A thesis submitted for the Degree of Doctor of Philosophy in the University of London

DISPOSITION OF THEOPHYLLINE AND AMINOPHYLLINE IN MAN

by

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ABSTRACT

The metabolism and pharmacokinetics of (^{14}C)-theophylline have been studied after intravenous doses of 100mg to healthy volunteers keeping to their usual diets, after 7 days abstention from methylxanthine-containing foods and beverages and again after such abstention while ingesting theophylline and caffeine in tablet form. The metabolism of orally administered (^{14}C)-theophylline has also been investigated. Metabolites were separated and quantitated by ion-exchange column chromatography, ion-exchange paper chromatography and liquid scintillation counting. Three major metabolites were found in urine in addition to theophylline, namely 3-methylxanthine, 1,3-dimethyluric acid and 1-methyluric acid, and two minor metabolites were detected but not identified.

The kinetics of the elimination of the metabolites were studied after intravenous administration, and theophylline, 1,3-dimethyluric acid and 1-methyluric acid were eliminated by first-order kinetics while that of 3-methylxanthine was described by Michaelis-Menten kinetics.

Abstention from methylxanthine-containing foods and beverages led to a significant decrease in the elimination half-life of ^{14}C due to increases in the pharmacokinetic constants describing the elimination of theophylline, 3-methylxanthine and 1,3-dimethyluric acid. When the methylxanthine content of the methylxanthine-containing foods and beverages was replaced by theophylline and caffeine in tablet form the pharmacokinetics and metabolism of intravenously administered (^{14}C)-theophylline were unchanged from those seen on the volunteers usual diets.

The metabolism and pharmacokinetics of intravenously administered (^{14}C)-theophylline ethylenediamine (aminophylline) have also been studied. The nature and elimination kinetics of the metabolites identified were the same as those observed after the intravenous administration of (^{14}C)-theophylline. Abstention from methylxanthine-containing foods and beverages had no effect on the disposition of intravenously administered (^{14}C)-aminophylline.

The implications of the results are discussed with reference to pharmacokinetics and drug metabolism, drug-diet interrelations and the effects of dietary methylxanthines on drug metabolism.

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Ethical approval for the studies with radiolabelled theophylline and aminophylline was obtained from the ethical committee of St. Mary's Hospital and Medical School.

Informed consent was obtained from all volunteers in each study. Details of the volunteers who received 100mg (10μCi) ^{14}C-theophylline orally are given below.

Volunteer	TJM	JC	MF	KL	JD	RLS
Age	24	30	36	22	24	41
Weight (kg)	67	87	72	70	56	90

The radiochemical purity of the [^{14}C]-theophylline was confirmed by thin layer ascending chromatography in isopropranol and 5% ammonia (4:1) and ion-exchange paper chromatography in butanol, acetic acid, water (4:1:1). The stability of the intravenous preparations was checked similarly. A single radioactive peak was observed in both systems with Rf's of 0.56 and 0.75 respectively. Reverse isotope dilution confirmed the radiochemical purity to be >99%.

For the intravenous studies the drugs were infused over a ten minute period into the brachial vein in the antecubital fossa of the left arm through a Millex-GS 0.22μM millipore filter (Millipore SA, 67 Molsheim-France) and via an Abbocath 18G intravenous cannula (Abbott Laboratories Ltd.) with a three way tap (K75a stopcock, Pharmaseal$^{(R)}$, AHS/International, Herstal/Belgique) and kept open with lithium heparin-saline 0.9%. A time zero blood sample was taken immediately following the completion of the ten minute infusion from the right antecubital vein.

The reproducibility of the analytical procedure was confirmed by carrying out random duplicate analyses on urine samples, variation between these duplicate determinations being less than 5%.

CHAPTER 1

Introduction

The Methylxanthines

Source and History: Caffeine, theophylline and theobromine are three closely related alkaloids that occur in certain plants widely distributed throughout the world (Table 1.1). From earliest times man has prepared beverages from aqueous extracts of these plants. The dried seeds of *Coffea arabica* contain between 0.7 to 2.0% caffeine and it is from these that coffee is made. The average cup of coffee contains between 100 and 150 mg caffeine, approximately a therapeutic dose. The dried leaves of *Thea sinensis* contain both theophylline and about 2% caffeine, and are used to prepare tea. Cocoa, obtained from the seeds of *Theobroma cacao*, contain both caffeine and theobromine and a cup of cocoa may contain as much as 200 mg theobromine. Maté, the national drink of many South American countries contains caffeine. Additionally some so-called soft drinks, particularly the cola flavoured drinks contain caffeine, A 360 ml.(120 g) bottle of a cola drink contains 35 to 55 mg caffeine, as they are made from the nuts of the tree *Cola acuminata*. These cola nuts, the guru nuts chewed by the natives of Sudan, contain about 2% caffeine. More than a billion kilograms of coffee are consumed annually in the United States alone.

There is little doubt that the popularity of the methylxanthine-containing beverages depends on their stimulant action, although most people are unaware of any stimulation. The degree to which an individual is stimulated by a given amount of caffeine varies. Some persons have the ability to drink several cups of coffee in the evening and yet "sleep like a log". However, there are rare individuals who are so sensitive to caffeine that a single cup of coffee will cause an almost toxic response. Decaffeinated coffee, containing only 1 to 6 mg caffeine per cup, may be an acceptable

Table 1.1

THE NATURAL AND DIETARY SOURCES OF THE METHYLXANTHINES

Methylxanthine	Natural Source	Dietary Source
Theophylline; 1,3-dimethylxanthine	*Thea sinensis*	Tea
Caffeine; 1,3,7-trimethylxanthine	*Coffea arabica*	Tea
	Thea sinensis	Coffee
	Theobroma cacao	Chocolate
		Cocoa
	Cola acuminata	Cola drinks
Theobromine 3,7-dimethylxanthine	*Theobroma cacao*	Chocolate
		Cocoa

replacement for such individuals.

The methylxanthine-containing beverages can cause problems in the therapeutic management of certain patients, as they are a dietary source of a stimulant of the C.N.S., as well as possessing other important pharmacological properties (Table 1.2). For this reason they may often be denied to individuals with hypertension because of their action on the cardiovascular and nervous systems. Obviously, in a disease in which sedation may be an important factor, stimulation by a methylxanthine would be undesirable. Over-indulgence in these beverages may lead to a condition that could be regarded as one of chronic poisoning. Central nervous system stimulation causes restlessness and disturbed sleep; persistent myocardial stimulation can be reflected in premature systoles and tachycardia. The essential oils of coffee may cause some gastrointestinal irritation, and diarrhoea is a common symptom. The high tannin content of tea and cocoa on the other hand can cause constipation.

There is no doubt that a certain degree of tolerance (Colton et al., 1968) and of psychic dependence (habituation) develops to the methylxanthine beverages. This is likely even in individuals who do not partake to excess, with withdrawal symptoms of headache, fatigue and irritability developing on its abstention (Goldstein et al., 1969; Johnson et al., 1972).

An acquired tolerance to two actions of caffeine has been demonstrated unequivocally in man, namely, diuresis (Eddy and Downs, 1928) and parotid gland secretion (Winsor and Strongin, 1933). An acquired tolerance to the diuretic effects of theobromine and theophylline has also been shown (Eddy and Downs, 1928). Although

Table 1.2 THE RELATIVE PHARMACOLOGICAL ACTIVITY OF THE METHYLXANTHINES

XANTHINE	CNS AND RESPIRATORY STIMULATION	CARDIAC STIMULATION	CORONARY DILATION	SMOOTH MUSCLE RELAXATION	SKELETAL MUSCLE RELAXATION	DIURESIS
CAFFEINE	1*	3	3	3	1	3
THEOPHYLLINE	2	1	1	1	2	1
THEOBROMINE	3	2	2	2	3	2

* 1 = most active.

acquired tolerance to the central nervous effects of caffeine has not been demonstrated convincingly in man (Goldstein et al., 1965) the sleep disturbing properties of this alkaloid are clearly more marked among non-users than among habitual users of coffee (Goldstein, 1964).

However, tolerance to caffeine in man appears to be of low magnitude. This is suggested by demonstrations that the tolerant individuals respond when the dose is raised only twofold or threefold (Eddy and Downs, 1928; Winsor and Strongin, 1933). Furthermore, many habitual coffee drinkers experience distressing signs and symptoms, including extrasystoles, when their daily consumption of caffeinated beverages is approximately doubled (Hawk, 1929) and habitual coffee drinkers also appear to maintain higher arterial blood pressures and lower heart rates than abstainers (Hadley, 1945). Caffeine has also been demonstrated to be a determinant of the frequency of coffee consumption, such that the higher the caffeine content of a cup of coffee, the less frequent is its consumption (Kozlowski, 1976).

However, the morning cup of tea or coffee is so much a part of the European and American dietary habit that one seldom looks upon its consumption as a drug habit, and there is no direct evidence that the practice is in any way harmful in average consumers. The feeling of well-being and the increased performance it affords, although possibly attained at the expense of decreased efficiency later in the day, are experiences that few would care to give up.

Chemistry

Caffeine, theophylline and theobromine are methylated xanthines. Caffeine is 1, 3,7-trimethylxanthine; theophylline, 1, 3-dimethylxanthine; and theobromine, 3, 7-dimethylxanthine. The structural formulas of purine, uric acid, xanthine and the three pharmacologically important xanthine derivatives are shown in figure 1.1. The oxypurines may form enol derivatives by migration of hydrogen to the oxygen substituents. This is illustrate by the so-called lactim (hydroxy) structure of uric acid, which is formed by enolisation of the lactam (oxy) form. However, the lactam (oxy) form is the predominant tautomer (Figure 1.2.). Some physical properties of the methylxanthines are listed in Table 1.3.

The methylxanthines are only sparingly soluble in water (Table 1.3) and have therefore been combined with a large number of agents to increase their water solubility for parenteral administration (Table 1.4). Theophylline and caffeine have also been shown to form stable complexes with a variety of compounds (Tables 1.5 and 1.6). The solubility properties of theophylline can be altered by a variety of agents but the agent with which it is most frequently combined for therapeutic use is ethylenediamine, to yield aminophylline (Figure 1.3).

Aminophylline (Theophylline ethylenediamine)

The first therapeutic use of a xanthine derivative (theobromine sodium salicylate) was as a diuretic in heart failure by Babcock in 1891. In 1895 Askanazy stated that diuretin could relieve the pain of angina pectoris, and the effects of xanthine derivatives in the treatment of this condition was verified by Breuer (1902). It was noted, however, that diuretin, being insoluble caused gastro-intestinal disturbances and attempts were made to find other xanthine

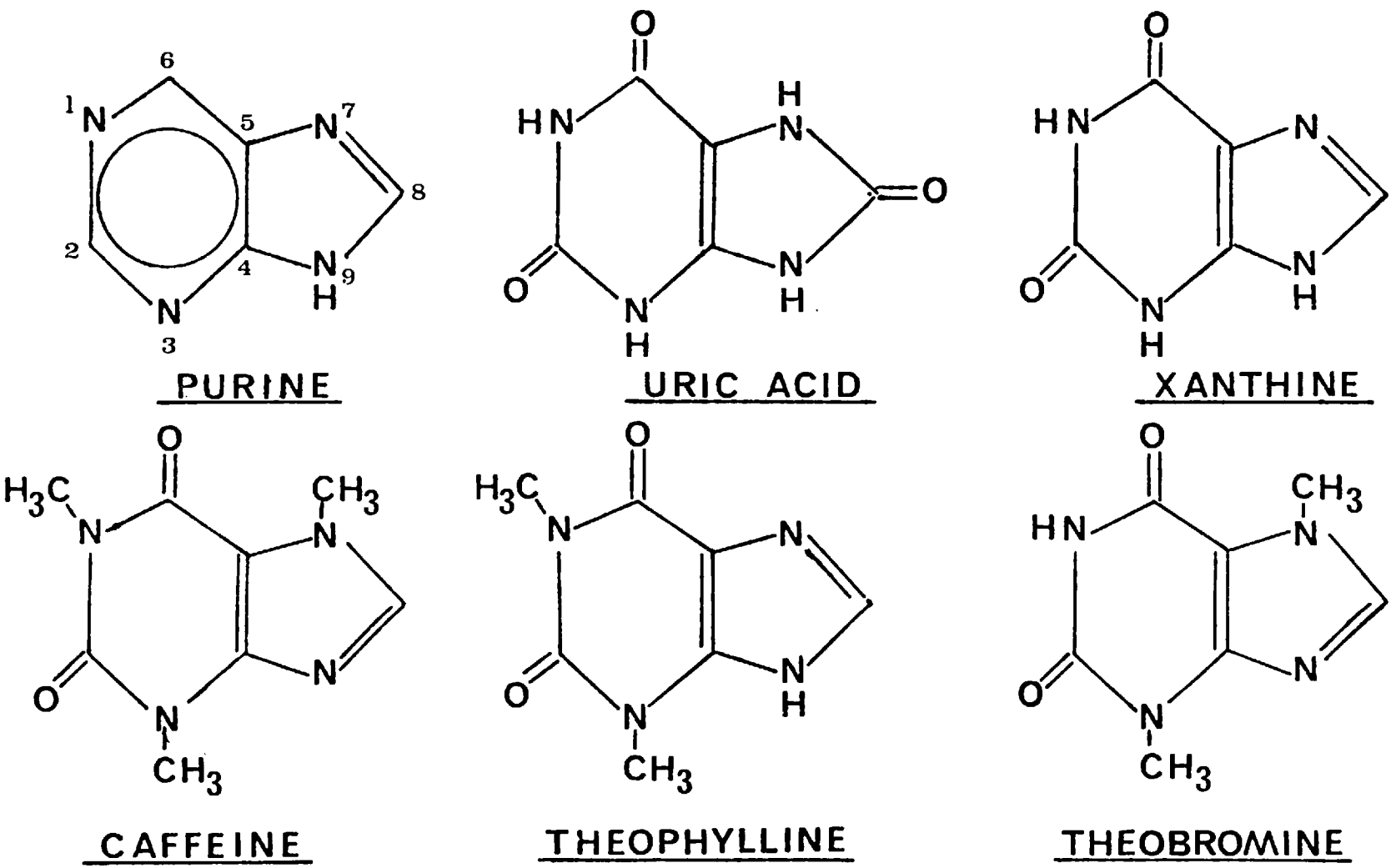
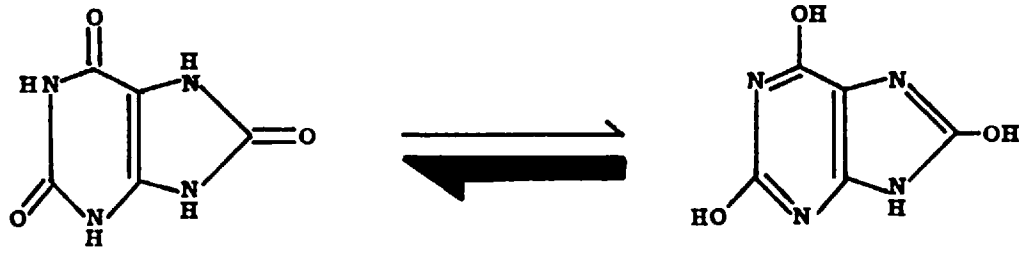


Figure 1.1 THE STRUCTURAL FORMULAE OF THE XANTHINES



URIC ACID ; LACTAM FORM
2,6,8-trioxypurine

URIC ACID ; LACTIM FORM
2,6,8-trihydroxypurine

Figure 1.2 THE TWO TAUTOMERIC FORMS OF URIC ACID

Table 1.3 SOME PHYSICAL PROPERTIES OF THE METHYLXANTHINES

PARAMETER	THEOPHYLLINE	CAFFEINE	THEOBROMINE
Mol. weight	180.17	194.19	180.17
Melting point	270 - 274°C	238°C	357°C
Solubility (mg/ml)			
Water	8.3	21.7	0.5
Boiling Water	Highly	667	6.67
Ethanol	12.5	15.15	0.45
Chloroform	9.09	181.8	Almost insoluble
Ether	Sparingly	1.89	Sparingly
pka	8.6	1.2	9.9
pkb	13.5 11.5	-	

Table 1.4 A SUMMARY OF SUBSTANCES USED TO SOLUBILISE METHYLXANTHINES

METHYLXANTHINE	SOLUBILISING AGENT	REFERENCE
Caffeine	Acid sodium metaphosphate	German Patent, 1907
Theophylline	Piperazine, ethylenediamine, hexamethyl diamine, lysidine.	German Patent, 1907.
Theophylline	Sodium salicylate, sodium benzoate, potassium benzoate.	German Patent, 1922.
N-Acetyltheobromine	Sodium salicylate, lithium salicylate, ammonium salicylate, potassium salicylate.	German Patent, 1922.
Theobromine	Sodium salicylate.	German Patent, 1922.
Theobromine or theophylline	Calcium or strontium hydroxide plus salicylic acid.	Austrian Patent, 1933.
Theophylline	Monoethanolamine, triethanolamine, glucamine, methylglucamine, ethylglucamine	U.S. Patent, 1932.
Theophylline	Diethanolamine	German Patent, 1933.

Table 1.4 cont.

METHYLXANTHINE	SOLUBILISING AGENT	REFERENCE
Theophylline	Diethanolamine	German Patent, 1933.
Caffeine	Mandelic acid	German Patent, 1941.
Theophylline, theobromine and caffeine	Adenosine	Belgian Patent, 1942.
Theophylline	Primary and secondary aliphatic monoamines.	German Patent, 1943.
Theophylline	Piperazine, ethylenediamine	U.S. Patent, 1909.
Theophylline	Mono-, di-, and tri-isopropanolamine	U.S. Patent, 1927.
Theophylline	Mono-aminopolyhydric alcohols	U.S. Patent, 1939
Theobromine or theophylline	Sodium salicylate, strontium salicylate, calcium salicylate.	Brit. Patent, 1924.
Caffeine, theobromine or theophylline	N-Methylbenzamide, N-N'-Dimethylbenzamide, p-Toluylic dimethylamide, α-, β-, and γ Pyridine carboxylic diethylamide	Brit. Patent, 1937.

Table 1.4 cont.

METHYLXANTHINE	SOLUBILISING AGENT	REFERENCE
Theophylline	Aminoisobutanol	Steinberg and Jensen, 1945.
Theophylline	2-carbamoylphenoxyacetic acid (sodium salt)	German Patent, 1961.
Theophylline	Nicotinamide	Reiss, 1961; Cohen, 1966.
Theophylline	Choline, sodium acetate.	Martindale, 1972.

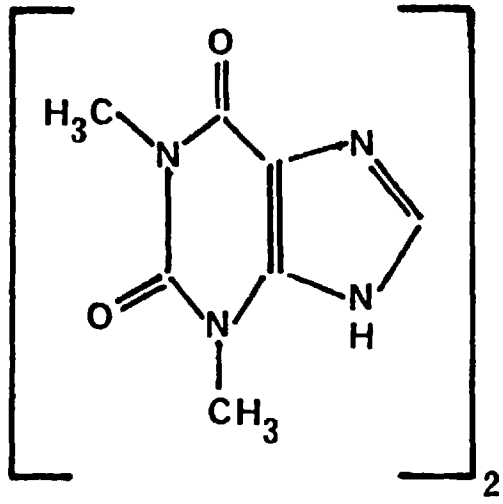
Table 1.5 SOME STABLE COMPLEXES OF THEOPHYLLINE

COMPOUND	REFERENCE
Saccharin	Brit. Patent, 1968.
Phenobarbital	U.S. Patent, 1935.
Papeverine	Mossini, 1939.
Caffeine	Reiss, 1961.
Sulphosalicylic acid	
Benzyl Alcohol	
α and β Naphthalene Acetate	

Table 1.6 SOME STABLE COMPLEXES OF CAFFEINE

COMPOUND	REFERENCE
Benzoic Acid	Higuchi and Zuck, 1953a
Aspirin	Higuchi and Zuck, 1953b
p-Hydroxybenzoic acid	
m-Hydroxybenzoic acid	
Salicylic acid	
Salicylate Ion	
Butyl Paraben	
Sulphathiazole	Higuchi and Zuck, 1954
Sulphadiazine	
p-Aminobenzoic acid	
Benzocaine	
Phenobarbital	
Barbital	

AMINOPHYLLINE



Theophylline

$C_2H_4(NH_2)_2$ $(H_2O)_2$

Ethylenediamine Water

MOL. WT. 456

Figure 1.3 THE COMPOSITION OF AMINOPHYLLINE ACCORDING TO THE BRITISH PHARMACOPOEIA

derivatives which were more soluble. Dessauer in 1908 introduced theophylline ethylenediamine, a more soluble preparation, and this stimulated further interest in the xanthine derivatives for the treatment of heart disease. Smith, Miller and Grabner (1925) compared the action of various xanthines on coronary blood flow in the isolated rabbit heart and found that theobromine increased the flow from 15 - 20%, theophylline 30 - 40% and theophylline ethylenediamine by 60 - 80%. Fowler, Hurevitz and Smith (1935) studied the effects of theophylline ethylenediamine on experimentally produced cardiac infarction in dogs and concluded that it was capable of improving collateral coronary circulation.

Cheyne-Stokes respiration occurs frequently in congestive heart failure, and by interfering with the patients rest and sleep, prevents improvement in the cardiac condition. Vogl (1932) was the first to use the xanthine derivatives (theophylline ethylenediamine) in the treatment of this condition. He found that by intravenously administering the drug it would abolish Cheyne-Stokes respiration in patients within a short time. His work was confirmed by many others, particularly Smith and his coworkers (1935;1938). Marais and McMichael (1937) claimed that the effect of theophylline ethylenediamine on Cheyne-Stokes respiration was due mainly to the ethylenediamine and that the theophylline itself was inactive. They found that 1 of 3 cases could be relieved by the administration of ethylenediamine alone. Later studies, particularly those of Nathanson and Fitzgibbon (1939) who repeated the experiments of Marais and McMichael, demonstrated that contrary to the above findings, ethylenediamine was completely ineffective in

abolishing Cheyne-Stokes breathing, and that theophylline was the active agent in theophylline ethylenediamine, and this has been the accepted explanation since.

The xanthine derivatives were used primarily for diuresis until Smith and his associates showed that these drugs were effective in dilating the coronary arteries. On the basis of this work, theophylline and theophylline ethylenediamine were used in congestive heart failure (Smith et al., 1935; Smith et al., 1926; Smith, 1928). The value of the xanthine derivatives, particularly of theophylline ethylenediamine in the treatment of congestive heart failure has since been verified by many workers.

While studying the effect of theophylline ethylenediamine in the treatment of congestive heart failure and Cheyne-Stokes respiration, it was found that it was also effective in relieving an attack of bronchial asthma (Hermann and Aynesworth, 1937). In the older age group of patients, a paroxysm of shortness of breath may be the result of bronchial asthma, emphysema or coronary artery disease. In such patients, with questionable cardiac status, adrenalin may precipitate left ventricular failure or other serious cardiac complications (Smith and Paul, 1938). Theophylline ethylenediamine therefore became the drug of choice at this time and was administered either as an intravenous solution or as a rectal suppository.

Pharmacological Properties

Caffeine, theophylline and theobromine share in common several pharmacological actions of therapeutic interest. They stimulate the central nervous system, act on the kidney to produce diuresis, stimulate cardiac muscle, and relax smooth muscle, notably bronchial muscle. Because the various methylxanthines differ markedly in the

intensity of their actions on various structures, one particular methylxanthine is usually more suitable than the others for any specific therapeutic effect. Table 1.2 denotes the relative potencies of the methylxanthines in producing their various effects.

Metabolism of the Xanthines and Methylxanthines

Xanthines: The extent of degradation of the purine ring system varies greatly from species to species. In man and other primates, the principal excretory product resulting from purine catabolism is uric acid, in which the purine ring system remains intact, whereas in some marine invertebrates, ammonia and carbon dioxide are the end products. Table 1.7 illustrates the metabolic route for the degradation of purines.

Methylxanthines: The major metabolic pathways of caffeine, theophylline and theobromine are illustrated in figure 1.4. Since the development of the enzymatic uricase method for the determination of uric acid, all studies have indicated that there is no appreciable increase in true uric acid excretion upon ingestion of caffeine, theophylline or theobromine. Increases in the excretion of material which give colour with uric acid reagents after the ingestion of caffeine and theophylline can be attributed to the presence of the chromogenic methyluric acids. After the ingestion of theobromine, the major excretory products found in urine are 7-methylxanthine, 3-methylxanthine, and unchanged theobromine. Small amounts of 7-methyluric acid are also excreted.

Theophylline differs from theobromine in that both of the methyl groups are on the pyrimidine ring, leaving the imidazole ring unsubstituted. The main excretory products are 1, 3-dimethyluric acid, 1-methyluric acid, 3-methylxanthine and unchanged theophylline. The oxidation of theophylline without demethylation appears to be the major metabolic pathway. In addition, a portion of the theophylline is demethylated at position one to give 3-methylxanthine. Two possible pathways for the formation of 1-methyluric acid are (1) demethylation of

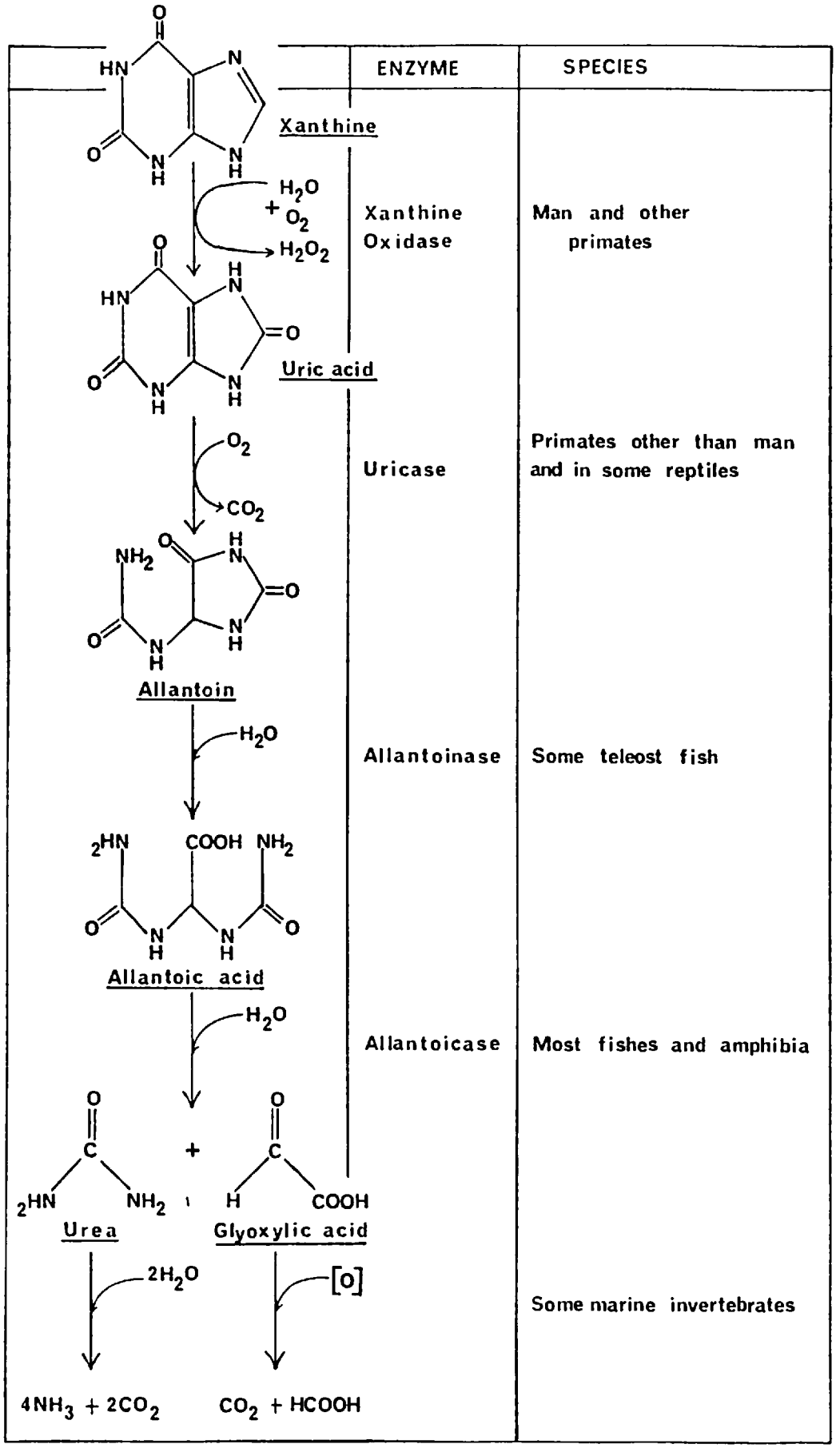


Table 1.7 THE FINAL STEP OF PURINE CATABOLISM IN VARIOUS SPECIES

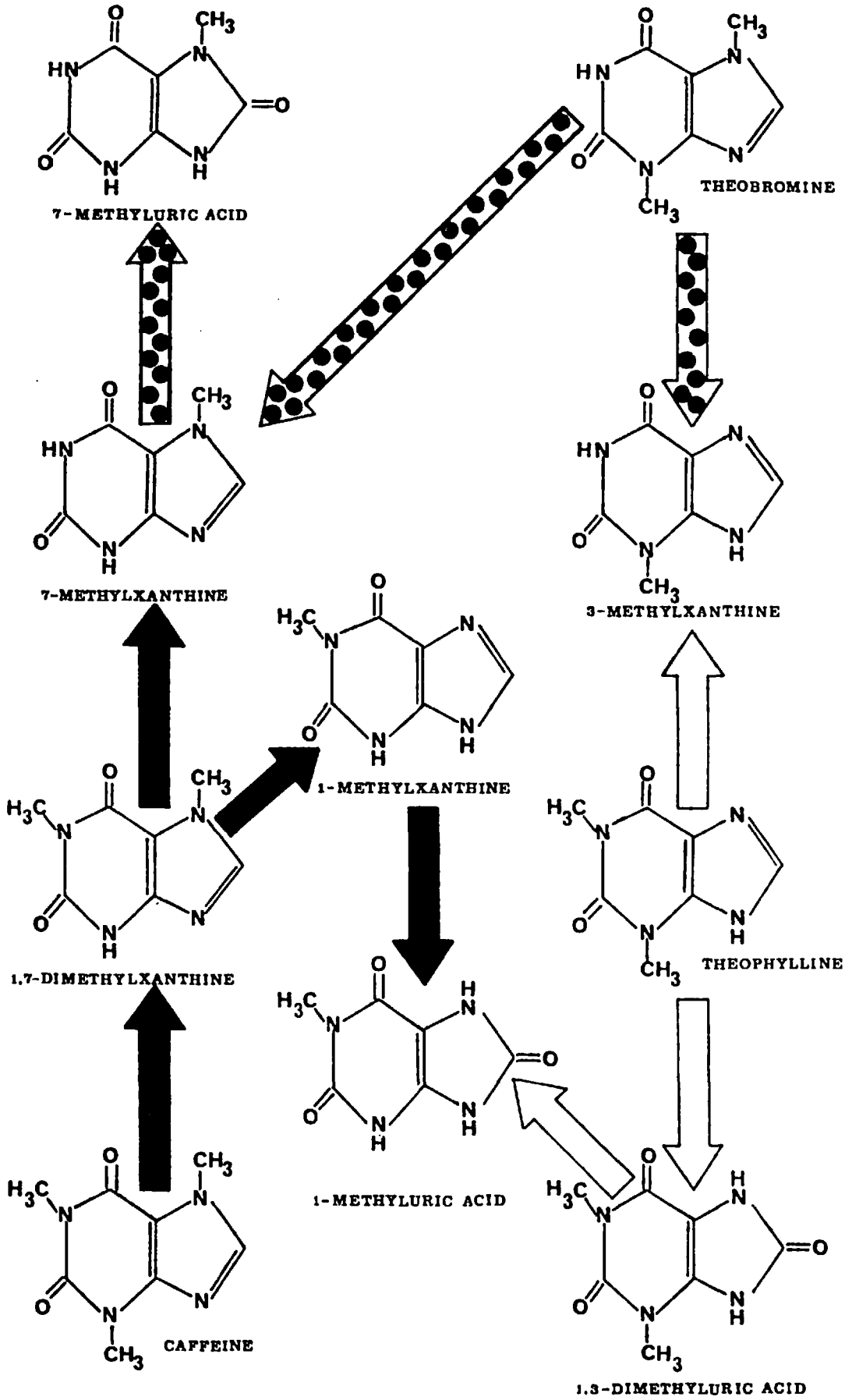


Figure 1.4 THE MAJOR METABOLIC ROUTES OF THE DIETARY METHYLXANTHINES, CAFFEINE (CLOSED ARROWS), THEOPHYLLINE (OPEN ARROWS) AND THEOBROMINE (DOTTED ARROWS)

1,3-dimethyluric acid or (2) direct oxidation of 1-methylxanthine. Although this latter compound has not been identified as one of the excretory products of theophylline metabolism, it may be an intermediate. Studies on theophylline metabolism by the rat liver microsomal system (Lohmann and Miech, 1976) have shown 1-methylxanthine to be generated, and that this metabolite is a substrate for xanthine oxidase and that its conversion to 1-methyluric acid is blocked by allopurinol. However, the urine content of 1,3-dimethyluric acid and 1-methyluric acid in man, has been shown to be directly related (Jenne et al., 1976) suggesting that the former may give rise to the latter.

It is clear that theobromine and theophylline follow somewhat different pathways of metabolism. The major products of theophylline metabolism in man are methyluric acids while the major products of theobromine metabolism are methylxanthines.

1-methyluric acid and 1-methylxanthine are the major metabolites found in urine after caffeine ingestion, with smaller amounts of 1,7-dimethylxanthine, 7-methylxanthine, 1,3-dimethyluric acid and unchanged caffeine. Caffeine is almost completely metabolised not only in man but in most other species in which it has been studied (Table 1.8). The plasma elimination half-life of caffeine in man and six other species is given in Table 1.9.

That demethylation of caffeine, theophylline and theobromine in man does not proceed beyond the monomethylxanthines is supported by the facts that no accumulation of xanthine in urine is observed and that insignificant, if any, increases in uric acid excretion occur (Cornish and Christman, 1957).

Table 1.8

EXCRETION OF UNCHANGED CAFFEINE AFTER ITS ORAL ADMINISTRATION TO VARIOUS SPECIES

SPECIES	% DOSE UNCHANGED	REFERENCE
Man	0.5 - 1.5	Axelrod and Reichenthal, 1952
	11.5	Cornish and Christman, 1957
	0.5 - 1.5	Schmidt and Schoyerer, 1966
Rat	2.0	Usanova and Shnol, 1958
	9.0	Rao et al., 1973
	4.1	Arnaud, 1976
Mouse	3.0 - 6.0	Burg and Stein, 1972
Rabbit	13.3	
Pig	6.0	Cunningham, 1970
Squirrel Monkey	4.0 - 6.0	Burg et al., 1974
Dog	Trace	Axelrod and Reichenthal, 1952

Table 1.9

PLASMA ELIMINATION HALF-LIFE OF CAFFEINE IN VARIOUS SPECIES

SPECIES	PLASMA $t_{\frac{1}{2}}$(HRS)	REFERENCE
Man	3.5	Axelrod and Reichenthal, 1952
Rhesus Monkey	2.4	Burg et al., 1974
	2.8	
Squirrel Monkey	11.0	Burg et al., 1974
Pig	10.0 - 15.0	Cunningham, 1970
Dog	5.0	Axelrod and Reichenthal, 1952
Rat	2.0	Czok et al., 1969
Mouse	0.6	Burg and Werner, 1972

Clinical Pharmacology of Theophylline

The wide variability of clinical response observed in patients receiving theophylline therapy has led to numerous studies attempting to correlate the observed clinical response to differing theophylline plasma levels. These studies have shown that there is a direct relation between theophylline plasma levels and the observed clinical response. However, there is a narrow margin between the therapeutic and toxic levels of theophylline such that relatively small variations in plasma levels can cause wide variations in the clinical response. For effective bronchodilation a serum or plasma concentration of about 10µg/ml is often required (Green, 1969; Jackson et al., 1964; Jenne et al., 1972; Mitenko and Ogilvie, 1973; Nicholson and Chick, 1973; Weinberger and Bronsky, 1974) although Mitenko and Ogilvie (1973) showed a dose-related improvement in pulmonary function in the range 5 to 20µg/ml plasma. At plasma levels above 20µg/ml serious toxic effects occur (Weinberger et al., 1976; Zwillich et al., 1975; Jacobs et al., 1976) such as agitation, convulsion (Bresnick et al., 1948; McKee and Haggerty, 1957) persistent nausea and vomiting or anorexia (Jenne et al., 1972).

Mitenko and Ogilvie (1973) and Jenne et al. (1972) quantitated the pharmacokinetic parameters for theophylline in humans, and dosage schedules based upon average values for these parameters have been widely recommended (Piafsky and Ogilvie, 1975). However, many patients receiving such regimens develop steady-state plasma theophylline levels outside the therapeutic range (Weinberger et al., 1976) and are therefore exposed to potentially toxic or subtherapeutic therapy, both of which represent a hazard.

What factors are responsible for the individual variation in response to theophylline therapy? While various pharmacokinetic parameters for theophylline have been determined, little overall difference has been noted between normal individuals and asthmatics. It is theophylline's widely varying elimination kinetics, manifested in terms of a variable β -phase half-life, and peak plasma levels, which account for the observed clinical variability in response. As only about 10% of an administered dose of theophylline is excreted from the body in unchanged form (Cornish and Christman, 1957; Jenne et al., 1976; Desiraju et al., 1977) and as the plasma clearance of theophylline is much smaller than liver blood flow, individual variation in the rate of theophylline metabolism is probably responsible for the clinical variability in response. Therefore, the clinical response to theophylline will be primarily dependent on factors influencing the activity of the hepatic microsomal enzyme system. Factors known to influence drug metabolism are listed in Table 1.10 and those that have been demonstrated to influence theophylline metabolism (in terms of changes in its plasma elimination half-life) are listed in Table 1.11.

Table 1.10

FACTORS KNOWN TO INFLUENCE DRUG METABOLISM

1. Genetic Factors
   - Species
   - Strain
   - Race (Caucasian, Oriental, Negroid etc.)
   - Sex

2. Environmental Factors
   a) Pharmacological variables
      - Acute or chronic administration
      - Bioavailability
      - Dose, size and form
      - Presence of other drugs
      - Routes of administration
      - Development of tolerance

   b) Dietary variables
      - Malnutrition, starvation, obesity
      - Protein/carbohydrate balance
      - Method of cooking
      - Food additives (intentional and unintentional)

3. Miscellaneous Factors
   - Age
   - Disease
   - Hormone balance (Pregnancy, estrous cycle)
   - Body weight
   - Others

Table 1.11 THEOPHYLLINE ELIMINATION RATES IN VARIOUS GROUPS

GROUP OR FACTOR	HALF-LIFE (HRS.) NORMAL GROUP	AFFECTED GROUP	REFERENCE
Asthmatic Children	-	2.65	Maselli et al., 1970
Hospitalised Patients	-	3.6	Jenne et al., 1975
Asthmatic Children	-	3.4	Loughnan et al., 1976
Asthmatic Children	5.8	3.7	Ellis et al., 1976
Smokers	7.2	4.1	Jenne et al., 1975
Smokers	7.0	4.3	Hunt et al., 1976
Adult Asthmatics	4.4	4.3	Mitenko and Ogilvie, 1973
Charcoal Broiled Beef Diet	6.0	4.7	Kappas et al., 1978
High Protein Low Carbohydrate Diet		5.2	Kappas et al., 1976
Pulmonary Diseased Adult Patients		5.2	Jenne et al., 1972

Table 1.11 cont.

