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Plasma and Salivary Pharmacokinetics of Caffeine in Man

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Summary. Plasma and salivary caffeine concentrations were measured by gas-liquid chromatography in 6 healthy caffeine-free volunteers following oral administration of 50, 300, 500 and 750 mg caffeine. Caffeine was also given to a single subject intravenously in doses of 300, 500 and 750 mg. Caffeine was rapidly absorbed and was completely available at all doses. The apparent first-order elimination rate constant decreased linearly with dose and was 0.163 ± 0.081 h^{-1} for 50 mg and 0.098 ± 0.027 h^{-1} for 750 mg. The total body clearance was unaffected by dose and was 0.98 ± 0.38 ml/min/kg. There was a trend towards increasing apparent volume of distribution with increasing dose. A linear relationship existed between the area under the plasma concentration, time curve and dose and dose-normalised plasma concentration, time plots were superimposable. These findings suggest that caffeine obeys linear pharmacokinetics over the dose range investigated. Despite significant inter-individual differences in pharmacokinetic parameters there was good reproducibility within 5 subjects given 300 mg caffeine orally on 3 occasions. Salivary caffeine levels probably reflect the unbound plasma caffeine concentration and can be used to estimate the pharmacokinetic parameters of the drug. Overall the saliva/plasma concentration ratio was 0.74 ± 0.08 but within subjects some time-dependence of the ratio was found with higher ratios initially (even after intravenous administration) and lower ratios at longer time intervals after the dose. Urinary elimination of caffeine was low and independent of dose: 1.83% of the dose was eliminated unchanged.

Key words: caffeine; pharmacokinetics, plasma, saliva, urinary elimination

Caffeine is probably the most widely ingested natural alkaloid. It is present in a variety of beverages: the average dosage being 83 mg/cup of brewed coffee and 41 mg/cup of tea (Burg 1975). It also occurs in cocoa, chocolate and soft drinks derived from nuts of Cola acuminata (up to 55 mg/12 oz bottle). The annual world consumption of coffee alone exceeds 4 million tons which in some countries implies a consumption of 100 g/person/year (Levi 1967). In addition it is included in many medications, particularly over-the-counter stimulants and headache mixtures. Attention has been drawn to possibly deleterious hypertensive effects of caffeine (Robertson et al. 1978) and an association has been suggested between coffee drinking and an increased risk of myocardial infarction (Jick et al. 1973). Recent epidemiological analyses, however, have indicated that heavy coffee consumption plays no significant role in the aetiology of cardiovascular diseases in general and myocardial infarction in particular (MacCornack 1977).

Investigations of caffeine pharmacokinetics in man have largely concentrated upon the induction and inhibition of its metabolism in animals and man (Welch et al. 1977; Parsons and Neims 1978; Patwardhan et al. 1980). There is, however, evidence for non-linear pharmacokinetic behaviour from animal studies. Thus in mice the elimination half-life is prolonged from 0.6 to 1.7 h following administration of 5 and 25 mg/kg caffeine respectively (Burg and Werner 1972). Similarly, studies in rats given oral or intravenous caffeine have indicated a non-linear increase of the area under the plasma concentration, time curve and deviations from first order kinetics of elimination (Aldridge et al. 1977; Latini et al. 1978). Although no detailed studies relating to caffeine have been reported in man, dose-dependent kinetics of the related xanthine theophylline have been demonstrated in asthmatic children (Weinberger and Gin-

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chansky 1977). It has also been shown that the elimination of theophylline metabolites follows parallel first-order and Michaelis-Menten kinetics and that the methylxanthines contained in foods and beverages may suppress its metabolism, presumably by competition for shared common metabolic pathways (Monks et al. 1979). Furthermore, loading subjects with theobromine by chronic administration prolongs the half-life of this xanthine (Drouillard et al. 1978). The data in this paper has, therefore, been examined particularly from the point of view of detecting non-linearity in caffeine pharmacokinetics.

