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PHARMACOKINETICS OF ORAL 500 mg PENICILLAMINE: EFFECT OF ANTACID ON ABSORPTION

by

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

MASTER OF SCIENCE
(Pharmacy)

at the

UNIVERSITY OF WISCONSIN-MADISON

1984

Pharmacy
AUM
IF25

Dedicated to the

Memory of my Mother

Mrs. Mwuese Ifan

... I love you, mom. And may your soul rest in perfect peace, Amen.

ACKNOWLEDGEMENTS

I thank my God whose goodness and mercy has brought me thus far. It is my prayer that His grace will follow me even as I venture into the next tier of my academic pursuit.

I wish to thank Dr. P. G. Welling for helping me realize my academic goal. I appreciate his kindness especially during my time of emotional stress, and for his thoughtful suggestions on this write-up. It has been nice working with him.

I wish also to thank Drs. K. A. Connors, W. R. Porter, and R. Burnette who, individually, contributed to the successful completion of this project, and for agreeing to serve on the committee.

I thank my wife, Mrs. Zanenge (Kwagh u sha Aōndo) Ifan, who has consistently provided the much needed inspiration for me to keep going. I enjoy being married to her.

To my colleagues, Florence W. Kwoḳ, Arzu Selen, Mark C. Rogge, and Diana Marrero, I wish all of them God's guidance and success in their professional careers.

PHARMACOKINETICS OF ORAL 500 mg PENICILLAMINE:
EFFECT OF ANTACID ON ABSORPTION

Agber Ifan

(Under the supervision of Professor P. G. Welling)

D-penicillamine (penicillamine) is the degradation product formed by the hydrolysis of penicillin. It is used for the treatment of a variety of disease conditions including: Wilson's disease, cystenuria, rheumatoid arthritis and biliary cirrhosis. It is administered orally, therefore, it is important that it be efficiently absorbed. It is important also to understand the absorption efficiency of penicillamine under the different conditions that it may be used.

It has been shown previously that plasma levels of penicillamine are markedly reduced when administered in the presence of iron salts and also by antacid. This study was carried out to identify the mechanism by which antacid preparation inhibit penicillamine absorption.

Penicillamine was administered to four healthy human volunteers in a randomized cross-over design. Three treatments, designated as treatments A, B, and C corresponding, respectively, to the fasted treatment

(control), the treatment immediately following Aludrox suspension, and to the treatment immediately following sodium bicarbonate solution. The doses were administered with the equivalent of 200 ml of total liquid intake. The four volunteers were each administered the three treatments at least one week apart. Blood and urine samples were collected and analyzed by high performance liquid chromatography with electrochemical detection.

Plasma penicillamine levels after dosing indicated that chelation, rather than pH change, plays the dominant role in the mechanism controlling the inhibition of the absorption of penicillamine when administered immediately following antacid preparation. However, in view of the poor agreement between urinary data obtained in this study and literature values, the pharmacokinetic analysis of the data was not pursued further.

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Professor of Pharmaceutics

Date: May 15th 1984

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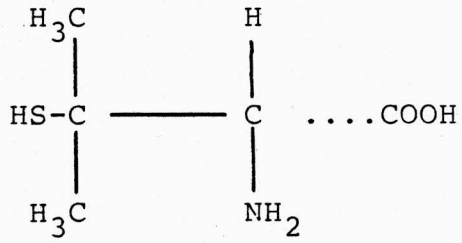
INTRODUCTION

1. Physical Properties and Chemical Structure:

D-3, 3-dimethylcysteine or D-penicillamine (Penicillamine) is the characteristic degradation product formed by the hydrolysis of penicillin (1). It is marketed under different trade names including: Cuprimine (MSD); Cuprenil, Depamine, Trolovol, DMC, and Distamine (Wallace) (2).

Penicillamine has the following chemical structure:



with a molecular weight of 149.21. It is a fine white or practically white crystalline powder having a slightly bitter taste (3). It is soluble in 9 parts of water, soluble in 530 parts of 96% ethanol, practically insoluble in chloroform and ether at 20°C (4).

The reported pK_a values of the carboxyl, α-amino, and β-thiol groups are 1.8, 7.9, and 10.5, respectively (1).

2. Uses and Chemical Reactivity:

Penicillamine is used clinically for the symptomatic management of Wilson's disease (5), cystenuria (6), lead poisoning (7), rheumatoid arthritis (8), and has recently been tried as a protective agent against radiation (9). It has also been used in cases of biliary cirrhosis (10), chronic hepatitis (11), and hyperbilirubinemia (12).

Penicillamine bears chemical similarity to a number of endogenous compounds, principal among these being cysteine and valine. Penicillamine differs from cysteine in that the two hydrogen atoms attached to the β-carbon atom of cysteine are replaced in penicillamine by methyl groups. It differs from valine in having a sulfhydryl group (13). This chemical similarity necessitates the use of an exceptionally specific assay method to accurately define penicillamine pharmacokmetics.

Penicillamine is a highly reactive molecule, the three functional groups undergoing characteristic reactions. These reactions include acid-base equilibria, nucleophilic addition and displacement, complexation with various metals, and oxidation (14, 15). It is likely, therefore, that factors such as pH and ionization (as specified by the pK_a values) will influence the various reactions of penicillamine. Penicillamine-disulfide formation,

especially, is known to be catalyzed by weakly alkaline environments (pH 8-9) (16). The pharmacological and therapeutic actions of penicillamine are, to a very large extent, explained on the basis of its chemical reactivity, namely its ability to chelate metal ions and take part in oxidation-reduction reactions, sulfhydryl-disulfide interchange and nucleophilic addition.

3. Metabolism:

Although penicillamine is able to take part in a variety of chemical reactions (17), only certain ones are biologically significant (18). These are presented in Scheme I. Penicillamine is capable of forming disulfide linkages either with another penicillamine molecule or with any other sulfhydryl-containing molecule, for example cysteine, giving rise to the so-called mixed D-penicillamine-cysteine disulfide. Disulfide formation does not require enzyme catalysis (18). The formation of metal chelates (Scheme II) and thiazolidines (Scheme III) are also spontaneous processes, not requiring catalysis. Methylation, however, is thought to be catalyzed by enzymes from intestinal bacteria (19). The primary path of penicillamine catabolism appears to be via disulfide formation unless there are significantly elevated levels of heavy metals present. In such

Complex Formation:

Pen - SH + Heavy Metal $\rightleftharpoons$ (Pen - SH) - Metal
(Cu, Zn, Al, etc.)

Pen + Pyridoxal $\rightleftharpoons$ Thiazolidine Derivative

Formation of Disulfides:

Pen - SH + Thiol - SH $\rightleftharpoons$ Pen - S - S - Thiol
(Pen - SH, Cysteine)

Pen - SH + Protein - SH $\rightleftharpoons$ Pen - S - S - Protein

Thiol-Disulfide Exchange:

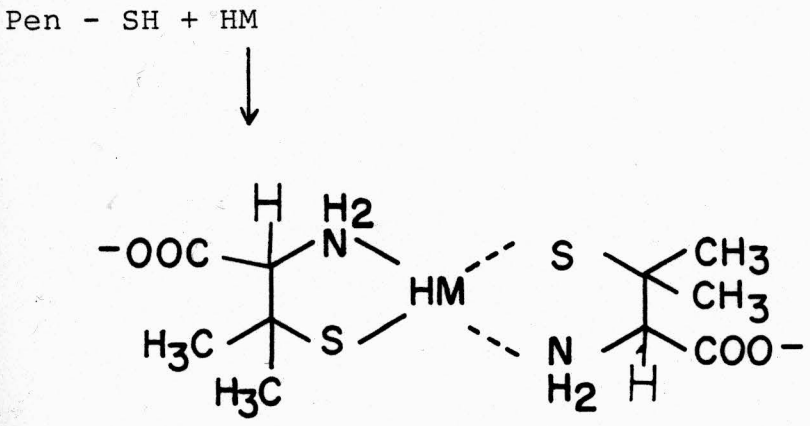
Pen - SH + Disulfide $\rightleftharpoons$ Pen - S - Thiol + Thiol - SH
(Thiol - S - S - Thiol)

Pen - SH + Protein - S - S - Thiol $\rightleftharpoons$ Protein - S - S -
Pen + Thiol - SH

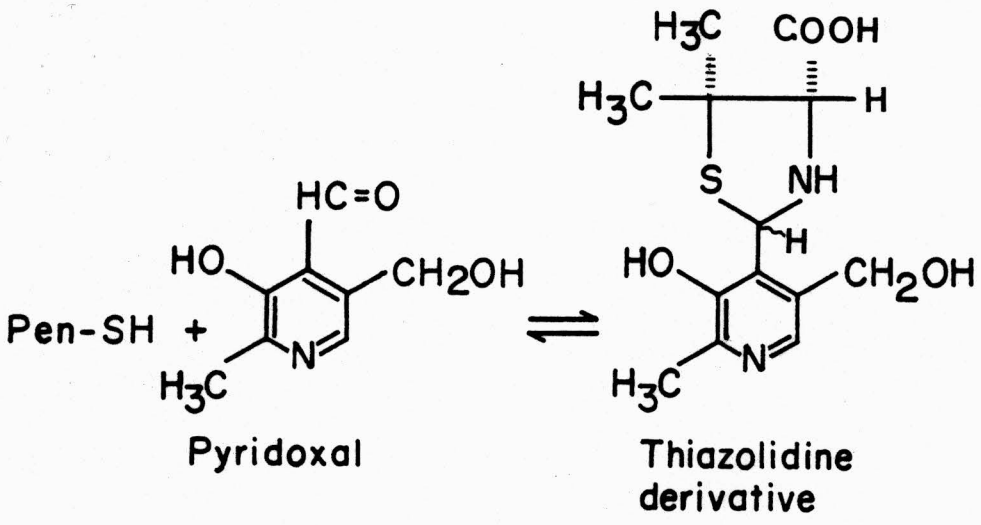
Methylation:

Pen - SH + $-CH_3$ (methyl donor) $\longrightarrow$ S - Methyl - Pen

Scheme I: Reactions of Penicillamine (Pen.)



Scheme II: Chelation of heavy metals (HM) by penicillamine. Two possible chelation structures are depicted. The heavy metal may interact with the amino and thiol groups of a single penicillamine molecule (solid lines to the left of HM) or it may be bound to two penicillamine molecules (entire structure around HM).



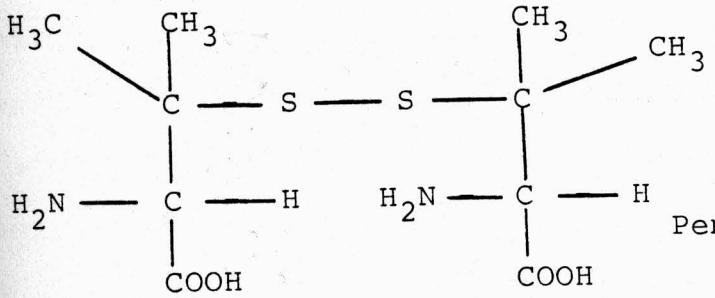
Scheme III: Reaction of Penicillamine with pyridoxal.

circumstances (e.g., Wilson's disease) more penicillamine would be excreted as a heavy metal chelate. Overall, the biochemical actions of penicillamine are attributed to its aminothiol properties, i.e., chelation of metals, reaction with carbonyl groups, and interference with sulfhydryl disulfide exchange reactions.

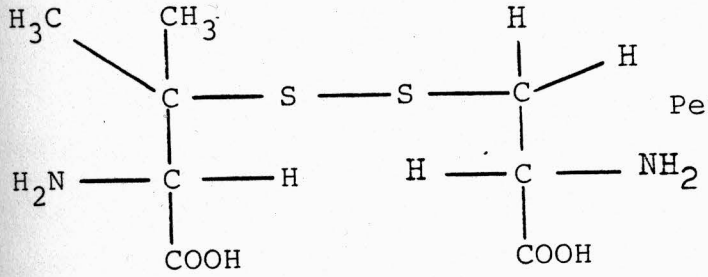
Penicillamine disulfide, penicillamine-cysteine disulfide, penicillamine homocysteine disulfide, and S-methyl-penicillamine constitute the major known metabolites of penicillamine in man. The chemical structures of these metabolites are presented in Scheme IV.

4. Absorption and Disposition:

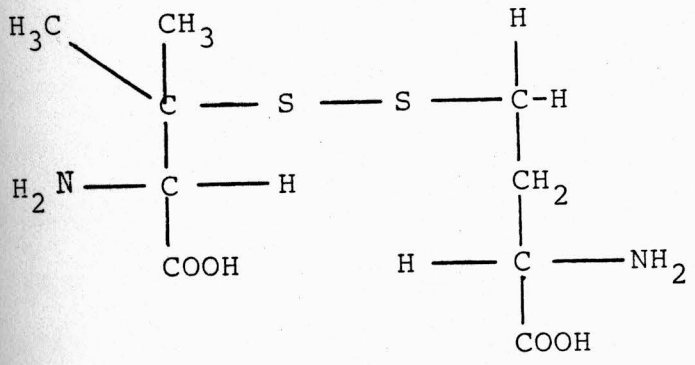
Penicillamine is taken orally and absorbed from the gut. The mechanism of penicillamine transport by the gut is not clear (18). It has been suggested, based on in-vitro studies with inverted sacs of rat intestine, that penicillamine is passively transported across the intestinal lumen (20). More recently, Perrett (21, 22) has proposed that penicillamine transport is enhanced by binding to gut proteins prior to absorption, and has quantified the uptake of the unchanged (reduced) penicillamine and its disulfide metabolites. He reports that less than 1% of the disulfide was absorbed from a perfusate whereas 37% of a 2m mole/liter solution of



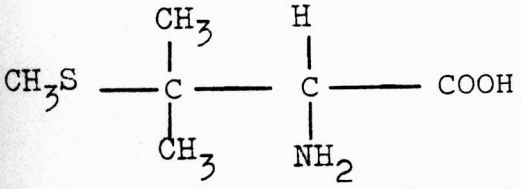
Penicillamine disulfide



Penicillamine-cysteine disulfide



Penicillamine homocysteine disulfide



S-methyl-penicillamine

Scheme IV: Chemical structures of known metabolites of penicillamine.

unchanged penicillamine was lost from a perfusate in both normal and cystenuric subjects (a finding which, the author claims, is consistent with the known stero-specificity of amino acid transport systems). This finding suggests that any preabsorption process favoring disulfide formation will decrease the systemic availability of penicillamine.

The rate of absorption of penicillamine is rapid, with peak plasma concentrations attained within 1.6 to 3.8 hr. (23 - 25). The initial clearance rate in the urine is correspondingly high, a finding which led Gibbs and Walshe (26) to conclude that penicillamine is rapidly absorbed from the gut and distributed throughout the body water space into urine until plasma protein binding reduces the rate of clearance. Consistent plasma patterns were obtained in human subjects, but there was marked intersubject variability in patterns and the kinetic parameters (23).

Very little penicillamine is found free in the plasma (18). Preliminary findings (27) placed the free penicillamine concentration below 20 μm. The remainder of the blood penicillamine, which may be as much as 90 percent of the total (26) appears to be bound to protein. Much of it is attached to albumin and some may

also be associated with α-globulins and ceruloplasmin. The erythrocytes only account for a small proportion of the blood penicillamine, (27) an observation consistent with the poor ability of penicillamine to penetrate red blood cells (28).

Animal studies with radiolabelled penicillamine (18) have shown that a variety of tissues can accumulate radioactivity. Radioactivity is, however, rapidly cleared from organs such as liver and kidney, while in tissues which are rich in collagen or elastin (skin, bone, cartilage) the label is lost at a much slower rate. The slow elimination of penicillamine from connective tissue may explain why penicillamine (or its metabolites) are only slowly excreted by the body (29).

Penicillamine is bacterially degraded in the intestine, presumably to the disulfide form, since this represents the predominant fecal excretion product (22). Part of an absorbed dose may also be lost in the so-called first-pass metabolic process (30), since penicillamine is oxidatively deaminated by D-aminoacid oxidase, an enzyme active in the liver and kidney for the detoxification of D-aminoacids (22). This may explain why the combined urinary and fecal recovery accounts for only approximately 65% of an administered dose.

5. Analytical Methods:

Despite its long clinical use, a complete pharmacokinetic characterization of penicillamine has been lacking. The lack of adequate pharmacokinetic studies may be attributed in part to the nonavailability of sensitive and specific assay methods for the compound.

Numerous methods have been developed for the analysis of penicillamine including colorimetry (15), gas-liquid chromatography (31), and radioimmunoassay (32, 33). But these methods are fraught with the possibility of interference from endogenous compounds or even penicillamine's metabolites. Also, these methods possess inherent limitations per se, especially regarding cost and the need for complex sample manipulation. Because of these limitations, most of the early pharmacokinetic studies were done utilizing radiolabelled penicillamine in animals (34 - 38), and humans (28, 39). Obviously, such studies represent both the unchanged penicillamine as well as penicillamine metabolites (19), and are inappropriate for characterizing the pharmacokinetics of unchanged penicillamine.

The development of high pressure liquid chromatography (HPLC) methods for the determination of penicillamine provided an assay method with the specificity

and sensitivity necessary to conduct pharmacokinetic studies. Assays for penicillamine using electrochemical detection have been described recently (27, 40). But even these methods are not without problems, which have tended to preclude their widespread use. More recently, Miners et. al (16) have described an HPLC method involving the fluorescence derivatization of the sulfhydryl group of penicillamine using N-[p-(2-benzoxazolyl)-phenyl] maleimide (BOPM), a reagent with specificity for the sulfhydryl group. The usefulness of this method for pharmacokinetic studies has not yet been established.

STATEMENT OF THE PROBLEM

Circulating levels of penicillamine have been shown (25) to be markedly reduced when administered in the presence of ferrous sulfate, an iron preparation containing the divalent cation Fe^{2+}, and also in the presence of an antacid preparation containing both Al^{3+} and Mg^{2+} cations, as compared to the fasting state. The reduction in plasma penicillamine levels, however, was more prounounced with the ferrous sulfate treatment. While the inhibition of penicillamine absorption by Fe^{2+} ion strongly suggests a chelation effect, as with the well-documented tretracycline-ion interaction, the mechanism by which antacid inhibits penicillamine absorption is not clear.

The involvement of chelation in the case of tetracycline is so well accepted that most reviews on drug interactions warn only against the concomitant use of tetracyclines and substances containing polyvalent cations. The absorption of tetracycline has now been known, however, to be reduced to a similar extent when administered immediately following sodium bicarbonate, an antacid which contains no polyvalent cations and cannot form chelates with tetracycline.

Therefore, while a chelation effect involving Al^{3+} and/or Mg^{2+} cations is possible, with regard to penicillamine-antacid interaction, the elevated pH due to the antacid may also be important. It has been reported (16) that penicillamine disulfide formation is increased at weakly alkaline pH. This can also inhibit the systemic availability of orally administered penicillamine. In order to resolve this question, the study which is the subject of this thesis, was designed to compare the systemic availability of penicillamine administered orally in the presence of a commercial antacid preparation containing polyvalent metal ions, solution of sodium bicarbonate, and also under controlled condition.

EXPERIMENTAL

1. Materials and Study Design

The subjects were four healthy human male volunteers, 23 to 26 (mean 24.5) years of age, weighing between 70 to 80 (mean 74) kg. Subject details appear in Table I. The subjects were shown to be physically fit by a medical examination; blood chemistry values were within the normal range. Normal blood chemistry values appear in Appendix I.

