Measurement of human growth hormone in urine: development and validation of a sensitive and specific assay

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ABSTRACT

A specific solid-phase immunoradiometric assay (IRMA), optimized for maximum sensitivity, has been developed for measurement of human GH (hGH) in urine. The sensitivity varied with sample size, giving a range of 0.001 to 0.003 mU/l for a sample volume of 2 ml. Recovery and dilution experiments, together with chromatography of urine samples, indicate that the method is specific for hGH. Added exogenous hGH was measured with a mean recovery of 101 ± 10% (s.d.) for 1 ml samples and 87 ± 8% for 2 ml samples. Measurements of samples diluted at 1:2 and 1:4 gave values of 97 ± 4 and 96 ± 6% respectively of those expected. Cross-reactions of human placental lactogen and prolactin were less than 0.008 and 0.04% respectively on a mol/mol basis. The assay was insensitive to the presence of NaCl (50–500 mmol/l), urea (50–1000 mmol/l), creatinine (1–20 mmol/l), Ca²⁺ ions (1–100 mmol/l), Mg²⁺ ions (0.05–50 mmol/l), 0.5–5% (w/v) glucose and a pH range of 6–9. Chromatography of unextracted samples showed that the immunoreactive material in urine eluted in a single homogeneous peak with a similar position to monomeric pituitary hGH (22 kDa). Administered hGH (0–002%) was recovered in urine collected over a 2-h period following an intravenous injection. The urine output of hGH showed a good correlation with serum hGH in 18 patients following routine insulin tolerance tests and in 25 patients following an oral glucose tolerance test. Urine from normal subjects showed a mean day-time excretion of 498 ± 150 nU/h (s.d.) and a mean night-time excretion of 132 ± 93 nU/h. Preliminary results suggest that the measurement of hGH using this assay reflects the variation in blood and could be a useful diagnostic procedure.

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INTRODUCTION

As pituitary human growth hormone (hGH) is secreted in a pulsatile manner, the serum concentration of spontaneously secreted hGH fluctuates widely over a 24-h period (Takahashi, Kipnis & Daughaday, 1968; Finkelstein, Roffwarg, Boyar et al. 1972; Drobny, Amburn & Baumann, 1983). For this reason the determination of serum hGH levels over 24 h or overnight hGH secretory pattern has been established in a number of studies (Spiliotis, August, Hung et al. 1984; Adlord, Buizi, Jones et al. 1987; Hindmarsh, Smith, Brook & Matthews, 1987).

The requirement for such frequent blood sampling demands a large investment of time, blood and labour and can be both uncomfortable and cumbersome for patient and staff alike. In contrast, a urine sample may be collected much more easily and, in its simplest fashion, the hGH level in urine may reflect the endogenous serum hGH level throughout the period of collection (Girard & Greenwood, 1968; Maack, Johnson, Kau et al. 1979; Hashida, Ishikawa, Kato et al. 1987; Okuno, Yano, Itoh et al. 1987). Consequently, the determination of the hGH output in urine may have many clinical applications; in particular, it may prove a useful monitor of physiological hGH levels during the treatment of impaired GH secretion.

The measurement of hGH in urine has, however, been hampered by two factors. (1) hGH is present in urine in a very low concentration in normal subjects (Chakmakjian & Langston, 1972; Sonksen, 1976). Indeed, Baumann & Abramson (1983) could extract only 180 ng hGH from 35 litres of normal urine and estimated that 0.01% of circulating hGH may appear in the urine. (2) There is a wide variety in the interfering factor content in urine, i.e. urea, salt and.
glucose (Girard & Greenwood, 1968; Hanssen, 1972; Baumann & Abramson, 1983). A highly sensitive and specific assay system would therefore be required to measure hGH concentrations down to approximately 0.001 mU/l. Most radioimmunoassay techniques are not sufficiently sensitive (or specific) to measure such levels (Miles & Hales, 1968; Jackson & Ekins, 1986) and therefore many attempts to measure hGH in urine to date have involved the concentration and subsequent dialysis of the urine sample (Bala & Beck, 1971; Hanssen, 1972). Here, we describe the development and validation of a direct, highly sensitive and specific immunoradiometric assay (IRMA) based on the use of solid-phase anti-hGH and a radioiodinated monoclonal antibody. A preliminary examination of the physiological hGH levels in non-extracted urine from normal subjects and in the non-extracted urine from patients undergoing a routine dynamic test is presented.

