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This Week's Citation Classic

Mattingly D. A simple fluorimetric method for the estimation of free 11-hydroxycorticoids in human plasma. J. *Clin. Pathol.* **15**: 374-9, 1962.

11-hydroxycorticoids have a structure which is unique to steroids of adrenal origin. Their plasma concentration is a valuable measure of adrenocortical activity, provided that the normal circadian rhythm is taken into account. This paper describes a simple fluorimetric assay which is ideal for routine clinical use. [The *SCI*® indicates that this paper has been cited 1,056 times since 1962.]

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"The dramatic introduction of the corticosteroids into clinical practice in 1948 provided an enormous impetus to the study of the role of the adrenal glands in health and disease. Despite their beneficial effects it soon became obvious that pharmacological doses of these drugs had many undesirable side-effects, not least being the inhibition of the normal pituitary adrenal response to stress. Attempts to investigate this phenomenon in individual patients were frustrated by the lack of suitable steroid assays which could be carried out in the routine laboratory.

"Cortisol, or hydrocortisone as it is better known to the clinician, is the main hormone secreted by the human adrenal cortex and accounts for most of the 11-hydroxycorticoids presented in the blood However, since the plasma levels of this life-maintaining steroid are extremely small, even in normal unstressed individuals, the techniques used to estimate it must be particularly sensitive. The reaction between these steroids and concentrated sulphuric acid produces highly potent fluorophores, but it was not until 1960 that DeMoor and his colleagues published the first practical fluorimetric method for the estimation of plasma 11-hydroxycorticoids in man.[1]

"At the time that their paper appeared I was working with Cuthbert Cope at Hammersmith Hospital and we were attracted by the potential advantages of fluorimetry over the much more laborious and less precise colorimetric methods which were then available. Unfortunately we could not duplicate the results obtained by DeMoor's group and our interest in their method waned until I discovered that this failure was largely due to impurities in the reagents available in Britain. The petroleum ether used in the preliminary defatting wash and the methylene chloride employed to extract the steroids from the plasma were particularly fertile sources of spurious fluorescence. Rigorous purification of the methylene chloride and omission of the petroleum ether and alkali washes eliminated most of the non-specific fluorescence, simplified the procedure considerably and, rather to our surprise, did not reduce the accuracy or precision of the method.

"The value of this simple and rapid steroid assay in the investigation of adrenal problems at the bedside soon became apparent, especially in cases of suspected adrenal failure. Since the synthetic corticosteroids did not fluoresce, it was possible to study endogenous adrenal activity during their administration.

"The fact that this paper has been chosen as a Citation Classic' shows that many workers have found this method of use in the elucidation of disorders of the adrenal cortex, and also indicates how the simplification of a more elaborate procedure can lead to quicker and more accurate diagnoses in clinical practice."

REFERENCE

1. DeMoor P, Steeno O, Raskin M & Hendrikx A. Fluorimetric determination of free plasma 11-hydroxycorticosteroids in man. *Acta Endocrinol.* **33:**297-307, 1960.