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The distribution of amylobarbitone, butobarbitone, pentobarbitone and quinalbarbitone and the hydroxylated metabolites in man‡

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Fluid and tissue specimens collected from 30 subjects at autopsy have been assayed for their content of common sedative barbiturates and the corresponding hydroxylated metabolites by g.l.c. Where one barbiturate had been ingested an inverse relationship between lipid solubility of the drug and the distribution in fluids and tissues was observed. In most cases the liver, and in the remainder the spleen, contained the highest concentrations of barbiturate. Bile concentrations were often in excess of those in the corresponding liver. The metabolites of the four sedative barbiturates were usually present in lower amounts than the parent drugs in the fluids and tissues of most subjects but urine often contained much higher concentrations of metabolites—sometimes exceeding that of the parent drug in the liver. Administration of two or more barbiturates together did not appear to affect the distribution and metabolism of the individual drugs.

Although the 5,5-disubstituted barbiturates have been in therapeutic use for over 70 years their distribution in man has previously been studied only with an assortment of specimens. These were analysed by non-specific spectrophotometric procedures that measured a total barbiturate concentration including mixtures of the drugs and any co-extracted metabolites (Bonnichsen et al 1961; Rehling 1967; Robinson 1967; Gee et al 1974).

The metabolism in man of amylobarbitone (Tang et al 1975), butobarbitone (Gilbert & Powell 1974), pentobarbitone (Tang et al 1977) and quinalbarbitone (Gilbert et al 1975) has been investigated by gas chromatography-mass spectrometry of urine extracts from volunteers given the respective drug. In each case the major metabolite isolated was hydroxylated on the longer side chain; however, hydroxyquinalbarbitone was excreted as a minor metabolite (Gilbert et al 1975).

Hydroxyamylobarbitone has been studied quantitatively in man by Kamm & Van Loon (1966) who followed its excretion into the urine of volunteers given the parent drug. Grove & Toseland (1970) developed an assay for this metabolite in plasma and urine and used the assay to measure the plasma half-life in subjects who had ingested 50 mg hydroxyamylobarbitone (Grove & Toseland 1971).

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We have measured the distribution of some sedative barbiturates and the corresponding hydroxylated metabolite in human fluid and tissue specimens by g.l.c. The specimens were from Coroners' autopsy cases where administration of barbiturates was suspected, although not necessarily in overdose amounts.

MATERIALS AND METHODS

Materials

All chemicals were reagent grade with the exception of concentrated hydrochloric acid, ammonium sulphate and sodium hydroxide, which were 'Analar' standard. An ethereal solution of diazomethane was prepared from *N*-methyl-*N*-nitroso-*p*-toluene sulphonamide (Diazald–Aldrich Chemical Co.) and was stable for four weeks at 4 °C. The analytical reference standards of the barbiturates were donated by May and Baker Ltd.; hydroxyquinalbarbitone and hydroxybutobarbitone were the kind gift of Dr J. N. T. Gilbert and the samples of hydroxyamylobarbitone and hydroxypentobarbitone were made available by Dr Robert E. Willette, National Institute of Drug Abuse, 11400 Rockville Pike, Rockville, Maryland USA. All tissues were homogenized without the addition of any fluid in a MSE Atomix blender then deep-frozen and all body fluids were refrigerated as soon as possible after collection pending analysis.

Methods

Preliminary qualitative analysis of the samples of stomach content and urine was according to Robin-

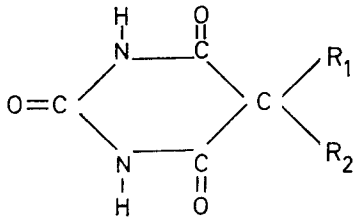

FIG. 1. The structure of Common sedative barbiturates and the Hydroxylated Metabolites.

R_1	R_2	
ethyl,	3′methylbutyl	Amylobarbitone
ethyl,	3′-hydroxy-3′-methylbutyl	Hydroxyamylobarbitone
ethyl,	n-butyl	Butobarbitone
ethyl,	3′-hydroxybutyl	Hydroxybutobarbitone
ethyl,	1′-methylbutyl	Pentobarbitone
ethyl,	1′-methyl-3′-hydroxybutyl	Hydroxypentobarbitone
allyl,	1′-methylbutyl	Quinalbarbitone
allyl,	1′-methyl-3′-hydroxybutyl	Hydroxyquinalbarbitone

son & Holder (1974); the barbiturates were measured by a g.c. assay (Robinson et al 1979).