GROUP OR FACTOR	HALF-LIFE (HRS.) NORMAL GROUP	AFFECTED GROUP	REFERENCE
Hospitalised Smokers	6.0	5.9	Piafsky et al., 1977a
Phenobarbitone	5.0	6.4	Piafsky et al., 1977b
Adult Males with Chronic-Obstructive Airways Disease		7.1	Elwood et al., 1978
Infants with Theophylline Poisoning		8.8	Vaucher et al., 1977
Obesity	6.0	8.6	Gal et al., 1978
Obese Smokers	9.0	8.2	Gal et al., 1978
Troleandomycin Administration	4.6	11.3	Weinberger et al., 1976
Congestive Heart Failure	8.6	16.1 - 64.9	Jenne et al., 1977
Acute Pulmonary Oedema	9.2	18.6	Piafsky et al., 1974
Apneic Infants		19.9	Giacoia et al., 1976

Table 1.11 cont.

GROUP OR FACTOR	HALF-LIFE (HRS.) NORMAL GROUP	AFFECTED GROUP	REFERENCE
Elderly Patients with Acute Pulmonary Oedema	6.7	22.9	Piafsky et al., 1977c
Hepatic Cirrhosis	6.7	25.6	Piafsky et al., 1977d
Cirrhosis	9.2	30.0	Piafsky et al., 1975
Female suffering from Seizure, Theophylline Neurotoxicity, Chronic Alcoholism, Jaundice & Increasing Ascites		54.5	Lawyer et al., 1978
Apneic Premature Newborns		30.2	Aranda et al., 1976

Factors Affecting Drug Metabolism

When a drug enters the body, one of three events may occur. Firstly, it may be metabolised by enzymes, secondly, be changed spontaneously without the involvement of enzymes or thirdly it could be excreted from the body in its unchanged form. The majority of drugs do in fact undergo enzyme-catalysed biotransformations in the body and the biochemical reactions that constitute the study of drug metabolism are numerous and diverse. The principal routes of drug metabolism were defined in detail by Williams (1959).

Most drugs undergo metabolism in the body in two phases (Table 1.12). The first phase involves reactions that can be classified as oxidations, reductions and hydrolyses. These reactions are catalysed by enzymes, many of which are located in the endoplasmic reticulum of the liver cell. The oxidative metabolism of many drugs and also of steroid hormones is carried out by these enzymes. If liver is homogenised and the homogenate centrifuged at 9,000 - 12,000 x g for 30 minutes, the supernatant collected and centrifuged at 105,000 x g for one hour, the sediment thus collected contains particles of fragmented endoplasmic reticulum, or microsomes. These microsomes contain a haemoprotein(s) known as cytochrome P450, which acts as the terminal oxidase for a variety of oxidative reactions that drugs undergo. The term P450 refers to the ability of the reduced form of the haemoprotein to react with carbon monoxide, yielding a complex with absorption peak at 450nm.

In the second phase reactions of drug metabolism, the products of the first phase undergo synthetic reactions again catalysed by enzymes occuring mainly in the liver but also to some extent in other tissues. These synthetic reactions are commonly referred to as conjugations and many kinds of molecules normally present in the body

Table 1.12 GENERAL PATHWAYS OF DRUG METABOLISM

Phase I Reactions:-

1. Oxidations

hydroxylations

dealkylations

oxide formation

desulphuration

dehalogenation

alcohol oxidation

aldehyde oxidation

2. Reductions

aldehyde reduction

azoreduction

nitroreduction

3. Hydrolyses

deesterification

deamidation

Phase II Reactions:-

glucuronic acid conjugation

acetylation

methylation

mercapturic acid formation

sulphate conjugation

various amino acid conjugations

can react with drugs or drug metabolites to form so-called conjugates (Table 1.13). Any factor that influences the phase I or phase II enzymes can subsequently affect the rate and route of drug metabolism.

Genetic Factors Affecting Drug Metabolism

The rate and manner in which an individual metabolises drugs is partly determined by his or her genetic inheritance. The genes concerned can be conveniently divided into two groups, those of large effect and those of small effect.

Genes of large effect

These are genes which, on their own, can determine a recognisable character in an individual. Their recognition enables the identification of different phenotypes within a given population and therefore the existence of polymorphism. That is, a frequency distribution curve of a large enough population sample, with respect to a measurable pharmacological variable (peak plasma levels, plasma half-life, ratio of unchanged drug to metabolites in urine) shows discrete groups corresponding to different phenotypes. These frequency distribution curves are called multimodal or discontinuous (Figure 1.5). Such genes influence drug metabolism by determining the amount and/or the activity of particular enzymes. The frequency with which such a genetically determined polymorphism occurs depends upon the particular gene frequency within the population studied. Such gene frequencies differ among different population groups and can lead to an uneven world distribution.

Several examples of polymorphic control of a drug metabolic pathway have been identified (Table 1.14), the most widely quoted example being that of isoniazid acetylation (Evans et al., 1960). Rapid inactivation (acetylation) is inherited as a dominant character which confers high levels of the inactivating acetyltransferase enzyme in homozygous individuals.

Table 1.13

SOME COMPOUNDS KNOWN TO ACT AS CONJUGATING AGENTS IN DRUG METABOLISM

Glucuronic acid	Ornithine
Sulphate	Glutamine
Glutathione	Taurine
Methylation	Glucose
Acetylation	
Mercapturic acid	
Glycine	

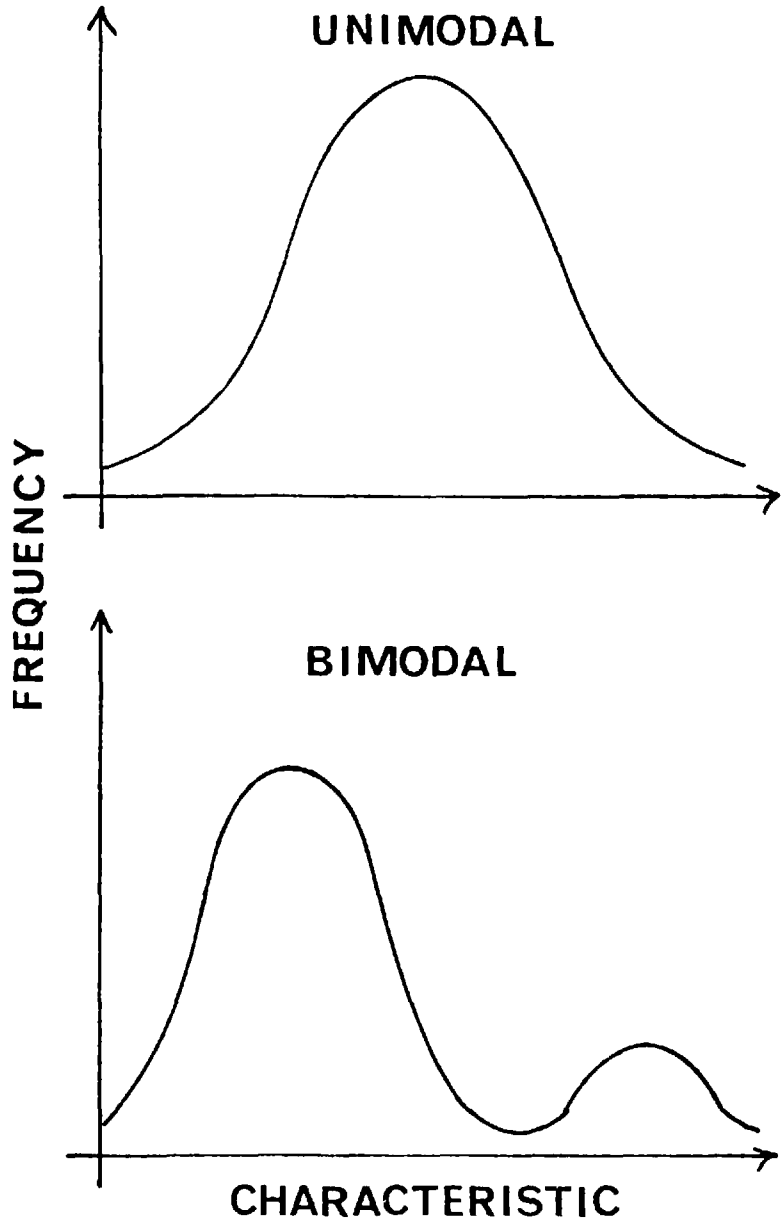


Figure 1.5 FREQUENCY DISTRIBUTION CURVES OF A UNIMODALLY AND BIMODALLY DISTRIBUTED CHARACTERISTIC

Table 1.14 DRUGS WHOSE METABOLISM IS DETERMINED BY GENES OF LARGE EFFECT

DRUG	MECHANISM OF METABOLISM	COMMENTS	REFERENCE
Isoniazid	Acetylation	Rapid inactivation of the drug is inherited as a dominant character which confers the presence of large amounts of the acetyltransferase concerned	Bonicke and Reif, 1953; Bonicke and Lisboa, 1957. Evans et al., 1960
Sulphamethazine and Hydrallazine	Acetylation	Authors demonstrated a coincidence of acetylation between isoniazid and sulphamethazine in vivo and hydrallazine in vitro	Evans and White, 1964
Hydrallazine	Acetylation	Slow inactivators of isoniazid require smaller doses of hydrallazine for blood pressure control, show toxic signs more commonly and develop antinuclear antibodies with the drug	Zacest and Koch-Weser, 1972 Perry et al., 1967
Phenelzine	Acetylation	Slow inactivators appear to be more susceptible to the drugs unwanted side effects	Evans et al., 1965

Table 1.14 cont.

DRUG	MECHANISM OF METABOLISM	COMMENTS	REFERENCE
Sulphamethoxy-pyridazine	Acetylation	Slow inactivators appear to be more susceptible to the drugs unwanted side effects	White and Evans, 1968
Dapsone	Acetylation	Slow inactivators appear to be more susceptible to the drugs unwanted side effects	Gelber et al, 1971
Diphenylhydantoin	Hydroxylation	Autosomal dominant trait in which affected individuals develop toxicity at "ordinary" dose levels, correlated to extremely high blood levels	Kutt et al., 1964
Debrisoquine	Hydroxylation	Ratio of debrisoquine and its major metabolite in urine was bimorphically distributed in study population. Family studies indicated that the alicyclic 4-hydroxylation of the drug is controlled by a single autosomal gene and that a defect in this metabolic step is caused by a recessive	Mahgoub et al., 1977

Table 1.14 cont.

DRUG	MECHANISM OF METABOLISM	COMMENTS	REFERENCE
Phenacetin	Dealkylation	Unusual reaction in two sisters. Deficient in liver microsomal enzyme for dealkylation. Alternative metabolic route, deacylation followed by hydroxylation, generates toxic metabolite causing haemolysis and methaemaglobinemia	Shahidi, 1968
Phenacetin and Guanoxan	Dealkylation and aromatic hydroxylation	The alicyclic hydroxylation defect that has previously been demonstrated for debrisoquine metabolism carries over to the oxidative metabolism of phenacetin and guanoxan	Sloan et al., 1978
Suxamethonium	Hydrolysis	In rare individuals the drug induces neuromuscular blockade of several hours duration. Many of these individuals possess an atypical plasma cholinesterase which is responsible for the metabolism of the drug. The atypical cholinesterase hydrolyses various substrates at considerably reduced rates	Harris, 1964 Kalow, 1962

Table 1.14 cont.

DRUG	MECHANISM OF METABOLISM	COMMENTS	REFERENCE
Sparteine	N-oxidation	Conversion to dehydrosparteine is determined by two autosomal allegic genes at a single locus, where non-metabolisers are homozygous for an autosomal recessive gene. The defect does not extend to either amobarbital metabolism or the conversion of clozapin to its N-oxide	Eichelbaum et al., 1978
Amobarbital	C-hydroxylation and N-glucosidation	Volunteers of oriental origin produce more of the N-glucoside than of the hydroxylation product. However, in Caucasians the reverse holds true. A possible deficiency in the N-glucoside pathway in both groups inherited as a recessive trait controlled by a single pair of allelic autosomal genes	Kalow et al., 1978

This acetylation polymorphism affects the metabolism of other drugs (Table 1.14). However, sulphanilamide and p-aminobenzoic acid which appear in urine in acetylated form, do not show this polymorphism and presumably a different enzyme is responsible for the acetylation of these drugs (Evans and White, 1964). The gene responsible for rapid isoniazid inactivation is thought to be of mongoloid origin, as its population frequency is greatest among Eskimos and Japanese and lowest among some Mediterranean Jews (Motulsky, 1964).

The adrenergic blocking agent, debrisoquine, undergoes significant hepatic first-pass metabolism, the extent of which is variable, and has recently been shown to be subject to genetic control. This drug is metabolised by hydroxylation mainly in the alicyclic 4-position, but also, to a smaller extent, in the aromatic 5-, 6-, 7- and 8- positions (Angelo et al., 1978; Mahgoub et al., 1977). The alicyclic 4-hydroxylation of debrisoquine in man is polymorphic, since one group excrete some 30 - 40% of an oral dose of the antihypertensive as the 4-hydroxy metabolite, while a much smaller group excrete only 3 - 4% in this form. The extent to which a subject effects the 4-hydroxylation of the drug appears to be an inherited characteristic (Mahgoub et al., 1977) under the control of a single autosomal gene, with the defect in the metabolic step being caused by a recessive allele. Furthermore, there is a correlation between the hypotensive response to debrisoquine and the extent of its metabolism in normotensive subjects (Angelo et al., 1975). The hypotensive responses are most marked in subjects who are poor metabolisers of the drug and the converse appears to hold for extensive metabolisers (Lancaster et al., 1978).

Genes of small effect

The metabolism of most oxidised drugs follow a continuous unimodal distribution (Vesell, 1972). Such curves are typical of variables which are multifactorially determined. Thus genes of small effect do not produce recognisable characters on their own but, acting together, a large number of them contribute to individual deviation in a normally distributed population (Figure 1.5). Although inheritance is a determinant of drug metabolism, the degree to which it is responsible for the variability within a population is uncertain. Indeed, as environmental influences differ both from one population to another and from one individual to another, it will be expected that the apparent heritability will also differ somewhat.

Environmental Factors Affecting Drug Metabolism in Man

During the 1960's it became clear that the phenomenon of enzyme induction, that being the ability of some compounds to stimulate the synthesis of drug-metabolising enzymes, could be of clinical significance in man (Conney, 1967). Table 1.15 lists some of those drugs known to induce drug metabolism in man. Several studies demonstrated that drugs of the phenobarbitone class of inducers could produce clinically important interactions with other drugs by increasing their oxidative metabolism. Such interactions are of the greatest importance with drugs which require regulation within narrow plasma concentration limits, for example anticoagulants, antiepileptics, hypoglycaemics and theophylline. Phenobarbitone has been shown to increase the rate of metabolism of theophylline *in vitro* (Jenne, 1975; Williams *et al.*, 1975; Lohmann and Meich, 1976) but studies in normal male subjects have shown no significant effects of phenobarbitone on theophylline kinetics (Piafsky *et al.*, 1977b).

Table 1.15 SOME DRUGS KNOWN TO CAUSE MICROSOMAL ENZYME INDUCTION IN MAN

Inducer	Drug Affected	Reference
Barbiturates	Tricyclic Antidepressants	Alexanderson et al., 1969 Hammer et al., 1967
	Griseofulvin	Busfield et al., 1963
	Phenytoin	Kristensen et al., 1969 Morselli et al., 1971
	Digitoxin	Jelliffe and Blankenhorn, 1966
	Anticoagulants	Burns and Conney, 1965 Cucinell et al., 1965 Dayton et al., 1961 Goss and Cickhaus, 1965 Robinson and MacDonald, 1966
Dichloralphenazone	Coumarin Type Anticoagulants	Breckenridge et al., 1971

Table 1.15 cont.

Inducer	Drug Affected	Reference
Antipyrine		
Carbamazepine	Coumarin type anticoagulants	Hansen et al., 1971
Griseofulvin	Coumarin type anticoagulants	Catalona and Cullen, 1966
Phenylbutazone	Aminopyrine	Chen et al., 1962
Glutethimide	Glutethimide	Schmid et al., 1964
	Warfarin	MacDonald et al., 1969
	Dipyrone	Remmer, 1962
Chloral Hydrate	Bishydroxycoumarin	Cucinell et al., 1966

Inhibition of metabolism by drugs which are themselves substrates of the cytochrome P450 enzyme system also occurs in clinical practice (Table 1.16). For example, diphenylhydantoin metabolism is inhibited by a number of drugs and warfarin metabolism can also be inhibited by several drugs (Breckenridge and Orme, 1971). Troleandomycin, a macrolide antibiotic, has been shown to inhibit theophylline clearance (Weinberger et al., 1977) probably by either decreasing hepatic uptake or altering enzymatic degradation in the liver, and another antibiotic, chloramphenicol, has been reported to inhibit the metabolism of a variety of co-administered drugs (Christensen and Skovsted, 1969; Petitpierre and Fabre, 1970).

Environmental Chemicals

The effects of non-drug inducing agents present in the environment on drug metabolism has also been studied. A number of environmental chemicals, particularly insecticides and industrial toxins have been shown to affect drug metabolism in both man and animals (Conney and Burns, 1972). Table 1.17 lists some environmental chemicals known to affect drug metabolism in man.

Socially Used Drugs

The "socially accepted" drugs tobacco, fermented beverages, the oral contraceptive pill and the methylxanthine-containing beverages, have all been shown to have significant effects on drug metabolism. Cigarette smoke contains 3-methylcholanthrene, 3-4 benzo(a)pyrene and other polycyclic hydrocarbons and these substances as well as nicotine are all drug metabolising enzyme inducers in animals (Wenzel and Broadie, 1966; Yamamoto, et al., 1966; Mitoma et al., 1968). The activity of benzo(a)pyrene hydroxylase for example is increased in the lungs, livers, intestines and placentas of human cigarette smokers (Welch et al., 1969). In vitro studies (Miech and Lohmann, 1975) have shown that pretreatment of rat liver microsomes with 3-methylcholanthrene can increase the rate of theophylline degradation,

Table 1.16 SOME DRUGS KNOWN TO CAUSE INHIBITION OF DRUG METABOLISM IN MAN

Inhibitor	Drug Affected	Reference
Disulphiram	Antipyrine	Vesell et al., 1971
	Warfarin	Rothstein, 1968
	Phenytoin	Olesen, 1966
Sulthiame	Phenytoin	Olesen and Jensen, 1969
		Hansen et al., 1968
Dicoumarol	Phenytoin	Hansen et al., 1966
	Tolbutamide	Kristensen and Hansen, 1967
Chloramphenicol	Tolbutamide	Christensen and Skovsted, 1969
	Chlorpropamide	Petitpierre and Fabre, 1970
Sulphaphenazole	Tolbutamide	Christensen et al., 1963
		Schulz and Schmidt, 1970
Para-Aminosalicylate	Isoniazid	Hanngren et al., 1970
Allopurinol	Antipyrine	Vesell et al., 1970
	Dicoumarol	

Table 1.16 cont.

Inhibitor	Drug Affected	Reference
Nortriptyline	Antipyrine, Dicoumarol	Weinberger et al., 1977
Troleandomycin	Theophylline	

Table 1.17 ENVIRONMENTAL CHEMICALS KNOWN TO AFFECT DRUG METABOLISM IN MAN

Agent	Drug Affected	Comments	Reference
1. Insecticides Chlorinated hydrocarbons Lindane D.D.T. Chlordane Endrin Dieldrin	Antipyrine	Antipyrine half-lives in male workers in an insecticide factory were significantly shorter than in office workers from the same factory (7.7 ± 2.6 hours, 13.1 ± 7.5 hours respectively.	Kolmodin et al., 1969
D.D.T.	Phenylbutazone	D.D.T. factory workers with D.D.T. and D.D.E. levels twenty times those of controls, eliminated phenyl-butazone faster than controls.	Poland et al., 1970

Table 1.17 cont.

Agent	Drug Affected	Comments	Reference
Aroclor 1016	Antipyrine	Five workers occupationally exposed to Aroclor 1016 showed significantly lower mean antipyrine half-lives (10.8h) than the mean half-life in non-PCB's-exposed normal subjects	Alvares and Kappas, 1977
Lindane	Phenylbutazone	A decrease in the plasma elimination half-lives of phenylbutazone in workers	Kolmodin-Hedman, 1973
D.D.T.	Antipyrine	The accelerated elimination of some drugs induced by chlorinated pesticides was long-lasting, the antipyrine half-life in 15 conifer workers exposed to D.D.T. being 9.8 ± 2.8hr during the spraying season and 10.9 ± 3.8 hr 8 months after exposure	Alvares et al., 1975; 1976

Table 1.17 cont.

Agent	Drug Affected	Comments	Reference
2. Heavy Metals Lead	Antipyrine	Exposure to lead in industry shown to prolong antipyrine half-life, although only clinically overt poisoning produces a degree of impairment likely to be clinically significant	Alvares et al., 1975; 1976

stimulation of caffeine metabolism in the rat, *in vivo*, by 3-methylcholanthrene also occurs (Aldridge *et al.*, 1977). The plasma theophylline half-life of adult cigarette smokers is less than that of normal adult non-smokers (Jenne *et al.*, 1975; Hunt *et al.*, 1976).

The effects of these "socially accepted" drugs on drug metabolism are summarised in Table 1.18.

Nutritional Factors Affecting Drug Metabolism

Many aspects of the impact which nutrition may have on drug metabolism have been studied (Campbell, 1977; Conney *et al.*, 1977) and there is ample evidence that it is a significant determinant of drug action (Table 1.19). Both the overall nutritional status of an individual and the composition of his diet can affect drug metabolism. Three types of dietary influence have been discerned. The first arises from variations in macronutrient intake (the balance of protein, carbohydrate and fat in the diet) the second results from variations in micronutrient intake (vitamins and minerals etc.) and the third from anutrient chemicals present in food stuffs *per se* (colourings, flavourings, antioxidants, preservatives etc.) or produced as a result of cooking processes.

The metabolism of theophylline, as assessed by its plasma elimination half-life, is increased in normal volunteers by changing from a low carbohydrate, high protein diet ($t_{\frac{1}{2}}$ 5.2h) to a high carbohydrate, low protein diet ($t_{\frac{1}{2}}$ 8.1h) (Kappas *et al.*, 1976). When beef is cooked by broiling over charcoal, steroids in the meat are converted to polycyclic aromatic hydrocarbons which are powerful inducers of the drug metabolising enzymes (Lijinsky and Shubik, 1964). Diets rich in charcoal broiled beef accelerate the metabolism of a range of drugs in animals and man (Conney *et al.*, 1977). Feeding a charcoal-broiled beef diet to human volunteers for five days decreases the plasma elimination half-life of theophylline (Kappas *et al.*, 1978).

Table 1.18

SOCIALLY-ACCEPTED DRUGS KNOWN TO AFFECT DRUG METABOLISM IN MAN

AGENT	DRUG AFFECTED	COMMENTS	REFERENCE
1. Cigarette smoke 3-methylcholanthrene 3-4, benzo(a)pyrene Nicotine Various polycyclic hydrocarbons	Propoxyphene	A dose of either 65mg in 80% of patients or 32mg in 20% was less effective among smokers	Boston Collaborative Drug Surveillance Program, 1973a
	Chlordiazepoxide and Diazepam	A strong association between adverse reactions to the two drugs and smoking. The frequency of drowsiness was highest in non-smokers, intermediate in light smokers and lowest in heavy smokers	Boston Collaborative Drug Surveillance Program, 1973b
	Chlorpromazine	Among recipients of the drug in a psychiatric population, the frequency of drowsiness was highest in 130 non-smokers (16%)	Swett, 1974 Jick, 1974

Table 1.18 cont.

AGENT	DRUG AFFECTED	COMMENTS	REFERENCE
		intermediate in 201 light smokers (11%) and lowest in 72 heavy smokers (3%)	
	Nicotine	Cigarette smokers have faster elimination rates both for nicotine and other drugs	Beckett and Triggs, 1967; Beckett et al., 1971
	Theophylline	Plasma elimination half-life is significantly shorter in smokers than in non-smokers	Jenne et al., 1975 Hunt et al., 1976 Piafsky et al., 1977
	Antipyrine	Antipyrine clearance was increased by 25% in moderate smokers, but heavy smoking produced no further increase	Vestal et al., 1975
		An increase of 23% was seen in the antipyrine half-life of 8 subjects who stopped smoking and were restudied two months later	Hart et al., 1976

Table 1.18 cont.

AGENT	DRUG AFFECTED	COMMENTS	REFERENCE
	Phenacetin	Plasma concentration of phenacetin in smokers was lower than that in non-smokers, whereas the ratio of the concentration of N-acetyl-p-aminophenol (APAP) to that of phenacetin was increased severalfold in the smokers. No difference in the plasma elimination half-life or in the amount of APAP excreted was found between smokers and non-smokers	Kuntzman et al., 1977 Pantuck et al., 1974
2. Fermented Beverages Alcohol	Tolbutamide, Warfarin and Phenytoin	All three drugs show accelerated clearance rates in heavy drinking, unemployed, male alcoholics when compared	Iber, 1977

Table 1.18 cont.

AGENT	DRUG AFFECTED	COMMENTS	REFERENCE
		to both normal subjects and to patients with cirrhosis who are not drinking	
	Antipyrine	Long-term ethanol injestion increased metabolism of drugs in some, but not all subjects	Vesell et al., 1971
	Ethanol, Pentobarbitone and Meprobamate	Chronic administration of ethanol accelerates elimination of the three drugs	Misra et al., 1971
	Phenobarbitone and Meprobamate	Acute doses large enough to maintain blood concentrations in the region 0.5 - 1.0 mg/ml prolong the half-lives of the two drugs	Rubin et al., 1970

Table 1.18 cont.

AGENT	DRUG AFFECTED	COMMENTS	REFERENCE
3. Steroid oral contraceptive	Antipyrine	Slows the elimination of antipyrine by increasing the volume of distribution (after long term use) and apparently decreasing the rate of metabolism.	O'Malley et al., 1972 Carter et al., 1974; 1975
		Increases total liver size and inhibits antipyrine metabolism.	Homeida et al., 1978

Table 1.19 NUTRITIONAL FACTORS KNOWN TO AFFECT DRUG METABOLISM IN MAN

FACTOR	DRUG AFFECTED	COMMENTS	REFERENCE
1. Macronutrient Intake Malnutrition	Antipyrine	Antipyrine half-life was shortened in undernourished subjects who were smokers and drank alcohol and exposed to various pesticides. However, half the subjects with severe protein/calorie malnutrition had half-lives prolonged by 12-13%.	Krishnaswamy and Naidu, 1977
	Testosterone and Estradiol	Metabolism of labelled testosterone and estradiol altered in young women with anorexia nervosa.	Fishman and Bradlow, 1977
Obesity	Theophylline	Plasma elimination half-life prolonged.	Gal et al., 1978

Table 1.19 cont.

FACTOR	DRUG AFFECTED	COMMENTS	REFERENCE
Protein/Carbohydrate Balance	Antipyrine and Theophylline	The average plasma half-life of antipyrine and theophylline decreased by 41% to 36% respectively when the diets of normal volunteers were changed from their customary diets to diets that contained high protein and low carbohydrate. When shifted to a high carbohydrate low protein diet the average half-lives increased 63% and 43% respectively	Conney et al., 1977
2. Anutrients Charcoal broiled beef containing various indoles	Antipyrine	Plasma half-life significantly decreased on feeding volunteers a charcoal-broiled beef containing diet for 4 days.	Conney et al., 1976

Table 1.19 cont.

FACTOR	DRUG AFFECTED	COMMENTS	REFERENCE
	Phenacetin	A similar diet decreased plasma levels of phenacetin without altering plasma concentrations of its major metabolite, N-acetyl-p-aminophenol, or the plasma half-life of phenacetin. Suggests that such a diet enhances phenacetin metabolism in the gastro-intestinal tract and/or during its first-pass through the liver.	Pantuck et al., 1976
	Theophylline	Mean plasma half-life decreased by 22% after subjects were fed the charcoal broiled beef-containing diet. Considerable variation in degree of responsiveness to the diet.	Kappas et al, 1978

Many plant foods may also cause enzyme induction, notably cabbage and Brussel sprouts which contain a number of indoles (indole-3-acetonitrile, indole-3-carbinol and 3,3'-diindolylmethane) which are potent inducers of aryl carbon hydroxylase in the rat (Loub et al., 1975).

Animal studies have demonstrated extensive and varied changes in drug metabolism caused by calorific deprivation (Campbell and Hayes, 1974), whereas Reidenberg (1977) has shown that the half-life values for a group of five drugs was normal in obese, but otherwise healthy volunteers, and unchanged by fasting. However, mean theophylline plasma half-lives have been shown to be longer in obese subjects ($t_{\frac{1}{2}}$ 8.6h) than in normals ($t_{\frac{1}{2}}$ - 6h) (Gal et al., 1978).

Other Factors

Table 1.20 lists a number of other factors which have been shown to affect drug metabolism.

Various drug-metabolising enzyme systems are impaired in the neonate (Vest and Rossier, 1963; Nyhan and Lambert, 1965). Prolonged plasma theophylline half-lives of 19.9 hours have been reported in both newborn infants (Loughnan et al., 1976) and in apneic infants (Giacoia et al., 1976).

Disease states can markedly change an individuals basal, genetically determined rate of drug elimination. Since the liver is the primary site of metabolism for the majority of drugs, any factor causing impairment of liver function or hepatic blood flow rate may be expected to affect the extent and rate of drug metabolism. A severe reduction in hepatic blood flow caused by cirrhosis has been reported to reduce both the plasma clearance and half-life of theophylline (Piafsky et al., 1975). Prolonged theophylline half-lives have also been reported in patients with acute pulmonary oedema (Piafsky et al., 1974) and a patient with severe congestive heart failure showed a marked increase in theophylline half-life which

Table 1.20 MISCELLANEOUS FACTORS KNOWN TO INFLUENCE DRUG METABOLISM IN MAN

FACTOR AND EFFECT	DRUG AFFECTED	REFERENCE
1. Age a) Neonate: It has been recognised for some time that young humans may be more sensitive to drugs than are adults. Many of the drug-metabolising microsomal enzymes are absent or present in negligible amounts in early life.	Acetanilide	Vest, 1959
		Rane and Sjoquist, 1972
	Chloramphenicol	Weiss et al., 1960
	Pethidine	Rudofsky and Crawford, 1966
		Caldwell et al., 1978
	Promazine	Rudofsky and Crawford, 1966
	Theophylline	Loughnan et al., 1976
		Giacoia et al., 1976
	Sulphobromopthalein	Wichmann et al., 1968
	Para-aminobenzoic acid	Vest and Salzberg, 1965
b) Old Age: The effect of old age on drug metabolism has not been extensively studied but recent work suggests that some elderly patients may have defective enzyme activity	Antipyrine	O'Malley et al., 1971
	Phenylbutazone	
	Amobarbital	O'Malley et al., 1977
	Aminopyrine	
	Warfarin	

Table 1.20 cont.

FACTOR AND EFFECT	DRUG AFFECTED	REFERENCE
2. Disease States a) Liver disease: As the liver is the major site of drug metabolism, any damage to the integrity of the liver will influence drug metabolism. This is usually manifested in terms of impairment of drug metabolising enzyme systems, with prolonged pharmacological drug effects.	Chloramphenicol Tolbutamide Rifamycin Prednisone Antipyrine Theophylline	Konin et al., 1959 Ueda et al., 1963 Acocella et al., 1962 Cooksley et al., 1971 Branch et al., 1973 Andreasen et al., 1974 Piafsky et al., 1975 Zwillich et al., 1975
b) Kidney disease: Certain pathways of drug metabolism are impaired in patients with uraemia. The mechanisms are unclear. By contrast metabolism of some drugs may be enhanced due to the reduction in protein binding, particularly those which are highly protein bound.	Sulphafurazole Phenytoin	Reidenberg et al., 1969 Lund et al., 1971

Table 1.20 cont.

FACTOR AND EFFECT	DRUG AFFECTED	REFERENCE
3. Route of Administration: After oral administration peak blood levels of parent drug and its pharmacologically active metabolite, 4-hydroxypropranolol, occur at about one hour after administration. After intravenous administration no 4-hydroxypropranolol is seen in blood because the first-pass contribution of the liver has been by-passed.	Propranolol	Paterson et al., 1970

returned to normal as the patients haemodynamic status improved (Jenne et al., 1974). Hepatic dysfunction, congestive heart disease and cor pulmonale were diagnoses commonly found in a series of patients with serum theophylline concentrations over 25ug/ml who manifested theophylline toxicity while receiving i.v. aminophylline (Zwillich et al., 1975).

Random intrapatient variation in theophylline kinetics has also been demonstrated in asthmatic adult smokers (Hunt et al., 1976) and asthmatic children (Leung et al., 1977).

These and many other factors represent the diverse ways in which drug metabolism can be altered and the study of factors affecting drug metabolism is becoming increasingly important as a means of investigation to maximise the therapeutic effects of drugs and minimise the risk of toxicity.

Aims and Scope of the Thesis

The major metabolic pathways followed by theophylline were elucidated by Cornish and Christman, (1957) and have since been confirmed by other workers (Thompson, et al., 1976). However, the ingestion of large amounts of methylxanthines in tea, coffee, chocolate and cola drinks might be expected to influence the disposition of theophylline, as these chemically related methylxanthines share some of the metabolic pathways of theophylline (Figure 1.4; Cornish and Christman, 1957; Sved et al., 1976). Although some workers have commented on the desirability of withdrawing methylxanthine-containing beverages for 24 hours prior to studies of theophylline disposition, this has been recommended on analytical grounds to reduce interference with the assay by dietary constituents, rather than as a systematic study of the influence of diet on theophylline disposition. Therefore, with the aid of ^{14}C-labelled theophylline it is possible to determine the metabolic fate of the drug without interference from dietary methylxanthines and by the collection of serial urine samples, to relate the kinetics of theophylline metabolism to its overall elimination.

The optimum therapeutic effect of theophylline is related to plasma concentrations between about 10 - 20 μg/ml. Little therapeutic effect is observed below this level with toxic effects occurring above this range. However, the therapeutic effectiveness of theophylline is hindered by wide inter-individual variations in plasma levels encountered in patients on identical dose regimens. As theophylline is eliminated from the body principally by metabolism, inter-individual differences in the rate and extent of metabolism of the drug will account for the observed variations in both plasma-levels and in the plasma elimination half-lives of theophylline. The large inter-individual variations which occur in drug metabolism

in man are determined by a variety of interacting genetic, environmental and physiological factors. Recently, attention has focused on the influence of diet on drug metabolism, and with respect to theophylline this may be significant due to the widespread ingestion of large amounts of chemically related methylxanthines. Therefore, with the aid of (^{14}C) labelled theophylline, it is possible to determine the influence of the methylxanthine-containing beverages on theophylline metabolism and to assess the extent to which they are responsible for the observed variations in theophylline disposition.

As theophylline is poorly soluble in aqueous media, it is frequently combined with agents to enhance its water solubility for parenteral administration. The most widely used agent is ethylene-diamine, which in combination with theophylline yields aminophylline, and it is frequently assumed that theophylline and aminophylline are pharmacologically equivalent. However in view of the apparent chemical differences between the two compounds and due to the widespread therapeutic use of aminophylline, a comparison of its metabolism and urinary kinetics with that of theophylline will provide valuable evidence as to the equivalence or non-equivalence of the two compounds.

CHAPTER 2

Materials and Methods

Materials and Methods

Compounds Compounds were purchased as follows: theophylline as anhydrous crystals (m.p. 273°C), (Sigma Chemical Co., Kingston-upon-Thames, U.K.), 3-methylxanthine (m.p. 350°C) and uric acid (m.p. 400°C), (Aldrich Chemical Co., Gillingham, Dorset, U.K.), 1-methylxanthine (m.p. 350°C), 3-methyluric acid (m.p. 350°C), (Fluka A.G., Buchs, Switzerland), 1-methyluric acid (m.p. approx 400°C) and 1,3-dimethyluric acid (m.p. 350°C),(Adams Chemical Co., Round Lake Beach, Ill., U.S.A.). 8-(^{14}C)-Theophylline, specific activity 34mCi/mmol, was purchased from the Radiochemical Centre, Amersham, U.K.

Experimental Protocol

Oral Administration

Adult healthy male volunteers received (^{14}C)-theophylline (100mg : 10µCi) orally in a hard gelatin capsule. Urine samples were collected and pooled at 24 hours and 48 hours.

Intravenous Administration:- Theophylline: Normal Diet

Four male subjects were given an intravenous infusion over a ten minute period of (^{14}C)-theophylline (100mg : 10µCi) which was dissolved in sterile normal saline (0.9% w/v) with the aid of 0.2N NH_4OH and sterilised by ultrafiltration. Urine samples were collected at regular intervals for 48 hours and blood samples at 0, 5, 10, 15, 30, 45, 60, 90, 120 minutes and then hourly to either 7 or 8 hours.

: Methylxanthine-deprived diet

In the second part of this study the same four volunteers abstained from the intake of foods containing methylxanthines (coffee, tea, cola drinks and chocolate) for seven days prior to the intravenous infusion of (^{14}C)-theophylline (100mg 10µCi). It has been shown that five to nine days are required for caffeine to disappear from the blood of "normal" coffee drinkers (Sved, Hossie and McGilveray, 1976; Warren, 1969). Blood and urine samples were collected as described above. Volunteers did not resume their normal diets until after the

last urine collection.

Methylxanthine-Supplemented diet

The diets of three male volunteers, who had participated in the previous studies, were supplemented by adding six bottles per day of Pepsi-cola (35 to 55mg caffeine per bottle) to their normal intake of methylxanthine-containing beverages. This diet was maintained for a period of seven days prior to the intravenous infusion of 100mg (10μCi) (^{14}C)-theophylline. Serial urine and blood samples were collected as described previously.

Caffeine/Theophylline Replacement diet

Methylxanthine-containing foods and beverages were withdrawn from the diets of three male volunteers. Their normal daily intake of caffeine and theophylline from these sources was estimated and replaced by the alternative ingestion of caffeine tablets (Pro-Plus, Ashe Laboratories Ltd., Leatherhead, U.K.)or theophylline tablets (Nuelin, Riker Laboratories, Loughborough, U.K.). This diet was maintained for a period of seven days prior to the intravenous infusion of 100mg (10μCi) (^{14}C)-theophylline. Serial urine and blood samples were collected as described previously.

Aminophylline

(^{14}C)-Aminophylline injection was prepared from 8-(^{14}C)-theophylline and ethylenediamine as described in the British Pharmaceutical Codex (1963), sterilised by ultrafiltration and administered to three male volunteers by intravenous infusion over a ten minute period (125mg aminophylline, 10μCi; being equivalent to 100mg theophylline). The volunteers were investigated on both their normal diets and on a methylxanthine-deprived diet according to the protocol described above. Serial urine and blood samples were collected as described previously.

Treatment of Urine Samples

The volume, pH and radioactive content of each sample was determined before storage at -20°C until required for further analysis.

Dowex 2X8-100 Anion Exchange Column Chromatography

Dowex 2X8-100 anion exchange resin (Sigma Chemical Co., Poole, Dorset) was treated to the following clean-up procedure:-

1)	wash with distilled water	200ml	30 minutes
2)	wash with 1N NaOH	200ml	60 minutes
3)	wash with distilled water	200ml	30 minutes
4)	wash with 1N HCl	200ml	60 minutes
5)	wash with 1N HCl	200ml	60 minutes

The resin was then packed as an aqueous slurry into a clean glass burette (int. diam. 1.0cm) to a height of 7.5 - 10.0cm with a glass wool plug above and below the resin bed.

