Effect of Dietary Purine Restriction, Allopurinol, and Oxipurinol on Urinary Excretion of Ultraviolet-Absorbing Compounds

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We demonstrate that purine restriction is associated with a decreased excretion of a number of uv-absorbing compounds—including uric acid, hypoxanthine, xanthine, adenine, pseudouridine, and 5-acetylamino-6-amino-3-methyluracil—indicating that these compounds originate, at least partly, exogenously. Administration of allopurinol and oxipurinol is associated with a decreased excretion of uric acid and an increased excretion of hypoxanthine and xanthine, as well as the excretion of the previously identified products of allopurinol and oxipurinol metabolism. Moreover, these drugs further decrease excretion of 5-acetylamino-6-amino-3-methyluracil, increase uracil excretion slightly, and strikingly increase orotic acid and orotidine excretion. No change in pseudouridine excretion was observed. The ultraviolet-analyzer is a valuable tool for detecting qualitative changes in the excretion of ultraviolet-absorbing compounds in the urine; with it, identified peaks can be semiquantitated.

Additional Keyphrases • 5-acetyl-6-amino-3-methyluracil • orotic acid • orotidine • pseudouridine • uric acid and its metabolic precursors • adenine • allopurinol ribonucleoside • anion-exchange chromatography • normal values

The potential importance of identifying and quantitating the purine and pyrimidine derivatives in human urine has become increasingly apparent. Unusually large quantities of some of these compounds are known to be excreted in a variety of inborn errors of metabolism such as gout (1, 2), the Lesch-Nyhan syndrome (3, 4), xanthinuria (5), and orotic aciduria (6), as well as in several acquired disorders, including leukemia (7, 8) and vitamin B₁₂ or folic acid deficiency (9).

However, under normal circumstances, most purines and pyrimidines other than uric acid appear to be excreted in relatively small quantities, and methods for quantitating their excretion are laborious. These methods generally include specific enzymatic assays (10–12); less specific chemical reactions (9, 13); or, more commonly, multiple-separation techniques with final identification by paper, column, or thin-layer chromatography (14–16). In addition to the tediousness of most of these techniques, the first two methods would not detect unusual or unexpected compounds in the urine; the last method is frequently only semiquantitative.

Development of the uv analyzer by members of the Body Fluids Analyses Program of the Oak Ridge National Laboratory (17) has provided a promising new tool, which may circumvent many of the technical problems that hinder progress in this field. The device consists of an automated, high-resolution anion-exchange chromatographic column coupled with an ultraviolet detection system and recorder. By using this system, one can resolve a 2-ml urine sample into 100 or more uv-absorbing peaks. At the present time, seven purine and pyrimidine compounds have been identified in normal urine with this instrument: pseudouridine, uracil, 5-acetylamino-6-amino-3-methyluracil, hypoxanthine, xanthine, adenine, and uric acid (17) (Table 1).

Here, we have evaluated the qualitative effect on the uv chromatographic pattern obtained for urine after restriction of dietary purines and administration of two purine analogs, allopurinol, and oxipurinol. In addition, the quantitative effect of these manipulations on the excretion of these seven purine and pyrimidine compounds has been assessed.

Materials and Methods

The uv analyzer, described elsewhere (18, 19), will not be discussed at length here. The proto-
Table 1. Peak Identification on Chromatograms from the UV Analyzera

<table>
<thead>
<tr>
<th>Compound</th>
<th>Elution vol, ml</th>
<th>Method of ident.a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudouridine</td>
<td>42</td>
<td>EP, UV, GC</td>
</tr>
<tr>
<td>Uracil</td>
<td>69</td>
<td>EP, UV, GC, MS</td>
</tr>
<tr>
<td>5-Acetylalino-6-amino-3-</td>
<td>75</td>
<td>EP, UV, GC, MS</td>
</tr>
<tr>
<td>methyluracilc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>142</td>
<td>EP, UV</td>
</tr>
<tr>
<td>Allopurinol ribonucleoside</td>
<td>210</td>
<td>EP, UV</td>
</tr>
<tr>
<td>Xanthine</td>
<td>239</td>
<td>EP, UV</td>
</tr>
<tr>
<td>Adenine</td>
<td>256</td>
<td>EP, UV</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>310</td>
<td>EP, UV, GC, MS</td>
</tr>
<tr>
<td>Oxpipurinol</td>
<td>324</td>
<td>EP, UV, GC, MS</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>424</td>
<td>EP, UV, GC, MS, IR</td>
</tr>
</tbody>
</table>

a Revised from (19).

