Measurements of Glutathione S-Transferase B<sub>1</sub> in Plasma after Birth Asphyxia: an Early Indication of Hepatocellular Damage

Geoffrey J. Beckett,¹ Amanda J. Hussey,¹ Ian Laing,² A. Forbes Howie,¹ John D. Hayes,¹ Richard C. Strange,³ Charles G. Faulder,² and Robert Humes²

Concentrations of glutathione S-transferase (glutathione transferase; EC 2.5.1.18) B<sub>1</sub> and B<sub>2</sub> subunits (B<sub>1</sub> and B<sub>2</sub>) and activity of alanine aminotransferase (ALT; EC 2.6.1.2) were measured in sequential plasma samples taken from 14 infants with birth asphyxia. Within 6 h of asphyxia, abnormal concentrations of B<sub>1</sub> were found in 11 infants, whereas only seven infants showed abnormal ALT activities at this time. In plasma sampled 24 h after birth, values for ALT were abnormal in 10, whereas values for B<sub>1</sub> were abnormal in six. Abnormal concentrations of B<sub>2</sub> were found in relatively few of these infants, apparently because this monomer is poorly expressed in liver samples obtained up to 41 weeks after conception. We conclude that measurement of B<sub>1</sub> may provide a useful index of hepatic impairment in birth-asphyxiated infants.

It is common practice to assess hepatic function in neonates who have suffered transient asphyxia at birth, usually by measuring the activities of aspartate aminotransferase (AST; EC 2.6.1.1) or alanine aminotransferase (ALT; EC 2.6.1.2) in plasma.4 In adults, determination of plasma glutathione S-transferase (GST; EC 2.5.1.18) concentrations as measured by RIA appears to provide a more-sensitive index of hepatocellular damage than does either AST or ALT (1), but the value of this assay in the newborn has not been assessed.

The GSTs are a group of dimeric proteins that constitute about 5% of the total hepatic cytosolic protein. They catalyze the conjugation of reduced glutathione with a wide spectrum of electrophiles, and appear to be important in the detoxification of some potential carcinogens (2). In humans, the GSTs may be divided into basic, neutral, and acidic forms, according to their isoelectric points; adult liver contains predominantly the basic GST (3). There are at least two immunologically distinct basic subunits (B<sub>1</sub> and B<sub>2</sub>), which may hybridize to form B<sub>1</sub>B<sub>1</sub>, B<sub>1</sub>B<sub>2</sub>, and B<sub>2</sub>B<sub>2</sub> subunit combinations (4); these appear to correspond to transferases ε, δ, and γ, respectively, described originally by Kamisaka et al. (5). The B<sub>1</sub> and B<sub>2</sub> monomers appear to be the products of separate genes situated on chromosome band 6p12 (6). Recent immunohistochemical studies of the hepatic distribution of GST in human fetuses, neonates, and adults have shown that basic and acidic GST are equally expressed in both periportal and centrilobular hepatocytes (7); this contrasts with the aminotransferases, which are found mainly in periportal cells. It is the centrilobular hepatocytes that are most easily damaged by hypoxia and toxins.

Determinations of B<sub>1</sub> or B<sub>2</sub> in plasma appear to be equally sensitive for use in detecting acute hepatic damage after paracetamol (acetaminophen) poisoning in adults, and both are superior to ALT measurements in this respect (8). The usefulness of these measurements in the assessment of hepatic dysfunction in neonates may be complicated, however, by the marked changes in GST expression that occur during gestation. Thus, in early fetal life the acidic form of GST is strongly expressed in liver, but with increasing gestational age this gene is down-regulated until at term the basic forms usually predominate (9, 10). In this study we have compared the concentrations of ALT, B<sub>1</sub>, and B<sub>2</sub> in plasma from a group of neonates who experienced transient hypoxia at birth. Because the data obtained suggested that B<sub>1</sub> and B<sub>2</sub> are differentially expressed, we have also examined the developmental expression of the enzyme monomers in liver.

