We describe biochemical and clinical features of 11 subjects (ages, 1.2–84 years, nine females and two males) with transient 5-oxoprolinuria (0.6–23.6 mol/mol of creatinine, reference range <0.07). A variety of conditions preceded the onset of acidosis, and all had taken acetaminophen (paracetamol), although in therapeutic amounts in most subjects. Metabolic acidosis was documented in nine subjects, and all had an increased anion gap and abnormal liver functions. 5-Oxoproline was the major urinary organic acid in five subjects, whereas the rest had more complex profiles comprising 5-oxoproline and other organic acids, such as lactate, 3-hydroxybutyrate, and 4-hydroxyphenyl lactate. The 5-oxoproline was predominantly of the L-configuration. One subject died during an acidotic episode, and the rest recovered with no apparent long-term ill effects. Urinary 5-oxoproline was within the reference range in six subjects that were re-tested after the anion gap normalized. These findings suggest that acetaminophen, in association with other unidentified factors, is involved in the development of this condition through a mechanism of depletion of liver glutathione stores.

High anion gap metabolic acidosis (HAGMA) is an occasional finding in subjects admitted to hospital emergency departments. Increases in plasma L-lactate (due to hypoxia, ischemia, or trauma) or ketones (due to inadequate intake or diabetic ketoacidosis) are the usual causes for the increase in anion gap. However, two adult subjects with metabolic acidosis, one with an increased anion gap, have been described in whom 5-oxoproline (pyroglutamic acid) was responsible for the acidosis (1, 2). 5-Oxoproline is an intermediate in the γ-glutamyl cycle (Fig. 1), which is involved in the uptake of amino acids into cells. Two rare genetic diseases of the γ-glutamyl cycle, glutathione synthetase deficiency and 5-oxoprolinase deficiency, that have been described lead to the accumulation of 5-oxoproline in plasma and urine (3). The two adult subjects with 5-oxoprolinuria presented acutely with symptoms not associated with either of these two genetic diseases. Furthermore, the enzyme activities of 5-oxoprolinase and glutathione synthetase were found to be normal in one subject (1). Although it was not possible to determine the exact causes of the acute onset 5-oxoprolinuria seen in these two subjects, a possible association with acetaminophen (paracetamol) was suggested (1). Here, we describe the clinical and biochemical details of 11 additional subjects with transient 5-oxoprolinuria and present additional evidence that strengthens the proposed association with acetaminophen.

**Materials and Methods**

**CLINICAL DETAILS OF SUBJECTS**

Subject 1 presented at 32 weeks of pregnancy with respiratory distress and HAGMA. Two weeks before admission, she developed lower back pain requiring acetaminophen after minor injury. She deteriorated rapidly on admission to hospital, where pneumococcal septicemia and fetal death in utero were diagnosed. She was treated with intravenous penicillin, and the fetus was delivered 36 h after admission. Her condition improved, although the back pain persisted. This was subsequently diagnosed as left sacroiliac osteomyelitis. The patient recovered, and a subsequent challenge with acetaminophen produced no apparent adverse clinical or biochemical effects.

Subject 2 presented with chills, vomiting, hematemesis, and confusion after a urinary tract infection. She had a past history of alcohol abuse and was on acetaminophen for back pain for the last 3 days. Infection was suspected, and she was commenced on antibiotics and intravenous fluids. She slowly improved on antibiotics despite negative bacterial cultures and was discharged after 6 days.

Subject 3 presented after a 1-month history of intermit-
tent vomiting, anorexia, malaise, and confusion. Acetaminophen had been taken immediately before admission. She had a past history of chronic obstructive lung disease, congestive cardiac failure, and subtotal gastrectomy for duodenal ulcer disease. She recently had been twice investigated at another hospital because of metabolic acidosis, but no cause was identified. She was treated for metabolic acidosis with intravenous bicarbonate and discharged 15 days later.

Subject 4 had a past history of hypothyroidism and multiple abdominal operations and presented after 7 days of abdominal pain requiring acetaminophen and opiate analgesia. She had been vomiting for \(\sim 12\) h before admission. An urgent laparotomy was performed with no abnormal findings, but she required admission to the intensive care unit because of pulmonary edema and myxedema crisis. Her acidosis gradually resolved without specific therapy after 7 days.

Subject 5 was a non-insulin-dependent diabetic with schizophrenia who was found semicomatose and hypothermic after an overdose, which included substantial acetaminophen ingestion. She was intubated, ventilated, and placed on hemofiltration. A renal biopsy showed acute tubular necrosis, and the subject died despite ongoing treatment.

