Measuring Bile-Salt Concentrations Lacks Clinical Value for Detecting Hepatic Dysfunction in Infants Receiving Parenteral Nutrition

Geoffrey J. Beckett,² E. Jean Glass,¹ M. Odile Callaghan,¹ Robert A. Elton,³ and Robert Hume⁴

Concentrations of conjugated cholate, chenodeoxycholate, direct bilirubin, and alanine aminotransferase (ALT, EC 2.6.1.2) were measured in plasma of 122 low-birthweight infants receiving parenteral nutrition. Eighteen (15%) of them developed hepatic dysfunction. We observed two distinct biochemical patterns in these infants. In the Type A pattern (12 infants), concentrations of direct-reading bilirubin and bile salts increased with no change in ALT activity. In the Type B pattern (six infants), increases in the concentrations of bile salt and direct bilirubin were followed by increases in ALT activity. Hepatic dysfunction persisted significantly longer in infants who developed the Type B pattern. The two patterns did not differ significantly in the times at which values for bile salts or direct bilirubin in plasma became abnormal or became normal at resolution, nor did maximal concentrations of bile salts in plasma differ significantly. Maximal concentrations of direct bilirubin were higher in the Type B infants. We conclude that, in such infants, measurement of bile-salt concentrations in plasma offers no advantages for detecting hepatic dysfunction over the more conventional measurement of direct bilirubin in plasma.

Additional Keyphrases: bilirubin • alanine aminotransferase • low-birthweight newborns • factors predisposing to liver dysfunction • two types of dysfunction

Serial measurement of direct-reading bilirubin, and of alanine aminotransferase (ALT), aspartate aminotransferase, and alkaline phosphatase activities in plasma has been advocated for monitoring hepatic function in infants who are being fed parenterally (1–3). In adults and children, measurement of bile-salt concentrations in serum or plasma is considered to be a sensitive and specific indicator of hepatobiliary dysfunction, diagnostically superior to measurement of bilirubin in plasma (4–7). However, the value of bile-salt measurements in assessing hepatobiliary function in the pre-term infant is not clearly established (8, 9).

We have compared the usefulness of measurements of conjugated cholate and conjugated chenodeoxycholate in plasma vs measurements of direct bilirubin concentration and ALT activity for monitoring hepatic function in prematurely-born infants who are receiving parenteral nutrition.

Patients and Methods

During two years we monitored 122 surviving low-birthweight infants who received parenteral nutrition (Table 1). The clinical practice consisted of early introduction of parenteral nutrition for all infants weighing <1.5 kg and in other low-birthweight infants with significant respiratory distress. Parenteral nutrition was given to all these patients and consisted of an amino acid solution (Vamin-Glucone; Kabi Vitrum Ltd., U.K.), a dextrose-electrolyte solution, and a lipid emulsion (Intralipid 20%; Kabi Vitrum Ltd.) as previously described (10). During parenteral nutrition, liver function was assessed at least weekly by measuring the concentrations of bilirubin and bile salt and the ALT activity in plasma. Infants with hepatic dysfunction were monitored until the problems resolved. We measured urinary sugar, plasma amino acids, and α1-antitrypsin and performed liver ultrasound scans to exclude other causes of neonatal hepatic dysfunction.

Plasma total and direct bilirubin were measured by the method of Malloy and Evelyn (11), the reading for direct bilirubin being taken after 1 min. Concentrations exceeding 25 µmol/L were considered significantly above normal (12). ALT activity in plasma was measured at 37 °C in a continuous-flow system (SMAC; Technicon Instruments Corp., Basingstoke, U.K.). The reference range, derived from data on 290 low-birthweight infants who were not receiving parenteral nutrition, was 10 to 30 U/L.

Conjugated cholate and conjugated chenodeoxycholate concentrations were determined by radioimmunoassay (13, 14). In these assays, taurine and glycine conjugates react equally, and there is approximately 15% cross reactivity with unconjugated bile salts. The bile-salt measurements were summed to give total concentrations of conjugated primary bile salts ("total bile salt"). We also calculated the molar ratio of conjugated chenodeoxycholate to cholate for each sample.

For all assays, CVs were <10%.

For statistical analysis of the incidence of infection and the number of transfusions of erythrocyte concentrate we used the Wilcoxon rank-sum tests; for analysis of the effect of blood-product transfusions we used the Kruskal–Wallis test (15). For the remaining analyses we used Student’s t-test.

Results

Hepatic Dysfunction and Predisposing Factors

Eighteen (15%) of these 122 infants who were being fed parenterally developed hepatic dysfunction (Table 1). For infants weighing <1.5 kg at birth the incidence was 35% (14/45 infants). Predisposing factors were low birthweight, shorter gestation, prolonged parenteral nutrition, delayed onset and establishment of enteral feeding, and presence of congenital and acquired bacterial infection (Table 1) (p <0.001). Other predisposing factors included a higher number of transfusions with blood and blood products (p <0.001) and a higher incidence of acquired viral infections (p <0.001).

