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Expression of glutathione *S*-transferase during rat liver development

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The ontogeny of rat liver glutathione *S*-transferase (EC 2.5.1.18) (GSTs) during foetal and postnatal development was investigated. The GSTs are dimers, the subunits of which belong to three multigene families, Alpha (subunits 1, 2, 8 and 10), Mu (subunits 3, 4, 6, 9 and 11) and Pi (subunit 7) [Mannervik, Ålin, Guthenberg, Jennsson, Tahir, Warholm & Jörnvall (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 7202–7206; Kispert, Meyer, Lalor, Coles & Ketterer (1989) Biochem. J. **260**, 789–793]. There is considerable structural homology within each gene family, with the result that whereas reverse-phase h.p.l.c. successfully differentiates individual subunits, immunocytochemical and Northern-blotting analyses may only differentiate families. Enzymic activity, h.p.l.c. and Northern blotting indicated that expression of GST increased from very low levels at 12 days of foetal growth to substantial amounts at day 21. At birth, GST concentrations underwent a dramatic decline and remained low until 5–10 days *post partum*, after which they increased to adult levels. During the period under study, GST subunits underwent differential expression. The Mu family had a lower level of expression than the Alpha family, and, within the Alpha family, subunit 1 was more dominant in the adult than the foetus. Subunit 2 is the major form in the foetus. Most noteworthy were subunits 7 and 10, which were prominent in the foetus, but present at low levels *post partum*. Immunocytochemical analysis of the 17-day foetal and newborn rat livers showed marked differences in the distribution of GSTs in hepatocytes. In the 17-day foetal liver Pi > Alpha > Mu whereas in the newborns Alpha > Mu ≫ Pi. Erythropoietic cells were not stained for any of the three GST families. Steady-state mRNA concentrations in the foetus correlated with the relative transcription of the Alpha, Mu and Pi class genes. However, in those genes expressed *post partum*, namely the Alpha and Mu class, low transcriptional activity was associated with high concentrations of mRNA. This suggests that there is a switch from transcriptional control to post-transcriptional control at birth. GST 7–7 appears to be regulated predominantly by transcription throughout the period of liver development under observation.

INTRODUCTION

The glutathione *S*-transferases (GSTs) are a family of multifunctional dimeric proteins which catalyse the conjugation of GSH with electrophiles, the reduction of organic hydroperoxides and certain GSH-dependent isomerizations, and are binding proteins for a number of lipophiles (Mannervik & Danielson, 1988; Ketterer *et al.*, 1988). GSTs play important roles in the detoxication of metabolites of drugs and carcinogens, lipid and DNA hydroperoxides. They are also involved in the hepatic uptake of bilirubin and the intracellular transport of haem and steroid hormones (Litwack *et al.*, 1971).

At least 12 GST subunits have been identified and shown to be members of four families referred to as Alpha, Mu, Pi and Theta. Using a numerical nomenclature, subunits 1, 2, 8 and 10 are members of the Alpha family, subunits 3, 4, 6, 9 and 11 are members of the Mu family, subunit 7 is the only member of the Pi family and subunits 5 and 12 are members of the Theta family (Mannervik *et al.*, 1985; Ketterer *et al.*, 1988; Kispert *et al.*, 1989, Meyer *et al.*, 1991*a*,*b*). In the adult rat the distribution of subunits varies from tissue to tissue (Hales *et al.*, 1978; Guthenberg *et al.*, 1985; Ketterer *et al.*, 1988; Ostlund Farrants *et al.*, 1987; Tahir *et al.*, 1988). The liver, which contains at least 100 μM total GST, is rich in subunits 1, 2, 3 and 4 and also contains subunit 8. Little is known about GST expression in the liver during foetal and early postnatal development. The present work shows the sequential expression of hepatic Alpha, Mu and Pi class GSTs from the 13-day foetus through the first 10 days *post partum* to the adult rat. GST subunit 7, which is expressed in the adult only during hepatocarcinogenesis (Kitahara *et al.*, 1984; Meyer *et al.*, 1985; Sato *et al.*, 1987), is shown to display a distinct expression during foetal development and may be considered to be an oncofoetal protein.

MATERIALS AND METHODS

Animals

Albino Wistar rats from 13 to 22 days gestation (newborns), 1–10 days old and female adults (approx. 250 g, 60–70 days old) were supplied by the Animal Resources Centre, Murdoch University, Western Australia, Australia.

Chemicals

All chemicals were of analytical grade. SDS and Protein Dye Reagent Concentrate were supplied by Bio-Rad Laboratories, Richmond, CA, U.S.A. EDTA, Tris, phenylmethanesulphonyl

Abbreviations used: GST, glutathione *S*-transferase; PMSF, phenylmethanesulphonyl fluoride; DTT, dithiothreitol; CDNB, 1-chloro-2,4-dinitrobenzene; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PBS, phosphate-buffered saline (0.15 M-NaCl/0.4 mM-sodium phosphate buffer, pH 7.4); 1 × SSC, standard saline citrate (0.15 M-NaCl/15 mM-sodium citrate buffer, pH 7).

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fluoride (PMSF), 3,5-diaminobenzoic acid, GSH, dithiothreitol (DTT), 2-mercaptoethanol, 1-chloro-2,4-dinitrobenzene (CDNB) and GSH–agarose were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. [^{32}P]dCTP was obtained from Amersham Australia Pty. Ltd., Sydney, N.S.W., Australia. Gene Screen was obtained from New England Nuclear, Sydney, N.S.W., Australia. Antibodies to GST subunit 1 (cross-reacting with other members of the Alpha family), subunit 3 (cross-reacting with other members of the Mu family) and subunit 7 were supplied by Medlabs, Dublin, Ireland. Antiserum to albumin was prepared by immunization of rabbits with purified albumin.

GST activity towards CDNB

CDNB is a substrate for all GSTs (except GST subunits 5 and 12, which are minor forms in liver) and was used as a measure of total GST activity. Liver soluble supernatant fraction was prepared by homogenizing 1:2 (w/v) of liver in 0.01 M-KCl/50 μM-PMSF/1 mM-EDTA/10 mM-sodium phosphate buffer, pH 7.0, and homogenate was centrifuged at 10000 *g* for 1 h at 4 °C. GST activity towards CDNB was determined as described by Habig *et al.* (1974). The protein content of cytosol was determined by the dye-binding method of Bradford (1976), with BSA as a standard.

Analysis of subunits of GST by reverse-phase h.p.l.c.