The assay for the hydroxylated metabolites was adapted from the method of Grove & Toseland (1970); the unchanged barbiturates were also extracted by this method but the resulting concentrations of the parent drugs in the tissues and fluids were lower and more variable by this method compared with the values measured by the method of Robinson et al (1979).

To tissue homogenate (3–5 g) (in duplicate) and fluid samples (1–5 ml) (not urine) in 50 ml polypropylene centrifuge tubes was added 8 ml of freshly prepared tungstic acid (Grove & Toseland 1970) and 100 μg of barbitone internal standard in 100 μl ethanol.

To urine (1–5 ml) in similar tubes and with the same quantity of internal standard, was added sufficient lead acetate solution to precipitate the urinary pigments (Willcox 1913).

For subject 28 who had ingested barbitone, 100 μg quinalbarbitone was used as the internal standard.

The tubes were mixed for 60 s each and allowed to stand for 10 min, centrifuged at 6000 rev min^{-1} for 10 min and the supernatant transfered to a 250 ml conical flask. The pH value of the aqueous phase was adjusted to pH 1 with concentrated hydrochloric acid, then ammonium sulphate (5 g) and chloroform (80 ml) were added to the flask which was shaken for 5 min by a wrist action mechanical shaker. The organic phase was separated through Whatman 41 filter paper and evaporated to dryness; the residue was methylated with 1 ml of an ethereal solution of diazomethane for 1 h (Gilbert et al 1970). After the removal of excess reagent and solvent, the sample was reconstituted with 100 μl ethanol and injected in 1 μl aliquots into the gas chromatograph.

Recoveries of the hydroxylated metabolites from aqueous solution were: 19·4 μg hydroxyamylobarbitone 92% recovered, 10·7 μg hydroxybutobarbitone 74% recovered, 11·2 μg hydroxypentobarbitone 89% recovered, and 12·7 μg hydroxyquinalbarbitone 88% recovered. Recoveries from 'spiked' liver homogenate were 10·7 μg hydroxybutobarbitone 89% recovered and 12·7 μg hydroxyquinalbarbitone 103% recovered, the available amounts of the other two drug metabolites were insufficient to perform this recovery.

G.l.c. apparatus

A Hewlett-Packard 5750 gas chromatograph was equipped with a thermionic detector, the 6′ × $\frac{1}{8}$″ i.d. glass column was packed with 3·8% W98 on 80–100 mesh Diatomite CQ. Operating temperatures: oven 180°, injection port 265° C and detector 400 °C; the gas flow rates: helium carrier 60, air 180 and hydrogen 33 ml min^{-1}.

The retention times of the barbiturates and hydroxylated metabolites as *NN′*-dimethyl derivatives on this column are barbitone (internal standard) 2·2 min, butobarbitone 4 min, amylobarbitone 4·8 min, pentobarbitone 5·3 min, quinalbarbitone 6·3 min, hydroxybutobarbitone 8·2 min, hydroxyamylobarbitone 8·7 min, hydroxypentobarbitone 10·3 min, hydroxyquinalbarbitone 11·9 min, and phenobarbitone 12·4 min. The sensitivity for the methylated compounds was 0·1 μg ml^{-1} fluid or g^{-1} tissue and the detector gave a linear response within the range 0–20 μg for the hydroxylated metabolites.

At attenuations used to detect the parent drugs the peak heights of the metabolites were inadequate for measurement so the attenuator sensitivity was increased 4–16 fold.

The identity of the hydroxylated metabolites was confirmed using an LKB 2091 gas chromatograph-mass spectrometer with a glass column 6′ × $\frac{1}{8}$″ i.d. filled with 3% OV1 on 80–100 mesh Diatomite CQ and operating at oven 160°, injection port 210 °C, separator 300 °C, source 250 °C and 70 eV electron impact ionization. The mass range of the detector is 0–350, carrier gas: helium 40 ml min^{-1}. The retention times of the *NN′*-dimethylbarbiturates and hydroxylated metabolites are (min): barbitone 2·5, butobarbitone 5·0, amylobarbitone 6·0, pentobarbitone 7·0, quinalbarbitone 8·5, hydroxybutobarbitone 12·0,

hydroxyamylobarbitone 12·5, hydroxypentobarbitone 16·0 and hydroxyquinalbarbitone 19·0.