Subjects were instructed not to take any other drugs for at least one week before or during the study. Subjects were also instructed not to ingest caffeine-containing beverages or eat chocolate for two days before, and also during, the study.

Commercial capsules of penicillamine, Cuprimine,[(a)] containing 250 mg of active ingredient per capsule, were utilized in the study. Two forms of antacid preparations were used: Aludrox[(b)] suspension, containing 307 mg aluminum hydroxide as a gel and 103 mg of magnesium hydroxide per 5 ml; and sodium bicarbonate,[(c)] 7.056 gm of water solution.

(a) Cuprimine® 250 mg Capsules, Lot No. J)532, Merck Sharp and Dohme, Division of Merck and Company, Inc., West Point, PA 19486.

(b) Aludrox® Suspension, Lot No. 3830026, Wyeth Laboratories Inc., Philadelphia, PA 19101.

(c) Sodium Bicarbonate Powder, Lot No. 221211, Columbus Chemical Industries Inc., Columbus, WI 53925.

Table I: Subject details.

Subject	Age	Height	Weight(kg)
1	25	5' 11"	70
2	24	6' 2"	80
3	23	5' 10"	75
4	26	5' 10"	72
Mean	24.5	5' 11"	74

All studies were carried out at the premises of School of Pharmacy, University of Wisconsin, Madison. Penicillamine was administered in a randomized crossover design as follows:

Subject	Treatment		
1	A	B	C
2	B	C	A
3	C	A	B
4	B	A	C

where treatments A, B, and C refer, respectively, to the fasting dose, the dose immediately following the ingestion of Aludrox suspension, and the dose immediately following the ingestion of sodium bicarbonate solution. The treatments were administered at least one week apart to each subject. Because of the need to process the samples immediately upon collection, the subjects were divided into two groups each consisting of two subjects. Groups one and two each received the three treatments on the first and second days, respectively, of any week.

2. Protocol

Subjects received single 500 mg (2 x 250 mg) oral doses of penicillamine under three different conditions designated as treatments A, B, and C corresponding,

respectively, to the fasted dose, the dose immediately following 30 ml of Aludrox suspension, and the dose immediately following a 7.056 gm per 30 ml aqueous solution of sodium bicarbonate. All doses were administered such as to ensure the intake of exactly 200 ml fluid volume.

The doses of antacid used in treatments B and C were calculated so that both had the same acid-neutralizing capacity.

Dosage calculation

According to production information[d] available, 10 ml of a suspension of Aludrox has the capacity for neutralizing 28 mEq of acid. Based on this information and a molecular weight of 84 for sodium bicarbonate, the amount of sodium bicarbonate to be used was calculated to be 7.056 gm.

Subjects were instructed to eat no solid food after 8.00 p.m. and to drink no liquid after 10.00 p.m. on the day before each penicillamine dose. On a study day, subjects drank 200 ml of water on rising (at least one hour before dosing). The 2 x 250 mg capsules of Cuprimine were swallowed whole without chewing. No

(d) Physicians' Desk Reference (PDR), 32nd Edition, p. 1811, 1978.

additional food or other liquid intake was permitted. Normal eating and drinking was resumed four hours after dosing but the caffeine and chocolate restrictions continued throughout the sampling period.

Blood samples (approximately 8 ml) were drawn from a forearm vein into EDTA (K_3)-containing Vacutainers[e] immediately before and then at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, and 12.0 hr after penicillamine administration. Urine samples were collected immediately before and then quantitatively during 0-2, 2-4, 4-8, 8-12, and 12-24 hr after dosing. Urine volumes were recorded and 10 ml samples retained for assay.

3. Analysis

Sample Preparation

The instability of penicillamine in plasma and other blood components as well as in urine has been reported previously (27, 41). As a result of the significant loss of penicillamine over a short period of time, blood and urine samples were treated immediately to precipitate the proteins and to decrease the pH. The penicillamine molecule has been reported (41) to be more stable in the acidic pH range.

(e) Vacutainer® Brand, Lot No. 3F039, Becton-Dickinson, Rutherford, N.J.

Blood samples were immediately separated into eight 1-ml micro test tubes and centrifuged at 15,000 r.p.m., using an Eppendorf Micro Centrifuge,(f) for approximately 1 min. The supernatant was pipetted into a clean test tube containing 1 ml of 15% w/v trichloroacetic acid to precipitate the proteins. The test tube was chilled in ice for 2-3 min and centrifuged(g) at 2,500 r.p.m. for approximately 5 min. The supernatant was filtered through a 0.45 μM membrane(h) filter and the filtrate was stored at 1-4°C. The samples were generally analyzed within 1-5 days of collection.

Urine samples were diluted two-fold with 0.4 M citric acid and stored at 1-4°C. Thawed urine samples were diluted five-fold (ten-fold in some instances) in 0.4 M citric acid, a 0.7 ml sample was then treated with 0.1 ml, 0.1% aqueous EDTA and electrolyzed. On the average, urine samples were analyzed within one week of collection.

(f) Eppendorf Micro Centrifuge, Model 5414, Brunkmann Instruments, Inc., Westbury, N.Y. 11590.

(g) Dynac Centrifuge, Becton, Dickinson and Company, Parsippany, NJ 07054.

(h) Whatman Limited, Maidstone, England.

The electrolytic method for the reduction of penicillamine disulfides to penicillamine described by Saetre and Rabenstein (27) was used. A diagram of the electrolytic cell appears in Figure I. The cell consisted of a 1.5 x 5 cm tube with an inverted U-shaped side arm (1 mm in diameter attached to the side approximately 1 cm from the bottom. A Hg pool of approximately 0.7 cm depth was used. Electrical contact to the Hg was made via a platinum wire sealed into the bottom. The side arm was filled with saturated sodium chloride/2% Agar and dipped into a beaker containing saturated sodium chloride and a platinum wire counter electrode. A small magnetic stirrer bar was placed on top of the Hg pool, and was used to ensure a homogenous mix of the sample.

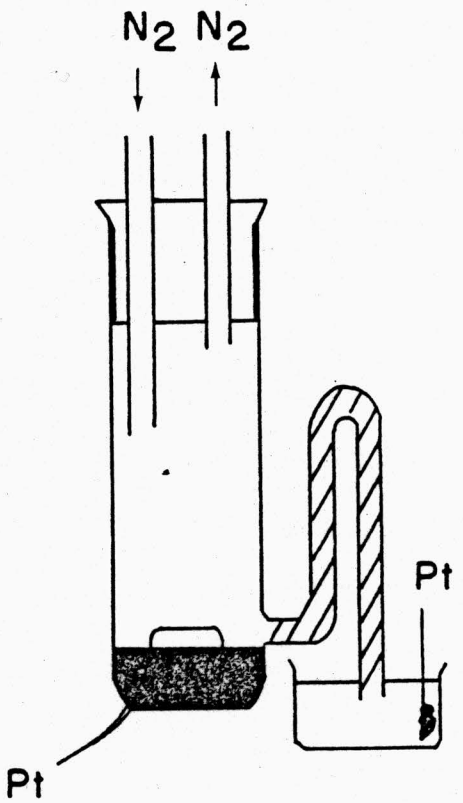
A Hewlett-Packard 6218 A power supply unit was used to supply a current of 10 m Amps to the electrolytic cell for approximately 5 min.

Chromatography

The plasma and urine samples were analyzed by the high performance liquid chromatographic method recently described by Saetre and Rabenstein (27), and by Bergstrom

Figure I: Electrolysis cell for the reduction of penicillamine disulfides. Details are given in the text.

FIG. I



ELECTROLYSIS CELL

et al. (40). The chromatographic system consisted of a 5 x 0.4 cm guard column and a 30 x 0.4 cm analytical column, both packed with Cation exchange resin.(i) The mobile phase consisting of a 0.03 M citric acid/ 0.01 M dibasic sodium phosphate buffer, pH 2.8, was de-aerated with nitrogen for approximately 15 min. This was pumped through the columns at a flow rate of 2.5 ml per min by means of an Altex model 110 A solvent metering pump. At this flow rate, the back pressure amounted to approximately 1.2×10^3 p.s.i. The mobile phase was maintained under a nitrogen atmosphere during chromatography.

Penicillamine concentrations were determined by means of a LC-3 amperometric detector coupled with an LC-25 system monitor.(j) The detector was comprised of a mercury/gold (Hg/Au) amalgam working electrode surface, a silver/silver chloride (Ag/AgCl) reference electrode, and an auxillary electrode, placed directly across from the working electrode surface. Some details of electrochemical detection appear in Appendix II. The magnitude of the working electrode potential was

(i) Zipax SCX®, strong cation exchange resin made of a fluoropolymer base. It has $-SO_3^-$ functional groups, Du Pont Instrument, Welmington, Del. 19898.

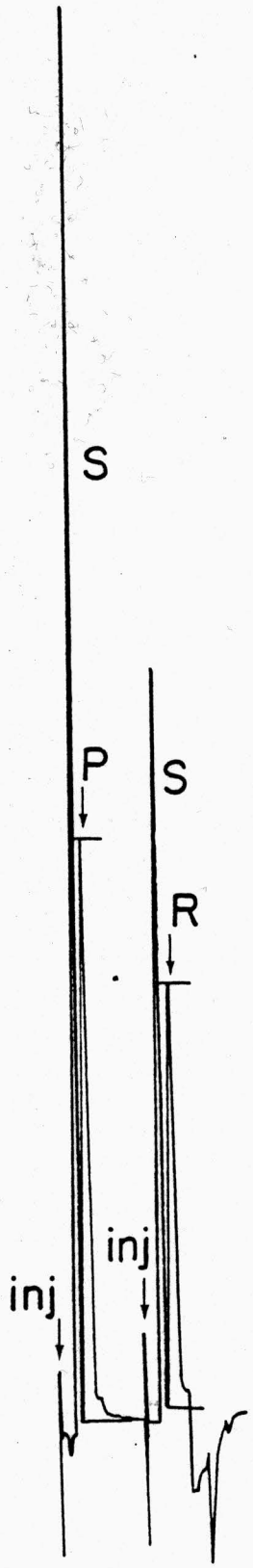
(j) LC-3 Amperometric Detector and LC-25 system monitors®, Bioanalytical System Inc., West Lafayette, IN 47906.

maintained at +0.1 volt relative to the reference electrode by means of the system monitor. The background current was generally less than 10 n Amps. The Linear[(k)] recorder input was set at 1 volt and the chart speed at 20 cm per hr. In this chromatographic system, penicillamine was eluted as a clear sharp peak. See Figure II. The concentration of penicillamine was determined from the peak height ratio of the unknown sample and a standard sample injected immediately after each unknown sample.

(k) Linear® Recorder, model 255. Linear Instrument Corp., Irvine, Cal.

Figure II: A chromatogram of penicillamine. S = solvent front, P = penicillamine peak from unknown sample, and R = penicillamine peak from standard solution prepared by dissolving penicillamine in EDTA (1g/l).

FIG. II



RESULTS

A. Plasma Drug Concentration

1. Pharmacokinetic Analysis:

Preliminary estimates of pharmacokinetic parameters were obtained by methods previously described by Wagner (43) and Wagner and Metzler (44). Elimination rate constants for the disappearance of penicillamine from plasma in individual subjects were calculated by linear regression of the log-linear terminal plasma concentration data. The elimination rate constants were obtained as the product of the slopes of these linear regression lines and -2.303. From the extrapolation to time t = 0 and subsequent curve stripping, or feathering, estimates of the first-order absorption rate constants were obtained. The intercepts on the log-concentration axes were used to calculate FD_o/V ratios:

$$\text{Intercept} = \frac{FD_o}{V} \left[\frac{k_a}{k_a - k_{el}} \right] \text{(a)}$$

(a) Parameters of this expression are defined in the discussion section.

These preliminary estimates of the absorption and elimination rate constants were used to fit individual plasma penicillamine concentration data sets by the method of least squares. The method uses an iterative nonlinear regression analysis program, NREG[b] on a digital computer,[c] and the procedure was done so as to conform to the equation for the one-compartment open model analysis of pharmacokinetic data. Details of this equation are presented in the discussion section.

The elimination half-life, $t_{½}$ (el), and the absorption half-time, $t_{½}$ (abs), were calculated as:

$$t_{½}\ (el) = \frac{0.693}{k_{el}}$$

and

$$t_{½}(abs) = \frac{0.693}{k_{a}}$$

The areas under the plasma penicillamine concentration-time profiles, AUC's, were obtained by the trapezoidal method.

2. Plasma Penicillamine Concentration-Time Data:

Tables II, III, and IV show individual plasma penicillamine concentration data corresponding to treatments A, B, and C, respectively. Semilogarithmic

(b) With user supplied subroutine. A typical NREG output is shown in Appendix III.

(c) Univac 1110 digital computer, Madison Academic Computing Center, University of Wisconsin, Madison, Wisconsin.

Table II: Plasma Penicillamine Concentrations

Following Treatment A

	Drug Concentration mcg/ml								
Subject	0.0HR	0.5HR	1.0HR	2.0HR	3.0HR	4.0HR	6.0HR	8.0HR	12.0HR
1	0.00	1.38	0.95	1.15	1.57	1.76	0.94	0.45	0.00
2	0.00	0.47	0.47	0.53	1.11	0.72	0.60	0.29	0.00
3	0.00	0.38	0.81	1.33	1.09	0.86	0.36	0.03	0.00
4	0.00	0.31	0.23	1.64	1.75	1.33	0.95	0.46	0.00

Table III: Plasma Penicillamine Concentrations

Following Treatment B

	Drug Concentration mcg/ml								
Subject	0.0HR	0.5HR	1.0HR	2.0HR	3.0HR	4.0HR	6.0HR	8.0HR	12.0HR
1	0.00	0.75	1.21	1.21	1.05	0.91	0.66	0.28	0.10
2	0.00	0.22	0.17	0.15	0.31	0.26	0.08	0.00	0.00
3	0.00	0.02	0.14	0.99	0.69	0.72	0.16	0.05	0.00
4	0.00	0.00	1.16	1.21	1.35	1.41	0.48	0.09	0.00

Table IV: Plasma Penicillamine Concentrations Following Treatment C

	Drug Concentration mcg/ml								
Subject	0.0HR	0.5HR	1.0HR	2.0HR	3.0HR	4.0HR	6.0HR	8.0HR	12.0HR
1	0.00	0.55	0.89	2.21	1.29	0.80	0.25	0.12	0.00
2	0.00	0.55	1.69	1.63	1.07	1.03	0.35	0.01	0.00
3	0.00	0.30	0.93	1.10	1.15	0.54	0.25	0.00	0.00
4	0.00	0.62	0.86	1.36	1.89	1.97	0.41	0.00	0.00

Figure III: Semilogarithmic plot of plasma penicillamine concentration versus time for subject No. 1. (▲) Treatment A., (■) Treatment B., (●) Treatment C.

FIG. III

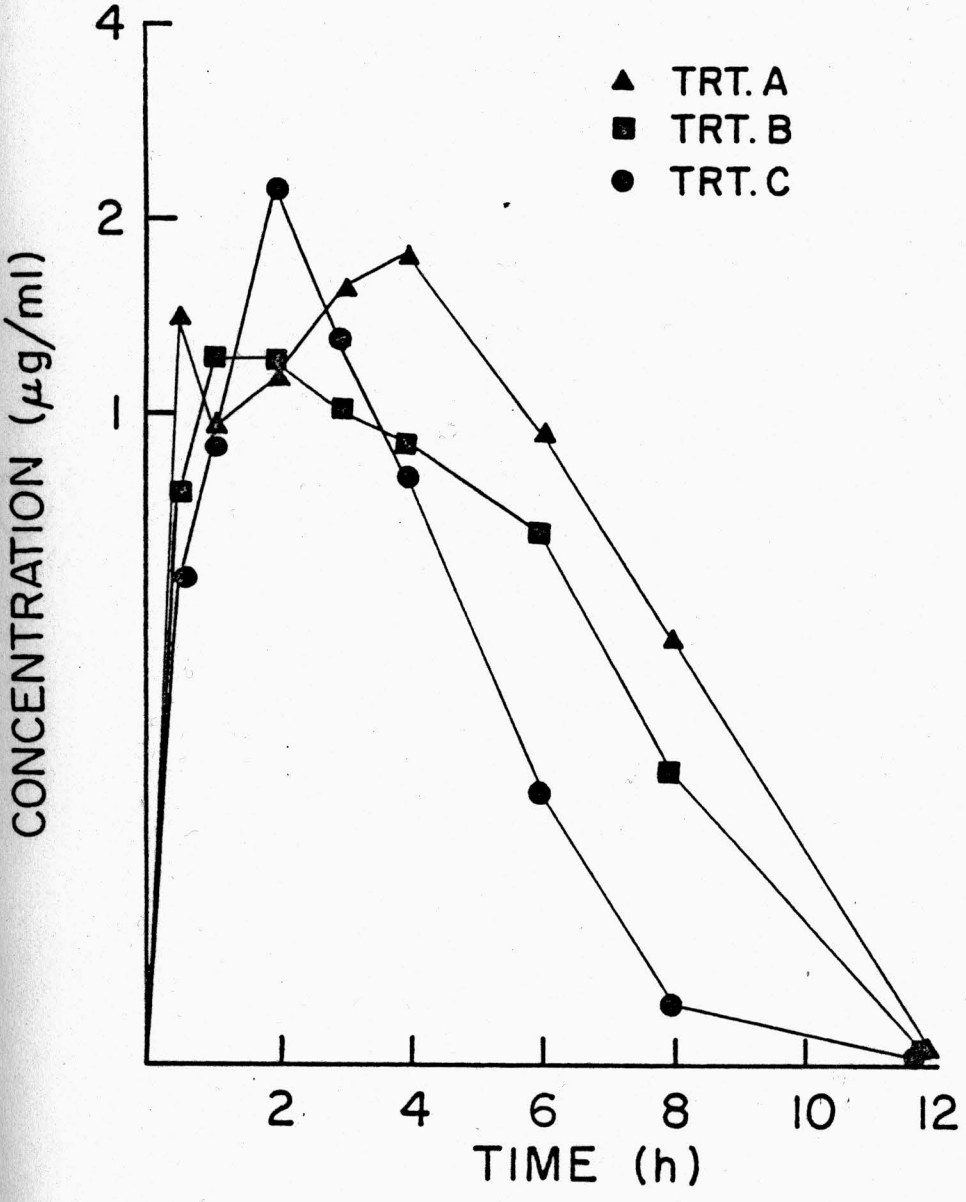


Figure IV: Semilogarithmic plot of plasma penicillamine concentration versus time for subject No. 2. (▲) Treatment A., (■) Treatment B., (●) Treatment C.

