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Studies of the development of basic, neutral and acidic isoenzymes of glutathione *S*-transferase in human liver, adrenal, kidney and spleen

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The ontogeny of basic, near-neutral and acidic glutathione *S*-transferase isoenzymes was studied by using chromatofocusing and ion-exchange chromatography. These isoenzyme sets demonstrated tissue-specific patterns of expression. For example, whereas basic isoenzymes were identified in all liver and adrenal cytosols obtained after 10 weeks gestation, these forms were not detected in kidney until 10 weeks post-natal age and in spleen until about 40 weeks post-natal age. Our data indicate that the basic monomers B_1 and B_2 are present in liver cytosol at 21 weeks gestation. Expression of the near-neutral isoenzymes was usually weak; for example, they were not generally expressed in liver until 30 weeks gestation, and no developmental patterns in their expression could be identified in adrenal, kidney and spleen. The acidic isoenzymes were usually strongly expressed in adrenal, kidney and spleen, although there was a decline in the level of expression in kidney after birth.

INTRODUCTION

The glutathione *S*-transferases (GST, EC 2.5.1.18) are dimeric enzymes that catalyse the conjugation of GSH with a variety of electrophiles, including some carcinogens (Mannervik, 1985).

Various GST isoenzymes have been identified in humans, and although the enzyme composition of many tissues appears complex (Strange *et al.*, 1984; Vander Jagt *et al.*, 1985) the different forms can be readily classified, on the basis of their isoelectric points, as basic, near-neutral or acidic (Mannervik *et al.*, 1985). Five basic isoenzymes (pI values 7.8–8.8) termed α, β, γ, δ and ϵ were first described in liver by Kamisaka *et al.* (1975), who suggested that they comprised homodimers and were the products of a single locus. More recently, however, Stockman *et al.* (1985) identified two enzyme monomers (B_1 and B_2) that appear to be the products of separate genes. They proposed that α, β and γ are a group of B_2B_2 transferases that includes a primary product as well as modified forms, that δ is a B_1B_2 heterodimer and that ϵ is a B_1B_1 transferase. A near-neutral isoenzyme, μ (pI 6.5), that is the product of a further gene has also been described (Warholm *et al.*, 1983), and several acidic isoenzymes (pI values approx. 5.0) have been identified, although it is not clear whether these are the products of a single locus.

The classification of the GST into basic, near-neutral and acidic isoenzymes is in broad agreement with the genetic model proposed by Board (1981). He suggested that the human enzymes were the products of three loci, GST1, GST2 and GST3. The products of GST2 correspond to α, β, γ, δ and ϵ and one of the products of GST1 to μ. Experiments using starch-gel electrophoresis, however, indicate that GST1 exhibits genetic variation, and the four phenotypes identified by using this technique have been attributed to combinations of the *GST1*0*, *GST1*1* and *GST1*2* alleles (Board, 1981). Individuals with the GST1 0 phenotype (*GST1*0/GST1*0*) are identified by an absence of near-neutral activity and those with the GST1 1 (*GST1*1/GST1*1*; *GST1*1/GST1*0*) and GST1 2 (*GST1*2/GST1*2*; *GST1*2/GST1*0*) phenotypes by isoenzymes that differ slightly in their anodal mobilities. It is not clear which of these corresponds to μ. Subjects who are heterozygous for the *GST1*1* and *GST1*2* alleles (GST1 2-1 phenotype) demonstrate three isoenzymes that are the result of hetero- and homo-dimeric combinations of the products of the two alleles. The products of GST3 correspond to the acidic isoenzymes.

The development of GST is of interest because of their putative importance in the detoxication of xenobiotics and organic peroxides. We have previously described the qualitative developmental expression of GST in several human tissues with the use of starch-gel electrophoresis (Strange *et al.*, 1985) and now describe further studies to quantify expression of this enzyme in liver, adrenal, kidney and spleen. The aims of the study were, firstly, to separate the basic, near-neutral and acidic GST isoenzymes present in these tissues by using chromatofocusing and thereby quantify the developmental expression of each of the enzyme sets. The basic set comprises various isoenzymes, and the second aim was to determine the ontogeny of α, β, γ, δ and ϵ and thereby deduce whether B_1 and B_2 are present in foetal tissues. These isoenzymes were resolved by using ion-exchange chromatography

Abbreviation used: GST, glutathione *S*-transferase.

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as described by Kamisaka *et al.* (1975) and Stockman *et al.* (1985). The ontogeny of the near-neutral isoenzymes has been studied assuming that these enzymes result from homo- and hetero-dimeric combinations of the products of the *GST1*1* and *GST1*2* alleles. The third aim of the study was therefore to confirm this assumption by hybridization *in vitro* of the isoenzymes obtained from an individual with the GST1 2-1 phenotype.

MATERIALS AND METHODS

Preparation of tissue cytosol

Samples of liver, adrenal, kidney and spleen were obtained within 4 h of death from aborted foetuses (10–24 weeks gestation) following termination of pregnancy, premature and term infants (26–42 weeks gestation) who died in the neonatal period and infants who suffered sudden-infant-death syndrome (2–67 weeks of postnatal age). Approval was obtained from the Reproductive Medicine Ethics Committee of the Simpson Memorial Maternity Pavilion, Royal Infirmary, Edinburgh. Adult tissues were also obtained at autopsy.

To prepare cytosol, 5 ml of 20 mM-Tris/HCl buffer, pH 7.20, containing sucrose (250 mM), EDTA (0.1 mM) and GSH (1 mM) was added to pieces of tissue (0.25–2 g). The tissue was cut into small pieces, homogenized and centrifuged (20000 *g* for 20 min at 4 °C). The supernatant was re-centrifuged (150000 *g* for 60 min at 4 °C) and this supernatant was termed cytosol.

