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URINARY HYDROXYPROLINE EXCRETION IN ORTHOPEDIC DISEASE, WITH SPECIAL REFERENCE TO SYSTEMIC BONE DISEASE AND BONE TUMOR (SECONDARY REPORT)

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It has been gradually recognised that urinary hydroxyproline might be a good index of collagen break-down or turnover, but the details have not been completely clarified. The following three points are discussed in this report; Variations of urinary total hydroxyproline excretion (1) in the human aging process, (2) in patients with growth disturbance of bone, and (3) in patients with malignant bone tumor, with observations made on the urine specimens of 134 normal healthy subjects consisting of 73 males and 61 females and their values classified by age and sex. The highest excretion was shown in the group of 10 to 14 years of age, with statistical significances noted between the highest excretion group and that of 6 to 9 years age group, as well as between the former and the group of 15 to 19 years of age. In patients with systemic bone disease lower excretion was observed in pituitary dwarfism, hypothyroidism, osteogenesis imperfecta. However, in cases with positively advanced bone age, excretion was elevated as in adrenogenital syndrome and pituitary dwarfism during therapy. It has been proved that there occurs an extremely increased excretion of hydroxyproline in patients with malignant bone tumor, especially in osteogenic sarcoma, and the changes in excretion were observed to parallel the clinical progress of the patient after surgical intervention. In the experimental study using Walker carcinosarcoma 256 urinary excretion markedly increased with aggravation of bone lesion and after amputation no elevated excretion was observed. As reported in this paper the change in urinary hydroxyproline could be a good index of bone collagen metabolism, showing elevated excretion in growing children, decrease in patients with hormonal growth disturbances and alteration in those with bone tumor or systemic bone disease.

Metabolic study of collagen, an important organic component of bone and cartilage, has been limitted for a long time on account of lack of specific methods for representation of tissue collagen, in spite of a number of detailed

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studies on mineral metabolism.

In recent years the physical and chemical properties of collagen have been distinctly explained by various methods. Hydroxyproline, which constitutes approximately 13 per cent of total amino acids in tissue collagen is almost absent in the composition of other tissue proteins with the exception of gelatine, and plays a significant role in the stability of the collagen macromolecule. Collagen hydroxyproline is said to be formed by the irreversible hydroxylation of peptide-linked proline and no free hydroxyproline is incorporated directly into this protein. Hence, the changes of collagen in tissues can be directly measured by determining urinary hydroxyproline excretion, and the highly important significance of this imino acid in collagen break-down or turnover is gradually being recognized.

In references concerning the body composition of the normal adult it has been reported that the total collagen content in the human adult is approximately 3,500 g, of which 57 per cent (about 2,000 g) are bone collagen, 34 per cent (about 1,200 g) skin collagen, and 9 per cent (about 300 g) other tissue collagen[1)2)]. Moreover it had been proved by isotopic technique that metabolic activity of bone collagen is several times greater than that of skin collagen[3)4)], so that it might be said that at least 70–80 per cent of the total urinary hydroxyproline are derived from bone collagen.

With the above facts in mind a continuous study was made on patients with various orthopedic diseases, as the present author and co-workers have found that urinary hydroxyproline excretion was increased in patients with severe bone lesions such as malignant bone tumor and in primary hyperparathyroid adenoma as well as in those with congenital systemic bone disease[5)]. In this report the following three points are discussed; namely, variations of urinary total hydroxyproline excretion in (1) the human aging process, (2) in patients with growth disturbance of bone, and (3) in patients with bone tumor.

A) CLINICAL STUDIES

a) Materials and Methods:

Twenty-four hour urine specimens were collected under toluene in a glass container and refrigerated. All subjects were placed on a diet free from gelatin containing foods 48 hrs prior to the experiment. The urinary total hydroxyproline content was measured by the colorimetric method of Prockop, D. J. and Udenfriend, S.[7)] No modification was used. Hydroxyproline is excreted in the urine almost entirely in the form of peptides, only 3 per cent of the total hydroxyproline being found as free amino acid. The values were expressed in mean values of duplicate methods or several determinations at different intervals. The urine of 134 normal healthy subjects consisting of 73 males and 61 females were

TABLE 1. Urinary Total Hydroxyproline Excretion in Normal Subjects Classified by Age

Age	Sex	No. of Cases	Total Hydroxyproline Excretion		
			Range (mg/24 hr)	Mean±S.D. (mg/24 hr)	Classified by Age Group Mean±S.D. (mg/24 hr)
−12 mo.	m	3	25.3-30.2	28.0± 3.1	28.0±3.1
	f	2	25.0-32.4		
1 yr.					30.5±12.0
2 yr.	m	1	13.0	14.5	
	f	1	16.0		
3 yr.	m	3	21.7-28.6	24.0± 4.7	
	f	2	17.2-24.5		
4 yr.	m	1	22.8-	27.4± 7.7	
	f	2	23.0-37.4		
5 yr.	m	4	23.2-29.9	38.7±11.9	
	f	5	39.8-55.2		
6 yr.	m	6	32.2-57.6	48.7±12.7	59.0±15.8*
	f	4	33.5-71.4		
7 yr.	m	2	68.1-75.0	71.6	
	f	0			
8 yr.	m	2	60.3-65.0	59.2± 6.7	
	f	3	51.8-65.6		
9 yr.	m	5	37.8-71.3	68.7±17.0	
	f	3	62.3-98.2		
10 yr.	m	2	74.2-90.6	87.6±17.3	87.0±19.2*
	f	2	76.5-108.9		
11 yr.	m	4	69.4-112.0	84.2±15.4	
	f	2	78.4-78.8		
12 yr.	m	7	52.2-127.6	91.2±27.2	
	f	3	79.4-97.5		
13 yr.	m	4	70.8-162.2	99.9±29.9	
	f	5	65.1-128.0		
14 yr.	m	2	93.7-113.3	103.5	
	f	0			
15-19 yr.	m	4	29.6-57.8	45.0± 7.3	45.0±7.3*
	f	5	35.1-59.0		
20-29 yr.	m	5	24.8-38.2	24.4± 4.9	
	f	7	14.3-27.8		
30-39 yr.	m	4	11.8-27.0	22.0± 5.8	
	f	5	19.2-33.9		

TABLE 1 (*Continued*)

Age	Sex	No. of Cases	Total Hydroxyproline Excretion		
			Range (mg/24 hr)	Mean±S.D. (mg/24 hr)	Classified by Age Group Mean±S.D. (mg/24 hr)
40-49 yr.	m	3	20.5-47.7	24.5±8.0	
	f	3	17.3-21.4		
50-59 yr.	m	4	25.7-38.6	25.5±4.6	23.5±7.6
	f	3	17.5-23.6		
60 yr.-	m	5	9.4-35.7	21.2±7.9	
	f	4	10.3-31.2		
Total		134			

* Between the highest excretion group of 10 to 14 year-old and the group of 6 to 9 year-old, and between the highests and the group of 15 to 19 year-old, statistical significances existed ($p \leqq 0.01$).

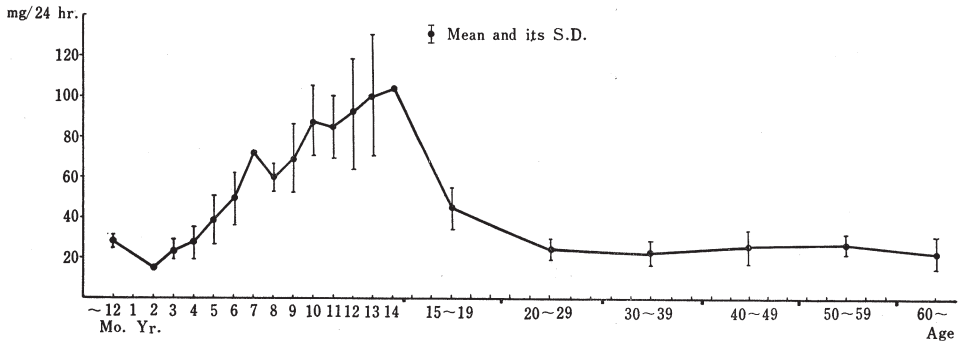

FIG. 1. Urinary total hydroxyproline excretion in normal subjects classified by age.
Between mean values of 9 and 10 year old cases there was about 27.5 per cent increase; and between 14 and 15 year old cases approximately 50 per cent decrease. There was no significant difference in normal values classified by decade over 20 year old adults.

examined and their values classified by age and sex (Table 1, Fig. 1).

