intravenous sites where furosemide has been administered, because contamination of the sample by even small volumes of furosemide significantly affected thyroxine results. The differences in sensitivity to furosemide interference among assays appear to reflect the serum dilution used in each method. This concern may apply to interference from other drugs (e.g., salicylates and fenofenac) as well as to other assays (e.g., free triiodothyronine).

This study does not address the in vivo effect of furosemide administration on thyroxine measurement. Because oral furosemide may influence measurement of the free thyroxine index (4), the time interval between doses of oral furosemide and blood sampling should be considered in result interpretation. Both the Vitros and AxSYM assays use sample volume:total volume ratios greater than the ratio (0.09) used by the assay in that study (4). Thus, it is likely that the in vivo effect from oral furosemide will be even greater in the Vitros and AxSYM assay systems. Furosemide concentrations of 6.6–73.0 μmol/L reported with routine therapeutic doses of furosemide (5) fall within the range of furosemide concentrations examined here. Clinicians thus may need greater awareness to potential interference from drugs such as furosemide when interpreting results from newer free thyroxine assays that use large sample volume:total volume ratios. TSH measurement may be a more useful test for assessing thyroid function in such cases.

References

Bilirubin in the Premature: Toxic Waste or Natural Defense? Cathy Hammerman,1 Robert Goldstein,2 Michael Kaplan,1,3 Maya Eran,2 Doris Goldschmidt,1 Arthur I. Eidelman,1,3 and Lawrence M. Gartner4 (1Department of Neonatology, Shaare Zedek Medical Center, Jerusalem, Israel 91031;2Gastroenterology Metabolism Laboratory, Shaare Zedek Medical Center, Jerusalem, Israel 91031;3Hebrew University, Hadassah School of Medicine, Jerusalem, Israel 91031;4Department of Pediatrics, University of Chicago, Chicago, IL 60637;*address correspondence to this author at: Department of Neonatology, P.O. Box 3235, Shaare Zedek Medical Center, Jerusalem, Israel 91031;fax 972-02-652-0689, e-mail cathy@cc.huji.ac.il)

Potentially toxic oxygen free radicals (OFRs) are generated continuously in neonates. Under physiological conditions, the human body has developed a complex network of antioxidant defenses sufficient to protect cells against oxidative damage. Oxidative stress results from a loss of this protective balance either because of overproduction of free radicals or because of inadequate antioxidant defenses. In prematures, the concentrations of most antioxidant enzymes are reduced, particularly during the early neonatal period (1). Impaired antioxidant defenses, occurring at a time when OFR production is both frequent and severe, render the premature neonate extremely susceptible to the development of OFR-mediated diseases.

During the first days of life when antioxidant defenses are reduced, serum bilirubin is increased physiologically. Because bile pigments protect easily oxidizable substances from destruction (2), it has been suggested that bilirubin functions as an antioxidant in term neonates (3). Nevertheless, neonatal hyperbilirubinemia is still widely regarded as clinically problematic, and bilirubin is regarded as a toxic metabolic waste. However, the physiologic early neonatal increase in serum bilirubin may provide a protective antioxidant defense mechanism to compensate for otherwise deficient antioxidant enzymes, especially in premature neonates with increased susceptibility to OFR-mediated diseases. We tested this possibility by examining the relationship between serum bilirubin concentration and antioxidant activity in the blood of premature infants.

We studied 41 premature neonates (<36 weeks gestational age) born consecutively from September 1996 to March 1997 at the Shaare Zedek Medical Center during their first week of life. All infants with indwelling arterial catheters were potential candidates for study. Blood samples (50 μL) were taken for measurement of total antioxidant status concurrently with the clinical bilirubin samples. Samples were taken beginning on day 2 of life and every other day for as long as an indwelling catheter was in place. Infants with Coombs-positive hemolytic anemia and/or glucose-6-phosphate dehydrogenase deficiency were excluded. Serum total bilirubin was measured by reflectance spectrophotometry (Kodak Ekcthem). The study was approved by the Institutional Review Board.

We measured the peroxyl radical-trapping capability (Randox Laboratories) of human blood as an indicator of total antioxidant activity (TAA) (4). This method, based on the method of Miller et al. (5), uses the peroxidase activity of metmyoglobin combined with its interaction with a phenothiazine compound to form a radical cation intermediate as a measure of antioxidant status. The method derives from the observation that when 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) is incubated with a peroxidase (such as metmyoglobin) and hydrogen peroxide, the relatively long-lived radical cation, ABTS·+ is produced. In the presence of antioxidant reductants and hydrogen donors in plasma, the absorbance of this radical cation is quenched to an extent related to the antioxidant capacity of the fluid. The system is calibrated with a 2.5 mmol/L solution of Trolox (an α-tocopherol analog with good water solubility). Results
correlate with the sum of the individual radical-trapping capabilities of the major antioxidants in plasma.

Mean values ± SD were calculated for continuous variables and compared by the Student t-test. Correlations between clinical variables were analyzed using a Pearson correlation. Combined analysis was performed using a best subset regression model, followed by a Spearman correlation to define correlations between variables.

The mean birth weight of the infants was 1374 ± 659 g, and the mean gestational age was 30.4 ± 3.6 weeks. The mean Apgar scores were 8 ± 2 and 8 ± 1 at 1 and 5 min, respectively. A total of 85 samples were taken, with 11 of the infants having three or more serial samples taken.

