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Research Article

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Urinary Excretion of Hydroxylysine and its Glycosides as an Index of Collagen Degradation

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ABSTRACT Urinary excretion of hydroxyproline (Hyp) is one index of total collagen degradation, from all sources. Since some of the Hyp released from collagen may be further metabolized before it is excreted, other markers are necessary to measure collagen breakdown. Excretion of the glycosides of hydroxylysine (Hyl), glucosyl galactosyl hydroxylysine (Hyl[GlcGal]), and galactosyl hydroxylysine (Hyl-[Gal]), more accurately reflects collagen metabolism since these products occur in specific ratios in different tissue collagens and are themselves metabolized only to a minor degree.

The ratios of total Hyl/Hyp and Hyl(GlcGal)/Hyl-(Gal) were measured in the urine of normal subjects and of patients with Paget's disease of bone, hyperphosphatasia, and extensive thermal burns. In patients with extensive thermal burns the pattern of urinary Hyl and its glycosides was consistent with degradation of collagen in dermis and fascia. When bone collagen degradation was dominant, the pattern of urinary metabolites reflected that source. Pagetic bone collagen has an amino acid composition similar to normal bone and Hyl(GlcGal)/Hyl(Gal) of 0.396–0.743, vs. normal of 0.474±0.088. In untreated patients with severe Paget's disease of bone or hyperphosphatasia (urinary Hyp > 2.0 μmol/mg creatinine) urinary Hyl/Hyp averaged 0.052±0.002 (0.042±0.009 in normal bone) and Hyl(GlcGal)/Hyl(Gal) 0.601 ±0.017 (0.47±0.009 in normal bone). When bone resorption was decreased sufficiently with calcitonin or disodium etidronate in these patients, both the urinary ratios of Hyl/Hyp and Hyl(GlcGal)/Hyl(Gal) rose. In normal subjects treated with calcitonin and excreting relatively little Hyp, the ratio of Hyl/Hyp approached 0.7 and Hyl(GlycGal)/Hyl(Gal) approached 3.5. These increased ratios reveal the existence of a source of collagen breakdown other than skin or bone. The first subcomponent of complement, Clq, which has collagen-like sequences, relatively high amounts of Hyl, and most of the glycosylated Hyl as Hyl(GlcGal), could be the source of these metabolites.

INTRODUCTION

Collagens contain unique, modified amino acids, the most abundant of which is 4-hydroxyproline (Hyp).[1] Measurement of urinary excretion of total Hyp (free Hyp plus Hyp-containing peptides) has been used extensively as an index of collagen degradation (1). It is not possible to obtain quantitative estimations of collagen breakdown by this measurement since most of the free Hyp is further metabolized to carbon dioxide and other products not included in the Hyp determination and only a portion appears in the urine. Woessner found that less than 15% of the Hyp of degraded collagen appears in the urine of the pospartum rat (2). Weiss and Klein (3) determined that when collagen peptides were administered to rats, 75% were metabolized to respiratory carbon dioxide and 25% excreted. In their studies of a patient with deficiency in Hyp oxidase (hydroxyprolinemia), Efron et al. (4) found that only 20% of the urinary Hyp was peptide bound whereas 80% was free. An additional deficiency in the use of measurement of Hyp excretion to assess

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[1] *Abbreviations used in this paper*: EHDP, disodium etidronate; Gal, galactosyl; Hyl, hydroxylysine; Hyp, 4-hydroxyproline; Hyl(Gal),β-1-galactosyl-0-hydroxylysine; (Hyl[GlcGal]),α-1-2-glucosyl-galactosyl-0-hydroxylysine; MRC, Medical Research Council; SCT, salmon calcitonin.

collagen degradation is that its estimation provides no information concerning the source of collagen since its content varies little from one tissue collagen to another.

Hydroxylysine (Hyl) is another amino acid unique to collagens and proteins containing collagen-like sequences. Like Hyp, Hyl is not reutilized for collagen biosynthesis and although it is considerably less abundant than Hyp, it is a potential marker for collagen metabolism. A variable proportion of Hyl residues in collagen are glycosylated as β-1-galactosyl-0-hydroxylysine (Hyl[Gal]) and some of these residues may be glucosylated as α-1-2-glucosyl-galactosyl-0-hydroxylysine (Hyl[GlcGal]) (5, 6). The relative proportion and total content of glycosylated Hyl are different for different collagens. For example the ratio Hyl(GlcGal)/Hyl(Gal) is 0.47±0.09 in human bone, but 2.06±0.47 in human skin (7). Cunningham et al. (5) and Kakimoto and Akazawa (8) identified these Hyl glycosides in normal human urine and Segrest and Cunningham (9) suggested that since they were not metabolized to the same extent as Hyp, they could serve as more quantitative markers for collagen breakdown as well as provide information as to the source of the collagen metabolites.

In the present study we have measured the urinary excretion of Hyp, Hyl, and Hyl glycosides in normal subjects and patients with accelerated collagen breakdown, particularly in disorders of the skeleton, to determine relative contributions of different collagenous tissues to the urinary pool of collagen degradation products. At high rates of bone collagen breakdown the excretion of Hyl and Hyl glycosides reflects the major contribution of bone collagen, but at low rates of turnover, induced by agents which suppress bone resorption, the contribution of components other than bone and skin becomes evident.

