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The excretion of hydroxyamylobarbitone in man after oral administration of amylobarbitone and hydroxyamylobarbitone

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The excretion of hydroxyamylobarbitone in man has been measured over six days after an oral dose of 200 mg of sodium amylobarbitone. The biological half-life of hydroxyamylobarbitone determined from "Sigma-minus" plots ranged between 16·8 and 22 h in seven subjects, in another subject the half-life was 34·4 h. The effects of increasing urine flow on the amount of hydroxyamylobarbitone excreted after ingestion of 200 mg of sodium amylobarbitone were assessed. A subject normally excreting 34% of the dose as hydroxyamylobarbitone excreted 45% of the dose as metabolite while taking chlorothiazide as a diuretic. With the same subject taking increased fluids to produce a greater urine flow 41% of the dose was excreted as hydroxyamylobarbitone. Hydroxyamylobarbitone is not bound to plasma proteins and when an aqueous solution of 50 mg of hydroxyamylobarbitone was taken by mouth, 57% of the dose was eliminated in the first 8 h and 91% in the first 24 h. The half-life for ingested hydroxyamylobarbitone was 5·7 h, showing that the rate of elimination of this metabolite is faster than its rate of formation when amylobarbitone is ingested.

Information about the formation and elimination of hydroxyamylobarbitone, derived from amylobarbitone, is incomplete in man because of lack of sensitive analytical techniques. Measurements of hydroxyamylobarbitone in urine have been made using ^{15}N labelled amylobarbitone (Maynert & Van Dyke, 1950), ultraviolet spectrophotometry (Moss, 1965) and gas-liquid chromatography (Kamm & Van Loon, 1966; Balasubramanian, Mawer & Rodgers, 1969). Kamm & Van Loon (1966) after giving their subjects amylobarbitone followed the excretion of the hydroxyamylobarbitone in the urine for five days by chromatographing the chlorotrimethylsilane derivative of the metabolite, but the limit of detection was only 5 μg/ml of sample. A more sensitive gas chromatographic assay, described by Grove & Toseland (1970), employs the new stationary polar phase FFAP and allows unchanged hydroxyamylobarbitone to be chromatographed with a limit of detection of 2 μg (total) from an extract of either plasma or urine.

Mawer & Lee (1968) have suggested that "rapid removal of metabolites might increase the rate of barbiturate hydroxylation" and, if forced diuresis increased the excretion of metabolites, a case be made for this treatment in the management of overdosage. We therefore investigated the effect of increasing the urine flow on the amounts of hydroxyamylobarbitone excreted, using the method of Grove & Toseland (1970).

METHODS

Excretion of hydroxyamylobarbitone after oral administration of sodium amylobarbitone

Each of eight healthy adult volunteers were given a capsule containing 200 mg of

sodium amylobarbitone. Complete urine collections were made for the six ensuing days and 20 ml of blood withdrawn into heparinized tubes at intervals over the first 15 h. The blood was centrifuged and the plasma was separated. The plasma samples and an aliquot from each 24 h urine was then analysed for hydroxyamylobarbitone by the method of Grove & Toseland (1970).

Excretion of hydroxyamylobarbitone after oral administration of sodium amylobarbitone with a diuretic

Sodium amylobarbitone (200 mg) was taken by one volunteer who also was given 500 mg of chlorothiazide to take at 8, 12, 16 and 20 h for the first and second day, the sodium amylobarbitone capsule being taken at 10 h on the first day. This resulted in a urine output for the two days of 6200 ml compared with 2840 ml without a diuretic. Urine was collected for six days and aliquots analysed as before.

Excretion of hydroxyamylobarbitone after oral administration of sodium amylobarbitone with increased fluid intake

The experiment was repeated a month later on the same volunteer, who was given sodium amylobarbitone (200 mg), and who increased his fluid intake over the first two days without taking the diuretic. The urine output for the 2 days was 4490 ml. All the urine was collected for six days and an aliquot taken from each 24-h sample for analysis.

Plasma binding of hydroxyamylobarbitone

Dialysis bags were made by knotting one end of each of a 25 cm length of 26/32 visking tubing (Scientific Supplies Ltd., Vine Hill, London, E.C.4) previously soaked in 1% acetic acid for 5 min. Three ml of fresh, heparinized plasma and 0·1 ml of a 0·4 mg% of hydroxyamylobarbitone in Tyrode solution (equivalent to 40 μg of hydroxyamylobarbitone) were added and the other end of the tube knotted. The bags were equilibrated with 3 ml of Tyrode solution for $\frac{1}{2}$, 1, 2, $2\frac{1}{2}$ and 3 h respectively in a metabolic shaker at 37°. The whole of the plasma and 2 ml of the Tyrode solution were analysed for hydroxyamylobarbitone.