Samples of soluble supernatant fraction were freeze-dried for transport and solubilized before use. The total GST fraction of these samples was obtained using a GSH–agarose affinity matrix and analysed for GST subunits by h.p.l.c. according to the method of Ostlund Farrants *et al.* (1987) on a Dynamax C_{18} column (30 cm × 4.6 mm) (Rainin Instrument Co., Woburn, MA, U.S.A.). Elution was monitored at 214 nm and peaks were integrated by using a Hewlett–Packard 3390A integrator. H.p.l.c. analysis separated and quantified subunits 1a, 1b, 1c, 2, 3, 4, 6, 7, 8, 9, 10 and 11 (Kispert *et al.*, 1989; Meyer *et al.*, 1991*a*,*b*).

Immunocytochemistry

Whole livers of 17-day gestation foetuses and newborn rats were fixed in Carnoy's solution for 6 h and then embedded in paraffin wax. Liver sections (4 μm thick) were attached to glass slides, on which all subsequent staining procedures were performed. The indirect immunoperoxidase detection of Alpha, Mu and Pi GSTs, and albumin was performed according to the method of Clement *et al.* (1985). Endogenous peroxidase in the samples was blocked by treatment with aq. 2.5% aq. (w/v) H_5IO_6 for 5 min and 0.02% $NaBH_4$ for 2 min (Heyderman, 1979). This was followed by a 1 h incubation with 10% (v/v) foetal calf serum in phosphate-buffered saline (PBS). The liver sections were then allowed to react with a dilution of 1:200 of the non-immune rabbit serum, 1:400 of the albumin antibody, 1:500 of the Alpha class and Mu class GST antibodies and 1:100 of the Pi class GST antibody for 1 h. After three washed with PBS, the sample was exposed to a 1:200 dilution of the second antibody (peroxidase-coupled goat IgG directed against rabbit IgG) for 1 h. The washing procedure was repeated, followed by a final wash in 50 mM-Tris/HCl buffer, pH 7.5. Localization was revealed by reaction with 0.05% diaminobenzidine and 0.01% H_2O_2 in 50 mM-Tris/HCl buffer, pH 7.5, for 20 min. In all experiments liver sections treated with non-immune serum were incorporated as negative controls and those treated with albumin antibodies were used as positive control for hepatocytes.

Run-on transcription in isolated nuclei

Nuclei were isolated from 0.5 mg of foetal, postnatal and adult liver according to the method of Becker *et al.* (1984). Isolated nuclei were suspended in 50% (v/v) glycerol/5 mM-$MnCl_2$/1 mM-$MgCl_2$/5 mM-DTT/20 mM-Tris/HCl buffer, pH 7.4, frozen in liquid N_2 and stored at −80 °C until use. Run-on transcription in isolated nuclei yielded ^{32}P-labelled RNA, which was immobilized on nitrocellulose membranes and hydridized to the following cDNA probes: pGSTr 155 (Taylor *et al.*, 1984), a subunit 1 clone that is known to hydridize to subunit 2 and is assumed to hybridize to the other members of the Alpha family, namely subunits 8 and 10; JTL9 (S. E. Pemble & J. B. Taylor, unpublished work), a clone for subunit 3 that is assumed to hybridize to other members of the Mu family such as subunits 4 and 11; pGSTr 7 (Pemble *et al.*, 1986), which is a subunit 7 clone and therefore represents the Pi family. Albumin, a liver-specific protein that has been shown by several workers (Tilghman & Belayew, 1982; Powell *et al.*, 1984; Panduro *et al.*, 1987) to be regulated predominantly at the transcriptional level during foetal development, is used in this study as a reference gene.

The method used was a modification of that of Shelly *et al.* (1989). Isolated nuclei (approx. 5×10^6) were incubated in the presence of 50 mM-Hepes/NaOH buffer, pH 8.0, 150 mM-NH_4Cl, 1 mg of nuclease-free BSA/ml, 1 mM-$MnCl_2$, 12.5% (v/v) glycerol, 0.1 mg of heparin/ml, 3.5 mM-$MgCl_2$, 60 units of RNasin, 0.25 mM-DTT, 0.5 mM-ATP, 0.5 mM-GTP, 0.5 mM-CTP and 100 μCi [α-^{32}*P*]UTP (specific radioactivity approx. 800 Ci/mmol) in 5 mM-Tris/HCl buffer, pH 7.5, for 20 min at 25 °C. The reaction was terminated by addition of 1 unit of RQ1 DNAase I and further incubation for 5 min at 37 °C. Labelled RNA was isolated by phenol/chloroform extraction followed by ethanol precipitation and was subsequently hybridized to 0.25 pmol of each of the following nitrocellulose-bound cDNA inserts: (i) albumin genomic subclones 'B', 'C' and 'D' (Sargent *et al.*, 1981); (ii) GST cDNA pGSTr 155 (Taylor *et al.*, 1984); (iii) JTL9 (S. E. Pemble & J. B. Taylor, unpublished work); (iv) pGSTr 7 (Pemble *et al.*, 1986); (v) glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA pRGAPDH-13 (Fort *et al.*, 1985), (vi) pBR322 for 72 h at 42 °C in a solution containing 55% formamide, 4 × SSC, 0.1 M-sodium phosphate buffer, pH 6.8, 5 × Denhardt's solution, 0.1% SDS, 100 μg of salmon sperm DNA/ml and 10% dextran sulphate (1 × Denhardt's solution is 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% BSA). After three washes in 1 × SSC/0.1% SDS, and two washes

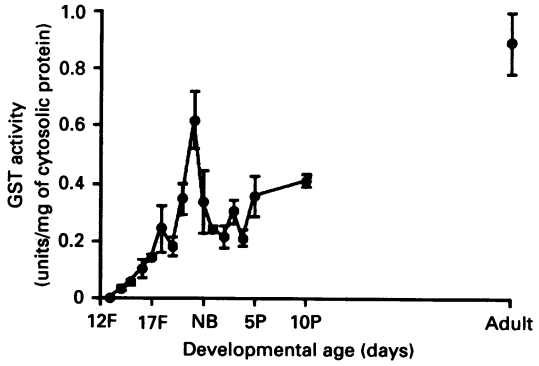

Fig. 1. GST activities towards CDNB in foetal, newborn and postnatal rat liver

Activities were measured in liver cytosolic extracts prepared from 13–21-day foetuses (13F–21F), newborn rats (NB) and 1–10-day-old rats (1P–10P). Results are means ± S.E.M. of three to six different cytosolic preparations. Peak value at day 21 is significantly different from value at day 20 ($P < 0.005$) and at birth, NB ($P < 0.05$).

