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URINARY HYDROXYPROLINE EXCRETION IN ORTHOPEDIC DISEASE

PRELIMINARY REPORT

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The significance of urinary hydroxyproline has not been completely clarified. It appears, however, that this amino acid excretion is correlated with either synthesis or destruction of collagen. Collagen hydroxyproline is reported to be formed by the irreversible hydroxylation of peptid-linked proline and no free hydroxyproline is incorporated directly into this protein. Therefore, the changes of collagen in the tissues can be indirectly measured by determining urinary hydroxyproline excretion. Although hydroxyproline has been variously studied concerning its significance in physiology and distribution in the tissues, metabolic study of this amino acid has been limited.

Then, urinary hydroxyproline excretion in various orthopedic diseases was investigated. The following observations have been made. Patients with severe bone lesion such as that arising from malignant bone tumor and primary parathyroid adenoma as well as those with congenital systemic bone disease were found in this study to excrete significantly increased amounts of hydroxyproline in the urine. This observation that hydroxyproline excretion is elevated in destructive bone tumor, in primary hyperparathyroidism, in rats receiving parathyroid extract, and in congenital systemic bone disease supports the view that the urinary hydroxyproline reflects the rapid rate of bone collagen degradation. At any rate, urinary hydroxyproline appears to be a good index of metabolic activity in bone diseases.

Most of the studies on connective tissue have been histological, since the metabolic activity in this tissue is believed to be lower than in other tissues. Recent introduction of the collagen disease concept, however, has provoked biochemical studies of this tissue. New information has been gained on such vital physiologic processes as wound healing, calcification and aging of the bone and immune reaction. Utilization of electron microscope, X-ray diffraction and isotope technique has produced clarification of the physical and chemical properties of collagen, the main constituent of connective tissue[1)-8)]. Amino acid analysis of collagen has disclosed that glycine, proline and hydroxyproline constitute one quarter of the total amino acid content of collagen.

Hydroxyproline, which constitutes approximately 13 per cent of amino acid

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in collagen, is almost absent in the composition of other tissue protein with the exception of gelatine.

Collagen hydroxyproline is reported to be formed by the irreversible hydroxylation of peptid-linked proline and no free hydroxyproline is incorporated directly into this protein[9)-11)]. Therefore, the changes of collagen in the tissues can be indirectly measured by determining urinary hydroxyproline excretion. Although hydroxyproline has been variously studied concerning its significance in physiology and amounts in the tissues, metabolic study of this amino acid has been limited.

The present authors[12)-24)] have been engaged in the investigation of metabolism in bone tissue and aging process of the bone and joint. Since 1962 metabolism of hydroxyproline has been studied in various orthopedic diseases[25)-27)].

The following observations have been made.

I. CLINICAL STUDIES

A) Methods: In the early stage of the present study determination of hydroxyproline was performed according to the method described by Neuman and Logan[10)]. Later, however, the method of Prockop and Udenfriend[28)] was employed since this achieved better recovery rate. During the forty-eight hours prior to experiment all patients were placed on a diet free from fish, gelatin, icecream, candy and animal skins. In the following twenty-four hours total urine was collected while the first urine in the morning was discarded. The urine was collected under toluene in a glass container and refrigerated. Hydroxyproline is excreted in human urine in either free or bound form. The free form constitutes only 4 per cent of total hydroxyproline, and the remaining can be released from its bound form (polypeptide) by acid hydrolysis at 126°F for three hours. Several determinations at different intervals were done on the same patient.

B) Results: Normal Values

The mean urinary hydroxyproline excretion found in 14 normal adults was 29.4 ± 3.2 mg/24 hr (bound) and 1.20 ± 0.1 mg/24 hr (free), whereas the mean for 10 normal children between 5 and 10 years of age was 63.2 ± 3.4 mg/24 hr (bound) and 2.08 ± 0.12 mg/24 hr (free). The high urinary excretion observed in children is probably due to increased collagen formation in the growing organism. As it is found that the free form of hydroxyproline is only 4 per cent of the total and it does not show significant variation from the normal in this study, only the value of bound form is given in the following table.

