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The kinetics of amylobarbitone metabolism in healthy men and women

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Summary

1. Sodium amylobarbitone (3·54 mg/kg) was given by intravenous injection to seven healthy men and nine healthy women who were not receiving other drugs. Serum amylobarbitone and urine hydroxyamylobarbitone concentrations were measured by gas-liquid chromatography. There was no significant difference between the groups either in the serum amylobarbitone concentration/time curves or in the urinary excretion of hydroxyamylobarbitone.

2. The serum amylobarbitone concentration decayed over 48 h as a double exponential function of time ; the first exponential component had a mean half-time of 0·6 h (males $0{\cdot}56 \pm 0{\cdot}06$ h, females $0{\cdot}62 \pm 0{\cdot}08$ h, $\pm$S.E.) and the second exponential component had a mean half time of 21 h (males $22{\cdot}7 \pm 1{\cdot}6$ h, females $20{\cdot}0 \pm 1{\cdot}0$ h, $\pm$S.E.).

3. The urinary excretion of hydroxyamylobarbitone over 48 h accounted for 34% of the dose (males $33{\cdot}8 \pm 3{\cdot}2\%$, females $35{\cdot}2 \pm 3{\cdot}0\%$, $\pm$S.E.). One male and two female subjects excreted hydroxyamylobarbitone partly as a conjugate which was readily hydrolysed in acid.

4. An elimination constant (k_{el}) derived from the serum concentration/time curve by the application of a two compartment model was approximately proportional to β (h^{-1}), the rate constant of the second exponential component. There was a positive correlation ($r=0{\cdot}78$, $P<0{\cdot}001$) between β and the mean rate of urinary excretion of hydroxyamylobarbitone during the 24 to 48 h period.

Introduction

Amylobarbitone provides a suitable substrate for the study of barbiturate oxidation in man. These experiments were performed to measure individual variation in amylobarbitone metabolism in a group of healthy adults and to detect any sex dependent differences.

The elimination of amylobarbitone is due almost entirely to biotransformation ; the urinary excretion of unchanged amylobarbitone after an overdose is very small (Mawer & Lee, 1968) and after a hypnotic dose it is negligible (Kamm & van Loon, 1966). However, the published evidence does not establish that the elimination of amylobarbitone is due entirely to oxidation.

Maynert (1952, 1965) showed that amylobarbitone is oxidized *in vivo* to hydroxyamylobarbitone (5 ethyl, 5(3′hydroxyisoamyl) barbituric acid), but the urinary excretion of this polar derivative accounts for only half the dose. The form in which

the remainder is eliminated has not been established. If the elimination of amylobarbitone is to be used as an *in vivo* measure of the activity of the enzyme system responsible for barbiturate oxidation, it is necessary to demonstrate parallel excretion of the oxidized product.

Sex differences in barbiturate oxidation have been observed in the rat using pentobarbitone and hexobarbitone as substrates (Kato & Gillette, 1965; Schenkman, Frey, Remmer & Estabrook, 1967). Oxidation in adult males was about three times as rapid as in females. Differences in the opposite direction have been observed with hexobarbitone in certain strains of mice (Gillette, 1967).

Corresponding studies in man have not been described in the main reviews of barbiturate metabolism (Bush & Sanders, 1967; Mark, 1963; Raventos, 1954; Richards & Taylor, 1956). Kamm & van Loon (1966) did not compare hydroxyamylobarbitone excretion rates in men and women. There seems to be no published information on the relative rates of barbiturate oxidation in men and women.

Methods

Hypnotic doses of amylobarbitone were given intravenously to healthy male and female subjects. The disappearance of amylobarbitone from the blood and excretion of hydroxyamylobarbitone in the urine were measured during the next 48 h.

Subjects

Twenty-six experiments with intravenous amylobarbitone were carried out on seven healthy men and nine healthy women whose ages and weights are given in Table 1. Duplicate experiments in any one subject were separated by at least 4 weeks. Two male subjects were given oral amylobarbitone nightly for 3 weeks. All the experiments were explained in detail and each subject gave informed consent.

