PHARMACOKINETIC AND CLINICAL STUDIES WITH CAFFEINE

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Master of Philosophy

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THE UNIVERSITY OF ASTON IN BIRMINGHAM

Pharmacokinetic and Clinical Studies with Caffeine

A project presented by Janet Elizabeth Hemingway
for the degree of M.Phil. 1986.

SUMMARY

A preliminary study was carried out to investigate the pharmacokinetics of caffeine. Four healthy adult volunteers each received 200mg, 400mg and 600mg doses of caffeine on separate mornings, having abstained from food and drink containing methylxanthines for the previous 24 hours. Blood, saliva and urine samples were taken at intervals during the next 24 hours. Determinations of caffeine, paraxanthine, theobromine and theophylline concentrations in the samples were made by high performance liquid chromatography. Caffeine concentrations in plasma and saliva decreased in a log-linear relationship with time, indicative of first-order kinetics, however this relationship did not hold for the metabolites. Mean ± standard deviation plasma caffeine clearance was 1.10 ± 0.45ml/min/kg and mean half-life was 7.1 ± 3.2 hours. Saliva/plasma caffeine concentration ratios showed time dependence with high values initially. Only 2.8 ± 0.5% of the caffeine dose was excreted unchanged in urine.

The clinical study aimed to compare the bronchodilator effects of caffeine and theophylline in patients with reversible airflow obstruction. This was a double-blind within-patient cross-over study. Patients were asked to fill in a diary card and take twice daily peak flow readings for ten weeks. FEV_1 and FVC were measured at each clinic visit. There was an initial two-week single blind placebo period then the patients were randomly allocated to one of the two treatments. There was a two-week "run-in" period during which the dose was adjusted to achieve plasma concentrations of 10-20µg/ml. The patient continued with the treatment for a further two weeks and the procedure was repeated with the other treatment. Due to recruiting difficulties only 17 patients entered the study and of these only ten completed it. Reasons for withdrawal included worsening of condition when changing from usual theophylline therapy to placebo, severe gastric irritation and medical problems unconnected with the study. Data from the ten patients who completed the study were analysed by ANOVA. No significant difference at the 5% level was found in either the pulmonary function tests or the subjective assessments. Possible explanations for this might be too few subjects, too short a study period, an entry criterion of 10% reversibility, exclusion of patients not tolerating the placebo period or maximal therapy with concurrent medications.

Keywords: Caffeine, Pharmacokinetics, Methylxanthine, Bronchodilator

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I. INTRODUCTION

I.1 Consumption and Source

The methylxanthine alkaloids caffeine, theophylline and theobromine have been constituents of the human diet for many centuries. Their use is believed to date back as far as the paleolithic period. Written evidence of use of a beverage containing methylxanthines, tea, dates back to about 350 AD where it is listed in a Chinese dictionary. Coffee may have been cultivated in Ethiopia as early as 575 AD but there is no written record until the 10th century.

A wide variety of plants naturally contain these purines, for example over 60 plant species throughout the world have been identified as containing caffeine[1]. Coffee is extracted from the fruit of Coffea arabica and related species. Tea is prepared from the leaves of Thea sinensis, a bush native to Southern China. Extract of the nuts of Cola acuminata are used to produce cola-flavoured drinks although 50% of the caffeine content is added by the manufacturer as the alkaloid. Cocoa and chocolate are derived from the seeds of Theobroma cacao. Other beverages, of South American origin, containing caffeine include Guarana (from the seeds of either Paullinia cupana or Paullinia sorbilis), yoco (from the bark of Paullinia yoco) and maté (from Ilex paraguariensis, a species of holly)[2].

Many attempts have been made to quantify the methylxanthine content of various beverages and foods. However, there is considerable variation

in the values reported due to difficulty in standardising the size of portion and strength of brew[3].

Coffee was found to account for 77% of total caffeine intake in an American study[4].

TABLE 1: Approximate caffeine content of foods and drinks

Food/drink		Caffeine content (mg)
Tea (140ml, 1 cup)		20-50
Coffee (140ml, 1 cup)	- percolated	100-120
	- instant	50-60
	- decaffeinated	2
Cocoa (230ml, 1 mug)		13
Drinking chocolate (230ml, 1 mug)		5-8
Soft drinks (340ml, 1 can)	- Coca Cola	50-60
	- Diet Pepsi	30
	- Pepsi Cola	37
Lucozade (140ml, 1 cup)		25
Milk chocolate (120g)		20-30
Plain chocolate (120g)		70-80

The caffeine content of coffee varies with species of coffee plant, the product form (eg, ground roasted vs instant), the method of brewing (eg, percolator vs filter), the amount of coffee used and brewing time. Freshly ground roasted coffee beans contain caffeine concentrations ranging from 0.8% to 1.8%. Theobromine and theophylline concentrations are relatively low in coffee. The values in green coffee beans are respectively 20 and 5 mg per kg. In addition to caffeine, coffee contains a variety of other substances (protein, carbohydrates, lipids, volatile oils and non-volatile oils) and some of these may have pharmacological actions which must be distinguished from those attributed to caffeine. For example chlorogenic acids and quinic acids may affect intestinal motility.

Tea is consumed by at least half the population of the world. The caffeine content of tea is affected by a variety of factors including seasonal variation, leaf position on the plant, genetic origin and use of nitrogen fertilisers. In addition to caffeine (20-50mg per cup) tea contains significant amounts of theobromine and theophylline (2 and 1mg respectively).

Cocoa is a better source of theobromine than caffeine (250mg and 5mg per cup respectively). Together, theobromine and caffeine account for up to 99% of the alkaloid content of Theobroma cacao with only trace amounts of theophylline. Cocoa powder contains about 1.9% theobromine and 0.21% caffeine.

Soft drinks account for only 4% of caffeine intake in a middle-aged sample group, however they were the primary source (about 50%) of dietary caffeine among young adults (18-24 years of age)[4]. In general, only cola-type drinks contain caffeine although some non-colas do also. Recently, caffeine-free cola type drinks have been introduced.

Maté constitutes the primary source of methylxanthines in the diet for a group of people in Argentina, Brazil, Paraguay, Uraguay and Chile. Worldwide annual production is in excess of 200,000 tons. Caffeine concentrations vary with age from 0.9% to 2.2%.

In addition to dietary sources, caffeine is found in a variety of non-prescription medicines such as compound analgesics, cold remedies and tonics at up to 100mg per dose. Theophylline is also a constituent of

some over-the-counter remedies for chest conditions although it is more widely obtained on prescription.

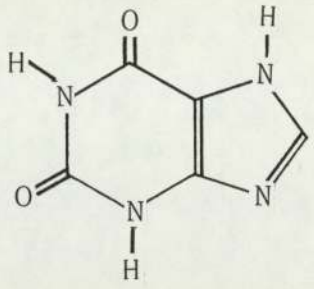
The annual worldwide consumption of caffeine is estimated at 120 000 tons, equivalent to 70mg per day for each inhabitant[5]. In the USA average caffeine intake per capita is above 200mg daily. Clearly any pharmacological effects of the methylxanthines will have wide-ranging consequences throughout the world.

I.2 Chemistry

Caffeine, theophylline and theobromine are derivatives of xanthine and are structurally related to uric acid. All three are white odourless crystalline powders with a bitter taste. Solubility is low and may be increased by the formation of complexes. Aminophylline is a complex of theophylline and ethylenediamine containing 78-84% anhydrous theophylline, 13-14% ethylenediamine with variation in the quantity of water. It is soluble 1 in 5 in water and the solution has a pH of 9.2-9.6.

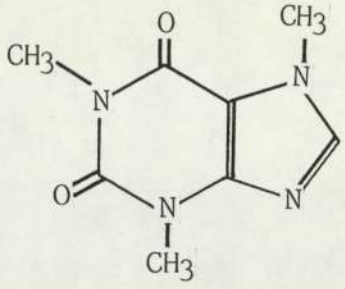
True salts of theophylline have also been used to increase aqueous solubility, eg, choline theophyllinate. These salts and complexes dissociate in biological fluids to yield the parent methylxanthine. Caffeine may be rendered more soluble in water by addition of an equal quantity of citric acid, sodium salicylate or sodium benzoate as complex double salts are formed.

FIGURE 1: The structural formulae of xanthine and the three naturally occurring xanthine derivatives



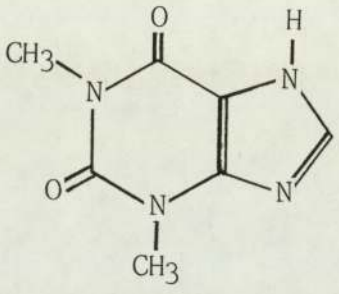
Xanthine

MW = 152.1



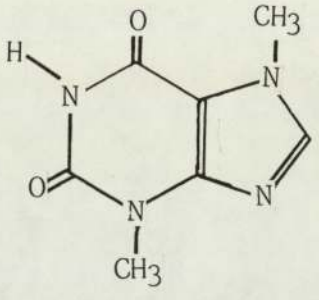
Caffeine (1,3,7-trimethylxanthine)

MW = 194.2



Theophylline (1,3-dimethylxanthine)

MW = 180.2



Theobromine (3,7-dimetylxanthine)

MW = 180.2

TABLE 2: Solubility data for the methylxanthines

Solvent	Caffeine	Theophylline	Theobromine
Water	1 in 60	1 in 120	1 in 2000
Boiling water	1 in 1	-	1 in 150
Alcohol	1 in 130	1 in 80	1 in 2500
Chloroform	1 in 7	1 in 200	1 in 6000

Caffeine is a very weak base and reacts with acids; the salts produced are very readily hydrolysed. Theobromine and theophylline are weakly amphoteric and behave more distinctly as acids or bases than caffeine does and hence will dissolve in aqueous acids and bases.

TABLE 3: Acidic and basic equilibrium constants for the methylxanthines

Compound	pKa	pKb
Caffeine (19°C)		14.2
Caffeine (25°C)	14 approx	
Theobromine (18°C)	10.0	13.9
Theophylline (25°C)	8.8	13.7

The methylxanthines have similar λ_{max} values (see Table 4).

TABLE 4: Optimum absorbance values for the methylxanthines

Methylxanthine	Solvent	λ_{max} (nm)	E 1% 1cm
Caffeine	aqueous acid	275	490
Caffeine	aqueous alkali	275	
Caffeine	alcoholic solution	275	
Theophylline	aqueous acid	272	530
Theophylline	aqueous alkali	277	650
Theobromine	aqueous acid	274	543
Theobromine	aqueous alkali	274	
Theobromine	alcoholic solution	274	524

I.3 Pharmacology

The principle pharmacological actions of the methylxanthines are relaxation of smooth muscle, stimulation of the nervous system, stimulation of cardiac muscle and a diuretic effect.

The methylxanthines relax various smooth muscles, most notably those of the bronchi, leading to the use of theophylline in the treatment of asthma. Other smooth muscles which have been demonstrated to exhibit theophylline-induced relaxation include the bladder, ureter, uterus and intestine[2]. Theophylline also antagonises spasm of the biliary

tract caused by opioids.

The stimulant effect of the methylxanthines is well recognised and are a major reason for coffee consumption among heavy coffee drinkers. While "liking the taste" was given as the reason for drinking coffee by three-quarters of subjects asked, nearly all heavy drinkers in a study stressed positive psychotropic effects such as "makes me feel alert", "wakes me up" and "gives me a feeling of well-being"[6]. Caffeine increases vigilance and decreases motor reaction time in response to both visual and auditory stimuli. Tasks requiring delicate muscular coordination and accurate timing may, however, be adversely affected by caffeine consumption[7].

The effect of caffeine on mood and on disturbance of sleep shows wide individual variation. A greater proportion of non-drinkers of coffee than habitual heavy coffee drinkers reported an increased sleep latency and decreased quality of sleep after coffee consumption. In a study designed to assess behavioural effects of caffeine in normal boys, side effects such as insomnia and nervousness were significantly higher only for the habitual low caffeine users, not for those whose normal use was similar to that given in the study (10mg per kg per day)[8]. It is uncertain whether those with a high consumption of caffeine-containing beverages became tolerant to its effects or conversely whether persons with high sensitivity to those effects tend to avoid coffee.

Although caffeine is traditionally considered to be more potent in its CNS effects than theophylline there is little experimental data to

support this and Rall[2] states that "theophylline produces more profound and potentially more dangerous CNS stimulation than does caffeine."

The methylxanthines also act as respiratory stimulants,[9, 10] an effect which may be useful when aminophylline is used in acute respiratory failure secondary to airways obstruction. The mechanism of action appears to be increased sensitivity of medullary centres to the stimulatory actions of CO_2[11]. Despite this stimulant effect caffeine did not cause an increase in breathlessness in patients with airways obstruction[12].

The cardiovascular effects of the methylxanthines are complex and depend on the conditions present at the time of administration and the dose used. In a therapeutic concentration theophylline reduces left ventricular ejection time and isovolumetric contraction time suggesting increased contractility. The effect of dietary caffeine intake on cardiac contractility is insignificant[13].

Low concentrations of caffeine may produce small decreases in heart rate by stimulation of vagal centres directly or via baroreceptor reflex mechanisms. At higher concentrations both caffeine and theophylline produce definite tachycardia. Arrythmias may also develop in some individuals[14, 15].

Effects of the methylxanthines on blood pressure depend on route of administration and dose. When a large dose is given intravenously the response may be biphasic with an initial fall followed by a secondary

rise[16]. However at lower doses or with oral administration the initial fall is not seen. The pressor effect of caffeine has been suggested to be useful in the treatment of orthostatic hypotension due to autonomic failure especially in the post-prandial state[17]. The cardiovascular effects of coffee have been shown to be almost entirely due to the caffeine content[18, 19].

The methylxanthines cause increased cerebrovascular resistance and this vasoconstriction is thought to be responsible for the relief of some forms of headache by caffeine and theophylline. The headache experienced by subjects habituated to high doses of caffeine following discontinuation of caffeine use may be explained by sudden vasodilation of the cerebral blood vessels[16].

The effect of the xanthines on coronary blood vessels is dilatation and increased coronary blood flow, however this increased oxygen supply is balanced by increased demand caused by greater work of the heart.

The methylxanthines have a diuretic effect[20, 21] with the order of potency theophylline > caffeine > theobromine. The patterns of enhanced excretion of water and electrolytes are similar to those of the thiazides. The mechanism of the diuretic effect is thought to be two-fold, an elevation in glomerular filtration rate and a decrease in renal tubular resorption of sodium and water[22]. The diuretic effect is short-lived as tolerance quickly develops.

The endocrine responses caused by the methylxanthines in experimental

animals include raised serum corticosterone and β-endorphin and decreased serum growth hormone and thyroid stimulating hormone. However most of these studies have used very high doses[23]. In humans a 500mg dose of caffeine elevated plasma concentrations of β-endorphin but had no significant effect on plasma concentrations of cortisol, TSH, growth hormone, prolactin or triiodothyronine and a 250mg dose induced no significant changes in plasma concentrations of any of the hormones measured[24]. A slight increase in basal metabolic rate can be noticed after administration of methylxanthines and may be accompanied by increased plasma concentrations of free fatty acids[25] and glucose. There is however no evidence that chronic methylxanthine consumption influences metabolic rate.

I.4 Therapeutic Use

Theophylline has an important role in the treatment of asthma. It is used as a prophylactic agent and also in the treatment of status asthmaticus. Therapeutic response is dependent on blood concentrations. The therapeutic range for theophylline is often quoted as 10-20μg/ml, however some benefit may be obtained from lower concentrations[26, 27] and concentrations above 15μg/ml may produce adverse effects such as gastrointestinal upset in susceptible individuals[28].

For treatment of acute symptoms aminophylline given intravenously provides the most rapid and assured delivery of medication. A loading dose of 5mg/kg given over a period of at least 10-15 minutes is recommended for patients who have not previously been treated with xanthines, followed by a maintenance infusion of 500μg/kg/h.

TABLE 5: Relationship between plasma theophylline concentrations and clinical effects

Concentration μmol/l	Concentration mg/l	Efficacy	Toxicity
17	3		
28	5	minimal	
55	10		
83	15		gastrointestinal upset
		maximal	nervousness
110	20		increased heart rate
220	40		tachyarrhythmias
			convulsions
330	60		cardiorespiratory arrest

For chronic oral therapy a range of sustained release preparations is available, with varying release characteristics and bioavailability[29, 30]. Dose should be adjusted to give optimal plasma concentrations by starting with a low dose eg, 16mg/kg/day up to a maximum of 400mg and increasing by 25% increments every three days if tolerated until optimal concentrations are achieved[31].

The availability of sustained release formulations makes theophylline particularly useful in the treatment of nocturnal asthma[32-34]. A single dose may be given at night, although this may lead to greater fluctuation in concentration than conventional regimens[35]. Patients with exercise-induced asthma may also benefit from regular prophylactic theophylline[36, 37].

The use of β-adrenergic agonists with theophylline may have an additive or possibly synergistic effect enabling lower doses of theophylline to be used and reducing side effects[38].

The use of theophylline in the treatment of chronic obstructive lung disease is less well defined. Some studies conclude that xanthines improve respiratory functions[39, 40], while others find no significant effects[41]. In some studies the patients feel subjective improvement[39] but in others they cannot discriminate between theophylline and placebo[40, 42]. The combination of β-agonists and theophylline may be less beneficial in chronic obstructive pulmonary disease (COPD) than in the treatment of asthma[43].

In addition to the well-known bronchodilator effects of theophylline other beneficial effects have been proposed including enhanced mucociliary transport, inhibition of mediator release and reduced fatigue of the muscles involved in respiration. Theophylline has been demonstrated to increase mucus transport rates in the ciliated airways of healthy subjects at low therapeutic doses (4mg/kg twice daily)[44]. This effect on mucus transport is additive with β-agonists[45]. Theophylline has been shown to inhibit histamine release from mast cells isolated from human adenoid tissues at therapeutic concentrations[46].

Since dyspnoea and respiratory failure may be partly due to respiratory muscle fatigue, reduced fatigue could lead to clinical improvement. Aminophylline has been demonstrated to improve diaphragmatic contractility and render it less susceptible to fatigue[47, 48], however at therapeutic concentrations no significant effect on contractility or fatigue of the normal human sternomastoid muscle was seen[49]. Caffeine has been shown to augment respiratory muscle contractility and its use in the treatment of selected patients with respiratory

muscle weakness has been suggested[50].

Theophylline in therapeutic doses improved gas exchange, increased arterial oxygen saturation and decreased arterial carbon dioxide concentration in patients with sleep disorders and chronic obstructive lung disease, however quality of sleep was reduced with decreased total sleep time and rapid-eye-movement sleep[51].

In patients with Cheyne-Stokes respiration theophylline is used to restore normal ventilatory patterns. Although diuretics and narcotic analgesics are more frequently used to treat left ventricular failure aminophylline may have a role in patients who are also suffering from asthma or bronchitis.

Another area where theophylline and caffeine have an important role is the treatment of apnoea of preterm infants[52, 53]. Caffeine has the advantage that it is easier to administer and requires less monitoring[54, 55].

Caffeine is included as an adjuvant in compound analgesic preparations containing aspirin and paracetamol, however the synergistic effect is difficult to prove[56]. In the treatment of migraine caffeine is included with some ergot derivatives due to its ability to constrict cerebral blood vessels.

A number of other therapeutic uses have been proposed for caffeine including increasing the motility of hypokinetic sperm, as an alternative to methylphenindate and dextroamphetamine in children with

minimal brain dysfunction (hyperkinetic syndrome) and as an adjuvant in cancer chemotherapy. However subsequent studies have not shown caffeine to be of use for these indications[7]. Topical treatment with 30% caffeine in a hydrophilic base has been shown to improve pruritis, erythema and scaling in patients with atopic dermatitis, and has been suggested as a treatment for allergic rhinitis, although further studies would be needed to confirm this indication[57].

I.5 Mode of Action

Several mechanisms have been proposed to explain the actions of the methylxanthines at a cellular level including the following:

1. Inhibition of cyclic nucleotide phosphodiesterases
2. Inhibition of 5'nucleotidase and alkaline phosphatase
3. Antagonism of adenosine
4. Mobilisation of calcium from intracellular depots
5. Stimulation of endogenous catecholamine release
6. Inhibition of xanthine oxidase
7. Prostaglandin antagonism

For many years phosphodiesterase inhibition giving rise to increased concentrations of cyclic AMP was the accepted mechanism of action for the methylxanthines. However more recently it has been shown that there are at least 3 enzymes responsible for cyclic nucleotide hydrolysis. Some of these forms show higher activity against cyclic GMP than cyclic AMP while others show the reverse. Theophylline and caffeine do not antagonise any form selectively[16]. This explanation

however is unlikely to be the sole effect since concentrations of theophylline sufficient to substantially inhibit cyclic nucleotide breakdown are rarely reached in therapeutic use. Furthermore other phosphodiesterase inhibitors such as papaverine and dipyridamole have no detectable relaxant effect on tracheal tone. According to this mechanism theophylline would be expected to act synergistically with the β-agonists, however most studies claim only an additive effect[58].

Alkaline phosphatase and 5'nucleotidase are inhibited by methylxanthines but again these effects are only seen at higher concentrations than would be achieved with normal therapeutic doses.

Antagonism of adenosine has been proposed as the mechanism for a number of the pharmacological effects of the methylxanthines. Adenosine produces bronchoconstriction in asthmatic subjects, an effect which is completely antagonised by therapeutic concentrations of theophylline[59]. Similarly, theophylline antagonises the adenosine mediated cardiovascular responses to dipyridamole in man[60]. However a more potent analogue of theophylline, enprofylline, which has a propyl group substituted for the methyl group at the 3 position lacks adenosine inhibitory activity[61]. Adenosine antagonism is now thought to be responsible for most of the effects of theophylline and caffeine including metabolic stimulation, central excitation and diuresis[62] however the notable exception is the effect on smooth muscle. Since only xanthines with a methyl group in the 1 position consistently antagonise adenosine, it should be possible to develop a xanthine without the side effects of theophylline[61, 63]. The picture is further complicated by the fact that two adenosine receptors may be

present in the lung as in the brain, A_1 which is inhibitory and A_2 which is stimulatory. None of the xanthine derivatives so far studied show a definite selectivity towards A_1 or A_2 receptors.

Extracellular calcium is necessary for aminophylline-induced increase in diaphragmatic contractility in dogs[64]. Calcium flux has also been implicated in the diuretic effects of theophylline[65]. However most studies concerned with the transport of calcium use xanthine concentrations higher than the maximum used therapeutically and use striated or cardiac muscle. The mechanism of action is thought to be release of calcium from stores in the sarcoplasmic reticulum[66].

The effects of the methylxanthines on plasma catecholamine concentrations have been used to explain their mechanism of action. Caffeine causes a significant increase in plasma adrenaline and noradrenaline when given as a 250mg dose to subjects who had abstained from food and drink containing methylxanthines for three weeks[67]. Further evidence for this explanation is that the effect of theophylline on normal airway conductance is partially blocked by propranolol, suggesting that theophylline may augment the output from adrenergic terminals to smooth muscle[68].

The action of the methylxanthines at a cellular level remains unknown, and may be a combination of several factors. The explanation currently most popular for actions other than bronchodilation is that of adenosine antagonism.

I.6 Pharmacokinetics

Absorption of both caffeine and theophylline after oral administration in the form of a solution or nonsustained release tablets is rapid and complete since they are essentially un-ionised under physiological conditions and are sufficiently lipophilic in nature to readily cross biological membranes. After fasting, maximal concentrations of theophylline are produced within the first two hours and maximal concentrations of caffeine within the first hour. Caffeine from soft drinks is absorbed more slowly than from tea and coffee due to the lower pH and higher sugar content. Absorption of theophylline from sustained-release preparations may be variable and incomplete, and may be greatly affected by food intake[69, 70]. Only preparations where absorption is reliable and unaffected by food should be considered for routine clinical use. Circadian variation has been noted in the absorption of theophylline with slower absorption at night. Suggested reasons for this include changes in gastric emptying, effect of food and posture[71, 72]. In premature infants peak blood caffeine concentration may be reached 4-5 hours after an oral dose due to delayed gastric emptying time. Neither the rate nor the extent of absorption are decreased in old age[73].

Intravenous aminophylline should be given over at least 15 minutes to prevent toxicity. Intramuscular injection of theophylline produces long-lasting local pain and should not be used. Caffeine, however, has been formulated as an injection for intramuscular use with sodium benzoate (caffeine and sodium benzoate injection USP 500mg/2ml equivalent to 250mg caffeine). Rectal absorption of theophylline may be

slow and erratic[74]. Administration of xanthine derivatives by inhalation is impractical as it produces coughing and has an unpleasant taste[75].

The ranges of apparent volume of distribution for caffeine and theophylline in both adults and children are quoted as 0.5-0.6 and 0.3-0.7l/kg respectively. These data are consistent with distribution into total body water. For each mg of theophylline absorbed the plasma concentration will increase by approximately 2μg/ml. Volume of distribution for theophylline is not affected by gender, smoking, pulmonary oedema or asthma although it is increased in the presence of hepatic cirrhosis since protein binding is reduced in these patients. Advanced age does not affect theophylline distribution, however when plasma protein binding was taken into account a reduction was observed[76]. Caffeine volume of distribution was decreased in elderly subjects and this is consistent with the general reduction in total body water and lean muscle mass[77]. Caffeine distribution was found to increase in direct relation to body weight[78], however ideal body weight was recommended when calculating a loading dose of theophylline[79]. Volume of distribution of both caffeine and theophylline is increased in premature neonates. Protein binding for caffeine and theophylline is 15-35% and 55-67% respectively, although theophylline has been reported to be as little as 40% bound[80]. These values indicate that binding effects on drug interactions and therapeutic monitoring will be more important for theophylline than caffeine. Caffeine binding was similar for young and elderly subjects and was suggested to be mainly due to albumin[81].

The methylxanthines can pass into saliva, breast milk, amniotic fluid and can cross the placenta. Caffeine and to a lesser extent theophylline cross the blood-brain barrier into cerebrospinal fluid. Caffeine saliva concentration varies from 60-95% of plasma concentration and the ratio may show time dependence[82]. Saliva monitoring of theophylline has been suggested as a noninvasive alternative to plasma determinations[83-85], however some workers feel that the variation is too great to make this method reliable[86]. In premature neonates saliva monitoring of theophylline and caffeine has been suggested as a screening procedure with serum concentrations being determined if the saliva concentration is above 8μg/ml[87].

The methylxanthines are eliminated mainly by hepatic metabolism[88] by the mixed function oxidase system, particularly the cytochrome P-450 oxidases. Only about 7-13% of theophylline and 0.5-3.7% of caffeine are excreted unchanged in urine.

In adults the major metabolite of theophylline is 1,3-dimethyluric acid, formed by hydroxylation. 3-Methylxanthine and 1-methylxanthine result from N-demethylation, and the latter is rapidly oxidised to 1-methyluric acid (see Fig. 2). These metabolites possess some degree of pharmacological activity of their own[89]. About 6% of a dose of theophylline is converted to caffeine, which is further metabolised[90].

Caffeine undergoes demethylation to form paraxanthine, theophylline and theobromine. Other metabolites found in lesser amounts are 1,3,7-trimethyluric acid, 1-methyluric acid, 1-methylxanthine and 5-acetylamino-6-formylamino-3-methyluracil (AFMU) (see Figs. 3, 4).