"On-column"Conditioning

1) The resin was conditioned with 50ml 6NHCl followed by
2) a water wash to neutrality
3) immediately prior to use a further 200ml distilled water was passed down the column.

By this procedure, xanthines are not retained by the column and are washed through with water, while uric acids are retained and require elution with acid.

Application of Urine

A volume of urine containing some 350,000dpm was adjusted to pH 7.1 - 7.2 and transferred to the pre-conditioned column with the aid of an equal volume of water. The eluate was collected and the column washed with water until the radioactivity eluting from the column approached background, this required 200 - 250ml. The column was then eluted with 0.05N HCl until the radioactivity eluting from

the column again approached background, this required 300 - 350ml (see Figure 2.1). The total recovery of radioactivity from the column approached 95 - 100% of that applied to the column. The two fractions were then concentrated by rotary evaporation and used for ion-exchange paper chromatography.

By this procedure, the urine was divided into a water fraction (xanthine containing) and an acid fraction (uric acid containing). This separation is essential as all published thin-layer and paper chromatographic systems for the separation of theophylline and its metabolites report identical Rf values for both 3-methylxanthine and 1,3-dimethyluric acid.

Ion-Exchange Paper Chromatography

Whatman DE 81 ion-exchange paper was soaked in Tris-phosphate buffer (0.2M) pH 6.6 and air dried.

Samples from the Dowex-concentrated fractions are applied in the normal way and developed with n-butanol-glacial acetic acid-water (4:1:1 by vol.).

Standard compounds were visualised under U.V. light (254nm) and Rf values were theophylline 0.75; 1-methylxanthine 0.64; 3-methylxanthine 0.56; 1,3-dimethyluric acid 0.59; 1-methyluric acid 0.38 and 3-methyluric acid 0.32.

Radiochemical Techniques

The (^{14}C) content of urine and other solutions was determined by liquid scintillation counting using either a dioxan-based scintillator or a Triton/toluene based scintillator, with a Packard Tri-Carb liquid scintillation spectrometer, Model 3385. Radioactivity on paper chromatograms was located by radiochromatogram scanning (Packard Model 7201 Scanner) and quantitated by scintillation counting (Caldwell et al., 1975).

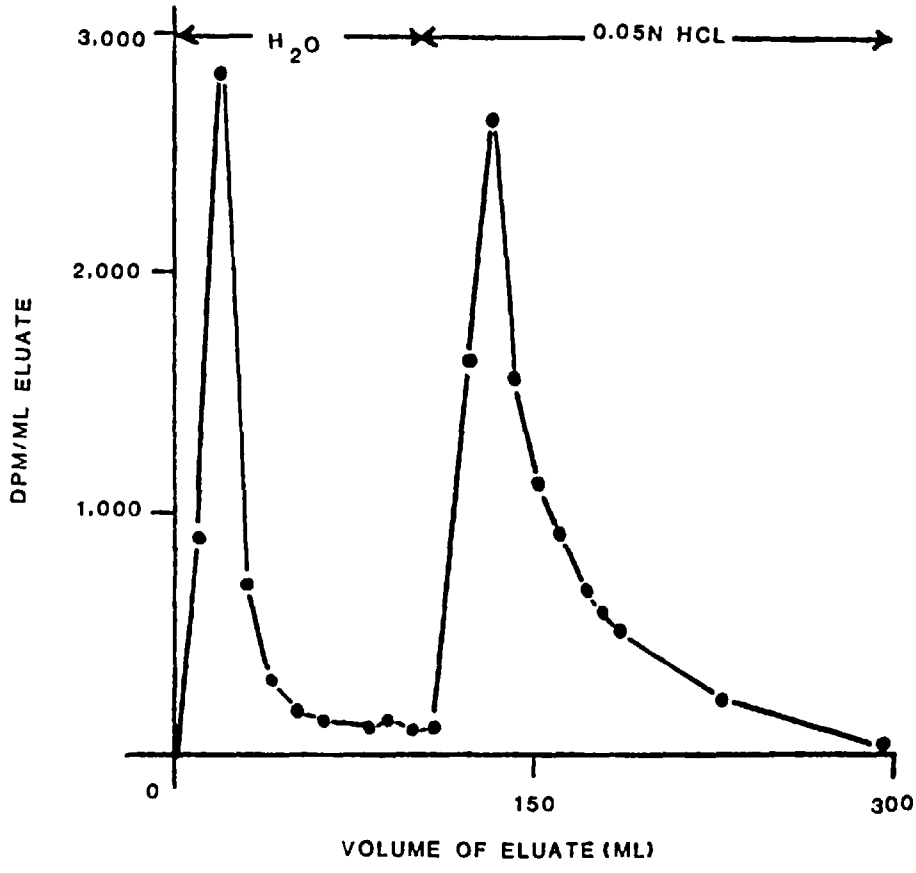


Figure 2.1 ELUTION OF RADIOACTIVITY FROM A DOWEX 2 X8-100 ANION EXCHANGE COLUMN FOLLOWING THE APPLICATION OF URINE (CONTAINING APPROX. 350,000 dpm) FROM AN INDIVIDUAL DOSED WITH (^{14}C)-THEOPHYLLINE

Treatment of Blood Samples *

Blood samples were centrifuged immediately after collection at 3,500 rpm. The total (^{14}C) content of plasma was then determined by liquid scintillation counting using a Triton/toluene based scintillant.

Reverse Isotope Dilutions

Theophylline or 3-methylxanthine (1g) (dissolved in methanol) was added to urine (50ml). The mixture was evaporated to dryness, the residue taken up in a small volume of methanol and filtered. The filtrate was then treated with ether to precipitate the compound, which was filtered and recrystallised from hot water to constant specific radioactivity. The presence of 1-methyluric acid and 1,3-dimethyluric acid in urine samples was also confirmed by reverse isotope dilution. A small amount of either compound was dissolved in boiling water. On cooling, the crystals formed were filtered and then re-crystallised from hot water to constant specific activity.

Kinetic Analysis of Urinary Metabolite Data

1. Hanes-type plots for the elimination of theophylline and its metabolites

All metabolite data were plotted according to one of the linearised forms of the Michaelis-Menten equation

$$\frac{S}{v} = \frac{Km}{V_{max}} + \frac{1}{V_{max}} S \quad \ldots\ldots\ldots \quad (1)$$

where S is the substrate concentration, v is the observed rate of metabolite formation, Km is the Michaelis constant and V_{max} is the theoretical maximum rate of the metabolic process. Applied to the conditions of this study, S is the amount of unmetabolised theophylline in the body, v is the rate of appearance in the urine of a given metabolite (which, in the case of theophylline metabolites, is assumed to be rate limited by formation, and therefore reflects the rate of formation), Km is the apparent in vivo Michaelis constant (expressed as the amount of drug in the body rather than as a concentration)

and V_{max} is the theoretical maximum rate of metabolite formation (amount per unit time).

According to equation (1) a plot of S/v versus S should be linear with a slope of $1/V_{max}$ and an intercept of Km/V_{max}, permitting the determination of V_{max} and Km. Data which does not show Michaelis-Menten kinetics but are apparent first-order, will yield a slope of zero in a S/v versus S plot since:-

$$v = kS \qquad \ldots\ldots \qquad (2)$$

and therefore

$$S/v = 1/k \qquad \ldots\ldots \qquad (3)$$

where k is the apparent first-order formation rate constant.

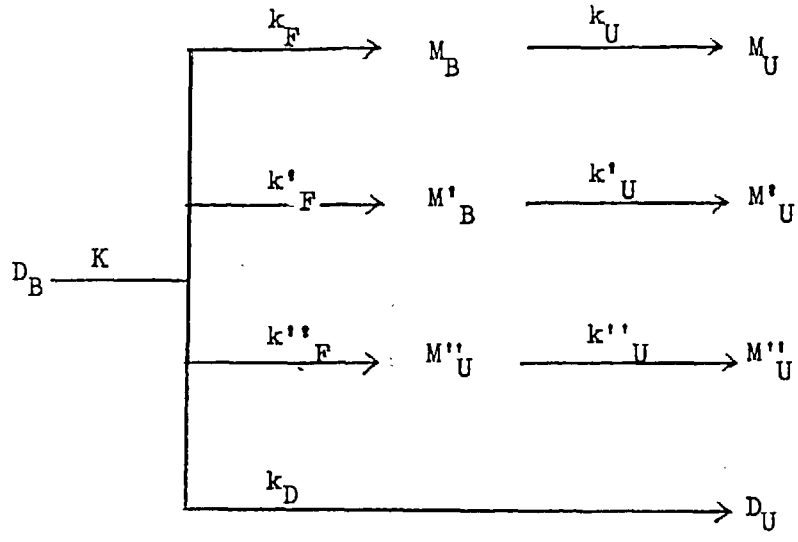
2. Determination of Elimination Rate Constants for Theophylline and its Metabolites by the "Sigma-Minus" Method. (Cummings et al.,1967)

In this method a first-order rate law is assumed for all the processes involved in the elimination of the drug and its metabolites. The drug is considered to undergo elimination by excretion of unchanged drug with the rate constant k_D, and by the simultaneous formation of a number of metabolites M, M', M'' ..., with the rate constants k_F, k'_F, k''_F ..., respectively. The overall rate constant for the elimination of drug by all routes is K, where:-

$$K = k_D + k_F + k'_F + k''_F \qquad \ldots\ldots \qquad (4)$$

The metabolites are considered to be instantaneously equilibrated in the body water and to undergo no further metabolism. They are excreted unchanged with the rate constants k_U, k'_U, k''_U ..., respectively.

The elimination of the drug and the excretion of its metabolites may be represented as follows:-



The following equations for these processes are presented with respectto the unchanged drug, but they may also be applied to the metabolites when the appropriate rate constants are substituted. In these equations:-

D_o = Amount of drug in the body at zero time,

D_B = Amount of drug in the body at time t,

D_U = Amount of drug excreted in the urine at time t,

M_F = Amount of metabolite formed at time t,

M_B = Amount of metabolite in the body at time t,

M_U = Amount of metabolite excreted in the urine at time t

so that $D_o = D_B + D_U + M_F$ and $M_F = M_B + M_U$

thus $$\frac{dD_B}{dt} = -KD_B \quad \ldots\ldots\ldots \quad (5)$$

$$D_{U\infty} - D_U = \frac{k_D D_o e^{-Kt}}{K} \quad \ldots\ldots\ldots \quad (6)$$

$$\text{so} \quad \ln (D_{U\infty} - D_U) = \frac{\ln k_D D_o}{K} - Kt \quad \ldots\ldots\ldots \quad (7)$$

To enable a plot of the above type it is necessary to first determine the amount of drug which is ultimately excreted ($D_{U\infty}$) in the unchanged form. This is achieved by a plot of the logarithm of the rate of excretion of the drug against the mid-point of the collection period. This plot generates a straight line which is described by

$$\ln \frac{dD_U}{dt} = \ln k_D D_o - Kt \quad \ldots\ldots\ldots \quad (8)$$

which has an intercept on the ordinate equal to $k_D D_o$ and a slope equal to -K.

($D_{U\infty}$) is calculated by estimating the rate of drug elimination at the end of the last collection period and dividing by the slope of the line:

$$D_{U\infty} = D_U \text{ collected} + \frac{\text{Rate at time t}}{\text{Slope}}$$

Equations of the same form as (8) are also obtained for the metabolites.

*Only the total radioactive content of plasma was estimated. Therefore no further analysis of plasma data was performed.

CHAPTER 3

Metabolism of Orally Administered (^{14}C)-Theophylline

Introduction

The major metabolic pathways of the methylxanthines, caffeine, theophylline and theobromine were elucidated by Cornish and Christman (1957) and the metabolism of theophylline has since been confirmed by various workers (Thompson et al., 1974; Jenne et al., 1976; Desiraju and Sugita, 1977).

However, in the investigations cited above, theophylline and its metabolites were identified by ultraviolet spectrophotometry techniques. Thus the presence of other U.V. absorbing materials present in urine might be expected to interfere with the estimation of theophylline and its metabolites. Furthermore, the ingestion of large amounts of methylxanthines (caffeine, theophylline and theobromine) in tea, coffee, chocolate and cola drinks might also influence both the disposition of theophylline and the estimations of its metabolites in urine since these chemically related methylxanthines share some of the metabolic pathways of theophylline (Cornish and Christman, 1957; Sved et al., 1976). Many workers have therefore commented on the desirability of withdrawing methylxanthine-containing beverages prior to studies on theophylline disposition, and this has been recommended on analytical grounds to reduce interference with the assay by dietary constituents.

However, with the aid of (^{14}C)-theophylline it is possible to determine the metabolism of theophylline without interference in the assay by endogenous methylxanthines. This should enable a more accurate quantitative estimate of theophylline and its metabolites, arising from an orally administered dose, than those previously reported.

Results

Oral (^{14}C)-Theophylline

After the oral administration of 100mg (10μCi) (^{14}C)-theophylline to six healthy male volunteers, 72.3% (64.1 - 83.5% Mean and range) of the dose was excreted in the urine after 24 hours and 87.2% (81.1 - 96.4%) after 48 hours (Table 3.1).

Ion-exchange paper chromatography of the concentrated aqueous eluates obtained after Dowex 2X8 - 100 anion exchange column chromatography (See Ch.2) showed two major radioactive peaks, with Rf values 0.55 and 0.74. One very minor radioactive peak with Rf 0.13 was observed in all but two of the subjects (Figure 3.1). The two major peaks corresponded to 3-methylxanthine and theophylline, whose presence was confirmed by reverse isotope dilution, while the minor peak was not identified. Chromatograms of the acid eluates obtained from the same Dowex column as above (see Ch.2) showed two radioactive peaks, with Rf values of 0.37 and 0.57, corresponding to 1-methyluric acid and 1,3-dimethyluric acid respectively (Figure 3.2) and confirmed by reverse isotope dilution.

The quantitation of theophylline and its metabolites in 0-24 hour urine is given in Table 3.1 and these are compared with results published by other laboratories employing different methods of analysis (Table 3.2). Thus the major metabolite in all six subjects studied was the 8-hydroxylated product, 1,3-dimethyluric acid, which accounts for 30.7 $\pm$ 2.5% (Mean $\pm$ S.D.) of the dose. N-demethylation in the 1 position gives rise to 3-methylxanthine (14.2 $\pm$ 2.4%) and the 8 hydroxylated/3-N demethylated product, 1-methyluric acid accounts for a further 15.2 $\pm$ 5.8% of the dose. Therefore the major routes of metabolism after the oral administration of (^{14}C)-theophylline involve

Table 3.1 QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN 0-24 HOUR URINE OF VOLUNTEERS AFTER THE ORAL ADMINISTRATION OF 100mg (10μCi) THEOPHYLLINE

SUBJECT	T.J.M.	J.C.	M.F.	K.L.	J.D.	R.L.S.	MEAN	S.D.	r**	p
METABOLITE										
THEOPHYLLINE	10.2*	14.5	7.4	11.1	11.7	10.9	11.0	2.3	0.29	n-s
1,3-DIMETHYLURIC ACID	35.3	30.4	31.0	30.1	27.8	29.3	30.7	2.5	0.77	n-s
1-METHYLURIC ACID	22.2	22.4	14.2	8.7	11.3	12.1	15.2	5.8	0.93	0.01
3-METHYLXANTHINE	16.5	14.5	15.4	15.8	13.2	9.9	14.2	2.4	0.62	n-s
UNKNOWN XANTHINE	-	-	2.1	2.5	2.0	2.0	1.4	1.1	-	-
TOTAL 0-24 HOURS	83.5	81.8	70.2	68.3	65.9	64.1	72.3	8.3		
TOTAL 24-48 HOURS	12.9	12.6	15.1	16.0	15.9	17.0	14.9	1.8		
TOTAL	96.4	94.4	85.3	84.3	81.8	81.1	87.2	6.6		

*All figures expressed as % of the dose recovered as that compound in the 0-24 hour urine

**r = correlation coefficient between % of the dose excreted as a particular metabolite and the overall elimination of total radioactivity in the 0-24 hour urine

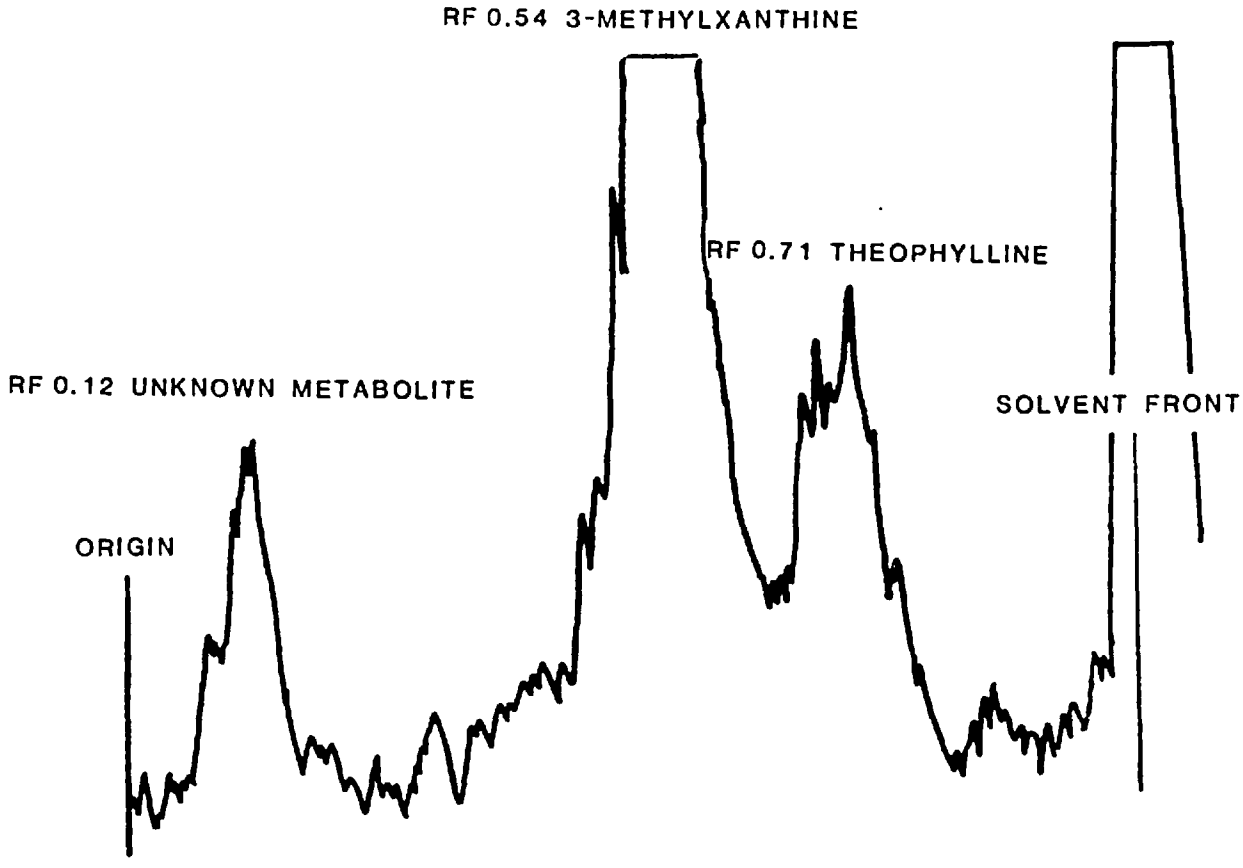


Figure 3.1 DE 81 ION-EXCHANGE PAPER RADIOCHROMATOGRAM SCAN OF THE CONCENTRATED DOWEX 2 X8-100 ANION EXCHANGE COLUMN WATER ELUATE (ORAL (^{14}C)-THEOPHYLLINE)

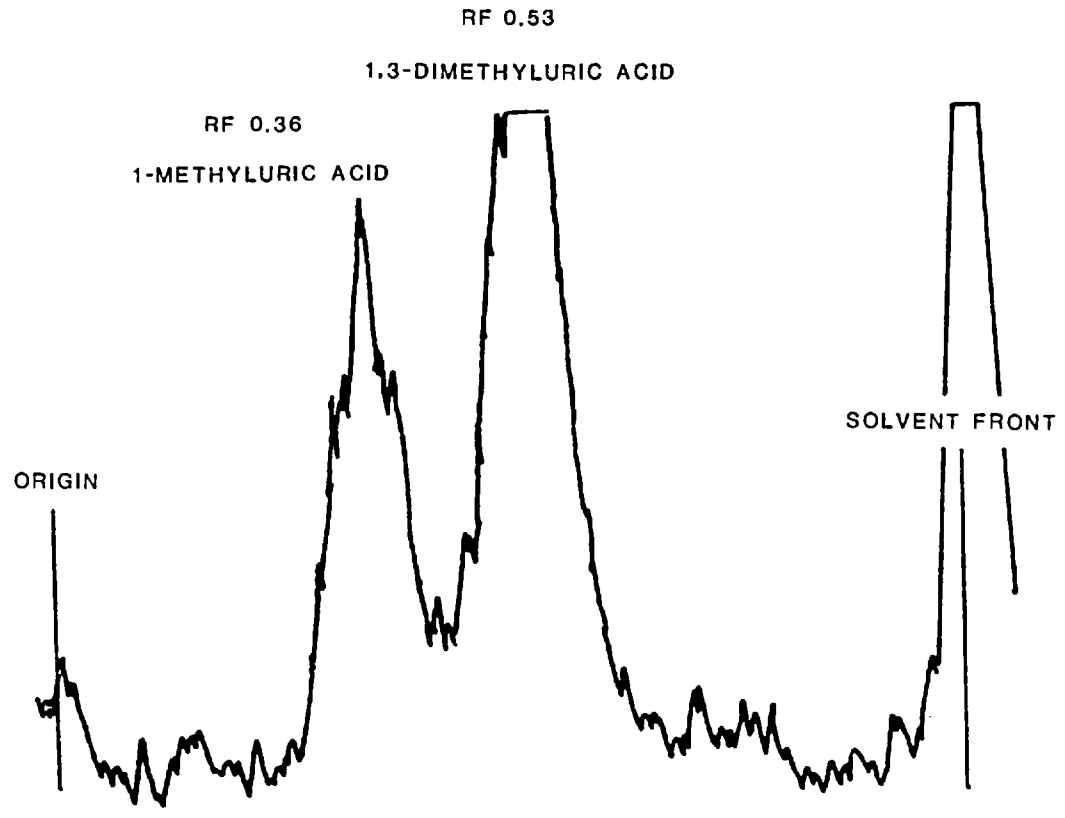


Figure 3.2 ION-EXCHANGE PAPER RADIOCHROMATOGRAM SCAN OF THE CONCENTRATED DOWEX 2 X8-100 ANION EXCHANGE COLUMN ACID ELUATE (ORAL (^{14}C)-THEOPHYLLINE)

Table 3.2 QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES BY VARIOUS AUTHORS

1,3-diMeX	3MeX	1,3-diMeU	1-MeU	Analysis and Protocol	Reference
11 *10	14 *12	31 *26	15 *12	Ion-exchange column chromatography, ion-exchange paper chromatography. 100mg (^{14}C)-theophylline. Oral. *Reverse isotope dilution	Present study
10	13	35	19	Ion-exchange column chromatography, u.v. absorption. 2x500mg taken orally over a 4 hour period.	Cornish and Christman, 1957
14	9	31	12	Paper chromatography and scintillation counting. Administered as 100mg (100μCi) Fenetylline (^{3}H-theophylline).	Ellison et al., 1970
8	36	40	17	High-pressure liquid chromatography. Patients administered 800 or 1,200mg aminophylline/24 hours in divided doses, either in a plain form or as a slow-release preparation.	Jenne et al., 1976
10	9	46	19	High-pressure liquid chromatography. Aminophylline administered to two subjects at a dose of 5 or 7mg/kg.	Desiraju et al., 1977

N-demethylation and 8-hydroxylation, with only 11.0 ± 2.3% of the dose being excreted unchanged in 24 hours.

Discussion

Although the oral route is the most convenient route for the administration of most drugs, the absorption of the xanthine alkaloids (caffeine, theophylline and theobromine) from the gastro-intestinal tract can be erratic (Ritchie, 1975) probably a consequence of their poor aqueous solubility (2% for caffeine, 1% for theophylline and less than 0.1% for theobromine). These results suggest that, at least in the six volunteers studied, theophylline is readily absorbed after oral administration, although the rate constant for absorption cannot be calculated from these studies.

The largest interindividual variation in the metabolism of orally administered (^{14}C)-theophylline is in its conversion to 1-methyluric acid, via the formation of 1,3-dimethyluric acid (see Ch.4). This metabolite accounts for between 8.7 - 22.4% of the dose, a two and a half fold difference between the six volunteers. However, the amounts of this metabolite excreted by each volunteer correlates ($p<0.01$) with the overall amount of total radioactivity each volunteer excretes in their 0-24 hour urine samples. Thus the 8-hydroxylation, N-demethylation of (^{14}C)-theophylline to 1-methyluric acid appears to be the major determinant of the overall elimination of radioactivity following oral (^{14}C)-theophylline. Two of these subjects R.L.S. and J.D., who excrete small amounts of 1-methyluric acid (12.1 and 11.3% respectively) are also known to be slow metabolisers of debrisoquine (Mahgoub et al., 1977) which is eliminated from the body both unchanged and as its 4-hydroxylated metabolite. Perhaps the metabolite defect in these two subjects, namely that of alicyclic hydroxylation, extends to the oxidation of a wider range of drugs, in this instance the 8-hydroxylation, N-demethylation of theophylline. That it is the

N-demethylation step which is principally affected is illustrated by the amounts of 1,3-dimethyluric acid which these two subjects excrete. The amounts of this metabolite, formed by hydroxylation of the 8-position of theophylline, are comparable with those excreted by the other four subjects (Table 3.2). Thus it is the lower levels of the N-demethylated product in these subjects which account for the overall lower recovery of radioactivity in the urine.

However, subject K.L., who also excretes small amounts of 1-methyluric acid (8.7%) has been phenotyped as an extensive metaboliser of debrisoquine. This subject is also a smoker and perhaps the differential induction of the two pathways (alicyclic hydroxylation and N-demethylation) caused by the cigarette smoke is responsible for the observed difference in drug metabolism by this subject. That the N-demethylation of theophylline to 3-methylxanthine is not affected to the same extent in these subjects suggests that enzyme(s) responsible for catalysing the two N-demethylation reactions are different. Whether there is a significant genetic component as a determinant of theophylline metabolism is being investigated in a parallel study.

The quantitation of theophylline and its metabolites by the analytical procedure employed in this study, compare well with those reported for theophylline metabolism using different analytical techniques (Table 3.2). The one inconsistent figure in this table is the value of 36% assigned to 3-methylxanthine by Jenne et al.,(1976). This value is approximately twice that of other published data, and in this study they report a 116% recovery of theophylline and its metabolites in 24 hour urine samples. This overestimation in the recovery of 3-methylxanthine might arise from two errors. The first involves interference in the high pressure liquid chromatographic assay by other u.v. absorbing material in urine; the second involves the estimation of theophylline and its metabolites arising from

sources other than that of the dose administered. Van Gennip et al (1973) reported that children excrete large quantities of 3-methylxanthine in their urine, and that this arises from the dietary intake of methylxanthines, as does the presence of 1-methylxanthine, a metabolite of caffeine. As the patients studied in the Jenne experiments apparently did not abstain from the dietary ingestion of methylxanthines prior to the study, and as they were already on maintainance aminophylline therapy, it is reasonable to assume that the theophylline and metabolites quantified in the 0-24 hour urine samples arose from sources other than that of the single administered dose. This stresses the value of employing ^{14}C-labelled theophylline in a study of its disposition in man, which enables the determination of theophylline and its metabolites without interference from endogenous methylxanthines.

The estimates of theophylline and its metabolites by reverse isotope dilution (Table 3.2) in urine samples are consistently lower than the values obtained by the ion-exchange procedures. This is probably due to the "diluting" of the radioactive metabolites by the presence of cold material in the urine, and arising from the dietary intake of non-labelled methylxanthines.

CHAPTER 4

Metabolism and Pharmacokinetics of Intravenously Administered (^{14}C)-Theophylline to Volunteers on their Normal Diets

Introduction

In the preceeding chapter the first study of theophylline metabolism in man using (^{14}C)-labelled drug was reported. This obviates the analytical problems associated with the presence of unlabelled methylxanthines derived from the diet. However, whilst such a study enables an accurate quantitative estimate of theophylline and its metabolites in 0-24 hour pooled urine samples, it gives no indication of the rate at which these metabolites are formed and eliminated from the body. Knowledge of the kinetic aspects of drug metabolism is as important as defining the qualitative aspects (Ch.7).

Therefore, after the intravenous administration of (^{14}C)-theophylline and the subsequent collection of regular urine samples, by determining the amounts of theophylline and its metabolites in each sample, a more detailed picture of theophylline metabolism will emerge.

A qualitative comparison of theophylline metabolism after both oral and intravenous administration will also be possible. By the intravenous administration of (^{14}C)-theophylline, any interindividual differences in the rate of drug absorption, which might affect the rate of metabolism, are precluded.

Results

Intravenous (^{14}C)-Theophylline:- Normal Diet

After the intravenous administration of 100 mg (10μCi) (^{14}C)-theophylline to four healthy male volunteers, 76.6 ± 5.7% (Mean ± S.D.) of the administered radioactivity was recovered in the urine after 24 hours and 85.3 ± 3.7% after 48 hours (Table 4.1).

Table 4.1:- Recovery of radioactivity in urine following the intravenous administration of (^{14}C)-theophylline

SUBJECT	T.J.M.	J.C.	L.A.W.	C.A.L.	MEAN	S.D.
0-24 Hrs.	68.6*	78.4	81.9	77.3	76.6	5.7
24-48 Hrs.	14.9	3.1	8.1	8.7	8.7	4.8
Total	83.5	81.5	90.0	86.0	85.3	3.7

*All values expressed as % of dose administered.

Ion-exchange paper chromatography of the concentrated aqueous eluates obtained after Dowex 2X8-100 anion exchange chromatography of the urine samples showed two major radioactive peaks with the same Rf values as those obtained after oral theophylline and corresponding to 3-methylxanthine and theophylline. However, all four volunteers excreted small amounts of an unknown metabolite with Rf 0.13, which was also present in the urine of subject R.L.S. after oral administration of theophylline (Figure 4.1). Ion-exchange paper radiochromatogram scanning of the acid eluates showed two major radioactive peaks corresponding to 1-methyluric acid and 1-3, dimethyluric acid. However, a minor peak Rf 0.70, which was not identified, was also found in the urine of all four volunteers (Figure 4.2).

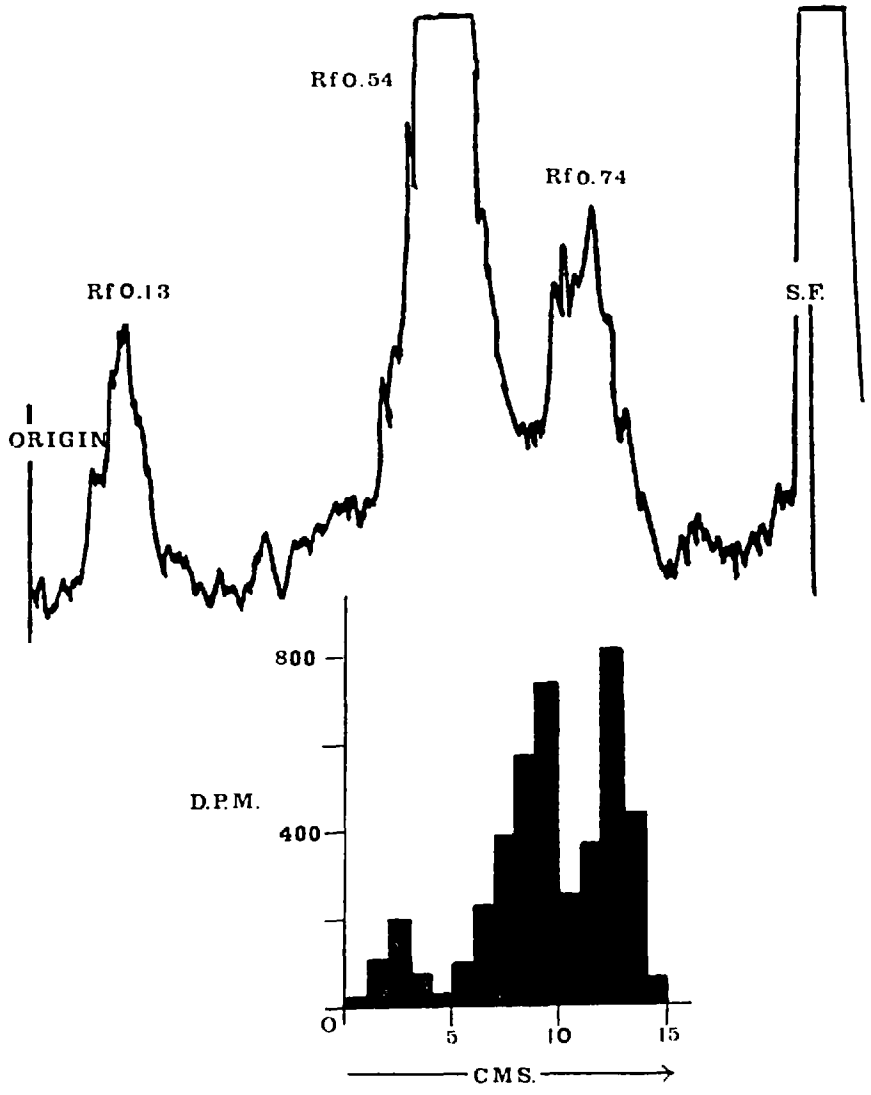


Figure 4.1 DE 81 ION-EXCHANGE PAPER RADIOCHROMATOGRAM SCAN OF THE CONCENTRATED DOWEX 2 X8-100 ANION EXCHANGE COLUMN WATER ELUATE (INTRAVENOUS (^{14}C)-THEOPHYLLINE)

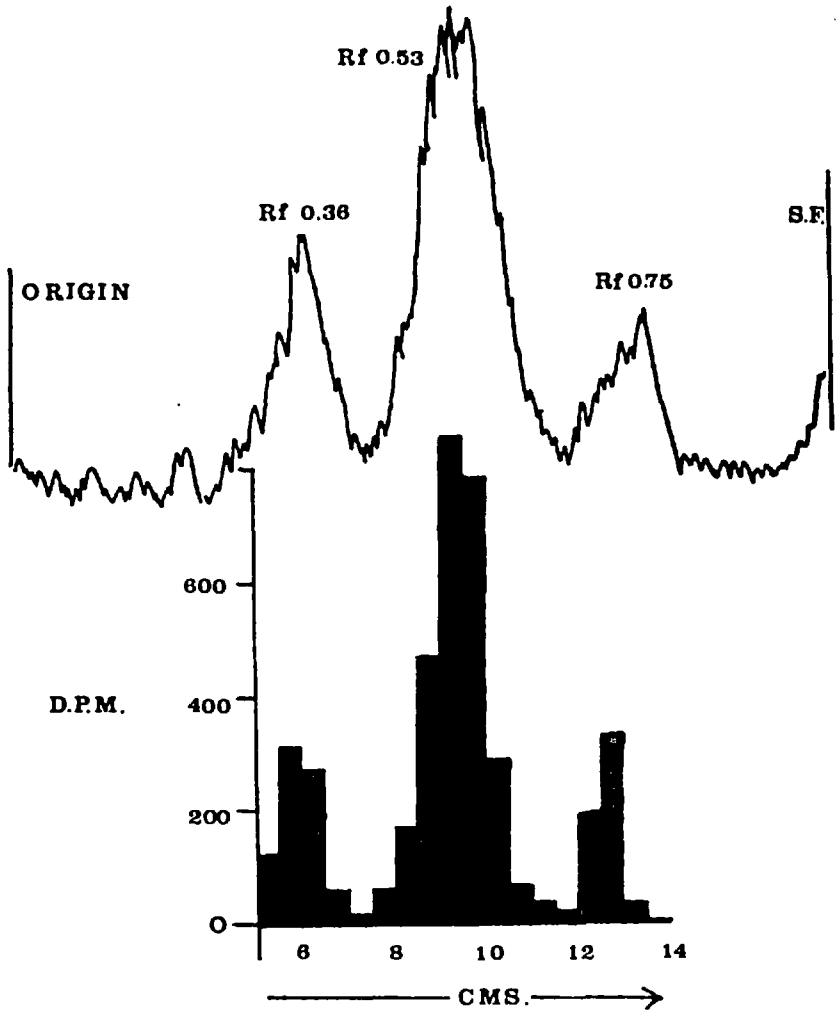


Figure 4.2 DE 81 ION-EXCHANGE PAPER RADIOCHROMATOGRAM SCAN OF THE CONCENTRATED DOWEX 2X8-100 ANION EXCHANGE COLUMN ACID ELUATE (INTRAVENOUS (^{14}C)-THEOPHYLLINE)

Quantitation of theophylline and its metabolites in serial urine samples are given in the Appendix. The cumulative excretion patterns of theophylline and its metabolites in each volunteer are illustrated in Figure 4.3.

Quantitation of theophylline and its metabolites in the total 24 hour urine of each volunteer is given in Table 4.2

Table 4.2 Quantitation of theophylline and its metabolites in 0-24h urine following the intravenous administration of (^{14}C)-theophylline.

SUBJECT	T.J.M.	J.C.	L.A.W.	C.A.L.	MEAN	S.D.
METABOLITE						
THEOPHYLLINE	7.51	11.14	6.47	4.91	7.51	2.65
3-METHYLX-ANTHINE	15.84	16.19	17.44	19.04	17.13	1.45
1,3-DIMETHYLURIC ACID	30.07	26.03	27.88	34.62	29.65	3.70
1-METHYLURIC ACID	12.90	18.75	25.00	16.63	18.32	5.70
UNKNOWN XANTHINE	1.67	0.95	1.08	1.09	1.20	0.32
UNKNOWN URIC ACID	0.61	5.31	4.11	0.35	2.60	2.49

*All values expressed as % of dose administered.