Chromatofocusing

Chromatofocusing was used to separate the isoenzymes of GST in liver, adrenal, kidney and spleen cytosol. Cytosols (1–3 ml; approx. 15 mg of protein/ml) were eluted (30 ml/h) (4 °C) from columns (40 cm × 1 cm) containing Polybuffer Exchanger 94 (Pharmacia Fine Chemicals) equilibrated with start buffer (25 mM-imidazole/HCl buffer, pH 7.30). The pH gradient was formed by using Polybuffer 74 adjusted to pH 4.00 with 5 M-HCl. Fractions (2.5 ml) were assayed for GST activity and pH. The basic isoenzymes (GST2) were usually not retained on the column, although in some samples small amounts of activity were eluted at pH values as low as 6.90. The near-neutral isoenzymes (μ) were eluted between pH 6.70 and 6.20. The main peak of activity of the acidic isoenzymes was eluted at a pH value of approx. 5.00. In some samples, particularly kidney and spleen, a small amount of activity was also eluted at a pH value of approx. 5.80. Since these two tissue acidic isoenzymes have similar electrophoretic mobilities in starch gels to the two GST isoenzymes found in human erythrocytes, we have proposed that they are all products of GST3 (Strange *et al.*, 1985).

DEAE-cellulose chromatography

Cytosols (3 ml; approx. 15 mg of protein/ml) were eluted (30 ml/h) (4 °C) from columns (30 cm × 1.5 cm) containing DEAE-cellulose (DE 52) equilibrated with 20 mM-Tris/HCl buffer, pH 7.30. Fractions (2.5 ml) were collected. Flow-through fractions containing GST activity were pooled and dialysed against 10 mM-sodium phosphate buffer, pH 6.70.

CM-cellulose chromatography

The dialysed pool from the DEAE-cellulose chromatography was eluted (4 °C) from a column (40 cm × 1.4 cm) containing CM-cellulose (CM 52) equilibrated with 10 mM-sodium phosphate buffer, pH 6.70. This buffer was pumped (30 ml/h) on to the column for 2 h following addition of the sample, and a linear NaCl gradient (15–110 mM-Na^+) was then formed by eluting the column with phosphate buffer containing increasing concentrations of NaCl. Fractions (2.0 ml) were collected.

Hybridization studies

Basic enzymes. The basic isoenzymes in tissue cytosols were separated by elution from DEAE-cellulose and then CM-cellulose, and the fractions corresponding to α, β, γ, δ and ϵ were separately combined. The enzymes were dissociated into monomers by incubation (25 °C for 20 min) in 25 mM-imidazole/HCl buffer, pH 7.3, containing guanidinium chloride (5 M) and then dialysed (20 h at 4 °C) against four changes (each of 2 litres) of 10 mM-sodium phosphate buffer, pH 6.7, containing GSH (1 mM) and EDTA (0.1 mM). The dialysed solutions were then eluted from CM-cellulose as described above.

Near-neutral isoenzymes. Cytosol (3 ml; 37 mg of protein/ml) from an adult subject with the GST1 2-1 phenotype was chromatofocused, and the fractions containing the GST1 2 enzyme (elution pH 6.60), GST1 2-1 enzyme (elution pH 6.35) and GST1 1 enzyme (elution pH 6.15) were separately pooled. Dissociation of the dimers was achieved by incubation (25 °C for 20 min) in 25 mM-imidazole/HCl buffer, pH 7.30, containing guanidinium chloride (5 M). The monomers were allowed to recombine by dialysis (20 h at 4 °C) against four changes (each of 2 litres) of 25 mM-imidazole/HCl buffer, pH 7.30, containing GSH (1 mM) and EDTA (0.1 mM). The resulting dimeric enzymes were then chromatofocused as described above.

Starch-gel electrophoresis

Horizontal starch-gel electrophoresis performed as described previously (Strange *et al.*, 1985) was used to classify peaks of enzyme activity from the chromatofocusing column as products of the GST1, GST2 or GST3 loci.

Inhibition studies

To assist in the identification of the enzymes eluted from the chromatofocusing column, the effects of three inhibitors, bromosulphophthalein, *N*-ethylmaleimide and cholate, on activities were determined. Portions of the enzyme-containing peaks from the column were incubated (25 °C for 30 min) with *N*-ethylmaleimide (0–2 mM) in 100 mM-sodium phosphate buffer, pH 6.45, and also mixed with bromosulphophthalein (0–200 μM) and sodium cholate (0–200 μM) without incubation. The concentration of inhibitor giving 50% inhibition (I_{50}) was determined from plots of remaining activity versus inhibitor concentration.

Activity

GST activity was determined at 30 °C with GSH and 1-chloro-2,4-dinitrobenzene as substrates, total protein concentrations in cytosols were determined by using a biuret method (Strange *et al.*, 1985) and Na^+ concentrations were determined by using flame photometry.

Table 1. GST activity in foetal, neonatal and infant tissues

GST activity was determined in tissue cytosols obtained during development. The results shown are means ± S.D. with the numbers of samples shown in parentheses.

	GST activity (μmol/min per mg of cytosol protein)			
	10–20 weeks	21–30 weeks	31–40 weeks	40 weeks onwards
Adrenal	0.45 ± 0.23 (9)	0.42 ± 0.14 (4)	0.85 ± 0.48 (4)	0.42 ± 0.28 (8)
Kidney	0.39 ± 0.80 (9)	1.05 ± 0.93 (5)	0.28 ± 0.02 (4)	0.66 ± 0.46 (9)
Liver	0.63 ± 0.27 (14)	0.43 ± 0.21 (12)	0.60 ± 0.19 (6)	1.32 ± 1.65 (16)
Spleen	0.28 ± 0.20 (6)	0.24 ± 0.19 (6)	0.18 ± 0.09 (4)	0.29 ± 0.27 (9)