METHODS

Sample collection. All 24-h urine samples were collected after the subjects had been on a low gelatin diet for at least 24 h. In some instances only first morning postabsorptive urinary samples were obtained. Samples were stored under toluene at 2–5°C and when collections were completed they were filtered and kept at −20°C. Samples were freeze-dried either before or after storage at −20°C. 24-h urine samples were obtained from 21 untreated subjects with Paget's disease of bone. Five of these subjects were subsequently treated with the diphosphonate disodium etidronate (EHDP) (10) at doses of 1–20 mg/kg for up to 12 mo and urine samples were again collected at 3–6 mo intervals. Four subjects with Paget's disease were treated with 50–200 Medical Research Council (MRC) U per day of salmon calcitonin (11) for 16–32 mo; pre and post-treatment urinary samples were collected from these individuals at various intervals. Five pagetic and two normal control subjects were given single intravenous or subcutaneous injections of calcitonin and urines collected at 30–60 min intervals. 24-h urine samples were collected from four patients with burns covering an estimated 50–88% of body surface. Multiple urine samples from two subjects with hereditary hyperphosphatasia were supplied through the courtesy of Dr. Melvin Horwith (New York Hospital-Cornell Medical Center, New York). 12-h specimens were collected before and during human calcitonin therapy in one of these. Samples of bone from pagetic and normal subjects were obtained at time of hip surgery for pagetic coxopathy or at necropsy. Fascia and skin were obtained at necropsy from two additional subjects who did not have obvious disease of collagenous tissues.

Preparation of samples

Bone. Fragments of normal and pagetic bone were cleaned and stripped of periosteum. Specimens were demineralized with three changes of 0.5 M disodium EDTA in 0.1 M Tris-HCl, pH 8.0, 20 ml/g of tissue, over a period of 3 days, at 5°C with constant agitation by means of a magnetic stirrer (7). The samples were rinsed with distilled water and freeze-dried. No Hyl or Hyp was found on amino acid analysis of acid hydrolysates of the supernatant solutions.

Skin. Fat and hair were stripped by dissection and the skin placed in 2 M NaBr at 20°C for 12 h, allowing the epidermis to be peeled away from the dermis (12, 13). The dermis was rinsed with distilled water and freeze-dried.

Fascia. Any muscle was stripped off by dissection and the fascia rinsed with distilled water and freeze-dried.

Analysis of total Hyl and Hyp. Samples of bone, skin, and fascia (approximately 8 mg dry weight) and 1–5 ml equivalents of freeze-dried urine were hydrolyzed under nitrogen in sealed tubes in constant boiling HCl at 108°C for 20 h. The samples were then dried in a desiccator containing solid NaOH under vacuum. Dried hydrolysates were quantitatively transferred with 0.1 N HCl to 5-ml volumetric flasks and brought to final volume. 500 μl of solution was used for amino acid anlysis. Quantitative amino acid analyses (14) were performed with a modified model 117 Beckman-Spinco automatic amino acid analyzer (Beckman Instruments Inc., Spinco Div., Palo Alto, Calif.).

Base hydrolysis and Hyl glycoside determination. Dry tissues (15–20 mg) and appropriate amounts of urine (7) (according to concentration) were used. Specimens were hydrolyzed in 2 N KOH in alkali-resistant boron-free hydrolysis tubes at 108°C for 20 h. The solutions were neutralized to pH 7with 1 M $HClO_4$. Samples were centrifuged at 1,000 rpm at room temperature for 5 min; the supernates were removed and freeze-dried. Samples were quantitatively transferred to a Bio Gel P-2 (200–400 mesh) gel filtration column (100 × 1.8 cm) (Bio-Rad Laboratories, Richmond, Calif.) in a total of 2 ml of 0.1 N acetic acid. 150 fractions (each 2.2 ml) were collected with an LKB fraction collector (LKB Instruments Inc., Rockville, Md.). Fractions numbered 51–75 (containing Hyl and its glycosides) were pooled and freeze-dried. These were then placed on the amino acid analyzer column.

Other methods. Creatinine was determined by the method of Folin (15). Determinations of C1q by radioimmunodiffusion were performed through the courtesy of Dr. Peter Schur at the Robert Breck Brigham Hospital.

