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RELATIONSHIP BETWEEN URINARY HYDROXYPROLINE AND GROWTH *

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Beginning with the report of Ziff, Kibrick, Dresner, and Gribetz (2), evidence accumulated in recent years has indicated that urinary hydroxyproline excretion reflects collagen metabolism, since collagen is the only protein in the body that contains hydroxyproline in significant amounts (3). Ziff and co-workers, observing that the excretion of this amino acid in peptide form was greater in children than in adults, suggested that the level of urinary hydroxyproline might be related to the size of the metabolically active, soluble collagen pool available for breakdown. The results of a number of investigations (4–7) have supported this suggestion.

In the present paper, further investigation of the relationship between growth and hydroxyproline excretion has been carried out in man. Urinary hydroxyproline has been determined in patients with normal, arrested, and accelerated growth, and in cases in which arrested growth has been stimulated by hormonal therapy. The data obtained indicate that the level of excretion of hydroxyproline shows a good correlation with rate of growth under all circumstances studied and, therefore, offers a useful and readily measured index of this phenomenon.

METHODS AND MATERIALS

Normal controls. Urines from normal children and adults were collected at home. In the 0- to 1-year group, the urine was collected in absorbent diapers with a special tissue paper sheet to isolate the feces. Diapers were changed six times in 24 hours. Upon removal, they were immersed in water saturated with toluene, and at the end of a 24-hour collection period, centrifuged in a washing machine and then extracted three times with more water. The pooled extract and washings had a final volume of about four liters. A suitable sample was then taken for determination of hydroxyproline. Recoveries of this amino acid, carried out by soaking diapers with a known amount of urine, averaged 97 per cent.

Hospitalized controls. Individuals with diseases in which marked alteration of collagen synthesis, breakdown, or excretion might be present were not accepted as controls. Accordingly, patients with anorexia, malnutrition, acute infection, renal disease, dystrophy, and inborn errors of connective tissue metabolism were excluded. Urines from hospitalized infants were collected by means of a metabolic bed or by use of a finger cot attached to polyethylene tubing.

In eleven hospitalized, adult control subjects, the breakdown of diseases was as follows: myocardial infarction, 1; convalescent rheumatic fever, 1; inactive pulmonary tuberculosis, 2; post-delirium tremens, 1; osteoarthritis, 2; compensated alcoholic cirrhosis, 1; hypertensive cardiovascular disease, 1; arteriosclerotic heart disease, 1; and scleroderma, 1. In 44 hospitalized children, the following conditions were represented: club foot, 4; post-poliomyelitis under rehabilitation, 14; convalescent salmonellosis, 1; convulsive disorder, 3; inactive rheumatic fever, 1; convalescent meningitis, 4; post-arsenic poisoning, 1; convalescent pneumonia, 3; pes planus, 1; cystic fibrosis, 1; scoliosis, 1; croup, 1; inactive tuberculosis, 1; bronchitis, 3; rheumatoid arthritis, 1; infectious mononucleosis, 1; cellulitis, 1; psychiatric disorder, 1; and sickle-cell anemia, 1.

A gelatin-free diet was instituted at least one day before the collection period. Urines were collected under toluene and samples stored at $-15°$ C. Unless otherwise specified, values represent averages of two consecutive 24-hour collections.

Total and free hydroxyproline were determined by the method of Prockop and Udenfriend (8) with the following modifications. To a 2-ml sample containing 2 to 10 μg of hydroxyproline, there were added 2 g of solid potassium chloride, 0.3 ml of a 10 per cent alanine solution, pH 8.7, and 0.7 ml of 0.25 N borate buffer, pH 8.7. Oxidation was carried out with 0.6 ml of 0.2 M chloramine-T in methyl Cellosolve for 20 minutes at room temperature. The reaction was stopped by addition of 2 ml of 3.6 M sodium thiosulfate. The samples were then extracted once with 3.3 ml of toluene and the toluene layer discarded. A second volume of 3.3 ml of toluene was

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then added and the mixture allowed to stand in a boiling water bath for 30 minutes. Color development was carried out by adding 0.6 ml of para-dimethylaminobenzaldehyde reagent to 1.7 ml of the toluene layer. Samples were analyzed in duplicate. Unless otherwise specified, the values given in the text represent total hydroxyproline.

