

This Week's Citation Classic®

Smith A D & Winkler H. A simple method for the isolation of adrenal chromaffin granules on a large scale. *Biochem. J.* 103:480-2, 1967.
[Department of Pharmacology, University of Oxford, England]

The catecholamine-storing particles of the adrenal medulla can be obtained on a large scale in a highly purified form, relatively free from mitochondria and lysosomes, by a simple centrifugation procedure. [The SC¹® indicates that this paper has been cited in more than 375 publications.]

Lots of Pure Pink Chromaffin Granules

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The project Hugh Blaschko set me (A.D. Smith) for my D. Phil. research was to characterize the soluble protein of adrenal chromaffin granules that was thought to bind the catecholamines. After a frustrating first year, during which I convinced myself that the protein did not significantly bind the amines, Hans Winkler joined me from Innsbruck. Since both of us were going to work on the chromaffin granule, we would have competed for the single swinging-bucket ultracentrifuge rotor. Instead, we decided to collaborate and devised a much simpler procedure that did not involve making up sucrose density gradients (done manually then): The large granule fraction from adrenal medulla was layered on to 1.6 M sucrose in a centrifuge tube for the angle rotor. The bright pink sediment contained quite pure chromaffin granules; the mitochondria floated on the top of the dense sucrose in which the lysosomes were suspended.

When we discussed publishing the method, our supervisor remarked: "You are publishing too many papers." Nevertheless, we kept this report separate from the accompanying paper¹ on the proteins that we were later to call

chromogranins,² and it seems that we were right. Incidentally, we gave copies of the two manuscripts prior to submission to Bill Paton, head of the Department of Pharmacology, on a Friday for him to read: The following Monday he returned them, something today's department heads rarely achieve.

We used the method to analyze the chemical composition of chromaffin granules (reviewed in another *Citation Classic*³) that allowed us to demonstrate that the secretion of catecholamines and proteins from the adrenal medulla occurred quantitatively by exocytosis.⁴ It was then shown that noradrenaline is secreted together with chromogranin A and dopamine β-hydroxylase from sympathetic nerves by exocytosis.⁵ Others used the procedure to study the processing of neuropeptides such as enkephalin. We are pleased that the method has proved so useful to other workers. Perhaps the choice of adjectives in the title has had something to do with the success of this paper: It is only human to be attracted by the words "simple" and "large scale."

The ability to study the chemical composition of a secretory organelle gave the impetus for much of our subsequent work. Examples include finding a soluble secretory form of acetylcholinesterase in the adrenal medulla and in the brain, its application to the antenatal diagnosis of spina bifida,⁶ and, possibly, its application to the antemortem diagnosis of Alzheimer's disease.⁷ I (Smith) was awarded the Seventh Gaddum Memorial prize by the British Pharmacological Society in 1979 for work on the mechanism of secretion of catecholamines, and Winkler was awarded the Fritz Kütz prize in 1970. We were each fortunate to succeed to the chair of pharmacology in our respective universities, and we anticipate a happy reunion after nearly 30 years when Winkler returns to Oxford as the Newton-Abraham visiting professor in 1993 with Hugh Blaschko in the audience for the lectures.

1. Smith A D & Winkler H. Purification and properties of an acidic protein from chromaffin granules of bovine adrenal medulla. *Biochem. J.* 103:483-92, 1967. (Cited 205 times.)
2. Blaschko H, Comline R S, Schneider F H, Silver M & Smith A D. Secretion of a chromaffin granule protein, chromogranin, from the adrenal gland after splanchnic stimulation. *Nature* 215:58-9, 1967. (Cited 235 times.)
3. Winkler H. The composition of adrenal chromaffin granules: an assessment of controversial results. *Neuroscience* 1:65-80, 1976. (Cited 345 times.) [See also: Winkler H. *Citation Classic. Current Contents/Life Sciences* 30(16):15, 20 April 1987.]
4. Schneider F H, Smith A D & Winkler H. Secretion from the adrenal medulla: biochemical evidence for exocytosis. *Brit. J. Pharmacol.* 31:94-104, 1967. (Cited 210 times.)
5. Smith A D. Mechanisms involved in the release of noradrenaline from sympathetic nerves. *Brit. Med. Bull.* 29:123-9, 1973. (Cited 120 times.)
6. Smith A D, Wald N J, Cuckle H S, Stirrat G M, Bobrow M & Lagercrantz H. Amniotic fluid acetylcholinesterase as a possible diagnostic test for neural-tube defects in early pregnancy. *Lancet* 1:685-8, 1979. (Cited 170 times.)
7. Navaratnam D S, Priddle J D, McDonald B, Esiri M M, Robinson J R & Smith A D. Anomalous molecular form of acetylcholinesterase in cerebrospinal fluid in histologically diagnosed Alzheimer's disease. *Lancet* 337:447-50, 1991.

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