FIGURE 2 Metabolic Pathways of Theophylline

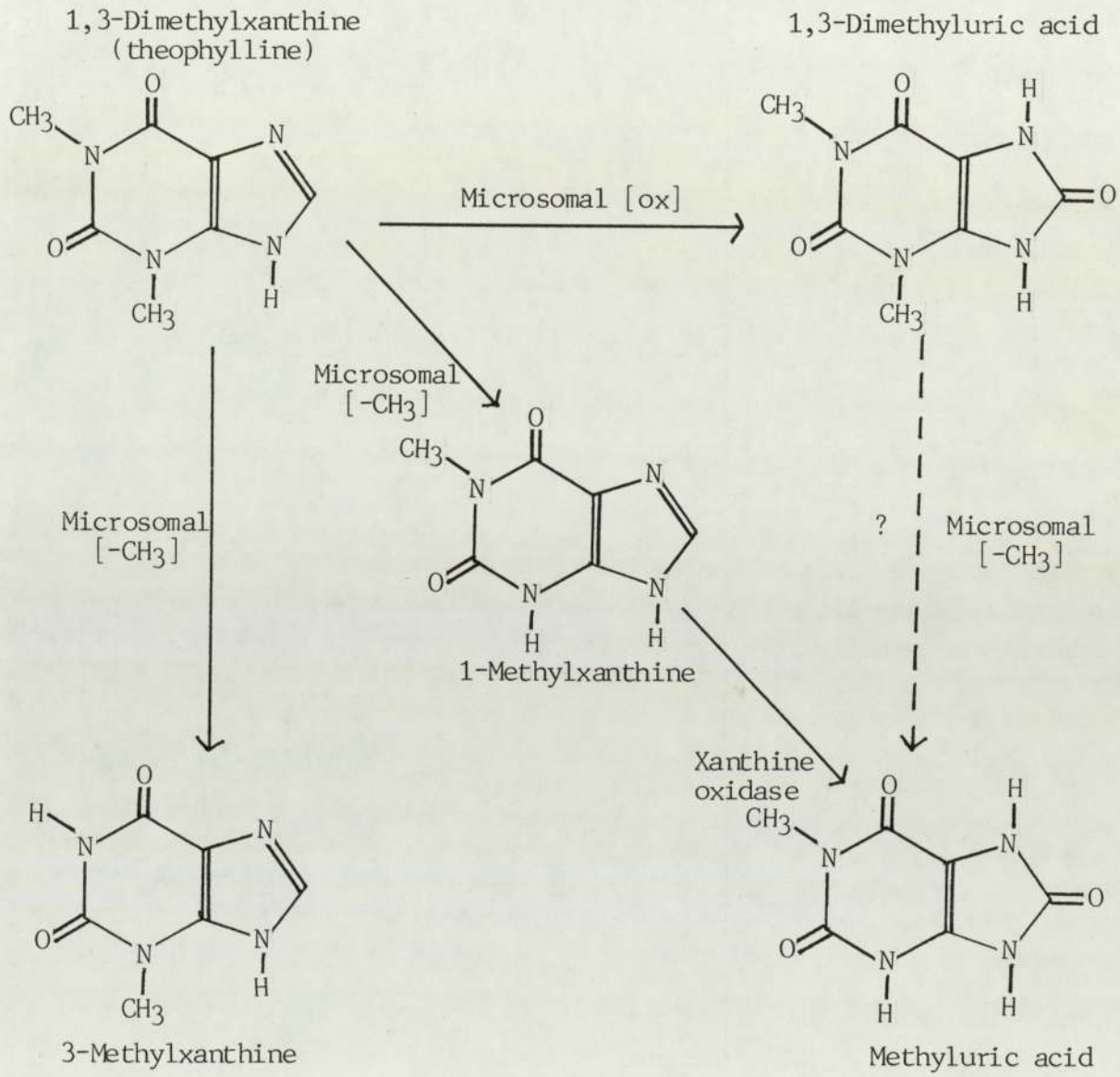
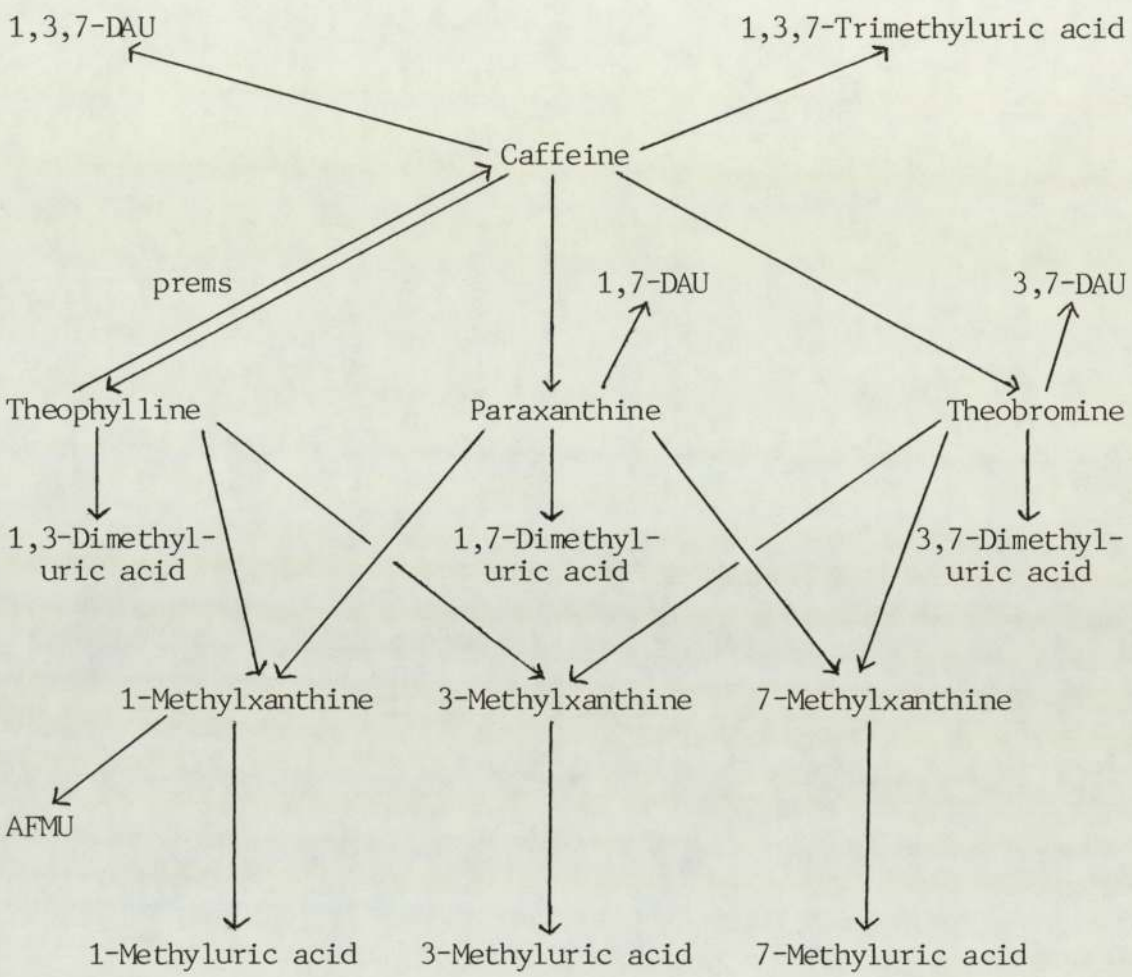
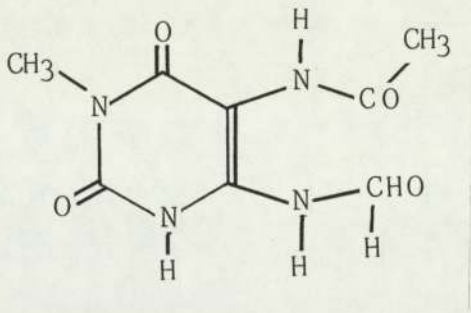


FIGURE 3 Metabolic Pathways of Caffeine



1,3,7-DAU	6-amino-5-(N-formylmethylamino)-1,3-dimethyluracil
3,7-DAU	6-amino-5-(N-formylmethylamino)-1-methyluracil
1,7-DAU	6-amino-5-(N-formylmethylamino)-3-methyluracil
AFMU	5-acetylamino-6-formylamino-3-methyluracil

FIGURE 4 5-Acetylamino-6-formylamino-3-methyluracil (AFMU)



The level of AFMU production has been found to be bimodally distributed and has been proposed as a measure of acetylator status[91].

Theophylline clearance varies with age, disease state, concomitant medication and diet. In premature infants the hepatic enzyme system responsible for the metabolism of theophylline is not fully developed, the half-life is prolonged (30.2 $\pm$ 6.5 hours)[52] and conversion to caffeine is the major metabolic pathway[92-94]. Young children rapidly eliminate theophylline (mean $t_{1/2}$ 3.5 hours) but clearance decreases during adolescence to reach adult values (mean $t_{1/2}$ 8-9 hours) and may be further reduced in old age[76]. Decreased theophylline clearance may result from hepatic cirrhosis, acute hepatitis, congestive cardiac failure and acute viral illness.

The half life of caffeine in adults ranges from 3 to 7.5 hours with metabolism being faster in females than males[95]. In preterm infants the elimination of caffeine, like theophylline, is slow due to immaturity of the metabolising enzymes. Advanced age has not been shown to have a significant effect on caffeine half life[77].

The methylxanthines are generally thought to obey first-order

kinetics, ie plasma concentration follows a log-linear decay[96], however there is some evidence that theophylline may exhibit dose-dependent kinetics particularly in children[97]. It has been shown that for both caffeine and theophylline the formation of each metabolite is actually capacity limited, however excretion of the administered xanthine appears to follow first order kinetics due to the effect of the xanthine on urine flow[98-99].

Cigarette smoking has been shown to significantly increase the clearance of both caffeine and theophylline[100-101], and is thought to be due to induction of metabolism by components of the smoke. A variety of interactions between the methylxanthines and other drugs have been noted (see Table 6). A diet low in protein but high in carbohydrate gives rise to lower rates of metabolism and vice versa, however this would only be important clinically if a patient changed his diet drastically. The effect of removal of caffeine from the diet leads to faster metabolism of theophylline[102], however increasing the dietary intake was not shown to have the reverse effect[103].

I.7 Toxicity

Non life-threatening adverse effects of theophylline and caffeine include nausea, symptoms of the gastrointestinal tract and central nervous stimulation including irritability, insomnia and tremor. For theophylline a clear relationship has been demonstrated betweeen severity of toxicity and plasma theophylline concentration[104-105]. Minor side effects occur at therapeutic or even sub-therapeutic concentrations.

TABLE 6 Clinically important drug interactions with caffeine and theophylline

Drug	Significance
Interactions with caffeine	
Alcohol	caffeine does not antagonise the deleterious effects of alcohol on the performance of psychomotor skill tests.
Barbiturates	the hypnotic effects of pentobarbital are antagonised by the concurrent use of caffeine.
Monoamine oxidase inhibitors	a single report claims that MAOIs can enhance the CNS stimulant effects of caffeine.
Interactions with theophylline	
Cimetidine	average clearance reduction is 40%. The interaction begins within 24 hours of initiating cimetidine and is gone within 3 days of discontinuing it.
Erythromycin	theophylline clearance decreases by 25% on average after 5 days of concurrent therapy. The effect is related to serum level of erythromycin.
Oral contraceptives	theophylline clearance is reduced by 30% on average.
Allopurinol	on average a 600mg/day dose reduces clearance by 25% but a 300mg/day dose has no effect.
Phenytoin	may increase theophylline clearance by 50-75% after 10 days of theophylline therapy. Theophylline may also inhibit phenytoin absorption.
Phenobarbitone	may increase theophylline clearance by 25% after 3-4 weeks phenobarbitone therapy.
Carbamazepine	theophylline clearance may double.
Rifampicin	may increase theophylline clearance by 50-75%.
Lithium	theophylline may increase lithium clearance and decrease its effectiveness.

Nausea is probably due to both a central effect and a direct effect on the gastric mucosa. Other gastrointestinal symptoms include acid indigestion, heartburn, abdominal pain, diarrhoea, flatulence or constipation. Coffee has been shown to cause a decrease in fasting and postcibal lower oesophageal sphincter pressure in normal volunteers and patients with reflux oesophagitis[106]. Caffeine produced a significant increase in gastric acid output 15 minutes after administration[107]. However subjects with symptoms associated with drinking coffee and low basal lower oesophageal sphincter pressure showed less acid response to coffee than normal subjects, suggesting that gastro-oesophageal reflux rather than acid hypersecretion may be responsible for symptoms[108]. An important consideration is the effect of substances other than caffeine present in coffee since decaffeinated coffee can induce acid secretion[109]. Theophylline at higher serum concentrations may cause gastric ulceration[110]. Gastrointestinal side effects may be minimised by initiating therapy with a low dose and building up slowly.

In a cross-sectional study involving 4558 Australians approximately one quarter of the reported prevalence of palpitations, tremor headache and insomnia were attributed to caffeine consumption[111]. Subjects who reported caffeine-attributed insomnia were found to have higher plasma caffeine concentrations at midnight, 8 hours after afternoon coffee, as a result of slower plasma clearance[112]. Although patients taking theophylline perceived the quality of their sleep to be improved, electroencephalography showed that sleep quality in fact deteriorated, with an increase in wakefulness and a decrease in non-rapid-eye-movement sleep[113].

Serum theophylline concentrations above 40μg/ml are associated with seizures which may occur without previous signs of toxicity and may be refractory to usual anticonvulsant therapy. The mortality rate with theophylline-induced seizures may be as high as 50% or permanent neurological damage may be sustained. Potentially fatal cardiac arrhythmias may occur at theophylline concentrations greater than 35μg/ml. Hypokalaemia is thought to be important in the mechanisms of theophylline toxicity[114]. The acute lethal dose of caffeine in adults appears to be approximately 5-10g either intravenously or orally although untoward reaction may be observed following the ingestion of 1g (15mg/kg) leading to plasma concentrations above 30μg/ml. These reactions include restlessness, excitement, mild delirium, sensory disturbances such as ringing in the ears and flashes of light, muscle tremor, extrasystoles and tachypnoea. Caffeine as a toxin does not entirely follow the generalisation that parenteral administration is more toxic than oral since absorption after oral administration is rapid[115]. One report documents the survival of an adult who ingested 24g of caffeine, leading to a maximal plasma concentration of 200μg/ml[116]. Adverse effects in this patient included haematemesis, tachycardia, hyperventilation, hyperglycaemia and ketonuria. The occurrence of metabolic acidosis and hypokalaemia was also noted. Shortly after ingestion of the caffeine a plasma theophylline concentration of 17.2μg/ml was noted, however caution is advised when measuring theophylline concentrations after caffeine overdose since non-specific methods can lead to overestimation[117]. Caffeine poisoning in children is rare, however there is a report of a twelve-month-old girl who survived after ingesting 1-1.5g of caffeine (105-158mg/kg), which produced a plasma concentration of 46μg/ml[118].

Epidemiological studies on the role of caffeine in cardiovascular disease mainly show no increase in risk and the few that do, usually do not demonstrate a dose response relationship[119]. Although a positive relationship was found between coffee consumption and both total and low density lipoprotein cholesterol the association was not seen with tea or cola consumption suggesting that caffeine alone does not exert a direct effect on lipid concentrations and the relationship may be due to confounding effects of other aspects of the diet[120]. Caffeine has been investigated as a possible teratogen, however animal studies have frequently involved the use of very high doses and are difficult to extrapolate to humans. From the human studies available it seems caffeine consumption has a minimal effect, if any, on the outcome of pregnancy[7]. Although caffeine consumption has been linked with cancer of the urinary bladder, pancreas and ovaries when animal studies and epidemiological studies are evaluated together they fail to provide substantive evidence of a link between caffeine consumption and cancer in man or other species[121].

I.8 Therapeutic Monitoring

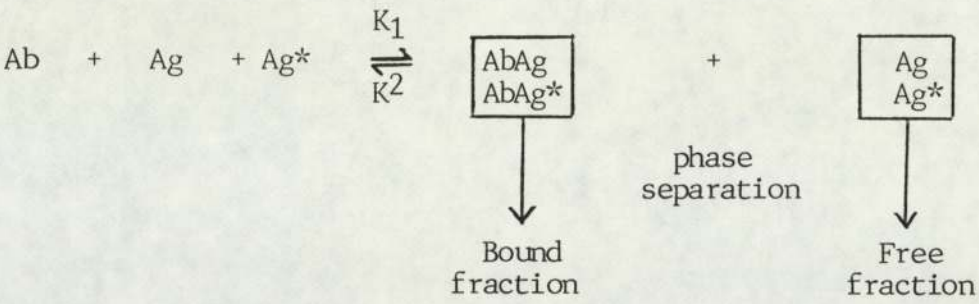
The narrow therapeutic range of theophylline, often quoted as 10-20μg/ml, and the wide variation in clearance make plasma concentration monitoring useful in selected cases both to prevent toxicity and to correct subtherapeutic dosing. When used in the treatment of neonatal apnoea, the concentration of theophylline is maintained within the range 6-12μg/ml[92]. The optimal caffeine plasma range is 8-20μg/ml[122], although caffeine concentrations as low as 3-4μg/ml abolish apnoea, and only transient jitteriness was observed with a plasma

concentration of 55μg/ml[123]. This indicates that the therapeutic index of caffeine is wider than that of theophylline for the treatment of neonatal apnoea. Methods of determining urinary concentration of caffeine have been developed, since, due to its stimulant properties, it has been used by sportsmen to enhance performance. A maximum allowable concentration of 15μg/ml in urine has been proposed[124].

A wide variety of assays have been developed for determining the concentrations of caffeine, theophylline and their metabolites in various biological fluids. UV spectrophotometry has been used, however early methods required 5ml of blood and there may be interference from a large number of other drugs, particularly weak acids such as barbiturates, salicylates and sulphonamides[125]. Theophylline assays may suffer interference from theobromine and caffeine. A large number of gas chromatography assays have been described, however derivatisation is required for most methods[124, 126]. Gas chromatography may be useful in conjunction with mass spectrometry for determining the structures of metabolites[127]. Thin layer chromatography has been used[128] but may be too cumbersome. High performance liquid chromatography (HPLC) has been widely used for methylxanthine assay and many methods are documented. The rate of N-demethylation of caffeine can be determined by the CO_2 breath test[129]. This involves the administration of caffeine labelled with ^{13}C or ^{14}C and the measurement of rate of appearance of exhaled CO_2 by mass spectrometry or liquid scintillation counting.

A variety of immunoassays has been developed for xanthine concentration determination. The general principle is shown in Fig. 5. The

FIGURE 5 The Principle of Immunoassays



where Ag represents the xanthine to be measured; Ag* represents the labelled xanthine, either radiolabelled or enzyme bound; Ab represents the specific antibody capable of binding Ag and Ag*; and K^1 and K^2 represent the association and dissociation constants, respectively.

xanthine in the biological sample and the labelled xanthine compete for a limited number of binding sites on the antibody partitioning themselves according to their relative abundance. The result is then compared to a standard dose response curve to obtain the initial concentration.

Radioimmunoassays have been developed for both caffeine and theophylline[126, 130] and have the advantage that, due to the specificity of the antibody, interference is low and will only occur from structurally similar compounds. The enzyme multiplied immunoassay technique (EMIT^R) is widely used for routine theophylline assay, having the advantage of small sample volume, minimum interference and automation. An EMIT assay has also been developed for measurement of serum caffeine in neonates, however this cannot be used in children or adults as the antiserum reacts with paraxanthine[131]. Another method used routinely for theophylline assay uses a fluorescent marker[132]. A refinement of a fluoroimmunoassay determines the theophylline concentration in the dried filter paper blood spots of patients for whom geographical location has previously prohibited monitoring[133]. An

immunoassay with a reagent strip format, the Ames Seralyser, has recently been developed for theophylline assay, and has the advantages of speed and ease of use[134-135]. Another interesting advance is the Acculevel, a disposable device which uses enzyme immunochromatography to indicate visually the theophylline concentration in a small capillary blood sample[136]. The advantages of ease of use, speed and being portable must be weighed against the higher cost of this method.

II. EXPERIMENTAL

II.1 HPLC

A vast number of HPLC assays for theophylline, caffeine and their metabolites have been described. Straight phase HPLC has been used[137], however reversed phase methods are now favoured due to the lower cost of the mainly aqueous mobile phase and the greater flexibility. Some methods use an ion pairing reagent to improve resolution and overcome interference[138]. Sample preparation frequently involves solvent extraction with a chloroform-isopropanol mixture or protein precipitation with ammonium sulphate or methanol since direct injection of the sample may damage the column. Various internal standards have been used including propoxyphylline[139], 8-chlorotheophylline[140] and β-hydroxyethyltheophylline[141]. Systems which involve gradient elution have been recommended for pharmacokinetic studies[142-143].

The initial extraction procedure employed in this study was based on that used by Hartley et al.[144] while the chromatographic method was modified from that of Muir et al.[145].

II.2 Apparatus and Reagents

A Pye Unicam LC3 chromatograph and variable wavelength UV detector were used in conjunction with a Phillips PM8251 single pen recorder and a computing integrator model 308 (LDC, Milton Roy). A Co:Pell ODS

(Whatman) precolumn (70mm x 2mm ID) was used with an Ultrasphere ODS 5μm (150mm x 4.6mm ID) analytical column.

The methanol and dichloromethane, HPLC grade, and isopropyl alcohol, tetrahydrofuran, sodium acetate and theobromine, analytical grade, were obtained from BDH. Tetrabutylammonium hydrogen sulphate, β-hydroxyethyltheophylline, caffeine, theophylline, uric acid, 3-methylxanthine, 1,7-dimethylxanthine, 1,3-dimethyluric acid and 1-methyluric acid were all obtained from Sigma. The 95% IMS was produced by the non-sterile manufacturing department, Churchill Hospital, Oxford.

The Sep-Pak C_{18} cartridges were supplied by Waters Associates. Separation of the free from protein bound caffeine was achieved using the Micropartition System MPS-1 (Amicon).

The mobile phase consisted of 0.01mol/l sodium acetate and 0.005mol/l tetrabutylammonium hydrogen sulphate, with the pH adjusted to 4.75 with either 0.1mol/l acetic acid or 0.1mol/l sodium hydroxide. 125ml methanol per litre was added and the mobile phase was filtered.

II.3 Method

A 300μl aliquot of the sample material, plasma, saliva or urine, was added to 20μl of IMS containing the internal standard, β-hydroxyethyl-theophylline, in a conical glass tube. The concentration of internal standard was sufficient to produce a concentration in the sample within the middle of the range of concentrations of the methylxanthine

to be measured. For example, if the anticipated range of caffeine concentrations in the samples was 0-20µg/ml then the concentration of internal standard used would be 10µg/ml. 3µg would be needed to produce a concentration of 10µg/ml in 300µl of plasma. Since the 3µg was added in 20µl the strength of the solution of internal standard in IMS was 150µg/ml.

The concentration of internal standard solution was always kept constant for a series of samples and the corresponding calibration curve. 6ml of the organic solvent (dichloromethane 90%, isopropanol 10%) was added to the tube and vortexed for 1 minute. The resultant mixture was centrifuged for 10 minutes and 5ml of the organic layer was transferred to a clean tube, evaporated to dryness at 55°C under a stream of air and reconstituted in 200µl of mobile phase.

A series of calibration standards were prepared in the same matrix as the test sample obtained from a volunteer who had abstained from methylxanthine-containing foods and beverages for 24h. The standards covered the range of concentrations expected in the test sample. For example, if plasma samples were anticipated to contain caffeine 0-20µg/ml the calibration points was 0, 5, 10, 15, 20 and 25µg/ml. Six sample tubes were labelled accordingly and 300µl of blank plasma would be added to each. Each sample was spiked with 20µl of caffeine solution with strengths as shown in Table 7.

TABLE 7: Concentrations of caffeine solutions for calibrators

Calibrator Strength (µg/ml)	0	5	10	15	20	25
Concentration of Caffeine Solution used (µg/ml)	0	75	150	225	300	375

FIGURE 6: Example of a calibration curve for caffeine

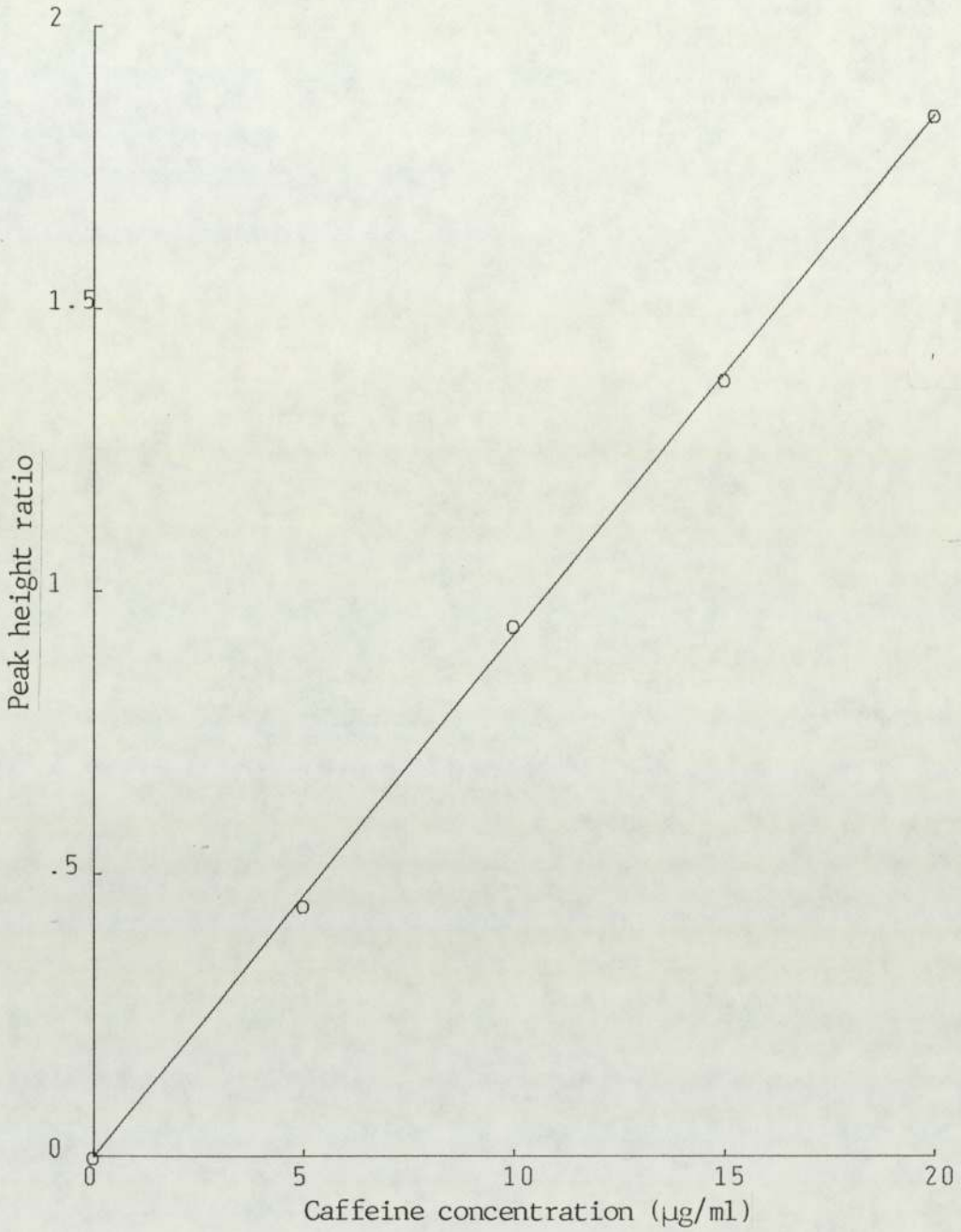
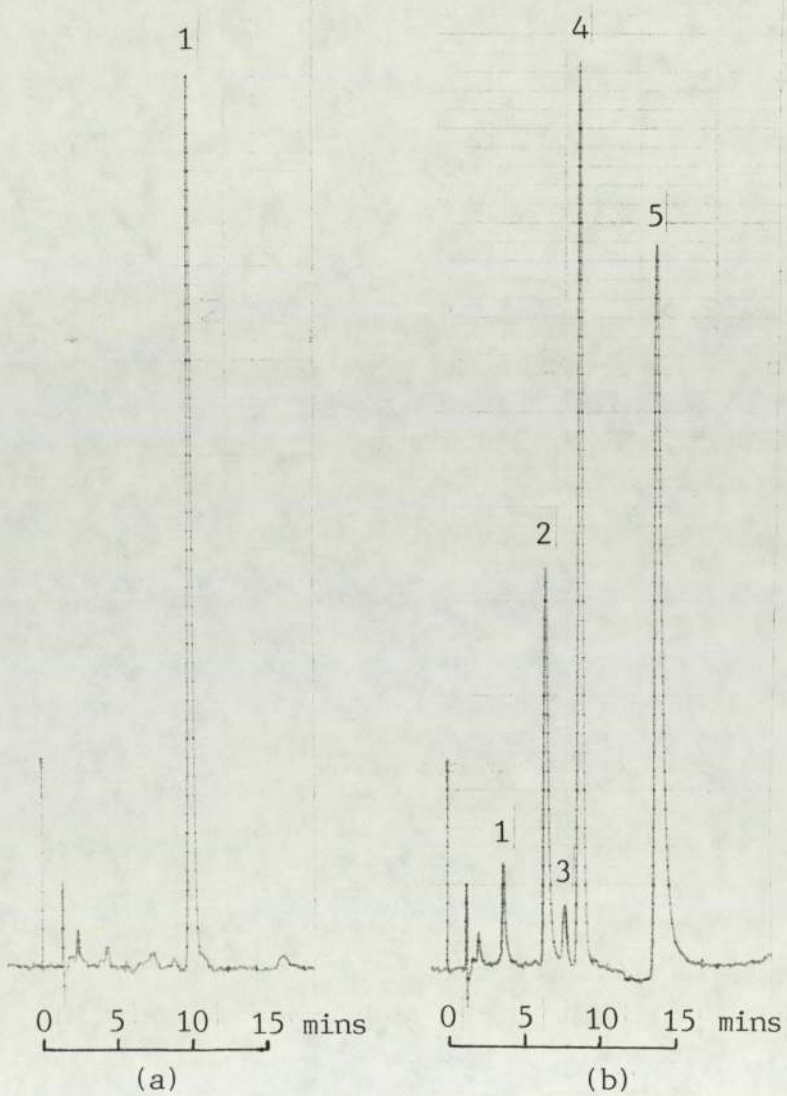


FIGURE 7: (a) Chromatogram of blank plasma (from a subject who had abstained from methylxanthine-containing foods and beverages for 24 hours) spiked with internal standard, β-hydroxyethyltheophylline to 10μg/ml (1); (b) Plasma taken from a volunteer 4 hours after a 600mg dose of caffeine and spiked with internal standard to 10μg/ml (4). Peaks are (1) theobromine, (2) paraxanthine, (3) theophylline and (5) caffeine.



