References

Burgen, A. S. V., Dickens, F. & Zatwas, L. J. (1949). *J. Physiol., Lond.*, **109**, 10–24.
Buterbaugh, G. G. & Spratt, J. L. (1968). *J. Pharmac. exp. Ther.*, **159**, 255–263.
MacIntosh, F. C. (1963). *Can. J. Biochem. Physiol.*, **41**, 2567.
Straughan, D. W. (1960). *Br. J. Pharmac. Chemother.*, **15**, 417–422.
Thies, R. E. & Brooks, V. B. (1961). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **20**, 569–578.
Wilson, H. & Long, J. P. (1959). *Archs int. Pharmacodyn. Ther.*, **120**, 343–352.

Modified method for the estimation of metaraminol and α-methyl-*m*-tyramine

SIR,—In recent years the catecholamine-depleting action of DL-α-methyl-*m*-tyrosine has attracted attention. Much evidence exists to suggest that the depleting agent is metaraminol, which is formed *in vivo* from methyltyrosine* via the intermediate α-methyl-*m*-tyramine (Carlsson & Lindquist, 1962). It has been postulated that metaraminol, which is taken up and stored within sympathetic nerves, may replace the noradrenaline and serve as a false transmitter (Crout, Alpers & others, 1964; Andén, 1964).

The *o*-phthalaldehyde method for the determination of metaraminol (Shore & Alpers, 1964) is specific for primary *m*-hydroxyphenylethyl amines, but will also produce fluorescent reactions with α-methyl-*m*-tyrosine and α-methyl-*m*-tyramine. It is therefore necessary to separate the three substances before condensation with *o*-phthalaldehyde. This is possible by passing the compounds through a Dowex-50 resin which allows the methyltyrosine to run through while retaining both the methyltyramine and metaraminol. Subsequent differential elution with N and 2N HCl allows a separation of the latter two amines.

We wish to report a method based on the above principles, sufficiently sensitive to allow for the analysis of single rat hearts. Columns of Dowex 50W, X-4, 450 mm by 60 mm, are prepared with sodium hydroxide, hydrochloric acid and 0·1M phosphate buffer, pH 6·5, as described by Carlsson & Lindquist (1962).

Single hearts, removed from rats treated with the methyltyrosine, are homogenized at high speed (VirTis "23" Homogenizer) in 25 ml of 0·4N perchloric acid. The supernatant is neutralized with cold 5N potassium carbonate and the ensuing precipitate removed by centrifugation (8000 rev/min for 10 min). The total amount of the supernatant remaining (approx. 25 ml) is forced through the Dowex column at a rate of 17 drops/min. The column is then washed with 10 ml of double distilled water and eluted first with 25 ml of 1N HCl and then with 25 ml of 2N HCl. The eluate is collected in 2·5 ml fractions which are then condensed with *o*-phthalaldehyde according to Shore & Alpers (1964).

The above method makes it possible to separately elute metaraminol and the methyltyramine (Fig. 1). The first curve, seen after the injection of the methyltyrosine or metaraminol, is metaraminol, and the second curve, observed only after methyltyrosine administration, is methyltyramine. Either curve can be selectively produced by the addition respectively of metaraminol or methyltyramine to extracts of hearts removed from untreated rats.

Quantitative estimation of the levels of metaraminol and methyltyramine present in the hearts is routinely obtained by summing the concentrations measured in eluates 3 to 8 inclusive, for metaraminol, and 12 to 19 inclusive for methyltyramine. We found the percentage recovery using this method to be 60·6 ± 5·3 for metaraminol and 62·8 ± 4·6 for methyltyramine.

* In this text methyltyrosine is used as an abbreviation for DL-α-methyl-*m*-tyrosine and methyltyramine for α-methyl-*m*-tyramine.

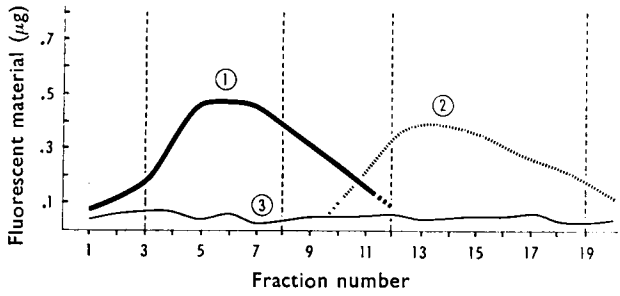

FIG. 1. Elution curves showing the separation of metaraminol (curve 1) and α-methyl-*m*-tyramine (curve 2) after the addition of 3·3 μg of each to extracts of hearts from untreated rats. Curve 3 was obtained from control hearts. Curves represent means of 6 analyses.

The procedure reported here involves principles previously employed (Andén, 1964). However, it differs from the previous method by describing the patterns of elution for metaraminol and methyltyramine and thereby demonstrating the feasibility of separating the two amines by differential elution. In addition, the sensitivity of the method reported here allows for the analysis of single hearts instead of pooled hearts. Results compare closely with earlier published values from pooled organs. For example, 4 hr after injection 400 mg/kg of DL-α-methyl-*m*-tyrosine we measured 2·5 nmole of metaraminol and 2·2 nmole of methyltyramine per g of heart tissue, compared with previously reported concentrations of 2·8 and 2·4 nmole respectively of the amines 6 hr after treatment (Andén, 1964).

We have also used the method to separate the α-methylated amines excreted in the urine. The procedure used is as follows: 10 ml of urine is adjusted to pH 9 with sodium hydroxide and then shaken with 30 ml of 1 : 1 mixture of n-butanol and heptane for 6 min. After centrifugation, 25 ml of the organic phase is shaken with 10 ml of 0·01N HCl for 2 min. 5 ml of the acid phase is adjusted to pH 6·5 and passed through the Dowex resin. After elutions with N and 2N HCl, as previously described, metaraminol and methyltyramine are condensed with *o*-phthalaldehyde and their fluorescence read.

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Department of Pharmacology,
University of Toronto,
Toronto 5, Canada.

T. A. PUGSLEY
G. E. JOHNSON

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References

Andén, N.-E. (1964). *Acta pharmac. tox.*, **21**, 260–271.
Carlsson, A. & Lindquist, M. (1962). *Acta physiol. scand.*, **54**, 87–94.
Crout, J. R., Alpers, H. S., Tatum, E. L. & Shore, P. A. (1964). *Science, N.Y.*, **145**, 828–829.
Shore, P. A. & Alpers, H. S. (1964). *Life Sci.*, **3**, 551–554.