

Follett B K, Scanes C G & Cunningham F J. A radioimmunoassay for avian luteinizing hormone. *J. Endocrinology* 52:359-78, 1972.

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Antibodies raised to gonadotrophins isolated from chicken pituitaries neutralized gonadotrophic activity *in vivo*. A radioimmunoassay for luteinizing hormone (LH) is described for the measurement of LH in chicken and quail plasma. Immunoassay concentrations correlate well with physiological responses. [The SC[®] indicates that this paper has been cited in over 460 publications.]

A Thoroughly Enjoyable Collaboration

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The impetus for this work was our total inability to measure circulating levels of reproductive hormones. Both of us were assaying pituitary hormones biologically, but the insensitivity and inaccuracy and our inability to process more than a few samples each week proved a continuing frustration: The problems to be solved were clear, the techniques were not to hand. Immunological methods for hormone assay—both isotopic and nonisotopic—were starting to emerge and the way forward seemed clear.

Progress began with a grant from the British Egg Marketing Board, then chaired by Sir Alan Parkes, which allowed a collection of almost half a million broiler chicken pituitary glands. This tissue was purified by Dr. Anne Stockell Hartree at Cambridge and the fractions assayed in Reading using the ovarian augmentation assay for follicle-stimulating hormone (FSH), the ovarian ascorbic acid depletion assay for luteinizing hormone (LH), the uterine weight assay in immature mice, and the uptake of ³²P into the testes of day-old chicks. Independently, Brian K. Follett and Colin G. Scanes in Bangor had begun a similar program.

Our interests were complementary, and this provided the glue to a thoroughly enjoyable collaboration. In Reading interests lay in the control of ovula-

tion in the domestic hen, whilst in Bangor they concentrated upon the photoperiodic control of reproduction in quail and other wild birds. The only risk came from developing radioimmunoassays (RIAs) using purified chicken hormones: There was no guarantee that the methods would measure LH in another galliform bird, the quail! In the end, however, the antisera have proved useful in all birds yet tested, and this wide species specificity has added to the value not only of this particular assay, but also of the next generation of LH assays developed in the US and Japan.

Our good fortune lay in developing the assay at an early stage in RIA technology. The LH fractions proved highly antigenic, and the most pure fraction was iodinated using the chloramine T method developed by Bill Hunter and Fred Greenwood.¹ Much effort was expended on breaking the "closed loop" in the original system (the first assay used the same fraction for raising an antiserum, for iodination, and as standard), but after some months it was clear that the measured levels in quail and chicken samples were similar with any combination of chicken LH reagents. In the years subsequent to establishing the original method, much effort has been expended on further validation and the assay remains valuable although under some conditions it can detect thyroid-stimulating hormone (TSH). Surprisingly, it has proved much more difficult to develop assays for chicken FSH and TSH. There are assays available for the former but the reagents are scarce, whilst avian TSH remains a continuing problem.

Having developed the assay, there was a fruitful period when it was used to tackle the original physiological problems. In the domestic hen, the ovulatory surge was found to occur six hours prior to ovulation and to be closely associated with peaks in progesterone and oestradiol.² Subsequently, Susan Wilson and Peter Sharp in Edinburgh established that progesterone is the positive feedback agent in birds and drives the LH surge.

The first step in analysing the photoperiodic control of reproduction was to characterize the output of LH as quail were moved to long daylengths. This was followed by the demonstration (impossible without RIA) that the photoperiodic response system operates normally in castrated birds: important in proving that the process is driven by the central nervous system alone. In quail the daylength response is rapid and LH secretion is increased after only one long day. That allowed experiments on the photoperiodic time-measuring machinery, the brain photoreceptor used by birds to detect light and the neural wiring within the brain.³

The assay can measure LH in 2-20 microlitres of plasma and with its wide cross-reaction can be used in small song birds. The assay has been used in nearly 50 species (ranging from albatrosses through penguins to starlings and sparrows!).

1. Hunter W M & Greenwood F C. Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature* 194:495-6, 1962. (Cited 6,610 times.) [See also: Greenwood F C. Longevity of immunochemical methods that work. *Citation Classic. Current Contents/Clinical Medicine* 17(26):16, 26 June 1989; *CC/Engineering, Technology & Applied Sciences* 20(26):16, 26 June 1989; *CC/Physical, Chemical & Earth Sciences* 29(26):16, 26 June 1989; and *CC/Life Sciences* 32(26):16, 26 June 1989.]
2. Cunningham F J. Ovulation in the hen: neuroendocrine control. *Oxford Rev. Reprod. Biol.* 9:96-136, 1987.
3. Follett B K. Birds. (Lamming C E, ed.) *Marshall's physiology of reproduction. Volume 1. Reproductive cycles of vertebrates.* New York: Churchill Livingstone, 1984. p. 283-350. (Cited 15 times.)