Ethanol was measured by the method of Curry et al (1966) using a Perkin Elmer F11 gas chromatograph with flame ionization detection and a 6′ × ⅛″ i.d. stainless steel column filled with 10% polyethylene glycol 400 on 80–100 mesh Chromosorb W AW DMCS. Oven temperature 90°C, gas flow rates: nitrogen carrier 60, air 500, and hydrogen 50 ml min^{-1}. The retention times of ethanol and n-propanol (internal standard) are 1·2 and 2·4 min respectively.

RESULTS AND DISCUSSION

Subjects who ingested a single barbituric acid derivative (Subjects 1–12)

The distribution of a sedative barbiturate and the corresponding hydroxylated metabolite in 12 subjects is presented in Table 1.

Stomach. Over 200 mg of unabsorbed barbiturate was detected in the stomachs of subjects 1, 2, 4, 5, 8 and 9, this inferred that the blood barbiturate concentration was still rising; butobarbitone appeared to have been completely absorbed from the gastric contents of subjects 10–12.

Blood. The peripheral blood barbiturate concentrations were generally consistent with the ranges of fatal poisonings (Bonnichsen et al 1961; Rehling 1967; Robinson 1967; Gee et al 1974).

The corresponding hydroxylated metabolite of the ingested barbiturate, when detected, was present at 2–4 mg $litre^{-1}$ which compares with a peak plasma concentration of 5·6 mg hydroxyamylobarbitone $litre^{-1}$ 20 h after 200 mg of the parent drug (Grove & Toseland 1970).

Bile. Bile specimens contained concentrations of the individual barbiturates within the range 27–198 mg $litre^{-1}$, these concentrations were up to five fold higher than those in the corresponding peripheral blood. Biliary metabolite concentrations varied from 0–312 mg $litre^{-1}$.

Urine. Eleven subjects excreted the barbiturate taken unchanged at 3–65 mg $litre^{-1}$ which is in excess of values following individual therapeutic doses of these barbiturates found by Parker et al 1970. Subject 10, had a butobarbitone urine concentration equivalent to the reported therapeutic value (Table 1). The hydroxylated metabolites were present at 0–259 mg $litre^{-1}$.

Organs. The livers contained the highest concentration (20–224 mg kg^{-1}) of the parent barbiturates in nine and the spleen (32–150 mg kg^{-1}) in three subjects. Analysis of left and right kidneys and lungs gave relatively uniform results but in subject 7 there was a difference in distribution of pentobarbitone consistent with a postural element in the immediate post-mortem interval.

Hydroxyamylobarbitone and hydroxybutobarbitone were measurable only in the livers of subjects 4 and 10 respectively. The concentrations of hydroxylated barbiturate metabolites throughout the organs of the remaining subjects were below (1–25 mg kg^{-1}) those of the corresponding parent drug; their distribution appeared uniform.

Subjects who ingested Tuinal (amylobarbitone + quinalbarbitone) capsules: (Subjects 13–26; Table 2)

Stomach. The barbiturates remaining unabsorbed in the stomach contents of these subjects were consistent with the ingestion of Tuinal, and in subjects 16, 17, 20, 21, 22, 24 and 26 over 200 mg barbiturate remained.

Blood. Neither barbiturate appeared to influence the absorption and distribution of the other, since their blood concentrations (peripheral) in subject 24 were equivalent and in subjects 14 and 15 they were similar.

All but subject 25 had blood barbiturate concentrations within the range observed by Gee et al (1974) which were values for the total amount of barbiturate present. Blood or plasma concentrations of the two barbiturates after therapeutic doses of Tuinal have not been reported. An estimate of the peak total barbiturate concentration based on a dose of 200 mg would be about 2–3 mg $litre^{-1}$ within 1–2 h.

When detected, hydroxyamylobarbitone concentrations were from 2–5 mg $litre^{-1}$ and hydroxyquinalbarbitone from 2–6 mg $litre^{-1}$.