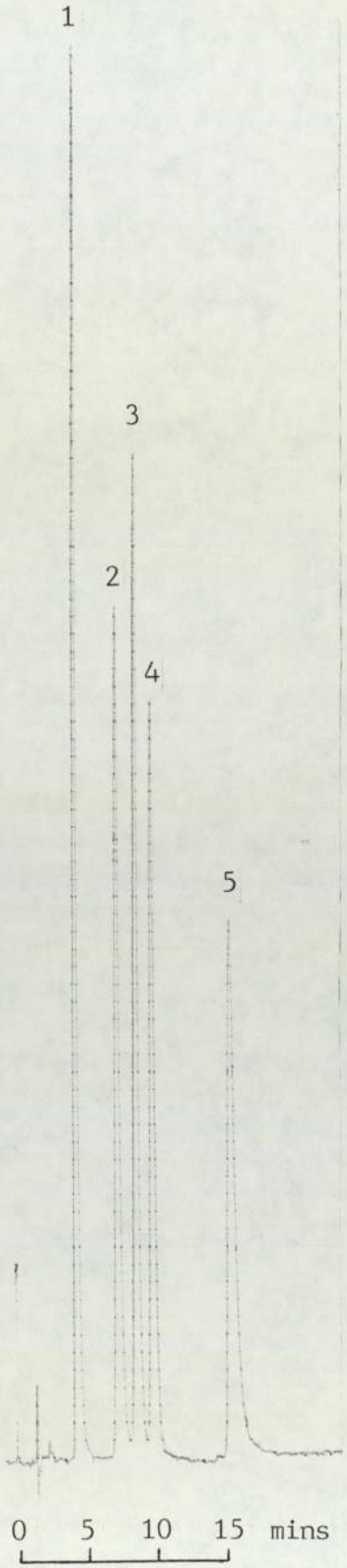
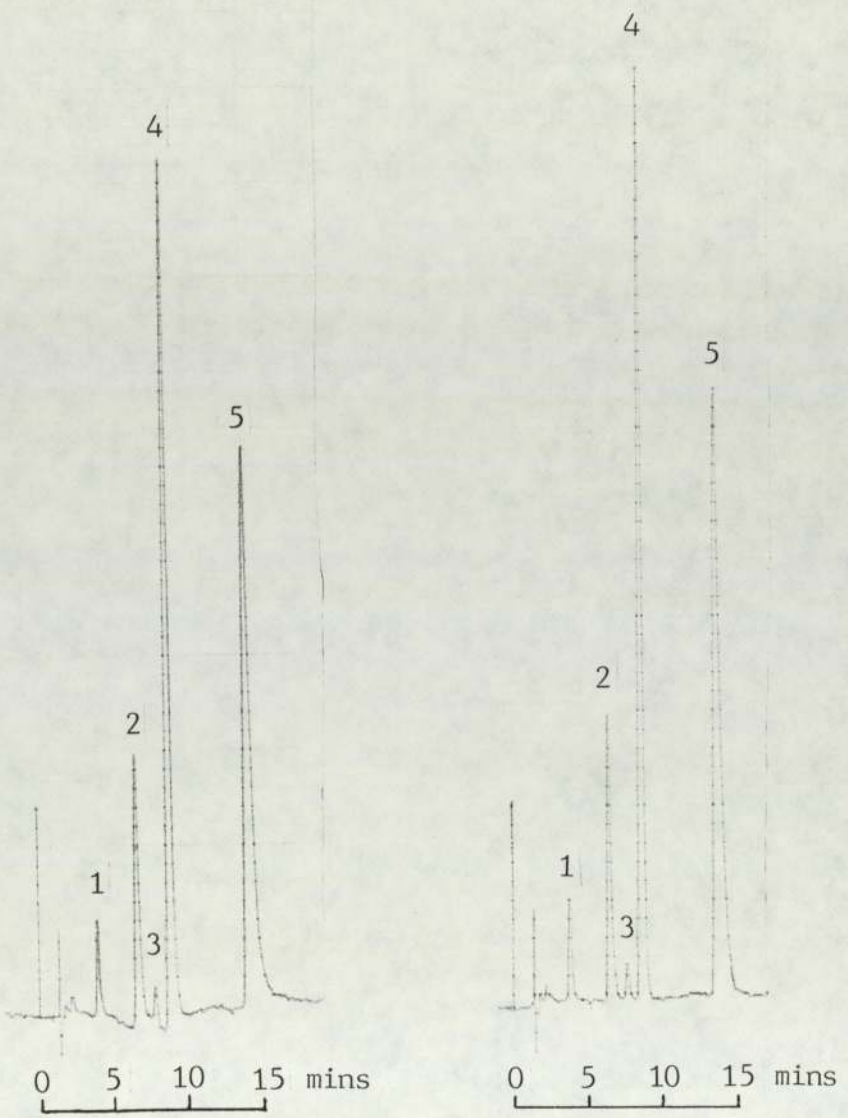


FIGURE 8: Chromatogram of blank plasma spiked to 10µg/ml with each of the following: (1) theobromine, (2) paraxanthine, (3) theophylline, (4) internal standard, (5) caffeine

FIGURE 9: Chromatograms of two separate extractions of saliva from a volunteer 4 hours after a 600mg dose of caffeine and spiked with internal standard to 10µg/ml (4). Peaks are (1) theobromine, (2) paraxanthine, (3) theophylline, (5) caffeine



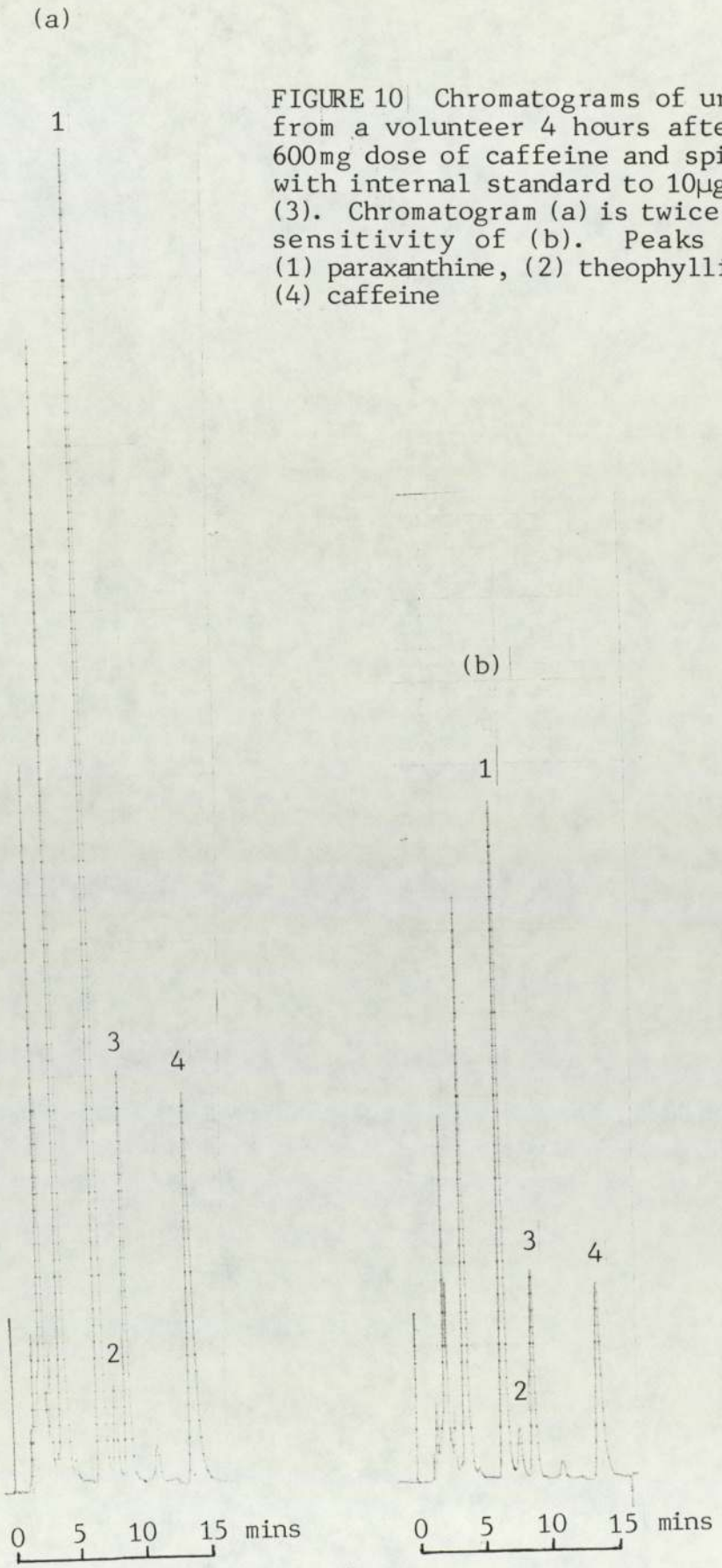


FIGURE 10 Chromatograms of urine from a volunteer 4 hours after a 600mg dose of caffeine and spiked with internal standard to 10μg/ml (3). Chromatogram (a) is twice the sensitivity of (b). Peaks are (1) paraxanthine, (2) theophylline, (4) caffeine

Each standard was subjected to the extraction procedure as above. 20µl of the solution in mobile phase was injected. A flow rate of 1.25ml/min (setting 1.4) was used at room temperature, generating a back pressure of 130bar. The detection wavelength was 274nm and the chart speed was 120mm/h.

Graphs comparing the peak height ratio of the calibration standards with actual concentration of methylxanthine present were constructed and used to determine the concentration in test samples. Each test sample was extracted in duplicate and repeated if results obtained were not within 10% of each other. For example, Fig. 6 shows a typical calibration curve for caffeine (correlation coefficient 0.9998, slope 0.0926 and intercept -0.0032).

Spiked samples were used for internal quality control; if the value obtained from the chromatogram differed by more than 10% from the value to which the sample had been spiked the batch was repeated until this figure was reached. The Heathcontrol scheme run by the University of Wales College of Medicine provided an external performance check for theophylline. Samples of unknown strength were received from the Heathcontrol centre, assayed and reported values compared with those from other laboratories.

II.4 Method Improvements

Since measurements of a volume as small as 20µl could be a source of error, the volume of internal standard solution was increased to 100-300µl and the concentration reduced accordingly. An aqueous solution

was used. Similarly, the volume of calibration standard was increased from 20µl to 100-300µl. Since this significant volume was added only to the calibration standards an equal volume of distilled water was added to the samples to avoid concentration effects on the calibration standards.

When reconstituting in mobile phase the tubes were left in a water bath at 55°C for 2 minutes to avoid potential problems due to the poor solubility of the xanthines. The tubes were then vortexed for 30 seconds.

II.5 Coefficient of Variation

Coefficients of variation were obtained by spiking 6ml of plasma, saliva or urine with the methylxanthine and taking six 300µl samples which were extracted and chromatographed as above. The remainder of the 6ml was stored at -20°C for one week and reassayed. The results are shown in Table 8.

Throughout these determinations peak area ratio was measured using the integrator, however the results achieved by this method were so similar to those obtained by measuring peak heights that the use of peak area ratios was discontinued.

II.6 Recovery

Six samples of plasma, saliva and urine were spiked to 10µg/ml, internal standard was added and they were extracted and

TABLE 8: Within-day and between-day coefficient of variation (CV) for caffeine and theophylline

Methylxanthine	Matrix	Concentration (μg/ml)	CV (%)	
			Within day	Between day
Theophylline	plasma	5	1.8	3.4
			3.5	
		20	1.8	5.1
			4.2	
Caffeine	plasma	5	4.5	4.3
			3.0	
		20	2.6	4.1
			4.9	
Caffeine	urine	5	3.3	3.4
			3.5	
		20	1.8	2.5
			3.0	
Caffeine	saliva	5	4.5	3.9
			3.2	
		20	2.7	3.6
			4.3	
Theophylline	plasma	0.15	3.4	
Theobromine	plasma	0.30	5.6	

chromatographed. A solution of the methylxanthine and internal standard in mobile phase containing maximum amounts recoverable was chromatographed six times. The results are shown in Table 9.

TABLE 9: Recovery of caffeine, theophylline and internal standard

Methylxanthine	Matrix	Recovery (%)	
		methylxanthine	internal standard
Theophylline	plasma	96.1	91.1
Caffeine	plasma	104.0	94.3
Caffeine	urine	98.4	92.1
Caffeine	saliva	85.0	86.1

II.7 Separation of Metabolites

Solutions of each of the methylxanthines and uric acid derivatives listed in Table 10, concentration 10µg/ml, were chromatographed with conditions as above. Since this did not separate theobromine and 1-methyluric acid the concentration of methanol was reduced to 5%. This prolonged elution time but did not improve separation. Adjusting the pH to 5.5 improved early separation but later metabolites coeluted. Doubling the concentration of ion-pairing reagent had no appreciable effect. An alternative mobile phase[146] containing 4% methanol and 1% tetrahydrofuran in water adjusted to pH 4 with phosphoric acid was used. This separated theobromine and 1-methyluric acid at the expense of the separation of 3-methylxanthine and 1-methyluric acid.

TABLE 10: Retention time for methylxanthines and uric acid derivatives

Methylxanthine or uric acid derivative	Retention time for different mobile phases (min)			
	12.5% MeOH pH 4.75 1.25ml/min	5% MeOH pH 4.75 1.5ml/min	5% MeOH pH 5.5 1.5ml/min	Ref 146 pH 4.0 1.25ml/min
Uric acid	2.4	2.1	3.3	1.5
3-methylxanthine	3.3	3.1	4.0	2.8
1-methyluric acid	4.4	4.6	7.3	2.8
Theobromine	4.3	4.6	6.1	3.7
1,3-dimethyluric acid	5.5	6.5	11.6	4.0
1,7-dimethylxanthine	7.4	8.6	11.6	6.2
Theophylline	8.7	10.4	13.8	
Internal standard	9.7	13.4	17.6	
Caffeine	14.9	20.6		

II.8 Storage of Samples

The stability of theophylline was investigated by Jonkman et al.[147] From this work it was concluded that storage of the sample as whole blood at either 6°C or 25°C for one day or as serum at 6°C for one week or at -20°C for one month did not significantly influence the accuracy of the final theophylline serum assay, however storage for three months at -20°C led to a small decrease in concentration. Caffeine in plasma samples stored at -20°C was stable for 4.5 months[77]. In the present study, all samples were assayed within one month of collection.

II.9 Sep-Pak Cartridges

An alternative sample preparation to solvent extraction is the use of small disposable cartridges such as Sep-Pak (see Fig. 11). A number

of methods using the C_{18} reversed phase cartridge have been described[148-150], but other packing materials may be used such as silica or florisil, depending on the chemical characteristics of the substance to be assayed. A method for rapid extraction of caffeine and the dimethylxanthines using Bond-Elute C_{18} columns has been described[151].

In this study Sep-Pak C_{18} cartridges were used. The cartridge was activated with 10ml methanol and 10ml distilled water, then since no solvent sequence was documented using Sep-Pak cartridges for caffeine the following sequence was investigated:-

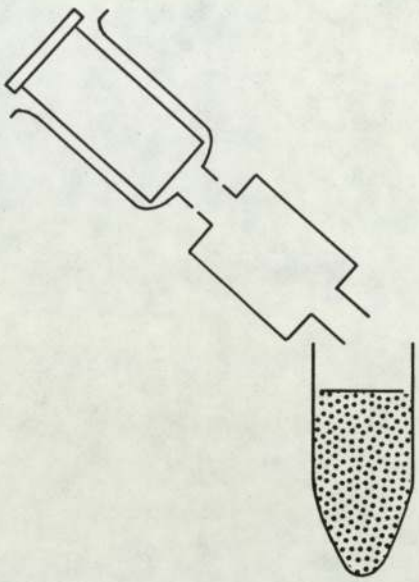
1. 300µl plasma spiked with caffeine, theophylline and internal standard to 10µg/ml
2. 1ml aqueous mobile phase used to rinse the sample tube
3. 2ml aqueous mobile phase
4. 2ml aqueous mobile phase with 25% methanol
5. 2ml aqueous mobile phase with 50% methanol
6. 2ml aqueous mobile phase with 75% methanol
7. 2ml methanol

The cartridge was then regenerated using the following sequence:-

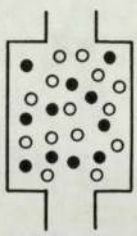
8. 2ml tetrahydrofuran
9. 2ml methanol
10. 10ml distilled water

Fractions 4 and 5 contained the caffeine, theophylline and internal

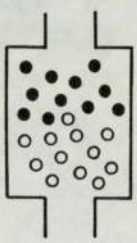
FIGURE 11: Sep-Pak C_{18} cartridges



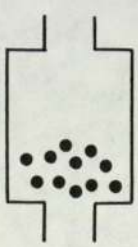
The sample is pumped through the cartridge from a luer-lock syringe. The eluate is collected in a sample tube



The plasma sample spiked with internal standard is loaded onto a prepared Sep-Pak C_{18} cartridge.



The cartridge is eluted with a weak solvent water to remove unwanted material.



The cartridge is eluted with a strong solvent, methanol, and the eluate is collected in a sample tube.

standard. The sequence was simplified to the following:-

1. 300µl spiked plasma
2. 1ml aqueous mobile phase used to rinse the sample tube
3. 2ml aqueous mobile phase
4. 2ml methanol

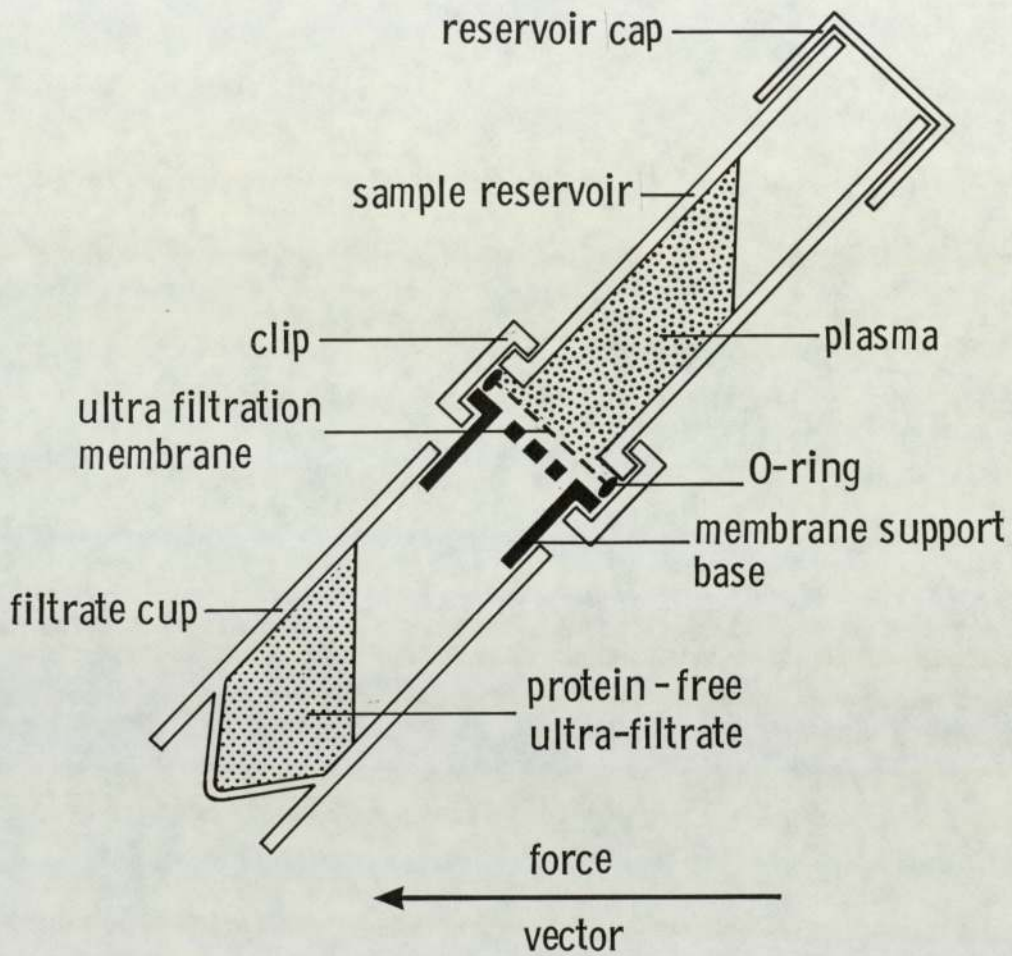
The cartridge was regenerated as before. Fraction 4 contained the methylxanthines and could be injected straight onto the column, however being a very dilute solution a high sensitivity had to be used. This was overcome by evaporating to dryness and reconstituting in mobile phase.

Coefficients of variation for six samples purified by this method were 5.2% and 1.9% for caffeine and theophylline respectively, ie, comparable with the organic extraction method. Although there is some time saving this is reduced when the cartridges are regenerated to avoid high cost.

II.10 Determination of Unbound Fraction

600µl of plasma was introduced into the sample reservoir of the Amicon micropartition system (see Fig. 12) and centrifuged with a fixed arm rotor for 40 minutes. Two 150µl aliquots of the microsolute were extracted by the organic solvent method and calibration standards were made using 150µl distilled water in place of the microsolute. The extracts were chromatographed in the usual way.

FIGURE 12: Micropartition system MPS-1 for separation of free from protein-bound microsolute



II.11 Disintegration Testing

Six caffeine capsules 400mg and six theophylline capsules 400mg were each tested for disintegration time using the method given in the British Pharmacopoea[152]. In all capsules, disintegration was well advanced by 2 minutes and complete by 6 minutes. This indicates that the capsules used were satisfactory for the purpose of this study, since the time limit stated in the British Pharmacopoea is 30 minutes.

II.12 Ames Seralyser

In the clinical study the Ames Seralyser was used according to the manufacturers instructions for the rapid determination of plasma theophylline concentrations. The results were confirmed using HPLC.

III. A PHARMACOKINETIC STUDY OF CAFFEINE AND ITS METABOLITES

III.1 Aims

This study was designed to provide pharmacokinetic data as a basis for the clinical study described in the following section.

III.2 Objectives

The objectives were:

1. To determine the concentration-time profiles for caffeine and its metabolites in healthy adult volunteers using increasing doses of caffeine.

2. To examine the relationship between saliva concentration and total and unbound plasma concentrations of caffeine.

3. To determine the urinary excretion of unchanged caffeine and paraxanthine (one of the major metabolites).

III.3 Method

The protocol for this study was submitted to and approved by the Central Oxford Research Ethics Committee. Four healthy adult volunteers, two males and two females, took part in this study. Each

received a summary of the protocol (Appendix 1) and a verbal explanation. The format of the protocol was used rather than a simplified letter of explanation since all volunteers had a scientific background. All volunteers were non-smokers for at least the past 6 months. No medication was taken by any subject throughout the study period, with the exception of oral contraceptives. Details of the volunteers are given in Table 11.

TABLE 11: Parameters for each volunteer influencing pharmacokinetics

Parameter	Subject 1	2	3	4	Mean	Standard deviation
Age	24	43	25	25	29.2	9.2
Sex	F	M	F	M		
Height (m)	1.65	1.77	1.62	1.75	1.70	0.07
Weight (kg)	61	67	60	61	62.2	3.2

Each subject took doses of caffeine of 200mg, 400mg, and 600mg on separate mornings, with at least two week intervals between doses. These subjects abstained from food and drink containing methyl-xanthines for 24 hours before and after the dose of caffeine was taken. Caffeine was taken in the form of filled hard gelatine capsules prepared by the investigator. The dose of caffeine was taken one hour after a light breakfast. No further food was taken for 3 hours but fluid intake was not restricted. Blood, saliva and urine samples were taken immediately before the dose of caffeine was administered and at intervals over the following 24 hours. 5ml blood samples were taken via an indwelling heparinised cannula at the following times after dosage: 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and

12 hours and by venepuncture at 24 hours. The blood was collected into heparinised vacutainers and centrifuged to obtain plasma within one hour of collection. Mixed saliva samples (ie not from one particular gland) were collected at the same time as blood samples. Urine samples were collected at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 hours after dosage. All samples were stored at -20°C while awaiting analysis. It was not considered necessary to keep the order in which the doses were taken constant since there was at least a 2-week time interval between doses, avoiding carryover effects, however all subjects took the 600mg dose last, having ensured that the two lower doses were well tolerated.

III.4 Results

Towards the end of the 24-hour pre-dose period during which subjects had abstained from dietary xanthines, all four reported symptoms including headache, tiredness and lethargy. These symptoms disappeared within one hour of taking the dose of caffeine. One to four hours after the 600mg dose subjects 1 and 3 reported feelings of light-headedness and tremor, particularly in the hands.

The metabolites of caffeine found were paraxanthine, theobromine and theophylline in order of abundance. Caffeine and paraxanthine were measured in plasma, saliva and urine after each dose. Theobromine and theophylline concentrations in plasma were measured after the 400mg dose only. The plasma concentration versus time data for caffeine and the three metabolites, after the 400mg dose, are represented in Figs. 13-16 for the period up to 12 hours. The 24 hour data is incomplete

FIGURE 13 Time profile of caffeine and its metabolites in Subject 1 after a 400mg dose of caffeine.

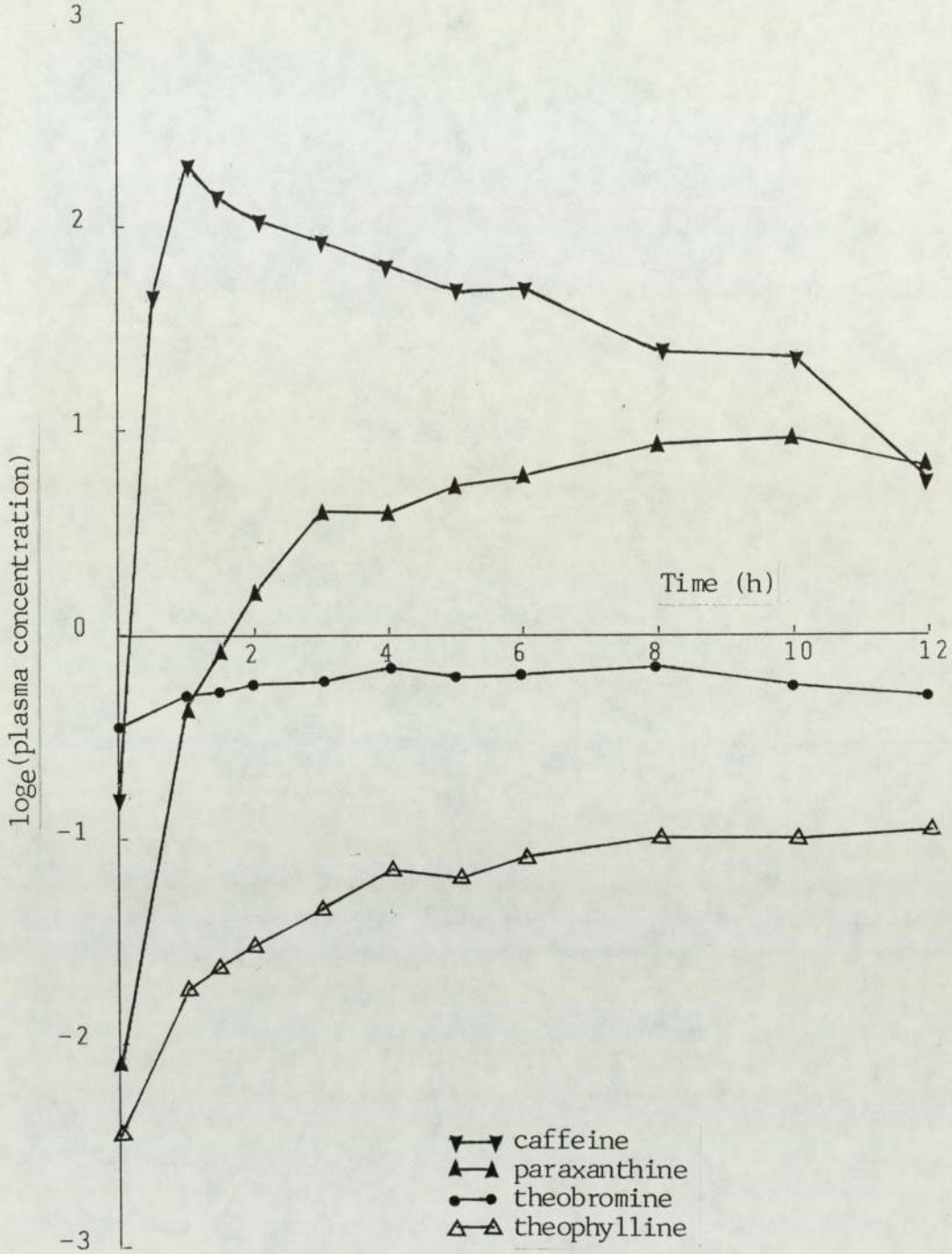


FIGURE 14 Time profile of caffeine and its metabolites in Subject 2 after a 400mg dose of caffeine.