In the patients with malignant bone tumor whose urine was measured continuously, the limitation of gelatin free diet was not always assured.

b) Results:

Normal Values.

Urinary total hydroxyproline excretion classified by age showed the highest value in the group of 10 to 14 years of age, and its mean value was 87.0 ± 19.2 mg/24 hr (Table 1, Fig. 1). In this group the values of the 13 and 14 years old were much higher. Nine subjects of the 13 year old (4 males, 5 females)

had the mean value of 99.9 mg/24 hr (S.D. ± 29.9, with a range of 65.1 to 162.2), and 2 subjects 14 years old (both males) a mean of 103.5 mg/24 hr. From 2 to 14 years of age, the excretion increased with age, but decreased after 15 years of age. The mean value of children 9 years of age was 68.7 ± 17.0 mg/24 hr and that of 10 years of age 87.6 ± 17.3 mg/24 hr. An increase of 27.5 per cent was noted between the 9 and 10 year old groups. The mean value of the 14 years old was 103.5 mg/24 hr and that of the 15 years old 51.7 ± 8.1 mg/24 hr. A decrease of approximately 50 per cent was noted between the 14 and 15 year old groups. The changes of urinary excretion in male and female subjects showed no significant difference (Fig. 2).

From these observations the urinary excretion in normal healthy subjects was classified according to the following age groups; 0 to 12 months, 1 to 5, 6 to 9, 10 to 14, 15 to 19 and over 20 years of age. The highest excretion was found in the group of 10 to 14 years of age, with a value of 87.0 mg/24 hr (S.D. ± 19.2, with a range of 52.2 to 162.2), in the group of 6 to 9 years of age 59.0 mg/24 hr (S.D. ± 15.8, with a range of 32.2 to 98.2), in the group of 15 to 19 years of age 45.0 mg/24 hr (S.D. ± 7.3, with a range of 29.6 to 59.0), and in the group of over 20 years of age 23.5 mg/24 hr (S.D. ± 7.6, with a range of 9.4 to 47.7). Statistical significance at 0.01 probability level was noted between the highest excretion group of 10 to 14 years of age and that of 6 to 9 years of age, as also between the former and the group of 15 to 19 years of age. In subjects over 20 years of age there were no significant variations in the mean values and their standard deviations classified by decade (Table 1, Fig. 1).

There are several studies concerning the relationship between aging process and urinary hydroxyproline excretion, such as those of Jones, C. R., Bergman, M. W., Kittner, R. J. and Pigman, W. W.[8]; Lenzi, F., Ravenni, G., Rubegni,

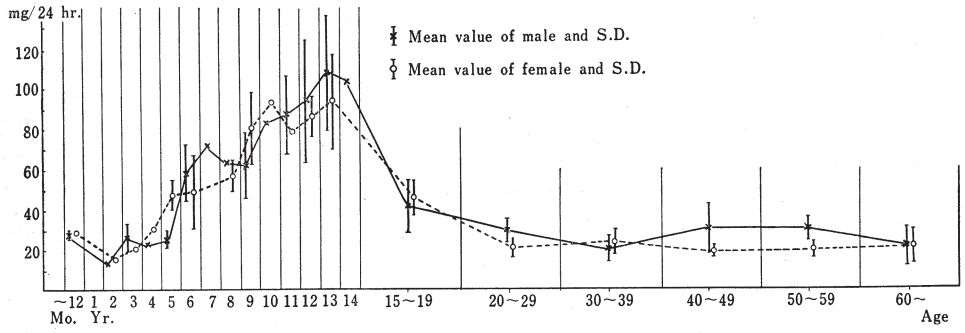

FIG. 2. Urinary total hydroxyproline excretion in normal subjects classified by age and sex.

Urinary total hydroxyproline excretion of males and females according to aging. No significant difference was observed between them.

M. and L. Del. Govane[9]; and Jasin, H. E., Fink, C. W., Wise, W. and Ziff, M.[10] In these reports the control values are shown grossly for two groups of adults and children compared with the values in disease or in experimental conditions. The changes in excretion are not shown according to age or age group. As high urinary excretion of hydroxyproline in children reflects increased collagen formation, it is believed that the age of most active collagen metabolism in normal subjects is 13 or 14 years of age. In relation to this Jones, C. R., Bergman, M. W., Kittner, R. J. and Pigman, W. W. reported it to be 14 years of age[8] and Jasin, H. E., Fink, C. W., Wise, W. and Ziff, M. in the group of 10 to 14 years of age[10]. In the group of over 20 years of age urinary excretion showed no significant variations and its mean value was 23.5 ± 7.6 mg/24 hr.

Diseased States.

I) Patients with Systemic Bone Disease

Studies of urinary hydroxyproline excretion in patients with systemic bone disease have been reported by Sjoerdsma, A., Davidson, J D., Udenfriend, S. and Mitoma, C. (1958)[11]; Jasin, H. E., Fink, C. W., Wise, W. and Ziff, M. (1962)[10]; Keiser, H. R., Gill, J. R., Sjoerdsma, A. and Bartter, F. C. (1963)[12]; Dull, T. A. and Henneman, P. H. (1963)[13]; Benoit, F. L., Theil, G. B. and Watten, R. H. (1963)[14]; Nakagawa, M. and Tamaki, T. (1965)[5]; and Kivirikko, K. I., Leitinen, O. and Lamberg, B. A. (1965)[15]. The present authors studied the urinary excretion in 58 patients with systemic bone disease and compared the values with excretion of normal subjects classified by age (Table 2, Fig. 3).

(1) Pituitary dwarfism

The mean excretion of 4 cases between 6 and 9 years of age (3 males, 1 female) was 28.9 ± 18.5 mg/24 hr, and in 3 cases (all males) 21.8 ± 11.0 mg/24 hr. These values were lower than the mean values or the lower limits of standard deviation (59.0 ± 15.8 mg/24 hr) for normal subjects of the same age group.

A high excretion of 134.0 mg/24 hr was observed in Case 6, a 12 year old girl, height 129.6 cm, body weight 34.0 kg. The urine of this patient was examined during anabolic and thyroid hormone therapy. With the exception of Case 6 described above, the others in the group of 10 to 14 years of age showed the mean value of 38.6 ± 8.2 mg/24 hr which was lower than the mean value of 87.0 ± 19.2 mg/24 hr for the same age of the control group.

