A NOTE ON A URINARY METABOLITE OF PHENOBARBITONE

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Recently a report of two spots forming a paper chromatographic pattern characteristic of acute phenobarbitone poisoning drew attention to the first metabolite of this compound found in human urine[1]. The material from the slower running spot has now been isolated and identified as *p*-hydroxyphenobarbitone. This is the same compound recently isolated from dog urine by Butler[2].

The paper chromatographic separation of phenobarbitone from its metabolite offered a ready, if tedious, method for isolating the metabolite. The first crystals were obtained by this method from several pooled samples of post-mortem urine. It was observed during these experiments that the metabolite was much less soluble in chloroform than was phenobarbitone. When recently nearly one litre of ante-mortem urine was submitted to the laboratory in a case of acute phenobarbitone poisoning it was decided to attempt to use this differing solubility as a means of separating the two compounds on a macro scale. The urine, acidified with dilute hydrochloric acid, was therefore extracted first with chloroform in a continuous extractor for seven hours and then four times with 1000 ml. quantities of ether. The chloroform was evaporated and the residue examined by paper chromatography. Both metabolite and phenobarbitone were present. In the ether extract, however, there was only the slower-running spot of the metabolite. Crystals were obtained by evaporation of an ether/light petroleum (40-60°) solution of this second extract. These, decolourised with charcoal and recrystallised from water, had a melting point = 222° C. (15 mg.). Found; C, 53·2; H, 5·3; N, 10·2 per cent. $C_{12}H_{14}N_2O_5$ requires C, 54·1; H, 5·3; N, 10·5 per cent. Melting point 222–3° C.

The crystals were examined by paper chromatography in the ascending, *n*-butanol saturated with 5N ammonia, system. No other ultra-violet absorbent material was present as shown by a contact print of the chromatogram on Ilford Reflex Contact Paper No. 50 using as the light source a mercury arc ultra-violet lamp, with no filters. The spot at $R_F = 0{\cdot}17$ reacted with the ferric chloride/potassium ferricyanide spray reagent for phenols[3] to give a blue spot. It gave no reaction with 2:6-dichloroquinone-chloroimide confirming the substituted *para*-hydroxy position.*

Observations in the ultra-violet are shown in Figures 1 and 2.

These show significant differences between phenobarbitone and this metabolite and are a valuable criterion for demonstrating phenobarbitone ingestion. The wavelength maxima and minima for the metabolite are:

*Note added in proof. Comparison of this compound with an authentic sample kindly provided by Dr. T. C. Butler, has confirmed its identity.

At *p*H 13, $\lambda_{max.}$ = 249 mμ, 290 mμ: $\lambda_{min.}$ = 231 mμ.
At *p*H 10, $\lambda_{max.}$ = 244·5 mμ, 290 mμ.
At *p*H 2, $\lambda_{max.}$ = approximately 273 mμ.

Apart from these frequency differences, in contrast with phenobarbitone, decreasing the *p*H from 13 to 10 does not significantly increase the maximum extinction reading. Because of the very small amount of material

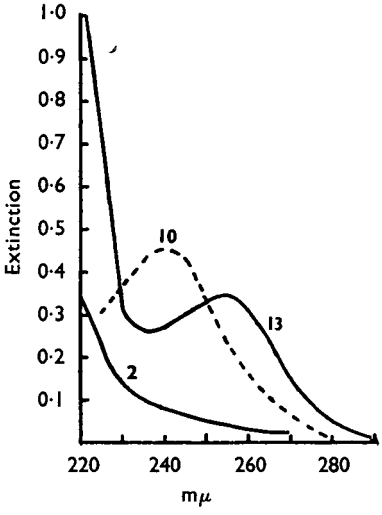

FIG. 1. Phenobarbitone showing ultra-violet observations at *p*H 2, 10 and 13.

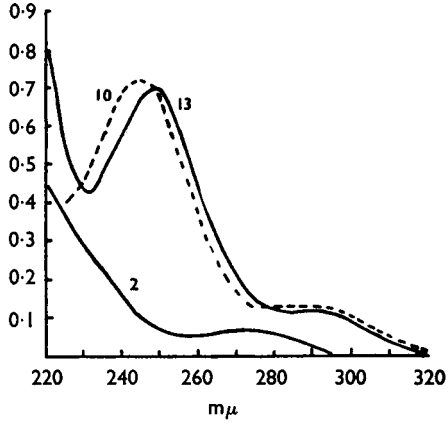

FIG. 2. *p*-Hydroxyphenobarbitone showing ultra-violet observations at *p*H 2, 10 and 13.

available, the extinction coefficient of *p*-hydroxyphenobarbitone has not been accurately determined. Solutions were made in 0·1 N sodium hydroxide and the *p*H lowered by the addition of micro drops of 6N sulphuric acid.

It was suggested by Butler that this compound was, in the dog, excreted in a conjugated form. After these first extractions the urine was therefore boiled with hydrochloric acid for six hours and then re-extracted with ether. No more *p*-hydroxy compound was obtained. This suggests that this metabolite is excreted in man in the free form.

Carbon hydrogen and nitrogen analyses were by Drs. Weiler and Strauss, Oxford.

REFERENCES

1. Curry, *J. Pharm. and Pharmacol.*, 1955, **7**, 604.
2. Butler, *Science*, 1954, **120**, 494.
3. Barton, Evans and Gardner, *Nature Lond.*, 1952, **170**, 249.