URINARY EXCRETION OF HYDROXYPROLINE IN LEPROSY*

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SUMMARY

Urinary excretion of hydroxyproline has been studied in patients with different types of leprosy. The mean average daily excretion is highest in lepromatous leprosy as compared with other forms of leprosy or normal controls. The most striking feature is the "spiky" pattern of the excretion with very wide fluctuations in the daily output observed in lepromatous leprosy patients in reaction. This pattern was not seen in similar investigations in patients with a stable form of leprosy and in a normal subject. The increase in hydroxyproline excretion in lepromatous leprosy was observed in both urinary hydroxyproline fractions studied (HP Fraction 1 and HP Fraction 2) but the increase in HP Fraction 2 was more significant.

Hydroxyproline, a non-essential imino acid occurs in the body almost exclusively in collagen and it may be considered an in vivo label of collagen. In the absence of an exogenous source of collagen, the urinary excretion of hydroxyproline in the adult human is more or less constant, reflecting the small turnover rates of collagen in a normal individual. In growing children there is a higher excretion of hydroxyproline. Thus, many investigators have studied urinary hydroxyproline and have employed it as an important index of collagen metabolism.

It was previously found that there are at least two bound hydroxyproline-containing fractions in human urine. One of these fractions was retained on a cationic exchange resin (Dowex-50, H+) and it was found to be the major urinary fraction of hydroxyproline (designated as HP Fraction 1). A small fraction of bound hydroxyproline which was designated as HP Fraction 2, was not retained on a cationic exchange resin and a glycopeptide was isolated from this fraction.

In view of the well recognized similarities between the clinical and immunological features of acute rheumatoid arthritis and reactive phases of lepromatous leprosy, a study of the pattern of urinary excretion of hydroxyproline in these reactive phases of lepromatous leprosy was undertaken. In addition, it was thought

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desirable to study the urinary excretion pattern on a longitudinal basis in different phases of the disease to see if there is a basic change in those patients with clinically unstable forms of the disease as compared with those with clinically stable forms. The present paper deals with the excretion of the two urinary fractions cited above in various forms of leprosy.

MATERIALS AND METHODS

The patients were hospitalized and were on a standard hospital vegetarian diet. The normal control subjects belonged to the hospital staff. Urine samples (24 h) were collected using toluene and chloroform as preservatives. Suitable aliquots of the urine were processed after acidification by chromatography on Dowex-50×8, H⁺ resin columns to obtain HP Fraction 1 and HP Fraction 2 (ref. 8). The amount of hydroxyproline in the two different fractions was estimated by the Neuman and Logan method after hydrolysis in sealed tubes with 6 N HCl at 140° for 3 h. In some of the experiments the hydrolyzates of whole urine were used for hydroxyproline estimation.

RESULTS

Long-term studies in a normal control subject and in a tuberculoid leprosy patient

The urinary excretion of hydroxyproline on consecutive days was studied in a normal subject and in a patient with tuberculoid leprosy, a stable form of the disease.

![Graph](image1)

Fig. 1. Urinary hydroxyproline excretion in a normal control subject. Closed circles: total hydroxyproline excretion. Open circles: hydroxyproline in HP Fraction 2.

![Graph](image2)

Fig. 2. Urinary hydroxyproline excretion in a tuberculoid leprosy patient. Only the total excretion is given.

The results are given in Fig. 1 for the normal subject and in Fig. 2 for the patient. It was found that the daily excretion of hydroxyproline did not show much variation although the values were somewhat higher in the tuberculoid leprosy patient.

**Long-term studies in lepromatous leprosy**

It was initially observed in a few random analyses that patients with lepromatous leprosy in reaction excreted very large quantities of hydroxyproline in the urine. However, when compared to the excretion in a normal subject or in a leprosy patient with a stable form of the disease, there were very wide fluctuations in the daily output. This was confirmed in a series of detailed follow-up studies. The results on the daily excretion of hydroxyproline in four cases out of a total of seven studied are presented in Figs. 3–6. It can be seen from this data that there is a very marked varia-
Fig. 5. Hydroxyproline excretion in a lepromatous leprosy patient with history of chronic erythema nodosum leprosum. Day 1 to day 11 had numerous erythema nodosum leprosum which gradually subsided. Only the total excretion is given.