Total urinary amino acids were determined by the method of Troll and Cannan (9).

Nitrogen and potassium balance. The pituitary dwarf S. B. was subjected to a 5-week balance study. The food supply was obtained in advance to assure uniformity. The diet was gelatin-free and afforded 700 kg-cal daily. Urine and feces were collected each day and frozen. Nitrogen analysis of food, urine, and feces was carried out by the combined macro-Kjeldahl and Conway diffusion techniques (10). Potassium was determined on the flame photometer with internal standards.

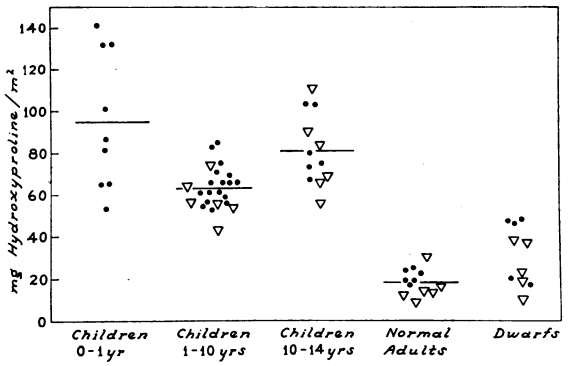

FIG. 1. DAILY HYDROXYPROLINE EXCRETION IN CHILDREN, ADULTS, AND DWARFS. Horizontal lines represent mean values, ●, males, and ▽, females.

RESULTS

Normal growth. Table I gives the daily total urinary hydroxyproline excretion observed in adult control subjects, both normal and hospitalized (Figure 1), and Table II, the corresponding values for control children. In the latter, the 24-hour excretion increased with age, but decreased again in the adult age group, where the values were similar to those of 0- to 1-year-old children. In the 10- to 14-year group, in which the factor of size in relation to the adult group was least significant, the average excretion was three times greater than in the adults.

When excretion was calculated per square meter of body surface to correct for differences in size, there was no overlap between adults and children in any age group, with results statistically significant at the 0.001 probability level for all age groups. It is interesting to note that the higher values obtained in children in the 0- to 1- and 10- to 14-year groups, when compared to the 1- to 10-

TABLE I

Daily urinary hydroxyproline in control adults

Group	Number	Body surface area	Hydroxyproline in mg		Hydroxyproline in mg/m²	
			Mean	Range	Mean	Range
		m^2				
Normal adults*	12	1.49–2.19	32.9	14.5–54.6	18.1	9.3–30.6
Hospitalized adults	11		34.7	19.3–59.1		

* One 24-hour specimen was analyzed.

TABLE II

Daily urinary hydroxyproline in control children

Group	Number	Age	Body surface area	Hydroxyproline in mg		Hydroxyproline in mg/m²	
				Mean	Range	Mean	Range
		years	m^2				
Normal *	7	0–1	0.20–0.39	32.7	17.6– 43.0	102.2	48.0–130.0
	21	1–10	0.44–1.30	49.0	14.9–150.0	66.1	41.9–145.0
Hospitalized	9	0–1	0.25–0.45	31.2	21.3– 52.3	95.1†	53.0–141.2
	23	1–10	0.49–1.24	47.7	24.0–102.2	63.1	42.5– 85.0
	12	10–14	0.99–1.64	103.7	67.5–169.0	80.5†	55.0–111.5

* One 24-hour urine specimen was analyzed.
† $p < 0.01$ with respect to excretion in the 1- to 10-year-old group, calculated by t test.

