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Effect of paracetamol on amylobarbitone hydroxylation in man: a gas chromatographic method for simultaneous estimation of underivatized paracetamol and barbiturates

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A rapid and specific technique for the simultaneous gas chromatographic estimation of underivatized paracetamol and barbiturates using an alkali flame ionization detector is described which is suitable for both forensic and pharmacokinetic investigations. An improved method for estimation of 3-hydroxyamylobarbitone is also detailed. These techniques were used in an investigation of the effects of oral administration of 1 g paracetamol 8 hourly on the formation of 3-hydroxyamylobarbitone from a single oral dose of 200 mg sodium amylobarbitone. No significant changes were found in the plasma concentrations and total body clearance of amylobarbitone nor was there any alteration in the urinary elimination of 3-hydroxyamylobarbitone.

Multiple drug ingestion is a common toxicological problem and paracetamol and barbiturates are often involved in drug overdosage. Barbiturates interfere with the estimation of paracetamol by ultraviolet spectrometry and gas-liquid chromatography (Street 1975). Furthermore, the highly polar nature of paracetamol produces unsatisfactory gas chromatographic behaviour on most separation phases giving rise to marked 'tailing' of the peak. Previously published methods have therefore relied upon the formation of various derivatives to improve chromatographic performance and thereby allow more accurate quantitation. With such procedures the presence of barbiturates may still interfere with quantitation (Street 1975). We present a rapid assay for underivatized paracetamol in the presence of most commonly encountered barbiturates which allows accurate estimations of both compounds to be made within the therapeutic range. This method was employed in a study of concurrent administration of these drugs in therapeutic doses.

Amylobarbitone has been proposed as a probe of hepatic drug oxidation in man since it is completely absorbed when administered orally, undergoes no first-pass hepatic metabolism and is innocuous in low doses (Inaba et al 1976). Only 1–2% unchanged amylobarbitone appears in the urine and the major metabolite is 3′-hydroxyamylobarbitone (Grove & Toseland 1970). Recently it has been suggested that another metabolite is derived by an unusual ring hydroxylation: *N*-hydroxyamylobarbitone (Tang et al 1975). The major paracetamol metabolites are the glucuronide and the sulphate but some 15–20% of the drug is metabolized (like amylobarbitone) via the mixed function oxidase system to a reactive alkylating metabolite of undetermined structure which in therapeutic doses is conjugated with hepatic glutathione but at high doses is thought to be responsible for hepatic necrosis (Davis et al 1976). It is known that patients taking a combined overdose of paracetamol and amylobarbitone show reduced hydroxylation of amylobarbitone (Grove 1976; Prescott & Stevenson 1972) which is usually associated with grossly deranged hepatic function tests. Reversible interference with amylobarbitone side-chain hydroxylation may occur in such cases when the liver damage is only mild (Toseland, personal observations). A study was therefore carried out using this assay for rapid and simultaneous determination of amylobarbitone and paracetamol to determine whether alterations in amylobarbitone hydroxylation can be detected when therapeutic doses of amylobarbitone and paracetamol are administered to normal subjects.

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MATERIALS AND METHODS

Simultaneous estimation of barbiturates and paracetamol in plasma

To 1 ml of plasma in a glass-stoppered tube was added 0·2 ml of a methanolic internal standard solution (for amylobarbitone at therapeutic doses 1 μg secobarbitone was used) 2 ml 0·5 M citrate

buffer (pH 5·5) and 5 ml redistilled ethyl acetate (Analar). After 10 min mechanical shaking and centrifugation at 750 g for 5 min the organic phase was transfered to a tapered glass tube and evaporated to dryness under a stream of nitrogen in a water bath at 70 °C. The residue was dissolved in 20 μl acetone and 1–3 μl injected into the gas chromatograph, a Hewlett-Packard 5750 G model with a high sensitivity alkali-flame ionization detector. Column, borosilicate glass (120 cm × 0·2 cm i.d.) packed with 0·35% CDMS on Chromosorb W (HP) 60–80 mesh. Injection port temp. 290 °C; detector temp. 400 °C; column oven, 170 °C for 4 min rising at 10° min^{-1} to 225 °C. Gas flows (ml min^{-1}): nitrogen 30; hydrogen 28 and air 250.

Estimation of amylobarbitone in urine. To 1 ml of urine in a glass-stoppered tube was added 1 μg secobarbitone (internal standard), 0·5 ml 0·5 M sulphuric acid and 5 ml of redistilled diethyl ether. After mixing and centrifugation the ether was transferred to a second tube and extracted with 2 ml of 0·025 M sodium hydroxide. After discarding the ether, 2 ml of 0·5 M sulphuric acid and 5 ml ether were added to the aqueous phase. Following mixing the ether was transferred to a conical tube and evaporated to dryness at 35 °C under a current of nitrogen. The dried extract was dissolved in 20 μl methanol and 1–3 μl was injected into the gas chromatograph [fitted with a glass column (180 cm × 0·2 cm) packed with 3% OV-17 on Gas-Chrom Q (60–80 mesh)]. Injections were made on column, which was isothermal at 190 °C.