Bile Salts

The mean bile-salt concentration was 18.1 µmol/L in plasma collected from 34 infants five to 10 days postnatally, who were being fed parenterally and whose liver function subsequently remained normal. The mean bile-salt concentration in 18 infants of similar age, who subsequently...
developed hepatic dysfunction after parenteral nutrition was begun, was not significantly different: 14.7 μmol/L. We considered the upper limit of the normal reference range for total bile salts for such subjects to be 30 μmol/L.

Patterns of Hepatic Dysfunction

We observed two distinct biochemical patterns in infants who developed hepatic dysfunction.

In "Type A," concentrations of conjugated bilirubin and bile salt were increased, then later resolved. These infants showed no significant changes in ALT activity, either before or after the changes in bilirubin and bile salts (Figure 1a).

In the "Type B" pattern, ALT initially remained within the reference interval but subsequently increased (Figure 1b). This increase in ALT was significantly (p < 0.001) slower than that in either direct bilirubin or plasma bile salts. During resolution of hepatic dysfunction the intervals required for ALT, bile salts, and direct bilirubin to reach normal values did not differ significantly.

The Type A pattern developed in 12 infants, Type B in six. Abnormalities in bile-salt and direct-bilirubin concentrations were significantly prolonged in infants who developed a Type B pattern as compared with infants who developed the Type A pattern (p < 0.001). However, the age at which these abnormalities first developed was not significantly different in the two groups (Table 2).

Both in Type A and Type B patterns, the increase in bile salts paralleled that in direct bilirubin. However, in Type A infants, the former became maximal significantly (p < 0.05) later than the latter, and maximal values for the former did not differ significantly between the two groups, but maximal direct bilirubin concentrations were significantly (p < 0.001) higher in the Type B infants.

Bile Salt Ratios

The mean ratio of conjugated chenodeoxycholate to cholate in the initial plasma samples (postnatal days five to 10) from infants who subsequently developed hepatic dysfunction was not significantly different from that for infants whose liver function remained normal: 0.90 (SD 0.25) and 0.85 (SD ± 0.31), respectively. Nor did we see any differences in the bile-salt ratios in infants who subsequently developed either Type A or Type B patterns.

Discussion

In the adult and child, plasma bile-salt measurements sensitively reflect hepatic function (4-7). However, the postprandial increase in plasma bile-salt concentrations (16) necessitates blood sampling while the subject is fasting or at specified times postprandially (17). If this is done, the measured bile-salt concentrations may be referred to a predetermined reference range. In the infant, however, there are further problems in relating an isolated bile-salt measurement to a reference interval.

There are developmental changes in the synthesis, conjugation, metabolism, excretion, and the enterohepatic circulation of bile salts in the first few postnatal months (18). Thus bile-acid metabolism during this period is complex, and is neither fully developed nor as yet fully elucidated. Bile acids are mostly conjugated with taurine during infancy, but glycine conjugates predominate in the adult. In the fetus, many monohydroxylated and atypical bile acids are conjugated with glucuronic acid or sulfate (19). Evidently

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**Table 1. Clinical Features of the Parenterally Fed Infants**

<table>
<thead>
<tr>
<th></th>
<th>Without hepatic dysfunction</th>
<th>With type A</th>
<th>With type B</th>
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<tbody>
<tr>
<td>No. of infants</td>
<td>104</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Birth weight, g (and range)</td>
<td>1755 (880-2500)</td>
<td>1552 (730-1990)</td>
<td>1319 (1020-1590)</td>
</tr>
<tr>
<td>Gestation, weeks (and range)</td>
<td>32.5 (26-37)</td>
<td>27.6 (26-35)</td>
<td>30.0 (28-32)</td>
</tr>
</tbody>
</table>

**Parenteral nutrition:**

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<tbody>
<tr>
<td>Onset, days (and range)</td>
<td>2.8 (1-13)</td>
<td>2.9 (1-5)</td>
<td>2.2 (1-3)</td>
</tr>
<tr>
<td>Duration, days (and range)</td>
<td>6.9 (1-35)</td>
<td>25.6 (4-68)</td>
<td>55.8 (35-84)</td>
</tr>
</tbody>
</table>

**Enteral nutrition:**

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</thead>
<tbody>
<tr>
<td>Onset, days (and range)</td>
<td>5.9 (1-39)</td>
<td>11.9 (1-32)</td>
<td>43.8 (35-64)</td>
</tr>
<tr>
<td>Fully established, days (and range)</td>
<td>11.7 (3-60)</td>
<td>31.6 (8-67)</td>
<td>58.8 (40-85)</td>
</tr>
</tbody>
</table>

No. of episodes of acquired bacterial infection: 21 14 8

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**Fig. 1.** Concentrations of direct bilirubin [■], ALT [●], conjugated cholate [○], and chenodeoxycholate [●] in plasma from infants with Type A (left) and Type B (right) patterns of hepatic dysfunction.