A detailed drug history was taken from each subject: none had received a course of treatment with any drug during the 6 months before the experiment. The groups were matched approximately for alcohol intake. Four of the men and three of the women had an average daily intake equivalent to about half a pint of beer. One man and one woman had a greater intake and the remainder were occasional drinkers. None of the women were taking oral contraceptives. The experiments were not restricted to any one phase of the menstrual cycle.

TABLE 1. *Serum amylobarbitone concentrations (μg/ml) after the intravenous administration of the sodium salt (3·54 mg/kg) to male and female subjects*

Time (h)	Male subjects Mean±S.E.	*n*	Female subjects Mean±S.E.	*n*
0·1	7·30±0·28	6	7·72±0·39	6
0·5	5·14±0·11	6	6·00±0·44	6
1·0	4·14±0·13	6	4·74±0·36	6
2·0	3·44±0·16	6	3·40±0·20	6
4·0	2·60±0·13	6	2·66±0·16	6
12·0	2·17±0·23	7	2·24±0·13	9
24·0	1·54±0·16	7	1·38±0·10	9
36·0	1·04±0·09	7	0·94±0·06	9
48·0	0·71±0·07	7	0·60±0·04	9
	Mean (range)		Mean (range)	
Age (years)	30 (24–41)		27 (20–43)	
Weight (kg)	74 (61–87)		57 (49–68)	

Amylobarbitone

In the single dose experiments, sodium amylobarbitone (sodium amytal for injection: Eli Lilly and Co. Ltd., 3·54 mg/kg) was given intravenously over 3 min. In eight early experiments the same dose was given more cautiously by infusion over 30 or 60 min.

In the multiple dose experiments one 200 mg tablet of sodium amylobarbitone (Eli Lilly and Co. Ltd.) was taken orally at 22.00–24.00 hours.

Both preparations gave one peak on gas-liquid chromatography. Trace amounts of barbitone (<0·1%) were detected.

Hydroxyamylobarbitone

A sample of pure hydroxyamylobarbitone was obtained by crystallization from ether extracts of urine excreted by amylobarbitone-treated subjects. The sample had the ultraviolet absorption characteristics described by Maynert (1952) and gave a single peak on gas-liquid chromatography. The mass spectrum (M.S. 12; 70 eV) demonstrated the molecular weight and structure proposed by Maynert (1952, 1965).

Collection of samples

In the single dose experiments blood was collected from an antecubital vein at the following times after the start of the injection: 0·1, 0·5, 1·0, 2·0, 4·0, 12, 24, 36 and 48 h. Blood was not taken from the arm which had received the intravenous dose. Four 12 h urine collections were made during the same period.

In the multiple dose experiments blood was collected daily at 12.00 hours.

Chromatography

Amylobarbitone and hydroxyamylobarbitone were estimated separately but by very similar procedures (Balasubramaniam, Mawer & Rodgers, 1969).

Known amounts of internal marker (quinalbarbitone in the case of amylobarbitone and cyclobarbitone in the case of hydroxyamylobarbitone) were added to serum or urine. The samples were acidified, saturated with ammonium sulphate and extracted by diethylether. The ether extracts were concentrated and purified by two stage thin-layer chromatography on silica gel. The purified barbiturates were extracted from the gel into acetone, concentrated and estimated by gas liquid chromatography (Pye 104, model 24, N_2 50 ml/min, Chromosorb W, Neopentylglycol adipate 3% w/w).

Calibration curves were prepared by extracting samples of serum to which known amounts of standard had been added. The threshold for measurement was about 0·2 μg/ml of sample for both amylobarbitone and hydroxyamylobarbitone. There was an 0·2 μg/ml error (S.D., n=30) in the measurement of an amylobarbitone concentration of 1·5 μg/ml of serum.

The concentrations of hydroxyamylobarbitone in urine were relatively high (Table 3) and in some cases it was possible to omit the stage of purification by thin-layer chromatography.