Another high excretion was observed in Case 12, a female 22 years of age. The value was 60.5 mg/24 hr, and her height was 138.5 cm, showing only 70 per cent growth in height of normal adult, and a bone age of 15 years. This was the only case in which urinary excretion was higher than that of normal

TABLE 2. Urinary Total Hydroxyproline Excretion in Patients with Systemic Bone Disease

Diagnosis	Case No.	Patient	Age	Sex	Total Hydroxyproline Excretion
			years		mg/24 hr
1. Pituitary dwarfism	1	S.E.	6	m	40.6
	2	A.S.	7	m	29.1
	3	K.K.	7	f	2.7
	4	Y.S.	9	m	43.0
	5	M.G.	10	m	29.3
	6	T.M.	12	f	134.0
	7	S.M.	13	m	41.9
	8	A.T.	14	f	44.6
	9	M.O.	16	m	23.1
	10	S.T.	16	m	32.1
	11	M.S.	18	m	10.3
	12	S.K.	22	f	60.5
2. Hypothyroidism	13	I.S.	5	m	15.0
3. Turner's syndrome	14	T.I.	10	f	10.3
	15	A.K.	14	f	71.7
4. Adrenogenital syndrome	16	T.Y.	5	f	80.6
	17	M.A.	8	f	126.2
	18	K.H.	9	f	91.9
	19	H.S.	10	m	34.1
	20	M.S.	11	m	38.2
5. Cushing's syndrome	21	T.T.	41	m	20.3
6. Marfan's syndrome	22	Y.U.	13	f	54.5
	23	N.M.	13	f	20.6
	24	T.U.	17	m	97.3
	25	Y.F.	18	f	41.0
7. Osteogenesis imperfecta	26	M.M.	6	m	25.7
	27	K.U.	7	m	35.4
8. Hurler's syndrome	28	A.H.	12	f	30.9
	29	A.F.	13	m	108.7
9. Polytope enchondral dysostosis	30	T.O.	3	m	40.3
	31	K.O.	5	f	37 8
	32	T.Y.	5	f	31.2
	33	K.M.	6	m	51.0
	34	C.I.	7	f	5.7
	35	M.H.	8	f	15.4
	36	K.Y.	10	f	19.9
	37	T.U.	15	m	141.8
	38	T.N.	17	m	59.7
	39	S.N.	31	m	22.7
10. Chondrodystrophia foetalis	40	M.K.	8	f	73.1
11. Multiple exostosis	41	T.M.	6	f	37.6
	42	M.Y.	6	f	76.7
	43	T.N.	7	m	62.4
	44	T.Y.	8	m	41.4
	45	K.K.	8	m	37.7
	46	K.A.	10	f	136.0
	47	H.M.	11	m	71.4
	48	M.T.	11	f	8.9
	49	I.T.	13	m	61.9
	50	Y.O.	13	f	11.2
	51	H.I.	14	m	6.2
	52	M M.	16	m	61.6
	53	S.W.	18	m	52.5
	54	K.M.	19	m	50.8
	55	T.H.	22	m	27.2
	56	M.M.	25	m	32.8
	57	G.M.	33	m	23.5
	58	Y.T.	41	f	3.9

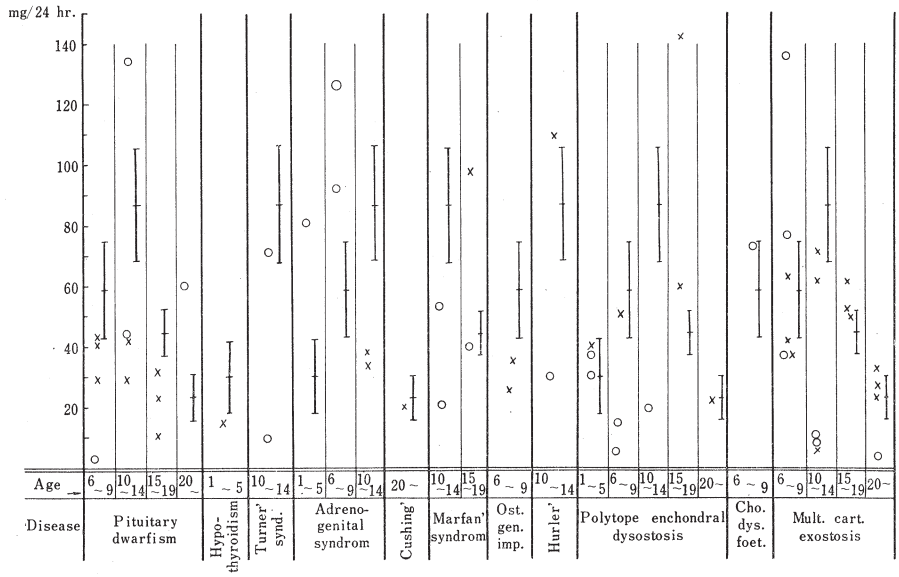

FIG. 3. Urinary total hydroxyproline excretion in patients with systemic bone disease.
×—male
○—female
—mean value and its s.d. in normal subjects classified by age group

subjects of the same age.

A summary of the above mentioned 10 of 12 cases, excepting Cases 6 and 12, revealed average values lower than those of corresponding ages of the control group.

(2) Hypothyroidism

In this 5 year old boy the urinary excretion was 15.0 mg/24 hr lower than the value of 38.7 ± 11.9 mg/24 hr for the control group of the same age.

(3) Turner's Syndrome

Case 15 was a 14 years old girl, 115.0 cm high, weight 22.5 kg, with bone age approximately 11 years of age. The urinary excretion 71.7 mg/24 hr coincided with the lower limit of the control value for the same age group. In Case 14, a 10 year old girl. height 111.6 cm, weight 19.6 kg, the value was 10.3 mg/24 hr and lower than that of the control.

(4) Adrenogenital Syndrome

With the exception of 2 cases in the group of 10 to 14 years of age, 3 cases showed much higher excretion than in the control groups. Brothers of 10 and 11 years of age (Cases 19 and 20) had bone ages of 15 years old. Their excretions became low during predonisolon therapy. Cases excreting higher values

without therapy had bone ages of 3 to 5 years greater than their chronological ages.

(5) Cushing's Syndrome

The case was a 41 year old male without therapy. Excretion was 20.3 mg/24 hr and was within normal range.

(6) Marfan's Syndrome

It has been reported by Sjoerdsma, A., Davidson, J. D., Udenfriend, S. and Mitoma, C. that elevated excretion occurs in some cases of Marfan's syndrome[11]. One of 4 cases, Case 24, a 17 year old boy, had higher excretion of 97.3 mg/24 hr. An 18 year old girl (Case 25) excreted 41.0 mg/24 hr which was within normal mean value. The remaining 2 cases (Cases 22 and 23) 13 year old girls, excreted 54.5 and 20.6 mg/24 hr respectively which were lower than the mean value of 99.9 ± 29.9 mg/24 hr of the control group of the same year of age.

(7) Osteogenesis imperfecta

The cases were 6 and 7 year old boys. Urinary excretions were 25.7 and 35.4 mg/24 hr respectively. These values were lower than the mean values of the same age group of controls, 48.7 ± 12.7 mg/24 hr (control of 6 yr.) and 71.6 mg/24 hr (control of 7 yr.).

(8) Hurler's Syndrome

The excretion in a 13 year old boy (Case 29) was 108.7 mg/24 hr, and was greater than the 87.0 ± 19.2 mg/24 hr for the same age group. However, it was within the upper limit (99.9 ± 29.9 mg/24 hr) of the mean value for the same age subjects. In the other case, a 12 year old girl (Case 28), the excretion was 30.9 mg/24 hr and was lower than that of the control group of the same age.

(9) Polytope Enchondral Dysostosis

Two of 10 cases excreted more than the controls (Cases 37 and 38), 5 within normal limit (Cases 30, 31, 32, 33 and 39), and the remaining 3 cases lower than the controls (Cases 34, 35 and 36). Patients with higher excretion were Case 37, 15 year old boy, and Case 38, 17 year old boy, with values of 141.8 mg/24 hr and 59.7 mg/24 hr respectively. These two patients appeared to have more marked muscle spasticity than the others.

(10) Chondrodystrophia Foetalis

An 8 year old girl, height 94 cm, showed excretion of 73.1 mg/24 hr which was within normal limits (Case 40).

(11) Multiple exostosis

Five of 18 cases excreted more than the controls (Cases 42, 46, 52, 53 and

56), 5 within normal limits (Cases 43, 47, 54, 55 and 57) and the remaining 8 lower than the controls.

Patients showing a lower bone age than their chronological ages, as in hormonal growth disturbance such as pituitary dwarfism, hypothyroidism, and Turner's syndrome, showed lower urinary hydroxyproline excretion than the control, already reported[10)13)].

In cases of pituitary dwarfism under growth hormone therapy it has been reported that with development of growth a corresponding increase of urinary hydroxyproline excretion occurs. We also observed increased excretion of 134.0 mg/24 hr in a case of pituitary dwarfism treated with thyroid extract. In contrast to the above, in cases of adrenogenital syndrome where growth is accelerated with endogenous hormonal effects, the urinary excretions were markedly elevated and the bone ages were 3 to 5 years greater than of the chronological ages.

