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Urinary Total Hydroxyproline: Creatinine Ratio

Range of Normal, and Clinical Application in British Children

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Wharton, B. A., Gough, G., Williams, A., Kitts, S., and Pennock, C. A. (1972). *Archives of Disease in Childhood*, **47, 74. Urinary total hydroxyproline: creatinine ratio. Range of normal, and clinical application in British children.** The total hydroxyproline : creatinine ratio has been determined in random samples of urine collected from 1577 normal Bristol children and from children with hypothyroidism, growth hormone deficiency, coeliac disease, and rickets.

The results in normal children had a profile similar to that of length velocity. When specific therapy was given to the children with growth failure there was a prompt increase in the total hydroxyproline : creatinine ratio, which occurred well before a growth spurt could be detected by anthropometry.

The ratio could be a useful chemical adjunct to anthropometry and radiology in the assessment of normal and abnormal growth. The prompt response of the ratio to changes in growth rate suggests that it may be particularly useful in studying physiological variation in growth over short periods of time and in close monitoring of treatment regimens for growth failure.

Random samples of urine may be used, automated methods of analysis are available, and other specialities find the investigation a useful one, so that for the paediatrician the urine total hydroxyproline : creatinine ratio has a potentially wide application.

Assessment of growth is an important physical sign in paediatrics. The excretion of hydroxyproline peptides in the urine is therefore of some clinical interest to paediatricians, since it is related to collagen turnover and so reflects the rate of growth in children (Jasin *et al.*, 1962; Smiley and Ziff, 1964; Zorab *et al.*, 1970) including infants (Younoszai *et al.*, 1967) and is also altered when disease of collagen-containing tissue occurs, e.g. rickets (Klein and Curtiss, 1963) and the Ehlers-Danlos syndrome (Straus and Tejaratchi, 1966).

Allison, Walker, and Smith (1966) have shown that, as a measure of growth, the ratio of hydroxyproline : creatinine in a 24-hour collection of urine is an improvement on the hydroxyproline excretion alone because it gives more consistent results for subjects of the same age, and subsequently it has been shown that the ratio determined in random samples of urine is similar to that found in a 24-hour collection (Howells, Wharton, and McCance, 1967; D. S. McLaren, personal communication; Younoszai *et al.*, 1969). The use of random samples of urine for this parameter of growth considerably increases its possible use in childhood.

Whitehead (1965) was the first person to use the hydroxyproline : creatinine ratio extensively. It was developed particularly as a screening test for marginal malnutrition (Rutishauser and Whitehead, 1969), and so most studies, including the range of normal, have been concerned with the paediatric problems of developing countries such as India (Anasuya and Rao, 1966; Mohanram *et al.*, 1969) and Uganda (Whitehead, 1967, 1969; Howells and Whitehead, 1967; Howells *et al.*, 1967; Crowne, Wharton, and McCance, 1969). Apart from a study of babies during the first week of life (Younoszai *et al.*, 1969), the use of the

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hydroxyproline : creatinine ratio in the management of children's disorders in technologically mature countries has not been examined.

This investigation was planned to establish the range of normal in an *adequate* sample of children living in this country; and to explore the clinical use of the ratio.

Method

Normal children. After obtaining the parent's consent, 1740 random samples of urine were collected from 1577 normal Bristol children as follows.

(a) *First week of life:* 206 samples from 134 babies born at Southmead Hospital, 37–41 weeks' gestation, birthweight 2721 g–3632 g.

(b) *Infancy*: 128 samples from 17 normal babies born at Southmead Hospital and subsequently visited at home.

(c) *Toddlers (1–4) years old*): single samples from 131 boys and 114 girls attending day nurseries of the City Department of Public Health and Social Service and who had been regarded as healthy on admission to the nursery.

(d) '*Schoolchildren*' (*5–18 years old*): single samples from 302 boys and 278 girls at primary schools (5–11 years) of the City Local Education Authority and from 301 boys and 300 girls (12–18 years) attending secondary schools, university, or an army medical examination. Children known to be attending hospital or to have any chronic illness were excluded.

Ill children. Random urine samples were collected from other children in the course of normal clinical practice whenever it was considered that the urinary ratio would be of interest. The results were not clinically available and so in no way affected the management of the child.

Urine samples. A 5–15 ml aliquot of each specimen was immediately transferred to bottles containing 1·0 ml 10% hydrochloric acid which after a few hours were stored at −20 °C until analysis. Total hydroxyproline concentration (i.e. free plus peptide hydroxyproline) was estimated by an autoanalyser method (Pennock, Moore, and Hoyle, 1970) on one portion of the sample following acid hydrolysis in an autoclave at 1·05 kg/cm^2 pressure for 1½ hours. Creatinine concentration was determined on another portion of the specimen by autoanalyser (Technicon Method File N-11). Results are expressed as mg hydroxyproline per g creatinine.