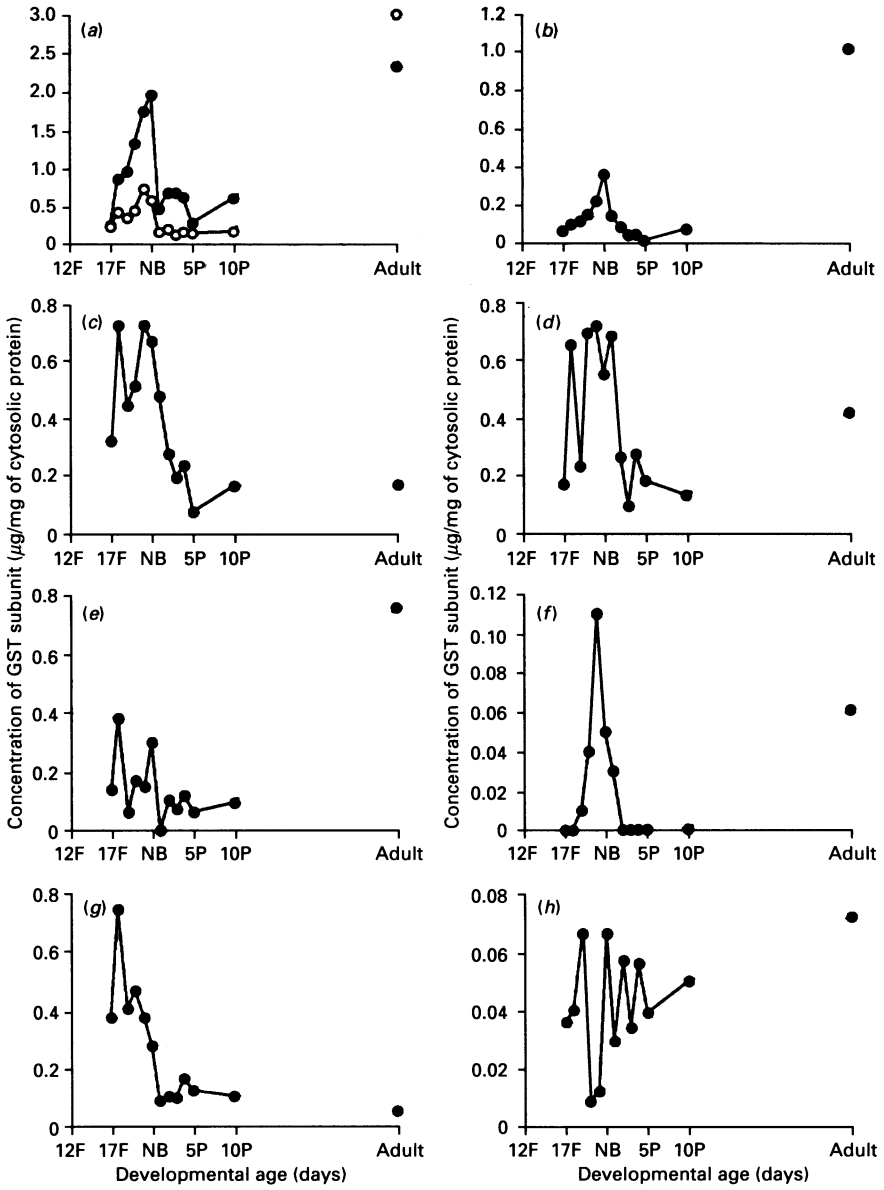

Fig. 2. Concentrations of GST subunits during rat liver development

Liver cytosolic extracts were prepared from 17–21-day foetuses (17F–21F), newborn rats (NB) and 1–10-day-old rats (1P–10P). The GST fraction was obtained by affinity chromatography and concentrations of GST subunit were analysed by reverse-phase h.p.l.c. as described in the text. Concentrations of subunits in the Alpha class GST are shown in (*a*) subunit 1 (○) and subunit 2 (●), (*b*) subunit 8 and (*c*) subunit 10. Concentrations of subunits in the Mu class GST are shown in (*d*) subunit 3, (*e*) subunit 4 and (*f*) subunit 11. Subunit 7 (*g*) is the only member in the Pi class GST. Concentrations of subunit 5 are shown in (*h*). Results are representative of analyses from two different liver cytosolic extracts. Each cytosolic extract was prepared from 250 mg of liver, which consists of whole liver from one to five foetuses.

in 0.1 × SSC/0.1 % SDS at 65 °C, associated radiolabel on the nitrocellulose was detected by fluorography and assessed by optical densitometry. The rate of transcription of the Alpha, Mu and Pi GSTs and albumin was calculated relative to transcription of the consitutive gene for GAPDH. Thus densitometry signals were converted into relative transcription rates by correcting for the fraction of primary transcript hybridizable to the recombinant genomic DNA and thereafter normalizing the corrected rate for the GSTs and albumin against the corrected rate for GAPDH for the respective filters. To enable comparison of rates of transcription between the GSTs and albumin over the same unit length, the corrected and normalized values were divided by the gene length, thereby giving the relative transcription of the GSTs and albumin genes per unit time to the GAPDH gene per unit time.

Isolation of total RNA and hybridization with cDNA representing Alpha, Mu and Pi class GSTs

Total RNA was isolated from whole rat liver by using the method of LeMeur *et al.* (1981). Sample was homogenized in 3 M-LiCl/6 M-urea/10 mM-sodium acetate buffer, pH 5.0, containing 200 μg of heparin/ml, 0.1 % SDS and 1 % 2-mercaptoethanol, and stored overnight at 4 °C. The precipitated RNA was sedimented by centrifugation at 15000 *g* for 20 min, washed three times with 4 M-LiCl/8 M-urea/1 % 2-mercaptoethanol in water dissolved in 1 % SDS/50 mM-sodium acetate buffer,