Subject 6 was a child who presented with a 4-day history of fever, vomiting, lethargy, and abdominal pain. Right lower lobe pneumonia was diagnosed, and she was commenced on intravenous penicillin. She deteriorated shortly after admission with abnormal liver function tests, encephalopathy, hypoglycemia, and coagulopathy. Because acetaminophen toxicity was suspected, the subject was placed on N-acetylcysteine in addition to cardiovascular and respiratory support. Liver function tests slowly normalized, and she was discharged after 10 days.

Subject 7 was a girl with spina bifida complicated by bilateral vesico-ureteric reflux and hypertension who presented with an infected ventriculo-peritoneal shunt. She was taking acetaminophen and developed renal failure and compensated metabolic acidosis after surgery for revision of the shunt. Two days later, she collapsed with septic shock and required cardiovascular and respiratory support. A metabolic acidosis persisted for 22 days while she was on acetaminophen. Substitution of codeine for acetaminophen resolved the acidosis.

Subject 8 was a child who presented with fever, diarrhea, and lethargy in the previous 24 h. She became unrousable and was intubated and transferred to the intensive care unit with a possible diagnosis of septic shock. She was placed on intravenous antibiotics and acetaminophen and made a rapid recovery. Bacterial cultures were negative.

Subject 9 presented after 3 days of abdominal pain. She had a past history of recurrent abdominal pain thought to
be diverticulitis and frequently ingested acetaminophen and codeine. At the time of admission, she was taking ~4 g of acetaminophen per day. A provisional diagnosis of acute diverticulitis was made, but a laparotomy revealed no abnormality. She was monitored postoperatively in the intensive care unit, where HAGMA was noticed. Fever and hypotension developed, and a repeat laparotomy was performed, but no ischemic bowel was found.

Subject 10 presented after an episode of collapse. He had a past history of mitral valve prolapse with severe mitral regurgitation. A coagulase-negative Staphylococcus was grown from blood cultures, and subacute bacterial endocarditis was diagnosed. He was commenced on a 4-week course of dicloxacillin. After he was commenced on acetaminophen for temperature control, the patient became unwell with severe HAGMA. Acetaminophen administration was ceased, and he was given an infusion of N-acetylcysteine. He made a rapid recovery and was discharged from hospital.

Subject 11 had a past history of pancreatitis secondary to alcohol abuse and presented with an acute onset abdominal pain and breathlessness after 1 week of constant epigastric pain for which he had been taking acetaminophen. No cause for his acute presentation could be found, but HAGMA was present. He was treated with intravenous N-acetylcysteine, and acetaminophen was ceased. He made an uneventful recovery.

**METABOLITE MEASUREMENTS**

Urine and plasma organic acids profiles were obtained after extraction with ethyl acetate, conversion to trimethylsilyl derivatives, and analysis by gas chromatography–mass spectrometry (GC-MS) (4). This method measures total 5-oxoproline concentrations (sum of D- and L-isomers), as well as detecting numerous other organic acids. The interbatch coefficient of variation for the measurement of total 5-oxoproline was 13%, with a mean recovery of 92% using this technique. The reference range for urine total 5-oxoproline was derived from 342 samples submitted for routine metabolic screening in which no evidence of inherited metabolic diseases was found. The reference range for blood total 5-oxoproline was derived from six healthy controls. Urines were also analyzed by an enzymic method specific for L-5-oxoproline. Urines were diluted to a creatinine concentration of 1.0 mmol/L, acidified with hydrochloric acid, saturated with sodium chloride, and extracted with ethyl acetate/isopropanol (5:1, by volume). The solvents were removed with a stream of air at 60 °C, and the residue was dissolved in 3 mL of 2 mol/L hydrochloric acid and heated at 100 °C for 90 min to convert L-5-oxoproline to L-glutamate. Samples were then neutralized with 1 mL of 6 mol/L sodium hydroxide. The L-glutamate concentration was then measured spectrophotometrically, using L-glutamate dehydrogenase (5). There was no detectable interference from D-5-oxoproline or L-glutamate present in the original sample, using this procedure. The interbatch coefficient of variation for the measurement of urine L-5-oxoproline was 8%, with a mean recovery of 96%.

