for part of the small-for-gestational-age group, and for the rest it was thought that malnutrition was significant.

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Urinary hydroxyproline in children with growth hormone deficiency

Clinical value in diagnosis and prognosis

Although a course of exogenous human growth hormone (HGH) is known to cause a rise in the total hydroxyproline excretion of growth hormone-deficient children (see Kivirikko, 1970, for review), there have been few assessments of the value of total hydroxyproline excretion in diagnosis of growth hormone deficiency (Teller *et al.*, 1973, 3 children; Van den Brande *et al.*, 1973, 4 children), and we are not aware of any studies concerning its use in the subsequent management of these children.

The potential use of the measurement has increased recently because autoanalyser methods are available, and the total hydroxyproline:creatinine ratio (THP:Cr) in single samples of urine correlates significantly with growth velocity (Wharton, Gough, and Pennock, 1973).

This investigation was planned to study the prognostic as well as the diagnostic value of urinary THP:Cr in a further 7 growth hormone-deficient children.

Methods

Two groups of children attending the endocrine clinic at Birmingham Children's Hospital were studied.

Group 1. In this group were 7 children in whom growth hormone deficiency was suspected. Urine was collected from these children during a nitrogen retention test consisting of three equal periods; (a) baseline, (b) 3 or 5 days when the patients received HGH daily (M.R.C. Raben, batches 6-9, 10 mg nominally), (c) post-HGH period (Brown, Stimmler, and Lines, 1967). The children received a constant previously self-selected daily diet of known protein content starting 2 or 3 days before the pre-HGH period and lasting throughout the test. The dietary protein and hydroxyproline remained constant throughout the test. All urine passed was saved either in 24-hour pooled collections, starting and finishing at 10.00 a.m. daily, or as individual urine specimens. The plasma growth hormone levels after insulin hypoglycaemia (Stimmler and Brown, 1967) were measured in each child. 4 children believed to be growth hormone deficient (i.e. maximum growth hormone level below 10 μ IU/ml, a fall in nitrogen excretion during HGH greater than 30%) were given Raben HGH therapeutically for 1 year (10 IU twice weekly) and their subsequent height velocity was observed.

Group 2. In this group were 3 children receiving HGH. Random urine samples were collected from 3

growth hormone-deficient children (as defined above) before treatment and at monthly intervals during treatment with M.R.C. Raben HGH 10 IU twice weekly.

Technical methods. Urine from group 1 was stored at 20°C with acetic acid until analysis in Uganda (total hydroxyproline, Prockop and Udenfriend, 1960; creatinine, Bonsnes and Taussky, 1965). Samples from group 2 were stored at 20°C with hydrochloric acid until analysis in Bristol (hydroxyproline, Pennock, Moore, and Hoyle, 1970; creatinine, Technicon Method File N-11). Patients were measured (P.H.W.R.) using a Harpenden stadiometer and skeletal maturity was determined by the method of Tanner, Whitehouse, and Healy (1962).

Results

Group 1. Table I shows the details of the children studied and the effect of HGH on the THP:Cr ratio. After HGH the greater the reduction in urinary nitrogen (column 6) the greater was the mean increase in THP:Cr (column 12). Compared to values before the HGH course (column 10), there was a significant rise in the ratio after HGH (column 11) in the 5 children (Cases 1-5) with the greatest reduction in nitrogen excretion, but one of these children (Case 5) had normal growth hormone levels. The subsequent acceleration in length velocity of the 4 growth hormone-deficient children during one year's therapeutic course of HGH (column 14) was proportional to the increase

in THP:Cr during the initial short-term diagnostic course of HGH (column 12).

Group 2. The Fig. and Table II show the

FIG.—Effect of long-term HGH on urinary THP:Cr of 3 GH-deficient children. Shaded area shows range (10-90th centiles) for normal children of same bone age (Wharton *et al.*, 1972).

TABLE I
Effect of HGH on the urinary total hydroxyproline : creatinine ratio (THP:Cr) in 7 small children

Details of children studied							Before HGH therapy	
Cases*	Sex	Chronological age (yr)	Bone age (yr)	Diagnosis	Maximum GH after insulin (μ IU/ml)	Reduction in nitrogen excretion during HGH† (%)	No. of urine samples	Urine THP:Cr (mean $\pm$ SD)
1	F	15.3	10.0	Panhypopituitary	4	47	12	48 $\pm$ 12
2	M	11.7	12.1‡	GH TSH deficiency	9	40	11	154 $\pm$ 27
3	M	10.0	6.0	Isolated GH deficiency	6	38	11	114 $\pm$ 18
4	M	11.5	10.0	Isolated GH deficiency	1	35	10	34 $\pm$ 10
5	M	8.5	8.0	Intrauterine dwarf	28	19	13	118 $\pm$ 22
6	M	5.6	5.0	Constitutional short stature	54	10	13	132 $\pm$ 20
7	F	8.8	7.5	Constitutional short stature	112	4	5	100 $\pm$ 15
Column no.	1	2	3	4	5	6	7	8

*Cases 1-4 were regarded as GH deficient as defined by GH response to insulin hypoglycaemia and nitrogen retention during a short course of HGH.

†A-B/B $\times$ 100 where A = urinary nitrogen before HGH, B = urinary nitrogen during HGH.

‡Had received androgens previously.

§Increase in THP:Cr (column 12) correlates significantly with reduction in nitrogen excretion (column 6) Spearman rank correlation coefficient = 0.85, P < 0.01.