Conjugated hydroxyamylobarbitone

Acid hydrolysis of conjugated hydroxyamylobarbitone was carried out by refluxing 5·0 ml urine with 5·0 ml 4 N HCl for 20–30 min. Release was incomplete at 15 min and loss of hydroxyamylobarbitone was detectable at 60 min.

Aliquots of urine were also incubated with purified mollusc β-glucuronidase (580,000 Fishman units/g). Samples of urine were boiled for 5 min to inactivate enzyme inhibitors. 2·0 ml aliquots were mixed with 2·0 ml sodium acetate buffer, (0·1 M, pH 4·0), 0·5 ml distilled water and 0·5 ml glucuronidase solution (90,000 Fishman units/ml). The mixture was incubated at 37° C for 48 h. Hydroxyamylobarbitone was extracted and estimated as described above.

The activity of the glucuronidase preparation was assayed by the hydrolysis of phenolphthalein monoglucuronide.

Fitted curves

Experimental concentration/time curves were fitted by the equation for a double exponential function (Riggs, 1963).

$$C_t = A.e^{-\alpha t} + B.e^{-\beta t}$$

C_t (μg/ml) represents the serum concentration at t h, A (μg/ml) the intercept of the first exponential function, B (μg/ml) the intercept of the second exponential function and α and β (h^{-1}) the corresponding rate constants. The intercepts and rate constants were obtained by weighted regression analysis of the log. concentration time curves.

Two compartment model

The dimensions of a hypothetical two compartment model were calculated by substitution of A, B, α and β into the equations derived by Riegelman, Loo & Rowland (1968). The calculations were checked by establishing an analogue computer model using a Solartron, SCD 10 computer and plotting the output on a Bryan's model 26,000-A4, X-Y plotter. The dimensions of the hypothetical central compartment (V_1, ml), peripheral compartment (V_2, ml), two transfer constants ($k_{1,2}$ and $k_{2,1}$, h^{-1}) and an elimination constant (k_{el}, h^{-1}) were presented to the computer. Three outputs were obtained representing (i) the drug concentration in the central compartment (C_1, μg/ml), (ii) the drug concentration in the peripheral compartment (C_2, μg/ml) and (iii) the amount of drug (% of dose) eliminated from the two compartment system between 0 and 48 h.

In eight subjects the second part (12–48 h) of the serum amylobarbitone concentration/time curve was determined after the slow intravenous infusion of the sodium salt over 30 or 60 min. This precaution proved unnecessary; most subjects slept for 1 or 2 h but were easily roused. In four of these subjects the first part (0·1–4·0 h) of the concentration/time curve was determined after the rapid intravenous injection of the same dose on a separate occasion.

The two compartment model was applied only to data from four men and four women in whom both parts of the concentration/time curve had been determined on the same occasion after the rapid intravenous injection of sodium amylobarbitone.

Results

The serum concentrations of amylobarbitone after the intravenous injection of the sodium salt into seven male and nine female subjects are recorded in Table 1. Data from four male and four female subjects were combined to give the concentration/time curve in Fig. 1.

The experimental concentrations were fitted by a double exponential function of time. The characteristics of the two exponential components are given in Table 2. In both sexes the first component decayed rapidly with a half-time ($t\frac{1}{2}$) of less than 1 h and the second component decayed slowly with a half-time of about 20 h.

The urinary concentrations of hydroxyamylobarbitone are recorded in Table 3. Acid treatment produced no change in the majority of subjects ; however, one male and two female subjects showed a two or three-fold increase in the apparent con-