Patients and Methods

Control Group

The control group, gestational age range 35–42 weeks, consisted of 62 infants who did not suffer birth asphyxia. Only one blood sample was usually available from these infants, taken at times ranging from 1 to 174 h after delivery. Venipuncture was performed as part of the investigation of unstable temperature (n = 10); inappropriate weight gain or loss (n = 6); jaundice (n = 6); suspected polycythemia (n = 6); blood-group typing (n = 6); group B beta hemolytic streptococcus detected in the mother (n = 5); cyanotic episodes (n = 5); maternal pyrexia in labor (n = 5); suspected anemia (n = 4); jitteriness (n = 4); prolonged rupture of membranes (n = 3); and suspected low blood glucose (n = 2). Infants were excluded from the control group if they were dysmorphic, had chromosomal abnormalities, or if results of the above investigations were abnormal. No infant in the control group required further treatment or investigation.

One infant was excluded from the control group because the GST B<sub>1</sub> concentration at 12 h was grossly above normal: 460 μg/L. There was no clinical evidence of any abnormality in this infant, and values for ALT and GST B<sub>2</sub> were within normal limits. There was insufficient sample to repeat the analyses.

Birth-Asphyxiated Infants

We studied 14 infants of 37–42 weeks gestation, consecutively delivered to the neonatal intensive-care unit with a clinical diagnosis of birth asphyxia. Meconium-stained fluid present at six deliveries and three infants had significant aspiration of meconium. Cardiotocographic

1 University Department of Clinical Chemistry, The Royal Infirmary, Edinburgh EH3 9YW, Scotland, U.K.
2 University Department of Child Life and Health, 17-19 Hatton Place, Edinburgh EH9 1UD, Scotland, U.K.
3 Clinical Biochemistry Research Laboratory, Department of Postgraduate Medicine, University of Keele, Hartshill Road, Stoke-on-Trent ST4 7PA, U.K.
4 Nonstandard abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; and GST, glutathione S-transferase.

Received March 2, 1988; accepted March 23, 1989.

CLINICAL CHEMISTRY, Vol. 35, No. 6, 1989 995
tracings were grossly abnormal in eight of the 11 for whom recordings were made. Four infants were delivered with significant cord compression. One infant was delivered after placental abruption, hemorrhage, and maternal hypotension, and one infant after spontaneous uterine rupture. All infants had Apgar scores of <4 at 1 min (mean 2, range 0–4) and <6 at 5 min (mean 5, range 1–6). All infants required resuscitation and ventilatory support for >15 min, and ventilatory support for 12 was continued for >24 h. The initial neurological features varied in severity, but hypotonia, decreased conscious level, decreased movement, and a disordered respiratory pattern were consistently present. The progression of neurological recovery through increased levels of consciousness, muscle tone, and movement was consistent with the diagnosis of hypoxic-ischemic encephalopathy (11). One infant showed progressive deterioration and died at 75 h.

A 1-mL blood specimen was collected from these infants into heparinized tubes within 1 h of delivery (time 0) and again at 6, 12, 24, 48, and 72 h later. Plasma was separated by centrifugation (1500 × g, 15 min) and ALT activity measured. The remaining plasma was stored at −20 °C until analyzed for GST B₁ and GST B₂.

Preparation of Liver Cytosols

Twenty-two livers were obtained within 4 h of death from aborted fetuses (10–24 weeks' gestation) after termination of pregnancy and from premature and term infants (27–41 weeks' gestation) who died in the neonatal period. These were used for quantification of B₁ and B₂. In addition, two livers were obtained from infants who suffered sudden infant death syndrome. The cytosols prepared from five livers were subjected to ion-exchange chromatography (see below). (Approval to take these tissue and blood samples was obtained from the Paediatric Reproductive Medicine Ethics of Medical Research Sub-Committee of the Lothian Health Board.)

Liver samples were also obtained within 6 h of death from 20 adults at routine autopsy (with the permission of Her Majesty’s Coroner). All showed normal histology and were from subjects without clinical evidence of liver disease.