Results

Range in normal children. The Table shows the total hydroxyproline : creatinine ratios (THP/Cr) in random urines from children of different ages. After birth the ratio rose rapidly reaching a peak towards the end of the neonatal period and thereafter fell rapidly until school age. During the primary school years (5–11 years) the ratio showed little change but was consistently higher in girls than boys. After the age of 11 years the ratio in girls gradually fell, but in boys there was a rise to a peak at 14 years of age before a fall occurred so that, during the secondary school years (11–18 years), the ratio was consistently higher in boys than in girls.

Serial results in normal children. The Figure shows the 10th and 90th centiles of the range observed in normal children together with serial results obtained from individual normal children as follows.

Cases 1 and 2. Two girls, birthweight 3·6 kg, 41 weeks' gestation, who subsequently developed normally during the year they were observed. The THP/Cr ratio remained within the normal range throughout.

Case 3. A boy of 13 years whose height velocity during the year's observation was 11·3 cm per year (97th centile) and,

Case 4. A boy aged 13 years whose height velocity over the same period was 2·6 cm per year (below the 3rd centile). The THP/Cr shows a similar profile in both boys during the year but the ratio is consistently higher in the boy with the faster growth rate.

Serial results in children with clinical abnormalities. The figure also shows serial results from various ill children.

Case 5. A girl with cretinism who presented at 5 months because she was not smiling (height 3rd centile, weight 10th centile, bone age 5th centile, serum thyroxine 1·2 mg, 1·0 mg, 0·5 mg/100 ml). The THP/Cr was extremely low and showed little change when the child was given 12·5 μg thyroxine daily. As the dose was doubled there was a dramatic rise in the ratio to just above the 10th centile, by which time the serum thyroxine had risen to 3·6 mg/100 ml.

Case 6. A boy with isolated growth hormone deficiency; before starting treatment with growth hormone the THP/Cr was low and the child had grown 2·7 cm (below 3rd centile) during the previous year. On starting HGH treatment the THP/Cr rose dramatically within one month and a growth spurt was confirmed by anthropometry (height velocity, 7·5 cm/year; >97th centile).

Case 7. A girl with no signs of malnutrition but whose jejunal biopsy showed subtotal villous

TABLE

Total Hydroxyproline : Creatinine Ratio in Random Urines Collected from Normal Bristol Children

Age	No. Studied	Total Hydroxyproline : Creatinine Ratio				
		Highest	Centiles			Lowest
			90	50	10	
Boys and Girls combined						
0 days after birth	28	488	335	170	107	74
1	36	680	498	220	112	58
2	31	760	630	310	204	171
3	23	855	720	485	200	47
4	24	1055	1000	500	224	115
5	16	1045	866	538	368	286
6	18	1525	1000	600	348	266
7	10	1080	1080	660	324	324
8–14	14	1390	1080	826	500	408
15–21	17	2000	1480	966	666	350
22–28	17	2060	1500	926	500	500
Completed months						
1	17	2340	1800	1150	608	514
2	15	942	832	638	475	380
3	16	930	880	600	352	253
6	17	350	332	248	204	160
12–13	15	365	260	178	86	60
Age (yr)						
Boys						
1	13	360	195	128	102	94
2	43	184	157	110	65	20
3	33	176	160	96	60	54
4	42	194	124	80	54	38
5	33	186	111	66	28	25
6	62	260	124	70	33	6
7	43	138	98	67	42	37
8	33	120	107	68	36	26
9	31	140	113	65	41	24
10	34	197	104	58	39	36
11	66	152	102	66	44	28
12	56	210	123	70	45	33
13	52	182	116	81	47	15
14	47	350	141	83	42	34
15	47	178	112	59	40	10
16	28	72	65	44	18	15
17	36	82	64	34	16	10
18	35	58	39	19	7	1
Girls						
1	24	306	254	173	67	29
2	31	203	146	111	55	50
3	30	204	170	96	51	45
4	29	136	127	80	52	46
5	47	170	133	78	48	38
6	49	200	102	67	43	40
7	28	129	110	78	51	38
8	28	130	121	80	45	43
9	33	164	123	69	37	31
10	28	134	120	70	56	42
11	65	151	112	73	51	38
12	51	157	118	61	34	29
13	40	186	92	54	22	19
14	46	240	94	45	22	12
14	49	106	78	35	15	9
16	45	78	41	20	10	5
17	24	160	102	18	9	8
18	45	32	23	12	5	2

