bined with the individual protein testing capacity of the WB. The assay can be tailored to the requirements of screening or confirmatory testing as well as to the needs of specific vaccine studies. Vaccine trials could benefit greatly from a PBA that differentiates protected from infected individuals. Because large-scale vaccine studies are likely to be conducted in settings where conditions may not permit easy handling of fresh blood specimens, the DBS technology constitutes an attractive alternative for specimen collection and the PBA appears to be well suited for this purpose. The HIV PBA offers substantial advantages over conventional immunoassays, but standardization, validation, and performance testing remain to be done before the assay can be used for clinical testing.

References

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Serum Copper Is Decreased in Premature Newborns and Increased in Newborns with Hemolytic Jaundice, Kleopatra H. Schulpis,1 Theodoros Karakonstantakis,2 Stavroula Gavrili, 3 Christos Costalos, 3 Eleftheria Roma, 3 and Ioannis Papassotiriou 2* (1 Institute of Child Health and 2 Department of Clinical Biochemistry, “Aghia Sophia” Children’s Hospital, Athens, Greece; 3 Neonatology Department, “Alexandra” Maternity Hospital, Athens, Greece; 4 First Department of Pediatrics, Athens University, Athens, Greece; 4) address correspondence to this author at: Department of Clinical Biochemistry, “Aghia Sophia” Children’s Hospital, 115 27 Athens, Greece; fax 30-210-7467171, e-mail biochem@paidon-agiasofia.gr or jppapasotiriou@ath.forthnet.gr

Copper is an active component of several enzyme systems, including cytochrome c oxidase and superoxide dismutase (1), and is essential for the prevention of anemia and leucopenia. It is important for the maturation of collagen and the maintenance of skeletal and vascular integrity because of its involvement in the lysyl oxidase enzyme system (2). Deficiency of copper is associated with an increased incidence of infection, as seen in patients with Menke kinky-hair disease, an inherited disease with low copper concentrations (3). The mechanism by which high copper concentrations interact with genetic
control systems, leading to liver damage, is still unknown (4). The normal term infant is born with a generous liver copper store, and copper deficiency in neonates is a rare event (5). Copper deficiency has been associated with anemia, neutropenia, and bone demineralization in both preterm and full-term infants (6–8).

Ceruloplasmin, the major serum copper-transporting protein, is synthesized in the liver, but its precise role in copper metabolism is unclear. Neonatal hepatic ceruloplasmin synthesis occurs after birth and is associated with a gradual increase in plasma concentrations (9).

We report the concentrations of copper in healthy and jaundiced full-term and premature infants and their correlations with hemoglobin, bilirubin, and liver enzymes.

The study was approved by the Greek Ethical Committee, and parental consent was obtained. The study comprised 303 neonates (full-term and premature). Newborns with obstructive jaundice or with infections or inflammatory process were excluded. All patients underwent routine laboratory tests to determine the etiology of their jaundice (see Table 1 in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol50/issue7/). Patients were divided into the following groups (Table 1).

Newborns with hemolytic jaundice (n = 179). The full-term group with hemolytic jaundice included 91 neonates, 2–3 days of age, with a mean (SD) birth weight of 3256 (360) g and a mean (SD) gestational age of 40 (2) weeks (range, 38–42 weeks). This group was divided into three subgroups according to serum total bilirubin (Table 1). The group of premature infants with hemolytic jaundice included 88 infants, 2–3 days of age, with a mean (SD) birth weight of 1350 (175) g and a mean (SD) gestational age of 32 (2.5) weeks. This group was also divided into three subgroups (A, B, and C) according to the above-mentioned total bilirubin concentrations (Table 1).

Newborns with nonhemolytic jaundice (n = 124). The full-term infants with nonhemolytic jaundice [n = 60 neonates; age, 2–3 days; mean (SD) birth weight, 3100 (268) g; mean (SD) gestational age, 40 (2) weeks (range, 38–42 weeks)] were divided into three groups according to the above-mentioned total bilirubin concentrations (Table 1). The group of premature infants with nonhemolytic jaundice included 64 preterm newborns with jaundice [age, 2–3 days; mean (SD) birth weight, 1280 (185) g; mean (SD) gestational age, 30 (4.5) weeks], who were also divided into three groups as above (Table 1).

Bilirubin, transaminases, γ-glutamyl transferase, and lactate dehydrogenase (LD) were measured on the Advia 1650 (Bayer Corporation). Ceruloplasmin oxidase activity was measured as described previously (12). Serum copper concentrations were measured in duplicate by flame atomic absorption spectroscopy (Perkin-Elmer Atomic Absorption Spectrophotometer A Analyst 800). Aliquots of human serum were used as control materials to assess the precision of the copper determinations. The interassay

### Table 1. Copper concentrations and hemolysis related biochemical tests in full-term and premature newborns with hemolytic or nonhemolytic jaundice.

