INTERFERENCE BY β-METHYL-β-ETHYL GLUTARIMIDE IN THE DETERMINATION OF BARBITURATES

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A rapid and reliable colorimetric method for the estimation of barbiturates is of importance to the clinical biochemist and the forensic chemist. The cobalt acetate-*iso*propylamine reaction was the main method used until recently when the widespread availability of ultra-violet spectrophotometers enabled measurements in the ultra-violet to form the basis of many methods. There are few compounds that interfere in practice with either of these methods[1] but it is the purpose of this paper to show that bemegride (β-methyl-β-ethyl glutarimide), which has recently been introduced as a treatment for acute barbiturate poisoning, can interfere in the quantitative estimation of barbiturates.

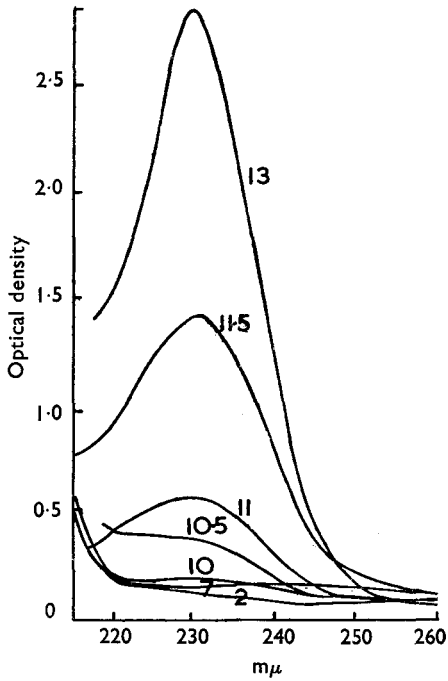

FIG. 1. β-Methyl-β-ethyl glutarimide 2 mg./100 ml. The figures are pH values; that of 11·5 is approximate and represents 0·5N ammonium hydroxide.

Recently in this laboratory it was observed that the absorption maximum of a blood-extract for barbiturate was to be found at 235 mμ in 0·5N ammonia solution instead of at 240 mμ. This observation led to the discovery that bemegride can interfere with the determination of barbiturates by measurements in the ultra-violet. Initial experiments with this compound showed that it was extracted by ether from aqueous acidic solution, that it was not extracted from ether by aqueous sodium bicarbonate solution, and that it was extracted from ether by 0·1N sodium hydroxide. It was thus being extracted together with the barbiturates. A positive cobalt-amine reaction was obtained and it appears that a false result is possible should this method be used to assay barbiturates in the presence of bemegride.

The 5:5-disubstituted barbiturates have absorption maxima at 255 mμ in 0·5N sodium hydroxide; this peak increases in intensity and moves to

240 mμ as the pH is decreased to 11·5. Between 11·5 and 9 the absorption is substantially unchanged but with further decrease of pH the absorption decreases until in acid solution it is negligible compared with its value at pH 9 to pH 11·5. The difference in absorption at pH 10–11 and pH 2 forms the basis of most ultra-violet methods of assay. Authors differ on the exact higher pH; McCallum[2] uses 0·5N ammonia solution, Walker and his colleagues adjust the pH of a sodium hydroxide solution to pH 10 with micro drops of sulphuric acid[3], while most other authors use borate buffer at approximately pH 10. Any compound whose absorption at 240 mμ decreases as the pH is decreased from pH 11 to pH 2 can interfere with this method. The ultra-violet spectrum of bemegride and its change with pH is shown in Figure 1.

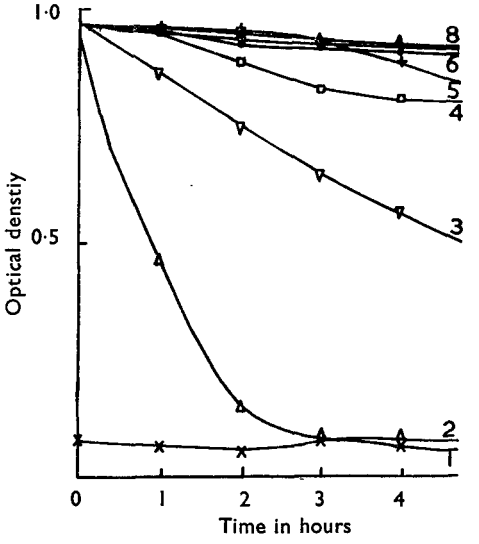

FIG. 2. Hydrolysis rate of bemegride and several barbiturates.

1. Bemegride in acid solution.
2. „ „ 0·5N NaOH.
3. Allobarbitone.
4. Phenobarbitone.
5. Amylobarbitone.
6. Butobarbitone.
7. (Between 6 and 8) Cyclobarbitone.
8. Quinalbarbitone.

It is apparent that, if present, bemegride can interfere with the assay of barbiturates. The Figure also shows that the variation of the spectrum of bemegride with pH is significantly different from that of the barbiturates. At pH 10 and pH 11·5 the absorption of the barbiturates is substantially the same but between these pHs the spectrum of bemegride undergoes radical changes. If, therefore, aliquots of an extract in equal volumes of 0·5N ammonia and borate buffer at pH 10 differ in their absorption at 240 mμ there is presumptive evidence for the presence of bemegride. If the whole spectra are then plotted at pH 9, pH 11·5 and pH 13 the resulting curves will enable an estimate to be made of the contribution of bemegride to the absorption of the barbiturate.

At the present time little is known of the distribution and nature of metabolites of bemegride. A urinary metabolite has been reported[4] and it appears that this compound may also interfere with the determination of barbiturates. A second method of estimating the barbiturate concentration seemed desirable and experiments showed that one method of some value was the hydrolysis of an aliquot under alkaline conditions. The glutarimide ring is apparently less stable than the barbiturate ring and consequently bemegride can be hydrolysed by

conditions affecting only a small proportion of most barbiturates. The hydrolysis products have negligible absorption at 240 mμ in acid and alkaline solution and the barbiturate may thus be assayed as usual after the hydrolysis. The suggested conditions are heating at 38° for 2 hours in 0·5N sodium hydroxide solution. Figure 2 shows the hydrolysis rates of bemegride and several barbiturates under these conditions. The concentrations of the glutarimide were obtained from readings at 230 mμ, while the barbiturate concentrations were followed from readings at 255 mμ.

Summary

1. Bemegride has been shown to interfere with the quantitative estimation of barbiturates.
2. A method which surmounts this difficulty is suggested.

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References

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