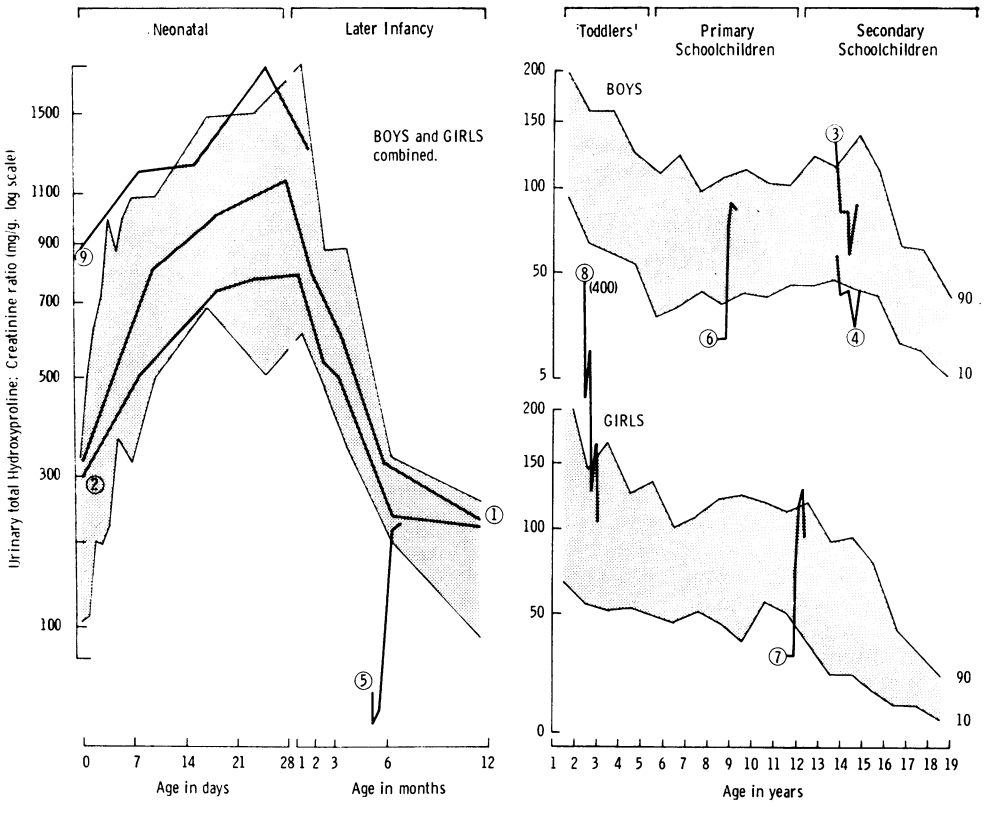

FIG.—*Total hydroxyproline : creatinine ratio in serial urines collected from normal children and those attending hospital. Cases 1 and 2: normal infants. Cases 3 and 4: normal adolescent boys. Case 5: cretinism, response to thyroxine. Case 6: hypopituitary dwarfism, response to growth hormone. Case 7: coeliac disease response to a gluten-free diet. Case 8: nutritional rickets, response to vitamin D. Case 9: pre-term baby. The shaded area shows the range (10–90 centiles) observed in normal Bristol children.*

atrophy. Introduction of a gluten-free diet was followed within 2 weeks by a substantial rise in THP/Cr, and growth during the subsequent year was 7·2 cm (75th centile) having been only 4·0 cm in the previous 2 years (<3rd centile).

Case 8. An Indian child with nutritional rickets. The THP/Cr was very high indeed but became normal on small curative doses of vitamin D. Though there was no radiological or biochemical evidence of rickets 3 months after starting therapy, the THP/Cr remained abnormally high for 3 months longer.

Case 9. A preterm baby (gestation 34 weeks, birthweight 1·5 kg) who thrived without any serious complication. THP/Cr was well above the normal range at birth and remained in the higher reaches of the normal range throughout the neonatal period.

Discussion

The THP/Cr in these Bristol children is similar to those found in smaller series of children from India (206 pre-schoolchildren, Mohanram *et al.*, 1969), from Uganda (137 pre-schoolchildren, Howells *et al.*, 1967; 195 of 9–19 years of age, Crowne *et al.*, 1969), and from Canada (14 newborn babies, Younoszai *et al.*, 1969). For most ages the numbers studied are sufficiently large for these results to form suitable standards of reference.