Bile. The range of amylobarbitone and quinalbarbitone concentrations were 13–530 and 5–370 mg $litre^{-1}$ respectively; these values were above those measured in the corresponding peripheral blood samples. Hydroxylated metabolites were generally lower than the parent drugs and varied (when detected) from 5–22 mg $litre^{-1}$ for hydroxyamylobarbitone $litre^{-1}$ and 8–14 mg $litre^{-1}$ for hydroxyquinalbarbitone.

Urine. In subjects 13–26 concentrations were in the range 1–12 mg $litre^{-1}$ amylobarbitone and 1–5 mg $litre^{-1}$ quinalbarbitone, these values were consistent with those in subjects 1–3 and 4–6 (Table 1) respectively.

Hydroxyamylobarbitone concentrations were in the range 0–56 mg $litre^{-1}$, and hydroxyquinalbarbitone 0–26 mg $litre^{-1}$. Where both metabolites were measured in a subject the hydroxyamylbarbitone concentration was the higher.

Table 1. The distribution of amylobarbitone (A), butobarbitone (B), pentobarbitone (P) and quinalbarbitone (Q) and the corresponding hydroxylated metabolites (H).

Subject				Amounts in: (mg litre^{-1})				Amounts in: (mg kg^{-1})						Stomach content mg
No.	Sex	Age		Blood	Liver blood	Bile	Urine	Liver	Left lung	Right lung	Left kidney	Right kidney	Spleen	
1	M	52	Q	12	57		6	77	28	27	37	69	41	824
			HQ	ND	ND		ND	4	2	2	3	4	4	
2	F	35	Q	9	22	42	3	51	17	23	25	24	150	552
3	F	32	Q	13	23	79	32	44	33	34	25	28	30	11
			HQ	2	4	ND	33	25	18	19	17	16	15	
4	M	17	A	36	41	198	12	71	46	40	33	31	77	1530
			HA	2	3	6	9	1	+	+	+	+	+	
5	M	28	A	35	45	178	12	79	41	38	52	65	63	908
			HA	ND	+	ND	ND	2	2	1	2	1	1	
6	F	74	A	25				224	85	82	88	86	104	
			HA	2				2	4	4	4	5	+	
7	F	85	P	51	67	152	7	165	51	93	46	72	61	· 74
			HP	ND				5	4	4	4	5	3	
8	M	21	P	10	14	148	5	20	16	42	18	13	32	550
			HP	ND	4		82	8	10	7	7	7	8	
9	M	49	P	25	37	59	62	46	38	29	20	29	32	351
			HP	4	5	13	65	9	10	10	7	8	8	
10	M	64	B	18	26	49	4	47	26	27	31	30	30	0·1
			HB	ND	10	204	259	2	+	+	+	+		
11	M	26	B	49	46	131	38	83	52	50	72	70	68	2
			HB	ND	1	312	5	17	19	16	12	13	10	
12	F	36	B	22++	19	27	26	58	19	23	23	24	23	0·4
			HB	3	4	4		10	8	9	8	6	9	

++ Heart blood sample. ND = Not detected. + = positive but not measurable.
Results are not corrected for recovery
Circumstances:

Subject 2	Blood ethanol	86 mg/100 ml	6	Blood ethanol 38 mg/100 ml
4	,,	,, 78 mg/100 ml	10	Evidence of chronic bronchitis and heart disease
5	,,	,, 103 mg/100 ml	12	Antemortem blood ethanol 75 mg/100 ml; survived 16 h

The ranges of barbiturate concentrations following ingestion of therapeutic doses are:

amylobarbitone	1 mg litre^{-1} plasma 2 h after 60 mg (Inaba & Kalow 1972).
butobarbitone	3·5–4 mg litre^{-1} plasma 1 h following 200 mg (Breimer 1974).
pentobarbitone	1·2–3·1 mg litre^{-1} plasma 0·5–2 h after 100 mg sodium salt (Sun & Chun 1977).
quinalbarbitone	0·9–3·7 mg litre^{-1} plasma 1 h after 150 mg sodium salt/70 kg (Dalton et al 1976).

Organs. Both amylobarbitone (7–288 mg kg^{-1}) and quinalbarbitone (5–339 mg kg^{-1}) were concentrated in the livers of most subjects and in the spleen in the remainder (16–345 mg amylobarbitone kg^{-1} and 15–454 mg quinalbarbitone kg^{-1}). Concentrations in lungs and kidneys were usually similar.