¶Increase in THP:Cr (column 12) correlates significantly with subsequent increase in height velocity (column 14) Spearman rank correlation coefficient = 0.85, P < 0.01.

TABLE II
Details of 3 GH-deficient children (Fig.)

Sex, chronological age (and bone age)	1. Boy 9.1 yr (4.6 yr)	2. Boy 3.6 yr (1.6 yr)	3. Girl 13.5 yr (10.9 yr)
Height velocity (cm/yr)			
Year preceding HGH	2.7	4.4	4.5
Year during HGH	7.5	11.2	7.1
Peak THP:Cr (mg/g)			
(a) Before HGH	29	129†	66
(b) During HGH	85	355	304
(c) Increase as % of (a)*	190%	170%	360%

*During one year variations of up to 100% are observed in normal children (Wharton *et al.*, 1973).

†Initial level within normal range; probably due to seasonal factors other than growth affecting collagen metabolism (Wharton *et al.*, 1973).

change in THP:Cr in urines collected at monthly intervals from growth hormone-deficient children receiving a therapeutic course of HGH. 2 patients showed a prompt rise in the urinary THP:Cr, and 1 year later a significant acceleration of height velocity was confirmed. The other patient received HGH for 4 months before the ratio rose.

Discussion

The change in THP:Cr did not differentiate completely the growth hormone-deficient children from the others; the one child with a significant rise

in THP:Cr despite normal growth hormone levels (Case 5), however, showed another substantial metabolic response to HGH, i.e. a 19% fall in nitrogen excretion. In retrospect, it might have been justified to give this child HGH therapeutically to see whether an increase in height velocity occurred, particularly since while Teller *et al.* (1973) observed a two- to eightfold increase in the 24-hour total hydroxyproline excretion of 3 growth hormone-deficient children given only one dose of HGH (2.5 mg), they also observed a twofold increase in the total hydroxyproline excretion of a primordial dwarf given a similar dose.

Since the rise in THP:Cr during the short course was related to the subsequent effect of a therapeutic course, it seems the ratio, unlike many other parameters studied (Clayton, Tanner, and Vince, 1971), may have a useful role in prognosis, but clearly larger numbers require study. The prompt rise in the ratio in 2 of the children receiving long-term growth hormone occurred well before an anthropometric response was apparent and, in the other child, a substantial rise occurred at 4 months, i.e. at an interval when anthropometric changes might still be difficult to interpret because of the normal variation in growth rate that occurs over short periods.

As a result of these preliminary observations, we feel urinary THP:Cr should be studied further in

and acceleration in height velocity during subsequent therapeutic course of growth hormone for 1 year

During 3-5 days of HGH					
No. of urine samples	Urinary THP:Cr ratio				Acceleration in growth velocity during 1-year course with HGH (cm/yr ²)
	Mean $\pm$ SD	Mean significantly higher than in pretreatment urines	Increase as % of pretreatment mean	No. of urines where ratio exceeded mean + 2SD in pretreatment urines (and %)	
10	78 $\pm$ 16	Yes P < 0.001	63	6 (60)	5.0
15	242 $\pm$ 66	Yes P < 0.001	57	14 (93)	4.2
8	134 $\pm$ 16	Yes P < 0.05	17	1 (12)	2.2
12	41 $\pm$ 8	Yes P < 0.05	20	2 (16)	3.0
9	144 $\pm$ 16	Yes P < 0.05	22	1 (11)	—
12	156 $\pm$ 40	No P < 0.10	18	1 (8)	—
9	104 $\pm$ 16	No P < 0.10	4	2 (23)	—
9	10	11	125¶	13	14¶

of HGH (see Methods).

$r_s = 0.76$, $P < 0.05$; or Pearson's correlation coefficient $r = 0.79$, $P < 0.05$.
coefficient $r_s = 1$, $P < 0.05$; or Pearson's correlation coefficient $r = 0.98$, $P < 0.02$).

children beginning growth hormone therapy in order to provide (a) confirmation of growth hormone deficiency depending on the biological response of the individual (rather than on the antigenicity of his own growth hormone), (b) some indication of the long-term response to HGH therapy, and (c) an early indication of successful treatment.

It is also possible that the change in urinary THP:Cr during 3 to 5 days of HGH might be useful in evaluating 'partial growth hormone deficiency', 'somatomedin deficiency', and also the smallest effective dose of HGH for a particular child; but these possibilities would require further evaluation.

Summary

The urinary total hydroxyproline:creatinine ratio was measured in 7 children in whom growth hormone deficiency was suspected and who were receiving a 3- to 5-day diagnostic course of growth hormone. The ratio rose significantly in the 4 growth hormone-deficient children, and this rise was proportional to their acceleration in height velocity for 1 year. One child, an intrauterine dwarf with normal growth hormone levels, also showed a significant rise in the ratio.

The ratio was also measured at regular intervals in 3 other growth hormone-deficient children receiving long-term hormone therapy. There was an early substantial rise in the ratio well before a response could be reliably detected by anthropometry.

It seems that serial determination of the ratio in any child starting growth hormone therapy should be useful since this would confirm the diagnosis, give some indication of the eventual acceleration, and would promptly detect a response to treatment. The ratio may be useful in assessing 'partial growth deficiency', 'somatomedin deficiency', and minimal requirements of growth hormone.

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