TABLE III

Daily urinary hydroxyproline in dwarfism

Diagnosis	Patient	Age	Body surface area	Hydroxyproline	
		years	m^2	*mg*	*mg/m²*
Pituitary dwarfism	S.B.	8.3	0.55	14.1	25.6
	W.R.	10.5	0.86	41.3	48.0
Cretinism	J.B.	4.3	0.53	5.5	10.4
	L.T.	0.3	0.29	5.6	19.6
	B.S.	5.3	0.70	11.8	16.9
Cockayne's syndrome (11)	C.E.	6.5	0.42	8.2	19.5
Arthrogryposis	G.L.	1.5	0.32	14.3	46.1
	B.L.	1.5	0.32	14.9	46.6
Intra-uterine runt	J.Y.	3.0	0.38	14.1	37.1
Hurler's syndrome	L.S.	3.8	0.45	17.3	38.4

year group, correspond to periods of more rapid growth.

Dwarfism. Table III lists the findings in ten children with dwarfism of various etiologies. Except for one of the pituitary dwarfs, W. R., all values are markedly decreased, both on an absolute basis and per square meter, when compared with those of children of the same age or size. The patient W. R. and the two patients with arthrogryposis, who were twins, were known to be growing slowly. As shown in Figure 1, the decreased hydroxyproline excretion values in the dwarf group approximated those of normal adults.

Effect of treatment of endocrine dwarfism on excretion

Pituitary dwarfism. (Case 1)—S. B., an 8.3-year-old white girl, had developed very slowly. On admission, height was 89.5 cm, weight, 12.4 kg, and bone age, 3.5 years. The patient was small but well proportioned. Physical examination was otherwise negative. Laboratory findings were: protein-bound iodine (PBI), 5.2 to 6.3 μg per cent (normal, 4 to 8 μg per cent); 24-hour radioactive iodine uptake (RAI), 14.9 per cent (normal, 10 to 30 per cent); urinary 17-ketosteroids, 1.1 mg per 24 hours (normal, 0.5 to 2 mg); 17-ketogenic steroids, 1.0 mg per 24 hours (normal, < 5 mg). After administration of 15 units of corticotrophin daily for 3 days, excretion of 17-ketosteroids was 2.1 mg and of 17-ketogenic steroids, 5.9 mg per 24 hours. The diagnosis was pituitary dwarfism, presumed to be due to isolated growth hormone deficiency.

The patient was placed on a controlled, gelatin-free diet, and after 2 weeks of weight stabilization, 2.5 mg of human growth hormone [1] was administered intramuscularly 6 days a week, for 4 weeks. At the end of treatment, the patient had gained 700 g and grown 1.5 cm. Potassium and nitrogen balance studies indicated an immediate increase in retention of these elements, more pronounced in the first week of treatment.

Figure 2 shows the levels of total urinary hydroxyproline in the periods before, during, and for three weeks after administration of growth hormone. Of note are the consistently low values, averaging 14.1 mg daily, observed during the control period, and the prompt rise in excretion after the start of treatment. At the end of the treatment period, the patient had achieved a urinary hydroxyproline level of 63.5 mg in 24 hours. The calculated value of 115.5 mg per m^2 corresponds to a level attained by children only during periods of rapid growth (see Table II). That this increase was not due to a nonspecific rise in urinary amino acids is shown by the fact that these increased only 50 per cent (see Figure 2), while hydroxyproline rose more than fourfold. Three weeks after growth hormone administration was discontinued, hydroxyproline values dropped to control levels. Free hydroxyproline of the urine

[1] We thank the Endocrinology Study Section, N.I.H., for generous supplies of this hormone.

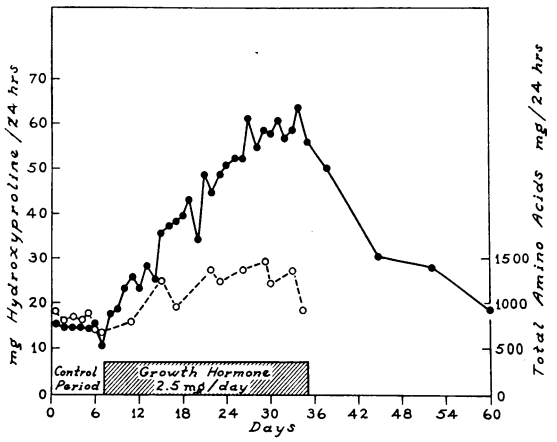

FIG. 2. EFFECT OF GROWTH HORMONE ON EXCRETION OF HYDROXYPROLINE AND TOTAL AMINO ACIDS IN PITUITARY DWARFISM (CASE 1, S. B., ♀, 8.3 YEARS). ●——●, represents hydroxyproline, and ○- - -○, total urinary amino acids.

remained unchanged during treatment, making up 1 to 3 per cent of the total.