Urine orotate was measured by anion exchange HPLC (6) or stable isotope dilution GC-MS (7). Acetaminophen was measured in blood, using a variety of commercial kits. Acetaminophen in urine was qualitatively detected by GC-MS, using the organic acid-profiling method described above or as a metabolite during amino acid screening, using high-voltage paper electrophoresis (8).

**Results**

Relevant biochemical data for each of the subjects are summarized in Table 1. Wherever possible, samples collected as close as possible to the acute episodes were analyzed. Metabolic acidosis, generally compensated, was documented in 9 subjects, and an increased anion gap was found in all 11 subjects. Total 5-oxoproline was grossly increased above the reference range in urine, ranging from 0.6 to 23.6 mol/mol of creatinine, and also in blood, ranging from 2.3 to 16.0 mmol/L, in seven subjects for whom blood samples were available. The enzymic measurement of L-5-oxoproline and comparison with the total 5-oxoproline values obtained by GC-MS in eight of the subjects showed that the excreted 5-oxoproline was predominantly of the L-configuration, which is the physiological form involved in the γ-glutamyl cycle. No other abnormal urinary organic acids were evident in subjects 2, 5, 7, 9, and 11, but the other subjects exhibited more complex profiles, with 3-hydroxybutyrate, lactate, and 4-hydroxyphenyl lactate also being increased in urine or blood. Increased excretion of the last organic acid may sometimes occur secondarily to abnormal liver function, which was a finding in all of the subjects. Urine orotate concentrations were within the reference range in all cases where data were available.

Acetaminophen or its metabolite was detected in all urines that had marked increases of total 5-oxoproline, and blood concentrations of acetaminophen were determined in some patients (Table 1). Subject 1 was given an acetaminophen challenge after recovery, and the excretion of 5-oxoproline was within the reference range before and after the challenge. Repeat testing was performed on subjects 6, 8, 9, and 11, all of whom had concentrations of urinary total 5-oxoproline within the reference range after recovery. Subject 7 was being treated with acetaminophen for analgesia, and consistently increased L-5-oxoproline excretion was found on four occasions during this time. Substitution of codeine for acetaminophen produced normalization of L-5-oxoproline excretion (Fig. 2).

**Discussion**

A number of conditions have been associated with increased urinary excretion of 5-oxoproline. Two genetic deficiencies of the γ-glutamyl cycle, glutathione synthetase deficiency and 5-oxoprolinase deficiency (3), lead to very large increases in 5-oxoproline excretion. Situations in which the availability of glycine is limited, such as
malnutrition (9, 10) and pregnancy (11), may also lead to increased 5-oxopoline but at more modest concentrations. Excretion of 5-oxopoline may also be increased secondary to some inborn errors of metabolism (12–15), type 2 diabetes (16), artificial feeds (17), and by medications such as vigabatrin (18).

The subjects with 5-oxoprolinuria described in this report clearly represent a heterogeneous and distinct group compared with subjects with 5-oxoprolinuria produced by genetic enzyme deficiencies in the γ-glutamyl cycle, although the concentrations of 5-oxopoline were similar to those seen in the latter group (19). The transient nature of the 5-oxoprolinuria, its association with a variety of symptoms, its generally acute onset, and the lack of chronic symptoms such as anemia and neurological impairment are distinguishing features. None of the subjects were related, and none had a family history that would suggest an underlying genetic cause of their condition. Finally, a previous investigation of another subject with acute onset 5-oxoprolinuria yielded normal activities of glutathione synthetase and 5-oxoprolinase (1).

None of the subjects were taking vigabatrin or on artificial feeds known to induce 5-oxoprolinuria. Although it was not possible to completely exclude functional glycine deficiency in all subjects, it was considered unlikely to be the primary cause of the 5-oxoprolinuria. First, the concentrations of 5-oxopoline excretion were much higher than those reported previously for conditions in which functional glycine deficiency has been considered to be a factor, such as malnutrition (9) or normal pregnancy (11). Second, there was no evidence of overt malnutrition in any of the subjects, and other conditions that might further limit glycine availability, such as postoperative stress, childhood growth, or diabetes, were notably lacking in subjects 2, 3, and 11.

All subjects were known or suspected to be taking acetaminophen at the time of their presentation. This was confirmed in all subjects during the analysis of urine and/or blood samples. Although some of the subjects were on multiple medications, acetaminophen was the one medication that appeared to be common in all subjects. Two previously reported subjects with transient 5-oxoprolinuria had also received acetaminophen (1, 2). As previously suggested (1), the 5-oxoprolinuria observed in these subjects may be related to the well-known phenomenon of depletion of glutathione stores in the liver.