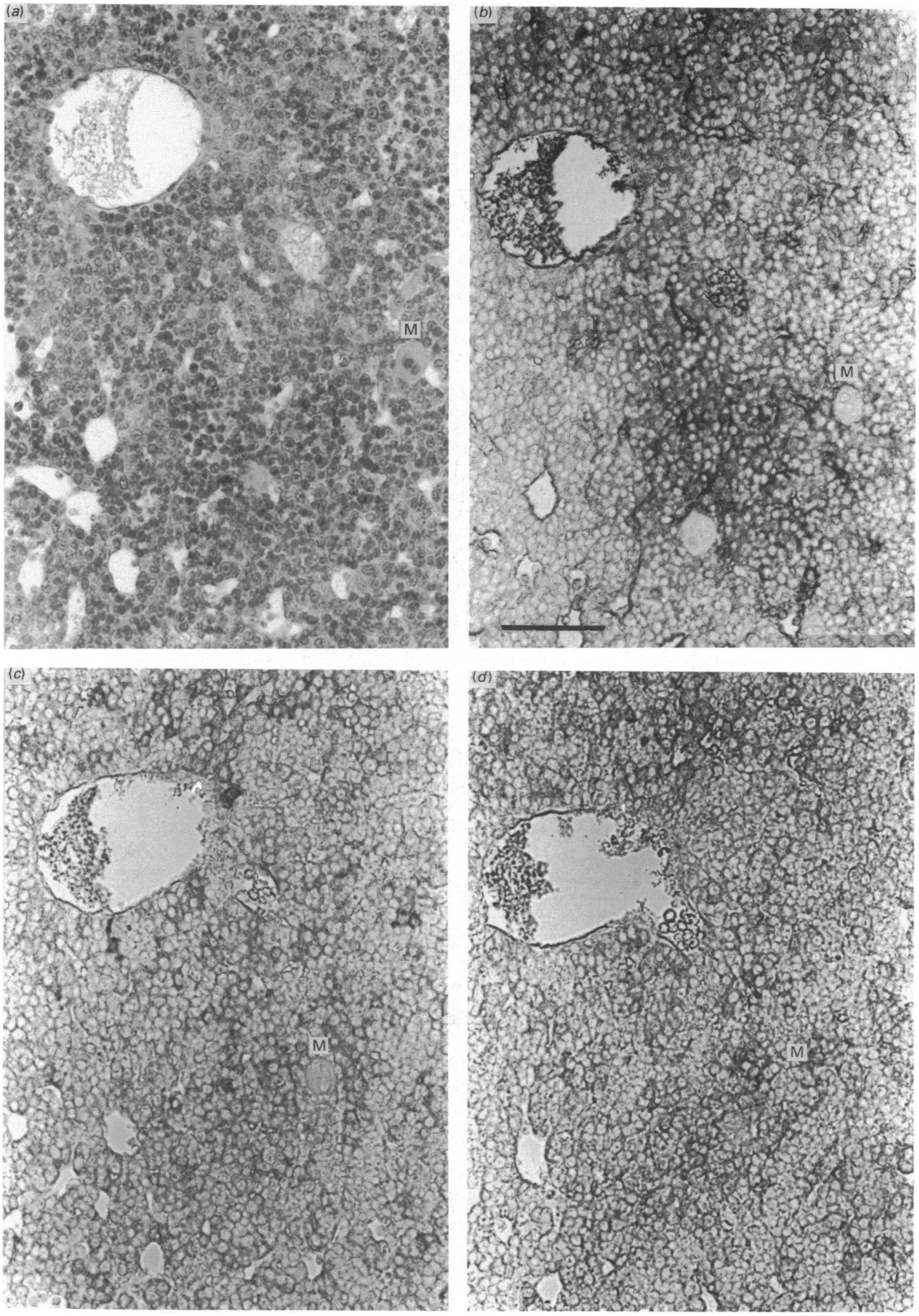

Fig. 3. Cellular distribution of GSTs in 17-day foetal liver

Whole liver of 17-day gestation foetus was fixed in Carnoy's solution and serial paraffin-embedded liver sections were stained with (*a*) haematoxylin and eosin, and antibodies against (*b*) Pi, (*c*) Mu and (*d*) Alpha class GSTs are described in the text. M, megakaryocyte. Results are representative of four livers. Magnification bar represents 200 μm.