However 2 cases, Cases 19 and 20, under corticoid therapy sohwed lower values, of 34.1 and 38.2 mg/24 hr than that of the control. A case of Cushing's syndrome showed excretion within normal limits. In the cases of Marfan's syndrome, one showed higher excretion, but the other 3 cases were within normal limits or showed lower values than the control. Hurler's syndrome (Case 29) showed a slightlye levated value when compared with the control. In the cases of polytope enchondral dysostosis, cases excreting more than the control were observed in 2 with muscle spasticity.

II) Patients with Bone Tumor

The urinary total hydroxyproline excretion in patients with bone tumor is

TABLE 3. Urinary Total Hydroxyproline Excretion in Patients with Bone Tumor

Group	No. of Cases	No. of Analysis	Total Hydroxyproline Excretion	
			Range mg/24 hr	Mean±S.D. mg/24 hr
Malignant Bone Tumor				
Osteogenic Sarcoma	10	58	7.5-276.0	80.9±19.1
Before Treatment	6	29	9.6-207.8	100.1±59.4
After Amputation	5	12	10.2-146.9	83.6±24.1
Died within a Year	3	12	21.0-172.0	58.7±47.5
Survival for>5 Years	2	10	22.4- 45.2	28.4± 6.0
Chondrosarcoma	2	17	16.8-107.5	58.3± 6.2
Metastatic Carcinoma of Bone	26	66	10.1-292.0	45.7± 5.9
Carcinoma of Soft Tissue without Bone Lesion	13	29	12.0- 40.0	26 0± 9.2
Multiple Myeloma	1	5	21.6- 81.0	41.8± 8.0
Benign Bone Tumor				
Giant Cell Tumor	5	25	13.3- 84.0	35.1± 6.3
Fibrous Dysplasia	2	10	14.0- 82.6	75.7±10.7
Bone Cyst	4		21.3- 54.9	38.8± 7.4

shown in Table 3.

The malignant bone tumor cases consisted of 10 osteogenic sarcomas, 3 males and 7 females. There were 2 cases each, aged 12, 15 and 16 years, and one case each, aged 19, 20, 25 and 31 years. Two of these cases survived more than 5 years after surgical intervention.

Most elevated excretion showed a value of 100.1 mg/24 hr (S.D. ± 59.4, with a range of 9.6 to 207.8) before amputation of the osteogenic sarcoma. In comparing these values with the controls of the same age, all excepting Case 5 showed greater excretion (Fig. 4). In Case 5 the initial examination showed a very low value of 10.4 mg/24 hr but after bone lesion advancement urinary excretion increased to 35.2 mg/24 hr.

Two cases of chondrosarcoma were secondary chondrosarcoma in a 33 year old male and a 34 year old female, and both showed malignant alteration at the pelvis from hereditary multiple exostosis. The excretion was 58.3 mg/24 hr (S.D. ± 6.2, with a range of 16.8 to 107.5) which was more than twice the value of 22.0 mg/24 hr (S.D. ± 5.8, with a range of 11.8 to 33.9) for the same decade of the normal adult.

Cases of metastatic carcinoma in the skeleton were all over 30 years old. The mean value was 45.7 mg/24 hr (S.D. ± 5.9, with a range of 10.1 to 292.0)

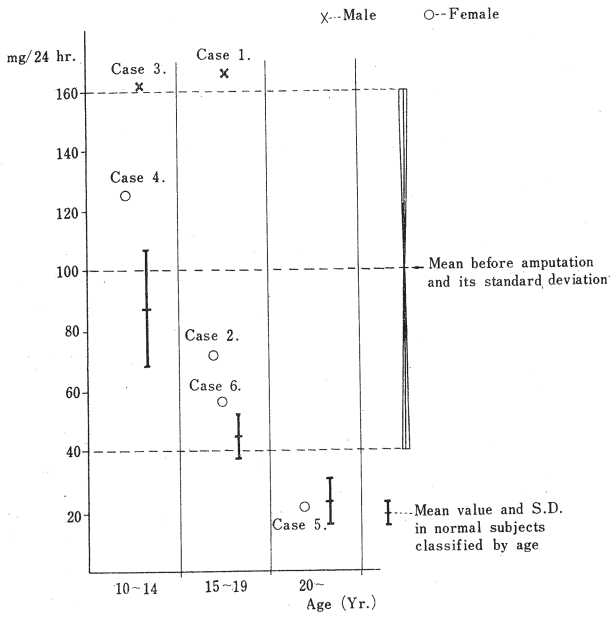

FIG. 4. Urinary total hydroxyproline excretion.
Before amputation of patients with osteogenic sarcoma, compared with normal subjects classified by age.

which was about twice the value of 23.5 mg/24 hr (S.D. ± 7.6, with a range of 9.4 to 47.7) for the control adult. However, the excretion in soft tissue carcinoma without bone lesion was 26.0 mg/24 hr (S.D. ± 9.2, with a range of 12.0 to 40.0) and within normal limits.

In the case of multiple myeloma in a 44 year old female the excretion was 41.8 ± 8.0 mg/24 hr.

Patients with malignant bone tumor showed greater excretion of this amino acid than in the controls classified by age.

In the group of benign bone tumors 2 cases each of giant cell tumor were observed in the 2nd and 3rd decades respectively, and one in the 4th decade; they were 3 males and 2 females. The mean excretion was 35.1 mg/24 hr (S.D. ± 6.3, with a range of 13.3 to 84.0).

In 2 cases of fibrous dysplasia, both had polyostotic bone lesion; they were 30 and 13 years old. The mean excretion was 75.7 mg/24 hr (S.D. ± 10.7, with a range of 14.0 to 82.6). In case of the 13 year old boy excretion was within normal limits. In the cases of bone cyst 2 cases were in the 2nd decade (1 male and 1 female), one was a 15 year old boy, and the other a 2 year old child.

In patients with benign bone tumor elevated excretion was also observed but was lower than in malignant bone tumor.

i) Relation between urinary total hydroxyproline excretion and surgical treatment of malignant bone tumor

The mean excretion after amputation in 5 cases with osteogenic sarcoma was 83.6 mg/24 hr (S.D. ± 24.1, with a range of 10.2 to 146.9) and lower than the 100.1 mg/24 hr before operation. As the processes of excretion appeared to be variable from case to case, each case will be described individually.

(Case 1) H. K. 17 year old boy, osteogenic sarcoma. An osteolytic lesion was found roentgenologically at the upper portion of the right tibia. The patient was admitted on Dec. 23, 1965. On Dec. 28, a biopsy was done and treated with arterial infusion of Mitomycine C for 3 consecutive days. On Jan. 7, 1966, amputation was accomplished at the distal portion of the right thigh. At the present time, 6 months after surgical treatment, a lung metastasis has been found in the chest roentgen film. The mean excretion value before treatment showed a very high excretion of 165.4 mg/24 hr (S.D.±14.2, with a range of 120.0 to 184.7) as compared with the mean value of 45.0 mg/24 hr (S.D.±7.3, with a range of 29.6 to 59.0) in the controls of the same age. After surgery the excretion showed a tendency to decrease, and a month after surgery the mean became 93.5 mg/24 hr (S.D.±12.1, with a range of 44.5 to 135.6) (Fig. 5).

