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Potentiometric


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Quantification of Acylcarnitines

To the Editor:

We read with interest the recent paper by Fishlock et al. (1) describing problems in quantification of carnitine and acylcarnitines, but we believe that they misrepresent the use of the perchloric acid fractionation technique. Their conclusion that perchloric acid fractionation may lead to contamination of the acid-insoluble fraction (long-chain acylcarnitines) with acid-soluble components (carnitine and short-chain acylcarnitines) is an important issue, and one that all workers in this field need to be aware of. As they point out, the importance for clinical diagnosis and interpretation of metabolic studies in differentiating carnitine and groups of acylcarnitines makes this a valid issue (2-7). However, we feel that the quantitative conclusions reached in the manuscript are misleading. The authors concluded that "fluid trapped in the protein precipitation by perchloric acid is approximately 24% of the total fluid volume and thus contains 24% of free carnitine and short-chain acylcarnitines" (1). If this figure could be generally extrapolated it would indeed invalidate the technique and call into question several previous studies. Their conclusion is based in part on 3H2O trapping done by using a 3200 x g perchloric acid fractionation of 250-μL samples. We (2) have used conical tubes and a much higher centrifugation rate (>10 000 x g) to handle small volumes, to ensure good packing of the pellet, and so facilitate separation. Indeed, when Fishlock et al. (1) used 20 000 x g separations, less than 1% of acetyl-L-[3H]carnitine was found in the acid-insoluble fraction. The centrifugation force used for their carnitine recoveries from human plasma is not noted, but based on 24% carnitine in the acid-insoluble fraction, we presume that inadequate low-speed separation was used. However, their final value, after multiple washings, of 3 μmol/L for human plasma long-chain acylcarnitines agrees well with the value of 4 μmol/L obtained by Gennuth and Hopkins (3,4), who used our standard perchloric acid fractionation.

We have had considerable experience with the perchloric acid fractionation method in studying carnitine metabolism under various conditions. Table 1, which summarizes some of our previously published results with this technique, allows several points to be made. First, if the 24% value for carryover of acylcarnitine to the acid-insoluble fraction is correct, then the ratio of measured long-chain acylcarnitine to total carnitine could never be less than 24% (24%/100%), even if the actual long-chain acylcarnitine concentration was zero. As can be seen from the table, the percentage of total carnitine present as long-chain acylcarnitine is always lower than 24%, and is as low as 6 or 7% under some conditions. Thus, the 24% carnitine trapping reported by Fishlock et al. (1) represents a significant overestimation. The utility of the perchloric acid fractionation is demonstrated by comparing samples assayed for carnitines under varied metabolic conditions. For example, during the transition from the fed to fasted state, acid-soluble carnitines in rat plasma declined by 29%, from 45 μmol/L to 32 μmol/L, while acid-insoluble acylcarnitines were unchanged at 8 μmol/L. If acid-insoluble acylcarnitines were largely the result of acid-soluble trapping, a 29% decrease in acid-soluble carnitines should have been accompanied by a corresponding fall in acid-insoluble acylcarnitines, but this was not seen. A more dramatic example was seen when livers from fed and fasted rats sacrificed 10 to 20 min after intravenous injection of 60 μmol of L-carnitine per 100 g body weight. In these studies, the content of acid-soluble carnitines was similar in the fed and fasted rats, but the acid-insoluble acylcarnitines were much higher in the fasted state (Table 1). Again, if simple carryover was responsible for the measured acid-insoluble acylcarnitines, no difference should have been seen in the two states. Moreover, the larger acid-insoluble acylcarnitine content seen in fasting is expected physiologically, and the changes after exogenous carnitine administration correspond to the carnitine distribution prior to injection (but maintaining the difference between fed and fasted animals), lending internal consistency to the measurements.

Thus, we share the concerns of Fishlock et al. but we do not agree with their quantitative conclusions and implied criticism of previous studies. With proper attention to laboratory technique—the use of high-force centrifugation when fractionating small volumes and the use of large volumes to facilitate separation if low speed centrifugation is used—the perchloric acid fractionation technique has proven extremely valuable in addressing problems of carnitine metabolism. To facilitate the quantitative separation of carnitine and acylcarnitines, we have recently developed a liquid chromatographic technique that is applicable to compounds of this class. These results will be published in detail separately, but here we state that, under conditions that provide complete baseline resolution of long-chain acylcarnitines from other carnitines (even when acid-soluble carnitines are present in large excess), quantification of the long-chain acylcarnitines have been in excellent agreement with our previously published values obtained by the perchloric acid fractionation method (2, 8).