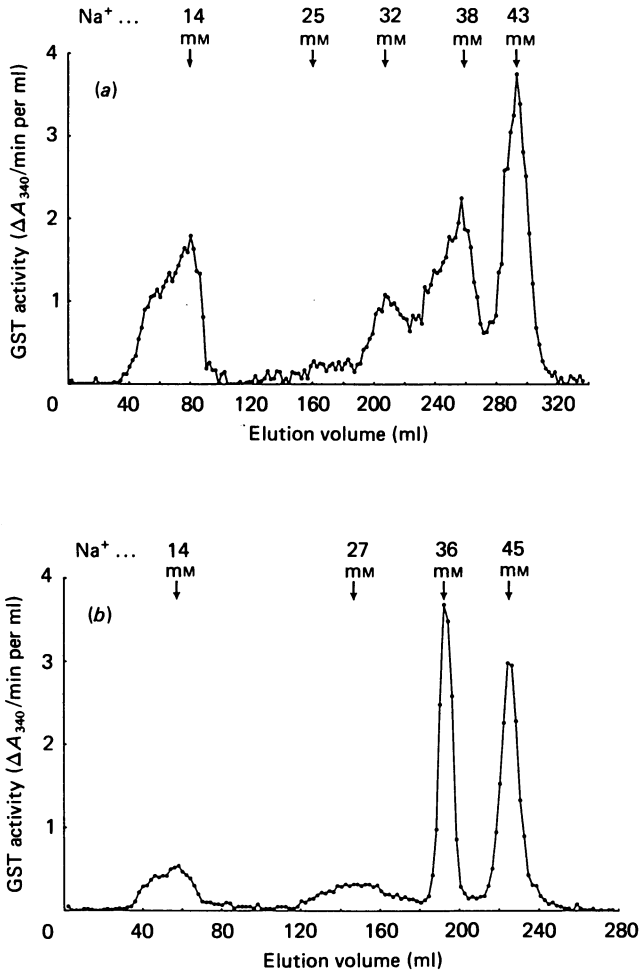

Fig. 1. CM-cellulose chromatography of basic GST isoenzymes in liver cytosol

(*a*) Adult liver cytosol was eluted from DEAE-cellulose, and GST activity eluted in the flow-through fractions was applied to CM-cellulose and eluted with 10 mM-phosphate buffer, pH 6.70. Basic GST isoenzymes were identified by measuring activity (●) and Na^+. Elution positions of isoenzymes: α (14 mM), β (25 mM), γ (32 mM), δ (38 mM) and ε (42 mM). (*b*) A second adult cytosol was similarly eluted from CM-cellulose. ● shows the elution of GST activity from the ion-exchanger. Fractions eluted at Na^+ concentrations of 14, 36 and 45 mM were pooled, incubated in guanidinium chloride, and, after dialysis, again eluted from CM-cellulose.

RESULTS

Before separation of the GST isoenzyme sets, enzyme activities in cytosols were determined. Table 1 shows that GST activity demonstrated considerable inter-individual variation, and no developmental trends were identified. It follows that in the data shown in Figs. 2, 4 and 5 the percentage of the total activity present in an isoenzyme set is proportional to the total activity of that set.

The basic, near-neutral and acidic GST isoenzymes in foetal, neonatal, infant and adult tissues were separated by using chromatofocusing. The identity of the eluted enzymes was then checked by starch-gel electrophoresis (Strange *et al.*, 1984, 1985). This allowed each peak to be classified as the product of either GST1, GST2 or GST3 and also the phenotype of the GST1 isoenzymes to be determined.

Development of basic GST isoenzymes (α, β, γ, δ and ε; GST2)

Liver. Starch-gel electrophoretic and chromatofocusing studies have shown that basic isoenzymes are consistently expressed in liver after 10 weeks of gestation (Strange *et al.*, 1985). However, although these techniques resolved this set into two or three partially resolved fractions, at least five isoenzymes have been identified after elution of adult cytosol from CM-cellulose. We therefore studied the development of this set by using ion-exchange chromatography.

Two adult liver cytosols were eluted from DEAE-cellulose, and the enzymes in the flow-through fractions were resolved by using CM-cellulose (Figs. 1*a* and 1*b*). The elution pattern shown in Fig. 1(*a*) is similar to that described by Hayes *et al.* (1983). Peaks of activity corresponding, in order of elution, to α, β, γ, δ and ε were identified. The concentrations of Na^+ at which these isoenzymes were eluted were similar to those described previously.

The elution profile of the second sample (Fig. 1*b*) was rather different and resembled that described by Kamisaka *et al.* (1975). Activity was eluted in the flow-through fractions and also at Na^+ concentrations of 27, 36 and 45 mM. To determine whether this elution pattern represents a different individual characteristic, a series of hybridization experiments was carried out. Pooled fractions from the peak eluted at an Na^+ concentration of 36 mM were hybridized and eluted from CM-cellulose. The elution profile demonstrated a peak of activity that was eluted in the flow-through fractions and

Table 2. Development of the basic isoenzyme set in liver

Samples of liver cytosol were obtained during development and the various isoenzymes were separated by using DEAE-cellulose and CM-cellulose chromatography. Patterns 1 and 2 are shown in Figs. 1(*a*) and 1(*b*) respectively.

Weeks of gestation	Na^+ 14–16 mM	Na^+ 27 mM	Na^+ 34–36 mM	Na^+ 39–41 mM	Na^+ 43–47 mM
Adult 1	+	+	←Adult pattern 1→		
Adult 2	+	++	←Adult pattern 2→		
21	−	−	←Adult pattern 2→		
28	−	−	←Adult pattern 1→		
30	Trace	−	←Adult pattern 1→		
39	Trace	−	←Adult pattern 1→		
40+12 weeks post-natal	+	−	←Adult pattern 1→		
40+38 weeks post-natal	Trace	−	←Adult pattern 1→		
40+61 weeks post-natal	+	−	←Adult pattern 2→		

also a peak (at Na^+ 41 mM) with partially resolved shoulders of activity that were eluted at Na^+ concentrations of approx. 36 mM and 45 mM. We interpret these data as indicating that the pooled fractions contain δ (B_1B_2) that is dissociated into γ (B_2B_2), δ (B_1B_2) and ϵ (B_1B_1).