b EP, elution position; UV, ultraviolet spectroscopy; GC, gas chromatography; MS, mass spectrometry; IR, infrared spectroscopy; CT, chemical testing.

c Purity of peak must be regarded as tentative.

Type used in the present study is the Mark II. Briefly, a 2-ml sample of urine, which has been treated only by filtration through a 0.2-μ Millipore filter, is loaded onto the column with the automatic sample-injection valve. The sample is then eluted from the strongly basic anion-exchange resin by slowly increasing the concentration of ammonium acetate, pH 4.4, from 0.015 to 6 mol/liter. The total elution process takes 40 h at a flow rate of 35–40 ml/h. Ambient temperature is used during the first 11.5 h of the run; for the remainder of the run the column jacket temperature is 60°C.

The eluate from the column passes through a recording double-beam spectrophotometer (1-cm light path cuvets) that has been modified to operate sequentially at four different wavelengths, and then via a volumetric siphon into waste or fraction collector. Solute concentration for a 2-ml sample was estimated by the method of Anderson (20) as follows:

\[
\text{Solute concentration (μg/ml)} = \frac{\text{MW} \times \text{c}}{2 \times \text{H} \times \text{w} \times \text{min} \times \text{ml/min} \times \text{c}}
\]

where \(\text{MW}\) is the molecular weight of compound; \(\text{H}\), the height in absorbance units; \(\text{c}\), the molar absorptivity at wavelength used; \(\text{w}\), min, the width of peak at half height, in min; \(\text{c}\) is 1.064.

5-Acetylalino-6-amino-3-methyluracil was a generous gift from Dr. Kay Fink. Allopurinol [4-hydroxypyrazolo-(3,4-d)-pyrimidine], oxipurinol [4,6-dihydroxypyrazolo-(3,4-d)-pyrimidine] and allopurinol ribonucleoside were obtained from Dr. Gertrude Elion (Burroughs Wellcome Research Laboratories, Tuckahoe, N.Y.). Hypoxanthine, xanthine, adenine, uric acid, uracil, and pseudouridine were obtained from commercial sources. Uricase and xanthine oxidase were purchased from Worthington Biochemical Corp., Freehold, N.J. Urinary uric acid and oxypurines (hypoxanthine plus xanthine) were determined by specific enzymatic assays (10, 11). Creatinine content of urine was determined by the method of Taussky (21).

The three patients in our study were admitted to a clinical research facility. Each patient had a long history of gouty arthritis but no tophi were present; each had essentially normal renal function as assessed by creatinine clearance. The pathogenesis of their hyperuricemia had not been established although previous studies had indicated that they excreted normal quantities of uric acid. Each patient was instructed to discontinue all medication one week before admission. On admission to the hospital, each was given a diet containing 2600 calories, and 70 g of protein, with no protein restriction. After two days, a diet essentially free of purines but containing the same caloric and protein content was substituted. After allowing at least five days for equilibration on this diet, each patient was started on allopurinol (200 mg/day). After two or three days, this was increased to 400 mg/day in two patients. After an additional three days, allopurinol was discontinued, and oxipurinol was started at a dose of 200–400 mg/day. All medications were given in equally divided doses every 6 h. All urine was collected at room temperature with 3 ml of toluene as preservative and analyzed for uric acid and creatinine. Selected samples were assayed for oxypurines. Aliquots of urine to be analyzed on the uv analyzer were frozen at −20°C for later study.