Organ distribution of the metabolites was usually uniform, the ranges detected were hydroxyamylobarbitone 0·4–9 mg kg^{-1} and hydroxyquinalbarbitone 0·2–5 mg kg^{-1}.

These values were similar to those drawn from subjects 1–3 and 4–6 (Table 1). Thus the distribution of the two barbiturates following overdoses of Tuinal appeared to follow the distribution of the individual barbiturates.

The differences between the amylobarbitone and quinalbarbitone concentrations in fluids and organs varied from being equivalent (subject 24) to quinalbarbitone blood concentration approximately a third of the amylobarbitone value (subject 25). The lipid solubility and metabolism of quinalbarbitone is greater than for amylobarbitone (Mark et al 1958; Tang et al 1975; Gilbert et al 1975), so if death occurred rapidly following ingestion, the quinalbarbitone would not be distributed and metabolized to the same degree as when death was delayed.

Poisoning with unformulated mixtures of barbiturates (Subjects 27–30; Table 3).

Blood. The concentrations of amylobarbitone (subject 27) and the barbiturates found in subjects 29 and 30 were consistent with previous fatalities (Bonnichsen et al 1961; Rehling 1967; Robinson 1967; Gee et al 1974) Phenobarbitone, 4 mg litre^{-1} blood, (subject 27) was less than the peak serum concentration, 12–30 mg litre^{-1}, measured by Louis (1954) after 750 mg. The blood barbitone, 39 mg litre^{-1}, in subject

Table 2. The distribution of amylobarbitone (A), quinalbarbitone (Q) and the metabolites hydroxyamylobarbitone (HA) and hydroxyquinalbarbitone (HQ)

Subject				Amounts in: (mg litre^{-1})				Amounts in: (mg kg^{-1})						Stomach content mg
No.	Sex	Age		Blood	Liver blood	Bile	Urine	Liver	Left lung	Right lung	Left kidney	Right kidney	Spleen	
13	F	27	A	9		13		22	3	4	10		8	I
			HA	ND		ND		1	1	1	2		1	
			Q	5		5		20	1	3	6		6	I
			HQ	ND		ND		2	2	2	3		1	
14	M	24	A	5	12		4	14	6	9	11		5	ND
			HA					1	1	1	1		1	
			Q	4	6		2	6	3	4	6		3	ND
			HQ					2	1	1	4		1	
15	M	24	A	7	9	25	4	11	7	6	9	10	12	+
			HA	ND	ND		2	1	ND	ND	1	2	ND	
			Q	6	6	10	3	10	6	6	8	9	8	+
			HQ	ND	ND		1	+	ND	ND	ND	ND	ND	
16	F	78	A	13	130			288	67	45	183	65	345	315
			HA	2	10	8		2	1	1	2	3	1	
			Q	10	103			339	68	40	246	61	454	405
			HQ	ND	18	ND		3	2	1	3	2	4	
17	F	63	A	9	44	134	3	78	18	18	51		62	450
			HA	4	3		56	2	2	2	4		3	ND
			Q	5	26	91	1	93	16	14	47		59	350
			HQ	3	2		26	2	2	1	2		2	ND
18	F	34	A	8	18	44	4	7	4	4	8	3	7	60
			HA	2	2	ND	29	2	1	1	1	1	1	ND
			Q	6	16	19	2	5	3	3	4	2	9	58
			HQ	2	2	ND	14	2	1	1	0·5	1	1	ND
19	M	23	A	12		91	7		30	22	9	38	29	ND
			HA	2			3		2	2	4	2	1	ND
			Q	7		39	3		24	24	6	32	24	ND
			HQ	3			1		2	2	2	2	1	ND
20	M	63	A	14	24	66	6	56	24	24	26	31	30	604
			HA	2	5	22	I	3	2	3	5	6	2	7
			Q	12	19	27	5	45	19	18	24	26	26	923
			HQ	I	4	18	I	3	2	2	3	4	1	ND
21	F	69	A	5	6	60	3	49	16	17	19	20	42	265
			HA	5		22	I	9	3	3	4	5	4	0·5
			Q	4		38	2	49	12	14	15	16	40	275
			HQ	3		14	I	2	2	2	2	3	3	ND
22	M	41	A	12	31	52	5	37	17	17	16	17	37	191
			HA	ND	ND	ND	ND	0·7	0·4	0·5	0·6	1	0·6	ND
			Q	13	24	44	3	49	17	15	16	16	44	182
			HQ	ND	ND	ND	ND	0·2	ND	ND	ND	ND	ND	ND
23	F	27	A	18	62	531	9	123	35	41	52	63	62	60
			HA	2	3	5	66	2	2	2	3	3	2	ND
			Q	12	67	371	5	125	35	34	55	60	94	83
			HQ	I	4	I	34	3	2	2	2	3	2	ND
24	F	66	A	5		207		45	43	47	32	43	82	185
			HA	+		+		1	1	1	0·4	+	1	ND
			Q	5		123		51	48	44	29	40	148	175
			HQ	ND		ND		4	2	2	2	1	3	ND
25	F	70	A	3			1	13	9	7	9	8	16	2
			HA	4		14	25	7	8	4	5	5	5	0·2
			Q	1			1	12	7	4	5	6	15	3
			HQ	6		9	9	5	I	2	3	3	3	I
26	M	33	A	19	26	159	12	42	20	18	26	25	36	1878
			HA	1	1	ND	32	2	2	1	1	1	2	ND
			Q	14	22	87	I	54	23	21	29	28	37	1214
			HQ	1	1	ND	23	3	2	1	1	1	3	ND