(Case 2)—W. R., a 10.5-year-old white boy grew normally during the first year of life. Beginning at 14 months of age, he had a number of hospital admissions because of convulsions associated with hypoglycemia. At 3.7 years, treatment with 10 mg of hydrocortisone daily was begun and continued intermittently until 4 weeks before admission, when steroid treatment was discontinued. On admission, height was 106.8 cm and weight, 30.0 kg. Growth rate during the 3 years before admission had been half the normal rate. At 7.5 years, RAI uptake was 28.1 per cent, and urinary 17-ketosteroids, 2.3 mg per 24 hours. Laboratory findings on admission were: urinary 17-hydroxy-corticosteroid excretion, 1.4 to 1.8 mg per 24 hours (normal, 2 to 8 mg); urinary 11-desoxy-corticosteroids (normal, 0 to 2 mg per 24 hours); and plasma 11-desoxy-corticosteroids (normal, 0 to 2.5 μg per cent), undetectable. After oral administration of 1 g of methopyrapone (SU-4885, Ciba) in 4 divided doses over a 24-hour period, urinary 17-hydroxy-corticosteroid excretion was 3.0 mg per 24 hours; urinary 11-desoxy-corticosteroids, 0.9 to 1.5 mg per 24 hours (normal, > 8 mg); and plasma 11-desoxy-corticosteroids, 1.0 μg. per cent (normal, > 8 μg per cent). After administration of 15 units of corticotrophin gel daily for 3 days, urinary 17-hydroxy-corticosteroid excretion was 7.4 mg per 24 hours. The diagnosis was pituitary dwarfism with combined growth hormone and adrenocorticotrophin deficiency.

After a 2-week control period on a gelatin-free diet, 2.5 mg of human growth hormone was administered intramuscularly, 6 days a week, for 4 weeks. At the end of this period, the patient had gained 900 g and grown 1.2 cm.

Daily excretion of hydroxyproline averaged 41.3 mg before administration of growth hormone (Figure 3). The peak values reached during the treatment period were three times greater. Three weeks after the hormone was discontinued, excretion had fallen to near that of the control period. The hydroxyproline values of 100 to 155 mg per m² attained during the treatment period corresponded to those of very actively growing children.

Cretinism. (Case 3)—J. B., a 4.3-year-old white girl, grew normally until age 2, when growth appeared to stop. On admission, physical findings were typical of severe hypothyroidism. Height was 88.5 cm and weight, 11.7 kg. Laboratory findings: PBI, 1.1 μg per cent; RAI uptake, 4.2 per cent; and RAI uptake after administration of 5 units of thyrotrophin daily for 3 days, 1.1 per cent. The diagnosis was primary hypothyroidism. Nine weeks after the start of treatment with desiccated thyroid, symptoms had disappeared, and the patient had grown 4.0 cm.

From a very low value of 5.5 mg per 24 hours, the excretion of hydroxyproline had risen 17-fold after 10 weeks of treatment (Figure 4). The value of 180 mg per m² is greater than the high-

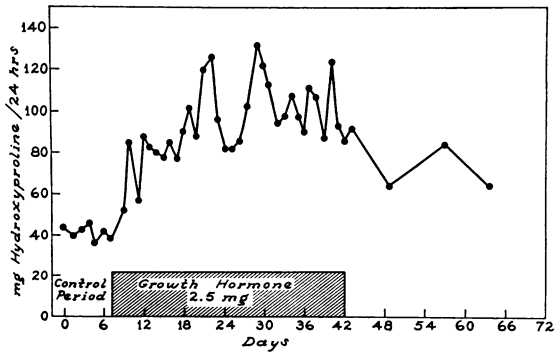

FIG. 3. EFFECT OF GROWTH HORMONE ON EXCRETION OF HYDROXYPROLINE IN PITUITARY DWARFISM (CASE 2, W. R., ♂, 10.5 YEARS).