RESULTS

The values for urinary excretion of Hyl and each of its glycosides by five normal adults before calcitonin

TABLE I
Urinary Excretion of Collagen Metabolites in Normal Subjects

	Number	Total Hyp	Total Hyl	Hyl(GlcGal)	Hyl	Hyl(GlcGal)	Hyl(Gal)
		creatinine	Hyp	Hyl(Gal)	creatinine	creatinine	creatinine
		μmol/mg	*mol/mol*	*mol/mol*		*μmol/mg*	
Adults*	5						
mean		0.173	0.216	1.44	0.0073	0.0115	0.0082
(range)		(0.075–0.300)	(0.096–0.418)	(0.94–2.53)	(0.0225–0.0343)	(0.0087–0.0193)	(0.0069–0.0093)
Children‡	8						
mean		1.18	0.117	0.94	0.0361	0.0484	0.0517
(range)		(0.95–1.37)	(0.088–1.36)	(0.87–1.12)	(0.0232–0.0701)	(0.0416–0.0562)	(0.0435–0.0593)

* Present study.
‡ Calculated from Pinnell et al. (12).

treatment are shown in Table I. The values for Hyl(Gal) are within the range of normal adult males (0.004–0.021 μmol/mg creatinine) calculated from the data of Kakimoto and Akazawa (8). The levels of Hyl(GlcGal) are in the lower portion of the range calculated from the data of Kakimoto and Akazawa (8) although it is not known if the latter subjects were on gelatin-free diets. In their two patients on protein restriction the levels of Hyl(GlcGal) (0.010 μmol/mg creatinine in both) were similar to those shown in Table I. Values for normal children, ages 7–12, calculated from previously reported data (12) are also shown. The excretion of total Hyl is greater in children than in adults, as is the excretion of total Hyp, and the ratio of Hyl(GlcGal)/Hyl(Gal) is lower in children than in adults. Each of our patients with untreated bone disease excreted Hyl and Hyl glycosides at levels outside the ranges of those of the normal subjects. The levels in patients with bone disease were proportional to the total Hyp excretion when Hyp/creatinine was greater than 2.0 μmol/mg. In the patients with Paget's disease these levels were also correlated with extent and activity of the disease (16). In untreated patients, there was little variation in the urinary excretion of Hyp, Hyl, and its glycosides when sampled at intervals during the day, and little variation (±10% of mean) in untreated patients from day to day when the values were normalized to creatinine excretion. Therefore, in some subjects only first morning postabsorptive urine samples were included for analysis. When normal subjects were treated with calcitonin and patients with Paget's disease treated with calcitonin or EHDP, the total excretion of Hyl and its glycosides decreased as did the excretion of Hyp. The fraction of total Hyl in the urine that was glycosylated was similar in all samples (n = 67) and averaged 0.754±0.078 (SD), in agreement with the value of 0.78 calculated from the data of Kakimoto and Akazawa (8).

In two urine samples with high total Hyp and Hyl, Hyl and glycosides were determined before and after alkaline hydrolysis. Levels of free Hyl(GlcGal) were 94 and 73% of the total Hyl(GlcGal), of Hyl-(Gal) 95 and 89% of total Hyl(Gal) and of Hyl 47 and 32% of total Hyl. In samples with low total Hyp and Hyl, determination of free Hyl and glycosides would necessitate placing large volumes of unhydrolyzed urine on the gel filtration and analyzer columns. To avoid clogging the columns we continued to analyze samples after alkaline hydrolysis.

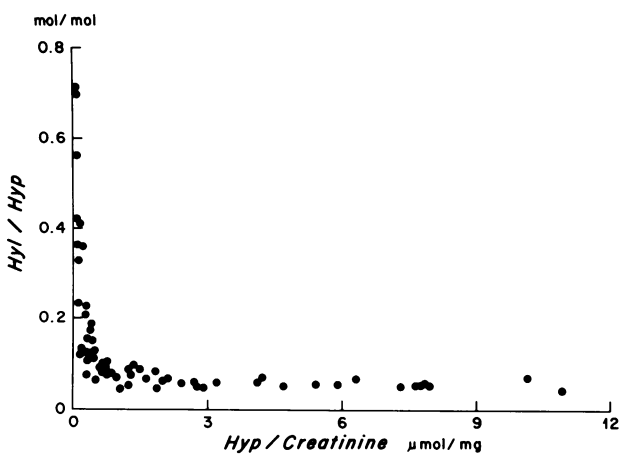

FIGURE 1 The molar ratio of urinary Hyl/Hyp as a function of urinary total Hyp normalized to creatinine excretion (Hyp/creatinine) in patients with Paget's disease (treated and untreated) and in normal subjects (with and without calcitonin treatment). Values include data from acute as well as chronic administration of calcitonin. Not included are values for two children with hyperphosphatasia with control excretion of Hyp/creatinine of 27.1 and 19.0 μmol/mg and Hyl/Hyp ratios of 0.034 and 0.047, respectively.

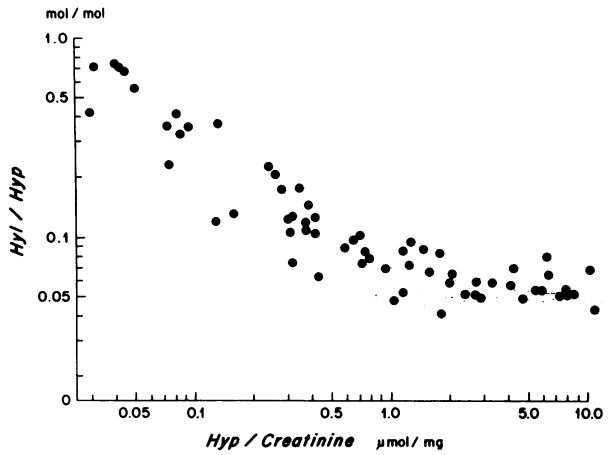

FIGURE 2 Data from Fig. 1 plotted on a log-log scale. At lowest values of Hyp/creatinine, ratios of Hyl/Hyp can be extrapolated to approximately 0.5–0.7.

