Citation Classics

Greenwood F C, Hunter W M & Glover J S. The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. *Biochemical Journal* 89:114-23, 1963.

The radio-immunoelectrophoretic technique for the assay of insulin in human sera developed by Yalow & Berson has been applied to glucagon and to growth hormone. This method uses low amounts of carrier-free [^{131}I] iodide. No prior treatment of the iodide sample is required, and the iodide's high isotope content makes possible high specific radioactivity with a low degree of chemical substitution. [The *SCI*® indicates that this paper was cited 1,779 times in the period 1961-1975.]

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"Some 15 years ago our aim was to develop a radioimmunoassay for human growth hormone. We regarded the development of a method for radio-iodinating growth hormone as a necessary prerequisite to achieve our goal. After watching with some amazement over the last 15 years the widespread application of our radio-iodination method, my memory of 'how it was' may be somewhat faulty. On many occasions I have been labelled as an expert on iodine chemistry, radio-iodine chemistry, and chemistry-labels of doubtful specificity. My expertise was in recognizing the importance of the classical radioimmunoassay paper of Berson and Yalow in I960.[1]

"As a then full-time researcher with the Imperial Cancer Research Fund [London, England], my goal was to measure human growth hormone and human prolactin in plasma. With the assistance of Professor C.A. Gemzell in Stockholm, I had initiated isolation of growth hormone and its dreadful bioassay. Thence, having acquired some of the arts of protein chemistry from Professor H.F. Deutsch in Madison, Wisconsin, and particularly some feeling for immunochemistry, I applied the tanned cell hemagglutination method developed for growth hormone by Professor C. Read of Iowa. In parallel studies Mr. Hunter had the back-breaking job of the bioassay for growth hormone. When it became apparent that neither the bioassay nor the tanned cell assay would work in human plasma we approached radioimmunoassay and radio-iodination with vim and vigor—it had to succeed.

"A critical point was our early visit to Amersham [The Radiochemical Centre, Bucks, England] to see how to handle 80 mCi of iodine 131 as then required by the Berson and Yalow technique, in the context of a normal lab in the center of London, only to be told shortly, but kindly, by Dr. Glover, 'don't.' Dr. Glover pointed out that Amersham was just making available carrier-free iodine 131. It may have been Dr. Glover, Mr. Hunter, or myself who picked out chloramine-T as a possible oxidizing agent. I am certain that the theoretical advantages of carrier-free iodine, together with an agent which would make available all the iodine for labelling, did not fully occur to me at the time. In this case, serendipity, motivation, and hard work substituted successfully for a *priori* reasoning.

"Certainly, I remember the initial experiments carried out by Mr. Hunter. We inadvertently radio-iodinated Sephadex, before realizing that we had to kill the reaction by metabisulfite, or risk losing, by absorption, microgram amounts of labelled material on columns. That was before we learned to presaturate with albumin. In one experiment we recovered 100% of the radioactivity by chasing with added potassium iodide. However, we repeatedly failed to show radio-iodination using *calculated* amounts of chloramine-T. The decision to start 'spooning in' chloramine-T was a desperate, but successful, last resort. Thereafter it was a case of smoothing out the methodology since we felt that without a routine method of radio-iodination, radioimmunoassay would become only a passing fad in a few research labs…"

1. **Yalow R S & Berson S A.** Immunoassay of endogenous plasma insulin in man. *Journal of Clinical Investigation* 39:1157-75, 1960.