Table 1. Carnitine and Acylcarnitines as Measured by Fractionation with Perchloric Acid

<table>
<thead>
<tr>
<th>Sample</th>
<th>Acid-soluble carnitines (free carnitine and short-chain acylcarnitines)</th>
<th>Acid-insoluble acylcarnitines (Long-chain acylcarnitines)</th>
<th>(Long chain acylcarnitines/total carnitine) x 100</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma from fed rat</td>
<td>45 μmol/L</td>
<td>8 μmol/L</td>
<td>15%</td>
<td>(2)</td>
</tr>
<tr>
<td>Plasma from rat fasted 24 hours</td>
<td>32 μmol/L</td>
<td>8 μmol/L</td>
<td>20%</td>
<td>(2)</td>
</tr>
<tr>
<td>Liver from fed rat</td>
<td>240 nmol/g</td>
<td>19 nmol/g</td>
<td>7%</td>
<td>(6)</td>
</tr>
<tr>
<td>Liver from fed rat, sampled 10 min after 60 μmol L-carnitine/100 g, i.v.</td>
<td>1220 nmol/g</td>
<td>79 nmol/g</td>
<td>6%</td>
<td>(6)</td>
</tr>
<tr>
<td>Liver from fasted rat, sampled 20 min after 60 μmol L-carnitine/100 g, i.v.</td>
<td>1150 nmol/g</td>
<td>320 nmol/g</td>
<td>22%</td>
<td>(6)</td>
</tr>
<tr>
<td>Liver from fasted rat</td>
<td>400 nmol/g</td>
<td>87 nmol/g</td>
<td>18%</td>
<td>(6)</td>
</tr>
<tr>
<td>Plasma from fed rat</td>
<td>42 μmol/L</td>
<td>4 μmol/L</td>
<td>9%</td>
<td>(3)</td>
</tr>
<tr>
<td>Plasma from patients with hepatic cirrhosis</td>
<td>65 μmol/L</td>
<td>8 μmol/L</td>
<td>11%</td>
<td>(3)</td>
</tr>
</tbody>
</table>
Asymptomatic Xanthinuria Detected as a Result of Routine Analysis of Serum for Urate

To the Editor:

Biochemical "profiling" is now a common first line of patient investigation, its major advantage being early, pre-symptomatic detection of some diseases—in particular, primary hyperparathyroidism, owing to the inclusion of analysis for serum calcium in the profile. Recently, the inclusion of serum glucose has proved to be an effective means of early diagnosis of diabetes mellitus (1). Serum urate is often included in the profile, detection of hyperuricemic states being the prime concern. We have further investigated patients with hyperuricemia and as a result have diagnosed two unrelated patients with xanthine oxidase (EC 1.2.3.2) deficiency.

A total of seven patients with profound hyperuricemia (serum urate <80 μmol/L) were followed up initially with serum urate estimation. Of these, five showed normal urate excretion, suggesting a renal tubular etiology of the x-uricemia. The other two patients had a very low rate of urinary urate excretion, <20 μmol/L. Neither patient was on dietary purine restriction or was being treated with any xanthine oxidase inhibitor. Liquid-chromatographic analysis (2) demonstrated greatly increased concentrations of xanthine and hypoxanthine in the blood and urine, thus confirming the diagnosis of hereditary xanthinuria (Table 1).

Deficiency of xanthine oxidase is a rare inborn error of purine metabolism. To date, fewer than 50 individuals with this disorder have been described in the literature, most of them Jewish (3–5). Because the condition is relatively benign, asymptomatic cases may remain undiagnosed and the true prevalence may be somewhat higher, although in a recent retrospective study of 47 420 patients no such cases were found (4). Early diagnosis permits therapy aimed at prevention of xanthine calculi to be begun, and avoidance of very vigorous exercise may decrease the deposition of xanthine crystals in muscle, thus diminishing the risk of development of myopathy (6).

References

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"Tandem ICON hCG" Urine Pregnancy Test Evaluated

To the Editor:

The Tandem ICON pregnancy test for detecting human chorionadotropin (hCG) in urine is a visual immunoenzymometric assay, produced by Hybritech, Inc., San Diego, CA 92121. The sensitivity of this filter-membrane method is stated to be 50 int. units/L and total assay time is 5 min.

We have evaluated the test on 241 urine specimens. Of these, 185 were from the walk-in pregnancy clinic of the Department of Gynecology and Obstetrics at the Milwaukee County Medical Complex, where they were analyzed fresh with either the Sensi-Tex (Roche Diagnostic Systems, Nutley, NJ 07100), the Tandem-Visual (Hybritech, Inc.) hCG urine pregnancy test, or both, according to the manufacturers’ instructions. The Sensi-Tex latex agglutination-inhibition test has a sensitivity of 250 int. units/L and a total assay time of about 90 min. The Tandem-Visual test has a sensitivity of 50 int. units/L and a total assay time of about 45 min. The remaining 56 urine specimens were from patients undergoing treatment in the infertility clinic at the Milwaukee County Medical Complex, and were not analyzed with either the Tandem-Visual or Sensi-Tex assay.

Table 1. Clinical Details and Data on Purines

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at diagnosis, years</th>
<th>Reason for profile</th>
<th>Serum*</th>
<th>Urine</th>
<th>Serum*</th>
<th>Urine</th>
<th>Serum Urine</th>
<th>Serum Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.B.*</td>
<td>♀</td>
<td>16</td>
<td>Anemia</td>
<td>20</td>
<td>17</td>
<td>69 (3)</td>
<td>326 (30)</td>
<td>29 (2)</td>
<td>600 (40)</td>
</tr>
<tr>
<td>K.B.*</td>
<td>♂</td>
<td>35</td>
<td>Abdominal pain</td>
<td>34</td>
<td>15</td>
<td>25</td>
<td>556</td>
<td>20</td>
<td>1600</td>
</tr>
</tbody>
</table>

* Reference range 150–450 μmol/L. ** S.B. is the second of five daughters of Pakistani parents. Other siblings are not affected. *Control figures in parentheses.

References