<table>
<thead>
<tr>
<th></th>
<th>Hemolytic jaundice groups</th>
<th>Nonhemolytic jaundice groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td><strong>Full-term infants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>38</td>
<td>25</td>
</tr>
<tr>
<td>Hb, g/L</td>
<td>198 (20) *</td>
<td>156 (30) *</td>
</tr>
<tr>
<td>Total bilirubin, mg/L</td>
<td>35 (6) *</td>
<td>104 (18) *</td>
</tr>
<tr>
<td>Conjugated bilirubin, mg/L</td>
<td>15 (10) *</td>
<td>88 (12) *</td>
</tr>
<tr>
<td>LD, U/L</td>
<td>150 (36) *</td>
<td>350 (80) *</td>
</tr>
<tr>
<td>Copper, mg/L</td>
<td>0.64 (0.10) *</td>
<td>1.10 (0.18) *</td>
</tr>
<tr>
<td><strong>Premature infants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>35</td>
<td>25</td>
</tr>
<tr>
<td>Hb, g/L</td>
<td>148 (25) *</td>
<td>105 (18) *</td>
</tr>
<tr>
<td>Total bilirubin, mg/L</td>
<td>30 (10) *</td>
<td>108 (12) *</td>
</tr>
<tr>
<td>Conjugated bilirubin, mg/L</td>
<td>21 (10) *</td>
<td>82 (10) *</td>
</tr>
<tr>
<td>LD, U/L</td>
<td>162 (36) *</td>
<td>478 (90) *</td>
</tr>
<tr>
<td>Copper, mg/L</td>
<td>0.44 (0.05) *</td>
<td>0.92 (0.08) *</td>
</tr>
</tbody>
</table>

* Results are the mean (SD).

a Hemolytic jaundice groups: full-term infants, n = 91; premature infants, n = 88.

b Nonhemolytic jaundice groups: full-term infants, n = 60; premature infants, n = 64.

Comparisons among groups:

- Hb, P < 0.01 for d/e, d/f, d/h, d/i, e/f, e/g, e/h, e/i, f/g, f/h, and f/i.
- Total bilirubin, P < 0.01 for d/e, d/f, e/g, d/h, d/i, e/i, f/g, t/h, and t/f.
- Conjugated bilirubin, P < 0.001 for d/e, d/f, d/i, e/f, d/h, e/h, e/i, f/g, e/h, t/g, f/h, g/h, g/i, and h/i.
- LD, P < 0.01 for d/e, d/f, e/g, e/h, e/i, h/g, f/h, and f/i.
- Copper, P < 0.01 for d/e, d/f, d/h, d/i, e/f, e/g, e/h, e/i, f/g, f/h, and f/i.
- Copper, full-term/premature infants, P < 0.01 for d/d, e/e, f/f, g/g, h/h, and i/i.
Copper concentrations were increased in all groups of full-term and preterm newborns with moderate or severe hemolytic jaundice compared with group A and with newborns with nonhemolytic jaundice (Table 1). Serum copper concentrations in premature infants with both hemolytic and nonhemolytic jaundice were significantly lower (P <0.001) than in full-term newborns. LD was increased in all groups with hemolytic jaundice. Transaminases and γ-glutamyl transferase were mostly within reference values (see Table 2 in the online Data Supplement). Ceruloplasmin was slightly but not significantly increased in full-term infants compared with premature infants (P >0.05). The serum copper concentration was positively correlated with conjugated bilirubin and LD and negatively with hemoglobin (Hb) in newborns with hemolytic jaundice (see Table 3 in the online Data Supplement).

The essential functions and potential toxicities of copper in humans have been reviewed (14). Animal studies showed that an excess of copper can accumulate within liver cells (15). Reported toxic effects associated with increased chronic exposure to copper are rare, and cases seem to occur as clusters in specific geographic areas. It is unclear whether copper excess in infants is in fact restricted to certain geographic areas or whether its occurrence has been underdetected or not reported in other areas, such as Mediterranean countries, in which hemolytic jaundice is very common among neonates (16, 17).

Jaundice is a common problem in apparently healthy newborns; it presents in the first week of life and persists beyond 14 days in 15–40% of those who are breast-fed (11). Hemolytic neonatal jaundice, which is mainly attributable to glucose-6-phosphate dehydrogenase deficiency, is a major problem in Greece and in most Mediterranean countries (16, 17).

Copper concentrations in all groups of premature infants are lower than in full-term infants (2, 7, 18). This is expected because fetal serum copper concentrations reach a maximum at the end of the last trimester of pregnancy, whereas the livers of premature infants are immature and cannot accumulate the metal (18). On the other hand, serum copper was increased twofold in the neonates with moderate hemolytic jaundice (group B) and almost threefold in premature and full-term infants with severe hemolytic jaundice (group C). Copper overload may result from intentional or accidental ingestion and hemolysis, such as glucose-6-phosphate dehydrogenase deficiency hemolysis (19), in which cases nonceruloplasmin copper is greatly increased in plasma (14). Because the liver has a marked capacity to excrete copper, chronic copper toxicity is rare. In individuals with liver disease, however, a moderate, sudden increase in plasma copper may lead to accumulation of the metal, but the precise role of the latter in subsequent hepatic injury is unclear (20, 21). Additionally, the eventual hepatocyte dysfunction from copper overload may lead to cell death with release of copper into the blood (9, 20). Suzuki et al. (22) assumed that the so-called “free copper ions” (nonceruloplasmin copper pool) that leak from damaged hepatocytes are bound to albumin and/or taken up by the erythrocytes and vice versa.