Estimation of 3-hydroxyamylobarbitone in urine. The first method for estimation of underivatized 3-hydroxyamylobarbitone was described by Grove & Toseland (1970) and employed the highly polar liquid free-fatty acid phase (FFAP). This assay can be improved by using the even more polar WG 11 (Werner Gunter Analysen Technik, Dusseldorf-Nord, West Germany). Interfering endogenous compounds were removed by passing 1 ml urine through a small column (10 cm × 0·5 cm) of Florisil 60–100 mesh (B.D.H. Chemicals, Poole, Dorset) which does not absorb barbiturates. 0·5 ml of treated urine was placed in a glass stoppered tube and 1 ml 0·5 M phosphate buffer (pH 4·5), 1 μg cyclobarbitone (internal standard) and 5 ml redistilled ether added. After vortexing for 30 s enough solid ammonium sulphate (previously washed in methanol) to saturate both phases was added. The ether layer was transferred to a conical tube, evaporated to dryness and the residue taken up in 20 μl acetone (Analar). 1–3 μl of this was injected on column into the gas chromatograph [fitted with a 120 cm × 0·2 cm glass column packed with 0·2% WG 11 on Chromosorb W (HP), 60–80 mesh operating isothermally at 225 °C].

Experimental regime. Two female and three male normal healthy volunteers ages 20–22 years, 52–73 kg received 200 mg sodium amylobarbitone (Eli Lilly) after fasting overnight on two occasions. Venous blood samples were taken at 0, 0·5, 1, 2, 4, 6, 8, 10, 24 and 30 h. Complete urine collections were made for up to 48 h. A light meal, constant within subjects, was taken at 4 h. On one occasion barbiturate administration was preceded by dosing with 1 g paracetamol (Winthrop) every 8 h for 24 h. On the experimental day, two further 1 g paracetamol doses were given: one simultaneously with the barbiturate and one at 8 h. The order of these regimes was determined randomly and an interval of two weeks intervened between them. Three other volunteers, one female and two male (ages 34, 33 and 45 years; 57, 76 and 71 kg respectively) provided only urine samples. The protocol was approved by the Guy's Hospital Ethical Committee. The elimination rate constant k was determined from the linear regression of the terminal phase of the plot of the logarithm of the plasma concentration against time. The half-life was found from $t_{\frac{1}{2}} = 0{\cdot}693/k$. The area under the plasma concentration, time curve (AUC) was estimated by the trapezoidal rule with the addition of the term: plasma concentration at 30 h/k to approximate the tail region of the curve. The apparent volume of distribution was determined from

$$Vd = \frac{\text{Dose}}{k\,(AUC)} \text{ and clearance from } Cl = \frac{\text{Dose}}{AUC}$$

RESULTS

No endogenous compounds were found to interfere with quantitation using this technique. Calibration curves were linear over the ranges investigated: amylobarbitone 0–5 μg ml^{-1} and paracetamol 0–50 μg ml^{-1}. The coefficients of variation were for amylobarbitone 9·2% (within assay) and 7·9% (between assay) at 4 μg ml^{-1} and for paracetamol 8·2% (within assay) and 5·6% (between assay) at 10 μg ml^{-1}. The lower limits of detection were 0·2 μg ml^{-1} for both compounds. For 3-hydroxyamylobarbitone on the WG-11 column, the calibration curve was linear over the range 0–40 μg ml^{-1} and the coefficient of variation (within assay) was 7·6%. The lower limit of detection was 0·7 μg ml^{-1}.

Table 1 gives the estimated pharmacokinetic parameters based upon analysis of the plasma

Table 1. Kinetic parameters determined from plasma estimations of amylobarbitone in 5 subjects receiving 200 mg sodium amylobarbitone ± paracetamol.

	Amylobarbitone		Amylobarbitone + paracetamol	
	Mean	s.d.	Mean	s.d.
k (h^{-1})	0·038	0·016	0·041	0·020
$t_{\frac{1}{2}}\beta$ (h)	20·5	7·3	20·3	9·3
Vd (litre kg^{-1})	1·55	0·53	1·32	0·62
Clearance (litre h^{-1} kg^{-1})	0·054	0·015	0·046	0·011

concentration data. Student's paired *t*-test showed none of these parameters to be significantly altered by administration of paracetamol. Table 2 summarises the urinary excretion data. There was no significant difference either in the percentage of the dose excreted as metabolite or in the ratio of metabolite to unchanged drug in the presence and absence of paracetamol.

