Sensitive, Optimized Assay for Serum AMP Deaminase

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A sensitive, optimized assay for serum AMP deaminase (EC 3.5.4.6) is presented, which is based on a colorimetric indophenol determination of ammonia liberated during the enzyme reaction. An average enzyme activity in serum of 2.7 U/liter (n = 36, range 0–6.2) for healthy adults was established. Both the enzyme and colorimetric reactions were optimized to give an assay sensitivity of 0.2 U/liter of serum, a 98–99% analytical recovery in the normal serum enzyme concentration range, and a CV of 7.9% in-run and 10.6% between-run. This optimization included studies on specific buffer, pH, ionic strength, and potential activators. The \( K_m \) for the enzyme in serum was determined to be \( 1.4 \times 10^{-3} \) mol/liter, which agrees well with the value reported for human skeletal muscle enzyme.

Above-normal serum AMP deaminase activity may be present in various myopathies and could be useful in detecting carriers of the X-linked dystrophies.

Additional Keyphrases: ammonia determination · indophenol reaction · muscle disease · normal values · Duchenne muscular dystrophy · enzyme activity · genetics · simplex optimization

Creatine kinase (ATP:creatine phosphotransferase, EC 2.7.3.2) is the serum enzyme usually used to confirm and monitor Duchenne muscular dystrophy and to detect female carriers of this X-linked disorder (1–4). A dramatic and continuously increased serum creatine kinase—as much as 100-fold normal—in the very young male is virtually pathognomic for Duchenne muscular dystrophy, but the activity in the serum of carriers is usually less pronounced and in some cases is normal (1, 5).

Furthermore, the increased serum creatine kinase activity after exercise (6), the reported presence of a dissociable serum creatine kinase inhibitor in patients with various muscle disorders (7), and the inverse correlation of serum creatine kinase activity with age in carriers (1) all detract from the predictive value of this screening method. Studies suggest that serum creatine kinase activity is above the normal range in only 60–68% of known carriers and 30% of possible carriers (1, 5). Similar results have been reported for carriers of Becker dystrophy (8), another myopathy with sex-linked inheritance. Interpretation of the results from possible carriers is complicated, however, by the high rate of spontaneous mutation with which Duchenne muscular dystrophy is believed to be associated (9).

For these reasons, the screening of suspected carriers is widely encouraged (10, 11) but only partly successful. We believe that another enzyme—AMP deaminase (AMP aminohydrolase, EC 3.5.4.6)—which is highly specific for skeletal muscle (12), may offer a suitable alternative (or adjunct) to serum creatine kinase determination in Duchenne muscular dystrophy carriers.

It catalyzes the reaction

\[ \text{AMP} + \text{H}_2\text{O} \rightarrow \text{IMP} + \text{NH}_3 \]

as part of the purine nucleotide cycle reported by Lowenstein (12). In addition to its tissue specificity, its activity is relatively high in normal skeletal muscle (13) and decreased in Duchenne muscular dystrophy (14, 15). This decrease is characteristic of many muscle enzymes associated with glycolysis and energy transfer in dystrophy, but a significant increase in serum activity, frequently associated with tissue decrease, has not previously been demonstrated for AMP deaminase.

We report here the development of a very sensitive, optimized assay for serum AMP deaminase and the establishment of a normal range. Preliminary results suggest substantially increased activity in serum in Duchenne muscular dystrophy, a variety of other myopathies, and Duchenne muscular dystrophy carriers (manuscript in preparation).

Materials and Methods

Reagents

Rabbit muscle AMP deaminase (grade IV; Sigma Chemical Co., St. Louis, Mo. 63187) was shipped in glycerol/water (66/34 by vol), 0.33 mol/liter KCl, pH 7.4, at 4 °C. The enzyme was stored at −20 °C in 50 mmol/liter sodium citrate buffer, pH 6.50, 1 mol/liter KCl, and 1 mmol/liter 2-mercaptoethanol. Sodium AMP (yeast, Type II), Na₂ATP (equine muscle), imidazole, and adenosine (Sigma grade, anhydrous) were also from

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Sigma. Na AMP, Na₂ATP, and adenosine were stored at −20 °C until used. Grade A dithiothreitol was from Calbiochem, La Jolla, Calif. 92037. “Rapid-Stat” inorganic orthophosphate assay kits were purchased from Pierce Chemical Co., Rockford, Ill. 61105. The alkaline hypochlorite solution used was a commercial household bleach (5.25% solution of NaOCl). All other materials were analytical reagent grade. Ammonia-free water was prepared by passing distilled water through a Universal Model 1 ion-exchange column (Illinois Water Treatment Co., Rockford, Ill. 61105).

