Purine metabolism and immunodeficiency: urinary purine excretion as a diagnostic screening test in adenosine deaminase and purine nucleoside phosphorylase deficiency

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Summary
1. We have compared urinary purine excretion by two different methods in three separate pediatric disorders of purine metabolism: purine nucleoside phosphorylase deficiency, adenosine deaminase deficiency and adenine phosphoribosyltransferase deficiency.

2. The abnormal purines identified in each case were specific for the defect and directly related to it: adenine in adenine phosphoribosyltransferase deficiency; the abnormal nucleosides inosine, guanosine and their corresponding deoxyribosides in purine nucleoside phosphorylase deficiency; deoxyadenosine in adenosine deaminase deficiency, the latter having previously been identified erroneously as adenine after degradation in the acidic conditions used.

3. Deoxyriboside excretion was specific for the two defects associated with immunodeficiency: adenine for adenine phosphoribosyltransferase deficiency and 2,8-dihydroxyadenine urolithiasis. The results obtained by a quantitative method were reflected in a simple rapid qualitative technique, isotachophoresis of the urine.

4. Purine overexcretion (principally inosine) was evident only in purine nucleoside phosphorylase deficiency, which emphasizes the importance of hypoxanthine salvage for the overall control of purine production in man. In none of these disorders was any inhibition of pyrimidine biosynthesis as reflected by oroticaciduria noted. Orotic acid excretion was within normal limits in all cases.

5. From these results it is suggested that the associated immunodeficiency in adenosine deaminase deficiency and purine nucleoside phosphorylase deficiency relates directly to the intracellular accumulation of 2'-deoxyribosides and not indirectly to an inhibitory effect on pyrimidine metabolism.

Key words: adenine, adenine phosphoribosyltransferase, adenosine deaminase, deoxyadenosine, deoxyguanosine, deoxyinosine, immunodeficiency, nucleoside phosphorylase.

Abbreviations: ADA, adenosine deaminase; APRT, adenine phosphoribosyltransferase; PNP, purine nucleoside phosphorylase.

Introduction
Within the past 5 years complete deficiencies of three different enzymes concerned with the catabolism and 'salvage' of purines have been described in children; two have been associated with severe immunodeficiency (Giblett, Anderson, Cohen, Pollara & Meuwissen, 1972; Giblett, Amman, Sandman, Wara & Diamond, 1975) and one with urolithiasis (Van Acker, Simmonds, Potter &...
Cameron, 1977). Of the former, adenosine deaminase (EC 3.5.4.4, ADA) deficiency is accompanied by severe impairment of both T and B cell lymphocyte function, whereas purine nucleoside phosphorylase (EC 2.4.2.1, PNP) deficiency is associated predominantly with defective T cell response. Affected children are leucopenic and may die within the first year of life of overwhelming infection unless successful transplant or therapy with immunocompetent cells can be achieved. By contrast, children with adenine phosphoribosyltransferase (EC 2.4.2.7, APRT) deficiency are clinically normal, apart from urolithiasis: they are not immunodeficient and the stone formation can be treated successfully with allopurinol (Van Acker et al., 1977).

The diagnostic biochemical feature of homozygotes for APRT deficiency is the excretion of adenine and its hydroxy metabolites in quantity in the urine: the 2,8-dihydroxymetabolite is even more insoluble than uric acid and is responsible for the stone formation (Simmonds, Van Acker, Cameron & Snedden, 1976).

The first definitive reports of the metabolites excreted in urine in the two defects associated with immunodeficiency, ADA deficiency and PNP deficiency, were published recently by workers in the Netherlands and the United States. The feature characteristic of PNP deficiency was the replacement of the normal purine end product, uric acid (Fig. 1), by the precursor nucleosides, inosine, guanosine and their deoxy-analogues (Stoop, Zegers, Hendrickx, Siegenbeek van Heukelom, Staal, de Bree, Wadman & Ballieux, 1977; Cohen, Doyle, Martin & Amman, 1976a); the deoxynucleosides having earlier escaped detection because of their instability in the acidic conditions used (Cohen, Martin & Amman, 1976b). The abnormal metabolite found in ADA deficiency was not adenosine but adenine (Mills, Schmaulstieg, Trimmer, Goldman & Goldblum, 1976). This finding was interesting because we had been developing a new technique, isotachophoresis of the urine, which could detect homozygotes for APRT deficiency by the adenine excreted. The method was simple, rapid and produced a result from only a few microlitres of sample in less than an hour. In view of the above report it was obvious that this technique might also be useful in distinguishing the small subgroup of ADA-deficient children from other patients with severe combined immunodeficiency and from children with PNP deficiency.

