To reuse the enzyme, the glass beads were washed with reaction buffer two or three times. One unit (1 U) of immobilized enzyme is defined as the amount of enzyme bound to glass beads that produces 1 μmol of H₂O₂ per minute under the standard assay conditions. The enzyme protein bound to the glass beads was estimated by determining the loss of protein from the solution during immobilization (4).

To determine the urinary oxalate concentration, we analyzed 24-h urine samples collected from healthy individuals; the final pH was adjusted to 2.5 by addition of HCl and they were stored at 4 °C until use. The pretreatment of urine samples was done according to Butter and Pannell (5) with modifications: 1.0 mL of each acidified urine sample was put into a 15-mL graduated centrifuge tube, 1.0 mL of aqueous CaC₂O₄ (5 g/L) solution was added, and the final pH was adjusted to 6.5–7.0 by adding NaOH; 8.0 mL of ethanol was added immediately and the tubes were covered with aluminum foil and kept overnight at room temperature (25 ± 3 °C) for precipitation. The next day, tubes were centrifuged at 3000 × g for 5 min and the precipitates were dissolved in 1.0 mL of 0.1 mol/L HCl. Urinary oxalate was assayed as described for immobilized enzyme except that oxalate solution was replaced by 0.1 mL of dissolved urinary oxalate. The value of urinary oxalate was extrapolated from the calibration curve for oxalate concentration vs enzyme activity.

Color intensity varied linearly with oxalate concentration up to an absorbance of 0.11. The lower detection limit was 5 μmol/L. The mean ± SD analytical recovery of added oxalate (17.5 and 35 mg/L) was 96.7% ± 3.4%. To evaluate the accuracy of the method, we compared the oxalate results of 20 urine samples by the Sigma kit method (x) with modifications as follows: 1 mL of urine sample was diluted with an equal volume of phosphate buffer (0.1 mol/L, pH 7.5) containing EDTA (0.01 mol/L), and its pH was adjusted to 5.0–7.0. We added 200 mg of activated charcoal to the diluted urine, mixed for 5 min, and filtered it through Whatman no. 1 filter paper. To 0.05 mL of filtered urine, 0.1 mL each of oxalate reagent A (3.2 mmol of 3-(dimethylamino)benzoic acid and 0.2 mmol of 3-methyl-2-benzothiazoline in sodium succinate buffer, pH 3.5) and oxalate reagent B (3000 U of barley oxalate oxidase and 100 000 U of horseradish peroxidase per liter of distilled H₂O) were added and mixed. The mixture was incubated at 37 °C for 5 min and its absorbance at 590 nm was read. The regression equation for the oxalate values obtained by the Sigma kit method (x) and by the present method (y) is y = 1.130x – 0.0012 for an oxalate value of 27.0 ± 5.4 mg/L (mean ± SD).

References

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Cysteine Proteinase Activity in Synovial Fluids Measured with a Centrifugal Analyzer

To the Editor:

The destruction of cartilage and bone in rheumatoid arthritis (RA) is thought to be mediated by proteolytic degradation. RA synovial fluids contain high concentrations of several proteinases, e.g., elastase (1-4), collagenase (2, 5), stromelysine (5), and lysosomal cysteine proteinases (CP) (6-8). In particular, several observations suggest potential roles for the lysosomal cathepsins B and L in cartilage degradation.

Cathepsins B and L are able to degrade cartilage collagens type II, IX, and XI (9) and cartilage proteoglycans (10, 11). Furthermore, inhibitors of cathepsin B are able to reduce the cartilage and bone degradation in experimental induction of arthritis in rats (12, 13). Huet et al. (8) previously reported that the cysteine proteinase activity determined by using a manual fluorometric method with the substrates (N-α-benzoxycarbonyl-L-phenylalanyl-L-arginine-7-amido-4-methylcoumarin, Z-Phe-Arg-AMC) or Z-Arg-Arg-AMC was significantly greater in synovial fluids of RA patients than in patients with osteoarthritis (OA). Therefore, CP activities in synovial fluid may reflect a potential degradative activity against the articular cartilage of a particular joint.

We describe here an automated kinetic method for measuring the CP activity in synovial fluids by using a Cobas Fara II centrifugal analyzer (F Hoffman-La Roche Co., Ltd., Basel, Switzerland). The CP activity was determined at 37 °C with the fluorogenic substrate Z-Phe-Arg-AMC (purchased from Bachem, Bubendorf, Switzerland), the specific inhibitor E64 [l-trans-epoxysoycuciln-1-leucylamido-(4-guanidino)-butane; Sigma Chemical Co., St. Louis, MO] and AMC (7-amino-4-methylcoumarin; Serva, Heidelberg, Germany) as an internal standard.

