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This Week's Citation Classic

Bush I E. Methods of paper chromatography of steroids applicable to the study of steroids in mammalian blood and tissues. *Biochemical J.* **50**:370-8, 1952. [National Institute for Medical Research, Mill Hill, London, England]

Using aqueous methanol as stationary phase, the separation of steroids by paper partition chromatography is achieved without special treatment of the paper. A family of solvent systems is described capable of separating the full range of biologically active steroid hormones and their metabolites, as are a new fluorescence reaction for Δ^4-3-ketosteroids and the 'wick' and 'running-up' techniques for complete transfer of extracts to paper chromatograms. [The *SCI*® indicates that this paper has been cited in over 1,580 publications since 1955.]

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"My main goal as a graduate student at the University of Cambridge was to identify the hormone(s) secreted by the adrenal cortex. Vogt's discovery of the high biological activity of adrenal venous blood[1] made this an ambitious but feasible task. But no one knew which, if any, of the steroids isolated from gland extracts was secreted into the blood. The big risk was that the activity measured by Vogt was due to an unidentified steroid in the 'amorphous fraction' of adrenal extract, whose biologicat activity was so high that it would be chemically undetectable in adrenal venous blood.

"In 1949, paper partition chromatography had been successfully applied to most of the important classes of hydrophilic biochemicals. Lipids, however, were another matter. In theory, the adrenal steroids should have been easily separable with simple benzene/water systems, but they weren't! Impatient with theory, I wasted a year with alumina-impregnated paper[2] proving that my first supervisor, P.R. Lewis, had been right; adsorption chromatography gave pretty pictures with pure steroids, but was almost useless with blood extracts. Duly humbled, I returned to theory suspecting that my first failures with partition systems had been due to the low solubilities of steroids, their adsorption by cellulose, and the low temperature in Cambridge. At the National Institute for Medical Research (London), with its resources (including radiators!), I decided to try systems based on hydrocarbon/methanol/water – horribly volatile in all senses compared with the stolid reliability of butanol, phenol, and collidine. In the first experiment (30 percent methanol/benzene at 35°C), adsorption was still evident but the major active adrenal steroids were separated as short 'comets.' Two days later, perfect 'spots' were obtained with 40 percent methanol as stationary phase. In a few more weeks, I had devised a 'family' of solvent systems based on aqueous methanol capable of separating the less polar androgens, estrogens, progesterone, and their metabolites. Attempting to get the Zimmerman reaction to work with Δ^4-3-ketosteroids, I noticed a dim orange fluorescence which led to the extremely sensitive soda fluorescence reaction for this group.

"The frequent citation is probably due to the very wide applicability of this rather simple family of solvent systems and later variants.[3] More convenient than Zaffaroni's alternatives[4] (impregnation of paper with formamide or propylene glycol), they played a major role in the discovery of aldosterone (Simpson, Tait, and Reichstein[5]), hitherto unknown estrogens (G.F. Marrian[6]), and the adrenal steroids in the blood of many vertebrate species.

"My overwhelming memory is of the role of luck and friendliness in making this work possible. Luck in having Lewis, R.K. Callow, and W. Feldberg as supervisors; and the generous provision of reference compounds and advice by Reichstein, Vogt, C.S. Hanes, A.J.P. Martin, and others to a somewhat rambunctious graduate student who would not be allowed to attempt such a project today. The spirit of the times is best illustrated by Feldberg's first advice to me after a disquisition on the virtues of chloralose as an anesthetic: 'And remember, Bush, when you are in research, you need plenty of holidays!' "

1. **Vogt M.** The output of cortical hormone by the mammalian suprarenal. *J. Physiology* **102**:341-56. 1943.
2. **Datta S P, Overell B G & Stack-Dunne M.** Chromatography on alumina-impregnated filter paper. *Nature* **164**:673-4. 1949.
3. **Sherma J & Zweig G.** Steroids, bile acids, and cardiac glycosides. *Paper chromatography and electrophoresis. Volume II. Paper chromatography.* New York: Academic Press, 1971. p. 200-47.
4. **Zaffaroni A, Burton R B & Keutmann E H.** Adrenal cortical hormones: analysis by paper partition chromatography and occurrence in the urine of normal persons. *Science* **111**:6-8, 1950. (Cited 250 times since 1955.)
5. **Simpson S A, Tait J F, Wettstein A, Neher R, von Euw I, Schindler O & Reichstein T.** Aldosteron. Isolierung und Eigenschaften. Über Bestandteile der Nebennierenrinde und verwandte Stoffe. *Helv. Chim. Acta* **37**:1163-200, 1954. (Cited 180 times since 1955.)
6. **Loke K H, Marrian G F & Watson E J D.** The isolation of a sixth Kober chromogen from the urine of pregnant women and its identification as 18-hydroxyoestrone. *Biochemical J.* **71**:43-8, 1959. (Cited 35 times since 1959.)