Urinary growth hormone excretion as a screening test for growth hormone deficiency

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Abstract
Overnight urinary growth hormone secretion was measured by an immunoradiometric assay incorporating commercially available reagents, in 41 normal prepubertal schoolchildren from three age groups: 3–5 years, 6–7 years, and 9–10 years. There was no significant difference between the groups expressing the results as total μU/specimen and so they have been combined to provide a prepubertal reference range of 2.25–10.50 μU/night. Prepubertal children with growth hormone deficiency who had not been receiving growth hormone treatment for two days had overnight urinary growth hormone concentrations well below this range. Urinary growth hormone was assayed in 49 children undergoing investigation for short stature with conventional provocative testing, and those shown to have growth hormone deficiency had correspondingly low overnight urinary growth hormone concentrations. There was, in addition, a strong correlation between overnight urinary growth hormone concentrations and peak serum response to provocation. This simplicity may provide a useful screening test for growth hormone deficiency.

Secretion of growth hormone from the anterior pituitary gland is pulsatile and greatest during slow wave sleep. Screening tests for growth hormone deficiency in current use are based on this observation, and therefore require overnight tests. Alternatively, secretion during the day can be stimulated by drugs. Both types of test require repeated blood sampling from an indwelling cannula, are time consuming, are expensive in terms of manpower and bed occupancy and, above all, are unpleasant and potentially dangerous. An alternative would be the measurement of urinary growth hormone concentration, which would assess integrated growth hormone secretion. Samples could be collected at home, which would have both physiological and psychological advantages. In the past, growth hormone assays lacked the sensitivity and specificity to measure accurately the concentration of growth hormone in urine, which is only approximately 0.01% of that in serum. In recent years, however, methods have improved and several immunometric methods have been described that use either enzyme or 125I tracers.

We describe the application of an immunoradiometric assay (IRMA) that uses an adapted commercially available kit, to derive a reference range for urinary growth hormone, to validate the assay, and to establish the correlation between urinary growth hormone excretion and serum response.

Patients and methods
T洎ed overnight collections of urine were obtained from three groups of children. Group 1 comprised healthy local primary schoolchildren with heights between the 3rd and 97th standard centiles. The children were from three age ranges; 3 to 4.9 years (n=16), 5.8 to 7.9 years (n=12), and 9.3 to 10.0 years (n=13). All were prepubertal, Tanner stage 1.

Group 2 comprised 14 prepubertal children in the age range 5.8 to 18.2 years who were attending the growth clinic at this hospital for treatment of growth hormone deficiency. They had been diagnosed because at least two conventional provocation tests had shown serum concentrations of less than 20 μU/l. All had normal renal function, serum was negative for growth hormone antibodies, and they had not been receiving treatment for two days.

Group 3 comprised all prepubertal children in the age range 3.7 to 14.6 years (n=49) admitted over a 15 month period for investigation of short stature by conventional provocation tests. Five of these children were highly likely to have growth hormone deficiency, as they had typical clinical features or causative features such as cranial irradiation or a cranio-pharyngioma.

Nine children in group 2 and 14 in group 3 were older than the upper limit set in defining the reference range, but all were prepubertal Tanner stage 1. Those from group 3 who were more than 10 years old were primed with sex steroids before provocation testing.

The urine samples were collected into plain plastic bottles and transported to the laboratory on the day of collection. Aliquots were taken from the measured urine volume and mixed with bovine serum albumin (1 g/l) and sodium azide (1 g/l) before storage at −20°C until assayed. A separate aliquot was taken for estimation of creatinine concentration using the Jaffe method on an autoanalyser (Technicon Ltd).

ASSAY

The assay used the reagents of the Boots-Celtech kit for estimation of serum growth hormone concentration. Before assay the urine was thawed and dialysed overnight against a phosphate/azide buffer (50 mm l/ phosphate, pH 7.0, containing sodium azide 1 g/l). The growth...
hormone was then extracted overnight on to a monoclonal antibody coupled to solid phase particles that were then centrifuged to remove excess urine. Iodinated antibody was added and after 3 to 5 hours the resulting sandwich of solid phase linked antibody, urinary growth hormone, and iodinated antibody was separated from excess label with a standard 'Sucrose' sucrose separation.

**STATISTICAL ANALYSIS**
The results were transformed logarithmically as the data were not normally distributed, and the non-parametric Wilcoxon rank sum and Mann-Whitney U tests were used to assess the significance of differences between groups. Correlation coefficients were calculated using the least squares regression analysis.

**Results**
Group 1—normal children: results were plotted for each of the three age groups with the 95% confidence intervals expressing the urinary growth hormone concentrations as total μU/night (graph A) and μU/mmol urinary creatinine (graph B) (fig 1). For clarity the y axes are logarithmic. There was no significant difference between sexes and no correlation between urinary growth hormone and height within these age bands. In graph A there was no significant difference between the three age groups (p>0.05) but when the results were expressed as μU/mmol urinary creatinine (graph B) the difference between the youngest and the oldest children was significant (0.05<p<0.01).

Expressing the results/unit time gave no better discrimination between normal and short children than the simple total μU/night. Thus these latter results were combined to provide a reference range (95% confidence intervals) for normal prepubertal children aged more than 3 years of 2.25–10.50 μU/night. This was the reference range used in the subsequent studies.