Materials and Methods

1. Estimation of caffeine concentrations in biological samples was by gas chromatography: to 0.5 ml plasma or saliva or 0.2 ml urine in a ground glass stoppered tube were added 20 µl of a 100 µg/ml methanol solution of phenacetin (internal standard), 0.1 ml 0.1 M sodium hydroxide and 3 ml redistilled ethyl acetate (Analar). This was vortexed for 20 s and the ethyl acetate was separated by centrifugation and quantitatively transferred to a conical tube and evaporated to dryness in a stream of dry nitrogen. The residue was dissolved in 20 µl methanol (50 µl for urine) and 1–3 µl injected into the gas chromatograph (Pye-Unicam series 104 with an alkali flame ionisation detector). The column was 2.4 m × 4 mm borosilicate glass column packed with 3% Poly S 179 (Supelco Inc.) on Gas Chrom Q 60–80 mesh (DCMS treated). Operating conditions were: temperatures – detector 330 °C, column 225 °C, and gas flow rates – air 480 ml/min, hydrogen 37 ml/min, nitrogen 75 ml/min. The retention times for phenacetin and caffeine were 1.5 and 3 min, respectively. The extraction efficiency of ethyl acetate for caffeine was 76% at 1 µg/ml and 71% at 5 µg/ml. Calibration curves for plasma, saliva and urine were run during each analysis and were linear over the range 0.5–50 µg/ml. The sensitivity of the assay was 0.05 µg/ml and the coefficient of variation was 6.5% at 1 µg/ml and 1.6% at 10 µg/ml. The correlation coefficient for assayed and actual caffeine concentrations in spiked samples was 0.9993.

2. Pharmacokinetic study 6 healthy non-smoking adult volunteers (one female, five male; mean age 25 years, range 21 to 36 years; mean body weight 69 kg, range 54 to 84 kg) consented to participate in the experiment. The protocol was approved by the Guy's Hospital Ethical Committee. Subjects abstained from caffeine-containing foods and beverages for 72 h before the study and throughout the investigation. To ensure that caffeine was absent from the plasma prior to commencement of the study, a blood sample was analysed for caffeine on the evening preceding the study. After an overnight fast, subjects reported to the laboratory and blood and mixed saliva samples were taken for analytical blanks. Each subject received in random order on separate occasions caffeine base (Sigma Chemicals) made up in gelatine capsules as doses of 50, 300, 500 or 750 mg. The capsules were swallowed with 100 ml water. One subject received only the 300, 500 and 700 mg doses: this subject also failed to produce salivary samples for the 500 mg caffeine dose. Subjects were permitted to eat a light meal (which was standard within subjects) 4 h after dosing. Venous blood samples (5 ml) were taken at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 24 and 48 h and additional samples were taken at 0.25 and 0.75 h after the 750 mg dose. Mixed saliva samples (stimulated by chewing paraffin film) were taken at these times. Complete 0–24 and 24–48 h urine collections were made. An interval of at least 4 days elapsed before beginning the next trial. Plasma, saliva and urine samples were stored at −20 °C pending analysis.

3. Caffeine Bioavailability was investigated in a single healthy male subject (age 36 years; weight 73 kg) who received 300, 500 and 750 mg anhydrous caffeine base in gelatine capsules orally on separate occasions. Intravenous infusions of a caffeine and sodium benzoate solution BPC 1954 (prepared by Guy's Hospital Pharmacy) to give similar doses of caffeine were also administered on separate occasions. Oral and intravenous doses of caffeine were given at not less than one week intervals, on each occasion the subject abstained from caffeine as described above. Blood samples, taken at appropriate intervals following drug administration, were analysed for caffeine. The durations of the caffeine infusions were 21, 41 and 68 min for the 300, 500 and 750 mg doses respectively. The bioavailability fraction F was calculated from

$$F = \frac{k_e^{po}(AUC)_{po}}{k_e^{iv}(AUC)_{iv}},$$

where $k^{po}{}_e$ and $k^{iv}{}_e$ are the first order elimination rate constants for oral and intravenous dosing and $(AUC)_{po}$ and $(AUC)_{iv}$ are the areas under the plasma concentration, time curve following oral and intravenous dosing.

4. Consistency of caffeine pharmacokinetics was determined by oral administration of 300 mg caffeine to two female and three male volunteers (aged

20–21 years; weights 55–73.2 kg). Caffeine was given on three occasions to the caffeine-free subjects with at least one week between each drug administration. Blood samples were taken at 0, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12 and 24 h and analysed for caffeine.

5. Pharmacokinetic Analysis. Plasma and salivary caffeine concentrations were fitted to a one-compartment open pharmacokinetic model with first-order absorption and lag time by an iterative non-linear optimisation program utilising the observations in their original scale of measurement by the simplex method (Nelder and Mead 1965). Some of the data, particularly at the lowest dose, suggested the possibility that fitting to a two compartment model might be appropriate. This was attempted in all such cases but in none was there an improved fit as reflected by a decreased residual sum of squared derivations.