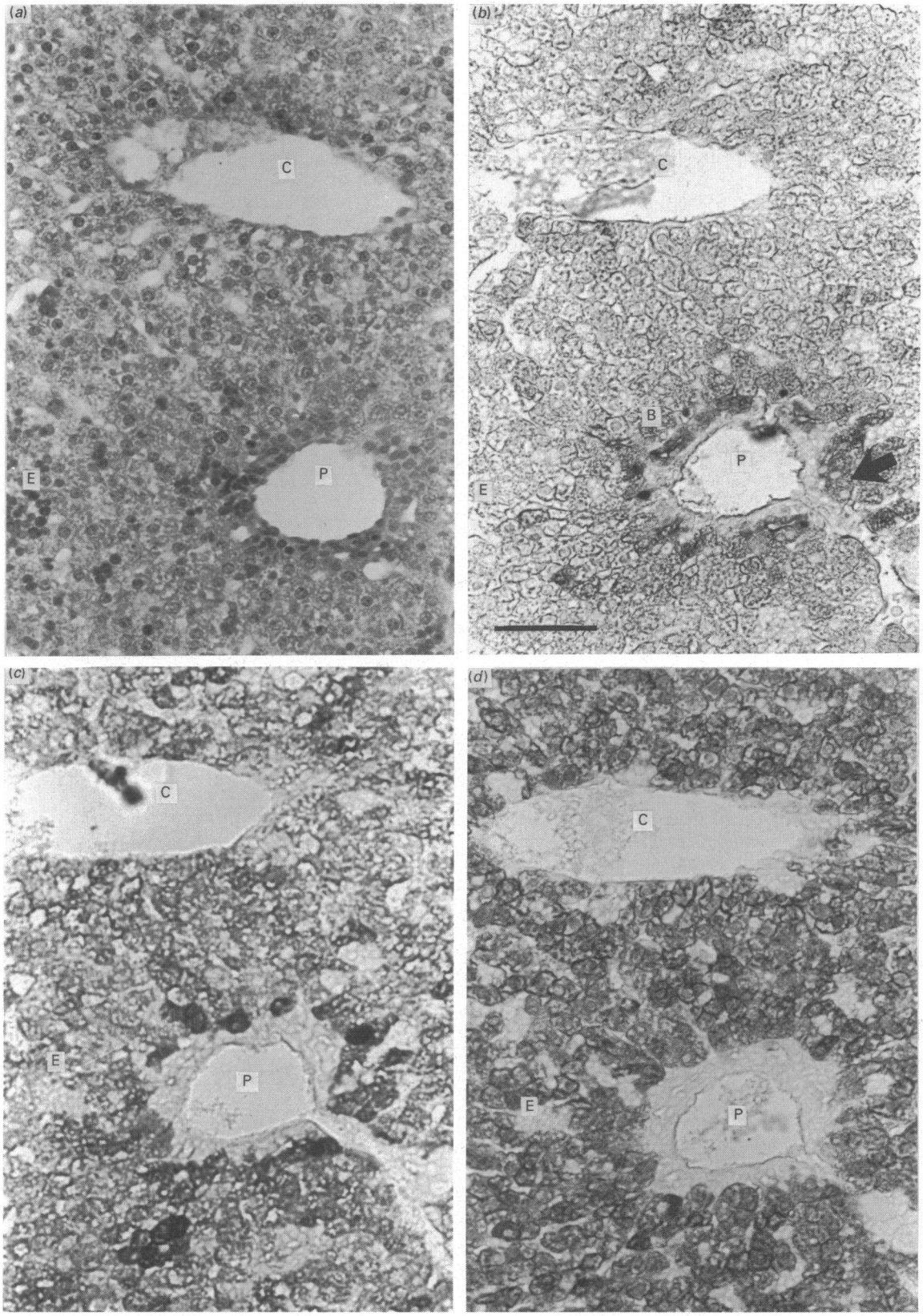

Fig. 4. Cellular distribution of GSTs in newborn rat liver

Whole liver of newborn rat was fixed in Carnoy's solution and serial paraffin-embedded liver sections were stained with (*a*) haematoxylin and eosin, and antibodies against (*b*) Pi, (*c*) Mu and (*d*) Alpha class GSTs as described in the text. P, portal tract; C, central vein; E, erythropoietic cells; B, bile duct; ←, hepatocytes. Results are representative of four livers. Magnification bar represents 200 μm.

pH 5.0, and extracted with phenol/chloroform (1:1, v/v) and diethyl ether. The RNA in the aqueous phase was then precipitated with ethanol and was finally dissolved in water. The concentration of RNA was estimated from A_{260}. For Northern-blot analysis, 10 μg of RNA was electrophoresed according to the method of Lehrach *et al.* (1977) and then electrophoretically transferred to Gene Screen by using a Bio-Rad Transblot apparatus.

As above, Alpha, Mu and Pi class GST mRNAs were detected by hybridization to ^{32}P-labelled probes pGSTr 155 (Taylor *et al.*, 1984), JTL9 (S. E. Pemble & J. B. Taylor, unpublished work) and pGSTr 7 (Pemble *et al.*, 1986) respectively. Radioactivity was detected by autoradiography. The cDNA probes were labelled by nick translation. Hybridization in 50% deionized formamide/5 × SSC/0.1% SDS/5 × Denhardt's solution containing 250 μg of sonicated salmon sperm DNA/ml was performed at 45 °C for 24 h after 18 h of prehybridization. The intensity of the image produced on X-ray film was quantified by using a Bio-Rad model 620 video densitometer and is expressed as a ratio of the values for GAPDH.

RESULTS

Enzymological and h.p.l.c. analyses

Total GST activity estimated by its activity towards CDNB showed two phases during development (Fig. 1). In the foetus, it was undetectable in 13-day liver, first observed at 14-day gestation and increased to a maximum (78% of adult value) by day 21 then declined towards birth (22 days). Postnatally, activity remained low at about 30% of the adult value during the first 4 days after birth and increased gradually to reach maximal levels observed in the adult (Fig. 1). H.p.l.c. analyses of GST purified from liver cytosolic fraction by using a GSH–agarose affinity column differentiated and quantified the individual GST subunits in foetal liver extracts. The method detects subunits 1a, 1b, 1c, 2, 3, 4, 7, 8, 10 and 11 (Kispert *et al.*, 1989). By applying this technique to cytosolic extracts of liver at different stages of development, it is possible to determine changes that occur with respect to each subunit as well as estimate their relative contributions to the total amount of each family (Fig. 2).

Within the Alpha family, subunit 2 > subunit 10 = subunit 1 > subunit 8 in foetal liver, but subunit 1 > subunit 2 > subunit 8 ⩾ subunit 10 in adult liver. Subunit 10 is more abundant in foetal liver whereas subunit 1 is more abundant in adult liver. Subunit 2 is prominent in both foetal and adult liver.

In the case of the Mu family, subunit 3 > subunit 4 > subunit 11 in the foetus, but subunit 4 > subunit 3 > subunit 11 in the adult. In the Pi family, subunit 7 is expressed only in the foetus. The small amount in homogenates of the normal adult liver are contributed by bile-duct epithelial cells, not the hepatocytes (Tatematsu *et al.*, 1985).

There is a rapid diminution in the content of all subunits at birth with levels remaining low until between the fifth and tenth days *post partum*, after which levels increase to adult values in all cases except subunits 7 and 10, which are only expressed in the foetus.

The expression of subunit 7 reaches a maximum at day 18 of gestation and falls rapidly to low levels at birth. Subunit 10 on the other hand is still expressed at birth and does not reach its minimum level until 5 days *post partum*.

Cellular distribution of GSTs

Whole liver from 17-day foetuses and newborn rats was fixed in Carnoy's solution. Haematoxylin and eosin staining revealed the presence of a significant number of erythropoietic cells arranged in large islands among the liver parenchyma in the 17-day foetal liver (Fig. 3*a*). Erythropoietic cells, which contain dense nuclei, differ from the hepatocytes, which contain pale nuclei and a higher proportion of cytoplasm. In contrast with 17-day foetal liver, fewer erythropoietic cells arranged in small islands are observed in the newborn rat liver (Fig. 4*a*, E). In the 17-day foetal liver not all hepatocytes were stained for the GSTs and the extent of staining was Pi > Alpha > Mu class GST (Figs. 3*b*–3*d*). The pattern of heterogeneity was different for each class of GST. Large groups of hepatocytes were positive for Pi class GST (Fig. 3*b*), whereas clusters of two to four cells were positive for the Mu class (Fig. 3*c*) and up to ten cells for the Alpha class (Fig. 3*d*). In the newborn rat liver, only a few hepatocytes around the portal region (Fig. 4*b*, arrow), and the epithelium of developing bile ducts (Fig. 4*b*, B) were stained for the Pi class GST. The epithelium of developing bile ducts and the portal tract were not stained for Mu class (Fig. 4*c*) and Alpha class (Fig. 4*d*) GSTs. Homogeneous staining of hepatocytes was