Fig. 6. A long-term study of the daily excretion of hydroxyproline in a fourth case of lepromatous leprosy and recurrent attacks of erythema nodosum leprosum. Closed circles: Total hydroxyproline excretion. Open circles: Hydroxyproline excretion in HP Fraction 2.

The average daily excretion of hydroxyproline in the urine in a total of 7 cases of lepromatous leprosy studied is given in Table I. The daily excretion in each case covered a very wide range of values and the mean average daily excretion was about 76 mg/day.

Hydroxyproline excretion in other types of leprosy

The excretion of hydroxyproline was also studied in other types of leprosy and in two groups of controls-normal subjects and patients with congenital sensory radicular neuropathy. The results are summarized in Table II. The mean average daily excretion of hydroxyproline was 75.9 mg/day in lepromatous leprosy and this value is very much higher than the values obtained for the controls and patients with other types of leprosy.

From the foregoing results it is clear that the total urinary output of hydroxyproline is markedly increased in lepromatous leprosy patients in reaction. This increase occurred in both urinary fractions studied, since the amount of hydroxyproline in HP fraction 2 closely paralleled the total excretion, as can be seen from Figs. 3–6. It should also be noted that some of the peak values in lepromatous leprosy represent

TABLE I

AVERAGE DAILY EXCRETION OF HYDROXYPROLINE IN LEPROMATOUS LEPROSY PATIENTS IN REACTION

<table>
<thead>
<tr>
<th>Case No.</th>
<th>No. of daily specimens</th>
<th>Hydroxyproline mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg/day</td>
</tr>
<tr>
<td>1</td>
<td>37</td>
<td>61 (10-132)</td>
</tr>
<tr>
<td>2</td>
<td>41</td>
<td>74 (0-123)</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>72 (10-254)</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>41 (10-100)</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>60 (44-81)</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>103 (24-143)</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>120 (91-153)</td>
</tr>
</tbody>
</table>

The data for Cases 1, 2, 3 and 4 is taken from Figs. 3-6 and the other values were obtained in a separate series from consecutive 24-h urine specimens. The mean average daily excretion of the 7 cases was 75.9 mg/day.

TABLE II

URINARY HYDROXYPROLINE EXCRETION IN VARIOUS TYPES OF LEPROSY AND IN CONTROLS

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>Hydroxyproline mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (normals)</td>
<td>18</td>
</tr>
<tr>
<td>Congenital sensory radicular neuropathy</td>
<td>5</td>
</tr>
<tr>
<td>Leprosy</td>
<td></td>
</tr>
<tr>
<td>Borderline</td>
<td>11</td>
</tr>
<tr>
<td>Tuberculoid</td>
<td>11</td>
</tr>
<tr>
<td>Indeterminant</td>
<td>4</td>
</tr>
<tr>
<td>Lepromatous (not in reaction)</td>
<td>9</td>
</tr>
<tr>
<td>Lepromatous (in reaction)*</td>
<td>7</td>
</tr>
</tbody>
</table>

* The mean of the average daily excretion was calculated from the data in Table I.

remarkably high levels of urinary hydroxyproline. The amount of hydroxyproline in HP Fraction 2 in the urine of lepromatous leprosy patients (mean, 16 mg/day) was found to be higher (P < 0.01) than in the control leprosy subjects (mean, 8 mg/day) and in the normal controls (mean, 5.3 mg/day).

During the course of this study the patients were under close clinical surveillance to see if there was a correlation between the clinical state and the elevated excretion of hydroxyproline. It may be mentioned that the plateau regions in Figs. 3 and 5 roughly corresponded to a quiescent period of the patients' erythema nodosum leprosum.