We assayed synovial fluid samples that had been drawn by aspiration from knee joints during therapy. We centrifuged the samples for 5 min at 3000 × g within an hour of collection and stored the supernates frozen at −20 °C until use. Stock and working solutions were prepared as follows: Z-Phe-Arg-AMC (10 mmol/L) was dissolved in dimethyl sulfoxide, and the working solution was obtained by diluting 50 μL of stock solution with 1950 μL of 1 g/L aqueous Brj 35 (polyoxyethylene monolauryl ether, from Serva). The stock solution of AMC (0.25 mmol/L) was diluted 100-fold with 0.3 mol/L acetate buffer, pH 5.5, just before use. The E64 stock solution (10 mmol/L) was dissolved in dimethyl sulfoxide and the working solution was obtained by diluting 5 μL of stock solution with 1516 μL of the aqueous Brj 35 to give a final concentration of 0.003 mmol/L.

The various settings of the apparatus are listed in Table 1. Briefly, the apparatus distributes successively: (a) 12.5 μL of sample and 214 μL of 0.3 mol/L acetate buffer pH 5.5, (b) 12.5 μL of the AMC working solution as internal standard, (c) 31 μL of substrate working solution, (d) 20 μL of
Table 1. Cobas Fara II Settings for Assay of Cysteine Proteinase Activity

<table>
<thead>
<tr>
<th>General</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measurement mode</td>
</tr>
<tr>
<td>Calibration mode</td>
</tr>
<tr>
<td>Reagent blank</td>
</tr>
<tr>
<td>Wavelength</td>
</tr>
<tr>
<td>Emission filter</td>
</tr>
<tr>
<td>PM-limit</td>
</tr>
<tr>
<td>Unit</td>
</tr>
</tbody>
</table>

**Analysis**

| Sample vol | 12.5 μL |
| Diluent vol (H2O) | 10 μL |
| Reagent vol | 214 μL |
| Incubation | 60 s |
| Intermediate reading M1 | 30 s |
| Start reagent 1 (AMC) | 12.5 μL |
| Diluent vol (H2O) | 10 μL |
| Incubation | 60 s |
| Intermediate reading M2 | 30 s |
| Start reagent 2 (Substrate) | 31 μL |
| Diluent (H2O) | 12.5 μL |
| Readings first | 30 s |
| No. | 5 |
| Interval | 60 s |
| Reagent 1 vol (DTT) | 20 μL |
| Readings first | 30 s |
| No. | 5 |
| Interval | 60 s |
| Reagent 2 vol (E64) | 20 μL |
| Readings first | 30 s |
| No. | 5 |
| Interval | 60 s |

**Calculation**

- No. of steps: 2
- Calculation step A: Endpoint
  - First: M1
  - Last: M2
- Calculation step B: Kinetic
  - For activity with DTT
    - First: 6
    - Last: 10
  - For activity with E64
    - First: 11
    - Last: 15
- Formula: B/A

**Calibration**

- For DTT Factor 1: 3112
- For E64 Factor 2: 3306

30 mmol/L dithiothreitol (DTT) in the acetate buffer, and (e) 20 μL of E64 working solution. The first reading (M1) is taken 30 s after mixing the sample and the acetate buffer, and the second reading (M2) 30 s after adding AMC. Five further readings were taken at 1-min intervals after succes-

ively adding the substrate, DTT, and E64. The fluorescence (F) was measured with excitation at 340 nm and emission at 450 nm.

The CP activity was calculated from the difference (DF) between the activities measured without and with E64, and expressed in mU/L (1 mU of activity corresponds to the release of 1 nmol of AMC per minute (14)). The details for the calculation are as follows.

**Total activity measured after addition of DTT (mU/L):**

\[
\text{DF} = \frac{\text{DF1 sample/min}}{\text{Factor 1}}
\]

where Factor 1 = AMC (μmol/L) × (V DTT/V AMC) × 1000, DF1 sample = fluorescence of the sample at step B (see Table 1), V DTT = final volume after DTT addition, and V AMC = final volume after AMC addition.

**Residual activity measured after addition of E64 (mU/L):**

\[
\text{DF2 sample/min}/\text{Factor 2}
\]

where Factor 2 = AMC (μmol/L) × (V E64/V AMC) × 1000, DF2 sample = fluorescence of the sample at step B, and V E64 = final volume after E64 addition.

The CP activity is the difference between the activities measured without and with E64.