Group 2—prepubertal children with growth hormone deficiency: in all cases the results were well below the reference range and easily distinguished from it (fig 2).

Group 3—prepubertal children with short stature: results from 49 children who underwent a sleep test and a clonidine or insulin stress test, or both, are shown in fig 3. All those children who were subsequently found to have growth hormone deficiency (defined as a serum growth hormone concentration of less than 20 mU/l in response to provocation) had correspondingly low overnight urinary growth hormone concentration. This was in obvious contrast to those whose serum response was more than 20 mU/l, who all had urinary growth hormone concentrations within the reference range. The urinary growth hormone excretion in these latter children aged under 10 years was, however, significantly less (0.01>p>0.001) than that of the normal schoolchildren.

Fig 4 shows that the correlation between overnight urinary growth hormone excretion and peak serum response to either clonidine or insulin was highly significant (r=0.64, p<0.001). Again the urinary growth hormone
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Discussion

If this assay is to be a good screening test it has to fulfil certain criteria. Firstly, it should be easily applicable to the appropriate population both in terms of aquisition of samples and laboratory handling. An overnight urine collection is non-invasive and simple to make at home, and thus is ideal for children. Moreover, the results can be expressed simply as μU/overnight specimen and there seems to be no advantage in calculating the results as a ratio of time or creatinine excretion. Previously reported assays have usually expressed their results by unit of urinary creatinine to compensate for possible inaccuracy of collection,10 but our results show that this produced the only significant difference between the age groups. This fall in urinary growth hormone/mmol creatinine with age is almost certainly a consequence of the known increase in urinary creatinine with age and body mass.11 Conversely, relating urinary growth hormone to creatinine may produce falsely high concentrations in patients with hypopituitarism, because protein synthesis and creatinine excretion are reduced.

Our assay also has the important advantage of using an adaptation of a commercially available kit. Most other groups have used 'in house' monoclonal antibodies, which few NHS laboratories will have the time, resources, or expertise to produce; many, however, are fully aquiredn with Boots-Celltech kits.4 5

Secondly the method needs to be highly sensitive and specific. This assay can detect 0-50 μU of growth hormone and is valid in terms of recovery, parallelism, and precision.8

Finally, results must show good discrimination between normal and abnormal subjects. This is not the first study to show differences in urinary growth hormone excretion between children with normal and abnormal growth. Hashida et al, using a highly sensitive and specific immunoenzymatic assay, reported five patients with hypopituitarism and urinary growth hormone concentrations below their range for normal subjects.10 In deriving the latter, however, they combined pubertal and prepubertal children (as have other groups3 9) despite the known rise in serum growth hormone in puberty.12 Recently this rise has also been seen in urinary growth hormone excretion with a peak in stage 4 puberty.7 Two other groups have found the mean excretion of urinary growth hormone to be significantly greater in normal children than in those with growth hormone deficiency, but they showed up to 25% overlap between the populations.5 6 Neither of these studies used preservatives when storing the samples and one also omitted dialysis.5 Both of these steps are considered essential by us6 and others,3 13 and their omission may affect the sensitivity of the assay, producing less good discrimination especially at the lower end of the range.

The diagnosis of growth hormone deficiency is controversial and there is probably a range of growth hormone secretion, with no clearly defined cut-off between normal and abnormal. Tall children become so by secreting more growth hormone and growing faster than short children.14 This is reflected in the significantly lower, but apparently normal, urinary growth hormone excretion in the children under 10 years old who were of short stature but who did not have classical growth hormone deficiency on provocation testing (fig 3). The trend towards higher urinary growth hormone concentrations in those over 10 years old but still prepubertal, may have been the result of the priming with sex steroids. Some overlap at the bottom end of the range could be anticipated, and indeed a few of the younger children had values at the lower limit. The comparatively large number of children with low urinary growth hormone concentrations is partly explained by our study population containing several children who could be predicted to have growth hormone deficiency. Any overlap may become more obvious when further data are available for analysis, but it would not detract from the potential value of measurement of urinary growth hormone excretion as a screening test.

The positive correlation between peak serum response to provocation and overnight urinary growth hormone excretion (fig 4) confirms that the urine test may be as good as blood tests in identifying children whose growth problems are caused by a relative deficiency of growth hormone. The correlation almost exactly mirrors that in another comparison of a physiological and a pharmacological stimulus to growth hormone release, in this instance a sleep test monitored by electroencephalography with peak response to an insulin stimulation test.15 The advantages of an overnight urine collection at home need no emphasis.

The results also support the correlation between urinary growth hormone excretion rate
and mean plasma growth hormone concentration, the sum of growth hormone peaks, and the area under the growth hormone curve during overnight secretory profiles. It does not signify that a high serum concentration produced in response to powerful drugs is associated with faster or better growth. Indeed, the advent of recombinant growth hormone (Gentotropin, KabiVitrum) means that an adequate response to provocation does not preclude treatment of a slowly growing child on clinical grounds, especially if 24 hour serum sampling confirms the suspicion of ‘neurosecretory dysfunction’. Far commoner, however, in the setting of a general paediatric clinic are short children with anthropometric data indicating possible growth hormone deficiency. A simple screening test to exclude growth hormone deficiency and thus identify the minority of children who require invasive investigations and referral to a regional growth centre would be an important advance and should result in a considerable financial saving; this urinary growth hormone assay may well be that test.

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