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THE BIOAVAILABILITY AND PHARMACOKINETICS OF METHOTREXATE.

THE UNIVERSITY OF ARIZONA,

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**THE BIOAVAILABILITY AND PHARMACOKINETICS  
OF METHOTREXATE**

by

**Mark Alan Campbell**

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**A Thesis Submitted to the Faculty of the  
DEPARTMENT OF PHARMACY PRACTICE  
In Partial Fulfillment of the Requirements  
For the Degree of  
MASTER OF SCIENCE  
WITH A MAJOR IN PHARMACY  
In the Graduate College  
THE UNIVERSITY OF ARIZONA**

**1 9 8 2**

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## APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

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Date

### DEDICATION

I wish to dedicate this thesis to the people who mean the most to me: my parents, Malcolm and Lois; my family; my wife, Sue; and all of my friends. Their continuous love, concern, guidance, encouragement, and support made this project a reality.

### ACKNOWLEDGMENTS

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## ABSTRACT

A study with a randomized factorial design was conducted to determine the pharmacokinetic disposition parameters of methotrexate following intravenous administration and the absolute intramuscular and oral bioavailability of methotrexate. Plasma concentration versus time data were collected from six patients with advanced squamous cell carcinoma of the head and neck. These data were analyzed by a "model independent" method to obtain the following disposition parameters: elimination half-life ( $t_{1/2}$ ), plasma clearance ( $Cl_g$ ), volume of distribution at steady-state ( $V_{ss}$ ), and volume of distribution in the terminal elimination phase ( $V_\lambda$ ). Elimination half-lives after intravenous, intramuscular and oral administration of methotrexate were not significantly different. The mean absolute intramuscular bioavailability of methotrexate was found to be 0.93. The mean absolute bioavailability of methotrexate from the commercial oral tablets was found to be 0.36. The preferred routes for methotrexate administration are the intravenous and intramuscular routes. Administration of methotrexate by the oral route is discussed.

## CHAPTER 1

### INTRODUCTION

Methotrexate, a folic acid antagonist, has been used as a chemotherapeutic tool since its introduction in the late 1940's. Initially, it was used to induce remission in children with acute lymphocytic leukemia. Other tumors were found to be sensitive to the effect of methotrexate including breast, head and neck, and gestational trophoblastic neoplasms. It was also found to be effective in non-cancerous, hyperplastic conditions such as psoriasis.

The dosage regimens of methotrexate have changed dramatically since its early use. Initially, very low doses were given at daily intervals and now very large doses are given experimentally in combination with citrovorum factor in the treatment of various neoplastic disorders. Studies have been conducted that associate the cytotoxic effectiveness of methotrexate to a tissue-dependent threshold concentration and duration of exposure to the drug (Goldman 1975). Therefore the goal of methotrexate therapy is to expose the tumor to a high enough concentration of methotrexate for a long enough period of time to obtain cell kill without incurring severe toxicity to normal tissue.

Methotrexate has been administered by the intravenous, intramuscular, and oral routes of administration. Only one study has been conducted comparing the therapeutic effectiveness of methotrexate given in the same dose by these three routes (Freeman-Narro et al. 1975).

They found that antimetabolic effects appeared sooner in those patients who received methotrexate intramuscularly as their serum concentrations were higher at 24 hours following the dose.

The intravenous route is most commonly used to administer methotrexate. There may be situations, however, when administration by the intramuscular or oral route is indicated. A patient whose veins have been compromised by previous chemotherapy and diagnostic venipunctures may be a candidate for intramuscular methotrexate. The oral route would be an alternative for those patients with minimal muscle mass. Oral administration also offers the advantage of self-administration on an outpatient basis for those patients on maintenance therapy. To provide equivalent therapeutic effectiveness when changing from one route of administration to another, the oncologist must know the equivalent dose for each route. This information is not currently available.

#### Purpose

Although methotrexate has been used in chemotherapy for several years, there has never been a study to determine the bioavailability of methotrexate from oral tablets. Also, no studies have been conducted to compare the absolute bioavailability of methotrexate from the oral and intramuscular route in the same patient. There have been several reports of methotrexate's disposition kinetics in the literature, however the results have been conflicting, partially because of the different analytical methods used to measure methotrexate in the plasma.

One purpose of this study is to report on the pharmacokinetic parameters obtained from the plasma concentration versus time profile of methotrexate following intravenous administration of a conventional dose. The second purpose is to assess the extent to which methotrexate is absorbed via the intramuscular and oral routes following a conventional dose so that therapeutically equivalent doses can be recommended.

#### Assumptions

The tumor type, age, or sex of the individuals in the study population are assumed not to have significant influence on the kinetic parameters or bioavailability of methotrexate. Inpatient and interpatient differences in urinary pH are assumed not to have significant influence on the renal clearance of methotrexate. The dietary intake of the patients before and after the fasting periods is assumed not to have a significant effect on the bioavailability and pharmacokinetics of methotrexate. The assignment of patients into one of the six random administration sequences is assumed to remove any effects that may be created by the sequence in which methotrexate is administered. The parenteral and oral dosage forms used in this study are assumed to be pure and homogenous. The pharmacokinetics of methotrexate are assumed to be linear at the doses being administered in this study.

#### Limitations

The analytical method used to quantitate the concentration of methotrexate in each patient's blood is accepted as being sensitive, precise, and specific. The urinary pH of the patients is determined

to be uncontrollable. The applicability of the information generated by this study to other populations is unknown.

#### Definition of Terms

**Pharmacokinetics:** Pharmacokinetics is the study and characterization of the time course of drug absorption, distribution, metabolism, and excretion, and the relation of these processes to pharmacological effects.

**Bioavailability:** Bioavailability is the rate and extent to which an intact drug is absorbed into the body (i.e., systemic circulation) from a pharmaceutical dosage form. When a drug is administered intravenously, it is by definition 100% available to the systemic circulation.

**Plasma Methotrexate Concentration:** The plasma methotrexate concentration is the amount of methotrexate in the plasma (the liquid portion of the blood in which the particulate components are suspended) and is expressed as nanograms per milliliter.

**Elimination Half-Life:** The elimination half-life is the amount of time required for the plasma drug concentration to decrease by 50% after absorption is complete and distribution equilibrium has been reached. It is generally independent of dose, concentration, or route of administration at therapeutic drug concentrations.

**Apparent Volume of Distribution:** The apparent volume of distribution is a pharmacokinetic parameter relating the amount of drug in the body to the plasma concentration. It does not necessarily represent

a real body space, however, it does give insight into the extent of extravascular distribution of a drug.

**Total Body Clearance:** Total body clearance is a measure of the ability of the body to remove all drug from a given volume of plasma per unit time. For a drug that is eliminated by renal excretion and hepatic metabolism, the total body clearance would be the sum of the renal clearance and the hepatic clearance.

## CHAPTER 2

### REVIEW OF THE LITERATURE

The concept of antifolate therapy was developed in the late 1940's when dietary measures were used to induce folate deficiency in patients with leukemia. In 1948, Farber and associates (1948) found the newly synthesized compound, 4-aminopteroylglutamic acid (aminopterin), an analogue of folic acid, to be effective in producing temporary remissions in children with acute leukemia. The severe myelosuppressive toxicity produced by aminopterin stimulated research to discover a less toxic and possibly more potent antagonist of folic acid. The development of methotrexate (amethopterin), a less toxic and less potent analogue of aminopterin, followed soon after. Clinical studies with methotrexate began in 1949 (Berman et al. 1949; Dameshek 1949). Since it was introduced, doses and dosing schedules have changed dramatically. Initially it was administered in daily doses of 1 to 5 mg/m<sup>2</sup>. It became evident that tumor cells did not respond to these lower doses so the use of higher doses of methotrexate developed. It was thought that the unresponsive tumor cells were resistant to the effects of methotrexate, the resistance being either natural or acquired. Currently methotrexate is given experimentally in doses as high as 600 mg/kg, in an attempt to overcome the resistance in the treatment of various neoplastic disorders, primarily osteogenic sarcoma (Penta 1975).

Citrovorum factor is administered concurrently as a source of reduced folate to "rescue" normal tissue cells.

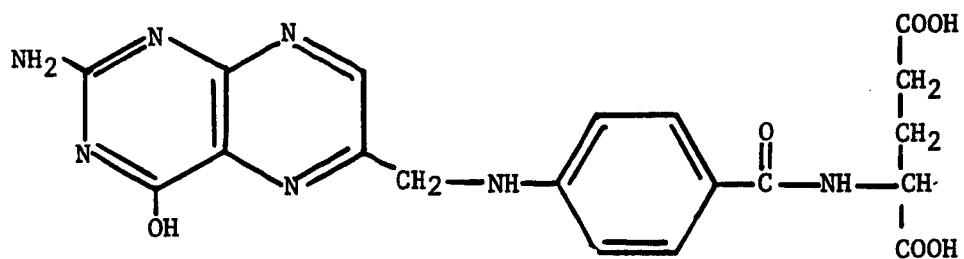
### Chemistry

Methotrexate (amethopterin) is 4-amino-N<sup>10</sup>-methylpteroylglutamic acid (Figure 1). Its structure differs from folic acid only in that it has an amino group instead of a 4-hydroxy group on the pteridine ring and methotrexate is methylated at the N<sup>10</sup> position. The molecular weight is 454 grams per mole. Methotrexate is a dicarboxylic weak acid with pKa's of 4.8 and 5.5. As predicted by these parameters, methotrexate is predominantly ionized and poorly lipid soluble at physiologic pH. The solubility of methotrexate in urine is  $2.2 \times 10^{-3}$  M at pH 5.7 and  $22 \times 10^{-3}$  M at pH 6.9 (Stoller et al. 1975).

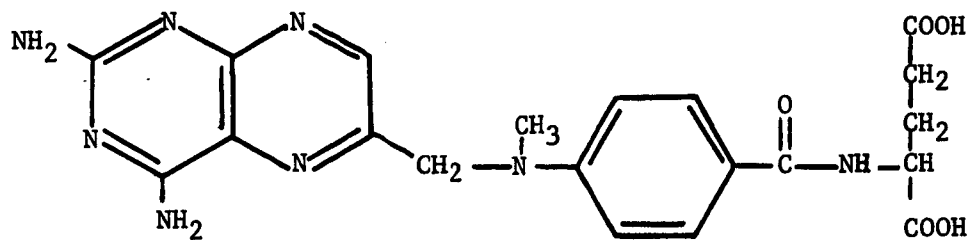
### Pharmacology

#### Folic Acid Metabolism

Figure 2 shows a simplified scheme of folic acid metabolism. The folic acid series are naturally occurring coenzymes. Their physiologic role is the transfer and utilization of one-carbon moieties for the biosynthesis of amino acids, and purine and pyrimidine units of DNA and RNA. Before it can be utilized, dietary folic acid is first reduced to dihydrofolic acid, then to tetrahydrofolic acid. These reductive steps are catalyzed by the enzyme dihydrofolate reductase using NADPH as hydrogen donor. Dihydrofolate is also a product of the conversion of uridylate to thymidylate by the enzyme thymidylate synthetase.



**FOLIC ACID**



**METHOTREXATE**

Figure 1. Chemical structures of folic acid and methotrexate.

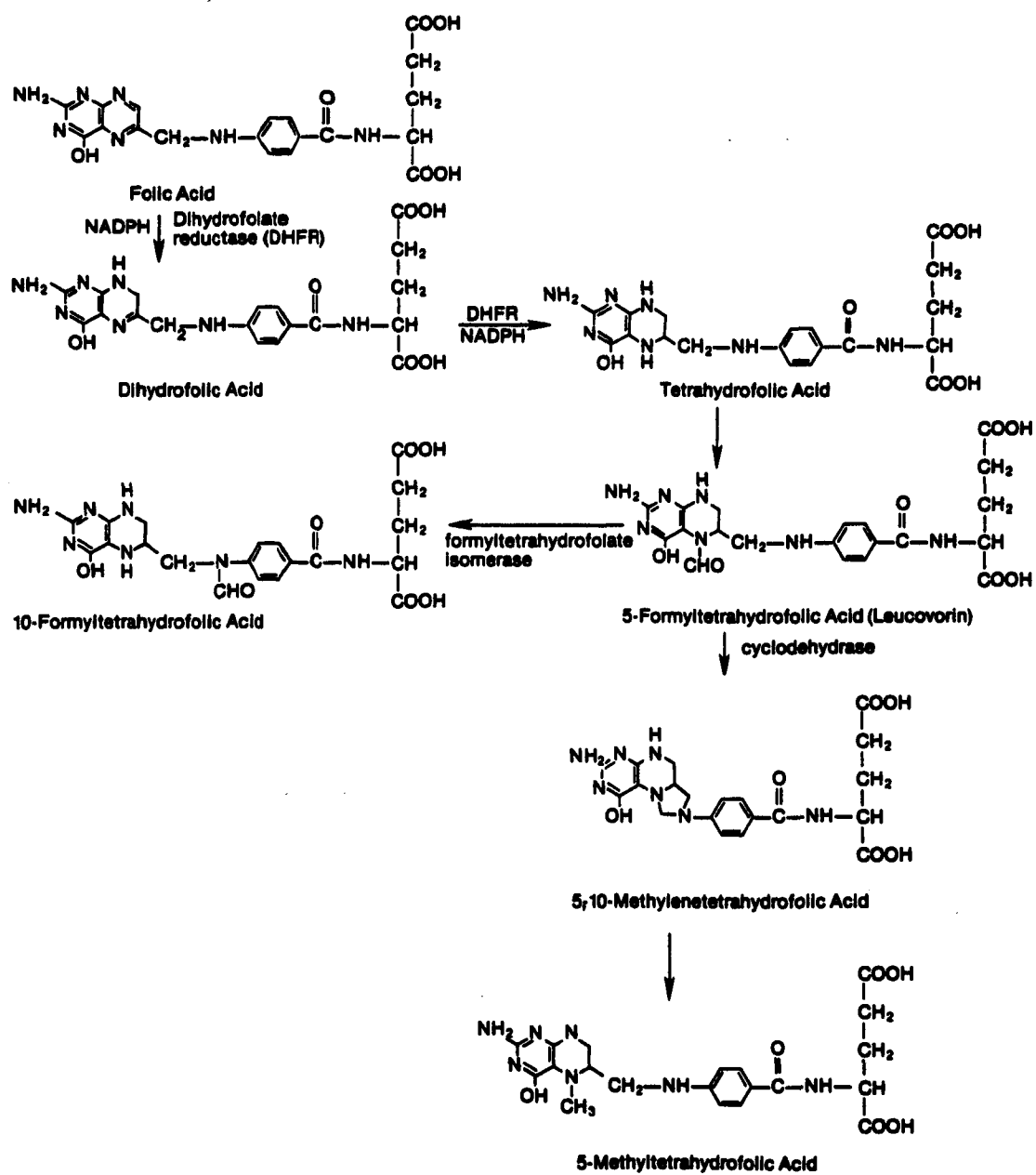


Figure 2. Schematic Diagram of Folate Metabolism.

Tetrahydrofolic acid is further metabolized to 5-formyltetrahydrofolic acid (leucovorin), a folate coenzyme found in relatively low concentrations in human tissue. Leucovorin is in turn converted to other active folate coenzymes. The primary folate coenzyme found in the plasma is 5-methyltetrahydrofolic acid. In fact, when doses of leucovorin are administered, a large proportion is metabolized to 5-methyltetrahydrofolate (Nixon 1979). It is the methyl group on the 5-position that is transferred in the de novo synthesis of purines and pyrimidines.

#### Mechanism of Action

The research of Nichol and Welch (1950), using rat liver slices, showed that aminopterin blocked the conversion of folic acid to 5-formyltetrahydrofolic acid. Following the identification of the enzyme dihydrofolate reductase (Futtermann 1957; Osborn et al. 1958), it was determined that the mechanism of action of methotrexate and other folate antagonists was the inhibition of dihydrofolate reductase, preventing the formation of tetrahydrofolic acid. Without reduced folate coenzymes necessary for the synthesis of purine and pyrimidine nucleotides, DNA and RNA synthesis ceases and cell death occurs.

Important biochemical reactions inhibited by methotrexate include the synthesis of the DNA-specific nucleotide thymidylic acid and the synthesis of inosinic acid, the precursor of purines required for both DNA and RNA synthesis. Certain studies (Schrecker et al. 1960; Skipper et al. 1952) indicated that the effect of methotrexate on purine synthesis is responsible for cell death in certain tissues. Conflicting

research, including a study by Winzler (1957) in human chronic myelogenous leukemia cells, suggested that the inhibition of thymidylate synthesis by folate antagonists is the primary factor leading to cell death. This theory is supported by the work of Hofflerend and Tripp (1972) which indicated that the inhibition of thymidylate synthesis is the most important mechanism of methotrexate cytotoxicity because DNA synthesis was inhibited to a greater extent than RNA synthesis in humans. Figure 3 shows how methotrexate blocks thymidylate synthesis.

Because of its inhibitory effect on DNA synthesis, methotrexate is considered to be cell-cycle specific in the S-phase, when DNA synthesis occurs. Ernst and Killman (1971) found that doses of methotrexate less than  $20 \text{ mg/m}^2$  arrested human leukemic myeloblasts in S-phase for about 20 hours with little effect on cells in the  $G_1$ ,  $G_2$ , or M phases. Doses higher than  $30 \text{ mg/m}^2$  arrested leukemic myeloblasts in S-phase for more than 48 hours and slowed the entry of cells from  $G_1$  into the S-phase. Tissues most sensitive to the cytotoxic effects of methotrexate are those undergoing rapid cellular turnover with a large proportion of cells in the dividing cell cycle.

