Gas Chromatography of Barbiturates II. Application to the Study of Their Metabolism and Excretion in Humans

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Five different barbiturates were administered to adult humans and their urine analyzed for the unchanged and metabolized products by gas chromatography. Barbital was easily identified in urine collected 16 days after administration. The demethylation of N-methyl substituted barbiturates could be followed. *p*-Hydroxy phenobarbital was detected by gas chromatography after administration of phenobarbital and mephobarbital.

DEVELOPMENT of new and more specific methods of analysis has repeatedly led to reinvestigation of problems associated with the metabolism and excretion of barbiturates. The earliest studies were based on gravimetric methods which, for the most part, lacked specificity and sensitivity (1–7). More recently, ultraviolet spectrophotometry has been extensively used (7–23). Occasionally, erroneous results have been reported due to the fact that the spectrophotometric method usually does not differentiate between an unchanged barbiturate and its metabolic product. Paper chromatography (24–26) and liquid-liquid partition systems (20, 27) have been used for separation of such mixtures. Gas chromatography has been shown to be a rapid and selective method for analysis of barbiturates in complex mixtures (28–30). It seemed to be of interest to study the applicability of this method to biological systems, particularly with regard to the urinary excretion of certain barbiturates and their metabolites.

EXPERIMENTAL

The experiments were carried out with adult humans. A single, therapeutic dose of the barbiturate in powder or tablet form was taken in the evening, and the urine collected the next morning and at intervals during the following 24 to 40 hours. In the case of barbital, urine samples were also collected 6 and 16 days after administration.

Purification.—The total volume collected during each time interval was acidified with dilute hydrochloric acid and extracted several times with ether. The combined ether extracts were washed with a 1% solution of sodium bicarbonate which removed much of the colored impurities. The bicarbonate wash was shaken once with a little ether, which was then combined with the total ether extract. The barbiturates were extracted from the ether solution with 0.1 *N* sodium hydroxide. Each alkaline extract was filtered through a pledget of cotton into an excess of dilute hydrochloric acid. The combined and acidified aqueous extracts were shaken several times with ether, the ether extracts dried over anhydrous sodium sulfate and evaporated to dryness in a rotating vacuum evaporator. The residue was dissolved in 1 to 5 ml. acetone, and this solution was used for analysis.

Gas Chromatographic Analysis. The instrument and experimental conditions described previously were used (30). The quantitative determinations were based on planimetric measurements of peak areas and internal standardization. Most of the work was carried out on a column containing 1.7% of neopentyl glycol sebacate on acid- and alkali-washed Chromosorb W, 60–80 mesh. However, *p*-hydroxy phenobarbital could not be eluted from this column. Apiezon L was found to be a satisfactory stationary phase for separation of phenobarbital and its *p*-hydroxy derivative.

The results are given in Tables I to V.

DISCUSSION

Although the present work is based on only a single administration of each drug, the results are in good agreement with earlier reports. Of the five barbiturates investigated, only barbital appears to be excreted quantitatively in unchanged form. Thus, following a single dose of 1500 mg., Lous (15) reported a recovery from urine of 33% after 48 hours and of 75 to 95% after 10 to 14 days. Our own work also confirms this slow elimination of barbital. Even after 16 days, detectable amounts were present in the urine.

No metabolite has ever been isolated from aprobarbital. Although Fabre (4) has reported to have recovered 46 to 90% of the dose after 6 to 10 days, more recent work (15) shows recoveries of less than 20% after 5 to 8 days. In our experiment, 8% of the dose was found unchanged after 48 hours. These low recoveries of aprobarbital would lead one to suspect that a major portion of this barbiturate may be metabolized to substances that are not detected by the methods used so far.

Butler, *et al.* (21), have shown that dogs excrete phenobarbital mainly as the *p*-hydroxy derivative. Later Curry (24, 25), Algeri and McBay (26), and Butler (20) have found that this conversion also takes place in humans, although to a lesser extent. In dogs, *p*-hydroxy phenobarbital is almost completely conjugated, mainly with glucuronic acid, while humans excrete about one-half of the metabolite in the free form, the other half as a conjugate other than the glucuronide (20). *p*-Hydroxy phenobarbital interferes with the U.V. spectrophotometric determination of phenobarbital unless a separation is carried out prior to the determination (27). By gas chromatography on a polyester column, only phenobarbital was eluted and determined. However, a column containing Apiezon L as the stationary phase (1.4% on Chromosorb W, 60–80 mesh) gave a well-defined peak for the *p*-

