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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-


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The authors of the paper referred to reply:

To the Editor:

We have the following comments in response to the letter of Iles et al.:

Sample choice for diagnosing inborn errors of metabolism. Most papers on NMR spectroscopy in body fluids of patients with inherited metabolic disease have used urine as a sample. In our experience, however, for many patients whose clinical signs and symptoms suggest an inborn error of metabolism, our laboratory is unable to make the diagnosis by using conventional techniques. Thus we decided to study urine, blood plasma, and CSF from this group of patients by NMR spectroscopy. Each of these body fluids contains some metabolites that do not occur in the other two fluids or occur only in a much lower concentration. The many as-yet unassigned resonances that we have found both in blood plasma and in CSF illustrate that much is still to be learned (1, 2). At 600 MHz, urine spectra may contain >500 resonances outside the glucose area (3.30–3.95 ppm). Furthermore, the spectra of urine samples are complicated by baseline problems. Under the conditions used in our studies (1, 2), CSF and plasma spectra contain fewer resonances than urine spectra and can therefore be interpreted more easily. A metabolic disturbance relevant for the diagnosis may remain unnoticed in the spectrum of the patient's urine but may be readily observed in the plasma or CSF spectrum. Such is not the case for organic acidurias, where the metabolites of interest generally occur in high concentrations in the urine. Our group, however, also aims to diagnose other known and as-yet unknown inborn errors of metabolism with $^1$H-NMR spectroscopy. Such diseases may be characterized by abnormal metabolite concentrations in the low micromolar range, and the relevant resonances may be easily overlooked among the many resonances present in a urine spectrum.

We think no general rule makes urine more suited than other body fluids for diagnosing an inborn error of metabolism with NMR spectroscopy. Further studies will have to show whether or not NMR spectroscopy of blood plasma and CSF can provide additional diagnostic information that cannot be found in the urine of these patients.

About sample preparation. We agree that NMR spectroscopy can detect various inborn errors of metabolism with intact urine samples. Blood plasma samples may also be analyzed without any sample pretreatment. Such studies often used the spin-echo technique, which has drawbacks for quantification of metabolites. The study of Foxall et al. (3; see their Fig. 1a) shows poor spectral resolution even at 750 MHz. Spin-echo and two-dimensional J-resolved NMR spectra are required to resolve and to recognize the resonances. Quantification, however, remains problematic because of potential T$_2$-differences between metabolite resonances and because of residual broad components (Fig. 1b of Foxall et al.). We decided (a) to eliminate proteins and H$_2$O to improve spectral quality (1, 2) and (b) to use single-pulse NMR spectroscopy instead of spin-echo NMR spectroscopy. We are convinced that this was a decisive step forward because it allowed reliable quantification of almost every metabolite in the spectrum.
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 Lehner 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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