The areas under the plasma concentration, time curves (AUC) were estimated by the trapezoidal approximation with appropriate extrapolation for the infinite portion of the curve. Plasma and salivary half-lives, apparent volume of distribution (V_d), renal clearance and total body clearance (Cl) were determined by standard methods (Gibaldi and Perrier 1975).

Statistical analysis was by analysis of variance (ANOVA) using the statistical package GENSTAT (Nelder 1973). Orthogonal comparisons were made to determine whether a linear, quadratic or cubic regression could account for the observations.

Results

Plasma Concentrations Following Oral Administration

The plasma concentration, time profiles (for example, Fig. 1) showed rapid caffeine absorption, the peak plasma concentration being attained at 30–90 min. Slower absorption was consistently noted in one subject in whom maximum plasma levels were reached at 2 to 3 h.

Table 1 shows the estimated values for the elimination rate constant (k_e) and half-life ($t_{1/2}$). A trend of decrease of elimination rate constant with oral dose was noted and ANOVA showed that dose had a significant effect ($p < 0.05$) on the elimination rate. A linear regression of plasma elimination rate constant upon dose accounts for these observations better than a cubic or quadratic relationship.

If caffeine obeys linear kinetics the curves produced by plotting the observed plasma level divided by dose versus time should be superimposable. This is a critical test of kinetic linearity. Despite some variation, which was not dose-related, this was found to be the case (Fig. 2) and no significant difference was found between the areas under each curve for each individual at the different doses.

The relationship of the AUC and dose is shown in Fig. 3. ANOVA indicates that the differences between individuals are significantly ($P < 0.01$) greater

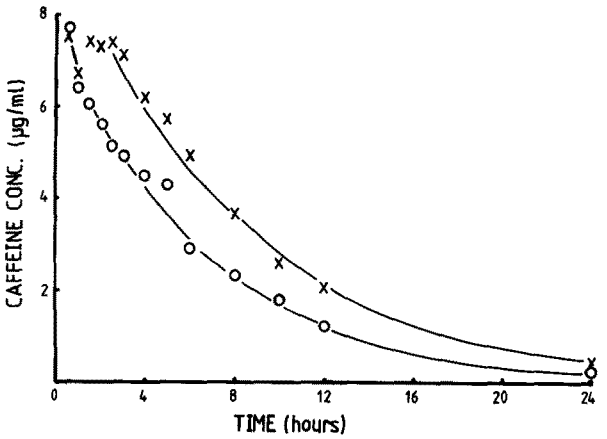

Fig. 1. Concentration of caffeine in plasma (x) and saliva (o) following 300 mg caffeine given orally to subject 1. Solid lines represent predicted concentrations from computer-fitted model.

Table 1. Plasma and saliva estimates of apparent first order elimination rate constant (k_e) and half-life ($t_{1/2}$) for different oral doses of caffeine

Caffeine dose [mg]	Plasma					Saliva				
	No of subjects	k_e [h^{-1}]	SD [h^{-1}]	$t_{1/2}$ [h]	Range [h]	No of subjects	k_e [h^{-1}]	SD [h^{-1}]	$t_{1/2}$ [h]	Range [h]
50	5	0.163	0.081	5.7	2.7–12.4	5	0.189	0.049	3.7	2.8–5.7
300	6	0.124	0.034	6.0	4.2–9.2	6	0.138	0.037	5.4	3.9–8.6
500	6	0.115	0.020	5.7	3.6–7.2	5	0.143	0.047	4.9	3.3–7.9
750	6	0.098	0.027	7.5	5.0–10.9	6	0.112	0.032	6.8	4.4–11.6

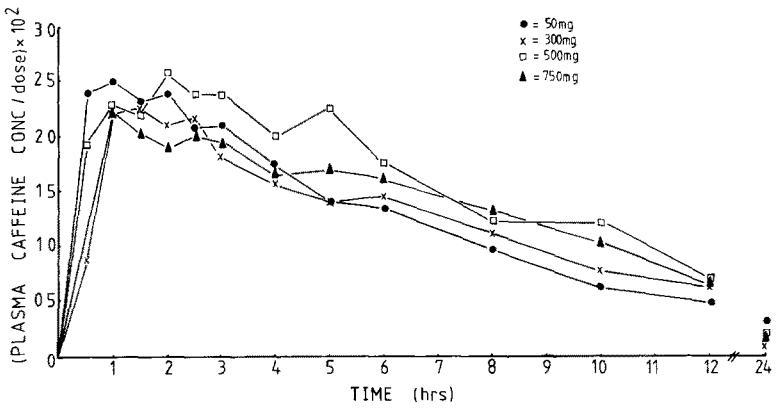

Fig. 2. Median values of plasma caffeine concentration/dose plotted against time for subject 1. Doses: ● = 50 mg; x = 300 mg; □ = 500 mg, and ▲ = 750 mg.