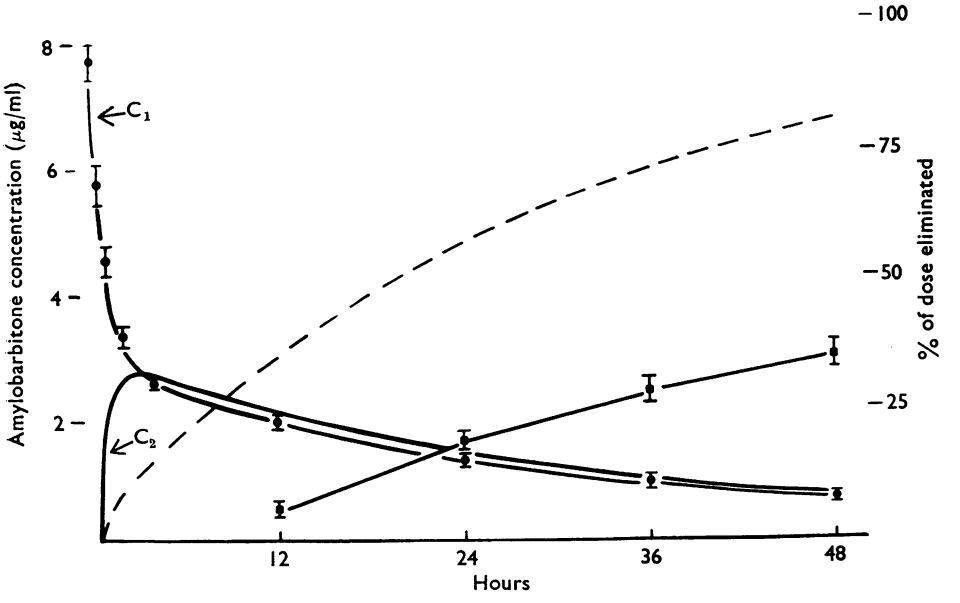

FIG. 1. Decay of serum amylobarbitone concentration (●——●, μg/ml ± S.E.M.) and the cumulative urinary excretion of hydroxyamylobarbitone (■——■, % of dose ± S.E.M.) during the first 48 h after the intravenous administration of sodium amylobarbitone (3·54 mg/kg) to four male and four female subjects. The curves C_1 and C_2 represent the concentrations of amylobarbitone (μg/ml) in hypothetical central and peripheral body compartments (Table 5). The broken line represents the fraction (%) of the dose eliminated from these compartments. The curves were printed directly on the graph by the analogue computer.

TABLE 2. *Characteristics of the two exponential components of the serum amylobarbitone concentration/time curve after the intravenous administration of the sodium salt* (3·54 *mg/kg*) *to male and female subjects*

		Male subjects		Female subjects	
		Mean ± S.E.	*n*	Mean ± S.E.	*n*
First exponential component	A (μg/ml)	4·84 ±0·30	6	5·13 ±0·57	6
	α (h^{-1})	1·33 ±0·15	6	1·24 ±0·18	6
	$t\frac{1}{2}$ (h)	0·56 ±0·06	6	0·62 ±0·08	6
Second exponential component	B (μg/ml)	3·28 ±0·40	7	3·30 ±0·18	9
	β (h^{-1})	0·032±0·002	7	0·035±0·002	9
	$t\frac{1}{2}$ (h)	22·7 ±1·60	7	20·00 ±1·00	9

centration of hydroxyamylobarbitone after acid hydrolysis. This increase was not observed when aliquots of the same urine samples were incubated with purified mollusc β-glucuronidase.

The cumulative excretion of total hydroxyamylobarbitone (% of dose ± S.E.M.) by seven male and nine female subjects between 0 and 48 h is shown in Table 4. Combined values for males and females are shown in Fig. 1.

The 48 h urinary excretion of unchanged amylobarbitone accounted for less than 1% of the dose.

The concentrations of hydroxyamylobarbitone in serum remained close to the threshold for measurement during the 48 h period.

The subjects (K.B. and G.M.) each received 4·0 g sodium amylobarbitone in divided doses over 3 weeks. The cumulative urinary excretion of hydroxyamylobarbitone over 4 weeks represented 54% and 52% of the total dose.

Discussion

There was no significant difference between the male and female subjects with respect either to the serum amylobarbitone concentration/time curves (Tables 1 and 2) or to the urinary excretion of hydroxyamylobarbitone (Table 4). Man seems to resemble the majority of animal species studied with the exception of the rat (Gillette, 1967).