Tissue samples were immediately frozen and stored at −70 °C until use. Portions (about 5 g) were washed with Tris HCl buffer (20 mmol/L, pH 7.2) to remove as much blood as possible, then cut into small pieces, homogenized in three volumes of the Tris HCl buffer containing sucrose (250 mmol/L), and centrifuged (20 min, 4 °C, 20 000 × g). The supernatant fluid was drawn off and recentrifuged (20 min, 4 °C, 150 000 × g); the resulting supernate is termed “cytosol.”

Ion-Exchange Chromatography

α, β, γ, δ, and ε GSTs were identified in five cytosols (28, 30, 39, 52, and 78 weeks of gestation) after elution from diethylaminoethyl- and carboxymethyl-cellulose (10).

Analytical Procedure

The concentration of B₁ and B₂ subunits in plasma and hepatic cytosols was measured by use of specific RIAs. There was no detectable cross-reactivity with the acidic and neutral forms of GST when we used these antisera: <1% of a cross-reactivity of B₂ was apparent in the B₁ assay, and vice versa (12).

ALT activity in plasma was measured in a Cobas-Fara centrifugal analyzer (Roche Diagnostics, Welwyn Garden City, Herts., U.K.) with kit reagents obtained from Boehringer Mannheim Diagnostics, Lewes, Sussex, U.K.

The concentration of protein in hepatic cytosols was measured in the Cobas-Fara by a biuret method.

GST activity in fractions obtained from ion-exchange chromatography was determined at 30 °C by use of reduced glutathione and 1-chloro-2,4-dinitrobenzene as substrates (10).

For all measurements the between-assay CV was <10% over the ranges measured.

Statistical Analysis

The significance of temporal changes in plasma analytes was assessed by using the Wilcoxon paired test or the Mann-Whitney U test for unpaired data as appropriate. We computed the Pearson product moment correlations (r) for the 22 liver cytosols, to investigate the relationship between B₁ and B₂ concentrations and post-conceptional age.

Results

Plasma Measurements

Control group. There were no significant temporal changes in ALT or GST B₂ in the control infants during the 174-h study period (Figure 1). Therefore we combined the data from the 62 infants to define the reference intervals. Because the data showed a skewed distribution, we calculated the reference intervals by assuming a log-normal

![Fig. 1. Plasma GST B₁ and GST B₂ concentrations in 62 control infants (one sample per infant, not one sample per infant at each time)](image)
distribution. The 5th–95th percentile ranges for ALT and GST B2 were calculated as 5–30 U/L and 0.7–7.3 μg/L, respectively.

The concentrations of GST B1 in the plasma samples from the 31 infants <24 h after delivery were significantly higher (Mann–Whitney \( P < 0.005 \)) than the GST B1 concentration in the 31 infants between the 24th and 174th postnatal hours. These smaller numbers made it impossible to identify the type of population distribution; we therefore chose reference intervals to include all the infants studied (Figure 1). The upper reference limits for GST B1 were taken as 100 μg/L for infants <24 h postpartum and 70 μg/L for infants between 24 h and 174 h postpartum.

Birth asphyxia group. Figure 2 shows the median concentrations of B1, B2, and ALT in plasma, with the 25th to 75th percentile range.

The values for B1 and B2 were significantly higher in the 0- and 6-h postpartum blood samples compared with values measured at 24 h (Wilcoxon paired test), but we saw no significant change in ALT during the initial 24-h period. The increase in plasma B1 in the first 6 h exceeded that of ALT, median values being 2.1 times the upper limit of the reference interval. In contrast, values for B2 in the initial sample and at 6-h post-delivery showed little or no abnormality.

After 24 h there was no significant change in the concentration of either B1 or B2, but ALT increased significantly (\( P < 0.01 \)), reaching peak median values of 2.1 times the upper limit of the reference interval by 48 h postpartum.

Within the first 24 h, values for B1 (Table 1) were the most frequently abnormal (11 of 14 infants). However, in the 24- to 48-h period, abnormalities in ALT were most frequently recorded (10 of 14 infants). Of the three infants who showed no abnormality in B1 in the first 24 h, one (patient 8; Table 1) showed no abnormalities in either B2 or ALT at any time during the 72 h of the study. In the remaining two infants (patients 3 and 11, Table 1) increases in ALT were seen between 24 and 72 h. Few infants exhibited abnormalities in B2 at any time.