Instrumentation
Spectrophotometric measurements were made with a Perkin-Elmer/Coleman 124 double-beam spectrophotometer with a Coleman 124-801 scale expander (Perkin-Elmer Corp., Norwalk, Conn. 06856). Standard quartz cuvettes, 1-cm pathlength, were used. Enzyme reactions were carried out in a water bath controlled to 0.1 °C. All standard pipets were Kimax class A. Microadditions were made with an Oxford to-deliver “Sampler” 20-μl micropipettor, accuracy determined to be 19.7 μl ±0.15 μl (Scientific Products) and with “to-contain” Dade “Accupette” micropipettes, accuracy ±0.5% (manufacturer’s specification, Scientific Products). All glassware was thoroughly rinsed with ammonia-free water before use. pH was measured with a Model 245 pH meter (Instrumentation Laboratory Inc., Lexington, Mass. 02173) after calibration with appropriate standards at 25 °C. For blood centrifugation we used a desk-top centrifuge.

Serum Collection, Handling, and Storage
Aliquots of pooled hospital serum were stored at −20 °C until needed during the course of assay development. To establish a normal range, we took blood samples from healthy adults, centrifuged, and stored the serum at 4 °C. Plain, silicone-coated, evacuated glass tubes were used for the collection and assays were run within 3 h of collection.

Assay for Ammonia
An 53 mmol/liter sodium citrate buffer (pH 6.20, μ = 0.270), was prepared from dried anhydrous citric acid and 1 mol/liter NaOH. A stock 1 mol/liter NaAMP aqueous substrate solution was adjusted to pH 6.20 with NaOH and aliquots were frozen until needed. Concentration was confirmed spectrophotometrically at 259 nm after appropriate dilution.

Ammonia production was assayed by a modification of the Chaney and Marbach indophenol method (16–18). Optimum concentrations of the indophenol reaction reagents for maximum sensitivity to ammonia were established simultaneously by using a simplex optimization procedure (19, 20). The initial reagent concentrations were those specified in the method modification of Belfield et al. (17). There were four variables, and step sizes (expressed as concentrations in the initial stock reagents) were: phenol, 8.0 g/liter; NaOH, 80 mmol/liter; sodium nitroprusside, 40 mg/liter; and commercial bleach (5.25% NaOCl), 4 ml/liter. Twenty-four steps were taken and optimization was reached at step 19 (data not shown).

On the basis of these results, a working phenol-nitroprusside solution was prepared from 15.25 g of crystalline phenol and 708 mg of sodium nitroprusside in 250 ml of water. Alkaline hypochlorite solution contained 150 mmol of NaOH and 7.2 ml of liquid household bleach (5.25% NaOCl) diluted to 250 ml with water. Both reagents were stored at 4 °C in brown glass bottles. Reported stability is six weeks (17).

Stock 2.000 mol/liter ammonia standard was prepared from 0.1322 g of dried (100 °C, 4 h) (NH₄)₂SO₄ diluted to 1 liter. The solution was stored at 4 °C throughout.

The optimized assay protocol appears in Table 1. Developed color was measured at 630 nm, the maximum absorption wavelength of indophenol at the assay pH. A serum control was run with each sample to correct for the background ammonia present in serum. AMP was added to these controls after quenching to correct for the slight increase that AMP imparts to the final absorbance (about 0.001 A per 1.0 mmol of AMP per liter).

AMP Deaminase Reaction Optimization
To study the effect of pH on the enzyme reaction, an 0.05 mol/liter sodium citrate buffer (pH 6.00, 25 °C) was prepared. NaOH was added and 5-ml aliquots were taken at 0.05 pH unit intervals up to pH 6.67. Assays were then run according to Table 1, at 37 °C. The reported pH is that of the final reaction mixture at enzyme reaction time tₒ, 25 °C, as determined from reaction duplicates.