The initial urine sample on patient E.D.B. contained large quantities of oxipurinol and, therefore, could not be used as a control sample as originally planned. In addition, the creatinine content of the 24-h urine obtained during the period of purine restriction without drugs in patient W.J. indicated that it had been an incomplete collection. To adjust for this, a correction factor, based on the patient's mean creatinine excretion during 10 days, was applied to the values obtained in this particular sample.

Results

Accuracy and Reproducibility

The recovery of five different compounds (hypoxanthine, adenine, allopurinol, oxipurinol, and allopurinol ribonucleoside) was determined at two or three different concentrations. The recovery of any one compound ranged from as high as 111% to as little as 64%. The mean recovery of the five compounds tested ranged from 78 to 92% (Table 2).

Uric acid excretion, determined by evaluation of peak area after elution from the anion-exchange column, was compared to values obtained by a
specific enzymatic spectrophotometric method on the same urine sample. In 13 different samples from three patients, the values obtained with the UV analyzer ranged from 49 to 91% of those determined by the enzymatic assay (Table 3). The former method, therefore, gave inconsistent results and, in each case, underestimated the true uric acid excretion.

Excretion of the oxypurines, hypoxanthine, and xanthine, was determined by an enzymatic assay that measures the total concentration of both compounds. The values obtained by this method were compared with the values obtained on the same urine sample for hypoxanthine and xanthine from the ultraviolet analyzer (Table 4). The amounts found, as measured by the UV analyzer, were 73 to 90% as great as the quantities determined to be present enzymatically.

Uric Acid

When the purine-free diet was begun uric acid excretion by two patients decreased 52 and 303 mg/day as assessed by enzymatic assay, 48 and 239 mg/day by the UV analyzer (Table 3). As expected, allopurinol therapy produced a further decrease in uric acid excretion, ranging from 64–87 mg/day. The changes found with the UV analyzer ranged from a decrease of 56 mg/day to an increase of 4 mg/day. If the dose of allopurinol was increased or oxipurinol substituted for allopurinol, the resulting small changes in uric acid excretion were detected equally well by the enzymatic method and the UV analyzer.
produced a striking increase in the excretion of this compound to values exceeding 20 mg/day.

Adenine

Urinary adenine excretion by two patients was 5.9 and 6.2 mg/day on a regular diet (Table 5), 0.9 and 4.6 mg/day while on the purine-free diet. The third patient excreted 5.0 mg/day on this diet. Allopurinol and oxipurinol had no discernible effect on the excretion of adenine in one patient but may have increased it slightly in the other two (W.J. and B.D.C.).

Pseudouridine

The major pyrimidine detected in the urine is pseudouridine. The excretion of this compound ranged from 54–113 mg/day on an unrestricted diet and from 36–56 mg/day on a purine-free diet, which represented a decrease in all three patients (Table 5). Allopurinol and oxipurinol had no discernible effect on pseudouridine excretion in two patients; its excretion may have increased in the third (W.J.).

Uracil

Uracil was undetected in the urine of two of the three patients on an unrestricted diet and in one of the three patients on a purine-free diet (Table 5). Small quantities of uracil were detectable in one patient on a regular diet and in two patients during purine restriction; values in these three urine samples ranged from 2.9–3.4 mg/day. Dietary manipulation had no discernible effect. Administration of allopurinol and oxipurinol produced a modest increase in uracil excretion in two of the three patients.

5-Acetylamino-6-amino-3-methyluracil

The presence of 5-acetylamino-6-amino-3-methyluracil has only recently been recognized as a major uv-absorbing component in human urine (22). The excretion of this compound in two patients on a regular diet ranged from 22.2–41.3 mg/day. On the purine-free diet excretion decreased to undetectable levels in one patient, 3.3 mg/day in another (Table 5). The third subject (E.D.B.) continued to excrete 26.5 mg/day even after five days of purine restriction. Allopurinol decreased the excretion of 5-acetylamino-6-amino-3-methyluracil to undetectable levels in both of these patients in whom this compound had been detected during dietary purine restriction; it could not be detected in any patient when oxipurinol was substituted for allopurinol in all three.