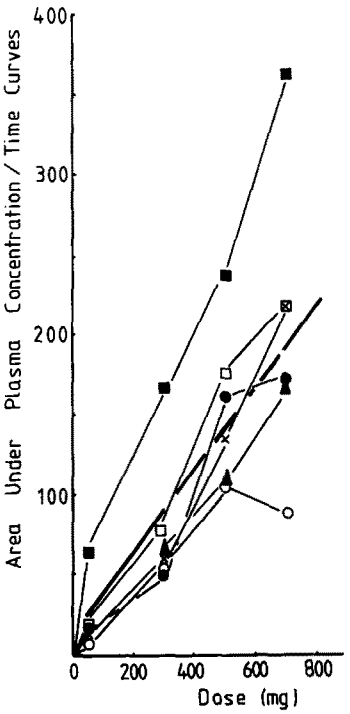

Fig. 3. Relationship between area under plasma caffeine concentration, time curve ($\mu g \cdot ml^{-1} \cdot h$) versus dose for each subject. Bold line is the linear regression of the means.

than experimental error and that dose significantly ($P < 0.01$) influences the area. A linear regression of the area under the curve with dose most satisfactorily accounts for the observations ($r = 0.78$). All but one of the individual subjects showed a linear increase of AUC with dose (Fig. 3). The mean total body clearance over all dose levels was 0.98 ml/min/kg (SD 0.38). ANOVA of the data appearing in Table 2 showed a greater inter-individual than within-individual variability of clearance. There was no significant relationship of clearance to dose. The overall mean apparent volume of distribution was 0.49 l/kg (SD 0.15) and there was a greater difference between individuals as compared to variation within each individual. Also a trend towards an increased apparent volume of distribution occurred with increasing dose and the V_d at 750 mg and 300 mg was significantly ($P < 0.01$) greater than 50 mg. The 500 mg dose, however, distributed in a volume not significantly larger than the 50 mg dose.

Bioavailability of caffeine was 0.92 at 300 mg, 0.91 at 500 mg and 1.06 at 750 mg. Corresponding bioavailability estimates from the salivary data were 1.01 for 300 mg and 0.92 for 750 mg. These estimates, given inherent experimental variation, suggest that the oral bioavailability of caffeine is essentially complete from this preparation and provide no evidence for dose-dependent bioavailability.

Consistency of Caffeine Pharmacokinetics. Table 3 shows estimates for elimination rate constant, apparent volume of distribution and systemic clearance of caffeine following oral administration of 300 mg to five subjects. There were significant ($P < 0.05$) inter-individual differences in these parameters but the estimates on each occasion within-individuals were not significantly different from one another. This demonstrates the consistency of the pharmacokinetic behaviour of caffeine within subjects not exposed to drugs or other agents from one occasion to another.

Salivary Concentrations. Figure 1 shows the computer-fitted curve to data from one subject. Table 1 again shows a decreasing elimination rate constant with dose. The differences between individuals were significant ($P < 0.01$) and influenced by dose ($P < 0.05$). A linear regression of the salivary elimination rate constant upon dose best accounted for the observations. Dose has a significant ($P < 0.01$) effect upon the area under the salivary caffeine concentration, time curve and this relationship is best accounted for by a linear regression.