MATERIALS AND METHODS

Human growth hormone (66/217) was obtained from NIBSC, Potters Bar, Herts, U.K. and was used as a reference material without further purification. Cellulose (Sigmacell type 20), carbodiimidazole, EDTA and Tween 20 were from Sigma Chemical Company, Poole, Dorset, U.K. All other chemicals were of analytical reagent grade and were purchased from BDH Chemicals Ltd, Dagenham, Essex, U.K. Bovine serum albumin (BSA) Fraction V and gamma globulin Fraction II were obtained from ICN Biomedicals Limited, High Wycombe, Bucks, U.K. Pig serum was from Sera Lab, Crawley Down, Sussex, U.K. Highly purified hormones were used for cross-reaction studies. Human placental lactogen (hPL) was obtained from Sigma Chemical Company. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin and thyroid stimulating hormone (TSH) were purified from frozen pituitaries (Hodgkinson & Lowry, 1981; McLean, Hodgkinson, Hope & Lowry, 1981).

Urine samples

Urine samples were collected at room temperature without added preservative. Volume and time were noted and aliquots assayed immediately or stored frozen at -20 °C. Repeated freezing and thawing was avoided. Urine specimens were centrifuged at 1000 g to remove particulate material. The supernatant was taken for assay. Samples were collected from patients undergoing a routine insulin tolerance test (ITT) or an oral glucose tolerance test (OGTT). Eighteen patients underwent an ITT; the first sample was taken immediately after injection, noting the time of the first voiding, and the second sample was taken 2-4 h after injection. An oral glucose load (50 g) was given to 25 patients and three samples were collected. The first sample was taken before the test, noting the time of voiding, the second 1 h and the third 2 h following the glucose load. Day- and night-time urine samples were collected from three normal adults, aged 27–30, on four separate days; neither diet nor activity were restricted. Samples were pooled for a 24 h hGH excretion measurement. Informed consent was obtained from all subjects and patients.

Blood samples

Blood samples were obtained before and 15, 30, 60, 90 and 120 min after i.v. administration of 0.1 U insulin/kg to 18 patients. Similarly blood was obtained before and 30, 60, 90, 120 and 150 min following an oral glucose load of 50 g in 25 resting, fasting patients. Samples were assayed for hGH in the serum IRMA (Knott, Hound & Edwards, 1985).

Buffers

Buffer A contained (per l) 0.45 mol sodium phosphate (NaH₂PO₄·2H₂O/Na₂HPO₄·2H₂O), pH 7.4, 30 g EDTA, 90 g BSA, 9 g gamma globulin, 1 g sodium azide and 3% (v/v) Tween 20. Buffer B contained (per l) 50 mmol sodium phosphate, pH 7.4, 6 g EDTA, 10 g BSA, 1 g sodium azide, 0.5% (v/v) Tween 20 and 10% (v/v) pig serum. Wash buffer contained (per l) 50 mmol sodium phosphate, pH 7.4, 1 g sodium azide and 0.5% (v/v) Tween 20.

Solid phase anti-hGH

A high-affinity (K=1.5×10¹⁰ litres/mol) sheep anti-GH antiserum (NETRIA S8/2), chosen from Scatchard analysis of six sheep anti-GH antisera was covalently coupled to carboxymidazole-activated micro-particulate cellulose (20 µm) (Chapman, Sutherland & Ratcliffe, 1983). The optimal concentration of solid-phase anti-hGH was obtained by titration. The reaction was carried out at room temperature over 24 h with ¹²⁵I-labelled monoclonal antibody in the presence and absence of hGH reference material. A concentration of 20 mg/ml (1 mg/tube) was chosen for assay use, as it maximized the signal to noise ratio (Miles & Hales, 1968).