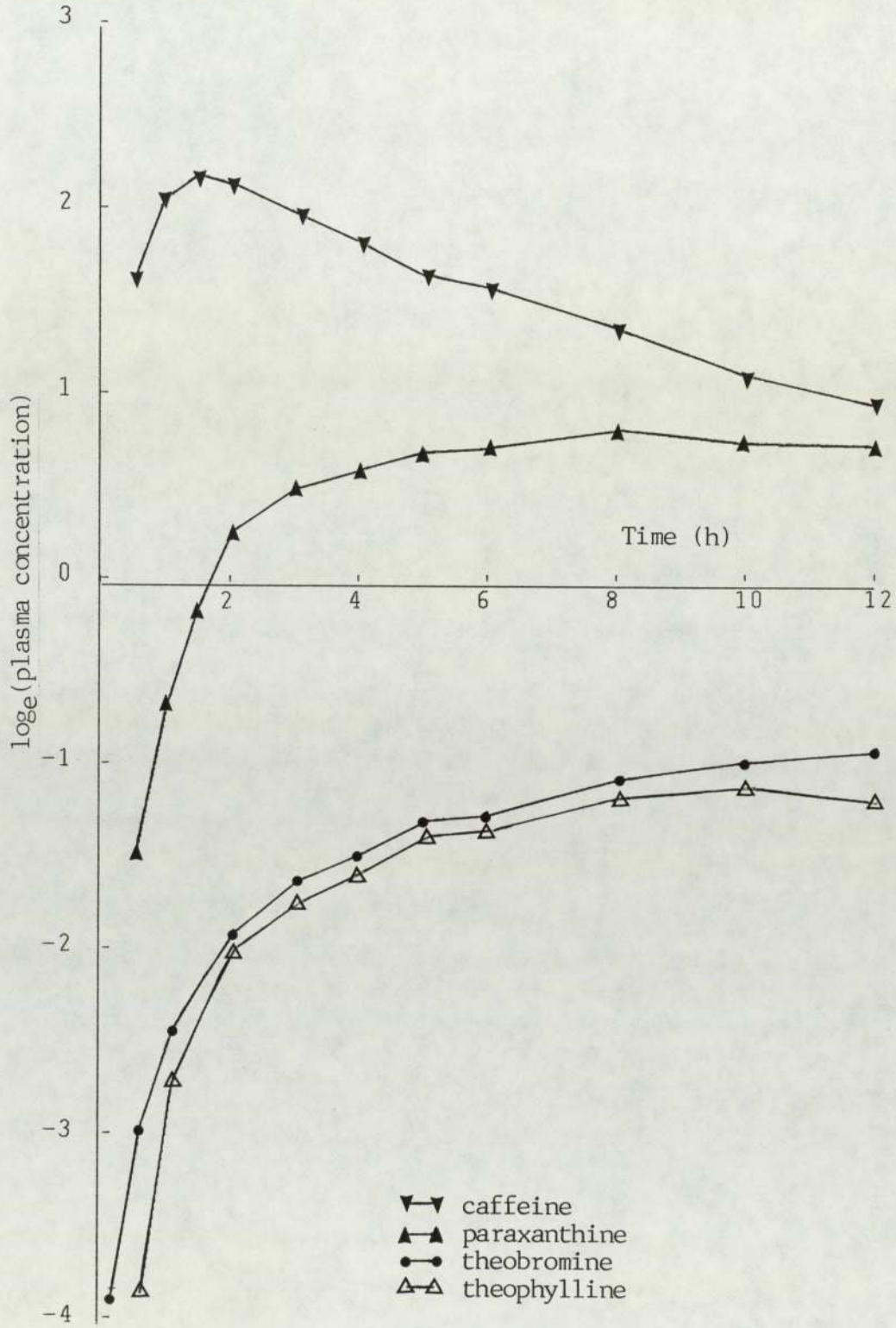


FIGURE 15 Time profile of caffeine and its metabolites in Subject 3 after a 400mg dose of caffeine.

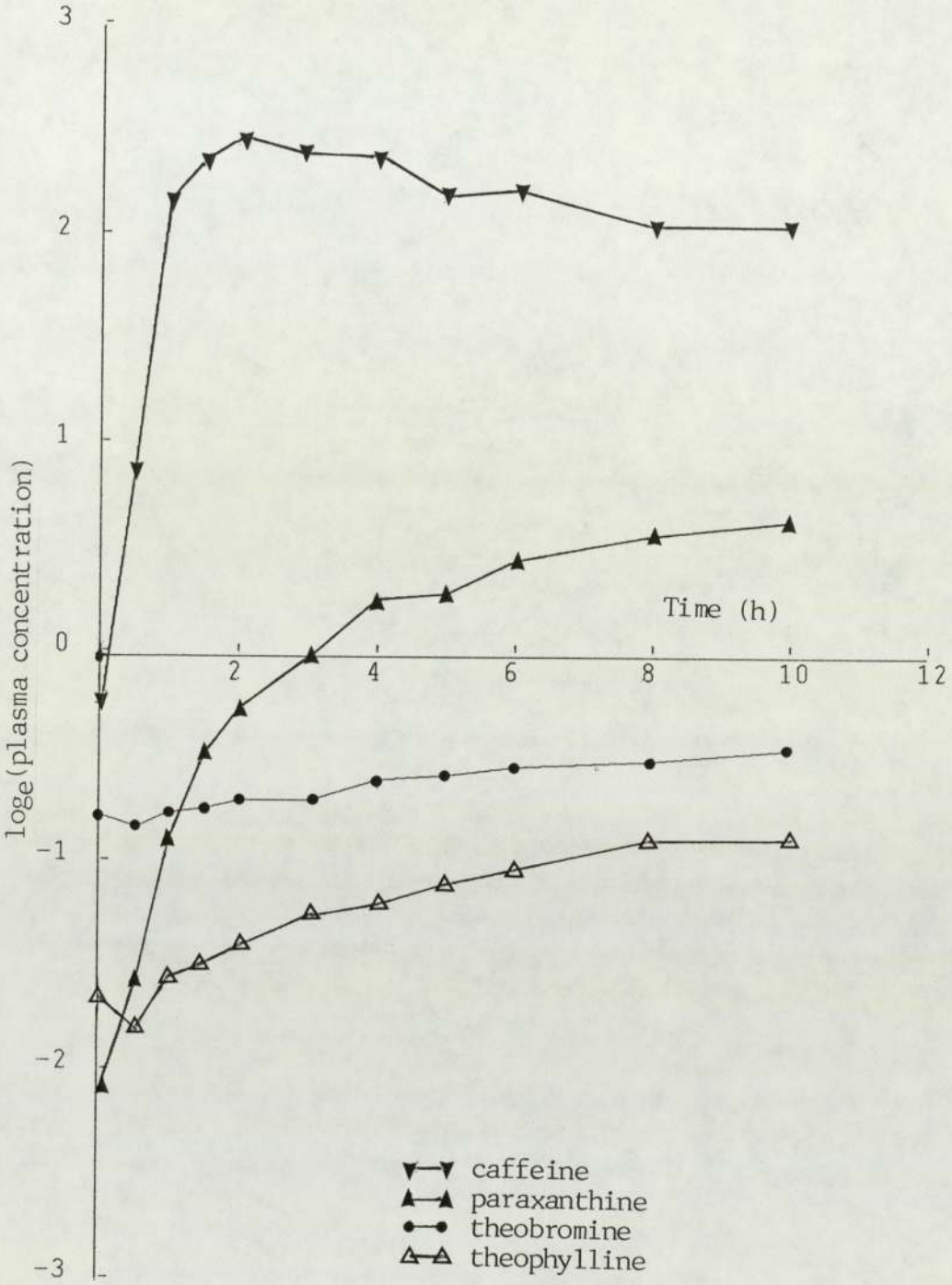
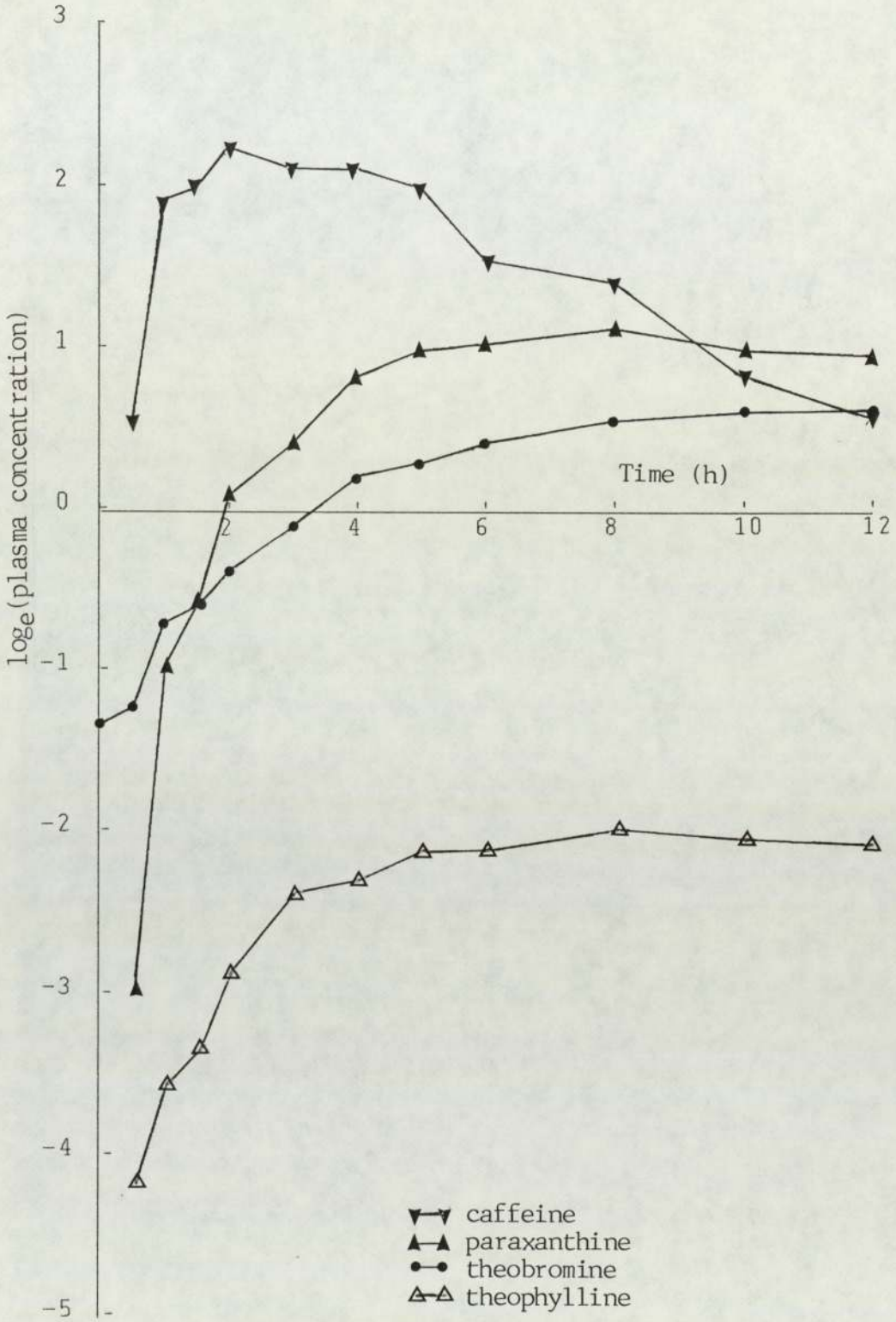


FIGURE 16 Time profile of caffeine and its metabolites in Subject 4 after a 400mg dose of caffeine.



and is not plotted.

Caffeine shows a linear relationship between the logarithm of concentration and time while the paths of the metabolites tend to be curved. From the linear relationship for caffeine the apparent volume of distribution and half-life can be calculated as follows:

$$\text{Plasma concentration} = \frac{\text{(amount in body)}}{\text{(volume of distribution)} \times \text{(bioavailability)}}$$

$$\text{or } V = Ab/(Cp \times F)$$

Example - For subject 1 after a 400mg dose of caffeine Ab is 400mg and Cp may be calculated from the intercept of the regression line on the graph of log(caffeine concentration) versus time.

$$\text{intercept of y axis} = 2.32$$

$$e^{2.32} = 10.2$$

$$V = 400/(10.2 \times 1) = 39.2 \text{ litres}$$

$$\text{half-life} = 0.693/k_e$$

where k_e is the elimination rate constant and is the gradient of the regression line for log(caffeine concentration) versus time in the elimination phase.

Example - For subject 1 after a 400mg dose of caffeine:

$$\text{gradient of slope is } -0.119$$

$$\text{half-life} = 0.693/0.119 = 5.8 \text{ hours}$$

Table 12 shows the calculation of apparent volume of distribution and half-life for each subject. The data of volume of distribution and half-life are summarised in Tables 13 and 14 where means are also given.

TABLE 12: Calculation of apparent volume of distribution and half-life.

Subject	Dose of caffeine (mg)	Correlation coefficient	Slope	Intercept	$e^{intercept}$	VD (l)	$t_{1/2}$ (h)
1	200	-0.985	-0.122	1.75	5.75	34.8	5.7
	400	-0.976	-0.119	2.32	10.2	39.2	5.8
	600	-0.972	-0.067	2.97	19.5	30.8	10.3
2	200	-0.990	-0.158	1.72	5.58	35.8	4.5
	400	-0.995	-0.125	2.32	10.2	39.2	5.5
	600	-0.985	-0.114	2.85	17.3	34.7	6.1
3	200	-0.979	-0.062	1.89	6.62	30.2	11.2
	400	-0.952	-0.058	2.52	12.7	31.5	11.9
	600	-0.978	-0.060	3.15	23.3	25.7	11.5
4	200	-0.980	-0.169	1.91	6.75	29.6	4.1
	400	-0.983	-0.182	2.70	14.9	26.8	3.8
	600	-0.993	-0.148	2.89	18.0	33.3	4.7

Calculations are based on the portion of the graph from the time 1h to 12h.

TABLE 13: Apparent volume of distribution (l)

Dose (mg)	Subject			
	1	2	3	4
200	34.8	35.8	30.2	29.6
400	39.2	39.2	31.5	26.8
600	30.8	34.7	25.7	33.3
Mean	34.9	36.6	29.1	29.9

TABLE 14: Half-life (h)

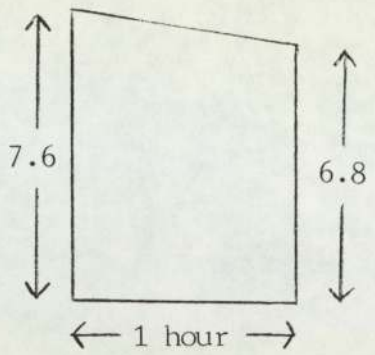
Dose (mg)	Subject			
	1	2	3	4
200	5.7	4.4	11.2	4.1
400	5.8	5.5	11.9	3.8
600	10.3	6.1	11.5	4.7
Mean	7.3	5.3	11.5	4.2

The area under the curve may be calculated using the trapezoidal rule. The calculation for all four subjects is shown in Table 15.

TABLE 15: Areas under the curve for caffeine ($mg.h.l^{-1}$)

Time interval (h)	Subject											
	1			2			3			4		
	Dose (mg)											
	200	400	600	200	400	600	200	400	600	200	400	600
0-0.5	1.6	1.4	0.1	0.2	1.2	1.3	0.3	0.8	2.0	0.1	0.4	0.7
0.5-1	3.0	3.8	2.5	1.2	3.1	4.3	1.3	2.8	6.3	0.8	2.1	3.9
1-1.5	2.6	4.6	6.7	2.0	4.1	6.6	2.3	4.7	9.5	1.7	3.5	6.4
1.5-2	2.2	4.0	8.4	2.0	4.2	7.0	2.8	5.5	10.4	2.1	4.1	6.6
2-3	4.0	7.2	16.3	3.7	7.7	12.7	6.0	11.2	20.4	4.1	8.6	12.4
3-4	3.6	6.4	15.8	3.2	6.6	11.2	5.3	10.6	19.2	3.8	8.0	10.3
4-5	3.4	5.7	15.5	2.9	5.5	10.5	4.8	9.7	18.0	3.4	7.6	9.2
5-6	3.1	5.3	14.7	2.5	4.8	9.8	4.6	8.9	16.7	3.0	5.8	8.2
6-8	4.8	9.3	24.6	4.0	8.4	17.0	8.5	16.6	29.3	4.7	8.4	13.5
8-10	3.7	7.7	20.1	2.9	6.6	12.1	7.5	15.1	26.2	2.7	6.1	10.4
10-12	3.1	5.9	18.4	2.0	5.3	9.4	6.9	13.8	25.0	2.0	3.8	7.3
12-24	9.7	16.1	77.7	5.5	17.5	34.6	28.3	56.8	105	0.7	11.1	19.8
24-36	2.2	4.4	33.9	0.9	3.7	8.5	13.4	28.4	49.8	0.8	1.3	3.7
36-48	0.5	1.0	15.2	0.1	0.8	2.2	6.4	14.1	24.2	0.1	0.1	0.6
Total	47	83	270	33	79	147	98	199	362	30	71	113

Example. For Subject 1 after a 400mg dose of caffeine for the time interval 2-3h.



Area of segment = $[(7.6 + 6.8) \cdot 1]/2 = 7.2 mg.h.l^{-1}$

The theoretical concentrations at 36h and 48h were obtained by extrapolation assuming that the relationship log-concentration against time remained linear.

The concentration-time profile is represented graphically for each subject (Figs. 17-20) and as the mean of all four (Fig. 21). For first order kinetics, the area under the curve should be proportional to dose (Fig. 22).

Plasma clearance may be calculated as follows:

$$\text{clearance} = \text{dose/AUC}$$

Example. For Subject 1 after a 400mg dose of caffeine

$$\text{clearance} = 400/82.9 = 4.821.h^{-1}$$

Tables 16 and 17 give values for clearance in units of $l.h^{-1}$ and $ml.min^{-1}.kg^{-1}$ respectively.

FIGURE 17 Time profile of caffeine in Subject 1 after 200mg, 400mg and 600mg doses of caffeine.

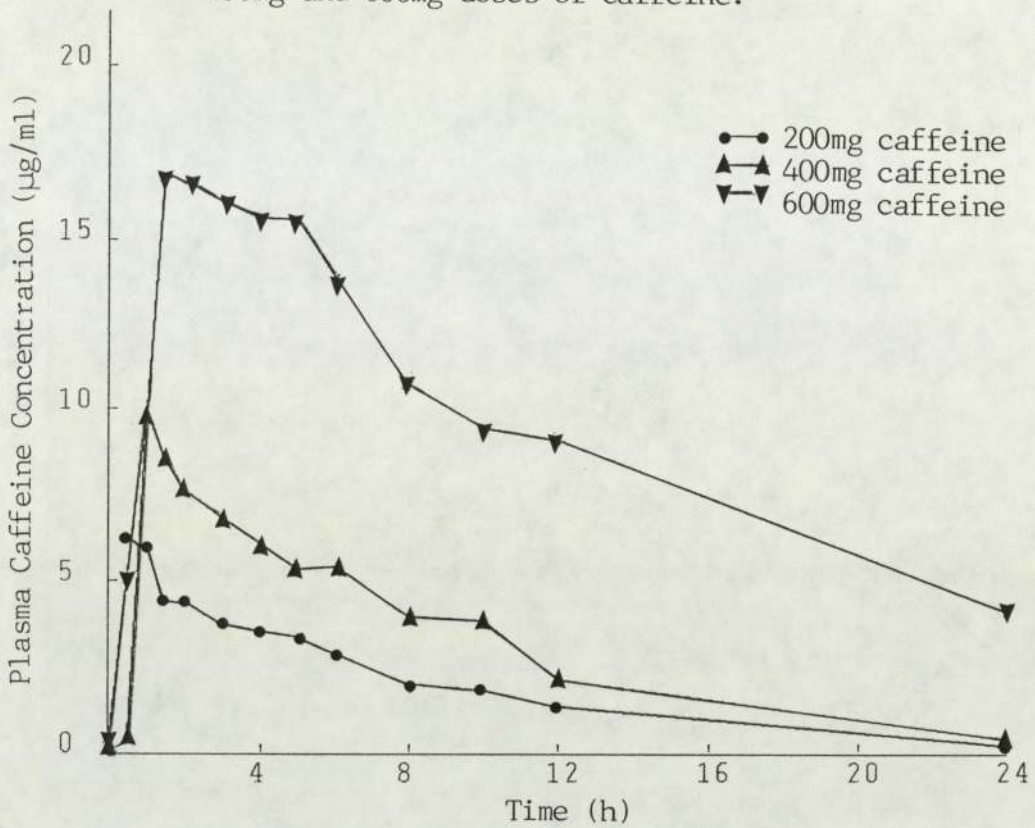


FIGURE 18 Time profile of caffeine in Subject 2 after 200mg, 400mg and 600mg doses of caffeine.

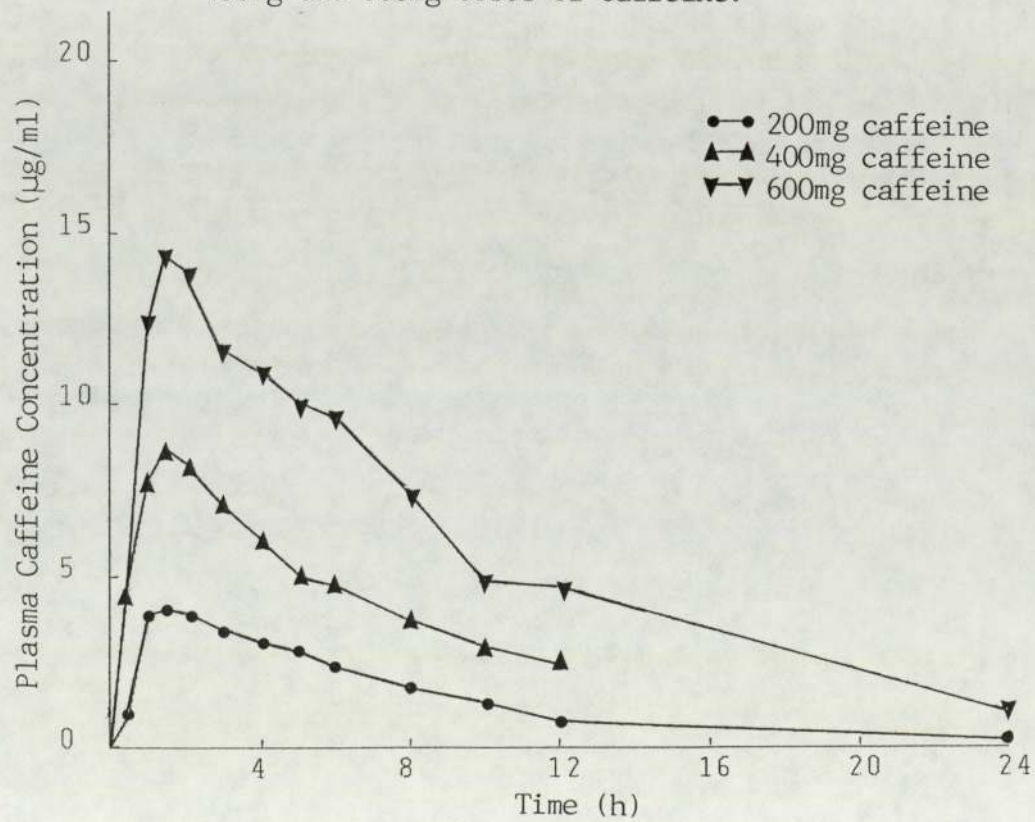


FIGURE 19 Time profile of caffeine in Subject 3 after 200mg, 400mg and 600mg doses of caffeine.

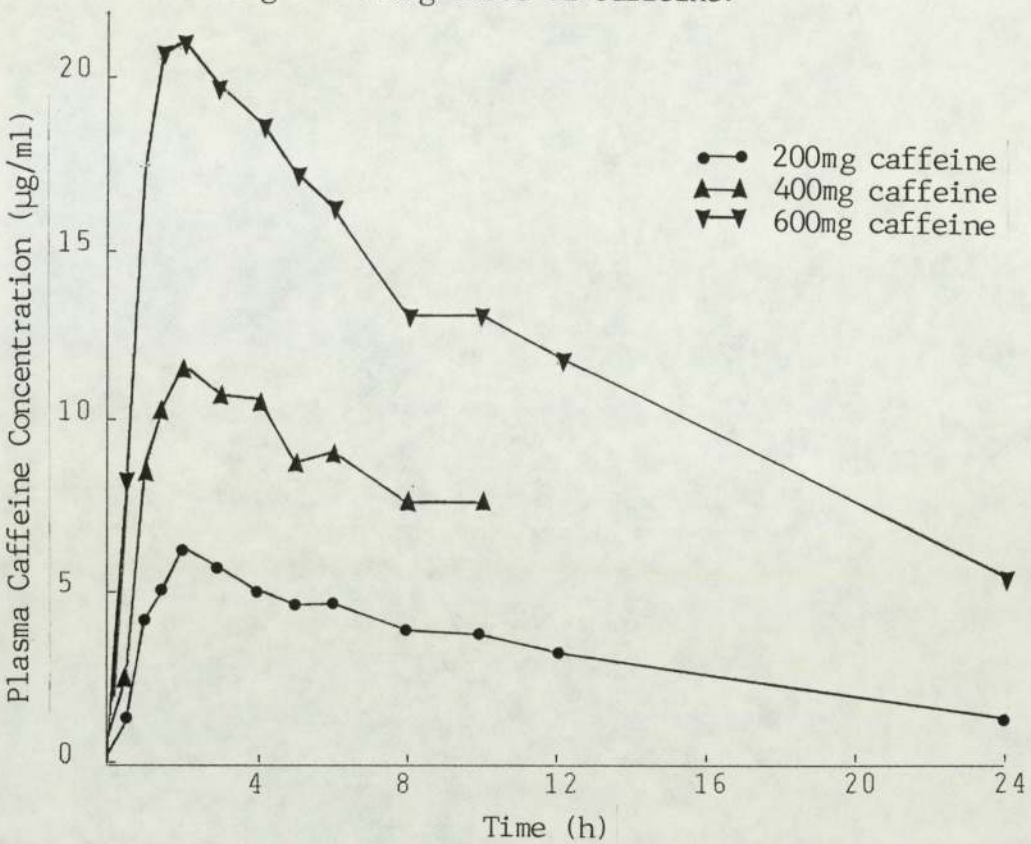


FIGURE 20 Time profile of caffeine in Subject 4 after 200mg, 400mg and 600mg doses of caffeine.

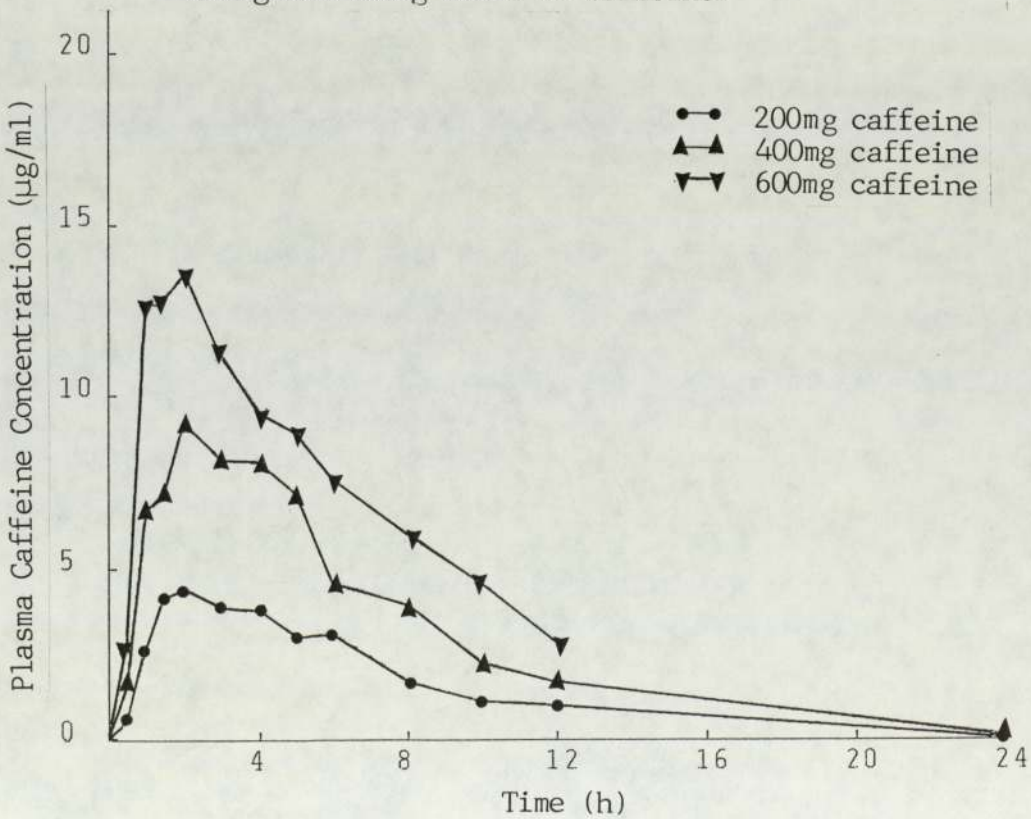


FIGURE 21 Mean time profile of caffeine after 200mg, 400mg and 600mg doses of caffeine.

