activity are desirable to characterise the active agent(s). Using Porapak Q columns, comparison of three of the published procedures for the gas chromatographic estimation of acetylcholine[1,2,4] indicated that, while in general the procedure of STAVINOHA AND RYAN[1] gives the largest detector response to a given amount of acetylcholine, the procedure of JENDEN *et al.*[2] is sensitive to the lowest concentration of acetylcholine.

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Rapid measurement of therapeutic levels of glutethimide in plasma

Several gas–liquid chromatographic methods for the measurement of glutethimide in whole blood or plasma have been presented[1–6]. Those applicable to cases of overdosage have tended to sacrifice sensitivity for speed; others, developed specifically for therapeutic studies, are somewhat lengthy and involved. The following report illustrates how the criteria of speed and sensitivity may be simultaneously satisfied by the use of improved instrumentation.

Materials and methods

Chromatography. A Varian Aerograph Series 2100 Gas Chromatograph, fitted with a flame ionisation detector, was used. The metal column (6 ft. × ¼ in. O.D.) contained 5% SE-30 on 70–80 mesh AW Chromosorb W, and was conditioned at 200° over a 24-h period prior to use.

Operating conditions. The operating conditions were the following: column oven, injection port, and detector temperatures, 195°, 220° and 240°, resp.; sensitivity setting, 1×10^{-10} A; carrier gas (nitrogen) flow rate, 60 ml/min.

Extraction. 2.0 ml of plasma were shaken for 10 min with 20 ml of re-distilled hexane in a 30-ml centrifuge tube. After centrifugation at 3,000 r.p.m. for 5 min, 18 ml of the organic layer were transferred to a second, dry 30-ml tube and evaporated under a stream of nitrogen to small bulk (2–3 ml), the tube being immersed in a water bath at 60°. The concentrate was transferred to a 10-ml conical test tube to which 1 ml of di-*n*-butyl phthalate internal standard (0.5 mg/100 ml acetone) had been added. The 30-ml tube was washed out with 1–2 ml of acetone and the washings were added to the contents of the conical tube. On evaporation to dryness, the residue was re-dissolved in 100 μl of dried ethanol and 3–5 μl of this extract injected on to the gas chromatograph.

Results and discussion

On the chromatographic system described, glutethimide and di-*n*-butyl phthalate had retention times of 3 and 4 min, respectively. The ratio of the peak height of glutethimide to that of di-*n*-butyl phthalate was linear over the range 0.01 to 1.0 mg % of glutethimide. Recoveries of glutethimide from plasma were of the order 60 $\pm$ 5 %, and the limit of measurement in plasma was 0.03 mg %. The omission of time-consuming purification and drying procedures on the organic extract enabled an analysis to be completed within 1 h. Further, the use of an internal standard eliminated the possibility of injection errors.

Limited therapeutic trials, in which subjects ingested 250 mg of glutethimide in tablet form, substantiated the findings of other workers[5] that peak levels in plasma ranged between 0.2 and 0.6 mg %.

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