Werkheiser (1961), using folic acid as the substrate at pH 6.1, concluded that the binding of folic acid to dihydrofolate reductase found in rat liver was stoichiometric. In a study examining the inhibition of this enzyme by methotrexate, Bertino et al. (1964) found that the binding of methotrexate to dihydrofolate reductase obtained from mice Ehrlich ascites carcinoma was stoichiometric. They reported  $K_m$  values of  $7.4 \times 10^{-6} \text{ M}$  and  $1.5 \times 10^{-6} \text{ M}$  for folate and dihydrofolate,

DEOXYURIDYLIC ACID  
(dUMP)

THYMIDINE MONOPHOSPHATE  
(TMP)

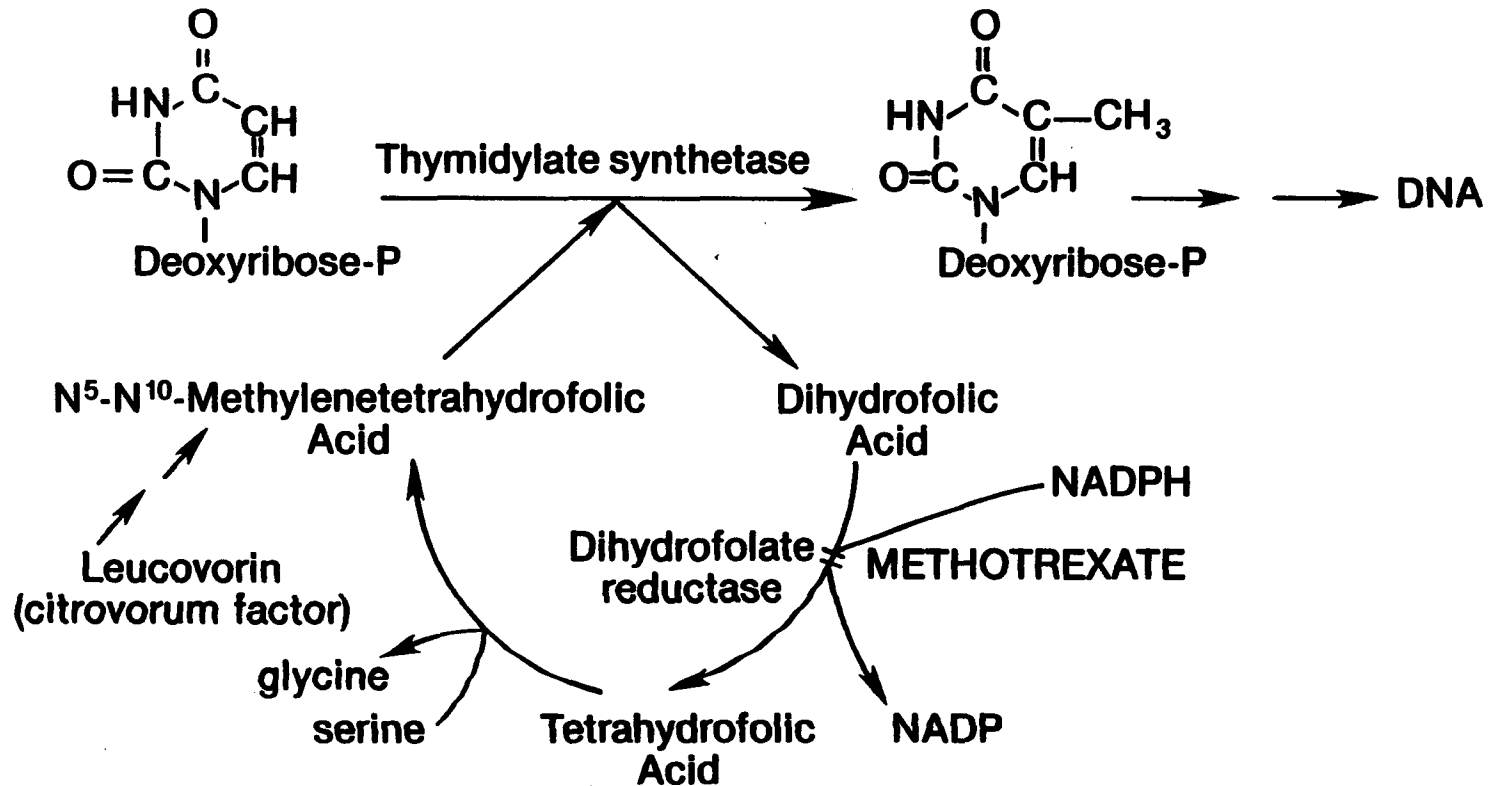


Figure 3. Schematic Diagram of the Inhibition of Thymidylate Synthetase by Methotrexate.

respectively, at pH 5.9, and a value of  $1.3 \times 10^{-6}$  M for dihydrofolate at pH 7.5. Another of their findings was that pH influenced the inhibition of the enzyme by methotrexate. Tighter binding and greater inhibition were produced by methotrexate at pH 5.9 than at pH 7.6. The inhibitor constant,  $K_i$ , for methotrexate was reported to be  $2.3 \times 10^{-9}$  M, using purified enzyme from chicken liver (Osborn et al. 1958).

It is difficult to correlate the plasma concentration with the pharmacologic effect of antineoplastic drugs because factors other than the pharmacologic action of the drug influence the therapeutic response and because the cytotoxic effects of these agents are irreversible. Such factors as tumor cell differentiation, metabolic activity, clonogenic potential, and degree of tumor vascularity also influence the therapeutic response to antineoplastic agents (Erllichman et al. 1980). However, a concentration dependency has been shown to exist with methotrexate. Efforts to quantitate the threshold concentration necessary to inhibit DNA synthesis in normal and tumorous tissues in mice were made by Chabner and Young (1973). They determined the threshold concentration to be  $1 \times 10^{-8}$  M for bone marrow and duodenal mucosa. This concentration, however, will vary depending on the tumor or tissue type and location. Goldman (1975) determined that intracellular methotrexate in excess of the threshold concentration is necessary for cytotoxicity. One hypothesis states that there exists dihydrofolate reductase with a low-affinity for methotrexate which continues to synthesize tetrahydrofolate although the high-affinity

sites are saturated. The presence of intracellular methotrexate in excess of the threshold concentration causes suppression of purine synthesis and inhibition of the influx of folate cofactors into the cell (Goldman 1975).

### Pharmacokinetics

#### Absorption

Only two studies (Halprin et al. 1971; Freeman Narrod et al. 1975) have investigated the pharmacokinetics of methotrexate following intramuscular administration. Both agreed that the absorption of methotrexate was rapid and complete. However, neither study administered the drug intravenously to the study patients so the true availability is unknown. Following single doses of 25 to 50 mg., Halprin et al. (1971) found that peak levels of methotrexate occurred at 2 hours following the dose. Freeman-Narrod et al. (1975) also reported the peak level to occur two hours following a 15 mg/m<sup>2</sup> dose.

Early studies of the absorption and excretion of methotrexate after oral administration gave conflicting results. Burchenal et al. (1951) and Werkheiser et al. (1961) could only recover 40-50% of a single 5-10 mg oral dose as unchanged drug in the urine after 24-48 hours, although Freeman (1958) was able to recover 100% of a 9 mg oral dose as parent drug in the urine after 12-24 hours. The reason for the inconsistent results may be the different assay procedures used by these investigators. Burchenal et al. (1951) used an insensitive microbial assay and Werkheiser et al. (1961) used an enzyme inhibition

method. Freeman (1958) used a fluorometric assay which is not as specific for methotrexate as the enzyme inhibition method. Therefore, Freeman's (1958) results are misleading because the fluorometric method measured both folate analogues and methotrexate. More recently, Henderson et al. (1965) and Wan et al. (1974) have shown that the absorption of methotrexate is dose-dependent following the administration of tritiated methotrexate. Henderson et al. (1965) administered methotrexate at two dosage levels, 0.1 mg/kg and 10 mg/kg. The excretion pattern following a 0.1 mg/kg oral dose, administered through a nasogastric tube, was similar to that of the same dose given intravenously, indicating that the absorption of the oral dose was comparable to that of the intravenous dose. The oral and intravenous plasma concentration versus time profiles were also similar, indicating that absorption from the gastrointestinal tract was comparable to that following intravenous administration although levels were only followed for six hours. At the higher dose level, it was concluded that absorption was incomplete because the plasma concentrations after the oral dose were only one-tenth of those seen with the same dose given intravenously. Wan et al. (1974) also administered tritiated methotrexate orally, via nasogastric tube, at two different dosage levels, 30 mg/m<sup>2</sup> and 80 mg/m<sup>2</sup>. In the five patients who received 30 mg/m<sup>2</sup> of methotrexate orally and intravenously two weeks apart, the average area under the oral plasma concentration-time curve was 47.7% of the area under the intravenous curve. The peak plasma concentration occurred at 1.5 hours after the dose. An oral dose of 80 mg/m<sup>2</sup> of methotrexate was administered to

one patient with only 31% of the dose recovered in the urine after 96 hours compared to 88% in the group of patients who received 30 mg/m<sup>2</sup> orally. An intravenous dose was not given to this patient, therefore the bioavailability could not be calculated. The results of these studies suggest saturable intestinal absorption as one mechanism that may be involved in the apparent nonlinear behavior of methotrexate.

Freeman-Narro et al. (1975) compared serum concentrations of methotrexate following intravenous or oral administration of a single 15 mg/m<sup>2</sup> dose to five patients. They found that higher and more prolonged serum concentrations occurred after intravenous administration indicating that either oral absorption was incomplete or that it was slow. However, their sampling times could have missed a substantial early peak or late peak methotrexate plasma concentration. Blood samples were collected at 1, 2, 4, 8, and 24 hours following the dose in this investigation.

Various factors have been shown to alter the gastrointestinal absorption of methotrexate in humans. Pinkerton et al. (1980) found that food had a negative influence on methotrexate absorption in ten children with acute lymphoblastic leukemia who received a dose of 15 mg/m<sup>2</sup> of methotrexate in tablet form. The peak serum concentration and area under the absorption curve were significantly reduced and the time to peak concentration increased when oral methotrexate was given with a "milky" meal (milk, cereal, and toast). Two reasons were suggested for the poor absorption of methotrexate after a "milky" meal. First, the pH-sensitive transport mechanism for methotrexate in the proximal

jejunum may have been negatively effected by the buffering action of milk, accounting for altered absorption of methotrexate. This buffering potential of milk is highly questionable, however, because the protein and calcium contained in milk are each stimulants of gastric-acid secretion (Ippoliti et al. 1976). The second reason is that methotrexate may bind to certain milk proteins, resulting in a reduction of methotrexate absorption. When methotrexate was taken with a citrus meal (orange juice, orange, and toast), the peak serum concentration and area under the absorption curve were reduced but to a smaller extent than with the "milky" meal. The mechanism of the citrus meal appeared to be delayed gastric emptying caused by the acidic content of the meal.

Concurrent therapy with prophylactic nonabsorbable antibiotics (polymyxin B, paromomycin, nystatin, and vancomycin) was shown to decrease the absorption of methotrexate ( $15 \text{ mg/m}^2$  twice weekly) from the gastrointestinal tract in 10 patients with small cell bronchogenic carcinoma (Cohen et al. 1976). The average decrease in absorption was 25%. The induction of an intestinal malabsorption syndrome by these agents was the mechanism of their effect on methotrexate absorption. This drug-induced malabsorption syndrome is characterized by the appearance of fat globules and muscle fibers in the stool, impaired absorption of D-xylose, and decreased serum carotene and folate levels indicating altered absorption of nutrients.

Despite the widespread use of methotrexate in chemotherapy for thirty years, there have been no studies in the literature concerned with the bioavailability of methotrexate from the commercial tablets.

This lack of information may be the reason for the infrequent use of oral methotrexate in chemotherapy regimens.

#### Distribution

After intravenous administration, methotrexate distributes rapidly within a volume of 18-21% of body weight (Leme et al. 1975; Freeman 1958), and then within a space of 76% of body weight. These volumes of distribution approximate those of extracellular water (20% of body weight) and total body water (60% of body weight), respectively.

In humans, methotrexate persisted in those tissues with high content of folic acid and citrovorum factor; liver, kidneys, and spleen (Charache et al. 1960). The lowest tissue levels of methotrexate were found in the brain, fat, and skeletal muscle (Anderson et al. 1970). Methotrexate is rapidly transported into human skin following parenteral administration (Comaish and Juhlin 1969). The distribution of methotrexate into interstitial fluid spaces, such as the cerebrospinal fluid, pleural cavity, and peritoneal cavity, occurs slowly and with characteristics similar to those of a passive transport mechanism (Dedrick et al. 1975).

Early studies of the protein-binding of methotrexate in humans reported that 45-50% of methotrexate in the plasma is bound to proteins (Henderson et al. 1965; Wan et al. 1974). Protein-binding, determined by an ultrafiltration technique (Toribara et al. 1957), was constant from concentrations of 0.1 to 1000 mcg/ml. More recently, however, the protein-binding in normal humans has been shown to be 95%, and constant

over the range of 1-30 micromoles/liter (Steele et al. 1979a).

The major binding protein was albumin which bound 87.3% of the protein-bound methotrexate in the serum. The reasons given for the discrepancy were problems with the techniques used by early investigators to determine protein-binding. Steele et al. (1979a) employed a continuous ultrafiltration technique using individual sera and measured methotrexate concentrations by a sensitive and specific radioimmunoassay method. In 14 patients with neoplastic disease, the binding of methotrexate to proteins was found to be 92%, not significantly different from protein-binding in normal subjects (Steele et al. 1981). The protein-binding of methotrexate was found to be nonlinear at methotrexate concentrations above 50 micromoles/liter, concentrations found only with high-dose methotrexate therapy (Steele et al. 1979a).

An extensive study was conducted by Paxton (1981) to investigate the discrepancies that exist in the literature with regard to the binding of methotrexate to plasma proteins. Protein-binding in normal subjects, determined by ultrafiltration, was found to be 46.5%, constant over the range of 0.1 nanomoles/liter to 100 micromoles/liter. This value is consistent with those reported by the previously mentioned investigators. Similar results were obtained when protein binding was measured by equilibrium dialysis and ultracentrifugation. Methotrexate concentrations were measured by the radioimmunoassay method of Paxton and Rowell (1977). Paxton suggested that, based on his data, the high value for protein binding obtained by Steele et al. (1979a) should be questioned as to its validity.

## Elimination

Renal excretion constitutes the major route of elimination for methotrexate (Freeman 1958; Huffman et al. 1973). Approximately 80% of a dose is excreted through the kidneys. Following an intravenous dose of 30 mg/m<sup>2</sup> in 22 patients, the average value for renal clearance was  $78 \pm 4.9$  ml/min in the measured plasma concentration range of 50 to 1000 ng/ml (Huffman et al. 1973). This value is less than the normal glomerular filtration rate suggesting that tubular reabsorption occurs at these plasma concentrations. In 15 patients with steady-state methotrexate concentrations of 90 to 200 ng/ml, the average renal clearance was 179 ml/min, indicating not only glomerular filtration but also active tubular secretion (Liegler et al. 1969). Lawrence et al. (1980) reported that the renal clearance of methotrexate following the intravenous administration of 100 mg to six patients was  $18 \pm 6$  ml/min compared to  $53 \pm 19$  ml/min following the administration of 25 mg intravenously to the same six patients. Plasma methotrexate concentrations following the 100 mg dose ranged from 10 to 40,000 ng/ml and from 10 to 6,000 ng/ml following the 25 mg dose. In preliminary studies with high-dose methotrexate, Shen and Azarnoff (1978) reported that renal clearance was 20-50 ml/min at plasma concentrations ranging from 100 to 500,000 ng/ml. Methotrexate tubular secretion utilizes the general organic acid transport mechanism as demonstrated by reduced methotrexate clearance in the presence of salicylates and para-aminohippurate (Liegler et al. 1969). The results of these studies suggest

that the mechanism for falling renal clearance with increased doses of methotrexate is saturability of the renal tubular secretion of the drug.

Probenecid was found to inhibit the renal tubular secretion of methotrexate when administered prior to or along with the methotrexate dose (Bourke et al. 1975; Aherne et al. 1978). In monkeys pretreated with 700 mg/m<sup>2</sup> of probenecid, the renal tubular transport of methotrexate was totally inhibited when intravenous doses of 1.8 to 621 mg/m<sup>2</sup> of methotrexate were administered (Bourke et al. 1975). The resultant steady-state methotrexate plasma concentrations of the probenecid-pretreated group were twice those determined in the non-probenecid treatment group over the range of methotrexate doses studied. Aherne et al. (1978) obtained similar results in human studies. The mean 24-hour plasma methotrexate concentration of the four patients who received a bolus of 200 mg/m<sup>2</sup> of methotrexate and probenecid (500 mg 60 minutes before and five hours after methotrexate) was 400 ng/ml compared to 90 ng/ml in the four patients who received 200 mg/m<sup>2</sup> of methotrexate and no probenecid. Also, the mean elimination half-life increased to 5.5 hours in the probenecid-treated group from 3.75 hours in the group not receiving probenecid. The authors of these studies suggested that the combination of methotrexate and probenecid be used clinically in order that lower doses of methotrexate may be used resulting in less toxicity while achieving the same therapeutic effect as higher doses of methotrexate given alone.