The corresponding elution profile of the peak eluted at Na^+ 45 mM (Fig. 1*b*) demonstrated a peak at an Na^+ concentration of 45 mM as well as a smaller peak that was eluted at an Na^+ concentration of 41 mM. The fractions (Fig. 1*b*) containing the activities eluted at Na^+ concentrations of 36 and 45 mM were also combined and allowed to dissociate. The elution profile resembled that shown in Fig. 1(*a*) rather than Fig. 1(*b*), with the relative activities of peaks corresponding to α, β, γ, δ and ϵ similar to those seen in Fig. 1(*a*). Dissociation and recombination of the peak in the flow-through fractions resulted in elution of a single peak of activity that was eluted in the same fractions. To determine whether multiple basic isoenzymes are present during development, a series of cytosols was eluted from the ion-exchangers (Table 2). The enzymes in these cytosols were eluted at similar concentrations of Na^+ to those for the adult forms, and no specifically foetal isoenzymes were identified. The α isoenzyme was weakly expressed in the last trimester but β was not detected up to 1 year post-natal age. The type of elution profile demonstrated by these samples was similar to that shown in either Fig. 1(*a*) or Fig. 1(*b*) and was not related to gestational age (Table 2).

Adrenal. Qualitative studies using starch-gel electrophoresis have shown that basic isoenzymes are strongly expressed in adrenal cytosol (Strange *et al.*, 1985). The ontogeny of this set of isoenzymes was quantified by chromatofocusing cytosols and determining their contribution to total activity. They were eluted as a single peak of activity in all samples studied, except for a single cytosol (61 weeks post-natal age) in which two peaks of activity were eluted at pH values of 7.42 and 7.30. Fig. 2 shows that expression of these isoenzymes was consistently high during development.

To determine whether isoenzymes corresponding to α, β, γ, δ and ϵ were present, a sample of adrenal cytosol from the adult shown in Fig. 1(*b*) was eluted from DEAE-cellulose and CM-cellulose. The profile, however, was more similar to that shown in Fig. 1(*a*); a prominent peak of activity was eluted at an Na^+ concentration of 44 mM (ϵ) with smaller peaks being eluted at Na^+ concentrations of 40 mM (δ) and 36 mM (γ). No activity was eluted at Na^+ concentrations of 14 mM (α) or 27 mM (β). Adrenal cytosol from the subject (28 weeks of gestation) shown in Table 2 was also eluted from the ion-exchangers. Although the elution profile of liver cytosol from CM-cellulose revealed a prominent peak at an Na^+ concentration of 47 mM (ϵ), other isoenzymes (γ and δ) were present in only trace amounts. The adrenal cytosol, however, contained substantial and approximately equal activities from enzymes eluted at Na^+ concentrations of 46 mM (ϵ) and 41 mM (δ). No other isoenzymes were detected.

Kidney. Four adult kidney cytosols were chromatofocused, and in each case the elution profile resembled that described for liver cytosol (Strange *et al.*, 1985), with two peaks of basic GST activity being eluted. Chromatofocusing of foetal and neonatal cytosols showed that although basic isoenzymes were present their contribution to activity in cytosol was small (Fig. 3*a*). Expression of these enzymes progressively increased thereafter (Fig. 3*b*), until adult levels of expression were attained about 1 year after birth (Fig. 2).

Spleen. Studies using starch-gel electrophoresis have shown that expression of basic GST is usually weak in adult spleen (Strange *et al.*, 1984). This finding was confirmed by chromatofocusing six adult spleen cytosols; trace amounts of activity were found in three subjects and relatively small amounts in a further two samples (Fig. 2). Trace amounts of basic isoenzymes were detected in spleen samples obtained before 40 weeks post-natal age, and even after this time three of the seven specimens studied demonstrated very low levels of expression. In the remaining samples the activities of basic isoenzymes appeared similar to those in the adult samples that demonstrated expression.

Because the level of expression of the basic isoenzymes was low in foetal kidney and spleen, studies to identify forms α, β, γ, δ and ϵ were not carried out.

Development of the near-neutral isoenzymes (μ; GST1)

Liver. Near-neutral isoenzymes are not usually expressed in liver cytosol until about 30 weeks of gestation (Strange *et al.*, 1985). Thereafter, GST1 activity