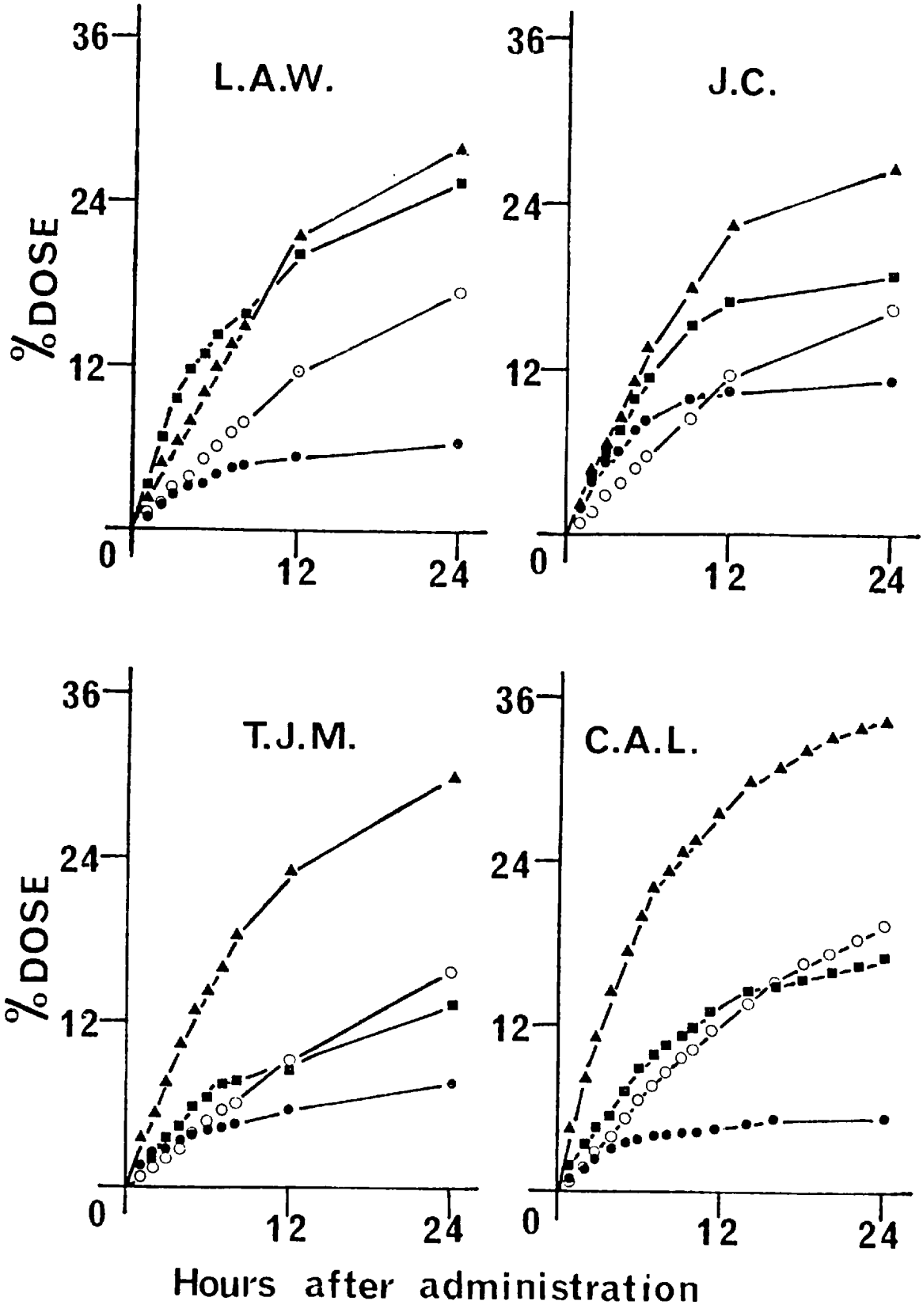


Figure 4.3 CUMULATIVE EXCRETION OF THEOPHYLLINE AND ITS METABOLITES AFTER THE INTRAVENOUS ADMINISTRATION OF (^{14}C)-THEOPHYLLINE (100mg 10μCi) TO FOUR VOLUNTEERS. THEOPHYLLINE (CLOSED CIRCLES), 3-METHYLXANTHINE (OPEN CIRCLES), 1-METHYLURIC ACID (SQUARES) AND 1,3-DIMETHYLURIC ACID (TRIANGLES).

The following empirical relationships were found between the urine components:-

1. There is an inverse relationship between the fractional urine content of 3-methylxanthine and 1,3-dimethyluric acid (Figure 4.4).

2. The amounts of 1,3-dimethyluric acid and 1-methyluric acid excreted in urine are directly correlated (Figure 4.5).

The amounts of theophylline and its metabolites excreted in urine were not affected by the urinary pH (Subject C.A.L.). However, it has been reported that there is a strong and highly statistically significant positive correlation between the renal clearance of theophylline and urine flow rate (Levy and Koysooko, 1976). These observations are confirmed in the present studies and Figure 4.6 shows the relationship between the urine flow rate and the amount of unchanged theophylline excreted, expressed as a percentage of the dose remaining in the body.

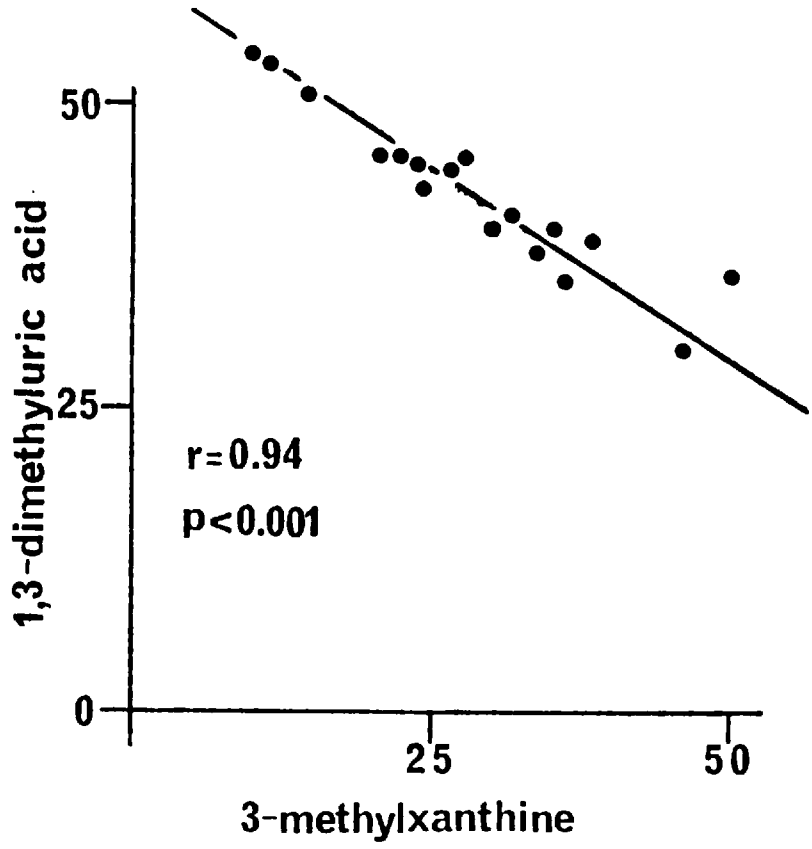


Figure 4.4 THE FRACTIONAL URINE CONTENT OF 3-METHYLXANTHINE AND 1,3-DIMETHYLURIC ACID. SUBJECT C.A.L.

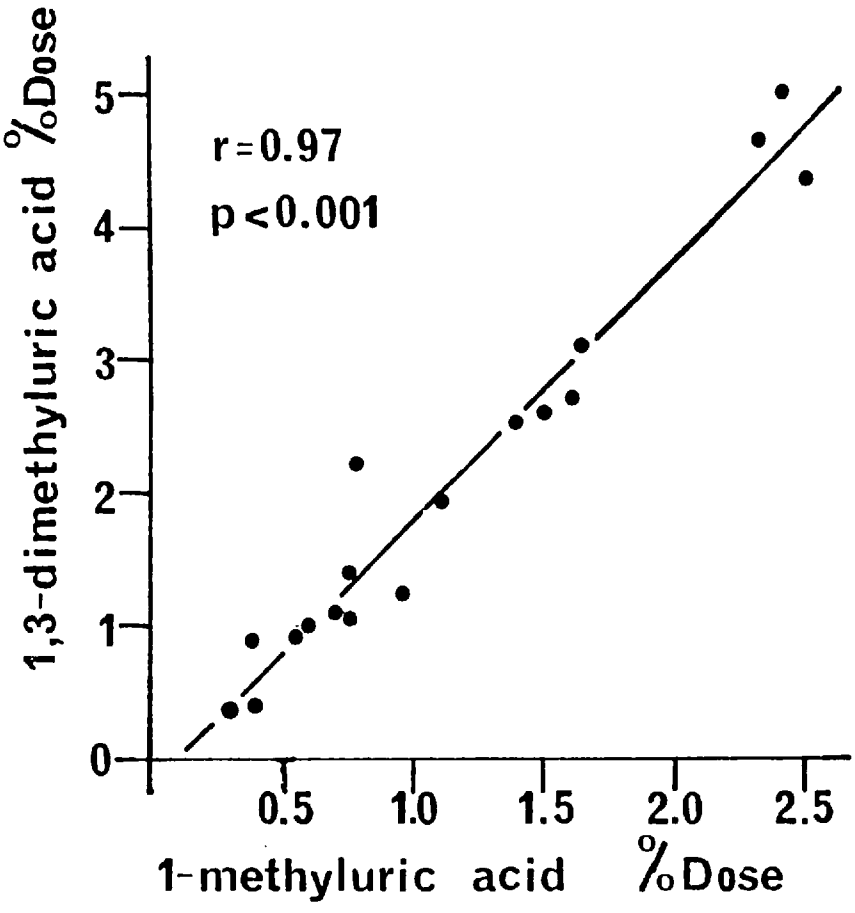


Figure 4.5 THE RELATIONSHIP BETWEEN THE AMOUNTS OF 1,3-DIMETHYLURIC ACID AND 1-METHYLURIC ACID EXCRETED IN URINE. SUBJECT C.A.L.

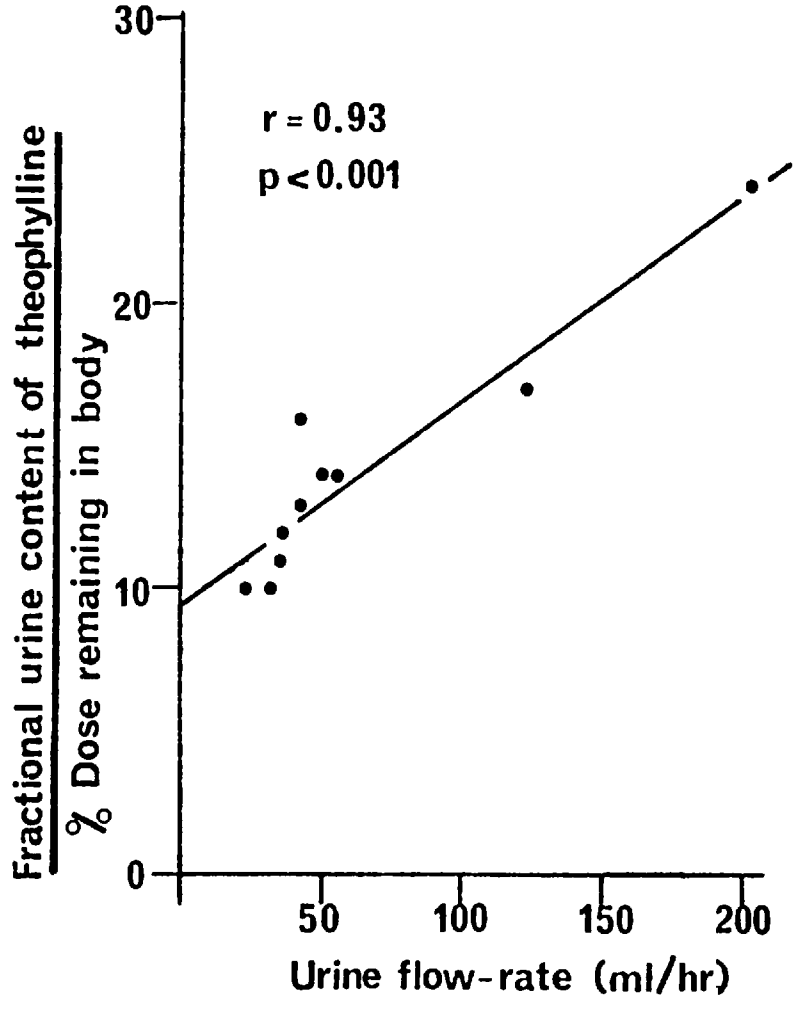


Figure 4.6 THE URINE FLOW-RATE DEPENDENCY OF THEOPHYLLINE EXCRETION. SUBJECT T.J.M.

Urinary Kinetics

The cumulative excretion plots for theophylline and its metabolites (Figure 4.3) show that the rates of excretion of theophylline, 1,3-dimethyluric acid and 1-methyluric acid decline at a constant rate with time after administration of (^{14}C)-theophylline This is reflected in the curvi-linear nature of the cumulative excretion plots, and is indicative of typical first-order elimination rate processes. However, the rate of excretion of 3-methylxanthine proceeds at a constant rate for about 12 hours post-administration. Only after this period of time does its rate of excretion start to decline. This is reflected in the straight line nature of the cumulative excretion plot for 3-methylxanthine (Figure 4.7) and is an indication of zero-order elimination rate processes.

All the urinary excretion data were therefore subjected to Hanes-type plots, as described in the methods. This analysis enabled the determination of first-order elimination rate constants for theophylline (k_{el}^{T}), 1,3-dimethyluric acid (k_{el}^{DMU}), 1-methyluric acid (k_{el}^{MU}) and for the unknown metabolites (k_{el}^{U}); and the determination of the two Michaelis parameters for 3-methylxanthine (V_{max}^{3MX} and Km^{3MX}). Data from subject C.A.L. is used to provide an example of this type of analysis (Figure 4.8). Table 4.3 summarises the various elimination rate constants for theophylline and its metabolites in the four volunteers, together with the time taken for each volunteer to excrete 50% of the administered radioactivity in urine ($t_{\frac{1}{2}}$).

Although the "Sigma-minus" method for the determination of the rate of elimination of a drug from the body, by all processes, (K), assumes elimination by first-order processes, this method of analysis was also carried out on the data. This enabled some comparison between the two analytical procedures to be made, and these will be discussed in Chapter 7. Table 4.4 lists those parameters determined

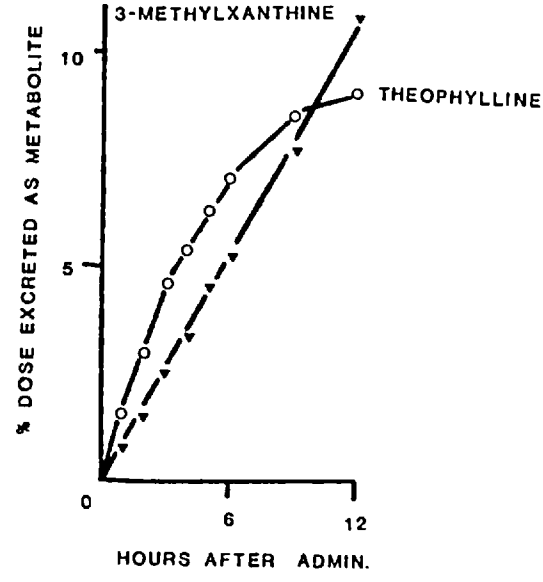


Figure 4.7 CUMULATIVE EXCRETION OF THEOPHYLLINE AND 3-METHYLXANTHINE TO ILLUSTRATE THE CONSTANT RATE OF EXCRETION OF 3-METHYLXANTHINE OVER THE 0-12H PERIOD POST DOSING. SUBJECT T.J.M.

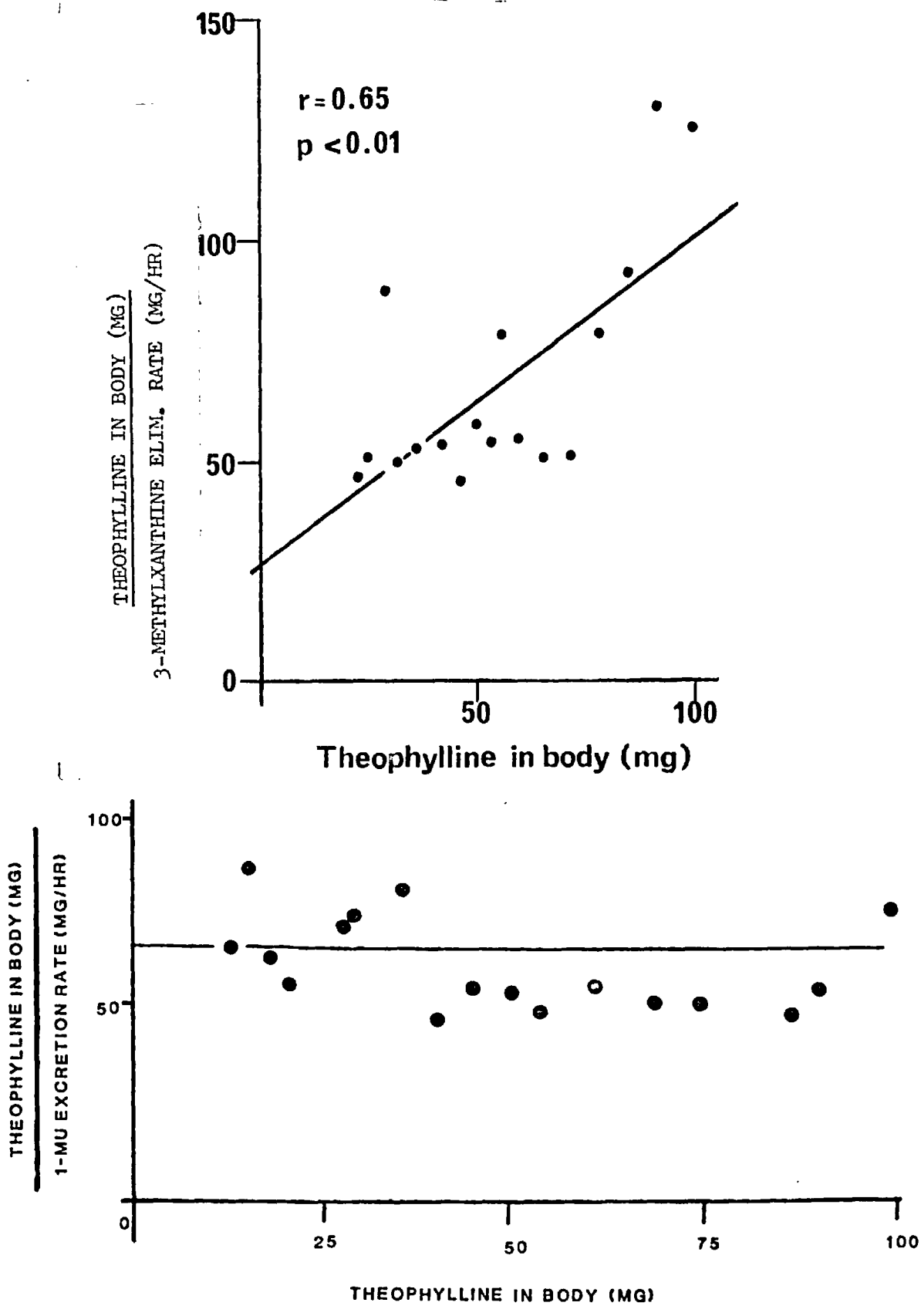
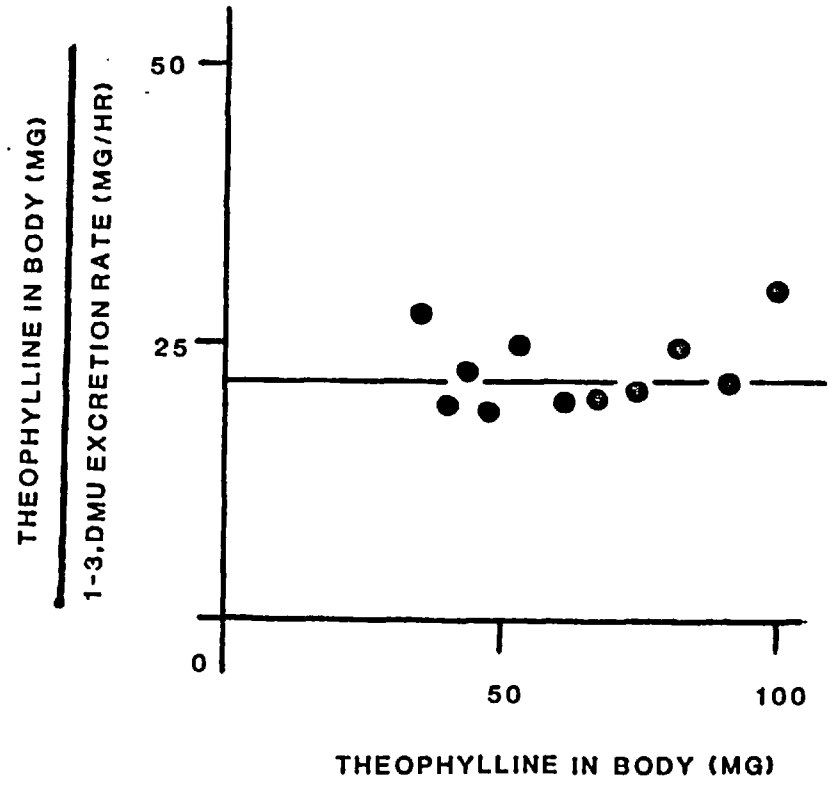


Figure 4.8 HANES-TYPE PLOTS (S/v AGAINST S) FOR THEOPHYLLINE AND ITS METABOLITES FROM SUBJECT C.A.L. FOLLOWING THE INTRAVENOUS ADMINISTRATION OF (^{14}C)-THEOPHYLLINE (100mg; 10μCi). ANY UNEXCRETED MATERIAL ASSUMED TO BE THEOPHYLLINE (LEVY ET AL., 1972).



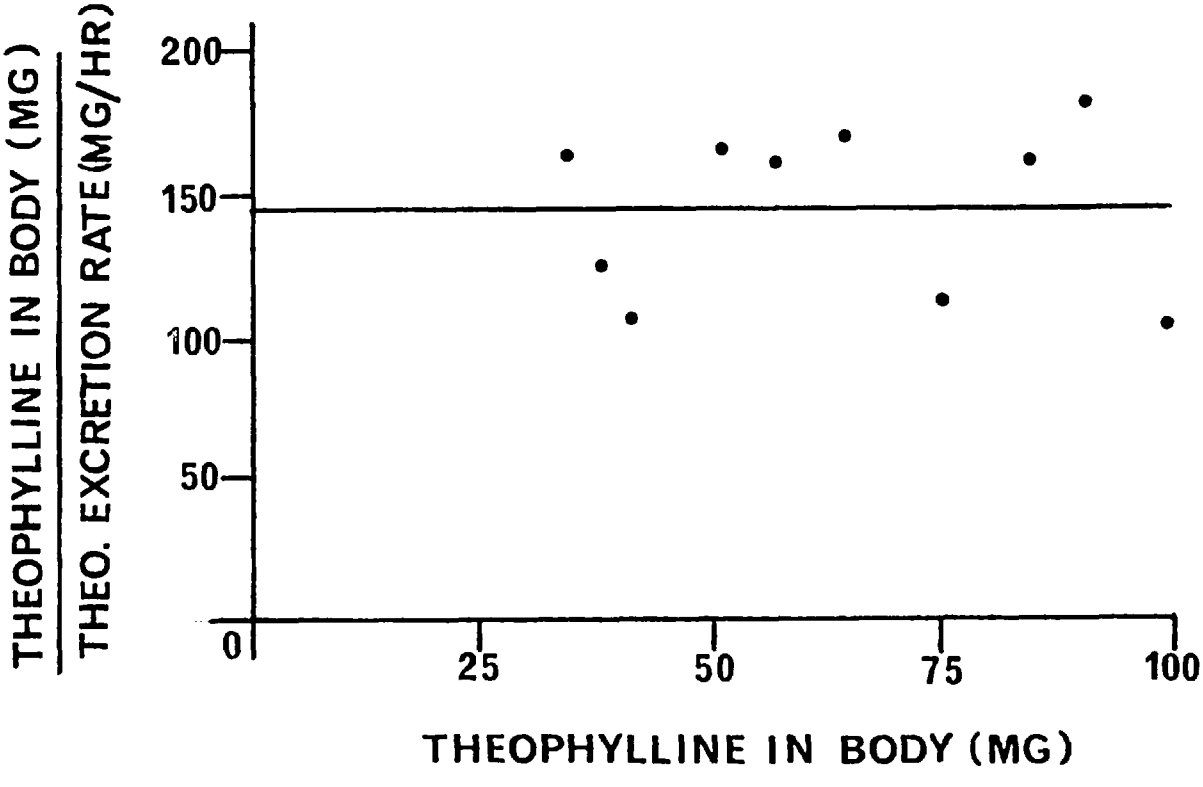


Figure 4.8 cont.

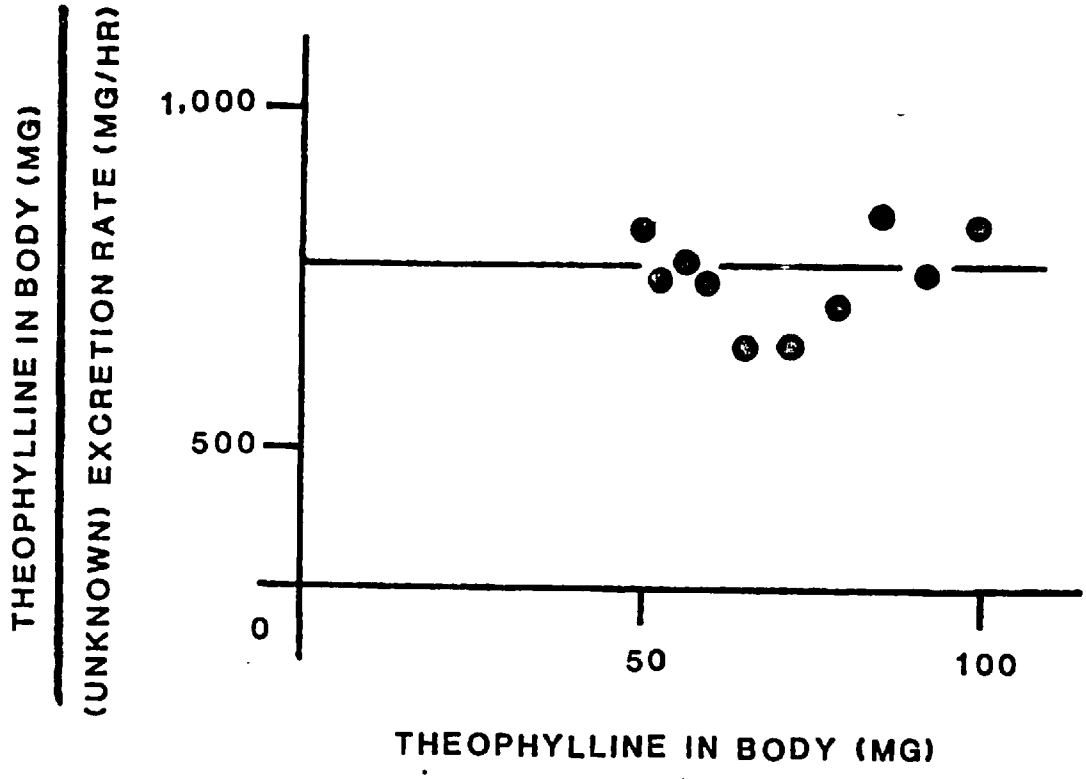


Figure 4.8 cont.

Table 4.3 PHARMACOKINETIC PARAMETERS DESCRIBING THE URINARY ELIMINATION OF (^{14}C)-THEOPHYLLINE

SUBJECT	T.J.M.	J.C.	C.A.L.	L.A.W.	MEAN	S.E..
k_{el}^{T}	0.005	0.016	0.007	0.007	0.009	0.002
k_{el}^{DMU}	0.026	0.027	0.032	0.025	0.028	0.002
k_{el}^{MU}	0.011	0.021	0.016	0.022	0.018	0.003
k_{el}^{U}	0.0017	0.008	0.0013	0.004	0.004	0.002
V_{max}^{3MX}	0.74	1.13	1.38	1.30	1.14	0.14
Km^{3MX}	3.1	21.7	36.9	29.6	22.8	7.3
$t_{\frac{1}{2}}$	13.3	8.4	8.9	8.4	9.8	1.2

k_{el}^{T}, k_{el}^{DMU}, k_{el}^{MU}, k_{el}^{U} are the first-order rate constants describing the urinary elimination of theophylline, 1,3-dimethyluric acid, 1-methyluric acid and the unknown metabolites respectively; Km^{3MX} and V_{max}^{3MX} are the Michaelis constants describing the urinary elimination of 3-methylxanthine; $t_{\frac{1}{2}}$ is the time taken for 50% of the administered dose to be recovered in the urine.

Table 4.4 PHARMACOKINETIC PARAMETERS DESCRIBING THE URINARY ELIMINATION OF (^{14}C)-THEOPHYLLINE DETERMINED BY THE "SIGMA-MINUS" METHOD.

PARAMETER / SUBJECT	$^{*}K(h^{-1})$	$^{**}k_D(h^{-1})$
J.C.	0.23	0.023
C.A.L.	0.18	0.009
L.A.W.	0.16	0.009
T.J.M.	0.11	0.008
MEAN	0.17	0.012

$^{*}K$ = rate of elimination of theophylline by all routes.

$^{**}k_D$ = rate of excretion of unchanged drug only.

by the "Sigma-minus" method for the four volunteers. Again data from subject C.A.L. is employed to provide an example of this type of analysis (Figure 4.9).

Using the kinetic parameters defined above and listed in Table 4.3, it becomes possible to construct an equation which describes the elimination of theophylline from the body, following its intravenous administration to man:-

$$\frac{dS}{dt} = (k_{el}^{T} + k_{el}^{DMU} + k_{el}^{MU} + k_{el}^{U})\ S + \frac{(V_{max}^{3MX}).S}{Km^{3MX}+S} \qquad \ldots\ldots \quad (1)$$

where k_{el}^{T}, k_{el}^{DMU}, k_{el}^{MU} and k_{el}^{U} are the previously defined first-order elimination rate constants; V_{max}^{3MX} and Km^{3MX} are the previously defined Michaelis constants; S is the amount of theophylline remaining in the body at time t; and the overall elimination rate of theophylline is given by $\frac{dS}{dt}$. Equation (1) can be simplified such that the elimination of theophylline can be described by the mixed-order equation shown below:-

$$\frac{dS}{dt} = A.S + \frac{B.S}{C+S} \qquad \ldots\ldots \quad (2)$$

where A is the combined first-order elimination rate constant and B and C are the apparent V_{max} and Km of the process(es) by which 3-methylxanthine is eliminated.

Equation (2) can be readily integrated to give t in terms of S_t,

$$t = \frac{B}{A(A.C+B)} \ln (A.C+B+A.S_o) + \frac{C}{A.C+B} \ln (S_o) \quad -$$

$$\frac{B}{A(A.C+B)} \ln (A.C+B+A.S_t) + \frac{C}{A.C+B} \ln (S_t) \qquad \ldots\ldots \quad (3)$$

where S_o is the amount of drug in the body at t = 0, i.e. the administered dose.

Substitution of the kinetic parameters from Table 4.3 into equation (3) and solution with a digital computer gave the results shown in Figure 4.10.

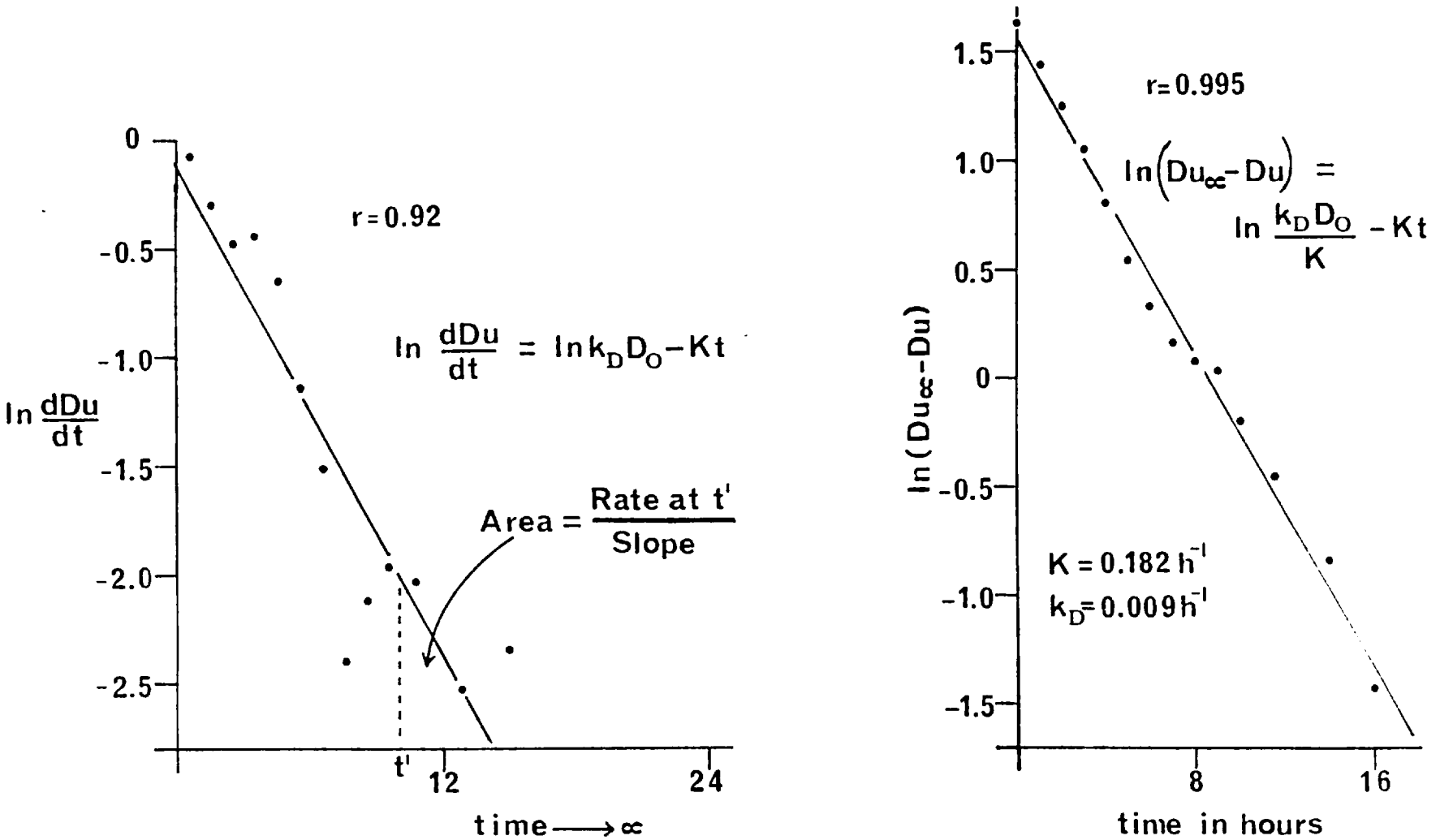


Figure 4.9 "SIGMA-MINUS" TYPE PLOT FOR THEOPHYLLINE FOLLOWING THE INTRAVENOUS ADMINISTRATION OF (^{14}C)-THEOPHYLLINE (100mg; 10μCi). SUBJECT C.A.L.

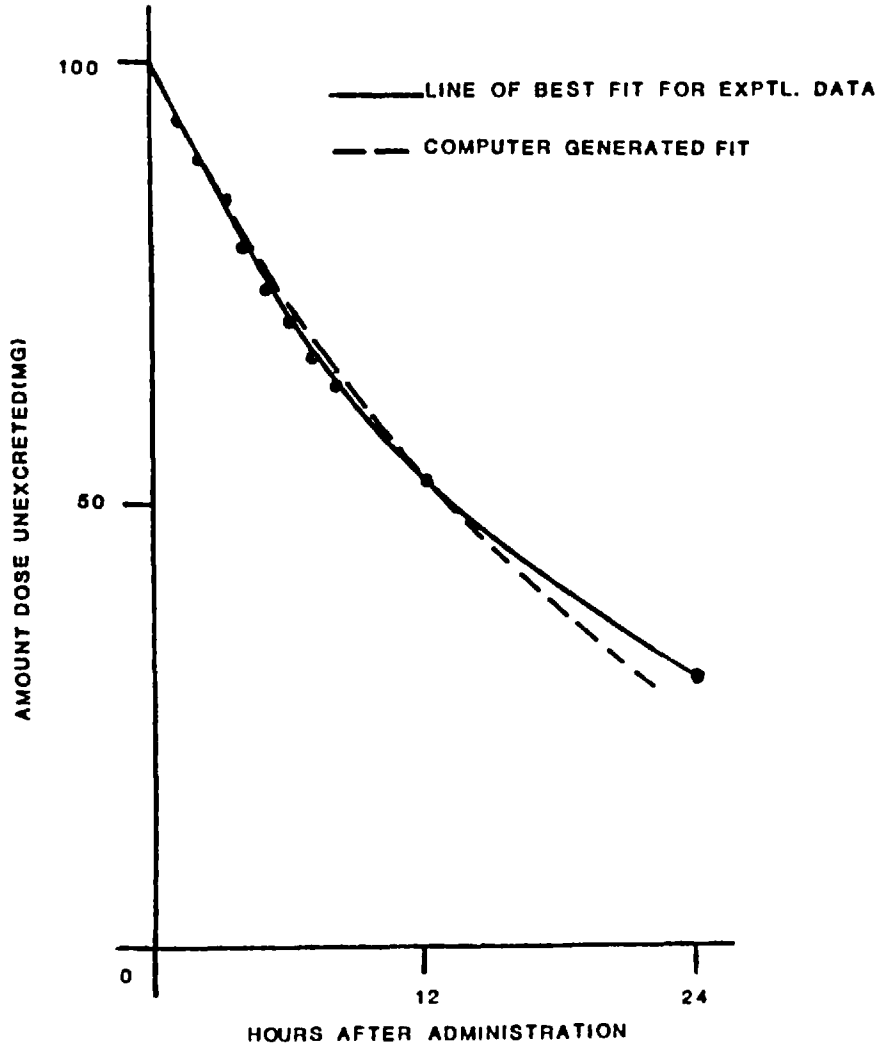


Figure 4.10 ATTEMPT TO FIT THE TIME COURSE OF TOTAL ^{14}C ELIMINATION, FOLLOWING THE INTRAVENOUS ADMINISTRATION OF (^{14}C)-THEOPHYLLINE TO A PHARMACOKINETIC MODEL CONSISTING OF 3-METHYLXANTHINE ELIMINATION BY MICHAELIS-MENTEN KINETICS AND APPARENT FIRST-ORDER PROCESS FOR 1,3-DIMETHYLURIC ACID, 1-METHYLURIC ACID THEOPHYLLINE AND THE UNKNOWN METABOLITE ELIMINATION

From equation (3), the elimination half-life ($t_{\frac{1}{2}}$) for any given value of S can be calculated:-

$$t_{\frac{1}{2}} = \frac{B}{A(A.C+B)} \ln \frac{A.C+B+A.S}{A.C+B+\frac{1}{2}A.S} + \frac{C}{A.C+B} \ln (2) \quad \ldots\ldots \quad (4)$$

It can be seen that $t_{\frac{1}{2}}$ is dependent upon the body load from which the measurements are begun, and is therefore not constant, as is the case where elimination is solely by first-order processes. Again substitution of the average kinetic parameters from Table 4.3 into equation (4) with varying values of S, gives rise to a graph of $t_{\frac{1}{2}}$ against S of the shape shown in Figure 4.11.