Early research with low doses of radioactive methotrexate in humans concluded that methotrexate was not metabolized (Freeman 1958; Henderson et al. 1965). Later, Valerino et al. (1972) found that

methotrexate was metabolized during enterohepatic circulation by intestinal bacteria, with the major metabolite being 4-amino-4-deoxy-N<sup>10</sup>-methylpteronic acid. Wan et al. (1974) found similar metabolites when methotrexate was incubated in human feces. Following the administration of 30 mg/m<sup>2</sup> intravenously, methotrexate metabolites found in the urine constituted 6% of the dose compared to 35% after oral administration of the same dose (Wan et al. 1974). The higher amount of metabolites excreted after oral doses is consistent with the theory that metabolism by intestinal bacterial in the gastrointestinal tract occurs during enterohepatic circulation. Since enterohepatic circulation also occurs after intravenous administration, intestinal metabolism of methotrexate prior to absorption may explain the larger amount of metabolites excreted after oral doses.

Jacobs et al. (1976) found the metabolite, 7-hydroxymethotrexate, in the urine of humans and monkeys after receiving doses of 50 mg/kg or more of methotrexate. This metabolite constituted as much as 1 to 11% of the dose found in the urine after 24 hours. Since the formation of this metabolite does not occur after lower doses, it was concluded that its formation is dose-dependent. It is postulated that this dose-dependence may be due to a low-affinity for methotrexate by the enzyme which catalyzes the conversion. In rabbits, this enzyme has been identified as the hepatic metalloflavoprotein aldehyde oxidase (Jacobs et al. 1976). However, it is not known whether or not this enzyme is responsible for the conversion in humans.

Biliary excretion accounts for the remainder of methotrexate elimination. From 0 to 9% of an intravenous dose, as determined by fecal

analysis, was excreted into the feces (Huffman et al. 1973). After a single intravenous dose of  $30 \text{ mg/m}^2$ , 6.7 to 9% of the administered dose was recovered by duodenal aspiration after 24 hours in 6 cancer patients (Shen and Azarnoff 1978). A study by Van Den Berg et al. (1980) pointed out the importance of enterohepatic circulation to the pharmacokinetics of methotrexate. They reported the case of a patient whom, after receiving a 200 mg intramuscular dose of methotrexate, began to have vomiting and diarrhea which persisted for 4 hours. Her renal clearance of methotrexate was 37 ml/min during this time but increased to 97 ml/min after these symptoms ceased, as determined by an increase in the slope of the line which plotted cumulative urinary excretion of methotrexate against the area under the plasma concentration-time curve up to the point of urine collection. They concluded that much of the drug was lost from the gastrointestinal tract during the vomiting and diarrhea as a result of enterohepatic circulation, although they did not attempt to measure the drug in the feces or vomitus. However, the large difference in renal clearance during and after the episode of vomiting and diarrhea is not actually due to the effect of enterohepatic circulation but probably due to the reduction of renal blood flow that occurs as a physiological response to vomiting and diarrhea.

Henderson et al. (1965) initially described the plasma concentration versus time course of methotrexate. The mean elimination half-life was reported to be 2.2 hours in ten patients following a 0.1 mg/kg dose of tritiated methotrexate administered either orally or intravenously. Plasma samples were collected every half-hour for two

hours following the dose and then every hour for four hours. In 26 patients with psoriasis, Halprin et al. (1971) reported that the methotrexate half-life ranged from 2 to 10 hours following single oral or intramuscular doses of 2.5 to 25 mg. Samples were collected at 2, 4, 8, 12, 16 and 24 hours after treatment and the samples were assayed by the fluorometric method.

Following the intravenous administration of  $30 \text{ mg/m}^2$  of tritiated methotrexate to 22 cancer patients, the plasma concentration versus time profile of methotrexate appeared to be triphasic (Huffman et al. 1973). The initial distribution phase was rapid with a half-life of  $0.45 \pm 0.11$  hours. The second phase, which is thought to represent renal clearance and biliary excretion, had a half-life of  $3.49 \pm 0.55$  hours. As plasma levels were followed for 4 days, they reported a third phase of elimination with a half-life of  $26.99 \pm 4.44$  hours. It was postulated that enterohepatic recycling was responsible for the third phase. Blood samples were collected at 1, 2, 4, 6, 8, 12, and 24 hours then every 12 hours up to 96 hours after the dose. The plasma was analyzed for methotrexate by determining total radioactivity using liquid scintillation spectrometry.

The plasma disposition kinetics of high-dose methotrexate have been studied by few investigators. Stoller et al. (1975) reported the plasma disappearance of methotrexate to be biexponential in six patients given 50 to 200 mg/kg of methotrexate infused intravenously over six hours followed by  $15 \text{ mg/m}^2$  of citrovorum factor intravenously every six hours for eight doses beginning two hours after the completion of the

methotrexate infusion. The half-life of the first phase was  $2.06 \pm 0.16$  hours and the second phase had a half-life of  $10.4 \pm 1.8$  hours. Plasma samples were collected for 72 hours following the dose and were analyzed by the dihydrofolate reductase enzyme inhibition method. The plasma disappearance of methotrexate following 4-hour infusions of 50 to 200 mg/kg in 172 infusions was also found to be biexponential by Isacoff et al. (1977). They reported the half-life of the first phase to be  $1.8 \pm 0.1$  hours and  $8.4 \pm 0.5$  hours for the second phase. Plasma samples were collected at 0, 2, 4, 8, 12, 24, 48, and 72 hours after the beginning of the infusion and were analyzed by a competitive protein-binding method. The authors concluded from their results that a meaningful dose-dependent elimination of methotrexate does not exist (Isacoff et al. 1977).

Certain pathophysiological conditions are known to influence the pharmacokinetics of methotrexate. Since the major route of methotrexate elimination is through the kidneys, it is logical that patients with impaired renal function will have impaired elimination of methotrexate. Kristensen et al. (1975) studied the influence of renal function on methotrexate elimination in 25 patients given an intravenous dose of 3.6-4.8 mg of tritiated methotrexate. The average half-life in patients with endogenous creatinine clearances less than 60 ml/min was 5.1 hours in contrast to 2.5 hours for patients with endogenous creatinine clearances greater than 60 ml/min. They reported the elimination half-life to be 23.1 to 46.2 hours in the anuric patient and 2.3 hours in a patient with a creatinine clearance of 100 ml/min.

The presence of fluid in a third-space reservoir (eg. ascites, pleural effusion) can also delay the disappearance of methotrexate from the plasma. Following a 30 mg/m<sup>2</sup> oral dose, Wan et al. (1974) found that a patient with a peritoneal effusion had a plasma half-life of 9.2 hours and an effusion half-life of 27.3 hours indicating a slow transfer of drug from the effusion into the plasma. Equilibration of plasma and effusion fluids is slow because of low blood perfusion of effusion fluid and inadequate mixing of these fluids. Evans and Pratt (1978) reported a similar occurrence in a 12-year old male who was given a 400 mg/kg infusion of methotrexate in the presence and absence of a pleural effusion. The terminal phase half-life was 6.7 hours without the pleural effusion and 14.4 hours in the presence of a pleural effusion, estimated by chest X-Ray to consist of 250 to 400 ml of pleural fluid. Pleural fluid methotrexate concentrations were consistently higher than serum concentrations. The increase in the elimination half-life in the presence of a pleural effusion (or any third-space reservoir) is a result of an increase in the volume of distribution of the drug.

#### Toxicity

Adverse reactions from methotrexate therapy are those associated with the effect of methotrexate on rapidly proliferating tissue. The principal sites of methotrexate toxicity are the bone marrow and the gastrointestinal tract. Myelosuppression, ulcerative stomatitis, and nausea and vomiting are the most common adverse reactions associated

with methotrexate. The occurrence of other symptoms of toxicity is dependent on the dosing regimen administered.

Gastrointestinal toxicity initially manifests itself with the development of buccal lesions which may become ulcers (Trier 1961). Oliff et al. (1979) studied ten patients receiving infusions of either 2760 or 5520 mg/m<sup>2</sup> of methotrexate with citrovorum factor rescue and found that salivary methotrexate concentrations did not correlate with the severity of oral mucositis. Salivary concentrations were 1-2% of the simultaneous plasma levels. The occurrence of diarrhea is associated with severe methotrexate toxicity as well as the development of extensive areas of denudation of the villus epithelium in the small intestine and morphologic mucosal ulceration along the lower gastrointestinal tract (Trier 1961). The occurrence of ulcerative stomatitis has been considered to be a sign of impending myelosuppression (Jaffe and Traggis 1975).

The hematologic effect of methotrexate was studied in 4 cancer patients who received 15 to 25 mg of methotrexate intramuscularly daily for 5 to 7 days (Speck et al. 1967). A moderate decrease in the cellularity of the bone marrow occurred during treatment. Cellular changes in the bone marrow included a severe depletion of the red-cell precursors and early granulocytes (myeloblasts, progranulocytes, and myelocytes), although there was an increase in late granulocytes (metamyelocytes and segmented neutrophils). An increase in the granulocyte-erythrocyte ratio was found suggesting that erythropoiesis was depressed more than granulopoiesis. Changes in the peripheral blood

were also noted. A reduction in the leukocyte count occurred in all of the patients and the hemoglobin concentration decreased slightly. Reticulocytopenia also occurred during therapy. The hematologic parameters of each patient returned to normal within a month following the cessation of therapy. The average nadirs for reticulocytes, leukocytes, and platelets were 4.6, 6.2, and 9.3 days, respectively, in ten patients who received single intravenous methotrexate doses of 6 to 16 mg/kg (Condit 1960). These values correspond to the life span of these cells indicating that methotrexate arrests the production or release of these cells from the bone marrow.

Chabner and Young (1973) studied the suppression of DNA synthesis by methotrexate in normal and tumor tissues of mice. They concluded that there are certain time-dependent and concentration-dependent relationships with regard to methotrexate toxicity. There is a critical minimal extracellular level for each target organ that must be exceeded before toxicity to that organ will occur. Also, there is a minimal duration of time that the tissue must be exposed to the critical methotrexate concentration before toxicity will occur. The critical extracellular concentration and minimum duration of exposure necessary for the occurrence of toxicity depend on the organ involved. For bone marrow and gastrointestinal epithelium, these values are  $2 \times 10^{-8}$  moles/liter and 42 hours, respectively (Young and Chabner 1973). Finally, the severity of toxicity depends primarily on the duration of exposure of the tissue to methotrexate. This finding is supported by Pitman et al. (1975) who found that there was a higher incidence of

myelosuppression if the plasma concentration of methotrexate was greater than  $1 \times 10^{-7}$  moles/liter 72 hours following a large dose of methotrexate. The development of these principles was an important step for the prevention of potential toxicity. Plasma levels are now being used to predict the occurrence of methotrexate toxicity (Stoller et al. 1977).

#### Antitumor Activity

Methotrexate has a broad spectrum of antineoplastic activity. Initial success with methotrexate occurred in the treatment of childhood leukemia. Methotrexate was able to induce complete remissions of acute leukemia with a median duration of 20 weeks, although resistance to therapy eventually developed and relapse occurred (Frei et al. 1961). This led to research to determine how to maintain remission with methotrexate and other agents. The Acute Leukemia Group B (1965) found that  $30 \text{ mg/m}^2$  of methotrexate administered intramuscularly twice a week was more effective than  $3 \text{ mg/m}^2$  given orally on a daily regimen for the maintenance of acute childhood leukemia. A later study by the Acute Leukemia Group B (1969) showed that a twice a week methotrexate dose of  $30 \text{ mg/m}^2$  administered orally maintained remissions as well as the same dose given intramuscularly. The median duration of remission was 10.5 months. Methotrexate was shown to be ineffective in the treatment of acute myelocytic leukemia in the adult (Frei et al. 1961). Complete or partial remission was attained in less than 10% of the patients studied.

Methotrexate is also effective in treating a major complication of acute leukemia, meningeal leukemia. Since methotrexate does not attain therapeutic concentrations in the cerebrospinal fluid with conventional doses given systemically, it must be given directly into the cerebrospinal fluid (Mellett 1977). Intrathecal methotrexate, administered in doses of 10 to 15 mg/m<sup>2</sup> every 2 to 5 days can result in complete remission of the disease (Rieselbach 1963). Bleyer and Dedrick (1977) suggest dosing on age-related standards which take into consideration the volume of the cerebrospinal fluid and the weight of the brain and the spinal cord.

Dramatic responses to methotrexate in patients with trophoblastic tumors were found in studies by Li et al. (1956). Hertz et al. (1961) reported their experiences with methotrexate therapy in trophoblastic disease. Methotrexate was administered intramuscularly in doses of 15 to 30 mg daily for five days and courses were repeated when toxicity had resolved. Complete remission was achieved in 48% of the patients with an average of five courses of therapy. Remission duration ranged from six months to over five years.

Methotrexate, in combination with cyclophosphamide and 5-fluorouracil (CMF regimen), has been used with success in the adjuvant treatment of breast cancer (Bonadonna et al. 1977). Treatment failure was compared in 207 patients who had a radical mastectomy and 12 monthly courses of CMF versus 179 patients whose primary therapy was only a radical mastectomy. Overall survival at 36 months was higher in the

mastectomy plus CMF group than in the mastectomy group (89.6% to 78.6%). The dose of methotrexate in the CMF regimen is 40 mg/m<sup>2</sup> on days 1 and 8.

Methotrexate has also been used since the early 1960's as standard therapy for advanced squamous cell carcinoma of the head and neck. An overall response rate of 63% was obtained with weekly intravenous doses of 60 mg/m<sup>2</sup> (Leone et al. 1968). With the advent of high-dose methotrexate, studies have been conducted comparing the efficacy of low-dose versus high-dose therapy. Woods et al. (1981) randomized 72 patients to receive weekly intravenous methotrexate doses of 50 mg/m<sup>2</sup>, 500 mg/m<sup>2</sup>, or 5 g/m<sup>2</sup>. Overall, a 45% response was obtained for the high dose, 26% for the medium dose, and 26% for the low dose. The higher response with the high methotrexate dose was offset by increased toxicity produced by this dose. There was no significant difference in survival among the three groups. DeConti and Schoenfeld (1981) conducted a prospective clinical trial involving 259 patients to evaluate three treatment programs, 1) weekly methotrexate (40 mg/m<sup>2</sup>), 2) biweekly methotrexate (240 mg/m<sup>2</sup>) with leucovorin rescue, and 3) biweekly methotrexate (240 mg/m<sup>2</sup>) with leucovorin rescue in combination with cyclophosphamide (500 mg/m<sup>2</sup>) and cytosine arabinoside (300 mg/m<sup>2</sup>). The overall response for each group was 26%, 24%, and 18%, respectively. The weekly low-dose methotrexate group had a median duration of response of 105 days compared to 42 and 49 days for the other groups. Survival was also better in the low-dose methotrexate group. These studies suggest the therapeutic equivalency, if not superiority, of weekly

low-dose methotrexate therapy compared to high-dose methotrexate treatment.

High-dose methotrexate, doses in excess of 20 mg/kg or 1 gram/m<sup>2</sup>, is being used experimentally to treat several types of neoplasms with variable success. Examples are osteogenic sarcoma, refractory acute leukemia, bronchogenic carcinoma, and head and neck cancer. Currently, high-dose therapy is only indicated in the adjuvant treatment of osteogenic sarcoma and the treatment of metastatic osteogenic sarcoma (Chabner and Slavik 1975).

#### Analytical Methods

Several pharmacokinetic studies of methotrexate have been conducted since its introduction. The results of these, however, have been conflicting. Differences in the sensitivity and specificity of the method used to measure methotrexate in body fluids can partially explain these conflicting results. The first analytical method used was a microbial assay, with Streptococcus faecalis as the test organism (Burchenal et al. 1951). A drawback of this method was that the drug available for use at that time contained 20 to 30% impurities. Some of these impure compounds both supported and inhibited the growth of S. faecalis. Also, the assay procedure was very time-consuming.

Freeman (1957) introduced the fluorometric method as a more specific method of measuring methotrexate in the plasma. The increase in fluorescence before and after a plasma sample was oxidized served as the basis for calculation of the methotrexate concentration. She reported a sensitivity of 5 ng/ml with this method. Fasting blank

plasma samples did not show an increase in fluorescence upon oxidation, and chromatographic analysis found that the oxidizable fluorescence in plasma after administration of the drug is methotrexate. From these results, it was concluded that the assay was specific for methotrexate. However, folic acid and other analogues undergo the same reaction, therefore they are also measured. Freeman's method was modified by Chakrabarti and Bernstein (1969) who measured the fluorescence after the plasma sample was oxidized. They found that the reproducibility of the assay was difficult below a concentration of 20 ng/ml.

The theory that methotrexate binds dihydrofolate reductase stoichiometrically stimulated the enzymological method for measuring methotrexate in biological fluids (Werkheiser et al. 1962). The colorimetric determination of tetrahydrofolic acid which indicates the degree of enzyme inhibition by methotrexate is the basis for this method. The sensitivity of this assay in serum was found to be 10 ng/ml. The reproducibility error was 10%. This method was determined to be specific and free from interference by the presence of growth factors (folic acid, folinic acid, thymine). A study comparing the fluorometric method with the enzymological method found that the enzymological method was the preferred procedure because of its greater sensitivity and specificity (Overdijk et al. 1975).