+ = positive but not measurable. I = interference.

Circumstances:

Subject: 13 Administered barbiturates by injection. The lungs contained aspirated stomach content*. 14 Lost balance, fell under a lorry*. 18 Blood ethanol 126 mg/100 ml. 19 Injection marks in elbows and forearms. 21 Blood ethanol 139 mg/100 ml. 22 Blood ethanol 252 mg/100 ml. 23 Dependent upon barbiturates for ten years. 25 Chronic bronchitis and emphysema observed*.

* = Significant in relation to death.

Table 3. The distribution of miscellaneous barbiturate mixtures and the associated hydroxylated metabolites other than amylobarbitone and quinalbarbitone

Subject				Amounts in: (mg litre^{-1})				Amounts in: (mg kg^{-1})						
No.	Sex	Age		Blood	Liver blood	Bile	Urine	Liver	Left lung	Right lung	Left kidney	Right kidney	Spleen	Stomach content mg
27	M	22	A	17		365	18	48	20	17	26	20	23	0·3
			HA	5		13	163	7	14	7	5	4	3	21
			Ph	4		1	8	8	3	1	4	3	7	1·8
28	F	85	A	7	13	I	11	15	7	6	7	8	6	0·2
			HA	6	7	I	I	11	7	9	14	12	8	ND
			Barb	39	60	I	289	41	37	34	30	32	37	1·3
29	F	26	B	23		24		57	36	41	58	39	137	178
			HB	ND		ND		+	+	+	+	+	+	ND
			Q	14		40		46	21	26	36	29	83	148
			HQ	ND		ND		ND	ND	ND	ND	ND	ND	ND
30	F	70	A	8	21	148	+	71	20	8	53	23	34	308
			HA	ND	ND	5	ND	1	1	+	1	1	3	ND
			P	15	36	138	+	133	54	17	96	47	72	374
			HP	ND	ND	5	ND	ND	ND	ND	ND	ND	ND	ND
			Q	6	16	86	ND	74	21	7	55	20	37	269
			HQ	ND	ND	10	ND	ND	ND	ND	ND	ND	ND	ND

+ = positive but not measurable.
Ph = phenobarbitone.
Barb = barbitone.
Circumstances:
Subject 27 Misused drugs and had a history of hepatitis
28 Survived 20 h in hospital
29 Blood ethanol 81 mg/100 ml
30 Blood ethanol 176 mg/100 ml

28 was similar to 43-1200 mg litre^{-1} serum found by Bailey & Jatlow (1975) in eight patients in coma; a peak serum concentration of 30 mg litre^{-1} was recorded following ingestion of 1500 mg (Louis 1954). The amylobarbitone blood concentration of 7 mg litre^{-1} in subject 28 was greater than therapeutic values (Table 1).

Concentrations of hydroxyamylobarbitone in subjects 27 and 28 (5 and 6 mg litre^{-1} respectively) were the highest recorded in this study. Hydroxylated metabolites were not detected in the blood of subjects 29 and 30.

