

Use of a Two-Site IRMA for GH in Identifying Children With GH-Dependent Growth Failure

Blethen and Chasalow compare the circulating growth hormone (GH) concentrations in adults of normal stature, endocrinologically normal short children with normal growth rates, and children with GH-dependent growth failure.

They used both the standard double antibody radioimmunoassay (RIA) method with a polyclonal guinea pig antiserum and a new immunoradiometric assay (IRMA). The former employs the standard National Hormone and Pituitary Program reagents and the latter uses two different monoclonal antibodies prepared against human growth hormone (hGH) (Hybritech, San Diego, CA). One antibody is covalently linked to a sepharose bead and the second is labeled with ^{125}I . These monoclonal antibodies were selected for IRMA on the basis of antibody competition for GH binding with the ^{125}I -labeled antibody. This assay is successful because each antibody binds to the GH molecule at a different epitope. The IRMA procedure is simpler and less time-consuming than the RIA technique.

The theoretic advantages of the IRMA method are: (1) linearity; (2) a relatively stable coefficient of variation over a greater range of antigen concentrations than in the classical RIA techniques; and (3) improved sensitivity and precision. With the use of monoclonal rather than polyclonal antibodies, several additional benefits are derived: (1) the amount of antibody is unlimited so that one can generate a very high capacity solid phase antibody system; and (2) since only a single type of antibody (selected for high affinity) is attached to the solid phase support, higher antigen concentrations can be tested.

The investigators sought to compare the results of circulating GH levels in normal adult volunteers, normal short children, and children with GH-dependent growth failure to determine if children in the last group (whose pharmacologic stimulation tests for GH secretion were normal) had an immunologically distinguishable circulating GH spe-

cies. Since samples that had no measurable GH by RIA were always unmeasurable with IRMA, samples for the IRMA were selected from samples with GH detectable by RIA.

When purified hGH was added either to human serum or the kit "zero calibrator," there was a strong correlation between the values found by RIA and IRMA (slope of the regression line = 0.86). In both normal individuals and children with GH-dependent growth failure, the ratio of IRMA-GH to RIA-GH was not affected by the time of sampling relative to the peak. The mean IRMA-GH to RIA-GH ratios were 0.48 ± 0.02 for normal subjects (slope = 0.62) v 0.35 ± 0.001 (slope = 0.39) for subjects with GH-dependent growth failure. These values are significantly different at $P < 0.001$.

These results indicate that both assays measured the authentic material with approximately equal effectiveness. For the normal group the slope of the regression line was less, indicating that there were differences in the folded structure of pituitary and circulating GH. However, the slope of the assay for those children with GH-dependent growth failure was even lower, indicating that their circulating forms of GH differed from those of normal subjects. The latter group of children is precisely the group that responded to exogenous replacement of GH.

Blethen SL, Chasalow FI: *JCE&M* 1983;57:1031.

Editor's comment—These data are exciting and, if confirmed, could materially aid physicians in deciding which children might respond to exogenous GH therapy. At present, some of these patients are considered to be at variance from normal, since they have normal levels of GH following physiologic or pharmacologic stimuli to GH secretion. The simple expedient of assaying their circulating levels of GH in two separate assays may enhance our knowledge of the syndrome of GH-dependent growth failure and target a group for a therapeutic trial with GH.