A radioimmunoassay procedure for methotrexate was developed in the early 1970's by Levine and Powers (1974). They reported that the sensitivity of this method was 0.1 ng/ml. The method was found to be specific for methotrexate because  $10^5$  or  $10^6$  times more folic acid,

tetrahydrofolic acid, or folinic acid were required to compete effectively with methotrexate for the antibodies. However, cross reaction tests with metabolites of methotrexate have not been performed. Intra-assay precision was reported to be 16% and interassay precision was 21% when measuring a test sample containing 5 ng of methotrexate (Bohuon et al. 1974). A disadvantage of this radioimmunoassay procedure was that it took 3 days to analyze a sample. A procedure requiring only 20 hours to perform was developed by Hendel and associates (1976). The intraassay coefficient of variation for this procedure was less than 1% and the interassay coefficient of variation was approximately 10%.

High-pressure liquid chromatography was introduced as a rapid and simple method to provide more specificity and equivalent sensitivity as compared to other analytical methods. The standard curve was reported to be linear down to a concentration of 45 ng/ml with an interassay coefficient of variation of 3% (Watson et al. 1978). This method was found to be specific for the parent compound.

The most recently developed method of measuring methotrexate in biological fluids is the "homogenous" enzyme immunoassay technique which was first applied to morphine (Rubenstein et al. 1972). The principle of this method involves the competition between methotrexate in a plasma sample and enzyme-labeled methotrexate for an anti-methotrexate antibody. Binding between the enzyme-labeled drug and the antibody renders the enzyme, glucose-6-phosphate dehydrogenase, inactive. Methotrexate concentrations are determined by the relative

change in absorbance due to the inhibition of the action of the enzyme and NAD on the substrate, glucose-6-phosphate. The standard curve is linear down to a concentration of 135 ng/ml (DePorceri-Morton et al. 1978). The value for intraassay and interassay precision was reported to be approximately 5%. However, Finley et al. (1980) have modified the procedure to increase the sensitivity to 5 ng/ml. The assay is specific to methotrexate and physiological concentrations of folic acid, folinic acid, or 7-hydroxymethotrexate do not interfere. Intraassay precision is 6.4% and interassay precision is 7.2% for this method. Comparative studies by DePorceri-Morton et al. (1978) found that the enzyme immunoassay technique correlated very highly with the enzyme inhibition method. A slightly lower correlation was obtained with the radioimmunoassay procedure which was explained to have occurred because there is a 7% crossreactivity with 7-hydroxymethotrexate for the radioimmunoassay procedure.

As mentioned previously, Finley et al. (1980) modified the "homogenous" enzyme immunoassay procedure to obtain better sensitivity with the same specificity. They reported the within-day coefficient of variation to be approximately 7%. This method was compared to the radioimmunoassay method and the two techniques were found to correlate very highly. The advantage of this method is that it is sensitive, specific, does not involve any extraction procedures, and is rapid and easy to perform.

### Methotrexate Impurities

Another reason for misleading results may be related to the purity of the methotrexate used in the study. Dosage forms of methotrexate may contain up to 15% impurities (United States Pharmacopoeia XX). The amount of impurities depends on the particular manufacturing process. The major impurities in commercial methotrexate are N<sup>10</sup>-methylpteroylglutamic acid and 2,4-diamino-N<sup>10</sup>-methylpteronic acid (Chatterji et al. 1978).

### Summary

Throughout the period of time that pharmacokinetic studies of methotrexate have been performed, reports of the disposition parameters and absorption of methotrexate from the intramuscular and oral routes have been conflicting. The primary reason for these discrepancies include differences between investigations in 1) assay procedure, 2) sampling times, and 3) length of sample collection. The design of studies that have attempted to assess the bioavailability of methotrexate from the oral and intramuscular route is questionable. Of the studies reported in the literature, there are not any in which each patient served as his own control; receiving methotrexate by the intravenous, intramuscular, and oral routes of administration. Also, in each of the studies performed to determine the absorption of methotrexate from the gastrointestinal tract, methotrexate has been administered as a solution through a nasogastric tube. There has not been an attempt to measure the bioavailability from commercial tablets.

## CHAPTER 3

### DESIGN OF STUDY

#### Introduction

In order to investigate the bioavailability and pharmacokinetics of methotrexate in man, approval for the investigation was obtained from the Human Subjects Ethical Review Committee at the University of Arizona, and the Research and Development Committee of the Tucson Veterans Administration Medical Center. After an explanation of the investigation, informed consent was obtained from each patient prior to their participation (see Appendix A). The parenteral and oral dosage forms of methotrexate used in this investigation are commercially available, therefore approval for their use by the Food and Drug Administration was unnecessary.

#### Methodology

All patients who participated in this investigation were adults with malignant neoplastic disease who were to receive methotrexate as part of standard care for their disease. The performance status of each patient was rated at a minimum of 6 on the Karnofsky Performance Scale (Appendix B) by their oncologist. The patients were judged to be in good physical condition despite their disease and with no renal or hepatic function abnormalities as determined by appropriate blood biochemistries. Table 1 lists the laboratory analyses that were conducted.

Table 1. Laboratory blood analyses.

WEEK ONE	WEEKS TWO AND THREE
COMPLETE BLOOD COUNT	COMPLETE BLOOD COUNT
White cell count	PLATELET COUNT
Red cell count	RENAL BATTERY
Hemoglobin	ALBUMIN-GLOBULIN RATIO
Hematocrit	
Mean corpuscular volume	
Mean corpuscular hemoglobin	
Mean corpuscular hemoglobin concentration	
PLATELET COUNT	
RENAL BATTERY	
Sodium	
Potassium	
Chloride	
CO <sub>2</sub> content	
Glucose	
Creatinine	
Urea Nitrogen	
LIVER PANEL	
Total bilirubin	
Direct bilirubin	
Serum glutamic oxaloacetic transaminase	
Alkaline phosphatase	
ALBUMIN-GLOBULIN RATIO	
Serum protein	
Serum albumin	
Albumin-globulin ratio	

Each of the six patients studied received a  $30 \text{ mg/m}^2$  dose of methotrexate by three different routes of administration: intravenously, intramuscularly, and orally (in tablet form). Methotrexate 2.5 mg tablets by Lederle, lot number 648-570, were used for the oral doses. Methotrexate sodium (25 mg methotrexate base/ml) for injection by Lederle, lot number 634-318, was used for the intravenous and intramuscular doses. Patients were sequentially entered into regimens which had been randomized by factorial design so that no two patients completed the three portions of the study in the same sequence (Table 2).

Each patient received their methotrexate dose one week apart for three consecutive weeks. Immediately prior to each administration of methotrexate, a heparin lock was placed in a hand or arm vein. The lock was flushed with 1 ml of a 50 unit/ml heparin solution after each sample was drawn. The heparin solution was withdrawn from the heparin lock tubing immediately before the blood sample was collected in order to avoid diluting the sample. Each patient was required to fast for 8 hours before and 4 hours after administration of each dose by the intravenous, intramuscular, and oral routes. The blood sample collection times for each route of administration were chosen in a manner that would permit the determination of the peak plasma methotrexate concentration and the total area under the plasma concentration versus time curve for a time period of at least three times the elimination half-life of methotrexate.

In the intravenous portion of the study, the dose of methotrexate sodium was administered by intravenous push over one minute

**Table 2. Methotrexate administration sequences.**

Patient number	Sequence*
1	PO-IV-IM
2	IV-PO-IM
3	PO-IM-IV
4	IV-IM-PO
5	IM-IV-PO
6	IM-PO-IV

\* IV: Intravenous  
IM: Intramuscular  
PO: Oral

followed by 10 ml of normal saline. Blood samples were obtained immediately prior to the dose and at the following times after the dose: 5, 15, 30, and 45 minutes; 1, 1.5, 2, 4, 6, 8, 12, and 24 hours. Blood samples (5 ml) for the first twelve hours were obtained through the heparin lock. After twelve hours, all samples were obtained by venipuncture and were collected in vacutainer tubes with anticoagulant. All samples were centrifuged immediately following collection. The plasma fraction was removed and kept frozen until assayed.

For the intramuscular portion of the investigation, the dose of methotrexate sodium was administered into the gluteal muscle. Blood samples were collected in the manner previously described, immediately prior to administration and at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, and 24 hours after the dose.

In the oral portion of the study, the methotrexate dose was given as whole tablets with 8 ounces of water. Blood samples were collected in the same manner described before, immediately prior to administration and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours after the dose. In all cases, the exact time of each collection was noted on both the collection tube and the data collection sheet.

All plasma samples were assayed for methotrexate concentration by the Clinical Laboratory at the Arizona Health Sciences Center under the direction of Paul Finley, M.D., by the homogenous enzyme immunoassay technique as modified by Finley et al. (1980) and using a centrifugal analyzer (COBAS BIO, Serial #18-2028). The commercially

available assay kit (EMIT<sup>®</sup>; Syva Corporation, Palo Alto, California) was used in the analysis. Reagent A (antibodies against methotrexate, glucose-6-phosphate, NAD) and Reagent B (glucose-6-phosphate dehydrogenase, chemically coupled to methotrexate) were each reconstituted with 3.0 ml of distilled water and stored at 25°C for one hour prior to use. Working Reagent A was prepared by diluting 1.0 ml of Reagent A with 9.0 ml of buffer solution. The buffer (0.055M Tris-HCl, pH 8.0) was prepared by diluting the buffer concentrate provided with the kit to 150 ml with distilled water.

Five standards, with concentrations of 0, 90, 225, 450, and 675 ng/ml were prepared by adding 1.0 ml of distilled water to the lyophilized powder provided with the assay kit to attain these concentrations. A sixth standard, with a concentration of 45 ng/ml, was prepared by adding 0.9 ml of a 5% albumin solution to 0.1 ml of the 450 ng/ml standard. Controls of 350 ng/ml and 454,000 ng/ml were prepared by adding 3.0 ml of distilled water to the lyophilized powder provided with the assay kit. A control of 150 ng/ml was prepared by adding 9.9 ml of a 5% albumin solution to 0.1 ml of the 454,000 ng/ml control, then adding 0.1 ml of this dilution to 2.9 ml of a 5% albumin solution. The 150 ng/ml and 350 ng/ml standards were run as controls in the assay. Patient samples that yielded concentrations above the range of the standard curve were diluted with a 5% albumin solution and reanalyzed.

The sample cups were loaded as follows: cup 1, water; cups 2-35, standards, controls, and patient samples. The automatic

sampler-diluter was set as follows: serum/standard pump, 7  $\mu$ L; flush pump buffer, 15  $\mu$ L; reagent volume, 170  $\mu$ L; and start reagent volume, 17  $\mu$ L. Automatically, the sample, buffer flush, and Reagent A were delivered to the larger middle well of the transfer disc. Then Reagent B was delivered to the small inner well of the transfer disc.

The transfer disc was placed on the centrifugal analyzer and the assay was begun immediately. The initial absorbance reading was taken at 5 seconds and the final reading 20 seconds later. Analyses were made at 30°C. The spectrophotometer was set at a wavelength of 340 nm to read the increase in absorbance of NADH.

The delta absorbances of the standards (except the 0 standard) were automatically entered in the computer (Hewlett-Packard 85) which calculated a cubic least-squares regression curve. The delta absorbances of the unknown samples were entered and the computer calculated the methotrexate concentration from the stored curve.

#### Data Analysis

The data obtained from the plasma concentration (C) versus time (t) profile of methotrexate following intravenous, intramuscular, and oral doses were analyzed by a "model-independent" method to obtain pharmacokinetic parameters. Assumptions of this method are that the data must be from a mammillary system describable by first-order processes with elimination from a central compartment (Benet and Galeazzi 1979).

Initially, the intravenous methotrexate data in the post-distribution phase of the plasma concentration-time plot were fit,

using linear regression, to the equation,

$$\ln C = \ln C^0 - \lambda_n t \quad (1)$$

where  $C^0$  is the extrapolated zero-time concentration expressed as ng/ml and  $\lambda_n$  is the terminal disposition rate constant in  $\text{hr}^{-1}$ . The half-life ( $t_{1/2}$ ) was determined using the relationship,

$$t_{1/2} = \frac{0.693}{\lambda_n} \quad (2)$$

To obtain total body clearance ( $Cl_g$ ) for methotrexate, it was necessary to obtain the total area under the plasma concentration versus time curve from time zero to infinity (AUC). The area from time zero to the first concentration-time point in the post-distribution phase was calculated using the trapezoidal rule as follows,

$$AUC_{t_1}^{t_2} = (t_2 - t_1) (C_2 + C_1)/2 \quad (3)$$

where  $AUC_{t_1}^{t_2}$  is the area under the curve between two consecutive concentration-time points,  $C_2 \cdot t_2$  and  $C_1 \cdot t_1$ . The area under the curve from the first concentration-time point in the post-distributive phase ( $C_t$ ) to time infinity ( $AUC_t^\infty$ ) was calculated from the equation,

$$AUC_t^\infty = \frac{C_t}{\lambda_n} \quad (4)$$

The concentration used for  $C_t$  was the theoretical value obtained from the linear regression equation, not the observed concentration. AUC was then obtained using the equation,

$$AUC = AUC_0^t + AUC_t^\infty \quad (5)$$

With the calculation of AUC, the volume of distribution in the terminal log-linear phase ( $V_\lambda$ ) and  $Cl_g$  were obtained from the following equations,

$$Cl_s = \text{Dose}/AUC \quad (6)$$

$$V = Cl_s/\lambda_n \quad (7)$$

The volume of distribution at steady-state ( $V_{ss}$ ) was calculated using the equation,

$$V_{ss} = \text{Dose} \frac{AUMC}{AUC^2} \quad (8)$$

where AUMC is the total area under the curve of the product of time and plasma concentration versus time from time zero to infinity (area under the first moment of the plasma curve). The area from time zero to the first time point in the post-distributive phase was calculated using the trapezoidal rule as follows,

$$AUMC_{t_1}^{t_2} = (t_2 - t_1) [(Ct)_2 + (Ct)_1]/2 \quad (9)$$

where  $AUMC_{t_1}^{t_2}$  is the area under the curve between two consecutive  $Ct \cdot t$  points,  $(Ct)_2 \cdot t_2$  and  $(Ct)_1 \cdot t_1$ . The area under the first moment of the plasma curve from the first time point in the post-distributive phase ( $tC_t$ ) to time infinity ( $AUMC_t^\infty$ ) was obtained from the equation,

$$AUMC_t^\infty = \frac{tC_t}{\lambda_n} + \frac{C_t}{\lambda_n^2} \quad (10)$$

where  $C_t$  is as defined previously. AUMC was calculated using the following equation,

$$AUMC = AUMC_0^t + AUMC_t^\infty \quad (11)$$

The total area under the plasma concentration versus time curve after intramuscular and oral administration of methotrexate was obtained in the same manner as for the intravenous data. The data in the post-absorptive, post-distribution portion of the plasma concentration-time curve were fit using linear regression from which the parameters,  $\lambda_n$

and  $t_{1/2}$ , were obtained. Area under the curve calculations for quantitating the extent of absorption were accomplished by determining the area from time zero to the first concentration-time point in the post-absorptive, post-distributive phase using the trapezoidal rule, and adding the area from this concentration-time point to time infinity calculated from equation (4).

The absolute intramuscular and oral bioavailability of methotrexate (F) was calculated by the following equations,

$$F = \frac{(AUC)_{IM}}{(AUC)_{IV}} \times \frac{X^{\circ}_{IV}}{X^{\circ}_{IM}} \quad (12)$$

and

$$F = \frac{(AUC)_{PO}}{(AUC)_{IV}} \times \frac{X^{\circ}_{IV}}{X^{\circ}_{PO}} \quad (13)$$

where  $X^{\circ}$  is the dose of methotrexate administered and the subscripts refer to the intravenous (IV), intramuscular (IM), and oral (PO) values.

### Statistics

The elimination half-lives and areas under the plasma concentration versus time curve after the intravenous, intramuscular, and oral administration of methotrexate were analyzed by analysis of variance, repeated measures design (Myers 1976). Each of the analyses of variance were performed by the Statistical Package for the Social Sciences (SPSS), RELIABILITY subprogram (Hull and Nie 1979). The maximum plasma concentration and the time to maximum plasma concentration after the intramuscular and oral administration of methotrexate were analyzed by

the paired t-test method. This technique is equivalent to analysis of variance with repeated measures in which there are only two treatments for each subject. The paired t-test analyses were performed by the SPSS subprogram, T-TEST (Nie et al. 1975).

Analysis of variance (ANOVA) is a statistical technique which partitions the total observed variation for experimental results into two components. First is the variation inherent within the individual being tested. This kind of variance is also known as error variance. The second component of variation is that which is attributed to the influence of differential treatments. The ratio of the treatment variation to the error variation is known as the "F statistic". The calculated F statistic is compared to tabulated values of F with regard to the appropriate degrees of freedom. The F distribution provides an estimate of the mathematical probability that the calculated F statistic has occurred by chance and not as a result of actual differences due to the experimental treatments applied. The F distribution is based on the following assumptions (Minium 1978):

1. The population of scores is normally distributed.
2. The treatment variation and error variation for the sample selected is the same as these variations in the population.
3. The scores for each subject tested represent a random sample of scores from the population.
4. The scores that are being tested for differences are the result of independent observations.

The assumption of independent observations cannot be met in an experimental design in which subjects are tested repetitively under different treatment conditions. This is the case with this study. In this type of study design, analysis of variance with repeated measures is the appropriate test for detecting significant differences (Winer 1978).