Allopurinol Metabolites

Treatment with allopurinol was associated with excretion in the urine of allopurinol and of allopurinol ribonucleoside and oxipurinol, known metabolites of the drug. Oxipurinol administration was followed by oxipurinol excretion in the urine, but allopurinol and allopurinol ribonucleoside were not detected. The only other known metabolite of allopurinol and oxipurinol therapy, oxipurinol ribonucleoside (23) was not detected in the urine after treatment of the three patients with either drug. Attempts to quantitate the excretion of these individual metabolites were unsuccessful.

On treatment with allopurinol and oxipurinol, two substantial new peaks appeared (26 and 123 ml after the elution of uric acid) that could not be attributed to the known effects of these drugs. These two peaks were identified as oortidine and orotic acid.

Discussion

Use of the uv analyzer to assess urinary excretion of minor purine and pyrimidine components represents a substantial improvement over the previous methods. The studies presented here indicate, however, that this technique should be considered semiquantitative at the present time. The most variable results were related to quantitation of uric acid excretion. Poor recovery of this com-
Table 6. Purines and Pyrimidines Excreted in Human Urine

<table>
<thead>
<tr>
<th>Compound</th>
<th>Range, present study, mg/day</th>
<th>Range, literature, mg/day</th>
<th>Ref. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudouridine</td>
<td>36-113</td>
<td>49-134</td>
<td>32, 25, 7, 31</td>
</tr>
<tr>
<td>Uracil</td>
<td>ND-3.4</td>
<td>3.7-13.7</td>
<td>32, 7, 31</td>
</tr>
<tr>
<td>5-Acetylamino-6-amino-3-methyluracil</td>
<td>22.2-41.3</td>
<td>Not quantitated</td>
<td>22, 25</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>1.7-9.7</td>
<td>3.1-12.5</td>
<td>38, 8, 24</td>
</tr>
<tr>
<td>Xanthine</td>
<td>2.3-8.6</td>
<td>3.7-8.7</td>
<td>8, 24, 31</td>
</tr>
<tr>
<td>Adenine</td>
<td>0.9-8.0</td>
<td>0.3-4.0</td>
<td>32, 8, 24</td>
</tr>
<tr>
<td>Uric acid</td>
<td>252-374</td>
<td>140-590</td>
<td>2</td>
</tr>
</tbody>
</table>

Not identified in present study*

- 7-Methylguanine
- 8-Hydroxy-7-methylguanine
- Guanine
- 1-Methylhypoxanthine
- 6-Succinoamino-purine
- N²-Methylguanine
- 4-Amino-5-imidazolecarboxamide

* Does not include known metabolites arising from caffeine ingestion.

Pseudouridine probably resulted from the high absorbance reflecting the high concentration of this compound in human urine. Peak height becomes impossible to estimate accurately when the absorbance exceeds 1000 units, as it often does for uric acid in this system. Attempts to resolve this problem compromise the overall versatility of the instrument in the analysis of the minor UV-absorbing components in the urine; such a compromise is unnecessary since other methods for uric acid determination are relatively simple and accurate. Measurements of minor components in urine made with this system are comparable in accuracy and reproducibility to those reported for other, longer methods that require both pretreatment of the urine and ion-exchange chromatography (14, 24).

The 24-h excretion of the seven purines and pyrimidines quantitated in the present study is compared with values reported in previous studies conducted under similar conditions (Table 6). Clearly, these seven compounds represent the major purine and pyrimidine end-products excreted in the urine, and our values agree well with those reported by others.

Seven purine bases detected in normal urine in earlier studies were not identified in the present study. With the exception of 7-methylguanine, these products are regularly excreted in quantities of less than 1 mg/day. The elution position of most of these latter compounds is known; and it is likely that if any were excreted in supranormal amounts, they would be relatively easy to detect.

