

# This Week's Citation Classic

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**Brown J B.** A chemical method for the determination of oestriol, oestrone and oestradiol in human urine. *Biochemical J.* **60**:185-93, 1955.  
I Clinical Endocrinology Research Unit, Medical Research Council, Univ. Edinburgh, Scotland]

**This paper described the first chemical method to be developed for the separate measurement of the three classical estrogens: estriol, estrone, and estradiol in the urine of men and nonpregnant women. The specificity, sensitivity, reproducibility, accuracy, convenience, and application of the method were discussed. [The SC<sup>i</sup>® indicates that this paper has been cited in over 980 publications since 1961.]**

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"Estrogen is the most important female hormone. Its measurement provides the key to many studies in human female reproduction including the monitoring of ovarian function in normal and infertile women, the identification of the times of fertility and ovulation during the cycle, the action of contraceptives, the use of fertility drugs, and the achievement of test-tube babies.

"Estrogen assay is also important in the study of cancers of the breast, endometrium, and ovary. The pressing need for a quantitative assay of estrogens in nonpregnant women was well recognized in 1950 and many other groups were engaged on the problem. The method cited was the first to be developed. It thus ranks as a classic biochemical procedure which helped to open up a whole new and important field of research.

"The method measured the then three known estrogen metabolites in human urine. It was developed in the Clinical Endocrinology Research Unit, MRC, University of Edinburgh, with the expert assistance of H.A.F. Blair. The work qualified me for a PhD degree and was supervised by G.F. Marrian. I was also involved in the development of methods for measuring

the other important female hormones and establishing their patterns of production.<sup>1</sup>

"The first step in developing the estrogen method involved a thorough study of the highly specific but notoriously unstable Kober color reaction. Four interdependent variables were identified and optimization of these for the two stages of the reaction and for each of the three estrogens provided an exceptionally stable system which became the accepted method for estrogen measurement for many years. Later, Ilttrich<sup>2</sup> introduced a solvent extraction step which, with fluorimetry, increased the sensitivity and specificity 10,000-fold.

"The new color method was applied to the development of optimum extraction and purification procedures from urine. A novel phase change procedure was included involving methylation of the estrogens. The methyl ethers were ideally suited to alumina chromatography by which they were separated from one another and further purified. Success was due to meticulous optimization of every step; the elimination of unnecessary manipulations; the recent availability of ground-glass joints, clean solvents, and a modern spectrophotometer; and Marrian's support. In collaboration with others, the method was further validated against bioassay, isotope methods, and gas-liquid chromatography. Workers involved included Bauld, Bulbrook, Greenwood, Diczfalussy, Gallagher, Fishman, Preedy, and Kellie. The method with modifications was widely applied,<sup>3</sup> and it was the only one which was clinically viable until the development of radioimmunoassays for plasma estradiol in the 1970s.<sup>4</sup> For myself, I started as an organic chemist in New Zealand, became a hospital biochemist, and then a reproductive endocrinologist. I am now a professor of obstetrics and gynecology, without ever having delivered a baby. The paper cited is my most important and the measurement of estrogens in body fluids is now widely used in still increasing numbers in the study and treatment of human infertility."

1. Brown J B, Klopper A & Loraine J A. The urinary excretion of oestrogens, pregnanediol and gonadotrophins during the menstrual cycle. *J. Endocrinology* **17**:401-10. 1958.
2. Ilttrich G. Eine neue Methode zur chemischen Bestimmung der oestrogenen Hormone im Harn. *Hoppe-Seyler's Z. Physiol. Chem.* **312**:1-14. 1958.
3. Brown J B & Beischer N A. Current status of estrogen assay in gynecology and obstetrics. *Obstet. Gynecol. Survey* **27**:205-35. 1972.
4. Abraham G E, Odell W D, Swerdloff R S & Hopper K. Simultaneous radioimmunoassay of plasma FSH, LH, progesterone, 17-hydroxyprogesterone, and estradiol-17 $\beta$  during the menstrual cycle. *J. Clin. Endocrinol. Metab.* **34**:312-18. 1972.