We measured the effect of ionic strength on the enzyme reaction by preparing a stock 22 mmol/liter citrate buffer (μ = 0.120, pH 6.20) and adding appropriate volumes of aqueous stock 2.000 mol/liter KCl to buffer aliquots to provide a graduated series of solutions of increasing ionic strength. The pH of each was readjusted with negligible volumes of NaOH to 6.20. Final ionic strength of the assay reaction mixtures, allowing for dilution and the NaAMP substrate present, ranged between 0.179 and 0.454. We then assayed serum AMP deaminase, using the protocol of Table 1.

Adenosine Deaminase Assay
Serum adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) was determined by substituting a 4.0 mmol/liter saturating concentration (21) of adenosine for NaAMP substrate and using the protocol of Table 1, otherwise unchanged.

Phosphatase Activity
We determined phosphatase activity on substrate AMP by measuring inorganic orthophosphate production. A Pierce “Rapid-Stat” kit, which makes use of a method based on the formation of a reduced phosphomolybdate chromogen, was used. Both citrate and AMP were observed to decrease color development,
Table 1. AMP Deaminase Assay Protocol

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Initial concn (mmol/liter)</th>
<th>Final concn (mmol/liter)</th>
<th>Volume of reagent added to:</th>
<th>Sample</th>
<th>Control</th>
<th>Standard</th>
<th>Reagent blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na citrate buffer, pH 6.20</td>
<td>53</td>
<td>51</td>
<td></td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td>Equilibrate at 37 °C for 10 min</td>
<td>0.020</td>
<td>0.020</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₂ stock standard</td>
<td>2.00</td>
<td>0.038</td>
<td>Incubate at 27 °C for 30 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td></td>
<td></td>
<td>reaction period</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaAMP stock substrate</td>
<td>1000</td>
<td>19</td>
<td></td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Phenol–nitroprusside</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NaAMP stock substrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline hypochlorite</td>
<td></td>
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</tr>
</tbody>
</table>

Calculation of activity

\[
\text{Activity (U/liter serum)} = \frac{(A_{\text{sample}} - A_{\text{blank}}) \times \text{standard (µmol)}}{(A_{\text{standard}} - A_{\text{blank}}) \times \text{reaction time (min)} \times \text{sample vol (liters)}}
\]

* 1 U is defined as the conversion of 1 × 10⁻⁸ mole of AMP per minute to ammonia

apparently because of an interaction with the molybdate reagent, which made it unavailable for phosphomolybdate complex formation. By substituting a saturated (NH₄)₂MoO₄ solution for the kit reagent, there was sufficient excess free molybdate to react with all inorganic phosphate present up to a concentration of 200 mg/liter, as demonstrated by a linear standard curve (not shown).

ATP and Thiol-Group Protectors

Fresh Na₃ATP stock solution was prepared in sodium citrate buffer, pH 6.20, to a final concentration of 20 mmol/liter and confirmed spectrophotometrically at 259 nm. The solution was made fresh daily and kept on crushed ice. Working aliquots of various concentrations were prepared by dilution with buffer as required. We assayed for the effect of ATP on serum AMP deaminase activity according to Table 1, with ATP/buffer solution substituting for buffer alone where appropriate.

Dithiothreitol was kept at 4 °C throughout. Fresh 2-mercaptoethanol and dithiothreitol solutions were each prepared in citrate buffer, pH 6.2, to final concentrations of 2.0, 5.0, and 8.0 mmol/liter. The protocol was altered to allow for a 45-min incubation of sera in these buffers before substrate addition.

