

# This Week's Citation Classic®

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Hartog M, Gaafar M A, Meisser B & Fraser R. Immunoassay of serum growth hormone in acromegalic patients. *Brit. Med. J.* 2:1229-32, 1964.  
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A radioimmunoassay of human growth hormone (HGH) is described using unextracted serum and a double antibody method for the separation of free and bound hormone. Serum HGH levels were measured in a group of acromegalic patients during glucose and insulin tolerance tests. [The *SCI*® indicates that this paper has been cited in over 195 publications.]

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In 1958 I was working as a locum [tenens] senior medical registrar at the West Middlesex Hospital, near London, and had developed a particular interest, amounting to an obsession, in trying to measure circulating human growth hormone (HGH). At that time the only methods were relatively insensitive biological ones, although W.D. Salmon and W.H. Daughaday of St. Louis had recently reported results of measuring "sulphation factor" that sounded very promising.<sup>1</sup>

An immunological approach to the measurement of serum HGH seemed possible, and the first step was to manufacture an antibody. In that respect I was extremely fortunate in receiving help from John Humphrey, then of the Department of Immunology at the Medical Research Council establishment at Mill Hill and recently, very sadly, deceased. The first antibodies when tested against HGH using Ouchterlony plates showed several lines of precipitation, indicating them to be impure. Later, purer HGH prepared by the Raben technique in Young's laboratories at the University of Cambridge became available. Eventually an antibody was found that only gave one line of precipitation with the antigen.

At about this time, I was again very lucky to receive an introduction to Russell Fraser, whose en-

doctrine/diabetes unit at the Royal Postgraduate Medical School, Hammersmith Hospital, London, was world renowned. Due to his extraordinarily busy timetable, the first time I met him was when he was doing a thyroid biopsy for which he used a high-speed drill. This made a considerable noise, but, despite this, we talked throughout the biopsy, and I explained my tentative plans for an HGH assay. By this time C.H. Read and D.B. Stone from Iowa City had reported preliminary results of measuring serum HGH using haemagglutination inhibition.<sup>2</sup> Fraser was wanting to develop the assay, and I subsequently joined his unit to begin a long and exciting period of work.

Initially, we applied the anti-HGH antibody to the measurement of HGH using the haemagglutination-inhibition technique and were able to get quite respectable looking standard curves and results on serum that were in agreement with clinical expectation. It was not long, however, before we began to experience serious nonspecific interference with the assay, and we had heard that other centres working on the method were experiencing similar problems. We arranged for samples of serum from a normal, a hypopituitary, and an acromegalic subject to be freeze-dried and sent blind to a number of these centres, the results of which showed no consistency between the different centres nor with the diagnoses.

By this time we had already decided to abandon the haemagglutination-inhibition technique and to try to adapt the method of radioimmunoassay developed by S. Berson and R.S. Yalow for insulin<sup>3</sup> and for which Yalow subsequently received the Nobel Prize, Berson, sadly, having died. The method involved labelling HGH with radioiodine and then separating free and bound hormone. W.M. Hunter and F.C. Greenwood, then at the Imperial Cancer Research Fund, London, had reported a novel method of iodination using chloramine-T<sup>4</sup> and were very generous in spending time explaining it to me. We used the double antibody method described by R.D. Utiger and his colleagues to separate HGH bound to rabbit anti-HGH serum by precipitation with an anti-rabbit gamma globulin serum.<sup>5</sup>

After several months we felt we had developed a viable method without the need of any preliminary extraction of serum and reported it in our article in the *British Medical Journal* at the same time as describing our results of applying the assay to patients with acromegaly. The simplicity of the assay has meant that it is still in use today.<sup>6</sup>

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