This sigmoid curve is characteristic of mixed-order elimination (Rowland, 1977), and the minimum and maximum $t_{\frac{1}{2}}$ values are given by:-

$$\text{min.} \quad t_{\frac{1}{2}} = 0.693 \frac{C}{A.C+B} \quad \text{as S approaches zero}$$

$$\text{max.} \quad t_{\frac{1}{2}} = 0.693 \frac{1}{A} \quad \text{as S approaches infinity}$$

Discussion

The metabolic fate of (^{14}C)-theophylline after its intravenous administration to four healthy male volunteers remained essentially the same as that after its oral administration. There appear to be no significant route differences in the metabolism of (^{14}C)-theophylline. Thus it is converted to three major metabolites, 1,3-dimethyluric acid, 1-methyluric acid and 3-methylxanthine. However, two minor metabolites are excreted in the urine of each volunteer after intravenous administration, one having the properties of a basic compound and the second the properties of an acid. At present these metabolites have not been identified, but recently several novel metabolites have been identified in the urine of various species following methylxanthine administration, (Table 4.5; Figure 4.12). Furthermore, both ribonucleosides and ribonucleotides are formed with analogues of purines and pyrimidines, probably by the same enzyme system (in the soluble fraction of the cell) responsible for synthesising nucleosides and

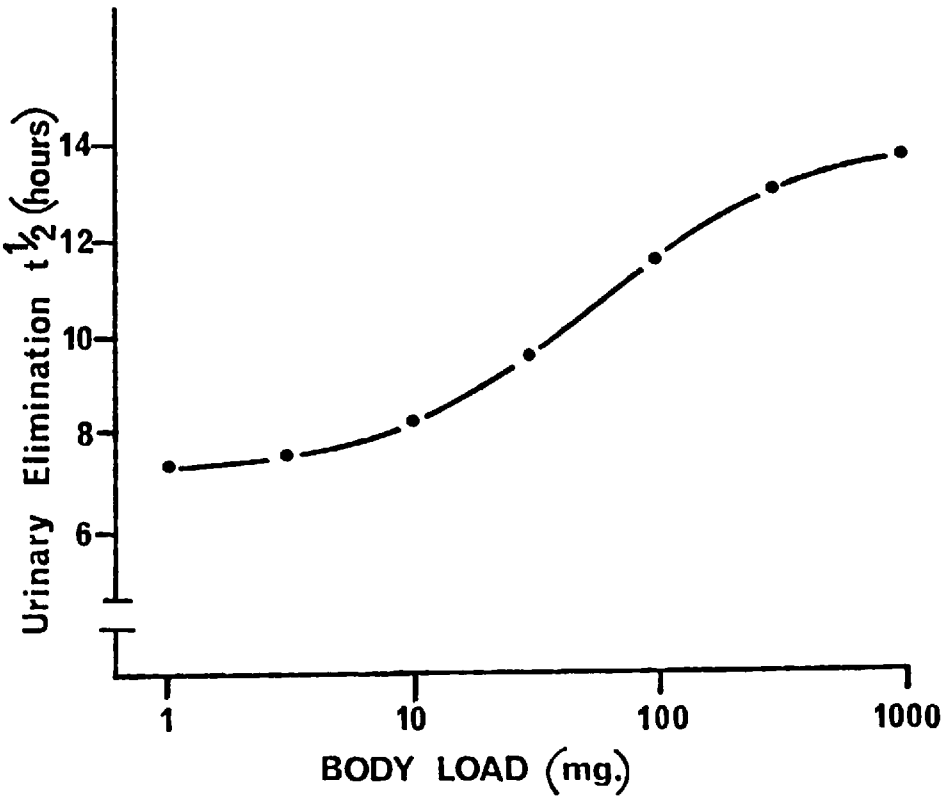


Figure 4.11 RELATIONSHIP BETWEEN THE URINARY ELIMINATION HALF-LIFE OF TOTAL ^{14}C AND THE BODY LOAD OF METHYLXANTHINES

Table 4.5 SOME NOVEL PRODUCTS OF METHYLXANTHINE METABOLISM

METHYLXANTHINE	METABOLITE	REFERENCE
Caffeine	In the rat 1,3,7-trimethyldihydrouric acid (I)* accounts for 1.3% of the dose and appears in equilibrium with its open chain N-formyl analog (II)	Rao et al., 1973 Arnaud, 1976
	3,6,8-trimethylallantoin (III) accounts for 11.4% of the dose in the rat	Rao et al., 1973
	N-methylurea (IV) and NN'-dimethylurea (V) account for less than 0.5% of the dose in rats	Arnaud, 1976
	A sulphur containing metabolite, isolated and identified in the urine of the horse, rabbit, rat and mouse as - (7-1 (1,3-dimethylxanthinyl)) methyl sulphoxide (VI). In mouse urine the corresponding sulphide and sulphone were also identified	Kamei et al., 1975
Theobromine	In the urine of rats 36% of the dose identified as 4-amino-5(N-methylformylamino) 3-methyluracil (VII). This is formed via the formation of 3,7-dimethyldihydrouric acid.	Arnaud, 1978

Table 4.5 cont.

METHYLXANTHINE	METABOLITE	REFERENCE
Theophylline	Caffeine has been shown to be a biotransformation product of theophylline in premature newborns treated with theophylline for apnea. Caffeine was authentified by high-pressure liquid chromatography and mass spectrometry	Baltassat et al., 1978 a, b.
	* I, II, III, IV, V, VI and VII refer to the structures of the various compounds which are illustrated in Figure 4.12.	

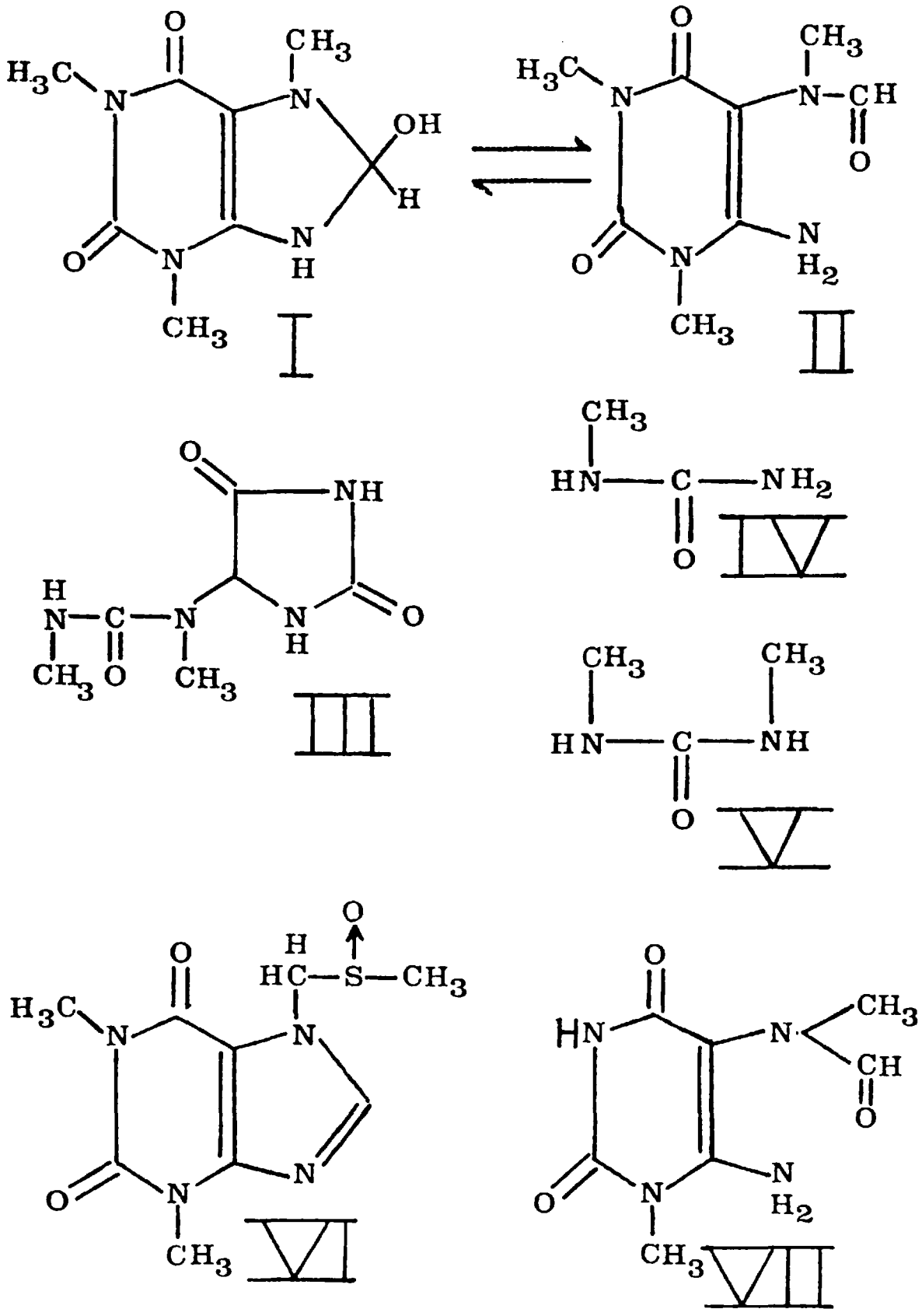


Figure 4.12 THE STRUCTURES OF SOME NOVEL PRODUCTS OF METHYLXANTHINE METABOLISM. (SEE TABLE 4.5).

nucleotides of the naturally occuring purines and pyrimidines. Many analogues of these compounds of interest as anti-cancer agents have been studied, and in almost every instance the biologically active compounds are the phosphorylated ribonucleoside derivatives (Montgomery, 1965). This type of reaction is exemplified by the conversion of 6-mercaptopurine to a ribonucleotide by reaction with 5-phosphoribosyl 1-pyrophosphate (PRPP), catalysed by a purine phosphoribosyl transferase (Lukens and Herrington, 1957) (Figure 4.13). Allopurinol (Figure 4.13) used in the treatment of gout, undergoes a similar reaction.

The structural similarity between these compounds and theophylline are clearly evident. Furthermore, it is the nitrogen atom in position nine of the imidazole ring in these compounds which participates in the reaction with PRPP. This position remains free in theophylline to participate in such a reaction, but whether the methyl group in position three, or the carbonyl groups in positions two or six hinder the reaction is not known. Alternatively, purines, pyrimidines and some of their analogues may react with α-D-ribose-1-phosphate; the phosphate group is split out and a ribonucleoside results. This type of reaction is catalysed by a nucleoside phosphorylase, of which theophylline might be a substrate.

The possible natures of the two unidentified metabolites in the present study are therefore numerous, with the potential opening of the imidazole ring, as observed by Arnaud (1978) after the administration of theobromine to the rat, offering further interesting alternatives.

The rate of elimination of theophylline, 1,3-dimethyluric acid and 1-methyluric acid have been shown to be described by first-order kinetics. However, the rate of elimination of 3-methylxanthine remains constant for a period of about 12 hours post administration, after which it declines at a constant rate with time. This observation can

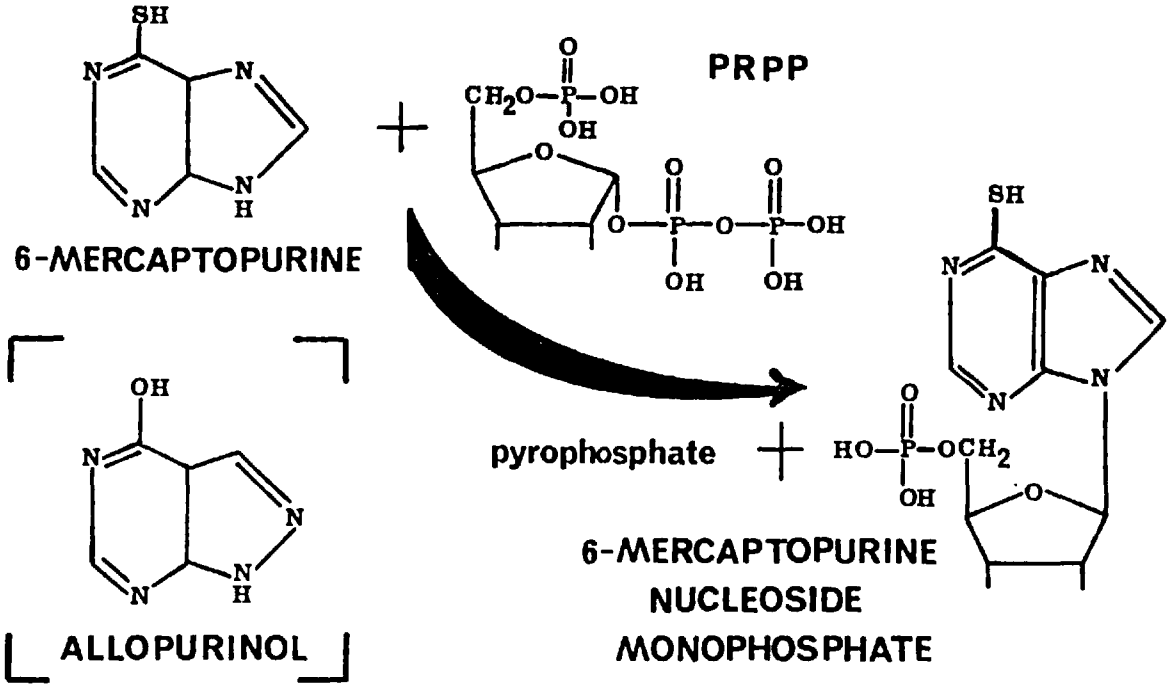


Figure 4.13 THE PHOSPHORYLATION OF THE NATURALLY OCCURRING PURINES AND PYRIMIDINES AS EXEMPLIFIED BY THE REACTION OF 6-MERCAPTOPURINE WITH 5-PHOSPHORIBOSYL 1-PYROPHOSPHATE. INSET: ALLOPURINOL.

be explained by one or both of two processes. Either the rate of formation of 3-methylxanthine is capacity limited, that is the enzyme system which catalyses the 1-N demethylation of theophylline is saturated; or the rate of excretion of 3-methylxanthine is capacity limited. The former explanation appears to be more acceptable as Van Gennip et al. (1973) have shown that, at least in the children they studied, man can excrete relatively large amounts of 3-methylxanthine in urine over a 24 hour period. Methylated xanthines could theoretically be formed endogenously from 1-methyladenine, 3-methyladenine and 7-methylguanine, components of transfer RNA (McPharlane and Lee, 1970; Craddock, 1970). However, any excessive methylxanthinuria is probably of dietary origin, for when the dietary methylxanthines are omitted from the diet the excretion decreases to very low levels or even zero (Van Gennip et al., 1973).

It therefore appears that man has a limited capacity for the conversion of theophylline to 3-methylxanthine, and that this reaction is probably rate-limiting for the overall elimination of theophylline. This finding is supported by the observations of Jenne et al. (1976) who noted an inverse correlation between the adjusted serum theophylline concentration and the fraction of 3-methylxanthine in the urine. Thus as more 3-methylxanthine appears in the urine, indicating removal of theophylline from serum by the process of metabolism, lower serum concentrations of theophylline are observed. On the basis of these findings Jenne et al. (1976) concluded that the 1-N demethylation of theophylline was the dominant reaction determining serum theophylline concentrations. The present study readily explains the basis of their findings.

The rate of theophylline elimination has previously been assumed to be directly proportional to its serum concentration (Mitenko and Ogilvie, 1973; Ellis et al., 1976) that is elimination by first-order

processes. Vaucher et al. (1977) have stated that the disappearance of theophylline from serum follows first-order kinetics even at high serum theophylline levels. While this is usually typical for drug elimination, some drugs do exhibit saturation of elimination mechanisms (Table 4.6) as concentrations in the body increase, resulting in disproportionate increases in their rates of elimination. In contrast to the work of Vaucher et al. (1977) Weinberger and Ginchansky (1977) while investigating the pharmacokinetics of theophylline in children discovered that increases in dosage were associated with serum concentrations which increased to a greater degree than was predicted by assuming first-order kinetics. Their data supported the presence of dose-dependent kinetics for theophylline elimination. They subsequently fitted their data to the Michaelis-Menten model over the range for which data was obtained and derived a plot relating steady-state serum theophylline concentrations against dose.

However, present results suggest the elimination of theophylline is now best described by parallel first-order and Michaelis-Menten kinetics. The mathematical model describing theophylline and subsequent determination of urinary half-life variations with dose, has important clinical implications. Such a system is characterised by apparent overall first-order elimination at concentrations well below Km since $S \ll km$ and saturation effects do not become evident. It also approaches a first-order process when the concentration is well above Km since $k.S \gg V_{max}$. This situation explains the observations and conclusions of Vaucher et al. (1977). However between these two extremes the apparent elimination half-life of theophylline continually changes with concentration (Figure 4.11).

The consequences of both saturable and non-saturable drug elimination are illustrated in Figure 4.14. In a non-saturable

Table 4.6 EVIDENCE FOR CAPACITY-LIMITED DRUG-METABOLISM *

DRUG	COMMENTS	REFERENCE
Salicylic acid	Intensive investigation of capacity-limited formation of salicylurate	Levy et al., (1969) Levy and Yamada (1970)
	Capacity-limited formation of salicyl phenolic glucuronide	Levy (1971) Levy et al., (1972)
	Complete model to explain salicylate pharmacokinetics	Levy et al., (1972)
Salicylamide	Conjugation with sulphate limited by the availability of sulphate	Levy and Matsuzawa (1966)
	Effect of capacity-limited metabolism on plasma levels of unchanged drug	Barr and Riegelman (1968)
Phenacetin and salicylamide or salicylic acid	Biotransformation interaction	Levy and Yamada (1971) Levy and Regardh (1971)
p-Aminobenzoic acid	Percent acetylation related to dose, rate of administration and nutritional factors	Drucker et al., (1964)

Table 4.6 cont.

DRUG	COMMENTS	REFERENCE
Diphenylhydantoin Phenylbutazone Biscoumacetate Probenecid	Changes in elimination half-life and apparent volume of distribution with dose	Dayton et al., (1967)
Bishydroxycoumarin	Elimination kinetics in several species	Nagashima et al., (1968)
Warfarin	Elimination kinetics in several species	Nagashima et al., (1969)
Novobiocin	Elimination half-life dependent on dose in adults and children	Wagner et al., (1968)
Heparin	Change in elimination half-life and apparent volume of distribution with dose	Estes et al., (1969)
Tetracycline	Apparent increase in elimination half-life after multiple dosing	Dolvisio et al., (1969)
Acetanilide	Formation of 4-hydroxyacetanilide shown to obey Michaelis-Menten kinetics	Shibasaki et al., (1968)
Diphenylhydantoin	Intravenous administration in man indicated dose-dependent kinetics did not occur	Suzuki et al., (1970) Blum et al., (1971)

Table 4.6 contd.

DRUG	COMMENTS	REFERENCE
	Oral administration in man indicated dose-dependent kinetics did occur	Arnold and Gerber (1970)
	Studies in mice and rats indicated dose-dependent kinetics did occur	Gerber and Arnold (1969) Gerber et al., (1971)
	Human and rat data fit with integrated form of Michaelis-Menten equation	Gerber and Wagner (1972)
Amylobarbitone	Influence of dose on distribution and elimination kinetics	Balasubramaniam et al., (1970)

*Table adapted from and references cited in the review by Wagner 1974.

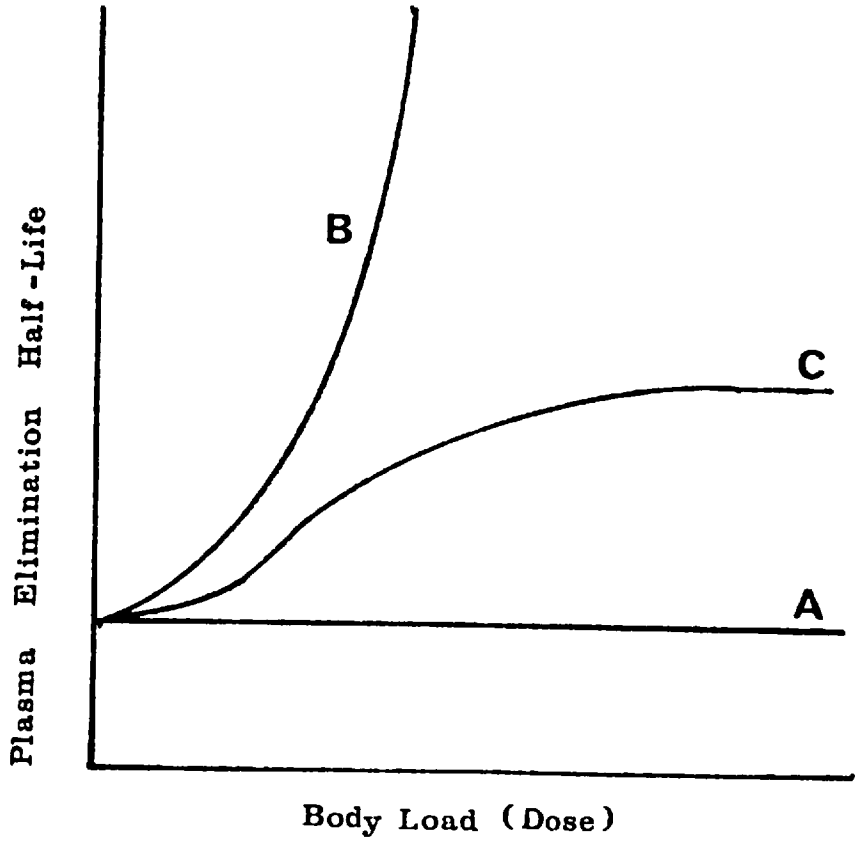


Figure 4.14 THE CONSEQUENCES OF BOTH SATURABLE AND NON-SATURABLE DRUG ELIMINATION AS EXEMPLIFIED BY CHANGES IN THE PLASMA ELIMINATION HALF-LIFE WITH DOSE. CURVE A REPRESENTS ELIMINATION BY FIRST-ORDER PROCESSES ONLY. CURVE B REPRESENTS ELIMINATION BY ZERO-ORDER PROCESSES ONLY. CURVE C REPRESENTS ELIMINATION BY MIXED-ORDER PROCESSES.

eliminating system (Case A) or when $S \ll Km$ in a saturable system, the plateau concentration is proportional to the dose S, and the half-life is independent of concentration. By contrast, in a saturable system as S approaches Km, theplateau concentration rises disproportionately with changes in S (Cases B and C). In Case B (that described by Weinberger and Ginchansky (1977) for theophylline elimination) where the non-saturable process in a negligible component of elimination, the increase is rapid and since the elimination half-life also increases as S approaches Km each new plateau concentration takes longer to reach. In Case C (that described by present data for theophylline elimination) where there is a significant non-saturable as well as a saturable process elimination kinetics become first-order as S exceeds Km. The elimination of phenytoin (Gerber and Wagner, 1972) in man appears to closely approximate Case B; the elimination of salicylic acid in man (Levy and Tsuchiya, 1972) and of theophylline more closely approximates Case C. With all three drugs, saturation of the enzymes occurs within the dosage range normally administered, thereby making it difficult to predict the outcome when the dosage regimen is altered. The same situation will hold for any drug whose Km lies within the therapeutic plasma concentration range and that range is narrow, with a minor increase in the dose causing a disproportionate increase in toxicity. The implications of both first-order and capacity-limited drug elimination are summarised in Tables 4.7 and 4.8.

Table 4.7 Consequences of first-order drug elimination

1. Biological half-life is independent of dose
2. Composition of excretion products is independent of dose
3. Area under blood level versus time curves are proportional to the amount of drug absorbed
4. The principle of superimposition applies; that is both plasma concentration/amount absorbed; and excretion rate of unchanged drug/amount absorbed versus time are independent of dose
5. Absorption kinetics have no effect on points 1 to 3

Table 4.8 Implications of capacity-limited drug elimination

1. Decline of drug levels in the body is not exponential
2. Time required to eliminate 50% of a dose increases with increasing dose
3. Area under blood level versus time curve is not proportional to the amount of drug absorbed
4. Composition of excretion products can be affected by dose and dosage form
5. Competitive inhibition of the capacity-limited process by other drugs that are metabolised by the same enzyme system or that require the same rate-limiting substance is likely.

The results, therefore, offer some explanation for the observed wide inter-individual variation in theophylline disposition. Differences in the various dose regimens suggested for theophylline therapy will in themselves result in varying theophylline plasma levels and elimination rates. In addition to those environmental factors already defined as affecting theophylline disposition (Chapter 1, Table 1.11), some genetic component must also be expected to influence drug disposition. The large inter-individual variation in theophylline disposition observed in patients on the same dose regimens might principally be a result of differences in the rates of 3-methylxanthine formation. Thus inter-individual differences in the genetically determined Michaelis-Menten parameters describing the conversion of theophylline to 3-methylxanthine, the major determinant of theophylline serum levels within the therapeutic dose range, will substantially influence the overall rate of elimination in any individual. Individuals with relatively high rates of 3-methylxanthine formation (high V_{max}) will eliminate theophylline at a faster rate than individuals who form 3-methylxanthine at a slower rate (low V_{max}). That is individuals with lower Km's for this reaction will become saturated at substrate concentrations lower than individuals with high Km's. This supposition is supported by the correlation observed between the Michaelis-Menten parameters for 3-methylxanthine elimination and the overall urinary elimination $t_{\frac{1}{2}}$ of radioactivity (Table 4.3). The subject with the lowest Km and V_{max} (TJM) exhibits the longest elimination $t_{\frac{1}{2}}$, whereas the remaining three subjects all exhibit similar Km, V_{max} and $t_{\frac{1}{2}}$'s.

There is a significant inverse relationship observed between the fractional urine content of 1,3-dimethyluric acid and 3-methylxanthine (Figure 4.4). This implies that the enzymes responsible for the generation of these metabolites are competing for the common precursor, theophylline. Thus, when relatively high levels of 1,3-dimethyluric acid are excreted, lower levels of 3-methylxanthine are found in urine,

and the converse is true. What factors are responsible for determining the relative activities of these two pathways? As the capacity for the production of 3-methylxanthine becomes saturated, it appears that the alternate pathway for the metabolism of theophylline can compensate, and thus more substrate is channeled down this second pathway. This is reflected in the urine content of the two products of these pathways such that as levels of 3-methylxanthine reach a maximum, fractionally more 1,3-dimethyluric acid is produced. As substrate levels fall, the conversion of theophylline to 3-methylxanthine is no longer saturated, and the relative amounts of substrate being channeled down each pathway changes such that fractionally more 3-methylxanthine is recovered in urine than 1,3-dimethyluric acid. This implies that at non-saturable levels of theophylline, the preferential pathway is 1-N-demethylation, but at saturable substrate levels the 8-hydroxylation pathway takes over as the dominant pathway of theophylline metabolism.

There is also exhibited a direct relationship between the urinary content of 1,3-dimethyluric acid and 1-methyluric acid. As more 1,3-dimethyluric acid is produced, subsequently more 1-methyluric acid is produced (Figure 4.5). This may mean that the former gives rise to the latter, but whether the intervening 3-N-demethylation step is rate-limiting, as suggested by Jenne et al. (1976) is debatable. Certainly kinetic analysis of the data in this study shows no evidence of saturation. This point is of interest in that the only other apparent route for the formation of 1-methyluric acid is via the formation of 1-methylxanthine as an intermediate, with such a rapid conversion to 1-methyluric acid that it does not appear in urine. This is consistent with the fact that 1-methylxanthine has a high affinity for xanthine oxidase, comparable to xanthine itself, in contrast to 3-methylxanthine, which is not a substrate for this enzyme (Krenitsky et al., 1972). Furthermore, it has been demonstrated in rat liver slices that the appearance of 1-methylxanthine, with a concominant fall in 1-methyluric acid, only occurs following the addition of the xanthine oxidase

inhibitor, allopurinol. The absence of 3-methylxanthine as a metabolite in rat urine suggests a species difference in theophylline metabolism. However, as relatively large amounts of 1-methylxanthine have been found in urine following caffeine administration (Cornish and Christman, 1957) and as yet 1-methylxanthine has not been identified in urine following theophylline administration, the most likely route of 1-methyluric acid formation is via the preceding formation of 1,3-dimethyluric acid.

The proposed mathematical model describing the elimination of theophylline provides a good fit with the observed and computer-generated decline curves (Figure 4.10). However, this model does not take into account the urine flow-rate dependence of theophylline renal excretion. A closer fit between the observed data and computer-generated data might be achieved if the above factor was incorporated into the original mathematical model.

CHAPTER 5

Metabolism and Pharmacokinetics of Intravenously Administered (^{14}C)-Theophylline to Volunteers on Various Diets

Introduction

The optimum therapeutic effect of theophylline is related to plasma concentrations between about 10-20μg/ml (Green 1969; Jackson et al., 1964; Jenne et al., 1972; Mitenko and Ogilvie, 1973; Nicholson and Chick, 1973; Weinberger and Bronsky, 1974) although Mitenko and Ogilvie (1973) showed a dose-related improvement in pulmonary function in the range of 5-20μg/ml plasma. However, the therapeutic effectiveness of theophylline is hindered by wide inter-individual variations in plasma levels encountered in patients on identical dose regimens. As theophylline is eliminated from the body principally by metabolism, inter-individual differences in the rate and extent of metabolism of the drug will account for the observed variations in both plasma levels and in the plasma elimination half-lives of theophylline. The large inter-individual variations which occur in drug metabolism in man are determined by a variety of interacting genetic, environmental and physiological factors (see Ch.1). Recently, attention has focused on the diet as a determinant of drug metabolism, and with respect to theophylline this may be significant due to the widespread ingestion of large amounts of chemically related methylxanthines.

Therefore with the aid of (^{14}C)-theophylline it is possible to determine the metabolic fate of theophylline without interference from endogenous methylxanthines as described in the preceeding chapter. However, whilst the use of (^{14}C)-theophylline enables an accurate quantitative estimate of theophylline and its metabolites arising solely from the administered dose, it does not preclude the interference of dietary methylxanthines on the rate of theophylline metabolism. Therefore, those kinetic parameters determined in the presence of endogenous methylxanthines may not necessarily reflect the true rate of the metabolic processes. As the metabolism of theophylline has been shown to be best described by parallel first-order and Michaelis-Menten kinetics (Ch.4) it might be expected that altering the body load of methylxanthines will cause changes in the kinetic

parameters, since such a mixed-order system will be expected to exhibit dose-dependency (Wagner, 1974; Rowland, 1977).

Thus, again with the aid of (^{14}C)-theophylline it is possible to determine the effect of the methylxanthine-containing foods on theophylline metabolism, and to assess the extent to which they influence the rate of theophylline metabolism and the extent to which they are responsible for the observed variations in theophylline disposition.

Results

The quantitation of (^{14}C)-theophylline and its metabolites in serial urine samples after the intravenous administration of 100mg (10μCi) (^{14}C)-theophylline to healthy male volunteers on a caffeine/theophylline replacement diet, methylxanthine-supplemented diet and a methylxanthine-deprived diet are given in the Appendix.

Quantitation of (^{14}C)-theophylline and its metabolites and the total radioactivity excreted in the total 0-24 hour urine of each volunteer on each of the above three diets is given in Table 5.1 and compared to the results obtained in volunteers on their normal diets. Statistical analysis of the data in Table 5.1 was carried out by performing an analysis of variance with a digital computer.

The metabolic pattern observed in all volunteers remained essentially the same on all the diets studied, the only qualitative difference being that the small amounts of the unknown acid metabolite could not be detected in any of the volunteers on a methylxanthine-deprived diet. After the administration of (^{14}C)-theophylline to four volunteers on a methylxanthine-deprived diet 85.8 ± 2.6% (Mean ± S.D.) of the dose was recovered in the 0-24 hour urine, significantly greater ($p < 0.01$) than that recovered on a volunteers normal diet. This increase was accounted for by significant increases in the 0-24 hour urinary recovery of both 3-methylxanthine ($p < 0.01$) and 1,3-dimethyluric acid ($p < 0.01$) (Figure 5.1).

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN 0 - 24 HOUR URINE FOLLOWING THE INTRAVENOUS

Table 5.1 ADMINISTRATION OF (^{14}C)-THEOPHYLLINE TO VOLUNTEERS ON VARIOUS DIETS

SUBJECT	T.J.M.				C.A.L.				J.C.				L.A.W.				MEAN			
DIET / METABOLITE	N	R	XD	XS	N	R	XD	XS	N	R	XD	XS	N	R	XD	XS	N	R	XD	XS
THEOPHYLLINE	7.5	6.9	9.8	9.6	4.9	7.9	9.2	14.5	11.1	10.6	10.9	13.9	6.5	-	5.1	-	7.5	8.5	8.8	12.7
3-METHYL-XANTHINE	15.8	16.4	20.3	17.2	19.0	18.7	22.8	17.5	16.2	18.4	18.4	17.7	17.4	-	22.3	-	17.1	17.8	21.0	17.5
1,3-DIMETHYL-URIC ACID	30.1	30.4	35.3	31.3	34.6	31.0	38.3	36.2	26.0	29.6	24.4	31.8	27.9	-	37.4	-	29.7	30.3	36.4	33.1
1-METHYLURIC ACID	12.9	11.5	15.3	12.5	16.6	14.5	16.8	17.0	18.8	18.0	19.2	13.4	25.0	-	20.6	-	18.3	14.7	18.0	14.3
UNKNOWN XANTHINE	1.7	2.0	2.2	1.8	1.1	0.1	1.1	0.9	1.0	0.1	0.9	-	1.1	-	0.9	-	1.2	0.7	1.3	0.9
UNKNOWN URIC ACID	0.6	0.1	-	0.8	0.4	1.0	-	1.3	5.3	1.5	-	2.1	4.1	-	-	-	2.6	0.9	-	1.4
TOTAL RADIO-ACTIVITY	68.6	67.4	82.8	73.4	77.3	72.9	88.7	87.6	78.4	78.0	84.4	78.9	81.9		87.1		76.6	72.8	85.8	80.0

N = Normal diet XD = Methylxanthine-deprived diet XS = Methylxanthine-supplemented diet

R = Caffeine/theophylline replacement diet

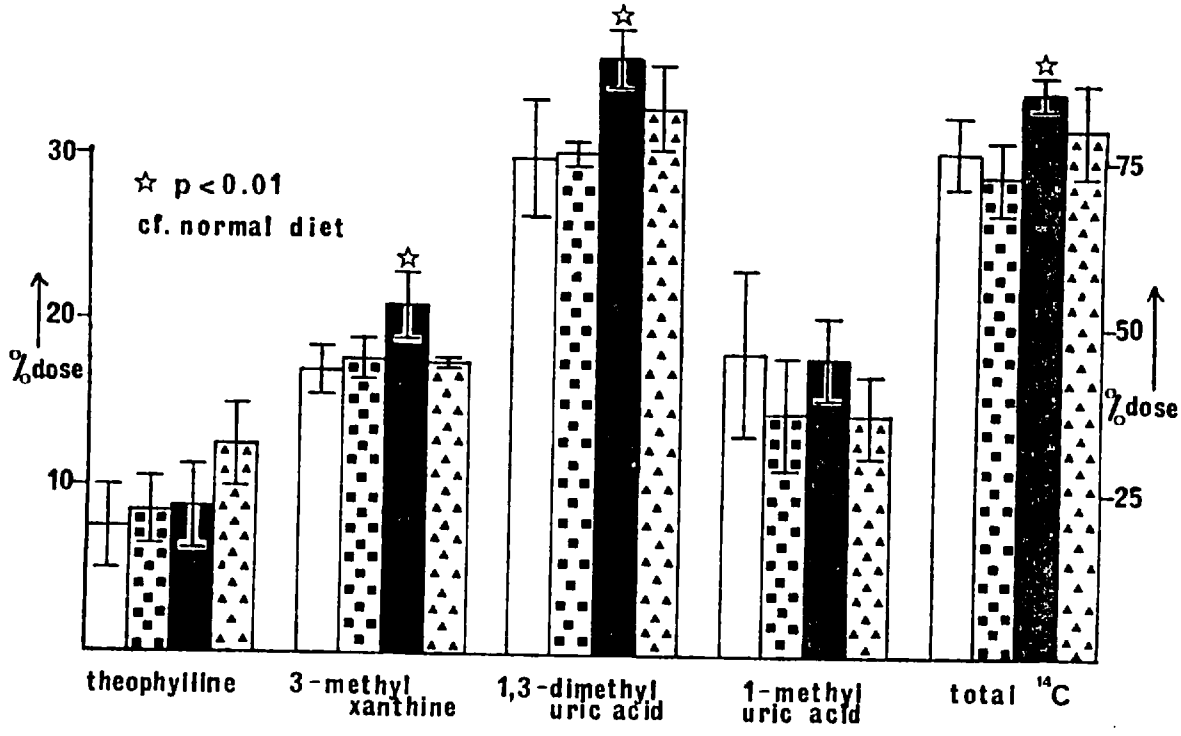


Figure 5.1 QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES (MEAN VALUES ± S.D.) EXCRETED BY VOLUNTEERS ON A NORMAL (OPEN BARS), METHYLXANTHINE-REPLACEMENT (HATCHED BARS), METHYLXANTHINE-DEPRIVED (CLOSED BARS) AND METHYLXANTHINE-SUPPLEMENTED (TRIANGULATED BARS) DIETS.

The recovery of total radioactivity and of metabolites in 0-24 hour urine of volunteers on a caffeine/theophylline replacement diet or a methylxanthine-supplemented diet was not significantly different from that observed in volunteers on their normal diets.

The relationships described in Chapter 4 after the intravenous administration of (^{14}C)-theophylline to volunteers on their normal diets were also observed in the volunteers in each of the three experimental diets, namely:-

1. The fractional urine content of 1,3-dimethyluric acid and 3-methylxanthine (Figure 5.2).
2. The rate of elimination of 1,3-dimethyluric acid and 1-methyluric acid (Figure 5.3)
3. The urine flow-rate dependency of theophylline elimination (Figure 5.4)

The pharmacokinetic parameters describing the urinary elimination of (^{14}C)-theophylline and its metabolites in volunteers on a caffeine/theophylline replacement diet, methylxanthine-deprived diet and a methylxanthine-supplemented diet are given in Table 5.2 and compared to the results obtained in volunteers on their normal diets.

Statistical analysis of the data in Table 5.2 was carried out by performing an analysis of variance with a digital computer. Again, the only statistically significant differences observed between those parameters determined on a volunteers normal diet and those on the three experimental diets were in parameters determined in volunteers on a methylxanthine-deprived diet. On such a diet both the V_{max} and Km of the Michaelis-Menten expression describing the elimination of 3-methylxanthine and the first-order rate constant describing the elimination of 1,3-dimethyluric acid were all significantly higher from those values obtained on the volunteer's normal diets ($p < 0.001$; $p < 0.01$; $p < 0.01$ respectively). These increases also accounted for

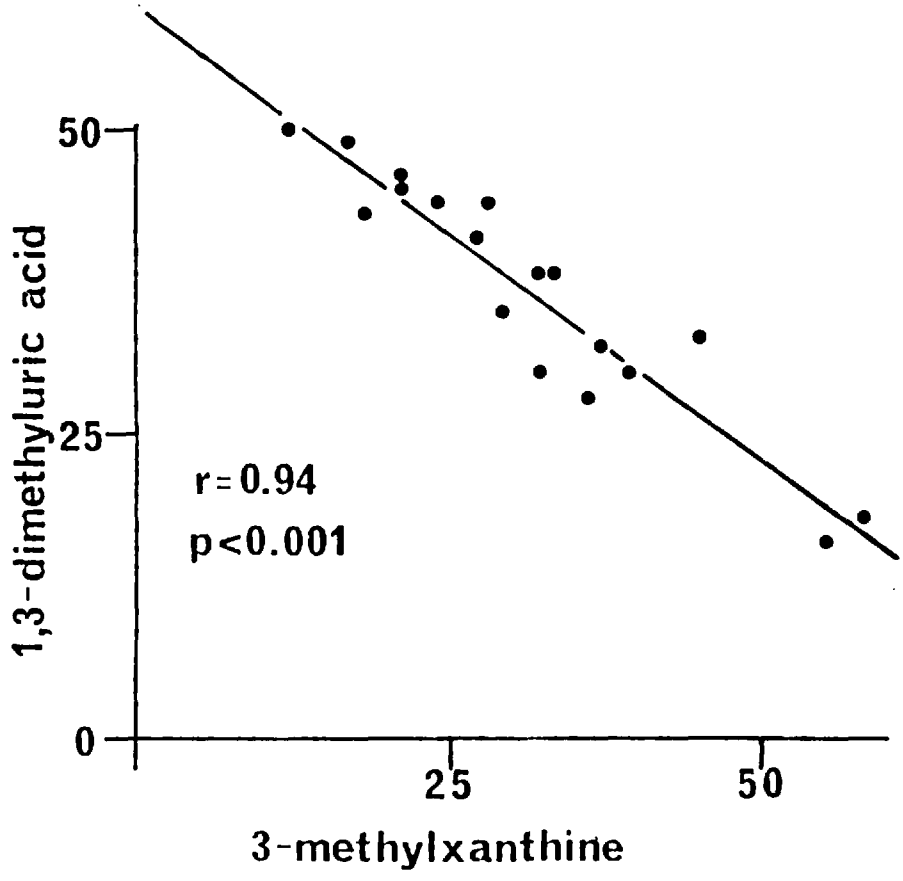


Figure 5.2 THE FRACTIONAL URINE CONTENT OF 3-METHYLXANTHINE AND 1,3-DIMETHYLURIC ACID AFTER THE INTRAVENOUS ADMINISTRATION OF (^{14}C)-THEOPHYLLINE (100mg; 1μCi) TO A VOLUNTEER ON A METHYLXANTHINE-DEPRIVED DIET. SUBJECT L.A.W.