No difficulty was encountered in fitting the equation for a double exponential function to the observed concentrations of amylobarbitone in serum. The rate constant (α, h^{-1}) of the first exponential component was greater than the rate constant (β, h^{-1}) of the second exponential component by a factor of more than 30 (Table 2).

Substitution of the intercepts and rate constants A, B, α and β into the equations of Riegelman *et al.* (1968) gave the dimensions of a hypothetical two compartment model shown in Table 5. The computer output corresponding with the amylo-

TABLE 3. *Influence of acid treatment and of β-glucuronidase treatment on the apparent concentration of hydroxyamylobarbitone (μg/ml) in aliquots of the total 48 h urine collected after intravenous sodium amylobarbitone (3·54 mg/kg)*

Urine from male subjects				Urine from female subjects			
	Untreated	Acid-treated	Enzyme-treated		Untreated	Acid-treated	Enzyme-treated
G.P.	56	50		T.F.	54	58	
K.B.	27	30		J.V.	37	39	
R.F.	22	12		V.H.	23	25	
M.H.	19	18		J.T.	20	18	
G.M.	19	17		H.D.	16	17	
J.R.	7	6		S.G.	16	15	
				E.B.	15	14	
G.Y.	13	26	10	M.A.	35	66	34
				H.E.	7	20	8

TABLE 4. *Urinary excretion of hydroxyamylobarbitone (% of dose) after the intravenous administration of sodium amylobarbitone (3·54 mg/kg) to seven male and nine female subjects*

Period (h)	Male subjects Mean ± S.E.	Female subjects Mean ± S.E.
0–24	15·7 ± 1·4	17·9 ± 1·5
24–48	18·1 ± 1·9	17·3 ± 1·8
0–48	33·8 ± 3·2	35·2 ± 3·0

barbitone concentration (C_1) in a hypothetical central compartment fitted the experimental serum concentrations closely (Fig. 1). The concentration (C_2) in the hypothetical peripheral compartment rose initially due to redistribution. There was a transient steady state at about 3 h and during the remainder of the experiment C_2 exceeded C_1. The two curves were however very close and almost parallel. The model suggests that during the slow decay phase the total body amylobarbitone is approximately proportional to the serum amylobarbitone concentration and that the rate constant for the elimination of amylobarbitone is approximately proportional to the rate constant β of the slow exponential decay.

The same conclusion is reached from consideration of the expression for the elimination constant (k_{el}) (Riegelman *et al.*, 1968).

$$k_{el} = \frac{A+B}{\frac{A}{\alpha}+\frac{B}{\beta}}$$

TABLE 5. *Dimensions of a hypothetical two compartment model* which fits the serum amylobarbitone concentration/time curve observed after the intravenous administration of sodium amylobarbitone (3·54 mg/kg) to four male and four female subjects*

Experimental values			
Mean body weight		64·2	kg
Mean amylobarbitone dose		206	mg
First exponential component	A	5·32	μg/ml
	α	1·20	h^{-1}
Second exponential component	B	2·91	μg/ml
	β	0·033	h^{-1}
Two compartment model			
Central compartment volume	V_p	25,030	ml
Steady state distribution volume	V_{dss}	64,320	ml
First transfer constant	$k_{1,2}$	0·699	h^{-1}
Second transfer constant	$k_{2,1}$	0·445	h^{-1}
Elimination constant	k_{el}	0·089	h^{-1}

* After Riegelman *et al.* (1968).