Relationship of urinary Hyl to Hyp. In Fig. 1 the molar ratio of urinary Hyl/Hyp in control and untreated and treated patients with bone disease is plotted as a function of total Hyp excretion normalized to urinary creatinine. The ratio Hyl/Hyp fell with increasing levels of Hyp excretion. Patients with Paget's disease or hyperphosphatasia and very high urinary Hyp, i.e. > 2.0 µmol/mg creatinine, had a mean molar ratio of urinary Hyl/Hyp of 0.052±0.002 (SEM). This value remained constant with increasing levels of Hyp over a wide range (to 28.0 µmol Hyp/mg creatinine). In patients with milder disease, both treated and untreated, the urinary ratio of Hyl/Hyp rose with decreasing excretion of Hyp. The highest values were reached in normal subjects given calcitonin acutely.

In Fig. 2 the data shown in Fig. 1 are replotted on a log-log scale to illustrate better the two components of the curve. A plateau is reached at levels of Hyp excretion greater than approximately 2 µmol/mg creatinine. At very low values of Hyp the ratio is extrapolated to approximately 0.5–0.7.

Relationship of urinary Hyl(GlcGal) and Hyl(Gal) to Hyp. In Fig. 3 the molar ratio of urinary Hyl(GlcGal)/Hyl(Gal) is plotted as a function of total urinary Hyp in normal subjects and untreated and treated patients with bone disease. The ratio fell with increasing levels of Hyp excretion, with a mean of 0.601±0.017 (SEM) at levels of urinary Hyp/creatinine >2.0 µmol/mg. The decrease in the ratio Hyl(GlcGal)/Hyl(Gal) is accounted for by a relatively larger contribution of Hyl(Gal) at the high excretions of Hyp. This is illustrated in Fig. 4 where the excretion of Hyl glycosides is plotted as a function of total Hyl excretion in treated and untreated patients with Paget's disease. The slope for the Hyl(Gal) data is steeper; therefore, at increasing concentrations of total Hyl in patients with Paget's disease,

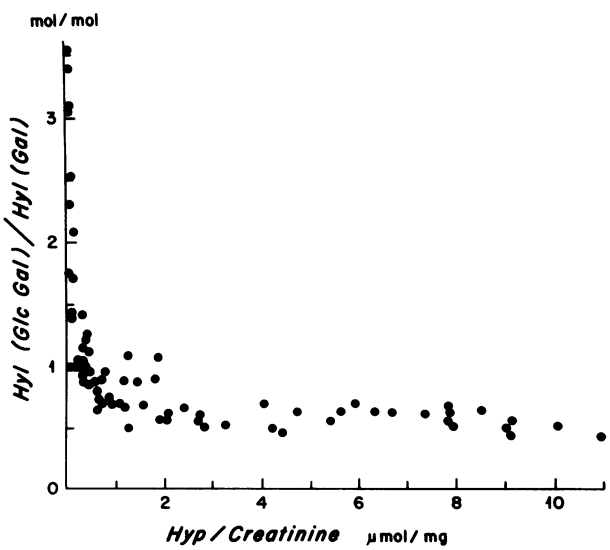

FIGURE 3 The molar ratio of urinary Hyl(GlcGal)/Hyl(Gal) as a function of urinary total Hyp normalized to creatinine excretion (Hyp/creatinine) in same patient population described in Fig. 1. Not included are values for the two children with hyperphosphatasia with control excretion of Hyp/creatinine of 27.1 and 19.0 µmol/mg and Hyl(GlcGal)/Hyl(Gal) ratios of 0.67 and 0.69, respectively.

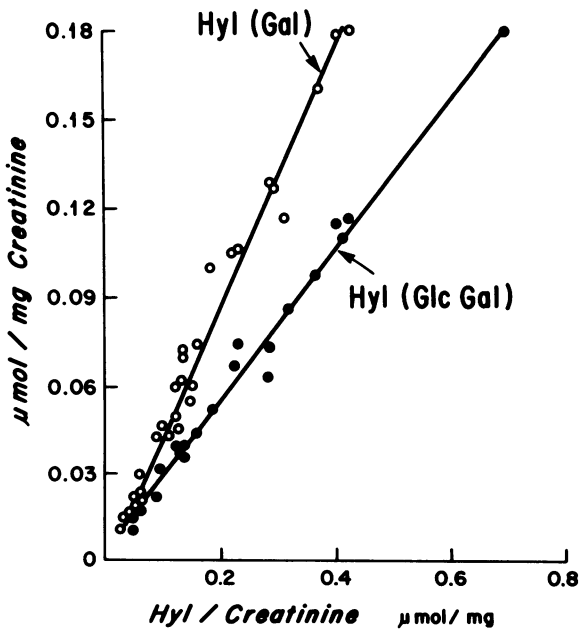

FIGURE 4 Urinary excretion of Hyl(Gal) (open circles) and Hyl(GlcGal) (closed circles) normalized to creatinine as a function of urinary excretion of total Hyl normalized to creatinine in patients with Paget's disease, treated and untreated.