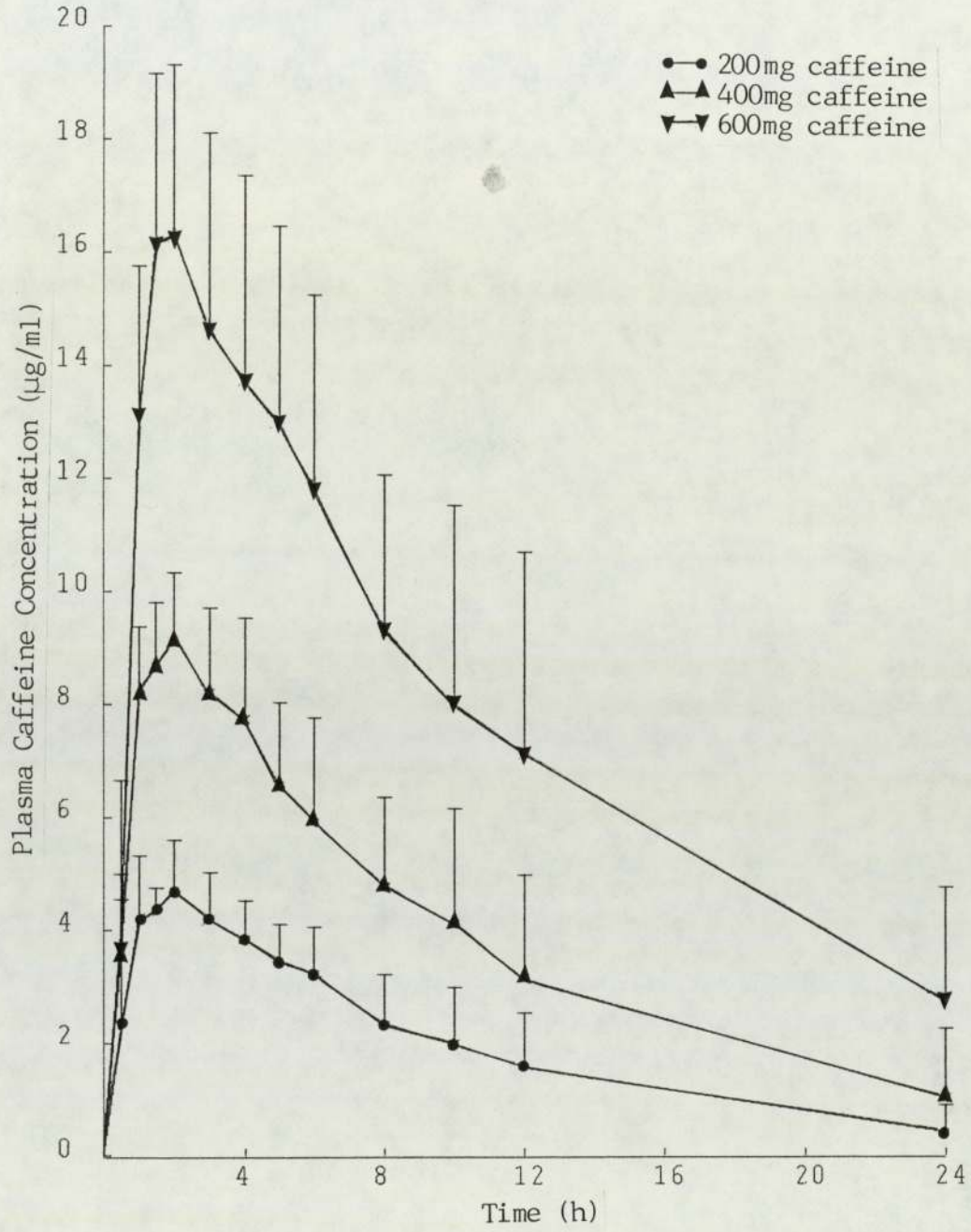


FIGURE 22 Relationship between area under plasma caffeine concentration time curve and dose for each subject

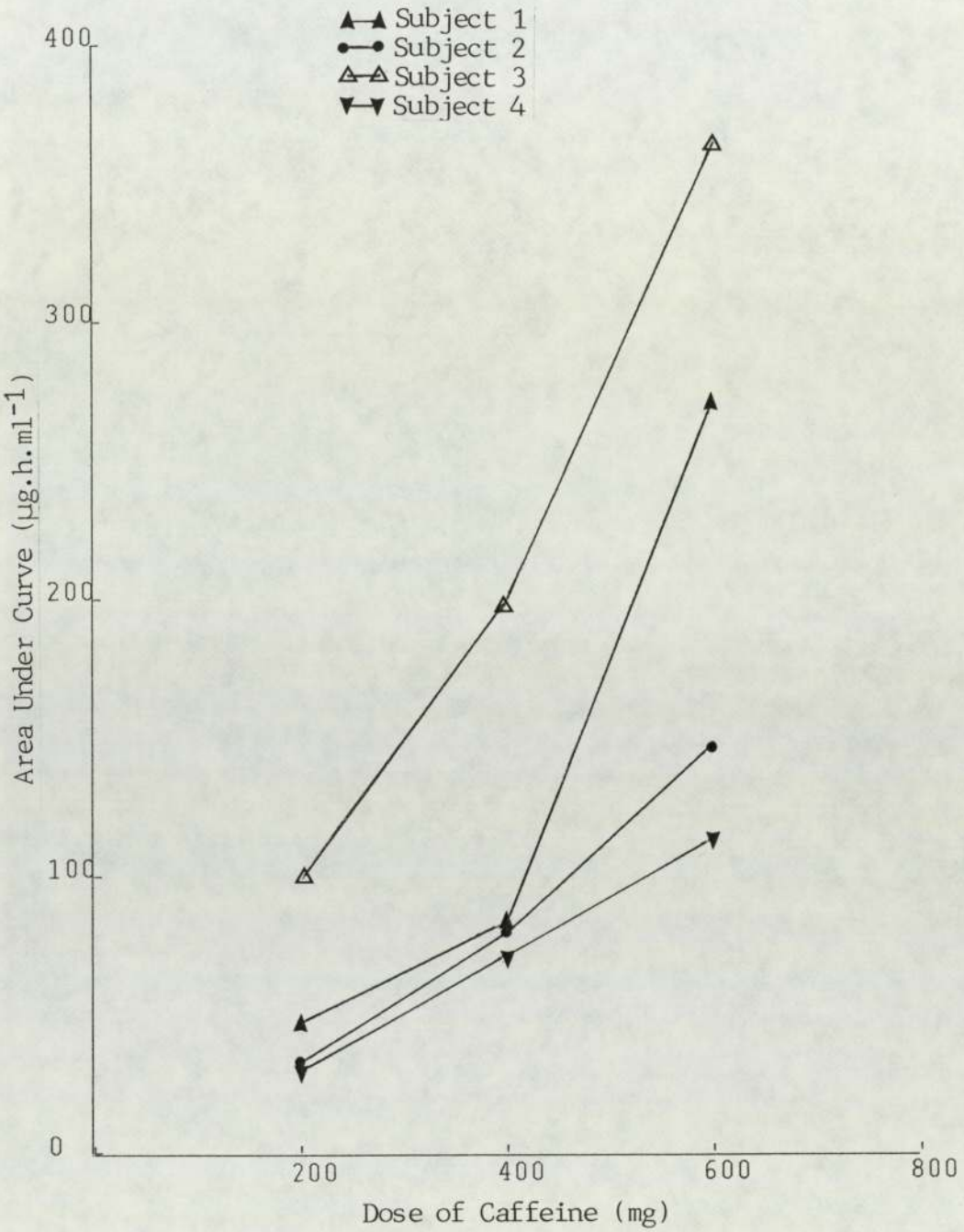


TABLE 16: Plasma clearance ($l.h^{-1}$)

Dose (mg)	Subject			
	1	2	3	4
200	4.21	6.04	2.03	6.62
400	4.82	5.03	2.01	5.64
600	2.22	4.08	1.66	5.32
Mean	3.75	5.05	1.90	5.86

TABLE 17: Plasma clearance ($ml.min^{-1}.kg^{-1}$)

Dose (mg)	Subject			
	1	2	3	4
200	1.15	1.50	0.56	1.81
400	1.32	1.25	0.56	1.54
600	0.61	1.01	0.44	1.45
Mean	1.03	1.25	0.52	1.60

Caffeine saliva concentration is compared with plasma concentration for each subject after the 600mg dose in Figs. 23-26. The saliva/plasma ratio for caffeine is shown in Table 18 and for paraxanthine in Table 19. The saliva/plasma ratios for both caffeine and paraxanthine decreased over the first two hours and then remained approximately constant.

The unbound caffeine concentration was measured for Subject 3 for the 400mg dose of caffeine (Fig. 27). The concentrations measured are compared with total concentration and saliva concentration in Table 20.

FIGURE 23 Plasma and saliva concentrations of caffeine in Subject 1 after a 600mg dose of caffeine

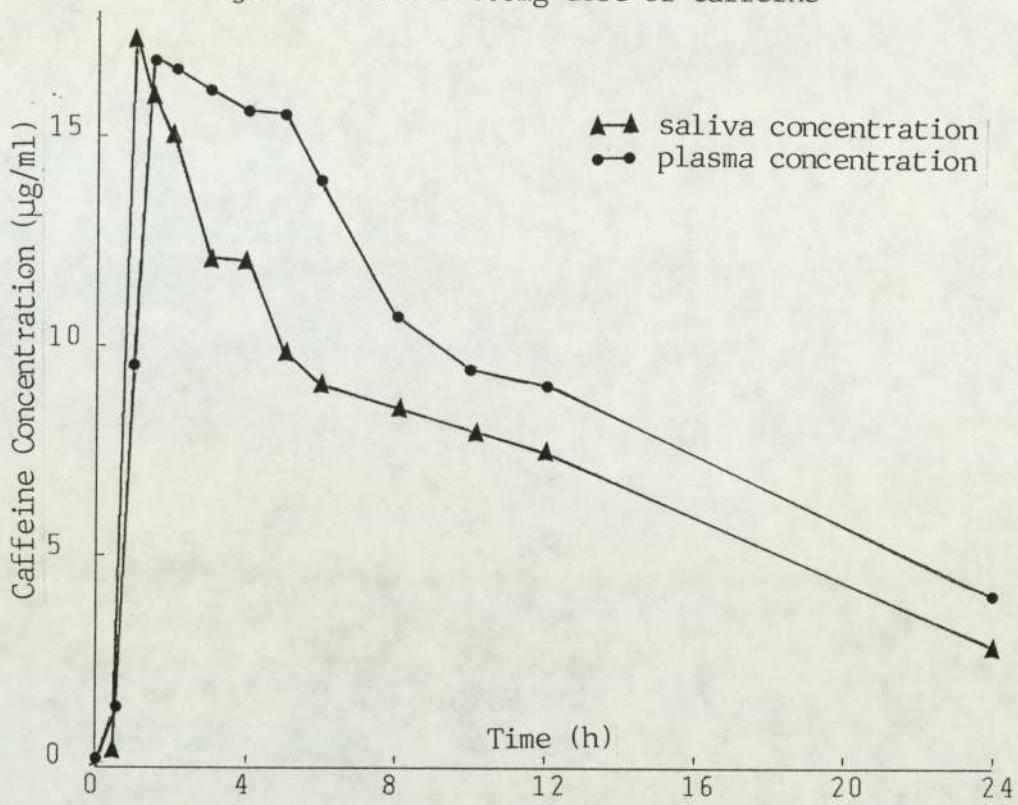


FIGURE 24 Plasma and saliva concentrations of caffeine in Subject 2 after a 600mg dose of caffeine

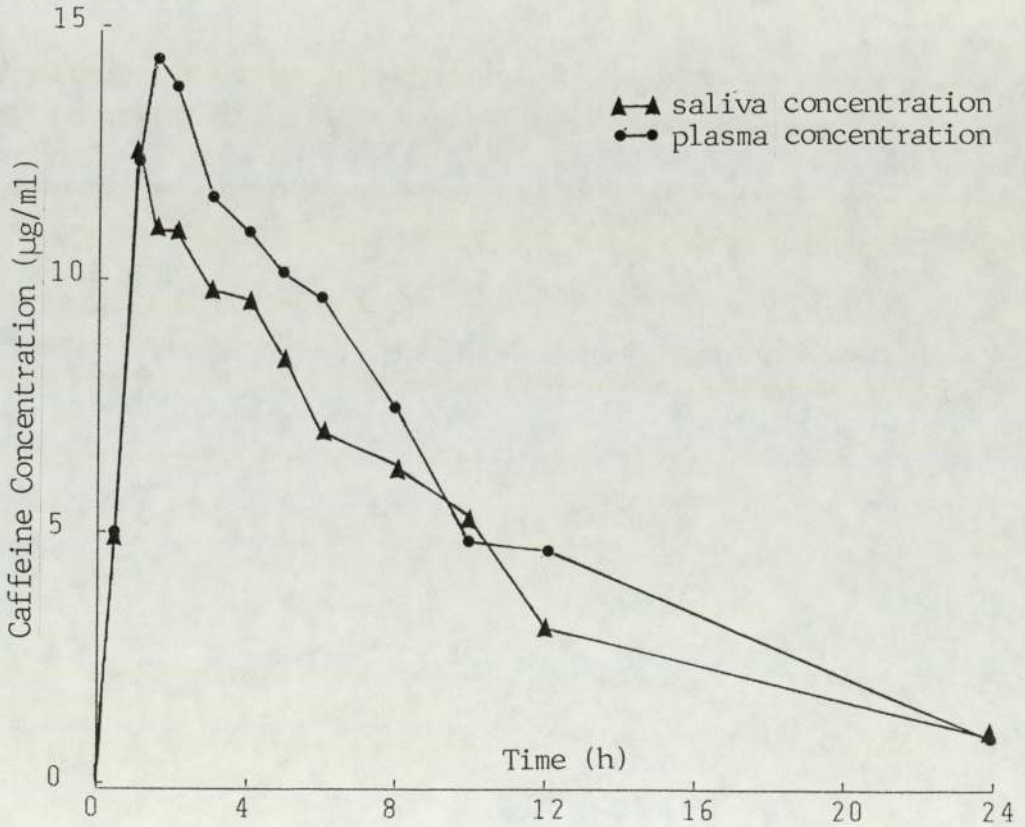


FIGURE 25 Plasma and saliva concentrations of caffeine in Subject 3 after a 600mg dose of caffeine

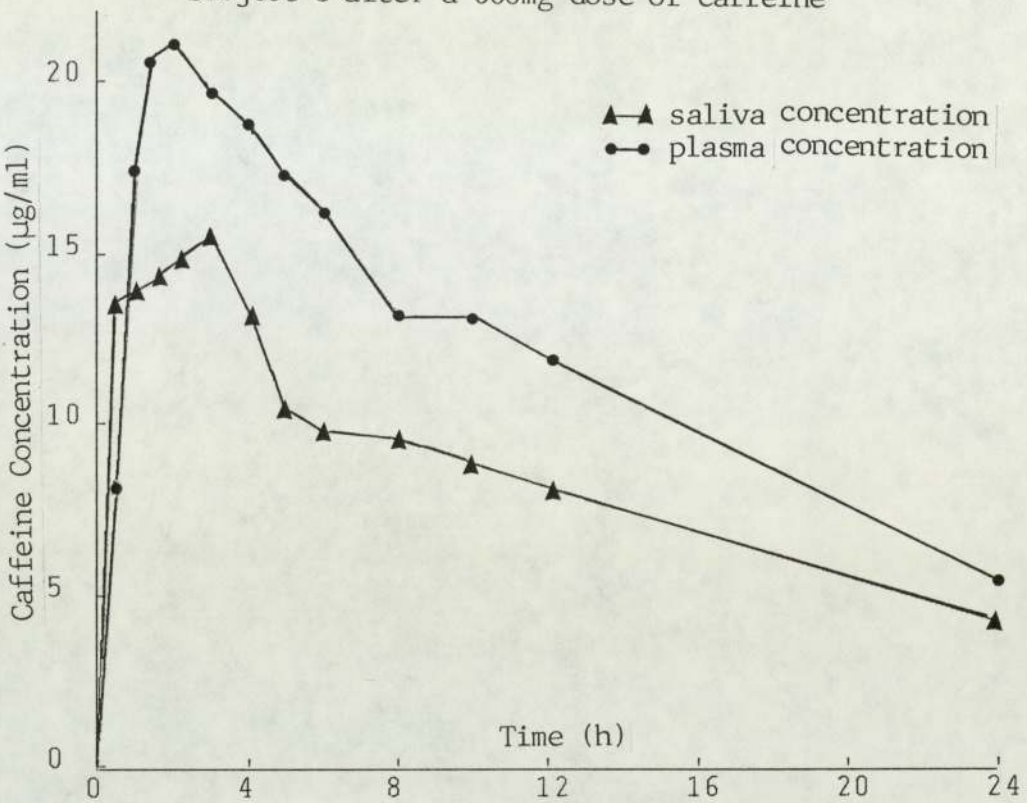


FIGURE 26 Plasma and saliva concentrations of caffeine in Subject 4 after a 600mg dose of caffeine

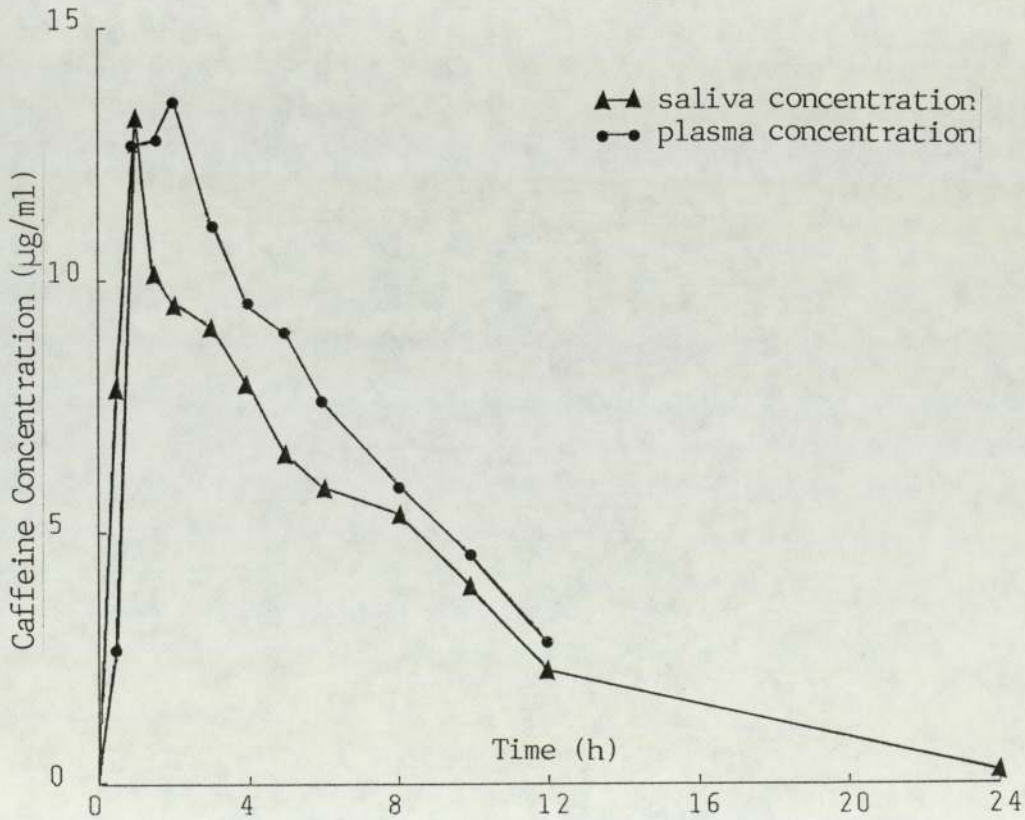


TABLE 18: Saliva/plasma ratio for caffeine

Time (h)	Subject 1			2			3			4			Mean and S.D.
	Caffeine dose (mg)												
	200	400	600	200	400	600	200	400	600	200	400	600	
0.5	0.83	0.91	4.06	2.03	1.04	0.97	2.24	1.75	1.78	4.86	3.27	2.78	2.21 1.31
1.0	0.69	0.92	1.76	0.88	0.93	1.03	0.90	1.18	0.80	2.13	1.41	1.04	1.14 0.43
1.5	0.97	0.85	0.95	1.10	0.83	0.76	0.84	1.07	0.69	1.10	1.11	0.79	0.92 0.15
2.0	0.90	0.80	0.90	1.10	0.70	0.79	0.74	1.00	0.69	0.88	0.81	0.70	0.83 0.13
3.0	0.92	0.85	0.75	0.84	0.78	0.84	0.70	1.02	0.79	0.92	0.83	0.81	0.84 0.08
4.0	0.77	0.91	0.77	0.94	0.89	0.87	0.84	0.84	0.71	0.76	0.78	0.84	0.83 0.07
5.0	0.79	0.96	0.63	0.72	0.99	0.83	0.74	0.95	0.60	0.88	0.68	0.73	0.79 0.13
6.0	0.77	0.74	0.65	0.88	0.99	0.72	0.63	0.79	0.60	0.70	0.82	0.77	0.75 0.11
8.0	0.87	0.78	0.79	0.75	0.90	0.85	0.73	0.74	0.73	1.00	0.53	0.90	0.80 0.12
10.0	0.78	0.71	0.84	0.89	0.84	1.09	0.68	0.66	0.67	0.99	0.95	0.85	0.83 0.14
12.0	0.91	0.91	0.82	1.21	0.85	0.67	0.72		0.68	0.92	0.95	1.34	0.91 0.21

TABLE 19: Saliva/plasma ratio for paraxanthine

Time (h)	Subject 1			2			3			4			Mean and S.D.
	Caffeine dose (mg)												
	200	400	600	200	400	600	200	400	600	200	400	600	
0.5			5.00	1.37	1.50	0.83	1.50	1.70	0.76	5.00	5.60	2.44	2.57 1.88
1.0	0.80	1.07	1.87	1.64	1.20	0.86	1.12	0.85	0.69	1.61	1.76	1.01	1.21 0.41
1.5			1.19	0.91	0.99	0.66	0.86	1.07	0.66	1.04	1.44	0.81	0.96 0.24
2.0	0.99		1.10	0.99	0.67	0.70	1.05	1.11	0.75	0.97	0.96	0.84	0.92 0.16
3.0		1.02	0.86	0.79	0.76	0.63	0.96	1.02	1.02	1.13	0.96	0.71	0.89 0.16
4.0	1.17	1.08	0.94	0.86	0.93	0.73	1.00	0.79	0.92	0.77	0.77	0.73	0.89 0.14
5.0		1.01	0.86	0.82	0.97	0.64	0.97	0.88	0.81	0.76	0.76	0.67	0.83 0.12
6.0	0.80	0.98	0.86	1.08	1.01	0.64	0.81	0.75	0.83	0.65	0.67	0.82	0.82 0.14
8.0	0.96		0.78	0.83	0.81	0.71	0.74	0.71	0.96	0.66	0.62	0.76	0.77 0.11
10.0		0.85	0.97	0.78	0.84	0.78	0.96	0.83	0.91	0.62	0.72	0.81	0.82 0.10
12.0	0.79	0.87	0.96	0.80	0.85	0.64	0.85		0.97	0.59	0.74	0.75	0.80 0.12

FIGURE 27 Unbound and total plasma concentrations and saliva concentrations of caffeine in Subject 3 after a 400mg dose of caffeine.

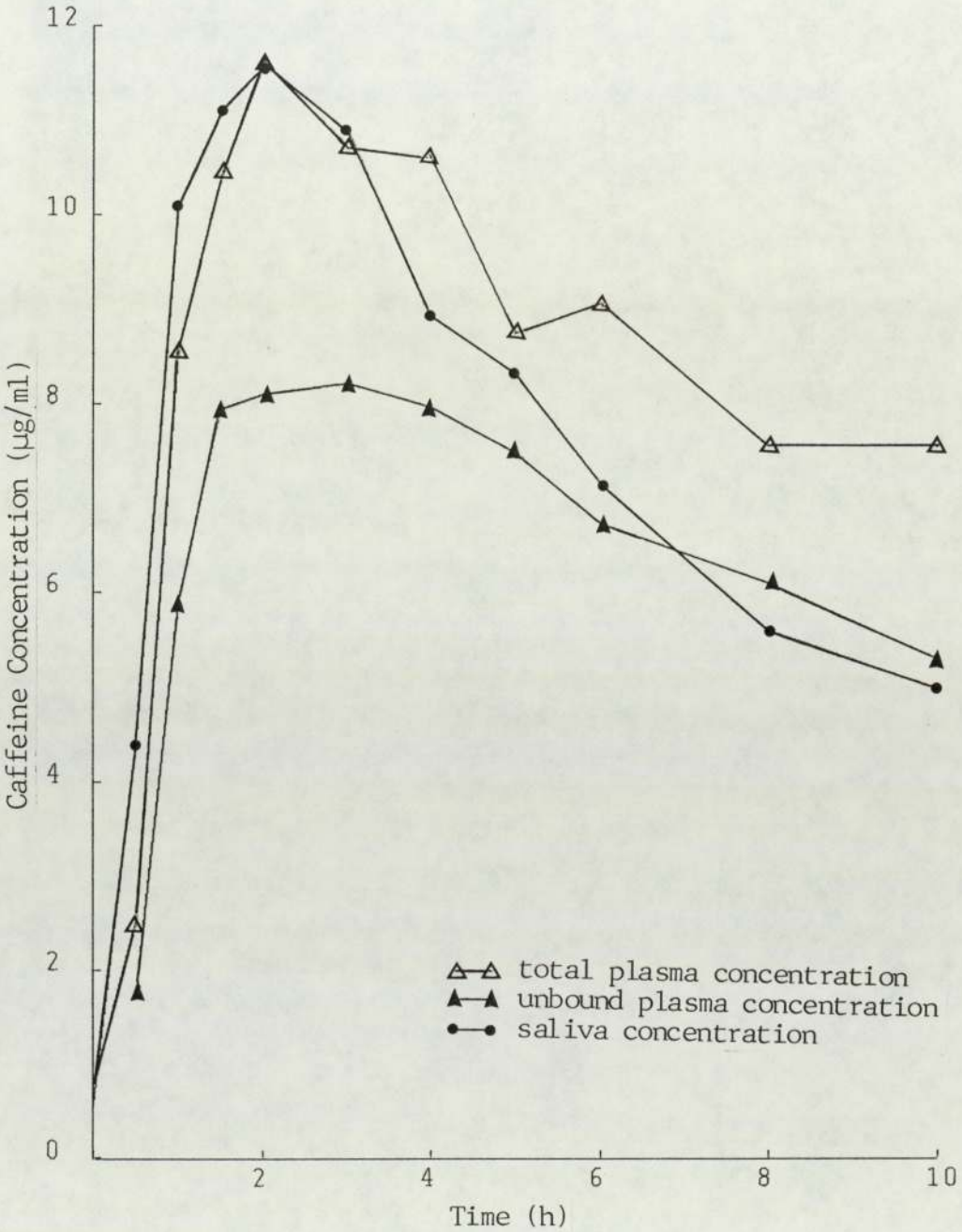


TABLE 20: The relationship between total and unbound plasma concentration and saliva concentration of caffeine in Subject 3 after a 400mg dose of caffeine

Time (h)	Total plasma concentration (μg/ml)	Saliva concentration (μg/ml)	Free plasma concentration (μg/ml)	Free/total (%)	Free/saliva (%)
0.5	2.52	4.41	1.73	68.6	39.2
1.0	8.57	10.10	5.90	68.8	58.4
1.5	10.40	11.09	7.96	76.5	71.8
2.0	11.61	11.58	8.10	69.8	69.9
3.0	10.72	10.89	8.22	76.7	75.5
4.0	10.59	8.91	7.96	75.2	89.3
5.0	8.73	8.32	7.47	85.6	89.8
6.0	9.06	7.14	6.73	74.3	94.2
8.0	7.55	5.59	6.09	80.7	108.9
10.0	7.55	4.97	5.28	69.9	106.2

Urine concentration of caffeine and paraxanthine was measured for each subject after each dose; as examples the urine data for Subject 1 is given in Tables 21 and 22 and the cumulative excretion for each subject after the 600mg dose of caffeine is shown in Figs. 28-31. The cumulative caffeine excretion was calculated as follows:

Example for Subject 1 after the 600mg dose of caffeine:-
At 4 hours urine volume was 200ml and concentration was 15.17μg/ml.
The total caffeine content was therefore 200 x 15.17 = 3034μg or 3mg.
Cumulative excretion up to 3 hours is 24.7mg + 3mg gives a cumulative excretion after 4 hours of 27.7mg.

Since caffeine excretion in urine is nearly complete after 24 hours the percentage caffeine excreted unchanged may be calculated as follows:

FIGURE 28 The urinary excretion of caffeine and paraxanthine in Subject 1 after a 600mg dose of caffeine.

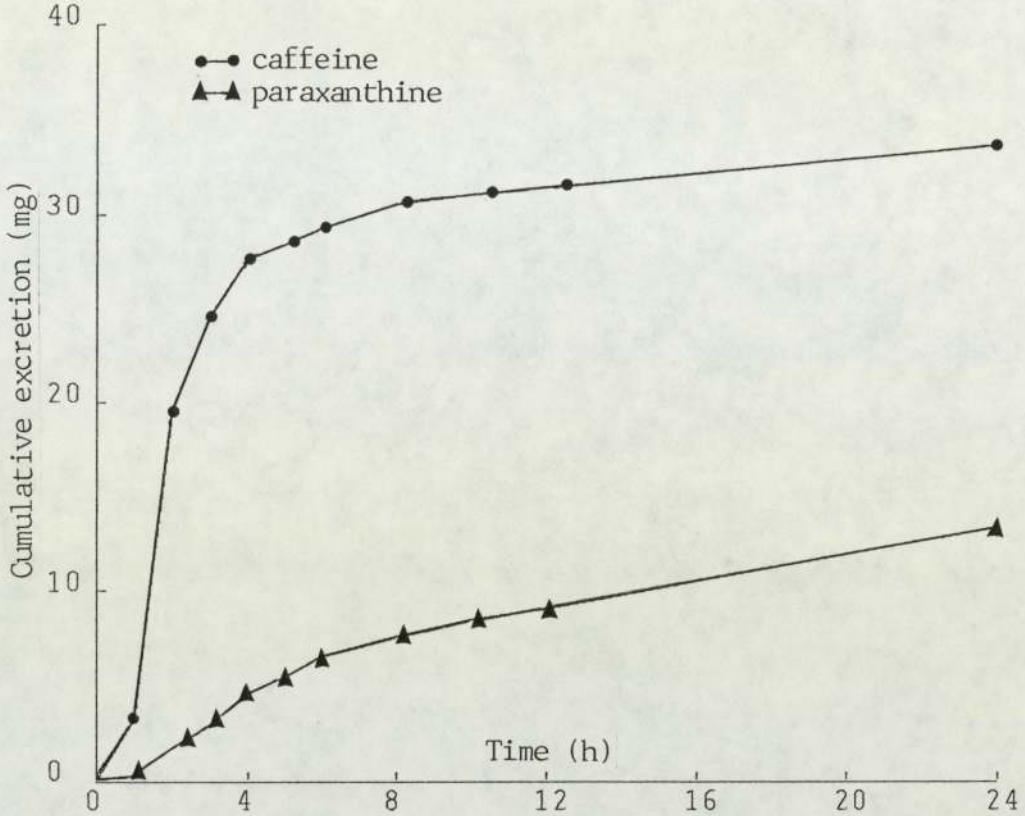


FIGURE 29 The urinary excretion of caffeine and paraxanthine in Subject 2 after a 600mg dose of caffeine.