FIG. IV

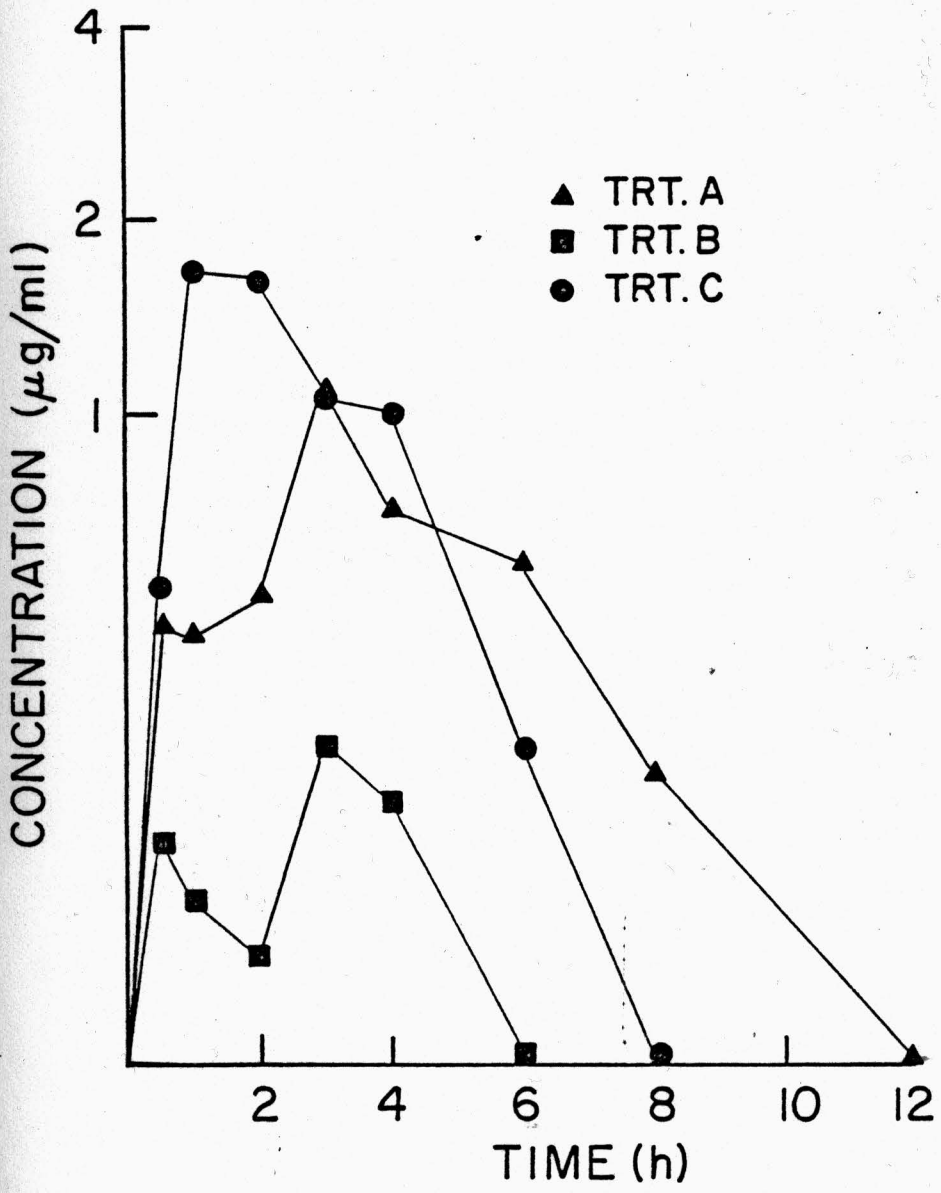


Figure V: Semilogarithmic plot of plasma penicillamine concentration versus time for subject No. 3. (▲) Treatment A., (■) Treatment B., (●) Treatment C.

FIG. V

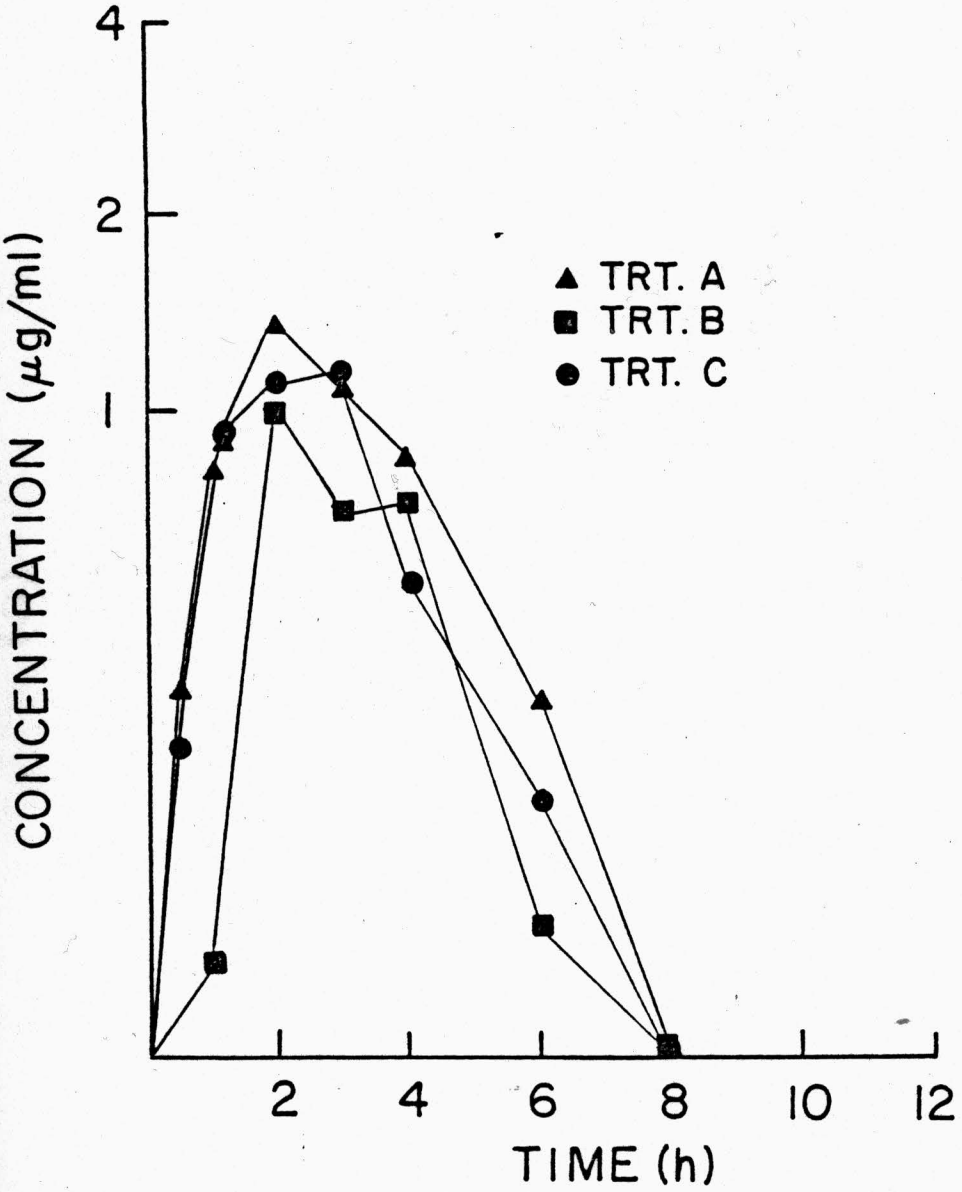
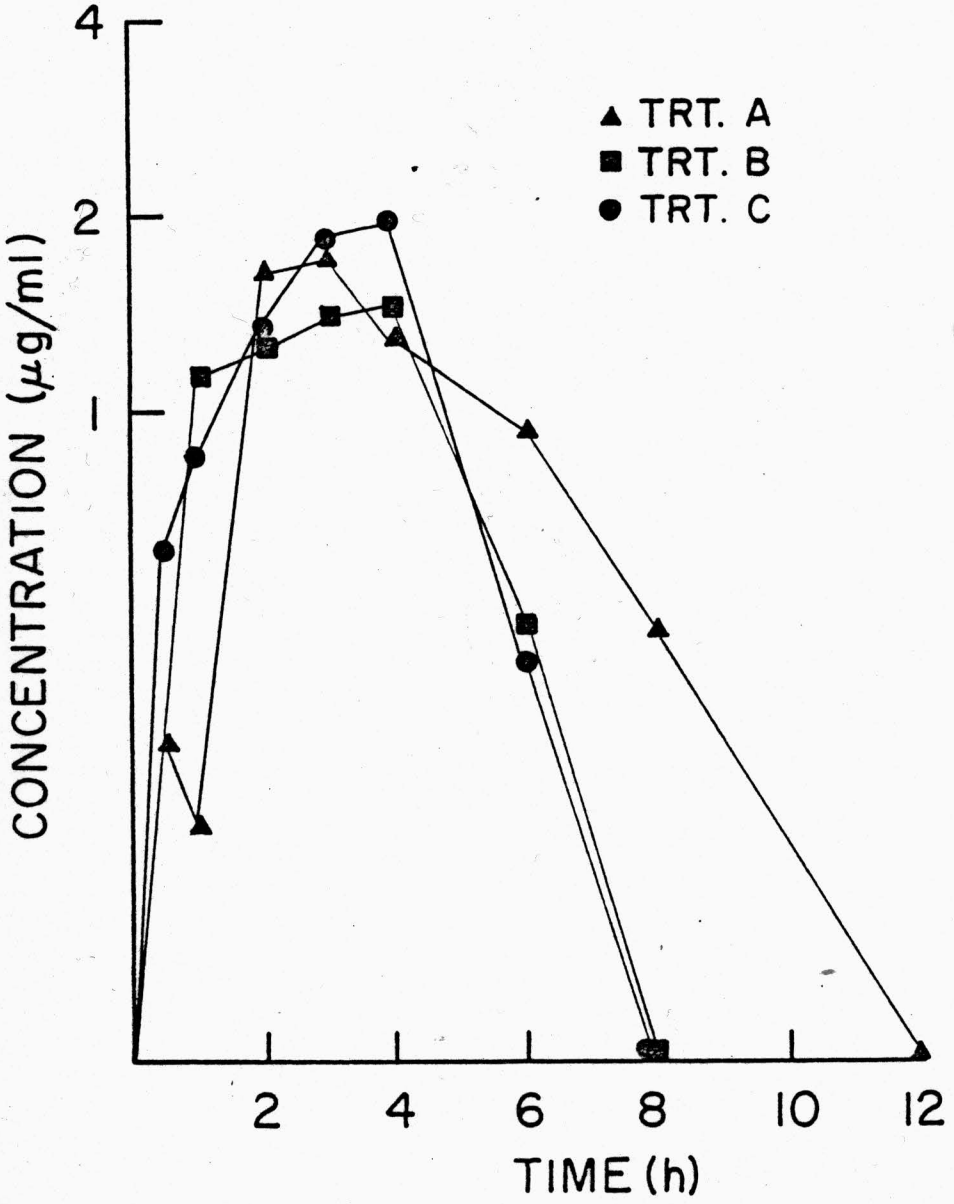


Figure VI: Semilogarithmic plot of plasma penicillamine concentration versus time for subject No. 4. (▲) Treatment A., (■) Treatment B., (●) Treatment C.

FIG. VI



plots of plasma penicillamine concentration-time profiles are shown in Figures III to VI. The phenomenon of double peaks following the fasted dose previously reported by Bergstrom et al. (23) and Osman et al. (25), was observed with three subjects. In these subjects, however, there was no double peak following the Aludrox and bicarbonate treatments.

Mean plasma penicillamine concentrations are shown in Table V with the corresponding semilogarithmic plots shown in Figures VII to IX. The plasma penicillamine levels reached a mean peak value of 1.35 mcg/ml at 3.0 hr following treatment A and declined to 0.01 mcg/ml at 12.0 hr. The plasma penicillamine levels from treatments B and C both had an earlier decline to approximately 0.01 mcg/ml at 8.0 hr. The mean peak plasma penicillamine concentration after treatment C was 1.55 mcg/ml at 2.0 hr, while that for treatment B only amounted to 0.89 mcg/ml at 2.0 hr. The time to peak in both treatments B and C thus occurred earlier than in the fasted treatment.

3. Summary of Pharmacokinetic Parameters

The least squares estimates of individual pharmacokinetic parameters are presented in Table VI to VIII. The parameters were compared among the three

Table V: Mean Plasma Penicillamine Concentrations Following Treatments A, B, and C.

	Drug Concentration mcg/ml								
Treatment	0.0HR	0.5HR	1.0HR	2.0HR	3.0HR	4.0HR	6.0HR	8.0HR	12.0HR
A	0.00	0.64 (0.50)[a]	0.62 (0.33)	1.16 (0.47)	1.36 (0.36)	1.17 (0.36)	0.71 (0.29)	0.38 (0.10)	0.00
B	0.00	0.25 (0.35)	0.67 (0.60)	0.89 (0.50)	0.85 (0.45)	0.83 (0.48)	0.35 (0.27)	0.10 (0.13)	0.00
C	0.00	0.51 (0.15)	1.10 (0.40)	1.58 (0.48)	1.35 (0.37)	1.09 (0.62)	0.32 (0.08)	0.03 (0.06)	0.00

a ± One Standard Deviation

Figure VII: Semilogarithmic plot of mean plasma penicilammine concentration versus time for Treatment A. Error bars represent 1 S.D.

FIG. VII

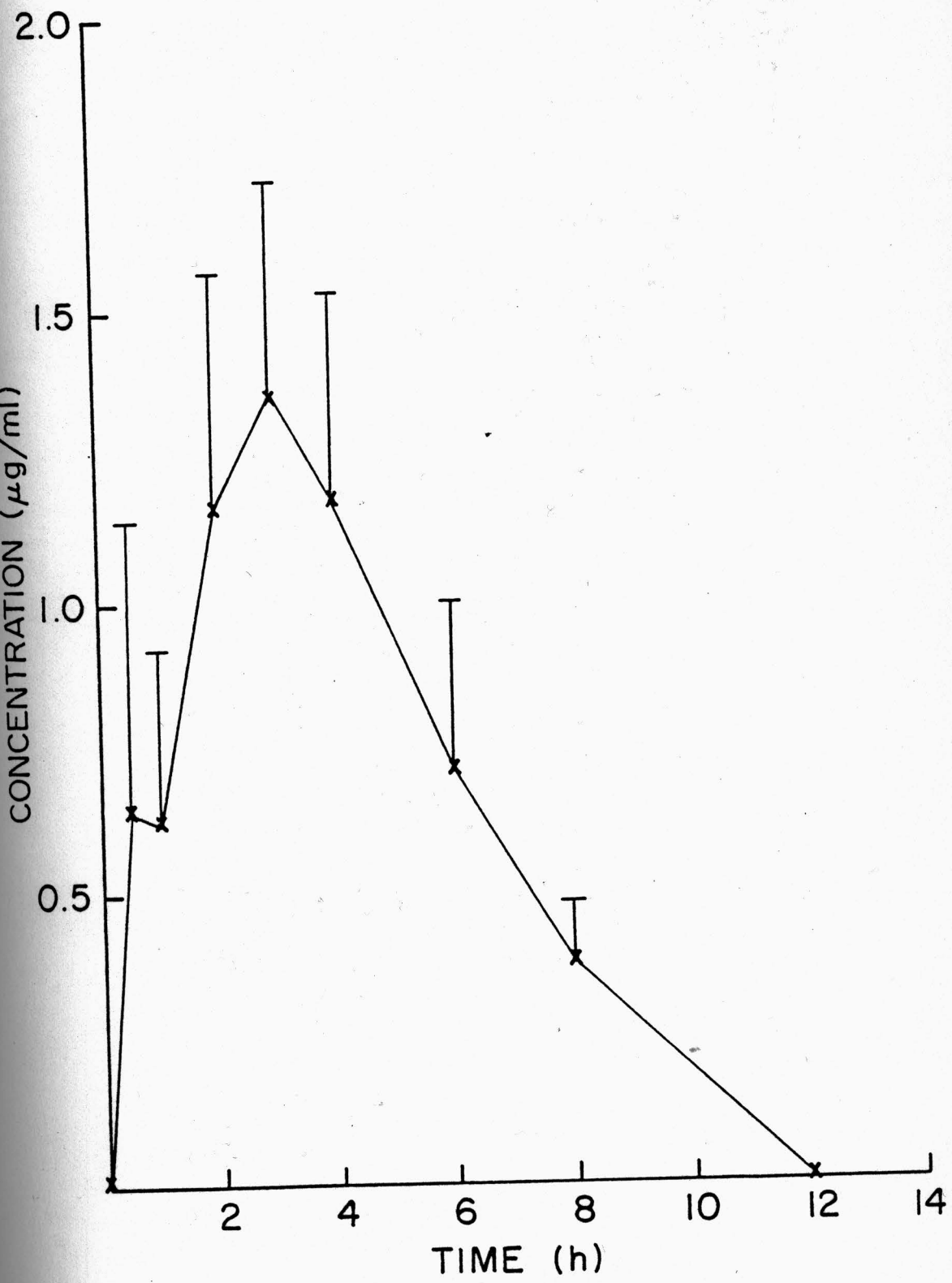


Figure VIII: Semilogarithmic plot of mean plasma penicillamine concentration *versus* time for Treatment B. Error bars represent 1 S.D.

FIG. VIII

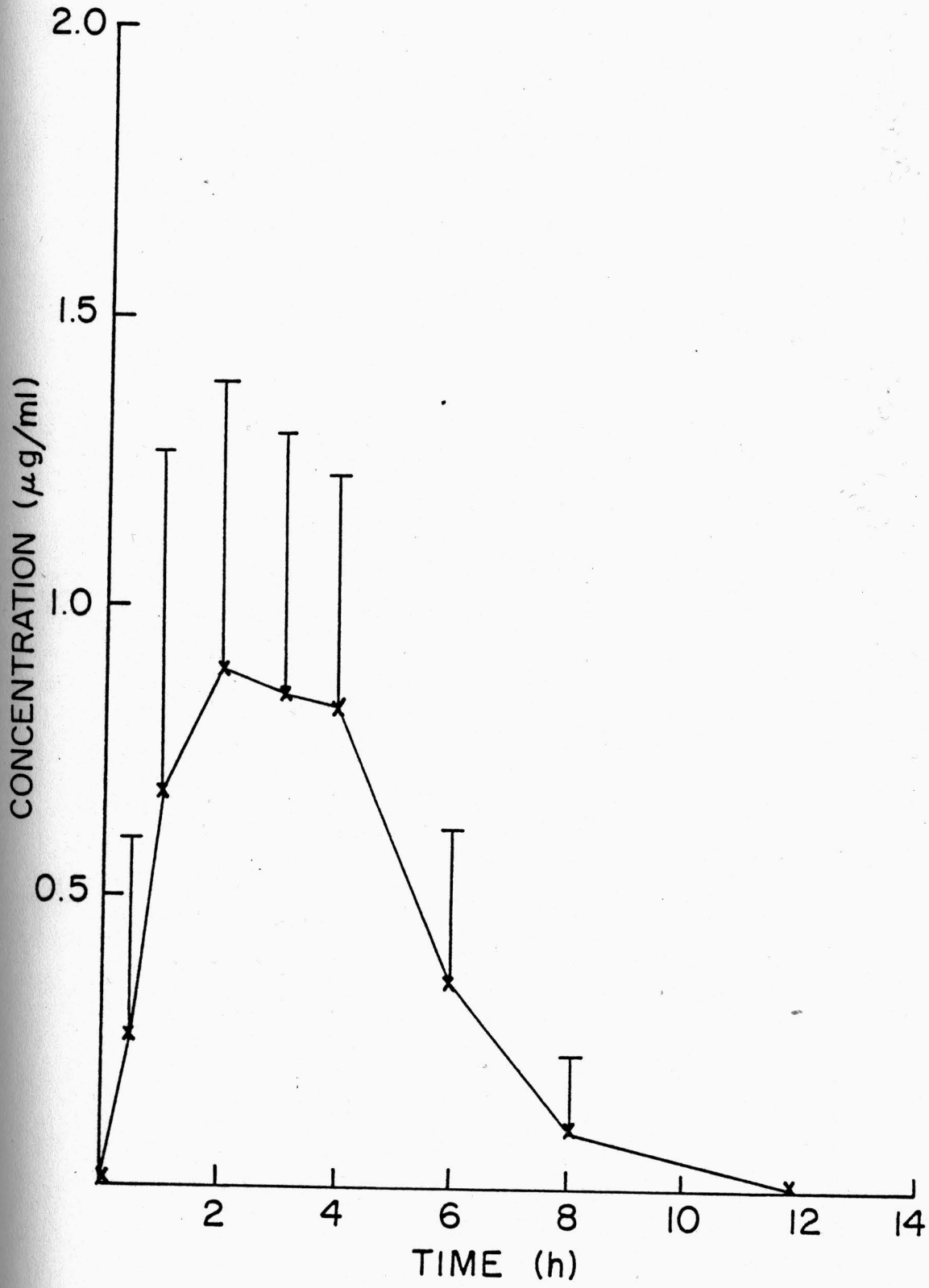


Figure IX: Semilogarithmic plot of mean plasma penicillamine concentration *versus* time for Treatment C. Error bars represent 1 S.D.

