

This Week's Citation Classic

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Hwang P, Guyda H & Friesen H. A radioimmunoassay for human prolactin. *Proc. Nat. Acad. Sci. US* 68:1902-6, 1971.
[McGill University Clinic, Royal Victoria Hosp., Montreal, Canada]

This paper reported the development of a radioimmunoassay for human prolactin, providing a simple, specific, and sensitive method of measuring prolactin concentrations in human circulation under various physiological and pathological conditions and facilitating the subsequent purification of human prolactin. [The SC[®] indicates that this paper has been cited in over 645 publications since 1971.]

Peter Hwang
Department of Physiology
Faculty of Medicine
National University of Singapore
Republic of Singapore 0511

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"Up to about 1970, there was still considerable doubt about the existence of a lactogenic hormone in man. Repeated attempts to purify prolactin from human pituitary glands had been unsuccessful. This failure, together with the observation that highly purified human growth hormone exhibited intrinsic lactogenic activity, led some investigators to propose that, perhaps, prolactin did not exist in man, its function being subserved by growth hormone.¹ This suggestion, however, was incompatible with other observations, such as the normal lactation seen in growth hormone deficient dwarfs.

"In 1970, the laboratory of Henry Friesen, among others, was attempting to demonstrate the separate existence of prolactin in primates. Harvey Guyda, working in the same laboratory, had by then shown that monkey pituitary glands secreted a lactogenic substance which was distinguishable immunologically from growth hormone. He had also succeeded in purifying a small amount of what was thought to be monkey prolactin from pituitary incubation media with an estimated purity of about ten percent by bioassay.²

"Soon after I joined Friesen's laboratory, I was asked to give some thought to the devel-

opment of a radioimmunoassay for primate prolactin. This represented an act of considerable generosity and some courage on the part of Friesen, for I was then fresh out of the University of Singapore Medical School with no research experience whatever. The problem at that time was how one could develop a radioimmunoassay for a substance which was contaminated to the extent of 90 percent by other proteins, and which was available only in microgram quantities.

"Two rabbits were immunized with several hundred micrograms of the crude monkey prolactin preparation; what remained, however, was insufficient for us to attempt further purification in order to achieve a degree of purity suitable for radioiodination. Scaling up with a large number of monkey pituitaries would have been logical but prohibitively expensive.

"A way to bypass this difficulty was suggested by a paper published by Herbert and Hayashida³ indicating that monkey prolactin and sheep prolactin might be immunologically related. Following up on this observation, we iodinated 5 µg of the crude monkey prolactin and applied the mixture, containing iodinated prolactin as well as other iodinated proteins, to an affinity column prepared by linking anti-sheep prolactin antibodies to Sepharose. Only the iodinated prolactin was retained by the column, yielding, on elution, an essentially homogeneous label suitable for use in a radioimmunoassay.

"The radioimmunoassay thus developed permitted easy measurement of circulating prolactin concentrations in humans for the first time. Perhaps more importantly, it also facilitated the subsequent chemical purification of human prolactin⁴ which, together with a similar achievement by U.J. Lewis *et al.*,⁵ not only firmly established the separate existence of human prolactin, but also made the radioimmunoassay available to many other investigators for extensive studies on the role of prolactin in human health and disease.⁶ These factors have no doubt contributed to the number of citations."

1. Li C H. The chemistry of human pituitary growth hormone: 1956-1966. (Pecile A & Müller E E, eds.) *Growth hormone*. Amsterdam: Excerpta Medica, 1968. p. 3-28.
2. Guyda H & Friesen H. The separation of monkey prolactin from monkey growth hormone by affinity chromatography. *Biochem. Biophys. Res. Commun.* 42:1068-75, 1971.
3. Herbert D C & Hayashida T. Prolactin localisation in the primate pituitary by immunofluorescence. *Science* 169:378-9, 1970.
4. Hwang P, Guyda H & Friesen H. Purification of human prolactin. *J. Biol. Chem.* 247:1955-8, 1972.
5. Lewis U J, Singh R N & Seavey B K. Human prolactin: isolation and some properties. *Biochem. Biophys. Res. Commun.* 44:1169-76, 1971.
6. Flückiger E, del Pozo E & von Werder K. Prolactin: physiology, pharmacology and clinical findings. *Monogr. Endocrinol.* 23:1-224, 1982.