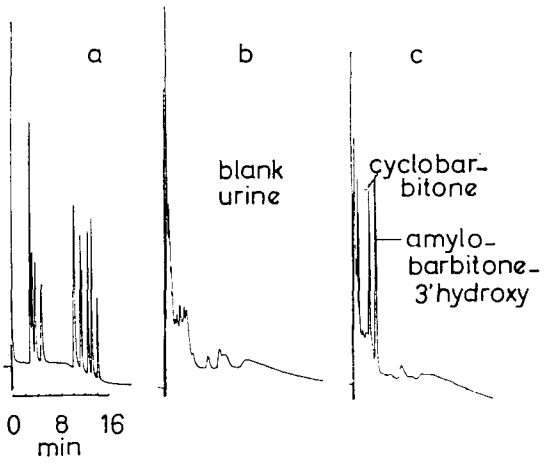

FIG. 1. Representative gas-chromatogram illustrating: a, relative positions of peaks due to (in order of appearance) 1, butobarbitone; 2, amylobarbitone; 3, pentobarbitone; 4, quinalbarbitone; 5, paracetamol; 6, cyclobarbitone; 7, heptabarbitone; 8, phenobarbitone; 9, 5-ethyl 5 *p*-tolyl barbituric acid. b and c, blank urine and urine containing 12·5 μg ml^{-1} 3-hydroxyamylobarbitone.

The mean plasma paracetamol concentrations were 4·9 μg ml^{-1} just prior to administration of amylobarbitone and paracetamol and this rose to a mean peak (usually at 1 h) of 32·8 μg ml^{-1}. The mean concentration at 10 h (the second peak following paracetamol administration at 8 h) was 26·3 μg ml^{-1} which fell to a mean level of 1·0 μg ml^{-1} at 30 h.

Table 2. Urinary excretion of amylobarbitone and 3-hydroxyamylobarbitone by 8 subjects following 200 mg sodium amylobarbitone with and without paracetamol.

	Amylobarbitone		Amylobarbitone + paracetamol	
	Mean	s.d.	Mean	s.d,
% dose excreted as 3-hydroxyamylobarbitone	22·16	8·39	18·57	8·14
% dose excreted as amylobarbitone	0·95	0·71	0·90	0·62
Ratio of metabolite to unchanged amylobarbitone	34·5	17·3	32·3	23·8

DISCUSSION

The use of the alkali flame ionization detector allows a relatively simple extraction procedure to produce chromatograms with a relatively small contribution to the detector response from endogenous compounds. The technique described above obviates the need to employ two separate columns for the estimation of paracetamol and barbiturate in the same sample as suggested by Stewart & Willis (1975). It also has the advantage of allowing chromatography of the underivatized compounds without the necessity of synthesizing *O*-benzoyl (Street 1975), trimethyl silyl (Prescott 1971a) or acetyl derivatives (Prescott 1971b). There is accordingly a saving of time without sacrifice in specificity. The method possesses sufficient sensitivity to estimate therapeutic plasma concentrations of both drugs and could find application for the identification and quantitation of these compounds in forensic science. The method for underivatized 3-hydroxyamylobarbitone is a modification of the method of Grove & Toseland (1970) but has greater sensitivity.

The half lives for amylobarbitone found in our subjects fall within the range 23·8 (6·7 s.d.) h published for a larger group of subjects by Inaba et al (1976) and is consistent with the range of 16·8–22 h determined from the urinary elimination of amylobarbitone (Grove & Toseland 1971a). The overall mean value of 0·92% of the dose excreted in the urine as unchanged amylobarbitone is comparable with the 0·7% found by Grove & Toseland (1971b) in 5 subjects. The mean value of 21·5% of the dose excreted as 3-hydroxyamylobarbitone is a little lower than the 30% (Grove & Toseland 1971b) or 25% (Irvine et al 1974) reported elsewhere. The $t_{\frac{1}{2}}$ of 3-hydroxyamylobarbitone is 5·7 h and its rate of elimination is faster than its rate of formation following ingestion of amylobarbitone (Grove & Toseland 1971a). As emphasized by Inaba et al (1976) the parameters of amylobarbitone elimination exhibit much interindividual variation but are relatively constant within individuals which makes

amylobarbitone an excellent monitor of hepatic function. One important determinant is known to be age (Irvine et al 1974). The 3-hydroxyamylobarbitone: amylobarbitone ratio has been suggested as being of value in assessing the extent of amylobarbitone metabolism by an individual (Grove & Toseland 1971b). No effect of paracetamol on this ratio was detected and the variation of the ratio within subjects over the 0–24 and 24–48 h periods was greater than the total hydroxyamylobarbitone ratio. The ratio may, however, be useful following overdosage of amylobarbitone when there is no knowledge of the dose ingested. Under these circumstances an isolated measurement of hydroxyamylobarbitone output would be meaningless and this ratio could indicate metabolic efficiency. From the results presented it may be concluded that therapeutic doses of paracetamol do not alter the formation of 3-hydroxyamylobarbitone from a therapeutic dose of amylobarbitone.

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