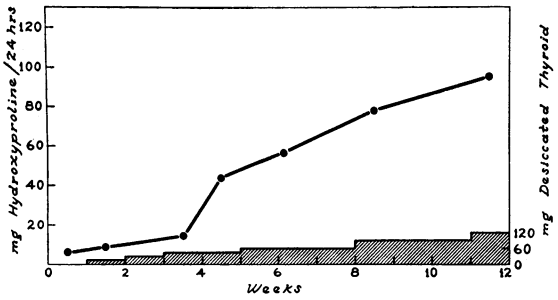

FIG. 4. EFFECT OF DESICCATED THYROID ON EXCRETION OF HYDROXYPROLINE IN PRIMARY HYPOTHYROIDISM (CASE 3, J. B., ♀, 4.3 YEARS).

est observed in the entire childhood control group (see Table II).

(Case 4)—L. T., a 4-month-old white girl, was admitted because of decreased physical activity and peculiar facies. Physical examination showed characteristic findings of severe hypothyroidism. PBI was 1.0 μg per cent. The diagnosis was congenital cretinism. Desiccated thyroid was administered in a daily dosage of 15 mg for 2 weeks, 30 mg for 2 weeks, 45 mg for 2 weeks, and 60 mg thereafter. After 7 weeks of treatment, her length was 65.0 cm and weight, 6.9 kg.

After 8 weeks of treatment (Table IV), hydroxyproline excretion had increased from 5.6 mg to 47.5 mg. The latter value expressed per square meter was 167.0 mg, and is markedly elevated when compared with the corresponding control group.

(Case 5)—B. S., a 5.3-year-old white boy, had received desiccated thyroid for symptoms of hypothyroidism intermittently since age 2. Eight months before admission, treatment was interrupted. On admission, height was 94.0 cm, weight, 21.0 kg, and bone age, 1.5 years. Serum PBI was 1.7 μg per cent and cholesterol, 454 mg per cent. After treatment with thyroid extract was resumed, the symptoms improved promptly, and 10 weeks later the patient had grown 2.25 cm. The diagnosis was cretinism.

TABLE IV

Daily urinary hydroxyproline in cretinism treated with desiccated thyroid (L. T., ♀, age 4 months)

Weeks of treatment	Hydroxyproline	
	mg	*mg/m²*
0	5.6	19.6
1	15.6	55.0
8	47.5	167.0

Urinary hydroxyproline excretion averaged 11.8 mg per 24 hours before treatment. Thyroid extract was administered in dosages from 15 increasing to 150 mg daily. At the end of 8 weeks, the excretion had risen to 71.7 mg, or 93.0 mg per m², a value characteristic of the excretion of actively growing children.

Acromegaly. (Case 6)—T. B., a 43-year-old white man, was admitted because of unremitting headaches of 4 years' and muscle weakness of 3 years' duration. During the preceding 9 years, he had noticed gradual enlargement of the nose and fingers. Physical examination showed typical acromegalic features. Pertinent laboratory laboratory findings were: FBS, 109 mg per cent; serum phosphorus, 3.9 to 4.6 mg per cent; PBI, 5.4 μg per cent; and RAI uptake, 15.1 per cent in 24 hours. Glucose tolerance curve was of the diabetic type. X-ray findings were characteristic of acromegaly.

The patient was treated with irradiation over the sella turcica, with a 3,200-roentgen tumor dose over a 1-month period.