Monoclonal antibody

A high-affinity monoclonal antibody (K=5.2×10⁹ litres/mol) was radioiodinated to a specific activity of 1 atom ¹²⁵I/1 mol IgG, using a modification of the chorlamine T method (Hunter & Greenwood, 1962). The products were purified on a Sephacryl S-300 column (0.9×60 cm) which was pre-equilibrated and
eluted with sodium phosphate buffer (50 mmol/l), pH 7.4, containing BSA (10 g/l) and sodium azide (1 g/l). The $^{125}$I-labelled monoclonal antibody was eluted as a single peak. This material was lyophilized and stored at 4 °C. The concentration of labelled monoclonal antibody was optimized for maximum assay sensitivity according to the theoretical immunoradiometric assay model of Jackson, Marshall & Ekins (1983). Assessed from precision profiles (not shown) an optimal concentration of radiolabelled monoclonal antibody of 0.75 nmol/l was chosen.

Chromatography

Samples of urine were applied to a Sephadex G-100 Superfine (0.9 x 60 cm) column which was pre-equilibrated and eluted with sodium phosphate buffer (50 mmol/l), pH 7.4 containing BSA (10 g/l) and sodium azide (1 g/l). Eluates were collected in 1 ml fractions at a rate of 3 ml/h. Fractions were then assayed directly.

Assay protocol

Duplicate tubes (64 x 11 mm polystyrene) containing hGH reference preparation or urine, 50 μl solid-phase anti-hGH (1 mg/tube) and 200 μl buffer A were vortex-mixed, capped, and continually mixed end-over-end for 16 h at room temperature. Tubes were then centrifuged for 5 min at 2000 g and the supernatant decanted to waste. Wash buffer (2 ml) was added to all tubes which were then centrifuged again. Supernatants were decanted to waste and the wash procedure repeated. Buffer B (400 μl), followed by 50 μl labelled monoclonal antibody at 50 000 c.p.m., were then added to every tube. These were vortex-mixed and then continually mixed on a vertical rotation system (Edwards, Cann, Howes et al. 1986) for 16 h at room temperature. The wash procedure, as detailed above, was repeated. Following two further washes, tubes containing the cellulose pellet were counted in a NE1600 multthead gamma counter for 10 min. Calculation of results was accomplished from linear regression analysis.

RESULTS

The assay was based on the optimal concentration of monoclonal antibody and solid-phase anti-hGH, as detailed above. The sensitivity was further improved by the use of a variable sample volume (0.6-5 ml), sequential addition of reagents, increased counting time and reduction of the non-specific signal (Addison, 1973; Jackson & Ekins, 1986).

Sensitivity

The sensitivity, defined as 2.5 s.d. from zero concentration (Raab & McKenzie, 1981) is directly related to the sample size (Table 1). Figure 1 shows the linear correlation between hGH concentration and $^{125}$I-labelled monoclonal antibody bound to solid-phase over the range of 6.25-400 μU/l.

<table>
<thead>
<tr>
<th>Sample volume (ml)</th>
<th>Sensitivity (μU/l)</th>
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<tbody>
<tr>
<td>0.6</td>
<td>8.2</td>
</tr>
<tr>
<td>1</td>
<td>2.7</td>
</tr>
<tr>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td>5</td>
<td>0.7</td>
</tr>
<tr>
<td>10</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Precision

Intra-assay precision was determined from precision profiles (Ekins, 1983). Considerable improvement in precision was achieved with two or three wash cycles. The effect of the washing procedure on the precision profile is shown in Fig. 2. Interassay (n = 10) coefficients of variation were calculated from the repeated analysis of samples containing 10, 25 and 50 μU hGH/l and were 12, 9 and 8% respectively.

Recovery

Various urine samples were assayed before and after addition of known amounts of hGH (IRP 66/217). Recoveries were calculated as a percentage of the amount added (Table 2).