### Table 1. Biochemical data* for subjects with transient 5-oxoprolinuria.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>F F F F F F F F M M</td>
</tr>
<tr>
<td>Age, years</td>
<td>33.2 54.4 60.1 56.6 64.8 5.8 17.1 1.2 73.1 84.2 57.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.12 7.23 7.14 7.16 6.80 7.49 7.38 7.29 7.31 7.15 7.09 7.35–7.45</td>
</tr>
<tr>
<td>Bicarbonate, mmol/L</td>
<td>6.0 11.0 6.0 5.0 2.0 14.0 22.0 15.0 37.0 23.0 7.0 9.0 35–45</td>
</tr>
<tr>
<td>Po2 mmHg</td>
<td>11.0 11.0 17.0 15.0 14.0 22.0 15.0 37.0 23.0 7.0 9.0 35–45</td>
</tr>
<tr>
<td>Anion gap, mmol/L</td>
<td>33.1 26.3 38.8 31.3 38.3 29.0 31.2 16.4 30.5 20.8 37.1 &lt;15</td>
</tr>
<tr>
<td>Total 5-oxoprine, mmol/L^c</td>
<td>ND 6.7 6.6 6.6 8.0 ND ND ND 16.0 11.3 2.3 &lt;0.07</td>
</tr>
<tr>
<td>Urine total 5-oxoprine, mmol/ mmol creatinine^c</td>
<td>17.0 13.8 11.0 10.0 4.0 5.9 20.4 0.6 22.0 23.6 5.7 &lt;0.07</td>
</tr>
<tr>
<td>L-5-Oxoprin, % of total 5-oxoprine^d</td>
<td>ND 82 125 112 ND 102 122 ND 93 97 91</td>
</tr>
<tr>
<td>Other urine organic acids^e</td>
<td>LAC 3HB, LAC, 3HB LAC, 4HPL LAC, 3HB LAC, 3HB</td>
</tr>
<tr>
<td>Acetaminophen, μmol/L</td>
<td>&lt;20 &lt;20 147.0 34.0 40.0 146.0 ND ND 33.0 ND 596.0 &lt;200^f</td>
</tr>
<tr>
<td>Urine orotate, mmol/mol creatinine</td>
<td>ND 1 2 5 &lt;1 4 2 3 &lt;1 &lt;1 2 &lt;5</td>
</tr>
<tr>
<td>AST, U/L</td>
<td>23 134 42 ND 385 10888 31 42 21 72 ND &lt;40</td>
</tr>
<tr>
<td>ALP, U/L</td>
<td>520 227 240 147 366 ND ND ND 126 158 218 35–100</td>
</tr>
<tr>
<td>GGT, U/L</td>
<td>ND 732 692 197 4070 94 304 13 47 137 254 &lt;50</td>
</tr>
<tr>
<td>Ammonium, m mol/L</td>
<td>ND ND ND ND ND 161 37 21 ND ND ND &lt;50</td>
</tr>
</tbody>
</table>

* Measured in blood unless stated otherwise.
^a Total 5-oxoprine measured by GC-MS.
^b ND, not determined.
^c L-5-Oxoprine was measured enzymatically. Values >100% are due to experimental uncertainties.
^d Acetaminophen, μmol/L
^e LAC, lactate; 3HB, 3-hydroxybutyrate; 4HPL, 4-hydroxyphenyl lactate; AST, aspartate transaminase; ALP, alkaline phosphatase; and GGT, γ-glutamyl transpeptidase.
^f Therapeutic range.
that occurs during acetaminophen toxicity (20). An acetaminophen metabolite, N-acetylbenoquinoneimine, reacts irreversibly with glutathione. The activity of γ-glutamyl cysteine synthetase is negatively influenced by concentrations of glutathione (21), and depletion of glutathione stores would be expected to produce increased activity of γ-glutamyl cysteine synthetase and excessive production of γ-glutamyl cysteine. This would normally be converted to glutathione through the action of glutathione synthetase. However, it is possible that, under the altered conditions resulting from acetaminophen ingestion, glutathione synthetase becomes rate limiting or is inhibited, leading to the accumulation of γ-glutamyl cysteine, which can then be acted on by γ-glutamyl cyclotransferase to produce L-5-oxoproline (Fig. 1). This mechanism is similar to that proposed to account for the excessive production of L-5-oxoproline in glutathione synthetase deficiency (3, 21) and the resulting HAGMA seen in these subjects.