FIG. IX

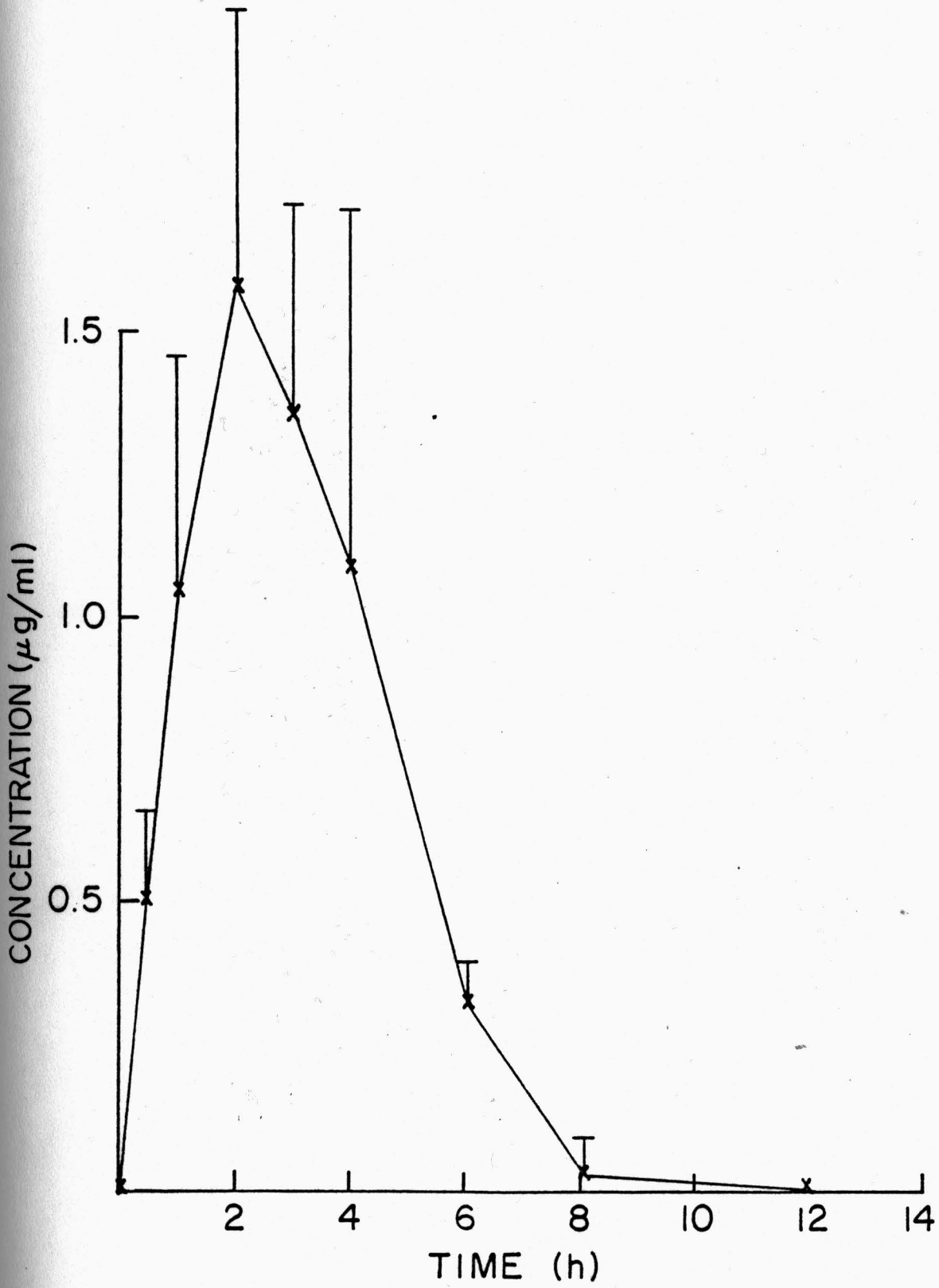


Table VI: Summary of Pharmacokinetic Parameters

Following Treatment A

Subject	FD/V mcg/ml	AUC mcg hr/ml	C_{max}* mcg/ml	T_{max}* hr	k_{a} hr^{-1}	k_{el} hr^{-1}	$t_{½}$(abs) hr	$t_{½}$(el) hr	t_{o} hr	R^2
1	3.99	10.0	1.80	4	0.44	0.37	1.56	1.87	0.10	0.92
2	2.20	5.38	1.20	3	0.37	0.36	1.89	1.93	0.10	0.93
3	2.70	5.32	1.30	2	0.57	0.44	1.22	1.58	1.00	0.97
4	3.28	9.03	1.80	3	0.86	0.36	0.80	1.93	0.91	0.98
Mean	3.04	7.43	1.53	3	0.56	0.38	1.37	1.83	0.53	0.95
± S.D.	0.77	2.44	0.32	0.82	0.22	0.04	0.47	0.17	0.50	0.03
% CV	25	33	21	27	39	10	34	9.2	93	3

*Represent observed values.

Table VII: Summary of Pharmacokinetic Parameters

Following Treatment B

Subject	FD/V mcg/ml	AUC mcg hr/ml	C_{max} mcg/ml	T_{max} hr	k_a hr^{-1}	k_{el} hr^{-1}	$t_{½}$(abs) hr	$t_{½}$(el) hr	t_o hr	R^2
1	1.79	7.07	1.20	1	0.13	0.23	2.54	3.08	0.11	1.00
2	0.52	1.26	0.31	3	0.66	0.35	1.05	1.98	0.10	0.87
3	1.77	3.35	0.98	2	0.78	0.75	0.89	0.92	0.92	0.98
4	1.75	6.77	1.40	4	0.57	0.56	1.22	1.23	0.46	0.96
Mean	1.46	4.61	0.97	2.50	0.53	0.47	1.43	1.80	0.4	0.95
± S.D.	0.62	2.80	0.47	1.29	0.29	0.23	0.75	0.96	0.4	0.06
% CV	43	61	48	51.6	53.6	49	53	53.3	97	6

Table VIII: Summary of Pharmacokinetic Parameters

Following Treatment C

Subject	FD/V mcg/ml	AUC mcg hr/ml	C_{max} mcg/ml	T_{max} hr	k_{a} hr^{-1}	k_{el} hr	$t_{½}$(abs) hr	$t_{½}$(el) hr	t_{o} hr	R^2
1	3.89	6.50	2.40	2	0.86	0.57	0.80	1.22	0.38	0.94
2	3.58	6.52	1.70	1	0.74	0.51	0.94	1.36	0.90	0.96
3	2.12	4.41	1.15	3	0.74	0.40	0.94	1.73	0.90	0.94
4	5.02	7.98	2.0	4	0.91	0.69	0.76	1.00	0.16	0.87
Mean	3.65	6.35	1.81	2.5	0.81	0.54	0.86	1.33	0.58	0.93
± S.D.	1.19	1.47	0.53	1.29	0.04	0.12	0.09	0.31	0.37	0.04
% CV	33	23	29	51.6	11	22	11	23	64	4

Table IX: Statistics of Penicillamine Kinetic Values

Parameter	Treatment A	Treatment B	Treatment C	Statistic
$\frac{FD}{V}$	3.04 ± 0.77	1.46 ± 0.62	3.65 ± 1.19	B < AC [a]
AUC	7.43 ± 2.44	4.61 ± 2.80	6.35 ± 1.47	B < AC [b]
C_{max}	1.53 ± 0.32	0.97 ± 0.47	1.81 ± 0.53	B < AC [c]
T_{max}	3 ± 0.82	2.50 ± 1.29	2.5 ± 1.29	NS
$t_{½}$(el)	1.83 ± 0.17	1.80 ± 0.4	1.33 ± 0.31	NS

a. Level of significance set at $P < 0.01$ (n = 4)

b. Level of significance set at $P < 0.10$ (n = 4)

c. Level of significance set at $P < 0.05$ (n = 4)

NS = not significant at the 95% confidence limit.

treatments by analysis of variance (ANOVA). Differences between individual treatments were examined by Tukey's test (45). A summary of the statistical evaluation of the pharmacokinetic parameters appears in Table IX.

4. Percent Absorbed-Time Plots (Wagner-Nelson Method):

The mean time course of penicillamine concentration profile appeared to indicate a difference in the rate at which drug was absorbed following the different treatments. Penicillamine is generally administered over long periods of time for subchronic conditions, and it is associated with many adverse side-effects. It is important from a clinical viewpoint, therefore, to be able to estimate the rate of its absorption. A very rapid absorption rate may produce transiently high drug concentrations in plasma, and this could precipitate adverse side-effects.

The problems associated with the characterization of absorption kinetics by curve-fitting revolve around the uncertainty in estimating the absorption rate constant, k_a. Absorption rate constants obtained by curve-fitting are at best estimates of the first-order loss of drug from the gastrointestinal tract, not of the first-order appearance of the drug in the systemic circulation (42). As pointed out in the introductory section, penicillamine

is bacterially degraded in the gut, presumably to the disulfide form. It is also known to undergo oxidation at slightly alkaline pH's. The apparent absorption rate constant, k_a, obtained by curve-fitting, therefore, is the sum of these various degradative pathways in addition to other factors such as gastric emptying or gastrointestinal mobility. Problems similar to this prompted the search for better methods of evaluating absorption kinetics. One of such methods is based on the construction and evaluation of percent absorbed-time plots. These plots do not rely on the assumption of zero- or first-order absorption.

Equation 1 is an expression for the ratio of the amount of drug absorbed to time t, $(X_A)_t$, to the amount of drug ultimately absorbed, $(X_A)_\infty$. The amount of drug ultimately absorbed in this case is the same as $(X_A)_{12\ hr}$.

$$\frac{(X_A)_t}{(X_A)_\infty} = \frac{C_t + k_{el} \int_o^t C\ dt}{k_{el} \int_o^\infty C\ dt} \qquad 1^{(d)}$$

where the parameters are as defined in the discussion section. Plots of percent absorbed versus time are shown

(d) See reference 43, pp 149-155 for the derivation and discussion of this equation.

in Figures X to XII. These plots indicate a relatively faster absorption rate following the sodium bicarbonate treatment. At approximately 2.0 hr post-dosing, absorption from treatment C is virtually complete.

5. Cumulative Relative Fraction Absorbed, CRFA:

The relative absorption of penicillamine from treatments A, B, and C in individual subjects was estimated by calculating the absorption of drug from treatments B and C relative to treatment A. The cumulative relative fraction absorbed, CRFA was estimated using equation 2, (46):

$$CRFA = \frac{C_t + k_{el}{}^{A} (AUC)_{0-t}}{k_{el}{}^{a} (AUC)^{A}_{0-\infty}} \qquad 2$$

where C_t is the concentration of penicillamine in plasma at time t, $(AUC)_{0-t}$ is the area under the penicillamine concentration versus time profile to time t_a calculated by the trapezoidal rule, k_{el} is the overall first-order elimination rate constant following treatment A, and $(AUC)^{A}_{0-\infty}$ is the area under the penicillamine concentration versus time profile to time ∞. This area was set equal to $(AUC)^{A}_{0-12\ hr}$. Figure XIII to XVI depict individual CRFA profiles, and the cumulative mean relative fraction absorbed, CMRFA, profile is shown in Figure XVII.

Figure X: Mean percent penicillamine absorbed versus time plot for Treatment A.

FIG. X

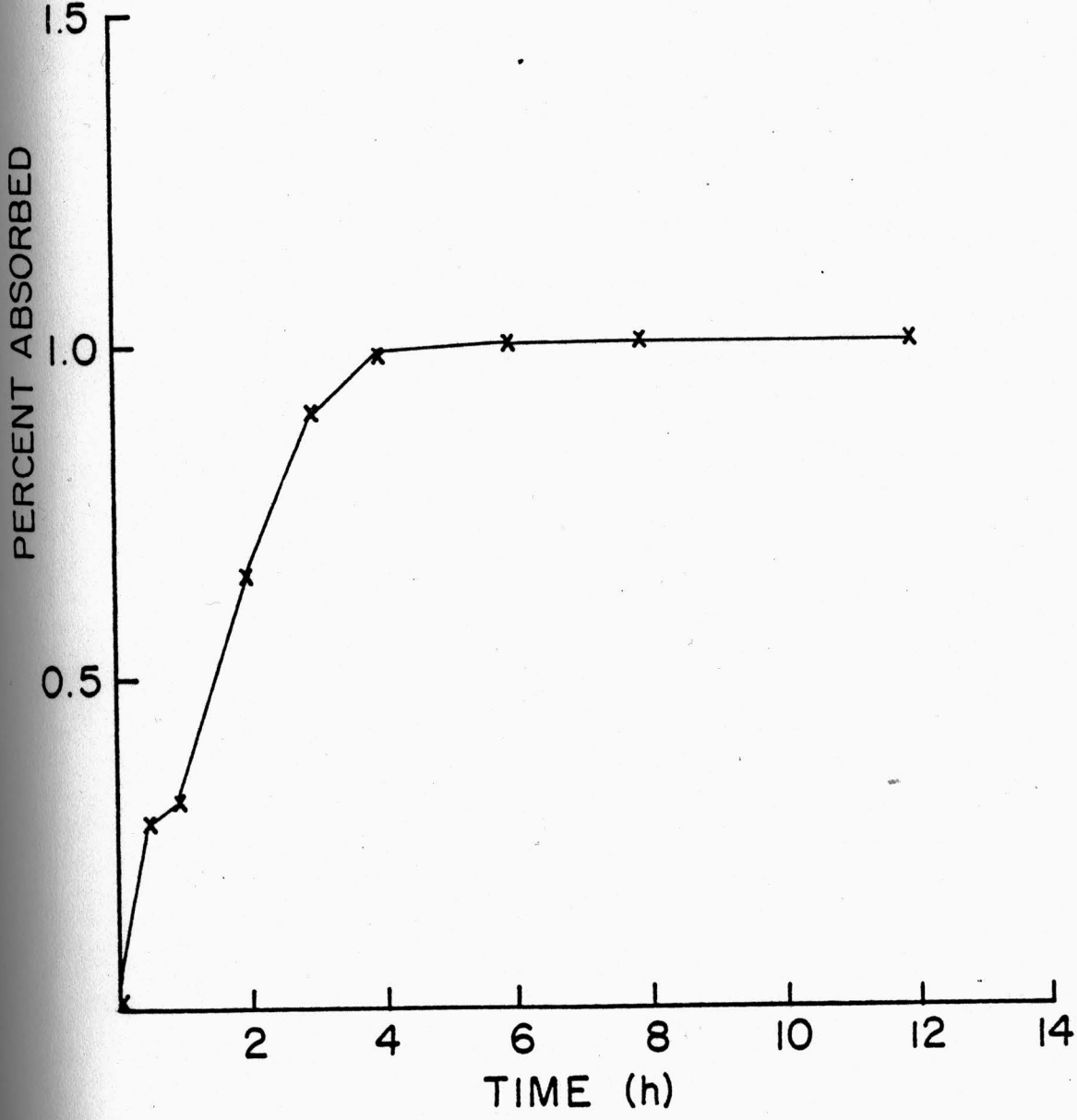


Figure XI: Mean percent penicillamine absorbed versus time plot for Treatment B.

FIG. XI

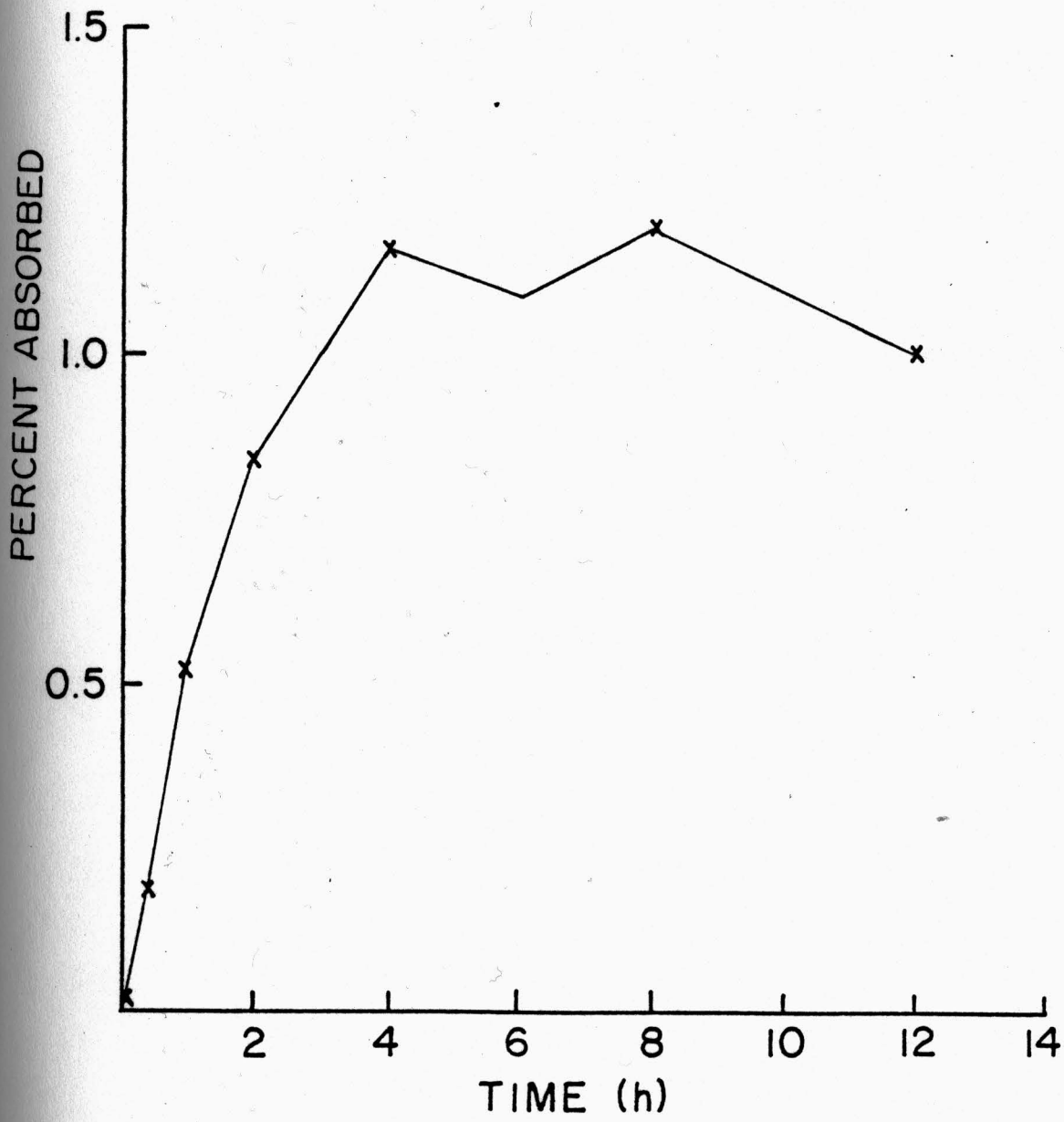


Figure XII: Mean percent penicillamine absorbed versus time plots for Treatment C.