The mean value of urinary hydroxyproline observed before irradiation, 107 mg per 24 hours, or 52.0 mg per m², is well above the highest level

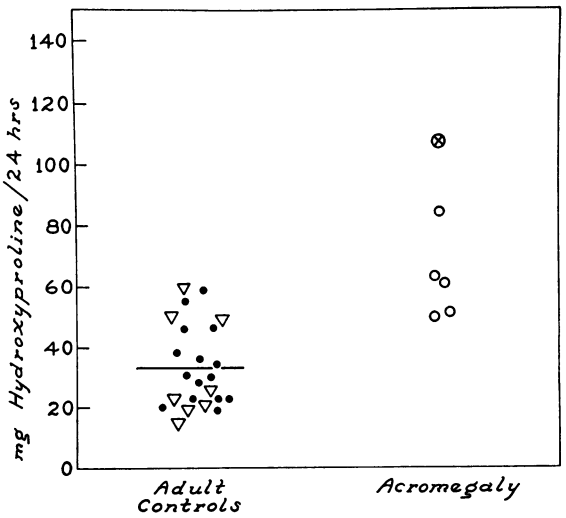

FIG. 5. URINARY HYDROXYPROLINE IN PATIENTS WITH ACTIVE AND INACTIVE ACROMEGALY AND IN ADULT CONTROL SUBJECTS. Horizontal lines represent mean values; ⊗, male with active acromegaly; ○, males with inactive acromegaly; ●, control males; and ▽, control females.

of excretion found in the group of normal adults (Figure 5). Three months after the course of radiation therapy ended, the excretion had decreased to 58.3 mg, or 28.3 mg per m^2, and a similar value of 60.8 mg, or 29.5 mg per m^2, was obtained 6 months later. Both post-treatment values were in the upper range of normal. Free urinary hydroxyproline in the pretreatment urines represented 1 to 2 per cent of the total.

Five additional patients with the diagnosis of acromegaly were studied in whom there was no evidence of clinical activity. One had an elevated total excretion of 84.1 mg per 24 hours, and four showed excretion values in the upper range of normal (see Figure 5).

DISCUSSION

Growth in childhood expressed in increment of body surface area per year is especially rapid in the first year of life and above age 10 for girls and 12 for boys, and relatively slower in the intervening period (12).[2] Accordingly, the excretion data in children representing the combined values of both sexes were grouped in 0- to 1-, 1- to 10- and 10- to 14-year age groups. Significant differences in absolute excretion between children and adults were noted only in the 10- to 14-year group, where size difference is less marked. When excretion was calculated per unit surface area, however, to correct for the influence of size, significant elevation above the adult average was noted in all childhood age groups. Surface area was chosen as a parameter of size because it was thought to be a more reliable index of connective tissue mass than body weight.

The excretion values per square meter given in Table II are significantly greater in the 0- to 1- and 10- to 14-year groups than in the 1- to 10-year group, indicating a correlation between hydroxyproline excretion per unit surface area and growth rate, since, as mentioned above, growth is known to be more rapid in these two age periods. These findings suggest that when sufficient data are available, it will be possible to relate excretion per unit surface area to the normal growth curve.

All ten children with dwarfism showed low excretion of hydroxyproline. On an absolute basis, the values in nine of these fell between 5.5 and 17.3 mg, or below the range of the control values for normal children. Seven of the ten had low values per unit surface area, and the remaining three, who were known to be growing slowly, had borderline values. The low excretion observed in non-growing or slowly growing children is further evidence for a relationship between hydroxyproline excretion and growth.

Most impressive was the rise in excretion of hydroxyproline following hormonal treatment of non-growing endocrine dwarfs. When growth hormone was administered to two pituitary dwarfs and desiccated thyroid to three cretins, excretion of hydroxyproline rose dramatically in all cases, and this was accompanied by objective evidence of growth. All five dwarfs attained levels of excretion that were characteristic of actively growing children. In the case of two of the cretins, the values calculated per square meter were the highest ever noted by this laboratory in any age group. When, in the case of the two pituitary dwarfs, administration of growth hormone was halted, excretion returned to pretreatment values within 3 weeks.

Further evidence of a relationship between hydroxyproline excretion and growth was seen in the markedly elevated values observed in the patient with active acromegaly. Before treatment, these were three times the adult average; 3 months after irradiation, they had fallen to near normal levels. The data in five patients with clinically inactive acromegaly were more difficult to interpret. Excretion ranged between 49.7 and 84.1 mg, that is, from above normal to the upper limits of normal. Whether this indicates residual activity, undetectable by the usual criteria, is not clear.