TABLE II

Effect of Chronic Therapy with Salmon Calcitonin (SCT) on Urinary Collagen Metabolites in Patients with Paget's Disease of Bone

Patient	Treatment	Hyp / creatinine	Hyl / Hyp	Hyl(GlcGal) / Hyl(Gal)
		μmol/mg	*mol/mol*	*mol/mol*
1	None	2.88	0.048	0.51
	SCT 100 MRC U/day × 15 mo	0.95	0.069	0.70
2	None	3.24	0.057	0.53
	SCT 50 MRC U/day × 24 mo	0.62	0.078	0.65
3	None	1.05	0.047	0.70
	SCT 50 MRC U/day × 8 mo	0.32	0.073	1.43

monosaccharide Hyl contributes an increasing percentage of the total. In normal subjects and paients with total urinary Hyp < 2.0 μmol/mg creatinine, the ratio of urinary Hyl(GlcGal)/Hyl(Gal) rose with decreasing excretion of Hyp. Highest values, approaching 3.5, were reached in normal subjects given calcitonin acutely.

Effects of calcitonin therapy. Measurements of urinary excretion of Hyl and its glycosides were obtained in three patients with Paget's disease who were treated chronically with salmon calcitonin for 16–32 mo at doses of 50–100 MRC units per day. As long as total Hyp was >2 μmol/mg creatinine the ratios of Hyl/Hyp and Hyl(GlcGal)/Hyl(Gal) remained constant over a wide range of Hyp excretion (2 – >10 μmol/mg creatinine); changes in ratios were observed only when the post-therapy Hyp was decreased to <2.0 μmol/mg creatinine. Pre and post-treatment values are shown in Table II. The ratio of Hyl/Hyp rose by an average of 0.023 and that of Hyl(GlcGal)/Hyl(Gal) by an average of 0.023 and that of Hyl(GlcGal)/Hyl(Gal) by an average of 0.347 as a result of treatment.

Five pagetic patients were given salmon calcitonin intravenously (8–20 μg) and acute changes measured. All exhibited decreases in urinary Hyp (average 2.97 μmol/mg creatinine) accompanied by increases in the ratio of Hyl(GlcGal)/Hyl(Gal) (average 0.25) and that of Hyl/Hyp (average 0.057). Within 2 h in one patient, urinary Hyp had fallen from 0.81 to 0.36 μmol/mg creatinine, while the Hyl/Hyp ratio rose from 0.081 to 1.30 and the Hyl(GlcGal)/Hyl(Gal) ratio rose from 0.66 to 0.94. A typical response is shown in Fig. 5.

In Table III are shown the values for urinary Hyp, Hyl, and Hyl glycosides in samples from a patient with hereditary hyperphosphatasia. The ratios of Hyl/

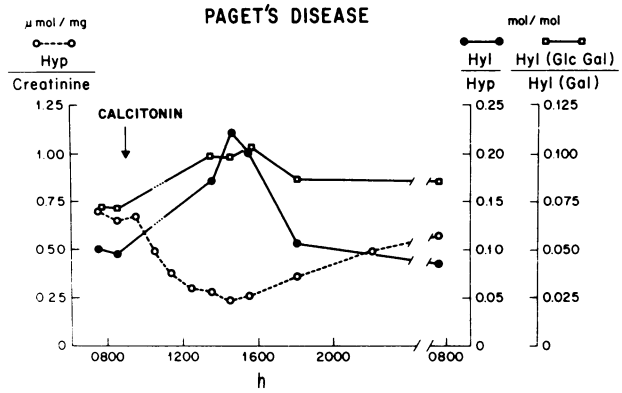

FIGURE 5 Acute response of urinary excretion of total Hyp/creatinine and molar ratios of Hyl/Hyp and Hyl(GlcGal)/Hyl(Gal) to administration of 10 μg of salmon calcitonin intravenously to a patient with Paget's disease.

Hyp and Hyl(GlcGal)/Hyl(Gal) in this patient represent the highest values for total Hyp excretion shown in Figs. 1–3 and indicate the constancy of these ratios in bone disease even at extreme values of Hyp excretion. After treatment with human calcitonin (1 mg/day) Hyp excretion fell sharply in the first 12-h samples collected each day. Values returned to pretreatment levels during the second 12 h. The Hyl-derived ratios were not significantly altered.

Two normal subjects were given calcitonin intravenously. Their post-treatment ratios of Hyl/Hyp and Hyl(GlcGal)/Hyl(Gal) rose to 0.71 and 0.72 and 2.29 and 3.54, respectively, at the nadir of Hyp excretion.