Table 2. Total body clearance and apparent volume of distribution at each dose level of caffeine

Dose [mg]	No of subjects	Total body clearance [ml/min/kg]		Apparent volume of distribution [l/kg]	
		Mean	SD	Mean	SD
50	5	1.12	0.30	0.35	0.10
300	6	0.98	0.34	0.52	0.07
500	6	0.75	0.19	0.44	0.12
750	6	1.09	0.56	0.64	0.14

Comparison of Plasma and Salivary Concentrations. In examining the relationship between these observations a number of comparisons were undertaken. The difference between the saliva and plasma elimination rate constants was not significantly different at the several dosages and the plasma and saliva elimination rate constants at each dose level demonstrated no significant difference. The time at which the peak caffeine level was reached in plasma and saliva (t_{max}) was calculated from the pharmacokinetic model for each individual and dose. No significant difference was demonstrated at the 50, 300 or 500 mg doses but at 750 mg there was a significant ($P < 0.05$) difference with mean t_{max} for plasma at 1.00 h and for saliva at 0.68 h. No relationship existed between t_{max} and dose.

The mean saliva to plasma (S/P) concentration ratio for simultaneously obtained observations is plotted against time in Fig. 4. It is seen that early values of the S/P ratio approach unity. This is possibly due to insufficient cleansing of the mouth of caffeine derived directly from the dosage form although the caffeine powder was administered as closed gelatine capsules which were swallowed whole with a small amount of water. This peak was most marked with the higher doses of caffeine. Over all the studies carried out in 11 subjects and 39 separate caffeine administrations the overall S/P ratio was 0.74 ± 0.08. Within individuals there was some fluctuation in the S/P ratio between 1 and 24 h, the ratio showing a trend towards decrease as the plasma concentration falls. The mean coefficient of variation for these ratios was 28%. In the subject receiving 750 mg caffeine intravenously the S/P ratio was 0.60 after 10 min infusion (plasma level 6.3 μg/ml) which rose to 0.82 at 20 min peaking at 0.92 at 40 min before achieving a mean level of 0.76 over the next 24 h. This suggests a rapid entry of drug into the saliva and also that the higher S/P ratio noted during the early phase of the experiments with orally administrated caffeine may not entirely result from local contamination by solid drug.

Table 3. Estimates of pharmacokinetic parameters following administration of 300 mg caffeine orally to 5 subjects on three separate occasions

Occasion	k_e [h^{-1}]		V_d [l/kg]		Cl [l/kg/min]	
	Mean	SD	Mean	SD	Mean	SD
1	0.167	0.084	0.555	0.053	1.59	0.91
2	0.167	0.080	0.588	0.045	1.62	0.81
3	0.162	0.055	0.567	0.076	1.57	0.66
Mean and SD	0.165	0.069	0.567	0.058	1.59	0.74

Table 4. Urinary elimination of unchanged caffeine over 48 hours (mean ± SD) for 5 subjects

Oral dose [mg]	Total caffeine elimination in urine [mg]	% dose eliminated	Renal clearance [ml/min]
50	1.72±2.74	3.5±5.5	2.17±3.90
300	3.16±0.80	1.1±0.3	0.71±0.24
500	5.95±3.75	1.2±0.8	0.74±0.50
750	12.45±9.02	1.1±0.4	0.96±0.30

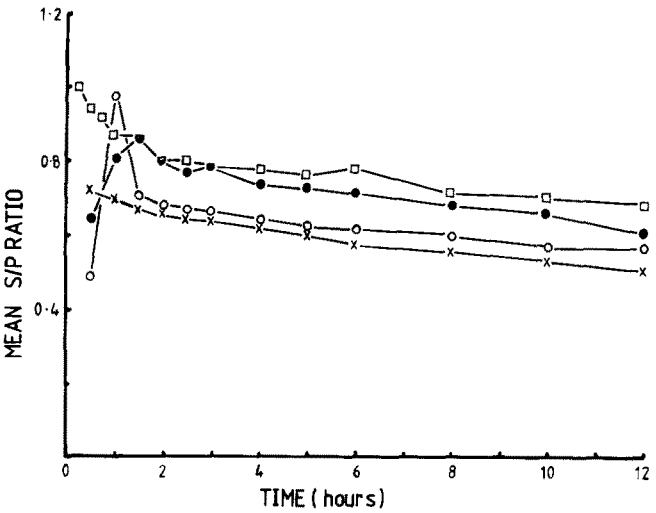

Fig. 4. Mean saliva/plasma concentration ratios of caffeine versus time elapsed following oral doses (x = 50 mg; ● = 300 mg; ○ = 500 mg and □ = 750 mg) of caffeine

Renal Elimination of Unchanged Caffeine. Urinary caffeine elimination was estimated in five subjects at four doses. The mean percentage of the dose excreted unchanged over 48 h was 1.83 (range 0.26–13.12%) (Table 4). The mean renal clearance of caffeine for all subjects and doses was 1.15 ml/min (range 0.09–9.17): ANOVA showed that renal caffeine clearance was independent of the dose administered.