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TABLE I.—URINARY EXCRETION AFTER SINGLE ADMINISTRATION OF 300 MG. OF BARBITAL

Time After Administration	Urine Volume, ml.	Barbital Found— mg.	Barbital Found— Cum. % of Dose
0–8.5 hr.	300	6.6	2.2
8.5–17 hr.	250	12.1	6.2
17–24 hr.	300	14.7	11.1
24–32 hr.	300	15.9	16.4
6 days (8-hr. sample)	300	5.2	...
16 days (24-hr. sample)	900	Trace	...

TABLE II.—URINARY EXCRETION AFTER SINGLE ADMINISTRATION OF 200 MG. OF METHARBITAL

Time After Administration, hr.	Urine Volume, ml.	Found— Metharbital— mg.	Metharbital— Cum. % of Dose	Barbital— mg.	Barbital— Cum. % of Dose
0–9.5	500	0.54	0.27	0.26	0.13
9.5–24	550	0.66	0.6	3.18	1.72
24–48	900	1.17	1.18	15.1	9.37

TABLE III.—URINARY EXCRETION AFTER SINGLE ADMINISTRATION OF 200 MG. OF PHENOBARBITAL

Time After Administration, hr.	Urine Volume, ml.	Found— Phenobarbital— mg.	Phenobarbital— Cum. % of Dose	*p*-OH-phenobarbital
0–9	400	6.3	3.65	+
9–24	550	15.5	10.9	+
24–48	900	25.0	23.4	+

TABLE IV.—URINARY EXCRETION AFTER SINGLE ADMINISTRATION OF 150 MG. OF MEPHOBARBITAL

Time After Administration, hr.	Urine Volume, ml.	Found— Mephobarbital mg.	Mephobarbital Cum. % of Dose	Phenobarbital mg.	Phenobarbital Cum. % of Dose	*p*-OH-Phenobarbital
0–9	400	1.2	0.8	6.4	4.3	+
9–24	300	Trace	...	6.3	8.5	+
17–32	500	Trace	...	6.8	13.0	+

TABLE V.—URINARY EXCRETION AFTER SINGLE ADMINISTRATION OF 150 MG. OF APROBARBITAL

Time After Administration, hr.	Urine Volume, ml.	Aprobarbital Found mg.	Aprobarbital Found Cum. % of Dose
0–9.5	300	1.7	1.1
9.5–24	350	1.8	2.3
24–48	1,350	8.7	8.1

hydroxy derivative. At 187° the retention time was 5.5 minutes for phenobarbital and 20 minutes for the metabolite. Authentic samples of *p*-hydroxy phenobarbital isolated from dog urine[1] and synthesized according to Butler (20) were used to identify the metabolite in urine collected after administration of phenobarbital and mephobarbital. No attempts were made to determine the amounts of the *p*-hydroxy compound quantitatively. However, a visual inspection of the chromatograms showed the amounts of the unconjugated material to be smaller than those of phenobarbital.

[1] The authors are indebted to Dr. Thomas C. Butler for supplying us with a sample of this material.

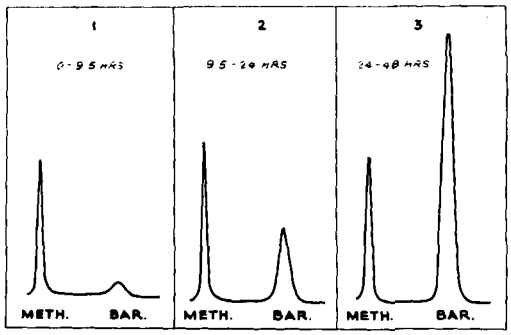

Fig. 1.—Gas chromatograms of urine extracts after administration of metharbital. METH., metharbital; BAR., barbital.

N-Methyl derivatives of barbiturates are demethylated in the body. Butler (16, 18, 22) has shown that mephobarbital is much more rapidly demethylated than metharbital. Our results confirm this (Tables II and IV, Fig. 1).

CONCLUSION

The gas chromatographic method of analysis has been found to be suitable for identification and quantitative determination of barbiturates and their metabolites excreted in the urine. Results obtained with five barbiturates are in good agreement with earlier reports.

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