RADIOIMMUNOASSAY OF HUMAN GROWTH HORMONE

By J. S. Cheah and A. H. Nakhoda

SYNOPSIS

Radioimmunoassay of human growth hormone was carried out using the HGH-125 Imusay™ (Abbott); this procedure being based on the solid phase radioimmunoassay technique described by Catt and Tregear (1967).

In 10 normal adult males the mean fasting serum growth hormone was 9·2 ng./ml. (standard deviation: 5·7; range: 1·2 to 18·1 ng./ml.). Following insulin hypoglycaemia, the mean peak serum growth hormone was 38·1 ng./ml. (standard deviation: 28·9; range: 7·6 to 108 ng./ml.).

In 6 patients with hypopituitarism, the mean fasting serum growth hormone was 2·5 ng./ml. (standard deviation: 1·4; range: 1·1 to 4·0 ng./ml.); following insulin hypoglycaemia the mean peak serum growth hormone was 2·5 ng./ml. (standard deviation: 1·5; range: 1·1 to 4·4 ng./ml).

In a further 6 normal male controls the mean fasting serum growth hormone was 7·8 ng./ml.; following an oral glucose tolerance test the mean serum growth fell to 1·3 ng./ml. In 5 cases of acromegaly the mean fasting serum growth hormone was 82·6 ng./ml. (range: 45 to 190 ng./ml.). In one case the serum growth hormone was partially suppressed by glucose while in the other 4 cases there was a paradoxical rise.

The HGH-125 Imusay™ (Abbott) provides a relatively simple procedure for the radioimmunoassay of human growth hormone; the results obtained correlate very well with the clinical state of the patients.

The radioimmunoassay (abbreviated to RIA in the rest of the text) of a protein hormone was introduced by Yalow and Berson in 1960 when they described the RIA of insulin. This established an important new technique for clinical investigations involving the measurement of minute quantities of protein hormones present in body fluids. The RIA of human growth hormone (referred to as GH in rest of text) was first described by Hunter and Greenwood (1964) and Glick *et al* (1963). Catt and Tregear (1967) reported a simplified RIA of HGH using a solid phase technique. This solid phase method has been incorporated into an HGH Immunoassay kit (HGH-125 Imusay™—Abbott). This paper describes our initial experience of the RIA of HGH using the HGH-125 Imusay™ kit.

METHODS AND MATERIALS

The radioimmunoassay (RIA) of human growth hormone (GH) was carried out using the HGH Immunoassay kit (HGH-125 Imusay™) produced by Abbott Laboratories, North Chicago, United States of America. The HGH was labelled with ^{125} Iodine. The assay was simple and rapid (an assay took 48 hours). A typical standard curve is shown in Fig. 1.

Department of Medicine (Unit I), University of Singapore.
J. S. CHEAH, F.R.A.C.P., Senior Lecturer.
A. H. NAKHODA, Ph.D., Biochemist.