We therefore investigated the potential of isotachophoresis as a single screening test for these disorders, in collaboration with Dr G. C. Mills of the University of Texas, and Dr S. K. Wadman of the University of Utrecht. The results obtained have been confirmed by an established quantitative technique (Simmonds, 1969a) and form the subject of this report.

**Methods**

Urinary purines were measured by the two methods in single samples shipped by air in solid carbon dioxide and stored at —20°C. Clinical details of the two children [L.L. with ADA deficiency (Mills et al., 1976) and R.V. with PNP deficiency (Stoop et al., 1977)] have been pub-

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**Fig. 1 Metabolic pathways for the catabolism of deoxyribonucleotides as distinct from ribonucleotides in man:**

the absence of an alternative catabolic route for deoxyadenosine at nucleotide level could result in dATP accumulation and inhibition of DNA synthesis in adenosine deaminase (ADA) deficiency. 1, Adenylate deaminase; 2, 5'-nucleotidase; 3, adenosine kinase; 4, deoxycytidine kinase. APRT, adenine phosphoribosyltransferase; HGPRT, hypoxanthine—guanine phosphoribosyltransferase; NP, purine nucleoside phosphorylase.
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FIG. 2. Scans obtained at 254 nm by isotachophoresis of the urine over a 45 min period (chart speed 8 cm/min): (a) in a control child; (b) in purine nucleoside phosphorylase deficiency; (c) in adenine phosphoribosyltransferase deficiency; (d) in adenosine deaminase deficiency. Details of the conditions used are given in the Methods section.

lished, as have the details for the patient (B.Dh.) with APRT deficiency (Van Acker et al., 1977).

Details of the anion-exchange chromatography method (which was modified to include an HCl gradient (0-001–0-1 mol/l), and in which deoxyadenosine was eluted before the gradient and thus also separately from adenine) have been published (Simmonds, 1969a, b). Orotic acid was estimated by an automated colorimetric method (unpublished) which did not distinguish orotic acid from orotidine.

The instrument used for isotachophoresis was an LKB 2127 Tachophor (LKB Instruments Ltd, Selsdon, South Croydon, U.K.). The application of the technique of isotachophoresis for the identification of urinary purines and pyrimidines has been worked out by us (A. Sahota, H. A. Simmonds & R. Payne, unpublished work). In this technique the urinary components separate according to their net mobility, being moved at constant velocity by voltage increment in a jacketed Teflon capillary (43 cm in length, 0.5 mm internal diameter). The apparatus has few moving parts and does not require an expert for operation. Urine (diluted if necessary; 1–5 μl) was introduced through a septum at the terminating electrode with a Hamilton syringe between two electrolytes; the terminating electrolyte was β-alanine (20 mmol/l), pH 10-6, and the leading electrolyte was Tris/HCl (2-5 mmol/l), pH 7-9, made up in 0-3% methylcellulose. The urine samples were applied directly without further preparation, apart from warming to ensure complete solution of purines of limited solubility. Pseudouridine, a pyrimidine excreted with relative constancy, was used as an internal marker for adequate sample dilution (Fig. 2). Separations required 40–45 min and were started with a current of 50 μA, which was reduced to 20 μA before the detection of the separated components by their U.V. absorbance at 254 nm.

Results

Quantification of urinary purines by anion-exchange chromatography

Urinary purines excretion by the immunodeficient children has been compared with earlier results from a control and an APRT-deficient child of comparable weight (Table 1).

The total purine end product was abnormal in relation to body weight only in PNP deficiency. Adenine was not present in the sample of L.L. (ADA deficiency) yet adenine was readily identified by this technique previously in APRT deficiency (B.Dh.). Deoxyribosides excreted in PNP deficiency were not degraded in this analytical method (in contrast to previous results obtained by others using more acidic cation-exchange systems: Cohen et al., 1976b). A new deoxy-compound, deoxyadenosine, was identified in ADA deficiency. The excretion of deoxyribosides was therefore found only in the immunodeficient children; none was excreted by the other two subjects. Pyrimidine excretion was not increased in either ADA or PNP deficiency.