When the patients were fed a diet free of purines, excretion of a number of compounds markedly decreased. It is well established that purine restriction decreases serum and urinary uric acid values, and it is not surprising that hypoxanthine, xanthine, and adenine excretion are similarly affected since at least two of these three compounds are immediate precursors of uric acid. Urinary pseudouridine excretion was decreased in two of the three patients by this diet and this is consistent with the observations of others (7).

The most striking effect of dietary purine restriction, however, was on the excretion of 5-acetylamino-6-amino-3-methyluracil. This compound, only recently identified as a component of normal urine (22, 25), appeared to originate almost entirely exogenously in two patients since its excretion diminished from 20-40 mg/day to nearly undetectable amounts in these subjects while they were on the purine-free diet. The significance of the apparent persistence of this compound in the urine of one patient on purine restriction is now unknown. In addition to the measured effects of dietary purine and pyrimidine restriction, excretion of many other substances, yet to be identified, was also apparently decreased. This rather striking effect of diet on the urinary excretion of UV-absorbing compounds served to re-emphasize the concept that diet and drug intake must be rigidly controlled if this system is to be used effectively.

Allopurinol, an analog of hypoxanthine, is an effective inhibitor of xanthine oxidase (xanthine: oxygen oxidoreductase, EC 1.2.3.2) (26); this enzyme catalyzes the conversion of hypoxanthine to xanthine and xanthine to uric acid (Figure 1), and has been widely and successfully used to treat gout and other hyperuricemic states (27, 28). However, in addition to its well-known effect on xanthine oxidase, recent in vitro studies suggest that it may also affect other enzyme systems and biochemical pathways (29, 30). For this reason, the effect of this compound on the excretion of UV-absorbing compounds in the urine was assessed in three patients. Its inhibition of xanthine oxidase was confirmed: uric acid excretion decreased and xanthine excretion increased. The widely variable effect of allopurinol on hypoxanthine excretion is not readily explained. Although the conversion of hypoxanthine to xanthine is also inhibited by allopurinol, hypoxanthine usually does not accumulate because it is rapidly converted to inosinic acid by the enzyme hypoxanthineguanine-phosphoribosyltransferase (IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8). Patients who lack this enzyme will markedly increase their hypoxanthine excretion when given allopurinol (4).
However, the activity of erythrocyte hypoxanthine–guanine phosphoribosyltransferase was within the normal limits in all three of these patients, which indicates that this is not the source of the variability observed.

The major products of allopurinol metabolism include oxipurinol and allopurinol ribonucleoside (Figure 2). Allopurinol and these two metabolites were identified in the urine from the three patients studied. A minor metabolite, oxipurinol ribonucleoside, was not identified in any of the urine samples obtained after allopurinol therapy was started.

On allopurinol administration, uracil excretion increased in all three patients. A similar effect of allopurinol has also recently been noted by Simmonds (31) in two patients, although the magnitude of the increased uracil excretion was apparently much greater in her patients than we observed. In contrast to her findings, however, we observed no decrease in pseudouridine excretion.

The excretion of 5-acetylamino-6-amino-3-methyluracil was measurable in only two of the three patients on a purine-free diet. However, in both of these patients, allopurinol therapy decreased the excretion of this compound until it could not be detected in the urine. This suggests that xanthine oxidase may be important in the formation of this compound. This is supported by the observation that a patient with xanthinuria, which is characterized by a genetic deficiency of xanthine oxidase, failed to excrete this compound in the urine, even on an unrestricted diet (31).

Two UV-absorbing peaks, which could not be accounted for by the known metabolism or metabolic effects of allopurinol, were observed in all three patients after they began allopurinol therapy. These peaks represent a striking increase in the urinary excretion of orotidine and orotic acid. Although the exact mechanism responsible for these changes has not been established, it is clear that allopurinol or, more likely, one of its metabolites, significantly interferes with de novo pyrimidine biosynthesis (33, 34).

Oxipurinol, one of the major products of allopurinol metabolism, produced changes similar to those observed after allopurinol therapy except that its administration, as expected, was not followed by the excretion of allopurinol or allopurinol ribonucleoside.

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References