The repeated measures ANOVA partitions total observed variation into variation between all subjects under a given treatment condition and variation within a given subject across all treatment conditions. This variation within subjects is then partitioned into variation which results from the different treatments (i.e., treatment effect) and the variation which a given subject inherently exhibits between repeated treatments regardless of the treatment applied. This last source of variation is referred to as residual variation. The residual variation can be partitioned further into a nonadditivity component which represents the variation that is due to an interaction of subject and treatment level and the balance component which constitutes the remainder of the residual variation. The partitioning of these variations is diagrammed in Figure 4. Under the conditions of repeated measures ANOVA, the F statistic becomes the ratio of the treatment variation to the residual variation (Myers 1976). Because of the computation of the residual variation, it is possible to compute an F statistic which estimates the variation due to differential treatment for a subject or group of subjects when repetitively tested under all treatment conditions. Thus the assumption of independent observations which is necessary for elementary ANOVA is not required for repeated measures

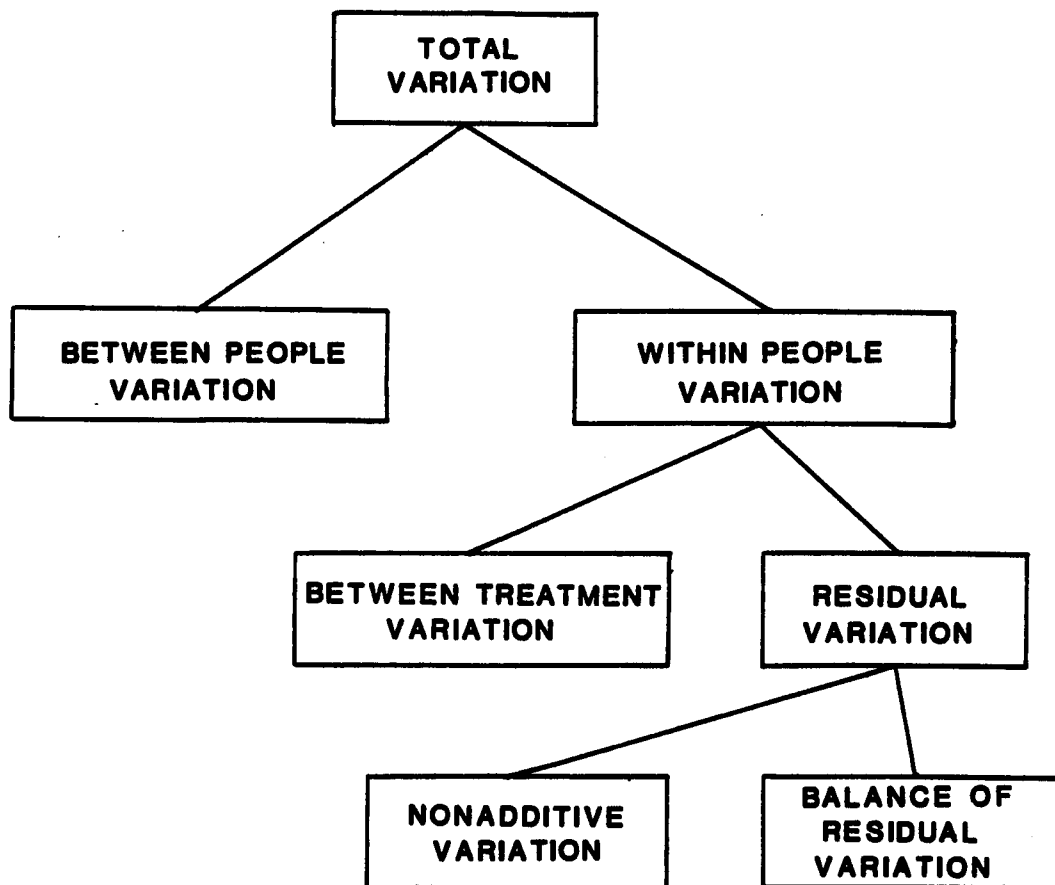


Figure 4. Partitioning of total observed variation under repeated measures analysis of variance.

ANOVA. A probability level of .05 was considered necessary to demonstrate an appropriate level of statistical significance for each repeated measures ANOVA.

With repeated measures ANOVA there are two models to be considered, the additive model and the nonadditive model. The difference between these two models is the nonadditive model includes an interaction term which represents the contribution made to a measurement due to an interaction between the subject and treatment level under observation. Nonadditive data which is treated as additive would result in a less efficient F test of the treatment effect or possibly, a biased F test, therefore it is important to know which model the data fits. In order to determine whether a data set is additive or nonadditive Tukey proposed a single degree of freedom test for additivity in which the null hypothesis being tested is that the interaction variance is zero. The F statistic is the ratio of the nonadditive variation to the balance variation. A significant F statistic implies that the data is nonadditive. Tukey's test for additivity, performed by the SPSS subprogram RELIABILITY, was applied to the elimination half-life and area under the plasma concentration versus time curve data at a probability level of .01.

Whenever significant differences among treatments were detected by repeated measures ANOVA, the a posteriori test of Scheffe was employed to determine where specific differences existed (Myers 1976). Of the several a posteriori tests available, the Scheffe is the most powerful test for complex contrast involving more than two treatment

means as is the case with this study. The Scheffe procedure is also the most conservative with respect to committing a Type I error (rejecting a true null hypothesis). This method will lead to the smallest number of significant differences, a reasonable approach to clinical research in which one wants to minimize the possibility that observed differences between treatments occurred by chance alone. The Scheffe method was applied in this study, with a probability of .05 being the level required to demonstrate statistical significance.

## CHAPTER 4

### RESULTS AND DISCUSSION

Data collection for this study began with the first patient on August 4, 1981 and was completed on May 21, 1982. Each course of chemotherapy was administered in the Hematology-Oncology outpatient clinic at the Arizona Health Sciences Center except for one patient who was treated while an inpatient at the Tucson Veterans Administration Medical Center.

#### Patients

A total of six patients completed the three courses of treatment with methotrexate. Each of these patients met the previously stated inclusion criteria. All of the patients were to undergo methotrexate therapy for advanced squamous cell carcinoma of the head and neck. A summary of their characteristics is found in Table 3.

The average age of the patients was 63 years and ranged from 57 years to 76 years of age. Their average calculated creatinine clearance was 70 ml/min and ranged from 61 ml/min to 91 ml/min.

Each patient obeyed the fasting requirement required for each course of therapy. Because of the anatomical restrictions caused by their disease, most of the patients in this study were on a liquid or pureed diet, with the main source of calories being from a dietary

**Table 3. Summary of patient characteristics.**

Patient	Sex	Age (years)	Weight (kg)	Body Surface Area (m <sup>2</sup> )	Karnofsky Score	Mean Creatinine Clearance* (ml/min)	Tumor Type	Prior Therapy
1	M	57	47.4	1.50	8	62	Squamous cell carcinoma of parotid gland	Surgery Radiation Cis-platinum Bleomycin
2	F	68	56.8	1.33	8	63	Squamous cell carcinoma of buccal mucosa	Radiation Surgery Vinblastine 5-Fluorouracil Bleomycin
3	M	59	47.1	1.50	6	70	Squamous cell carcinoma of middle esophagus	Surgery Radiation
4	M	59	54.7	1.57	7	74	Squamous cell carcinoma of oropharynx	Bleomycin Cis-platinum
5	M	76	55.0	1.64	7	61	Squamous cell carcinoma of tongue	Bleomycin Cis-platinum Radiation
6	M	59	67.3	1.80	9	91	Squamous cell carcinoma of epiglottis	Surgery Radiation

\* Creatinine  
clearance =  $\frac{\text{Weight (144 - Age)}}{71 \times \text{serum creatinine}}$   
(males)

Creatinine  
clearance =  $0.85 \left[ \frac{\text{Weight (144 - Age)}}{71 \times \text{serum creatinine}} \right]$   
(females)

supplement such as Isocal® or Sustacal®. Patient 6, however, was able to consume a regular diet.

All of the patients were taking one analgesic on an "as needed" basis to control their pain during the course of the study. These analgesics were acetaminophen, acetaminophen with codeine, and acetaminophen with oxycodone. Patient 2 was taking diazepam infrequently for anxiety. Patient 6 was taking ampicillin for a wound infection but it was discontinued prior to each course of therapy. Patient 3 was receiving digoxin for an atrial arrhythmia which developed due to the metastasis of his disease. No other medications were known to have been consumed by any of the patients.

#### Methotrexate Assay

Plasma sample analyses to determine methotrexate concentration were performed in the Clinical Laboratory at the Arizona Health Sciences Center by technician Jane Williams, under the direction of Paul R. Finley, M.D. The samples were submitted to the laboratory in single patient groups. Each plasma sample was numbered randomly in order to eliminate the potential for technician bias.

A standard curve, which plotted the delta absorbance rate versus concentration of the calibrators, was calculated for each run. The methotrexate concentration of the calibrators ranged from 0 ng/ml to 675 ng/ml. Figure 5 shows an example of a standard curve. The standard deviation of each standard curve was calculated and these values are summarized in tabular form in Table 4. These low standard deviation values indicate that a very small portion of the difference between

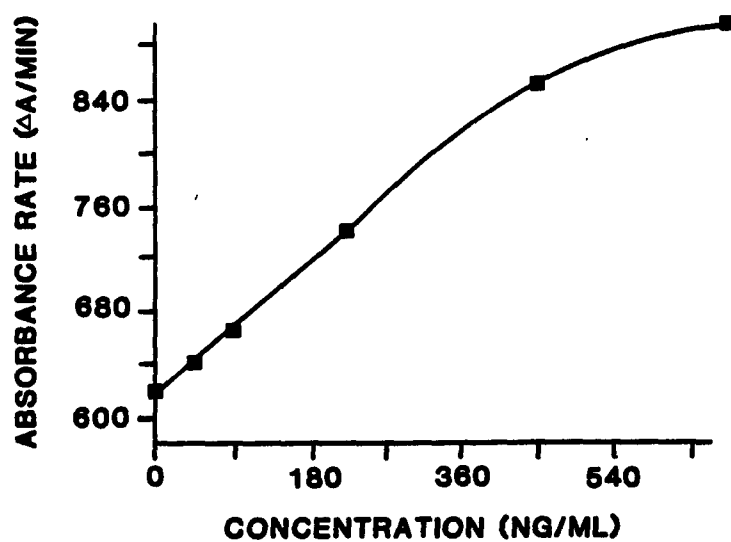


Figure 5. Example of a standard curve for methotrexate performed on the COBAS BIO (Patient 1, Curve 2).

Table 4. Standard deviations for the methotrexate standard curves.

Date	Group	Average Standard Deviation* ( $\Delta A/\text{min}$ )**
9-28-81	Patient 1	5.11 0.05
10-20-81	Patient 2	3.85 0.74 1.63
1-28-82	Patient 3	1.05 4.93
2-16-82	Patient 4	0.38 0.61 5.19
3-29-82	Patient 5	3.50 3.13
5-24-82	Patient 6	0.62
4-27-82	Reanalysis	2.45 1.70 0.72

\* These values represent the standard deviations of each standard curve that was run

\*\* Equals the change in absorbance per minute

the observed and actual methotrexate concentration of the calibrators was due to error variance of the assay procedure. This implies that the values obtained for methotrexate plasma concentrations were reliable and accurate. The lowest methotrexate concentration that could be measured accurately and precisely was 45 ng/ml.

#### Intravenous Methotrexate

Plasma methotrexate concentration versus time data were analyzed by a "model-independent" method to obtain the pharmacokinetic parameters. Individual patient plasma methotrexate concentration versus time data are found in Appendix C. Graphical displays of these data including the line of best fit derived from a linear regression of points in the post-absorptive, post-distributive phase are shown in Appendix D. The pharmacokinetic disposition parameters of methotrexate following the intravenous administration of a 30 mg/m<sup>2</sup> dose are listed in Table 5.

The data for Patient 4 from the intravenous course of therapy was excluded from statistical analysis. Although the disposition rate constants after intramuscular and oral administration were in close agreement, the intravenous value differed from these values by three-fold. Also, the disposition rate constant obtained for this patient following intravenous administration differed tremendously from the intravenous values obtained for the other patients. To our knowledge, this patient was not taking any drugs that would inhibit the renal elimination of methotrexate and his dietary intake was similar to that

**Table 5. Pharmacokinetic parameters of methotrexate following the intravenous administration of methotrexate.**

Patient	$Cl_s$ (ml/min)	$V_{ss}$ (L/kg)	$V_\lambda$ (L/kg)	$\lambda$ (hr <sup>-1</sup> )	$t_{1/2}$ (hr)
1	75	0.60	0.68	0.1406	4.93
2	114	0.28	0.32	0.3754	1.85
3	133	0.77	0.99	0.1768	3.92
4	58*	0.88*	1.05*	0.0610*	11.36*
5	123	0.56	0.72	0.1841	3.76
6	175	0.58	0.76	0.2054	3.37
MEAN	124	0.56	0.69	0.2165	
$\pm$ S.D.	$\pm 36$	$\pm 0.18$	$\pm 0.24$		

\* Not used in the calculation of the mean and standard deviation

of the other patients. His renal function was within the range of the other patients also. Plasma samples from the intravenous course of therapy were submitted to the laboratory for reanalysis and the plasma concentration values which resulted were equivalent to those obtained after the first analysis.

The mean volume of distribution at steady-state ( $V_{ss}$ ) was calculated by equation (8) to be  $0.56 \pm 0.18$  L/kg (mean  $\pm$  S.D.) while the mean volume of distribution in the terminal elimination phase ( $V_{\lambda}$ ) was  $0.69 \pm 0.24$  L/kg. Our values for  $V_{ss}$  ranged from 15.9 L to 38.7 L with a mean of 29.9 L. Huffman et al. (1973) reported  $V_{ss}$  to be  $73.3 \pm 15.6$  L following the intravenous administration of 30 mg/m<sup>2</sup> of methotrexate to 22 patients. Although Huffman and his associates did not indicate the body weight of their patients, it is readily apparent that a difference exists. A partial explanation for this difference may be that the body weights of the subjects in Huffman's investigation were higher than those in our study. The mean volume of distribution in the terminal elimination phase was found to be 76% (i.e., 0.76 L/kg) by Henderson et al. (1965) following the intravenous administration of a 0.1 to 10 mg/kg dose to 5 patients. This value was obtained by dividing the dose by the plasma concentration at time zero extrapolated from the line which best fits the points in the terminal elimination phase ( $C^{\circ}$ ). Henderson's value is in close agreement with our value for  $V_{\lambda}$ , however the value obtained by Henderson and coworkers may actually be an overestimation of the true volume of distribution based on their method of calculation which gives an estimate of  $V_{\lambda}$  for a one-compartmental model,

a model which is characterized by instantaneous distribution of the drug into tissues to which it is accessible. The overestimation occurs because the one-compartment equation does not account for the non-instantaneous distribution of the drug.

The mean plasma clearance of methotrexate was found to be  $124 \pm 36$  ml/min. The values reported after similar intravenous doses by Lawrence et al. (1980) and Huffman et al. (1973) were values of renal clearance, not plasma clearance, which partially explains why our value is higher because renal clearance only accounts for 80-90% of the plasma clearance of methotrexate. Lawrence and his associates (1980) reported the plasma clearance to be  $62 \pm 19$  ml/min after the 25 mg dose and  $31 \pm 6$  ml/min after the 100 mg dose. There are three other plausible reasons for the differences in clearance values. First, the length of sampling time differs between investigations. Huffman and associates (1973) collected samples for 96 hours compared to 48 hours by Lawrence and coworkers (1980) and 24 hours in this study. The shorter length of sampling time in this investigation may have caused an underestimation of the area under plasma concentration-time curve resulting in an overestimation of the plasma clearance. However, collecting samples for only 24 hours can be justified because there were only a few cases in which a measurable methotrexate concentration could be detected at 24 hours. This point leads us to the second reason for the disparity in results. Each of these investigations employed a different analytical method of measuring methotrexate plasma concentrations. The method used by Huffman et al. (1973), which measures the radioactivity of a sample

after the administration of tritiated methotrexate, is known to be nonspecific for the parent drug therefore metabolites and hence folate analogues were also measured as methotrexate. Because of this occurrence, the higher and more prolonged plasma methotrexate concentrations found in Huffman's study are misleading and would cause the area under the plasma concentration versus time curve to be overestimated resulting in an underestimation of the plasma clearance. The third reason may partially explain the discrepancy between our value for plasma clearance and that reported by Lawrence et al. (1980). Plasma clearance is inversely proportional to the elimination half-life of a drug, therefore if the elimination half-life found in Lawrence's investigation was longer than the value obtained in this study (3.20 hours), the value obtained for plasma clearance by Lawrence and his associates would be lower than our plasma clearance value. However, Lawrence et al. (1980) did not report a value for the elimination half-life.

The mean elimination half-life ( $t_{1/2}$ ) for methotrexate following intravenous administration, calculated from the mean terminal disposition rate constant ( $\lambda$ ), was 3.20 hours, ranging from 1.85 hours to 4.93 hours. This value is in agreement with the half-life for the second phase of elimination (renal clearance and biliary excretion) observed by Huffman and associates (1973) in their investigation (3.49 hours). It is also within the range of half-life values reported by Halprin et al. (1971). The mean half-life of methotrexate in this study is longer than the half-life observed by Henderson et al. (1965), however, samples were collected for only six hours after the dose in their

investigation. To obtain an accurate estimate of the half-life, blood samples should be collected for at least three half-lives.