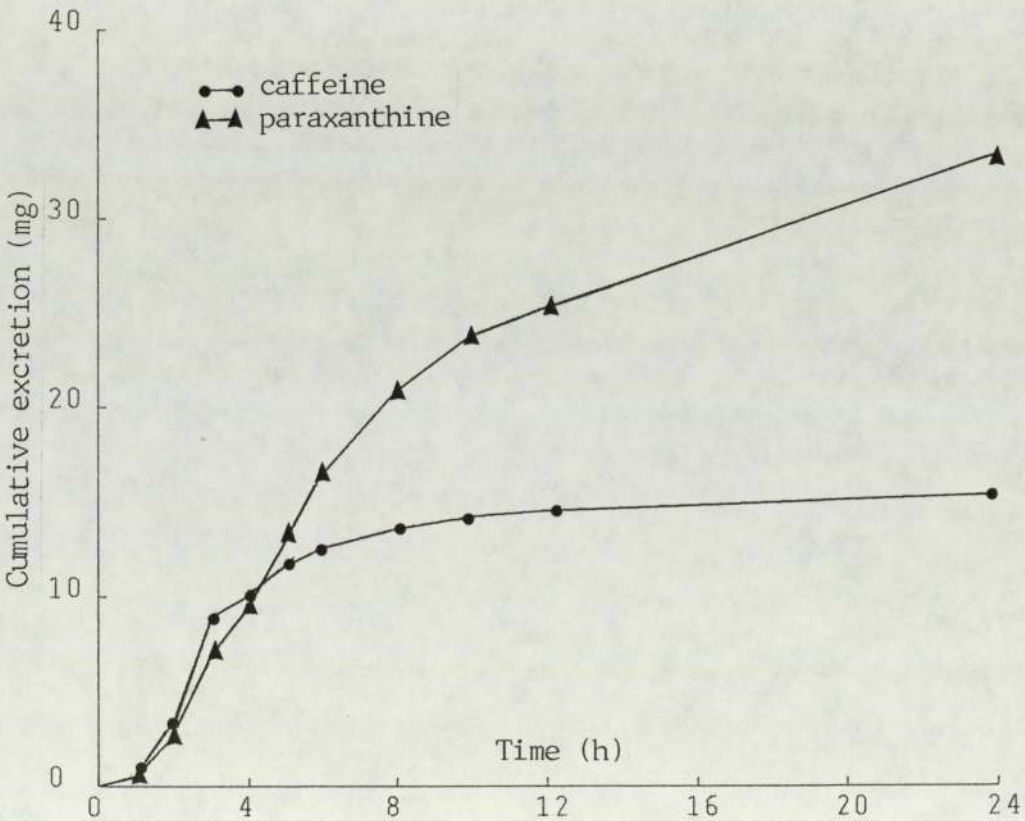


FIGURE 30 The urinary excretion of caffeine and paraxanthine in Subject 3 after a 600mg dose of caffeine.

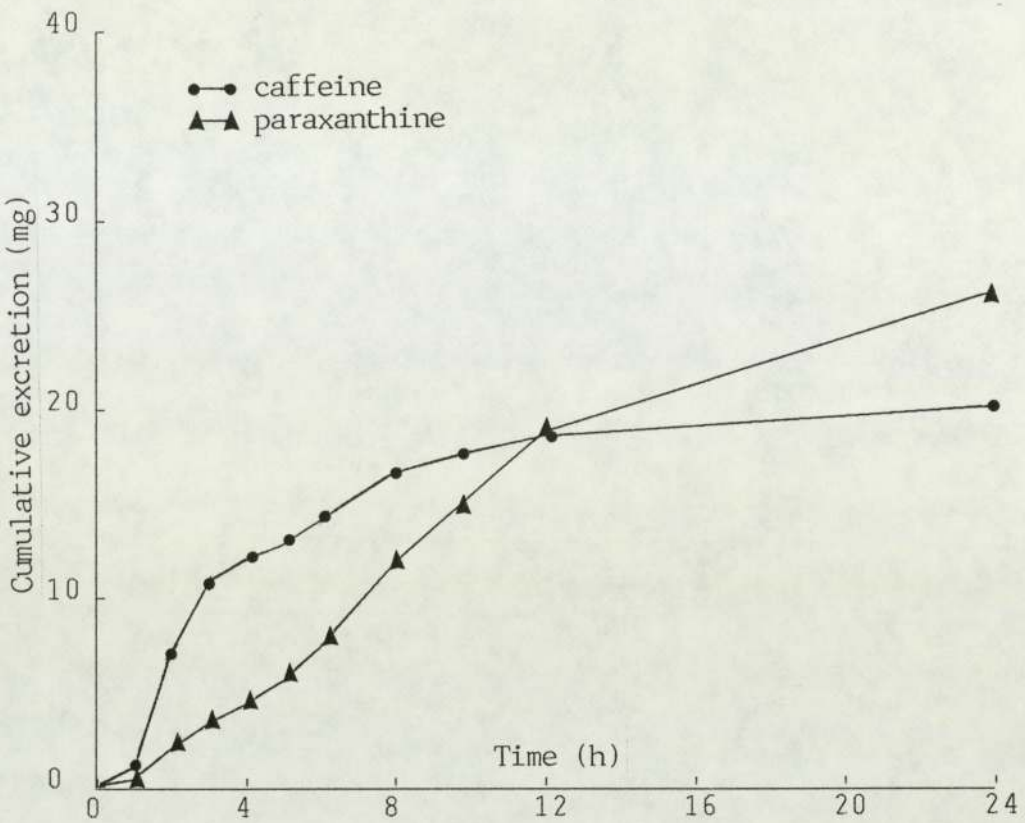
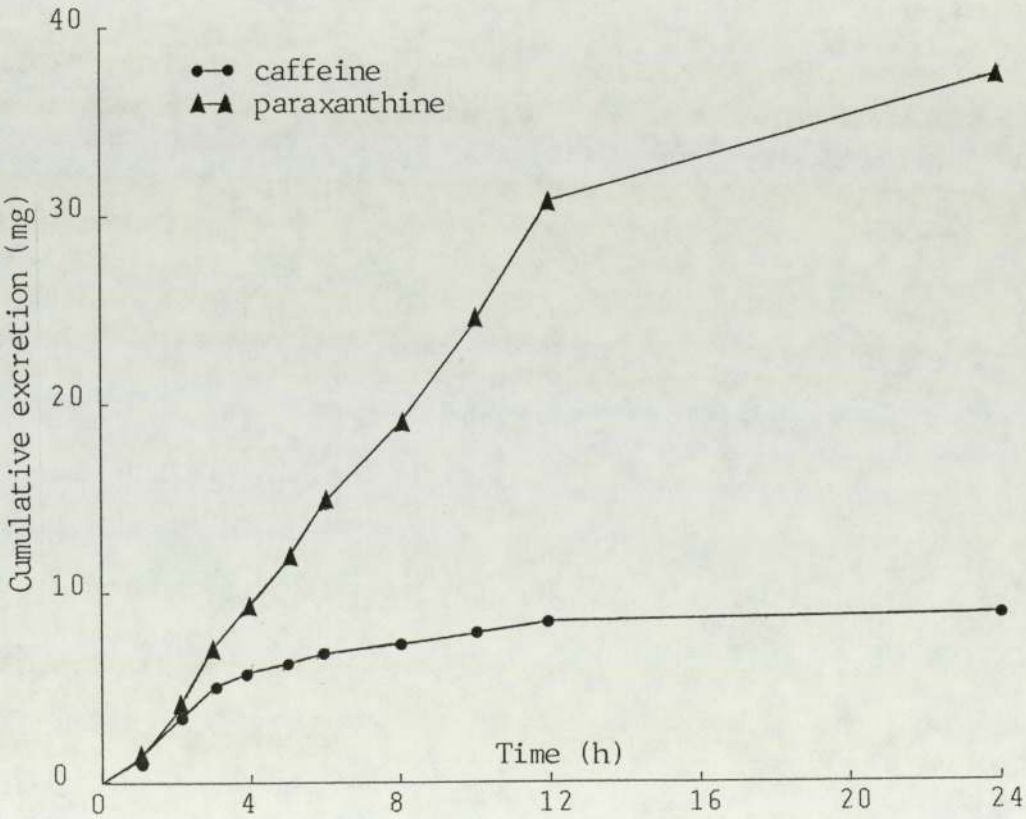


FIGURE 31 The urinary excretion of caffeine and paraxanthine in Subject 4 after a 600mg dose of caffeine.



Example for Subject 1 after the 600mg dose of caffeine:-

33.6mg of caffeine had been excreted in urine after 24 hours

Percentage excreted unchanged = (33.6/600) x 100 = 5.6%

TABLE 21 Urinary caffeine excretion for Subject 1

Time (h)	Caffeine dose (mg)											
	200				400				600			
	Vol ml	Conc µg/ml	CxV	Cumu excr mg	Vol ml	Conc µg/ml	CxV	Cumu excr mg	Vol ml	Conc µg/ml	CxV	Cumu excr mg
0	100	0.8	76	0.0	25	0.8	20	0.0	100	0.2	16	0.0
1	450	3.9	1751	1.7	175	17.4	3045	3.0	350	9.5	3321	3.3
2	600	4.1	2447	4.2	150	19.3	2895	5.9	800	20.3	16280	19.6
3	300	2.2	648	4.8	225	21.0	4725	10.7	450	11.2	5058	24.7
4	300	2.8	843	5.7	200	12.7	2540	13.2	200	15.2	3034	27.7
5	200	1.7	338	6.0	50	3.4	170	13.4	75	9.6	721	28.4
6	50	2.0	102	6.1	100	4.9	490	13.9	100	8.7	875	29.3
8	125	1.6	200	6.3	150	7.8	1170	15.0	150	8.7	1303	30.6
10	75	1.8	134	6.5	160	4.8	768	15.8	100	5.4	538	31.1
12	150	0.9	130	6.6	100	1.4	140	15.9	100	3.5	355	31.5
12-24	850		946	7.5	850		595	16.5	500		1677	33.2
24	300	1.4	408	7.9	50	0.0	0	16.5	150	3.2	474	33.6

TABLE 22 Urinary paraxanthine excretion for Subject 1

Time (h)	Caffeine dose (mg)											
	200				400				600			
	Vol ml	Conc µg/ml	CxV	Cumu excr mg	Vol ml	Conc µg/ml	CxV	Cumu excr mg	Vol ml	Conc µg/ml	CxV	Cumu excr mg
0	100	0.0	0	0.0	25	2.0	50	0.0	100	1.1	108	0.0
1	400	0.0	0	0.0	175	6.1	1067	1.1	350	0.9	329	0.3
2	600	0.5	277	0.3	150	17.3	2595	3.7	800	1.7	1336	1.7
3	300	1.4	430	0.7	225	14.5	3262	6.9	450	2.9	1323	3.0
4	300	3.2	954	1.7	200	11.0	2200	9.1	200	8.5	1698	4.7
5	200	1.2	249	1.9	50	10.5	525	9.6	75	11.5	866	5.5
6	50	4.0	201	2.1	100	16.1	1610	11.2	100	10.2	1022	6.6
8	125	3.7	469	2.6	150	42.8	6420	17.7	150	6.7	1011	7.6
10	75	7.9	596	3.2	160	22.8	3648	21.3	100	9.3	935	8.5
12	150	5.3	802	4.0	100	14.3	1430	22.8	100	6.0	597	9.1
12-24	850		2474	6.4	850		10922	33.7	500		3267	12.4
24	300	0.5	143	6.6	50	11.4	570	34.2	150	7.1	1065	13.4

The percentage caffeine excreted unchanged for each subject is given in Table 23.

The renal clearance may be calculated using the following equation:

Clearance = (urine concentration x urine flow)/plasma concentration

Example for Subject 1 after the 600mg dose of caffeine:-

200ml urine of concentration 15.2µg/ml was produced in the time interval 2-3 hours after dose. During this time the mean plasma

TABLE 23: Percentage caffeine excreted unchanged in urine.

Dose (mg)	Subject			
	1	2	3	4
200	4.0	1.3	4.3	1.1
400	4.1	2.1	2.4	1.1
600	5.6	2.6	3.3	1.5
Mean	4.6	2.0	3.3	1.2

concentration was 15.8μg/ml. Therefore the renal clearance for caffeine at this time was

$$(200 \times 15.2)/15.8 = 190\text{ml/h} = 3.2\text{ml/min}$$

The renal clearance for Subject 1 after each dose of caffeine is shown in Table 24. Figure 32 shows a weak relationship (correlation coefficient 0.83) between clearance and urine flow. The renal clearance for paraxanthine was calculated in a similar manner and the values obtained for Subject 1 are shown in Table 25.

TABLE 24: Renal caffeine clearance for Subject 1

Time interval (h)	Renal clearance (ml/min)		
	200mg dose	400mg dose	600mg dose
0-1	9.7	9.8	11.3
1-2	8.0	5.5	20.0
2-3	2.7	11.0	5.2
3-4	3.8	6.7	3.2
4-5	1.7	0.4	0.8
5-6	0.5	1.5	1.0
6-8	0.7	2.2	0.9
8-10	0.6	1.6	0.4
10-12	0.7	0.4	0.3
12-24	2.5	0.7	0.4

FIGURE 32 The effect of urine flow on the renal clearance of caffeine

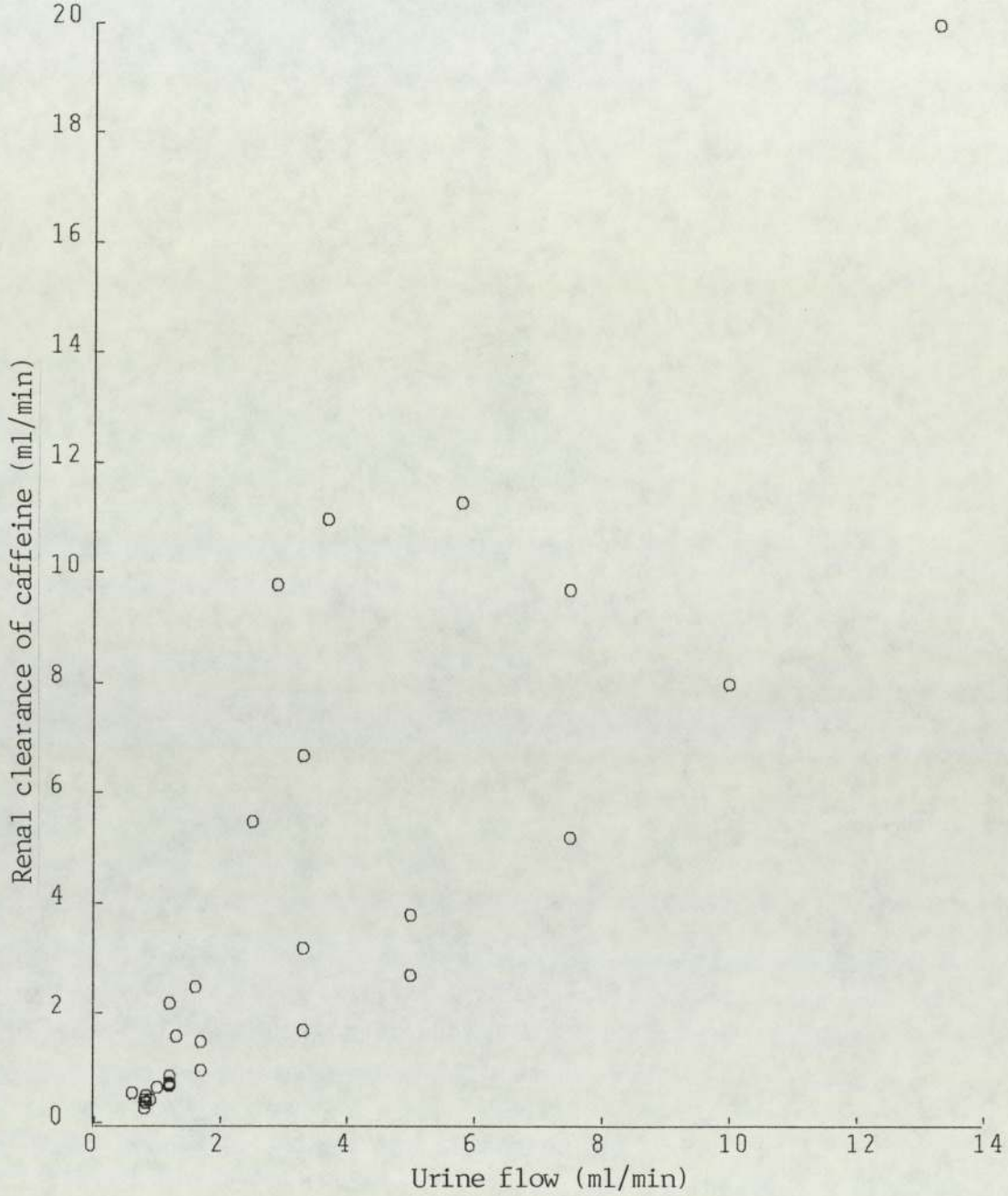


TABLE 25: Renal paraxanthine clearance for Subject 1

Time interval (h)	Renal clearance (ml/min)		
	200mg dose	400mg dose	600mg dose
0-1	0.0	43.4	45.7
1-2	6.9	45.0	42.8
2-3	7.4	43.7	21.2
3-4	15.7	20.2	19.4
4-5	3.7	4.5	8.2
5-6	2.8	12.7	9.0
6-8	3.3	22.9	4.2
8-10	4.3	12.0	3.7
10-12	6.1	5.0	2.3
12-24	1.1	10.0	3.5

III.5 Discussion

The symptoms of headache, tiredness and lethargy experienced by all four subjects on abstaining from dietary xanthines are typical of caffeine withdrawal. A mechanism has been suggested to explain the cause of the headaches[16]. During long-term caffeine use tolerance develops and is associated with an increase in the number of adenosine receptors in the cerebral vessels. Adenosine dilates blood vessels directly and by inhibiting noradrenaline release. When caffeine is suddenly discontinued adenosine acts unopposed at an increased number of receptors leading to vasodilation and causing headache. Administration of caffeine quickly reduces the headache. Plasma caffeine levels were not measured during normal dietary methylxanthine intake in this study, however this was done in the clinical study. None of the volunteers had an abnormally high dietary caffeine intake, suggesting that a large number of people may have developed caffeine tolerance but may not be aware of it.

Two of the subjects experienced tremor and lightheadedness after the highest dose of caffeine. It may be noted that these were the subjects who reached the highest plasma caffeine levels and that the time when symptoms were noted corresponded to peak plasma concentrations.

Major metabolites of caffeine found in plasma in this study were the dimethylxanthines. This is in agreement with previous studies[82, 88, 99]. Trace amounts of other metabolites have been found including 5-acetylamino-6-amino-3-methyluracil, 1-methyluric acid and 1-methylxanthine, however this study concentrated on the metabolites found in greatest abundance. Although some of the minor metabolites eg 1-methylxanthine, posess their own spectra of pharmacological activity[89], this would not normally be clinically significant due to the low concentration. However in patients with impaired renal function but normal hepatic function these metabolites might accumulate to concentrations which did have a significant effect. In the present study, a linear relationship was consistently seen between log(plasma caffeine concentration) with time (all correlation coefficients greater than 0.95, mean 0.98) indicating first-order kinetics. However, the concentrations of the dimethylxanthine metabolites did not show this relationship over the times measured, increasing more slowly and peaking later (after 8-12 hours). This agrees with Tang-Lui[99] who reported that the plasma concentrations of the methylxanthines remained relatively constant for at least 10 hours before declining in a non log-linear fashion, becoming convex descending at low concentrations. This indicates that clearance increases at lower concentrations and suggests saturation of the

enzyme systems involved in the metabolism of caffeine. Further evidence for dose dependence is the disproportionately high peak plasma concentration in Subject 3 after the 600mg dose of caffeine.

Apparent volume of distribution could be calculated after oral dosing since it is well recognised that caffeine absorption is rapid and complete after oral dosing[77]. Newton[82] reported that bioavailability was 0.92 after a 300mg dose, 0.91 after a 500mg dose and 1.06 after 750mg of caffeine. The mean apparent volume of distribution found in the present study was 0.52±0.041/kg. This is consistent with values previously reported in the literature eg Blanchard[77] 0.61±0.021/kg for young men and 0.52±0.021/kg for elderly men, Abernethy[78] 0.65±0.041/kg for obese subjects and 0.69±0.041/kg for non-obese subjects and Tang-Lui[99] 0.52±0.041/kg for healthy adults. These values are consistent with distribution throughout total body fluid, with no accumulation in any particular tissue.

The mean half-life for caffeine found in this study was 7.1±3.2h. Literature values show considerable variation: Levy[112] 7.4h for caffeine wakeful subjects, 4.2h for subjects whose sleep was not disturbed by caffeine; Parsons[100] reported 3.5h for smokers, 6.0h for non-smokers; Blanchard[77] 4.3h for young men, 3.6h for elderly men and Callahan[95] 3.1±0.9h for females, 4.5±1.1h for males and 10.4±2.4h for females taking oral contraceptives. Thus it can be seen that smoking reduces half-life probably by induction of hepatic aryl hydrocarbon hydroxylase activity, while use of oral contraceptives increases half-life. None of the subjects in this study had smoked for at least six months, however Subject 3 was taking an oral contraceptive and Subject

1 commenced oral contraceptives between taking the 400mg dose and the 600mg dose, a fact which is reflected in the increased half-life of 10.3h after the 600mg dose from 5.8h after the 400mg dose. The increase in half-life observed with oral contraceptives results from an impairment of plasma clearance, while volume of distribution[153] and the proportions of metabolites formed[95] are not changed. Oral contraceptive steroids appear to decrease microsomal caffeine metabolism, however the exact mechanism by which this occurs remains unclear[154]. In view of the long half-lives in Subjects 1 and 3 a longer period of abstention from dietary xanthines, eg three days, would have been more appropriate and continuing the sample collection for a further 24h would have been useful. The limitation of the period of abstention from dietary xanthines to 24h resulted in some residual xanthines being present at the beginning of the study. This should be avoided in any future work.

A criterion for linear kinetics is that the area under the curve should be directly proportional to the dose. A linear relationship between dose and area under the curve was observed for two of the four subjects. In Subject 1 the deviation from linearity can be attributed to commencing oral contraceptives between taking the 400mg and 600mg doses of caffeine as previously discussed, and the resulting reduction in plasma clearance. In Subject 3 the deviation from linearity was less marked but suggests a decreased plasma clearance after the highest dose due to saturation of the enzymes responsible for metabolism.

The value of mean plasma clearance obtained in this study,

1.10±0.45ml/min/kg is consistent with previous work. Callahan[95] found the plasma clearance to be 1.38ml/min/kg for males, 1.82ml/min/kg for females and 0.62ml/min/kg for females taking oral contraceptives while Blanchard[77] reported values of 1.42 and 1.60ml/min/kg for young and elderly males respectively. Parsons[100] measured body clearance from saliva data and recorded 2.58ml/min/kg for smokers and 1.56ml/min/kg for non-smokers.

The saliva/plasma ratio for caffeine showed time dependence with high values initially but becoming steady after the first two hours. The mean saliva/plasma ratio from the first two hours onwards was 0.82. In premature infants, saliva caffeine concentration approximated 76-80% of serum concentration[87]. Newton[82] found the saliva/plasma ratio to be 0.74±0.08 and reported time dependence. The explanation for this is unlikely to be residual caffeine in the mouth after the dose is taken orally since this effect is also seen if caffeine is administered intravenously. Why the saliva/plasma ratio should be higher in the absorption and distribution phase than during elimination remains unclear. Caffeine is 15-35% bound to plasma proteins[7, 81], thus the saliva concentrations found in the present study reflect the unbound plasma concentration. When the unbound concentration was determined for Subject 3 the saliva concentration approached the unbound concentration after 4 hours, however initially the saliva concentration was over twice the unbound concentration. This suggests that using the saliva concentration as a guide to the unbound plasma concentration before four hours after dosing gives a gross overestimation and should not be used. In the saliva/plasma ratio for paraxanthine time dependence was even more marked and the

mean ratio after two hours was 0.84.

Only a small proportion of the dose of caffeine is excreted unchanged in urine, most of the filtered caffeine being reabsorbed in the renal tubules. This study found a mean value of 2.8±0.5% as compared with Newton[82], 1.83%; Cornish[88], 1.2%; and Tang-Lui[99], 3.7%. The latter study demonstrated that renal clearance was highly dependent on urine flow. Although this was seen in this study the relationship was not as strong (correlation coefficient 0.83). The values of renal clearance are greater for paraxanthine than caffeine. This would be expected since paraxanthine is less lipophilic than caffeine and thus is less readily reabsorbed in the kidney tubules. It was impossible to determine the total percentage of paraxanthine excreted since the excretion was not complete after 24h; further urine collection for an additional 24h would have made this possible. The ratio of caffeine to paraxanthine excreted over 24h varied widely from 0.2-2.5. Wide variation in ratios of urinary metabolites excreted after oral caffeine have been reported by Grant[91].

Although at first sight caffeine appears simply to obey first order kinetics, on closer examination the situation is more complex. For the dimethylxanthine metabolites the relationship between log(concentration) and time is not linear but convex at lower concentrations. This, however, is not the case with caffeine since its diuretic effect initially increases urine flow and thus renal clearance. A further consideration is recycling into the gut of caffeine secreted into saliva, and by implication into other intestinal secretions.

IV. A COMPARISON OF THE BRONCHODILATOR EFFECTS OF THEOPHYLLINE AND CAFFEINE IN ADULTS WITH REVERSIBLE AIRFLOW OBSTRUCTION

IV.1 Introduction

Becker[155] compared the bronchodilator effects of caffeine (10mg/kg) and theophylline (5mg/kg) in asthmatic patients aged 8-18 years in a double-blind single-dose study. The results of this study showed the bronchodilator effect of caffeine did not differ significantly from that of theophylline, although it must be noted that the plasma concentrations of theophylline were lower than the caffeine concentrations and lower than the desired concentration for maximum therapeutic effect. The conclusions drawn from these findings were that caffeine has an appreciable bronchodilator effect and should be avoided during investigations of the effects of any anti-asthma drug on lung functions. Caffeine was not recommended for regular use as a bronchodilator but could have temporary benefit when prescribed anti-asthmatic medications are not available.

The comparison of the bronchodilator effects of caffeine and theophylline was extended to adults in a study by Gong[156]. This was a double-blind, randomised crossover study in nine asthmatic adults who ingested decaffeinated coffee containing varying amounts of added caffeine (mean 0.2, 2.5, 5.6 and 7.2mg/kg body weight) on different days. The subjects also ingested decaffeinated coffee and aminophylline (200mg) on a separate day of the study. This study indicated that caffeine was an effective bronchodilator but was only

40% as active as an equivalent molar dose of theophylline. In conclusion the authors recommended that caffeine-containing products should be avoided for at least 12 hours prior to pulmonary function testing. The therapeutic value of caffeine as a bronchodilator is limited by its short duration of action, toxic effects and the possible development of tolerance, however the interactions and effects of concomitant use of caffeine and theophylline merit further investigation. This study was designed to extend the comparison still further from a single dose to a multiple dose study in adults.

IV.2 Aims

This study aimed to compare the bronchodilator effects of caffeine with theophylline in adults with reversible airflow obstruction over a period of 10 weeks using both pulmonary function tests and subjective assessment. The side effects of both treatments were also studied.

IV.3 Design

The protocol for this study was submitted to and approved by the Central Oxford Research Ethics Committee. It was a double-blind, within-patient crossover study. It was hoped that a total of 20 patients would take part. Out-patients from the Osler Chest Clinic at the Churchill Hospital, Oxford and the Chest Clinic at Abingdon Hospital who fulfilled the criteria listed below were given a verbal and written explanation of the study (Appendix 2). After an interval of at least two days (specified by the Ethics Committee) patients were contacted either by telephone or by letter and invited to participate

in the study. In the case of those who wished to participate, a letter was sent to their GP (Appendix 3).

Each patient started with a two-week single-blind placebo period. The patient was given a diary card (Fig. 33) and a mini peak flow meter. Throughout the total 10 weeks of the study the patient was required to make readings of peak flow, the best of three readings before any medications were taken in the morning and in the evening. The diary card had a box for every day for two weeks and was kept as simple as possible, a simple numerical scale being used for the subjective assessments. The patient continued with his normal medication throughout the study except for those subjects taking theophylline beforehand. Once the placebo period was complete each patient was then randomly allocated to one of the two treatments. A two-week "run-in" period followed during which the dose of the methylxanthine was adjusted to give plasma concentrations in the range 10-20μg/ml. The methylxanthine was taken in four divided doses daily in the form of capsules prepared by the investigator. After the "run-in" period the patient continued the treatment for a further two weeks and then the procedure was repeated for the other treatment, thus the study took the patient a total of ten weeks, two weeks placebo plus two four-week treatment periods. Spirometry was carried out in the chest clinic at each fortnightly visit.

