Simple, sensitive, fluorimetric methods for the estimation of physostigmine in tissue samples and solutions

SIR,—Previous methods for the determination of physostigmine in solutions are either insensitive, indirect or otherwise unsuitable for application to tissue samples (Ellis, Plachte & Straus, 1943; Teare & Taylor, 1967). A simple, sensitive, quantitative method for physostigmine in tissue samples utilizing its powerful natural fluorescence (Udenfriend, Duggan & others, 1957) and which can also be applied to solutions, has now been developed.

Approximately 0·5 g of rat brain or other tissue, to which was added 0·1 to 20·0 μg physostigmine, was homogenized in 5 ml 0·01N hydrochloric acid containing 0·05% ascorbic acid and 0·05% EDTA, and 2·5 ml of 1·2N perchloric acid was added. After mixing, the homogenate was centrifuged at 7000 *g* for 5 min. The supernatant was removed to a centrifuge tube to which 5N potassium carbonate solution was added to adjust the pH to 6. After 5 min centrifugation at 7,000 *g*, 1 ml of the supernatant was removed and the fluorescent intensity determined at excitation 290 mμ and emission 350 mμ wavelengths, in an Aminco Bowman fluorimeter. Internal standards and tissue blanks were also determined.

The method can be applied to solutions by removing a 1 ml sample after the appropriate dilution and adjustment to pH 6.

The method can detect 0·1 μg/g (linear range 0–40 μg/g) in tissue samples and 0·05 μg/ml (linear range 0–5 μg) in solutions with 95–100% recovery (14 values).

Samples are adjusted to pH 6 for fluorimetry because: (i) this pH is in the range pH 2–7 over which maximum fluorescence of physostigmine occurs; (ii) it is the pH of maximum stability (Gisvold, 1962); (iii) it allows the precipitation of potassium perchlorate thus reducing the ionic strength of the sample, which reduces quenching of fluorescence.

Decomposition products such as rubreserine, do not interfere with the assay but eseroline, the initial decomposition product, may interfere. However, with the mild, rapid technique little eseroline should be formed (Swallow, 1951).

While salicylate, a commonly used salt in physostigmine solutions, also possesses strong native fluorescence, it was shown not to interfere appreciably with physostigmine estimations as equivalent concentrations of physostigmine as a salicylate or sulphate salt, possessed equal fluorescence. The usual antioxidants, preservatives and other additives present in physostigmine preparations do not interfere with the estimation.

A much more sensitive fluorimetric technique for the estimation of physostigmine involves condensation with ethylenediamine and subsequent extraction of the fluorescent product with isobutanol (Laverty, Michaelson & others, 1963). Ethylenediamine will also condense with physostigmine degradation products, so the fluorescence of the isobutanol extract determined at excitation 420 mμ and emission 510 mμ wavelengths is a measure of physostigmine, eseroline and rubreserine. The method can detect 0·002 μg/ml physostigmine. In tissue samples, catecholamines will seriously interfere, which restricts the application of this reaction to pure solutions and tissue extracts containing little or no catecholamines.

The above methods provide simple, sensitive assay techniques for physostigmine and some of its degradation products in both tissues and solutions.

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α-Methyltyrosine: effects on fixed ratio schedules of reinforcement

SIR,—α-Methyltyrosine is an inhibitor of tyrosine hydroxylase (Nagatsu, Levitt & Udenfriend, 1964) and when administered to guinea-pigs causes a fall in tissue catecholamine levels without affecting 5-hydroxytryptamine (5-HT). α-Methyltyrosine causes a decrease in motor activity, rotorod performance and shuttle-box conditioned avoidance responding (Rech & Moore, 1965) as well as a decrease in conditioned avoidance responding in an operant situation (Hanson, 1965). These effects are attributed to a decrease in catecholamines in that the effects of α-methyltyrosine are decreased by pre-treatment with a monoamine oxidase inhibitor, which does not alter its brain levels (Moore & Rech, 1967a). In addition, the depression of the conditioned avoidance response is reversed by L-dihydroxyphenylalanine (Moore & Rech, 1967b); also, effects of reserpine and α-methyltyrosine on the conditioned avoidance response and motor activity are similar (Smith & Dews, 1962; Seiden & Carlsson, 1963). We now report the effect of α-methyltyrosine on operant behaviour utilizing positive reinforcement.

Six 80-day old, male, albino rats (Holtzman) were trained in a Lehigh Valley operant conditioning chamber on a fixed-ratio schedule of reinforcement (FR-10, i.e., every tenth lever press was reinforced with 0·01 ml of water) (Ferster & Skinner, 1957). Reinforcement contingencies were programmed by means of solid-state logic modules (Massey Dickinson Co.). Training continued until the total number of responses in a 30-min session did not exceed ±10% of the mean total number of responses from the five previous sessions. When animals reached this level of response, α-methyltyrosine (suspended in polyethylene-glycol-200 and saline, 1:1) or vehicle was injected (i.p.) 8 and 4 hr before the next daily session. One week later animals given the vehicle were given drug and vice versa.

Lever pressing performance was initially depressed by 36% during the first 4 min period (see Table 1). During subsequent time periods, performance was

TABLE 1. EFFECT OF α-METHYLTYROSINE ON LEVER PRESSING PERFORMANCE. Each value = mean % depression (± s.e.m.) calculated from each animal's previous day's performance.

	Time (min)						
Schedule	4*	8	12	16	20	24	Total
FR-10	36·2 ± 6·6	47·9 ± 6·9	56·9 ± 10·8	72·3 ± 12	77·4 ± 10	72·5 ± 10	62·6 ± 9
FR-20	36·1 ± 7·7	80·2 ± 6·8	92·2 ± 4·1	93·8 ± 4·5	99·4 ± 1	97·9 ± 2	84·6 ± 3

* All time periods significantly different at $P < 0.05$ except 4 min (Wilcoxon Rank Test).