#### Intramuscular and Oral Methotrexate

The plasma concentration versus time data following intramuscular and oral administration of methotrexate were also analyzed by a "model-independent" method to obtain the parameters, terminal disposition rate constant ( $\lambda$ ) and elimination half-life ( $t_{1/2}$ ).

Table 6 lists the individual half-lives of methotrexate for each route of administration. The mean elimination half-life for methotrexate after intramuscular administration, calculated from the mean terminal disposition rate constant, was 3.28 hours and ranged from 2.65 hours to 5.27 hours. After oral administration, the mean elimination half-life, calculated in the same manner, was 3.21 hours with a range of 1.58 hours to 4.29 hours. The results of the repeated measures analysis of variance for the elimination half-lives after the intravenous, intramuscular, and oral administration of methotrexate are shown in Table 7. The analyses were performed using one trial factor across which measurements were repeated. This factor was route of administration with three levels of measurement. The first F statistic reported in this table represents the effect of the route of methotrexate administration. The second F statistic in this table is the value calculated from Tukey's test for nonadditivity. In this data set, the null hypothesis was retained (i.e., this data fits the additive model).

Table 6. Comparison of the elimination half-life of methotrexate following intravenous, intramuscular, and oral administration of methotrexate.

PATIENT	INTRAVENOUS		INTRAMUSCULAR		ORAL	
	$\lambda$ (hr <sup>-1</sup> )	$t_{1/2}$ (hr)	$\lambda$ (hr <sup>-1</sup> )	$t_{1/2}$ (hr)	$\lambda$ (hr <sup>-1</sup> )	$t_{1/2}$ (hr)
1	0.1406	4.93	0.2016	3.44	0.1617	4.29
2	0.3754	1.85	0.2611	2.65	0.4373	1.58
3	0.1768	3.92	0.2315	2.99	0.1749	3.96
4	0.0610*	11.36*	0.1984	3.49	0.1638	4.23
5	0.1841	3.76	0.1316	5.27	0.1695	4.09
6	0.2054	3.37	0.2455	2.82	0.1884	3.68
MEAN	0.2165	3.20**	0.2116	3.28**	0.2159	3.21**
<u>+ S.D.</u>	<u>+ 0.0919</u>		<u>+ 0.0462</u>		<u>+ 0.1089</u>	

\* Not used in the calculation of the mean and standard deviation

\*\* From  $t_{1/2} = \frac{0.693}{\text{mean } \lambda}$

**Table 7. Results for repeated measures analysis of variance of the elimination half-lives after the intravenous, intramuscular, and oral administration of methotrexate.**

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	Probability
Between people	10.53273	4	2.63318		
Between people	3.97900	10	.39790		
Between routes	.04489	2	.02245	.04565	.9556
Residual	3.93411	8	.49176		
Nonadditivity	.15376	1	.15376	.28472	.6101
Balance	3.78034	7	.54055		

No statistically significant difference was found between the mean elimination half-lives after intravenous, intramuscular, and oral administration (Table 7). Although there have been no studies to compare the elimination half-life of methotrexate following these three routes of administration, there is no evidence to indicate that a difference should exist. These results suggest that, indeed, no difference does exist.

Absorption of methotrexate from both the muscle and the gastrointestinal tract appears to be rapid as measurable concentrations of methotrexate were detected in the plasma 15 minutes after the administration of the dose in most cases. Measurable concentrations of methotrexate were not observed until 30 minutes after an oral dose in Patients 1 and 3. The mean time to reach maximum plasma concentration ( $t_{\max}$ ) after intramuscular administration was  $0.85 \pm 0.49$  hours, ranging from 0.25 hours to 1.5 hours (Table 8). After oral administration, the mean time to attain maximum plasma concentration was  $1.55 \pm 0.51$  hours with a range of 0.75 hours to 2.0 hours. No statistically significant difference was found between the mean values of  $t_{\max}$  after intramuscular and oral administration (Table 9). The maximum plasma concentrations ( $C_{\max}$ ) of methotrexate following intramuscular and oral administration are also listed for each patient in Table 8. The values of  $t_{\max}$  and  $C_{\max}$  obtained after the oral administration of methotrexate to Patient 4 were excluded from the analysis because the patient vomited an unknown quantity of the dose after 5 minutes. At that point he was given another oral dose which was 75% of his original dose. Therefore a

Table 8. Comparison of the time to peak and peak concentrations of methotrexate following intramuscular and oral administration of methotrexate.

PATIENT	INTRAMUSCULAR		ORAL	
	Time to peak (hr)	Peak MTX Conc. (ng/ml)	Time to peak (hr)	Peak MTX Conc. (ng/ml)
1	1.5	1385	1.5	431
2	1.0	1294	0.75	749
3	0.25	1226	1.5	440
4	1.0	1180	4.23*	1317*
5	1.0	1634	2.0	531
6	0.5	1044	2.0	354
MEAN	0.85	1294	1.55	501
$\pm$ S.D.	$\pm$ 0.49	$\pm$ 202	$\pm$ 0.51	$\pm$ 152

\* Not used in the calculation of the mean and standard deviation

Table 9. Results for paired t-test of the difference between the times to maximum plasma concentration after the intramuscular and oral administration of methotrexate.

Variable	Number of Cases	Mean	Standard Deviation	Standard Error	T Value	Degrees of Freedom	Probability
Intramuscular	5	.85	.487	.218			
Oral	5	1.55	.512	.229			
Difference	5	-.70	.779	.348	-2.01	4	.115

violation of the study protocol had been committed. A statistically significant difference was found between the mean values of  $C_{\max}$  after the intramuscular and oral doses of methotrexate (Table 10). This difference implies that absorption is greater after intramuscular administration than following oral administration.

Total area under the plasma concentration versus time curves, from time zero to infinity ( $AUC_0^\infty$ ) for intravenous, intramuscular, and oral methotrexate were determined by equation (5). The areas following intramuscular and oral methotrexate administration were compared with the areas under the curve for intravenous administration of methotrexate to determine the absolute intramuscular and oral bioavailability of methotrexate. Individual patients' intramuscular bioavailability values are presented in Table 11. The mean absolute intramuscular bioavailability was  $0.93 \pm 0.14$ , ranging from 0.78 to 1.11. The value for Patient 4 was not included in the analysis since the data from the intravenous course of therapy was excluded due to the unusually long half-life obtained, therefore a comparison could not be made. In their studies of the intramuscular absorption of methotrexate, Halprin et al. (1971) and Freeman-Narro et al. (1975) did not measure a true absolute bioavailability, however, their research led them to conclude that the intramuscular absorption of methotrexate was rapid and complete. Based on our data, it appears that essentially the total intramuscular dose of methotrexate administered is available to the systemic circulation, a conclusion consistent with the previously mentioned studies.

Table 10. Results for paired t-test of the difference between the maximum plasma concentrations after the intramuscular and oral administration of methotrexate.

Variable	Number of Cases	Mean	Standard Deviation	Standard Error	T Value	Degrees of Freedom	Probability
Intramuscular	5	1316.6	217.055	97.070			
Oral	5	501.0	152.179	68.057			
Difference	5	815.6	218.877	97.885	8.33	4	.001

Table 11. Absolute intramuscular bioavailability of methotrexate.

PATIENT	$AUC_0^\infty$ (IM) (ng·hr/ml)	$AUC_0^\infty$ (IV) (ng·hr/ml)	DOSE(mg)		F
			IV	IM	
1	7798	9948	45	45	0.78
2	5928	5865	40	40	1.11
3	5320	5645	45	45	0.94
4	6977	13440	47	47	0.52*
5	7530	6763	50	50	1.11
6	4323	5250	55	55	0.82
MEAN					0.93
$\pm$ S.D.					$\pm$ 0.14

\* Not used in the calculation of the mean and standard deviation

The mean absolute oral bioavailability was found to be  $0.36 \pm 0.10$  with a range of 0.24 to 0.52. Individual patients' oral bioavailability values are listed in Table 12. The value for Patient 4 was excluded from the analysis because of the protocol violation committed during the course of therapy with oral methotrexate, as explained previously. Based on these calculations, it appears that approximately 60% of a  $30 \text{ mg/m}^2$  oral dose is not available to the systemic circulation due to incomplete absorption or pre-systemic metabolism. From our data it is not known whether pre-systemic metabolism, if it occurs, takes place in the liver or within the gastrointestinal tract. However, there is evidence in the literature to suggest that pre-systemic metabolism occurs. Wan et al. (1974) found that metabolites of methotrexate in the urine constituted 35% of a  $30 \text{ mg/m}^2$  oral dose compared to 6% for the same dose administered intravenously. These findings suggest that metabolism by intestinal bacteria in the gastrointestinal tract occurs during enterohepatic circulation and that metabolism by intestinal bacteria prior to gastrointestinal absorption may account for the larger amount of metabolites excreted after oral doses.

Dose-dependent absorption of methotrexate from the gastrointestinal tract may also contribute to the low availability found after oral administration. Chungi et al. (1978) have shown that the absorption of methotrexate from the lumen of the rat small intestine, in situ, is saturable at relatively low concentrations and obeys Michaelis-Menton kinetics. Henderson et al. (1965) and Wan et al.

Table 12. Absolute oral bioavailability of methotrexate.

PATIENT	$AUC_0^\infty$ (PO) (ng·hr/ml)	$AUC_0^\infty$ (IV) (ng·hr/ml)	DOSE(mg)		F
			IV	PO	
1	2396	9948	45	45	0.24
2	1916	5865	40	40	0.33
3	2193	5645	45	45	0.39
4	9719	13440	47	47.5	0.72*
5	3545	6763	50	50	0.52
6	1665	5250	55	55	0.32
MEAN					0.36
$\pm$ S.D.					$\pm$ 0.10

\* Not used in the calculation of the mean and standard deviation

(1974), administering methotrexate orally to humans at two dosage levels, found that absorption was significantly lower at the higher level indicating that incomplete absorption of methotrexate is partially due to dose-dependent gastrointestinal absorption.

The value obtained in this study for oral bioavailability of methotrexate is 25% lower than the value reported by Wan et al. (1974). In addition to the above explanations, the method by which methotrexate is administered orally would influence the availability from the gastrointestinal tract. Previous studies administered methotrexate as a solution through a nasogastric tube. In this investigation, methotrexate was administered by mouth as the commercial tablets. Dosage form constitutes a significant factor as solutions are generally absorbed more rapidly because they bypass the steps of disintegration and dissolution required for tablets before absorption can take place. Because of this physical property, solutions have the potential to be absorbed more extensively and to have greater bioavailability than tablets.

A statistically significant difference was found between the mean values of the area under the plasma concentration versus time curve after intravenous, intramuscular, and oral methotrexate (Table 13). The a posteriori test of Scheffe was applied to this data at the 95% confidence interval to determine between which pair(s) of means does a statistical difference exist. This test indicates that there are differences between oral and intravenous areas and oral and intramuscular areas under the plasma concentration versus time curve.

**Table 13. Results for repeated measures analysis of variance of the areas under the plasma concentration versus time curve after the intravenous, intramuscular, and oral administration of methotrexate.**

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F	Probability
Between people	17826497	4	4456624		
Within people	63986153	10	6398615		
Between routes	56541890	2	28270945	30.38145	.0002
Residual	7444264	8	930533		
Nonadditivity	3373177	1	3373177	5.79998	.0469
Balance	4071087	7	581584		

The intramuscular and intravenous areas under the curve were not significantly different. From this information, it is concluded that essentially 100% of the intramuscular dose administered to patients in this study is available to the systemic circulation. However, the same dose administered orally was absorbed to a significantly lesser extent than the intramuscular dose and the availability of methotrexate to the systemic circulation was significantly lower than after equal intravenous and intramuscular doses.

## CHAPTER 5

### SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

#### Summary

The objectives of this study were to determine 1) the pharmacokinetic disposition parameters obtained from the plasma concentration versus time profile of methotrexate following the intravenous administration of a 30 mg/m<sup>2</sup> dose and 2) the absolute intramuscular and oral bioavailability of methotrexate. A randomized factorial crossover study was designed to meet these objectives.

Methotrexate was administered, in a dosage of 30 mg/m<sup>2</sup>, by the intravenous, intramuscular, and oral routes to each patient. The subjects for this study were six patients of the Hematology-Oncology Clinic of the Arizona Health Sciences Center or the Tucson Veterans Administration Medical Center. All of the patients were between the ages of 57 and 76 with a diagnosis of advanced squamous cell carcinoma of the head and neck.

Each patient received a dose of methotrexate each week for three consecutive weeks. Blood counts were obtained immediately prior to each course of therapy to determine whether the patient was leukopenic ( $< 3000/\text{mm}^3$ ) or thrombocytopenic ( $< 100,000/\text{mm}^3$ ). The doses were administered as follows: 1) intravenous - slow push into a scalp vein followed by a saline flush, 2) intramuscular - into the gluteal muscle mass, and 3) oral - 2.5 mg tablets with 8 ounces of water. Blood

samples were collected for 24 hours following the dose at time intervals which were based on the route of administration of the therapy. Plasma samples were analyzed by the homogenous enzyme immunoassay method to determine the concentration of methotrexate in the sample.

The plasma concentration versus time data after intravenous, intramuscular, and oral administration were analyzed by a "model-independent" method to obtain the pharmacokinetic parameters. Area under the plasma concentration versus time curve and elimination half-life data were analyzed by repeated measures analysis of variance. The time to maximum plasma concentration and maximum plasma concentration data were analyzed by the paired t-test.

Pharmacokinetic disposition parameters of methotrexate after intravenous administration were reported. The elimination half-lives after intravenous, intramuscular, and oral administration of methotrexate were essentially the same. Statistically significant differences were not found between these values.

The maximum plasma concentration after intramuscular administration was found to be significantly higher than after oral administration. Although the time to maximum plasma concentration was longer after an oral dose than an intramuscular dose, the difference was not statistically significant.

Availability of methotrexate after intramuscular administration was less than after intravenous administration, however it was not a statistically significant difference. The oral availability of methotrexate was significantly less than the intravenous and intramuscular availability.

### Conclusions

The disposition parameters of methotrexate after intravenous administration are in agreement with those values reported in the literature after similar doses of methotrexate. The elimination half-life reported by the current study supports the value for the second phase of elimination obtained by Huffman et al. (1973) after intravenous administration of the same dose of methotrexate. We were unable to duplicate the prolonged third phase of elimination demonstrated in Huffman's investigation. Our data supports the belief that this prolonged phase of elimination exists only because the analytical method used in Huffman's study was not specific for methotrexate.

There is a large variation in the values obtained for the plasma clearance of methotrexate. Since greater than 80% of a dose of methotrexate is excreted by the kidneys as unchanged drug, the variability in renal function among the patients may partially account for the wide range of values obtained for plasma clearance. Indeed, there is a strong correlation ( $r=0.85$ ) between the patients' mean calculated creatinine clearance and their plasma clearance of methotrexate.

Prior to this study there were no reports in the literature of the absolute intramuscular bioavailability of methotrexate. In studies by Freeman-Narrodd et al. (1975) and Halprin et al. (1971), it was concluded that the intramuscular absorption of methotrexate was rapid and complete. Although the bioavailability was less than 100% in this study, it was statistically determined to be equivalent to the availability after an intravenous dose of the same magnitude.

The absorption of methotrexate from the gastrointestinal tract was shown to be dose-dependent by Henderson et al. (1965) and Wan et al. (1974). With respect to this observation, it is difficult to compare our results to those of other investigators. However, Wan et al. (1974) administered the same oral dose as in this study and found that the oral bioavailability was greater than that found in this investigation. It is important to recognize that in previous studies, methotrexate was administered as a solution through a nasogastric tube. This investigation is the first to report the absolute bioavailability of the commercial tablets.

#### Recommendations

On the basis of their significantly greater bioavailability, the intravenous and intramuscular routes appear to be the preferred routes of administration for methotrexate. The administration of equivalent doses of methotrexate by these routes will result in plasma levels which will remain in the therapeutic range for a longer period of time. This is important as the cytotoxicity of methotrexate is dependent upon both the extracellular concentration and the duration of exposure of the cell to methotrexate. Therapeutic levels are obtained after an equivalent oral dose of methotrexate, however because it is less available to the systemic circulation, cytotoxic plasma concentrations are maintained for a shorter period of time.

Caution should be taken in extrapolating the results of this study to other dosing ranges of methotrexate. There is much documentation in the literature to support the existence of both a saturable

intestinal absorption mechanism and a saturable renal elimination mechanism. The exact doses and plasma concentrations at which saturation of these transport mechanisms occurs still need to be identified.

Based on the absolute oral bioavailability and the unpredictable potential for saturable intestinal absorption, the administration of methotrexate by the oral route cannot be recommended as the preferred method of administration. Also, the absolute bioavailability found in this study was obtained under fasting conditions. Since food is known to alter the gastrointestinal absorption of methotrexate, oral absorption becomes even more unpredictable. If the clinical oncologist determines that the oral route is preferred, he should take these factors into consideration when deciding upon the dose to be administered. An adjustment of the dose based on the absolute bioavailability may not result in the desired plasma concentrations because of these factors.

There has been one study conducted that deals with the problem of saturable intestinal absorption. Steele et al. (1979b) administered an oral syrup of methotrexate by two regimens, 1) a single 100 mg dose and 2) 25 mg every 2 hours for 4 doses, to eight cancer patients. The elimination half-life of methotrexate was found to be the same after each regimen. Area under the plasma concentration versus time curve was significantly greater following the subdivided dose by a ratio of almost 2:1. Further research needs to be done in this area to determine whether subdividing the dose is a viable alternative for administering methotrexate orally.