### Table 1. Concentrations of Glutathione S-Transferase and Alanine Aminotransferase in Plasma of Birth-Asphyxiated Infants during the First 24 h and Subsequent 48 h after Delivery

<table>
<thead>
<tr>
<th>Infant</th>
<th>B1</th>
<th>B2</th>
<th>ALT</th>
<th>B1</th>
<th>B2</th>
<th>ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.5*</td>
<td>10.1*</td>
<td>9.0*</td>
<td>1.3*</td>
<td>0.9</td>
<td>4.4*</td>
</tr>
<tr>
<td>2</td>
<td>2.0*</td>
<td>0.9</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>3</td>
<td>0.9</td>
<td>0.2</td>
<td>0.3</td>
<td>0.5</td>
<td>0.4</td>
<td>1.5*</td>
</tr>
<tr>
<td>4</td>
<td>5.0*</td>
<td>1.5*</td>
<td>1.6*</td>
<td>3.7*</td>
<td>0.4</td>
<td>2.7*</td>
</tr>
<tr>
<td>5</td>
<td>8.1*</td>
<td>1.1*</td>
<td>0.9</td>
<td>1.5*</td>
<td>0.3</td>
<td>2.0*</td>
</tr>
<tr>
<td>6</td>
<td>3.1*</td>
<td>0.9</td>
<td>0.5</td>
<td>3.1*</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>7</td>
<td>1.9*</td>
<td>0.9</td>
<td>1.8*</td>
<td>0.2</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>8</td>
<td>0.4</td>
<td>0.6</td>
<td>0.6</td>
<td>0.9</td>
<td>0.9</td>
<td>0.5</td>
</tr>
<tr>
<td>9</td>
<td>2.6*</td>
<td>0.7</td>
<td>12.8*</td>
<td>0.2</td>
<td>0.5</td>
<td>8.4*</td>
</tr>
<tr>
<td>10</td>
<td>17.9*</td>
<td>0.9</td>
<td>1.2*</td>
<td>0.7</td>
<td>0.8</td>
<td>1.5*</td>
</tr>
<tr>
<td>11</td>
<td>0.1</td>
<td>0.4</td>
<td>0.6</td>
<td>0.4</td>
<td>0.8</td>
<td>1.9*</td>
</tr>
<tr>
<td>12</td>
<td>3.1*</td>
<td>1.0</td>
<td>1.7*</td>
<td>3.8*</td>
<td>2.0*</td>
<td>6.9*</td>
</tr>
<tr>
<td>13</td>
<td>1.1*</td>
<td>0.9</td>
<td>1.0</td>
<td>0.7</td>
<td>0.6</td>
<td>3.7*</td>
</tr>
<tr>
<td>14</td>
<td>8.2*</td>
<td>2.7*</td>
<td>1.9*</td>
<td>1.2</td>
<td>1.6*</td>
<td>1.7*</td>
</tr>
</tbody>
</table>

Mean abnormality\( a \) 4.4 1.5 2.4 1.3 0.7 2.8

No. of abnormal results 11 4 7 6 2 10

\( a \) Abnormal results. \( b \) Mean for the 14 infants.

### Determination of B1 and B2 in Hepatic Cytosol

Carboxymethyl-cellulose chromatography. Figure 3 shows the elution profile from carboxymethyl-cellulose of a liver obtained 78 weeks post-conception. An enzyme corresponding to \( \alpha \) can be identified in fractions eluted before the start of the Na+ gradient and to \( \gamma, \delta \), and \( \epsilon \) by the Na+ concentrations required for their elution (9); no enzyme corresponding to \( \beta \) was identified in this liver. Compared

![Table 1. Concentrations of Glutathione S-Transferase and Alanine Aminotransferase in Plasma of Birth-Asphyxiated Infants during the First 24 h and Subsequent 48 h after Delivery](image)

Fig. 2. Temporal changes in GST B1 and GST B2 concentrations and ALT activities in plasma from 14 birth-asphyxiated infants