Results

Serum AMP Deaminase Activity

Buffer. Several buffers were considered for use. Phosphate was excluded because a wide variety of inorganic and organic substances competitively inhibit AMP deaminase activity (22, 23). This inhibition is countered at saturating substrate concentration (13), but it was considered to be an inherently poor choice nonetheless. Tris-HCl has a color-quenching effect on the indophenol reaction (24) and has a pKₐ of two units above the reaction pH optimum, so it is unsuitable. We found that imidazole also interferes significantly with color development at 0.1 mol/liter concentration. Sulfamic buffer has a pKₐ too low for optimum buffer capacity in the 6.0–6.8 range, although it has been frequently used in studies of AMP deaminase. Citrate was selected because its pKₐ of 5.67 at 53 mmol/liter is reasonably close to reaction pH and its effect on indophenol color development negligible. Sodium and potassium ions activate the human muscle enzyme to about the same optimal extent (13); therefore sodium was arbitrarily chosen as the cation.

Serum concentration. Enzyme activity was linear with serum concentration between 0.0 and 83 µl of serum per milliliter of reaction volume (Figure 1). The serum concentration selected for AMP deaminase reaction optimization and normal range determination was 19 µl/ml (a 20-µl sample size).

Substrate concentration. The Kₘ of serum AMP deaminase was determined from a Lineweaver–Burk plot (Figure 2) to be 1.4 × 10⁻³ mol/liter, agreeing well with a value previously reported for the isolated human skeletal muscle enzyme at pH 6.5 in imidazole buffer (25). Enzyme cooperativity, reported at low AMP concentrations (26, 27), was not apparent, nor did there appear to be any substrate inhibition at 25 mmol of AMP per liter, the highest concentration studied. A final NaAMP concentration of 19 mmol/liter, which gives 98% of Vₘₐₓ, was chosen for further work. This represents 15 × Kₘ. Because enzyme activity is low, substrate depletion during the reaction is not a problem.

pH. Optimum serum enzyme activity occurred around pH 6.21 (Figure 3), and pH 6.20 was therefore
selected. The change in pH during the reaction was about +0.002/min.

**Ionic strength.** Optimum serum enzyme activity in the assay occurred when total ionic strength was 0.295–0.320 (Figure 4). There was a rather rapid decrease in activity on either side of this plateau. A value of 0.300 was chosen.

**Reaction time.** AMP deaminase activity was constant between 0 and 60 min (Figure 5). Thirty minutes was chosen for routine use in the assay, to provide both a
rapid procedure and a significant color difference between the serum blank and the sample.

Serum effect on AMP deaminase activity. No significant inhibition of commercial rabbit muscle AMP deaminase activity by pooled human serum under protocol conditions was found (Table 2).

Potentially interfering enzyme reactions. The generation of ammonia from the AMP substrate by a coupled reaction of serum 5'-nucleotidase (EC 3.1.3.5) and adenosine deaminase suggests itself as a potential interference in the assay. This would give falsely elevated values for apparent AMP deaminase activity.

\[
\text{AMP} \xrightarrow{5'-\text{nucleotidase}} \text{adenosine} + P_i
\]

\[
\text{Adenosine deaminase} \xrightarrow{} \text{inosine} + \text{NH}_3
\]

The total phosphatase activity in pooled serum under AMP deaminase assay conditions was 2.4 U/liter. The corresponding rate of adenosine production would have this value as an upper limit, because some of the measured phosphate could arise from dephosphorylation of other serum organic phosphate compounds by various phosphatases.

Activity of adenosine deaminase under the same reaction conditions, but with adenosine substituting for AMP, was 12.3 U/liter. Because both enzymes were active in serum under AMP deaminase assay conditions, even though these conditions are not optimal for either enzyme (28, 29), it was important to calculate the effect of their coupled activity on ammonia concentration during the 30-min AMP deaminase reaction period.

Several assumptions were necessary. We assumed that virtually no adenosine is present in serum at \( t_0 \), and therefore that all adenosine available for deamination results from phosphatase activity. The \( K_m \) for human adenosine deaminase was taken to be \( 7.3 \times 10^{-5} \) mol/liter (21). The increase in adenosine concentration was equated with phosphate increase, even though this may assign too high a value to 5'-nucleotidase activity. Finally, saturating conditions for the 5'-nucleotidase reaction, non-saturating conditions for that of adenosine deaminase, and Michaelis-Menten kinetics were assumed.