Adverse Effects. No serious adverse effects occurred during oral caffeine administration although some subjects noted headache, palpitations and flushing at the highest dose. Caffeine withdrawal from the diet prior to each trial produced dysphoria (headache, irritability, lethargy, yawning, inefficiency and nervousness) which began 12–20 h after withdrawal. A similar physical withdrawal syndrome was noted in a double blind investigation by Goldstein et al. (1969) and demonstrates the dependence on caffeine of our average British consumers of tea and coffee.

During intravenous caffeine administration the only adverse effects were sinus tachycardia and a subjective sensation of dyspnoea and anxiety with the 750 mg dose. These resolved when the infusion rate was decreased.

Discussion

Our results, like those of Axelrod and Reichenthal (1953), Robertson et al. (1978) and Patwardhan et al. (1980), show that orally administered caffeine is rapidly absorbed. Unlike Robertson et al. (1978) who administered a 250 mg dose and observed a six-fold variation in peak plasma levels, with 300 mg the variation in peak plasma concentration was only 2.3 fold (range 5.3 to 12.5 μg/ml). Possibly this difference results from two loading doses of 250 ml water given at hourly intervals prior to the caffeine by Robertson et al. (1978). The plasma half-lives reported here may be compared with 2.5–4.5 h after 7 mg/kg caffeine (Axelrod and Reichenthal 1953); a mean of 4 h in seven subjects (Cook et al. 1976); 3.7–9.4 h in thirteen non-smokers following 0.7 to 2.2 mg/kg (Parsons and Neims 1978) and 5.5 ± 2.6 (SD) h in thirteen normal males after 250 mg (Patwardhan et al. 1980).

For drugs with first-order pharmacokinetic behaviour the elimination rate constant is independent of dose but for Michaelis-Menten kinetics the half-life changes with dose. The linear relationship observed for plasma and salivary estimates of the elimination rate constant would therefore suggest that caffeine does not obey linear kinetics. Monks et al. (1979) investigated the urinary excretion kinetics following intravenous administration of radiolabelled theophylline and found that whilst theophylline, 1,3-dimethyluric acid and 1-methyluric acid were excreted by apparent first order kinetics, 3-methylxanthine elimination was described by Michaelis-Menten kinetics. The metabolic fate of caffeine in man is not completely understood. Cornish and Christman (1957) found the major urinary metabolites to be 1-methyluric acid, 1,3-dimethyluric acid, 7-methylxanthine, 1-methylxanthine and 3,7-dimethylxanthine, but could detect no 3-methylxanthine. Schmidt and Schoyerer (1966), however, found amounts of 3-methylxanthine present in approximately equal amounts to 7-methylxanthine. This presumably results via conversion of caffeine to theophylline (this pathway was described by Sved et al. (1976)), or to theobromine and the further metabolism of these dimethylxanthines to 3-methylxanthine. Monks et al. (1979) have proposed that caffeine competes with theophylline for metabolism since its omission from the diet significantly reduces the urinary half-life of theophylline. It is therefore possible that this saturable pathway might exist for caffeine.

The relationships between the AUC of both plasma and salivary caffeine concentrations versus time and dose are linear over the range investigated, however, suggesting linear or non-saturable kinetics. This criterion of linearity is inherently more reliable than that based on computer-estimated elimination rate constants since it utilises the original data and is model-independent. The appreciable amounts of caffeine in saliva result in recycling of drug into the gut. By analogy secretion may occur into other intestinal secretions. These processes are ignored by classical linear pharmacokinetic models and it therefore is theoretically desirable to use model-independent pharmacokinetic methods rather than to assume a simplified specific model (Wagner 1976).

Total body clearance is model-independent and a plot of AUC versus dose should yield a line through the origin of slope 1/Cl ie. total body clearance is a constant, independent of dose, if linear kinetics apply. This was confirmed for caffeine in the present study. Total body clearance is the product of the elimination rate constant and the apparent volume of distribution and if, as found in this study, the elimination rate constant decreases with increasing dose, it might be expected that the apparent volume of distribution would rise. This trend was found with increasing dose although this change was not entirely clear-cut (Table 3). Investigation of more subjects would be required to confirm this observation. The apparent overall volume of distribution determined

in our subjects may be compared with values of 0.61 ± 0.8 l/kg (Parsons and Neims 1978) and 0.54 ± 0.18 l/kg (Patwardhan et al. 1980). Such apparent volumes of distribution are consonant with the suggestion of Axelrod and Reichenthal (1953) that caffeine is distributed in organs in proportion to their water content since total body water as a percentage of body weight is around 55%.