Serum bilirubin was significantly correlated (P = 0.005) with total antioxidant status (Fig. 1). Other than through its effect on bilirubin concentrations, treatment with phototherapy had no independent effect on TAA (P = 0.24).

Gestational age did not affect TAA (P = 0.91; only the initial TAA value was considered for those infants with several measurements). Birth weight (P = 0.79), the sex of an infant, and the concentration of inspired oxygen (P = 0.12) were not significantly correlated with TAA.

Mean total antioxidant status values were related to postnatal day of life (DOL), with an initial increase from day 2 to 4, followed by a decrease during the remainder of the first week of life (Table 1).

Best subset regression of the entire data sample, with TAA as the dependent variable and an F-to-remove of 3.9, confirmed that TAA can be predicted from a linear combination of the bilirubin at time of sampling and the DOL studied and that both bilirubin and DOL have independent effects on TAA. Variables tested and found not to add to the predictability of TAA included birth weight, gestational age, sex, use of phototherapy, and FiO2. Spearman correlation revealed no correlation between the variables bilirubin and DOL.

Our present findings extend previous reports (3). Stocker et al. (6) showed that bilirubin at physiologic concentrations can protect linoleic acid from oxidation in vitro. Farrera et al. (7) compared the peroxy antioxidant trapping potential of various substances and found that free bile pigments showed higher activity than did the vitamin E analog Trolox. According to Frei et al. (8), bilirubin contributes ~10% of the total antioxidant status. Serum antioxidant potential in prematures increases over the first 4 days of life, as does serum bilirubin. However, a direct correlation between serum bilirubin and antioxidant potential has not been shown previously in prematures (9).

Animal studies support a protective effect of hyperbilirubinemia. Denorry et al. (10) exposed Gunn rats to hyperoxia and demonstrated that jaundiced rats had less oxidative damage, as evidenced by the lower concentrations of lipid peroxides, conjugated dienes, and carbonyl proteins in jaundiced rats than in nonjaundiced rats.

In term human infants, Belanger et al. (11) detected a decrease in antioxidant capacity after exchange transfusion, implying a correlation between bilirubin removal and antioxidant capacity. Gopinathan et al. (1) observed a direct correlation between serum bilirubin and total antioxidant potential at birth in term infants, which they did not find in prematures. However, as a result of aggressive phototherapy in these premature infants, their bilirubin at 5 days reached only a mean of 67 ± 47 mmol/L (3.9 ± 2.8 mg/dL). Thus, there was not a wide enough range of bilirubin values to demonstrate any correlation, even should one exist.

Benaron et al. (12) and Hegyi et al. (13) noted lower serum bilirubin concentrations in neonates with oxygen radical-mediated diseases as compared with control, age-matched infants. These data imply that bilirubin may be consumed in response to the generation of oxygen-derived free radicals, supporting an active clinical role for bilirubin as an antioxidant. Conversely, a retrospective study of early bilirubin concentrations in relation to the subsequent development of retinopathy of prematurity in prematures (4, 14) did not demonstrate any such correlation. However, all clinical studies of this type are hampered by the fact that clinical disease is multifactorial and that the relationship with bilirubin, if one exists, may well be more complex than a direct correlation. Oxidative stress may consume available antioxidant resources (10) as suggested above, or conversely, it may induce antioxidant release (15) or some combination thereof. We have, therefore, correlated serum bilirubin not with clinical disease, but rather with a biochemical index of antioxidant status, demonstrating that bilirubin does contribute to the total antioxidant potential of the premature neonate.

We recognize that the fact that some of our babies were

<table>
<thead>
<tr>
<th>Day of life</th>
<th>Bilirubin</th>
<th>TAA</th>
</tr>
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<tbody>
<tr>
<td>2 (n = 22)</td>
<td>6.1 ± 1.7</td>
<td>1.34 ± 0.34</td>
</tr>
<tr>
<td>4 (n = 22)</td>
<td>7.4 ± 3.6</td>
<td>1.41 ± 0.31</td>
</tr>
<tr>
<td>6 (n = 22)</td>
<td>5.9 ± 3.2</td>
<td>1.14 ± 0.36*</td>
</tr>
<tr>
<td>8 (n = 12)</td>
<td>6.3 ± 1.7</td>
<td>1.07 ± 0.37*</td>
</tr>
</tbody>
</table>

* P < 0.05 compared with day 4 of life.

Fig. 1. Correlation between serum bilirubin in premature neonates and antioxidant potential during the first week of life.

TAA = 1.01 ± 0.04 × serum bilirubin; P = 0.0049; r = 0.39.
sampled more than once whereas others only were sampled once represents a possible limitation to our study. As such, these data do not purport to represent the general antioxidant status of premature infants. Nevertheless, they do demonstrate a positive relationship between serum bilirubin and antioxidant status.

Bilirubin management in the term neonate is currently in a state of flux (16–22). Therapeutic recommendations that have been accepted for years are being reevaluated and liberalized. Our observations, if further validated, suggest that the time may have arrived to also reevaluate clinical bilirubin management of the preterm infant. However, these relationships are complex and multifaceted. Thus, any consideration of potential clinical applications of this information may be undertaken only with extreme caution and with additional supportive data.

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References