studies with magnetic resonance spectroscopy.

Finally, a 600-MHz spectrometer is currently very expensive for routine use, whereas a much lower-field magnet, e.g. 400 MHz, could be used in front-line screening of untreated urine for inherited disorders. We agree that for further characterization of certain specific disorders, especially where low concentrations of important metabolites are found, the superior sensitivity, resolution, and, for nonvolatile compounds, quantification demonstrated by Wevers et al. is justified.

Nonetheless, $^1$H-NMR analysis of unextracted urine offers a simple and rapid, noninvasive method for screening, diagnosis, and monitoring of metabolic disorders in children and infants, including neonates. In such work, time of analysis and ease of interpretation of the result are often of the essence. Although even at 400 MHz the technique is not yet inexpensive and widely available, it appears to us illogical to promote the application of even more expensive high-field instrumentation coupled with complex sample preparation procedures and invasive patient sampling when field-proven and simpler alternatives already exist.

References

7. Iles RA, Jago JR, Williams SR, Chalmers RA. 3-Hydroxy-3-methylglutaryl CoA lyase deficiency studied using 2-dimen-
Standardizing the pH of the sample can minimize intersample variation in the chemical shift of resonances (I, 2) and helped us in interpreting the plasma spectra. This step is even more beneficial for urine and CSF spectra because pH of these body fluids has a greater intersample variation (I, 2). Minimizing intersample variation of the chemical shift of a resonance will be of great help for future automation of spectral analysis (assignment and quantification of metabolites).

We agree with Iles et al. that there seems to be no ideal pH that can avoid overlap of resonances. In our choice of pH we were led by the arguments given in the earlier study by Lehnert and Hunkler (4) and by their already available database of relevant metabolites at this pH. Iles et al. suggest measuring the sample first at its natural pH, followed by repeat analyses at acidic (2.5) and alkaline pH (8.5) if necessary. Analytically, there are no reasons to prefer the physiological pH of the sample. Given the complexity of the spectra and the intersample variability in pH, it is not enough to record the pH of the sample. The natural pH of the sample simply has an unacceptable intersample variation (especially urine and CSF), which hampers the assignment of resonances. This may be why Iles et al. require the presence of both the doublet and the quartet resonances from methylnalonic acid for the diagnosis of methylmalonic aciduria from a urine sample. To be able to use the same model compound database for the interpretation of spectra from all body fluids, we decided to use a common pH (2.50) throughout our work (blood plasma, CSF, and urine). This seems more straightforward than working at three different pH values to avoid a simple two-step sample preparation (1). Remove proteins by filtration, 2. control pH before evaporation and reuptake in D2O. We do not consider this "a complex sample preparation" that uses "a lengthy extraction procedure" (I, 2).

The spectrometer. The increasing field strengths available in NMR spectroscopy open new possibilities that must be explored. At present, a 600-MHz machine is still very expensive, but prices may come down, just as they have for 400-MHz spectrometers. Clearly, studies at 600 MHz give better-resolved spectra and a higher sensitivity than studies at 400 MHz, thus providing more information and allowing easier interpretation and (automated) quantification. Even if one selects a lower field system (e.g., 400 MHz) as a front-line screening machine for metabolic disease, the NMR findings obtained at 600 MHz will be extremely relevant.

References


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Positive Interference from Homocystinuria Urine in a Spot Test for Molybdenum Cofactor Deficiency

To the Editor:

Molybdenum cofactor deficiency and homocystinuria share certain clinical features. The former, an autosomal recessive disorder, results from combined deficiency of sulfite oxidase, xanthine dehydrogenase, and aldehyde oxidase (I) and is characterized by severe neurological abnormalities, dislocated ocular lenses, mental retardation, and excessive urinary excretion of sulfite, thiosulfate, S-sulfocysteine, taurine, xanthine, and hypoxanthine. For simple diagnosis of molybdenum cofactor deficiency, urinary sulfite is easily detected in fresh urine specimens with a strip test (Merckquant® 10013, E. Merck, Darmstadt, Germany, or Macherey-Nagel Quantofox® SO3”, Gallard Schlesingers Industries, Carle Place, NY) (2). Homocystinuria, an inborn error of methionine metabolism most commonly caused by a deficiency of cystathionine β-synthase, is characterized by accumulated homocysteine and methionine in plasma (3). Patients present clinically with dislocated lenses, skeletal changes or osteoporosis, intravascular thrombosis, and sometimes mental retardation.

Duran et al. (4) reported a false-positive reaction for urinary sulfite after subjects were treated with the mucolytic drug 2-mercaptoethanesulfonate. We report here a strong positive interference when the test was applied to the urine of a vitamin B6-unresponsive homocystinuric patient on a regular diet.

The patient, a 9-year-old son of first cousins, had a history of some mental deficiency and speech problems. An ophthalmologic examination identified dislocated lens in the left eye and a slightly dislocated right lens. Physical examination noted a marfanoid stature with genu varum. The diagnosis of homocystinuria was established at age 9 years after a plasma amino acid analysis by HPLC on two occasions yielded methionine concentrations of 447 and 537 μmol/L (normal ≤37 μmol/L).

To evaluate the patient’s responsiveness to vitamin B6, we gave him a trial of 300-mg tablets of vitamin B6 three times daily for ~40 days. At the end of this trial, his fresh urine was tested with a sulfite test strip (Merckquant 10013), in an attempt to appreciate a possible enhancement of his deficient enzymatic activity (cystathionine β-synthase) with secondary excretion of sulfite. This test strip reaction was positive, although both parents and one brother gave negative reactions. His plasma methionine content was still high (483 μmol/L), and the patient was considered vitamin B6-unresponsive. Samples of plasma, urine, and skin biopsy were sent to Jean-Marie Saudubray (Necker Hospital, Paris, France) for confirmation of the diagnosis of homocystinuria. A later check of the patient’s urine while he was on a low-protein diet containing betaine citrate at 6 g daily gave a negative result for the sulfite test.

To identify the compound responsible for the positive sulfite test in this patient’s earlier urine, we investigated several prepared solutions. Only compounds with free-SH radicals, such as homocysteine, gave positive reactions (Table 1). In particular homocysteine gave a positive result at a concentration of 0.5 mmol/L in comparison, a fivefold higher concentration of sodium sulfite was required.