Bile. Barbiturates in the bile were in the range of 1–365 mg litre^{-1} and usually greatly exceeded amounts in the corresponding blood. The hydroxylated metabolites, measured only in subjects 27 and 30 (range 5–13 mg metabolite litre^{-1}), were in greater concentrations than metabolites in the corresponding blood.

Urine. Subjects 27 and 28 excreted unchanged amylobarbitone at 18 and 11 mg litre^{-1} respectively which is in agreement with other subjects who had taken the drug. The 289 mg litre^{-1} barbitone value (subject 28) could not be related to other reports. Amylobarbitone and pentobarbitone in subject 30 were excreted in unquantifiable amounts which is consistent with a rapid death.

Hydroxyamylobarbitone, 163 mg litre^{-1}, in the urine from subject 27 was the only metabolite measured.

Organs. The barbiturates were concentrated in the livers of three (8–133 mg barbiturate kg^{-1}) and in the spleen of the other subject (83–137 mg barbiturate kg^{-1}). Posture appeared to have influenced the distribution of the drugs in subjects 27 and 30. The distribution of individual barbiturates in the mixtures was similar to that found when the drugs were taken singly.

Hydroxyamylobarbitone in subjects 27, 28 and 30 was in the range 1–14 mg kg^{-1} and appeared to concentrate in the left lung (subject 27), both kidneys (subject 28) and the spleen (subject 30). Metabolites of the other barbiturates were not detected.

The high concentrations of hydroxyamylobarbitone in subject 27, who had a history of hepatitis, could not be correlated with Breimer's (1974) findings that the half-life of hexobarbitone increased from a mean of 261min in healthy people to 483min in patients with hepatitis.

DISCUSSION

The estimation of the ingested dose

A traditional method of assessing drug overdosage has been the calculation of the 'fatal dose' ingested by the 'r' factor of Widmark (Widmark 1932, cited by Harger 1961) from the blood concentration of the drug. Originally used for ethanol, it has been adapted for barbiturates (Gee et al 1974); however, this factor does not take account of metabolism or excretion of the drug. A better method is the comparison of peak blood or serum concentrations following the administration of therapeutic doses with the peripheral blood concentrations at autopsy, the latter were assumed to be maximal.

The estimation of the time between ingestion and death

The time between ingestion of barbiturates and death was often either not known or approximate. Curry & Sunshine (1961) found that a liver/blood barbiturate concentration ratio in excess of 4 correlated with death within 5 h; if the ratio was less, no specific time could be assigned, although death usually supervened after 5 h.

The liver/blood barbiturate concentration ratios (Table 4) from subjects 1–12 where a single barbiturate had been ingested, were difficult to relate to the conclusions of Curry & Sunshine (1961) because of insufficient information about the time of death, but quinalbarbitone generally gave higher ratios than the other barbiturates which correlated well with the lipid solubility of the molecule.

Application of the ratio to the Tuinal deaths also was difficult as Curry & Sunshine (1961) measured total barbiturate concentration.

Our results suggested a relative time scale between ingestion of Tuinal and death based upon the difference in concentrations of the barbiturate in the peripheral blood and tissues; where death occurred rapidly, there was little difference, but with time there was less quinalbarbitone because of its faster rate of metabolism and distribution. Thus the results for subjects 14, 15 and 22 were consistent with death a short time after intake and for the remainder a delay before death.

The amylobarbitone liver/blood ratios for subjects 27 and 28 were consistent with death in excess of 5 h which correlated with subject 28 who survived 20 h and for 29 and 30 the ratios were consistent with a delayed and a rapid death respectively.

Routes of drug administration

Most subjects had ingested the barbiturates but parenteral administration incurs the more rapid pharmacological effect and greater risk of toxicity. Balasubramaniam et al (1970) who injected 3·54 mg kg^{-1} of sodium amylobarbitone (equivalent to 225 mg free acid in a 70 kg subject) found within 10 min the serum concentration was 7·0–7·5 falling to 4·0–4·3 mg $litre^{-1}$ at 30 min, in comparison to 1 mg $litre^{-1}$ observed by Inaba & Kalow (1972) 1 h after a 60 mg oral dose.

Table 4. The ratio of individual liver/blood barbiturate concentrations from Subjects 1–30.

