

students answering Y, N and D, and the total number of students (i.e. answer sets in use).

This is a high-resistance, low-current system (requiring a stabilized ± 15 V supply) which works with 10-500 answer sets, is accurate to $\pm 2.5\%$ ($\pm 1\%$ if hand calibrated), and costs only £3-£3.50 per student to install plus £35 for the console (including ± 15 V supply). Following construction RV1

(Figure 1) is adjusted to give f.s.d. for 100%, and RV2 to calibrate the number-of-students scale on meter Y. The console may be upgraded for an additional £10 with extra circuitry to (i) improve the accuracy to 1% without the need for hand calibration, and (ii) to enable the console operator to 'freeze' the meter readings and eliminate any late answer changes which would otherwise alter the readings while being noted.

The determination of kynurenine by gas-liquid chromatography; evidence for its presence in rat brain

M.H. JOSEPH

Division of Psychiatry, Clinical Research Centre, Watford Road, Harrow, HA1 3UJ

Kynurenine is a major metabolite of tryptophan on a pathway initiated by the inducible liver enzyme tryptophan pyrrolase. This pathway is a route for the synthesis of NAD and it may compete with other pathways for available tryptophan, which may be limited. In particular competition with the synthesis of 5-hydroxytryptamine (5-HT) in the brain may be of importance in the precipitation of psychiatric depression (Curzon, 1969; Joseph, Young & Curzon, 1976).

The pyrrolase pathway has been studied in animals via the *in vitro* determination of tryptophan pyrrolase, and in animals and man via the urinary excretion of tryptophan metabolites, including kynurenine. Previously the relatively low sensitivity of analytical methods for the latter has required the use of loading doses of tryptophan. Recently we have described (Joseph & Risby, 1975) a more sensitive colourimetric method for the estimation of kynurenine in plasma (which requires the suppression of interference from tryptophan). The present demonstration shows that the *o*-amino acetophenone produced by heating kynurenine with strong alkali in that method can be conveniently and sensitively determined by gas liquid chromatography. Following extraction of the *o*-amino acetophenone into butyl acetate containing dichloro-*p*-xylene as internal standard it is derivatized with trifluoroacetic anhydride; excess reagent is hydrolysed with 0.2M borax in 1.5M sodium hydroxide. 1 μ l of supernatant is injected into a

Hewlett-Packard 5713A gas chromatograph. Injection port 250°C, column 10% OV1 on H.P. Chromosorb W at 120°C, detector 300°C ^{63}Ni electron capture, carrier gas argon/methane (95/5) at 60 ml/minute.

When this method was applied to brain tissue from male Sprague Dawley rats (200-250 g) levels of some 200 ng/g kynurenine were apparent. The identity of the *o*-amino acetophenone has been confirmed by mass spectrometry and further evidence that it is derived from kynurenine will be discussed. Until recently it has been thought that tryptophan pyrrolase was not present in brain, but Gal (1974) has reported the existence of an enzyme in rat brain homogenate capable of synthesizing labelled kynurenine from labelled tryptophan. If the kynurenine present in brain is indeed synthesized there, there would be a possibility of competition between the 5-HT and pyrrolase pathways within the brain itself. These questions are the subject of current investigations.

I thank Dr A. Lawson of the Division of Clinical Chemistry, Clinical Research Centre for Mass-Spectrometric analysis of *o*-aminoacetophenone.

References

- CURZON, G. (1969). Tryptophan pyrrolase—a biochemical factor in depressive illness? *Br. J. Psychiat.*, **115**, 1367-1374.
- GAL, E.M. (1974). Cerebral tryptophan-2,3-dioxygenase (pyrrolase) and its induction in rat brain. *J. Neurochem.*, **22**, 861-863.
- JOSEPH, M.H. & RISBY, D. (1975). The determination of kynurenine in plasma. *Clin. Chim. Acta*, **63**, 197-204.
- JOSEPH, M.H., YOUNG, S.N. & CURZON, G. (1976). The metabolism of a tryptophan load in rat brain and liver. The influence of hydrocortisone and allopurinol. *Biochem. Pharmacol.* (in press.)