Copper is an important metal in two enzymes with roles in antioxidant defense, intracellular copper zinc superoxide dismutase and cytochrome c oxidase, which plays an essential role in mitochondrial electron transport (23, 24). Evidence of lipid peroxidation and mitochondrial injury in experimental and clinical hepatic copper overload supports this hypothesis and suggests a potential therapeutic role of α-tocopherol and other antioxidants in situations of hepatic copper overload (25, 26).

In cases with hemolytic jaundice, copper is released via hemolysis and may exert toxic effects. A single measurement of indicators in blood is not the best way to detect the effect of the metal, but it does describe an acute copper load in relation to other useful biochemical markers, such as Hb and conjugated bilirubin. In addition, the large increase in LD suggests that there is a liver response to the oxidative stress probably induced by the copper (24, 25).

The majority of the neonatal jaundice cases in Greece and most Mediterranean countries are hemolytic (16). As a consequence, the observed high copper concentrations in the neonates, which correlated positively with the conjugated bilirubin and LD and negatively with Hb concentration, were probably attributable to the destroyed erythrocytes. The fact that the ceruloplasmin concentrations did not differ significantly among the studied groups supports this conclusion. These results suggest that the copper concentration may reflect the ongoing biological and toxicologic actions of copper in the liver and the severity of hemolysis (19).

Neurologic manifestations that reflect changes in the basal ganglia are observed in patients with severe copper excess (14, 27). The mechanisms leading to this specific involvement are still unknown. Newborns with hemolytic jaundice, especially premature infants with high conjugated bilirubin (group C), are at risk of developing kernicterus. A possible accumulation of copper in the brain may play an additional role in such an encephalopathy. Moreover, as with hepatic and neurologic manifestations, signs and symptoms may arise in any organ in which copper is deposited, such as kidneys (e.g., Fanconi syndrome) (14).

In neonates with hemolytic jaundice, the high serum copper may be of intracellular (erythrocyte) origin. This could be a factor mainly for premature infants with severe hemolytic jaundice, who are at risk of developing severe symptoms, as described above. Consequently, measuring serum copper could provide an additional useful marker for the differential diagnosis between moderate or severe hemolytic and nonhemolytic neonatal jaundice and identify high-risk premature infants. This study cannot assess the potential value of chelating agents for the treatment of...
patients with severe hemolytic jaundice and high copper, but many countries have limited use of chelation therapy to late childhood and adulthood. α-Tocopherol or other antioxidants may be administered as usual (25).

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References

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Trehalose Is a Potent PCR Enhancer: Lowering of DNA Melting Temperature and Thermal Stabilization of Tag Polymerase by the Disaccharide Trehalose, Andrej-Nikola Spiess,* Nadine Mueller, and Richard Ieell (Institute for Hormone and Fertility Research, Centre of Innovative Medicine, Falkenried 88, 20251 Hamburg, Germany; *) author for correspondence: fax 49-40-42803-1699)

Compatible solutes are a class of compounds that stabilize cells and cellular components exposed to extreme conditions. In bacterial systems, the uptake or synthesis of compatible solutes renders the cells and their enzymatic machinery more resistant to stress-inducing environmental conditions such as high osmolarity or high temperatures (1, 2). Compatible solutes comprise a heterogeneous group of compounds, covering amino acids and their derivatives (3), sugars (4), and more obscure compounds such as the pyrimidine derivative ectoine (5).

The compatible solute trehalose is a nonreducing disaccharide in which two α,α-1,1-glycosidic bond. It is synthesized by a variety of eukaryotic organisms, conferring tolerance against desiccation, dehydration, heat, cold, and oxidation (6). The addition of trehalose increases the enzymatic activity of several eutherian enzymes used for cDNA synthesis or restriction digestion of DNA (7, 8). Trehalose also enhances the priming specificity in differential-display reverse transcription-PCR (9) through high-temperature priming and a thermoactivated reverse transcriptase.

PCR amplifications are frequently impaired by high GC content of the target sequence, leading to low yield and specificity of products, with no product at all in the worst cases. Locally high-temperature melting regions within the template can act as permanent termination sites (10). Several low-molecular-weight products have been identified that enhance the PCR of difficult templates, e.g., dimethyl sulfoxide (11) and other sulfoxides (12), formamide (13), nonionic detergents (14), and compounds belonging to the family of compatible solutes, such as betaine (15–17). The latter is present in most of the commercially available PCR-enhancing solutions (18).

Here we report the application of trehalose as a potent PCR enhancer for GC-rich templates. This compound avoids false negatives in PCR typing (16). In this study, we used trehalose in real-time, reverse transcription-PCR amplification of the mouse oxotocin receptor (mOT-R) transcript, which has a very high GC content. The identified molecular properties of this compound that lead to its PCR-enhancing ability are based on (a) lowering the melting temperature of DNA and (b) thermostabilization of the Taq polymerase.

Total RNA was prepared from 100 mg of mouse brain