

# Urinary Hydroxyproline Excretion in Myelofibrosis

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**Urinary hydroxyproline measurements were performed in a group of health volunteers as well as patients with cancer and myelofibrosis. Patients in whom there was no metastatic involvement of bone marrow excreted an amount of hydroxyproline not different from that of the control group. Those who had marrow metastasis produced elevated levels of hydroxyproline; the highest excretions were observed when marrow fibrosis was associated with metastasis. These results contrasted with those observed in agnogenic myeloid metaplasia patients whose excretions were equivalent to the control group. The result suggests differences in the pathogenesis of myelofibrosis and a technique potentially useful for distinguishing between patients who may otherwise be diagnostic problems.**

**S**EVERAL published studies have indicated that measurement of urinary hydroxyproline (UHP) is valuable in determining whether osseous metastases are present in patients with various types of cancer.<sup>1-5</sup> Although UHP derives almost entirely from collagen biosynthesis in bone,<sup>6-8</sup> prior studies have not indicated whether secondary marrow fibrosis (secondary MF) was present. The current study examined whether patients with secondary MF have UHP excretion levels differing from those found in patients where fibrosis is absent. In order to evaluate the role of marrow fibrosis on UHP excretions, a group of patients with agnogenic myeloid metaplasia (primary MF) was also studied.

## MATERIALS AND METHODS

UHP was measured in 9 healthy volunteers and in 43 patients admitted to The Brookdale Hospital Medical Center between July 1975 and September 1977. All patients had clinical and laboratory evaluations that were indicated by their disease state, including bone scan and survey, as well as bone marrow aspiration and biopsy. Liver biopsy was obtained in patients if the presumptive diagnosis was primary MF or necessary for treatment planning. Marrow biopsy specimens were stained by Wilder's reticulin<sup>9</sup> and a modified trichrome stain.<sup>10</sup> Marrows having a 2+ or higher score by either method were diagnosed as myelofibrosis (MF).<sup>11</sup> The study was divided into five groups.

*Group A.* Group A was composed of nine normal volunteers with no evidence of malignancy.

*Group B.* Ten patients with biopsy proven malignancy but no demonstrable bone or marrow metastasis; three breast carcinomas, two colon carcinomas, one each had carcinoma of uterus, esophagus, lung, stomach, and Hodgkin disease.

*Group C.* Thirteen patients with proven malignancy and marrow metastasis, but no demonstrable MF; nine breast carcinomas, one each had carcinoma of esophagus, prostate, and of unknown primary site.

*Group D.* Nine patients with proven malignancy, marrow metastasis, and myelofibrosis (secondary MF); five breast carcinomas, and four carcinomas of prostate. There was no obvious difference in the extent of bone and marrow metastases in group C and group D patients as determined by bone scan, bone survey, and serum alkaline phosphatase.

*Group E.* Eleven patients with agnogenic myeloid metaplasia (primary MF); eight of them had not received any therapy at the time of study, while two patients were receiving Myleran and one was being treated with androgen and corticosteroid.

All subjects ingested a diet free of meat, meat products, fish,

gelatine, ice cream, and soft candy, beginning 24 hr prior to and until urine collection was completed 24 hr later. Urine specimens were frozen under toluene as a preservative and thawed when required for analysis.

Total urinary hydroxyproline determinations were performed employing the method described by Cleary and Saunders.<sup>12</sup> Briefly, hydrolyzed urine was passed through a column of ion retardation resin to remove acid, salts, and pigments. Oxidation and chromophore development followed directly on an aliquot from the column eluate. Urine creatinine determination was performed using standard technicon autoanalyzer method. Results (UHP) were expressed as a ratio of total urinary hydroxyproline (in  $\mu\text{g}$ ) divided by urinary creatinine (in mg). Each determination was performed in triplicate and the mean value calculated. The ranges and the mean values  $\pm$  1 standard deviation for each group are reported.

## RESULTS

The results and significant statistical evaluations are displayed in Table 1. Urinary excretions of hydroxyproline in the control, nonmetastatic cancer, and primary myelofibrosis groups were essentially the same. The patients with osseous metastases excreted significantly higher amounts of UHP, the highest values being observed in those with secondary myelofibrosis.

## DISCUSSION

Current concepts in collagen metabolism suggest that urinary hydroxyproline results from degradation of an intermediary product formed in the synthesis of collagen,<sup>4,14,15</sup> although intracellular collagen destruction may play a role as well.<sup>16</sup> Elevations of UHP have been reported in several circumstances, including osseous metastases,<sup>1,17,18</sup> but prior studies have not indicated whether myelofibrosis accompanies these

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Table 1. Ratios of Total Urinary Hydroxyproline to Urinary Creatinine

Number of Patients	Group A*	Group B	Group C	Group D	Group E
1	32.06	42.36	29.74	192.91	20.90
2	25.50	36.94	87.57	209.16	32.37
3	39.21	52.07	54.44	144.96	37.22
4	42.06	43.99	102.30	169.59	23.94
5	12.85	35.49	105.06	165.62	35.91
6	19.42	52.40	124.76	249.12	24.32
7	23.64	40.47	55.03	140.47	42.22
8	43.88	12.11	96.90	120.00	3.71
9	44.73	59.45	90.21	109.70	17.38
10		62.44	67.48		35.27
11			69.71		39.81
12			42.83		
13			96.40		
Mean $\pm$ SD	31.48 $\pm$ 11.67	43.77 $\pm$ 14.40	78.47 $\pm$ 27.87	166.83 $\pm$ 44.55	28.45 $\pm$ 11.45

Comparison among groups:

Group A versus group B: not significant.

Group B versus group C:  $p < 0.01$ .

Group C versus group D:  $p < 0.01$ .

Group D versus group E:  $p < 0.01$ .

Group A versus group E: not significant.

\*See text for characteristics of each group.

changes. The results reported here confirm the elevations occurring in osseous metastases and do indicate that the presence of secondary MF is accompanied by an even greater excretion of UHP. The cause for the elevations of UHP even in the absence of tinctorial evidence for secondary MF remains speculative. Under normal conditions, collagen synthesis appears restricted to fibroblasts, although in disease states other tissues may engage in such production, a possible event when tumor metastases to marrow occurs.<sup>19-22</sup> The higher levels observed in secondary MF may reflect synthesis and/or destruction by both reactive fibroblasts as well as tumor cells.

The differences in UHP observed between patients who have primary and secondary MF are significant and of interest. If substantiated by additional studies, a technique useful for differentiation between patients with primary MF and those with secondary MF in whom tumor cells are not readily demonstrated in marrow is available.<sup>23,24</sup> Such patients, as exemplified

by those with prostatic cancer, require very different therapeutic approaches, i.e., estrogens rather than androgens.

Although the fibroblasts in primary MF have generally been considered malignant,<sup>25</sup> a recent study casts some doubt on that thesis, since determination of a glucose-6-phosphate dehydrogenase marker revealed monoclonal enzyme production from extramedullary hematopoietic tissues, while fibroblasts produced a pleuriconal enzyme. Comparative data for enzymes in secondary MF is not available, and the explanations for differences in urinary hydroxyproline excretion remains unclear.

A more complete understanding of the mechanisms of variation in urinary hydroxyproline excretion observed in cancer patients, particularly those with secondary MF, and the normal values observed in primary MF requires study of a large number of patients and a more detailed analysis of the fibroblasts and collagen produced in those disturbances.

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