The results reported have been obtained in single samples from the two immunodeficient children. However, the identification of deoxyadenosine as the major adenine-containing metabolite has since
Table 1. Urinary purine and pyrimidine excretion quantified by anion-exchange in adenosine deaminase deficiency (ADA−), purine nucleoside phosphorylase deficiency (PNP−) and adenine phosphoribosyltransferase deficiency (APRT−), compared with values in a control subject of similar age, on a caffeine-free diet.

<table>
<thead>
<tr>
<th></th>
<th>Urinary purine and pyrimidine excretion (mmol/24 h)</th>
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<tbody>
<tr>
<td></td>
<td>ADA− (L.L.)</td>
</tr>
<tr>
<td>Pyrimidines</td>
<td></td>
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<tr>
<td>Pseudouridine</td>
<td>0.05</td>
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<tr>
<td>Orotic acid*</td>
<td>0.01</td>
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<tr>
<td>Uracil</td>
<td>UD</td>
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<td>Normal purines</td>
<td></td>
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<td>Bases</td>
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<tr>
<td>Uric acid</td>
<td>0.25</td>
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<tr>
<td>Xanthine</td>
<td>0.01</td>
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<tr>
<td>Hypoxanthine</td>
<td>0.01</td>
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<tr>
<td>7-Methylguanine</td>
<td>0.01</td>
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<tr>
<td>Abnormal purines</td>
<td></td>
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<tr>
<td>Bases</td>
<td></td>
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<tr>
<td>Guanine</td>
<td>UD</td>
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<tr>
<td>Adenine</td>
<td>UD</td>
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<tr>
<td>8-Hydroxyadenine</td>
<td>UD</td>
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<tr>
<td>2,8-Dihydroxyadenine</td>
<td>UD</td>
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<tr>
<td>Nucleosides</td>
<td></td>
</tr>
<tr>
<td>Inosine</td>
<td>UD</td>
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<tr>
<td>Guanosine</td>
<td>UD</td>
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<tr>
<td>Deoxyinosine</td>
<td>UD</td>
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<tr>
<td>Deoxyguanosine</td>
<td>UD</td>
</tr>
<tr>
<td>Deoxyadenosine</td>
<td>0.06</td>
</tr>
<tr>
<td>Total</td>
<td>0.34</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>4.50</td>
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<tr>
<td>Total purine excretion (mmol 24 h⁻¹ k⁻¹)</td>
<td>0.076</td>
</tr>
</tbody>
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* Orotic acid plus orotidine.

been confirmed by Dr G. C. Mills and Dr F. C. Schmalstieg (University of Texas, Galveston, U.S.A.) as have the amounts of the other purines and pseudouridine (Table 1) excreted in separate specimens from the same child with ADA deficiency. Likewise, many subsequent samples from the PNP child have been analysed in Holland and confirm the above data.

Isotachophoresis

The tachophor scans (Fig. 2) reflect the quantitative data. The only abnormality in the scan in APRT deficiency was a dominant adenine peak, which clearly distinguished it from the control (impurities migrating in the terminator contribute to the small peak in the adenine position in the control and other scans; 2,8-dihydroxyadenine and 8-hydroxyadenine were not detectable at 254 nm). Adenine was not present in L.L: no adenine peak was evident, yet the scan in ADA deficiency was obviously abnormal compared with the control; orotic acid excretion did not appear to be increased. The PNP-deficient scan showed an obvious increase in total metabolites and the unusual pattern due to the nucleosides and deoxy-nucleosides, particularly the guanine derivatives, extending the scan beyond pseudouridine. Again, orotic acid excretion was not increased.

Discussion

Techniques for the rapid identification of inborn errors of purine metabolism are not readily available, except in specialized centres. They normally depend on complicated techniques using radioiso-
topes for the identification of the enzyme defect in haemolysates. A screening method for the initial identification of these defects which would involve only a few drops of urine is desirable. Isotachophoresis of the urine may be such a technique. Although it is not quantitative, comparison with quantitative results show remarkably good agreement. Extension of our studies in APRT deficiency to the two inborn errors of purine metabolism associated with immunodeficiency, PNP and ADA deficiency, indicate that these defects may clearly be distinguished from the metabolites excreted in urine by both methods.