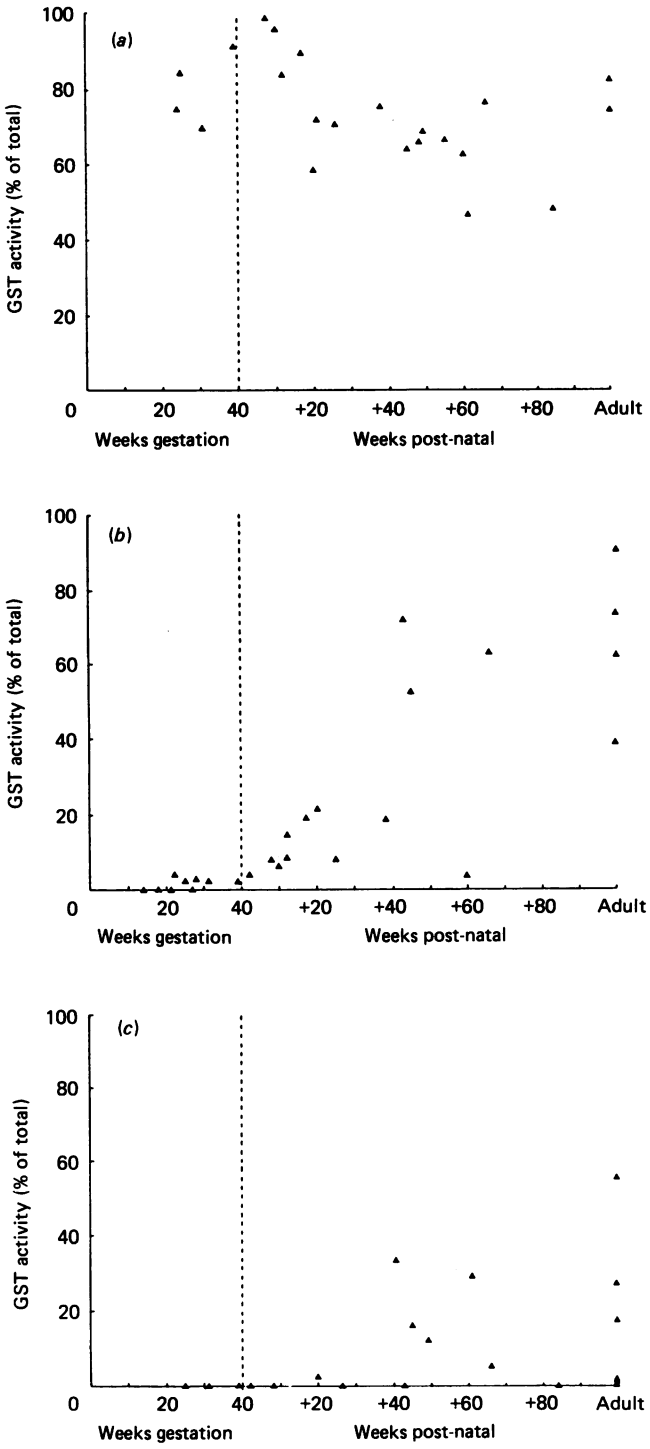

Fig. 2. Expression of basic GST isoenzymes in adrenal, kidney and spleen

Adrenal (*a*), kidney (*b*) and spleen (*c*) cytosols obtained during development were chromatofocused and the contributions of the basic (▲), near-neutral and acidic isoenzymes to activity were quantified.

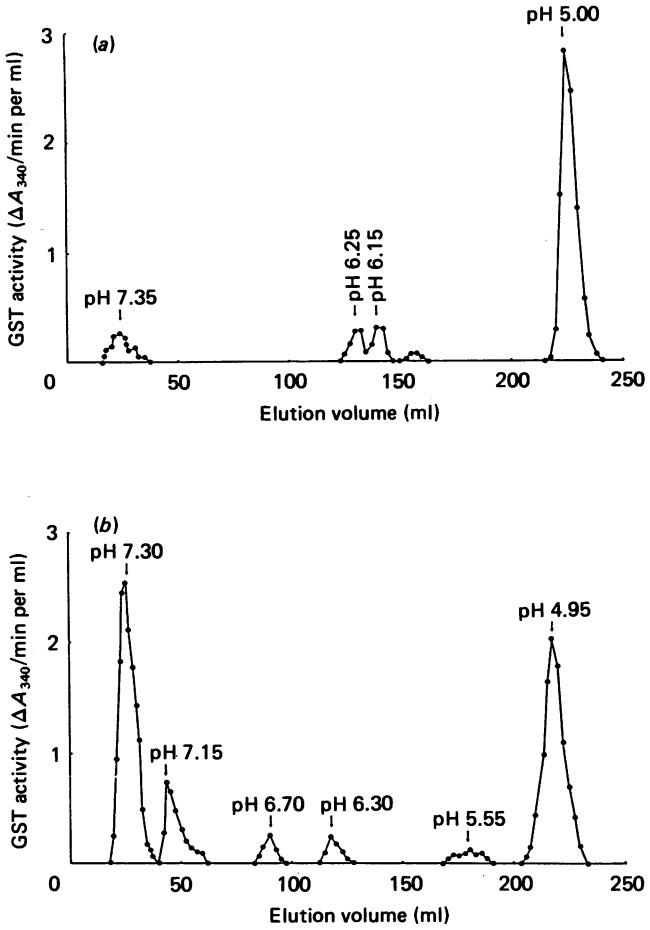

Fig. 3. Chromatofocusing of GST isoenzymes in kidney cytosol

Kidney cytosols obtained at 8 weeks post-natal age (*a*) and 45 weeks post-natal age (*b*) were chromatofocused. GST activity is given by ●.

increases similarly in individuals with GST1 1 or GST1 2 phenotypes.

A total of three enzymes have been identified in a series of liver cytosols from subjects with the GST1 1, GST1 2 or GST1 2-1 phenotypes (Strange *et al.*, 1985). Chromatofocusing was used to demonstrate that one of the three enzymes found in individuals with the GST1 2-1 phenotype is a heterodimer comprising monomers derived from two homodimeric enzymes. Liver cytosol from a subject with this phenotype was chromatofocused, and samples of the GST1 2 (*GST1*2/GST1*2*) and GST1 1 (*GST1*1/GST1*1*) enzymes were isolated and their identities confirmed by starch-gel electrophoresis (Strange *et al.*, 1985). The enzymes were mixed, incubated in guanidinium chloride and re-chromatofocused. The two original enzymes were identified as well as the expected hybrid GST1 2-1 enzyme. Similarly, dissociation and recombination of the GST1 2-1 enzyme resulted in the formation of three peaks of activity that were identified as the GST1 2, GST1 2-1 and GST1 1 enzymes.