	Barbiturate			
Subject	B*	A*	P*	Q*
1				6·4
2				5·7
3				3·4
4		2·0		
5		2·3		
6		9·0		
7			3·2	
8			2·0	
9			1·8	
10	2·6			
11	1·7			
12	2·6			
13		2·4		4·0
14		2·8		1·5
15		1·6		1·7
16		22·2		33·9
17		8·7		18·6
18		0·9		0·8
19		—		—
20		4·0		3·8
21		9·8		12·3
22		3·1		3·8
23		6·8		10·4
24		9·0		10·2
25		4·3		12·0
26		2·2		3·9
27		2·8		
28		2·1		
29	2·5			3·3
30		8·9	8·9	12·3

* See Table 1 for definitions.

The effect of ethanol with barbiturates

In none of 10 subjects in whom ethanol was found was the concentration of ethanol itself sufficient to cause death and in most there was a sufficient blood barbiturate concentration to account for death. In subject 2 (quinalbarbitone 9 mg $litre^{-1}$ and ethanol 100 ml blood) and subject 21 (total barbiturate 9 mg and $litre^{-1}$ ethanol/100 ml blood) the peripheral blood barbiturate concentrations were low in relation to other fatal cases and the c.n.s. depressant action of the ethanol was assumed to have caused the fatal outcome.

Table 5. Expression of barbiturate concentration in body fluids and tissues as a percentage of that in the liver.

Barbiturate	Quinalbarbitone			Amylobarbitone			Pentobarbitone			Butobarbitone			Barbitone
Subject	1	2	3	4	5	6	7	8	9	10	11	12	28
Blood	16	18	30	51	44	11	31	50	54	38	59	38	95
Liver blood	74	43	52	58	57		41	70	80	55	55	33	146
Bile		82	180	279	225		92	740	128	104	158	47	
Urine	8	6	73	17	15		4	25	135	9	46	45	705
Liver	100	100	100	100	100	100	100	100	100	100	100	100	100
Left lung	36	33	75	65	52	38	31	80	83	55	63	33	90
Right lung	35	45	77	56	48	37	56	210	63	57	60	40	83
Left kidney	48	49	57	47	69	39	28	90	44	66	87	40	73
Right kidney	90	47	64	44	82	38	44	65	63	64	84	42	78
Spleen	53	294	68	109	80	46	37	160	70	64	82	40	90

Enzyme induction

In subjects 27 and 28 the concentrations in fluids and tissues of hydroxyamylobarbitone, from amylobarbitone conjointly administered with phenobarbitone or barbitone were greater than the metabolite in subjects 4–6 who had taken amylobarbitone alone. Increased metabolism due to enzyme induction may explain this difference.

Chronic administration

Breimer (1974) observed that the plasma half-life of butobarbitone fell from 40 to 32 h during chronic administration of the drug. The history of subject 23 was consistent with chronic administration of barbiturates over 10 years; the urinary concentrations of the two Tuinal metabolites were greater than the other subjects which may have been due to increased metabolic turnover by the liver microsomal enzymes.

The high bile barbiturate concentrations of 531 mg amylobarbitone and quinalbarbitone observed in subject 23 raise the possibility of enterohepatic circulation as only 6% of a single dose of amylobarbitone is excreted in the faeces (Tang et al 1975).

The effects of the lipid solubility of barbiturates on distribution at autopsy

When the concentrations of the barbiturates in the fluids and tissues from subjects 1–12 and the barbitone from subject 28 were expressed as percentages of the amounts in liver (Table 5) trends were observed that could be correlated with the lipid solubility of the barbiturate ingested.

The values for peripheral blood were generally lowest for the most lipid soluble barbiturate, quinalbarbitone, and highest for the least lipid-soluble barbitone. The urinary values, in ascending order, were: quinalbarbitone, amylobarbitone, pentobarbitone, butobarbitone and barbitone; which correlated well with the partition coefficients reported by Mark et al (1958).

Comparisons between quinalbarbitone (subjects 1–3) and barbitone (subject 28) concentrations show differences between the organs, the values for most tissues from subjects 1–3 were 35–50% of the corresponding liver concentration with barbitone the comparable values were 70–90%. These differences in distribution appear to be related to the time needed to achieve a barbiturate concentration in brain sufficient to cause death by respiratory depression, and here lipid solubility would have affected diffusion through the blood brain barrier.

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