## Fluorimetric detection of tranylcypromine in urine

SIR,—Trans-2-phenylcyclopropylamine (tranylcypromine) is a potent inhibitor of monoamine oxidase (Maas & Nimmo, 1959) and is widely used as an antidepressant agent. At present there is no specific chemical method for its detection in the amounts in which it occurs in biological tissues. An investigation was made to determine whether the compound possessed fluorescent properties.

Fluorescence was measured by an Aminco-Bowman spectrophotofluorimeter (Cat. No. 4–8202B) fitted with Aminco thermoelectric cooler and X-Y recorder.

At an emission of 290 m$\mu$ the compound possessed two excitation peaks, at 220 and 270 m$\mu$. A small peak observable at 290 m$\mu$ was due to Rayleigh and Tyndall scatter (Udenfriend, 1962). When excited at 220 m$\mu$, there was a main peak at 290 m$\mu$ and a smaller second-order emission peak at 565 m$\mu$ (uncorrected). A similar spectrum was obtained with excitation at 265 m$\mu$.

When solutions of tranylcypromine sulphate were excited at 220 m$\mu$, the intensity of fluorescence measured at 290 m$\mu$, was proportional to concentration from 0·1–4 $\mu$g/ml. Above 4 $\mu$g/ml the curve deviated from linearity due to self-absorption. The intensity of fluorescence was increased by decreasing the temperature of the solution from 15–5°. In an investigation of the effect of pH on the intensity of fluorescence of a 10 $\mu$g/ml solution, it was maximal below pH 7, and at greater pH values fell away rapidly, no fluorescence being observable above pH 11. It was found that the pH at which the intensity had fallen to that equivalent to a 5 $\mu$g/ml solution was 8·4, which agrees well with the value for the pH of 8·55 determined by potentiometric titration. This suggests that it is the ionised species ($R \pm NH_3$) and not the free base ($R - NH_2$) which is the fluorescent form.

*Detection in urine.* Preliminary investigations showed that benzene was a suitable solvent for this extraction procedure.

Urine (15 ml) to which 75 $\mu$g of tranylcypromine had been added was placed in a 100 ml glass-stoppered centrifuge tube and adjusted to pH 11–14 with 5N NaOH. Benzene (30 ml) was added and the tube was shaken mechanically for 20 min. It was then centrifuged and the benzene layer removed by means of a dropping pipette and transferred to a tube containing 0·1N NaOH (5 ml). After shaking for 1 min to wash the benzene layer, the tube was centrifuged and a 25 ml aliquot of the benzene layer added to 0·1N $H_2SO_4$ (1 ml) in a 100 ml glass-stoppered centrifuge tube. This was shaken mechanically for 15 min and centrifuged. The acid layer was removed by means of a dropping pipette and transferred to a test tube. A further 1 ml of 0·1N $H_2SO_4$ was added to the benzene layer in the centrifuge tube which was shaken for another 15 min. It was then centrifuged and the acid layer transferred to the test tube containing the first acid extract. The bulked acid extracts were well mixed and a 0·5 ml aliquot removed and transferred to a test tube containing 0·1N $H_2SO_4$ (2 ml). After mixing this solution was cooled in a refrigerator for 30 min to 5°. Its fluorescence was then read at 220 m$\mu$ excitation and 290 m$\mu$ emission (uncorrected), and compared with that of a control blank urine which had been taken through the extraction procedure.

In 3 patients given single doses of tranylcypromine sulphate 20 mg, the drug appeared in the urine within 2 hr and disappeared after 12–16 hr. It was detected in all specimens from 8 patients receiving tranylcypromine sulphate, 10–20 mg daily, alone or in combination with trifluoperazine hydrochloride.

Quantitative estimations of tranylcypromine sulphate in urine are made difficult by interference in fluorescent activity by tyramine and tryptamine. Attempts to overcome this are being made.

*Acknowledgements.* Paul Turner is in receipt of a Wellcome Senior Research Fellowship in Clinical Science. We thank the Board of Governors of St. Bartholomew's Hospital for a grant to J. H. Young. Professor Taylor and Drs. Linford Rees and C. M. B. Pare kindly allowed us access to their patients. Smith Kline and French Laboratories Ltd., provided tranylcypromine sulphate (Parnate). Dr. D. M. G. Armstrong, Mr. C. J. Thompson and Miss E. P. Saunders gave valuable assistance.

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May 20, 1966

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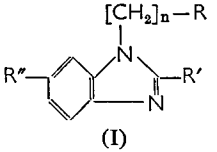
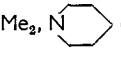
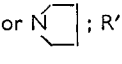
## Inhibitory effect of 1-alkylbenzimidazoles on gastric secretion in the rat

SIR,—While examining the pharmacological effects of a new series of benzimidazole derivatives of general structure (I) we observed that they exerted an inhibitory effect on secretion of gastric acid and gastric juice.



(I)

The most active compounds are those in which

$n = 2$; $R = NMe_2$,  or  ; $R' = H$ or $C_6H_5$; $R'' = H$ or Cl.

With the most active derivatives, the dose causing a 50% inhibition of secretion of gastric acid and gastric juice in the rat is 5 to 10 mg/kg intramuscularly, while the intramuscular LD50 values are about 200 mg/kg.

Some effects of one of the compounds lying in the middle of the range of activity are now described. The compound is 1-(2-piperidinoethyl)benzimidazole (H-635). The inhibitory effect of this compound on gastric secretion in the rat is seen in Table 1.

TABLE 1. THE EFFECT OF 1-(2-PIPERIDINOETHYL)BENZIMIDAZOLE ON GASTRIC SECRETION IN THE RAT

| No. of animals | Dose mg/kg i.m. | Inhibition (%) of secretion of: | | |
|---|---|---|---|---|
| | | Free acid | Total acid | Gastric juice |
| 12 | 9·0 | 38·9 | 42·2 | 26·4 |
| 25 | 12·0 | 74·2 | 60·6 | 51·6 |
| 27 | 25·0 | 78·8 | 61·6 | 66·3 |
| | ED50 mg/kg i.m. | 10·0 | 11·2 | 16·4 |