The involvement of acetaminophen in triggering 5-oxoprolinuria is further supported by the finding that rats chronically fed a diet containing acetaminophen develop gross excretion of 5-oxoproline in their urine (22). It is noteworthy that many of the subjects identified in the present study had been taking acetaminophen for some time before their presentation. In particular, subject 7 had a persistent increase in 5-oxoproline excretion while taking acetaminophen, which normalized after acetaminophen was withdrawn (Fig. 2). Furthermore, it is unlikely that the 5-oxoprolinuria was caused by HAGMA or abnormal liver function, with the presence of acetaminophen, a widely used analgesic, being merely a chance association. The population from which the reference range for urinary total 5-oxoproline was derived included a large number of hospital inpatients, including substantial numbers of subjects with HAGMA and abnormal liver function. Although it is not entirely possible to rule out other exogenous agents as being responsible for the 5-oxoprolinuria in these subjects, the evidence presented, when combined with evidence obtained from animal studies (22), points convincingly to acetaminophen being one of the causative agents of this condition.

One interesting feature was that 9 of the 11 subjects were female ($P = 0.03$) and that two other subjects with acute onset 5-oxoprolinuria reported in the literature have also been female (1, 2). Orotate measurements were also performed because secondary excretion of 5-oxoproline has been reported in some subjects with the X-linked disorder ornithine transcarbamylase deficiency (15). Female carriers of this disorder can occasionally develop hyperammonemia and increased orotate excretion during periods of high protein intake or increased catabolism. However, the finding of normal urine orotate concentrations and normal, or only modestly increased, concentrations of ammonia in the subjects described in the present report makes this an unlikely possibility. The cause of the sex bias in patients with transient 5-oxoprolinuria is uncertain. It may merely reflect different drug usage patterns between males and females or may represent some sex-related aspect of acetaminophen or glutathione metabolism. For example, glutathione-S-transferase enzymes are involved in the conjugation of acetaminophen with glutathione, and the expression and activity of a
It should be emphasized that acetaminophen ingestion alone is unlikely to account for the severity of the 5-oxoprolinuria seen in these subjects. Although increases in urinary 5-oxoproline concentrations have been reported in children taking small amounts of acetaminophen (28), the concentrations were orders of magnitude less than those seen in the subjects described in the present study. Furthermore, subject 1 was re-investigated after her recovery with a acetaminophen challenge, and no substantial increase in urinary 5-oxoproline concentrations was observed. With the exception of subjects 5, 11, and possibly 6, all subjects were on therapeutic doses of acetaminophen, and there did not appear to be any obvious dose-response relationship between acetaminophen and 5-oxoproline concentrations in plasma. Finally, subjects who have taken acetaminophen overdoses generally have much lower excretion of 5-oxoproline than the subjects described in this report (data not shown). It thus appears likely that there was a synergistic interaction between acetaminophen and other factors to produce the gross 5-oxoprolinuria observed in these subjects. Most of the subjects had preceding illnesses that would be expected to lead to other metabolic derangements, as reflected in the finding of lactate and ketones in the urine of some patients. Abnormal liver function was also a feature of the subjects. Although it is not entirely possible to disentangle the contribution of acetaminophen to these abnormal liver function tests, it appears that the concentration of acetaminophen was insufficient in most of the subjects to explain the extent of the abnormalities. From the data presented here, it is not possible to evaluate what factors, in addition to acetaminophen, may be necessary to cause excessive production of 5-oxoproline. It may be that conditions that lead to abnormal liver function or, as discussed above, limitations in the availability of glycine, when combined with depletion of liver glutathione due to acetaminophen ingestion, can trigger this condition.

It seems likely that this condition may be under-reported and that adult subjects with this condition may go unrecognized. Although analysis of urine organic acids by GC or GC-MS can quickly identify the condition, this test is usually performed only on children as part of investigations for genetic enzyme deficiencies and may only be available in tertiary pediatric hospitals. Clinicians in adult hospitals may be unaware of the availability of such tests for the investigation of HAGMA. It thus seems prudent to recommend that any subjects with HAGMA that cannot be adequately explained by increased lactate and/or 3-hydroxybutyrate concentrations should be investigated for the presence of 5-oxoproline in urine or plasma, and evidence of acetaminophen ingestion should be sought. Withdrawal of acetaminophen or substitution of alternative analgesic agents should be undertaken, and treatment with N-acetylcysteine should also be considered, even in those subjects receiving therapeutic doses of acetaminophen.

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References