Specificity

With respect to specific and non-specific binding the assay was insensitive to the following: NaCl (50-500 mmol/l), urea (50-1000 mmol/l), creatinine (1-20 mmol/l), Ca$^{2+}$ ions (CaCl$_2$) (1-20 mmol/l), SO$_4^{2-}$ ions (Na$_2$SO$_4$) (1-1000 mmol/l), Mg$^{2+}$ ions (MgCl$_2$) (0.05-50 mmol/l), 0.5-5% (w/v) glucose and a pH range of 6-9. Serial dilutions of hPL, prolactin, FSH, LH and TSH were assayed to test specificity. Cross-reactivity of these various hormones was assessed in terms of apparent hGH concentration in the presence (0.5 μg/l) and absence of hGH and expressed as a percentage cross-reaction and on a mol/mol basis (Table 3). Assay specificity was also
demonstrated by parallelism between dilutions of urine samples and hGH standards. Urine samples from ten subjects were assayed neat and at a dilution of 1:2 and 1:4. The observed result was expressed as a percentage of the calculated value. Dilutions were considered parallel to hGH standard if the observed result did not differ significantly from the calculated result. Parallelism was shown for all ten samples following a 1:2 dilution; results were within 1 s.d. of the mean (97.4% ± 5.3; mean ± s.d.). One sample, however, showed non-parallelism following a 1:4 dilution (71%), >3 s.d. from the mean (96.6% ± 7.3; mean ± s.d.). Precise estimation of parallelism is difficult due to the very low levels of hGH in urine, especially following dilution.

The binding of the polyclonal and monoclonal antibodies for different epitopes on the hGH molecule was assessed from titration and competition experiments. A sixfold excess of solid-phase showed no loss of monoclonal antibody binding to the bound GH. Preincubation of monoclonal antibody with hGH showed that a fivefold excess of monoclonal antibody did not reduce binding of GH to the solid-phase. A fivefold excess of unlabelled monoclonal antibody showed no loss of binding of 125I-labelled GH to solid-phase. These studies indicated that the antibodies were binding to different epitopes.

**Measurement of administered hGH**

One subject received an i.v. bolus injection of 2 IU hGH. Timed blood and urine samples were collected...
TABLE 3. Solutions of cross-reactants, at two concentrations, were assayed in the presence and absence of human GH (0-5 µg/l). The response, in terms of apparent hGH concentration, was expressed as a percentage, on a mol/mol basis, of the cross-reactant concentration.

<table>
<thead>
<tr>
<th>Cross-reactant</th>
<th>Concentration (µg/l)</th>
<th>Cross-reaction as % (mol/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hPL</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Prolactin</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>FSH</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>LH</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>TSH</td>
<td>0.1</td>
<td>0.2</td>
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<td></td>
<td>1.0</td>
<td>1.0</td>
</tr>
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hPL, human placental lactogen.

over a period of 5 h. The profile of serum and urine levels of hGH is shown in Fig. 3. A very small percentage (0-002% ± 0-002; mean ± s.d.) of injected hGH was excreted into the urine over a 2-h collection period. Figure 3 shows that the excretion of hGH in urine increased proportionately to the serum level.

Dynamic tests

To examine whether the urinary hGH level reflects the serum hGH level, the urinary output of immunoreactive hGH was compared with the level in serum following a routine ITT and an OGTT in 18 and 25 patients respectively. The urinary hGH output (nU/h) showed good correlation with the mean serum hGH concentration (r=0.84, P<0.001) following an OGTT. The urinary hGH output also showed excellent correlation with the mean serum hGH level (r=0.90, P<0.001) and the peak serum hGH level (r=0.90, P<0.001) following an ITT.

FIGURE 4. Elution pattern of GH immunoreactivity following chromatography of unextracted urine samples on Sephadex G-100S (0.9 × 60 cm). Samples were from two normal subjects (a and c) and one treated acromegalic patient (b). Recoveries from the column were 70, 75 and 90% respectively. The void volume (V0) and the elution position of monomeric pituitary hGH are shown by arrows.
Assay of hGH in urine

The effect of volume on GH excreted in urine was assessed in 64 samples. Urine volumes (ranging from 15 ml to 450 ml) were correlated with the amount of GH excreted. The correlation coefficient was 0.074 and was not significant (P > 0.5).

Normal values
Urine samples collected from normal individuals showed higher levels of hGH in the early morning sample (498 ± 150 (s.d.) nU/h), when compared with the day-time sample (132 ± 93 nU/h). In these subjects the 24-h excretion of hGH was 8.3 ± 5.5 µU and ranged from 3 to 22 µU for 12 samples.