Diseased States

In Table 1 total urinary hydroxyproline excretion in orthopedic diseases in adult patients is shown. Four patients with malignant bone tumor including 3 osteogenic sarcoma and 1 metastatic carcinoma demonstrated significantly

TABLE 1. Comparison of Hydroxyproline Excretion in Controls, and in Patients with Various Bone Disease (Adults)

Group	No. of Subjects	No. of Analyses	Hydroxyproline Excretion	
			Range mg/24 hr	Mean s. d mg/24 hr
Controls (Adults)	14	52	16.0- 36.0	29.4± 3.2
Malignant bone tumor	4		129.9-343.2	168.2± 9.2
Osteosarcoma	3	195	149.2-343.2	234.0±11.3
Multiple myeloma	1	13	129.9-246.0	148.4± 8.2
Benign bone tumor	3		56.8-271.2	126.9± 6.2
Bone cyst	1	5	56.8-158.2	98.2± 6.8
Eosinophilic granuloma	1	9	98.8-271.2	134.0± 7.0
Giant cell tumor	1	6	67.6-135.2	101.4± 5.9
Osteomyelitis	6	70	49.9-140.4	99.0± 3.7
Spinal tuberculosis	7	80	41.6-125.5	86.5± 4.3
Before operation	4		125.5-118.6	120.3± 1.2
After operation	4		88.4- 41.6	64.2± 4.6
Pulmonary tuberculosis	10	50	23.4- 36.8	32.5± 3.2
Rheumatoid arthritis	7	70	36.4- 98.2	67.5± 3.8
Fractures	7	90	17.3- 34.2	30.2± 2.6
Osteoporosis	6	60	18.6- 32.2	28.9± 2.6
Controls (Children)	10	30	28.3-103.0	63.2± 3.4

increased excretion, the mean value being 168.2±9.2 mg/24 hr. An 18-year-old boy with osteogenic sarcoma excreted 120 mg/24 hr, when the lesion was radiologically localized, whereas he excreted 330 mg in 24 hours 12 weeks later when the lesion had advanced. The mean urinary excretion in 3 patients with benign bone tumor was 126.9±6.2 mg/24 hr. Highest excretion was noted in a case of eosinophilic granuloma with 134.0±7.0 mg/24 hr. In a case of giant cell tumor the excretion was 101.4±5.9 mg/24 hr and 98.2±6.8 mg 24 hr in an instance of bone cyst. Patients with infection of the bone were found to excrete hydroxyproline at about 3 times the normal level. In 6 osteomyelitis patients the mean value was 99.0±3.7 mg/24 hr. In rheumatoid arthritis in which increased hydroxyproline excretion has been noted by other authors[29], the excretion was about 2 times the normal level, the mean for 7 instances being 67.5±3.8 mg/24 hr. Excretion in patients with fracture or osteoporosis was within normal limits.

In the children, 3 cases with congenital systemic bone disease including 1 Hurler syndrome showed significantly increased excretion, the mean being 180.0 ±8.6 mg/24 hr (Table 2). One child with primary hyperparathyroidism, in whom a rickets like lesion of the metaphysis of long bones was evidenced radiologically, excreted maximum 431.6 mg/24 hr. This was reduced to 50 mg/24 hr 5 weeks after surgical removal of the adenoma and has remained within normal limits since. Laboratory data in this patient were as follows: Serum Ca 8.38 meq/l, P 3.5 mg/dl, HCO^{-}_{3} 25 meq/l, alkaline phosphatase 29.7 Bod. U., Daily urinary output 2,000-6,000 ml (specific gravity 1,002-1,004, Ca 259.5 mg/day (mean), 1,000 mg/day (maximum)). Parathyroid function: PI 0.93%, TRP 83.5%.