Deoxadenosine has not previously been reported in quantity in human urine. The original erroneous identification of this compound as adenine (Mills et al., 1976) was due to the instability of the deoxy-compound in the cationic systems used in the original separation. Isotachophoresis proved invaluable in confirming that no adenine was detectable in the ADA-deficient urine; and that, apart from the presence of adenine, the excretion of u.v.-absorbing compounds was otherwise quite normal in APRT deficiency. The lability of deoxy-compounds in acidic conditions has also presented problems of identification in PNP deficiency. Cohen et al. (1976b) first reported inosine as the only nucleoside in the urine of their PNP-deficient child. However, Stoop et al. (1977) correctly identified both deoxyinosine and deoxyguanosine, after the unexpected finding of hypoxanthine, guanine and deoxyribose in the urine of a child with PNP deficiency.

These comparative studies have confirmed that only in PNP deficiency is the total purine endproduct increased some fivefold in relation to body weight. This obvious purine overproduction, with inosine the major metabolite, demonstrates extensive recycling of hypoxanthine in the normal state (Fig. 1) and the overall importance of this so-called hypoxanthine 'salvage' for nucleotide feedback control of purine production de novo in man: a fact previously noted in the Lesch Nyhan syndrome, where the salvage enzyme hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8) is absent. In contrast, the normal purine production in APRT deficiency indicates that endogenous adenine production and salvage by this route is relatively non-essential. The detection of inosine, guanosine and their deoxy-analogues, but not xanthosine, in PNP deficiency also suggests that the xanthosine pathway is not involved in purine nucleotide degradation in man.

We conclude that although the urinary excretion patterns of a single patient may not be representative per se of that disorder, the metabolites identified in this report were specific and directly related to the particular enzyme defect. Isotachophoresis has proved a useful confirmatory tool in that it has clearly established the presence of adenine in APRT deficiency and demonstrated its absence in ADA deficiency, while at the same time distinguishing the latter from PNP deficiency. The method is simple and rapid and may be of value in screening for these defects in the future.

Speculation

Our identification of deoxynribosides in the urine in two different inborn errors of purine metabolism both associated with immunodeficiency, and their absence from the urine of the control or immunologically competent APRT-deficient child, has led to the speculation (Simmonds, Panayi & Corrigal, 1978) that the integrity of these pathways may be vital to the immune response specifically for the 'detoxification' of 2'-deoxyribosides rather than the ribosides themselves (Fig. 1). Experiments to test this hypothesis have confirmed that deoxyadenosine is much more toxic than adenosine toward proliferating lymphocytes in vitro (Simmonds et al., 1978).

This hypothesis could explain why only cells of lymphoid origin, where the turnover of DNA is rapid, appear to be specifically affected. It would also be compatible with the observation that at physiological concentrations adenosine is preferentially converted into the nucleotide adenosine monophosphate (Perrett & Dean, 1977), the alternative route of deamination at the nucleotide level (Fig. 1) being preferred for the catabolism of adenine compounds in most tissues (Lomax & Henderson, 1973).

Previous experiments attempting to relate the associated immunodeficiency to the enzyme defect in both disorders have suggested inhibitory effects of adenosine in ADA deficiency on pyrimidine biosynthesis de novo (Carson & Seegmiller, 1976; Hovi, Smyth, Allison & Williams, 1976) as well as pyrimidine biosynthesis de novo (Ullman, Cohen & Martin, 1976). Cohen, Staal, Amman & Martin (1977) have reported oroticaciduria in children with PNP deficiency and considered this evidence in support of the latter hypothesis. The methods used, however, were not specific and we have been unable to confirm the presence of oroticaciduria in either ADA deficiency or PNP deficiency. The absence of oroticaciduria in these two defects supports the alternative suggestion that 2'-deoxy-
nucleosides exert their toxicity in rapidly dividing cells directly and not by a route involving interference with pyrimidine metabolism.

Acknowledgments

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References