FIG. XII

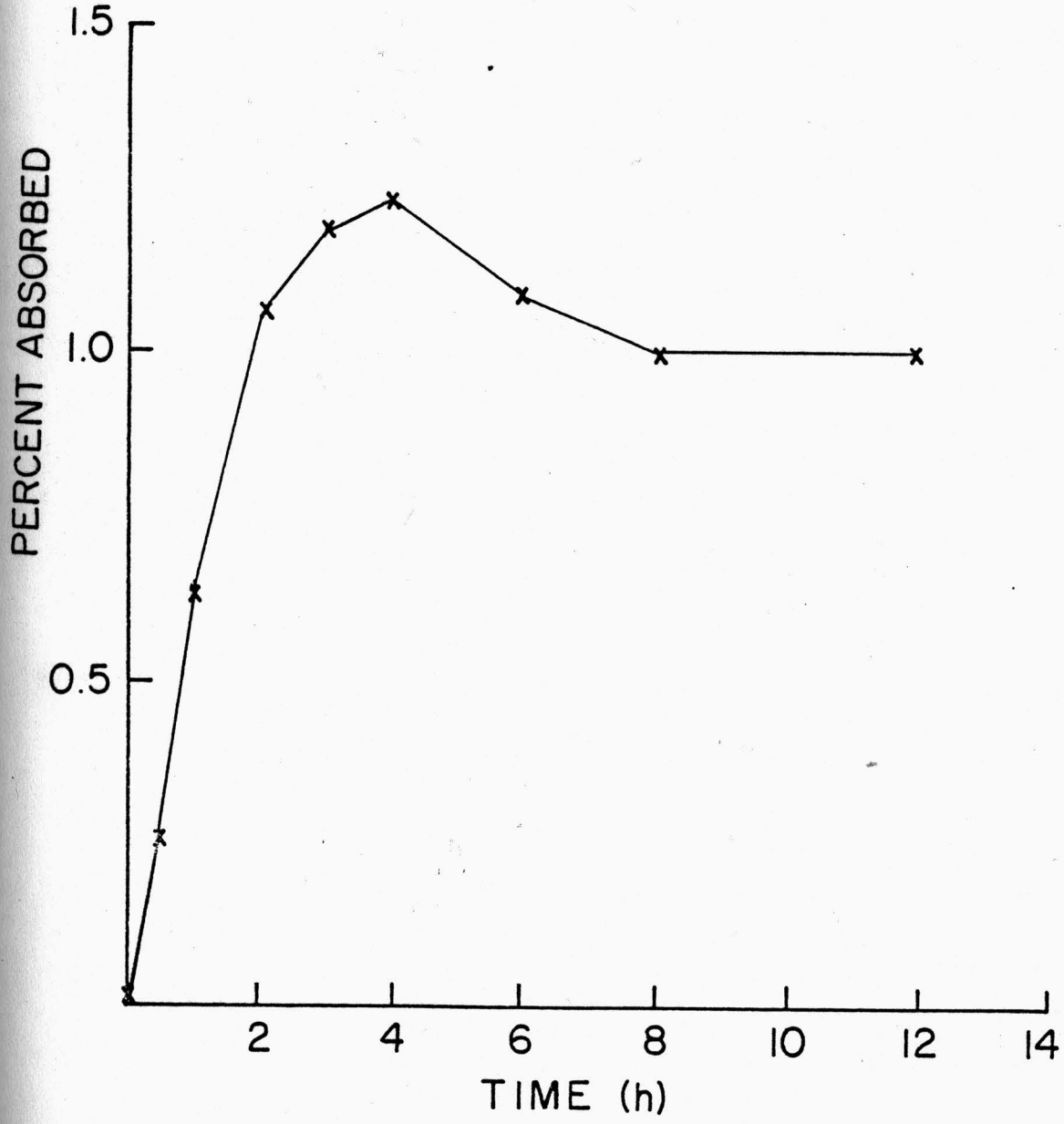


Figure XIII: Cumulative relative fractions of penicillamine absorbed as a function of time in subject No. 1. (▲) Treatment A., (■) Treatment B., (●) Treatment C.

FIG. XIII

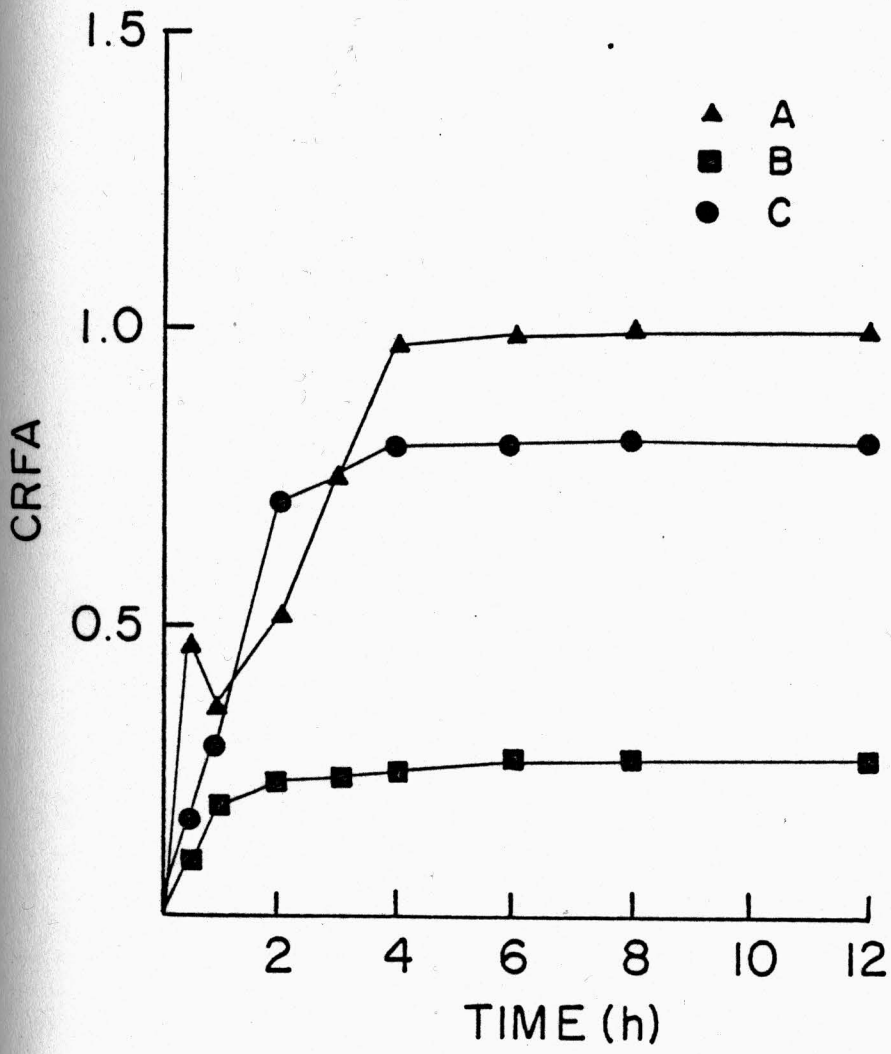


Figure XIV: Cumulative relative fractions of penicillamine absorbed as a function of time in subject No. 2. (▲) Treatment A., (■) Treatment B., (●) Treatment C.

FIG. XIV

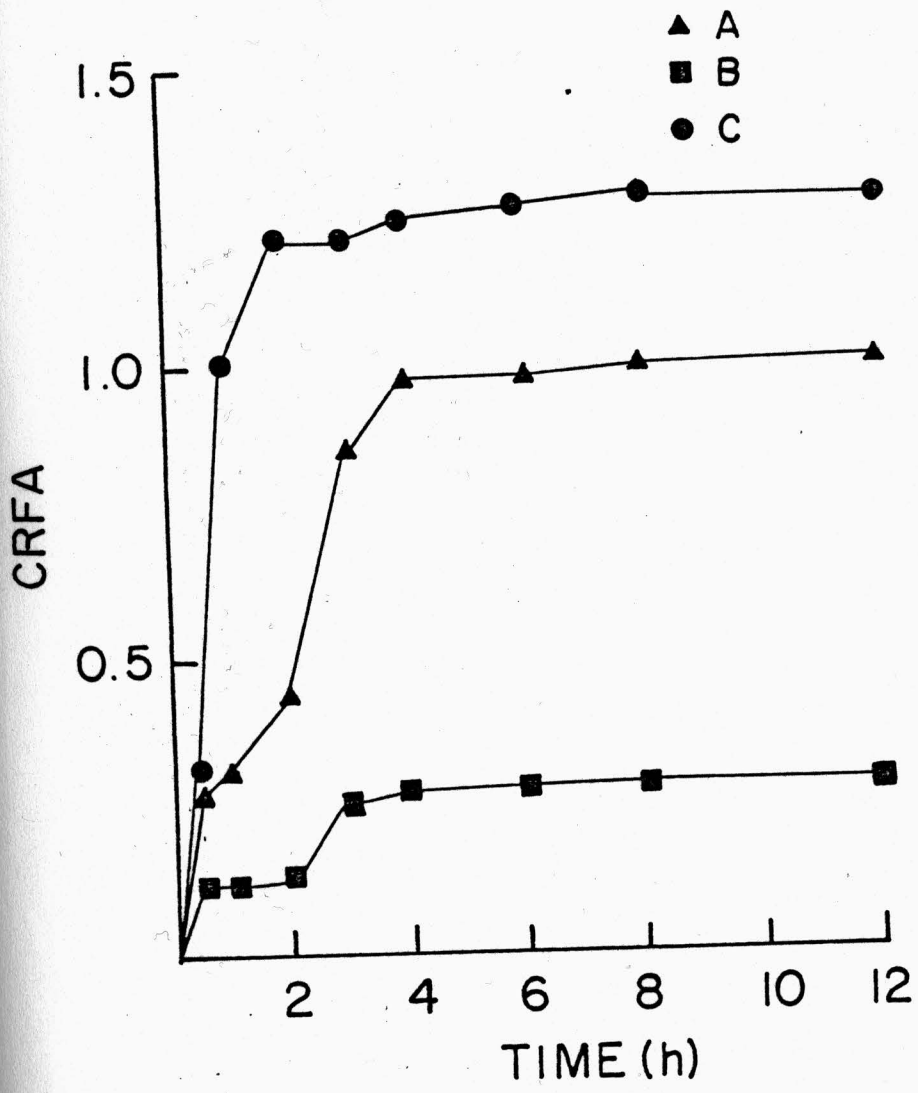


Figure XV: Cumulative relative fractions of penicillamine absorbed as a function of time in subject No. 3. (▲) Treatment A., (■) Treatment B., (●) Treatment C.

FIG. XV

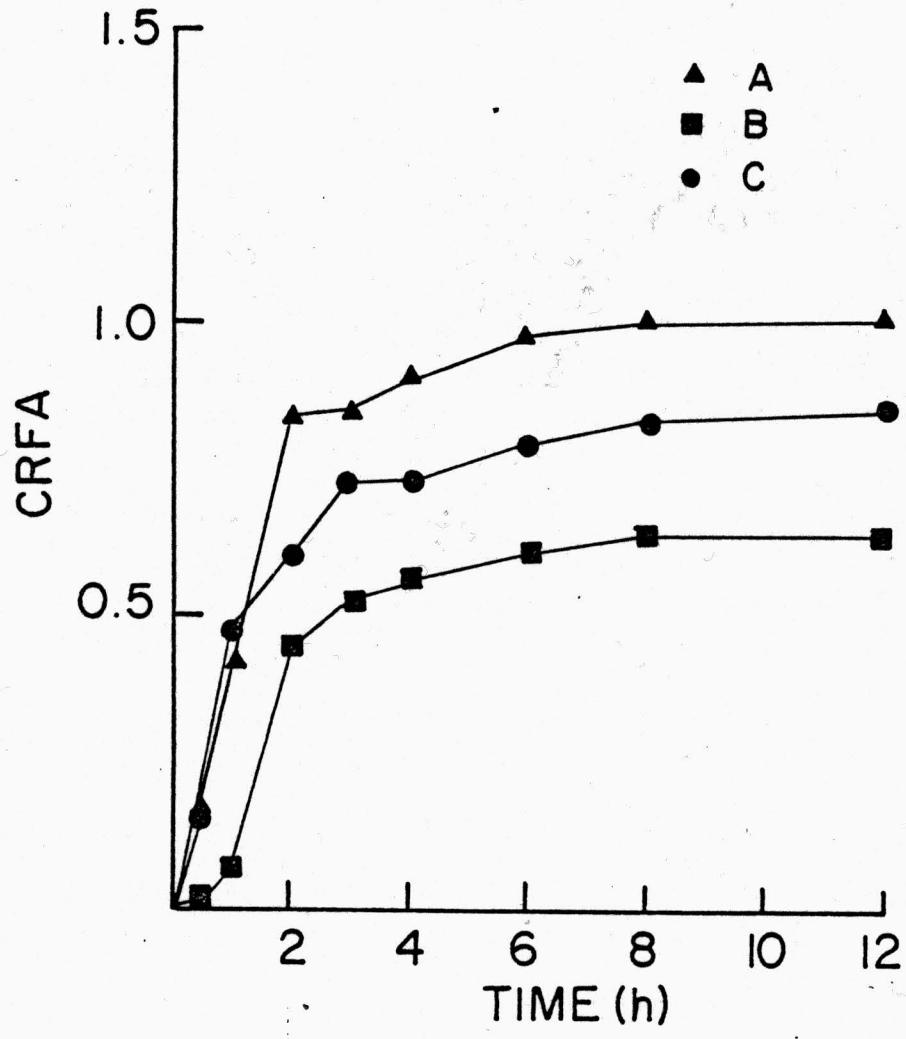


Figure XVI: Cumulative relative fractions of penicillamine absorbed as a function of time in subject No. 4. (▲) Treatment A., (■) Treatment B., (●) Treatment C.

FIG. XVI

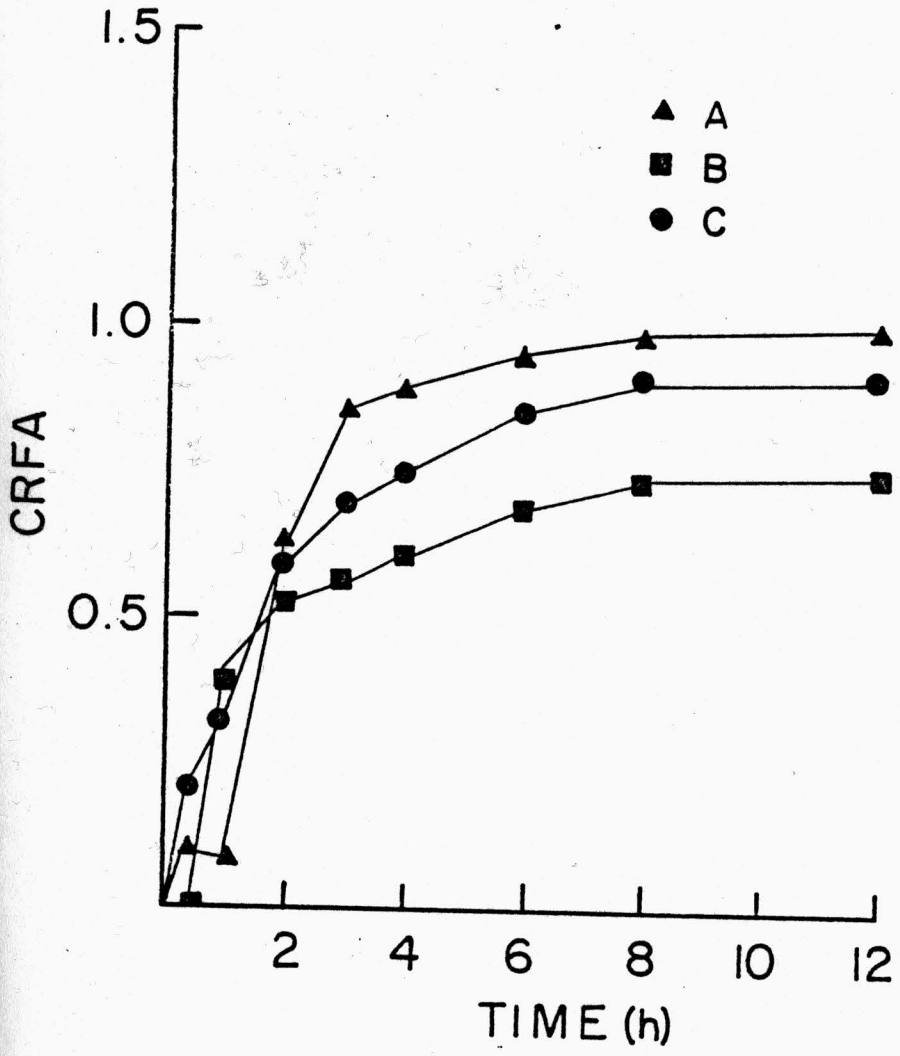
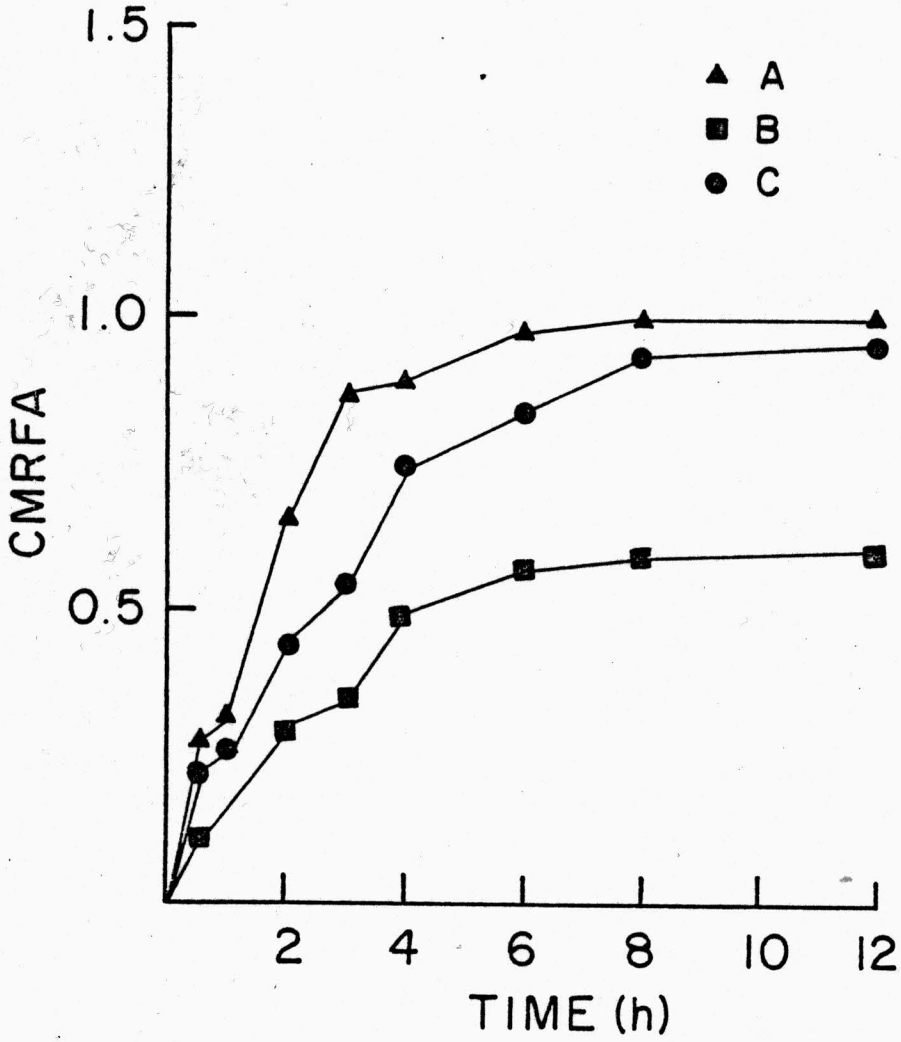


Figure XVII: Cumulative mean relative fractions of penicillamine absorbed as a function of time. (▲) Treatment A., (■) Treatment B., (●) Treatment C.