Compared with the manual end-point method previously described (8), this automated method is fast (60 samples/h) and needs a sample size of only 100 μL. The within-run precision was determined with a synovial fluid sample (n=28, mean ± SD 827 ± 12 mU/L, CV = 1.4%). The run-to-run precision was made with another sample, analyzed 30 times (788 ± 13 mU/L, CV = 1.6%). The linearity of the method was determined by assaying a synovial fluid sample at several dilutions in 0.3 mol/L acetate buffer, which showed that results between 150 and 7500 mU/L can be considered accurate. The CP activities extended up to 7500 mU/L in RA synovial fluids and up to 800 mU/L in OA synovial fluids.

Our data provide an adaptation for a kinetic determination of CP activity with a fluorogenic substrate on a Cobas Fara II centrifugal analyzer.

We thank R.M. Filpo for providing samples of synovial fluid.

**References**


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Xylose, Anti-Gliadin, and Anti-Endomysium Antibodies in Adult Celiac Disease

To the Editor:

The onset of celiac disease in adults is rare. The first symptoms usually appear during childhood (1). Xylose absorption tests have been used to evaluate small-intestinal absorption and to discriminate between normal subjects and patients with proximal intestinal malabsorption. Recently, determinations of serum concentrations of IgA-class anti-endomysium antibodies and of IgA and IgG anti-gliadin antibodies have shown high specificity and sensitivity for gluten-sensitive enteropathy (2).

We have studied a 57-year-old man with enteropathy-associated T-cell lymphoma and four men with adult celiac disease, ages 20 to 44 years, of whom three had iron-deficiency anemia unresponsive to iron therapy, and one had malabsorption. Serum and urine tests were performed 1 and 5 h, respectively, after ingestion of 25 g of xylose (3). Anti-gliadin antibodies were measured by enzyme immunoassay and anti-endomysium titers by immunofluorescence.

Our results (Table 1) demonstrate the usefulness of these tests for adults and constitute a screening method to select patients for whom a biopsy is appropriate. IgA-class anti-gliadin and anti-endomysium antibodies appear to provide the highest sensitivity and specificity in adult celiac disease.

Table 1. Xylose and Anti-Gliadin and Anti-Endomysium Antibodies in Five Patients and Others

<table>
<thead>
<tr>
<th>Patients</th>
<th>Serum xylose, g/L</th>
<th>Urinary xylose, g/L h</th>
<th>Anti-gliadin, units</th>
<th>Anti-endomysium, titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celiac disease</td>
<td>&lt;0.1</td>
<td>&lt;2.5</td>
<td>&gt;40</td>
<td>&gt;40</td>
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<tr>
<td>T-cell lymphoma</td>
<td>&lt;0.1</td>
<td>&lt;2.5</td>
<td>&gt;40</td>
<td>&gt;20</td>
</tr>
<tr>
<td>Other subjects</td>
<td>&gt;2.5</td>
<td>&gt;4.0</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>

References

Table 1.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Serum xylose, g/L</th>
<th>Urinary xylose, g/L h</th>
<th>Anti-gliadin, units</th>
<th>Anti-endomysium, titer</th>
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</table>

L). This infant had been investigated for a metabolic inherited disease, not for a chemically induced acidosi. The authors who reported the case (1) reminded clinicians investigating recurrent infantile metabolic acidosi to consider intentional chemical poisoning by substances such as EG.

In the second well-publicized case (2), a child who was thought to have died from EG poisoning was found to have had methylimidazole acidemia. In this patient, retrospective analysis of plasma collected earlier revealed increased concentrations of methylmalonic acid, propionic acid, acetoacetic acid, β-hydroxybutyrate, lactate, and β-hydroxyisovaleric acid. It was determined that the gas chromatographic retention times for EG and propionic acid were very similar. The actual peaks did not overlap but were sufficiently close to have been misidentified in this case. The relative retention times were 0.659 (EG) and 0.659 (propionic acid), compared with the retention time of 2.96 min for the internal standard, propylene glycol.

Careful attention to the relative retention times would have precluded the misidentification. No mention was made of whether the laboratory had performed a confirmation procedure for the presence of EG. The mother had been charged with murder but was exonerated when her second child was found to have the same metabolic disease and retrospective analysis was performed on frozen plasma from the first child, collected before his death.

The third case (3) involved the emergency-room admission of a multiple drug overdose patient. As part of the toxicological investigation, analysis for EG was performed. An instance with a retention time similar to the internal standard (propylene glycol) was detected. Propylene glycol was determined to be present in the intravenous solutions of two drugs administered to this patient. The authors recommended that another internal standard for EG analysis be used, such as 1,3-propanediol.

The misidentification of EG based upon reliance on a gas chromatographic retention time highlights the importance of confirming each toxicological procedure, as is standard practice for drug testing of urine.

It has been known for several years that the toxicity seen after EG poisoning is caused by EG metabolites, not by EG per se (4). Clinical laboratories have traditionally provided quantitative results for EG in serum as an index for whether dialysis should be initiated or discontinued. A better in-