Drug Monitoring and Clinical Toxicology (K. K. G. T.) were assayed by the present method (y). The results were $y = 1.09z + 0.894 (r = 0.998$ with a standard error of estimate of 0.326) for caffeine and $y = 1.06z - 0.125 (r = 0.999$, standard error of estimate $= 0.359)$ for theophylline.

All samples of this correlation experiment were also analyzed with the in-house HPLC method for the determination of these drugs. In the inhouse method (z), a solid-phase extraction procedure (4) is used, followed by $C_{18}$ reversed-phase chromatographic analysis with methanol in water (175/825, by vol) as eluent and ultraviolet detection. Linear regression of the results with those of the present method (y) were $z = 0.777y + 2.632 (r = 0.984$ with a standard error of estimate of 1.169) for caffeine and $z = 0.937y + 0.165 (r = 0.998$, standard error of estimate $= 0.375$) for theophylline.

We conclude that the described method is easy to perform and inexpensive. The method takes very small sample volumes, which could be reduced to 5 $\mu$L if necessary. Sample purification or extraction is not necessary. The presented technique is especially suitable for determining caffeine in serum samples of premature infants.

References

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Whole-Blood Lead Reference Intervals for Adults

To the Editor:

Since the Centers for Disease Control (CDC) issued revised recommendations for whole-blood lead concentrations for children in 1991 (1), there has been confusion about what values to consider as normal for adults. Blood lead concentrations are unusual in the sense that reference standards are defined by government agencies. Workplace standards, for example, are set by the federal Occupational Safety and Health Administration (OSHA) (2); the concentration of concern in the workplace starts at 400 $\mu$L and was set in 1978. In the US, >95% of normal blood concentrations of lead in adults result from workplace exposure (3). Outside the workplace, normal adult values for whole-blood lead are often cited as <250 $\mu$L; this limit appears to have been set by the CDC in 1985 (4) and was intended to apply to children. According to the new CDC guidelines, whole-blood lead in children <6 years old should be <100 $\mu$L (1); again there is a tendency to apply these limits to adults (3, 5). Information on lead concentrations in nonoccupationally exposed adults is scanty.

To determine a reference interval in adults, we tested 100 whole-blood specimens collected for routine blood counts from individuals of ages $\geq 18$ years. In this sample, the average whole-blood lead was 30 $\mu$L/L, the range was 3–131 $\mu$L/L, and the SD was 21 $\mu$L/L. The reference interval, determined by excluding the upper and lower 2.5% of the population, was 6–115 $\mu$L/L. Specimens collected from adults specifically for lead determinations show an average >30 $\mu$L, presumably reflecting increased occupational and (or) environmental exposure.

We measured whole-blood lead with a Perkin-Elmer (Norwalk, CT) Elan 500 inductively coupled plasma mass spectrometer (ICP-MS). The reference laboratory at the University of Utah has been using ICP-MS for the last 3 years, during which time we have been successfully enrolled in the College of American Pathologists and the New York State and California lead-testing proficiency programs. Specimens were collected for routine blood counts in purple-top containers rather than trace-metal collection tubes; although purple-top containers have been identified as a source of sporadic contamination, this was not a significant problem here. This study complies with the ethical standards of our institution.

The 1991 CDC recommendations for whole-blood lead in children consist of several categories (1): Class I (<100 $\mu$L/L) is "not lead-poisoned"; Class II (100–190 $\mu$L/L) suggests a need for "evaluation and more frequent screening"; Class III (200–440 $\mu$L) indicates a need for "evaluation and remediation"; Class IV (450–690 $\mu$L/L) requires "medical and environmental interventions"; and Class V (>690 $\mu$L/L) represents "a medical emergency. The application of these categories to adults has been limited. For example, although a whole-blood lead of 700 $\mu$L is an emergency in children because of the danger of lead encephalopathy, adults are not in jeopardy of encephalopathy until much higher concentrations are present. The use of <100 $\mu$L as the normal reference limit in nonoccupationally exposed adults appears appropriate for our population, with 3% of the study subjects falling outside the reference interval. However, the actions and interventions recommended for children with increased concentrations of blood lead should not be applied indiscriminately to adults, because adults are less vulnerable to lead exposure.

References

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Novel Homozygous Mutation of Phenylalanine Hydroxylase Gene in a Chinese Patient with Phenylketonuria

To the Editor:

A novel homozygous R158W mutation in exon 5 of the phenylalanine hydroxylase (PAH; EC 1.14.16.1) gene was detected by dideoxy sequencing of polymerase chain reaction (PCR)-amplified DNA fragments and solid-phase technology involving the biotin-streptavidin system (1).

Amplification primers and optimal protocols were selected experimentally according to previous publications (for review see 2) and prepared with a DNA synthesizer (Applied Biosystems, Inc., Foster City, CA). To amplify DNA fragments, we used Tth DNA polymerase kit (Toyobo, Osaka, Japan). Amplification primers for exon 5 were: 5A, 5'-TCTAGGTCCTTA-GAGCCGCAC-3', and 5B, 5'-XCAA-CAAGCAAGGCAGACTTAC-3', where X is biotin conjugated to the 5' end of antisense primer by the monomethoxytrityl (MMT) hexanamine linker (Millipore, Tokyo, Japan). After initial denaturation at 94 °C, thermal cycling was carried out for 30 cycles at the following conditions: denaturation for 60 s at 94 °C, 120 s annealing at 60 °C, and 50 s primer extension at 75 °C. Sequencing revealed a homozygous C→T transition at the first base of codon 158, resulting in a substitution of Trp for Arg (Figure 1A).

The allele-specific oligonucleotide probes, labeled with alkaline phosphatase, were designed for nonradioactive examination with the DNA synthesizer and labeling kits (Toyobo; or Cambridge Research Biochemicals, Cheshire, UK). DNA fragments of amplified exon 5 were hybridized with the probes after Southern blot transfer onto nylon membranes. The patient's sample hybridized with the mutant probe, but not with the normal one, whereas the normal control reacted just the opposite (Figure 1B).

The biochemical and clinical phenotype of the patient showed classical phenylketonuria (PKU) with severe hyperphenylalaninemia (pretreatment serum phenylalanine 316 mg/L).

The novel R158W substitution found in the Chinese patient affected the same codon as the R158Q mutation observed in Europeans (3, 4). The R158Q mutation is associated with classical PKU (5), and constitutes ~40% of the mutant alleles of haplotype 4 in the European population (4). In general, the C→T transition resulting from deamination of methylycytosine accounts for ~35% of the point mutations involved in the disease, thus constituting a hot spot for mutations (6). It has been suggested that Arg→Trp substitution in PKU is widely present in exon 7 and 12 but not in exon 5 (7). According to these facts as well as genetic and biochemical findings, we consider the R158W substitution in exon 5 a noteworthy mutation because it might be involved in PKU etiology among Orientals. Moreover, the occurrence of this molecular change may be expected regardless of race.

References

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Assesement of High-Density Lipoprotein Cholesterol in Hypertriglyceridemic Sera

To the Editor:

Severe hypertriglyceridemia interferes with the measurement of high-density lipoprotein cholesterol (HDL-C)