FIG. XVII



B. Urine Drug Concentration

Cumulative percentage recovery of total drug in urine is presented in Table X.

The values of cumulative urinary recovery of total drug from the three treatments are not in agreement with literature values (23, 25). A current of 10 m Amps was applied for 5 min to reduce penicillamine disulfine to penicillamine. Both Bergstrom et al. (23), and Osman et al. (25), used a current of 6.43 m Amps for 10 min to effect the reduction. (The reason for applying a current of 10 m Amps for 5 min is given in the discussion section). Because of the procedural difference, it seemed inappropriate to pursue the pharmacokinetic analysis of the urine data any further.

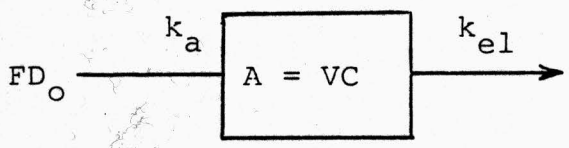
Table X: Percentage Cumulative Urinary Excretion of Penicillamine

	Urine Levels														
	Treatment A					Treatment B					Treatment C				
Subject	0-2HR	2-4HR	4-8HR	8-12HR	12-24HR	0-2HR	2-4HR	4-8HR	8-12HR	12-24HR	0-2HR	2-4HR	4-8HR	8-12HR	12-24HR
1	0.51	2.02	4.66	7.69	11.21	0.72	2.26	4.78	7.40	11.75	0.24	2.05	4.87	8.30	12.17
2	0.38	1.28	2.76	4.72	6.86	0.47	1.47	3.30	5.33	7.50	0.96	3.18	7.39	12.14	17.27
3	0.36	1.13	2.63	4.43	6.42	0.39	1.22	2.55	4.13	5.83	0.76	2.52	5.30	8.47	8.47
4	0.57	2.15	4.46	7.14	10.01	0.84	2.70	5.86	9.46	9.46	0.24	0.92	2.05	3.48	5.14
Mean	0.45	1.65	3.63	6.0	8.62	0.61	1.91	4.12	6.58	6.14	0.55	2.58	4.90	9.63	12.63
± S.D.	0.10	0.51	1.08	1.66	2.35	0.21	0.69	1.48	2.35	3.28	0.37	0.57	2.2	2.17	4.42
% CV	22	31	29.8	27.6	27	34.7	35.9	35.9	35.6	53	66	22	44.8	22.5	35

DISCUSSION

1. Analytical Model

Plasma penicillamine concentration data have previously been shown (23-25) to comply with the one-compartment open model scheme for pharmacokinetic analysis. Scheme V depicts the one-compartment open model:



Scheme V: Representation of one-compartment open model

where k_a is the apparent first-order absorption rate constant, and k_{el} is the apparent first-order elimination rate constant for the drug. The apparent first-order elimination rate constant, k_{el}, characterizes the overall elimination of a drug from a one-compartment model, and as written usually represents the sum of two or more rate constants characterizing individual elimination processes:

$$k_{el} = k_e + k_m + k_m^1 + k_b + \ldots$$

where k_e and k_b are apparent first-order elimination rate constants for renal and biliary excretion, respectively,

and k_m and k_m^1 are apparent first-order rate constants for two different biotransformation (metabolism) processes (42). The absorption rate constant, k_a, and the elimination rate constant, k_{el}, are referred to as apparent first-order rate constants to indicate that they may only be pseudo first-order rate constants. The numerical values of the absorption and elimination rate constants in this study were not very different from each other. The elimination rate constant, k_{el}, had to be fixed in certain instances to facilitate computer fitting of plasma penicillamine concentration data.

The symbols V, FD_o, and C represent, respectively, the volume of distribution of the drug in the body, the amount of drug at the absorption site at time $t = 0$, and the concentration of drug in plasma.

The amount of drug A in the body compartment is thus the product of the volume of distribution, V, and the plasma drug concentration, C. For a drug that enters the body by an apparent first-order absorption process, is eliminated by a first-order process, and is distributed in the body according to a one-compartment model, the following differential equation applies:

$$\frac{dA}{dt} = k_a FD - k_{el} A \qquad 3$$

where FD is the amount of drug or fraction of dose remaining to be absorbed after a finite time lapse, at the absorption site. The other parameters are as defined previously. The rate of loss of drug from the absorption site is:

$$\frac{dFD}{dt} = - k_a FD \qquad 4$$

This expression integrates to:

$$\text{In } FD = - k_a t + X \qquad 5$$

where X is the constant of integration.

Therefore,

$$FD = X_e^{-k_a t} \qquad 6$$

From the outset, however, i.e., at time t = 0, FD = FD_o, so that X = FD_o. Thus:

$$FD = FD_o e^{-k_a t} \qquad 7$$

Substituting 7 into 3 yields

$$\frac{dA}{dt} = k_a FD_o e^{-k_a t} - k_{el} A \qquad 8$$

or

$$\frac{dA}{dt} + k_{el} A = k_a FD_o e^{-k_a t} \qquad 9$$

Equation 9 is the standard form of a linear differential equation with constant coefficients. Multiplying equation 9 by the integrating factor $e^{k_{el}t}$ and rearranging gives:

$$\frac{dA}{dt} e^{k_{el}t} + e^{k_{el}t} k_{el}A = e^{k_{el}t} k_a F D_o e^{-k_a t}$$

This expression can be rewritten as:

$$\frac{d(Ae^{k_{el}t})}{dt} = k_a F D_o e^{(k_{el} - k_a)t} \qquad 10$$

Integration of equation 10 gives:

$$A e^{k_{el}t} = \frac{k_a F D_o e^{(k_{el} - k_a)t}}{k_{el} - k_a} + y \qquad 11$$

where y is the integration constant. To obtain the numerical value of y, equation 11 is divided through by the factor $e^{k_{el}t}$:

$$A = \frac{k_a F D_o e^{(k_{el} - k_a)t}}{e^{k_{el}t}(k_{el} - k_a)} + y e^{-k_{el}t} \qquad 12$$

At t = 0, A = 0, and the exponentials are unity,

$$y = - \frac{k_a F D_o}{k_{el} - k_a}$$

So that:

$$A = \frac{k_a F D_o \, e^{-k_a t}}{k_{el} - k_a} + \frac{k_a F D_o \, e^{-k_{el} t}}{k_{el} - k_a} \qquad 13$$

or

$$A = F D_o \left[\frac{k_a}{k_a - k_{el}}\right] \left[e^{-k_{el} t} - e^{-k_a t}\right] \qquad 14$$

Equation 14 is more conveniently expressed in the plasma drug concentration form. To do so, the equation is divided through by the volume of distribution, V:

$$C = \frac{F D_o}{V} \left[\frac{k_a}{k_a - k_{el}}\right] \left[e^{-k_{el} t} - e^{-k_a t}\right] \qquad 15$$

Equation 15 applies to the usual situation where k_a is larger than k_{el}. When a lag time, t_o, occurs before absorption, the lag time is subtracted from the t-terms appearing in equation 15:

$$C = \frac{F D_o}{V} \left[\frac{k_a}{k_a - k_{el}}\right] \left[e^{-k_{el}(t - t_o)} - e^{-k_a (t - t_o)}\right] \qquad 16$$

Equation 16 is the form of the one-compartment open model equation that was used to analyze plasma penicillamine data in this study.

2. Plasma Penicillamine Profile

Occurrence of double peaks in the blood level curves of penicillamine has been reported previously (23, 25, 47). The double peak phenomenon was observed also during this study. Other drug compounds such as phenytoin (48, 49), and cimetidine (50, 51) have also been reported to show double peaks in their blood level curves. A possible explanation for the occurrence of double peaks may involve enterohepatic recirculation of the drug (23). However, this explanation is unlikely as double peaks were observed only in fasted subjects, and have not been reported in subjects who were administered drug following a meal (23), or an antacid preparation such as in this study.

Hepatobiliary elimination of a drug frequently results in a greater part of the compound undergoing clearance from the body in the feces. Fecal excretion usually represents the end-point of the enterohepatic cycling of a drug or its metabolite (52). Furthermore, when secretion of a drug in the bile does occur, the drug compound appears in the feces irrespective of the route of administration, i.e., orally or by parenteral administration.

The excretion of large quantities of penicillamine disulfide in the feces has been documented (22). The

appearance of this metabolite in feces has so far been attributed to bacterial degradation of penicillamine in the gut. Perrett (22) has also reported that when penicillamine was administered intravenously to a cystenuric patient on two separate occasions, only 42% and 52% of the infused doses were identified in urine. He suggests, therefore, that loss of absorbed penicillamine back into the gastrointestinal tract may occur. It appears that the most probable way by which an infused dose could reenter the gastrointestinal tract is via the enterohepatic circulation. In order to fully characterize what appears to be happening, it may be necessary to conduct further studies using animal models.

Bergstrom et al. (23) have attributed the unusual plasma penicillamine concentration-time profiles to gastrointestinal transit time or an "absorption window" effect. This so-called absorption window may be interferred with when penicillamine is administered immediately following other drug substances or food.

The penicillamine molecule has three pKa values, 1.8, 7.9, and 10.5 corresponding, respectively, to the carboxyl group, the α-amino group, and to the β-thiol group (1). It seems reasonable to assume that the

molecule would exist as a charged species in the gastrointestinal tract. The "absorption window" theory, apart from its utility in explaining the phenomenon of double peaks, may also explain the transport of this charged species across the lipoidal epithelial lining of the gastrointestinal tract. Most probably penicillamine (D-penicillamine) cannot be transported across the epithelium of the gastrointestinal tract by the same transport system used for the natural amino acids. (The natural amino acids have the L-configuration).

Plasma penicillamine concentrations were significantly lowered when it was administered immediately following Aludrox suspension, which, in addition to being an antacid preparation, contained Al^{3+} and Mg^{2+} cations. There was no significant difference between the fasted treatment and the treatment involving administration of penicillamine immediately following sodium bicarbonate solution. See Figures VII to IX.

The administration of penicillamine immediately following sodium bicarbonate led to improved absorption rate. The clinical advantage to be gained from this might be improvement in the efficacy of penicillamine therapy. However, the improved absorption rate, with the consequent transiently high plasma drug concentration, could also

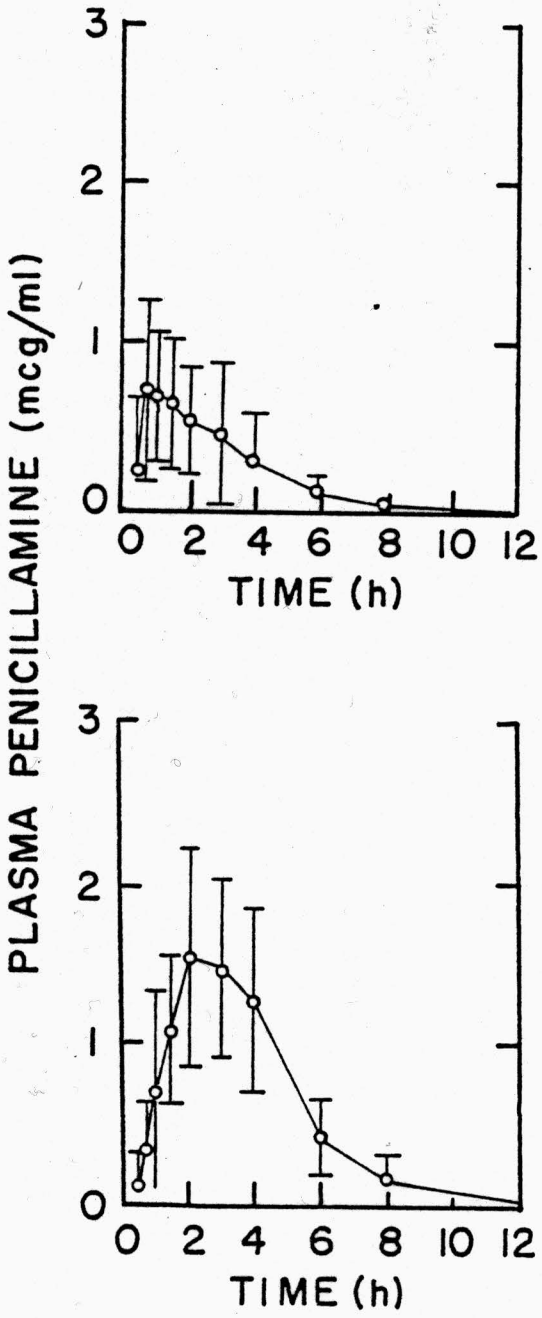
precipitate adverse side-effects. A large scale clinical study would be required to examine these factors.

The cumulative mean dose of penicillamine absorbed from treatments B and C relative to treatment A accounted for approximately 55 and 95% respectively, of the dose of penicillamine administered. See Figure XVII. The 95% absorption following treatment C, the sodium bicarbonate treatment, was not statistically different from treatment A, the fasted treatment. The hypothesis that sodium bicarbonate would also lower blood penicillamine significantly thus failed. It is not very clear, therefore, how a condition such as achlorhydria would influence penicillamine absorption. It is possible that the dose of sodium bicarbonate used may not have induced a transient alkaline pH environment capable of effecting penicillamine oxidation.

The elimination half-life, $t_{½}$ (el), and the peak plasma drug concentration, C_{max} values from this study compare well with literature values (23-25). On the average, a lag time, t_o, before absorption of approximately 6 min was observed. It is not clear how the lag time before absorption correlated with the onset of the antacid effect from both sodium bicarbonate solution and Aludrox suspension, and the rate at which

Figure XVIII: Mean plasma level of penicillamine after single 500-mg oral dose immediately following 300-mg ferrous sulfate (Purepec). Redrawn from Ref. No. 25

Figure XIX: Mean plasma level of penicillamine after single 500-mg oral dose immediately following 30 ml antacid suspension (Maalox-Plus).
Redrawn from Ref. No. 25


PLASMA PENICILLAMINE (mcg/ml)
3
2
1
0
0 2 4 6 8 10 12
TIME (h)
3
2
1
0
0 2 4 6 8 10 12
TIME (h)

polyvalent cations were made available for chelation from the latter antacid preparation. The onset of the antacid effect was assumed to be immediate since both antacid preparations were administered in liquid form.

Chelation apparently plays the dominant role in the reduction of penicillamine absorption from the gastrointestinal tract by antacid preparations. The fact that the effect with ferrous sulfate was found (25) to be more pronounced as compared to an antacid preparation, (see Figures XVIII and XIX) may, possibly, have to do with factors such as solution rate of the metal ion and, therefore, the rate at which it is being made available for chelation, the amount of metal ions actually present in the preparation, and the interaction of the metal ion with the gastrointestinal tract. The marked inhibition of penicillamine absorption by Fe^{2+} (25), is further support of the involvement of chelation. This evidence suggests that the absorption of penicillamine and possibily its therapeutic efficacy may be reduced by concurrent iron or antacid therapy.

3. Urinary Drug Elimination

Whether the therapeutic activity of penicillamine is due to the unchanged penicillamine molecule or to the oxidized forms (or both) has not been established (53).

It is not even clear what the modality of the penicillamine molecule is that ultimately gets absorbed (23). Circulating levels of total penicillamine may, therefore, remain unchanged. Given such a possibility, the urinary excretion of total penicillamine may remain relatively unchanged too. In that case the obvious question to be raised would be what constitutes the controlling mechanism(s) for the passage of the unchanged drug molecule and the oxidized forms of penicillamine from the blood stream into urine. If the mechanism (s) is such that it allows the uninterrupted passage of metabolite into urine, irrespective of the treatment (a situation which would probably be expected) then the urinary excretion of total drug should remain relatively the same. Otherwise, the problem translates into that of characterizing penicillamine absorption. The investigation and full characterization of the absorption process would probably lead to a better undertanding and characterization of urinary elimination of penicillamine. The absolute availability of penicillamine has not been studied.

Urinary data obtained in this study are not in agreement with literature values (23, 25). See Table XI for a comparison of cumulative percent urinary excretion.

Table XI: Comparison of Cumulative Urinary Excretion Data From Bergstrom et al.(23), Osman et al. (25), and from this work.

Treatment	Bergstrom et al.[b]	Osman et al.	This Work
Fasting	10.5 ± 4.0	38.3 ± 11.7	20.4 ± 5.7
Standard Breakfast	4.5 ± 1.5	22.3 ± 5.4	-
Commercial Antacid Suspension[a]	-	24.1 ± 4.6	19.5 ± 5.4
Sodium Bicarbonate Solution	-	-	24.4 ± 12.9
Ferrous Sulfate	-	12.9 ± 3.4	-

[a]Commercial antacid containing Al^{3+} and Mg^{2+} cation.

[b]Represents percentage urinary excretion of unchanged penicillamine, not of total drug.

As noted earlier, the present study used a current of 10 mAmps for 5 min to effect reduction of oxidized forms of penicillamine in urine. Others (23, 25) have used a current of 6.43 m Amps for 10 kin. The scale on the Hewlett Packard, Model 6218A power supply unit used in this study is calibrated to read 10 m Amps (and multiples thereof). It was envisaged that the selection of a current reading of 6.43 m Amp on such a scale would not possibly be reproduced well. Therefore, a current of 10 m Amps and a time of 5 min was decided upon. The product of current and reaction time in this study is less than that from Bergstrom et al. (23), and from Osman et al. (25). The voltage across the cell was not monitored during the study, therefore, it is not known if the reduction potential of all penicillamine disulfides present was exceeded or not. Hence it was not possible to determine if complete reduction of the disulfides had occurred.

However, one may have seen the same trend, that of plasma drug concentration lowering correlating with urinary excretion even with such electrolytic conditions. In this study all three treatments resulted in approximately 20% elimination of administered dose via the kidneys. It may be that neither of these conditions

(6.43 m Amps for 10 min, or 10 m Amps for 5 min) represents the optimal condition for the reduction of oxidized forms of penicillamine. However, in view of the poor agreement between urinary recovery pattern in this and the two previous studies (23, 25), further examination of the data in this study was not carried out.

Appendix I

Normal Blood Chemistry Values

Clinical Laboratories-University Hospitals*
University of Wisconsin-Madison

TEST	NORMAL VALUE	
	Males	Females
Red cell count (m/cu.mm)	4.6-5.8	4.0-5.2
Hematocrit (%)	41-51	36-46
Hemoglobin (gm%)	14.2-17.6	12.2-15.4
White cell count (k/cu.mm)	3.8-9.0	
White cell differential (%)		
Segmented neutrophils	36-73	
Bands	0-5	
Eosinophils	0-5	
Basophils	0-2	
Lymphocytes	18-54	
Monocytes	1-11	
Platelet count (k/cu.mm)	140-380	
BUN (mg%)	8-20	
Creatinine (mg%)	0.6-1.3	
SGOT (u/L)	20-45	
SGPT (u/L)	up to 35	
Alkaline phosphatase (u/L)	20-110	
Glucose (mg%)	75-110	
Urinalysis	-	

*State of Wisconsin Laboratory Identification Number 58-DDOBO.