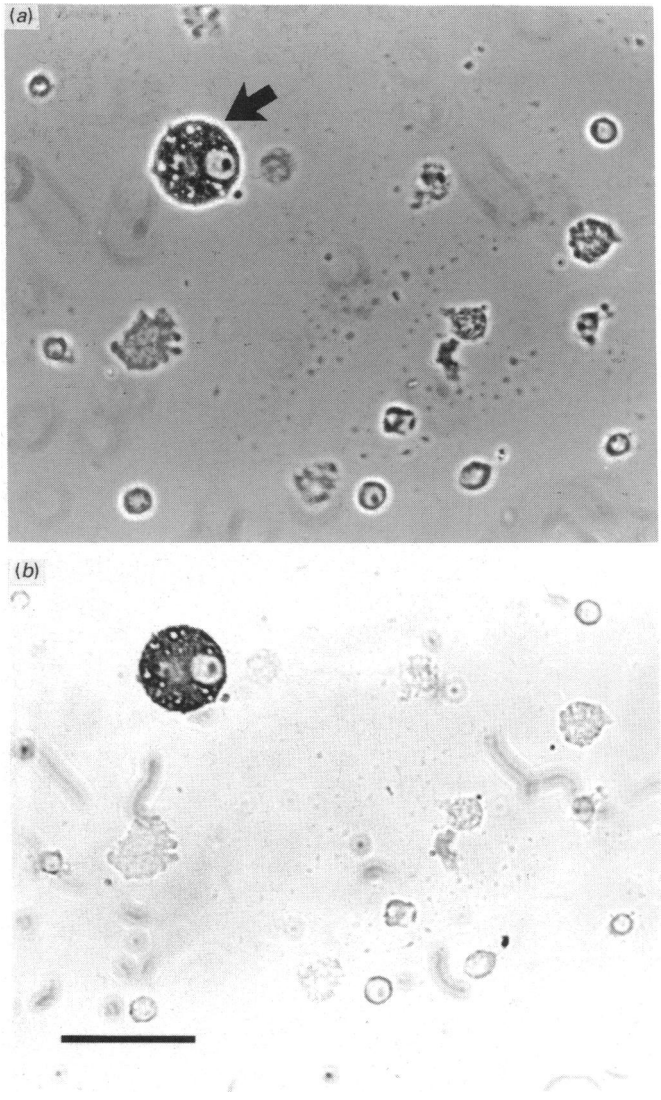

Fig. 5. Cellular distribution of Pi class GST in hepatocytes and erythropoietic cells isolated from 19-day foetal liver

Hepatocytes and erythropoietic cells were isolated from 19-day foetal liver and immunostained for Pi class GST as described in the text. Photomicrographs were taken under (*a*) phase and (*b*) bright-field illumination. Hepatocyte (←) is strongly stained whereas erythropoietic cells are not stained. Magnification bar represents 200 μm.

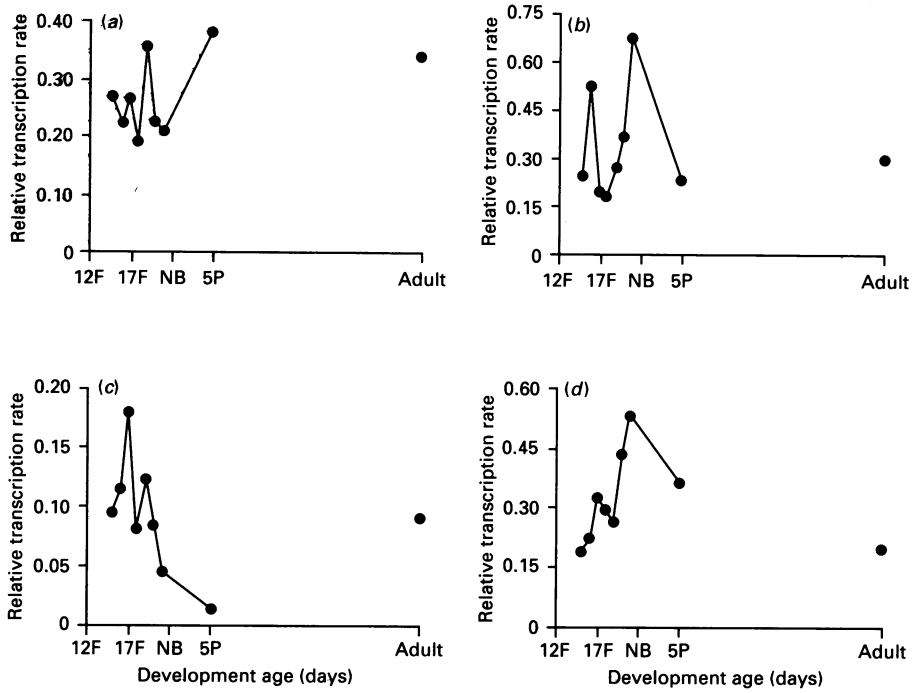

Fig. 6. Relative transcription rates of GSTs and albumin during rat liver development

Nuclei were prepared from foetal (F), postnatal (P) and adult livers and the relative rate of transcription of (*a*) Alpha class GST, (*b*) Mu class GST, (*c*) Pi class GST and (*d*) albumin in the nuclei samples was assessed as described in the text. Results are representative of two different nuclei preparations.

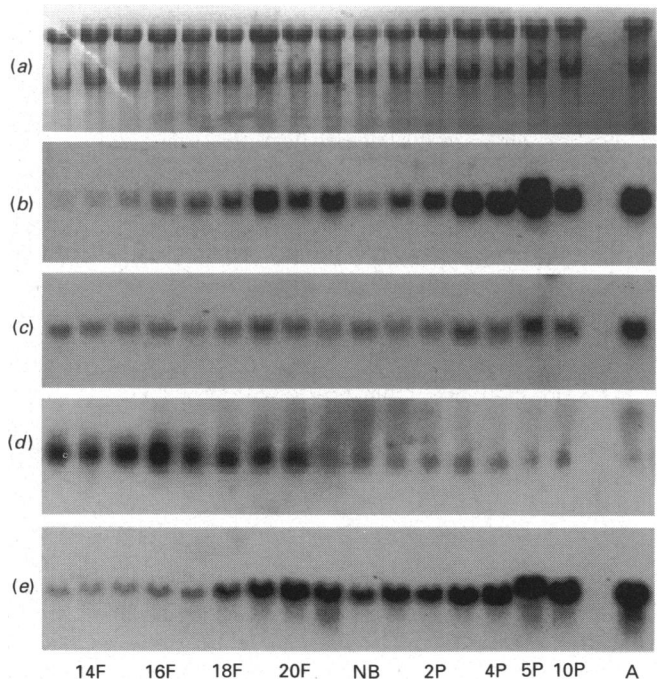

Fig. 7. Northern-blot analysis of total RNA prepared from foetal, newborn, postnatal and adult livers