Median values are shown by the line and the 25th to 75th percentile range is shown by shaded area. Normal ranges derived from the infants in Figure 1 are shown by the horizontal broken lines. Significant changes from the 24-h value are determined by the Wilcoxon matched-pair test and are shown by *\( P < 0.05 \) and **\( P < 0.01 \)

![Fig. 3. Carboxymethyl-cellulose chromatography of basic GST isoenzymes in liver cytosol](image)

Liver cytosol obtained 78 weeks post-conception was eluted from carboxymethyl-cellulose as described in the text. The elution volumes of enzyme activity (8) corresponding to \( \alpha \) (Na+ 14 mmol/L), \( \gamma \) (Na+ 34 mmol/L), \( \delta \) (Na+ 45 mmol/L), and \( \epsilon \) (Na+ 47 mmol/L) are shown. In this liver no activity was eluted in fractions expected to contain \( \beta \) (Na+ 27 mmol/L).
with samples from adults, the contributions of \( \gamma \) and \( \delta \) to total activity are smaller in infants (4, 5, 9). Determination of the concentrations of \( B_1 \) and \( B_2 \) monomers in fractions of the column eluate showed that most of the activity attributable to the basic GST resulted from an enzyme consisting only of \( B_1 \) monomers. \( B_1 \) and \( B_2 \) were eluted at similar Na\(^+\) concentrations in the other cytosols. Similar results were obtained from the other cytosols.

**Developmental expression of \( B_1 \) and \( B_2 \) in fetal liver cytosols.** We measured, by RIA, \( B_1 \) and \( B_2 \) in liver cytosols obtained between 10 and 41 weeks post-conception. During this period there was a significant correlation between the amounts of the two monomers (\( r = 0.56; P < 0.01 \)).

Neither the amounts of \( B_1 \) and \( B_2 \) nor the ratio \( B_2/B_1 \) changed significantly with time (\( r = -0.16, -0.17, \) and \( -0.05, \) respectively). There was substantially less \( B_2 \) than \( B_1 \) (mean \( 0.93 \pm 0.76 \) vs 9.0 \( \pm 3.1 \) \( \mu g/mg \) of cytosol protein) present in the samples, and the extent of interindividual variation (CV) for \( B_2 \) was also greater than for \( B_1 \) (81% and 34%, respectively).

We compared the concentrations of \( B_2 \) and \( B_1 \) measured with those found in cytosols from 20 adults; both \( B_1 \) (mean \( 12.8 \pm 5.5 \) \( \mu g/mg \) cytosol protein) and \( B_2 \) (mean \( 3.8 \pm 2.1 \) \( \mu g/mg \) cytosol protein) in adults were significantly greater than in the infants' samples.

**Discussion**

The human basic GST monomers \( B_1 \) and \( B_2 \) appear to be the products of at least two genes (6, 13). Recent studies suggest that determining the plasma concentrations of these proteins may be useful in the assessment of hepatic function in adults (1). Because no corresponding data have been published for neonates, we assessed the use of measuring plasma concentrations of these proteins in control and birth-asphyxiated infants and, because the proteins appeared to demonstrate differential expression, we studied their developmental expression in liver.

The changes in the plasma concentrations of \( B_1 \) and ALT in the birth-asphyxiated babies showed clearly different patterns. Abnormal values for \( B_1 \) were recorded most frequently within 6 h of asphyxiation, whereas ALT activities were most frequently abnormal in samples taken more than 24 h after asphyxiation. Generally, the increases in ALT were smaller than those of \( B_1 \) when results were expressed as multiples of the upper limit of the reference range.

The disparate pattern of temporal changes observed in ALT and GST \( B_1 \) is to be expected from the physicochemical properties and plasma half-lives of these enzymes. The GSTs have a low molecular mass (\( M_r 52,000 \)), and high intrahepatic concentration (1-5). They appear to be released more quickly into plasma than is ALT after an acute hepatic insult (1-8). The plasma half-life of \( B_1 \) and \( B_2 \) is short (<1 h) compared with that of ALT (~48 h); thus, GST concentration in plasma decreases quickly soon after active hepatic damage has ceased, whereas ALT remains increased (1-8).