Appendix II

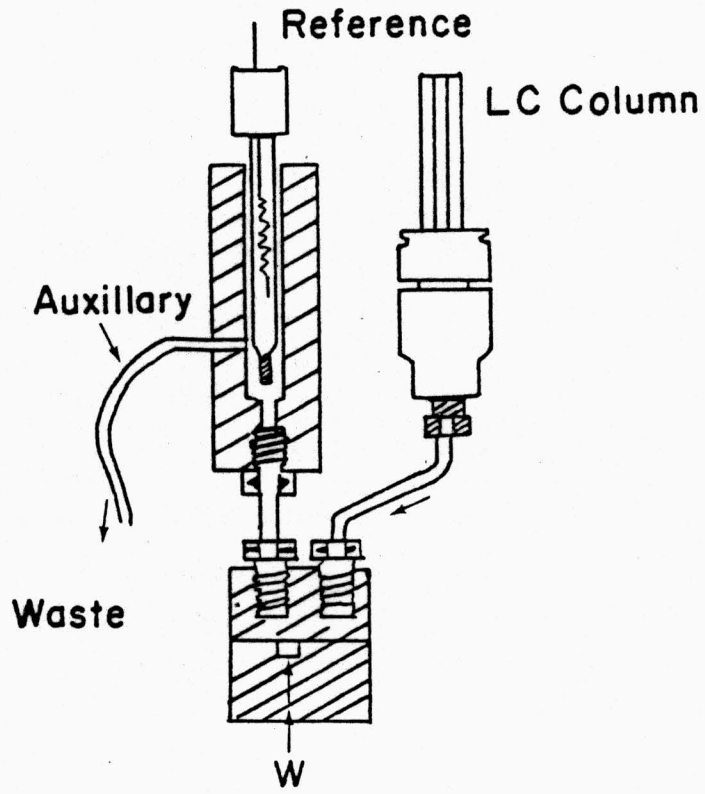
Electrochemical Detection

Electrochemical Detection (54, 55)

1. Thin-layer Amperometric Detector

Figure XX depicts a diagramatic representation of a thin-layer amperometric detector for high performance liquid chromatography. The working electrode surface is part of a channel wall formed by sandwiching a fluorocarbon gasket between two blocks made from stainless steel (upper half) and an engineering plastic, e.g., Plexiglass (lower half). The electrode material used may vary depending on application. Electrode materials include glassy carbon, platinum, or gold. A gold electrode was used in this study. The reference electrode is a silver wire, dipped into a silver chloride solution. It is called a reference electrode in the sense that the magnitude of the working electrode potential is set with respect to it. The third electrode, the auxilliary electrode, may be placed downstream; a convenient arrangement, which, unfortunately, results in non-linear response when large samples are injected. The auxilliary electrode may be placed directly across from the working electrode surface. This arrangement is said to reduce uncompensated resistance even when mobile phases with low ionic strengths are used. It is the preferred arrangement for most pharmacokinetic analysis.

FIG. XX



THIN-LAYER AMPEROMETRIC DETECTOR[b]

2. Scopes and Limitations

Electrochemical detection in liquid chromatography has three distinct advantages for applicable systems:

I. Selectivity,

II. Sensitivity, and

III. Economy.

The suitability of electrochemical detection ultimately depends on the voltammetric characteristics of the molecule(s), in a suitable mobile phase, and at a suitable electrode surface. It is imperative to attempt to simultaneously optimize both the column and detector performance by selecting appropriate mobile phase components. For penicillamine, these mobile phase components usually are citric acid and disodium hydrogen phosphate combined to give a buffer of a certain ionic strength. Bergstrom et al. (41) have listed different compositions of citric acid-phosphate buffer which may be used for the chromatography of penicillamine. This list does not, in any measure, substitute for what chromatographers may find workable in their various situations, since experience indicates that considerable differences between individual columns occur (41).

The amperometric detection of penicillamine involves a simple surface reaction:

$$Hg + RSH^{(a)} \rightarrow HgSR + e^{-} + H^{+}$$

The surface of the so-called working electrode is of gold-mercury (Au-Hg) amalgam (actually a mercury coat is applied onto the gold surface; details of how to apply this coat will be presented in the next section). A laminar flow profile of a solution, or mixture, containing the analyte is passed over the working electrode surface. Oxidation of the analyte (penicillamine, in this case) leads to the release of electrons to the surface. This current is subsequently converted to a voltage capable of driving a recorder. See Figure XXI.

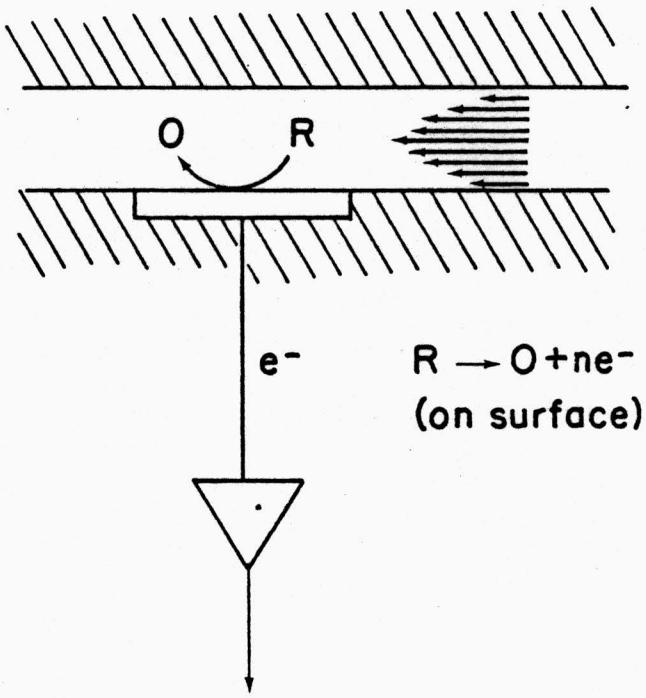
The oxidation reaction of penicillamine to penicillamine disulfide could also take place:

$$2RSH \rightarrow RSSR + 2e^{-} + 2H^{+}$$

But this reaction can be avoided by maintaining the magnitude of the working electrode potential at a value lower than that at which penicillamine disulfide formation takes place (penicillamine disulfide formation requires approximately + 1.0 V). The magnitude of the working electrode potential was maintained at + 0.1 V.

An important requirement of amperometric detection in liquid chromatography, especially in analysing biological samples, is ruggedness and long-term stability. Usually biological samples have to be analyzed within a few days of collection. It is important, therefore, to

FIG. XXI



ELECTRON TRANSFER AT ELECTRODE SURFACE

ensure that the chromatographic system is responding properly before initiating the process of sample collection. Usually many test runs are required in order to establish that the system is working properly. But this process in itself wears down the working electrode, which usually requires frequent re-conditioning. The response of one particular electrode surface to such factors as the ionic strength, pH, and electrochemical reactivity of the solvent and electrolyte, however, may not necessarily be the same as that of a re-conditioned electrode surface. The ionic strength, pH, electrochemical reactivity of the solvent and electrolyte, and the presence of electroactive impurities (dissolved oxygen, halides, trace metals) all constitute important considerations of factors that either have to be maintained constant, or eliminated, in any given run. With such a measurement step, random error, i.e., error involved with the actual mechanism and with the limitation of the measurement step (56), would be difficult to overcome. Even if calibration curves were to be constructed with each analysis, the transition between measurements made using one electrode surface and those measurements made using a reconditioned electrode surface would tend to be somewhat different. This can be a problem in any type of analysis.

3. Reconditioning the Electrode Surface

The surface reaction on the working electrode surface leads eventually to the destruction of the mercury coat. The mercury coat, therefore, has to be re-applied frequently. It is imperative (in order to minimize random error) to keep the technique for resurfacing the electrode as uniform as possible. The technique which proved most useful under the conditions for this study was as follows:

I. rinse the used-up mercury coat repeatedly with distilled water

II. clean with small volumes of 95% ethanol and again rinse with distilled water

III. apply one or two drops of 6 M nitric acid to the electrode surface insuring the acid does not touch either the gold pin or the inlet to the gold pin. It is advisable to work in an open space, and to set the lower half of the electrode block on a wooden test tube holder. Let stand for 1-2 hr. Do not attempt to scratch the surface of the electrode in order to hasten the removal of the mercury coat. When the nitric acid drop(s) takes on a rusty tinge, rinse repeatedly with distilled water. Apply more nitric acid to ensure complete removal of the mercury coat.

IV. rinse copiously with distilled water and wipe dry (using, for example, micro-wipes disposable wipers).

V. apply 1 or 2 drops of fresh mercury to the gold-like surface (the mercury should cover the whole surface) and let stand for 2-3 hr.

VI. carefully remove as much excess mercury as possible using a dull knife blade, but do not scrape. Polish lightly with soft tissue paper.

VII. allow surface to equilibrate overnight before using.

When a freshly conditioned electrode is hooked onto a chromatographic system, the background current is high (usually greater than 100 n Amps). The background current generally takes 2.0 to 3.0 hr to decay to below 10 n Amps, which generally is the acceptable background current for analysis.

The amperometric detector is a device that would try the patience of the most stalwart chromatographer. It is important not to allow your patience to run out. A little persistence at it usually pays off in the end.

[a]RSH indicates the penicillamine molecule.

[b]Redrawn from Ref. No. 54

Appendix III

Typical Computer Output Using the Program NREG

```
&RUN
$FOR,SI      ,MAIN
FORTRAN-MACC 1.17S-01/31/84-21:00:41          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
00104    10.         2D)
00104    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 ,6HX*E/1 ,6HDV/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',5D/
00124    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,I3,6X,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)
00164    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
00214    40.          CALL NREG(THINT,NAMES,BNDLO,BNDHI,FIX,DIFF,THFIN,YOBS,YPRD,
00214    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-01/31/84-21:00:43          SUBF
00101     1.          SUBROUTINE F(TH,Y,NEXP,NVAR,NP,NRPRD)
00104     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)-TH3).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                                    OTP
FORTRAN-NACC 1.17S-01/31/84-21:06:45
00101      1.            SUBROUTINE OUTPUT(NEXP,NEXP2,THFIN,NVAR,NF,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(1HC,' R-SQUARED FOR FUNCTION',I1,' =',F6.4/1X,'COR FOR FUNCT
00163     40.             1ION',I1,' =',F6.4)
00164     41.              IF (NEXP2.EQ.0) GO TO 66
00166     42.              IF(K.GT.1) GO TO 300
00170     43.              SYOBS1 = SYOBS
00171     44.              SDEV1 = SDEV
00172     45.              GO TO 200
00173     46.          300 SYOBS = SYOBS + SYOBS1
00174     47.              SDEV = SDEV + SDEV1
00175     48.              RS = (SYOBS-SDEV)/SYOBS
00176     49.              WRITE(6,111) RS
00201     50.          111 FORMAT(1X,'OVER ALL R-SQUARED=',F6.4)
00202     51.           66 CONTINUE
00203     52.              CALL PRPL (NEXP,NEXPO,THFIN,NVAR,NP,NRPRD,YPRD,YOBS)
00204     53.              RETURN
00205     54.              END

       END OF COMPILATION:         NO  DIAGNOSTICS.
$FOR,SI      ,PRPL
FORTRAN-NACC 1.175-01/31/84-21:05:46              PRPL
00101      1.              SUBROUTINE PRPL (NEXP,NEXPO,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(6HPRINTER)
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 + 1
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/(NEXPO-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,NEXPO
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 = NEXP2
00164     37.              K = NEXPO
```

```
00165     36.            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 = NEA1
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,'TIMES$','FU
00204     51.           1NCTION VALUES$$',4HAXES,'PLOT OF OBSERVED,O''S AND PREDICTED,P''S
00204     52.           2VALUES$$',12.0,12.0)
00205     53.            CALL GRAPH4(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
MAP, 30R1U08 SL1B 2 01/31/84 21:06:49
ABCM STATUS OF OUTPUT ELEMENT=UNKNOWN
QUARTER/THIRD WORD INSENSITIVE


BANKNAME(BDI)          ADDRESS LIMITS
$IBANK(4)              001000 113462      36195 IBANK WORDS DECIMAL
$DBANK(5)              114000 145333      13020 DBANK WORDS DECIMAL
STARTING ADDRESS  113265


            SEGMENT $MAIN$          001000 113462      114000 145333

UR1TRI/UW:CCO1              $(1)    001000 001165
DSHOLR/CC  ..IPSP           $(1)    001166 001225      $(0)    114000 114006
                                                       $(2)    000000
PLQQAG/GSPV3E               $(1)    001226 001523      $(0)    114007 114042
NFFT1B/FORIC                $(1)    001524 002567      $(2)    114043 114260
NR1HC/NAGNLINREGOO          $(1)    002570 003333      $(0)    114261 115003
NR1W33/NAGNLINREGOO         $(1)    003334 003622      $(0)    115004 115054
                                                       $(2)    BLANK$COMMON
NR1S.P/NAGNLINREGOO         $(1)    003623 004246      $(0)    115055 115122
                                                       $(2)    BLANK$COMMON
NEXPCB/NAGFORFUN12          $(1)    004247 004476      $(2)    115123 115202
SYMBOL/MAY81CSP             $(1)    004477 004660      $(0)    115203 115222
                                                       $(2)    GSPCTL
MCOR2/GSPV94                $(1)    004661 004703
DICTEG/FEB81GSP             $(1)    004704 006203      $(0)    115223 115340
                            $(3)    GSPCTL             $(2)    TCGCTL
UR3SRM/UW:CC                $(1)    006204 006262
NEXP1B/NAGFORFUN10          $(1)    006263 006332      $(0)    115341 115341
```

UR2MSK/UMCC	$(1)	006333 006464		$(0)	115342 115367
				$(2)	BLANK$COMMON
URSORT/NAGUTILITY02	$(1)	006465 007304		$(0)	115370 115527
EXP$/NAGFORFUN10	$(1)	007305 007407		$(2)	115530 115553
NR1CK/NAGNLINREG00	$(1)	007410 007425		$(0)	115554 115554
GRDLIN$GSP/W39	$(1)	007426 010143		$(0)	115555 115747
BASIS1/PSPRL00	$(1)	010144 010714		$(0)	115750 116153
SORT$/NAGFORFUN10	$(1)	010715 010764		$(2)	116154 116165
PLQQ07/PSPRL10	$(1)	010765 011036		$(0)	116166 116207
NFTCH$/FORIO	$(1)	011037 011337		$(2)	116210 116245
FORIO84/FORIO	$(1)	011340 011633		$(2)	116246 116270
ALOG1/NAGFORFUN10	$(1)	011634 011751		$(2)	116271 116344
PLQQ45GSP/W39	$(1)	011752 012101		$(0)	116345 116374
NR1FEV/NAGNLINREG00	$(1)	012102 012307		$(0)	116375 116502
				$(2)	BLANK$COMMON
PLQQ43GSP/W39	$(1)	012310 012346		$(0)	116503 116513
				$(2)	116514 116560
NTAB$/FORIO				$(2)	116561 121251
FORIO82/FORIO	$(1)	012347 014677		$(2)	121252 121260
FORIO83/FORIO	$(1)	014700 015266		$(3)	121261 121312
NR1SRT/NAGNLINREG00	$(1)	015267 015516		$(2)	BLANK$COMMON
				$(0)	121313 121336
NR1PLT/NAGNLINREG00	$(1)	015517 015652		$(2)	BLANK$COMMON
				$(0)	121337 121412
NR1RD/NAGNLINREG00	$(1)	015653 015754		$(2)	BLANK$COMMON
				$(0)	121413 121430
NR1DRV/NAGNLINREG00	$(1)	015755 016034		$(0)	121431 121464
NR1SKD/NAGNLINREG00	$(1)	016035 016175		$(2)	BLANK$COMMON
				$(0)	121465 121465
URTIME/NAGUTILITY00	$(1)	016176 016204		$(0)	121466 121501
NR1TP$/NAGNLINREG00	$(1)	016205 016271		$(2)	BLANK$COMMON
				$(0)	121502 121557
NR1WRT/NAGNLINREG00	$(1)	016272 016412		$(2)	BLANK$COMMON
				$(0)	121560 122374
NR1NST/NAGNLINREG00	$(1)	016413 020115		$(2)	BLANK$COMMON
	$(3)	NR1BLK		$(0)	122375 122455
NR1ENK/NAGNLINREG00	$(1)	020116 020357		$(2)	BLANK$COMMON
				$(0)	122456 123351
NR1HC/NAGNLINREG07	$(1)	020360 023126		$(0)	123352 123446
NR1DIF/NAGNLINREG00	$(1)	023127 023723		$(0)	123447 123660
NR1SWD/NAGNLINREG00	$(1)	023724 026043		$(2)	BLANK$COMMON
				$(0)	123661 123703
NR1LIZ/NAGNLINREG00	$(1)	026044 026130		$(2)	BLANK$COMMON
				$(0)	123704 123734
NR1LIN/NAGNLINREG00	$(1)	026131 026226		$(2)	BLANK$COMMON
				$(0)	123735 123766
NR1BES/NAGNLINREG00	$(1)	026227 026312		$(0)	123767 124020
NR1LIT/NAGNLINREG01	$(1)	026313 026463		$(2)	BLANK$COMMON
	$(3)	NR1BLK		$(0)	124021 124146
NR1PIV/NAGNLINREG00	$(1)	026464 027046		$(0)	124147 124324
NR1HV/NAGNLINREG00	$(1)	027047 030012		$(0)	124325 124340
NR1BLM/NAGNLINREG00	$(1)	030013 030070		$(2)	BLANK$COMMON
				$(0)	124341 124342
URDATE/NAGUTILITY00	$(1)	030071 030107		$(0)	124343 124477
NR1PST/NAGNLINREG00	$(1)	030110 030534		$(2)	BLANK$COMMON
				$(0)	124500 124525
ASINEQ$$/NAGFORFUN10	$(1)	030535 030760		$(0)	124526 124662
NR1IB$/NAGNLINREG00	$(1)	030761 031615			

```
NR1BLK(COMMONBLOCK)
NR1BLK/NAGNLINREG02     $(1)    031616 034261
                        $(3)    NR1BLK
NR1BDA/NAGNLINREG00     $(1)    034262 034333

NR1ECS/NAGNLINREG10     $(1)    034334 040101
PLQQQ2/GSPV35           $(1)    040102 040301
PLQQG1/GSPV35           $(1)    040302 040562
GSTXT/JAN82GSP          $(1)    040563 041124

GDASHSGSP/W39           $(1)    041125 041204

GRFUNC/GSPV76           $(1)    041205 041247
LOCATE/GSPW50           $(1)    041250 042043

LINE/MAY83GSP           $(1)    042044 043514

RSTRFS/MAY82CSP         $(1)    043515 044203
                        $(3)    GSPCTL
URXIIPS/NAGUTILITY01    $(1)    044204 044263
ROWVECSGSP/W39          $(1)    044264 044543
TCG/SEC82GSP            $(1)    044544 046517
                        $(3)    TCGCTL
OBJTRN/GSPV03           $(1)    046520 047043

NEFTCS/FORIO            $(1)    047044 047434
BASIS2-3/PSPRLOC        $(1)    047435 047557
PCOORD/GSPV04           $(1)    047560 047724
                        $(3)    GSPCTL
DEXPS/NAGFORFUN11       $(1)    047725 050105
NEXPSS/NAGFORFUN10      $(1)    050106 050246
ATANS/NAGFORFUN10       $(1)    050247 050507
GPAGE/GSPV04            $(1)    050510 050577

POSITN/GSPV54           $(1)    050600 050727

DSQRTS/NAGFORFUN10      $(1)    050730 051011
AXLINEGSP/NOV82CR       $(1)    051012 057760

DUBSGSP/W39             $(1)    057761 060001
PLQQQR/GSPW58           $(1)    060002 060231
NOSYMS/FORIO            $(1)    060232 060341
NREG/NAGNLINREG02       $(1)    060342 060465
SINCOSS/NAGFORFUN10     $(1)    060466 060633
PLQQGH/PSPRLC1          $(1)    060634 060723
GSPRNT/JUNE82           $(1)    060724 062003
                        $(3)    TCGCTL
STDWRT/APR83GSP         $(1)    062004 062216


NEXPSS/NAGFORFUN11      $(1)    062217 062406
DSHDLF/GSPRLFPSP        $(1)    062407 062642

GSPSET/JAN84GSP         $(1)    062643 063351
                        $(3)    TCGCTL
                        $(2)    BLANKSCOMMON
                                124863 124700
                        $(0)    124701 125147
                        $(2)    BLANKSCOMMON
                        $(0)    125150 125175
                        $(2)    BLANKSCOMMON
                        $(0)    125176 126565
                        $(0)    126566 126654
                        $(0)    126655 126752
                        $(0)    126753 127234
                        $(4)    GSPCTL
                        $(0)    127235 127241
                        $(2)    000000
                        $(0)    127242 127251
                        $(0)    127252 127415
                        $(2)    GSPCTL
                        $(0)    127416 127673
                        $(2)    GSPCTL
                        $(0)    127674 130010
                        $(2)    000000
                        $(2)    130011 130047
                        $(0)    130050 130107
                        $(0)    130110 130274
                        $(2)    GSPCTL
                        $(0)    130275 130364
                        $(2)    GSPCTL
                        $(2)    130365 130426
                        $(0)    130427 130433
                        $(0)    130434 130461
                        $(2)    000000
                        $(2)    130462 130520
                        $(2)    130521 130542
                        $(2)    130543 130602
                        $(0)    130603 130607
                        $(2)    000000
                        $(0)    130610 130626
                        $(2)    GSPCTL
                        $(2)    130627 130646
                        $(0)    130647 132202
                        $(2)    GSPCTL
                        $(0)    132203 132214
                        $(0)    132215 132267
                        $(2)    132270 132273
                        $(0)    132274 132306
                        $(2)    132307 132336
                        $(0)    132337 132377
                        $(0)    132400 132544
                        $(2)    GSPCTL
                        $(0)    132545 133276
                        $(4)    GSPCTL
                        $(6)    000000
                        $(2)    133277 133320
                        $(0)    133321 133406
                        $(2)    000000
                        $(0)    133407 134237
                        $(2)    GSPCTL
                        $(4)    000000
```

```
TRWRIT/NOV82GSP          $(1)    063352 063554    $(0)    134240 134332
                                                  $(4)    TCGCTL
                                                  $(6)    GSPCTL
PAGDEF/MAY82GSP          $(1)    063555 063766    $(0)    134333 134414
                         $(3)    GSPCTL           $(2)    000000
PAGE/MAY82GSP            $(1)    063767 064412    $(0)    134415 134541
                         $(3)    GSPCTL           $(2)    000000
ARGSET/GSPV38            $(1)    064413 064531    $(0)    134542 134567
WRTEXT/APR83GSP          $(1)    064532 065324    $(0)    134570 134675
                                                  $(2)    GSPCTL
UARNID/UWCC              $(1)    065325 065333    $(0)    134676 134676
ERU$
PLOGGE/JAN82GSP          $(1)    065334 065710    $(0)    134677 135001
                                                  $(2)    GSPCTL
STDOGEN/DEC83GSP         $(1)    065711 067512    $(0)    135002 135175
                         $(3)    000000           $(2)    GSPCTL
TCGCTL(COMMONBLOCK)                                       135176 135203
SHOIMG/JAN84GSPCR        $(1)    067513 077360    $(0)    135204 136461
                         $(3)    TCGCTL           $(2)    GSPCTL
VECTOR/GSPWN6            $(1)    077361 100110    $(0)    136462 136520
                                                  $(2)    GSPCTL
UWERR8/NAGFORFUN02       $(1)    100111 100701    $(2)    136521 137160
BLOGI/NAGFORFUN10        $(1)    100702 101094    $(2)    137161 137266
SCALE/GSPV38             $(1)    101095 103326    $(0)    137267 140043
$$$$$$(COMMONBLOCK)                                       140044 140125
GSPCTL(COMMONBLOCK)                                       140126 140572
GRAPH/NOV82GSP           $(1)    103327 107122    $(0)    140573 142077
                         $(3)    000000           $(2)    GSPCTL
FORIO81/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

SYS$*RLIBS. LEVEL 25
END MAP.  ERRORS: 0  TIME: 24.352  STORAGE: 20685/7/040777/0100777
$AQT
```