DISCUSSION

Urinary hydroxyproline excretion has been extensively employed as an index of collagen metabolism. In the general group of disorders known as collagen diseases, the urinary levels of hydroxyproline are commonly normal\textsuperscript{11}. But, increased excretion of hydroxyproline has been reported in cases of active rheumatoid arthritis and active scleroderma\textsuperscript{12}, dermatomyositis\textsuperscript{13}, acute rheumatic fever\textsuperscript{14}, in patients with

oversecretion of growth hormone\textsuperscript{14}, in hyperthyroidism\textsuperscript{18} and in hyperparathyroidism (ref. 16). Elevated levels of urinary hydroxyproline have also been observed in tropical sprue and in some malabsorptive states\textsuperscript{17,18}.

From the results presented in this paper it is clear that urinary excretion of hydroxyproline is markedly increased in lepromatous leprosy in reaction. Urinary hydroxyproline can be separated into at least two broad fractions by ion exchange chromatography\textsuperscript{6-8}. The acidic fraction, HP Fraction 2, constituted only a small percentage of the total urinary hydroxyproline in the normal subjects while it appears to be a significant fraction in lepromatous leprosy in reaction. The increase in the hydroxyproline excretion occurred in both urinary fractions, although in general the percentage increase was relatively greater in HP Fraction 2.

The most striking feature is the pattern of daily urinary hydroxyproline excretion observed in lepromatous leprosy patients in reaction. This "spiky" pattern was not observed in the normal control nor in patients with a stable form of leprosy. The diagnostic value of this observation has not yet been evaluated.

A casual inspection of Figs. 3-6 will show regions where the hydroxyproline excretion was much lower than the peak values and where the daily variation was minimal. In the limited survey made here it was found that these regions roughly corresponded to quiescent periods with no fever and with resolution of erythema nodosum leprosum. Further studies may be necessary to establish a more direct correlation.

The increased excretion of urinary hydroxyproline in leprosy patients suggests an enhanced breakdown or turnover of collagen in this disease. The marked increase and fluctuations in hydroxyproline excretion in lepromatous leprosy patients in reaction may be a reflection of altered collagen metabolism associated with the reactive phases of leprosy. It may be of interest to note that some of the peak values for urinary hydroxyproline observed in leprosy are similar in magnitude to those of thermal burns where massive collagen destruction is known to take place\textsuperscript{19,20}.

The actual mechanism of elevation of urinary hydroxyproline in the leprosy patients in reaction is not known. The following possibilities, at present speculative, may be considered: (a) during the acute reactive episode there are associated acute inflammatory lesions of skin, muscle and synovial membranes. This can be compared to the changes in rheumatoid arthritis\textsuperscript{10} and may result in accelerated breakdown of collagen; (b) the *Mycobacterium leprae* may act through a collagenase released by it, especially since reactive phases of leprosy are associated with breakdown of *M. leprae*. This possibility is suggested by the studies on Madura mycosis ("madura foot") by Rippon and Lorincz\textsuperscript{21} who traced the etiology of the disease to the elaboration of a collagenase by the associated organism (*Streptomyces madurae*) and consequent breakdown of body collagen. In two cases of madura foot which we studied (unpublished data, Cherian and Radhakrishnan) the excretion of hydroxyproline was about 130 mg/day, similar to the levels observed in leprosy; (c) the activity of the mammalian collagenase in the patients may be enhanced either through the mediation of *M. leprae* or through some other mechanism; and (d) both synthesis and breakdown of collagen may be affected. Such an accelerated turnover of collagen may be due especially to elevated protocollagen hydroxylase which is known to increase during wound healing\textsuperscript{22}. Work is in progress to check the validity of these hypotheses.

It has been previously reported\textsuperscript{7} that the urinary fraction HP Fraction 2

contains mainly an acidic glycopeptide. The pronounced increase of this glycopeptide fraction in leprosy may be of significance, since there are reports on the increased excretion of glycopeptides and glycoproteins in various inflammatory conditions.  

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Requests for reprints should be sent to A.N.R.

REFERENCES