One can then calculate from the appropriate rate equations for the two reactions that \( 6.5 \times 10^{-11} \) mol of ammonia was produced by the coupled activity in the reaction vessel during 30 min. This represents an apparent AMP deaminase activity of about 0.1 U/liter serum. An interference of this magnitude is not considered significant, because it is less than the standard deviation in the precision of the method and below the limit of assay sensitivity, as discussed below. Diversion of substrate AMP by the activity of phosphatases would represent a decrease of \( 2.0 \times 10^{-6} \) mol/liter in AMP concentration during the 30-min assay. This is negligible, 0.01% of the total substrate present at \( t_0 \).

Potential activators. Although ATP has an activating effect on AMP deaminase activity at physiological AMP concentrations in skeletal muscle (about 5 mmol/liter) (13, 26, 30), it reportedly inhibits activity when the substrate is saturating (31). Our results substantiate this inhibition with increasing ATP concentrations (Figure 6). ATP was therefore not included in the assay protocol.

No increase in enzyme activity was found with either dithiothreitol or 2-mercaptoethanol addition. Furthermore, both reagents have a color-quenching effect on the indophenol reaction, thereby decreasing the assay sensitivity to ammonia.

Enzyme stability. Serum AMP deaminase stability at 4 °C and at -20 °C as measured according to the protocol and expressed as a percentage of initial activity is shown in Figure 7. The enzyme loses about 10% of its activity in the first 24 h of refrigeration, but is then relatively stable until day 4. The enzyme in frozen serum loses less than 10% of its activity immediately and is then reasonably stable for about four weeks.

Colorimetric Indophenol Reaction

Standard curve. The absorbance of indophenol produced by reacted ammonia was linear with ammonia concentrations between 0 and \( 18.2 \times 10^{-5} \) mol/liter (Figure 8). The upper limit exceeds by a factor of two
the highest serum ammonia concentration seen during the course of our investigations.

Time for color development. Indophenol color development at 37 °C was complete within 10 min after alkaline hypochlorite reagent was added (Figure 9). Color was stable for at least 1 h.

Sensitivity. A change of 0.001 in indophenol absorbance corresponds to a change in ammonia concentration of $4.0 \times 10^{-8}$ mol/liter. This is therefore the lower detection limit of the method with most instruments, equal to $6.9 \times 10^{-10}$ g of NH₃/g of H₂O (or 0.20 U/liter serum AMP deaminase activity). These values represent a 59% lower reaction detection limit as compared with results obtained before simplex optimization of the indophenol reagent concentrations.

**Table 3. Analytical Recovery of Ammonia (NH₃) Added to Serum**

<table>
<thead>
<tr>
<th>Present in assay</th>
<th>Equivalents of NH₃ added</th>
<th>Calc activity</th>
<th>Observed activity</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pooled serum</td>
<td>—</td>
<td>—</td>
<td>3.1</td>
<td>—</td>
</tr>
<tr>
<td>Pooled serum + NH₃</td>
<td>9.8</td>
<td>12.9</td>
<td>12.8</td>
<td>99.2</td>
</tr>
<tr>
<td>Pooled serum + NH₃</td>
<td>19.5</td>
<td>22.6</td>
<td>22.2</td>
<td>98.2</td>
</tr>
<tr>
<td>Pooled serum + NH₃</td>
<td>31.3</td>
<td>34.4</td>
<td>32.5</td>
<td>94.5</td>
</tr>
<tr>
<td>Pooled serum + NH₃</td>
<td>48.8</td>
<td>51.9</td>
<td>48.7</td>
<td>93.8</td>
</tr>
</tbody>
</table>

*Mean of triplicate determinations.

*Present as (NH₄)₂SO₄.

Reliability and a Normal Range

Accuracy. Accuracy of the entire assay protocol, as measured by recovery of ammonia added to serum in the form of (NH₄)₂SO₄, is detailed in Table 3. Recovery of ammonia at concentrations representing normal serum AMP deaminase activity for 30 min ($3 \times 10^{-6}$ to $6 \times 10^{-6}$ mol/liter) was about 98–99%. At concentrations that would be reflected in serum of unusually high background ammonia concentration or with pathologically increased serum AMP deaminase activity, recovery was 94%.

Precision. The precision of this assay was measured in-run on a single serum specimen, and between-run over a period of three weeks with aliquots from the same serum pool but differing reagent preparations. The results appear in Table 4. The CV was 7.9% for in-run precision and 10.6% for between-run precision.