IV.4 Selection of Patients

To be eligible for participation in this study patients were required to be aged 18-65 and were already taking or thought likely to benefit

FIGURE 33

DIARY CARD

			WEEK 1							WEEK 2						
1. Wheeze last night.	Good night Slept well, slightly wheezy Woke 2-3 times wheezing Bad night, mostly awake	0 1 2 3														
2. Cough last night.	None Little Moderately bad Severe	0 1 2 3														
3. Wheeze today	None Little Moderately bad Severe	0 1 2 3														
4. Activity today	Quite normal Can only run short distance Can only walk due to chest Too breathless to walk	0 1 2 3														
5. Sputum volume	None A few small blobs Large amounts (Add Y if yellow, G if green)	0 1 2														
6. Meter Best of 3 blows.	Before breakfast medicines Before bedtime medicines															
7. Inhaler	Number of times used in past 24 hours															
8. Caffeine containing food and drink.	Cups of coffee Cups of tea Cups of drinking chocolate Glasses of coca cola ~~Bars of chocolate~~															
9. Comments	Note if you see your doctor (D), or stay away from work (W) because of your chest, and anything else important such as infection (I)															

from treatment with a xanthine bronchodilator. Only patients showing more than 10% reversibility in peak expiratory flow rate (PEFR) either spontaneously or after the use of a bronchodilator inhaler were admitted to the study. Patients were excluded if they were on maintenance oral steroids or had received intermittent courses with a total of more than 28 days in the previous three months. Patients with liver disease, heart failure, current infection or who were taking cimetidine were also excluded, as were patients with known hypersensitivity to theophylline. Those who were thought likely not to comply with the regimen or to miss appointments and those with excessive dietary methylxanthine intake were not invited to participate.

IV.5 Assessments

A vitalograph was used for the measurement of forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1). Patients were asked not to use any bronchodilator, either a β-2 adrenoceptor agonist or ipratropium bromide for four hours before spirometry to avoid erroneously high readings. Blood samples were taken by venepuncture using heparinised vacutainers. Plasma concentrations were measured one hour post dose for caffeine and two hours post dose for theophylline. Samples were centrifuged within one hour of collection and the serum harvested stored at -20°C until assayed. Each sample was assayed for caffeine and theophylline by the HPLC method described previously. The Ames Seralyser was used to confirm theophylline concentrations. A plasma sample was taken at the end of the placebo

period to indicate methylxanthine concentrations produced by dietary intake alone.

IV.6 Results

Due to recruiting difficulties, only 17 patients started the study. The demographic details and medications taken by the subjects are given in Table 26. The medications listed remained constant throughout the study. The results of each of the ten subjects who completed the study are given in Tables 27-36 with predicted values calculated from nomograms[157, 158]. Two of the parameters measured, FEV_1 and morning PEFR are represented as examples in Figs. 34 and 35. The means and standard deviations for the ten subjects are given in Table 37. One way analysis of variance (ANOVA) was used to determine the significance of the results using the StatsWorks program run on an Apple Macintosh computer (Table 38). No results were significant at the 5% level. The reasons why some subjects had to withdraw from the study are listed in Table 39.

The plasma sample taken at the end of the placebo period was to establish xanthine concentrations reached by dietary intake alone. The mean caffeine concentration was 2.47 $\pm$ 1.73μg/ml and the mean theophylline concentration was 0.10 $\pm$ 0.13μ/ml. The highest of these concentrations, a caffeine concentration of 6.16μg/ml and a theophylline concentration of 0.24μg/ml were found in Subject 5, who withdrew from the study. During the maintenance phases of the study following the "run-in" periods the mean peak concentrations measured were 11.4 $\pm$ 3.8μg/ml for caffeine and 11.9 $\pm$ 2.8μg/ml for

theophylline. In the placebo phase and the caffeine phase (i.e. when no theophylline, other than from dietary sources, was taken) a correlation was observed between the caffeine concentration and the theophylline concentration (see Fig. 36, correlation coefficient 0.938, intercept -0.101, slope 0.079). No similar relationship was found when the placebo phase and theophylline phase were considered (i.e. when no caffeine, other than dietary, was taken), the correlation coefficient was -0.032. A reasonable correlation was found between values of theophylline concentration determined by HPLC and values obtained using the Ames Seralyser (see Fig. 37, correlation coefficient 0.901, slope 1.000, intercept 0.227).

TABLE 26: Demographic data and concurrent medication for all patients who started the study

Subject	Age y	Sex	Ht cm	Wt kg	Duration of asthma y	Smoking history	Other medication	Completed study
1	58	M	185	87	40	5-6/day	BI[a] 2 puffs QDS VI[b] 2 puffs QDS	yes
2	65	M	183	83	60	ex[c]	Ventolin neb. 0.5ml QDS Atrovent neb. 0.5ml QDS Pulmicort inhaler 4 puffs BD	yes
3	50	M	168	68	30	none	Pulmicort inhaler 2 puffs QDS Ventolin rotacaps 800µg QDS Ventolin spandet 1 nocte VI PRN	yes (on 2nd attempt)
4	57	M	173	73	31	ex	BI 2 puffs QDS VI 2 puffs QDS	no
5	41	F	165	74	2	12/day	Bricanyl SA tabs 1 BD BI 4 puffs BD VI PRN	no
6	64	F	163	78	27	ex	BFI[d] 2 puffs TDS VI 2 puffs TDS Zaditen tabs 2 BD	yes
7	28	F	167	105	10	none	BFI 2 puffs TDS Bricanyl inhaler 2 puffs TDS Beconase spray 1 puff TDS	yes
8	59	F	160	70	42	none	BFI 2 puffs QDS VI PRN Calcium & vit D tabs Naproxen 250mg 5 OD Paramol PRN Nitrazepam 5mg nocte	no

TABLE 26: continued

Subject	Age y	Sex	Ht cm	Wt kg	Duration of asthma y	Smoking history	Other medication	Completed study
9	65	M	167	65	11	ex	BFI 2 puffs BD Duovent inhaler 2 puffs QDS PRN Zaditen tabs 1 nocte	yes
10	43	M	175	89	25	none	VI 2 puffs 6/day BI 2 puffs QDS	yes
11	50	M	180	74	3	ex	VI 2 puffs QDS BI 2 puffs QDS	yes
12	33	M	186	77	3	ex	VI PRN BI 2 puffs TDS	no
13	56	F	160	71	18	ex	BFI 2 puffs TDS Berotec inhaler 2 puffs QDS Lederfen tabs 300mg 1 TDS Gastrocote tabs PRN	no
14	49	F	163	>125	31	none	Becotide rotacaps 400µg QDS Ventolin rotacaps 800µg QDS VI PRN Zaditen tabs 1 BD	yes
15	60	M	169	73	2	occas.	Becotide rotacaps 400µg QDS Ventolin rotacaps 800µg QDS	no
16	39	F	163	76	15	ex	VI 2 puffs TDS Bextasol inhaler 1 puff TDS	yes
17	60	F	160	66	50	none	VI 2 puffs QDS BI 4 puffs TDS Dihydrocodeine tabs PRN for back pain	no

a, Becotide inhaler; b, Ventolin inhaler; c, has not smoked for at least 6 months prior to study; d, Becloforte inhaler.

TABLE 27: Study results for Subject 1

Predicted PEFR (l/min): 604
Predicted FEV_1 (l): 3.63
Predicted FVC (l): 5.12
Predicted FEF 25-75% (l/s): 3.4

Parameter	Placebo	Theoph. run in	Theoph.	Caffeine run in	Caffeine
FEV_1 (l)	1.08	1.30	1.25	1.12	1.35
FVC (l)	3.15	2.95	3.00	2.77	3.25
FEV_1/FVC (%)	34	44	42	40	41
FEF 25-75% (l/s)	0.31	0.35	0.35	0.34	0.35
PEFR (l/min) am	330	327	366	328	362
PEFR (l/min) pm	347	356	382	354	366
Inhaler use d^{-1}	6.5	6.3	5.3	6.6	5.4
Wheeze last night	11[a]	1	1	3	0
Cough last night	6	1	1	1	3
Wheeze today	16	9	3	18	8
Activity today	19	1	0	15	10
Sputum volume	10	13	2	13	9
Coffee (cups)	1.5	1.3	1.8	1.6	2.0
Tea (cups)	6.8	5.8	4.8	6.3	5.3
Chocolate (bars)	0	0.3	0.1	0	0.1
Comments	no signif. problems, no change	doing well, feels stable	static, "feels good"	no real change[b]	no change
Side effects	some flexural itching		some heart-burn in evenings		

a, between the first and second time periods Subject 1 changed his interpretation of "normal" from "normal in the population" to "normal for him": b, i.e. no change from previous period.
Values for lung functions and dietary xanthine intake are means.

Subjective scores are totals over the 2-week trial period.
No preference for a particular fortnight, most influence on chest from weather.

TABLE 28: Study results for Subject 2

Predicted PEFR (l/min): 580
Predicted FEV_1 (l): 3.30
Predicted FVC (l): 4.78
Predicted FEF 25-75% (l/s): 3.0

Parameter	Placebo	Caffeine run in	Caffeine	Theoph. run in	Theoph.
FEV_1 (l)	0.97	0.95	0.85		1.20
FVC (l)	2.88	2.55	2.52		3.54
FEV_1/FVC (%)	34	37	34		34
FEF 25-75% (l/s)	0.33	0.34	0.34		0.40
PEFR (l/min) am	173	194	174	175	171
PEFR (l/min) pm	228	241	202	239	226
Inhaler use d^{-1}	3.7	3.8	4.0	3.4	3.2
Wheeze last night	9	2	7	13	19
Cough last night	15	10	14	15	14
Wheeze today	10	11	13	14	14
Activity today	28	28	28	28	28
Sputum volume	21	15	23	28	28
Coffee (cups)	5.2	4.8	4.9	2.0	1.5
Tea (cups)				2.9	3.8
Comments	no change	well, no real change	no real change		
Side effects	some gum tenderness some flatulence no nausea	touch of nausea, sleeping well	upset stomach for 2 days, with pains and diarrhoea		slight nausea

No preference for any particular treatment period

TABLE 29: Study results for Subject 3

Predicted PEFR (l/min): 590
Predicted FEV_1 (l): 3.21
Predicted FVC (l): 4.29
Predicted FEF 25-75% (l/s): 3.3

Parameter	Placebo	Caffeine run in	Caffeine	Theoph. run in	Theoph.
FEV_1 (l)	1.72	1.00	1.10	1.55	0.96
FVC (l)	3.36	2.3	2.4	2.79	2.13
FEV_1/FVC (%)	51	43	46	55	45
FEF 25-75% (l/s)	0.67	0.46		0.73	
PEFR (l/min) am	207	284	260	256	241
PEFR (l/min) pm	276	325	277	288	282
Inhaler use d^{-1}	8.6	8.1	8.1	9.0	9.0
Wheeze last night	24	8	13	14	9
Cough last night	14	4	4	1	5
Wheeze today	14	8	12	14	13
Activity today	28	25	27	28	28
Sputum volume	2	0	7	6	9
Coffee (cups)	4.3	5.0	5.5	4.8	3.9
Tea (cups)	1.6	1.0	0.5	1.7	2.7
Comments	satis-factory	generally more short of breath	fair, chest tight for last 2 days	little tighter	no change
Side effects	none	none	none	none	

TABLE 30: Study results for Subject 6

Predicted PEFR (l/min): 430
Predicted FEV_1 (l): 2.22
Predicted FVC (l): 2.99
Predicted FEF 25-75% (l/s): 2.50

Parameter	Placebo	Caffeine run in	Caffeine	Theoph. run in	Theoph.
FEV_1 (l)	1.45	1.50	1.60	1.55	1.52
FVC (l)	2.00	2.12	1.75	2.03	2.19
FEV_1/FVC (%)	72	71	91	76	69
FEF 25-75% (l/s)	1.00	1.06	1.59	1.07	0.99
PEFR (l/min) am	174	223	238	266	267
PEFR (l/min) pm	246	283	267	280	295
Inhaler use d^{-1}	3.7	3.2	3.4	3.6	3.2
Wheeze last night	5	6	15	8	8
Cough last night	4	1	2	0	0
Wheeze today	6	3	7	6	4
Activity today	1	0	0	0	0
Sputum volume	0	0	0	0	0
Coffee (cups)	2.0	2.1	2.1	0.9	1.9
Tea (cups)	4.0	4.4	3.8	4.0	3.9
Chocolate (bars)	0.5	0.4	0.6	0.7	0.4
Coke (glasses)	0	0	0.1	0	0
Comments	some wheezy spells ? due to cold weather	sore throat last two days	less well slightly chesty cold	seems stable now	no problems
Side effects	headache on one day	mild headache last few days	restless at night dyspepsia flatulence headaches	still dyspepsia R_x Gaviscon	dyspepsia as usual

Stopped trial for one week between caffeine phase and theophylline run in due to dyspepsia, possibly aggravated by a short course of steroids for exacerbation due to chest infection.

TABLE 31: Study results for Subject 7

Predicted PEFR (l/min): 483
Predicted FEV_1 (l): 3.25
Predicted FVC (l): 4.06
Predicted FEF 25-75% (l/s): 3.7

Parameter	Placebo	Theoph. run in	Theoph.	Caffeine run in	Caffeine
FEV_1 (l)	2.78	2.85	2.65	3.18	3.00
FVC (l)	3.75	3.95	4.00	4.37	4.60
FEV_1/FVC (%)	74	72	66	73	65
FEF 25-75% (l/s)	2.34	2.47	2.00	2.73	
PEFR (l/min) am	294	276	270	271	294
PEFR (l/min) pm	253	298	305	305	349
Inhaler use d^{-1}	3.3	3.0	3.0	3.0	3.0
Wheeze last night	6	4	5	9	2
Cough last night	3	8	13	14	14
Wheeze today	5	5	6	11	13
Activity today	2	5	4	3	13
Sputum volume	1	0	0	0	0
Coffee (cups)	0.7	1.0	0.8	0.5	0.4
Tea (cups)	2.1	2.0	2.0	2.0	2.0
Coke (glasses)	0	0	0	0.1	0.1
Comments	very well	well	fairly good but heavy cold at present	chest is better	well, minor improvement
Side effects	heartburn first two days	none	none	heartburn "does not like these"	none

Stopped trial for 2 weeks between theophylline run in and theophylline due to heavy cold. Repeated one week of run in.

TABLE 32: Study results for Subject 9

Predicted PEFR (l/min): 553
Predicted FEV_1 (l): 2.70
Predicted FVC (l): 3.85
Predicted FEF 25-75% (l/s): 2.6

Parameter	Placebo	Theoph. run in	Theoph.	Caffeine run in	Caffeine
FEV_1 (l)	1.8	2.3[a]	1.9	1.72	1.94
FVC (l)	3.3	3.4	3.05	3.10	3.00
FEV_1/FVC (%)	54	68	62	55	65
FEF 25-75% (l/s)		1.54	1.05	0.74	1.20
PEFR (l/min) am	229	237	216	226	199
PEFR (l/min) pm	395	367	390	381	366
Inhaler use d^{-1}	8.1	6.6	7.8	8.1	9.1
Wheeze last night	24	10	5	4	18
Cough last night	13	6	0	0	5
Wheeze today	18	13	10	15	14
Activity today	16	0	0	0	0
Sputum volume	14	14	15	14	21
Coffee (cups)	4.5	4.2	4.6	5.5	5.0
Tea (cups)	1.3	1.3	0.5	0.3	0.3
Chocolate (bars)	0	0.1	0.1	0.1	0.2
Comments		no change	sleeping a little more soundly	sleeping well	no real change[b]
Side effects	none	none	irritation of skin R.elbow not clinically related to drug	minor dyspepsia	minor dyspepsia

a, patient unable to avoid using β-agonist inhaler for full four hours. Last used 3h 20 min before spirometry: b, i.e. no change from previous period.
No preference for any treatment period.

TABLE 33: Study results for Subject 10

Predicted PEFR (l/min): 615
Predicted FEV_1 (l): 3.68
Predicted FVC (l): 4.84
Predicted FEF 25-75% (l/s): 3.8

Parameter	Placebo	Caffeine run in	Caffeine	Theoph. run in	Theoph.
FEV_1 (l)	3.3	2.75	3.3	2.9	3.2
FVC (l)	4.9	4.80	4.17	3.9	4.3
FEV_1/FVC (%)	67	57	79	74	74
FEF 25-75% (l/s)			2.78		
PEFR (l/min) am	434	429	458	411	404
PEFR (l/min) pm	429	430	462	466	477
Inhaler use d^{-1}	4.6	5.0	5.0	5.1	5.6
Wheeze last night	22	28	29	28	29
Cough last night	1	0	0	8	11
Wheeze today	1	10	14	16	14
Activity today	0	0	0	8	14
Sputum volume	1	0	0	6	11
Coffee (cups)	6.7	6.7	6.1	7.1	7.4
Tea (cups)	2.1	2.1	2.2	2.1	1.4
Comments		no change		fairly good recovering from URTI	well
Side effects	none	more indiges-tion	patient reduced dose due to heart-burn	none	none

No preference for any treatment period.

TABLE 34: Study results for Subject 11

Predicted PEFR (l/min): 611
Predicted FEV_1 (l): 3.61
Predicted FVC (l): 4.94
Predicted FEF 25-75% (l/s): 3.5

Parameter	Placebo	Theoph. run in	Theoph.	Caffeine run in	Caffeine
FEV_1 (l)	2.95[a]	2.50	2.92	2.97	3.10
FVC (l)	4.25	4.12	4.34	4.28	4.00
FEV_1/FVC (%)	69	61	67	69	77
FEF 25-75% (l/s)	1.93	1.55	1.67	1.95	
PEFR (l/min) am	456	480	494	482	488
PEFR (l/min) pm	462	488	498	496	494
Inhaler use d^{-1}	9.8	10.3	9.2	11.3	11.1
Wheeze last night	15	9	6	13	11
Cough last night	14	7	2	7	6
Wheeze today	14	11	9	12	12
Activity today	14	13	13	13	14
Sputum volume	9	11	10	10	11
Coffee (cups)	1.9	2.1	2.0	2.1	1.9
Tea (cups)	3.8	4.2	4.0	3.6	3.1
Chocolate (bars)	0.9	0.6	0.8	0.9	0.3
Comments	fairly stable but not at best level	asthma seems better, easier nights	better nights, not needing to take Ventolin even if wakes	recent influenzal symptoms but mostly nasal	well
Side effects	none	indiges-tion as strength increased but settled	still indiges-tion but not as bad. Severe headache first 3 days	a little more tired than usual	none

a, spirometry 2 hours after β-agonist inhaler on this occasion.

TABLE 35: Study results for Subject 14

Predicted PEFR (l/min): 464
Predicted FEV_1 (l): 2.59
Predicted FVC (l): 3.35
Predicted FEF 25-75% (l/s): 2.9

Parameter	Placebo	Caffeine run in	Caffeine	Theoph. run in	Theoph.
FEV_1 (l)	1.08	1.05	1.15	1.02	1.10
FVC (l)	2.12	2.03	2.20	2.18	2.23
FEV_1/FVC (%)	51	52	52	47	49
FEF 25-75% (l/s)	0.54	0.46	0.48	0.48	0.52
PEFR (l/min) am	145	136	152	164	161
PEFR (l/min) pm	156	179	194	200	206
Inhaler use d^{-1}	6.3	5.1	4.4	4.4	4.4
Wheeze last night	14	14	0	4	8
Cough last night	6	4	5	4	4
Wheeze today	17	14	14	8	9
Activity today	20	14	15	8	9
Sputum volume	0	0	0	1	1
Coffee (cups)	6.1	5.9	5.6	5.5	5.1
Tea (cups)					
Comments	quite severely obstructed notices effect of recent wet weather	fine	feeling better could be due to more dry days	fine but hay fever season now	fine except for hay fever
Side effects	none	none	none	none	none

Overall preference for last 2 weeks.

TABLE 36: Study results for Subject 16

Predicted PEFR (l/min): 485
Predicted FEV_1 (l): 2.86
Predicted FVC (l): 3.62
Predicted FEF 25-75% (l/s): 3.3

Parameter	Placebo	Caffeine run in	Caffeine	Theoph. run in	Theoph.
FEV_1 (l)	2.61	2.42	2.61	2.58	2.63
FVC (l)	3.80	3.47	3.63	3.55	3.76
FEV_1/FVC (%)	69	70	72	73	70
FEF 25-75% (l/s)	1.17	1.73	2.02		
PEFR (l/min) am	262	288	301	290	297
PEFR (l/min) pm	240	284	303	292	305
Inhaler use d^{-1}	3.0	2.5	0.7	1.5	1.2
Wheeze last night	10	6	1	5	3
Cough last night	9	7	0	5	2
Wheeze today	12	8	2	3	4
Activity today	5	5	0	6	3
Sputum volume	0	0	0	0	0
Coffee (cups)	1.6	0.9	1.1	1.7	1.2
Tea (cups)	3.5	3.5	3.3	3.2	3.2
Comments	not too bad feeling tired	no change	feels better not having to use inhaler so much	feels better weather improving	no change
Side effects	tiredness headache 2-3 times a week but this is normal	irrit-ability for 1st few days	back pain cleared on its own probably not related	none	a little indiges-tion

FIGURE 34 Variation in FEV_1 for each subject over the course of the study

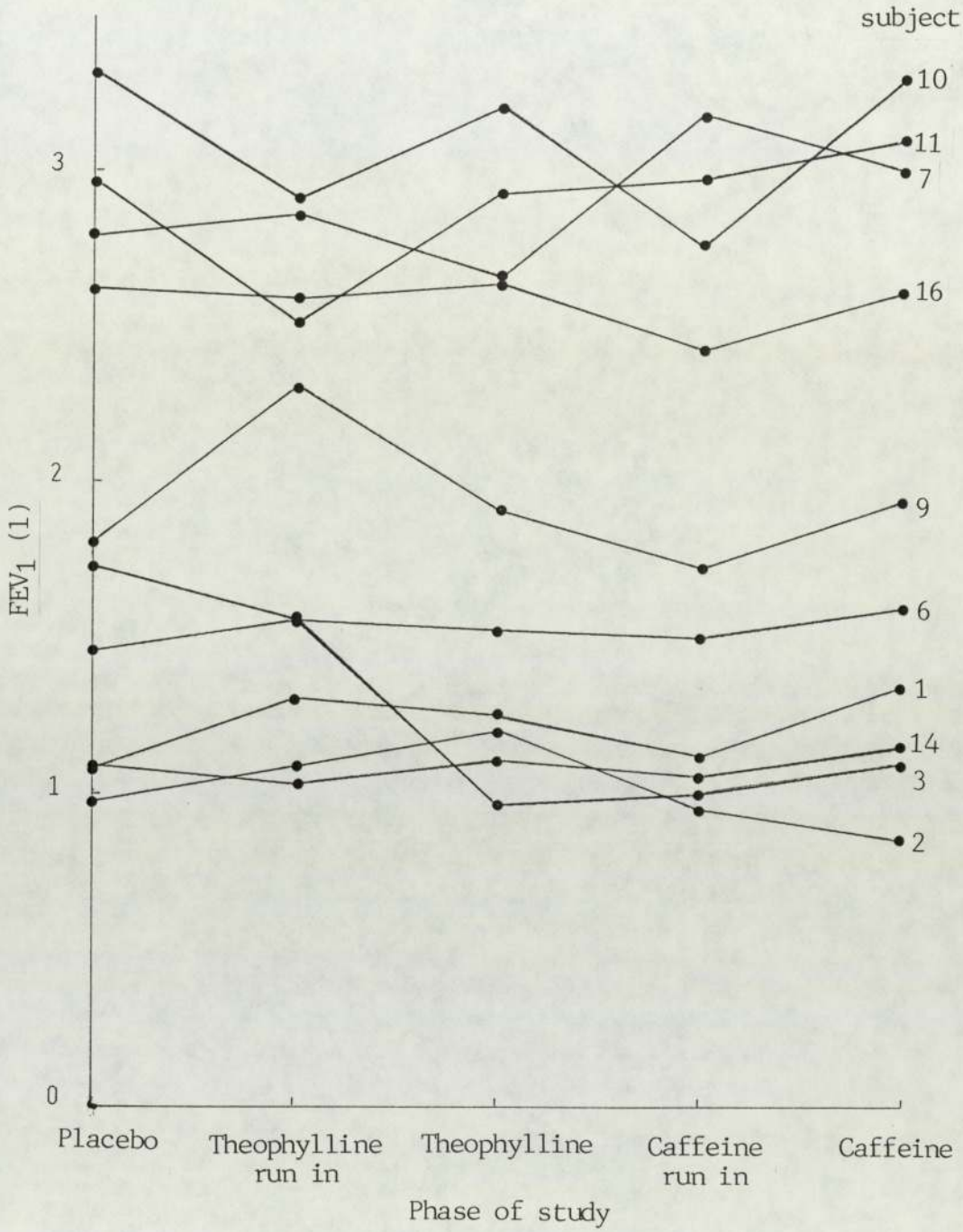


FIGURE 35 Variation in PEFR before morning medication for each subject over the course of the study

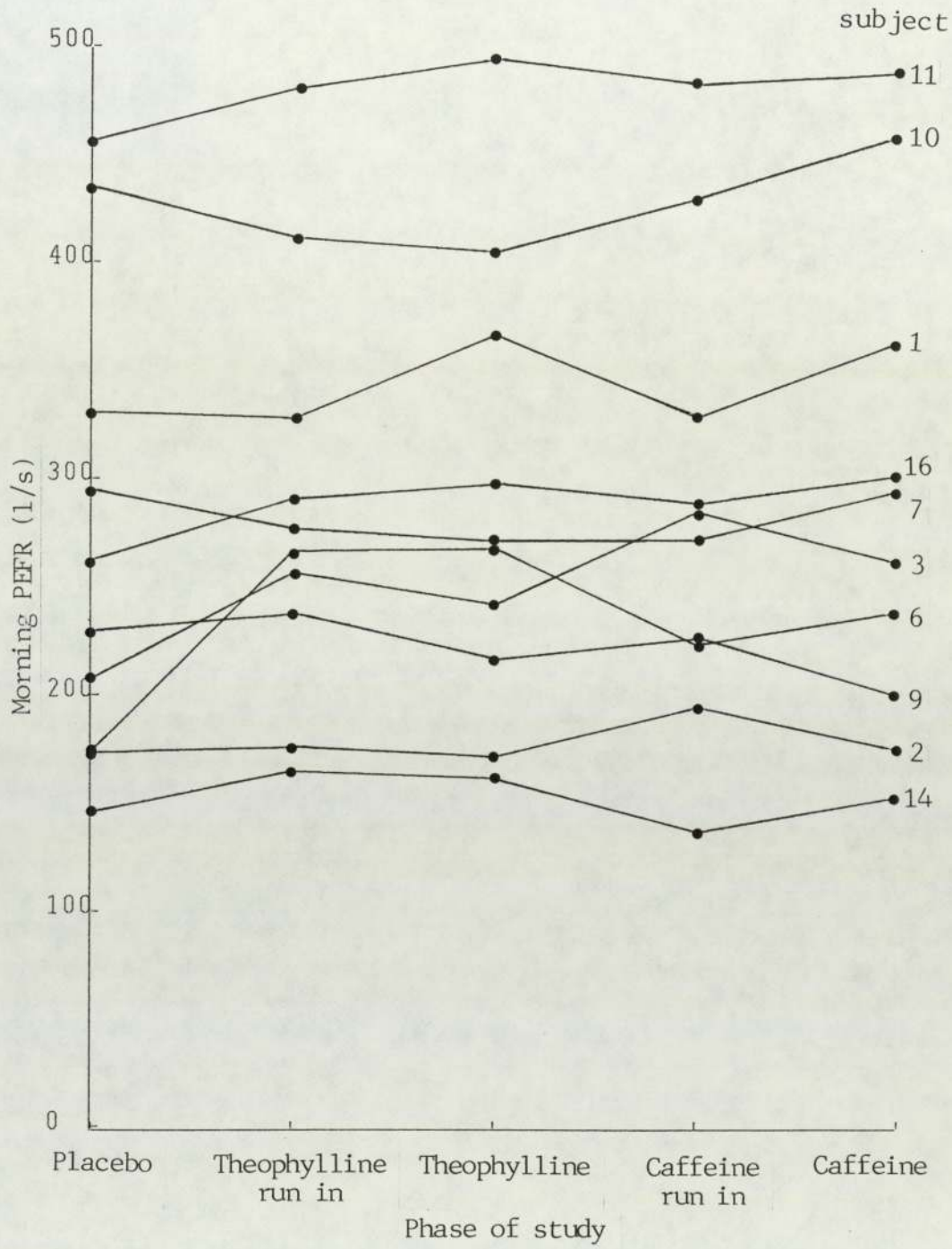


TABLE 37: Mean (and standard deviation) study results

Predicted PEFR (l/min): 541 (69)
Predicted FEV_1 (l): 3.10 (0.49)
Predicted FVC (l): 4.18 (0.73)
Predicted FEF 25-75% (l/s): 3.2 (0.4)

Parameter	Placebo	Theoph. run in	Theoph.	Caffeine run in	Caffeine
FEV_1 (l)	1.97 (0.86)	2.06 (0.71)	1.93 (0.84)	1.87 (0.88)	2.00 (0.92)
FVC (l)	3.35 (0.89)	3.21 (0.77)	3.25 (0.86)	3.18 (1.00)	3.15 (0.94)
FEV_1/FVC (%)	57 (15)	63 (12)	58 (14)	59 (15)	62 (18)
FEF 25-75% (l/s)	1.04 (0.75)	1.17 (0.74)	1.00 (0.64)	1.09 (0.86)	1.25 (0.94)
PEFR (l/min) am	270 (108)	288 (98)	289 (105)	286 (105)	293 (114)
PEFR (l/min) pm	303 (100)	327 (93)	337 (98)	328 (92)	328 (100)
Inhaler use d^{-1}	5.8 (2.4)	5.3 (2.8)	5.2 (2.7)	5.7 (2.8)	5.4 (3.1)
Wheeze last night	14.0 (7.1)	9.6 (7.7)	9.3 (8.4)	9.3 (7.7)	9.6 (9.5)
Cough last night	8.5 (5.2)	5.5 (4.4)	5.2 (5.4)	4.8 (4.7)	5.3 (5.0)
Wheeze today	11.3 (5.7)	9.9 (4.4)	8.6 (4.2)	10.4 (5.0)	10.9 (4.0)
Activity today	13.3 (10.7)	9.7 (10.5)	8.7 (11.1)	10.3 (10.4)	11.0 (10.5)
Sputum volume	5.8 (7.4)	8.9 (8.6)	7.6 (9.1)	5.2 (6.8)	7.1 (8.9)
Coffee (cups)	2.9 (2.0)	2.8 (2.1)	2.8 (2.1)	3.0 (2.3)	3.0 (2.2)
Tea (cups)	3.1 (1.8)	3.0 (1.4)	2.9 (1.4)	2.9 (1.9)	2.6 (1.7)
Chocolate (bars)	0.1 (0.3)	0.4 (0.6)	0.1 (0.3)	0.1 (0.3)	0.1 (0.2)

TABLE 38: The ANOVA of pulmonary function tests, subjective scores and dietary xanthine intake

Parameter	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	Prob. > F
FEV_1	between levels	0.02	2	0.01	0.01	0.985
	residual	20.85	27	0.77		
	total	20.87	29			
FVC	between levels	0.20	2	0.10	0.12	0.885
	residual	21.84	27	0.81		
	total	22.04	29			
FEV_1/FVC	between levels	138	2	69.23	0.27	0.763
	residual	6856	27	253.91		
	total	6994	29			
FEF 25-75%	between levels	0.27	2	0.13	0.22	0.807
	residual	11.67	19	0.61		
	total	11.94	21			
PEFR am	between levels	2810	2	1405	0.12	0.890
	residual	322725	27	11953		
	total	325535	29			
PEFR pm	between levels	6015	2	3008	0.31	0.739
	residual	265530	27	9834		
	total	271545	29			
Inhaler use	between levels	1.59	2	0.80	0.10	0.903
	residual	209.10	27	7.74		
	total	210.69	29			
Wheeze last night	between levels	138	2	69.23	0.98	0.390
	residual	1915	27	70.91		
	total	2053	29			
Cough last night	between levels	70.47	2	35.23	1.30	0.290
	residual	734.20	27	27.19		
	total	804.67	29			
Wheeze today	between levels	42.27	2	21.23	0.97	0.393
	residual	593.40	27	21.98		
	total	635.67	29			

TABLE 38: The ANOVA of pulmonary function tests, subjective scores and dietary xanthine intake (continued)

Parameter	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F ratio	Prob. > F
Activity today	between levels	63	2	31.60	0.27	0.765
	residual	3159	27	117.0		
	total	3222	29			
Sputum volume	between levels	17	2	8.63	0.12	0.887
	residual	1943	27	71.96		
	total	1960	29			
Coffee	between levels	0.21	2	0.11	0.02	0.977
	residual	98.82	22	4.49		
	total	99.03	24			
Tea	between levels	1.41	2	0.70	0.27	0.768
	residual	57.87	22	2.63		
	total	59.28	24			

TABLE 39: Reasons for subjects withdrawing from the study

Subject	Time from Start (days)	Reason
4	4	Prior to the study this subject had been taking Phyllocontin 450mg BD. Discontinuation of this treatment in the placebo phase rapidly lead to a worsening of the condition. Peak flows were 250, 150, 110 and 50l/min on successive mornings and 320, 170, 120 and 60l/min on successive evenings. There were increases in all subjective scores and in inhaler use.
5	21	This subject decided to withdraw from the study because she required medical attention for severe neck pains, a long-standing condition, and did not wish to attend two different clinics.
8	20	Theophylline 100mg QDS produced side effects of nausea and vomiting without reaching therapeutic blood concentrations. This subject was taking another gastric irritant drug, Naproxen, and had taken long-term oral steroids. On stopping the study she admitted "sometimes" taking Cimetidine tablets 400mg nocte, a fact which had not been revealed on previous questioning.
12	34	This subject failed to keep several appointments, possibly due a chest infection.
13	2	Prior to the study this subject had been taking Phyllocontin 225mg BD. On starting the placebo phase of the study this subject felt unwell with increased wheeze (score increased from 1 to 3) and headaches, and decided to revert back to the Phyllocontin.
15	1	This subject withdrew without explanation. He had complained that the diary card was too complicated.
17	17	This anxious lady withdrew due to feelings of tiredness, particularly in the legs. Before the study she had been taking Phyllocontin 450mg BD.