Results of therapy with EHDP. In five patients with Paget's disease treated with EHDP, post-therapy ratios of Hyl/Hyp and Hyl(GlcGal)/Hyl(Gal) rose as

TABLE III

Effect of Treatment with Human Calcitonin on Urinary Collagen Metabolites in a Patient with Hyperphosphatasia

Day	Time	Treatment	Hyp / Creatinine	Hyl / Hyp	Hyl-(GlcGal) / Hyl(Gal)
1	0830–2030 h	None	27.1	0.034	0.67
	2030–0830 h	None	29.2	0.033	0.73
2	0830–2030 h	None	29.2	0.036	0.73
	2030–0830 h	None	26.5	0.033	0.66
3	0900–2100 h	SCT 200 U	13.3	0.038	0.81
	2100–0900 h	0	25.2	0.038	0.73
4	0900–2100 h	SCT 200 U	15.2	0.046	0.79
	2100–0900 h	0	24.2	0.039	0.65

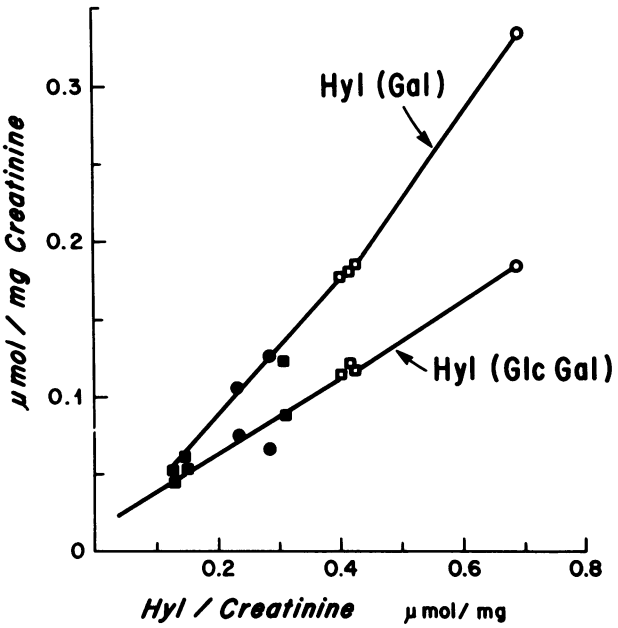

FIGURE 6 Urinary excretion of Hyl(Gal) and Hyl(GlcGal) as a function of urinary excretion of total Hyl, with all values normalized to creatinine. The open circles are the pretreatment values for a patient with Paget's disease treated with 100–200 MRC units daily of salmon calcitonin over a period of 2.5 yr. The closed circles are values obtained after 2.0 and 2.5 yr of therapy. The open squares are the pretreatment values for another patient with Paget's disease. Closed squares are values obtained while on daily therapy with EHDP showing progressive decline after 9 mo of 5 mg/kg, an additional 3 mo of 20 mg/kg, and an additional 6 mo of 20 mg/kg.

total Hyp excretion fell. These values are included in the data shown in Figs. 1–4.

In Fig. 6 the effect of therapy with EHDP is compared to that with salmon calcitonin on the urinary excretion of Hyl glycosides by two paients with Paget's disease. In both treatments there was a more profound decline in Hyl(Gal) than in Hyl(GlcGal). However the values for both therapies fell along similar curves.

Analysis of tissues from patients with Paget's disease. Since the patients with untreated Paget's disease had ratios of Hyl/Hyp and Hyl(GlcGal)/Hyl(Gal) in urine different from those in normal subjects, and since it is presumed that the collagen degradation products in pagetic patients are derived predominantly from bone, information on the composition of pagetic bone collagen is essential for interpretation of such data. Results of amino acid analysis and determination of Hyl and its glycosides in bone from five patients with Paget's disease are presented in Table IV. There was no significant difference in the amino acid composition of pagetic versus normal bone. The Hyl/Hyp ratio of pagetic bone was 0.050±0.010 (range 0.35–0.067), not significantly different from the value for normal bone of 0.042±0.009 (7). The ratio of Hyl(GlcGal)/Hyl(Gal) was 0.480±0.150, not significantly different from the normal value of 0.474±0.088. A single sample from a pagetic vertebral body did have a ratio of 0.743, significantly greater than normal. There have been reports contending that skin of pagetic subjects is abnormal, showing features of pseudoxanthoma elasticum (17). Analysis of samples of dermis from two of our patients revealed a normal amino acid composition including a Hyl/Hyp ratio of 0.049 and 0.064, not significantly different from the normal dermal value of 0.048±0.005 (12) whereas the ratio of Hyl(GlcGal)/Hyl(Gal) in the single specimen analyzed was 1.60, also within the range of normal dermis. Thus the low ratio of Hyl/Hyp in association with the low ratio of Hyl/GlcGal)/Hyl(Gal) found in the urine of patients with extensive Paget's disease is compatible with bone collagen as the major source of degradation.

Analysis of urine from patients with thermal burns. Since the ratio of Hyl/Hyp in normal dermis, 0.048 ±0.005, is similar to that of bone but the ratio of Hyl(GlcGal)/Hyl(Gal) very much higher than bone, it would be expected that patients with thermal burns would show a pattern of urinary degradation products different from patients with Paget's disease relative to total Hyp excretion. In three adults with extensive burns the results, shown in Table V, were as predicted. Hyl/Hyp ratios were somewhat higher than expected and could be accounted for by a contribution not only from dermis but from fascia which has a higher Hyl/Hyp ratio than skin (12). Indeed, normal deep fascia obtained at necropsy from two additional normal subjects showed the ratio of Hyl/Hyp to be 0.074 and 0.068 and that of Hyl(GlcGal)/Hyl(Gal) to be 1.35 and 1.89.