(Case 2) M. K. 16 year old girl. Osteogenic sarcoma. Roentgenological finding showed a mixed form of lesion at the lower portion of the right femur. Biopsy was done on Jan. 8, 1966, and treatment with arterial infusion of Mitomycine C for 3 consecutive days was carried out. On Jan. 21, amputation was accomplished. The excretion before treatment showed a very high excretion of 71.3 mg/24 hr (S.D.±10.5, with a range of

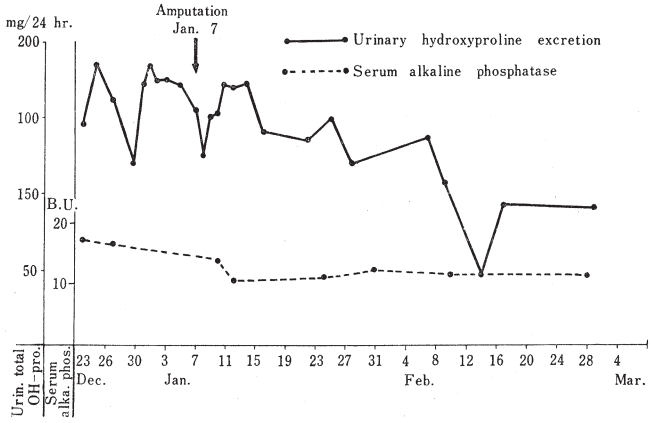

FIG. 5. Figure 5 Shows a decreasing type of urinary total hydroxyproline excretion in Case 1. H. K. a 17 year old boy with osteogenic sarcoma of the upper portion of the right tibia, originally mis-diagnosed as osteomyelitis. The roentgen finding showed osteolytic bone destruction.

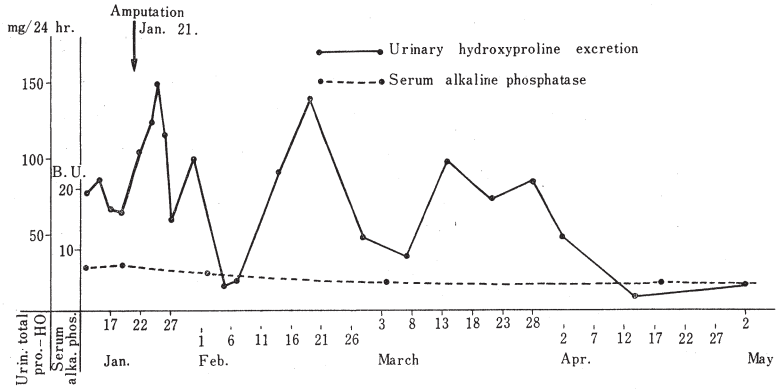

FIG. 6. Case 2. M. K. 16 year old girl, with osteogenic sarcoma of the lower portion of the right femur, a mixed form bone destruction was found roentgenelogically. This figure shows the decreasing type as observed in Case 1. The progress after surgical treatment was good, and 8 months after amputation no lung metastasis was found. The values of serum alkaline phosphatase were not elevated at the beginning, but after operation they were decreased.

64.8 to 87.0) when compared with the mean for the same age of the controls. After surgery the excretion tended to decrease and the mean 4 months after surgery was 35.3 mg/24 hr (S.D. $\pm$25.3, with a range of 9.3 to 62.7) (Fig. 6).

(Case 3) Y. O. 12 year old boy. Osteogenic sarcoma. Sclerotic bone lesion was found in the radiogram at the lower portion of the left femur. For 3 consecutive days from April 27, 1965, arterial infusion with Mitomycine C was made and on April 30,

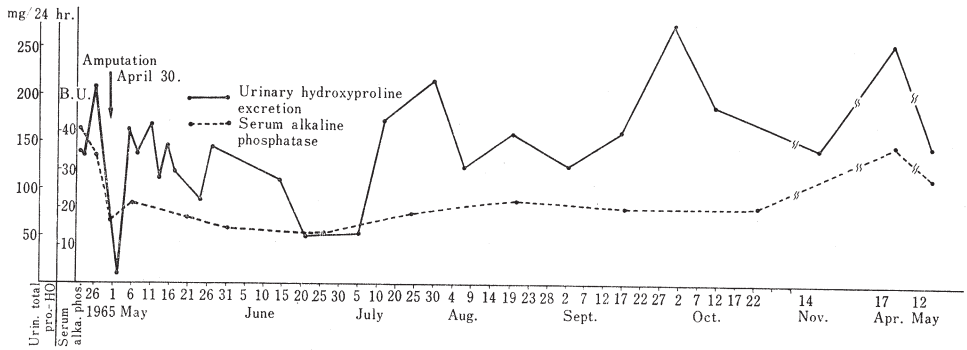

FIG. 7. This figure shows the excretion process in Case 3, 12 year old boy with osteogenic sarcoma. Roentgen showed sclerotic bone destruction. The mean of hydroxyproline excretion was 161.3 mg/24 hr before surgery, decreasing in about 2 months after treatment. Five months after surgery, the excretion increased with development of lung metastasis. The patient is alive but under ^{60}Co radiation therapy.

amputation was accomplished. The mean excretion before surgery was 161.3 mg/24 hr (S.D.±25.6, with a range of 139 8 to 207.8), and showed about 78 per cent increase as compared with the mean of 91.2 mg/24 hr (S.D.±27.2, with a range of 52.2 to 97.5) for the same age. However, 2 months after surgery excretion gradually decreased but after about 5 months following surgery the excretion showed a tendency to increase, and 8 months after surgery lung metastasis was found roentgenologically (Fig. 7).

(Case 4) S. Ts. 12 year old girl. Osteogenic sarcoma. Sclerotic bone lesion was roentgenologically found at the lower end of the left femur. From Jan. 11, 1966 arterial infusion was made for 3 consecutive days and on Jan. 14, amputation was accomplished. Several weeks after surgery lung metastases were found. Four months after surgery metastasis in the pelvis was also found and the child died 7 months after surgical treatment.

In the process of urinary hydroxyproline excretion the mean before surgery was

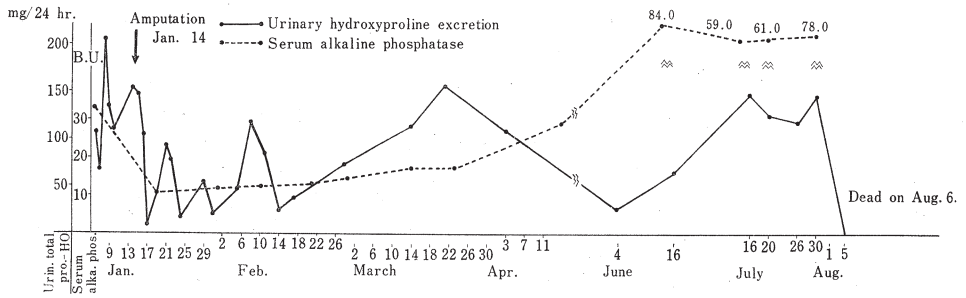

FIG. 8. The results of Case 4 12 year old girl, are shown in this figure. She suffered from an osteogenic sarcoma at the lower portion of the left femur. Lung metastases were found several weeks after amputation. In the terminal stage, the excretion increased, reaching a peak, then became zero on the day before death. Decrease and increase of urinary hydroxyproline excretion ran almost parallel with the value of serum alkaline phosphatase.

125.3 mg/24 hr (S.D.±50.3, with a range of 68 to 205.1). For about 2 weeks after operation the excretion showed a tendency to decrease but thereafter the tendency reversed towards increase. About 2 months before death a high excretion tendency appeared and continued. The excretion 3 weeks before death was 133.6 mg/24 hr (S.D.±15.0, with a range of 117.0 to 146.4) and the day before death the excretion fell to zero (Fig. 8). Variation of urinary hydroxyproline excretion during the entire course of the disease ran almost parallel with the value of the serum alkaline phosphatase.

(Case 5) S. T. 25 year old female. Osteogenic sarcoma. A sclerotic bone lesion was found at the upper portion of the right humerus. The patient was admitted to the hospital on Aug. 6, 1965. At the beginning the excretions ranged from 9.6 to 16.5 mg/24 hr which were lower than the 23.5±7.6 mg/24 hr for the control adults. An arterial infusion of Mitomycine C and intravenous injection of Endoxan were carried out. But as the bone lesion advanced the excretion increased to a range of from 33.9 to 35.2 mg/24 hr.

