A NOTE ON THE PAPER CHROMATOGRAPHIC SEPARATION OF PHENOBARBITONE METABOLITES

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Received June 20, 1955

BARBITURATES are the most common poisonous drugs in Britain at the present time. Death frequently involves a long period of coma and consequently little barbiturate remains in the body for the chemical toxicologist to identify. Any method which gives a lead to the particular barbiturate ingested is therefore of value. The identification of pentobarbitone from its urinary metabolites has been reported[1] and I wish to report a paper chromatographic pattern which has been frequently seen in this laboratory in the urinary barbiturate extract only in acute phenobarbitone poisoning. Figure 1 illustrates the pattern. The butanol: ammonia solvent system of Algeri and Walker[2] on Whatman No. 1 paper followed by their mercuric sulphate: diphenylcarbazone method of development has been used. The tungstic acid protein precipitation method used by Valov[3] followed by a sodium bicarbonate wash to remove acids has in all instances been used to extract the barbiturates. They have been then extracted from the ether solution by 0·5N sodium hydroxide when acidification and re-extraction into ether has given the crude barbiturate extract for chromatography.

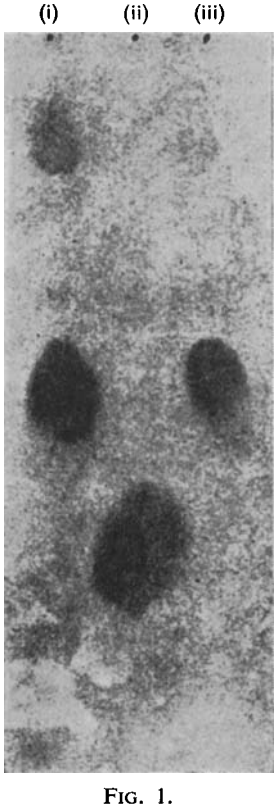

FIG. 1.

(i) Urinary extract.
(ii) Amylobarbitone 75 μg.
(iii) Phenobarbitone 50 μg.

Apart from a recent report of the isolation of *p*-hydroxy phenobarbitone[4] in the urine of dogs after ingestion of phenobarbitone no other metabolites have as yet to my knowledge been reported. The slower running spot $R_F = 0{\cdot}17$ has been extracted from the paper and shows ultra-violet absorption spectra at *p*H 13, 10 and 2 characteristic of the barbiturate ring. However, there is a slight variation. At *p*H 13 $\lambda_{\text{min.}} = 232{\cdot}5$ mμ and $\lambda_{\text{max.}} = 249{\cdot}5$ mμ as distinct from $\lambda_{\text{min.}} = 233$ mμ and $\lambda_{\text{max.}} = 255$ mμ for phenobarbitone. Otherwise the spectra, and the change in spectra with *p*H, follow those generally observed for all barbiturates. This laboratory investigates many instances of barbiturate poisoning, but we have not seen this pattern reproduced by any barbiturate other than phenobarbitone, nor does it normally appear in the blood, brain liver or

cerebrospinal fluid extracts. In only two instances of phenobarbitone poisoning has a slower running spot appeared in other than urine extracts. In both of these the liver extracts also showed a spot which agreed in R_F with the much larger quantity of slower running spot seen in the urine.

The paper chromatographic separation offers an easy method for the isolation of this metabolite and work is proceeding with its identification.

Thanks are accorded to Dr. F. G. Tryhorn for advice.

References

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