Developmental biology. In recent years there have been substantial reviews of the significance of urinary THP excretion, particularly from the European continent though only a few have concentrated on paediatric aspects (Kivirikko and Laitinen, 1965; Filliat *et al.*, 1965; Faglia, Norbiato,

and Tirinnanzi, 1966; Ziff, 1966; Emmrich, Häntzschel, and Häntzschel, 1967; LeRoy, 1967; van Gemund, Vio, and Giesberts, 1968). Briefly, total hydroxyproline excretion is related to the size of the individual, his growth rate, and the health of the collagen-containing tissues. Large stature, rapid growth rate, and disorders such as rickets all result in an increased THP. Variations due to stature may be allowed for by relating THP either to the surface area of the individual or to creatinine excretion. Due to the combined effects of stature and growth rate, excretion of THP over 24 hours is highest during adolescence and is as high in mature adults as in infants. When corrected for surface area, THP is greatest in infancy and falls during adolescence (see LeRoy, 1967, for review).

The figure based on a substantially larger series has shown that THP/Cr also corrects for the effects of stature and gives a profile very similar to length velocity (Tanner, Whitehouse, and Takaishi, 1966). The best evidence of a relation between THP/Cr and growth rate, however, is the clinical experience with Cases 5, 6, and 7 (Fig.), all of whom showed a rapid increase in THP/Cr and were subsequently shown to have grown at a much greater rate than previously.

It appears that if disease of collagen-containing tissue can be excluded then the THP/Cr of an individual child provides an indication of growth rate without having to correct for surface area or any other body measurement. Though direct mathematical relations between length velocity and THP have been shown, in our opinion it is probably unwise to attempt to convert an individual THP or THP/Cr result into anthropometric terms. It should rather be accepted as a chemical index of the overall growth rate. THP/Cr has the particular advantage of reflecting growth rate at *one point* in time whereas anthropometry can show only the end-point which has been reached so that calculation of anthropometric growth rates are necessarily in retrospect. In developmental studies, this may be useful for demonstrating growth rates over periods of time too short for anthropometric accuracy; for example though the two adolescent boys (Cases 3 and 4) shown on the Figure had very different length velocities during the year, the individual THP/Cr values obtained at 3-monthly intervals had the same profile of variation, i.e. higher levels at the beginning and end of the observation (summertime) and lower levels during the intervening autumn, winter, and spring. We are at present studying a larger number of boys to see whether the THP/Cr will detect seasonal variation in growth.

Clinical use. It seems probable that THP/Cr could be a useful adjunct to anthropometry and radiology as an assessment of growth in those conditions where growth charts are an essential part of diagnosis or management. The ratio *promptly* reflected the changes in growth rate, well before such a change was clinically apparent, so that its use could perhaps tend to *earlier* indication of successful treatment (e.g. Cases 5, 6, and 7), *earlier* diagnosis of treatment failure, or *earlier* and more appropriate variation in doses of hormones used for replacement therapy. It would be particularly valuable where the only easily observed signs of successful therapy are anthropometric (e.g. growth hormone deficiency, or coeliac disease in older children without gastrointestinal symptoms). However, the exact clinical use of THP/Cr in these conditions will need careful assessment in larger numbers of affected children and this is under way.

The validity of random samples increases the potential value of THP/Cr determination to the paediatrician considerably, and samples can easily be collected at home. In adults there is some diurnal variation in hydroxyproline and creatinine excretion (Mautalen, 1970), but for clinical purposes random samples from children are adequate (Howells *et al.*, 1967; Younoszai *et al.*, 1969). As a safeguard it is wise to collect more than one sample and to collect always at the same time of the day—usually mid-morning. Fortunately, colleagues in adult medicine also find THP determinations clinically useful, e.g. in the management of malignancy (Platt, Doolittle, and Hartshorn, 1964; Bonadonna *et al.*, 1966; Hosley *et al.*, 1966), bone and locomotor disease (Ziff *et al.*, 1956; Lee and Lloyd, 1964; Goidanich, Lenzi, and Silva, 1965; Le Jeune *et al.*, 1966), endocrine disorders (Benoit, Theil, and Watten, 1963), and skin disease (Brunish and Sørensen, 1965). Therefore, in a general hospital there may be sufficient demand from various specialties for the chemical pathologist to include the determination in the routine service. Autoanalyser methods are available (Grant, 1964; Pennock *et al.*, 1970).

Raised THP/Cr in children may sometimes indicate disease of collagen-containing tissues, e.g. Case 9 with rickets; such disorders are usually clinically obvious, however. If growth failure and connective tissue disease occur together there may be difficulty in interpretation of the THP/Cr; on the other hand a high value in the presence of clear growth failure may indicate connective tissue disorder which is not clinically apparent. Such a combination occurs in severe kwashiorkor and is associated with a significantly higher death rate

(Howells *et al.*, 1967; Whitehead, 1967), and we have observed a similar combination of a very high THP/Cr despite obvious growth failure in an infant with severe coeliac disease.

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