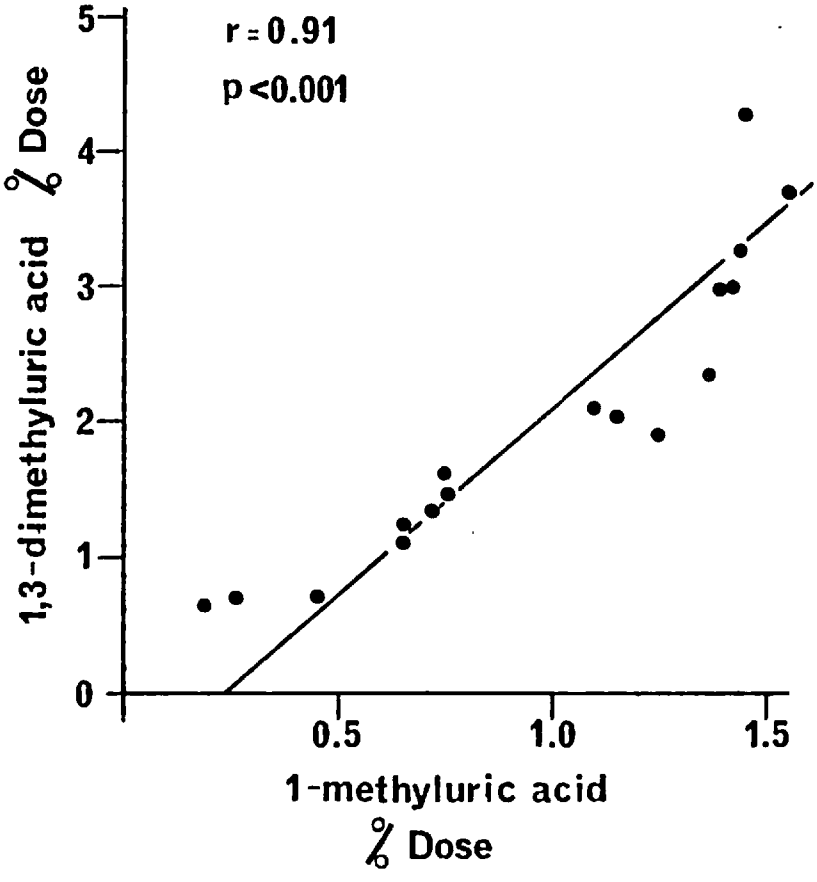


Figure 5.3 THE RELATIONSHIP BETWEEN THE AMOUNTS OF 1,3-DIMETHYLURIC ACID EXCRETED IN URINE AFTER THE INTRAVENOUS ADMINISTRATION OF (^{14}C)-THEOPHYLLINE (100mg; 10μCi) TO A VOLUNTEER ON A METHYLXANTHINE-DEPRIVED DIET. SUBJECT C.A.L.

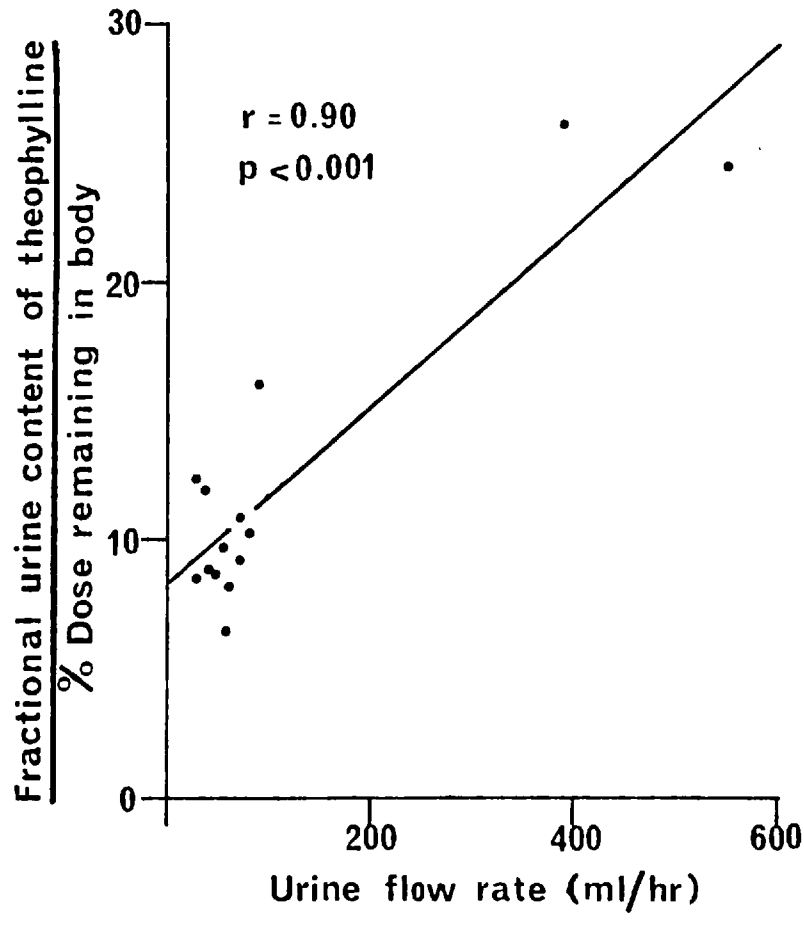


Figure 5.4. THE URINE FLOW-RATE DEPENDENCY OF THEOPHYLLINE EXCRETION AFTER THE INTRAVENOUS ADMINISTRATION OF (^{14}C)-THEOPHYLLINE (100mg; 10μCi) TO A VOLUNTEER ON A METHYLXANTHINE-DEPRIVED DIET. SUBJECT L.A.W.

Table 5.2 PHARMACOKINETIC PARAMETERS DESCRIBING THE URINARY ELIMINATION OF (^{14}C)-THEOPHYLLINE TO VOLUNTEERS ON VARIOUS DIETS

SUBJECT	PHARMACOKINETIC PARAMETERS							
	DIET	$k_{el}^{T}(h^{-1})$	$k_{el}^{DMU}(h^{-1})$	$k_{el}^{MU}(h^{-1})$	$k_{el}^{U}(h^{-1})$	V_{max}^{3MX}(mg/hr)	km^{3MX}(mg)	$t_{\frac{1}{2}}$(h)
T.J.M.	N	0.005	0.026	0.011	0.0017	0.74	3.1	13.3
	R	0.005	0.021	0.008	0.002	0.71	2.0	14.5
	XD	0.009	0.031	0.014	0.002	1.84	53.4	8.7
	XS	0.006	0.024	0.010	0.002	0.87	9.4	12.0
J.C.	N	0.016	0.027	0.021	0.008	1.13	21.7	8.4
	R	0.010	0.024	0.016	-	1.45	44.6	9.3
	XD	0.014	0.042	0.024	0.001	2.08	58.4	6.0
	XS	0.016	0.027	0.015	0.002	1.16	24.6	9.6
C.A.L.	N	0.007	0.032	0.016	0.0013	1.38	36.9	.8.9
	R	0.006	0.026	0.013	-	1.42	41.5	10.7
	XD	0.011	0.043	0.017	0.0015	2.27	57.5	6.9
	XS	0.016	0.036	0.019	0.002	1.54	39.7	6.8
L.A.W.	N	0.007	0.025	0.022	0.004	1.30	29.6	8.4
	XD	0.007	0.039	0.022	0.002	2.29	52.5	6.2
MEAN	N	0.009	0.028	0.018	0.004	1.14	22.8	9.8
	R	0.007	0.024	0.012	-	1.19	29.4	11.5
	XD	0.010	0.039	0.019	0.002	2.12	53.9	7.0
	XS	0.013	0.029	0.015	0.002	1.19	24.6	9.5
	r*	0.58	0.81	0.89	0.05	0.86	0.85	
	p	0.05	0.001	0.001	n.s.	0.001	0.001	

*correlation coefficient between pharmacokinetic parameters and urinary $t_{\frac{1}{2}}$

the significant decrease in the urinary elimination half-life of total radioactivity observed in volunteers on a methylxanthine-deprived diet ($p < 0.02$).

Table 5.2 also lists the correlation between the various pharmacokinetic parameters describing the urinary elimination of (^{14}C)-theophylline and the urinary elimination half-life of total radioactivity on all diets.

By employing the values obtained for the apparent Km of the Michaelis-Menten expression describing the elimination of 3-methylxanthine (Table 5.2) it becomes possible to estimate the degree of saturation of the enzyme system responsible for the theophylline/3-methylxanthine conversion according to the equation derived by Wagner (1973):-

$$\% \text{ saturation} = \frac{100.C}{Km + C}$$

where Km is the previously defined Michaelis constant for 3-methylxanthine elimination and C is the amount of substrate in the body (expressed either in absolute terms or as a concentration). Figure 5.5 shows the relationship between the administered dose or body load of methylxanthines and the % saturation of the enzyme system, using the mean Km value obtained in the volunteers on a methylxanthine-deprived diet. Assuming the apparent Km value obtained on volunteers methylxanthine-deprived diet to be a closer estimate of the actual Km than that determined in the presence of dietary methylxanthines (see introduction and discussion for justification) and substituting the observed % saturation calculated on each volunteers normal diet into the above equation the apparent C_o on each volunteers normal diet can be calculated (where C_o represents the body load of methylxanthines in each volunteer prior to the administered dose). This value and the % saturation of the enzyme system in each volunteer on a normal and

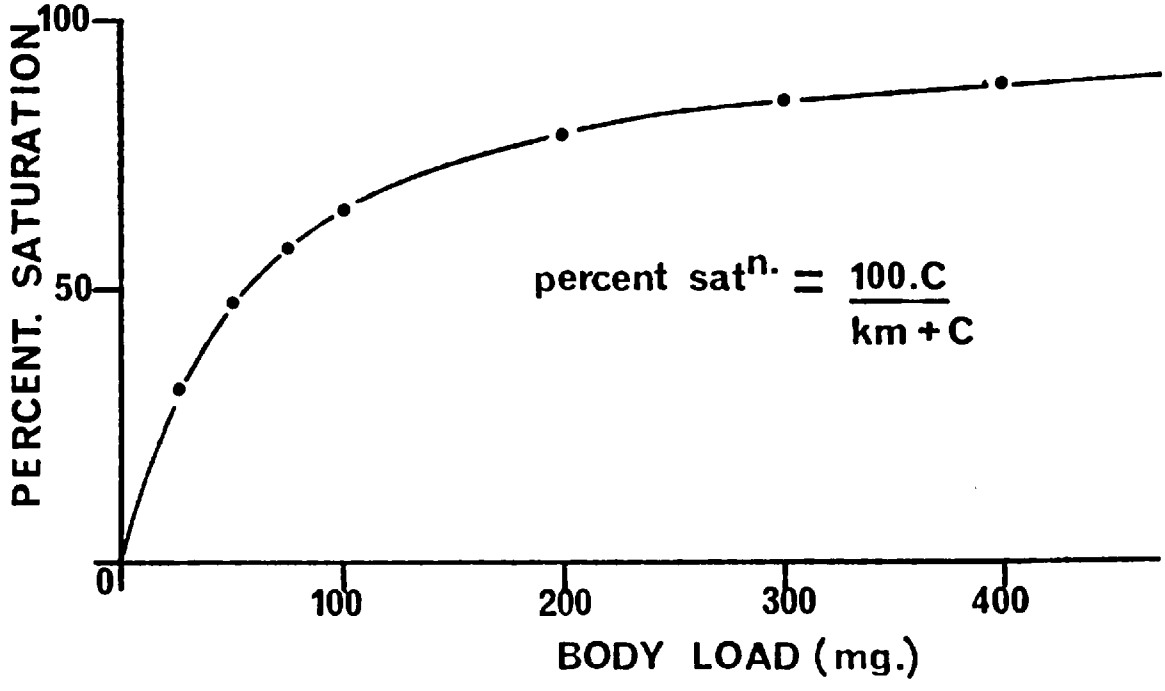


Figure 5.5 RELATIONSHIP BETWEEN THE % SATURATION OF THE ENZYME(S) RESPONSIBLE FOR THE CONVERSION OF THEOPHYLLINE TO 3-METHYLXANTHINE AND THE BODY LOAD OF METHYLXANTHINES

methylxanthine-deprived diet is given in Table 5.3.

Table 5.3 Percentage saturation of the enzyme system(s) responsible for the conversion of theophylline to 3-methylxanthine in volunteers on their normal and methylxanthine-deprived diets.

DIET / SUBJECT	NORMAL			METHYLXANTHINE-DEPRIVED	
	Km	% Saturation	Co(mg) Theoretical	Km	% Saturation
T.J.M.	3.1	97.0	1,727	53.4	65.2
J.C.	21.7	82.2	270	58.4	63.1
L.A.W.	29.6	77.2	178	52.5	65.6
C.A.L.	36.9	73.0	155	57.5	63.5

There is a direct correlation between the percentage increase in the V_{max} observed in volunteers on a methylxanthine-deprived diet and the subsequent percentage decrease in the urinary elimination half-life of total radioactivity ($p<0.05$) (Figure 5.6).

Table 5.4 lists the personal details of the volunteers participating in the study and their estimated dietary methylxanthine intake.

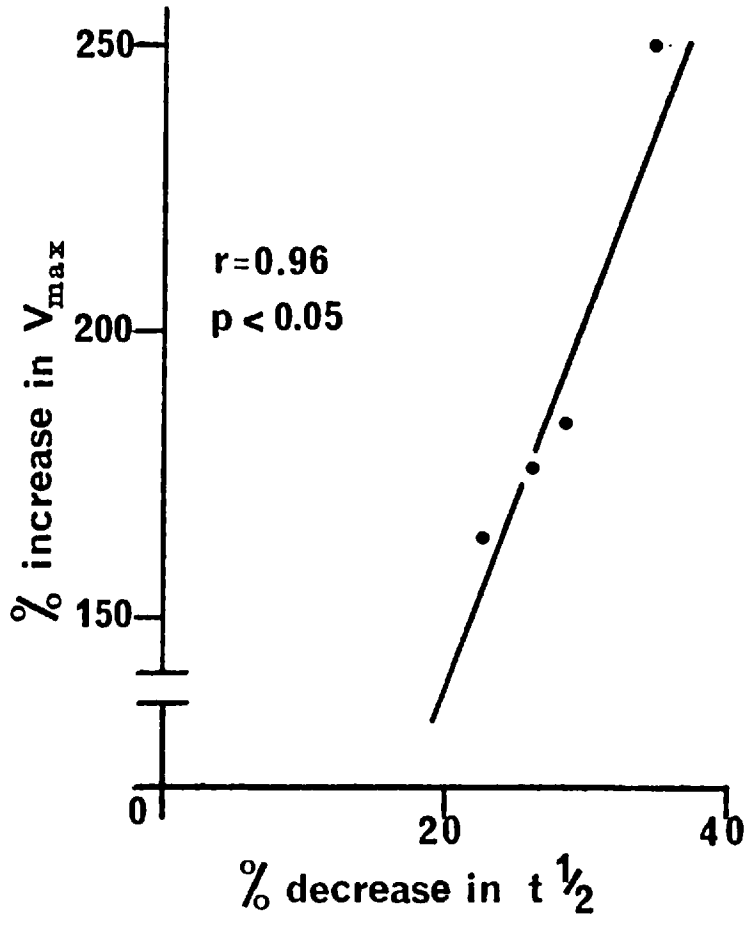


Figure 5.6 RELATIONSHIP BETWEEN THE % INCREASE IN THE V_{max} FOR 3-METHYLXANTHINE AND THE % DECREASE IN THE URINARY ELIMINATION HALF-LIFE

Table 5.4 PERSONAL DETAILS AND DIETARY METHYLXANTHINE INTAKE OF THE SUBJECTS

Subject	Age	Wt(kg)	Average daily consumption					Estimated daily Caffeine intake (Mg)*
			Smoking (cigarettes)	Tea (cups)	Coffee (cups)	Cola (cups)	Chocolate bars (50 g blocks)	
T.J.M.	24	67	0	3	3	0.5	1	500
J.C.	30	87	10	2	6	0	0	700
L.A.W.	27	82	0	2	2	0	1	350
C.A.L.	21	75	0	3	1	0	1	300

*Estimated caffeine content: coffee 90-120mg/cup; tea 50mg/cup; cola 55mg/cup; chocolate 50mg/50g block.

Discussion

The removal of methylxanthine-containing foods from the diets of volunteers receiving intravenous (^{14}C)-theophylline results in significant increases in both the rate and extent of (^{14}C)-theophylline metabolism. In Chapter 4 it was shown that the metabolism of (^{14}C)-theophylline was saturable, and that its elimination could be described by parallel first-order and Michaelis-Menten kinetics. One of the consequences of such a mixed-order system for the elimination of (^{14}C)-theophylline is that it would be expected to exhibit dose-dependency (Figure 4.14). The present results on the deprivation of dietary methylxanthines on the elimination of (^{14}C)-theophylline are consistent with the latter being dose-dependent.

How does the deprivation of methylxanthines from the diet of volunteers cause an increase in the rate and extent of (^{14}C)-theophylline metabolism? The dietary methylxanthines share common pathways of metabolism (Figure 1.4). Furthermore, the mechanisms of biotransformation are common to all three methylxanthines, namely N-demethylation and C-oxidation. It is possible then that theophylline, caffeine and theobromine are substrates for a common enzyme system(s). Thus the presence or absence of one methylxanthine would be expected to affect the metabolism of another. This clearly occurs in the case of theophylline when methylxanthines are removed from the diet. In addition, theophylline has been shown to be a metabolite of caffeine in man (Cornish and Christman, 1957; Midha et al., 1977) such that the ingestion of caffeine alone will result in the presence of theophylline. Thus two possible interrelated mechanisms are probably responsible for the observed effects of dietary deprivation of methylxanthines on (^{14}C)-theophylline metabolism. The presence of chemically related methylxanthines

which share both common mechanisms and pathways of metabolism will inhibit theophylline metabolism on a volunteers normal diet and theophylline generated as a metabolite of caffeine will be present in addition to the administered therapeutic dose. Consequently the absence of dietary methylxanthines removes a source of metabolic inhibition and as the metabolism of (^{14}C)-theophylline is dose-dependent, the rate of elimination of the administered dose increases.

That the observed effects of the dietary deprivation of the methylxanthine-containing beverages is due to the presence in these beverages of the chemically related caffeine, theophylline and theobromine and not by any of the five hundred or more other compounds identified in these beverages, is substantiated by the results obtained in volunteers on a caffeine/theophylline replacement diet. Both the metabolic and pharmacokinetic aspects of (^{14}C)-theophylline disposition on such a diet were not significantly different from its disposition on the volunteers normal diets (Tables 5.1 and 5.2; Figure 5.1). Thus when methylxanthines are removed from the diet and replaced by their ingestion in tablet form, the metabolism and elimination of (^{14}C)-theophylline is the same as that on the volunteers normal diets.

The dietary intake of methylxanthine-containing beverages can markedly influence the disposition of an intravenous dose of (^{14}C)-theophylline. The clinical implications of such an observation are numerous. The necessity to individualise dose regimens to maximise the therapeutic effectiveness of theophylline is already well established (Piafsky and Ogilvie, 1975). Present data suggest that patients who consume large quantities of dietary methylxanthines would eliminate a therapeutically administered dose of theophylline slower than those patients who only ingest small amounts of the methylxanthine-containing beverages. This is supported by estimations of the degree of saturation of the enzyme-system(s) responsible for the conversion of theophylline to 3-methylxanthine, and in the theoretical body load

of substrate available to the system(s) when the volunteers are on their normal diets (Table 5.3). Subject T.J.M., who exhibits the longest urinary elimination half-life of the four volunteers, has a body load of almost 1.75g. The remaining volunteers carry a body load of between 155 and 270mg, and consequently exhibit substantially shorter urinary elimination half-lives (Table 5.2). Furthermore, the removal of methylxanthines from the diet has its greatest effect on the subject who, on his normal diet, carried the highest body load of substrate (subject T.J.M.). This is reflected in the correlation between the percentage increase in V_{max}^{3MX} observed in the volunteers on a methylxanthine-deprived diet and the subsequent decrease in the urinary elimination half-life (Figure 5.6). Thus, subject T.J.M. exhibits an increase of 249% in the V_{max} for 3-methylxanthine, with a decrease of 34.6% in the urinary elimination half-life. Conversely, subject C.A.L. carried the lowest body load on his normal diet, also exhibits the smallest increase in the V_{max} for 3-methylxanthine and the smallest decrease in the urinary elimination half-life.

Therefore, patients who consume large amounts of methylxanthines might require their dose regimens to be adjusted accordingly. It is suggested that such patients require either a smaller dose or the same dose administered less frequently. It must also be taken into account that other factors are known to influence theophylline disposition (Table 1.11) and make a corresponding dosage adjustment. Clearly, knowledge of the extent to which patients are exposed to determinants of drug metabolism (smoking habits etc.) would be valuable to provide effective therapeutic management of patients receiving those drugs whose effectiveness is directly related to their serum or plasma concentration and where the therapeutic range is relatively narrow. With such information, and with a knowledge of the degree to which each individual factor can influence drug disposition, a simple nomogram could be constructed as an aid to the initial dose regimen required.

When the volunteers were placed on a methylxanthine-supplemented diet, both the rate and extent of (^{14}C)-theophylline metabolism remained the same as that on a volunteer's normal diet. For drugs which are eliminated solely by first-order processes, an increase in substrate levels would not be expected to alter the rate of drug elimination. However, theophylline is eliminated by parallel first-order and Michaelis-Menten kinetics, and a change in substrate levels would be expected to be reflected in changes in its overall rate of elimination (Wagner, 1974; Rowland, 1977). The present results can be interpreted in the light of our knowledge of mixed-order elimination.

If the Km of the saturable process (in this case the conversion of theophylline to 3-methylxanthine) lies within the therapeutic dose range then one would expect to see changes in the overall elimination kinetics over this dose range. Clearly the Km for the above process does lie within the therapeutic dose range (Mean Km = 54.0mg). However, depending upon the individuals body load of methylxanthines, those kinetic parameters determined in volunteers on their normal diets could lie on the plateau section of the relationship between the urinary elimination half-life and body load (Figure 5.7). Thus decreases in the body load (methylxanthine-deprived diet) will have profound effects on the observed elimination kinetics of theophylline, whereas increases in the body load (methylxanthine-supplemented diet) might have far less discernable effects. As the dose increases the contribution of the saturable process to the overall elimination of (^{14}C)-theophylline becomes negligible and the first-order rate processes become the principal determinant of overall elimination (i.e. when $S \gg Km$; Wagner,1974).

The volunteer in whom the methylxanthine-supplemented diet has the greatest effect is subject C.A.L. This subject excretes 87.6% of the

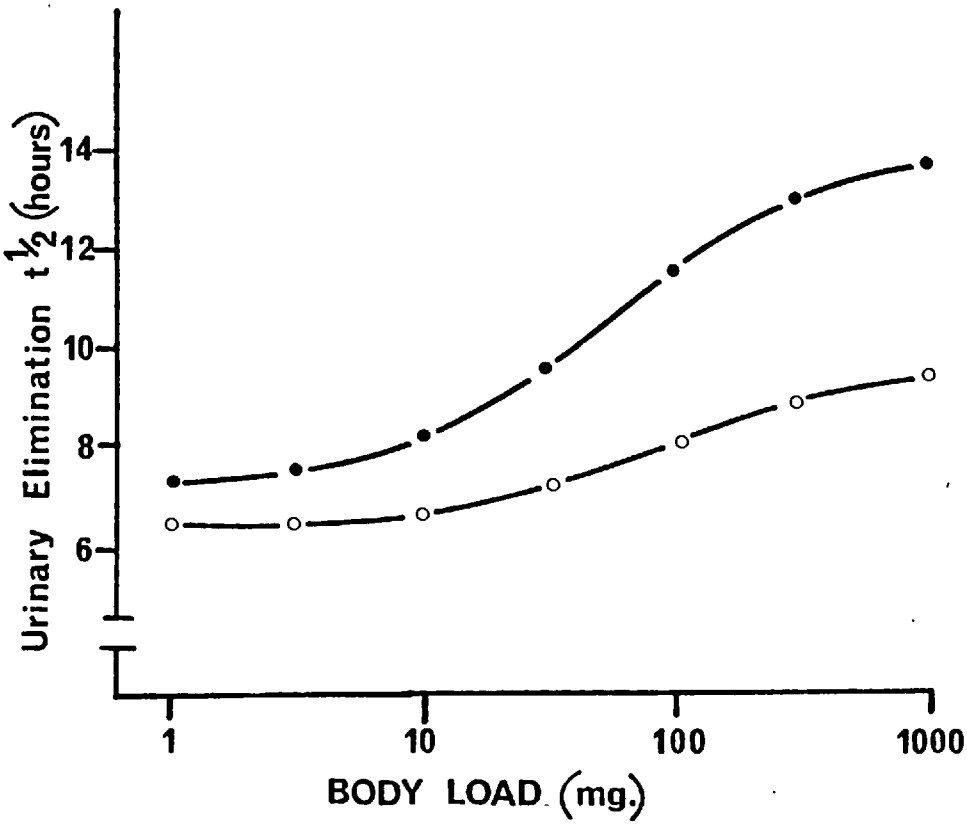


Figure 5.7 RELATIONSHIP BETWEEN THE URINARY ELIMINATION HALF-LIFE OF TOTAL ^{14}C AND THE BODY LOAD OF METHYLXANTHINES. CLOSED CIRCLES REPRESENT RELATIONSHIP DETERMINED BY EMPLOYING PARAMETERS FROM VOLUNTEERS ON A NORMAL DIET. OPEN CIRCLES REPRESENT RELATIONSHIP DETERMINED BY EMPLOYING PARAMETERS FROM VOLUNTEERS ON A METHYLXANTHINE-DEPRIVED DIET.

administered dose in the 0-24 hour urine on such a diet, compared to only 77.3% of the dose on his normal diet. Subsequently, the urinary elimination half-life falls from 8.9 hours on his normal diet to 6.8 hours on the methylxanthine-supplemented diet. Such a response appears to contradict expectations based on the present kinetic theory. However, the known pharmacological effects of the methylxanthines provide the basis of an explanation for the observed results. Subject C.A.L. was the volunteer with the lowest body load of methylxanthines on his normal diet. It is reasonable to assume that this subject therefore consumes less tea and coffee than the other volunteers (Table 5.4). Such a subject would be expected to have developed only a low level (if any) of tolerance to the pharmacological effects of the methylxanthines. Of particular importance here is the diuretic effects of the methylxanthines to which some degree of tolerance has been demonstrated (Eddy and Downs, 1928). Thus, subject C.A.L. produces 1.0ml/min of urine on his normal diet which increases to 2.2ml/min on the methylxanthine-supplemented diet. As the renal clearance of theophylline is urine flow-rate dependent (Levy and Koysooko, 1976) subject C.A.L. consequently excretes 14.5% of the administered dose as unchanged theophylline, compared to only 4.9% on the normal diet. This increase accounts for essentially all the increase in radioactivity which subject C.A.L. excretes in the 0-24 hour urine. Indeed the combined total of metabolites this subject excretes accounts for 73.1% of the dose on the methylxanthine-supplemented diet as compared to 72.4% on the normal diet.

Whilst subject C.A.L. maintains the lowest initial theoretical body load of methylxanthines and that the level of tolerance so developed will be of low magnitude, it has also been established that the degree to which any individual develops tolerance to the pharmacological effects of methylxanthines varies considerably (Ritchie, 1975). Subject C.A.L. might also be one of those individuals more

sensitive to the methylxanthines.

Dietary methylxanthines have therefore been shown to be a significant determinant of the disposition of (^{14}C)-theophylline. This rather specific dietary effect on theophylline disposition has not yet been investigated to discover if other drugs are affected in a similar manner. It would therefore be interesting to investigate the effects of dietary methylxanthines on the disposition of other drugs, particularly those which, like theophylline, are substrates for the cytochrome P_{448} enzyme system(s). Such drugs are usually typified by having their metabolism increased by the polycyclic hydrocarbons present in cigarette smoke, whilst the phenobarbitone class of enzyme inducers have little or no effect on the metabolism of these drugs.

Many dietary factors have been shown to influence drug metabolism (Table 1.19). Further research might discover more specific interactions between the diet and drug metabolism and some of these are discussed in Chapter 7.

CHAPTER 6

Metabolism and Pharmacokinetics of Intravenously Administered (^{14}C)-Aminophylline

Disposition of (^{14}C)-Aminophylline

Introduction

As theophylline is only sparingly soluble in water it has been combined with a large number of agents to increase its water solubility for parenteral administration (see Table 1.4). The combination of ethylenediamine with theophylline to yield aminophylline, is the most widely used form of the drug and it is frequently assumed that theophylline and aminophylline are pharmacologically equivalent. Indeed, throughout the literature, theophylline and aminophylline are used synonymously. That is, in the title of an article the word theophylline is used, whereas in the text one discovers that the form of the drug being administered is in fact aminophylline.

The British Pharmacopoeia states that aminophylline is comprised of two molecules of theophylline, one of ethylenediamine and two of water, yielding a molecular weight of 456, as compared to only 180 for theophylline, and represented by the formula $(C_7H_8N_4O_2)_2.C_2H_4(NH_2)_2.2H_2O$. However, for the preparation of aminophylline, the British Pharmacopoeia requires that as little as 78% and not more than 84% of anhydrous theophylline can be used, that not less than 13% and not more than 14% ethylenediamine be used, and that a variable quantity of water can be added. This suggests that the stoichiometric relationship between the constituents of aminophylline is not as simple as suggested. Beyond this expression of the percentage of the individual components present in aminophylline, the exact nature of the theophylline/ethylenediamine combination is unclear.

Thus in view of the apparent chemical differences between theophylline and aminophylline and the widespread therapeutic use of aminophylline, the metabolism of (^{14}C)-aminophylline was studied in adult male volunteers and compared with the metabolism of (^{14}C)-theophylline in the same volunteers (as presented in Ch.4).

Results

Normal Diet

After the intravenous administration of 125mg (^{14}C)-aminophylline (≡100mg theophylline) to three adult male volunteers maintaining their normal diets, 89.8 ± 2.0% (Mean ± S.D.) of the dose was recovered in the urine after 24 hours and 97.6 ± 1.3% after 48 hours. This was significantly greater ($p < 0.05$, by paired, two-tailed Student t test; $p < 0.02$ by analysis of variance) than the recovery of radioactivity following the intravenous administration of 100mg (^{14}C)-theophylline to the same three volunteers (Table 6.1). This is illustrated with data from subject T.J.M. (Figure 6.1).

Table 6.1 Urinary recovery of radioactivity following the i.v. administration of either (^{14}C)-aminophylline or (^{14}C)-theophylline to adult male volunteers.

SUBJECT	T.J.M.		J.C.		L.A.W.		MEAN	
DRUG	T	A	T	A	T	A	T	A
0-24h	68.6*	90.5	78.4	91.2	81.9	87.5	76.3	89.8
24-48h	14.9	8.2	3.1	6.7	8.1	8.6	8.7	7.8
TOTAL	83.5	98.7	81.5	97.9	90.0	96.1	85.0	97.6

T = (^{14}C)-theophylline; A = (^{14}C)-aminophylline

*All figures expressed as % dose recovered in urine.

Quantitation of theophylline and its metabolites in serial urine samples of each volunteer is given in the Appendix. The total 24 hour urinary recovery of theophylline and its metabolites are given in Table 6.2. The metabolic pattern after i.v. (^{14}C)-aminophylline

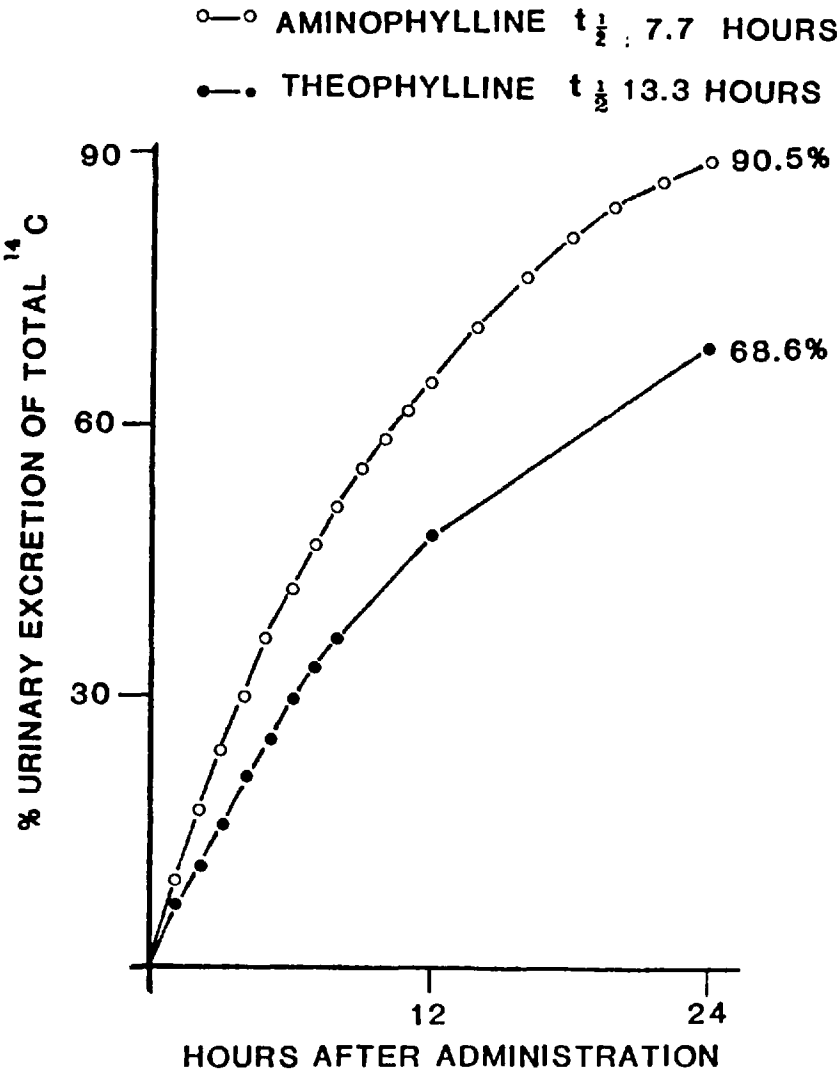


Figure 6.1 CUMULATIVE EXCRETION OF ^{14}C AFTER THE INTRAVENOUS ADMINISTRATION OF (^{14}C)-THEOPHYLLINE (100mg; 10μCi) AND (^{14}C)-AMINOPHYLLINE (125mg≡100mg THEOPHYLLINE: 10μCi) TO SUBJECT T.J.M. ON HIS NORMAL DIET

Table 6.2 QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN THE TOTAL 24 HOUR URINE AFTER EITHER i.v. (^{14}C)-AMINOPHYLLINE OR i.v. (^{14}C)-THEOPHYLLINE

SUBJECT	T.J.M.		J.C.		L.A.W.		MEAN	
	T	A	T	A	T	A	T	A
THEOPHYLLINE	7.5*	9.2	11.1	14.7	6.5	6.8	8.4	10.2
3-METHYLXANTHINE	15.8	22.1	16.2	21.7	17.4	19.9	16.5	21.2
1,3-DIMETHYLURIC ACID	30.1	36.4	26.0	34.4	27.9	40.1	28.0	37.0
1-METHYLURIC ACID	12.9	17.3	18.8	17.4	25.0	17.1	18.9	17.3
UNKNOWN XANTHINE	1.7	2.0	1.0	0.7	1.1	1.1	1.3	1.3
UNKNOWNURIC ACID	0.6	-	5.3	0.8	4.1	-	3.3	0.3

T = 100mg, i.v. (^{14}C)-theophylline; A = 125mg, i.v. (^{14}C)-aminophylline (≡ 100mg theophylline)

*All figures expressed as % of the dose recovered as that compound in the 0-24 hour urine

remained essentially the same as that following i.v. (^{14}C)-theophylline. Thus, in addition to some "unchanged" theophylline, three major and one minor metabolite are excreted, the only difference being that the small amounts of the unknown acid metabolite recovered in the urine of all three volunteers after i.v. (^{14}C)-theophylline is not detected after (^{14}C)-aminophylline. However, the quantities of two of the major metabolites, namely 3-methylxanthine and 1,3-dimethyluric acid, recovered in the 0-24 hour urine, are both significantly increased ($p < 0.05$, by paired, two-tailed Student t test) after (^{14}C)-aminophylline, and it is these increases which account for the greater urinary recovery of total radioactivity (Figure 6.1).

The various relationships observed after i.v. (^{14}C)-theophylline administration, namely:-

1. The fractional urine content of 1,3-dimethyluric acid and 3-methylxanthine (Figure 6.2)
2. The rate of elimination of 1,3-dimethyluric acid and 1-methyluric acid (Figure 6.3) and
3. The urine flow-rate dependency of theophylline elimination (Figure 6.4).

were all observed after (^{14}C)-aminophylline administration.