DISCUSSION
We have reported the development and validation of a sensitive and specific IRMA for the direct measurement of hGH in urine. It became apparent during this study that precise optimization of assay parameters is critical to the accurate measurement of a very low concentration of hGH in a sample of unextracted urine. The sensitivity of a two-site IRMA is essentially dependent on the non-specific binding of labelled antibody, the precision of the separation procedure and the affinity with which the antibody binds to analyte (i.e. the equilibrium constant of the reaction) (Jackson & Ekins, 1986). The affinity of the antibody reaction is constrained by available antibodies but the precision of separation and the non-specific binding parameter are subject to experimental manipulation. The optimization of this assay was therefore based on minimizing the experimental error and instrumental error significant in the 'zero' dose–response, following selection of antibodies with high equilibrium constants.

In the present study, the excretion of hGH in 24-h urine samples from normal adults ranged from 3 to 22 µU (1.5–11 ng). Proteins with the size and charge of monomeric GH undergo glomerular filtration and reabsorption in the proximal tubule (Maack et al. 1979). Based on a 24-h integrated plasma hGH concentration of 2–5 µg/l (Sonkson & West, 1979), a glomerular filtration rate of 180 litres/24 h, and assuming a glomerular sieving coefficient for GH of 0.65 (Johnson & Maack, 1977), it can be calculated that 234–585 µg hGH are filtered through the glomeruli in 24 h.

Our findings in normal adults indicate that less than 0.01% (0.00024–0.005%) of this amount is excreted into the urine. This estimate is lower than previously calculated by Baumann & Abramson (1983), although their estimate of 0.01% was based on only one pool of urine collected overnight from several normal subjects, and had undergone substantial extraction with poor recovery. In addition, we have shown that an estimated 0.002 ± 0.0002% (mean ± s.d.) of exogenously administered hGH is recovered in the urine from the normal subject studied.

The kidney plays an important role in the metabolism of circulating plasma proteins and several lines of evidence have suggested that this is true with respect to the turnover of hGH (Johnson & Maack, 1977). From our findings and those of Chakmakjian & Langston (1972), Hanssen (1972), Okuno et al. (1987) and particularly Baumann & Abramson (1983) it is clear that only a small amount of endogenous hGH is excreted into the urine. In order to detect very low levels of hGH in urine many investigators to date have found it necessary to adopt a method to concentrate the urine sample. These methods have mainly involved lyophilization or ultrafiltration (Geller & Loh, 1963; Girard & Greenwood, 1968; Hanssen, 1972; Hashida, Ishikawa, Nakagawa et al. 1985). It is evident from most of these studies that the methods for the measurement of hGH in urine have suffered from an inherent lack of sensitivity. The subsequent use of extraction procedures may be implicated in the widely discrepant results. Hashida et al. (1985) and Hashida, Ishikawa, Nakagawa et al. (1986) described a highly sensitive enzyme immunoassay for the measurement of hGH in dialysed normal urine concentrates. During the course of our study Hashida et al. (1987) described a modified assay with improved sensitivity, given as 130 pg/l. They were able to measure hGH in non-concentrated urine, although the urine sample still required dialysis. Dialysis of the urine sample is inconvenient and may give rise to variable recovery of endogenous hGH, particularly at low concentrations of hGH. In contrast, we have developed a simple, direct assay which does not require the prior concentration or dialysis of the urine sample and is sufficiently sensitive to measure hGH concentrations down to 0.6 µU/l (300 pg/l) in a 10 ml urine volume.

Recently a group of Swiss workers (Girard, Erb, Pampalone et al. 1987; Erb, Karolyi, Pampalone et al. 1988) have reported the development of a similar radiometric assay based on solid-phase immunoextraction. They have shown their method to be insensitive to NaCl, urea and pH and are similarly able to measure hGH levels down to 300 pg/l in a 10 ml urine sample.

Immobilization of antibody on a solid-phase support may offer the antibody–antigen reaction some protection against the widely variable physico-chemical environment of urine. In the present assay, we have demonstrated that the solid-phase antibody–antigen reaction is remarkably resistant to very high, non-physiological concentrations of urinary
constituents. This has also been shown by Girard et al. (1987). A negligible effect of cross-reacting or interfering substances was confirmed by dilution and recovery experiments in unextracted urine. Parallelism was demonstrated between two dilutions of various urine samples and hGH standards. The results of the recovery experiment show that there was a significantly reduced recovery when hGH was added to a 10 ml urine sample. Girard et al. (1987) found no such reduction in recovery for a 10 ml sample volume, although they recovered only one concentration of exogenous hGH and from apparently only one immunextracted ‘hormone free’ urine sample. The results of the recovery and dilution experiments indicate that the method is specific and is resistant to non-specific interference for a 0.6, 1, 2 or 5 ml sample volume. The data in this paper are based on assays using either a 1 ml or a 2 ml sample volume.