On Oct. 19, 1965, interscapulothoracic amputation of the right shoulder was accomplished. A highly elevated excretion of 97.9 mg/24 hr resulted but decreased later and lung metastases were found in the radiogram of the chest. Sixteen weeks after surgery, due to intra-cranial metastasis the patient died suddenly (Fig. 9).

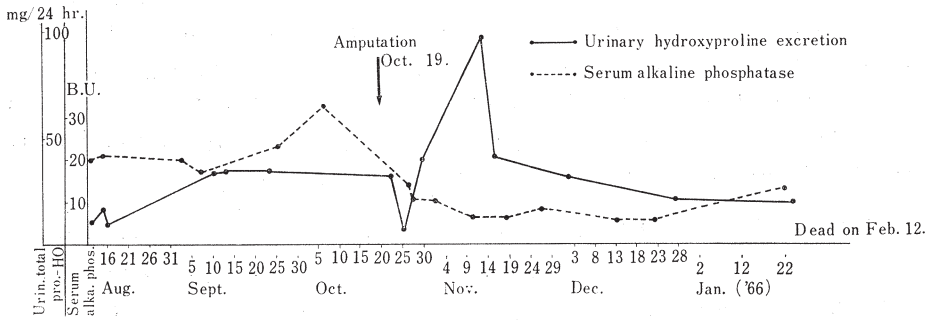

FIG. 9. Case 5 was a 25 year old female with osteogenic sarcoma at the upper portion of the right humerus. Excretion gradually increased with the development of the bone destruction and of the tumor. The excretion reached a peak of 97.9 mg/24 hr 3 months prior to death, gradually decreasing untill her death from lung and cerebral metastases causing cerebral bleeding.

(Case 7) M. M. 33 year old male. Chondrosarcoma. This was a case of secondary chondrosarcoma at the right portion of the pelvis, which became malignant from hereditary multiple exostosis. Malignant changes were found in the tumor from the right ishium. In Oct. 1964 radiation therapy with ^{60}Co was started and a total dose of 4,000 r was gvien. On April 14, 1965 he was admitted to our hospital. The excretion on admission was 51.9 mg/24 hr, and about twice the mean for the control group of the same age. With ^{60}Co radiation therapy the excretion decreased and after reaching a peak of 107.5 mg/24 hr a tendency towards decrease was seen and the patient died on Oct. 6 1965 (Fig. 10)

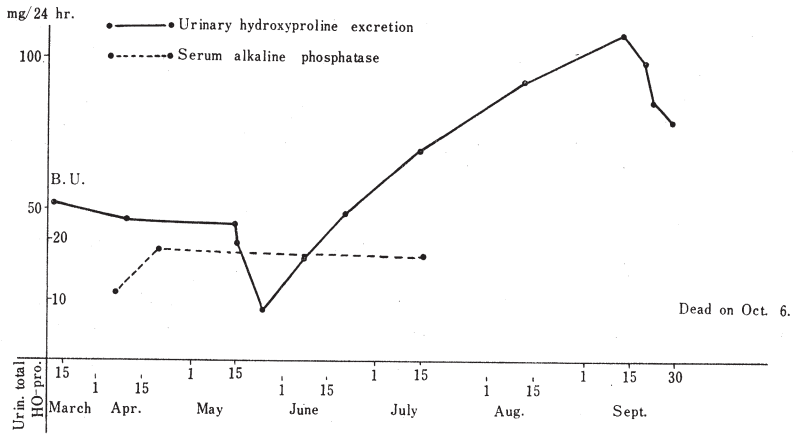

FIG. 10. Case 7 of secondary chondrosarcoma, 33 year old male, with increased excretion after ^{60}Co radiation therapy reaching a peak of 107.5 mg/24 hr one month before death.

In Case 1 and Case 2, showing a good clinical course after amputation, the excretions showed a tendency to decrease, but in Cases 4, 5 and 7 that died during the experiments, and Case 3 with lung metastasis, a tendency to increase was observed as their irreversible clinical courses progressed. In all cases above mentioned the relationship between the urinary hydroxyproline excretions and serum alkaline phosphatase showed almost parallel changes.

ii) Clinical state of malignant bone tumor and urinary hydroxyproline

During the past year of study 4 patients with osteogenic sarcoma, 1 with chondrosarcoma, 9 with metastatic carcinoma died. In cases in which continuous measurement was performed an increase of excretion was observed in the terminal state and after reaching a peak of excretion patients died (Cases 4, 5 and 7). The excretion in 9 cases with metastatic carcinoma of bone who died during the study was 77.4 ± 46.3 mg/24 hr which was higher than that of controls.

B) EXPERIMENTAL STUDIES

From the results observed in patients with malignant bone tumor the following experimental studies were undertaken.

a) Materials and Methods:

Small fragments of tumor mass of Walker carcinosarcoma 256, transplantable tumor to rats, were transplanted into the left tibial condyl of Splague Dawley strain male rats, weighing about 70 g. Twenty-four hour urine was collected daily or every two days and determination of hydroxyproline was made according to

the method of Prockop, D. J. and Udenfriend, S. as described above[7].

All rats used for experiments and controls were fed with regular solid food made by Asahi Electron Co. Ltd., and given water ad libitum during the experiment. Each rat was placed in a separate cage, thus separating urine and feces, and a 24 hr urine was collected. After urine collection the cage was washed with distilled water and the washing was mixed with the collected urine. The mean value of urinary total hydroxyproline of these rats weighing about 70 g was 0.42 ± 0.08 mg/24 hr.

The experimental studies were grouped as follows; group of (1) transplants into left tibial bone, 19 rats, (2) subcutaneous transplantation, 12 rats (control group), (3) sham operation; hole drilling at left tibial condyle, 6 rats.

b) Results:

In the subcutaneous transplanted and bone transplanted groups, rats with

TABLE 4. Experimental Bone Tumor Induced by Walker Carcinosarcoma 256
Urinary Total Hydroxyproline Excretion

	Induced Bone Tumor	Subcutaneous Transplanted	Sham Operation
	Total Hydroxyproline Excretion		
	Mean±S.D. (mg/24 hr)	Mean±S.D. (mg/24 hr)	Mean±S.D. (mg/24 hr)
Before Transplant.	0.42±0.08†	0.42±0.08	0.42±0.08
Day after Transplant.			
2	0.86±0.09	0.53±0.05	0.60±0.05
3	0.77±0.06		
4	0.77±0.05	0.65±0.11	0.52±0.06
5	0.76±0.06		
6	0.77±0.06	0.61±0.08	0.4 ±0.08
7	0.72±0.04		
8	0.62±0.05	0.47±0.07	0.32±0.05
9	0.56±0.04		
10	0.47±0.09	0.34±0.05	0.3 ±0.08
11	0.48±0.02		
12	0.46±0.05	0.66±0.09	0.4 ±0.05
13	0.64±0.1		
14	1.23±0.17	0.64±0.09	
15	1.39±0.11		
16	1.40±0.28†*	0.37±0.04*	0.46±0.08
17	0.77±0.05		
18	0.50±0.06		0.50±0.09
19	0.50±0.08		
20	0.43±0.07		0.49±0.05
21	0.4 ±0.07		
23	0.49±0.1		
27	0.46±0.06		

Splague-Dawley rats weighing 70 g were used. Their 24 hr urine specimens were collected separately. The cages were washed with distilled water after collection, and this water was mixed with the collected urine,

†, * There was a significant difference by the t-test ($p \leqq 0.01$)

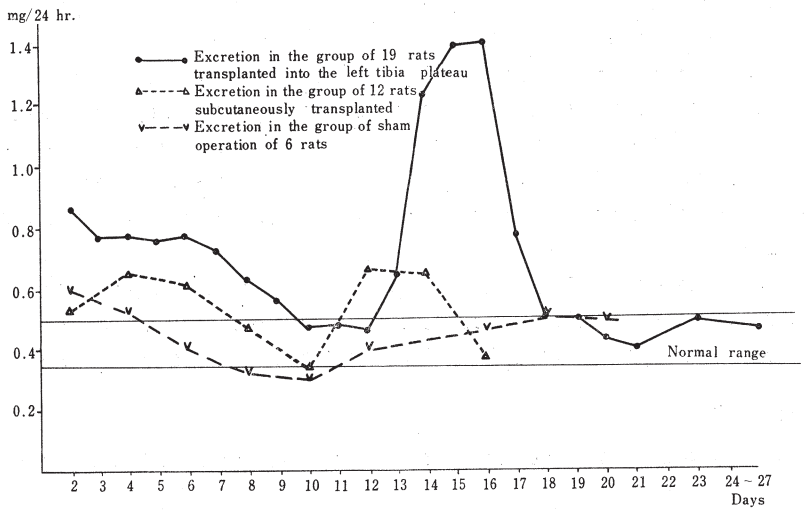

FIG. 11. Urinary total hydroxyproline excretion in experimental bone tumor induced by walker carcinosarcoma 256.