Total RNA was prepared from foetal (13F–14F), newborn (NB), postnatal (1P–10P) and adult (A) livers, electrophoresed and electrophoretically transferred to Gene Screen as described in the text. A photograph of the gel is shown in (*a*). The membrane was probed for (*b*) Alpha class GST, (*c*) Mu class GST, (*d*) Pi class GST and (*e*) albumin.

observed for the Alpha class GST (Fig. 4*d*), but heterogeneous staining with some very strongly stained hepatocytes around the portal region was observed for the Mu class GST (Fig. 4*c*). All hepatocytes appeared to be stained for albumin in the 17-day foetal and newborn rat liver (results not shown). Erythropoietic cells including the megakaryocytes were not stained for any of the GSTs and albumin (Figs. 3 and 4, 'E' and 'M'), and the staining was not different from the negative staining with non-immune serum (results not shown). To confirm that erythropoietic cells do not contribute significantly to the total GSTs in the liver, hepatocytes and erythropoietic cells were isolated from 19-day foetal liver and analysed by immunocytochemical staining. Hepatocytes were stained strongly for albumin and all GSTs whereas erythropoietic cells stained for none of these antigens. The staining pattern for Pi class GST is shown in Fig. 5.

Regulation of GST during liver development

Transcription of Alpha class GST during foetal development (Fig. 6*a*) was associated with a similar increase in steady-state mRNA concentrations (Figs. 7*b* and 8*a*). Five-day-old and adult rats displayed similar relative transcription rates and mRNA concentrations of Alpha class GST. However, the Alpha class GST mRNA concentrations in 5-day-old and adult liver were approximately 4-fold greater than that observed in 19-day foetal liver, although the relative transcription rates of the gene at these developmental stages were not different.

Steady-state Mu class GST mRNA concentrations were low during early gestation and increased by 2.5-fold towards late gestation (Figs. 7*c* and 8*b*). Transcriptional activity of class Mu GST genes correlated well with the mRNA concentration during foetal development, except at 16 days gestation, when tran-

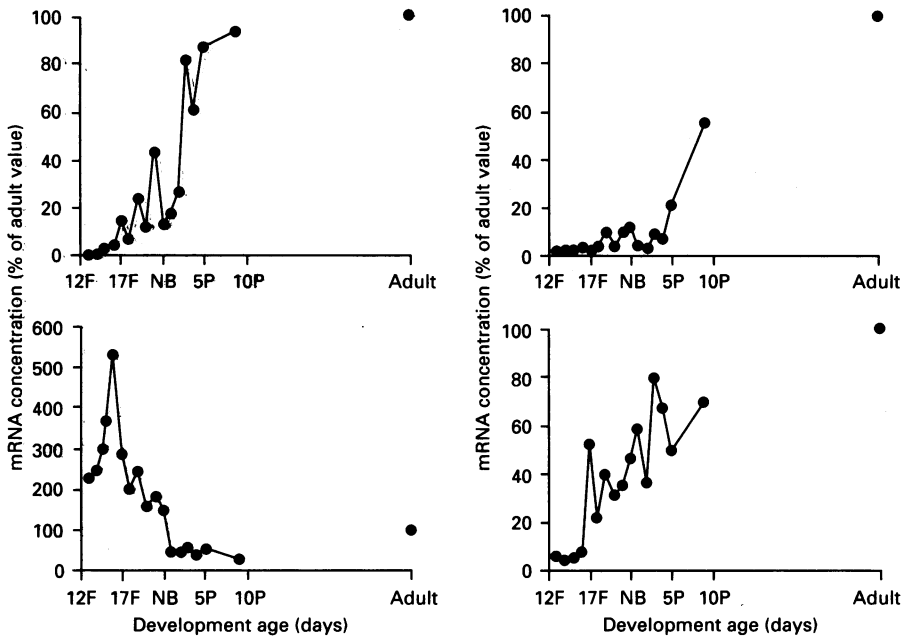

Fig. 8. Relative steady-state mRNA concentrations of GSTs and albumin during rat liver development

Relative steady-state mRNA concentrations of (*a*) Alpha class GST, (*b*) Mu class GST, (*c*) Pi class GST and (*d*) albumin were quantified by densitometry and expressed as a ratio of the values for GAPDH. Results are representative of two different RNA preparations and are expressed as a percentage of adult level.

scription was higher than the steady-state mRNA concentration (Fig. 6*b*). Steady-state Mu class GST mRNA concentrations in adult liver were at least 5-fold that of foetal and early postnatal (1–5 days after birth) liver. Although there was no difference in the relative transcription rate in 19-day foetal, 5-day postnatal and adult liver, a 5–10-fold difference in the steady-state mRNA concentration between the foetal and postnatal period was apparent.

Low but significant transcription and steady-state mRNA concentrations of the GST 7 gene was observed in foetal liver with a distinct peak in steady-state mRNA concentration and relative transcription rate occurring at 16 and 17 days of foetal development respectively (Figs. 6*c*, 7*d* and 8*c*). Transcription of GST 7 appears to correlate well with its concentration of mRNA throughout liver development.

Steady-state albumin mRNA concentration correlated well with its transcriptional activity during foetal development (Figs. 7*e* and 8*d*). Although there was no difference in transcription rate in 19-day foetal and adult liver (Fig. 6*d*), the mRNA concentration in adult liver was about twice that of the 19-day foetal liver (Fig. 8*d*).

DISCUSSION

This study shows the ontogeny of GST during three phases of liver development in the rat: (i) foetal, from 13 days of gestation until birth; (ii) early postnatal, from 1 to 10 days after birth; (iii) adulthood. Although an overall increase in total GST enzyme activity appeared to accompany liver development, a considerable variation in the expression of individual GST subunits was demonstrated. Large changes occurred during late gestation and the early postnatal period. This differential expression of GSTs was depicted by changes in protein, steady-state mRNA and relative transcription levels. Thus, whereas the Alpha class GSTs appeared to predominate throughout the three phases of liver development under observation, subunit 2 was found to be the major subunit in foetal liver and subunit 1 in the adult.

