

This Week's Citation Classic

Klopper A, Michie E A & Brown I B. A method for the determination of urinary pregnanediol. *J. Endocrinology* 12:209-19, 1955. [Clinical Endocrinology Research Unit, Medical Research Council, Univ. Edinburgh, Scotland]

Pregnanediol is the chief metabolite of the sex hormone progesterone. At the time when this work was done, it was not yet possible to measure progesterone itself. We managed to measure pregnanediol by boiling urine with hydrochloric acid to free the steroid from its combination with glucuronic acid. The free pregnanediol was extracted from the urine and the crude extract purified by chromatography on alumina columns. The purified material was acetylated and rechromatographed. The nearly pure pregnanediol acetate gave a yellow colour with sulphuric acid which enabled spectrophotometric measurements to be made. [The **SCI**[®] indicates that this paper has been cited over 455 times since 1961.]

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"Invent a new mousetrap and the world will beat a path to your door. The pregnanediol method was just that — a gimmick, no more, and it is this which accounts for the article's frequent citation. It was certainly no great trick, although it took me three years to work it out and fed me for a good many more. In one form or another the various parts of the technique had been worked out either for pregnanediol or for other steroids and it was just a case of fitting all the ideas together. Probably the most original idea was to make the derivative of pregnanediol and that was Jim Brown's sole contribution to the work, a notion produced over beer in the pub round the corner from the Medical Research Council laboratory where we worked. He was much too busy with his own classic work

on estrogen estimation to do more.¹ Brown's work has dominated the field for more than a quarter of a century. If that computer in Philadelphia does not identify that as a *Citation Classic*, it can't count.

"Although the pregnanediol method was really the centrepiece of my PhD thesis, Guy Marrian brought in Eileen Michie at the last stages to give bench expertise and biochemical respectability to the work. He had suffered some rude shocks by letting young medicals work unsupervised. In the end I got just the right amount of supervision from Marrian himself. He was a towering figure in the world of steroid chemistry, having himself been the first to isolate estriol and pregnanediol.² With the perspective of time I can see how difficult it must have been not to simply take over and tell us what to do. Happily he was too busy isolating and identifying half a dozen new estrogens himself. Indeed, if there is any single reason for the creativity of the laboratory at that time it was because everybody had their own thing to do.

"Marrian did do one thing for me. He had a passion for brief and factual writing. My first draft of the pregnanediol method contained everything that I had learned in three years and much besides. Marrian tore it up after a glance and told me to start again. The shock of seeing my brainchild destroyed before my eyes comes back to me every time I put pen to paper. The experience may not have put much into my writing but it certainly took a lot out.

"The pregnanediol method was to be a tool to elucidate the physiology of progesterone. In the end it took us only a little way down the road. We were analysing exhaust fumes and trying to deduce the nature of the internal combustion engine. The estimation of pregnanediol in urine has now largely been superseded by the measurement of the original active hormone, progesterone, in blood."

- Brown J B.** A chemical method for the determination of estriol, estrone, and estradiol in human urine. *Biochemical J.* 60:185-9, 1955. [The **SCI**[®] indicates that this paper has been cited over 1,010 times since 1961.]
- Marrian G F.** The chemistry of oestron. 1. Preparation from urine and the separation from an unidentified solid alcohol. *Biochemical J.* 23:1090-2, 1929.