DISCUSSION

Since the Hyl glycosides were first isolated from urine by Cunningham et al. in 1967 (5) they have been found in both invertebrate (18) and vertebrate collagens (6, 19). The urinary Hyl glycosides are metabolic products of endogenous collagen (19), and their excretion roughly parallels excretion of Hyp in normal and diseased subjects.

There is previous evidence that measurements of urinary Hyl glycosides provide more accurate reflections of collagen degradation than measurements of urinary Hyp (9, 20). Segrest and Cunningham (9) calculated collagen turnover equivalents from urinary Hyl glycoside levels to be two to four times larger than those calculated from levels of urinary Hyp. Assuming that these estimations are accurate, Hyl glucoside excretion would account for 50–100% of degraded collagen. An explanation could be that the gly-

TABLE IV
Chemical Composition of Pagetic Bone

	Pagetic Bone					Control
	1	2	3	4	5	
	Femur	Vertebra	Sacrum	Rib	Ilium	
Bone	Residues/1,000 residues					Vertebrae
4-Hydroxyproline	102	113	109	100	108	100.5*±5.9
Aspartic Acid	52	51	48	52	53	48.5±0.9
Threonine	21	21	19	21	21	18.0±0.9
Serine	37	38	36	39	38	36.2±0.8
Glutamic Acid	85	84	80	86	85	80.7±1.7
Proline	122	129	124	128	125	120.7±2.8
Glycine	290	277	307	281	282	311.0±3.8
Alanine	104	109	110	111	110	111.2±1.8
½ Cystine	1.4	0.6	0	0.5	0.9	0.3±0.1
Valine	26	25	23	20	26	24.2±1.7
Methionine	6.2	7.3	4.0	11.4	7.7	5.6±0.6
Isoleucine	11	11	10	11	12	10.2±0.8
Leucine	28	27	26	24	28	25.9±1.0
Tyrosine	6	4.8	2.5	5.5	5.3	4.0±0.2
Phenylalanine	15	15	13	18	16	13.6±1.0
Hydroxylysine	5.4	5.0	3.8	6.7	4.2	4.2±0.7
Lysine	28	24	28	28	20	29.0±2.8
Histidine	6.4	2.0	4.2	1.5	1.8	5.6±0.3
Arginine	52	55	51	55	56	48.5±2.4
Total hydroxylysine Glycosides, %	27	33	33	27	56	30‡
Hyl(GlcGal)/Hyl(Gal)	0.422	0.743	0.396	0.464	0.390	0.474‡±0.088
Hyl/Hyp	0.053	0.044	0.035	0.067	0.039	0.042*±0.009

* Mean±SD from Pinnell et al. (12).
‡ Pinnell et al. (7).

cosides are protected from enzymatic degradation, as are prolylhydroxyprolyl peptides (4), or, alternatively, that the specific enzymes involved in degradation are absent. Hiles and Henderson (21) found hydroxylysine kinase to be present in the liver and/or kidney of rats, mice, chickens, cow, rabbits, and two species of primates but undetectable in human liver or kidney. Measurable activity of phosphohydroxylysine phosphorylase, another enzyme which may participate in Hyl degradation, was observed in human tissues (22).

Our data support the thesis that Hyl glycosides are only partially degraded and could provide a better index of collagen metabolism than urinary Hyp by indicating the source of the degraded collagen. The fraction of Hyl that is glycosylated is approximately 0.3 in bone and skin, the putative major sources of collagen degradation. However, the urinary fraction of glycosylated Hyl/total Hyl is greater than 0.7. Since there is no other known source of Hyl glycosides other than in collagens or in collagen-like sequences, the major change in this fraction could be accounted for by a decrease in the free Hyl denominator.

Patients with severe resorptive bone disease such as Paget's disease, and urinary Hyp > 2.0 μmol/mg creatinine had a urinary Hyl(GlcGal)/Hyl(Gal) ratio of

TABLE V
Urinary Collagen Metabolites in Patients with Thermal Burns

Patient (age-sex)	Time of collection after burn	Estimated extent of burn	Hyp / creatinine	Hyl / Hyp	Hyl(GlcGal) / Hyl(Gal)
	days	%	*μmol/mg*	*mol/mol*	*mol/mol*
1 (60-M)	54	70	3.42	0.083	0.64
	85		3.01	0.108	0.71
2 (29-F)	16	50	1.96	0.185	0.60
3 (33-F)	2	88	1.48	0.087	0.71
4 (17-M)	18	60	4.80	0.064	0.68

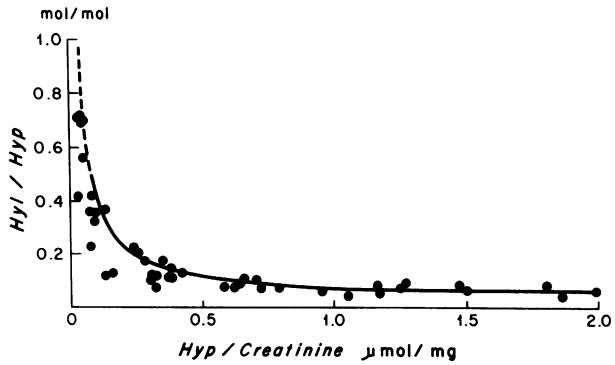

FIGURE 7 The molar ratio of urinary Hyl/Hyp as a function of urinary total Hyp normalized to creatinine. Curve is calculated assuming degradation products only from Clq and bone, as described in the text. Solid circles are data from subjects described in Figs. 1–3 but including only those with total urinary Hyp/creatinine <2.0 μmol/mg.