Adrenal. Although the genetical polymorphism of GST1 was demonstrable in adult adrenal (Strange *et al.*, 1984), the contribution of this set to activity in foetal and neonatal tissue was small (Fig. 4), and near-neutral isoenzymes were not always detected after 30 weeks gestation even in subjects shown to have GST1 1 or GST1 2 phenotypes by starch-gel electrophoresis or chromatofocusing of liver cytosol (Strange *et al.*, 1985). Only small amounts of adrenal tissue were available from most subjects, and even in samples that expressed GST1

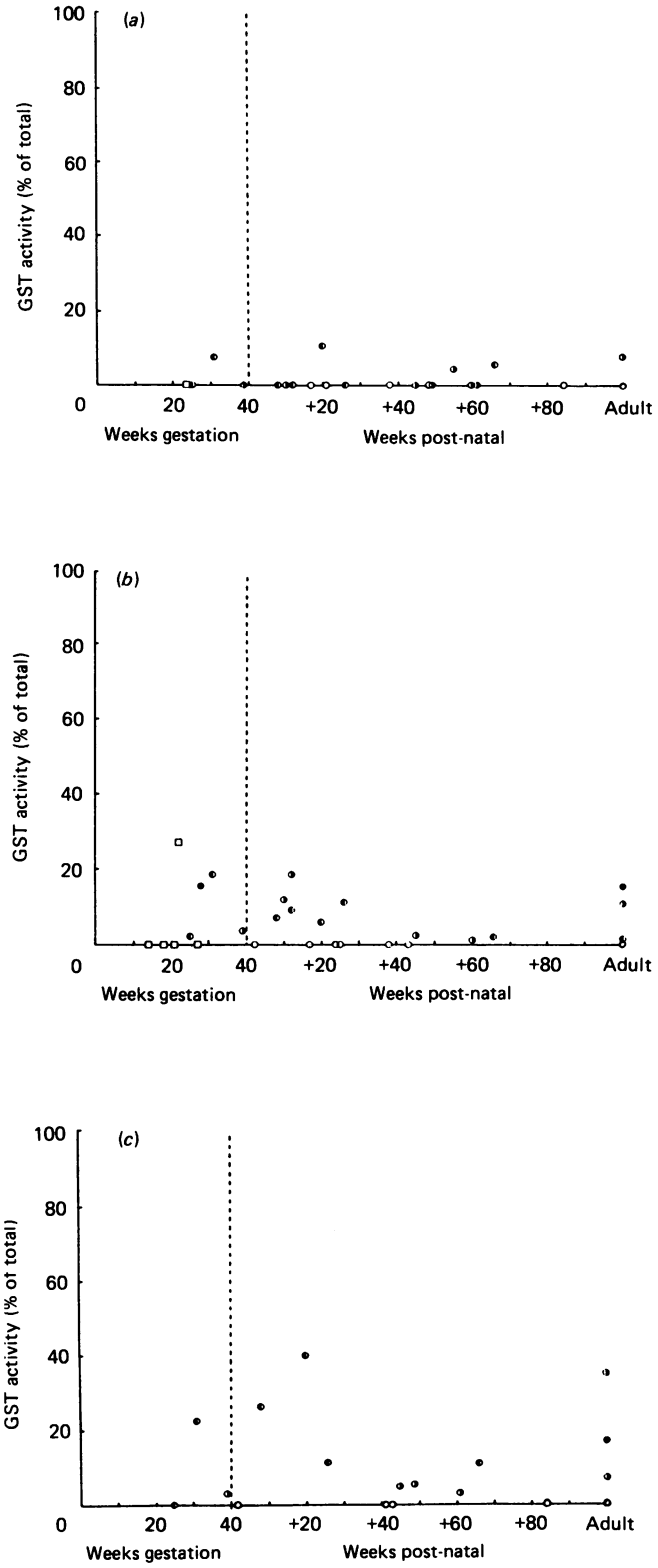

Fig. 4. Expression of near-neutral GST isoenzymes in adrenal, kidney and spleen

Adrenal (*a*), kidney (*b*) and spleen (*c*) cytosols obtained during development were chromatofocused and the contributions of the basic, near-neutral and acidic isoenzymes to activity were quantified. The GST1 phenotype is given by: ◐, GST1 1; ◑, GST1 2; ●, GST1 2-1; ○, GST1 0; □ indicates that no phenotype could be assigned.

the level of activity was often at the detection limits of the enzyme assay.

Kidney. Near-neutral isoenzymes were identified in kidney cytosol obtained after 22 weeks gestation. After this time the GST1 phenotype was as predicted from study of liver cytosol from the same subject. The contribution of these isoenzymes to cytosol activity was variable and no developmental trend was observed (Fig. 4). The elution of the near-neutral isoenzymes in kidney was more complex than that of the enzymes in the liver or adrenal from the same subject, since two further isoenzymes were eluted after the GST1 isoenzymes. Fig. 3(*a*) shows a representative example of the elution of a cytosol of GST1 1 phenotype. Although the levels of activity eluted were low, this profile was a consistent feature of cytosols with this phenotype. Studies using starch-gel electrophoresis showed that the GST1 1 isoenzyme was eluted first (elution pH approx. 6.20) and that the most acidic isoenzyme of this group (elution pH approx. 5.80) was a minor product of GST3 (Strange *et al.*, 1985). It is unclear whether the intermediate enzyme (elution pH 6.15) is a post-synthetic modification of the GST1 isoenzyme or a GST1/GST3 hybrid. Kidney samples with the GST1 2 phenotype also demonstrated activity that was eluted between the GST1 2 (elution pH approx. 6.70) and minor GST3 isoenzyme (Fig. 3*b*).

Spleen. Development of the near-neutral isoenzymes in spleen was similar to that in kidney. Activity was present after 30 weeks of gestation in all samples classified as GST1 1 or GST1 2 phenotypes. No developmental trends in expression were observed (Fig. 4). The chromatofocusing profiles of these isoenzymes were similar to those described for kidney cytosols from the same subjects.

Development of the acidic isoenzymes (ρ and ω; GST3)

Acidic isoenzymes have been identified in all human tissues. Board (1981) has suggested that in liver these isoenzymes are the products of GST3. It is not clear however, whether the corresponding enzymes in other tissues are the products of the same locus.