FIGURE 36 Plasma caffeine concentration vs plasma theophylline concentration for all patients during the placebo and caffeine phases of the study (the phase where the patients were taking theophylline is not included)

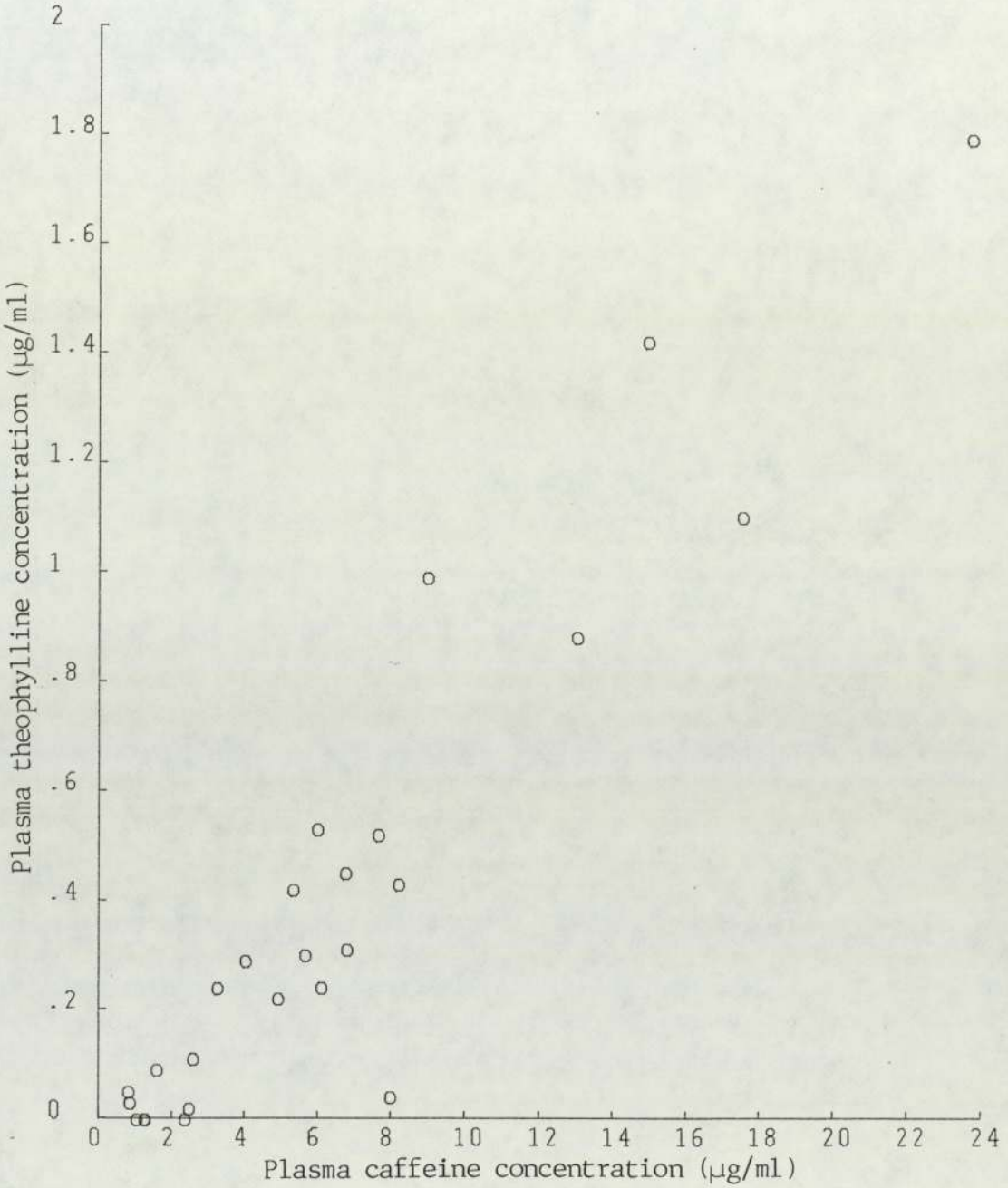
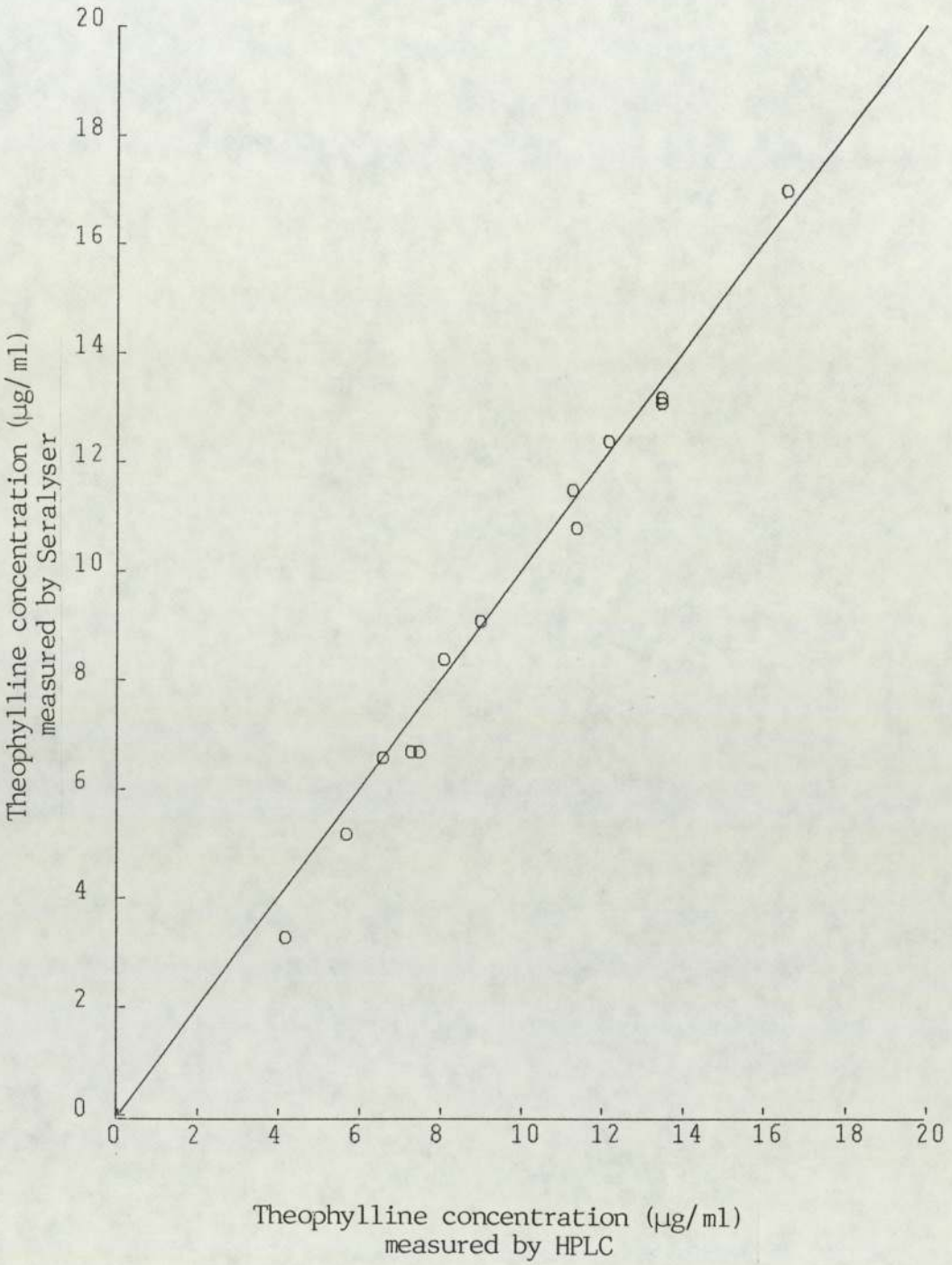


FIGURE 37 Plasma theophylline concentration obtained using a Seralyser compared with concentration obtained using HPLC. The line of identity is plotted.



IV.7 Discussion

The choice of ten weeks for the length of study was a balance between keeping it sufficiently short to be acceptable to the patients while being long enough for a stable period to be reached on each medication. Ideally the placebo period would not have been a 2-week single blind period at the start of the study but would have been double blind and of four weeks duration, of which two weeks was "run-in" and two weeks continuation, the placebo period being randomised with the two treatment periods. This, however, would have added an extra two weeks to the total length of the study, which might have resulted in fewer patients agreeing to participate or lack of compliance towards the end of the trial period. Since asthmatic patients experience fluctuations in their condition as a result of changes in the weather, amount of exercise taken, chest infections etc. each treatment period had to be sufficiently long that these changes did not influence the results. Most patients were adversely affected by cold damp weather, however randomisation of the order of treatment should have prevented seasonal variation from affecting outcomes. All except one of the patients, Subject 15 who withdrew from the study, found the diary card easy to understand and were so interested in monitoring their own performance that no peak flow readings were missed. Compliance with the four times daily regimen as determined by counts of capsules returned and backed up by blood concentration monitoring, was good, possibly because most patients had to remember to use inhalers at least four times daily. A sustained release preparation would have been more satisfactory than the simple non-sustained release capsules used, however no sustained release

preparation of caffeine is manufactured and it was necessary to keep the appearance identical and prevent recognition by either the patient or the clinician. Using a "double dummy" technique with a sustained release theophylline and simple caffeine-filled gelatin capsules would have conferred an advantage on theophylline in terms of a smoother plasma profile. A further disadvantage of using a non-sustained release preparation was the difficulty of timing the collection of plasma samples. The times for sampling, one hour after dosage for caffeine and two hours for theophylline, were chosen to be close to peak concentrations[2], since for the purpose of this study the maximum concentration, where side effects and therapeutic effects are greatest, were thought to be more important than trough concentrations. However it was realised that every sample may not have been taken at the exact peak level due to patient-to-patient variation in absorption and metabolism. Patients were advised always to take the capsules with or after food to minimise gastric irritation. Patients were asked not to use a β-agonist inhaler or rotacaps for four hours before spirometry. This again was a balance designed to be acceptable to the patients. If the time chosen had been longer some patients would have been too breathless to get to the clinic. Patients were asked that if they could not last the full four hours that they should make a note of the time they last used the inhaler. Had the time been any shorter residual effects would have made interpretation of lung function data with respect to xanthine treatment impossible.

The choice of the figure of 10% reversibility as an inclusion criterion, lower than that used in some studies, was to bar as few people as possible from entry into the study. Patients with an

irreversible component to their disease may benefit from methylxanthines due to a reduction in muscle fatigue[48]. Patients taking cimetidine were excluded from the study since the interaction can cause a decrease in theophylline metabolism and since this indicates a history of GI ulceration which could be aggravated either by caffeine or theophylline. The exclusion criteria, although necessary, may be a source of bias in the sample of patients. Patients who had already taken theophylline but had discontinued due to severity of side effects were not included in the study. Thus if these patients are considered a completely representative sample of the total population the incidence of side effects would be lower than it is in reality. Similarly patients on oral steroids were excluded to avoid the confounding effects that a short course of steroids in one particular treatment period could have. However this excludes some of the most severe patients and those who may be more sensitive to the gastric side effects of the xanthine since they are already taking one gastric irritant drug. On the other hand since the study involved several daytime clinic visits it tended to attract retired people, adding another source of bias, particularly if they had taken early retirement as a result of their COPD. The mean age of the patients completing the study was 51, ranging from 28 to 65. Other patients who were able to attend the clinic appointments were shift workers, part-time workers and housewives.

The pulmonary function tests used in this study are well established and used routinely in chest clinics throughout the country. They have the advantage of being quick, simple and not unpleasant to perform. The FEV_1 is relatively effort independent and has excellent

reproducibility. It is recommended, together with FVC, as the measurement of first choice for routine work[159]. The $FEF_{25-75\%}$ has been recommended as an early index of airway obstruction, although it is limited by wide variation within healthy, non-smoking populations, with low correlations for age and height[157]. PEFR has the advantage that the mini Wright peak flow meter is cheap and portable and so may be used at home. However a disadvantage is that this test is effort dependent and relies on patient motivation and absence of cheating for example by coughing into the peak flow meter. PEFR is more useful than FEV_1 in the measurement of variability of airways obstruction over a short timescale for example at different times of day. Thus measurements of peak flow and spirometry complement each other. Taking the best of three spirometric measurements at any one time has been shown to be sufficient to record a patients performance since the best test occurred with equal frequency among the first three tests but after that subjects tended to tire[160]. The predicted values were obtained using nomograms calculated from healthy, non-smoking populations. The percentage of the mean predicted values obtained during the placebo period are as follows: PEFR am 50%, FEV_1 63% and FVC 80%, indicating the severity of disease in these patients.

The study suffered from a high withdrawal rate, 41%, (see Table 39) for a variety of reasons including other medical problems, intolerable side effects and failure to keep clinic appointments. A significant point is that two patients withdrew from the study because they were unable tolerate discontinuation of their usual dose of theophylline when required to do so in the placebo phase. When considering the results it is necessary to take into account the fact that the two

patients who might have benefitted most from methylxanthine treatment had to be excluded from the study because they were unable to complete the placebo phase. Nevertheless the study would have been meaningless if a placebo phase had not been included.

One of the major criticisms of this study is that normal dietary methylxanthine intake was permitted. The reason for this was simply that very few patients would have been prepared to take part had this not been the case. The caffeine and theophylline concentrations due to dietary intake alone were 2.47 $\pm$ 1.73μg/ml and 0.10 $\pm$ 0.13μg/ml respectively. This compares with a mean plasma caffeine concentration of 2.12μg/ml found by Smith[161] in a study of 600 adults. The range was from <0.2μg/ml to 13.1μg/ml with no significant difference between men and women. In 95% of the population the concentration was less than 5.6μg/ml and in 5.8% the concentration was <0.2μg/ml. An attempt was made to quantify dietary xanthine intake by asking all patients to record in their diary cards the amount of coffee, tea, chocolate and Coca-Cola they had taken each day, however this could only be a rough approximation of caffeine intake due to variables such as size of cup, strength of brew and method of preparation. When the caffeine plasma concentration due to dietary intake alone, 2.47 $\pm$ 1.73μg/ml is compared with the peak achieved in the maintenance caffeine treatment phase of the study, 11.4 $\pm$ 3.8μg/ml it can be seen that dietary intake did not completely invalidate the study.

The plasma concentrations of both caffeine and theophylline were measured in all samples since it had been suggested that any broncho-dilation produced on treatment with caffeine might be due to

theophylline produced by its metabolism. This was clearly not the case since the theophylline concentration never exceeded 1.8μg/ml during the caffeine treatment phase, well below the therapeutic range. Gong[156] reported a maximum theophylline concentration of 1.9μg/ml 3 hours after ingestion of 450mg of caffeine. In the present study, a linear relationship was seen between plasma caffeine concentration and plasma theophylline concentration during the placebo and caffeine treatment phases of the study. Had this correlation only been seen in the placebo phase an explanation might have been that those patients who had a high dietary intake of caffeine also tended to have a higher dietary intake of theophylline. However this relationship persisted when the main source of caffeine intake was in the form of pure powder in the study capsules. The more likely explanation therefore seems to be a build up of theophylline as a product of caffeine metabolism. A linear relationship was not seen when the placebo and theophylline phases of the study were considered, although it might have been expected since caffeine is a minor metabolite of theophylline. A possible explanation is that dietary intake confused the picture, whereas in the previous case the dietary intake of theophylline was small enough to be insignificant.

The Ames Seralyser was found to be a quick and efficient method for analysing plasma theophylline concentration. It was particularly useful in situations where a sample was analysed before the patient left the clinic and a change in dose made after consideration of this information. A good correlation was found with HPLC, although only theophylline concentrations could be measured using the Seralyser. Good correlations between the Ames Seralyser and HPLC, substrate-

labelled fluorescent immunoassay and enzyme-multiplied immunoassay have previously been documented[162-165].

One way analysis of variance was considered the most appropriate statistical test to apply to the data since three groups were compared, placebo, treatment with caffeine and treatment with theophylline. This test compares a measure of variation or scatter within each treatment group with the scatter between each group. The resultant F value was compared with tabulated values to determine its significance. The last column in Table 38 gives the probability that the variation between treatment groups occurred by chance, eg there was a 98.5% probability that variation in FEV_1 between placebo, treatment with theophylline and treatment with caffeine occurred by chance. There was no significant difference between the three treatment periods at the 5% level in either pulmonary function tests or subjective scores. Although the subjective scores produced lower probabilities of variation being entirely due to chance than the pulmonary function tests, the lowest probability, that for "cough at night", was 29%, far too high to indicate a significant difference between the three treatment periods.

Even if the bronchodilator activity of caffeine proved too weak to stand up to statistical analysis a significant difference might have been expected between placebo and theophylline. There are a number of factors which may contribute to the results obtained. As previously mentioned two patients whose response to theophylline was good were excluded from the study since they could not tolerate the placebo period. The length of each study phase of two weeks may have been too

short to obviate the fluctuations in condition caused by factors other than the treatment. Since some of the patients did the study during the winter months several had chest infections for part of the trial. Theophylline is less beneficial where the airway obstruction is largely irreversible; had a reversibility or 15% rather than 10% been specified an improvement in pulmonary functions and subjective scoring for theophylline, even if not for caffeine, might have been seen. A further consideration is that all patients were already receiving multiple treatments, which might have been optimal, affording no room for further improvement. The number of patients recruited for this study was smaller than originally intended and together with a high withdrawal rate made the chance of a statistically significant result smaller. Reasons for poor recruitment are as follows: the entry criteria for the study were strict and excluded many patients; several daytime clinic visits were necessary, a factor which caused several patients with full-time jobs to decline; some patients although initially willing to take part were disuaded by anxious relatives; the Osler Chest Clinic is an active research centre, many patients have already taken part in at least one study or were currently involved in another study.

No formal studies have compared the ability of caffeine and theophylline to cause gastric irritation and heartburn, however in this study complaints of GI side effects were approximately equal for the two treatments. This is in contrast to the situation in neonates where caffeine has a much wider therapeutic index than theophylline. While theophylline may produce tachycardia at concentrations of 13μg/ml, no obvious cardiovascular neurologic or gastrointestinal

toxicity was observed at plasma caffeine concentrations up to 50μg/ml[122]. In Becker's study[155] in children aged 8 to 18 years there was no significant difference between the adverse effects in the caffeine-treated group and the theophylline-treated group. Side effects were mild and transient and included headache, dizziness, shakiness and tremor. However it must be noted that the mean theophylline plasma concentration (8.4 ± 1.7μg/ml) was lower than the mean caffeine plasma concentration (13.5 ± 2.9μg/ml).

Since in adults caffeine has no advantage over theophylline in terms of side effects and has a weaker bronchodilator activity[155, 156] it seems very unlikely that it has a place as an alternative to theophylline in the treatment of obstructive airways disease except perhaps in emergency situations when theophylline is not available.

V. CONCLUSION

With the wide dietary consumption of caffeine, the use of theophylline in the treatment of obstructive airways disease and the use of both caffeine and theophylline in neonatal apnoea the methylxanthines seem set to give cause for study and debate for years to come. More studies are needed to investigate the interactions between caffeine and theophylline since there are several applications where this may be important, such as the exact dietary advice given before pulmonary function tests, the effect of change in dietary caffeine intake on plasma theophylline concentration and the combined gastric irritant effect of therapeutic concentrations of theophylline and a high dietary intake of caffeine.

Despite advances in the use of theophylline with sustained release preparations and therapeutic monitoring neither it nor caffeine have the characteristics of an ideal bronchodilator. Much work has already been done[166-168] in an attempt to develop better bronchodilators related to theophylline but with fewer side effects. It is most important that this search should continue.

APPENDIX 1

PROTOCOL

A DOSE-RESPONSE STUDY OF CAFFEINE, WITH ANALYSIS OF METABOLITES

Aim

To determine the relationship between dose of caffeine and serum concentrations attained, and to obtain information about the kinetics of its metabolites.

Rationale

Some studies, for example that of Sarrazin et al.[1], have shown that theophylline, a xanthine derivative closely related to caffeine, exhibits dose-dependent kinetics. This is important as a small increase in dose may lead to an increase in serum concentrations much greater than expected and result in toxicity. Others however have not seen this effect within the normal therapeutic range of serum concentrations[2]. A similar disagreement exists for caffeine, with some evidence of linear kinetics[3, 4] but other results demonstrate dose dependence[5, 6]. It is hoped this study will help to resolve the issue and will provide further information on the kinetics of the main metabolites of caffeine.

Design

Four healthy adult volunteers will take part in this study. Each will receive doses of caffeine of 200mg, 300mg, 400mg and 500mg on separate mornings with blood, urine and saliva samples taken at intervals during the following 24h. Initially, one subject will be studied at one dosage level to determine the optimum times of sample collection for the rest of the study. Analysis of samples for caffeine and its metabolites will be performed using High Performance Liquid Chromatography.

Volunteers

These will be healthy non-smoking adults employed at the Churchill Hospital. Persons taking any medications except oral contraceptives will be excluded from the study.

Drugs and Dosage

Capsules containing anhydrous caffeine 200mg, 300mg, 400mg and 500mg prepared by the investigator will be taken by each volunteer on separate days.

Assessments

Blood, urine and saliva will be analysed for caffeine and its metabolites by HPLC using the method of Muir et al.[7] Any adverse effects experienced during the trial will be noted.

Possible Adverse Effects

Tremor, tachycardia and restlessness may be experienced but are expected to be mild.

Procedure

The first volunteer will abstain from caffeine containing foods and beverages for 24h. A capsule containing 300mg caffeine will be taken and washed down with 200ml water one hour after a light breakfast. Blood samples (5ml) will be collected via an indwelling heparinised cannula at the following times after dosage: 0, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, 480, 540, 600, 660, 720 and 1440 mins. All blood samples will be centrifuged within one hour of collection and the plasma harvested stored at -20°C until analysed for caffeine and its metabolites. Saliva samples will be collected at the same times as blood samples. Urine samples will be collected at hourly intervals for the first 12h and at 24h. Throughout the study period the volunteer will abstain from caffeine-containing foods and beverages and will report any side effects.

This method will be repeated in this volunteer at other dosage levels, and in the other three volunteers at each dosage level, with blood, urine and saliva samples taken at those times deemed necessary from the initial trial.

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APPENDIX 2

LETTER OF EXPLANATION TO PATIENTS

The Churchill Hospital
Headington
Oxford
OX3 7LJ

Dear Patient

You are being asked to participate in a drug study conducted at the Churchill Hospital to compare the effects of caffeine and theophylline on breathing. You have been selected for the study because you have asthma and should benefit from treatment with theophylline or caffeine.

The plan of the study is that you would attend the clinic to have your breathing measured and you will be given capsules to take four times a day. After three days we will take a blood sample to see if you have the right amount of drug in your blood. We hope over the period of a fortnight to find the dose of drug that is right for you. This may mean a further blood test and alteration of dose. During this fortnight we would like you to take a peak flow meter home and measure your peak flows twice a day and answer some questions on how you feel each day. After a fortnight we would like you to continue taking this drug for another fortnight, still making the peak flow readings. (We will check your breathing and the amount of drug in your blood in clinic at the end of the fortnight). After this you will change to the other drug using the same plan.

Of possible benefit to you is that we will find the best dose of drug for you and your breathing will be easier and asthmatic attacks will not occur or they will decrease in number.

Theophylline and caffeine can produce side effects of nausea, tremor, fast heart rate and restlessness, although the chances of any serious side effects occuring are extremely rare. In the event of any side effect please contact immediately either the persons mentioned below and/or report to the Chest Unit at the Churchill Hospital.

All records will be kept strictly confidential. In any publication of the results you will be referred to by initials or code. We suggest you keep this letter and show it to any doctor who may become involved in your care.

Names addresses and telephone numbers of investigators:

Dr D J Lane, Osler Chest Unit, Churchill Hospital, Headington, Oxford
Tel: Oxford 64841 ext 601
Mrs J E Hemingway, (work) Pharmacy Department, Churchill Hospital
(home) 9 Lime Ct, Lime Walk, Headington, Oxford
Tel: Oxford 60551

APPENDIX 3

LETTER OF EXPLANATION TO GENERAL PRACTITIONERS

The Churchill Hospital
Headington
Oxford
OX3 7LJ

Dear Doctor

Your patient ..... has agreed to participate in a drug study conducted at the Churchill Hospital to compare the clinical effects of caffeine and theophylline in asthmatic patients.

The plan of the study is that he/she will be treated with either theophylline or caffeine for four weeks, including a 2-week "run in" period when the dosage will be adjusted to achieve optimal blood concentrations. Throughout the four weeks he/she will be measuring peak flows twice daily and filling in a diary card. Blood concentrations and lung functions will be measured at two weeks and four weeks. This procedure will then be repeated with the other drug. The study will be carried out double blind. Any medications taken regularly will be continued throughout the trial period and use of a beta agonist inhaler when required will be permitted.

Side effects of theophylline and caffeine include nausea, vomiting, tachycardia, tremor, anxiety and rarely convulsions. If the need arises do not hesitate to contact us at the telephone numbers and addresses given below.

Dr D J Lane, Osler Chest Unit, Churchill Hospital, Headington, Oxford
Tel: Oxford 64841 ext 601
Mrs J E Hemingway, (work) Pharmacy Department, Churchill Hospital
(home) 9 Lime Ct, Lime Walk, Headington, Oxford
Tel: Oxford 60551

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