Any mechanism attempting to explain the observed relationship between growth rate and excretion of hydroxyproline ought to take into consideration the presence of increased amounts of soluble collagen in the tissues of growing animals (13–16), presumably as a consequence of an increased rate of synthesis of this protein. This fraction has a high turnover rate and is the precursor of fibrous collagen. It is also known that urinary hydroxyproline is increased in young animals as compared with adults (5, 6). These con-

[2] Values for the fiftieth percentile of height and weight in normal children (12) were used to calculate surface area.

siderations, as well as the results reported in this paper, agree with an earlier suggestion (2) that the increased excretion associated with growth represents peptides derived from soluble collagen.

Data recently obtained in this laboratory in rats with osteolathyrism (7) also support the point of view above. These animals had increased amounts of neutral salt-extractable collagen in the skin and excreted urinary hydroxyproline at elevated levels without gross differences in growth rate from control animals. This finding indicates that hydroxyproline peptide excretion rises with an increase in soluble collagen even when the latter change occurs as a result of factors other than growth. In this connection, an increased excretion of hydroxyproline has recently been reported in patients with Paget's disease and hyperparathyroidism (17), conditions in which the rate of formation of new collagen in bone is presumably increased.

Although the arguments presented strongly suggest that the increased excretion during growth depends on an increase in soluble collagen, it is difficult to evaluate the contribution of the fibrous collagen to urinary hydroxyproline. Although this fraction turns over very slowly (18), it is present in large amounts. Lindstedt and Prockop (5) have recently demonstrated a urinary hydroxyproline fraction in adult rats with a turnover rate of about 300 days, which they suggested was derived from the fibrous collagen pool. It is possible, therefore, that fibrous collagen does contribute to urinary hydroxyproline, and since there is little soluble collagen in adult tissue (19), the contribution from mature fiber would be proportionately greater in this group than in children.

In considering other possible explanations for the increased excretion of hyroxyproline during growth, one cannot rule out the possibility that renal factors may play a part in the phenomena described, since it is known that growth hormone (20, 21) and thyroid hormone (20, 22) may influence renal function. Against this possibility, however, is the well documented evidence that renal clearance and glomerular filtration rate expressed per unit surface area, in contrast to hydroxyproline excretion, are low in the first year of life and shortly thereafter attain adult levels that remain constant from then on (20, 23). Also, it may be pointed out, total amino acid excretion, mainly in the form of peptides (24), rose much less than hydroxyproline excretion when measured in one of the pituitary dwarfs. Finally, the increased excretion noted in osteolathyrism is evidence against a renal factor, since hormonal influences capable of affecting renal function were presumably not involved in the lathyritic process.

SUMMARY

Total hydroxyproline excretion has been determined in normal and hospitalized adults and children, in children with dwarfism, and in patients with acromegaly.

Total excretion was significantly greater in children in the 10- to 14-year group than in adults. When calculated per unit body surface area, it was greater in all childhood age groups. Peak levels of excretion per square meter coincided with periods of active growth.

Children with dwarfism showed decreased excretion of hydroxyproline. Administration of growth hormone to two children with pituitary dwarfism and of thyroid hormone to three children with cretinism resulted in a prompt rise in excretion, which attained levels characteristic of rapidly growing children. After cessation of growth hormone administration, excretion fell to pretreatment levels.

One patient with active acromegaly excreted markedly increased amounts of hydroxyproline. After irradiation therapy, these fell toward normal. Five patients with clinically inactive acromegaly showed levels elevated in one case and otherwise in the upper range of normal.

We conclude that there is a close relationship between rate of growth and urinary hydroxyproline excretion, and suggest that the increased excretion is a result of the presence during growth of an increased amount of metabolically active, soluble collagen in the tissues, which is available for breakdown. The results indicate that measurement of urinary hydroxyproline may provide a useful index of growth activity.

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