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Modification of Quantitative Colorimetric Estimation of Glutethimide in Toxicology

SIR,—In the course of an investigation of the vomitus of a patient containing glutethimide, certain snags were encountered in its quantitative estimation.

The measurement of the ultra-violet absorption spectrum and characteristic "half-life" in ethanolic potassium hydroxide (Goldbaum and Williams, 1960) works satisfactorily with urine and blood. Applied to the vomitus, there was enough fatty matter present in the chloroform extract to cause a turbid solution in the final ethanolic potassium hydroxide mixture, thus making the spectrophotometric reading impossible. Dissolving the extract in 10 per cent ethanol removed most of the fat and gave a qualitative identification of the glutethimide. However, the recovery of glutethimide was not quantitative.

The colorimetric method of (Sheppard, D'Asaro and Plummer, 1956) also gave a turbid solution and treatment with aluminium hydroxide column chromatography recommended by these authors did not remove the fatty impurities. We have now found that by extracting the purple colour into isobutanol the fatty impurities does not interfere. We also found the choice of 0·5 ml. of the ferric chloride reagent to be more satisfactory in imparting a less strong yellow colour to the isobutanol layer. Accurate and reproducible results were obtained as long as the readings were taken within half an hour.

Reagents. (*a*) Hydroxylamine hydrochloride 2M (store in refrigerator) (*b*) Sodium hydroxide 3·5N (*c*) Hydrochloric acid 3·5N (*d*) Ferric chloride 0·37M in 0·1N hydrochloric acid. (*e*) Isobutanol A.R. (*f*) Methanol A.R. (*g*) Chloroform A.R.

Method. Extract the specimen with chloroform. Filter and evaporate the solvent at a low temperature to 2–3 ml. and then at room temperature (30°) to dryness. Dissolve the residue in a small amount of methanol, so that it contains not more than 1 mg. of glutethimide per ml., for colour development.

Standard Graph. Introduce 1 ml. of methanol solution of glutethimide, containing respectively 0·25, 0·50, 0·75 and 1·00 mg. into 4 × 10 ml. glass stoppered cylinders. Add 1 ml. of hydroxylamine hydrochloride reagent and 1 ml. 3·5N of sodium hydroxide. Allow to stand for 30 min. Add 1·5 ml. 3·5N hydrochloric acid, 5 ml. isobutanol and then 0·5 ml. of ferric chloride reagent. Shake vigorously for 30 sec. and allow to separate. As soon as separation is complete, pipette off the isobutanol and filter through a 5 cm. No. 1 Whatman filter paper. Measure its optical extinction, without delay, at 510 mμ using the reagents as blanks.

Recovery experiments. We have investigated the recovery of glutethimide in vomitus by adding 1 mg. of pure glutethimide to four specimens of vomitus,

and obtained an average recovery of 90 per cent. Barbiturates and brominated ureides were found not to interfere.

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