0.601, similar to the value for bone and lower than that for skin. Thus the constant value reached at high excretions of Hyp approximates the normal value for bone, diluting out other influences. Since the ratios for urine and bone are similar, it is unlikely that the glycosides are metabolized to a significant degree. The composition of pagetic bone is similar to that of normal bone and therefore the ratio of urinary glycosides in Paget's disease is compatible with turnover of pagetic bone. In the paient with hyperphosphatasia calcitonin decreased urinary Hyp excretion during the first 12 h after injection but during the subsequent 12 h values returned to base line. However, the Hyp/creatinine ratio even on therapy remained extremely high, beyond the range of any of the untreated pagetic subjects. Thus, no significant rise in the ratios in Hyl/Hyp or Hyl(GlcGal)/Hyl(Gal) was observed.

In patients with mild Paget's disease, both treated and untreated, urinary ratios of Hyl/Hyp and Hyl(GlcGal)/Hyl(Gal) rose with decreasing excretion of Hyp. The highest values were found in normal subjects given calcitonin acutely. In the latter group, the Hyl/Hyp ratio approached 0.75, greater than ten times values for skin or bone, and the Hyl(GlcGal)/Hyl(Gal) ratio approached 3.5, also much higher than that for skin or bone. Therefore, in normal subjects whose bone resorption is decreased by calcitonin, a different pattern of collagen metabolites is revealed. Potential sources for these metabolites could include cartilage (23) and basement membrane (24) collagens which have relatively high contents of Hyl and its glycosides, present mostly as Hyl(GlcGal). It is unlikely, however, that these collagens turn over at any significant rate.

A more likely candidate is Clq, a subcomponent of complement. It has a mol wt of 410,000 daltons and is probably composed of six noncovalently linked subunits, each of approximately 65,000 mol wt (25–27). Each of these subunits contains three covalently linked polypeptide chains which differ in amino acid sequence. Included in the sequences are regions which contain the collagen-type repeat (Gly-X-Y), Hyp, and Hyl, as well as Hyl(GlcGal) (27, 28). The Hyl/Hyp ratio reported is usually greater than 0.5 and most of the Hyl glycosides are in the disaccharide form. Kohler and Müller-Eberhard studied the metabolism of Clq in normals and in various pathologic states (29). With their data, we have calculated that Clq could account for as much as 0.08 μmol of Hyp/mg creatinine in normals and 0.68 μmol in their patient with IgE myeloma.

An attempt was made to obtain data in individuals who might be expected to have high Clq turnover (patients with high serum globulins or febrile illness). None of these patients had ratios of Hyl(GlcGal)/Hyl(Gal) or Hyl/Hyp that deviated significantly from those plotted in Figs. 1 and 3, or elevated Clq values, although turnover of Clq was not measured. None of the patients studied by Kohler and Müller-Eberhard (29) had elevated serum Clq concentrations, although all had increased turnover. Based on our previous calculation that Clq could account for approximately 0.08 μmol Hyp/mg creatinine in normals, and assuming a ratio of Hyl/Hyp in Clq of approximately 0.5 (25) and that bone is the only other source of collagen breakdown products, a theoretical curve for Hyl/Hyp as a function of total Hyp could be calculated (Fig. 7). The observed data showed a reasonable fit with the calculated curve. Thus, it is possible but not proven that metabolism of Clq is responsible for the high values for Hyl(GlcGal)/Hyl(Gal) seen in our patients when bone collagen degradation is suppressed.

An additional possible explanation for the increase in the ratio of Hyl(GlcGal)/Hyl(Gal) is that the glycosides may be derived from the higher molecular weight portion of the urinary collagen metabolites (retentate) which reflect new collagen synthesis (30). Although the ratio we have determined in preliminary studies, 1.7, is higher than that of bone collagen, the presence of this fraction alone cannot account for changes in the composition of Hyl glycosides during treatment of Paget's disease, since the rise in that urinary fraction accompanying therapy was of insufficient magnitude to affect the glycoside ratio.

The results of therapy of Paget's disease with either calcitonin or EHDP are consistent with a specific decrease in bone collagen resorption rather than collagen resorption in general. The relatively high urinary ratio of Hyl/Hyp in patients with extensive burns probably reflects involvement of collagenous structures deep to the dermis such as fascia, which have higher Hyl/Hyp ratios than the dermis. The results are thus consistent with other observations (12) that

bone collagen is the major source of urinary collagen degradation products and that skin collagen contributes little. A rapidly turning over component such as Clq could be a major contributor when the magnitude of bone degradation becomes relatively less.

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