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CLINICAL CHEMISTRY, Vol. 31, No. 7, 1985 1189
the conjugation process is continually maturing during this period. In this study we have used antiserum that measure taurine and glycine conjugates equally; however, little or no unconjugated or sulfate or glucuronic acid conjugates of bile acids are measured. We cannot exclude the possibility that the measurement of either unconjugated or sulfated or glucuronide conjugates of bile acids may have some clinical value, although this appears unlikely.

Concentrations of conjugated cholate and chenodeoxycholate in plasma may be markedly increased, especially in the premature infant (20, 21), and vary widely from infant to infant. Significant differences in postpartum bile-salt concentrations in full-term, premature, and low-birthweight infants of similar postnatal age have also been observed, but no significant difference in alkaline phosphatase activity or bilirubin concentration in plasma was found (22). These findings have led others to postulate that the abnormal bile-salt concentrations measured in such infants are not indicative of cholestasis, but instead indicate a specific dysfunction in bile-salt metabolism and transport in the low-birthweight infant (22).

The variability in total bile-salt concentrations found in healthy low-birthweight infants makes it unrealistic to use such measurements clinically in the conventional way by relating the concentration of bile salt in a single sample to a reference range. The use of measurements of individual bile salts (e.g., conjugated chenodeoxycholate or conjugated cholate) in this way would present even greater problems, because the percentage contribution of the individual bile salts to the total bile-salt pool will change with the maturity of the infant, irrespective of the overall state of hepatic function.

The monitoring of sequential samples for bile-salt concentration should improve the diagnostic efficiency of bile-salt measurements in infants, because an increase in bile-salt concentration from a baseline value previously established for the same infant would indicate a hepatobiliary problem. However, we could find no advantage in bile-salt measurements over bilirubin measurements, because the two tend to parallel one another. Changes in the proportion of the two primary bile salts (the bile-salt ratio) that occurred either before, during, or after the period of hepatic dysfunction also provided no clinically useful data. Indeed, if only one of the primary bile salts had been measured, misleading information as to the progression of hepatic function would have been obtained.

We noted no differences between infants with Type A or Type B hepatic dysfunction with respect to characteristics such as birthweight, gestation, Apgar scores, incidence or severity of infection, or drugs prescribed. Parenteral nutrition was required significantly longer in infants with Type B than in those with Type A dysfunction, and Type B thus is probably a more severe form of Type A.

In conclusion, although the value of plasma bile-salt measurements has been demonstrated in the adult, such measurements appear to have no particular advantage over measurement of ALT and direct bilirubin in the early detection of hepatic dysfunction in pre-term, low-birthweight infants who are receiving parenteral nutrition. Similarly, in view of the increased incidence of rickets in such infants (23), the use of alkaline phosphatase measurements would appear to be of little value in the absence of isoenzyme studies.

Measurement of direct bilirubin gave the earliest biochemical indication of hepatic dysfunction in our patients, but the direct-reading component of plasma bilirubin, as measured here, may not correlate directly with values for conjugated bilirubin (24). Hepatic dysfunction may be detected earlier by more specific measurements of the conjugated bilirubin fraction (24, 25), but until such methods become routinely available it is necessary to be content with the approximate values for these pigments obtained by the tests which are currently in routine use. Many methods are available for estimation of direct bilirubin, so levels of significance can only be interpreted with a knowledge of local laboratory methods and experience (26).

We wish to thank Prof. L. G. Whitby for his help in the preparation of this manuscript, and Mrs. E. Ward for her secretarial assistance.

References

Table 2. Time Course and Maximal Values in Types A and B Patterns

<table>
<thead>
<tr>
<th>Time</th>
<th>No. of days (and range) to</th>
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<tbody>
<tr>
<td>Maximal concentration (range), µmol/L</td>
<td>Onset of clin. significant concn.</td>
</tr>
<tr>
<td><strong>Type A</strong></td>
<td></td>
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<tr>
<td>Direct bilirubin</td>
<td>60.8 (32–104)</td>
</tr>
<tr>
<td>Bile salts</td>
<td></td>
</tr>
<tr>
<td>t</td>
<td>128.8 (91–188)</td>
</tr>
<tr>
<td>cc</td>
<td>63.8 (31–95)</td>
</tr>
<tr>
<td>cdc</td>
<td>64.8 (25–94)</td>
</tr>
<tr>
<td><strong>Type B</strong></td>
<td></td>
</tr>
<tr>
<td>Direct bilirubin</td>
<td>101.2 (67–124)</td>
</tr>
<tr>
<td>Bile salts</td>
<td></td>
</tr>
<tr>
<td>t</td>
<td>158.4 (132–188)</td>
</tr>
<tr>
<td>cc</td>
<td>47.8 (28–60)</td>
</tr>
<tr>
<td>cdc</td>
<td>111.0 (86–134)</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>120.8 (80–260)</td>
</tr>
</tbody>
</table>

* t, total conjugated cholate plus conjugated chenodeoxycholate; cc, conjugated cholate; cdc, conjugated chenodeoxycholate. b In U/L.