Standardizing the pH of the sample can minimize intersample variation in the chemical shift of resonances (1, 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 (1, 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 lead 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 methymalonic acid for the diagnosis of methymalonic aciuria 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 D$_2$O. We do not consider this "a complex sample preparation" that uses "a lengthy extraction procedure" (1,2).

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 (1) 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 (Merckoquant$^®$ 10013, E. Merck, Darmstadt, Germany; or Macherey-Nagel Quantofix$^®$ SO$_2^-$, Gallard Schlesinger Industries, Carle Place, NY) (2). Homocystinuria, an inborn error of methionine metabolism most commonly caused by a deficiency of cystathionine $\beta$-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 B$_6$-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 subluxed right lens. Physical examination noted a marfanoid stature with genu valgum. 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 $\mu$mol/L (normal $\leq$37 $\mu$mol/L).

To evaluate the patient’s responsiveness to vitamin B$_6$, we gave him a trial of 300-mg tablets of vitamin B$_6$ three times daily for ~40 days. At the end of this trial, his fresh urine was tested with a sulfite test strip (Merckoquant 10013), in an attempt to appreciate a possible enhancement of his deficient enzymatic activity (cystathionine $\beta$-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 (433 $\mu$mol/L), and the patient was considered vitamin B$_6$-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.
Discordant CA 19.9 Serum Results by Microparticle Enzyme Immunoassay and Immunoradiometric Assay

To the Editor:

Recently, we observed abnormally high CA 19-9 values in some serum samples collected from a patient over a 4-month period. This patient was a 67-year-old woman with chronic hepatitis C and benign tumor of the spinal cord. The CA 19.9 was determined with the Abbott IMx® assay (cat. no. 2203-20; Abbott Diagnostics, Abbott Park, IL), a microparticle enzyme immunoassay that includes a mouse monoclonal antibody (1116-NS-19.9 from Centocor, Malvern, PA). As far as we know, such markedly high values have not been reported in benign diseases, except in cholelithiasis complicated by cholangitis (1-3).

As indicated in Table 1, all CA 19-9 concentrations estimated by the IMx kit were high, ranging from 165 to 3881 kIU/L (upper limit of normal = 37 kIU/L). The calibration curve ranges from 0 to 500 kIU/L, and serum samples yielding >500 kIU/L were diluted in the dilution buffer provided by the manufacturer. The investigations undertaken failed to find the usual causes. Thus, no pancreatic, liver, or gastrointestinal tract tumors could be detected by transabdominal and endoscopic ultrasonography, computed tomography, and endoscopic examination. Furthermore, neither cholelithiasis nor cholangitis was present. It is unlikely that the benign tumor of the spinal cord is responsible for this large increase in the CA 19.9 concentrations, as no such case has been reported to date.

As seen in Table 1, the pronounced variations in the results from one serum to another during follow-up and the discrepancies observed for sample no. 6, whether it was tested diluted or not, suggested false-positive results with the IMx kit. Therefore, we determined the CA 19.9 concentrations in these serum samples (kept frozen and then thawed for this purpose) with an IMx kit (CIS Bio International, Gif-sur-Yvette, France), a two-site method with an 125I-labeled antibody. All results obtained with this method were normal (upper limit of normal = 37 kIU/L) in undiluted samples as well as in 1:10 diluted samples, showing no hook effect. Serum no. 7 was also measured by the Enzymun® test CA 19.9 (Boehringer Mannheim, Mannheim, Germany), an ELISA test that includes streptavidin-coated tubes, biotinylated antibody, and peroxidase-labeled antibody. The result (obtained with undiluted sample) was also normal (upper limit of normal = 25 kIU/L).

These discrepant CA 19.9 results between kits are surprising, because the same mouse monoclonal antibody 1116-NS-19.9 is used in all three kits. Perhaps a serum component is responsible for the increased results obtained with the Abbott IMx kit: Interferences from circulating heterophile antibodies and from rheumatoid factors in two-site immunoassay procedures are well known (4, 5), though the latter was not reported on the Abbott CA 19.9 assay information leaflet.

When we tested whether human anti-murine antibodies (HAMA) and rheumatoid factors were responsible for these results, the screening for HAMA was negative; the screening for rheumatoid factors in serum sample no. 7 was positive (IgM class) by an in-house enzyme immunoassay.

We concluded, therefore, that rheumatoid factors probably interfere with the Abbott IMx kit and could be responsible for the high results obtained. Nevertheless, this interference should be confirmed by further studies of serum samples with known rheumatoid factors. Thus, possible interference of

Table 1. CA19.9 concentrations (kIU) measured by different immunoassays.

<table>
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<th>Draw date</th>
<th>Sample</th>
<th>IMx (Abbott) Undiluted</th>
<th>Diluted (1:10)</th>
<th>IRMA (CIS Bio Int.) Undiluted</th>
<th>ELISA (Boehringer Mannheim)</th>
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<td>—</td>
<td>10</td>
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<tr>
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<tr>
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<td>&gt;500</td>
<td>569</td>
<td>&lt;10</td>
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</table>

* Final result.

Despite the sensitivity of this reaction, both parents, who are obligate heterozygotes for homocystinuria, gave negative sulfite tests.

Because molybdenum cofactor deficiency and homocystinuria share similar clinical presentations, mainly dislocated ocular lenses, methods more specific for each disorder should be used for their differentiation.

References


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