

This Week's Citation Classic

Niswender G D, Midgley A R, Jr., Monroe S E & Reichert L E, Jr. Radioimmunoassay for rat luteinizing hormone with antiovine LH serum and ovine LH-¹³¹I. *Proc. Soc. Exp. Biol. Med.* **128**:807-11, 1968.

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A radioimmunoassay procedure was developed for quantification of luteinizing hormone (LH) in the blood of rats utilizing an immunological cross-reaction between rat LH and an unique antiserum produced in a rabbit against ovine LH. The assay was characterized carefully to demonstrate that other pituitary hormones or components of serum did not interfere with the measurement of LH. The extreme sensitivity of the assay (5 pg NIH-LH-S1) allowed quantification of the hormone in as little as 0.02 to 0.2 ml serum. [The *SCI*[®] indicates that this paper has been cited over 970 times since 1968.]

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"This paper was the first of approximately 15 describing the unique properties of an antiserum (from rabbit number 15) which has been proven specific for quantification of LH in serum obtained from a large number of mammals including primates and numerous wildlife species. Literally hundreds of antisera against ovine LH have been tested by ourselves and others and to date none has demonstrated the great degree of cross-reaction with LH from other species demonstrated by antiserum from rabbit number 15. Since this antiserum appeared to be uniquely useful we have made it available for radioimmunoassay purposes to all scientists who have requested it. This is probably the reason the original paper

is cited frequently. The assay system has been used by over 200 investigators in 33 countries to quantify LH in serum from over 30 species.

"Development of this antiserum is a classic example of serendipity in science. I had been funded by the National Institutes of Health for postdoctoral training with Rees Midgley at the University of Michigan. Therefore, while completing requirements for my PhD with Phillip Dzuik and A.V. Nalbandov at the University of Illinois, I immunized 25 rabbits with ovine LH. Based on present knowledge, the immunization techniques used were inappropriate and, even after repeated immunizations, only one rabbit (number 15) produced detectable antibodies to LH. I was allowed to move both the rabbit and the antisera I had collected to Michigan. Midgley was already recognized as one of the world's authorities on development of radioimmunoassay procedures and had been instrumental in establishing very rigid criteria for proving that radioimmunoassay procedures were reliable. Thus, the assay procedures which utilized this antiserum were all extensively characterized to demonstrate their reliability. Leo Reichert at Emory University supplied the highly purified LH for radioiodination. In subsequent research, he has supplied hormone preparations from a variety of species to test the hormonal specificity of several radioimmunoassay systems and has continued to be a valued colleague. Scott Monroe was a medical student working in the laboratory and had prepared the crude preparations of rat LH, follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH) used to test specificity of the radioimmunoassay procedure. He was also involved in development of the first assay for LH in Rhesus monkeys. Development of radioimmunoassay procedures gave reproductive biologists the capability to measure reproductive hormones in blood and was an important step in the advancement of our knowledge in reproductive biology."