TABLE 2. Comparison of Hydroxyproline Excretion in Controls, and in Patients with Various Bone Diseases (Children)

Group	No. of Subjects	No. of Analyses	Hydroxyproline Excretion	
			Range mg/24 hr	Mean s. d mg/24 hr
Controls (Children)	10	30	28.3-103.0	63.2±3.4
Hurler syndrome	1	6	135.6-228.3	190.2±8.3
Primary hyperparathyroidism				
Before operation	1		431.6-160.0	293.2±6.3
After operation	1		81.0- 44.2	58.1±3.4
Legg-Calvé-Perthes disease	3	40	28.6-123.4	84.7±4.3
Fractures	3	40	28.8- 86.2	68.2±2.6
Prog. musc. dystrophy	1	10	32.2- 68.4	56.3±3.7

Following removal of the adenoma, serum calcium level fell to 3.15 meq/l and this was normalized within 5 weeks postoperatively. Calcium in the urine was also normalized to 100-200 mg/day level. Alkaline phosphatase began to fall 14 weeks postoperatively and has remained on 15 Bod. U. level since. Radiologic evidence of improvement was first suggested in the 8th postoperative week and within 20 weeks postoperatively the bone was radiologically normal. Osteotomy for bilateral coxa vara was additionally performed on this patient 13 and 17 weeks respectively after removal of the adenoma. This patient made an uneventful recovery from the renewed intervention and excellent bone fusion in the osteotomy site was evidenced.

Patients with severe bone lesion such as that arising from malignant bone tumor and primary parathyroid adenoma as well as those with congenital systemic bone disease were found in this study to excrete significantly increased amounts of hydroxyproline in the urine. These findings suggest a probable correlation between urinary hydroxyproline and metabolism of bone tissue. Metabolic disorder and collagen destruction may influence urinary excretion of this amino acid.

II. EXPERIMENTAL STUDIES

The observation made on a patient with primary hyperparathyroidism exhibiting significantly augmented urinary hydroxyproline excretion was further studied in animals.

A) Methods: Female rats weighing 60 to 70 g received 50 or 100 units of parathyroid extract daily subcutaneously. Both experimental and control rats were maintained a 19 per cent casein diet during a 5 week observation period.

B) Results: Urinary total hydroxyproline excretion is shown in Table 3. An increase of hydroxyproline excretion was first noted at the 4th week in rats receiving 50 units of parathyroid extract daily, whereas in the rats receiving 100 units daily an increase was noted as early as the 2nd week. His-

TABLE 3. Hydroxyproline Excretion in Rats injected with Parathyroid Extract

Groups ↓ Weeks→	1	2	3	4	5
Controls	0.35±0.11	0.28±0.02	0.27±0.06	0.25±0.01	0.41±0.08
Extract injected (50 U.S.P. Units)	0.11±0.03	0.23±0.04	0.21±0.03	0.75±0.06	0.81±0.05
Extract injected (100 U.S.P. Units)	0.37±0.04	0.81±0.07	0.87±0.09	1.45±0.16	1.34±0.10

tologically, rats given 100 units of parathyroid extract daily for 5 weeks demonstrated narrowing of epiphyseal cartilage plate, fibrosis of the bone marrow, and bone absorption with augmented osteoclasts. It was found that the changes in the bone correlated with the increase in total urinary hydroxyproline excretion.

DISCUSSION

The significance of urinary hydroxyproline has not been completely clarified. It appears, however, that this amino acid excretion is correlated with either synthesis or destruction of collagen or with both. Using hydroxyproline-N^{15} Stetten[11] has found in animal experiment that hydroxyproline is derived not from dietary protein but from hydroxylation of body protein. Thus collagen hydroxyproline may be produced by irreversible hydroxylation of peptide-linked proline. The source of urinary peptide hydroxyproline (bound) should be sought in collagen or in its precursor. Ingestion of gelatin in large amount augments hydroxyproline excretion whereas ingestion of free hydroxyproline or free proline does not produce increased excretion of this amino acid[30)31)]. It was found by preceeding workers[32)33)] that increased urinary hydroxyproline excretion occurred in association with abnormal bone metabolism as in acromegaly, hyperthyroidism and in Paget's disease. Their findings may support an hypothesis that urinary hydroxyproline excretion reflects the rapid rate of bone collagen degradation.

