Miller, L. C. & Tainter, M. L. (1944). *Proc. Soc. exp. Biol., N.Y.*, **57**, 261–264.
Paton, W. D. M. & Zaimis, E. J. (1948). *Nature, Lond.*, **161**, 718.
Paton, W. D. M. & Zaimis, E. J. (1949). *Brit. J. Pharmacol.*, **3**, 381–400.
Rice, S. A. (1956). *J. Amer. chem. Soc.*, **78**, 5247–5252.
Rice, S. A. (1958). *Ibid.*, **80**, 3207–3214.
Thomson, R. E. (1946). *Endocrinology*, **39**, 62.
Waser, P. G. (1959). In *Curare and Curare-like Agents*, editors: Bovet, D., Bovet-Nitti, F. & Marini-Bettolo, G. B., p. 220, Amsterdam: Elsevier.
Waser, P. G. (1962). *Pflüg. Arch. ges. Physiol.*, **274**, 431–446.

Determination and identification of amphetamine in urine

SIR,—We report herein an improved and more convenient method than that reported previously, for the determination and identification of amphetamine in urine (cf. Beckett & Rowland, 1964). The determination is based on a modification of the method of Cartoni & Stefano (1963). Gas-liquid chromatography was used with *NN*-dimethylaniline as the internal standard, while amphetamine was identified and separated from related amines as the acetone derivative (cf. Brochmann-Hanssen & Svendsen, 1962).

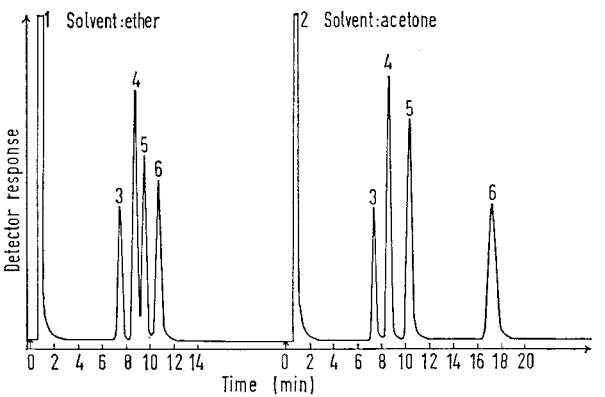

FIG. 1. Chromatograms of amphetamine, methylamphetamine, β-phenylethylamine and *NN*-dimethylaniline in the absence and presence of acetone: **1** ether, **2** acetone, **3** *NN*-dimethylaniline (t_R 7·5 min), **4** methylamphetamine (t_R 8·7 min), **5** amphetamine (t_R 9·5 min in ether, 10·4 min in acetone), **6** β-phenylethylamine (t_R 10·8 min in ether, 17·4 min in acetone).

Procedure. Urine (2-5 ml) was pipetted into a glass-stoppered centrifuge tube, neutralised with dilute hydrochloric acid or sodium hydroxide solution, as appropriate, and then 0·1 ml 5N hydrochloric acid added. The urine was extracted with freshly distilled Analar diethyl ether (3 × 2·5 ml), centrifuged and the ether extract rejected. Sodium hydroxide (0·5 ml, 5N) was added to the urine which was then extracted with ether (3 × 2·5 ml), centrifuging between each extraction. These ethereal extracts were transferred to a 15 ml Quickfit test tube, the base of which was finely tapered. 1 ml of *NN*-dimethylaniline solution in ether (5 μg base/ml) was added and the solution then concentrated on a water bath at 40° to about 50 μl. Approximately 3–5 μl of the concentrate was injected for analysis into the chromatograph. A calibration curve was obtained by measuring the ratio of peak heights of amphetamine to *NN*-dimethylaniline for known concentrations of amphetamine in urine. The curve was found to be linear over the range 0·1–10 μg amphetamine per ml of urine and the method had

an overall accuracy of $100 \pm 5\%$. Interfering peaks were not found on analysing many samples of urine from many subjects.

A Perkin Elmer F11 gas chromatograph was used. The column was a 100–120 mesh acid-washed celite 545, onto which was coated 5% potassium hydroxide, and 10% carbowax 6000, and packed into a 2 metre 1/8 in o.d. stainless steel tube. The column was silizanised with hexamethyldisilizane at the operating conditions which were as follows: column temperature 140°; injection block temperature 250°; nitrogen flow rate 20 ml/min (measured at room temperature); hydrogen pressure 15 lb/in^2; air pressure 25 lb/in.^{2}

Amphetamine was further identified by alteration of its retention time by conversion to its acetone derivative as follows. Acetone (0·5 ml) was added to the ether concentrate in a tapered test-tube and evaporated to about 50 μl on a water-bath at 60°. The concentrate (3–5 μl) was introduced into the chromatograph; a characteristic shift of the amphetamine peak to the amphetamine: acetone derivative peak was observed (see Fig. 1). The observed shift differentiates amphetamine from other related amines chromatographed in the presence and absence of acetone, e.g. see Fig. 1.

The total time necessary for the analysis of sixteen samples is about 6 hr, which is shorter than that for the previous method (Beckett & Rowland, 1964). Furthermore less sample is required and assays of 0·1 μg amphetamine base per ml urine may be made without interference from constituents in urine.

Acknowledgement. One of us (M.R.) thanks the Medical Research Council for a grant held whilst carrying out this research.

Chelsea School of Pharmacy,
Chelsea College of Science and Technology,
Manresa Road, London, S.W.3.

A. H. BECKETT
M. ROWLAND

November 17, 1964

References

Beckett, A. H. & Rowland, M. (1964). *J. Pharm. Pharmacol.*, **16**, *Suppl.*, 27*T*–31*T*.
Brochmann-Hanssen, E. & Svendsen, A. B. (1962). *J. pharm. Sci.*, **51**, 938–941.
Cartoni, G. P. & Stefano, F. de, (1963). *Italian. G. Biochim.*, **8**, 298–311.

The pharmacology of hippocampal neurones

SIR,—Little is known of the direct response of cells in the hippocampal cortex to potential cerebral neurotransmitters. We have applied various drugs microelectrophoretically into the environment of these cells and tested their effects on cell firing. This letter reports some preliminary findings.

Our experiments were made on nine adult cats under chloralose-urethane or pentobarbitone anaesthesia. The overlying neocortex was sucked away and the lateral ventricle was filled with 3% agar, and the exposure of the dorsal hippocampus completed. The activity of cells in the CA1 and CA3 fields was recorded extracellularly with the central 2·7 molar NaCl filled barrel of a 5-barrelled glass micropipette. The total tip diameter was 4–8 μ. Cell firing was monitored continuously on an oscilloscope and with an audioamplifier. The spikes were also led through a discriminator to a ratemeter the output of which was led to a penwriter.

The other 4 barrels of the microelectrode contained strong aqueous solutions of ionizable drugs. These were expelled from the electrode tip by the passage of appropriate currets.

Over 150 cells have been detected—these either fired spontaneously or could be excited by L-glutamate (confirming unpublished observations by Andersen,