Liver. The contribution of acidic isoenzymes to GST activity in cytosol declined rapidly during intra-uterine life, so that at full term this locus was weakly expressed (Strange *et al.*, 1985).

Adrenal. Expression of acidic isoenzymes in adrenal cytosol during development was variable, and no trends were identified (Fig. 5). Activity was eluted from the chromatofocusing column as a single peak in all but three samples, in these cytosols a small amount of activity being eluted between the main acidic and near-neutral isoenzymes, but there was no evidence of the putative GST1/GST3 hybrid.

Kidney. Examples of the elution of acidic GST isoenzymes in kidney cytosols from the chromatofocusing columns are shown in Fig. 3(*a*) and 3(*b*). They were eluted as a main peak of activity at pH values approx. 5.0, and, as described above, small amounts of activity were also eluted before this main peak. The expression of acidic isoenzymes in kidney cytosol was constant until about 40 weeks post-natal age. After this time expression declined to the levels observed in adult samples (Fig. 5).

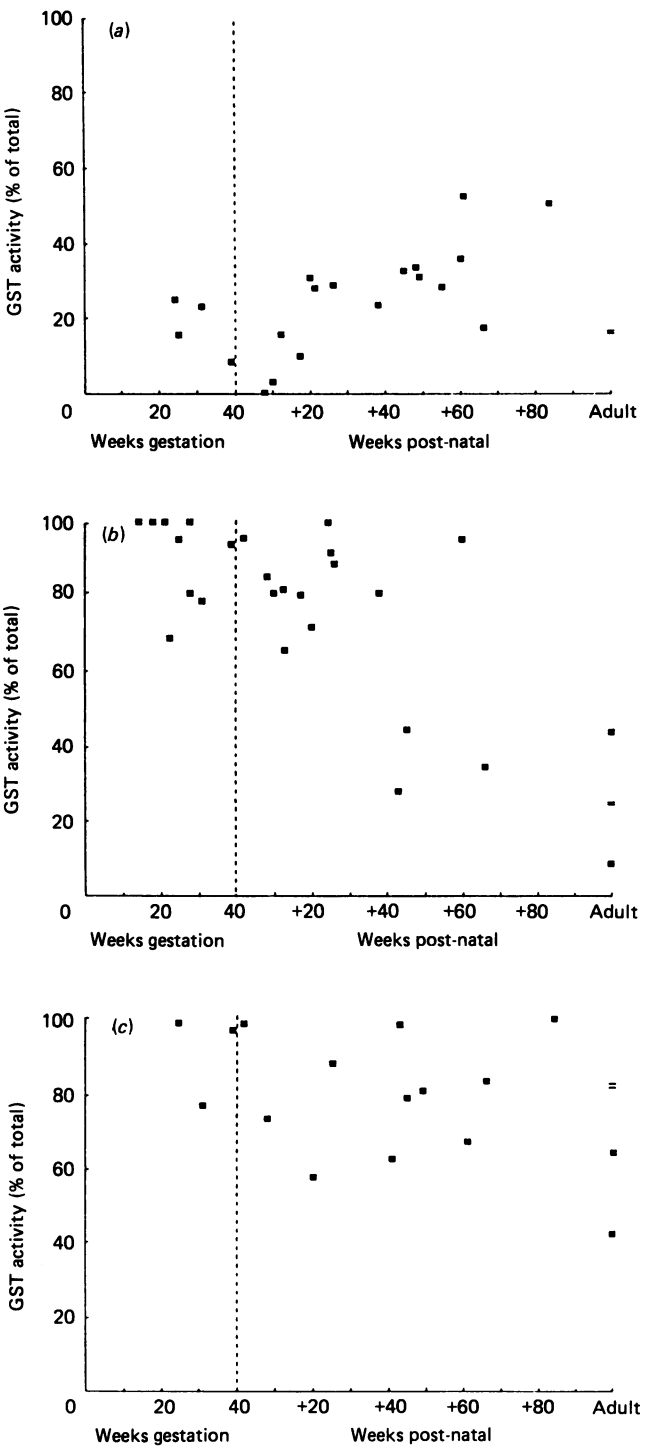

Fig. 5. Expression of acidic GST isoenzymes in adrenal, kidney and spleen

Adrenal (*a*), kidney (*b*) and spleen (*c*) cytosols obtained during development were chromatofocused and the contributions of the basic, near-neutral and acidic (■) isoenzymes to activity were quantified.

Spleen. Elution of acidic isoenzymes in spleen cytosol was similar to that in kidney. The enzymes were eluted as a main peak of activity at pH values between 5.2 and 4.8. The level of expression of these enzymes remained high during development, and no trends were observed, although expression did appear to be lower in the adult samples examined.

Inhibitor studies

The effects of sodium cholate, bromosulphophthalein and *N*-ethylmaleimide on the activities of basic, near-neutral and acidic isoenzymes obtained from foetuses and adults were compared to determine whether the enzymes within each set behaved similarly throughout development. In particular we wished to determine whether the small amounts of activity eluted at pH values between 6.70 and 6.20 could be classified as near-neutral and therefore products of GST1.

As expected from studies in adults (Warholm *et al.*, 1983), cholate inhibited the near-neutral isoenzymes from both foetal (31 weeks gestation) and adult liver, adrenal, kidney and spleen. The range of values of I_{50} was 100–200 μM. The inhibitor had no effect on the basic and acidic isoenzymes (Warholm *et al.*, 1983).

The values of I_{50} for bromosulphophthalein (3–7 μM) for the adult and foetal near-neutral isoenzymes were also similar and in agreement with those reported by Mannervik (1985). The values of I_{50} for the basic isoenzymes (10–19 μM) from the different tissues were also similar but were lower than those reported by Mannervik (1985). Values for the acidic isoenzymes (90–100 μM) were very similar to those previously reported (Mannervik, 1985).