Determination of a normal range. A group of 36 normal healthy adults were individually analyzed for
AMP deaminase activity and the results are presented in Table 5. No significant sex-related difference is seen, and there was no significant correlation between serum AMP deaminase and age.

Discussion

No method for serum AMP deaminase determination has previously been reported. We have therefore developed an accurate, precise, and highly sensitive assay and have established a normal range of activity in serum of healthy adults. This assay is based on a colorimetric quantitation of ammonia produced in a 30-min enzyme reaction period at 37 °C. The spectrophotometric method of Kalcottar (32) is more direct but is insufficiently sensitive for use on the activities of AMP deaminase found in serum.

The enzyme assay was made optional on a system consisting initially of sodium citrate buffer (0.10 mol/liter, pH 6.5) and a 5 mmol/liter final concentration of substrate AMP. Each variable except temperature was made optimal and then all experiments were rerun under these derived optimum conditions to ensure that earlier results remained valid.

An elegant, less tedious, and statistically sound method of simultaneously optimizing all continuous variables in an analytical procedure—the simplex optimization process (19)—was used to lower the detection limit of the indophenol reaction to ammonia.

We found the activity of AMP deaminase in normal serum to be low in comparison with that of some other clinically useful enzymes. This may reflect a slow efflux of AMP deaminase from the cell, either because of its probable high molecular weight or its close association with muscle myosin (33). Alternatively, it may represent an unusually high sensitivity of the enzyme to proteolytic inactivation within the cell itself or in the serum after efflux.

It therefore seemed worthwhile to explore the effect of various possible enzyme activators. However, all trials proved ineffective. For example, ATP, which is reported to activate the enzyme in vitro at low substrate concentra-

Table 5. AMP Deaminase Activity in Serum of Healthy Adults

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Range</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>20</td>
<td>0.0–6.2</td>
<td>2.7</td>
</tr>
<tr>
<td>Women</td>
<td>16</td>
<td>0.3–5.0</td>
<td>2.7</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>0.0–6.2</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Table 4. Precision of the AMP Deaminase Assay Procedure

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>(±SD)</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-run</td>
<td>6.76–8.93</td>
<td>7.97 ± 0.63</td>
<td>7.9</td>
</tr>
<tr>
<td>Between-run</td>
<td>6.41–9.02</td>
<td>7.64 ± 0.81</td>
<td>10.8</td>
</tr>
</tbody>
</table>

Furthermore, we find no measurable increase in activity when either dithiothreitol or 2-mercaptoethanol is incubated with the buffered serum sample before assay. Thiol-group protectors have been used in the published preparative methods (34, 35), but the necessity for them is put into question by recent evidence that either of two thiol-blocking reagents, N-ethylmaleimide or 5,5'-dithiobis(2-nitrobenzoate), do not inactivate the enzyme when reacted with the six exposed thiol groups in native rat skeletal muscle AMP deaminase (36).

Incubation of commercially-prepared rabbit muscle AMP deaminase with human serum does not affect enzyme activity, suggesting that no AMP-deaminase inhibitory factor is present in normal serum. The linearity of enzyme activity with reaction time is evidence that there is no appreciable temperature inactivation of the enzyme at 37 °C during the 30-min assay.

However, the enzyme stability is highly related to sample storage temperature. On refrigeration there is an average loss of activity of 10% in 24 h, but freezing at -20 °C maintains stability for about four weeks.

Interference from the coupled 5'-nucleotidase/adenosine deaminase reactions discussed above could be a problem. We calculate that this interference is not important when the activities of these enzymes in serum are normal. However, increased activity of either enzyme would increase ammonia production and thus cause a falsely elevated value for apparent AMP deaminase activity. We are therefore searching for some method of eliminating the potential for this effect.

Finally, it should be noted that the normal range established by our procedure does not display a gaussian distribution (not an uncommon situation) and therefore that the customary method for calculation of a standard deviation is not applicable. Preliminary data, not included here, also suggest that there is no significant correlation between serum AMP deaminase and creatine kinase activities in these 30 normal samples.

References