Another disadvantage of the oral route of methotrexate administration is a practical one. Since methotrexate is only available in tablet form in the 2.5 mg strength, this group of patients were required to take from 16 to 22 tablets for a  $30 \text{ mg/m}^2$  dose. This would pose a problem for a patient who has difficulty swallowing. The formulation of a tablet of a higher strength or the pharmaceutical compounding of methotrexate into a liquid dosage form would be alternative solutions to this problem.

Although the oral route of methotrexate administration is not recommended as the primary method of administration, it cannot be discounted totally as it is a viable alternative when administration by the intravenous or intramuscular routes is not indicated. By closely monitoring hematological parameters, the oncologist can qualitatively determine the oral absorption of methotrexate. If a nadir in the white blood cell or platelet count does not occur, the oncologist may adjust the dose upward. This method is not as exact as adjusting the dose based on bioavailability data but if the dosage were determined by combining these pieces of information, a more reliable therapeutic oral dose may be obtained.

**APPENDIX A**

**SUBJECT'S CONSENT FORM**

### SUBJECT'S CONSENT

#### The Bioavailability and Pharmacokinetics of Methotrexate

You are being asked to participate in a drug study conducted at the University of Arizona Health Sciences Center which is designed to compare the amount of methotrexate in your blood when given by three different routes of administration, (1) by mouth, (2) intramuscular injection, and (3) intravenous injection. You have been selected for the study because you have cancer and because you are receiving methotrexate as a part of your chemotherapy.

You understand that the study will last for three courses of your therapy. During one course of therapy, you will receive your methotrexate dose by intravenous injection. For another course, you will receive your methotrexate dose by intramuscular injection. You will also receive one methotrexate dose as oral tablets. You will be asked to fast for eight hours before and four hours after the administration of each dose. The methotrexate doses will be the same in these courses as it would be if you were not participating in the study. Before each dose, a heparin lock (a small vein needle) will be placed in a hand or arm vein for the purpose of drawing blood samples to measure the amount of methotrexate in your blood. You will be asked to remain in the clinic for the first two hours after each dose. On the first day of each course of therapy, 14 samples of one teaspoonful (5 ml) each will be drawn from the heparin lock. On the second, third, and fourth day, you will be asked to come to the clinic to have one teaspoonful of blood drawn by needlestick. A total of 8 1/2 ounces of blood will be collected during the study (about 3 ounces per course).

The side effects of methotrexate include nausea and vomiting, ulcers in the mouth, low white blood cell count, hair loss, itching, dizziness, fatigue, and blurred vision. You may also experience discomfort from the heparin lock and possible bruising and bleeding from the needlestick. Dr. Alberts will be available for immediate treatment should any of these side effects occur. You will not be charged for the methotrexate that you receive, the cost of analysis for the methotrexate blood levels, nor for the heparin lock, or any clinic visits solely for obtaining blood or for maintaining the heparin lock. You will continue to be responsible for the costs of your routine care including physician charges, standard laboratory tests necessary, and routine clinic visits. Only standard doses of methotrexate will be used in the study, therefore, excess drug toxicity is not anticipated. Rarely, this may occur anyway and treatment including hospitalization may be required. You should be aware that you must remain responsible for any charges that might accrue in this instance. We hope to learn from this study, the best method for giving an important anticancer drug by different routes. This could allow for oral dosing of methotrexate (sparing an injection),

and could help prevent drug toxicity or loss of effect when different routes of drug administration are needed.

The nature, demands, risks and benefits of the project have been explained to me as well as the type of treatment as known and available, and I understand what my participation involves. Furthermore, I understand that I am free to ask questions and withdraw from the project at any time without affecting my medical care.

I understand that in the event of physical injury resulting from the research procedures, financial compensation for wages or time lost and the costs of medical care and hospitalization is not available and must be borne by the subject.

I understand that \_\_\_\_\_ will provide more information upon my request.

I also understand that this consent form will be filed in an area designated by the Human Subjects Committee with access restricted to the principal investigator or authorized representatives of the particular department. A copy of this consent form is available to me upon request.

\_\_\_\_\_  
Subject's signature

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature of parent or legal  
representative

\_\_\_\_\_  
Date

I have carefully explained to the subject the nature of the above project. I hereby certify that to the best of my knowledge the subject signing this consent form understands clearly the nature, demands, benefits, and risks involved in participating in this study. A medical problem or language or educational barrier has not precluded a clear understanding of his/her involvement in this project.

\_\_\_\_\_  
Investigator's signature

\_\_\_\_\_  
Date

\_\_\_\_\_  
Signature of witness

\_\_\_\_\_  
Date

## **APPENDIX B**

### **KARNOFSKY PERFORMANCE SCALE**

## KARNOFSKY PERFORMANCE SCALE

Activity status	Point	Description
Normal activity	10	Normal, with no complaints or evidence of disease
	9	Able to carry on normal activity but with minor signs or symptoms of disease present
	8	Normal activity but requiring effort; signs and symptoms of disease more prominent
Self-care	7	Able to care for self, but unable to work or carry on other normal activities
	6	Able to care for most needs but requires occasional assistance
	5	Considerable assistance required, along with frequent medical care; some self-care still possible
Incapacitated	4	Disabled and requiring special care and assistance
	3	Severely disabled; hospitalization required but death from disease not imminent
	2	Extremely ill; supportive treatment, hospitalized care required
	1	Imminent death
	0	Death

## **APPENDIX C**

**METHOTREXATE PLASMA CONCENTRATION DATA FOR  
PATIENTS 1-6 AS A FUNCTION OF TIME AFTER  
THE INTRAVENOUS, INTRAMUSCULAR, AND  
ORAL ADMINISTRATION OF METHOTREXATE**

**(Tables C-1 to C-6)**

Table C-1. Methotrexate Plasma Concentration versus Time, Data for Patient 1.

INTRAVENOUS		INTRAMUSCULAR		ORAL	
Time (hr)	Plasma Concentration (ng/ml)	Time (hr)	Plasma Concentration (ng/ml)	Time (hr)	Plasma Concentration (ng/ml)
0.083	4903	0.25	227	0.33	*
0.25	2000	0.50	540	0.50	114
0.75	1566	0.75	658	0.75	277
1.00	1271	1.00	1158	1.00	354
1.50	1044	1.50	1385	1.50	431
2.00	1022	2.00	1203	2.00	395
3.00	817	3.00	1067	3.00	286
4.00	667	4.00	783	4.00	222
6.00	572	6.00	499	6.00	118
8.00	386	8.00	350	8.00	98
9.92	259	9.75	268	11.75	70
21.50	64	21.42	*	23.75	*

\* Undetectable

Table C-2. Methotrexate Plasma Concentration versus Time, Data for Patient 2.

INTRAVENOUS		INTRAMUSCULAR		ORAL	
Time (hr)	Plasma Concentration (ng/ml)	Time (hr)	Plasma Concentration (ng/ml)	Time (hr)	Plasma Concentration (ng/ml)
0.17	3269	0.37	931	0.25	82
0.33	2406	0.58	1180	0.50	377
0.58	2043	0.75	1226	0.75	749
0.75	1952	1.00	1294	1.00	704
1.00	1453	1.55	1203	1.50	613
1.55	1090	2.33	1067	2.22	386
1.97	863	3.00	1000	3.17	236
3.00	645	4.00	726	4.00	159
4.08	409	5.63	472	6.00	73
5.17	241	7.00	345	7.00	45
6.00	186	11.75	95	11.92	*
7.50	123	23.67	*	23.67	*
12.08	*				
24.00	*				

\* Undetectable

Table C-3. Methotrexate Plasma Concentration versus Time, Data for Patient 3.

INTRAVENOUS		INTRAMUSCULAR		ORAL	
Time (hr)	Plasma Concentration (ng/ml)	Time (hr)	Plasma Concentration (ng/ml)	Time (hr)	Plasma Concentration (ng/ml)
0.083	3814	0.25	1226	0.25	*
0.25	2769	0.50	1203	0.50	73
0.67	1044	0.78	1090	0.75	200
0.92	863	1.17	1112	1.00	390
1.08	726	1.58	1044	1.50	440
1.55	636	2.00	863	2.00	386
2.00	554	3.00	681	3.00	254
4.03	363	4.00	472	4.00	177
6.00	250	6.00	209	6.00	141
8.00	159	8.08	182	8.00	95
12.05	104	12.50	77	12.50	45
23.88	*	23.08	*	24.33	*

\* Undetectable

Table C-4. Methotrexate Plasma Concentration versus Time, Data for Patient 4.

INTRAVENOUS		INTRAMUSCULAR		ORAL	
Time (hr)	Plasma Concentration (ng/ml)	Time (hr)	Plasma Concentration (ng/ml)	Time (hr)	Plasma Concentration (ng/ml)
0.083	5493	0.25	590	0.33	50
0.25	2679	0.50	953	0.58	104
0.50	1816	0.75	1135	0.75	195
0.75	1544	1.00	1180	1.00	338
1.00	1362	1.50	1090	1.50	411
1.53	908	2.08	1044	2.00	531
2.08	681	3.10	772	3.00	953
3.00	597	4.03	590	4.23	1317
4.08	586	6.00	495	6.08	1085
6.05	461	7.08	427	7.83	590
8.05	384	11.97	127	12.00	204
12.07	254	23.17	*	25.00	45
24.38	177				

\* Undetectable

Table C-5. Methotrexate Plasma Concentration versus Time, Data for Patient 5.

INTRAVENOUS		INTRAMUSCULAR		ORAL	
Time (hr)	Plasma Concentration (ng/ml)	Time (hr)	Plasma Concentration (ng/ml)	Time (hr)	Plasma Concentration (ng/ml)
0.083	5902	0.25	863	0.25	82
0.25	3133	0.50	1453	0.50	182
0.50	1453	0.75	1634	0.75	304
0.75	1498	1.00	1634	1.00	450
1.03	649	1.50	1362	1.50	499
1.50	674	2.07	863	2.00	531
2.00	595	3.00	599	3.00	422
3.00	486	4.00	545	4.00	304
4.00	533	6.00	336	6.00	173
6.00	381	7.75	281	7.83	150
7.70	286	11.90	123	12.05	86
12.00	86	23.95	45	23.93	*
23.93	*				

\* Undetectable

Table C-6. Methotrexate Plasma Concentration versus Time, Data for Patient 6.

INTRAVENOUS		INTRAMUSCULAR		ORAL	
Time (hr)	Plasma Concentration (ng/ml)	Time (hr)	Plasma Concentration (ng/ml)	Time (hr)	Plasma Concentration (ng/ml)
0.083	4767	0.25	645	0.33	95
0.25	2542	0.50	1044	0.55	209
0.50	1226	0.75	1044	0.75	295
0.75	1000	1.00	795	1.00	313
1.00	885	1.50	772	1.50	341
1.50	663	2.00	658	2.00	354
2.00	604	3.00	449	3.00	213
3.00	390	4.00	449	4.00	213
4.00	313	6.00	331	6.00	114
6.00	218	8.00	154	7.67	77
8.00	145	12.00	54	11.83	45
11.95	77	23.93	*	23.75	*
23.92	*				

\* Undetectable

## APPENDIX D

### METHOTREXATE PLASMA CONCENTRATION AS A FUNCTION OF TIME AFTER THE INTRAVENOUS, INTRAMUSCULAR, AND ORAL ADMINISTRATION OF 30 MG/M<sup>2</sup> OF METHOTREXATE

The line of best fit from the linear regression of the points in the post-absorptive, post-distributive phase is drawn for each route of administration.

(Figures D-1 to D-6)

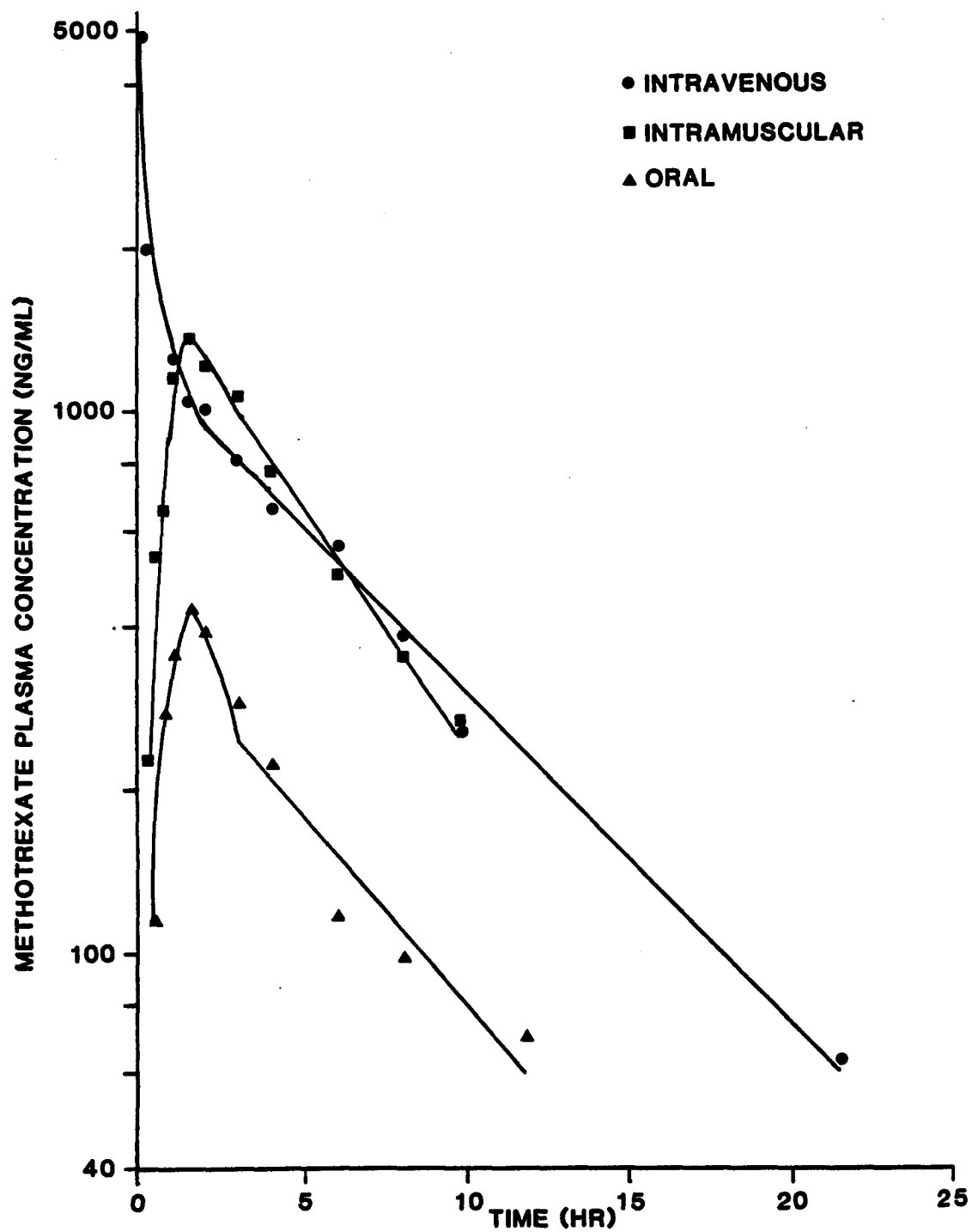


Figure D-1. Methotrexate plasma concentration as a function of time (Patient 1).