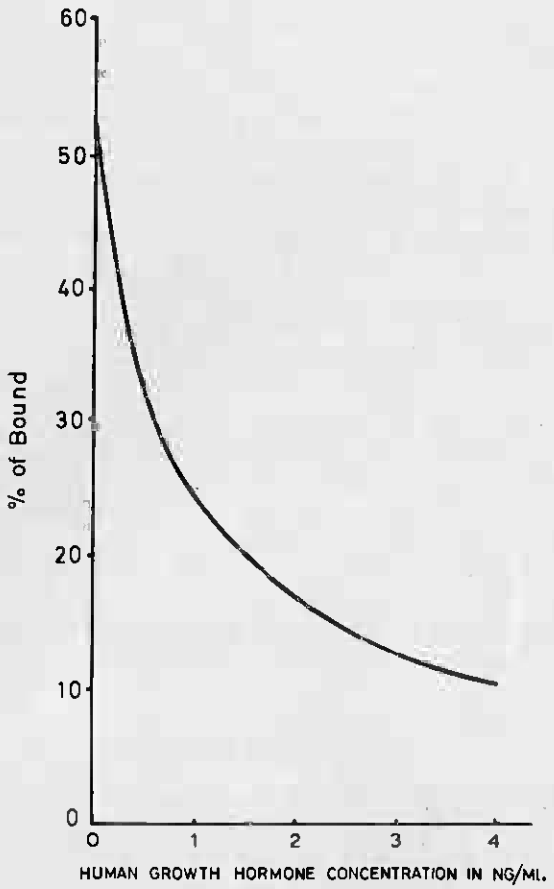

Fig. 1

In 10 normal subjects (all males) the fasting serum GH and the rise in GH following insulin hypoglycaemia were determined. After an overnight fast, the blood sugar was determined and 5 ml. of venous blood was taken for GH determination. Soluble insulin was then given intravenously in the dosage of 0·1 unit/Kg. body weight. Blood was taken for blood sugar and GH estimations at ½ hourly intervals for 2 hours. Similar tests were performed on 6 patients with hypopituitarism (2 patients had hypophysectomy for pituitary tumours; 3 patients had pituitary tumours and 1 patient had Sheehan's syndrome). All the tests were performed with the patients lying comfortably in bed. All the cases were watched carefully for symptoms of hypoglycaemia. None of the cases developed symptoms of hypoglycaemia that required premature termination of the tests.

In a further 6 normal male controls the fasting GH levels and the serum GH following an oral load of glucose were measured. After an overnight fast, the blood was taken for blood sugar and serum GH determinations. Oral glucose was then given in a dose of 50g. Blood was taken for blood sugar and serum GH measurements at ½ hourly intervals for 2 hours. Similar tests were done on 5 patients with acromegaly.

RESULTS

A typical example of the inverse relationship between the blood sugar and serum GH in a normal subject is shown in Fig. 2. In 10 normal subjects the mean fasting serum GH was 9·2 ng./ml. (standard deviation: 5·7; range 1·2 to 18·1 ng./ml.; see Table I and Fig. 3). Following insulin hypoglycaemia (the fall in blood sugar was

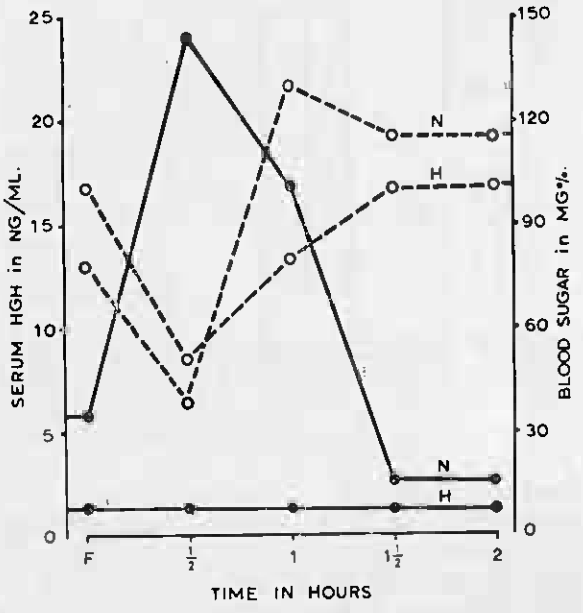

Fig. 2

SERUM H.G.H. (FASTING AND FOLLOWING INSULIN HYPOGLYCAEMIA) IN 10 NORMAL CASES.

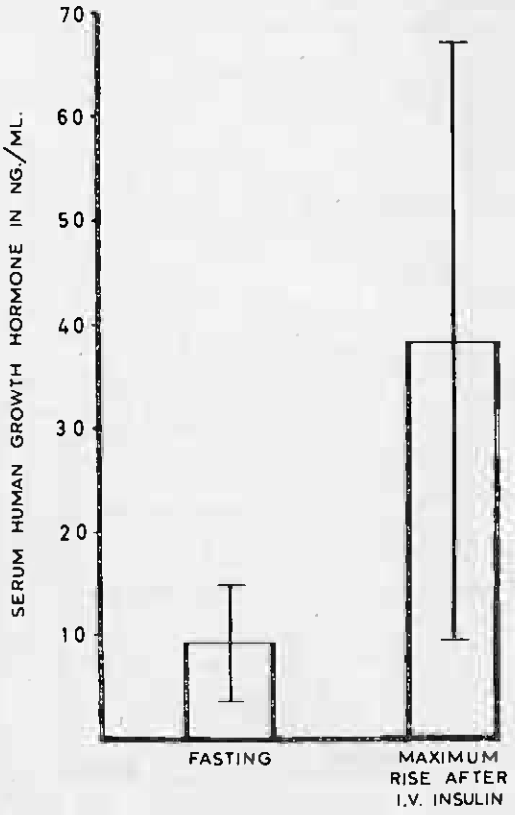

Fig. 3

TABLE I

FASTING AND PEAK SERUM GROWTH HORMONE (HGH) VALUES (MEAN ± 1 STANDARD DEVIATION) IN 10 NORMAL SUBJECTS AND 6 CASES WITH HYPOPITUITARISM

Type of Patient	Number of Patient	HGH in ng./ml. (Mean ± 1 S.D.)	
		Fasting	Peak After Insulin Hypoglycemia
Normal	10	9·2 ± 5·7	38·1 ± 28·9
Hypopituitarism	6	2·5 ± 1·4	2·5 ± 1·5

at least 50% of the fasting value), the mean peak serum GH rose to 38·1 ng./ml. (standard deviation 28·9; range 7·6 to 108·0 ng./ml.; see Table I and Fig. 3).