Prockop and Sjoerdsma, and Sjoerdsma, Davidson, Udenfriend and Mitoma[30)34)] found augmented excretion of urinary hydroxyproline in patients with Marfan syndrome. The present study demonstrated significantly increased hydroxyproline excretion in congenital systemic bone disease including Hurler syndrome. It is reported that hydroxyproline excretion is augmented following administration of growth or thyroid hormone, substances known to promote bone matrix formation or bone maturation[35)]. This finding suggests that the variation in urinary hydroxyproline can be a significant index reflecting metabolic activity of bone matrix in various diseases.

The augmented excretion in growing children well agrees with the observation in growing animals of an increase in tissue soluble collagen or fresh precursor of collagen. This is also supported by the report of Kao and Mc

Gavack[36] who found increased hydroxyproline excretion when there was increased tissue soluble collagen.

The present observation of augmented urinary hydroxyproline excretion in a patient with primary hyperparathyroidism as well as in rats receiving parathyroid extract is apparently of significance, since it suggests that this hormone affects bone collagen in a destructive manner.

The pressnt study further disclosed that urinary hydroxyproline excretion is significantly increased in patients with malignant bone tumor, in whom active bone destruction occurs. To date there has been only a related report by Platt, Dolitle and Hartshorn[37] who found augmented hydroxyproline excretion in patients with metastatic carcinoma of bone. Similarly an increased excretion is noted in patients with such bone infection as osteomyelitis and spinal tuberculosis.

The increased hydroxyproline excretion during bone infection as reported in this paper appears to arise from bone destruction and not from infection, since in patients with pulmonary tuberculosis there was no significant change in hydroxyproline excretion.

It seems probable that increased urinary hydroxyproline excretion in cases with bone destruction is correlated with bone collagen breakdown. However, the mechanism of collagen breakdown is not clear, nor is there evidence of collagenase presenting in man. Whether the urinary bound hydroxyproline is related to collagen formation or to mature collagen degradation remains to be clarified. The observation that hydroxyproline excretion is elevated in destructive bone tumor, in primary hyperparathyroidism, in rats receiving parathyroid extract, and in congenital systemic bone disease supports the view that the urinary hydroxyproline reflects the rapid rate of bone collagen degradation. On the other hand increased excretion in a case of acromegaly[31], hyperthyroidism, following administration of growth or thyroid hormone, and in children is indicative of its relationship with protein synthesis. Furthermore, increased excretion in Paget's disease as observed by other authors[30][34] suggests that here both collagen destruction and collagen synthesis are involved. At any rate, urinary hydroxyproline appears to be a good index of metabolic activity in bone diseases.

Urinary hydroxyproline excretion in other disorders is currently being investigated by the present authors. Further study of hydroxypyoline excretion in bone tumor patients is also underway to investigate its relationship with tumor type, influence of surgical and radiologic treatment, symptoms and prognosis.

SUMMARY

Urinary hydroxyproline excretion in various orthopedic diseases was investigated.

The following observations have been made.

1) Marked increase in hydroxyproline excretion was noted in patients with malignant bone tumor.

2) In a child with primary hyperparathyroidism accompanied by severe bone lesion significantly elevated hydroxyproline excretion is observed. There was rapid decrease in hydroxyproline excretion following removal of the adenoma returning to the normal level with symptomatic improvement. In rats receiving parathyroid extract urinary hydroxyproline excretion was increased. The degree of increase agrees with the dose and duration of hormone injection and changes in bone tissue.

3) In patients with osteomyelitis and spinal tuberculosis hydroxyproline excretion was elevated. The amount of excretion correlates positively with the degree of bone destruction and postoperative new bone formation.

4) In abnormal metabolic bone diseases such as Hurler syndrome, hydroxyproline excretion is significantly increased.

These observations suggest the following conclusion.

Increased urinary hydroxyproline excretion in chidren, patients with acromegaly and hyperthyroidism as well as in those receiving growth or thyroid hormone which promotes collagen formation, obviously resulted from enhanced collagen synthesis. On the other hand, collagen destruction appears to be responsible for increased excretion in patients with malignant bone tumor, hyperparathyroidism and in rats receiving parathyroid extract. Elevated urinary hydroxyproline excretion in patients with Paget's disease is reported by other authors. These combined findings suggest that urinary hydroxyproline excretion is influenced by both collagen synthesis and destruction. Further detailed investigation is needed.

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