The cross-reaction of hPL, prolactin, LH, FSH and TSH in the presence (0.5 μg/l) and absence of hGH was examined. Our results show that there is only a very low cross-reaction with these hormones and that there is no significant cross-reaction with them at concentrations found in urine (Sinha, Selby & Vanderlaan, 1973; Wide, 1976; Chard, 1979; Beastall, Ferguson, O’Reilly et al. 1987). In addition, the monoclonal and polyclonal antibodies used in this assay appear to show little competition for epitope binding sites on the hGH molecule. The specificity of this assay, in terms of recovery of exogenous hGH, parallel dilution of unextracted samples, cross-reactivity and its insensitivity to a wide range of urine constituents indicate a resolution to some of the problems encountered by previous investigators.

The identity of the ‘immunoreactive hGH’ material in urine, in this study, confirms the findings of previous workers (Chakmakjian & Langston, 1972; Baumann & Abramson, 1983; Hashida et al. 1985, 1986) that it has a molecular size similar to the monoclonal, 22 500 molecular weight form of pituitary hGH and may well represent the excretion of unmodified hGH. While much of the previous work has relied on concentrating the urine many fold before chromatography, we were able to quantify the chromatographic identity of hGH in urine on unextracted samples. Some extraction procedures, in particular lyophilization, may alter the molecular characteristics of hGH (Cheever & Lewis, 1969). Further work is required to clarify the precise nature of the immunoreactive hGH in urine, especially unextracted urine. Other hetero-geneic forms have so far remained undetected in unextracted urine although Baumann & Abramson (1983) have described the presence of a 20 000 molecular weight form and an unidentified acidic form in extensively concentrated urine. It is possible that oligomeric forms of hGH, by virtue of their molecular size, escape the renal degradation process and would not appear in urine in sufficiently quantifiable amounts (Maack et al. 1979; Baumann, Stolar & Buchaman, 1986).

Geller & Loh (1963), in a very early study, found hGH in urine to be biologically active, but no further studies seem to have been reported. If hGH in urine has maintained its biological activity on its passage through the kidney, then this may provide additional evidence for the lack of renal interconversion and the unmetabolized nature of hGH in urine.

The correlation of urine measurements of hGH with serum hGH levels under pharmacological stimulation has been demonstrated by Hashida et al. (1987) and Okuno et al. (1987). In our study, urine hGH levels were well correlated with integrated and peak serum levels following an ITT and with integrated serum levels during an OGTT. Both the administration of exogenous hGH and the dynamic studies confirm a significant correlation between variations in blood and those in urine, during the test period.

In a separate study we have confirmed that the measurement of hGH in urine accurately reflects pituitary hGH secretion during overnight secretory profiles in children (Edge, Hord, Edwards & Dunger, 1989).

Human GH has been shown to be secreted in distinct episodes during 24 h in adults and children, although much emphasis has been placed on the nocturnal increase in hGH secretion (Takahashi et al. 1968; Miller, Tannenbaum, Colle & Guyda, 1982). In a limited study, the level of hGH in urine from normal subjects clearly portrayed a similar circadian pattern to that reported to exist in blood.

Inherent in these measurements, however, is an important intra-individual variation. Girard et al. (1987) have shown a several-fold overnight variation in urinary hGH excretion in children although their results are based on early morning first voiding samples rather than timed collections. As part of a study of hGH excretion in children (Edge et al. 1989) we found much less overnight intra-individual variability (% coefficient of variation) in ten timed collections from three children (66.0, 32.1 and 40.4% respectively). It is clear, however, that such variability may have important implications for the diagnostic usefulness of urinary GH measurement and hence will require further study.

From these studies it is appreciated that a change in renal function could considerably affect the interpretation of urinary hGH results. A study of various changes in renal function, such as exercise, water loading and pathology, is in progress.

These preliminary results indicate that the measurement of hGH in urine can reflect variation of hGH in the blood and thus could be a useful procedure in physiological studies and clinical diagnosis.
REFERENCES