In 6 patients (5 males and 1 female) with hypopituitarism (2 patients had hypophysectomy for pituitary tumours; 3 cases had pituitary tumours and 1 patient had Sheehan's syndrome), the mean fasting serum GH was 2·5 ng./ml. (standard deviation 1·4; range 1·1 to 4·0 ng./ml.; see Table I, Figs. 3 and 4). In these patients, following insulin hypoglycaemia, the mean peak serum GH level reached was 2·5 ng./ml. (standard deviation 1·5; range 1·1 to 4·4 ng./ml.; see Table I and Fig. 4).

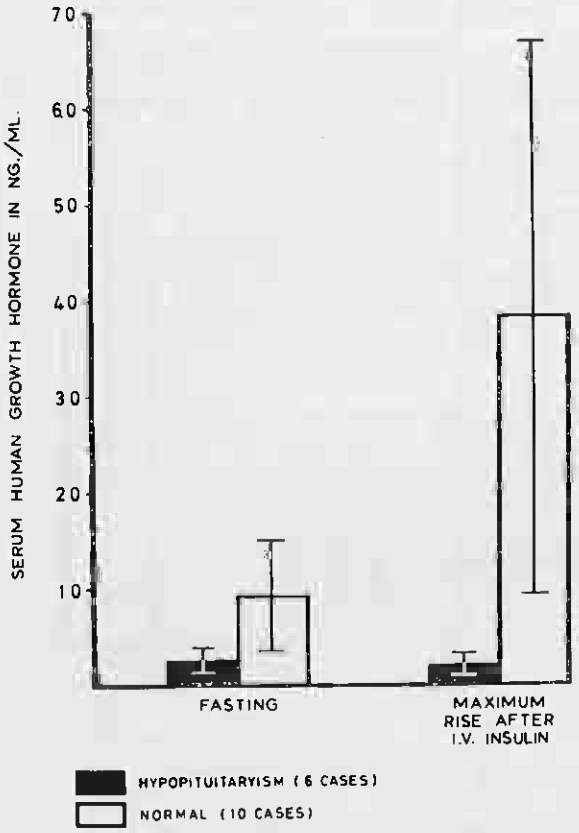

Fig. 4

In a further 6 normal males, the mean fasting serum GH was 7·8 ng./ml. (standard deviation 6·4; range: 2·0 to 18·5 ng./ml.); 2 hours after an oral load of 50 g. of glucose the mean serum GH was 1·3 ng./ml. (standard deviation: 0·6; range 1·0 to 2·4 ng./ml.; see Table II and Fig. 5). In 5 cases of acromegaly the mean fasting serum GH was 82·6 ng./ml. (standard deviation 67·2; range 45·0 to 190·0 ng./ml.; see Table II, Figs. 6 and 7). The GH levels during a glucose tolerance test in these subjects are shown in Fig. 7. The mean serum GH at 2 hours after a 50 g. load of glucose

TABLE II

THE FASTING SERUM GROWTH (HGH) AND THE SERUM GROWTH HORMONE 2 HOURS AFTER AN ORAL LOAD OF 50g. OF GLUCOSE IN 6 NORMAL SUBJECTS AND 5 CASES WITH ACROMEGALY

Type of Patient	Number of Patients	HGH in ng./ml. (Mean ± 1 S.D.)	
		Fasting	2 Hours After 50 g. of Oral Glucose
Normal	6	7·8 ± 6·4	1·3 ± 0·6
Acromegaly	5	82·6 ± 67·2	88·6 ± 54·5

SERUM H.G.H. (FASTING AND 2 HOURS AFTER 50 G. ORAL GLUCOSE) IN 6 NORMAL CASES.