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

ITEMS

ITEMS(1): NEXP = 9 NUMBER OF EXPERIMENTS
ITEMS(2): NVAR = 1 NUMBER OF VARIABLES
ITEMS(3): NP = 4 NUMBER OF PARAMETERS

ITEMS(4): NROBS = 9 ROW DIMENSION OF ARRAY YOBS
ITEMS(5): NPRD = 9 ROW DIMENSION OF ARRAY YPRD
ITEMS(6): NRWTS = 9 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 = 183

PARAMETERS

NO.	NAME	INITIAL VALUE	PROPORTION FOR DERIV. ESTIMATE	LOWER BOUND	UPPER BOUND
1	ABS/1	6.2000000-01	5.0000000-03	1.0000000-04	1.0000000+04
2	K+E/1	3.7000000-01	5.0000000-03	1.0000000-04	1.0000000+04
3	FDV/1	2.4700000+00	5.0000000-03	1.0000000-04	1.0000000+04
4	LAG/1	9.9999999-03	5.0000000-03	1.0000000-04	1.0000000+04

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

SING. VALUES		5.5717192+00	2.6610832+00	8.3723465-01	5.4122863-02
PAR.	1 ABS/1	-.0729936	.8514373	-.4639663	.2333702
	2 K+E/1	.9672779	.3652966	-.0918977	-.1121320
	3 FDV/1	-.1349672	.1691747	-.1532877	-.9841922
	4 LAG/1	-.0416916	-.4921084	-.8676364	.0574291

SETUP TIME = .041 SECONDS

T H E I T E R A T I O N S

ITERATION NO. 1		BASE POINT		TEST POINT
SUM OF SQUARES		9.8234750-01		9.6793146-01
L		0		0
LAMBDA		0.0000000		0.0000000
GAMMA		1.0000000+00		7.3984735-03
ANGLE IN DEGREES		.0000		86.8164
MAX. PIVOT REDUCTION		1.0000000+00		9.6561521-03
PAR. 1	ABS/1	6.2000000-01	S	5.9722105-01
2	K+E/1	3.7000000-01		3.7653549-01
3	FDV/1	3.4700000+00		3.5499074+00
4	LAG/1	9.9999999-03		1.0000000-04

CUMULATIVE NO. OF FUNCTION CALLS = 2 ITERATION TIME = .025 SECONDS CUMULATIVE TIME = .066 SECONDS

ITERATION NO. 10		BASE POINT		TEST POINT		TEST POINT		TEST POINT
SUM OF SQUARES		8.3232445-01		8.3407453-01		8.3243419-01		8.3227556-01
L		3		2		2		2
LAMBDA		2.4627529-03		1.1575756-03		1.1575756-03		1.1575756-03
GAMMA		1.0000000+00		1.0000000+00		5.0000000-01		2.5000000-01
ANGLE IN DEGREES		88.6183		8.8193		5.8193		8.8193
MAX. PIVOT REDUCTION		1.6317694-02		4.3875687-03		4.3875687-03		4.3875687-03
PAR. 1	ABS/1	4.1506377-01		3.6780103-01		3.9143240-01		4.0324808-01
2	K+E/1	3.9471767-01	S	4.3947243-01	S	4.1709490-01	S	4.0590614-01
3	FDV/1	4.2389145+00		4.7136986+00		4.4763065+00		4.3576105+00
4	LAG/1	1.0000000-04	B	1.0000000-04	B	1.0000000-04	B	1.0000000-04

CUMULATIVE NO. OF FUNCTION CALLS = 22 ITERATION TIME = .015 SECONDS CUMULATIVE TIME = .199 SECONDS

THE LAST ITERATION

		BASE POINT		TEST POINT
ITERATION NO. 15				
SUM OF SQUARES		8.3226731-01		8.3226731-01
L		8		8
LAMBDA		5.3307279+01		5.3307279+01
GAMMA		6.1035156-05		3.7252903-09
ANGLE IN DEGREES		7.0611		5.9501
MAX. PIVOT REDUCTION		2.7697951+00		2.7698723+00
PAR. 1	ADS/1	4.0324678-01		4.0324678-01
2	M+B/1	S 4.0590593-01	S	4.0590593-01
3	FDV/1	4.3576104+00		4.3576104+00
4	LAG/1	1.0053738-04		1.0053738-04

CUMULATIVE NO. OF FUNCTION CALLS = 64 ITERATION TIME = .024 SECONDS CUMULATIVE TIME = .316 SECONDS

ITERATION TERMINATES:
MAX. RELATIVE CHANGE IN A PARAMETER .LT. TOL(1) = 1.5000000-06

FINAL PARAMETER VALUES

FINAL VALUE SUM OF SQUARES = 8.3226732-01

AR.	1	ABS/1	4.0324678-01
	2	M+E/1	4.0598593-01
	3	FDV/1	4.3876104+01
	4	LAG/1	1.0053730-04

ITERATION SUMMARY

ITER NO.	SUM OF SQUARES	L	LAMBDA	GAMMA	ANGLE	NO. PIVOT FAILURES	NO. BOUNDS FAILURES	MAX. PIVOT REDUCTION	TIME IN SECONDS
0	9.5834750-01	0	0.0000000	1.0000000+00	.0000	0	0	1.0000000+00	.000
1	9.6793146-01	3	0.0000000	1.0000000+00	86.9164	0	0	9.6561521-03	.025
2	8.4905892-01	0	0.0000000	1.0000000+00	76.1074	0	1	7.3936596-03	.027
3	8.4765464-01	1	1.1668877-02	1.0000000+00	86.8075	0	1	3.7167816-02	.012
4	8.3824569-01	3	2.7917760-03	1.0000000+00	87.7279	0	1	9.5237674-03	.018
5	8.3503625-01	3	2.5427486-03	1.0000000+00	88.4593	0	1	6.0637011-03	.015
6	8.3476273-01	3	2.5107424-03	1.0000000+00	88.5765	0	1	8.9918076-03	.015
7	8.3364831-01	3	2.4786298-03	1.0000000+00	88.7210	0	1	5.6784851-03	.013
8	8.3283345-01	4	5.0736814-03	1.0000000+00	87.8827	0	1	1.7790862-02	.017
9	8.3282443-01	3	2.4627829-03	1.0000000+00	88.6183	0	1	1.6317694-02	.001
10	8.3227556-01	2	1.1978756-03	2.5000000-01	5.8193	0	1	4.3875687-03	.019
11	8.3227548-01	8	5.3307279+01	4.8826125-04	18.4014	0	0	1.9392669+00	.054
12	8.3227418-01	8	5.3307279+01	3.0517578-05	2.8656	0	0	2.7905793+00	.016
13	8.3227304-01	8	5.3307279+01	3.0517578-05	8.2101	0	0	2.5616423+00	.008
14	8.3226731-01	8	5.3307279+01	6.1035156-05	7.0611	0	0	2.7657951+00	.015
15	8.3226731-01	8	5.3307279+01	3.7252903-09	5.9501	0	0	2.7688723+00	.024

SINGULAR VALUES AND VECTORS AT TERMINAL POINT

SING. VALUES			5.9391304+00	4.2222235+00	2.9006486+00	5.4089035-04
PAR.	1	ABS/1	-.3e85933	.8091481	-.4782714	-.0914602
	2	M+E/1	.9576080	.1129695	-.4368987	.0922463
	3	FDV/1	-.1126644	.0642250	-.0033399	.9915269
	4	LAG/1	-.3020642	-.5730552	-.7618168	.0001692

NORMALIZING ELEMENTS AND CORRELATION MATRIX

PARAMETER NO.			1	2	3	4
NORM. ELTS.			1.6y09220+02	1.7054538+02	1.8331384+03	4.3340296-01
PAR.	1	ABS/1	1.0000000			
	2	K+E/1	-.9999973	1.0000000		
	3	FDV/1	-.9999988	.9999992	1.0000000	
	4	LAG/1	-.7214867	.7221446	.7217610	1.0000000

CONFIDENCE LIMITS ON LINEAR HYPOTHESIS

			LOWER LIMIT	FINAL VALUE	UPPER LIMIT
PAR.	1	ABS/1	-1.3757162+02	4.0324678-01	1.3837811+02
	2	K+E/1	-1.3675472+02	4.0590593-01	1.3956653+02
	3	FDV/1	-1.4914360+03	4.3576104+00	1.5001512+03
	4	LAG/1	-3.5354506-01	1.0053730-04	3.5374615-01

EXPLORATION

				LOWER TEST	SUM OF SQUARES	LINEAR EST. OF SUM OF SQUARES	UPPER TEST	SUM OF SQUARES	LINEAR EST. OF SUM OF SQUARES
PAR.	1	ABS/1	B	1.0000000-04	1.0767233+01	3.6621848+00	3.4896962+01	1.0779150+01	2.0717855+04
	2	K+E/1	B	1.0000000-04	4.9131135+01	5.7106206+00	3.5196062+01	1.0456797+01	3.5655792+04
	3	FDV/1	B	1.0000000-04	1.0777544+01	1.0702036+01	3.7230600+02	7.3128182+04	7.3129156+04
	4	LAG/1	B	1.0000000-04	8.3226731-01	8.3226731-01	8.8511942-02	9.3760011-01	9.4135379-01

NOTICE

THE SUM OF SQUARES AT THE LOWER TEST POINT 4 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 = 4.079870e-01
THIS IS THE SCALE UNIT IN THE GRAPH OF THE RESIDUALS.

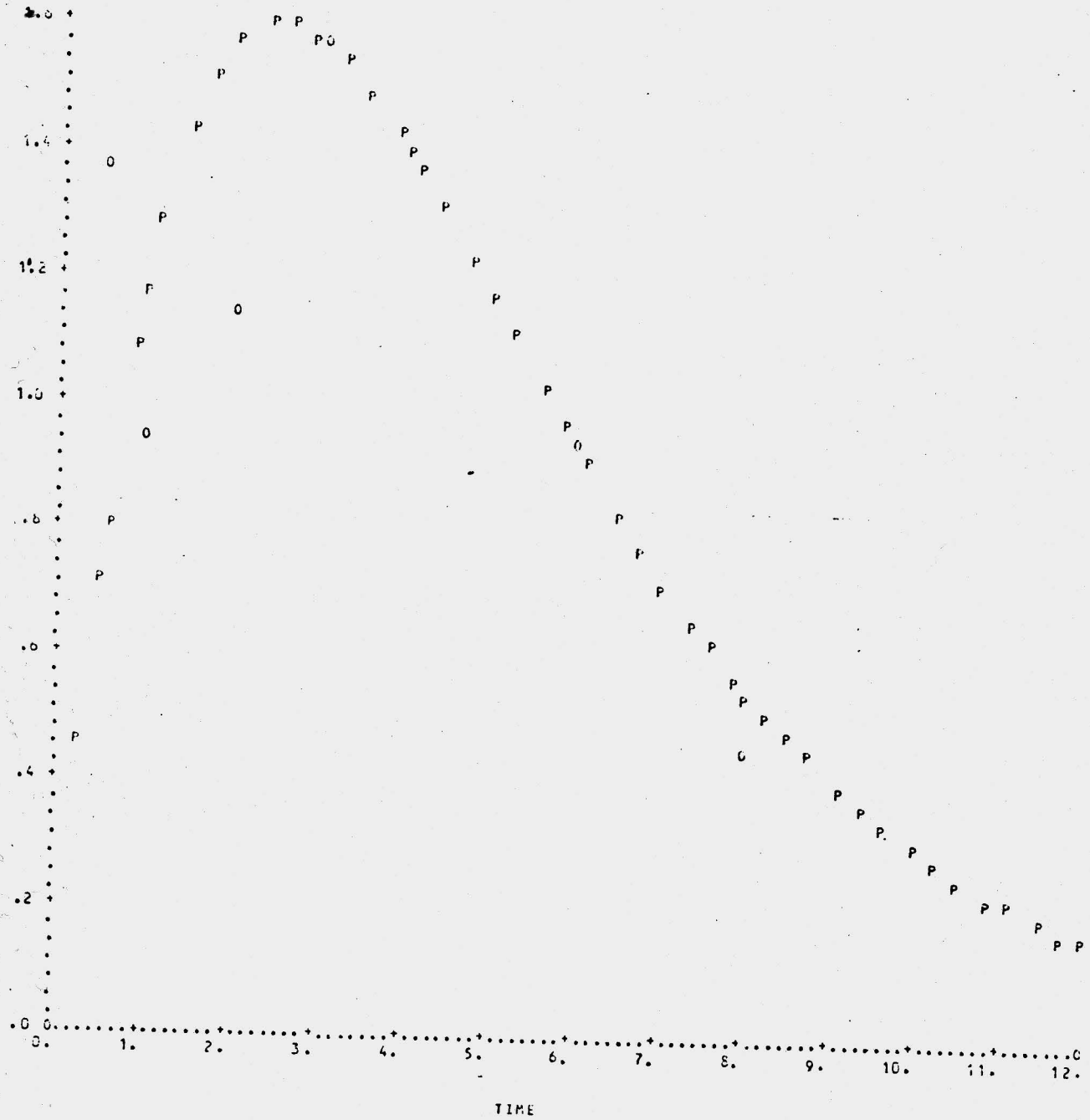
NO. DEGREES OF FREEDOM = 5

EXPT. NO.	VAR. NO.	PREDICTION	OBSERVATION	RESIDUAL	GRAPH OF RESIDUAL (-2 -1 0 +1 +2)
1	1	0.0000000	0.0000000	0.0000000	*
2	1	7.1757826-01	1.3800000+00	6.6242173-01	**********
3	1	1.1724631+00	9.5000000-01	-2.2246312-01	******
4	1	1.5647136+00	1.1500000+00	-4.1471360-01	**********
5	1	1.3661367+00	1.5700000+00	3.9633347-03	*
6	1	1.3933715+00	1.7600000+00	3.6662851-01	**********
7	1	9.3057946-01	9.4000000-01	9.4205365-03	*
8	1	5.5244285-01	4.5000000-01	-1.0244284-01	****
9	1	1.642743e-01	0.0000000	-1.642743e-01	****

TIME SINCE END OF THE LAST ITERATION = .130 SECONDS . TOTAL TIME = .446 SECONDS

. R-SQUARED FOR FUNCTION1 = .9228
. COR FOR FUNCTION1 = .8663

PLOT OF OBSERVED,O'S AND PREDICTED,P'S VALUES



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