The most important feature of linear kinetics is superimposition and Fig. 2 shows that this principle is not violated by caffeine over the dosage range considered here. In a preliminary abstract Christensen et al. (1980) have made similar observations and suggest that caffeine obeys linear kinetics over a dose range of "1 to 8 cups of coffee".

Superimposable plasma concentration, time curves after oral and intravenous caffeine were described by Axelrod and Reichenthal (1953) in man and by Welch et al. (1977) in rats. Also, virtually identical plasma concentrations were found after oral administration of 10 mg/kg caffeine to premature infants as were measured following similar doses intravenously to other babies (Aranda et al. 1979). These observations indicate virtual or complete bioavailability of the drug and complement our own findings.

The amount of unchanged caffeine excreted in the urine was very small, typically 0.5–3.5% of the dose. This may be compared with the results of Axelrod and Reichenthal (1953) who found that 0.5–1.5% of a 500 mg intravenous dose of caffeine was excreted unchanged in the urine over 24 h by three normal men and with the data of Cornish and Christman (1957) who found only 1% of a 1 g oral dose of caffeine in the urine of two normal subjects over 48 h. Renal clearance was also low and much lower than the systemic clearance which is consistent with the extensive biotransformation of caffeine. The elimination rate constant therefore approximates to the overall metabolic rate constant. Welch et al. (1977) showed that the caffeine metabolism in rats is accelerated by administration of enzyme inducing agents. Dietary control was not imposed upon our subjects between trials and such factors could possibly have contributed to the variability in systemic clearance noted within and between subjects.

Salivary caffeine measurements provide a non-invasive method of monitoring caffeine plasma levels but salivary concentrations are lower than those in plasma. They apparently passively reflect caffeine elimination from plasma. Since the times to peak caffeine concentration in plasma and saliva are not significantly different, it appears that such passive diffusion is relatively rapid. The S/P ratio of 0.74 found in the present work is less than the ratio of 1.02 determined by Cook et al. (1976) and 0.90 ± 0.17 (SD) observed by Parsons and Neims (1978): both these groups used radio-immunoassay and their studies did not extend beyond 8 hours. Parsons and Neims (1978), however, also noted a higher S/P ratio during the first 1–1.5 h ascribing this to residual caffeine in the mouth. In our single subject given intravenous caffeine, higher initial S/P ratios were observed. This tendency for the S/P ratio to be higher in the absorption and distribution phases than in the elimination phase has been observed for other drugs including theophylline following rectal administration (Knop et al. 1975). In neonates the S/P ratio for caffeine was 0.71 with a coefficient of variation of 44% over the therapeutic range (up to 13 μg/ml) for apnoea (Khanna et al. 1980). Horning et al. (1977) studied one patient measuring caffeine by gas chromatography-mass spectrometry and found a saliva/plasma ratio of 0.55.

If salivary and plasma caffeine concentrations are plotted in time order as suggested by Galeazzi et al. (1976) for the most part counter-clockwise hysteresis loops are obtained. This implies that the "salivary compartment" may be pharmacokinetically "deeper" than the "plasma compartment". Salivary caffeine probably represents the unbound fraction of drug in the plasma. Axelrod and Reichenthal (1953) found 15% binding to human plasma protein but a more recent study by Patwardhan et al. (1980) gave values of 31.4 ± 1.9% for males and 31.5 ± 4.5% for females. These latter values are consonant with the overall S/P ratio found in the present study.

Our results suggest that although there is an increase in plasma $t_{½}$ with increasing oral doses of caffeine, the total body clearance remains constant. This implies that the metabolism of caffeine in caffeine-free subjects over the dose range investigated obeys first-order kinetics. It seems probable, however, that caffeine demonstrates non-linear pharmacokinetic behaviour in rats and mice, possibly related to their different metabolism of this substance. This difference is important for toxicologists. Many of the dire predictions regarding the effects of this widely ingested compound on spermatogenesis, teratogenesis and other functions have been made, as has been pointed out by Palm et al. (1978), by extrapolation from the results obtained in these species at unrealistic dose levels.

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