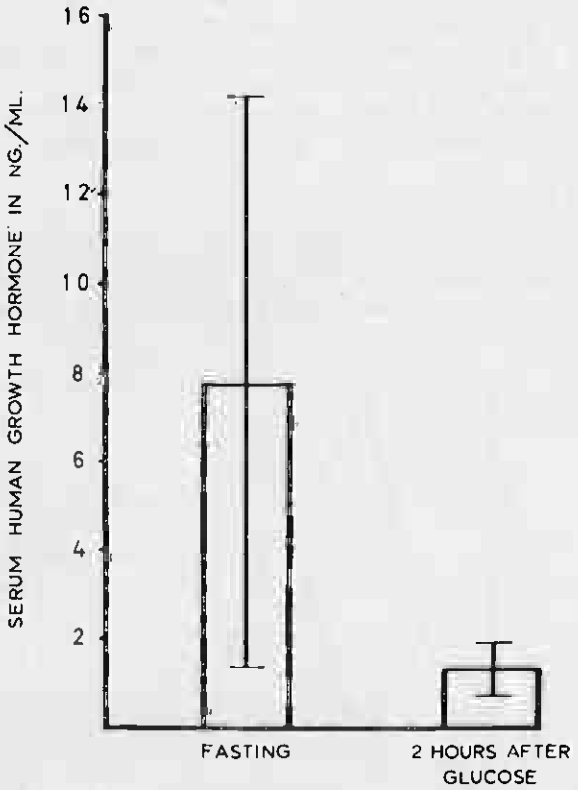

Fig. 5

was 88·6 ng./ml. (standard deviation 54·4; range 26·0 to 149 ng./ml.; see Table II, Figs. 6 and 7).