Fifty two male Splague-Dawley rats were transplanted with Walker carcinosarcoma 256 into the left tibia plateau. Of the 52 rats, 47 succumbed. Transplantation success rate was 90.4 per cent. Seventeen of the 47 rats had metastases in the lungs and lymph nodes. The mean survival rate was 23.1 days in the group of experimental bone tumor and 16.4 in the group of 12 rats subcutaneously transplanted. Those having metastases were not counted in either group. Statistical significances at 0.01 probability level are shown between the excretion peaks in the group of transplanted bone tumor and those subcutaneously transplanted and of the sham operations ($p \leqq 0.01$).

lung or lymph node metastases were omitted. Table 4 and Fig. 11 show changes of urinary excretion in each group.

In the group of bone transplantation markedly elevated excretion was observed on the 14th, 15th and 16th day after transplatation, with a peak of 1.40 ± 0.28 mg/24 hr on the 16th day. Thereafter a marked decreased excretion was observed and the rats died in increasing numbers on the 17th and 18th days when the excretion rate become near normal. On the 27th day only 3 out of 19 rats survived.

In the subcutaneous transplanted group the excretion on the 12th day was 0.66 ± 0.09 mg/24 hr and on the 16th day 0.37 ± 0.04 mg/24 hr, showing gradual decrease and two days later all rats died with tumor. In the group of drilling tibia (sham operation) it was within normal control limit.

Temporarily increased excretions which might be due to surgical intervention were observed in these three groups but return to normal value was seen from 8 to 10 days.

Marked elevated excretion seen in the group transplanted into bone was almost three-fold when compared with that of the sham operation and almost twice that of the subcutaneous group. The difference was statistically significant at the 0.01 probability level for these groups (Table 4, Fig. 11).

Moreover, to study the changes in urinary excretion with therapy the following experiment using 8 Splague-Dawley rats was made. On the 14th day the left hind leg with transplanted tumor was amputated at the upper portion of the femur. The changes in urinary excretion are shown in Table 5 and Fig. 12. The excretion after amputation decreased and all rats survived. This was statistically significant at the 0.01 probability level calculated by *t*-test (Table 5, Fig. 12).

TABLE 5. Urinary Hydroxyproline Excretion in the Group of "Amputation"

Day after Transplantation	Total Hydroxyproline Excretion (mg/24 hr)
	Mean±S.D.
13	0.70±0.09
14 (Amputation)	
15	0.44±0.06
16	0.62±0.08
17	0.66±0.05
20	0.58±0.07

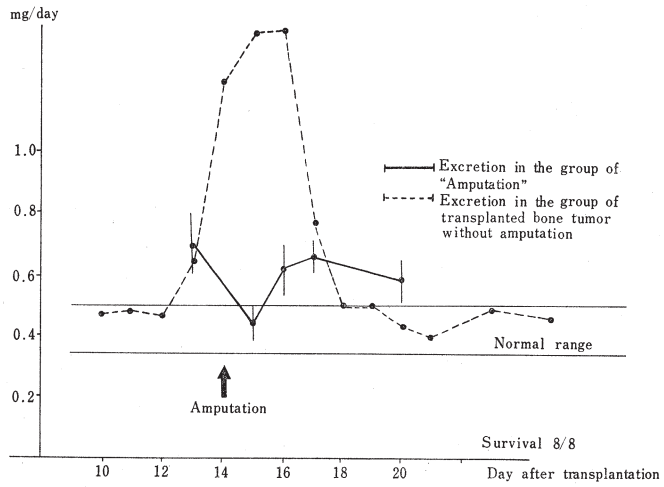

FIG. 12. Urinary total hydroxyproline excretion in the group of "amputation".

After amputation the excretion is not elevated, all survived. Statistical significance at 0.01 probability level is shown between the peak of transplanted bone tumor and the excretion of the amputation group ($p \leqq 0.01$).

DISCUSSION

It has been gradually recognised that urinary hydroxyproline might be a good index of collagen metabolism, but the details have not been completely clarified. According to the isotopic technique employed by Stetten, M. R. this amino acid was reported to be formed by the irreversible hydroxylation of peptide-linked proline and to be not derived from dietary protein[16].

In the study of low protein diet by Prockop, D. J. and Sjoerdsma, A. it has been proved that urinary hydroxyproline peptides are not derived from a pool of collagen precursers[17], and supported the hypothesis that urinary hydroxyproline is derived from the break down of body collagen. It is believed that the mechanism of hydroxyproline peptides and body collagen could be more precisely studied by physiological and biochemical researches on the structure of collagen.

There are references pertaining to increased excretion of hydroxyproline in growing children, though only few we have on excretion in normal subjects according to age, such as those by Jones, C. R., Bergman, M. W., Kittner, R. J. and Pigman, W. W.[8]; Lenzi, F., Ravenni, G., Rubegni, M. and L. Del. Govane[9]; and Jasin, H. E., Fink, C. W., Wise, W. and Ziff, M.[10] The present authors reported that urinary excretion rises in patients with severe bone lesions such as hyperparathyroid adenoma with bony lesions, malignant bone tumors, congenital systemic bone diseases, senile osteoporosis and in rats treated with parathyroid extract[5,6].

While the above study was being made we felt that there is keen need to know the age-dependent changes in normal excretion level, and hence, in this report the changes in urinary excretion were analyzed in detail by age and sex in 134 normal subjects of ages less than one year to 90 years, consisting of 73 males and 61 females.

Hydroxyproline excreted in urine may be derived mainly from soluble collagen as it has been reported that neutral soluble collagen is the most activated form and its half-life 2 to 3 days. The aggregation of cross-linking of collagen makes a half-life delay. The half-life of acid soluble collagen is said to be 20 to 25 days and that of insoluble collagen 150 to 300 days[18].

Increased excretion in normal children can be attributed to accelerated bone turnover with degradation or break down of collagen with growth[19,20]. It was also observed in this study that the highest excretion was seen in the age group from 10 to 14 years, as Jasin, H. E., Fink, C. W., Wise, W. and Ziff, M. have reported[10].

Decreased excretion of this amino acid occurred in patients with growth disturbance such as pituitary dwarfism, hypothyroidism, osteogenesis imperfecta. However, in cases of adrenogenital syndrome with bone age more developed than the chronological age, the excretion increased as it did in pituitary dwarfism

during therapy. From these results the conclusion seems justified that an increase of urinary hydroxyproline excretion is due to collagen synthesis as in case of the growing period.

In the preliminary report the present author and co-workers reported that increased excretions were found in primary hyperparathyroidism with bone lesion (child) and in rats injected with parathyroid extract[5]. It is very interesting to know that hyperparathyroidism or parathyroid extract act destructively on bone collagen. Among the studies on bone tumor and urinary hydroxyproline excretion a report published by Platt, W. D., Dolittle, L. H. and Hartshorn, J. W. S.[21] is the only one in which an increased excretion of this amino acid in patients with metastatic carcinoma was noted. The present authors had already observed a markedly elevated excretion of this amino acid in patients with osteogenic sarcoma in the preliminary report and carried out studies in detail on the excretion in patients with osteogenic sarcoma in this report. In this study it was confirmed that a marked increase of excretion occurs in the osteogenic sarcoma and changes in excretion proportional to the progress of patient after surgical amputation.