The kinetic parameters describing the urinary elimination of theophylline and its metabolites after i.v. (^{14}C)-aminophylline are given in Table 6.3 and these are compared with those derived after i.v. (^{14}C)-theophylline. There are significant increases in the V_{max} of the Michaelis-Menten expression describing the urinary elimination of 3-methylxanthine ($p < 0.05$, by paired two-tailed Student t test) after i.v. (^{14}C)-aminophylline than after i.v. (^{14}C)-theophylline. This is illustrated with data from subject T.J.M. (Figure 6.5). Although there is also an increase in the Km of the Michaelis-Menten expression, this does not reach statistical significance in the small group studied. There is also a significant

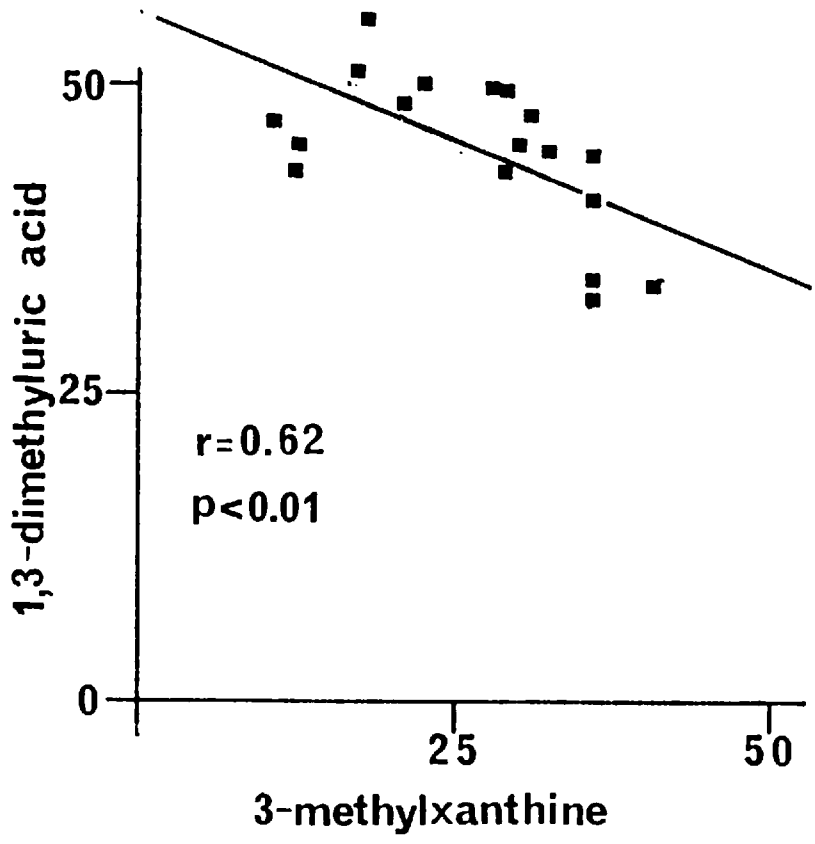


Figure 6.2 THE FRACTIONAL URINE CONTENT OF 3-METHYLXANTHINE AND 1,3-DIMETHYLURIC ACID AFTER THE INTRAVENOUS ADMINISTRATION OF (^{14}C)-AMINOPHYLLINE (125mg≡100mg THEOPHYLLINE; 10μCi) TO A VOLUNTEER ON HIS NORMAL DIET. SUBJECT L.A.W.

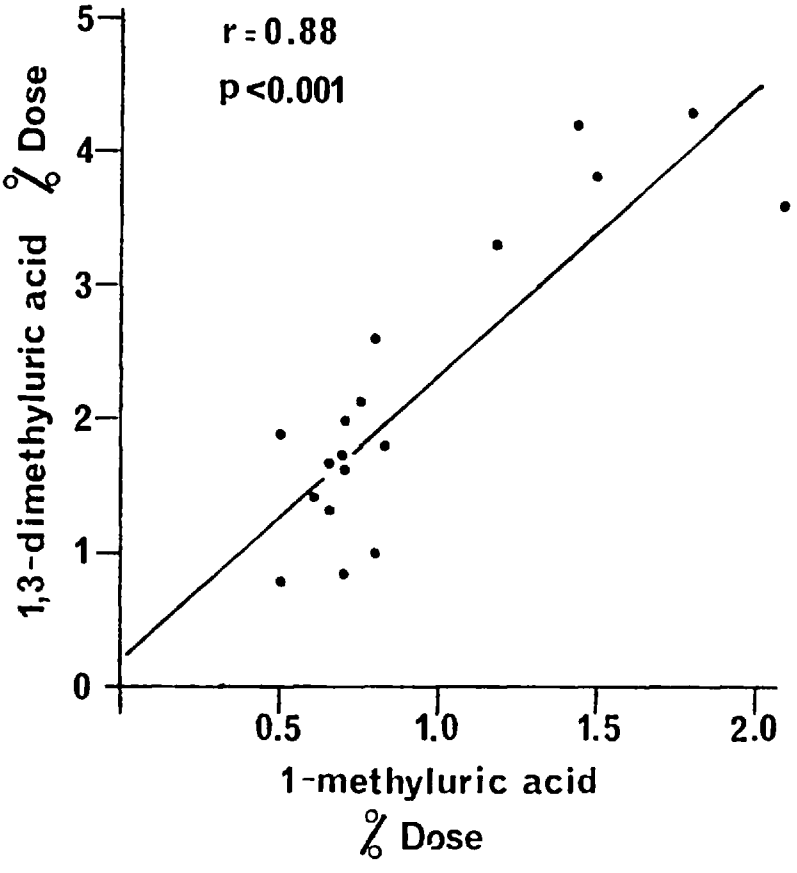


Figure 6.3 THE RELATIONSHIP BETWEEN THE AMOUNTS OF 1,3-DIMETHYLURIC ACID AND 1-METHYLURIC ACID EXCRETED IN URINE AFTER INTRAVENOUS (^{14}C)-AMINOPHYLLINE (125mg≡100mg THEOPHYLLINE; 10μCi). SUBJECT L.A.W.

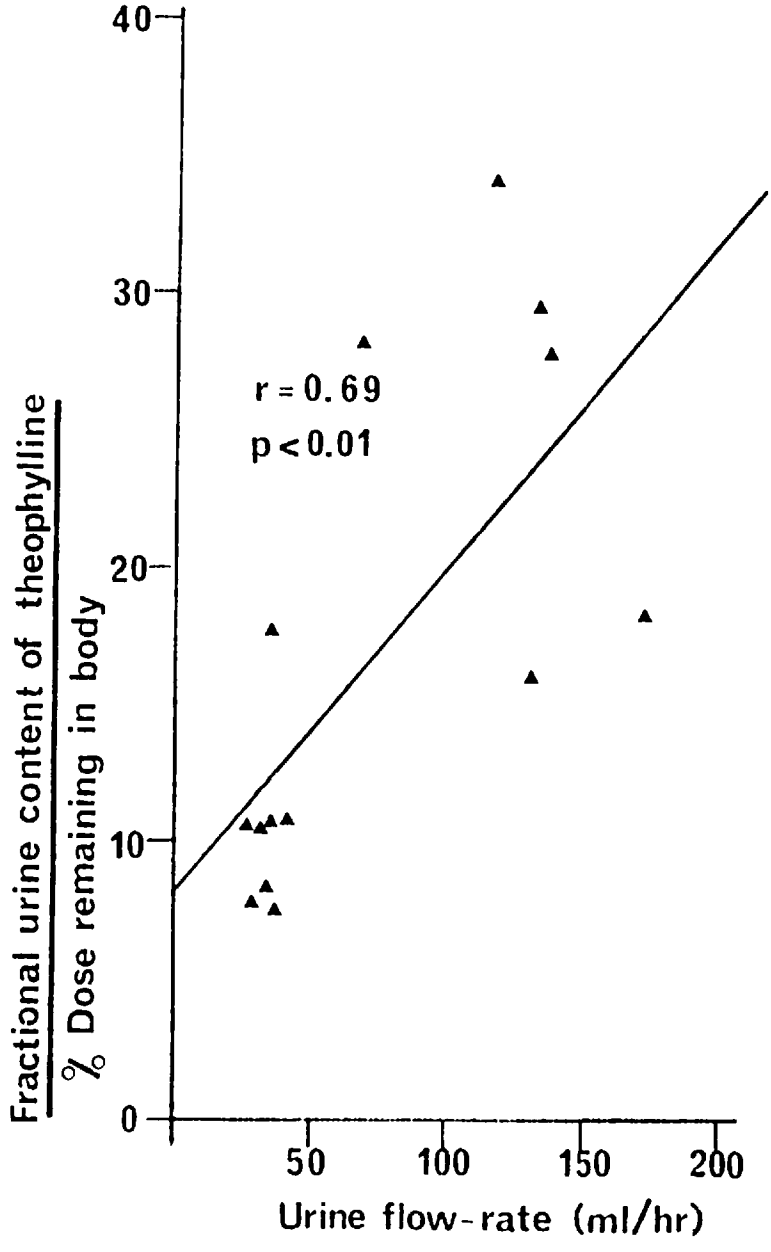


Figure 6.4. THE URINE FLOW-RATE DEPENDENCY OF THEOPHYLLINE EXCRETION AFTER INTRAVENOUS (^{14}C)-AMINOPHYLLINE (125mg ≡ 100mg THEOPHYLLINE; 10μCi). SUBJECT T.J.M.

Table 6.3

PHARMACOKINETIC PARAMETERS DESCRIBING THE URINARY ELIMINATION OF INTRAVENOUS (^{14}C)-AMINOPHYLLINE OR (^{14}C)-THEOPHYLLINE

		PHARMACOKINETIC PARAMETERS						
SUBJECT	DRUG	$k_{el}^{T}(h^{-1})$	$k_{el}^{DMU}(h^{-1})$	$k_{el}^{MU}(h^{-1})$	$k_{el}^{U}(h^{-1})$	V_{max}^{3MX}(mg/hr)	Km^{3MX}(mg)	$t_{\frac{1}{2}}$(h)
T.J.M.	A	0.009	0.034	0.016	0.002	1.59	26.3	7.7
	T	0.005	0.026	0.011	0.002	0.74	3.1	13.3
J.C.	A	0.011	0.031	0.015	0.001	1.96	42.7	6.9
	T	0.016	0.027	0.021	0.008	1.13	21.7	8.4
L.A.W.	A	0.009	0.036	0.016	0.001	1.43	25.6	7.5
	T	0.007	0.025	0.022	0.004	1.30	29.6	8.4
MEAN	A	0.010	0.034	0.016	0.001	1.66	31.5	7.4
	T	0.009	0.026	0.018	0.005	1.06	18.1	10.0

A = 125mg, i.v. (^{14}C)-aminophylline (≡ 100mg theophylline)

T = 100mg, i.v. (^{14}C)-theophylline.

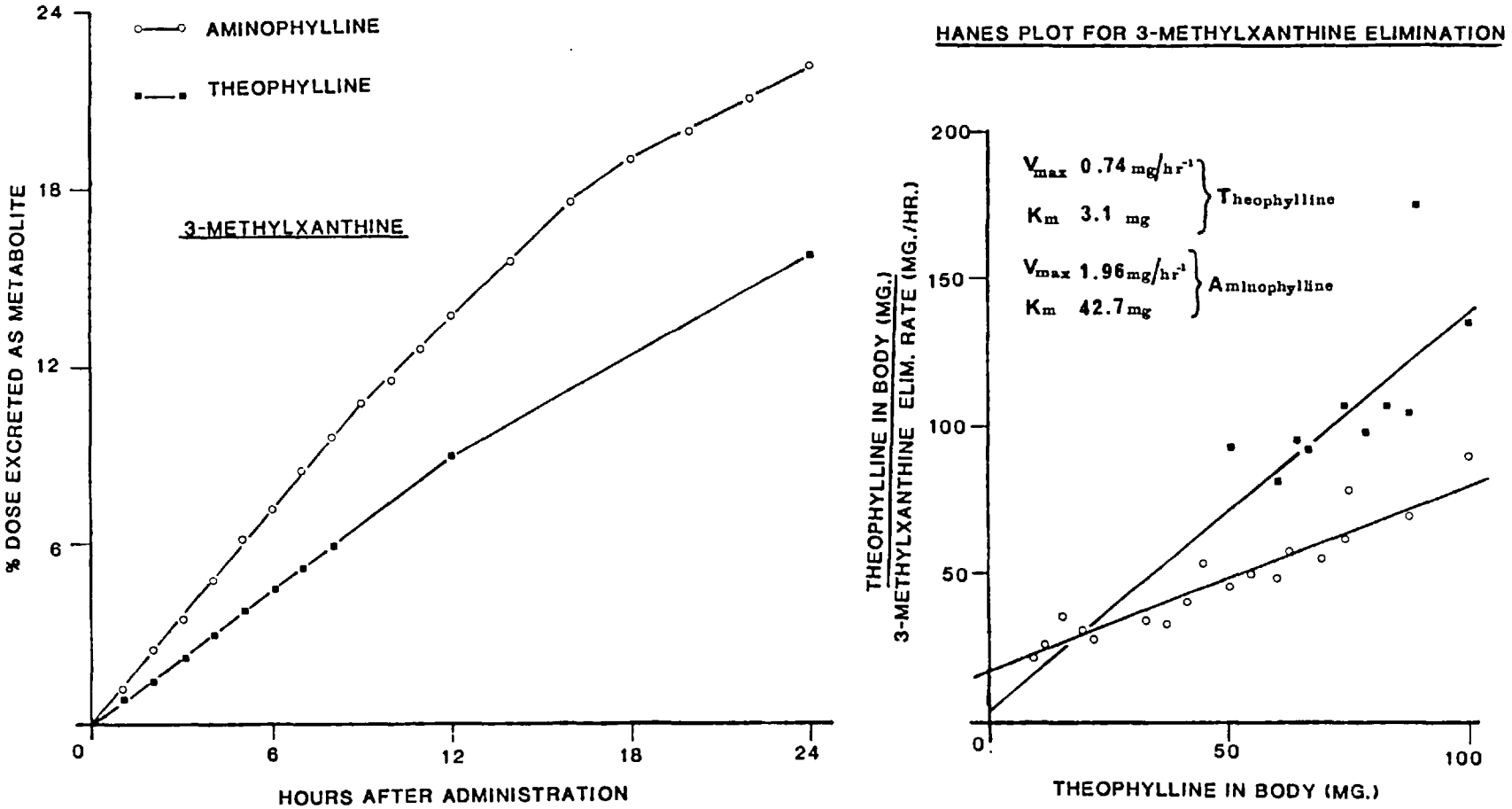


Figure 6.5 HANES-TYPE PLOTS FOR 3-METHYLXANTHINE ELIMINATION AFTER EITHER INTRAVENOUS (^{14}C)-THEOPHYLLINE (100mg; 10μCi) OR (^{14}C)-AMINOPHYLLINE (125mg≡100mg THEOPHYLLINE; 10μCi). SUBJECT T.J.M.

increase in the first-order elimination rate constant for 1,3-dimethyluric acid after i.v. (^{14}C)-aminophylline compared with that after i.v. (^{14}C)-theophylline ($p<0.05$, by paired two-tailed Student t test) (Figure 6.6). The increases in these kinetic parameters account for the significant decrease ($p<0.05$, by paired two-tailed Student t test) in the urinary elimination half-life of total radioactivity from 10.0 hours after i.v. (^{14}C)-theophylline to 7.4 hours after i.v. (^{14}C)-aminophylline (Table 6.3).

The mathematical model describing the urinary elimination of (^{14}C)-theophylline, derived in Chapter 4, was found appropriate to describe the urinary elimination of (^{14}C)-aminophylline.

Methylxanthine-Deprived Diet

After the intravenous administration of 125mg (^{14}C)-aminophylline (≡100mg theophylline) to three adult male volunteers on a methylxanthine-deprived diet, 89.9 ± 6.7% (Mean ± S.D.) of the dose was recovered in the urine after 24 hours and 96.7 ± 3.8% after 48 hours. This was not significantly different from that recovered after its administration to volunteers on their normal diets (Table 6.4).

Table 6.4 Urinary recovery of radioactivity following the i.v. administration of (^{14}C)-aminophylline to volunteers on their normal and methylxanthine-deprived diets

SUBJECT	T.J.M.		J.C.		L.A.W.		MEAN	
DIET	N	XD	N	XD	N	XD	N	XD
0-24h	90.5*	82.8	91.2	95.8	87.5	90.9	89.8	89.9
24-48h	8.2	11.4	6.7	5.2	8.6	3.9	7.8	6.8
TOTAL	98.7	94.2	97.9	101.0	96.1	94.8	97.6	96.7

N = Normal diet; XD = Methylxanthine-deprived diet

*All figures expressed as % dose recovered in urine.

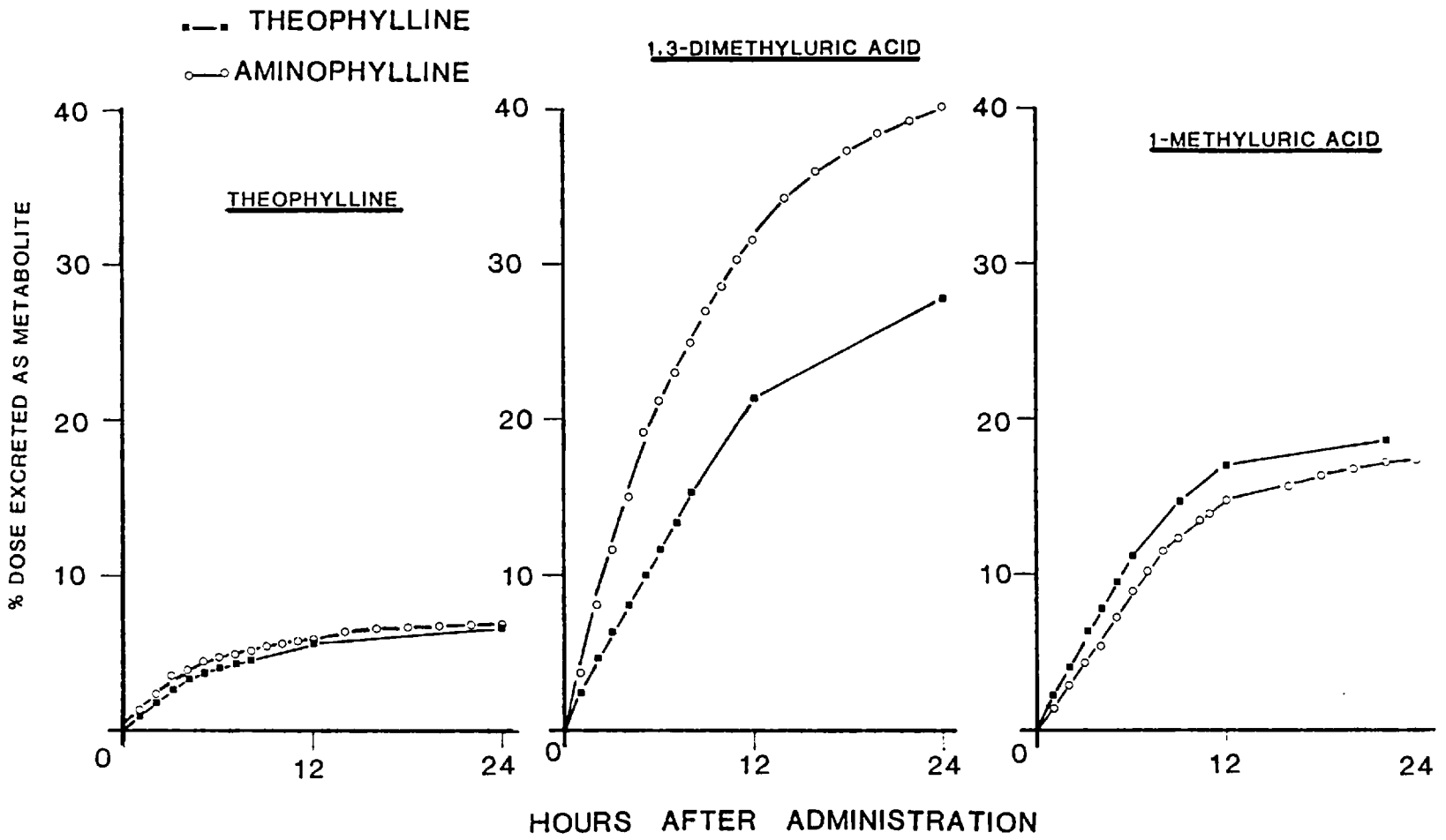


Figure 6.6 CUMULATIVE EXCRETION OF THEOPHYLLINE, 1,3-DIMETHYLURIC ACID AND 1-METHYLURIC ACID AFTER EITHER INTRAVENOUS (^{14}C)-THEOPHYLLINE (100mg; 10μCi) OR (^{14}C)-AMINOPHYLLINE (125mg≡100mg THEOPHYLLINE; 10μCi)

Quantitation of theophylline and its metabolites in serial urine samples of each volunteer is given in the Appendix. The total 24 hour urinary recovery of theophylline and its metabolites is given in Table 6.5. The metabolic pattern after i.v. (^{14}C)-aminophylline remained essentially the same on both a normal and a methylxanthine-deprived diet. The only difference was the appearance of small amounts of the unknown acid metabolite in the urine of two of the volunteers on a methylxanthine-deprived diet. However, the quantities of both theophylline and its three major metabolites recovered in 0-24 hour urine remained the same in volunteers on both a normal and methylxanthine-deprived diet.

The kinetic parameters describing the urinary elimination of theophylline and its metabolites following i.v. (^{14}C)-aminophylline to volunteers on a methylxanthine-deprived diet are given in Table 6.6. There are no differences between the values derived after administration to volunteers on either their normal or methylxanthine-deprived diets.

Discussion

In view of the aforementioned apparent chemical differences between theophylline and aminophylline, the previously held view of pharmacological equivalence may not be entirely justified. Present results suggest that the disposition of (^{14}C)-theophylline and (^{14}C)-aminophylline are not comparable. This is suggested by both the differences in the rate and extent of (^{14}C)-theophylline and (^{14}C)-aminophylline metabolism on volunteers' normal diets, and by the differing effects that the dietary methylxanthines have on the metabolism and elimination of both drugs. The complexation of (^{14}C)-theophylline with ethylenediamine results in significant increases in both the rate and extent of the former's conversion to 3-methylxanthine and 1,3-dimethyluric acid. These increases are responsible for the

Table 6.5 QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN THE TOTAL 24 HOUR URINE OF VOLUNTEERS RECEIVING i.v. (^{14}C)-AMINOPHYLLINE ON THEIR NORMAL AND METHYLXANTHINE-DEPRIVED DIETS

SUBJECT	T.J.M.		J.C.		L.A.W.		MEAN	
DIET / METABOLITE	N	XD	N	XD	N	XD	N	XD
THEOPHYLLINE	9.2*	11.7	14.7	13.1	6.8	7.1	10.2	10.6
3-METHYLXANTHINE	22.1	16.9	21.7	22.0	19.9	22.0	21.2	20.3
1,3-DIMETHYLURIC ACID	36.4	35.3	34.4	38.9	40.1	41.0	37.0	38.4
1-METHYLURIC ACID	17.3	12.2	17.4	17.8	17.1	17.4	17.3	15.8
UNKNOWN XANTHINE	2.0	2.5	0.7	0.2	1.1	0.9	1.3	1.2
UNKNOWN URIC ACID	-	0.5	0.8	0.8	-	-	-	-

N = Normal diet; XD = Methylxanthine-deprived diet. *All figures expressed as % of the dose recovered as that compound in 0-24 hour urine.

Table 6.6 PHARMACOKINETIC PARAMETERS DESCRIBING THE URINARY ELIMINATION OF (^{14}C)-AMINOPHYLLINE IN VOLUNTEERS ON THEIR NORMAL AND METHYLXANTHINE-DEPRIVED DIETS.

		PHARMACOKINETIC PARAMETERS						
SUBJECT	DIET	$k_{el}^{T}(h^{-1})$	$k_{el}^{DMU}(h^{-1})$	$k_{el}^{MU}(h^{-1})$	$k_{el}^{U}(h^{-1})$	V_{max}^{3MX}(mg/hr)	Km^{3MX}(mg)	$t_{\frac{1}{2}}$(h)
T.J.M.	N	0.009	0.034	0.016	0.002	1.59	26.3	7.7
	XD	0.009	0.029	0.010	0.002	1.03	23.1	9.9
J.C.	N	0.011	0.031	0.015	0.001	1.96	42.7	6.9
	XD	0.017	0.046	0.022	0.001	2.04	33.8	5.9
L.A.W.	N	0.009	0.036	0.016	0.001	1.43	25.6	7.5
	XD	0.009	0.051	0.019	0.001	3.13	77.0	5.7
MEAN	N	0.010	0.034	0.016	0.001	1.66	31.5	7.4
	XD	0.012	0.042	0.017	0.001	2.07	44.6	7.2

N = Normal diet; XD = Methylxanthine-deprived diet.

observed decrease in the urinary elimination half-life of total radioactivity from 10.0 hours after (^{14}C)-theophylline to 7.4 hours after (^{14}C)-aminophylline. The clinical implications of these observations are clear. A change in the therapeutic management of a patient receiving theophylline, to aminophylline, or vice versa, must take into account the differences in disposition of the two drugs. If the increased rate of metabolism and urinary elimination of (^{14}C)-aminophylline is reflected in lower plasma concentrations of theophylline then either aminophylline will have to be administered at a higher dose to maintain therapeutic plasma concentrations of theophylline or the same equivalent dose will have to be administered at more frequent intervals. Furthermore, these differences in the disposition of (^{14}C)-theophylline and (^{14}C)-aminophylline might explain some of the previously observed variations in plasma elimination half-lives of theophylline (Table 1.11). Patients receiving identical doses of either aminophylline or theophylline might be expected to exhibit differences in plasma concentrations and plasma elimination half-lives, irrespective of any other influencing factor.

Differences in the disposition of a drug administered in varying dosage forms have been noted previously, particularly in terms of altered bioavailability (Table 6.7) but differences in the rate and extent of drug metabolism arising solely from alterations in the dosage form appear to be rare. What are the possible mechanisms for the observed differences between (^{14}C)-theophylline and (^{14}C)-aminophylline metabolism? The exact reasons for the observed differences are not clear, but it is possible to speculate that it may be due to one or more of the following:-

1. Increased lipid solubility of the complex leading to higher concentrations of the parent drug in hepatocytes

Table 6.7

DRUGS FOR WHICH FORMULATION HAS BEEN SHOWN TO AFFECT BIOLOGICAL AVAILABILITY TO AN IMPORTANT EXTENT

Acetoheximide
Ampicillin
Aspirin
Chloramphenicol
Digoxin
Griseofulvin
Hydrochlorthiazide
Hydrocortisone
Nitrofurantoin
Penicillin V
Phenindione
Phenacetin
Phenylbutazone
Phenytoin
Prednisone
Prednisolone
Para-aminosalicylic acid
Quinidine
Riboflavine
Spironolactone
Triamterine
Sulphadiazine
Tolbutamide
Tetracyclines
Warfarin

From: Curry, 1977.

2. Greater affinity of the complex for the metabolising enzymes.

3. Ethylenediamine acting as an activator of the metabolising enzymes either as an allosteric activator rendering the active site more accessible for the parent drug, or as an enzyme inducer.

Which, if any, of these explanations is responsible for the observed variations depends upon the following as yet unanswered questions.

What is the exact nature of theophylline/ethylenediamine complex?

How stable or unstable is it?

At what stage, if any, does the complex dissociate after intravenous administration?

With respect to the first question, preliminary results with infra-red spectroscopy suggest that the two carbonyl groups of theophylline are involved in the interaction with ethylenediamine. The absence of involvement of the N-methyl groups of theophylline in the interaction is substantiated by nuclear magnetic resonance studies employed as an assay to determine the theophylline content of various aminophylline tablets (Turczan et al., 1972). In these studies the signals from the N-methyl protons in theophylline were unaffected by the presence of ethylenediamine. Further investigations are required to assess the contribution of both the N-methyl groups and the carbonyl groups of theophylline to the interaction with ethylenediamine. ^{13}C or ^{15}N nuclear magnetic resonance studies might be the most useful means of providing the answers.

With regard to the latter two questions, aminophylline is a combination of a strong base with a weak acid, such that a solution of aminophylline readily absorbs carbon dioxide from the atmosphere, the resulting carbonic acid subsequently displaces theophylline from the

complex and precipitates it. If carbon dioxide absorbed from the air can displace theophylline from the complex, then after the oral administration of aminophylline one would expect the complex to dissociate almost immediately on contact with the much stronger gastric acids. However, whether the complex dissociates after intravenous administration is questionable. A double-labelling experiment employing (^{14}C)-theophylline and (^{3}H)-ethylenediamine might provide the answer to this problem.

That (^{14}C)-theophylline and (^{14}C)-aminophylline behave differently in man is confirmed by the absence of an effect of a methylxanthine-deprived diet on (^{14}C)-aminophylline disposition. This form of dietary manipulation had significant effects on the metabolism of (^{14}C)-theophylline discussed in Chapter 5. However, no such diet-related differences were observed after intravenous (^{14}C)-aminophylline. Two of the subjects (J.C. and L.A.W.) excreted slightly more radioactivity on a methylxanthine-deprived diet but the third subject (T.J.M.) actually excreted less. The lower recovery of radioactivity in this subject was associated with smaller amounts of the two N-demethylated products excreted after intravenous (^{14}C)-aminophylline than after intravenous (^{14}C)-theophylline (Table 6.5). Thus the removal of methylxanthine-containing foods and beverages from the diet increases both the rate and extent of (^{14}C)-theophylline metabolism, but not of (^{14}C)-aminophylline metabolism. Perhaps the increase in metabolism that the complexation of (^{14}C)-theophylline with ethylenediamine causes overshadows any effects that the removal of methylxanthines from the diet might exert on (^{14}C)-aminophylline metabolism.

In conclusion, the disposition of (^{14}C)-theophylline and (^{14}C)-aminophylline is different. The rate and extent of conversion to both 3-methylxanthine and 1,3-dimethyluric acid is greater after (^{14}C)-aminophylline administration, and no diet-related differences are observed after (^{14}C)-aminophylline as after (^{14}C)-theophylline.

CHAPTER 7
Discussion

In this chapter it is proposed to discuss the implications of the methods and results presented in the preceeding chapters with reference to the following areas; the application of pharmacokinetics to the study of drug metabolism; drug-diet interrelations; and the effects of dietary methylxanthines on drug metabolism and their implications.

PHARMACOKINETICS AND DRUG METABOLISM

Plasma kinetics or urinary metabolite kinetics ?

Initial pharmacokinetic studies involved investigating the time-course of only the unchanged drug in the body. The limitations of such studies were soon realised. In particular, for drugs which are converted into active metabolites, knowledge of the time-course of the unchanged drug in the body imposes obvious limitations. However, with the knowledge of a drugs pharmacokinetic behaviour it has been demonstrated that there are pronounced interindividual differences in the elimination kinetics of various drugs. Interindividual variability in the response to drugs is a vital yet often neglected facet of drug treatment. Where the individual response has been shown to be related to plasma concentrations of the drug, the application of pharmacokinetic models describing the plasma concentration time-course of drugs, while useful as an aid to manipulate therapy, do not allow the identification of the mechanism(s) responsible for the observed variations in plasma elimination kinetics. Thus as metabolism is the major route of elimination for the majority of drugs, studies of the rate of elimination of both drugs and their metabolites will provide a more detailed examination of the mechanism(s) responsible for the interindividual differences observed in plasma elimination kinetics.

It is, therefore, important to define the kinetic aspects of drug metabolism. Obvious approximations can arise when one simply measures, for example, the total 24 hour urinary excretion of a

particular metabolite. Such data gives no indication of the rate of elimination of the metabolite. Both aspects are important in the handling of any drug. For example, three individuals (designated A, B and C; Figure 7.1) might excrete 30% of a drug in the form of a certain metabolite over 24 hours. On the basis of this information, it would be assumed that no differences occur between the three individuals. However, if the rates at which the three individuals eliminate the metabolite are examined, then profound interindividual differences are observed, although the total 24 hour output is identical. Indeed, the problem of species variations is subject to the same limitations. Again the total 24 hour excretion data might be the same in all three species (designated A, B and C; Figure 7.1) but as indicated the three species might exhibit remarkably different elimination rates. However, the problem of ensuring that experimental animals produce urine at sufficiently regular intervals might present practical difficulties for this type of study to be carried out successfully. Metabolism is clearly a dynamic process, since metabolites are continually being formed and eliminated. Defining the kinetic aspects of drug metabolism is therefore as important as defining the qualitative aspects.

The differences behind the two approaches, that of plasma pharmacokinetics and urinary kinetics, are simple. The former is concerned with measuring the rate of disappearance of substrate (drug) from the biological system, and does not account for those mechanisms responsible for the disappearance. The latter approach is involved with measuring the rate of appearance of one or more products generated from the biological mechanism(s) responsible for the disappearance of the substrate (drug) from the system. The application of either system depends upon the requirements of each particular study. From the clinical view point it is essential to be aware of the time-course of

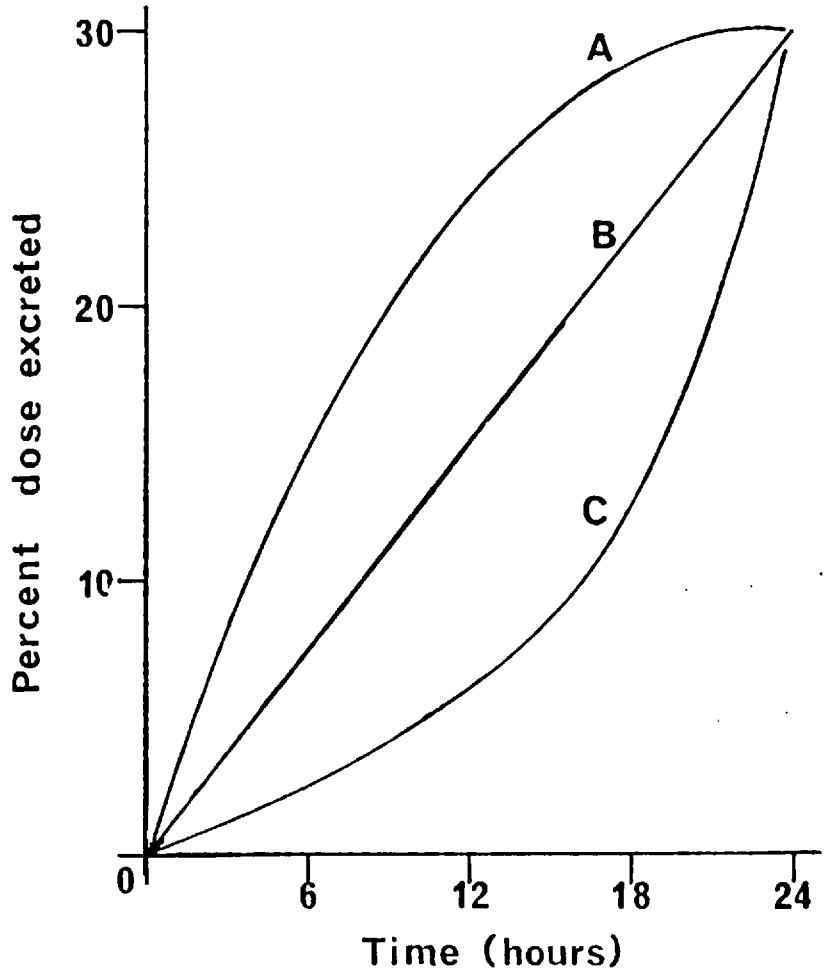


Figure 7.1 CUMULATIVE EXCRETION OF AN IMAGINARY COMPOUND IN EITHER THREE INDIVIDUALS OR THREE SPECIES (DESIGNATED A, B AND C)

unchanged drug in the body, but where individual variations in drug metabolism and response arise it becomes necessary to identify the source of variation. In such instances an examination of the urinary metabolite kinetics might provide the answer.

Which method of analysis?

In the present study two methods were available for the treatment of the serial urine sample data. One involved the treatment of the data according to a modification of the Michaelis-Menten equation as used by Levy et al. (1972) to describe the elimination of salicylate. The second concerned the application of the "Sigma-minus" method as devised by Cummings et al. (1967). Both methods base their theory on certain assumptions but both rely on data obtained by studying the appearance in urine of drug and metabolites. The Levy method can identify both first-order and zero-order elimination whereas the "Sigma-minus" method assumes that all processes responsible for the elimination of drug from the body are first-order. The Levy method assumes that the fraction of the dose remaining in the body at any time is present in the unchanged form whereas the "Sigma-minus" method assumes almost instantaneous drug metabolism and that the fraction of the dose remaining in the body exists as both unchanged drug and as metabolites not yet excreted.

There are, therefore, completely contrasting assumptions made by both methods. In terms of applicability to the present study the Levy method is the more suitable since one of the metabolic routes of theophylline metabolism is saturable within the therapeutic dose-range as discussed in Chapters 4 and 5. Thus application of the "Sigma-minus" method within this range will lead to an inaccurate estimate of the overall elimination rate constant. However, at theophylline levels above this range (i.e. when $S \gg Km$ for the saturable process) apparent overall first-order elimination kinetics are observed and the "Sigma-minus" method may be used as a method of estimating the

overall elimination rate-constant.

The second major difference between the two methods is over the nature of that fraction of the dose remaining unexcreted. By using the Levy method it is assumed that this fraction is present as unchanged drug. In terms of the present study this assumption is justifiable for several reasons. Firstly, the nature of the two methyluric acid metabolites ensures their rapid excretion from the body in urine, with little if any reabsorption by the kidney tubules. Indeed, these metabolites have yet to be identified in the plasma of individuals receiving methylxanthines either therapeutically or from the diet. The third major metabolite 3-methylxanthine, has been shown to be present in plasma samples (Thompson et al., 1974) though in smaller amounts than theophylline itself (approx. 3.5µg/ml at plasma theophylline concentrations of 15.0µg/ml.). However, the contribution of the diet towards this level rather than that being derived from the therapeutic source is not stated. Van Gennip et al. (1973) have shown that large amounts of 3-methylxanthine are formed and excreted on a daily basis. Therefore, as the metabolites of theophylline are readily excreted in urine and assuming that no tissue sequestration of the metabolites occurs, any of the dose remaining in the body is likely to be unchanged theophylline.

Considerations and Implications of data derived from urinary metabolite studies

Such kinetic studies have shown the metabolism of theophylline to be dose-dependent. This dose-dependency arises as a consequence of the conversion of theophylline to 3-methylxanthine being describable by Michaelis-Menten kinetics, that is, saturable. The clinical consequences of saturable drug metabolism can be illustrated by the results observed by Weinberger and Ginchansky (1977). By studying the plasma pharmacokinetics of theophylline in patients they noted that disproportionate increases in plasma concentrations of the drug occurred when the dose was increased. These increases could not be

predicted if drug elimination followed first-order kinetics. That unpredictable increases in the plasma concentrations of theophylline can occur on dosage adjustment means that the clinician must take extreme care in ensuring that plasma concentrations of theophylline reach the therapeutic range without reaching toxic concentrations. Thus plasma pharmacokinetics enabled the recognition of a clinical problem in dosage adjustment, whilst the present study has identified the specific biochemical mechanism responsible for the observed problem.

Interindividual differences in the activity of the saturable pathway of theophylline metabolism might be reflected in interindividual differences in the overall elimination of theophylline from the body. Interindividual differences in the activity of this pathway might be expected to be initially under genetic influence, in addition to the secondary influence of dietary methylxanthines discussed in Chapter 5. The possible role of a significant genetic component as a determinant of theophylline metabolism is being investigated in a parallel study.

If the initial observations discussed in Chapter 3 are confirmed, then theophylline becomes a remarkable example of a drug whose metabolism is significantly influenced by both environmental and genetic factors. Two pathways have been clearly shown to be affected by the environment (dietary methylxanthines) whilst the third pathway (production of 1-methyluric acid) appears to be non-responsive to environmental influence, and is under primarily genetic control. What confers protection of one pathway from environmental influence and not another is yet a further question which requires more detailed investigation.

It has therefore been demonstrated that in addition to those factors previously identified as influencing the plasma elimination half-life of theophylline (Table 1.11) dietary methylxanthines are a significant determinant of both the rate and extent of theophylline metabolism. Without a study of the rates of formation of theophylline

metabolites, the mechanism by which the dietary methylxanthines exert their influence on theophylline metabolism could not have been discerned (Ch. 5).

The influence of the diet on theophylline metabolism might be equivalent to the effect of chronic drug administration on drug metabolism. The influence of chronic drug administration on the metabolism and elimination of drugs is difficult to assess, particularly when only the plasma concentrations of the unchanged drug are monitored. The elimination of a single drug dosage during chronic administration of that drug can be followed by administering a radioactive tracer dose which then permits the determination of the rate of elimination of the tracer dose from plasma whilst plasma concentrations remain at steady state. This technique has already been used to investigate the pharmacokinetics of phenytoin in patients on maintainance phenytoin therapy (Houghton and Richens,1974). The determination of the rate of appearance of the major hydroxylated metabolite of phenytoin in urine would also reflect the influence of chronic drug administration on drug metabolism.

