CC/NUMBER 4
JANUARY 25, 1982

This Week's Citation Classic

Curzon G & Green A R. Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. *Brit. J. Pharmacol.* **39**:653-5, 1970.
[Dept. Chemical Pathology, Inst. Neurology, London, England]

A rapid and sensitive method for measuring 5-hydroxytryptamine (5HT) and 5-hydroxyindoleacetic acid (5HIAA), using o-phthalaldehyde (OPT) and L-cysteine, is presented, enabling both compounds to be measured in small areas of rat brain. [The *SCI*® indicates that this paper has been cited over 590 times since 1970.]

Gerald Curzon
Department of Neurochemistry
Institute of Neurology
London WC1N 2NS
England

October 9, 1981

"From 1966 onward, Richard Green was a PhD student in my laboratory and was determining 5HT and its metabolite 5HIAA in rat brain. The best fluorometric methods then available needed more or less a whole rat brain per determination. Regional work necessitated pooling material from a number of brains. This was a big restriction on 5HT studies. The large numbers of animals needed was a particular problem for us as our animal housing was limited to a boxed-in space under a bench! However, in 1968 Maickel *et al.*[1] described a 5HT method sensitive enough for brain regions of a single rat. This was based on solvent extraction, heating at 100° with OPT in ION hydrochloric acid, and fluorometry. It was clear from the literature that the extraction could be modified to separate 5HT from 5HIAA and as both fluoresced with OPT we thought that the method could be adapted for both substances. An afternoon's work early in 1970 confirmed this but the sensitivity was rather disappointing.

"Fortunately, at the bench next to Green a technician (Devi Kantamaneni) was trying out a new OPT method for 5HIAA in cerebrospinal fluid.[2] Its authors had noted that instead of forming the fluorescent complex, most of the 5HIAA was destroyed by the hot acid. They prevented this by cysteine. Green therefore repeated his determinations with cysteine present. Sensitivities for 5HT and 5HIAA were increased fourfold so that both substances were determinable in regions of a single rat brain.

"I had considerable misgivings about writing up the method as it had little originality, being essentially a combination of two previous papers.[1,2] Also, while we were testing it out, Maickel's group described a development of their method which permitted determination of both 5HT and 5HIAA in regions.[3] In the end, we decided that as we had found our procedure useful so also might others, and we sent it to the *British Journal of Pharmacology*.

"Why is the method cited so often? (a) It is more sensitive than other fluorimetric procedures and gives good recoveries. (b) It is robust, rapid, and simple. (Someone once told me that they interpreted failure to get it to work as a sign of incompetence.) (c) The residue of the extract containing 5HT can be used to determine tryptophan, its precursor. (d) Interest in 5HT remains high.

"Has the method had a crucial influence? I don't think so. If it had not existed, other methods would have been used more, though perhaps their lower sensitivity and/or greater laboriousness would have led to more effort being needed for the same return. Anyway, if we had not put these papers[1,2] together, others would probably have done so before long.

"Over the years, the method has been improved in various details. The present version was recently described and compared with high pressure liquid chromatography (HPLC).[4] To my relief, values correlate well. We still use the OPT method though we often use HPLC instead. Green is now at the MRC Clinical Pharmacology Unit, Oxford."

1. **Maickel R P, Cox R H, Saffiant J & Miller F P.** A method for the determination of serotonin and norepinephrine in discrete areas of rat brain. *Int. J. Neuropharmacol.* 7:275-81, 1968. [Citation Classic. *Current Contents/Life Sciences* **24**(27):18, 6 July 1981.]
2. **Korf J & Valkenburgh-Sikkema T.** Fluorimetric determination of 5-hydroxyindoleacetic acid in human urine and cerebrospinal fluid. *Clin. Chim. Acta* **26**:301-6, 1969.
3. **Miller F P, Cox R H, Snodgrass W R & Maickel R P.** Comparative effects of p-chlorophenylalanine, p-chloroamphetamine and p-chloro-N-methylamphetamine on rat brain norepinephrine, serotonin and 5-hydroxyindole-3-acetic acid. *Biochem. Pharmacol.* **19**:435-42, 1970.
4. **Curzon G, Kantamaneni B D & Tricklebank M D.** A comparison of an improved o-phthalaldehyde fluorometric method and high pressure liquid chromatography in the determination of brain 5-hydroxyindoles of rats treated with L-tryptophan and p-chlorophenylalanine. *Brit. J. Pharmacol.* **73**:555-61, 1981.