Oral administration of hydroxyamylobarbitone

A healthy adult volunteer ingested a single dose of 50 mg of hydroxyamylobarbitone in water. All the urine was collected over two days and 20 ml of blood withdrawn into heparinized tubes at intervals over the first 5 h. Aliquots of urine were analysed as before. The blood samples were centrifuged, the plasma separated and analysed for hydroxyamylobarbitone.

Calculation of the half-life of hydroxyamylobarbitone

The excretion data obtained from the eight subjects ingesting 200 mg of sodium amylobarbitone and the one subject ingesting 50 mg of hydroxyamylobarbitone were examined using the graphical treatment suggested by Cummings, Martin & Park (1967). This treatment, known as a "Sigma-minus" method, assumes first-order elimination and is based on the following equation:

$$M_{u\infty} - M_u = \frac{k_f D_o e^{-Kt}}{K} + M_B$$

Where $M_{u\infty}$ = Amount of the metabolite in the urine when excretion is complete; M_u = Amount of metabolite in the urine at time t; M_B = Amount of metabolite

in the body at time t; k_f = First order rate constant governing the formation of the metabolite; K is the overall elimination rate constant of the drug by all routes, i.e. unchanged drug and metabolites; D_0 is a constant related to the drug at zero time.

A plot of $\ln(M_{u\infty} - M_u)$ against time has a slope of $-K$. The value of the biological half-life may also be obtained from this graph since $t_{0.5} = 0.693/K$.

RESULTS AND DISCUSSION

Plasma concentrations of hydroxyamylobarbitone never exceeded 0·5 μg/ml in hourly samples taken over the 15 h after amylobarbitone had been taken. Attempts to measure the plasma half-life of the metabolite from these data were, therefore, unsuccessful.

The excretion pattern of hydroxyamylobarbitone in the urine of eight volunteers is shown in Table 1. These results are in good agreement with those findings of Kamm & Van Loon (1966). The $t_{0.5}$ for seven of our volunteers ranged from 16·8–22 h, an eighth volunteer with a $t_{0.5}$ of 34·4 h (subject B) had had her gall bladder removed some years previously but whether this might have interfered with biliary excretion is uncertain. There were no other clinical abnormalities in this subject.

Although our results showed that to obtain a more accurate slope ideally the urine collections should have been taken for smaller periods in the initial stages, the difference between the derived $t_{0.5}$ and that for hydroxyamylobarbitone (see later) was felt sufficient not to warrant repetition of the early analyses.

Effect of increased urine flow on the excretion of hydroxyamylobarbitone

Table 2 shows the excretion pattern of volunteer A with a normal urine flow, a diuretically augmented urine output and a urine output increased by extra fluid intake. A greater urine output is seen to be accompanied by a greater excretion of hydroxyamylobarbitone, lending some support to the proposition of Mawer & Lee (1968) that forced diuresis may be of value in cases of barbiturate overdose.

Protein binding

Since hydroxyamylobarbitone was excreted over a period of six days, we considered the possibility that the metabolite was bound to the plasma proteins. The distribution of hydroxyamylobarbitone between fresh plasma and Tyrode solution after equilibration for intervals up to 3 h revealed that complete equilibrium occurred after 2 h, indicating that protein binding of the hydroxyamylobarbitone had not taken place.

Excretion of hydroxyamylobarbitone after oral administration of the metabolite

Table 3 gives the hydroxyamylobarbitone concentration of the urine and plasma samples, collected after one volunteer had taken 50 mg of hydroxyamylobarbitone in a solution of 50 ml water. Clearances for hydroxyamylobarbitone and creatinine are also shown.

As expected, hydroxyamylobarbitone is rapidly excreted with 57% of the dose being eliminated in the first 8 h and 91% in the first 24 h.

Since the measurement of clearances depends on a constant plasma concentration, comparison between that of hydroxyamylobarbitone and creatinine is not strictly correct. Nevertheless, it would appear from our figures that, in the initial stages,

when a large amount of hydroxyamylobarbitone is still present in the body, it is excreted at the glomerular filtration rate. It was not possible to do this experiment on more subjects owing to the lack of pure hydroxyamylobarbitone.

