COST

CPU TIME                        00:00:00.507        $0.01
EXEC CHARGES                               9        $0.10
SOFTWARE SUPPORT                          15        $0.16
FILE I/O REQUESTS                        299        $0.05
FILE I/O WORDS                        328289        $0.02
MEMORY USAGE                           0.081        $0.03
CARDS IN                                  52        $0.01
PAGES PRINTED                              1        $0.02
SHARED FILES                               2        $0.22
JOB CHARGE                                 1        $0.04

TOTAL COST                                          $0.66

RUN TIME: 22:11:24 --> 22:11:35 JUN 12,84.  PREV: 22:10:51 JUN 12,84
THE $ AMOUNTS ARE APPROXIMATE
RATE MULTIPLIERS     0.50 OVERNIGHT
                     1.00 UW MADISON
```

```
* * * * * * SPERRY 1180 TIME/SHARING EXEC --- MULTI-PROCESSOR SYSTEM --- VER.  MACC 38.22  SITE *  MACC     * * * * * *
RUNID: AD2498        PROJECT: 05994       USER: 3908464260          CREATED ON JUN 12, 1984 AT 22:13:38    INPUT DEVICE: NACR3
          FILE NAME: PR@000AD2498          PART NUMBER: 00          PRINTED ON JUN 12, 1984 AT 22:13:53   OUTPUT DEVICE: NAPR2


@RUN SELEN,5994,3908464260,5M,500
@SYM PRINT$,,XEROX1
@ASG,AX PROBENECID.
@ERS PROBENECID.
FURPUR 28R3A U02 SLIB34 06/12/84 22:13:39
END ERS.
@FOR,IS ,PROBENECID.DIFEQ
FORTRAN-MACC 1.17S-06/12/84-22:13:40                DIFEQ
00101      1.            INTEGER N,METH,MITER,INDEX,IWK(1),IER,K
00102      2.            REAL Y(7),WK(19),X,TOL,XEND,H,YY(1)
00103      3.            DIMENSION X(8)
00104      4.            DATA X/0.0,2.0,4.0,8.0,12.0,16.0,24.0,36.0/
00106      5.            EXTERNAL FCN,FCNJ
00107      6.            N=1
00110      7.            TOL=0.001
00111      8.            H=0.01
00112      9.            METH=1
00113     10.            MITER=1
00114     11.            YY(1)=106.03
00115     12.            INDEX=1
00116     13.            DO 10 K=1,7
00121     14.            XX=X(K)
00122     15.            XEND=X(K+1)
00123     16.            CALL DGEAR (N,FCN,FCNJ,X,H,YY,XEND,TOL,METH,MITER,INDEX,IWK,WK,
00123     17.           *IER)
00124     18.            INDEX=2
00125     19.            WRITE(-,-)K,XEND,YY(1)
00132     20.            Y(K)=YY(1)
00133     21.        10  CONTINUE
00135     22.            STOP
00136     23.            END

      END OF COMPILATION:         NO  DIAGNOSTICS.
@FOR,IS ,PROBENECID.SUBFCN
FORTRAN-MACC 1.17S-06/12/84-22:13:41                SUBFCN
00101      1.            SUBROUTINE FCN(N,X,YY,DY)
00102      2.            DIMENSION DY(1),YY(1)
00103      3.            TH1=85.44
00104      4.            TH2=1.0
00105      5.            TH3=130.0
00106      6.            TH4=21.81
00107      7.            DY(1)=TH1*TH2*EXP(-TH2*X)-(TH4*YY(1))/(TH3+YY(1))
00110      8.            RETURN
00111      9.            END

      END OF COMPILATION:         NO  DIAGNOSTICS.
@FOR,IS ,PROBENECID.SUBFCNJ
FORTRAN-MACC 1.17S-06/12/84-22:13:43                SUBFCNJ
00101      1.            SUBROUTINE FCNJ(N,X,YY,PD)
00102      2.            REAL YY(N),PD(N,N)
00103      3.            TH3=130.0
00104      4.            TH4=21.81
00105      5.            PD(1,1)=(-TH4*TH3)/((TH3+YY(1))**2)
```

```
00106      6.            RETURN
00107      7.            END

      END OF COMPILATION:         NO  DIAGNOSTICS.
@MAP,IN ,PROBENECID.PROGRAM
Collector 31R1U04 1984 Jun 12 Tue 2213:44


END MAP.  ERRORS: 0  TIME: 7.506  STORAGE: 19459/7/042677/0101477
@P
 Ready
@XQT PROBENECID.PROGRAM
                1      2.0000        157.0736
                2      4.0000        143.5080
                3      8.0000        102.6993
                4     12.0000         68.3808
                5     16.0000         42.6149
                6     24.0000         13.8882
                7     36.0000          2.0637
@FIN

    RUNID:  AD2498   PROJECT: 05994              USER: 3908464260
22:13:51    AD2498 FIN

  ITEM                                  AMOUNT       COST

CPU TIME                        00:00:00.517        $0.01
EXEC CHARGES                               9        $0.10
SOFTWARE SUPPORT                          15        $0.16
FILE I/O REQUESTS                        299        $0.05
FILE I/O WORDS                        328290        $0.02
MEMORY USAGE                           0.081        $0.03
CARDS IN                                  52        $0.01
PAGES PRINTED                              1        $0.02
SHARED FILES                               2        $0.22
JOB CHARGE                                 1        $0.04

TOTAL COST                                          $0.66

RUN TIME: 22:13:38 --> 22:13:51 JUN 12,84.  PREV: 22:11:35 JUN 12,84
THE $ AMOUNTS ARE APPROXIMATE
RATE MULTIPLIERS    0.50 OVERNIGHT
                    1.00 UW MADISON
```


UNIVERSITY OF WISCONSIN COMPUTER CENTER

```
***EOF***        2 PAGES COST     $0.10      AD2498   56789012345678901234567890123456789O
```