The near-neutral and basic isoenzymes from both foetal and adult tissues were relatively insensitive to *N*-ethylmaleimide (I_{50} values in the range 1200–1750 μM), whereas the acidic enzymes were inhibited at low concentrations (I_{50} values 19–20 μM) of this agent.

DISCUSSION

We have described aspects of the development of the basic, near-neutral and acidic GST isoenzymes. The isoenzyme profiles were often complex because of a combination of factors: multiple allelism, the presence of more than one locus or a process of post-translational modification. GST activity was present in all cytosols studied, but, although major changes in expression occurred, no developmental trends in total activity were identified. Previous studies have shown large inter-individual variations in erythrocyte GST activity (Strange *et al.*, 1982), and it appears that this is a feature of the enzyme in human tissues.

Studies using starch-gel electrophoresis have indicated that the basic, near-neutral and acidic GST isoenzymes in foetal, neonatal and infant tissues are similar to those in adults. Furthermore the incidence of the various GST1 phenotypes in livers obtained after 30 weeks gestation is as expected from observations in adults (Strange *et al.*, 1984, 1985). The present studies show that the chromatographic behaviour and sensitivity to inhibitors of the enzymes within a set is also similar during development. These results, together with findings demonstrating extensive immunological similarities, firstly, between the basic isoenzymes from foetal and adult livers (Guthenberg *et al.*, 1986), as well as, secondly, between the acidic isoenzymes from various foetal tissues and those from placenta and adult erythrocytes (Pacifici *et al.*, 1986) suggests that, at least after the first trimester, no foetal-specific isoenzymes are synthesized.

The development of the basic set appears particularly complex since, although it was originally believed to

result from the post-synthetic modification of the primary product of a single locus (Kamisaka *et al.*, 1975), hybridization experiments (Stockman *et al.*, 1985) indicate at least two monomers. Since these are the most detailed investigations of this isoenzyme set, we used the same ion-exchangers to resolve the basic isoenzymes. We found that, whereas α and β were generally present in relatively small amounts in adult samples and virtually absent from cytosols obtained up to 1 year after birth, the relative activities of γ and δ varied markedly. Because of the closeness of their elution concentrations of Na^+, identification of γ and δ was often difficult. The elution profiles of six samples resembled that described by Hayes *et al.* (1983), whereas three samples demonstrated a pattern similar to that reported by Kamisaka *et al.* (1975). Since both types of elution profile were found in neonatal, infant and adult samples, the relative proportions of γ, δ and ϵ did not depend on the stage of development. This indicates that B_1 and B_2 are present at 21 weeks gestation.

The relationship between α, β, γ, δ and ϵ is unclear. Kamisaka *et al.* (1975) suggested that they were charge isomers produced by deamidation and disulphide bond formation. Since such modifications result in an increased negative charge on the protein, ϵ could be the primary gene product and α the most modified form.

The hybridization experiments revealed some interconvertibility of these enzymes. As expected (Stockman *et al.*, 1985), activity eluted at an Na^+ concentration of 14 mM (α) was generated from the isoenzyme that was eluted at an Na^+ concentration of 36 mM (δ) but not from that eluted at a concentration of 45 mM (ϵ). Dissociation of this latter enzyme also resulted in a smaller peak of activity. This peak apparently corresponds to the activity, eluted between δ and ϵ, described by Stockman *et al.* (1985). Interestingly, dissociation of combined fractions of the peaks eluted at Na^+ concentrations of 36 and 45 mM (δ and ϵ) resulted in an elution profile resembling that shown in Fig. 1(*a*) rather than that of the original cytosol. This indicates that the CM-cellulose profile is not a fundamental individual characteristic, a suggestion supported by the observation that the relative proportions of γ, δ and ϵ were not a constant individual characteristic in liver and adrenal.

The ontogeny of the basic isoenzymes varied in the tissues studied. Whereas in liver (Strange *et al.*, 1985) and adrenal the contribution of this set appeared constant, these enzymes were not expressed in kidney or spleen until after birth. Adult levels of expression were achieved at about 50 weeks post-natal age.

The similar chromatographic properties and sensitivities to inhibitors demonstrated by the near-neutral isoenzymes identified during development suggest that they are products of a single locus. Furthermore, the hybridization experiments supported previous suggestions that the three near-neutral enzymes found in subjects with the GST1 2-1 phenotype comprise combinations of two monomers. These were first detected in liver at about 30 weeks of gestation, although expression of the locus achieved adult levels only after about 10 weeks post-natal age. The adult levels of expression of these enzymes in adrenal, kidney and spleen were lower than in liver, and in many samples the activity of the near-neutral isoenzymes was at the detection limits of the assay method.

Acidic GST isoenzymes are expressed in all human tissues, and studies by Pacifici *et al.* (1986) suggest that they are the products of the same locus. We found that these isoenzymes were eluted as a major peak of activity with small amounts of activity being eluted at a slightly higher pH. Activity was also eluted between the near-neutral and acidic isoenzymes. Further studies are required before the origin of this previously unrecognized species is known, but, since it was not present in kidney and spleen samples with the GST1 0 phenotype, it could represent an inter-locus (GST1/GST3) hybrid or a post-synthetic modification of the near-neutral enzyme. Expression of the acidic isoenzyme was constant in adrenal and spleen, but declined after about 50 weeks post-natal age in kidney.

Data are now available on the ontogeny of basic, near-neutral and acidic GST isoenzymes in human lung (Fryer *et al.*, 1986), liver, adrenal, kidney and spleen. Expression of each of the sets was variable, but comparison of development in spleen and kidney, two tissues derived from mesoderm, with that in liver and lung (endoderm) and adrenal (mesoderm and ectoderm) suggests that development in mesoderm is associated with a post-natal increase in the expression of the basic isoenzymes as well as a slow decline in the relative expression of the acidic isoenzymes.

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