activity as measured by the Abbott ER-EIA is similar to that of estrogen binding activity as measured by the DCC method. Thus, the ER-EIA method is no substitute for careful handling of the tissue before the assay.

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References

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Urine Discoloration after Acetaminophen Overdose

P. M. S. Clark,¹ J. D. A. Clark,² and T. Wheatley²

Three patients with acetaminophen overdose were observed to have dark-brown urine at an early stage of their illness. Subsequently, acute anuric renal failure and hepatic dysfunction developed in all three. p-Aminophenol was identified by chromatographic and colorimetric methods in the urine of each case and is thought to be responsible for the discoloration.

Additional Keyphrases: p-aminophenol · enzymatic analysis · chromatography

Case Histories

Case 1: A 51-year-old woman was admitted to hospital 20 h after she had ingested 75 g of acetaminophen, 320 mg of codeine phosphate, and an unknown quantity of nitrazepam. Dark-brown urine was observed 16 h later, when she was already oliguric. She rapidly developed acute anuric renal failure, (plasma creatinine 337 μmol/L) and a moderately increased (776 U/L) serum alanine aminotransferase (EC 2.6.1.2, ALT, normal range <40 U/L) measured at 37°C. Despite supportive measures she died the following day.

Case 2: A 54-year-old man, admitted 15 h after ingesting 50 g of acetaminophen and 100 mg of diazepam, developed urinary discoloration 48 h after the overdose. At this stage he had both renal and hepatic dysfunction, which continued to deteriorate (plasma creatinine 486 μmol/L, normal range 35-125 μmol/L; serum ALT 7870 U/L), and he died four days after the overdose.

Case 3: A 26-year-old woman presented 36 h after ingesting 40 g of acetaminophen. Initial investigations confirmed hepatic damage and impaired renal function (plasma creatinine 224 μmol/L, serum ALT >6000 U/L) and she was observed to have brown urine. Despite full supportive management she developed anuric renal failure (plasma creatinine 908 μmol/L) and hepatic encephalopathy, but after ventilation and hemodialysis she recovered and was discharged three weeks later. Subsequent follow-up revealed normal renal and hepatic function.

Owing to the delay in presentation, none of these patients was treated with N-acetylcysteine. Their serum acetaminophen concentrations on admission were 4.8, 2.0, and 0.3

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mmol/L (a high risk of hepatotoxicity being associated with concentrations exceeding 0.3 mmol/L at 12 h after ingestion). Histopathological examination of the kidneys and livers in the first two cases demonstrated necrosis of the proximal tubular epithelium and centrilobular hepatic necrosis.

Materials and Methods

The urine was analyzed colorimetrically and by thin-layer chromatography. The colorimetric assay (1) we used (Porton Products, Wrexham, U.K.) measures both acetaminophen and p-aminophenol. Any p-aminophenol present can be determined by the second reaction of the assay with o- cresol and ammonia/copper ions without added enzyme. For thin-layer chromatographic analysis of the urine we used silica-coated F plates (Merck, Darmstadt, F.R.G.) and a mobile phase of butanol:acetic acid:water (3:1:1 by vol). Aqueous acetaminophen and p-aminophenol standards were also analyzed.

Results

p-Aminophenol was demonstrated in the urine of all three cases by the colorimetric assay (Table 1). Thin-layer chromatographic analysis of the urines (Figure 1, plate viewed at 254 nm) and standards indicated the presence of compounds with the same Rf values as acetaminophen (0.73) and p-aminophenol (0.69 and 0.83). The latter (B) showed two bands, both turning dark brown when exposed to visible light.

Discussion

There are several well-recognized causes of brown urine, including liver disease and hemolytic anemia, where bilirubin and urobilinogen are responsible for the color. The heme derivatives, methemoglobin and myoglobin, may also cause brown urine. Less commonly, brown urine may be due to melanin (oxidized from melanogen in malignant melanoma) and polymerized homogentisic acid (alkaptonuria). In our three cases the urinary discoloration may have been partly due to bile pigments, but the brown urine was considerably darker than expected with hepatic dysfunction and developed in case 1 before biochemical evidence of liver damage.

<table>
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<tr>
<th>Table 1. Concentrations of Acetaminophen (A) and p-Aminophenol (B) in Serum and Urine of Three Overdose Cases</th>
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<td>Day after admission</td>
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ND = not detected.

None of the patients had hemolytic anemia, melanoma, or alkaptonuria, and the urinary electrophoretogram showed no myoglobin in the first case. In cases 2 and 3, dipstick testing was positive for bilirubin and urobilinogen when these patients were first noted to have brown urine.

Brown urine has also been described in four cases of phenacetin overdose and, although the nature of the brown pigment was uncertain, acetaminophen and its metabolites might have been responsible (2).

In contrast, brown urine has not been documented in cases of acetaminophen overdose, although serum samples from such cases may become brown on storage (3). Solutions of p-aminophenol undergo oxidation in neutral or alkaline conditions and may in time polymerize to yield a brown color. Brown et al. (3) showed that the pigmentation of stored serum from overdose patients could be attributed to polymerization of p-aminophenol.

We therefore postulate that the brown urine in our three cases of acetaminophen overdose was ascribable to p-aminophenol and its polymerization products. The metabolism of acetaminophen is complex (4–5), and the mechanism for the production of p-aminophenol has yet to be determined.

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References