Greengard (1970) proposed that changes in expression of liver-specific proteins generally occurred at three specific developmental stages: (i) late gestation; (ii) at or directly after birth; (iii) just before weaning (approx. 3 weeks of age in the rat). During these periods the liver undergoes significant anatomical, morphological and physiological changes in response to (i) commitment of embryonal cells to become hepatocytes, (ii) preparation for extra-uterine function during late gestation and (iii) maturation of newborn liver. In this study, low hepatic GST activity was observed during early gestation followed by a rapid increase during late gestation. The dramatic increase in GST activity during late gestation was associated with an increase in the Alpha and Mu classes of GST. H.p.l.c. analyses differentiated between GST subunits and indicated that this increase in GST activity was attributed to an increase in subunits 1, 2, 8 and 10 in the Alpha family and subunits 3, 4 and 11 in the Mu family. A sudden decline in total hepatic GST activity occurred at birth and the early postnatal period. As expected this was associated with a decrease in subunits detected by h.p.l.c. A similar developmental pattern has been reported for three drug-metabolizing enzyme activities in the mouse, namely benzo[*a*]pyrene hydroxylase, epoxide hydrolase and total GST (Rouet *et al.*, 1984).

The Pi family, represented by only subunit 7, showed a distinct developmental pattern in the liver, being abundant in 18-day foetal liver but almost absent postnatally. This differential expression of subunit 7 was also reported by Abramovitz & Listowsky (1988). In this study, immunocytochemical staining revealed that GSTs were not uniformly distributed among the hepatocytes in 17-day foetal liver. The frequency of expression of classes at GSTs among these 17-day foetal hepatocytes was Pi > Alpha > Mu. At birth, Alpha class GST was uniformly distributed in the liver parenchyma, Mu class GST was heterogeneously distributed in the liver and Pi class GST was only found in a few hepatocytes and bile-duct epithelial cells. On the other hand, Alpha and Mu class enzymes were not found in the epithelium of developing bile ducts. The peak level of GST

subunit 7 occurs at the same period as peak erythropoiesis. Our results obtained using immunostaining analysis indicated that erythropoietic cells contributed minimally to the total GSTs of foetal liver. Strange *et al.* (1990) have reported similar findings in human foetal liver.

Although GSTs in adults can be regulated by xenobiotics and sex hormones (Ketterer *et al.*, 1988), the specific factors regulating GST enzyme concentrations during liver development are not known. During foetal life the liver receives its blood supply mainly from the umbilical vein and the portal vein, and the hepatic artery contributes minimally to liver blood flow. Immediately after birth, the newborn liver undergoes considerable adaptation to changes in liver circulation (Elderston, 1980; Elderston *et al.*, 1980) and this could contribute to changes in drug-metabolizing enzymes. Foetuses and weanling rats are subjected to maternal influences, which include exposure to maternal hormones and xenobiotics. In rodents, levels of several forms of cytochrome *P*-450 are controlled by hormones, and the developmental aspects (at least after birth) are closely associated with the concentrations of androgen, and at least four rat liver cytochrome *P*-450s can be modulated in this manner (Waxman *et al.*, 1985; Dannan *et al.*, 1986).

To establish the nature of the developmental regulation of the GST gene, the steady-state mRNA concentrations and relative transcription rates of these genes during the sequential stages of liver developmental were assessed. Steady-state concentrations of a particular mRNA reflect the final accumulation of the transcript and are not necessarily representative of the rate of transcription of the gene. Post-transcriptional mechanisms, including (i) the processing of the primary transcript, (ii) nucleocytoplasmic transfer of mRNA and (iii) a selective stabilization or degradation of the mRNA, may be involved in the regulation of mRNA conservation in the cell (Darnell, 1982; Raghow, 1987).

Our results for regulation of the albumin gene obtained using Northern blotting and 'run-out' transcription analyses are in agreement with other workers (Tilghman & Belayew, 1982; Powell *et al.*, 1984; Panduro *et al.*, 1987). We have shown that the albumin gene is regulated at the transcriptional level during foetal development but is predominantly controlled by a post-transcriptional mechanism in postnatal development.

The overall expression of the Alpha GST gene during foetal development is regulated predominantly at the transcriptional level. A switch from transcriptional control during foetal development to post-transcriptional control during postnatal development, including adulthood, was observed. A similar mode of developmental regulation of the Mu family GST gene during liver development was also observed. Thus, like Alpha class GST, the expression of Mu class GST appeared to be controlled by 'mixed' regulation, that is transcriptional control during foetal development and post-transcriptional control postnatally. A 'mixed' regulation for ligandin (an alternative name for GSTs containing subunit 1) during liver development has been reported by Panduro *et al.* (1987). They reported a transcriptional control of ligandin gene during foetal development and a post-transcriptional control durig the postnatal period. Although the Alpha and Mu class GSTs showed a similar pattern of gene regulation during liver development, it is not known whether they are regulated by the same mechanism. In contrast, GST 7–7 appears to be regulated predominantly by transcription throughout the period of liver development under observation. Thus the mechanisms regulating the expression of the three GST genes are separate and distinct, even though they are members of a multigene family. Panduro *et al.* (1987) have reported that isoenzymes R17 and TF-1, both of which are members of the cytochrome *P*-450 multigene family, showed differential regulation during liver development. The expression of R17 during foetal development is regulated at the transcriptional level whereas post-transcriptional controls operate postnatal expression of the gene. GST subunit 7, like cytochrome *P*-450 TF-1, is predominantly controlled at the transcriptional level throughout liver development.

In conclusion, our study reveals a differential expression of the three major classes of the GST multigene family, Alpha, Mu and Pi, during a sequential stage of liver development from the 13-day gestation to 10-day postnatal period. The Pi class GST is essentially a foetal protein and displays a unique expression during development and is regulated predominantly at the level of transcription. The Alpha and Mu class GSTs are essentially mature proteins and are regulated by 'mixed' mechanisms during liver development, displaying transcriptional control during foetal development and post-transcriptional control in the postnatal period. The significance of these changes in the expression of GSTs for the physiology of the developing animal are yet to be understood. The apparent vulnerability of the newborn rat to substances detoxified by GSTs is particularly interesting and may in part explain the susceptibility of this age group to carcinogens.

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