The measurement of \( B_2 \) concentrations in plasma appeared to be of no value in neonates, because few infants showed abnormalities in this GST. In addition, \( B_1 \) concentrations were abnormal in all infants who showed abnormal \( B_2 \) concentrations.

The use of ion-exchange chromatography to resolve the various basic isoenzymes in adult liver cytosols has shown that the \( B_1 \) subunit, present mainly as the homodimer, \( \epsilon \), predominates (4, 5, 10). We previously showed that enzymes apparently corresponding to \( \alpha \), \( \beta \), \( \gamma \), and \( \delta \) were very weakly expressed in cytosols from fetuses and neonates, although \( \epsilon \) was present at concentrations similar to those in adults (10). However, defining the expression of the basic GST by measuring the relative activities of \( \alpha \), \( \beta \), \( \gamma \), and \( \delta \) in cytosols is unsatisfactory. These enzymes, usually identified by their elution order and Na\(^+\) concentration from a cation-exchanger, are difficult to quantify, firstly because the principal \( B_2 \) homodimer (\( \gamma \)) and the GST \( B_2B_2 \) heterodimer (\( \delta \)) are only partly resolved, and secondly because interindividual variations in elution patterns can make identification of \( \gamma \) and \( \delta \) difficult (10). The elution of fetal and neonatal cytosols from carboxymethyl-cellulose followed by radioimmunoassay of the fractions for GST \( B_1 \) and \( B_2 \) confirmed that the proteins corresponding to \( \alpha \), \( \beta \), \( \gamma \), and \( \delta \) also made up the expected combination of \( B_1 \) and \( B_2 \) subunits. This indicates that there are no fetal-specific basic isoenzymes.

The concentrations of the two GST monomers did not change during the period between 10 and 41 weeks post-conception. The amounts of \( B_2 \) were much lower than those of \( B_1 \) and lower than those of \( B_2 \) in adult liver. The relatively low and variable concentrations of \( B_2 \) in hepatic cytosols from neonates may, in part, explain the poor performance of \( B_2 \) measurements in detecting hepatic damage. A close interrelationship between \( B_1 \) and \( B_2 \) is suggested by the finding that the amounts of each in liver were significantly correlated. However, whereas \( B_1 \) is consistently expressed, \( B_2 \) is weakly expressed until at least a year after birth. Other GST loci demonstrate time-specific expression during development (9, 10) and these, as well as other human enzymes, can take many months after birth before adult levels of expression are achieved (14).

The genes encoding the basic GST demonstrate restricted tissue expression, particularly during fetal life (10). Until 40 weeks of gestation, the enzymes are strongly expressed in only liver and adrenal; thus the finding of raised plasma concentrations of \( B_1 \) or \( B_2 \) is likely to reflect hepatic damage.

In adults with no evidence of hepatic dysfunction the plasma concentration of \( B_1 \) is normally below 4 \( \mu g/L \) (1, 8). In control infants, during the first 24 h after birth, the plasma concentrations of \( B_1 \) were up to 25 times greater than in adults. This suggests the possibility of increased hepatocellular permeability during the neonatal period that is not manifest by ALT measurements, and, therefore, measurements of \( B_1 \) in plasma appropriate to temporal reference ranges must be used.

At present, GST measurements are not widely available but there is evidence in adults that determination of \( B_1 \) concentrations in plasma may usefully complement the more-conventional measurements of aminotransferase activities (1). The data presented here show that measure-ment of plasma \( B_1 \) by RIA, may allow early recognition of hepatic damage in the birth-asphyxiated neonate. Because of the short plasma half-life of \( B_1 \), the finding of an increase in \( B_1 \) 24 h after delivery may suggest a continued hepatic injury.

This work was funded by a Scottish Home and Health Department Grant K/MR8/50/C570 awarded to J.J.B. and J.D.H. and Birthright-ROCOG awarded to R.H. We are grateful to Professor L. G. Whitby for his advice in the preparation of this manuscript, Mrs. E. Ward for her secretarial assistance, and Dr. P. Jones for statistical advice.
References

CLINICAL CHEMISTRY, Vol. 35, No. 6, 1989