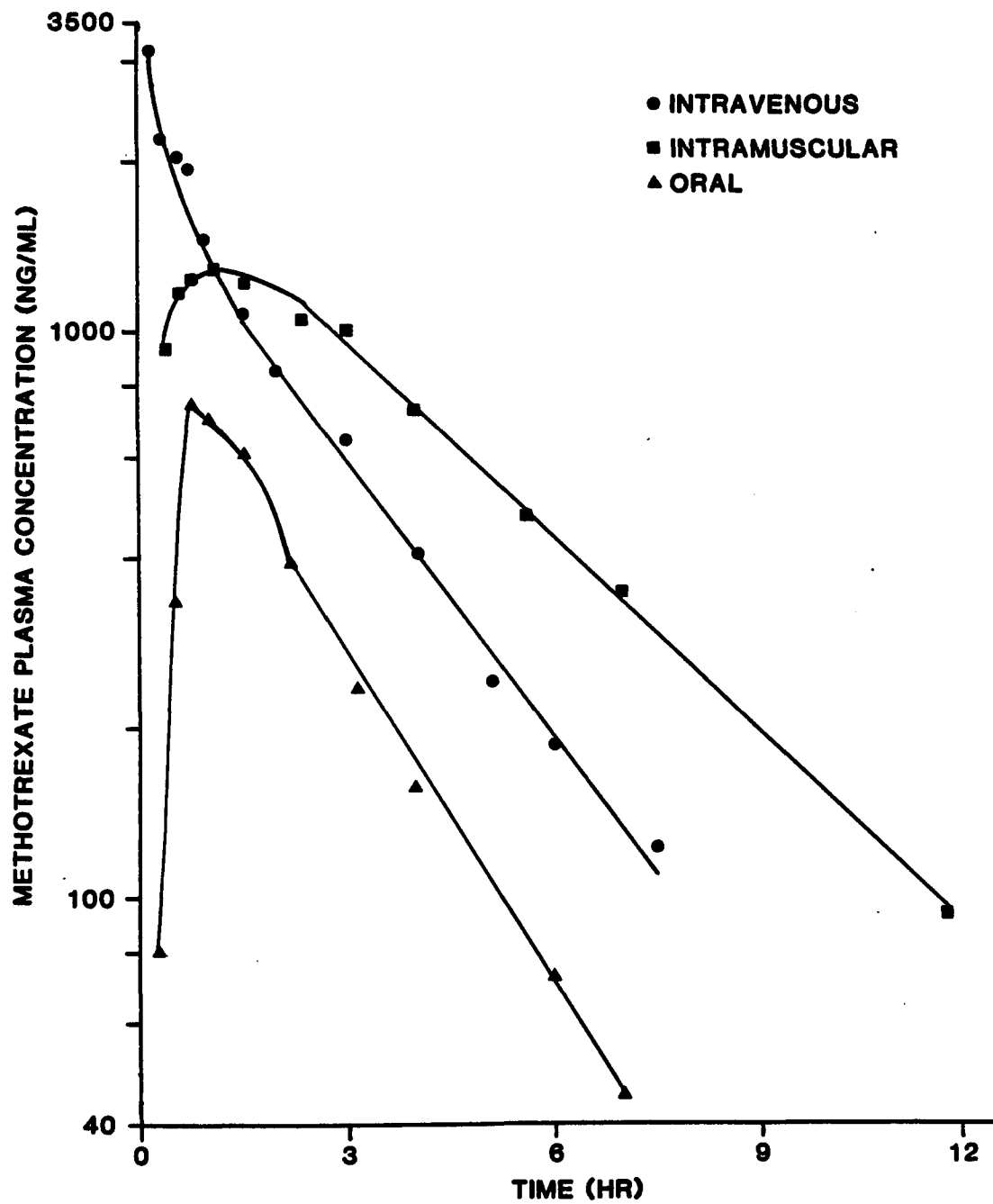


Figure D-2. Methotrexate plasma concentration as a function of time (Patient 2).

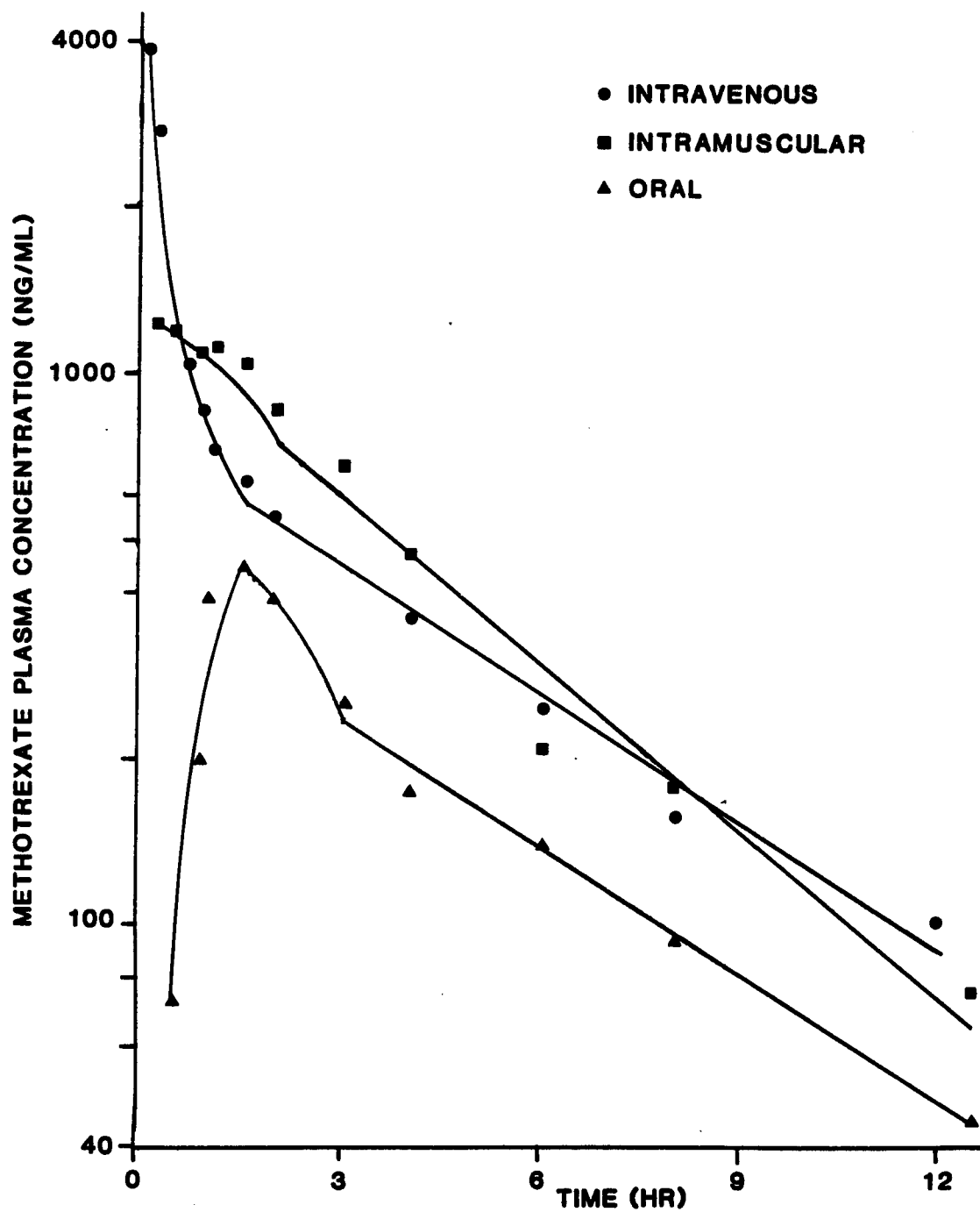


Figure D-3. Methotrexate plasma concentration as a function of time (Patient 3).

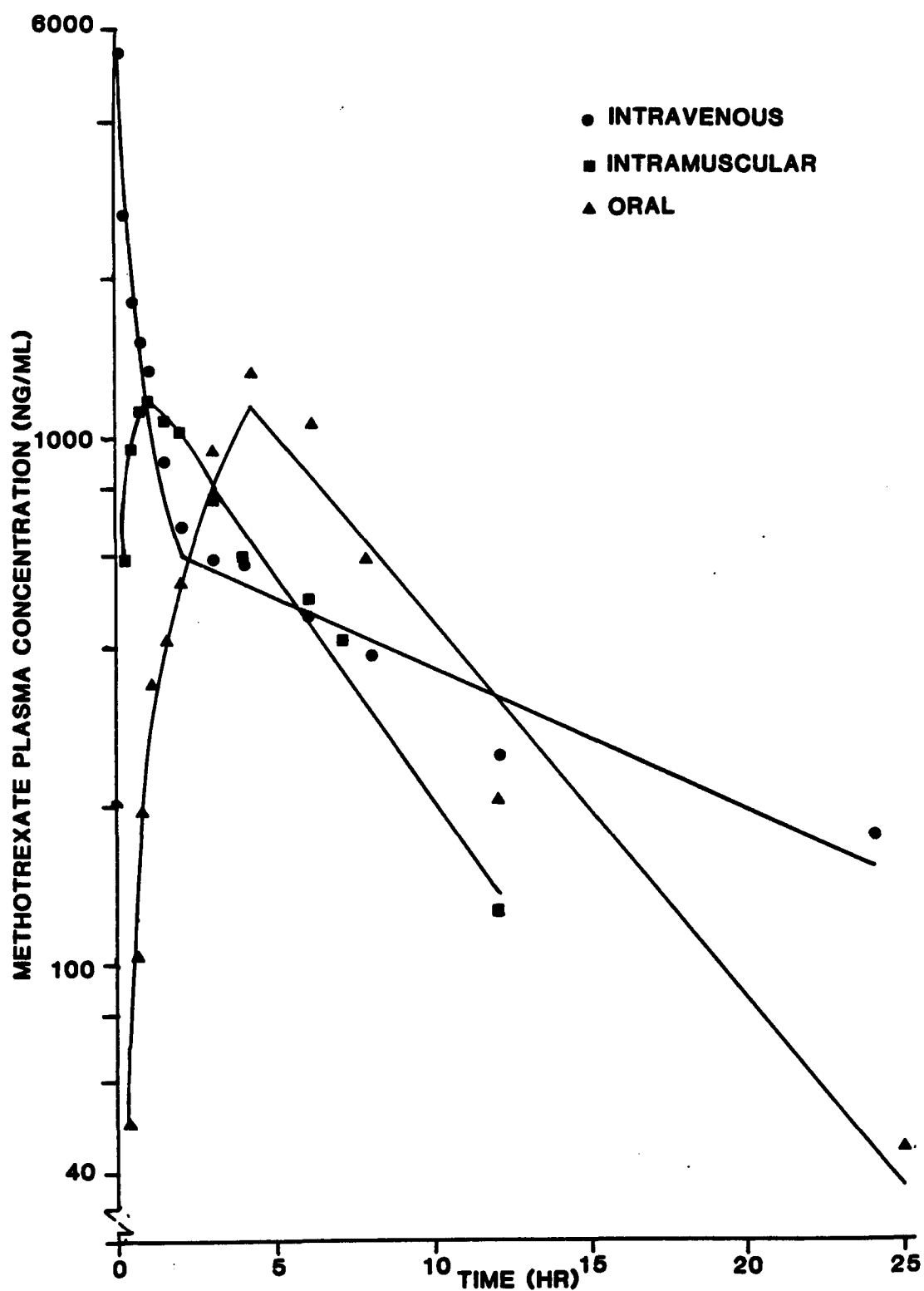


Figure D-4. Methotrexate plasma concentration as a function of time (Patient 4).

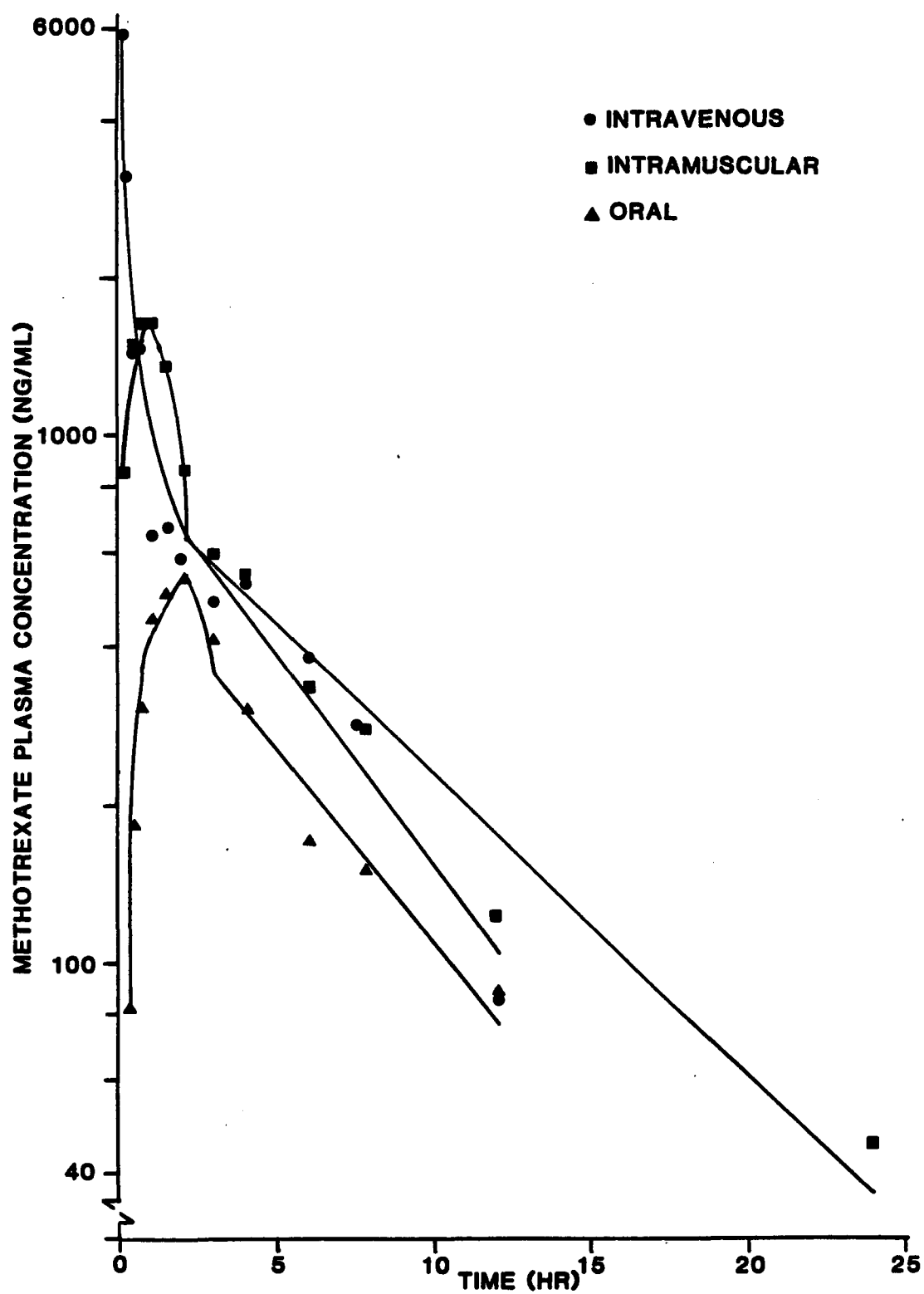


Figure D-5. Methotrexate plasma concentration as a function of time (Patient 5).

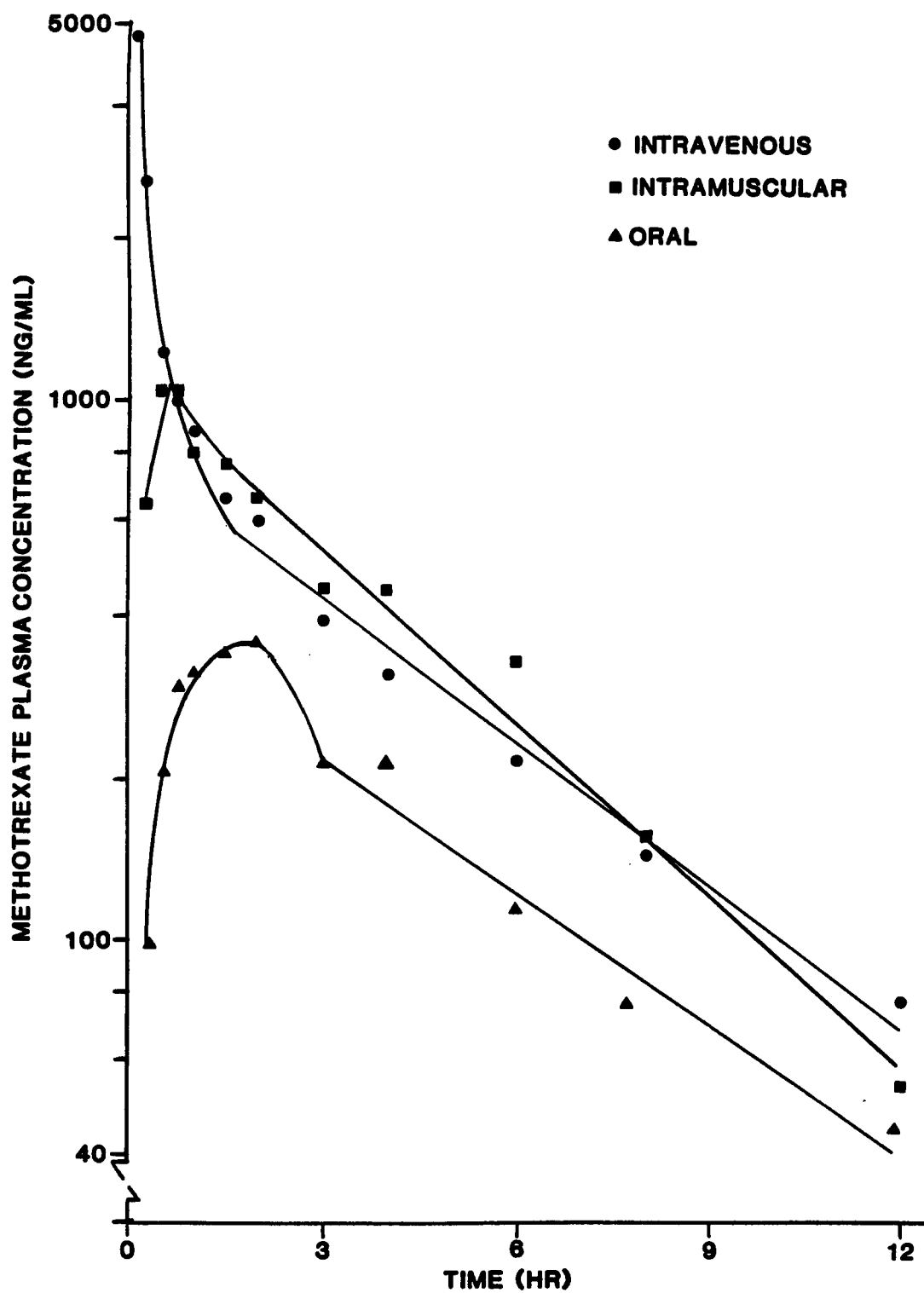


Figure D-6. Methotrexate plasma concentration as a function of time (Patient 6).

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