Table 1. *% dose of sodium amylobarbitone (200 mg) excreted as hydroxyamylobarbitone (HO-A) by eight subjects.*

Subject	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Total % dose as HO-A	$t_{0.5}$ (h)
A	12·32	11·87	5·73	2·66	1·40	0	33·98	19·4
B	9·68	9·26	6·63	3·97	3·17	2·46	35·17	34·4
C	18·36	19·21	5·88	1·51	2·59	0·95	48·50	22·0
D	13·06	13·69	9·41	4·15	2·42	2·33	45·06	18·4
E	10·26	13·43	10·01	4·67	1·14	0·67	40·18	20·0
F	17·21	16·01	5·15	1·46	1·30	—	41·13	16·8
G	9·93	10·11	8·60	2·70	1·90	0·29	33·56	22·0
H	18·42	13·07	4·19	6·70	2·02	0·81	45·21	21·6
Mean	13·66	13·33	6·95	3·48	1·99	1·07	40·35	21·8
± Standard deviation	±3·81	±2·98	±1·99	±1·65	±0·22	±0·26	±5·32	±4·99

Table 2. *The concentration of hydroxyamylobarbitone (HO-A) in the urine of subject A after ingesting 200 mg of sodium amylobarbitone with no diuretic, chlorothiazide and extra fluid.*

	No diuretic			500 mg chlorothiazide qds for first two days			Fluid intake increased		
HO-A on day:	Urine volume (ml)	μg/ml	% Dose	Urine volume (ml)	μg/ml	% Dose	Urine volume (ml)	μg/ml	% Dose
1	1000	22·46	12·32	4000	9·12	20·01	2150	14·0	16·51
2	1840	11·76	11·87	2200	6·62	8·0	2340	10·6	13·61
3	1630	8·7	5·73	1250	16·4	11·25	1750	5·12	4·91
4	1200	4·15	2·66	1110	7·2	4·38	2200	2·98	3·60
5	1170	2·0	1·40	1400	0·8	0·61	2550	1·02	1·43
6	1260	0	0	1580	0·64	0·55	1900	0·72	0·75
Total % dose as HO-A			33·98			44·8			40·81

Table 3. *Excretion of hydroxyamylobarbitone (HO-A) after ingestion of a 50 mg dose.*

Time (h)	Volume (ml)	Urine μg/ml	% Dose	Blood Time (h)	μg/ml plasma	Clearance (ml/min) HO-A	Creatinine
0–2	157	47·3	14·9	¾	0·55	112	107
				1¼	0·81	76	73
2–4	91	61·0	11·1	2¼	0·91	51	84
				2¾	1·25	37	61
4–6	236	40·0	18·9	4¼	1·0	79	50
6–8	288	20·4	11·7				
8–10	271	16·3	9·0				
10–12	432	10·6	9·1				
12–14	220	14·2	6·2				
14–24	475	10·4	9·9				
24–48	1180	3·2	9·5				
			100·3				

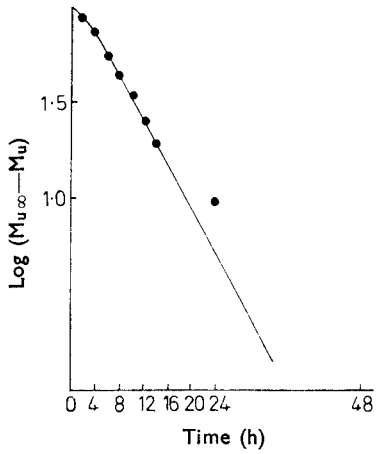

FIG. 1. The "Sigma-minus" plot of the excretion of hydroxyamylobarbitone after ingestion of 50 mg of hydroxyamylobarbitone. $t_{0.5}$ = 5·7 h.

When this data was plotted as a Sigma-minus function (Fig. 1) the rapid elimination is emphasized. The $t_{0.5}$ for hydroxyamylobarbitone is seen to be 5·7 h.

We can infer from the difference in the $t_{0.5}$ for ingested hydroxyamylobarbitone and the $t_{0.5}$ for hydroxyamylobarbitone derived from sodium amylobarbitone that the rate of elimination of this metabolite is much faster than its rate of formation. This explains the negligible accrual of the metabolite in the blood of patients ingesting amylobarbitone, indeed hydroxyamylobarbitone concentrations of any significance may only be found in the blood of patients in coma or renal failure. For these reasons we would not expect to find evidence of the conjugation of hydroxyamylobarbitone, although Balasubramanian, Lucas & others (1970) have reported three subjects excreting hydroxyamylobarbitone partly as a conjugate readily hydrolysed in acid. We have not been able to substantiate their findings. Analysis of twenty different urines before and after acid hydrolysis with 4N HCl failed to produce any increase in the hydroxyamylobarbitone concentration.

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