It is presumed that an abnormally increased urinary excretion of hydroxyproline in patients with malignant bone tumor is due to increased soluble collagen pool produced by extraordinal acceleration of collagen metabolism in the tumor host and simultaneously to one derived from insoluble collagen. These phenomena were also demonstrated in experimental rats. That is, urinary excretion in rats in which tumor fragments of Walker carcinosarcoma 256 had been transplanted into bone was about 3-fold that of the controls on the 14th and 16th days after transplantation. In the group in which surgical amputation was performed on the 14th day the excretion remained approximately normal and the rats survived.

Moreover, it was very interesting that in patients with malignant bone tumor and in experimental animals urinary excretion rose before death. We would like to call this "Terminal Increase Phenomenon" of urinary hydroxyproline excretion. From the fact that approximately 57 per cent of collagen in the human adult is contained in bone and its metabolic activity is higher than in skin and other tissues[1)2)3)], it is suggested that approximately 80 per cent of urinary total hydroxyproline is derived from bone collagen.

This is confirmed by the clinical results in which cases with bone metastasis showed more than double the urinary excretion of this amino acid as compared with normal values. Whereas increase of the urinary hydroxyproline did not occur in cases with soft tissue carcinoma without bone lesion. Experimental results in rats implanted with Walker carcinosarcoma 256 into bone tissue showed three times the amount of urinary hydroxyproline excretion as compared with the control group of rats while there was no significant increase of urinary

excretion of this amino acid in rats with this carcinosarcoma implanted into soft tissue.

As reported in this paper the changes in urinary hydroxyproline excretion could be a good index of bone collagen metabolism, showing elevated excretion in growing children, decrease in patients with hormonal growth disturbances and alteration in those with bone tumor or systemic bone disease.

Urinary total hydroxyproline excretion in bone disease is being investigated by the present authors by observing the decrease in hypophysectomized or thyroidectomized, corticoid administered and Vitamin C depleted rats and further studies are also underway to investigate senile change and calcium metabolism.

SUMMARY

1. Urinary total hydroxyproline excretion in normal subjects was classified by age and group, such as (1) under 12 months, (2) from 1 to 5 years old, (3) from 6 to 9 years, (4) from 10 to 14 years, (5) from 15 to 19 years and (6) over 20 years. The highest excretion was shown in the group of 10 to 14 years old.

2. In patients with systemic bone disease lower excretion was observed, such as in pituitary dwarfism, hypothyroidism, osteogenesis imperfecta. However, in cases with positively advanced bone age, excretion was elevated as in adrenogenital syndrome and pituitary dwarfism during therapy. In the groups of Marfan's syndrome and Hurler's syndrome one of each showed elevated excretion, and these values were compared with the controls of the same age, and discussed.

3. It has been proved that there extremely increased excretion of hydroxyproline in the patients with malignant bone tumor particularly in osteogenic sarcoma and the changes of excretion were observed to parallel the clinical progress of the patient after surgical amputation of the extremity. That is while the patient remained in good clinical condition the urinary excretion had a tendency to decrease to normal limit, on the contrary it appeared to be increased when his condition became worse on account of recurrence or metastasis of tumor. And before death, the excretion increased to a temporary peak, so we would like to call it "Terminal Increase Phenomenon".

4. In the experimental studies using Walker carcinosarcoma 256 urinary hydroxyproline excretion remarkably increased with aggravation of bone lesion and after amputation no elevated excretion was observed.

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REFERENCES

1) Forbes, R. M., Cooper, A. R. and Mitchell, H. H., The composition of the adult human body as determined by chemical analysis, *J. Biol. Chem.*, **203**, 359, 1953.
2) Forbes, R. M., Mitchell, H. H. and Cooper, A. R., Further studies on the gross composition and mineral elements of the adult human body, *J. Biol. Chem.*, **223**, 969, 1956.
3) Neuberger, A. and Slack, H. G. B., The metabolism of collagen from liver, bone, skin and tendon in the normal rat, *Biochem. J.*, **53**, 47, 1953.
4) Gerber, G., Gerber, G. and Altman, K. I., Studies on the metabolism of tissue proteins. I. Turnover of collagen labeled with proline-U-C^{14} in young rats, *J. Biol. Chem.*, **235**, 2653, 1960.
5) Nakagawa, M. and Tamaki, T., Urinary hydroxyproline excretion in orthopedic disease. Preliminary report., *Nagoya J. Med. Sci.*, **27**, 228, 1965.
6) Nakagawa, M., Natsume, R., Yoshida, T., Shiono, M., Kida, O., Iwata, H., Hirakoh, H. and Kasahara, K., Clinico-biochemical study on senile osteoporosis, *Nagoya J. Med. Sci.*, **28**, 110, 1966.
7) Prockop, D. J. and Udenfriend, S., A specific method for the analysis of hydroxyproline in tissues and urine, *Analytical Biochem.*, **1**, 228, 1960.
8) Jones, C. R., Bergman, M. W., Kittner, R. J. and Pigman, W. W., Urinary hydroxyproline excretion in normal children and adolescents, *Proc. Soc. Exp. Biol. Med.*, **115**, 85, 1964.
9) Lenzi, F., Ravenni, G., Rubegni, M. and L. Del. Govane., The significance of urinary hydroxyproline in osteoporosis, *Pan Minerva Med.*, **5**, 155, 1963.
10) Jasin, H. E., Fink, C. W., Wise, W. and Ziff, M., Relationship between urinary hydroxyproline and growth, *J. Clin. Invest.*, **41**, 1928, 1962.
11) Sjoerdsma, A., Davidson, J. D., Udenfriend, S. and Mitoma, C., Increased excretion of hydroxyproline in Marfan's syndrome, *Lancet*, **2**, 994, 1958.
12) Keiser, H. R., Gill, J. R., Sjoerdsma, A. and Bartter, F. C., The effect of parathyroid extract on hydroxyproline metabolism, *Clin. Res.*, **11**, 41, 1963.
13) Dull, T. A. and Henneman, P. H., Urinary hydroxyproline as an index of collagen turnover in bone, *New Eng. J. Med.*, **268**, 132, 1963.
14) Benoit, F. L., Theil, G. B. and Watten, R. H., Urinary hydroxyproline excretion in endocrine disease, *Clin. Res.*, **11**, 89, 1963.
15) Kivirikko, K. I., Leitinen, O. and Lamberg, B. A., Value of urine and serum hydroxyproline in the diagnosis of thyroid disease, *J. Clin. Endocr. Metab.*, **25**, 1347, 1965.
16) Stetten, M. R., Some aspects of metabolism of hydroxyproline studied with aid of isotopic nitrogen, *J. Biol. Chem.*, **181**, 31, 1949.
17) Prockop, D. J. and Sjoerdsma, A., Significance of urinary hydroxyproline in man, *J. Clin. Invest*, **40**, 842, 1961.
18) Jackson, D. S. and Bentley, J. P., On the significance of the extractable collagens, *J. Biophys. Biochem. Cytol.*, **7**, 37, 1960.
19) Ziff, M., Kibrick, A., Dresner, E. and Gribetz, H. J., Excretion of hydroxyproline in patients with rheumatic and non-rheumatic diseases, *J. Clin. Invest.*, **35**, 579, 1956.
20) Kao, K. Y. T. and McGavack, T. H., Connective tissue. I. Age and sex influence on protein composition of rat tissues, *Proc. Soc. Expel. Biol. Med.*, **101**, 153, 1959.
21) Platt, W. D., Dolittle, L. H. and Hartshorn, J. W. S., Urinary hydroxyproline excretion in metastatic cancer of bone, *New Eng. J. Med.*, **271**, 287, 1964.