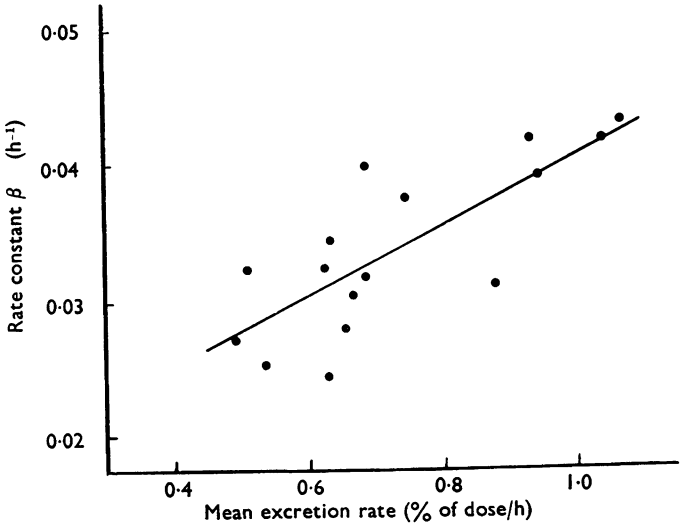

FIG. 2. Urinary excretion of hydroxyamylobarbitone between 24 and 48 h after the intravenous administration of sodium amylobarbitone (3·54 mg/kg). The graph shows the positive correlation ($r=0·78$, $P<0·001$) among sixteen subjects between mean excretion rate (% of dose/h) and the rate of elimination of amylobarbitone represented by β (h^{-1}), the rate constant for the slow decay of serum amylobarbitone concentration.

Substitution for example of the values for the four males and four female subjects (Table 5) gives

$$k_{el} = \frac{8{\cdot}2}{4{\cdot}4 + 103{\cdot}2}$$

The contribution of $\frac{A}{\alpha}$ to k_{el} is sufficiently small to neglect and the expression can be simplified to

$$k_{el} = \frac{A+B}{B} \cdot \beta ;$$

the elimination constant becomes proportional to β. The ratio $\frac{A+B}{B}$ approximately equals the ratio of the steady state distribution volume (V_{dss}) to the central compartment volume (V_p); the values obtained by Riegelman *et al.* (1968) for different drugs lay in the range 1·5 to 2·5. A value of 2·8 was obtained for amylobarbitone in this study.

The urinary excretion of hydroxyamylobarbitone in the long term experiments accounted for only half the dose and the 48 h excretion after injection of amylobarbitone accounted for only a third (Fig. 1). These fractions agree closely with the data of Maynert (1952, 1965) and of Kamm & van Loon (1966). The remainder of the dose may be eliminated as undetected conjugates of hydroxyamylobarbitone in faeces or urine or as further oxidation products not yet identified. It is also possible that the elimination of this portion of the dose is not dependent on the drug oxidase system; if this were the case there need be no positive correlation between the elimination of amylobarbitone and the rate of excretion of hydroxyamylobarbitone.

A positive correlation ($r=0{\cdot}78$, $P<0{\cdot}001$) was observed in the group of sixteen subjects between the rate constant for elimination of amylobarbitone, expressed as β, and the mean rate of urinary excretion of hydroxyamylobarbitone (% of dose per hour) from 24 to 48 h (Fig. 2). This justifies the use of the rate constant (β), or its reciprocal the half-time ($t_{\frac{1}{2}}$), of the serum amylobarbitone concentration/time curve as a measure of the rate of side chain oxidation. The correlation to hydroxyamylobarbitone excretion rate was weaker ($r=0{\cdot}51$, $P<0{\cdot}05$) during the first 24 h. This may have been the result of variation between individuals in the rate of amylobarbitone distribution.

The conjugation of hydroxyamylobarbitone by a proportion of healthy adults has not been described before. It was not reported by Maynert (1952, 1965). Kamm & van Loon (1966) did not observe release of further hydroxyamylobarbitone during incubation with β-glucuronidase, but they recognized that non-glucuronide conjugates may have been present. Butler (1956) observed the excretion of a sulphate of *p*-hydroxyphenobarbitone in human urine. The hydroxyamylobarbitone conjugate has not yet been characterized.

Dr. A. S. Curry generously provided the chromatographic standard of hydroxyamylobarbitone and Mr. D. Smith the purified β-glucuronidase. The mass spectra were determined in the laboratory of Dr. J. Wilson, Department of Chemistry, University of Manchester. Mrs. M. Rodgers provided skilled technical assistance. Dr. Balasubramaniam is on postgraduate study leave from the University of Ceylon. The investigation was assisted by the Research Grants Committee of the United Manchester Hospitals.

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