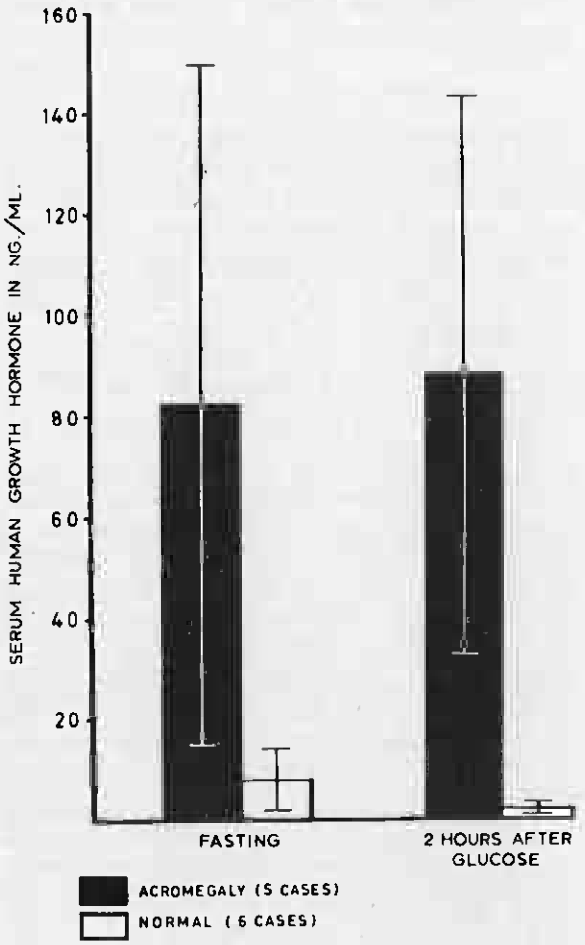

Fig. 6

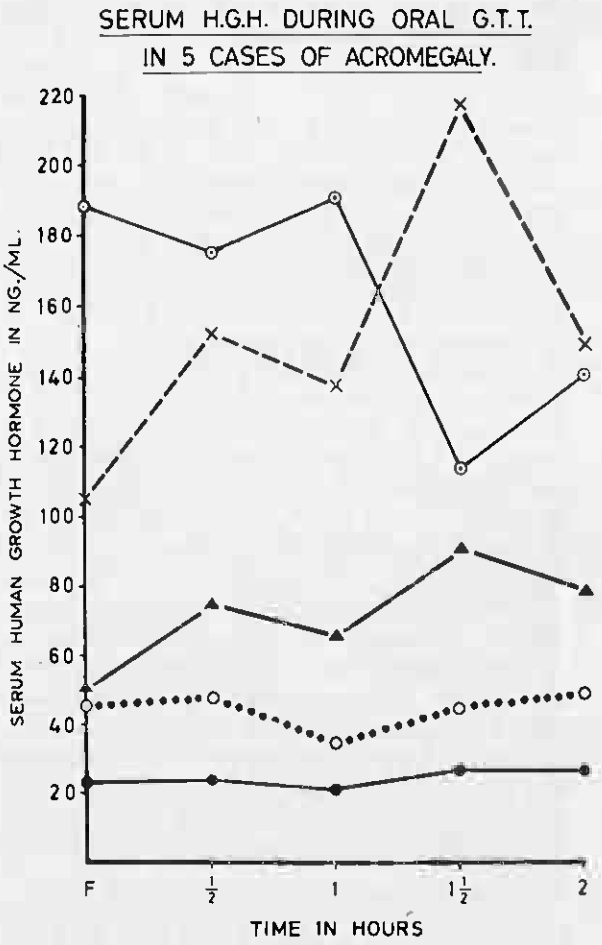

Fig. 7

Four of the acromegalies showed a paradoxical rise in the serum GH 2 hours after glucose. In one subject there was a partial suppression from 190 ng./ml. at fasting to 140 ng./ml. at 2 hours i.e. a suppression to 73·4% of the fasting level (see Fig. 7).

DISCUSSION

There are four requisites for RIA: a highly purified antigen; a specific antibody produced by injection of that antigen into another animal species; purified antigen labelled radioactively (usually iodine-125 or 131) having high specific activity and a method for separating the antigen-antibody complex from the free antigen. In the early RIA of GH, the antigen-antibody complex was separated from the free antigen by electrophoresis. This method, although accurate, is relatively tedious and time-consuming (Greenwood, 1967). In the method used in this study (HGH-125 Imusay™; Abbott), an antibody coated plastic tube is used to separate the free antigen from the antigen-antibody complexes formed on the tube (Catt and Tregear, 1967). This method is relatively rapid and less tedious than the immunoelectrophoretic methods. Our initial experience with the solid phase RIA shows that this method provides results that correlate very well with the clinical state of the patients and would therefore provide a rapid and easy method for the diagnosis of GH disorders.

There are many factors that influence the regulation of GH secretion in man (Catt, 1971). There are also many methods available in the RIA of HGH; the results from the various methods vary slightly (Greenwood, 1967). Thus it is not surprising that the normal range of serum HGH varies from laboratory to laboratory (Ewer and Deiss, Jr., 1969).

In the present series of 10 normal subjects, the mean fasting serum GH was 9·2 mg./ml. and

the range was 1·2 to 18·1 ng./ml. The range in the series reported by Ewer and Deiss Jr., (1969) was 1 to 4 ng./ml. and that reported by Catt (1971) was 1 to 5 ng./ml. The wider range in our present series is likely to be due to the fact that some of our cases were not in the true basal state; excitement being known to cause an elevation in the serum GH (Glick *et al*, 1965; Catt, 1971). Unger *et al* (1965) reported higher fasting level of GH in women than in men but this finding has not been confirmed (Ewer and Deiss, Jr., 1969).

Insulin-induced hypoglycaemia is the most potent and consistent stimulus to HGH secretion. In the present study, the mean serum GH in 10 normal subjects was 38·1 ng./ml. following insulin hypoglycaemia; the range being 7·6 to 108 ng./ml. This wide range in the serum GH following insulin hypoglycaemia in normal subjects has been widely recognised (Glick *et al*, 1965; Daughaday and Parker, 1965; Ewer and Deiss, Jr., 1969).

The demonstration of the failure of the serum GH to rise following insulin hypoglycaemia is required for the diagnosis of GH deficiency (Clayton, 1967). The clinical features of GH deficiency are not specific (Cheah and Tay, 1973). This response was exhibited by all the subjects tested in this study with clinically-proven hypopituitarism.

The results of the suppression of the serum GH by glucose during a glucose tolerance test in the normal subjects in the present series closely resembles those of other reported series (Earll, Sparks and Forsham, 1967; Ewer and Deiss, Jr., 1969). In one of our acromegalic cases, the serum GH was partially suppressed by glucose while in the other 4 cases there was a paradoxical rise in the serum GH. This paradoxical rise in the serum GH following oral glucose is a feature seen only in acromegaly or gigantism (Burger and Catt, 1969; Fraser, 1970). The measurement of the serum GH in acromegaly before and after treatment is essential for the proper management of these cases (Fraser, 1970). The old concept that acromegalies may burn themselves out in the course of time is probably incorrect (Fraser, 1970; Teo and Cheah, 1973).

The introduction of the RIA of GH has considerably advanced the understanding of the secretion of GH in health and diseases (Daughaday and Parker, 1965; Glick *et al*, 1965; Catt, 1970; Fraser, 1970).

This study has shown that the solid phase RIA of Catts and Trepar (1967), as modified by Abbotts for use with its kit, produces a simple and reliable method for both the diagnosis and management of subjects with defects in GH secretion.

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