Such metabolic studies have also shown the rate and extent of metabolism of theophylline and aminophylline to be different. That changes in drug formulation can affect the kinetics of drug metabolism is a novel and interesting finding, the clinical consequences of which are discussed in Chapter 6. That these differences might not have been observed unless a detailed study of drug metabolism was carried out, stresses the importance of studying both the plasma kinetics of the unchanged drug as an aid to therapeutic management, and in the kinetics of drug metabolism in identifying sources of variations in plasma elimination kinetics.

What potential use do the derived formation/elimination rate constants for various drugs and their metabolites have?

Are the rates at which metabolites are formed by the same mechanism comparable?

For example, is the rate at which theophylline is hydroxylated to 1,3-dimethyluric acid the same as the rate at which caffeine is hydroxylated to 1,3,7-trimethyluric acid? Although pathways of drug metabolism are diverse, the enzyme systems involved may not be as numerable

Why is the 1-N-demethylation of theophylline to 3-methylxanthine saturable whilst other drug demethylation mechanisms are non-saturable?

There will be, and are, differences in the rate of formation of metabolites generated by similar mechanisms. This is to be expected due to the vast differences in the structure and stereochemistry of the substrates (drugs) and thus their affinity and relationship to the active-site of the drug metabolising enzymes. When more data becomes available on the kinetics of drug elimination and metabolite formation might some pattern emerge?

Will some groups of drugs generate groups of similar metabolites at similar rates?

Are these drugs metabolised by the same enzyme?

If apparently similar drugs generate similar metabolites at different rates could the drugs be substrates for different forms of the same enzyme (isoenzymes) or are they really metabolised by different enzymes?

Thus the practical implications of using such methods to study drug metabolism are clear. As far as human metabolic studies are concerned, current investigative procedures can be ineffective in detecting interindividual variations in drug metabolism. The practice of defining

various hybrid pharmacokinetic parameters (e.g. plasma elimination half-lives, clearance rates etc.) without defining the major components of these parameters (e.g. metabolism) may serve to disguise important components of interindividual variation in handling drugs. These limitations are being recognised (Kalow et al., 1977 Sloan et al., 1978) and can be exemplified by two examples. Kuntzman et al. (1977) showed that cigarette smoking increased the metabolism of phenacetin in man, as exemplified by lower plasma concentrations of phenacetin in smokers and the ratio of the concentration of N-acetyl-p-aminophenol (the O-dealkylation product of phenacetin metabolism) to that of phenacetin was increased severalfold in the smokers. However, no difference was observed in the plasma elimination half-life of phenacetin between smokers and non-smokers. Secondly, the overall rate of elimination of debrisoquine is not statistically significantly different between extensive and poor metabolising phenotypes whereas the respective rates of formation of the major metabolite, 4-hydroxydebrisoquine is significantly higher in the extensive metabolisers.

Thus the importance of studying both the kinetics of drug metabolism as a means of identifying sources of individual variations in drug disposition, and the clinical importance of defining the pharmacokinetic aspects of the unchanged drug in plasma is being increasingly recognised. Progress in drug metabolism methodology, an enhanced understanding of how environmental and genetic factors contribute to determining individual differences in metabolism of, and responses to drugs, should lead to improvements in the therapeutic use of many drugs.

DRUG-DIET INTERRELATIONS

Many forms of drug-diet interactions are known, particularly the influence of food on drug absorption. Gastric absorption of any drug, but particularly acidic drugs, is reduced after food intake. For example, the intake of food decreases blood levels of concomitantly administered aspirin derivatives (Wood, 1967). The intestinal absorption of many drugs is slowed by concurrent food intake either because of delayed gastric emptying or because of dilution of the drug in the intestinal contents.

The reducing effect of food on the bioavailability of certain other drugs may seriously reduce therapeutic efficiency when the drug is given by the oral route. The absorption of theophylline itself is markedly reduced by food (Piafsky and Ogilvie, 1975). In studies conducted by Welling et al. (1975) it was found that the absorption of theophylline was faster when it was given after a high protein meal rather than a high fat or high carbohydrate meal. Peak levels of theophylline in these studies were highest when the drug was given dissolved in 500ml of water and taken fasting.

Utilisation of food substances or beverages as antidotes to drug poisoning has been a common practice formany years. Emesis has been induced by mustard and water or by raw eggs, boiled starch or flour and milk. These so-called demulcents were considered to diminish toxic drug absorption as was hot strong tea which was frequently given to precipitate, in the stomach, apomorphine, cinchona alkaloids, strychnine, veratrine, digitalis, antipyrine and colchicine as well as heavy metals (Boyd, 1958). Furthermore, tea can impair the absorption of nonheme iron and could therefore contribute to impaired utilisation of iron given to correct an iron deficiency. Disler et al. (1975) studied the affect of tea drinking on the absorption of iron among Indian housewives. The drinking of tea, with or without milk, was found to inhibit

iron absorption from various iron solutions. Tea also significantly inhibited the absorption of heme iron from a solution of uncooked rabbit haemoglobin in tomato juice. However, it was also shown that tea did not inhibit the absorption of haemoglobin iron if it had been cooked. The authors considered it likely that the inhibition caused by tea on iron absorption was due to the formation of tannates, which are non-absorbable. It is known that the formation of such iron complexes within the intestinal lumen may diminish iron absorption. Preliminary observations by the same authors indicate that coffee has a similar effect though the mechanism is unknown.

These observations may be extremely important. The effects of dietary methylxanthines consumed in tea and coffee on theophylline metabolism have already been described. However, do individuals who consume large amounts of tea and coffee suffer from a significant impairment of iron absorption? Such individuals might therefore possess limited amounts of iron for incorporation into the cytochrome enzyme systems, with a subsequent decreased capacity for drug metabolism.

Other drug-diet interactions arise from systemic reactions induced by drug-food incompatabilities. Some foods do contain chemicals with known pharmacological activity. In addition to the dietary methylxanthines for example, a number of foods have been shown to evoke hypertensive crises in patients receiving monoamine oxidase inhibitors related to the tyramine content of various foods prior to the attack. Whereas cheese was the most common food to produce pressor effects in patients on these drugs, a number of other foods and beverages were incriminated as well as amines other than tyramine. Several patients had attacks of hypertension and severe headaches after eating the British yeast extract Marmite, and Blackwell et al. (1965) showed that this food contained both tyramine and histamine. Other foods which have been recognised to produce hypertensive effects in those patients

on monoamine oxidase inhibitors are broad beans, pickled herrings, chicken livers and certain wines, as for example, the Italian wine Chianti. In broad beans the amino acid dopa, or its amine derivative dopamine, have been incriminated (Blomley, 1964). However, it is possible that many drugs might have their structural analogues present in the diet, and that there might exist as with the dietary methylxanthines, a more specific dietary effect on drug metabolism.

EFFECTS OF DIETARY METHYLXANTHINES ON DRUG METABOLISM:

Implications

Can dietary methylxanthines inhibit the generation of carcinogenic metabolites?

Do dietary methylxanthines offer protection from their potential carcinogenic activity?

Is the observed effect of the dietary methylxanthines on theophylline metabolism restricted to this one drug only? Are dietary methylxanthines a new class of inhibitors of drug metabolism? It is important to investigate whether this inhibition of drug metabolism extends to other drugs, particularly those which share the cytochrome P_{448} enzyme system(s) for which theophylline is a substrate. This cytochrome system is intimately involved in the metabolism of various polycyclic hydrocarbons, such as benzo(a)pyrene and 3-methylcholanthrene which are both known carcinogens and substrates for this enzyme system.

However, the aromatic amine derivative phenacetin, which is chemically related to the known carcinogens β-naphthylamine and certain fluorenes including N-2-fluorenylacetamide, has been associated with tumors of the renal pelvis or urinary bladder in patients who had chronically taken overdoses of compounds which contained caffeine

and phenazone in addition to phenacetin (Johansson et al., 1974). The question has therefore been raised of the contribution of caffeine and phenazone to the carcinogenicity of phenacetin-containing compounds (Editorial, 1969); the cocarcinogenic effects of caffeine may be significant (Schmauz and Cole, 1974). The purine analogue allopurinol has also been shown to be carcinogenic in rat urinary bladder (Wang et al., 1976). So can the dietary methylxanthines offer some sort of metabolic protection from the generation of carcinogenic metabolites or are they themselves carcinogens?

The nutritional condition of animals and their specific dietary intake are significant modifiers of the response to carcinogens (Rogers, 1978). Many nutrients influence the activity of hepatic and other tissue enzymes responsible for metabolism of foreign chemicals (Campbell and Hayes, 1974). Nonnutrient food components can also induce the activity of enzymes (Wattenberg, 1975). However, because of the complexity of metabolic pathways and, in most cases, lack of knowledge of the active forms of drugs and other chemicals, the effect on carcinogenesis of dietary manipulation of enzyme activity cannot be predicted (Gillette, 1976).

APPENDIX

Key to Appendix :-

1st. Column — Time of urine sample post-dosing (hrs.)

2nd. Column — Volume of sample (ml)

% dose excreted as

- I — Theophylline
- II — 3-Methylxanthine
- III — 1,3-Dimethyluric acid
- IV — 1-Methyluric acid

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES:
SUBJECT T.J.M.; NORMAL DIET.

SAMPLE	VOLUME	% DOSE	I	II	III	IV
0 - 1	202	6.26	1.51	0.74	3.09	0.89
1 - 2	42	4.42	0.65	0.54	2.20	1.23
2 - 3	36	4.47	0.48	0.86	2.12	0.80
3 - 4	50	5.33	0.66	0.79	2.73	1.14
4 - 5	43	4.71	0.49	0.81	2.35	1.49
5 - 6	36	3.77	0.29	0.70	1.54	0.79
6 - 7	30	3.93	0.29	0.77	1.88	0.78
7 - 8	27	3.64	0.25	0.71	2.53	0.30
8 - 12	206	10.89	0.99	3.12	4.54	2.20
12 - 24	1460	21.18	1.90	6.80	7.09	3.32
	TOTALS	68.60	7.51	15.84	30.07	12.90

UNKNOWN URIC ACID 0.61%

UNKNOWN XANTHINE 1.67%

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES:
SUBJECT J.C.; NORMAL DIET.

SAMPLE	VOLUME	% DOSE	I	II	III	IV
0 - 1	190	7.65	1.82	0.79	2.00	2.22
1 - 2	164	6.74	1.57	0.87	2.47	1.79
2 - 3	470	7.10	1.74	1.09	2.12	2.16
3 - 4	136	5.64	0.93	0.95	1.73	1.46
4 - 5	105	7.37	1.34	0.91	2.75	1.93
5 - 6	185	5.34	0.90	0.84	2.15	1.74
6 - 9	283	13.29	1.61	2.80	4.67	3.52
9 - 12	166	12.55	0.67	3.39	4.55	2.19
12 - 22	203	12.73	0.56	4.55	3.59	1.74
	TOTALS	78.41	11.14	16.19	26.03	18.75

UNKNOWN XANTHINE 0.95%

UNKNOWN URIC ACID 5.3%

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES:
SUBJECT L.A.W.; NORMAL DIET.

SAMPLE	VOLUME	% DOSE	I	II	III	IV
0 - 1	100	6.91	0.99	0.79	2.41	3.09
1 - 2	61	7.58	0.84	1.03	2.24	3.50
2 - 3	58	6.72	0.65	0.93	1.49	2.63
3 - 4	72	6.96	0.72	1.21	1.86	2.19
4 - 5	61	5.65	0.41	1.08	1.97	1.32
5 - 6	43	4.84	0.44	0.99	1.77	1.46
6 - 7	43	4.45	0.31	0.95	1.39	1.17
7 - 8	36	4.47	0.22	0.88	2.12	0.95
8 - 12	190	14.61	1.16	3.61	6.15	3.36
12 - 24	490	19.74	0.73	5.97	6.48	5.33
	TOTALS	81.93	6.47	17.44	27.88	25.00

UNKNOWN XANTHINE 1.08%

UNKNOWN URIC ACID 4.11%

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES:
SUBJECT C.A.L.; NORMAL DIET.

SAMPLE	VOLUME	pH	% DOSE	I	II	III	IV
0 - 1	180	7.5	7.99	0.92	0.80	4.35	1.44
1 - 2	68	8.0	6.79	0.74	0.71	3.69	1.55
2 - 3	55	7.5	6.36	0.62	0.93	3.26	1.44
3 - 4	71	7.0	6.48	0.64	1.33	2.97	1.43
4 - 5	62	6.5	6.51	0.52	1.41	3.00	1.41
5 - 6	49	5.5	5.46	0.32	1.30	2.34	1.38
6 - 7	39	5.7	4.61	0.22	1.09	2.08	1.11
7 - 8	22	5.5	2.73	0.09	0.71	1.22	0.65
8 - 9	30	5.2	3.56	0.12	0.98	1.62	0.77
9 - 10	30	5.5	2.81	0.14	0.84	1.12	0.65
10 - 11.5	55	5.4	4.96	0.19	1.56	2.04	1.16
11.5 - 14	83	5.5	5.40	0.20	1.95	1.91	1.24
14 - 16	164	6.0	3.96	0.19	1.36	1.51	0.78
16 - 18	154	5.5	3.40	-	1.30	1.33	0.72
18 - 20	67	5.5	1.84	-	0.65	0.74	0.45
20 - 22	266	5.5	2.54	-	1.16	0.75	0.28
22 - 24	100	6.5	1.92	-	0.96	0.69	0.17
		TOTALS	77.32	4.91	19.04	34.62	16.63

UNKNOWN XANTHINE 1.09%
UNKNOWN URIC ACID 0.35%

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES:
SUBJECT T.J.M.; REPLACEMENT DIET.

SAMPLE	VOLUME	pH	% DOSE	I	II	III	IV
0 - 1	122	6.5	4.75	1.30	1.83	1.37	0.13
1 - 2	39	5.8	4.53	0.57	0.52	2.71	0.60
2 - 3	31	5.6	4.24	0.45	0.56	2.27	0.79
3 - 4	29	5.1	3.99	0.41	0.73	1.94	0.74
4 - 5	26	5.3	3.92	0.34	0.85	1.68	0.88
5 - 6	33	5.4	4.54	0.42	1.01	2.01	0.87
6 - 7	40	5.5	3.52	0.28	0.89	1.62	0.60
7 - 8	44	5.9	3.23	0.33	0.72	1.37	0.68
8 - 10	454	5.7	5.40	0.57	1.14	2.36	1.18
10 - 11	242	5.8	2.50	0.43	0.69	1.01	0.37
11 - 12	46	5.3	2.52	0.20	0.72	1.06	0.42
12 - 14	72	6.4	4.73	0.45	1.50	1.60	0.94
14 - 20	162	6.1	12.92	0.75	3.45	6.56	1.72
20 - 22	78	5.6	4.17	0.26	1.73	1.56	0.62
22 - 24	126	6.2	2.41	0.13	1.01	0.78	0.49
		TOTALS	67.37	6.89	17.35	29.90	11.03

UNKNOWN XANTHINE 2.01%
UNKNOWN URIC ACID 0.11%

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES: SUBJECT J.C.; REPLACEMENT DIET.

SAMPLE	VOLUME	pH	% DOSE	I	II	III	IV
0 - 1	266	5.8	5.89	2.51	0.80	1.08	0.80
1 - 3	486	5.9	10.44	2.13	1.81	3.63	2.87
3 - 4	120	5.6	9.06	1.66	1.19	4.19	2.02
4 - 5.5	78	5.6	7.42	0.91	1.51	2.88	1.73
5.5 - 7	250	5.7	7.20	0.75	1.57	2.91	1.60
7 - 9	213	5.7	9.01	0.78	2.37	3.78	2.08
9 - 10	35	5.9	3.12	0.21	0.67	1.37	0.82
10 - 11	29	5.8	2.68	0.13	0.69	1.16	0.70
11 - 12.5	63	5.9	3.82	0.17	0.99	1.48	1.12
12.5 - 14	72	5.8	4.02	0.27	1.26	1.54	0.95
14 - 16	110	5.6	4.29	0.20	1.15	1.72	1.22
16 - 18	86	5.5	3.30	0.16	1.18	1.18	0.78
18 - 20	92	5.7	2.95	0.12	0.92	1.19	0.72
20 - 24	510	6.3	4.81	1.27	2.17	0.75	0.62
		TOTALS	78.01	11.27	18.28	28.76	18.03

UNKNOWN XANTHINE 0.12%

UNKNOWN URIC ACID 1.47%

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES:
SUBJECT C.A.L.; REPLACEMENT DIET.

SAMPLE	VOLUME	pH	% DOSE	I	II	III	IV
0 - 1	147	8.0	6.94	1.60	0.88	2.71	1.19
1 - 2	106	7.3	6.46	0.97	1.07	2.98	1.22
2 - 3	68	7.5	4.68	0.54	0.68	2.53	0.68
3 - 4	44	7.0	4.66	0.41	0.86	2.18	1.08
4 - 5	54	7.0	5.15	0.50	1.00	2.41	1.24
5 - 6	59	6.5	4.86	0.46	1.12	2.16	1.12
6 - 7	64	6.5	4.55	0.38	1.31	1.90	0.95
7 - 8	43	6.5	3.64	0.22	0.91	1.74	0.77
8 - 9	52	6.0	3.24	0.25	1.00	1.38	0.61
9 - 10	188	5.5	3.44	0.53	1.04	1.30	0.58
10 - 11	132	6.0	3.28	0.47	1.03	1.04	0.74
11 - 12	64	6.5	2.81	0.21	0.83	1.17	0.59
12 - 14	74	6.0	3.94	0.30	1.39	1.50	0.75
14 - 16	58	6.0	3.45	0.21	0.98	1.51	0.75
16 - 18	79	6.0	3.88	0.27	1.27	1.54	0.80
18 - 20	81	6.0	2.90	0.20	1.13	1.28	0.50
20 - 22	141	6.0	2.55	0.20	1.14	0.96	0.45
22 - 24	175	6.5	2.43	0.19	1.08	0.74	0.43
		TOTALS	72.86	7.91	18.72	31.03	14.45

UNKNOWN XANTHINE 0.13%

UNKNOWN URIC ACID 1.02%

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES:
SUBJECT T.J.M.; METHYLXANTHINE-SUPPLEMENTED DIET

SAMPLE	VOLUME	pH	% DOSE	I	II	III	IV
0 - 1	410	6.9	7.41	2.15	0.51	2.97	0.86
1 - 2	116	6.2	5.02	0.97	0.62	2.49	0.81
2 - 3	266	6.0	4.82	1.17	0.72	2.02	0.78
3 - 4	52	5.1	3.96	0.47	0.79	1.82	0.76
4 - 5	37	4.9	3.88	0.39	0.93	1.70	0.73
5 - 6	42	4.9	4.58	0.39	1.00	2.16	0.88
6 - 7	38	5.1	3.87	0.35	0.82	1.78	0.75
7 - 8	36	5.3	3.48	0.26	0.73	1.60	0.77
8 - 9	173	5.3	3.21	0.44	0.78	1.23	0.59
9 - 10	68	5.7	3.48	0.38	0.91	1.75	0.31
10 - 11	53	6.5	3.11	0.30	0.81	1.28	0.63
11 - 12	84	6.8	3.19	0.41	0.85	1.21	0.62
12 - 14	222	6.8	5.30	0.57	1.38	2.08	1.13
14 - 16	300	6.0	4.56	0.48	1.33	1.91	0.71
16 - 18	50	5.4	3.73	0.16	1.19	2.07	0.31
18 - 20	111	5.4	4.03	0.21	1.60	1.38	0.84
20 - 22	200	5.8	2.94	0.24	1.01	1.16	0.53
22 - 24	435	6.2	2.80	0.30	1.20	0.72	0.44
		TOTALS	73.37	9.64	17.18	31.33	12.45

UNKNOWN XANTHINE 1.79%
UNKNOWN URIC ACID 0.75%

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES:
SUBJECT J.C.; METHYLXANTHINE-SUPPLEMENTED DIET

SAMPLE	VOLUME	pH	% DOSE	I	II	III	IV
0 - 1.2	300	6.1	8.61	2.41	1.09	3.18	1.56
1.2 - 2	342	6.2	6.32	1.52	0.62	2.44	1.30
2 - 3	228	6.2	5.86	1.24	0.82	2.29	1.31
3 - 4	129	6.0	6.74	1.28	1.07	2.68	1.43
4 - 5	416	6.3	6.94	1.61	1.47	2.17	1.42
5 - 7	420	6.2	7.66	1.67	1.30	2.72	1.97
7 - 9.5	126	6.2	7.02	0.80	1.94	2.96	1.14
9.5 - 10	133	6.4	2.32	0.35	0.64	0.98	0.35
10 - 11	388	6.6	2.82	0.61	0.63	1.06	0.38
11 - 12	104	6.2	3.29	0.30	0.80	1.68	0.41
12 - 14	120	6.3	4.15	0.53	1.29	1.77	0.38
14 - 16	500	6.1	5.12	0.61	1.67	2.39	0.45
16 - 20.5	427	5.8	7.51	0.63	2.35	3.42	1.11
20.5 - 22	47	5.7	1.97	0.11	0.76	1.10	-
22 - 24	271	6.3	2.56	0.21	1.21	0.96	0.18
		TOTALS	78,89	13.88	17.66	31.80	13.39

UNKNOWN URIC ACID = 2.10%

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES:
SUBJECT C.A.L.; METHYLXANTHINE-SUPPLEMENTED DIET

SAMPLE	VOLUME	pH	% DOSE	I	II	III	IV
0 - 1	313	6.9	10.95	3.31	1.15	3.86	1.68
1 - 2	335	6.0	8.74	1.71	0.82	4.21	1.66
2 - 3	201	6.4	7.28	1.35	0.87	3.20	1.42
3 - 4	95	6.8	6.38	0.89	1.00	2.82	1.53
4 - 5	158	6.4	6.64	0.92	1.09	2.84	1.66
5 - 6	380	5.6	6.07	1.07	1.01	2.70	1.17
6 - 7	310	5.8	4.95	0.86	1.04	1.87	1.08
7 - 8	328	6.0	4.93	0.79	1.28	1.97	0.89
8 - 9	180	6.1	3.90	0.64	1.10	1.36	0.73
9 - 10.75	281	6.3	6.53	0.81	1.98	2.37	1.37
10.75 - 12	56	5.4	3.18	0.24	0.95	1.48	0.39
12 - 14	150	5.4	4.76	0.54	1.35	1.81	0.99
14 - 16	70	5.4	3.53	0.49	0.96	1.59	0.49
16 - 18	68	5.3	3.07	0.36	0.92	1.37	0.42
18 - 22	116	5.7	4.79	0.29	1.29	2.10	1.11
22 - 24	72	6.5	1.94	0.19	0.73	0.62	0.40
		TOTALS	87.64	14.46	17.54	36.17	16.99

UNKNOWN XANTHINE 0.89%

UNKNOWN URIC ACID 1.32%

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES:
SUBJECT T.J.M.; METHYLXANTHINE - DEPRIVED DIET.

SAMPLE	VOLUME	pH	% DOSE	I	II	III	IV
0 - 1	147	7.0	6.19	1.05	1.23	2.69	1.00
1 - 2	52	5.4	7.02	0.88	1.08	3.32	1.58
2 - 3	316	5.4	6.26	1.09	1.01	2.85	1.18
3 - 4	395	5.3	7.23	0.95	0.98	3.62	1.51
4 - 5	55	5.1	5.91	0.67	1.17	2.92	1.03
5 - 6	51	5.3	5.62	0.58	1.18	2.70	0.99
6 - 7	142	5.4	4.89	0.77	1.32	1.75	0.93
7 - 8	112	6.0	4.89	0.99	1.42	1.63	0.74
8 - 9	59	6.1	2.67	0.43	0.97	0.87	0.27
9 - 10	36	5.0	2.90	0.27	0.84	1.22	0.42
10 - 11	32	5.7	3.45	0.28	1.03	1.40	0.56
11 - 12	33	5.5	2.76	0.28	0.79	1.04	0.52
12 - 14	60	5.4	4.85	0.27	1.06	2.28	1.09
14 - 16	323	5.7	4.56	0.38	1.11	2.05	0.92
16 - 18	415	5.7	3.97	0.22	0.98	1.84	0.85
18 - 20	220	5.5	3.43	0.21	1.42	1.08	0.73
20 - 22	512	6.3	4.14	0.44	1.61	1.36	0.71
22 - 24	150	7.7	2.01	-	1.07	0.70	0.24
		TOTALS	82.75	9.76	20.27	35.32	15.27

UNKNOWN XANTHINE 2.18%

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES:
SUBJECT J.C.; METHYLXANTHINE DEPRIVED DIET

SAMPLE	VOLUME	pH	% DOSE	I	II	III	IV
0 - 1	960	6.1	12.53	3.09	1.12	5.07	2.80
1 - 2	566	6.1	6.37	1.24	0.80	2.67	1.48
2 - 3	277	6.3	10.77	1.41	1.61	4.63	2.54
3 - 4	121	5.7	7.45	0.84	1.23	3.40	1.88
4 - 5	109	5.3	7.08	0.86	1.48	3.14	1.62
5 - 6	169	5.9	5.58	0.64	1.35	2.07	1.38
6 - 7	157	6.8	4.61	0.47	1.19	1.66	1.29
7 - 8	130	7.8	5.22	0.66	1.14	2.38	0.96
8 - 9	232	7.2	6.22	0.62	1.56	2.54	1.44
9 - 10	23	9.0	1.43	0.14	0.42	0.61	0.25
10 - 11	93	6.5	3.09	0.24	1.01	1.11	0.75
11 - 12	289	6.5	2.85	0.26	0.92	1.09	0.63
12 - 14	320	6.3	1.77	0.13	0.56	0.68	0.38
14 - 17.5	660	6.4	5.14	0.19	1.79	2.02	1.06
17.5 - 20	490	6.1	2.14	-	0.92	0.79	0.43
20 - 22	246	6.4	1.20	0.07	0.72	0.26	0.15
22 - 24	163	6.1	0.98	-	0.55	0.27	0.16
		TOTALS	84.43	10.86	18.37	34.39	19.20

UNKNOWN XANTHINE 0.86%

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES:
SUBJECT L.A.W.; METHYLXANTHINE DEPRIVED DIET.

SAMPLE	VOLUME	pH	% DOSE	I	II	III	IV
0 - 1	79	6.1	9.87	1.01	1.17	4.97	2.41
1 - 2	46	5.4	6.24	0.49	1.14	2.70	1.63
2 - 3	58	4.7	9.43	0.51	1.58	4.66	2.34
3 - 4	69	4.9	9.65	0.66	1.98	4.34	2.50
4 - 5	42	5.4	6.69	0.38	1.41	3.07	1.64
5 - 6	53	5.9	6.37	0.36	1.71	2.61	1.49
6 - 7	69	5.7	5.57	0.31	1.33	2.56	1.37
7 - 8	60	5.0	4.50	0.17	1.24	2.20	0.77
8 - 9	392	5.4	3.58	0.39	1.03	1.37	0.75
9 - 10	550	6.3	3.18	0.30	1.01	1.05	0.76
10 - 11	90	6.2	3.75	0.21	1.36	1.23	0.95
11 - 12	31	5.6	2.34	0.09	0.78	0.93	0.54
12 - 14	74	5.4	4.68	0.16	1.49	1.92	1.11
14 - 16	50	5.6	2.91	-	1.09	1.10	0.72
16- 18	57	5.7	2.77	0.05	1.08	1.03	0.59
18 - 20	52	5.7	2.28	-	1.02	0.89	0.37
20 - 22	60	5.5	1.77	-	0.97	0.40	0.40
22 - 24	106	6.4	1.54	-	0.90	0.34	0.30
		TOTALS	87.12	5.09	22.29	37.37	20.64

UNKNOWN XANTHINE 0.88%

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES:
SUBJECT C.A.L.; METHYLXANTHINE - DEPRIVED DIET

SAMPLE	VOLUME	pH	% DOSE	I	II	III	IV
0 - 1	218	7.1	9.46	2.47	1.72	3.36	1.31
1 - 2	70	7.2	8.04	1.01	1.06	4.11	1.66
2 - 3	55	6.6	7.33	0.83	1.23	3.41	1.70
3 - 4	78	6.4	7.29	0.80	1.32	3.64	1.48
4 - 5	62	7.0	6.89	0.64	1.39	3.40	1.34
5 - 6	72	6.8	6.60	0.62	1.62	3.02	1.10
6 - 7	43	5.4	4.85	0.30	1.18	2.14	1.14
7 - 8	44	5.5	5.04	0.22	1.30	2.52	0.92
8 - 9	52	5.9	4.45	0.25	1.39	1.92	0.81
9 - 10	73	5.9	4.44	0.31	1.23	1.95	0.87
10 - 12	145	6.0	6.31	0.51	2.41	2.51	0.88
12 - 14	220	5.6	4.62	0.49	1.92	1.44	0.77
14 - 16	121	5.5	3.31	0.21	1.09	1.24	0.77
16 - 18	90	6.0	3.33	0.12	1.14	1.31	0.76
18 - 20	104	5.6	2.50	0.18	0.88	0.88	0.56
20.-22	323	6.0	2.29	0.11	1.03	0.80	0.35
22 - 24	128	6.7	1.98	0.10	0.86	0.63	0.39
		TOTALS	88.73	9.17	22.77	38.28	16.81

UNKNOWN XANTHINE 1.09%

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES AFTER I.V. AMINOPHYLLINE: SUBJECT T.J.M.; NORMAL DIET

SAMPLE	VOLUME	pH	% DOSE	I	II	III	IV
0 - 1	148	8.0	9.09	2.53	1.12	3.64	1.37
1 - 2	173	6.8	8.43	1.39	1.30	3.36	1.53
2 - 3	130	6.6	6.30	0.83	1.02	2.73	1.27
3 - 4	37	5.4	6.12	0.35	1.29	2.65	1.24
4 - 5	41	5.3	6.54	0.49	1.37	3.07	1.32
5 - 6	34	5.3	5.52	0.37	1.12	2.47	1.22
6 - 7	34	5.4	5.36	0.26	1.24	2.54	1.12
7 - 8	28	5.4	4.10	0.17	1.12	1.91	0.82
8 - 9	32	5.4	4.12	0.21	1.10	1.72	0.86
9 - 10	25	5.4	2.98	0.14	0.84	1.20	0.56
10 - 11	35	5.3	3.31	0.24	1.09	1.23	0.67
11 - 12	67	5.6	3.45	0.37	1.10	1.18	0.75
12 - 14	234	6.7	6.46	0.76	1.94	1.79	1.39
14 - 16	266	5.8	5.44	0.45	1.93	1.71	1.04
16 - 18	94	5.5	4.39	0.21	1.43	2.08	0.46
18 - 20	70	5.5	3.08	0.12	0.99	1.13	0.50
20 - 22	260	6.5	3.48	0.18	1.13	1.27	0.70
22 - 24	378	7.4	2.35	0.14	0.99	0.73	0.49
		TOTALS	90.52	9.21	22.12	36.41	17.31

UNKNOWN XANTHINE 2.04%

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES AFTER I.V. AMINOPHYLLINE: SUBJECT J.C.; NORMAL DIET

SAMPLE	VOLUME	pH	% DOSE	I	II	III	IV
0 - 1	652	6.6	9.91	4.01	1.79	2.80	1.17
1 - 2	113	7.1	8.93	1.97	1.24	3.77	1.68
2 - 3	143	6.2	6.80	1.22	1.19	2.88	1.35
3 - 4	97	6.7	6.34	0.99	1.29	2.85	1.06
4 - 5	287	6.5	4.60	0.97	0.88	1.59	0.86
5 - 6	348	6.2	8.32	1.31	1.90	3.36	1.75
6 - 7	54	6.9	4.32	0.44	0.93	1.20	1.32
7 - 8	92	6.3	5.86	0.42	1.31	2.36	1.27
8 - 9	28	5.8	3.34	0.25	0.81	1.35	0.65
9 - 10.3	55	6.6	5.15	0.37	1.25	1.70	1.43
10.3 - 11	26	6.2	2.06	0.12	0.47	0.86	0.39
11 - 12	71	6.7	3.35	0.29	0.86	1.09	0.79
12 - 16	1320	6.9	11.11	1.44	3.21	4.90	1.03
16 - 18	188	6.6	3.80	0.31	1.34	1.67	0.48
18 - 20	239	6.5	2.95	0.25	1.05	1.05	0.50
20 - 22	100	6.5	2.38	0.15	0.99	0.63	0.42
22 - 24	254	7.0	2.02	0.20	1.21	0.36	0.27
		TOTALS	91.24	14.71	21.72	34.42	16.42

UNKNOWN XANTHINE 0.66%

UNKNOWN URIC ACID 0.79%

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES
AFTER I.V. AMINOPHYLLINE: SUBJECT L.A.W.; NORMAL DIET.

SAMPLE	VOLUME	pH	% DOSE	I	II	III	IV
0 - 1	69	7.2	8.30	1.15	1.10	3.75	1.54
1 - 2	146	6.6	9.19	1.18	0.96	4.31	1.83
2 - 3	266	6.9	8.44	1.18	1.04	3.61	2.09
3 - 4	65	7.0	6.48	0.42	1.13	3.29	1.23
4 - 5.25	68	8.0	7.66	0.51	1.39	4.19	1.43
5.25 - 6	42	7.6	4.39	0.27	0.92	2.13	0.76
6 - 7	22	5.9	3.58	0.10	0.82	1.79	0.82
7 - 8	97	6.0	4.40	0.33	1.33	1.97	0.70
8 - 9	83	6.8	3.81	0.29	1.09	1.89	0.52
9 - 10	33	6.8	3.50	0.13	0.94	1.73	0.71
10 - 11	85	6.6	3.91	0.20	1.27	1.75	0.68
11 - 12	48	6.4	3.12	0.14	0.91	1.33	0.67
12 - 14	251	6.7	5.32	0.40	1.64	2.53	0.81
14 - 16	84	6.0	4.07	0.16	1.48	1.65	0.70
16 - 18	42	5.7	3.30	0.07	1.02	1.46	0.62
18.-20	119	5.7	3.06	0.10	1.11	1.00	0.80
20 - 22	129	6.9	2.54	0.11	0.92	0.86	0.69
22 - 24	137	7.5	2.45	0.06	1.00	0.82	0.53
		TOTALS	87.52	6.80	19.87	40.06	17.13

UNKNOWN XANTHINE 1.05%

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES AFTER I.V. AMINOPHYLLINE: SUBJECT T.J.M.; METHYLXANTHINE DEPRIVED DIET.

SAMPLE	VOLUME	pH	% DOSE	I	II	III	IV
0 - 1	233	7.4	8.13	1.92	1.86	2.41	0.66
1 - 2	58	6.4	5.30	1.08	0.44	2.74	0.79
2 - 3	46	5.3	4.84	0.61	0.62	2.11	0.75
3 - 4	85	5.5	5.41	0.74	0.91	2.58	0.89
4 - 5	50	5.3	4.94	0.56	0.92	2.24	0.77
5 - 6	378	5.5	4.85	0.78	0.73	1.81	1.04
6 - 7	265	5.6	4.76	0.56	0.85	2.14	0.75
7 - 8	68	6.0	4.48	0.41	0.95	2.04	0.68
8 - 9	55	6.8	3.80	0.43	0.98	1.50	0.49
9 - 10	84	6.3	3.49	0.41	0.90	1.44	0.52
10 - 11	60	6.2	3.45	0.40	0.76	1.61	0.51
11 - 12	158	6.9	3.62	0.60	0.87	1.56	0.50
12 - 14	362	7.2	6.58	1.16	1.39	2.74	0.98
14 - 16	273	6.9	4.98	0.69	1.00	2.33	0.67
16 - 20	342	5.6	8.15	0.82	2.02	3.74	1.22
20 - 22	325	6.2	3.27	0.38	0.85	1.22	0.50
22 - 24	106	6.3	2.77	0.15	0.82	1.13	0.48
		TOTALS	82.82	11.70	16.87	35.34	12.20

UNKNOWN XANTHINE 2.46% UNKNOWN URIC ACID 0.53%

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES AFTER I.V. AMINOPHYLLINE: SUBJECT J.C.; METHYLXANTHINE - DEPRIVED DIET.

SAMPLE	VOLUME	pH	% DOSE	I	II	III	IV
0 - 1	445	7.2	11.00	3.61	1.28	3.92	1.37
1 - 2	585	6.7	10.58	2.21	1.52	4.33	2.10
2 - 3	138	7.1	8.52	1.41	1.34	3.60	1.65
3 - 4	90	6.5	8.31	1.02	1.63	3.95	1.54
4 - 5	195	6.5	7.73	1.15	1.64	3.24	1.55
5 - 6	121	6.3	6.03	0.74	1.35	2.71	1.25
6 - 7	318	6.4	5.12	0.73	1.20	1.97	0.84
7 - 9	413	6.7	9.91	0.97	3.25	3.22	2.02
9 - 10	49	6.5	3.62	0.20	0.95	1.69	0.67
10 - 11	54	5.6	3.90	0.16	0.95	1.89	0.74
11 - 12	36	5.5	2.68	0.10	0.64	1.23	0.45
12 - 14	62	5.4	4.35	0.15	1.09	2.02	0.79
14 - 16	311	5.8	4.72	0.33	1.51	1.86	0.89
16 - 18	391	5.9	3.36	0.21	1.07	1.27	0.74
18 - 20	130	5.9	2.42	0.11	0.84	0.91	0.50
20 - 22	104	6.2	2.03	-	0.86	0.76	0.41
22 - 24	117	6.7	1.52	-	0.88	0.35	0.29
		TOTALS	95.80	13.12	22.01	38.92	17.80

UNKNOWN XANTHINE 0.19%
UNKNOWN URIC ACID 0.79%

QUANTITATION OF THEOPHYLLINE AND ITS METABOLITES IN SERIAL URINE SAMPLES:
AFTER I.V. AMINOPHYLLINE: SUBJECT L.A.W.; METHYLXANTHINE - DEPRIVED DIET.

SAMPLE	VOLUME	pH	% DOSE	I	II	III	IV
0 - 1	145	7.4	12.10	2.63	1.39	5.90	1.98
1 - 2	63	6.5	9.19	0.69	1.56	4.47	2.11
2 - 3	66	6.0	9.18	0.58	1.65	4.72	2.11
3 - 4	83	6.2	8.50	0.60	1.87	4.09	1.73
4 - 5	93	6.5	7.47	0.53	1.69	3.59	1.46
5 - 6	55	6.3	5.50	0.29	1.38	2.75	1.07
6 - 7	146	6.3	5.34	0.35	1.48	2.16	1.07
7 - 8	458	6.3	4.13	0.37	1.12	1.68	0.65
8 - 9	622	6.4	5.20	0.56	1.59	2.10	0.82
9 - 12	315	6.6	9.18	0.34	2.38	4.32	1.50
12 - 15	91	5.5	5.89	0.11	2.08	2.17	1.19
15 - 17	45	5.7	3.01	-	1.07	1.26	0.64
17 - 21	102	5.4	4.03	-	1.39	1.37	0.82
21 - 24	220	6.1	2.21	-	1.31	0.43	0.28
		TOTALS	90.93	7.05	21.96	41.01	17.43

UNKNOWN XANTHINE 0.94%

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